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EFFECTS OF WALNUT LEAF EXTRACTS PREPARED IN DIFFERENT SOLVENTS ON SEED GERMINATION AND SEEDLING GROWTH OF WHEAT

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Abstract. In this study, effects of walnut leaf extracts prepared in six different solvents, i. e. water, ethanol, methanol, aseton, tetrahydrofuran (THF) and dimethyl sulphoxide (DMSO), on seed germination and post-germinative seedling growth of wheat (*Triticum vulgare* L. cv Altay) have been investigated. Among the treatments, DMSO extract was only seen to inhibit germination percentage of the seeds at second day. It also decreased germination rate of the seeds significantly with respect to control. Root elongation was inhibited significantly by DMSO and water extracts, whereas, shoot elongation was inhibited by the treatments of DMSO, water, THF and methanol extracts in range of effectiveness grade from high to low. Root and shoot fresh weights were decreased by all the treatments. However, the highest inhibitory effect was generally found to be DMSO extract both in seed germination and seedling growth parameters. Thus, it was discussed in the manuscript that DMSO may be more efficient and proper solvent in extraction of walnut leaf.

Keywords: seed germination, seedling growth, solvents, walnut leaf extracts.

AIMS AND BACKGROUND

Chemical interactions 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 volatilisation from leaves and degradation of dead plant parts. Allelochemicals become stressful when they are toxic, and they are generally with 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[1–3].

The inhibitory effect of walnut on associated plants is one of the oldest examples of allelopathy. The chemical responsible for walnut allelopathy is juglone

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(5-hydroxy-1,4 naphthoquinone), and it has been isolated from many plant species in the walnut family including *Juglans nigra*, *Juglans regia* and others[4–7]. The juglone reduced and nontoxic form hydrojuglone is abundant in root and leaves of walnut tree. When exposed to the air or to some oxidising substance it is oxidised to 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[8,9]. Walnut has been reported to be toxic both to herbaceous and woody plants[10,11]. Juglon beneficial effect is rare that may be a few plant species. For example, muskmelon growth has been increased both by juglone and walnut leaf extracts[12].

Walnut leaf extracts are used in allelopathic research because of its juglone content, and it has also been shown to have herbicidal, antimicrobial, and antioxidant activities[2,3,13]. Several solvents such as distilled water, ethanol, methanol, acetone are being used in preparation of walnut leaf extract by different researchers. 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 6 different solvents and to find out the most efficient solvent.

EXPERIMENTAL

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[14]. Further 10 g dried leaf were homogenised using a Waring Blender in 100 ml of distilled water, ethanol, mathanol, acetone, 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 was dissolved in 50 ml distilled water. 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 kept in a refrigerator until treatment.

Application of treatments. Wheat seeds surface sterilised 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. Then the dishes were left in an incubator at 25°C in continuous dark. During 5 days, germination percentages were recorded daily, and the end of 5th day the postgerminative growth of the seeds was determined by measuring lengths and fresh weights of the seedlings.

The treatments were as follows:
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 acetone;
6. Walnut leaf extract prepared in THF;
7. Walnut leaf extract prepared in DMSO.

RESULTS AND DISCUSSION

Walnut leaf extracts prepared in 6 different solvents, i.e. water, ethanol, methanol, acetone, tetrahydrofuran (THF) and dimethyl sulphoxide (DMSO), on seed germination and post-germinative seedling growth of wheat (*Triticum vulgare* L. cv Altay) have been found to have inhibitory effect at different levels. Among the treatments, only DMSO extract was seen to inhibit significantly germination percentage of the seeds at second day (Table 1). It also decreased germination rate of the seeds significantly with respect to control. Root elongation was inhibited significantly by DMSO and water extracts, whereas shoot elongation was inhibited by the treatments of DMSO, water, THF and methanol extracts in range of effectiveness grade from high to low. Root and shoot fresh weights were decreased by all the treatments (Table 2). However, the highest inhibitory effect was generally found to be manifested by the DMSO extract both in seed germination and seedling growth parameters.

We preferred to use wheat seed in this study, because juglone and walnut leaf extracts were found to inhibit its germination and seedling growth in a previous study[12]. Our study proves the previous study, as well. In both studies, walnut leaf extracts have shown more inhibitory effect on seedling growth than on seed germination. This result is in agreement with the data of previous works that seedling growth is much more sensitive to the walnut extracts than seed germination[10,12,15].

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 has been found that the DMSO extract has the highest inhibitory effect on seed germination and seedling growth compared to other treatments (Tables 1 and 2). It is difficult to explain exactly why DMSO is the most efficient solvent among the other solvents used in this study. In addition, there is no study to compare our results. We suppose that DMSO may solve juglone existing in the walnut leaf more efficiently than the other solvents used in the study. We do not know the mechanism of the effect of DMSO. The efficient solubility of juglone in DMSO may be due to similarities of their chemical polarities. On the other hand,

in general, DMSO is being used to solve some dyes such as hemotoxylon which is used in dying cancer tissues[16]. In this respect, juglone chemical is a natural dye of walnut in brownish colour, as well.

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

Walnut leaf extracts	Days				
	1	2	3	4	5
Control (distilled water)	0	90a	95a	95a	95a
Water extract	0	85a	90a	95a	95a
Ethanol extract	0	75ab	90a	90a	90a
Methanol extract	0	75ab	85a	90a	90a
Acetone extract	0	75ab	85a	90a	90a
THF extract	0	75ab	85a	90a	90a
DMSO extract	0	70b	80a	80a	85a

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

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

Walnut leaf extracts	Germination rate	Root length (cm)	Shoot length (cm)	Root fresh w. (mg)	Shoot fresh w. (mg)
Control (distilled water)	75a	8.0a	5.5a	800a	1200a
Water extract	74a	3.4b	1.6b	300bc	200c
Ethanol extract	69ab	4.1ab	3.1ab	400b	600bc
Methanol extract	68ab	3.3ab	2.2b	300bc	400bc
Acetone extract	68ab	4.9ab	3.7ab	500b	900b
THF extract	68ab	4.1ab	1.7b	400b	500bc
DMSO extract	63b	2.0b	0.8c	200c	300c

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

As seen in Table 2, shoot elongation was inhibited much more than root by DMSO extract. That is, although root elongation was decreased 4-fold by DMSO extract according to control, the inhibition was about 7-fold in the case of shoot elongation. However, this inhibition difference did not reflect on fresh weight that both root and shoot fresh weights were inhibited 4-fold by DMSO extract. In former studies, it has been shown that inhibitory effects of allelochemicals are different on root and shoot growth because phytotoxicity is a function both of allelochemical and plant species studied[10,15,17].

In previous studies, juglone has been shown to have herbicidal effect on several weeds[18], antimicrobial effects on bacteria and fungi[19–21], anticancer effect on tumor cells[22–27], and antioxidant effect on related enzymes[13,28]. However, juglone use for these purposes is not an economical approach becuase it is a rather expensive chemical, whereas, since walnut leaf extract is rich in juglone, instead of

juglone it may be used for the practical purposes as mentioned above. In addition, as concluded in this study, DMSO may also be used in preparation of efficient walnut leaf extract.

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