



RESEARCH PAPER

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Assessment of hydro-priming on overcoming the allelopathic effect of walnut leave aqueous extract on seed germination and seedling growth

Hossein Khoshvaghti¹, Ramin Lotfi^{2*}

¹*Department of Agriculture, Payame Noor University (pnu), Iran*

²*Department of Plant Eco-physiology, Faculty of Agriculture, University of Tabriz, Iran*

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Abstract

Juglone (5-hydroxy-1,4-naphthoquinone) is an allelochemical responsible for walnut allelopathy. In order to assess the effects of hydro-priming on overcoming the allelopathic stress of walnut leave aqueous extract (control, 1.5, 3 and 6 percent) on seed germination and seedling growth of wheat and corn a laboratory experiment was conducted in University of Payame noor, Iran. Results showed that with increasing of walnut leave aqueous extract, especially sever concentration (6 %), germination percentage, shoot length of wheat and corn were inhibited. Root length and germination rate of corn and root dry weight of wheat were also declined with increasing of extract concentration. The highest germination percentage and root length of wheat were obtained by seeds hydro-primed. However, seeds of corn were not affected by hydro-priming. Significantly interaction of extract × hydro-priming on shoot dry weight suggested that hydro-priming can improve seedling growth of wheat under juglone allelochemical conditions. Therefore, hydro-priming can be used to promote seed germination and seedling growth of wheat seeds and may be beneficial to the arrangement of intercropping system(s) of juglone-sensitive species between walnut trees in the same field.

*Corresponding Author: Ramin Lotfi ✉ rlotfi67@yahoo.com

Introduction

In common definition, the allelopathy is any direct or indirect, useful or useless effects of plants on other ambient plants by germinating and growing via created chemical material and transmittal (Rice, 1984). Allelopathy is characterized by a reduction in plant emergence or growth reducing their performance in the association (Florentine *et al.*, 2006).

The readily visible effects of allelochemicals on the growth and development of plants include inhibited or retarded germination rate, root or radicle and shoot or coleoptile extension and led to production of swelling or necrosis of root tips and increased number of seminal roots (Rice, 1974). The chemicals have harmful effects on the crop in the eco-system resulting in the reduction and delaying of germination, mortality of seedlings and reduction in growth and yield (Mcworthier, 1984; Herro and Callaway, 2003).

Seed priming is soaking of seeds in a solution of any priming agent followed by drying of seeds that initiates germination related processes without radicle emergence (McDonald, 1999). Common priming techniques include osmo-priming (soaking seeds in osmotic solutions), halo-priming (soaking seeds in salt solutions) and hydro-priming (soaking seeds in water) (Ghassemi-Golezani *et al.*, 2008). Seed priming has been reported to improve seed germination and seedling emergence (Abdulrahmani *et al.*, 2007; Ghassemi-Golezani *et al.*, 2010).

Juglone (5-hydroxy-1,4-naphthoquinone) is an allelochemical responsible for walnut allelopathy, and the inhibitory effect of black walnut (*Juglans nigra*) on associated plant species is one of the oldest examples of allelopathy (Davis, 1928; Rice, 1984; Rizvi & Rizvi, 1992). Juglone has been isolated from many species in the walnut family (Juglandaceae), including *J. nigra* L., *J. regia* L., and some others (Prataviera *et al.*, 1983). Hydrojuglone, a colourless, non-toxic reduced form of juglone, is abundant, especially in the leaves, fruit hulls, and roots of

walnut. When exposed to air or some other oxidizing substances, hydrojuglone is oxidised to its toxic form, juglone (Segura-Aguilar *et al.*, 1992). Rain dissolves and washes juglone from the leaves and transfers it into the soil, where this toxic substance affects neighboring herbaceous and woody plants that absorb it into their roots (Rietveld, 1983). According to many studies (Ghassemi-Golezani *et al.*, 2008, 2010) that indicated priming improved germination and vigor of seed during water or salt stress, thus this experiment was tested to assessment the effect of hydro-priming on overcoming the allelopathic effect of walnut leave aqueous extract on seed germination and seedling growth.

Materials and methods

A factorial experiment in completely randomized block design with three replications was conducted under laboratory conditions to evaluate the effects of hydro-priming on overcoming the allelopathic stress of walnut leave aqueous extract on seed germination and seedling growth of wheat and corn, in the University of Payamnour in Iran. The Seeds of wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) were divided into two sub-samples, one of which was kept as control (unprimed) and another sub-sample was soaked in distill water for 4 h in an incubator adjusted to 15 °C.

Thirty grams of walnut leaves powder were suspended in 500 ml distilled water and shaken for 48 hours by a horizontal rotary shaker in room temperature to obtain 6 percent concentration of aqueous extract. The solution was diluted appropriately with distilled water to give the other extract concentrations of 3 and 1.5 percent.

In this experiment, 10 seeds of each species were placed on Whatman filter paper in 9 cm petri dishes. Four ml of distilled water and final walnut leaves aqueous extract concentrations were added to each petri dish. Their solutions were applied where required during the course of experiment. Then, their petri dishes were incubated at 20±°C and germinated seeds (protrusion of radicle by 2 mm)

were counted every day up to 7 days. Germination rate (GR) was calculated according to Ellis and Roberts (1980):

$$GR = \frac{\sum n}{\sum D.n}$$

Where n is the number of seeds germinated on day D, D is the number of days from the beginning of the test and GR is the mean germination rate. Then percentage of germination was also determined.

At the end of test, length of root and shoot were measured. Root and shoot of each sample were then

dried in an oven at 80 °C for 24 hours (Perry, 1977) and mean dry weight of root and shoot for each treatment at each replicate was determined.

All the data were analyzed on the basis of experimental design, using MSTATC and SPSS-16 software. The means of each trait were compared according to Duncan multiple range test at $P \leq 0.05$ and standard error values. Excel software was used to draw figures.

Table 1. Analysis of variance of the effects of hydro-priming on seed and seedling growth of wheat under different extract concentration of walnut leaves.

Source	df	Germination percentage	Germination rate	Root length	Soot length	Root dry weight	Shoot dry weight
Replication	2	329.167	0.006	3.617	0.555	0.0001	0.0001
Extract (A)	3	2681.944**	0.035	5.698	12.835*	0.003*	0.001
Hydro-riming (B)	1	2204.167**	0.001	20.167*	4.335	0.0001	0.001
A × B	3	381.944	0.119	0.938	1.922	0.0001	0.001*
Error	14	152.976	0.052	3.785	2.253	0.0001	0.0001

*, ** Significant at $p < 0.05$ and $p < 0.01$, respectively

Table 2. Analysis of variance of the effects of hydro-priming on seed and seedling growth of corn under different extract concentration of walnut leaves.

Source	df	Germination percentage	Germination rate	Root length	Soot length	Root dry weight	Shoot dry weight
Replication	2	129.167	0.001	48.012	2.337	0.001	0.0001
Extract (A)	3	594.44**	0.192**	65.907**	16.429**	0.0001	0.0001
Hydro-riming (B)	1	266.667	0.0001	0.023	0.753	0.0001	0.0001
A × B	3	44.44	0.013	11.357	6.343	0.0001	0.0001
Error	14	124.405	0.004	17.282	4.790	0.0001	0.0001

*, ** Significant at $p < 0.05$ and $p < 0.01$, respectively

Table 3. Means of the germination and seedling growth factors of corn for extract treatment.

Treatment	Germination percentage (%)	Germination rate (day ⁻¹)	Root length (cm)	Shoot length (cm)
Extract (%)				
Control	43.33 ^a	0.77 ^a	14.41 ^a	10.04 ^a
1.5	33.33 ^{ab}	0.71 ^a	13.33 ^a	9.37 ^{ab}
3	26.67 ^b	0.48 ^b	13.00 ^{ab}	7.33 ^{ab}
6	20.05 ^b	0.40 ^c	9.6 ^b	6.54 ^b

Different letter each column indicate significant difference at $p < 0.05$

Results

Analysis of variance of the data for germination and seedling growth factors indicated that germination percentage and shoot length of wheat and corn were

significantly affected by extract concentration. The effects of aqueous extract of walnut on root dry weight of wheat, and germination rate and root length of corn were also significant (Table 1 & 2). The

effect of hydro-priming was significant for germination percentage and root length of wheat. The interaction of extract \times hydro-priming was only significant for shoot dry weight of wheat seedling (Table 1).

Germination percentage and Germination rate

Germination percentage of wheat (Fig. 1) and corn (Table 3) were significantly inhibited with increasing of extract concentration of walnut leaves. In both species, germination percentage in extract concentration of 1.5 percent and control was statistically similar and had most germination than that of 3 and 6 percent concentration. Hydro-priming improved germination percentage of wheat seeds (Fig. 2).

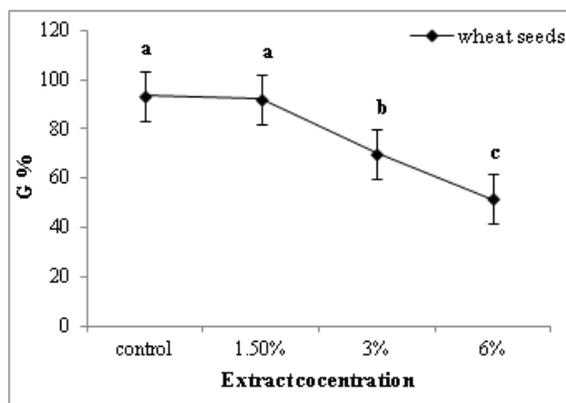


Fig. 1. Effects of different concentration (%) of walnut leaves extract on Germination percentage (G %) of wheat. Different letter indicate significant difference at $p \leq 0.05$. Bar = \pm SE, $P < 0.05$.

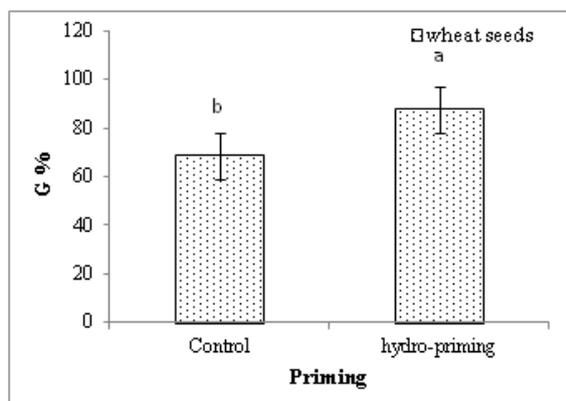


Fig. 2. Effects of hydro-priming on on Germination percentage (G %) of wheat. Different letter indicate significant difference at $p \leq 0.05$. Bar = \pm SE, $P < 0.05$.

Germination rate of corn (Table 3) was significantly decreased with increasing of extract concentration. The lowest germination rate was showed in the sever extract concentration (6%).

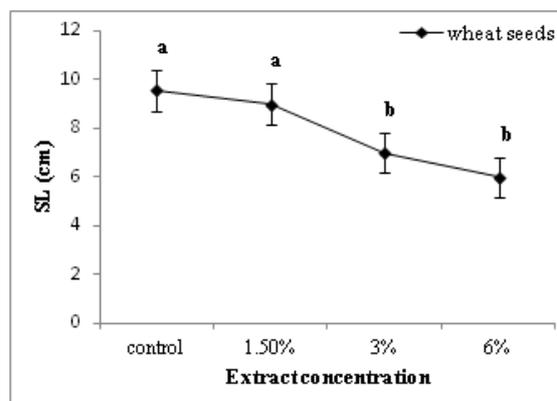


Fig. 3. Effects of different concentration (%) of walnut leaves extract on shoot length (SL) of wheat. Different letter indicate significant difference at $p \leq 0.05$. Bar = \pm SE, $P < 0.05$.

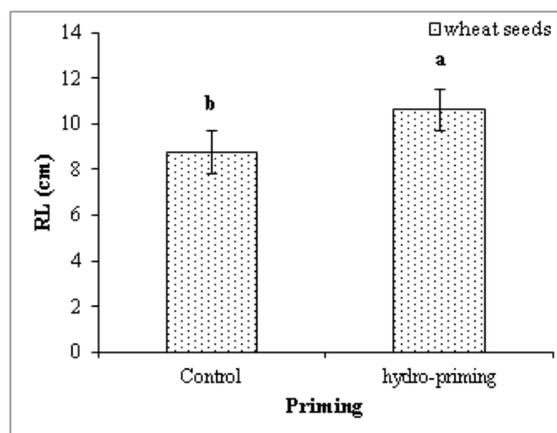


Fig. 4. Effects of hydro-priming on root length (RL) of wheat. Different letter indicate significant difference at $p \leq 0.05$. Bar = \pm SE, $P < 0.05$.

Root and shoot length

Root and shoot length of corn (Table 3) and shoot length of wheat (Fig. 3) were inhibited as a result of extract concentration increased. As, sever extract concentration (6 percent) had the least shoot and root length in both species. In corn seedling the difference between control, 1.5 and 3 percent of extract concentration was not significant.

Root length of wheat seedling was improved as a result of hydro-priming. As, seeds hydro-primed of

wheat had 21 percent more root length in comparison with unprimed seeds (Fig. 4).

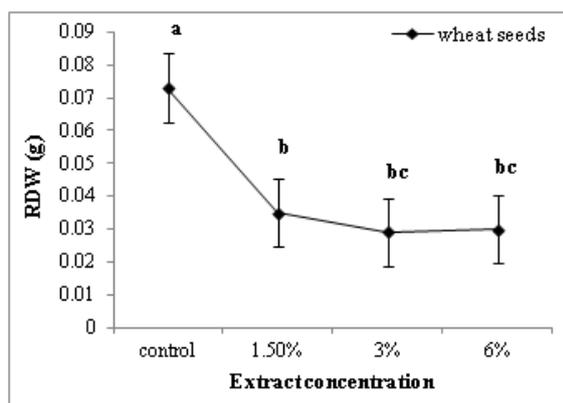


Fig. 5. Effects of different concentration (%) of walnut extract on root dry weight (RDW) of wheat. Different letter indicate significant difference at $p \leq 0.05$. Bar = \pm SE, $P < 0.05$.

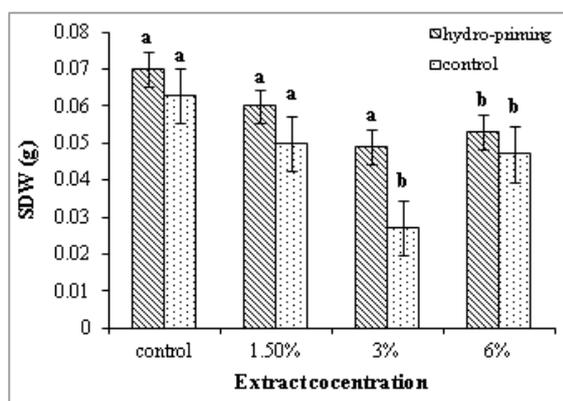


Fig. 6. Interaction of extract concentration and hydro-priming on shoot dry weight (SDW) of wheat. Different letter indicate significant difference at $p \leq 0.05$. Bar = \pm SE, $P < 0.05$.

Root and shoot dry weight

Root dry weight of wheat seedling was declined with usage extract of walnut leaves. As, the extract concentration of 1.5, 3 and 6 % had similarly the least root dry weight than that of control (Fig. 5).

The significantly interaction of extract \times hydro-priming (Fig. 6) showed that with increasing extract concentration, shoot dry weight was decreased. Also, hydro-primed seeds had quantitatively more shoot dry weight in compared to unprimed seeds. Especially, in extract concentration of 3 percent, hydro-primed seeds were produced higher shoot dry weight than seeds unprimed in this concentration.

Discussion

This study has shown that juglone inhibitory effects on germination of corn were more than that of seedling growth of this specie (Table 3). In generally, corn against juglone allelopathic effects was resistant in comparison with wheat. Experiments conducted by Jose and Gillespie (1998), showed that corn was less negatively affected by juglone than soybeans. This supports our conclusion that corn is more tolerant of nut allelochemicals than other vegetable varieties.

In generally, seedling growth was much more sensitive to juglone than seed germination that similarly reported by Terzi (2008, 2009). The literature on the physiological action of juglone shows that the mechanism of action is not well understood: juglone inhibits plant growth by reducing photosynthesis and respiration (Hejl *et al.*, 1993; Jose & Gillespie, 1998), increasing oxidative stress (Segura-Aguilar *et al.*, 1992), and blocking protein synthesis in the transcription stage by inhibiting RNA polymerases (Chao *et al.*, 2001).

The effect of hydro-priming on germination and seedling growth of corn was not significant (Table 2). Many study reported that effects of seed priming are clearly in weak and sensitive seeds. As, in this study wheat was more sensitive to juglone and significantly affected by hydro-priming (Table 1) and germination and seedling growth of this specie was improved (Fig. 2, 4, 6). It might be due to early synthesis of nucleic acids e.g. DNA, RNA and proteins during hydration process, which ultimately resulted in improved energy of germination of seeds (Bray *et al.*, 1989; Dell'Aquila and Bewley, 1989). Rapid germination of seeds ultimately could lead to the production of larger seedlings (Ghassemi-Golezani *et al.*, 2010).

In conclusion, the present study shows that pretreatment of seeds with hydro-priming alleviated the inhibitory effect of juglone on seed germination and seedling growth. This may be beneficial to the arrangement of intercropping system(s) of juglone-

sensitive species between walnut trees in the same field.

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