World Journal of Environmental Biosciences
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Available Online at: **www.environmentaljournals.org**

Volume 6, Supplementary: 1-5

ISSN 2277- 8047

Seed Priming to Overcome Autotoxicity of Alfalfa (Medicago sativa)

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ABSTRACT

Autotoxicity refers to any direct or indirect harmful effect of one plant on another plant of the same species through the production and release of different chemicals. Seed priming is a pre-sowing treatment to improve seedling development and increasing the rate of germination and plant resistance to various stresses. In order to determine the effect of different seed priming methods on reducing allelopathic property in alfalfa, a factorial experiment was conducted in a completely randomized design with four replications at Islamic Azad University of Karaj, Iran during 2016. Experimental factors consisted of various seed priming methods in five levels (hydro- priming, osmo-priming, chemo-priming, priming-hormone and control without priming), different concentrations of Alfalfa root extract in four levels (0, 33, 66 and 100%) and alfalfa cultivar in two levels (Hamadani and Gharah-Younjeh). The results of this study showed that the effect of various seed priming methods, extract and cultivar concentration, as well as the interaction between priming and extract concentration on the studied traits were meaningful. Among seed priming methods, hydro priming and osmo priming (PEG) were the most influential methods of priming. The results showed that, with increasing alfalfa extract concentrations, all the evaluated traits were decreased significantly. Comparison of the mean interactions between priming methods and the concentration of extracts showed that with increasing the concentration of extract in all priming methods, germination decreases; however, the slope of the decrease in different treatments was different so that hydro priming and osmo priming methods were more successful in reducing the harmful effects of high concentrations of alfalfa extract. In general, the results of this experiment showed that hydro priming and osmo-priming of alfalfa seeds can significantly reduce the negative effects of autotoxicity in this important plant.

Keywords: *Hydro-priming, osmo-priming, germination percentage, radicle length, seedling weight*

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INTRODUCTION

Autotoxicity refers to any direct or indirect harmful effect of one plant on another plant of the same species through the production and release of different chemicals (Kohli et al., 2001). These chemicals are part of the plant's secondary metabolites and have different effects depending on the plant species and various biochemical reactions. The chemical compounds are found in different parts of some plants such as root, leaf, stem, flower and fruits, and can affect germination, root and shoot growth of target plants and the number of soil microorganisms (Golzardi et al., 2015). Autotoxicity is a form of allelopathy which inhibits germination and growth of a species of a same type by self-produced toxins. In other words, autotoxicity refers to adverse effects of one species by self-produced toxins; but it should be noted that the majority of allelopathic research has been conducted regarding the effects of one species on another and for this reason, there is a little research on the effect of a species on its own species or a plant on similar plants (Mondani et al., 2010). Plant allelopathy is a component of plant interactions in the ecosystems to control weeds in the crop rotation; however, it should be noted that allelopathy may exert negative effects on the growth and yield of crops as well. For this reason, the role of allelopathy should be considered in selecting the type of plants that are planted in the crop rotation. Many plants have generally been considered to be allelopathic and secretions from live tissue or plant residue decomposition after death can affect adjacent plant life (Baghestani, 1999). So far, extensive research has been done on the effects of allelopathic crops which indicates the negative and adverse impacts of crops on weeds (Sabahie et al., 2014; Golzardi et al., 2009) and other crops (Golzardi et al. 2014; Faridmarandi et al., 2014). Studies show that residues of cultivated alfalfa also leave adverse effects on the future crops. It seems that the toxicity level of the residues is related to the effect of environmental and seasonal factors on plant growth, such as day length, temperature, and access to nutrients (Mason-Sedun and Jessop, 1989). In addition, the survival and durability of toxic compounds of alfalfa residues depend on climatic factors (Mason-Sedun and Jessop, 1988). Most examples regarding the autotoxicitic effects can be found in perennial plants such as pine and perennial grasses such as darnel and alfalfa, which may be due to the long establishment of these plants; therefore, it seems that the release of secondary compounds and long-term survival in the surrounding area of these plants can lead to autotoxicitic effects on the plants being

established from the same plants (Mondani et al., 2010). Priming is one of the most significant treatment methods increasing seeds' germination potential (Farooq et al., 2006). Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre germination metabolic activity prior to emergence of the radicle and generally enhances germination rate and plant performance (Taylor & Harman, 1990). During priming, the seeds are partially hydrated to promote the metabolic activities before germination, while preventing roots from rising. They then dried back to initial moisture content (McDonald, 2000). Various priming treatments are used to increase the speed and synergy of seeds germination (Hesabi et al., 2014). Among different priming treatments we can name osmo-priming (having seeds soaked in osmotic solutions such as poly ethylene glycol (PEG)), halo priming (salt treated seeds), hydro-priming (seeds soaked in water). After being placed in their bed, the primed seeds are germinated sooner and as a result, the establishment of the plants from these seeds takes place faster, better and more uniformly. The realization of such conditions makes it possible to bio-ecologically provide a special position for plants derived from primed seeds (Farooq et al., 2006). Hessabi et al. (2014) reported that hydro- priming and osmo-priming increased significantly the percentage and speed of germination as well as alfalfa seedling growth. Therefore, regarding alfalfa's allelopathic potential and the significance of this strategic plant, it seems necessary to find ways of reducing the inhibiting factors. Since it is proved that priming treatments can affect various stresses, the methods may be helpful in reducing alfalfa's allelopathic potential. Hence, this experiment was carried out to assess this possibility by implementing different priming techniques.

MATERIALS AND METHODS

In order to evaluate the effect of different priming treatments on reducing allelopathic potential of alfalfa, an experiment was conducted with a factorial arrangement based on completely randomized design with four replications at Research Laboratory, Faculty of Agriculture, Islamic Azad University, Karaj Branch during 2014. Factors of test consisted of various seed priming methods in five levels (hydro- priming, osmo-priming, chemo-priming, priming-hormone and control without priming), different concentrations of Alfalfa root extract in four levels (0, 33, 66 and 100%) and alfalfa cultivar in two levels (Hamadani and Gharah-Younjeh).

Preparation of alfalfa root extract

initially,alfalfa roots were randomly selected and collected from farms around Karaj. Then the roots were completely dried at 80 ° C for 5 days.In the next step, the roots were grinded until they were completely powdered. 75 g of powdered roots was mixed with 1.5 liters of distilled water to make alfalfa extract. The resulting solution was poured into a Arlene and Arlene was placed on a shaker for 24 hours at a speed of 200 rpm (Golzardi et al., 2014). The obtained liquid extract, after passing through four Whatman filter paper, was centrifuged for about 10 minutes at 5000 rpm. The final extract was poured inside the cleaned glass container and was kept in a refrigerator to be used. The final extract was set as 100% concentration and was prepared by adding distilled water to concentrations of 66% and 33%. Distilled water was used for zero concentrations (Golzardi et al., 2015).

Seed Priming Methods

For hydro-priming, we used distilled water to soak seeds for 12 hours at 25°C; then, the seeds were removed from water. For osmo-priming with poly ethylene glycol (PEG), the seeds were placed in PEG solution with potential 0.2 M for 12 hours at 25°C; then, the seeds were removed from the solution. For hormone-priming, 1 g of gibberellin was dissolved in 100 cc of distilled water and the seeds were placed inside it for 12 hours. For chemo-priming, we put the seeds in 500 ppm NO3 for 12 hours at 25°C and the seeds were then removed from the solution and dried to their original weight. After priming, the seeds were washed with distilled water and were exposed them to air for 24 hours for its humidity returns to initial state, before priming (Hesabi et al., 2014).

Germination Test

A petri dish (8 cm diameter) was selected for the experiment on which 2 paper filters were placed for seed cultivation. In each dish, 25 seeds are placed in which 6 ml extract was added. As a control experiment, petri dishes containing distilled water are also prepared. To prevent evaporation of the extract, petri dishes were closed by Parafilm and were transferred to a germinator set by 15 °C (day and night) with 16 hour running cycle. Seedling count and aeration were conducted on a daily basis according to ISTA instruction. The final counting and measurement of root and shoot length, fresh and dry weight, was also performed separately for each species again according to ISTA instruction. To obtain the dry weight of seedlings, the samples were dried and weighed after 4 hours at 60°C (Golzardi et al., 2015; Mondani et al., 2010). For statistical analysis of the data, SAS software was used at the end of the experiment and the LSD test was used at 5% level to compare the averages. Graphs were plotted with Excel.

RESULTS AND DISCUSSION

Effect of experimental treatments on alfalfa germination percentage

The results of analysis of variance showed that the effect of priming method, extract concentration and cultivar on germination percentage of alfalfa seed was significant at 1% probability level. In addition, the interaction effect of priming method × concentration of extract was significant at 1% probability level, but other interactions were insignificant (Table 1). The results of comparison of mean showed that seed germination percentage was decreased with increasing concentration of alfalfa extract (Table 2). This will prove the existence of autotoxicity in alfalfa. Miller (1983) conducted an experiment to determine the effects of autotoxicity in alfalfa and its sustainability. The results showed that the toxic factors from the previous crop contributed to the re-growth of alfalfa. The highest seed germination percentage (94.5%) was observed at zero concentration of extract (distilled water) and hormone priming. Additionally, at zero concentrations of extract, osmo-priming and hydro-priming were placed in the top statistical group (a) with 93% and 94% germination, respectively. Hassabi et al. (2014) reported that hydro-priming and osmo-priming significantly increased alfalfa seed germination percentage. At a concentration of 100% alfalfa extract, the maximum seed germination percentage was also observed in hydro-priming and osmo-priming treatments in 100% alfalfa extract (35% and 37% seed germination respectively). The lowest seed germination percentage (1.5%) was obtained in non-priming treatment and 100% alfalfas extract (Table 2). Golzardi and Aghamollaei (2017) reported that different methods of seed priming had different effects on autotoxicity reduction in

forage rape, so that the hydro-priming and osmo-priming methods with polyethylene glycol were the most effective priming methods for decreasing autotoxicity in forage rape. In order to investigate the impact of hydro-priming on overcoming of allelopathic effect of the aqueous extract of walnut leaves, Khoshvaghti and Lotfi (2013) suggested that the highest germination percentage in wheat occurred when the seeds were placed under the influence of hydro-priming.

Table 1. Analysis of variance for the effect of experimental treatments on seed germination and seedling growth of alfalfa

S.O.V.	Mean of Squares (M.S.)				
	d.f.	Germination percentage	Root length	Shoot length	Seedling fresh weight
Seed priming (a)	4	20993.10**	628.52**	1323.61**	0.7724 **
Extract concentration (b)	3	27635.30**	1353.76**	3440.01**	1.5388 **
Alfalfa cultivar (c)	1	6400.90**	2.35 n.s.	134.37*	0.1778 **
axb	12	844.30**	532.41**	120.23**	0.0636 **
axc	4	292.90 n.s.	24.20 n.s.	22.99 n.s.	0.0161 n.s.
axb	3	242.23 n.s.	4.40 n.s.	1.34 n.s.	0.0069 n.s.
axbxc	12	54.90 n.s.	214.68 n.s.	26.05 n.s.	0.0075 n.s.
Error (E)	120	126.36	13.63	31.01	0.0082
C.V.	-	10.51	9.27	8.46	7.35

** and * significant at 0.05 and 0.01 probability level respectively, n.s. = non-significant

Effect of experimental treatments on root length of alfalfa

The results of analysis of variance showed that the effect of priming method, extract concentration on root length of alfalfa was significant at 1% probability level. In addition, the interaction effect of priming method × concentration of extract was significant at 1% probability level, but other interactions were insignificant (Table 1). With increasing concentration of alfalfa extract, the root length was significantly decreased (Table 2). In examining the effects of autotoxicityin rapeseed, Mondani et al., 2010, demonstrated that the length of root of rapeseed was reduced in the presence of rapeseed root extract. The highest root length percentage (20.4%) was observed at zero concentration of extract (distilled water) and hydro priming treatment. Additionally, at zero concentrations of extract, chemo priming and hydro-priming were placed in the top statistical group (a) with 18.9% and 17.3% root length, respectively. Hassabi et al. (2014) reported that hydro-priming and osmo-priming significantly increased alfalfa root length percentage. At a concentration of 100% alfalfa extract, there was no significant difference between different seed priming methods in stem length. Whereas, the lowest root length (0.2 mm) was obtained in non-priming treatment and 100% alfalfa extract (Table 2). Golzardi and Aghamollaei (2017) reported in different concentrations of extract, the highest root length of forage rape was observed in hydro-priming and osmo-priming treatments. In order to investigate the impact of hydro-priming on overcoming of allelopathic effect of the aqueous extract of walnut leaves, Khoshvaghti and Lotfi (2013) suggested that the highest percentage of root length in wheat occurred when the seeds were placed under the influence of hydro-priming.

Effect of experimental treatments on shoot length of alfalfa

The results of analysis of variance showed that the effect of priming method, extract concentration on shoot length of alfalfa was significant at 1% probability level. In addition, the interaction effect of priming method × concentration of extract was significant at 1% probability level, but other interactions were insignificant (Table 1). With increasing concentration of alfalfa extract, the shoot length was significantly decreased (Table 2). In examining the effect of autotoxicityin rapeseed, Mondani et al., 2010, demonstrated that the shoot length of rapeseed was reduced in the presence of rapeseed root extract. The highest shoot length percentage (29.7%) was observed at a concentration of 33% extract and hydro priming treatment. Additionally, at zero concentration of extract (distilled water), chemo-priming and hydro-priming were placed in the top statistical group (a) with 28.3% and 27.6% of shoot length, respectively. Hassabi et al. (2014) reported that hydro-priming and osmo-priming significantly increased shoot length in alfalfa. At a concentration of 100% alfalfa extract, there was no significant difference between different seed priming methods in shoot length. Whereas, the lowest shoot length was obtained in non-priming treatment and 100% concentration of alfalfa extract (Table 2). Golzardi and Aghamollaei (2017) reported that in different concentrations of extract, the highest shoot length of forage rape was observed in hydro-priming treatment. In order to investigate the impact of hydro-priming on overcoming of allelopathic effect of the aqueous extract of walnut leaves, Khoshvaghti and Lotfi (2013) suggested that the highest percentage of shoot length in wheat occurred when the seeds were placed under the influence of hydro-priming.

Table 2. Interaction effect of seed priming method and extract concentration on seed germination and seedling growth of alfalfa

Treatment		Germination percentage	Length of root (mm)	Length of shoot (mm)	Seedling fresh weight (g)
Priming method	Alfalfa extract concentration				
Control	0	35.00 e	4.538 e	8.455 fg	0.11238 e
	33%	23.50 f	4.456 e	9.913 f	0.092388 efg
	66%	3.00 g	0.666 fg	0.749 h	0.00913 fg
	100%	1.50 g	0.208 g	0.000 h	0.00388 g
Hydro-priming	0	93.00 a	18.915 abc	28.371 ab	0.53838 ab
	33%	91.00a	16.330 c	29.704 a	0.62488 a
	66%	84.00 a-d	10.914 d	22.081 cde	0.43775 cd
	100%	37.00 e	3.123 efg	2.330 h	0.08450 efg
Osmo-priming	0	94.00 a	20.455 a	25.121 a-d	0.57900 ab
	33%	89.00 abc	17.330 abc	26.080 abc	0.55763 ab
	66%	78.00 cd	10.079 d	19.039 e	0.39875 d
	100%	35.00 e	4.205 ef	4.289 gh	0.09425 ef
Chemo-priming	0	90.00ab	19.995 ab	27.623 ab	0.57600 ab
	33%	91.00 ab	16.371 bc	23.495 b-e	0.50763 bc
	66%	81.00 bcd	11.539 d	21.829 cde	0.40288 d
	100%	26.00 ef	2.958 efg	3.083 gh	0.05875 efg
Priming hormone	0	94.50 a	16.581 bc	26.373 abc	0.56563 ab
	33%	86.00 a-d	11.673 d	19.744 de	0.57350 ab
	66%	76.00 d	9.455 d	19.289 e	0.35950 d
	100%	17.00 f	3.499 efg	4.624 fgh	0.04300 efg

In each column, means denoted by the same letter did not significantly differ at $P < 0.05$ according to LSD test.

Effect of experimental treatments on fresh weight of alfalfa seedling

The results of analysis of variance showed that the effect of priming method, extract concentration and cultivar on the fresh weight of alfalfa seedling was significant at 1% probability level. In addition, the interaction effect of priming method × concentration of extract was significant at 1% probability level, but other interactions were insignificant (Table 1). With increasing concentration of alfalfa extract, the fresh weight of alfalfa seedling was significantly decreased (Table 2). In examining the effect of autotoxicityin rapeseed, Mondani et al., 2010, demonstrated that the fresh weight of rapeseed seedling was reduced in the presence of rapeseed root extract. The highest fresh weight of alfalfa seedling (624.0%) was observed at a concentration of 33% extract and hydro priming treatment. Additionally, at zero concentration of extract (distilled water), osmo-priming and chemo-priming were placed in the top statistical group (a) with 576.0% and 573.0% of fresh weight of seedling, respectively. At a concentration of 100% alfalfa extract, the maximum weight of seedling (0.0924%) was observed in osmo-priming treatment. The lowest fresh weight of seedling (0.0038) was obtained in non-priming treatment and 100% concentration of alfalfa extract (Table 2). Golzardi and Aghamollaei (2017) reported that different methods of seed priming had different effects on autotoxicity reduction in forage rape, so that the hydro-priming and osmo-priming methods with polyethylene glycol were the most effective priming methods for decreasing autotoxicity in forage rape. In order to investigate the impact of hydro-priming on overcoming of allelopathic effect of the aqueous extract of walnut leaves, Khoshvaghti and Lotfi (2013) suggested that the highest seedling weight in wheat occurred when the seeds were placed under the influence of hydro-priming.

CONCLUSION

The results of this study showed that the effect of various seed priming methods, concentration of alfalfa root extract and cultivar, as well as the interaction between priming and extract concentration on the studied traits (germination percentage, shoot length and root, seedling fresh weight) were meaningful. Among seed priming methods, hydro-priming and osmo-priming (PEG) were the most influential methods of priming, so that in a concentration of zero (distilled water), the percentage of germination was 81.3% and in the concentration of 100% alfalfa extract, it was 23.3%. The comparison of the mean interaction between priming methods and the concentration of extract showed that with increasing the concentration of extract, germination decreases in all priming methods; however, the slope of the decrease in different treatments was different, so that hydro priming and osmo priming methods were more successful in reducing the harmful effects of high concentrations of alfalfa extract. In general, the results of this experiment showed that hydro priming and osmo-priming methods of alfalfa seeds can significantly reduce the negative effects of autotoxicity in this important plant.

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