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Using allelopathic effects of drought-tolerant plant extracts to produce natural weed controllers

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ABSTRACT

Environmental and human health requirements necessitate replacing chemical herbicides with natural ones for weed control. Allelopathy is a form of interaction among plants that may assist in producing natural herbicides, but less research has been conducted in this important area. Allelopathic effects of extracts of five drought-tolerant plant species widely used in urban landscapes were examined using five completely randomized design experiments with four replications. The species were *Rosmarinus officinalis*, *Lavandula officinalis*, *Salvia sclarea*, *Atriplex halimus*, and *Atriplex canescens*. The plant species' soluble extracts were prepared at 0, 2.5, 5, and 7.5%. The seeds' germination percentage and rates of *Taraxacum officinale*, *Conyza canadensis*, and *Tragopogon major* were measured daily after being affected by these extracts. Increasing the concentrations of all the extract types was associated with germination percentages and rate declines. The most outstanding allelopathic effects were related to *Atriplex halimus* and *Salvia sclarea* extracts, respectively. *Tragopogon major* was considered the most sensitive weed species in reducing its germination rate when affected by the landscape plant extracts, except for *R. officinalis* extract. The reduction in germination percentage of *T. major* and *C. canadensis* under *A. halimus* extract compared to the control was the highest. *A. halimus* and *S. sclarea* extracts can be used as natural weed controllers for important weeds such as *Taraxacum officinale*.

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Introduction

In agricultural and urban landscape ecosystems, plants, instead of coexistence, primarily interfere with each other. If the plants have not passed their critical growth stage, weed density can usually reduce their growth and yield (Hayashi and Numata, 1971). To manage these ecosystems, the most common method of weed control is using chemical herbicides. However, a significant number of research has been published on the different side effects of chemical herbicides on people, animals, crops, and the environment (Hasanuzzaman *et al*., 2020; Ngozi *et al*., 2020; Van Bruggen *et al*., 2018). For example, some chemical and physical properties of the pesticides such as their persistence in soil and water have caused the permanent presence of these pesticides in surface and groundwater which may cause damage to several non-target organisms (Cleary *et al*., 2019).

In research considering the use of combinations of atrazine and glyphosate, and that both can coexist in different environmental matrices (Mahler *et al*., 2017), the toxicity of these herbicides alone and in a mixture were evaluated on multiple bioindicator organisms (García-Espiñeira *et al*., 2018). Aquatic invertebrate organisms were exposed to numerous pollutants, in concentrations that vary according to multiple factors, including climatic conditions, losses through aerial drift, and application handling. The results showed higher concentrations of atrazine cause potential risks of embryotoxicity for zebra fish (Blahova *et al*., 2020).

Research shows an intensive application of chemical herbicides along with the lack of knowledge about the interaction between the product molecules and soil particles and their influence on the environment that can cause contaminations in surface and underground water sources (Andrade *et al*., 2010). The leading destination of herbicides in the environment is soil, regardless of whether they are applied pre- or post-emergence (Dos Reis *et al*., 2017). When reaching the soil, herbicides will be subject to retention, transformation, and transport processes that influence their behavior (Silva *et al*., 2007). The occurrence of rainfall or irrigation after pesticide application may cause leaching losses to groundwater that vary according to the intensity (Lewan *et al*., 2009) .On the other hand, when the herbicide molecules are leached to deeper soil layers, there is a decrease in the control efficiency, and they can also reach the water tables and promote water contamination (Amim *et al*., 2016). Therefore, excessive use of synthetic herbicides leads to tremendous environmental hazards (Aktar *et al*., 2009) including the development of herbicide-resistant weed biotypes. Therefore, for the sake of a pollution-free earth ecosystem and sustainability in crop productivity, conventional agriculture has to be improved by limiting the use of synthetic agrochemicals including chemical herbicides.

In addition to having significant negative effects on the environment, chemical herbicides have negative health effects on the human. For example, Glyphosate which is a widely used herbicide for the control of broad- and narrow-leaf weeds is considered toxicologically harmful and presents a potential association with human carcinogenesis and other chronic diseases, including mental and reproductive behaviors (Valle *et al.*, 2019). Although there have been very few studies for adequate assessment of the risk of cancer at other sites, some findings have linked herbicide exposure with colon, lung, nose, prostate, and ovary cancers as well as to leukemia and multiple myeloma. Future studies must better identify and quantify the nature of herbicide exposures. In the interim, it seems only important to monitor and promote safety practices among persons occupationally exposed to phenoxy herbicides, particularly farmers and professional sprayers (Morrison *et al.*, 1992). More safely, the use of such chemicals has to be replaced with safer weed controllers for people and the environment.

For a long time, researchers have realized the positive and negative interactions of plants on each other, although they have found that these interaction effects are generally negative (Hayashi and Numata, 1971). In this regard, the concept of allelopathy has been developed and defined as a phenomenon through which plants and organisms can produce biochemicals that affect the growth, survival, or germination of other plants (Zimdahl, 2018).

Allelopathy is considered as both beneficial or deleterious biochemical interaction between weeds or plants and micro-organisms through the production of chemical compounds that are released into the environment, and subsequently influence the growth and development of the neighboring plants (Zhang, 2003). For example, invasive alien species or weeds can distinctly inhibit seed germination and seedling growth performance of indigenous species through allelopathy. The progressively increased drought stress can potentially affect the allelopathy of invasive alien species. Thus, it is significant to explain the allelopathy of invasive alien species on seed germination and seedling growth performance of indigenous species under drought stress to obtain a deeper explanation for the main driving mechanism attributed to the successful invasion (Wu *et al.*, 2020). On the other hand, allelopathy has recently been considered an eco-friendly and biological weed control mechanism for weed management. However, there is limited research that addresses this critical research area. The recent increased interest in biological weed control methods is reasonable since its improvement and expansion will reduce the excessive use of chemical herbicides, reduce their harmful effects, and support the successful implementation of weed control (Baybordi *et al.*, 2000).

Many weed species of urban green spaces are from the Asteraceae family, but *Taraxacum officinale, Conyza Canadensis,* and *Tragopogon major* are the most commonly distributed species. *Taraxacum officinale* is a perennial plant propagated by seeds. Germination of the seeds usually occurs at depths of less than 2 cm. *Conyza canadensis,* which is an annual plant, is propagated by seeds. The fruits are nuts and covered with a hairy rough cover. The seeds are thin and have pappus, and their size is 2.1 mm. *Tragopogon major* is a biennial plant with 30 to 90 cm height and large amounts of seeds. The seeds are slim, with a length of twelve millimeters and rough tips attached to an umbrella body called pappus, which helps transfer them by winds (Macias, 1995).

These weed species are largely distributed in urban green spaces around the world. On the other hand, using chemical herbicides to control these weeds endangers citizens’ health. Biological control to reduce these species' seed banks in urban green spaces in addition to providing environmentally safe herbicides is highly recommended. Due to the importance of biological control of weeds and the lack of information on this topic for urban landscaping, this research evaluated the allelopathic impacts of some green space plant extracts on seed germination of selected weed species from the Asteraceae family.

This study specifically examined the allelopathic effects of five plant species' extracts widely used in urban landscapes, including *Rosmarinus officinalis*, *Lavandula officinalis*, *Salvia sclarea*, *Atriplex halimus,* and *Atriplex canescens,* on three weed species of *Taraxacum officinale*, *Conyza canadensis,* and *Tragopogon major.* The primary aim was to develop knowledge in the five species' allelopathic potential for future environmentally-friendly weed management plans in urban landscapes.

Material and methods

Plant materials and preparation of the extracts

The experiments were conducted in five trials. The seeds of *Tragopogon major*, *Taraxacum officinale*, and *Conyza canadensis* were collected from the seed bank of Mashhad botanic garden in Iran, and were disinfected with sodium hypochlorite 1%, and were washed with distilled water three times. A fermentation operation was performed to remove the mucilage around the seeds of *C. canadensis*.

Shoots of *Rosmarinus officinalis*, *Lavandula officinalis*, *Salvia sclarea*, *Atriplex canescens,* and *Atriplex halimus* were collected, washed, and disinfected, and then were dried at room temperature in the dark. Treatment concentrations of 2.5, 5, 7.5 g per 100 ml of distilled water were soaked for 24 hours, and distilled water was used as the control treatment to make the extracts.

The study was conducted in five separate trials for each type of extract in a completely randomized design with four replications. Fifty seeds of each weed species were placed in each petri dish as the experimental units. Filter papers were impregnated with the prepared soluble extracts and were set in room conditions at 25 °C in the dark. The seeds were daily counted to determine the percentage of their germination and speed of their germination for ten days.

Measurements

The germination percentages were recorded every day, starting from the first day after the seeds were initially placed in the petri dishes (Khayyat et al., 2014). The germination percentages were measured using the following formula:

$$GP = (Ni / N) \times 100 \quad (1)$$

Where; GP is the percentage of germination, Ni, is the number of germinated seeds on the last day of counting, and N is the total number of seeds. Also, the germination rate (RS) was measured using the method explained by Khan and Yungar (1997). The formula is as follows:

$$RS = \sum_{i=1}^{n} Si/Di \quad (2)$$

RS is the germination rate (number of germinated seeds per day), Si was the number of transduced seeds in each count, and Di was the number of days until counting the number of germinated seeds.

The data were analyzed using the SAS software package, and the graphs were drawn using an Excel software package. The means were compared using Duncan tests with a probability level of 5%.

Results

Effects of Rosmarinus officinalis extract

The results of the analysis of variance showed that the weed species, the concentration levels, and the interactions between the weed species and the concentration of *Rosmarinus officinalis* extract on germination percentage and germination rate of the weeds seeds were significant ($p \leq 0.01$) (Table 1).

Germination percentage

The effect of different concentrations of the extracts of *R.officinalis* shoots on weed germination percentage showed that the seed germination (%) was significantly ($p \leq 0.01$) reduced compared to the control by increasing the concentration of the extracts. The survey results also showed that among the three studied species, the seeds of *T. major* with the concentration level of 7.5% had the least sensitivity. The seeds of *T. officinale* had the highest sensitivity to the allelopathic effects of *R. officinalis* extract. The results showed that the tolerance threshold for the seeds of *T.major* was in the higher concentration levels of the extracts compared to that in the other two species. Also, in *C. Canadensis*, in concentration levels of 5 and 7.5% of the extract, significant differences in germination rates were not observed (Fig 1).

Table 1. Analysis of variance (mean of squares) related to the effects of *Rosmarinus officinalis* extract

S.V.	Df	Mean of squares: Germination percentage	Mean of squares: Germination rate
Weed type	2	245.58**	50.56**
Concentration	3	2414**	107.45**
Weed type×concentration	6	126.91**	9.88**
Error	36	144	1.28
Cv		2.04	1.31

**: significant at a probability level of 1%.

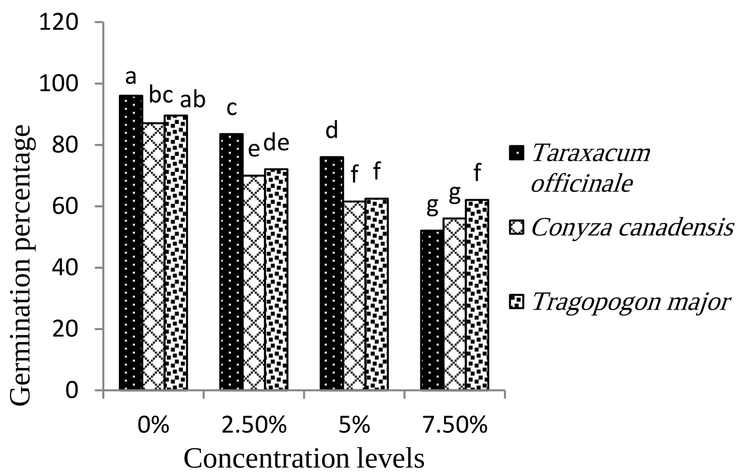

Figure 1. Interaction effects between four concentration levels of *Rosmarinus officinalis* extracts and the seeds of different weed species on their germination percentage. The same letters in each column indicate non-significant differences

Germination rate

The results showed that the highest germination rate was in the control treatment. The germination rate decreased with increasing the concentration of *R.officinalis* extract. *C.canadensis* showed the highest germination rate, and *T.major* and *T.officinale* had the lowest rates of germination significantly ($p \leq 0.01$). The most sensitive species were *T. major* and *T.officinale* and in concentrations of 7.5%, their germination rate was 9.87% to 9.9%. There were no significant differences in concentrations of 5% and 7.5% in the germination rate of *T.officinale* and concentrations of 2.5% and the control in the germination rate of *C.canadensis* (Fig 2).

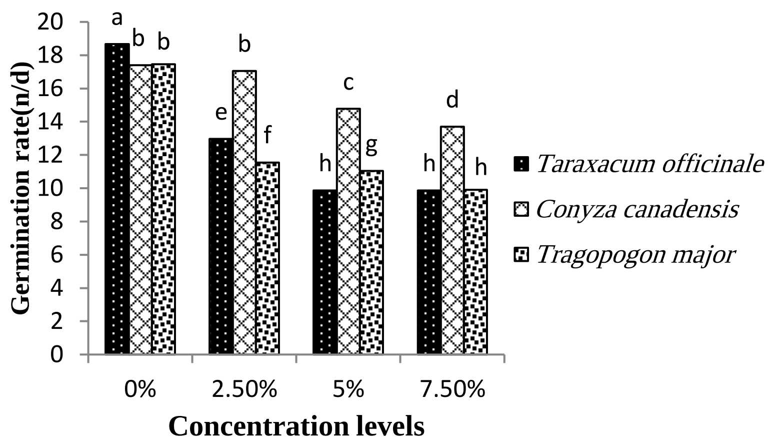

Figure 2. Interaction effects between concentration levels of *Rosmarinus officinalis* extracts and the seeds of three weed species on the germination rate. The same letter in each column indicates non-significant differences

Effects of Lavandula officinalis extract

The results of the analysis of variance showed that weed species, concentration levels, and the interaction between weed species and concentration of *Lavandula officinalis* extract on germination percentage, and germination rate of the weeds seeds was significant ($p \leq 0.01$) (Table 2).

Table 2. Analysis of variance (mean of squares) related to the effects of *Lavandula officinalis* extract

S. V.	df	Mean of squares	
		Germination percentage	Germination rate
Weed type	2	242.02**	9.50**
Concentration	3	4220.91**	120.99**
Concentration × weed type	6	40.27**	0.36**
Error	36	2.10	3.26
cv		1.90	1.50

**: significant at a probability level of 1% .

Germination percentage

The effect of different concentration levels of the aerial parts of lavender extracts on seed germination of the weeds showed that germination percentage decreased with increasing the extracts' concentration level significantly ($p \leq 0.01$). The survey results also showed that *T. officinale* had the least sensitivity to the allelopathic properties of lavender, and *T. major* had the highest sensitivity among the three studied species. The results showed that the tolerance threshold for *T. offisinale* was at a higher level of the extracts compared to that in the other two species, while the tolerance threshold for *C. canadensis* and *T. major* was at the lowest levels of the concentrations. Also, significant differences were not found in concentrations of 2.5% and 5% in the germination percentage of the seeds of *T. major* (Fig 3).

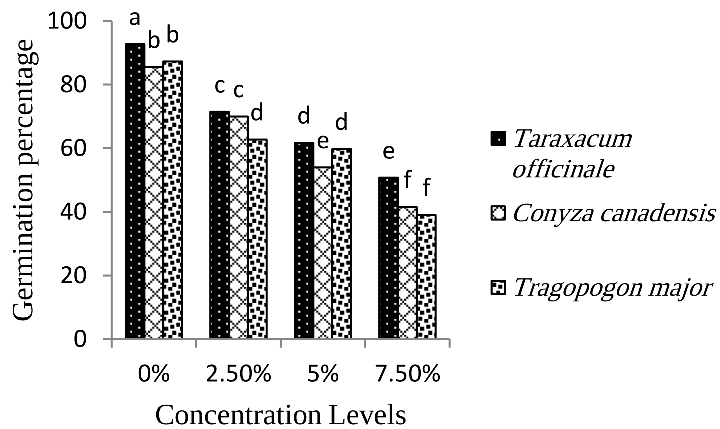

Figure 3. Interaction effects between concentrations of *Lavandula officinalis* extracts and the seeds of weeds species on germination percentage. The same letter in each column indicates non-significant differences

Germination rate

Comparing the germination rates showed that the highest germination rate in all three species was related to the control treatment significantly (p≤0.01). In all three weed species, the germination rate was decreased with increasing the concentrations of lavender extract. At the 7.5% concentration level, *T. officinale* showed the highest germination rate, and *T. major,* and *C. canadensis* had the lowest germination rates (Fig 4).

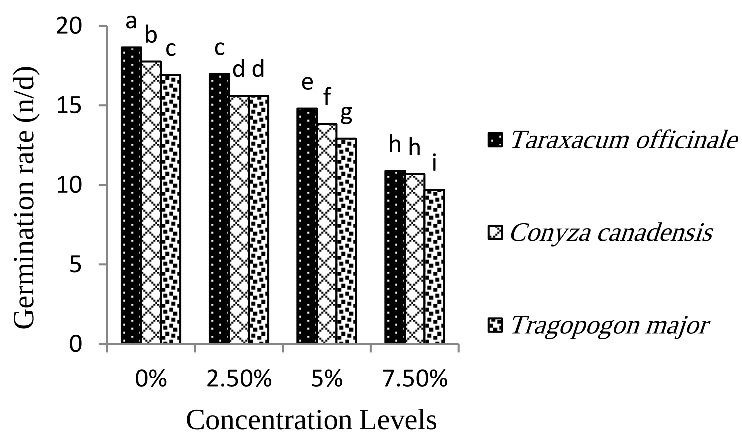

Figure 4. Interaction effects between concentrations of *Lavandula officinalis* extract and the seeds of weeds species on germination rate. The same letter in each column indicates non-significant differences

Effects of Salvia sclarea extract

The results of the analysis of variance showed that weed species, concentration levels, and the interaction effect between the weed species and concentration levels of *Salvia sclarea* extract on germination percentage and germination rate of the weed seeds were significant (p≤0.01) (Table 3).

Table 3. Analysis of variance (mean of squares) related to the effects of *Salvia sclarea* extract

S. V.	df	Mean of squares	
		Germination percentage	Germination rate
Weed type	2	99.77**	13.96**
Concentration	3	4958.24**	156.33**
Concentration × weed type	6	26.91**	1.88**
Error	36	1.38	0.04
cv		2.23	2.07

**: significant at a probability level of 1%.

Germination percentage

The effect of different concentrations of the extract of aerial parts of *Salvia sclarea* on germination of the weed seeds showed that by increasing the concentration level of the extracts, the seed germination was significant (p≤0.01). reduced compared to the control treatment. The survey results also found that *C. canadensis* were the least sensitive to the allelopathic effects among the three species examined, and *T.major* and *T. officinale* were the most sensitive in this aspect at 7.5% concentration level. Therefore, the tolerance threshold to allelopathic effects for *C. canadensis* was at the highest levels of the extracts' concentration (Fig 5).

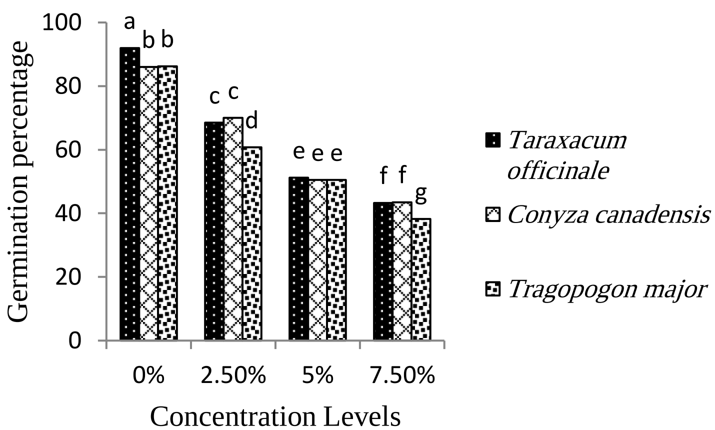

Figure 5. Interaction effects between concentrations of *Salvia sclarea* extract and the seeds of weeds species on germination percentage. The same letter in each column indicates non-significant differences

Germination rate

Comparing the germination rate showed that the highest germination rates for all three species were related to the control treatment. At a 7.5% concentration level, *T. major* had the least germination rate, and then *C. canadensis* and *T. officinale* were the next in the ranking numbers significantly (p≤0.01) (Fig 6).

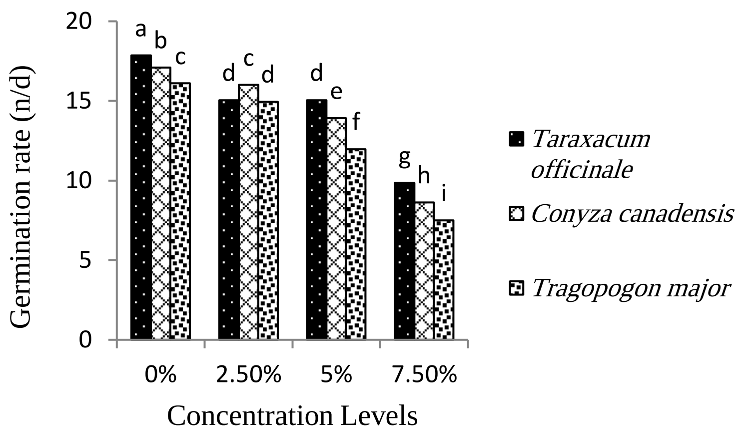

Figure 6. Interaction effects between Salvia sclarea extract's concentration levels and the seeds of weeds species on germination rate. The same letter in each column indicates non-significant differences

Effects of Atriplex halimus extract

The results of the analysis of variance showed that weed species, concentration levels, and the interaction between the weed species and concentration levels of *Atriplex halimus* extract on germination percentage and germination rate of the weed seeds were significant ($p \leq 0.01$) (Table 4).

Table 4. Analysis of variance (mean of squares) related to the effects of *Atriplex halimus* extract

S. V.	df	Mean of squares	
		Germination percentage	Germination rate
Weed type	2	260.08**	17.01**
Concentration	3	7224.52**	152.47**
Concentration × weed type	6	55.58**	3.27**
Error	36	1.34	0.03
cv		2.77	1.38

**: significant at a probability level of 1%.

Germination percentage

Different concentrations of the extracts of the aerial parts of *A.halimus* on the germination of weed seeds showed that with increasing the concentration of the extracts, the seed germinations were reduced significantly ($p \leq 0.01$). The survey results also showed that among the three studied species, seeds of *T.major* had the highest sensitivity to the allelopathic effects, and seeds of *T. officinale* were the least sensitive ones. The results showed that the tolerance threshold to the concentration of the extracts for *T. officinale* was at higher levels than that in the other two species. They were in the lowest levels in *T. major* and the middle levels in *C. canadensis* (Fig 7).

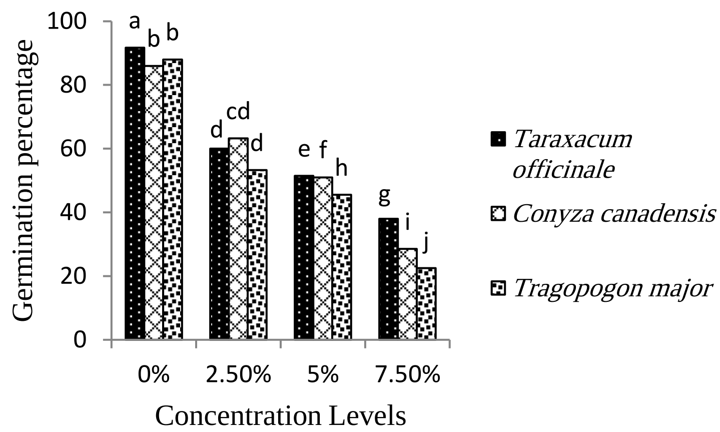

Figure 7. Interaction effects between the concentrations of *Atriplex halimus* extracts and the seeds of weeds species on germination percentage. The same letter in each column indicates non-significant differences

Germination rate

Comparing the effects of the *A. halimus* extract on germination rate shows the highest germination rate in all the species of weeds is the control treatment. *T.officinale* showed the highest germination rate, and the lowest germination speed was in *T. major* and then in *C. canadensis* significantly ($p \leq 0.01$) (Fig 8).

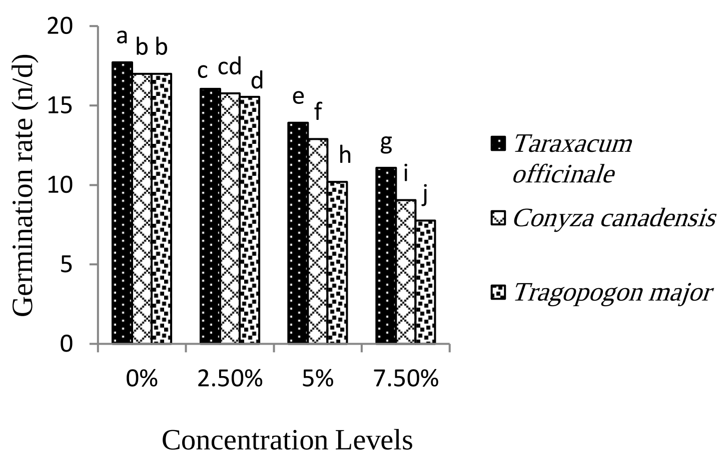

Figure 8. Interaction effects between concentrations of *Atriplex halimus* extract and the seeds of weeds species on germination rate. The same letter in each column indicates non-significant differences

Effects of Atriplex canescens extract

The results of the analysis of variance showed that weed species, concentration, and the interaction between the weed species and concentration of *Atriplex canescens* extract on germination percentage and germination rate of the weed seeds were significant ($p \leq 0.01$) (Table 5).

Table 5. Analysis of variance (mean of squares) related to the effects of *Atriplex canescens* extract

S. V.	df	Mean of squares	
		Germination percentage	Germination rate
Weed type	2	365.68**	5.12**
Concentration	3	1815.63**	30.99**
Concentration × weed type	6	44.57**	1.67**
Error	36	0.98	0.01
cv		1.33	0.76

**: significant at a probability level of 1%.

Germination percentage

The effect of different concentrations of the extracts of the aerial parts of *A. canescens* on seed germination of the weeds showed that with increasing the concentration of the extracts the germinations of the seeds were significantly reduced compared to the control treatments ($p \leq 0.01$). The survey results also showed that among the three studied species, the seeds of *T. officinale* were the least sensitive to the allelopathic effects. The seeds of *T. major* were the most susceptible to this aspect. The results showed that the tolerance threshold for the seeds of *T. officinale* was at higher levels than the tolerance of the seeds of *T. major*. However, *C. canadensis* was located in the middle levels of the two other species in terms of this factor (Fig 9).

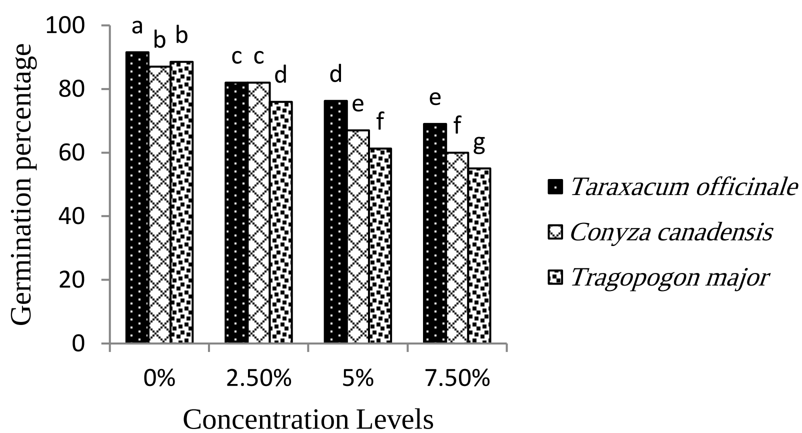

Figure 9. Interaction effects between the concentrations of *Atriplex canescens* extract and the seeds of weeds species on germination percentage. The same letter in each column indicates non-significant differences

Germination rate

The comparison of the germination rate due to the effect of *A.canescens* extracts showed the highest seed germination rate in all three species was higher than that in the control treatment. The concentration level of 7.5% *C. canadensis* and *T. major* showed the highest germination rate, and *T. officinale* had the lowest germination rate significantly ($p \leq 0.01$).

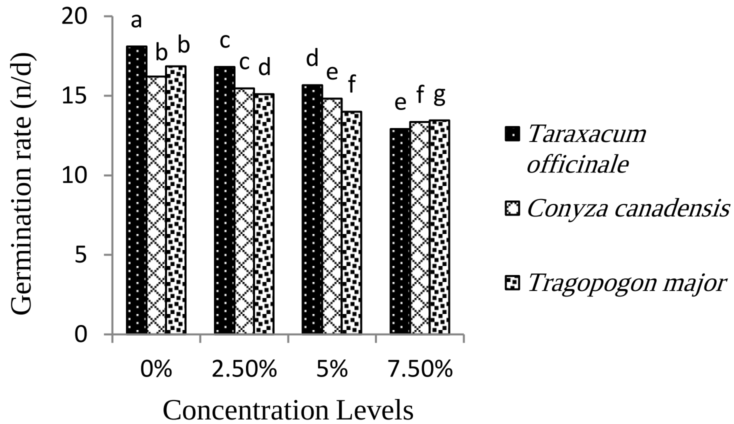

Figure 10. Interaction effects between concentrations of *Atriplex canescens* extract and the seeds of weeds species on germination rate. The same letter in each column indicates non-significant differences

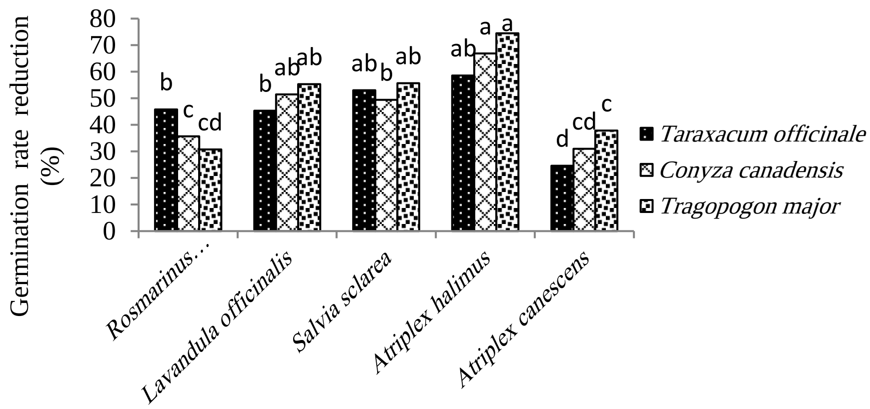

Figure 11. Germination percentage reduction in response to the extracts of different landscape plants

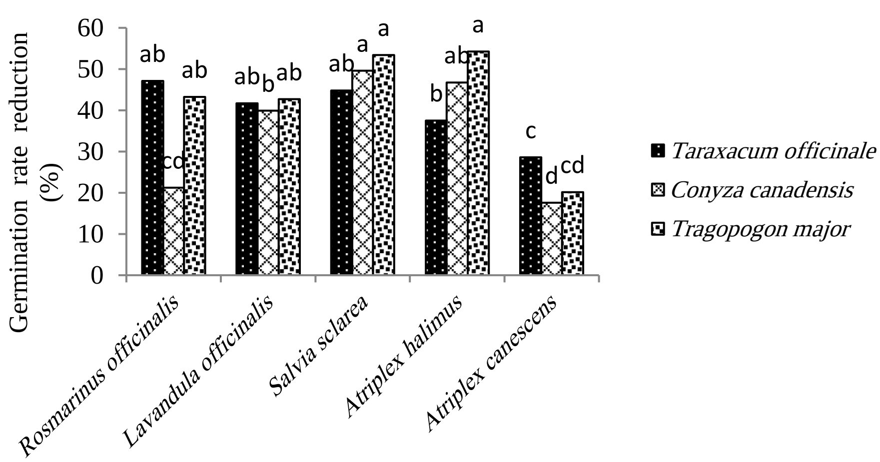

Figure 12. Germination rate reduction (%) in response to the extracts of different landscape plants

Discussion

Among the harmful biotic stresses for crops, weeds rank first as they cause severe loss of crop yields, even more than the combined losses caused by plant pathogens and insect pests worldwide. Around 33-53% of crop production is damaged if weeds are not controlled in the crop fields (Weir *et al.*, 2004).

Based on the results of this experiment, among the three plant species of *R. officinalis*, *L. officinale*, and *S. sclarea*, which belong to Lamiaceae family, *S. sclarea* showed higher allelopathic properties. When treated with a 7.5% concentration of the extract of this plant, the weeds' germination declined more than when treated with the extract of the other two species. Therefore, regarding allelopathic properties, *S. sclarea* was considered the strongest, and *L. officinalis* and *R. officinalis* gained the next scores. Lamiaceae family bears some plant species containing biochemicals with allelopathic activities proven for the Salvia genus (Molisch, 1937). Allelopathic products have a molecular and genetic basis (Om *et al.*, 2002). In previous research, Azizi *et al*. (2006) examined 56 aromatic medicinal plants of 22 families from Iran for their allelopathic potential and found 51 species inhibited the seedling growth of lettuce. Gilani *et al*. (2010) also evaluated 81 plant species in Pakistan and reported 78 species with allelopathic potential inhibiting the root growth of lettuce.

Salvia sclarea, as a source of natural substances, is a good candidate for developing natural pesticides (Jasicka-Misiak *et al.*, 2018). *Salvia sclarea* extract's main components are Lemonen, Alphapinen, Spatholenol, Myersen, Betapinin, Beta Caryophyllene, which have important inhibitory effects on the germination rate and root length of the seeds (Yilar *et al.*, 2020). The use of synthetic chemicals to control weeds is a concern related to the environment and human health. Oils found in plants from Lamiaceae, Asteraceae, and Anacardiaceae families can serve as safe and natural herbicides for humans and the environment (Macias, 1995).

The next plant with a significant allelopathic impact was *Atriplex halimus*, which possessed antioxidant activities. The butanolic and ethyl acetate extracts from the leaves have more hydrogen donation (Kadan *et al.*, 2013). Ebrahimi *et al.* (2016) examined the inhibitory effect of different species of Atriplex. Their studies showed allopathic effects of allochymic compounds were present in all Atriplex species. These compounds were mainly present in the studied species' leaves and fruits. Other researchers have expressed that plants with allelopathic properties are viable options for alternative weed management under sustainable agricultural schemes (Macías *et al*., 2007).
These plants could be exploited in weed management through several processes. For example, they can be used as cover/smother crops (Milchunas *et al*., 2011) or rotational/companion crops (Urbano *et al*., 2006). Their extracts (Jabran *et al*., 2010) can be used, or their residues can be used as mulches. Their allelopathic substances can be used as natural herbicides (Iqbal and Cheema, 2008), or their allelopathic extracts can be mixed with chemical herbicides (Iqbal and Cheema, 2008). Finally, the allelopathic crop cultivars can be used through breeding programs (Islam *et al*., 2018).

In terms of weed seeds' tolerance threshold to allelopathic effects, we may conclude that the seeds of *T. major* were the most sensitive to the species' extracts except for the extract of *R. officinalis.* Therefore, it had the lowest threshold and germination percentage reduction compared to the control treatment. More recognizable results were observed in *T. major* and *C. canadensis* under the effect of *A. halimus*. Therefore, it seems under the impact of *A. halimus* and *S. sclarea*, the seeds of *T. officinale* had the highest reduction in its germination percentage compared to the decrease in the germination of the control treatment (Fig 11).

In reducing the germination rate compared to the control, the most significant decrease in germination rate for *T. major* and *C. canadensis* was under treatments with *A. halimus* and *S. sclarea.* Such a decrease for *T. officinale* was under the effect of *R. officinalis* and *S. sclarea* (Fig 12). These results are consistent with the findings of Mushtaq et al. (2010), who stated that herbicide use in wheat farms could be reduced by 75% using sorghum and sunflower extracts without reducing the net yield of wheat.

As the output of this study, we have provided scientific evidence of the potential of some landscape plant extracts for weed controls and their use in producing bio-herbicides based on the allelopathic effects of plants. Using such effects and interactions in plants can guide us towards more knowledge-based mixed plantings and growing plants together and can assist us to more naturally manage weeds in crop fields and urban landscapes. Such an approach has partially been followed in some previous research work (Azirak and Karaman, 2008, Saharkhiz et al., 2009, Ungar and Khan, 2001).

Conclusion

Some plant species' potential to affect their surrounding plants has been well documented. In this study, the reduction in germination percentage of *T.major* and *C. canadensis* under *A. halimus* extract compared to the control treatment was the highest. The seeds of *Taraxacum officinale,* when affected by *A. halimus* and *S. sclarea* extracts, showed the highest reduction in their germination percentage compared to the seeds of the control treatment. While some hazardous influences have been reported from plants' allelopathic effects (Jiao *et al.*, 2021), there can be some positive effects of such plant interactions in weed control management, as investigated in this study. However, future studies should employ such allelopathic impact on the construction of natural herbicides.

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