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Research Article

Environmental factors affecting seed germination and seedling emergence of waxy-leaved mustard *Boreava orientalis*

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Abstract: Waxy-leaved mustard *Boreava orientalis* Jaub. and Spach. has recently become farmland invasive weed in the Kurdistan province, Iran. Hence this study was conducted better to understand the seed germination ecology of *B. orientalis.* Therefore, in this study, the effect of temperature and osmotic potential, salinity, and burial depth on seed germination and seedling emergence of *B. orientalis* was investigated in the Weed Research Laboratory of the University of Kurdistan in 2020. Seed germination of *B. orientalis* was determined at 10, 20, 30, and 35 °C. *Boreava orientalis* seeds were germinated in an aqueous solution with the osmotic potential of 0, 3, 6, 9, and 12 MPa. The levels of salt stress consisted of control 0, 50, 100, 150, and 200 mM. The burial depths were 0, 3, 6, 9, and 12 cm. The results revealed that germination started at 5 ºC with 0 osmotic potential, and no germination occurred at 30 and 35 °C with different osmotic potentials. The Lowest germination was at 30 and 35 °C; the highest was at 0 Mm NaCl concentration, and the lowest was at 200 Mm of NaCl. The highest germination was at 0 depth, and the lowest was at 12 cm. These results indicate that the germination of this weed species is sensitive and thus greatly reduced under drought and high-temperature conditions. It was also sensitive to both salinity stress and deep burial.

Keywords: dormancy, invasion, invasive weed, ecological factors

Introduction

Recently, an invasive weed called waxy leaved mustard (*Boreava orientalis* Jaub. and Spach.) from the Brassicaceae family has been observed in the wheat and barley fields and some of the summer crops of Kurdistan province, which is spreading so fast that it has completely infected the wheat fields. This invasive weed has been spreading in agricultural fields rapidly in some cities of Kurdistan province for about four years (Babaei *et al*., 2020). For the first time, this species in Iran was recorded by Mozafarian (1985). Invasive weeds (weed invaders) are adapted plants that emerge, sometimes in vast numbers, at substantial spaces from parent plants and can expand over a large area. Invasive weeds can affect the invaded natural or semi-natural population in various ways. Invasive weeds can also majorly affect artificial and human-made habitats and directly affect economic effects (Kikodze *et al*., 2014, Babaei *et al*., 2021). This weed has entered the province of Kurdistan, and in recent years, the pollution has increased as high as 40 plants per

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square meter (Babaei *et al*., 2020).

Waxy-leaved mustard (Yellow weed) is a widespread occurrence in barley and wheat fields in the middle of Turkey, Konya, and other localities in which barley and wheat are cultivated as a winter crop weed is also distributed all over the world. This species is also known as *Isatis quadrualata*, which is also introduced into the flora of Iran. (Moazzeni *et al*., 2010, GBIF).

Knowledge of seed ecology and biology would be beneficial in better management and control of weeds; germination is one of the most critical steps in plant growth. Each plant species has special conditions for germination that are different from other species (Lu *et al.*, 2011, Babaei and Mahmoudi, 2021). The most critical environmental factors affecting seed germination and emergence are optimal temperatures, pH, light, and burial depth, which differ among species (Chachalis and Reddy, 2008). Various ecological factors affect germination; temperature is one of the most important and effective factors. Temperature is a significant determinant of seed germination when other factors (soil moisture, soil salinity, and acidity) are not limiting. Moisture stress may also delay, reduce or prevent seed germination and growth of plants (Asif Tanveer *et al*., 2012). Some conditions and environmental factors control seed dormancy to regulate the germination process. Seeds with non-deep physiological dormancy variously respond to warm or cold stratification in dry or moist conditions to transition from a dormant to a less or non-dormant state capable of relatively rapid onset and germination rate (Foley, 2008). In some seeds, the mechanism of seed dormancy is regulated by temperature, while in some other seeds, germination occurs in a wide range of different temperatures (MacDonald *et al*., 1992). Depending on the soil burial depth, the seeds are exposed to different humidity, temperature, and light conditions that affect germination and emergence (Chauhan and Johnson, 2008; Babaei and Mahmoudi, 2021).

Information on seed germination and seedling emergence is needed to improve weed management (Mennan *et al*., 2006). The implementation of weed control and management operations depends on when the seedling emerges intensively (Masin *et al*., 2011; Babaei *et al*., 2022). Therefore, this experiment and research aimed to investigate and determine the effect of temperature, osmotic potential, burial depth, and salinity on the germination of *B. orientalis* seeds.

Materials and Methods

Seed collection

Mature seeds of *B. orientalis* were collected from 3 fields of wheat at Dehgolan (35.3172º N, 46.9989° E). The seeds were kept at 4 °C for 10 days to induce seed dormancy (Chauhan and Johnson, 2009c), and then experiments were performed on them. The seeds were cleaned and dried for seven days at 25 ºC and kept in paper pockets until initial experiments were used.

Seed germination procedure

The seeds were disinfected using (NaOCl 10%) for 5 minutes and then washed with distilled water. Seed germination was tested by putting 25 seeds evenly in 9 cm diameter Petri plates containing filter paper Whatman No. 10. The control was moistened with 5 mL of distilled water, and for the other treatments, aqueous solutions of salt at different concentrations were used.

Effect of temperature and the osmotic potential on germination

B. orientalis seeds germinated in an aqueous solutions with the osmotic potentials of 0, 3, 6, 9, and 12 bar. These solutions were prepared by dissolving Poly Ethylene Glycol1 6000 in distilled water (Kaufman et al., 1973). Tested constant temperatures were 0, 5, 10, 15, 20, 25, 30, and 35 ºC. To break the dormancy of the seeds, they were exposed to 4 °C before being placed under experimental conditions. The germinated seeds (with minimum radicle growth of 2 mm long) were counted daily for two weeks.

Salinity effect on germination

The seeds were placed in Petri dishes containing 5 ml of 0, 50, 100, 150, and 200 NaCl solution.

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The control sample (0 m M) was placed in a petri dish containing distilled water. Therefore, to prepare 5 ml of 50 mM NaCl, 2.92 gr of pure salt was dissolved in distilled water. Finally, the volume was applied according to the required amounts. Salt concentrations were 0, 50, 100, 150 and 200 mM.

Burial depth effect of on seed emergence

Cold Stratification is used to break the seed dormancy. Seeds were placed in cups of 10 cm diameter and height of 15 cm. The buried depth of the seeds was 0, 3, 6, 9, and 12 cm, performed in four replications. The burial depth was marked and determined on the cups by a ruler. The soil used consisted of clay, loam sand, and manure in equal proportions

Data analysis

The study was a factorial experiment with four replications based on a completely randomized block design. The data from the repeated experiments were combined. Data were analyzed using SAS (9.2) software, and then the graphs were fitted using a linear model. The graphs were drawn using (SigmaPlot ver.12) software. The graphs were fitted using a Linear model by using Equation 1. Diagrams related to the effect of temperature and potential osmotic factors fitted using a three-parameter sigmoidal model (Chauhan *et a*l., 2014). Data obtained from salinity and burial depths were fitted using following polynomial model.

$$G\ (\%) = T50 + G_{max}x \qquad \text{Eq. 1}$$

Where *G* (%) represents cumulative emergence at burial depth or residue amount x, while G_{max} is maximum emergence.

$$G\ (\%) = \frac{G_{max}}{1+e^{-(\frac{x-T50}{G_{rate}})}} \qquad \text{Eq. 2}$$

Where G(%) cumulative germination percentage, while X is maximum germination, T_{50} is time required for 50% germination, G_{rate} is The slope

Results

The seed germination highest value was at 5 °C and the osmotic potential of 0 bar, 85% (Fig. 1). The habitat of this weed is in cold and mountainous areas, and the reason for its cold need for germination and dormancy deprivation of its seeds is cold. By increasing temperature and osmotic potential, the germination percentage decreased and completely stopped at 30 ºC beyond it. The cold was a stimulant for germination. Seed germination was reduced by high temperature and high osmotic potential simultaneously. Figure 1 shows the germination process during the day, which is affected by temperature and osmotic potential. The effect of cold on germination was significant. This plant needs cold shock and has high germination at 5 °C. A three-parameter sigmoid model fitted the emergence data (Fig. 1). germination has also decreased with increasing osmotic potential, indicating that many seeds were dormant at the beginning of the experiment. Germination occurred at 10 °C and decreased with increasing osmotic potential (Fig. 2). Seed germination took place at 10 °C, indicating the plant's need for low temperature, and this trend continued has continued. Germination continued at 15 °C and was similar to 10 °C (Fig. 3), and this value decreased at 20 °C (Fig. 4). Germination was slow at 25 °C. Moreover, seeds at osmotic potentials of 0.3, 0.9, and 1.2 MPa did not germinate (Fig. 5).

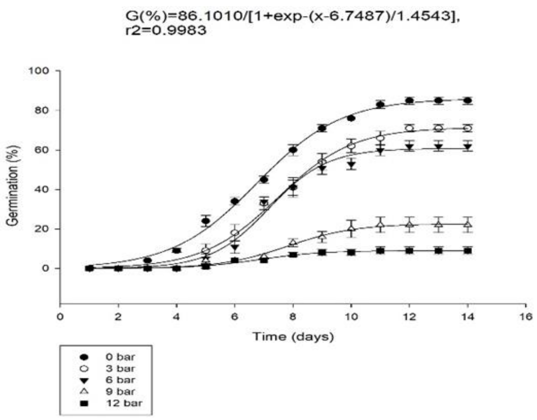

Figure 1 Temperature 5 ºC and different osmotic potential effect on germination percentage of seeds of *BOREAVA orientalis* (days after sowing). The data are fitted with three parameters sigmoidal equation. The bars indicate standard error.

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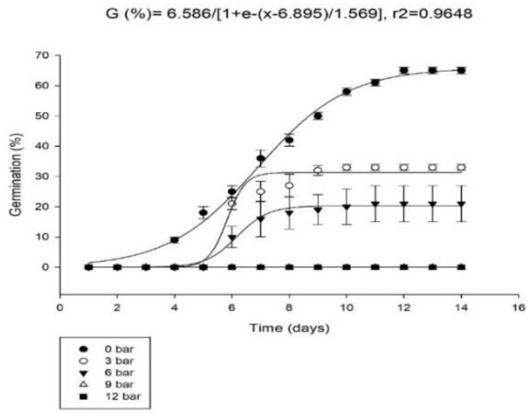

Figure 2 Temperature 10 ºC and osmotic potential effect on germination percentage seed of *Boreava orientalis* (days after sowing). The data are fitted with three parameters sigmoidal equation. The bars indicate standard error.

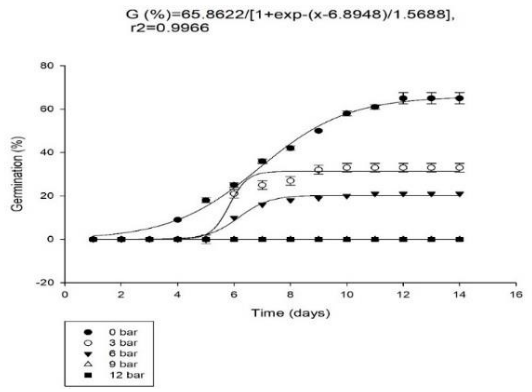

Figure 3 Temperature 15 ºC and osmotic potential effect on germination percentage of *Boreava orientalis* seeds (days after sowing). The data are fitted with three parameters sigmoidal equation.. The bars indicate standard error.

Based on the results of the experiments, it is concluded that the waxy-leaved mustard is a cold-season plant. Cold leads to the breaking of the seed dormancy, and with the start of the warm season and obtaining the necessary temperature for growth, it begins to germinate. This weed has a higher tolerance to cold than to heat, which makes the weed resistant to cold conditions. *B. orientalis* has a base of 4 °C, starting to germinate at this temperature, and can germinate up to 30 °C. However with increasing temperature, the growth indicators are reduced, and the physiological functions of the plant are disturbed. The germination percentage, rate, and seedling vigor index are affected and reduced. The highest value in this table of Gmax is related to 0 bar at 5 °C, and the lowest value is related to 12 bar treatment, which cannot germinate due to weed seeds' osmotic potential and drought stress.

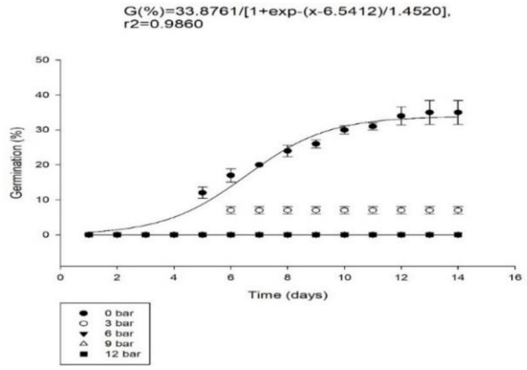

Figure 4 Temperature 20 ºC and osmotic potential effect on germination percentage of *Boreava orientalis* seeds (days after sowing). The data are fitted with three parameters sigmoidal equation. The bars indicate standard error.

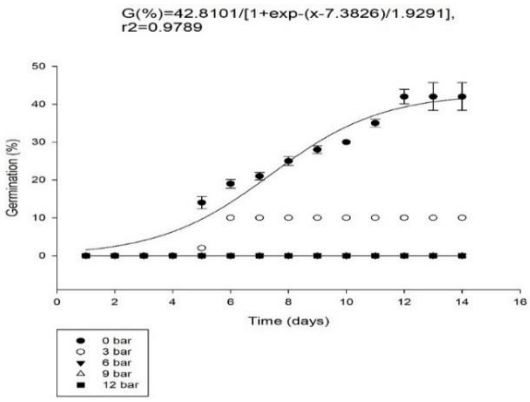

Figure 5 Temperature 25 ºC and osmotic potential effect on germination percentage seed of *Boreava orientalis* (days after sowing). The data are fitted with three parameters sigmoidal. The bars indicated standard error.

The treatment of 0 bar without drought stress had the minimum time to reach 50% (T50) germination, and the maximum time was related to the potential of 9 bar. The germination percentage was the least at the osmotic potential 12 bar (Table 1). mustard. *B. orientalis* is sensitive to dehydration, and osmotic stress prevents it from germinating. In this weed, low temperature caused germination to begin. If there is enough moisture, the seed absorbs it, and the germination process is completed, but this process is not complete in drought stress. The lowest treatment is related to 6, 9, and 12 bar osmotic potentials, which were zero and did not germinate. T50 also had the shortest germination time in the 0-bar treatment, and the longest treatment time was that of 3 bars. There was no germination in the other treatments (Table 2). According to Table 3, the highest value of G_{max} is related to the zero-bar treatment at 15 °C due to the absence of drought stress that the seeds can absorb moisture and germinate. The lowest treatment is related to 6, 9, and 12 bar osmotic potentials, which were zero. T50 also had the shortest germination time in the 0-bar treatment; the longest treatment time was 3 bars. It was zero in the other treatments. Table 4 shows that the highest amount of G_{max} is related to the 0-bar treatment at 15 °C, due to the absence of drought stress that the seeds can absorb moisture and germinate. The lowest germination belonged to 6, 9, and 12 bar osmotic potentials, which prevented any germination, and there was none. T50 had the shortest germination time in the 0-bar treatment, and the longest treatment time was at 3 bars. In Table 5, the highest value of G_{max} is related to the 0-bar treatment at 15 °C. The seeds can absorb moisture and germinate. The lowest treatment was related to 6, 9, and 12 bar osmotic potentials, which were zero and did not germinate. T50 also had the shortest germination time in the 0-bar treatment, and the longest treatment time was at 3 bars. There does not make sense in the other treatments.

Table 1 Summary of three-parameter sigmoid model fitted germination percentage data of *BOREAVA orientalis* at different osmotic potentials at 5 ºC.

Osmotic potential (bar)	Parameter estimates (± SE)				
	G_{max}	T50	G_{rate}	R_2	F_{value}
0	86.10 ± 1.33	6.74 ± 0.10	1.45 ± 0.08	0.99	< 0.0001
3	71.39 ± 1.33	7.46 ± 1.09	1.28 ± 0.09	0.99	< 0.0001
6	60.82 ± 1.47	7.16 ± 0.13	0.99 ± 0.11	0.99	< 0.0001
9	22.56 ± 0.58	7.81 ± 0.14	1.15 ± 0.11	0.99	< 0.0001
12	8.17 ± 0.27	6.83 ± 0.17	1.07 ± 0.15	0.98	< 0.0001

Table 2 Summary of three-parameter sigmoid model fit germination percentage data of *Boreava orientalis* at different osmotic potentials at 10 ºC.

Osmotic potential	Parameter estimates (± SE)				
	G_{max}	T50	G_{rate}	R_2	F_{value}
0	31.32 ± 0.91	5.82 ± 0.11	0.32 ± 0.12	0.97	< 0.0001
3	20.25 ± 0.43	6.17 ± 0.10	0.54 ± 0.09	0.98	< 0.0001
6	0	1	1	0.99	< 0.0001
9	0	0	0	1	
12	0	0	0	0	

Table 3 Summary of three-parameter sigmoid model fitted germination percentage data of *Boreava orientalis* at different osmotic potentials at 5 ºC.

Osmotic potential (bar)	Parameter estimates (± SE)				
	G_{max}	T50	G_{rate}	R_2	F_{value}
0	31.32 ± 0.91	5.82 ± 0.11	0.32 ± 0.12	0.97	< 0.0001
3	20.25 ± 0.43	6.17 ± 0.10	0.54 ± 0.09	0.98	< 0.0001
6	0	1	1	0.99	< 0.0001
9	0	0	0	1	
12	0	0	0	0	

Table 4 Summary of three-parameter sigmoid model fit germination percentage data of *Boreava orientalis* at different osmotic potentials at 20 ºC.

Osmotic potential (bar)	Parameter estimates (± SE)				
	G_{max}	T50	G_{rate}	R_2	F_{value}
0	33.87 ± 1.51†	6.54 ± 0.29	1.45 ± 0.24	0.97	< 0.0001
3	0	1	1	1	< 0.0001
6	0	0	0	0	
9	0	0	0	0	
12	0	0	0	0	

Table 5 Summary of three-parameter sigmoid model fitted germination percentage data of *Boreava orientalis* at different osmotic potentials 25 ºC.

Osmotic potential (bar)	Parameter estimates (± SE)				
	G_{max}	T50	G_{rate}	R_2	F_{value}
0	42.81 ± 3.28	7.37 ± 0.51	1.92 ± 0.38	0.97	< 0.0001
3	0	1	1	1	< 0.0001
6	0	0	0	0	
9	0	0	0	0	
12	0	0	0	0	

Seed germination of *B.orientalis* is negatively affected by salinity stress (Fig. 6). The highest germination rate was for 0 Mm treatment with 63% germination, and the lowest germination was related to treatment with 200 mM concentration with 0% germination. Seedling size decreases with increasing salinity (Fig. 7). Salinity stress reduces seedling size reduces germination, and inhibits seedling growth.

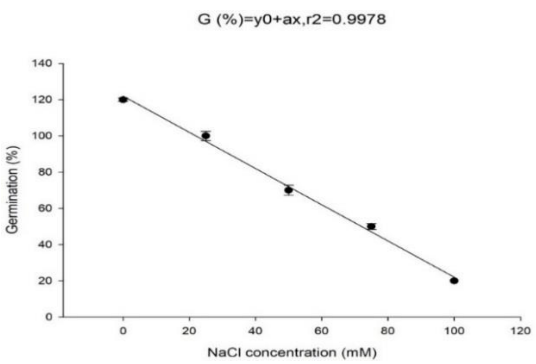

Figure 6 Salinity effect on germination percentage seed of *Boreava orientalis*. The line represents a linear model fitted to the data. The bars indicate standard error.

Seeds of *B. orientalis* germinate from low soil depths and have a high germination power. The seeds were germinated at six and nine cm deep after 54 and 69 days; however, there was no germination at 12 cm (Fig. 7).

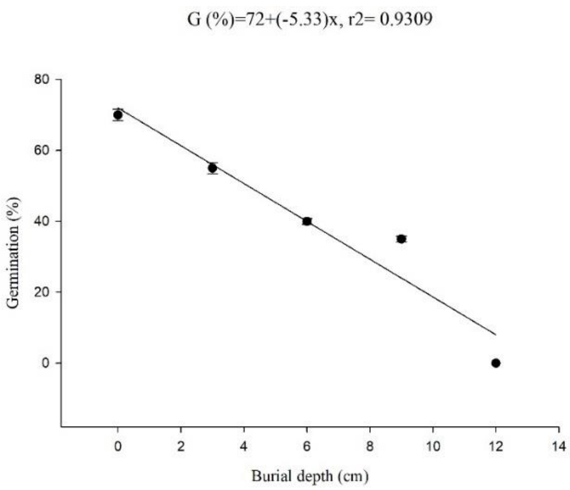

Figure 7 Seed burial effect at different burial depths on germination of *Boreava orientalis* seeds. The line represents a linear model fitted to the data. The bars indicate standard error.

Discussion

Temperature and osmotic potential

One of the most influential factors in germination is temperature, with a number of species that can germinate over an extensive temperature range; others require critical levels of relatively high temperature (Chauhan and Johnson, 2008). Synedrella *Synedrella nodiflora* germinated over a wide temperature range. Thus, the germination percentage was meager at 25/15 °C (Chauhan and Johnson, 2009b). Seed germination in *Centaurea balsamita* occurred over a range of 5 °C to 35 °C with the highest germination at 25 °C (Nosratti *et al*., 2017).

Buffalobur *Solanum rostratum* germination was 98, 91, and 28% at osmotic potentials of 0, 20.5, and 21.1 MPa. Optimum germination was at osmotic potentials between 0 and 20.2 MPa, where germination exceeded 95%. These findings indicate that Buffalobur seed is relatively tolerant to low water potential. Data were similar to those reported for hairy nightshade (Zhou *et al*., 2009); in contrast, other weed species were susceptible to low osmotic potentials, such as trumpet creeper (Chachalis and Reddy, 2008), Texasweed *Caperonia palustris* L. (Koger *et al*., 2004), American slough grass (Rao *et al*., 2008), and Cadillo (Wang *et al*., 2009). Experiments have shown that Buffalobur can survive in poor drainage and drought stress in adverse soil conditions. Plants under osmotic pressure and different temperatures show different reactions.

In research in water at 8 °C, the black seed (*Brassica napus*) germinated faster than the yellow seed line (P = 0.008). The saline and PEG solutions inhibited the germination of the yellow seed genotype to a larger extent than the black seed (Zhang and Gusta, 2010).

A study by (Chachalis *et al*., 2008) measured a significant decline in seed germination as the osmotic potential increased; germination was 22% at osmotic potentials up to 20.8 MPa. In addition, germination was inhibited at osmotic potentials, 21.3 MPa. These findings indicate that Venice mallow seed germination is tolerant to drought stress and osmotic potential

Some environmental factors are known to affect weed seed germination. The optimum temperature osmotic potential mainly depends on the species. in one study On wild oat seed germination, as the osmotic potential of the solution increased, wild oat germination increased (Boyd and Acker, 2004).

This weed is sensitive to high temperatures. Increasing the temperature causes temperature stress, and the germination percentage of the seed should decrease. The response to drought stress is different in different species. In this weed species that grows in humid regions, the sensitivity to drought stress and temperature that leads to drought stress is high.

Redroot *Amaranthus retroflexus* L. and Palmer amaranth *A. palmeri* S. Wats. need critical levels of nearly high temperatures. Germination of both *Amaranthus* spp. was similar at 30/20 and 35/25 °C temperatures (Chauhan and Johnson, 2009a).

Salt stress

This weed is sensitive to salinity and has a low salinity tolerance threshold. The spreading areas of this weed are in places with no soil salinity or very low salinity. In this experiment, waxy-leaved mustard showed that the physiological activities of the plant decreased with the increase in salinity. Salinity stress is a type of osmotic stress that causes a decrease in the amount of moisture in the plant. Drought stress follows it and causes disturbance in activities of the plant.

(Di Tommaso, 2004) reported that NaCl in salt can change germination phase by replacing calcium and magnesium in the anion-exchange process, leading to nutrient and water stress. Another study suggests that, in saline conditions, a proportion of *Eclipta* seed can germinate, which could be a vital feature of this species that enables it to colonize saline areas rice is germinated (Chauhan and Johnson, 2008). Factors like salinity and pH affect seed germination of rigid Ryegrass (Chauhan *et al*., 2006a). Soil salinity may affect seed germination as it decreases the moisture absorption of seeds and facilitates the entry of ions in higher amounts that influence seed health. The salinity level at which seed germination is reduced varies with species, genotype, environmental conditions, osmotic potentials, and specific ions (Gomez *et al*., 2008). Germination of little mallow seeds was inversely related to NaCl concentration, although seeds germinated at all concentrations up to 160 mM NaCl (Chauhan *et al*., 2006b). In *Amaranthus spinosus* and *A. viridis*, germination was more than 83% up to the concentration of 150 mM NaCl; 37% germination occurred at 200 mM NaCl, and was inhibited at 250 mM NaCl. The concentration required for 50% inhibition of maximum germination was 194 mM NaCl. When non-germinated seeds were removed from 250 mM NaCl and placed in distilled water, germination was 88%, indicating that the saline solutions had not conversely affected seed viability (Chauhan *et al*., 2008).

Burial depth

Germination happened when the seeds of Slender and Spiny amaranth were placed on the soil surface. 7% of Spiny amaranth seedlings emerged from 0.5 cm. For Slender amaranth, 6% emergence was achieved at 4 cm depth, and no emergence was observed at 6 cm (Chauhan and Johnson, 2009b). Restoration of catch weed bedstraw was sensitive to soil moisture levels and burial depth (Boyd and Van Acker, 2003).

Conclusions

Based on our results, this weed is sensitive to high temperatures, and high humidity is also one of the needs of this plant. It is susceptible to salinity. It can also germinate at great depths and emerge when buried deep up to 9 cm. Information on seed ecology can facilitate weed management. This research revealed the effect of ecological factors on waxy-leaved mustard. Further investigation into the biology and ecology of this weed can help prevent it from spreading to other similar areas. The result can help manage this invasive weed and prevent it from spreading to other areas.

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Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

Author's contributions

All authors contributed to the study's conception and design. TA, SB, performed material preparation, data collection, and analysis. TA wrote the first draft of the manuscript, and all authors commented on previous versions. All authors read and approved the final manuscript.

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عوامل محیطی مؤثر بر جوانه‌زنی و سبز شدن بذر خردل برگ مومی *Boreava orientalis*

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چکیده: خردل برگ مومی *Boreava orientalis* Jaub. Spuch. اخیراً به علف هرز مهاجم مزارع استان کردستان-ایران تبدیل شده است. از این‌رو این مطالعه به‌منظور درک بهتر بوم‌شناسی جوانه‌زنی بذر *B. orientalis* انجام شد. دانش در مورد ویژگی‌های جوانه‌زنی علف‌های هرز برنامه‌ریزی کنترل و مدیریت مؤثر را تسهیل می‌کند. بنابراین تأثیر دما و پتانسیل اسمزی، شوری و دفن بر جوانه‌زنی بذر و سبز شدن گیاهچه در آزمایشگاه تحقیقات علف‌های هرز دانشگاه کردستان در سال ۲۰۲۰ مورد بررسی قرار گرفت. آزمایش از دمای صفر تا ۳۵ درجه سلسیوس انجام شد. بذور *B. orientalis* در محلول آبی با پتانسیل اسمزی ۰، ۳، ٦، ۹ و ۱۲ بار قرار داده شدند. سطوح شوری شامل ۰، ۵۰، ۱۰۰، ۱۵۰ و ۲۰۰ میلی‌مولار بود. عمق دفن ۰، ۳ ، ٦، ۹ و ۱۲ سانتی‌متر بود. نتایج نشان داد که جوانه‌زنی در دمای ۵ درجه سلسیوس با پتانسیل اسمزی صفر شروع شد و در دمای ۳۰ و ۳۵ درجه سلسیوس با پتانسیل‌های اسمزی متفاوت، جوانه‌زنی رخ نداد. کم‌ترین جوانه‌زنی در دمای ۳۰ و ۳۵ درجه سلسیوس و بیش‌ترین آن در ۰ میلی‌مولار شوری و کم‌ترین آن در ۲۰۰ میلی‌مولار NaCl بود. بیش‌ترین جوانه‌زنی در عمق صفر و کم‌ترین آن ۱۲ سانتی‌متر بود. این نتایج نشان می‌دهد که جوانه‌زنی این‌گونه علف هرز حساس است و بنابراین در شرایط خشکی و دمای بالا به‌شدت کاهش می‌یابد. همین‌طور این علف هرز به تنش شوری و دفن عمیق بسیار حساس بود.

واژگان کلیدی: خواب بذر، تهاجم، علف‌های هرز مهاجم، عوامل اکولوژیکی

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