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Article

Germination Ecology of Ivy-Leaved Morning-Glory: an Invasive Weed in Soybean Fields, Iran

Ecologia de Germinação de Glória-da-Manhã com Folhas de Hera: uma Erva Invasora em Campos de Soja, Irã

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ABSTRACT - Ivy-leaved morning-glory (*Ipomoea hederacea* Jacq.) is an exotic species that is becoming an increasing problem in soybean fields of Golestan province, Iran. Because little information is available on the biology of this weed species in Iran, experiments were conducted to investigate the effects of different factors on seed germination and emergence of Ivy-leaved morning-glory. Maximum germination occurred at constant temperature of 20 °C (82%) and alternating temperature of 15/25 °C (94%). Germination was reduced with increasing salinity and drought stresses. Sodium chloride concentration and osmotic potential that inhibited 50% maximum germination were -1.64 and -1.03 MPa, respectively. Acidity was a limiting factor for the germination, due to inhibiting effect of alkaline conditions on germination. However, the results showed that high temperature pretreatment >100 °C decreased seed germination. Germination decreased from 82 to 3% as temperature increased from 100 to 130 °C. There was no significant difference between seedling emergence in burial depths of 1 to 10 cm, but emergence reduced with increasing burial depth from 10 to 14 cm, and no seedling was emerged from a depth of 15 cm. The results of the flooding experiment also revealed that the seeds of this species are sensitive to this stress, so that emergence was 9% after 3 d flooding.

Keywords: biotic stress, alternating temperatures, exotic species, pH.

*RESUMO - **Ipomoea hederaceae** Jacq. é uma espécie exótica que está se tornando um problema crescente em lavouras de soja da província de Golestan, Irã. Considerando que há pouca informação disponível sobre a biologia dessa espécie no Irã, experimentos foram conduzidos para investigar os efeitos de diferentes fatores sobre a germinação de sementes e emergência de Ipomoea hederaceae. A máxima germinação ocorreu em temperatura constante de 20 °C (82%) e em temperatura alternada de 15/25 °C (94%). A germinação foi reduzida com o aumento da salinidade e do estresse hídrico. A concentração de cloreto de sódio e o potencial osmótico que inibiram 50% da máxima germinação foram de -1,64 e -1,03 Mpa, respectivamente. O pH foi um fator limitante para a germinação, pois houve efeitos inibitórios das condições alcalinas sobre a germinação. Os resultados demonstraram que o pré-tratamento das sementes com temperatura elevada, maior que 100 °C, resultou em decréscimo da germinação. A germinação decresceu de 82% para 3% com o aumento da temperatura de 100 °C para 130 °C. Não houve diferenças de emergência de plântulas em profundidades de enterrio entre 1 e 10 cm, mas a emergência foi reduzida com o aumento da profundidade de enterrio de 10 para 14 cm, e nenhuma plântula emergiu a 15 cm de profundidade. Os resultados de experimentos com inundação também revelaram que as sementes dessa espécie são sensíveis a esse estresse, tanto que a emergência após três dias de inundação foi de 9%.*

Palavras-chave: estresse abiótico, temperaturas alternadas, espécies exóticas, pH.

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INTRODUCTION

Ivy-leaved morning-glory (*Ipomoea hederacea* Jacq.) belongs to Convolvulaceae family. It is a summer climbing vine with alternate, ivy-shaped leaves and erect hairs on both surfaces of leaves. Flowers are large, showy, and white to pink to dark purple (Tenaglia, 2017). This plant is native to tropical America (Anonymous, 2018). In some Asian countries such as India and Pakistan, its seeds are used for the medicinal properties (Singh and Singh, 2012; Ul-Haq et al., 2012). But this species is known as an important weed in subtropical areas. It is considered as one of 10 worst weeds in soybean, cotton, and tobacco in the southeast of USA. This weed is also observed in Africa (Ghana), South America (Brazil), and it is reported as a quarantine weed from Australia. As well as, it was reported in 2010 for the first time from Soybean fields of Aliabad region, Gorgan, Golestan province in Iran (Pahlevani and Sajedi, 2011).

Ipomea spp. are soybean competitive weed, as Culpepper et al. (2001) stated that these weeds can reduce soybean yield 25-43% at densities of 2 to 8 plants m^{-2}. Furthermore, various species of morning-glory may result in the crop lodging, interfere with harvesting operations that reduce harvesting efficiency, increase foreign matter in the harvested product and reduce product quality. Since the primary introduction of weeds to new areas takes place mainly through seeds, understanding the seed ecology is required. Also, studying the ecology of seed germination of various species is very useful to recognize and explain plant evolution and ecological adaptation (Baskin et al., 2004). Biological and ecological information, specifically germination ecology of a specific weeds is necessary to develop long-term management strategies (Mennan and Ngouajio, 2006; Nakamura and Hossain, 2009; Ali et al., 2013; Tanveer et al., 2014). Peters et al. (2000) reported that knowledge of the influence of environmental factors on the germination and emergence behavior of seeds would be helpful to prevent their invasion to new areas and determine its management practices.

Germination of weed seed influenced by many environmental factors, such as the temperature, pH, soil salinity and moisture (Koger et al., 2004; Chauhan et al., 2006a, b; Eslami et al., 2010; Altop et al., 2015; Asgarpour et al., 2015; Sadeghloo et al., 2013). Seed germination under adverse environmental conditions such as drought and salinity stresses might enable weeds to take advantage of the limiting conditions of the growth of other species (Chauhan and Johnson, 2008).

Because of the increasing range of this weed distribution in soybean fields in Golestan province, we investigated different environmental factors including of temperature (constant, alternating and high temperatures), osmotic and salinity stresses, pH, burial depth and flooding duration on seed germination and seedling emergence of Ivy-leaved morning-glory.

MATERIALS AND METHODS

Experiments were conducted to evaluate the effect of constant temperature, alternating temperature, pH, salinity stress, drought stress, and high temperature pretreatment on germination, and the influence of planting depth and flooding on emergence of Ivy-leaved morning-glory.

Seed collection

Seeds collected from multiple plants through soybean fields of Gorgan (36°50’29” N, 54°26’36” E, 143 m above sea level) in fall 2013. They stored at 4 °C until experiment initiation. The mean 100 seed weight was 2.93 g. A preliminary test of germination indicated that seeds had dormancy. So, treatment of concentrated sulphuric acid (98% H_2SO_4) for 25 min was used to break seed dormancy.

General protocol for germination tests

Four replications of 25 seeds of Ivy-leaved morning-glory were placed in 9 cm petri dishes on a layer of Whatman No. 1 filter paper, moistened with either 5 mL distilled water or treatment solution when required. Germination tests were conducted in an incubator at 25 °C in darkness (optimum temperature for germination, data not shown), except for the experiment of the effect of temperature on seed germination. The seeds were checked daily for germination. Seeds were considered germinated when the radicle was 2 mm in length (Ghaderi-Far et al., 2010; Maraghni et al., 2010; Sing et al., 2012). Seed germination was counted until 14 d after the start of the experiment.

Effect of constant and alternating temperatures on germination

Seven constant temperatures (10, 15, 20, 25, 30, 35 and 40 °C) and eight fluctuating 12/12 h night/day temperatures (7.5/12.5, 10/15, 12.5/20, 15/25, 20/30, 25/37.5, 30/42.5, and 35/45 °C) in darkness were selected to determine the effect of temperature on germination.

Effect of high temperature pretreatment on germination

Seeds were subjected to high temperature for short duration to simulate the fire effects. Seven various temperatures (80, 100, 120, 130, 160, 200, and 240 °C) for 5 min were studied along with a control (no treatment). A drying oven was utilized for high temperature treatment of seeds. Each treatment consisted of four replicate petri dishes with 25 seeds. Seeds were then set to germinate using the optimum temperature of 25 °C.

Effect of osmotic and salinity stresses on germination

Osmotic solutions with 0, -0.2, -0.4, -0.6, -0.8, -1.0, -1.1, -1.2, and -1.4 MPa potentials were prepared by dissolving measured quantities of PEG 8000 in distilled water (Michel, 1983).

Salt stress on seed germination was evaluated by placing the seeds in dishes containing sodium chloride (NaCl) solutions of 0, -0.2, -0.4, -0.6, -0.8, -1.0, -1.1, -1.2, -1.4, -1.5, -1.8, and -2.0 MPa, which were prepared by dissolving 0, 2.35 , 4.70, 7.06, 9.41, 11.77, 14.12, 16.47, 18.83, 21.18 and 23.54 g NaCl in 1 L of distilled water.

Effect of pH on germination

The effect of pH on seed germination was evaluated by placing seeds in buffer solutions with pH 3 to 9 prepared according to Susko et al. (1999). Distilled water (pH=6.6) was used as the control.

Effect of burial depth on seedling emergence

Twenty-five seeds of Ivy-leaved morning-glory were buried in soil in 19 cm diam plastic pots at depths of 1, 3, 5, 7, 10, 13, 14, and 15 cm. The soil used was loam with an organic matter content of 0.95% and pH of 7.2. Pots were placed in outdoor from June 26 to July 16, 2015. The average minimum and maximum temperatures and the rainfall during the experiment were 23.5 °C, 35.5 °C, and 39.5 mm, respectively. Pots were irrigated throughout the study to provide optimal moisture for seed germination. Seedling emergence was recorded daily until the seedlings ceased to emerge about 20 d after planting. Emergence was defined as the appearance of a part of seedling on the soil surface.

Effect of flooding on seedling emergence

Twenty-five seeds were buried at 1 cm soil depth in each pot (16 cm diam. by 19 cm depth). According to the results of seed depth experiment, maximum germination was observed in seeds buried at this depth. The pots were filled with the same soil as in the burial depth experiment. Pots containing the seeds were placed inside larger plastic pots (20 cm diam. by 32 cm depth) to maintain 3 cm water above the soil.

Statistical Analysis

In all experiments, treatments were laid out in a completely randomized design with four replications. The germination percentage data were transformed prior to analysis to prevent variance heterogeneity, but this did not improve homogeneity. Therefore, non-transformed percentage germination data were applied for ANOVA and regression analysis. Data were subjected to analysis of variance and the treatment means were separated using LSD at P≤0.01. All the statistical analyses were conducted with SAS (version 9.1).

Regression analysis was utilized to determine the effect of osmotic and salinity stresses, planting depth, and high temperature pretreatment. Data were fitted to a three-parameter sigmoidal function sing Sigma Plot (version 11). The model fitted was as follows:

$$G = G_{max}/\{1 + \exp[-(x - x_{50})]/G_{rate}]\} \qquad \text{(eq. 1)}$$

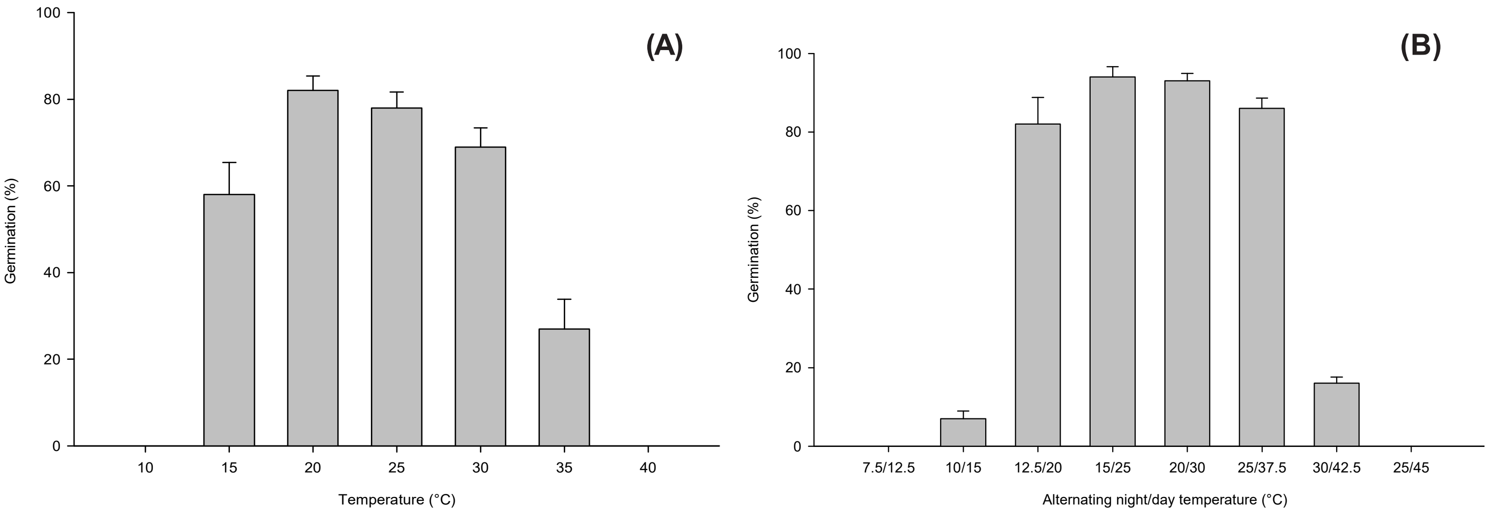
where G is the total germination (%) at different concentrations of salt and osmotic potential, high temperature, or burial depth; G_{max} is the maximum germination (%), x_{50} is the concentration of salt and osmotic potential, high temperature or planting depth required for 50% inhibition of the maximum germination, and G_{rate} indicates the slope.

RESULTS AND DISCUSSION

Effect of constant and alternating temperatures on germination

Germination percentage changes at different temperatures were illustrated in Figure 1. No germination occurred at 10 °C. Fifty-eight percent of seeds germinated at 15 °C. With increasing the temperature from 15 to 20 °C, germination increased, so that the highest germination (82%) was observed at 20 °C. Thereafter, seed germination reduced and the seed germination ranged from 25-78% for the temperature range of 25-35 °C. Seeds did not germinate at 40 °C (Figure 1A).

Maximum germination (94%) of Ivy-leaved morning-glory was observed at night/day temperature of 15/25 °C. However, there was no significant difference between 15/25 °C and 20/30 °C (Figure 1B). A substantial reduction in germination was observed at temperatures below 12.5/20 °C or above 30/42.5 °C. These results indicated that alternating temperatures could lead to a greater seed germination percentage than that at constant temperatures. These results were similar to those obtained by Nakamura and Hossain (2009). They observed that germination of redflower ragleaf (Crassocephalum crepidioides) at alternating temperatures was higher than that at constant temperatures.



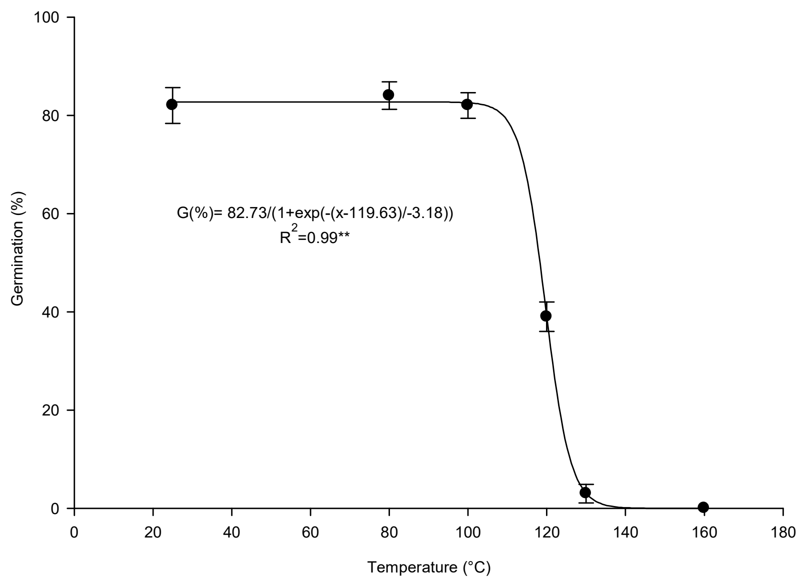
Vertical bars represent standard error of the means.

Figure 1 - Effect of constant temperature (A) and alternating temperature (B) on germination of Ivy-leaved morning-glory seeds.

The results of experiment of Thullen and Keeley (1983) showed that ivy leaf morning-glory seed germinated the most at the night/day air temperature regime of 27/32 °C, but germination decreased as temperature increased up to 32/38 °C. No germination occurred at 10/15 °C. Oliveira and Norsworthy (2006) reported that the optimum germination of Pitted morning-glory (*Ipomoea lacunose*) was between 20 and 25 °C. The highest germination of Tall morning-glory (*Ipomoea* purpurea) was observed at alternating temperatures of25/15 °C to 30/20 °C (Singh et al., 2012). Chauhan and Abugho (2012) stated that maximum germination of Three lobe morning-glory (*Ipomoea triloba*) was obtained in complete darkness at night/day temperatures of 15/25 °C and 25/35 °C, and the germination decreased to <10% and <20% at below 20/12.5 °C and above 35/25 °C, respectively.

Effect of high temperature pretreatment on germination

A three-parameter sigmoidal model described the effect of heating on seed germin ability. Germination was greater than 80% up to temperatures of 100 °C for 5 min, but the increasing temperature from 100 °C to 120 °C decreased severely germination of Ivy-leaved morning-glory, with no germination at >130 °C. On the basis of the fitted model, a temperature required for 50% reduction of maximum germination was estimated 119.63 °C (Figure 2). This suggests that high temperatures caused by fire can destroy part of the seeds. So, burning of crop residue after harvesting to prepare the land for planting the next crop in some areas could be effective to control this invasive weed by removing some seeds in the soil surface.



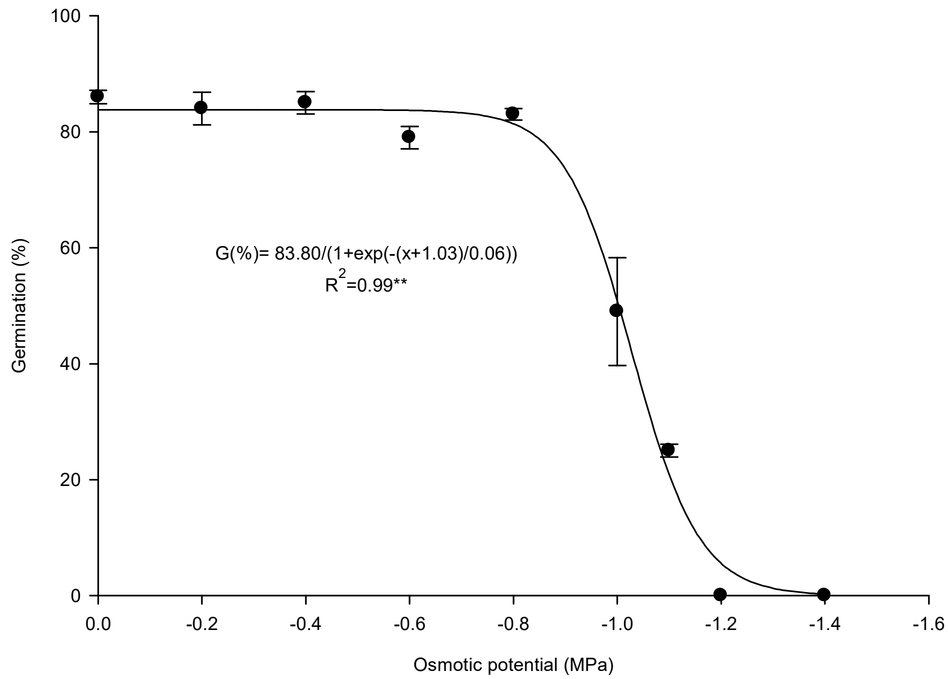
Vertical bars represent standard error of mean.

Figure 2 - Effect of high temperature on germination of Ivy-leaved morning-glory seeds.

Vermeire and Rinella (2009) in the study of fire effect on emergence of invasive species of leafy spurge (*Euphorbia esula*), Japanese brome (*Bromus japonicas*), Russian knapweed (*Acroptilon repens*), and spotted knapweed (*Centaurea maculosa*) observed that emergence was reduced from 79 to 88% in comparison to unburned treatment. Seed germination of Horse purslane (*Trianthema portulacastrum*) reduced up to 50% when exposed to temperature of 119 °C for 5 min (Lee et al., 2011).

Effect of osmotic stress on germination

Osmotic stress significantly affect seed germination of Ivy-leaved morning-glory, as provided a good fit by three-parameter sigmoidal model (G (%) = 83.8/{1+exp[-(x+1.02)]/0.06}) (Figure 3). Germination declined from 85%to 25% as osmotic potential dropped from 0 to -1.1 MPa and no



Vertical bars represent standard error of mean.

Figure 3 - Effect of osmotic potential on germination of Ivy-leaved morning-glory seeds.

germination was occurred at ≥-1.2 MPa. Germination decreased by 50% of the maximum at an osmotic potential of -1.03 MPa. The results show that this weed has tolerant to low water potential in the germination stage.

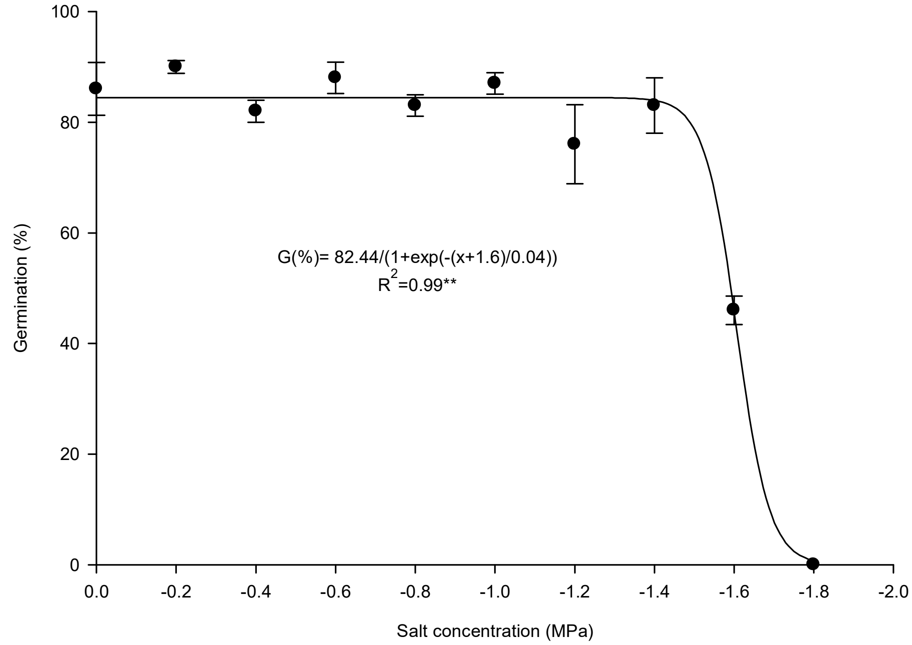
Three lobe morning-glory germination was reduced to 6% (from 90% in the non-treated control) at an osmotic potential of -0.6 MPa, and it was completely inhibited at -0.8 MPa (Chauhan and Abugho, 2012).The germination of Tall morning-glory was very low (15%) at an osmotic stress of -0.3 and -0.4 MPa, and no germination was observed above that (Singh et al., 2012). Germination of Pitted morning-glory (Oliveira and Norsworthy, 2006) decreased with an increase in drought stress. Seed germination of *Ipomoea asarifolia* declined linearly with decreasing osmotic potential, and germination ceased at osmotic potential <-0.8 MPa (Dias-Filho, 1996). This suggests that morning-glory species had varying sensitivity to osmotic stress. The results of our study indicated that Ivy-leaved morning-glory was more tolerant to water stress than other weed species of morning-glory. These results were similar to those reported by Crowley and Buchanan (1978).

Effect of salinity stress on germination

Osmotic potential caused by sodium chloride, ranging from 0 to -1 MPa, did not influence germination of the seeds of Ivy-leaved morning-glory. This species had excellent tolerance to salinity with 86 and 46% germination at 0 and -1.6 MPa, respectively. The osmotic potential due to NaCl required for 50% reduction of maximum germination (x_{50}) was estimated -1.6 MPa (Figure 4), indicating that the inhibitory effect on seed germination was larger in PEG treatment than in NaCl treatment. Thus, this exotic species could pose an invasion threat to saline soil habitats.

Germination of Three lobe morning-glory occurred under a wide range of salt concentration, as seeds germinated 96-99% across a NaCl range of 0 to 250 mM (Chauhan and Abugho, 2012).

Ninety percent of Tall morning-glory seeds germinated in distilled water, whereas germination dropped to less than 40% at a salt concentration of 50 mM, and complete inhibition of its germination occurred at 80 mM (Singh et al., 2012).

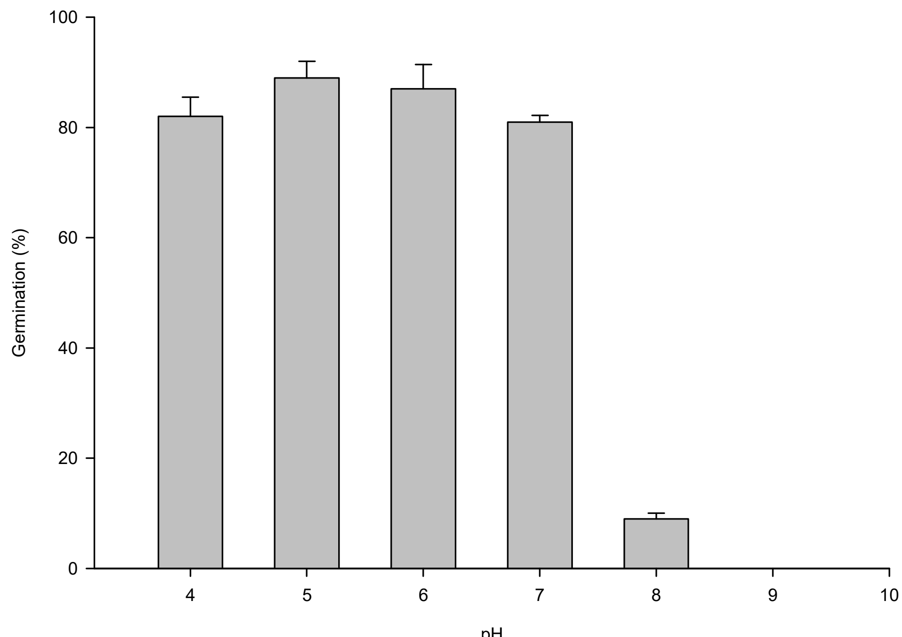


Vertical bars represent standard error of mean.

Figure 4 - Effect of salt concentration on germination of Ivy-leaved morning-glory seeds.

Effect of pH on germination

Germination of Ivy-leaved morning-glory seeds was significantly influenced by pH (Figure 5). Maximum (89%) and minimum (9%) germination were observed at pH 5 and 8, respectively. There was no significant difference in germination between the pH levels of 4 to 7. However, treatments of pH above 7 significantly decreased seed germination compared to control in which germination was 85%. No germination was observed at pH>8. This suggests that acidic and neutral conditions were favorable for germination of Ivy-leaved morning-glory. Most Gorgan soils fall in the acidic-to-neutral range, although some soils have pH values greater than 7. Hence, soil pH factor may affect the distribution of this weed.



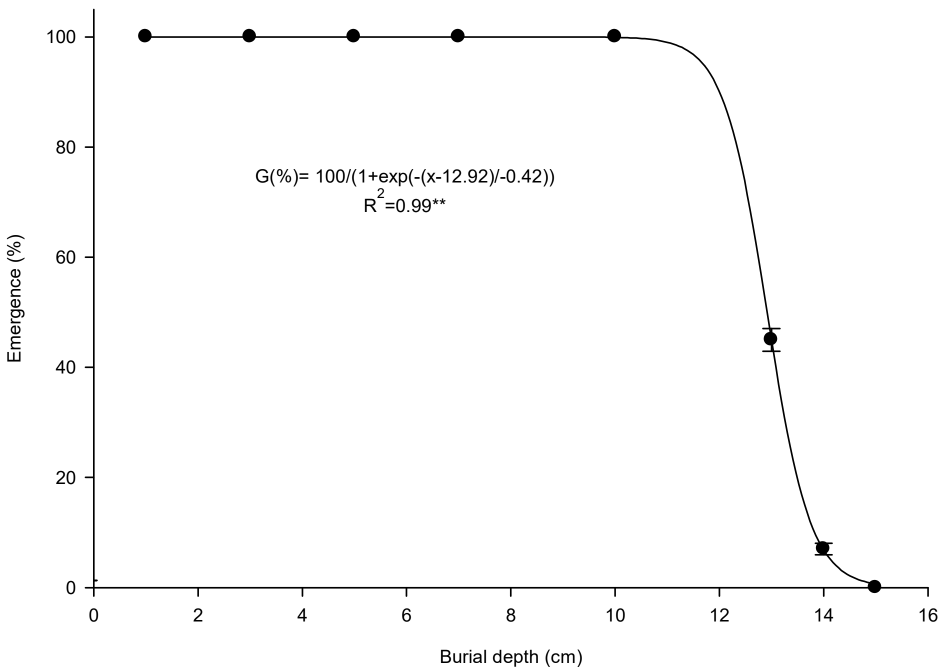
Vertical bars represent standard error of mean.

Figure 5 - Effect of pH on germination of Ivy-leaved morning-glory seeds.

The highest Pitted morning-glory germination was observed at pH 3 with optimal germination in a range from 6 to 8 of buffered pH solutions (Oliveira and Norsworthy, 2006). In contrast, maximum germination of Tall morning-glory (75%) was occurred at pH 6 with 31% germination at pH 5, and there was no germination at pH 4 (Singh et al., 2012). The pH range for the germination of different morning-glory species in studies show that soil pH is a limiting factor for germination of these weed species.

Effect of burial depth on seedling emergence

Ivy-leaved morning-glory emergence as a function of a range of planting depths is presented inFigure6. Emergence was at its maximum for seeds planted at depths of 1 to 10 cm, and dramatically decreased with increasing the depth of burial to 45% at 13 cm. No emergence observed at a planting depth of 15 cm. The burial depth which caused 50% inhibition of the maximum emergence was 12.93 cm (Figure 6).



Vertical bars represent standard error of mean.

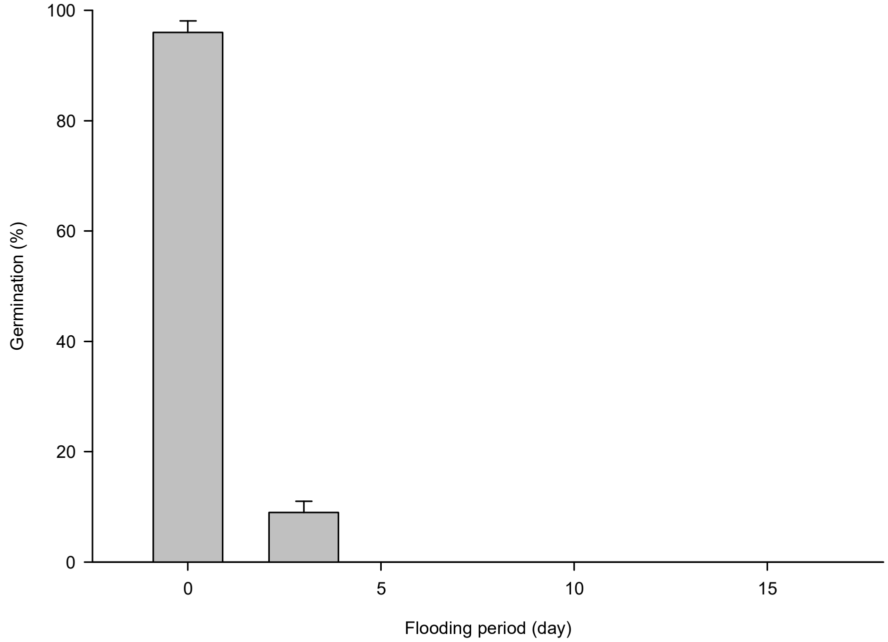
Figure 6 - Effect of seed burial depth on emergence of Ivy-leaved morning-glory seeds.

Since less than 1% of the light can penetrate greater than 4 mm soil deep (Benvenuti et al., 2001), the results explained that Ivy-leaved morning-glory seeds are not a light requirement for emergence. Hence, this species is able to emerge even after the closure of canopy of crop.

Investigation of unemerged seeds at the end of the experiment (14 d) showed that they had decayed (fatal germination). It seems that seeds buried in depths of ≥13 cm germinated, but the *seedling* died before reaching the *soil* surface. Considering the fatal germination at these depths, burial of Ivy-leaved morning-glory seeds to a 15 cm, or possibly greater, depth with tillage operations may be an appropriate method of reducing Ivy-leaved morning-glory seed banks through fatal germination.

Effect of flooding on seedling emergence

Flooding had a suppressive effect on the emergence of Ivy-leaved morning-glory. Only 9% of seeds produced seedlings when flooding duration was for three days, whilst under normal (no flooding) conditions, emergence was 96%. Seeds of Ivy-leaved morning-glory buried in flooded soil for over three days failed to emerge (Figure 7). These findings indicated that Ivy-leaved



Vertical bars represent standard error of mean.

Figure 7 - Effect of flooding period on emergence of Ivy-leaved morning-glory seeds.

morning-glory was sensitive to flooding. The results of the present study agree with similar studies showing that emergence of other Morning-glory species [such as Palm leaf morning-glory (*Ipomoea wrightii*), Pitted morning-glory and Tall morning-glory] were also inhibited at flooding conditions (Gealy, 1998; Singh et al., 2012). It suggests that this species is not capable to be established in flooded or low drained soils. According to the impact of flooding on the emergence of this plant, flooding can be a suitable method for the management of this weed.

In conclusion, the results of our study indicated that environmental conditions had substantial effects on seed germination and emergence of Ivy-leaved morning-glory. This species is a tropical plant that its germination occurred from temperatures of 15 °C to 35 °C. However, seeds germinated greater at alternating temperatures. This weed had excellent tolerance to salinity and drought stresses. Therefore, these two environmental factors are not limiting factors of this species spread in dry farmlands of semi-arid regions in Iran. Seed germination was affected by pH. The results suggest that Ivy-leaved morning-glory tends to germinate better in acidic soils compared with neutral-to-alkaline soil environments. All seeds buried at 1-10 cm depths were able to emerge, and no germinable seeds were found at depths greater than 15 cm. So, burying seeds deep enough (>15 cm) can be beneficial to prevent morning-glory emergence. However, subsequent tillage operations must be at less depths to avoid transferring seeds near to the soil surface. Seedling emergence was inhibited by flooding >3 d. Thus, this technique could be exploited in the management of this weed. High temperatures also decreased seed germination of this species; therefore, residue burning can be effective in reducing the population of the seed bank.

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