

# SEED GERMINATION AND SEEDLING EMERGENCE OF VELVETLEAF (*Abutilon theophrasti*) AND BARNYARDGRASS (*Echinochloa crus-galli*)<sup>1</sup>

*Germinação de Sementes e Emergência de Plântulas de Abutilon theophrasti e Echinochloa crus-galli*

SADEGHLOO, A.<sup>2,4</sup>, ASGHARI, J.<sup>2</sup>, and GHADERI-FAR, F.<sup>3</sup>

**ABSTRACT** - *Abutilon theophrasti* and Barnyardgrass (*Echinochloa crus-galli*) are major weeds that affect cropping systems worldwide. Laboratory and greenhouse studies were conducted to determine the effects of temperature, pH, water and salinity stress, and planting depth on seed germination and seedling emergence of Velvetleaf and Barnyardgrass. For Velvetleaf, the base, optimum and ceiling germination temperatures were estimated as 5, 35 and 48 °C, respectively. Seed germination was sensitive to drought stress and completely inhibited by a potential of -0.6 MPa, but it was tolerant to salinity. Salinity stress up to 45 mM had no effect on the germination of Velvetleaf, but germination decreased with increasing salt concentration. Drought and salinity levels for 50% inhibition of maximum germination were -0.3 MPa and 110 mM, respectively. Seed germination of Velvetleaf was tolerant to a wide range of pH levels. For Barnyardgrass, the base, optimum and ceiling germination temperatures were estimated as 5, 38 and 45 °C, respectively. Seed germination was tolerant to drought stress and completely inhibited by a potential of -1.0 MPa. Salinity stress up to 250 mM had no effect on seed germination. Drought and salinity levels for 50% inhibition of maximum germination were -0.5 MPa and 307 mM, respectively. A high percentage of seed germination was observed at pH=5 and decreased to 61.5% at acidic medium (pH 4) and to 11% at alkaline medium (pH 9). Maximum seedling emergence of Velvetleaf and Barnyardgrass occurred when the seeds were placed on the surface of the soil or at a depth of 1 cm.

**Keywords:** pH, planting depth, seed germination, temperature, weed.

**RESUMO** - *Abutilon theophrasti* e *Echinochloa crus-galli* são as principais plantas daninhas que afetam os sistemas de cultivo em muitas partes do mundo. Estudos em laboratório e em casa de vegetação foram conduzidos para determinar os efeitos da temperatura, pH da água e estresse salino e da profundidade de semeadura na germinação e na emergência das plântulas de *A. theophrasti* e *E. crus-galli*. Para *A. theophrasti*, as temperaturas de base, ótima e de teto de germinação foram estimadas em 5, 35 e 48 °C, respectivamente. A germinação das sementes foi sensível ao estresse hídrico e inibida completamente por um potencial de -0,6 MPa, mas foi tolerante à salinidade. Estresse salino de até 45 mM não teve nenhum efeito sobre a germinação de *A. theophrasti*, porém esta diminuiu com o aumento da concentração salina. Os níveis de seca e de salinidade para 50% de inibição da germinação máxima foram de -0,3 MPa e 110 mM, respectivamente. A germinação das sementes de *A. theophrasti* foi tolerante a uma vasta gama de níveis de pH. Para *E. crus-galli*, as temperaturas de base, ótima e de teto de germinação foram estimadas em 5, 38 e 45 °C, respectivamente. A germinação das sementes foi tolerante ao estresse hídrico e inibida completamente a um potencial de -1,0 MPa. Estresse salino de até 250 mM não teve efeito sobre a germinação das sementes. Os níveis de seca e de salinidade para 50% de inibição da germinação máxima foram de -0,5 MPa e 307 mM, respectivamente. Elevada porcentagem de germinação das sementes foi observada a pH=5, com diminuição para 61% em meio ácido (pH=4) e para 11% em meio alcalino (pH=9). A máxima emergência das plântulas de *A. theophrasti* e *E. crus-galli* ocorreu quando as sementes foram colocadas sobre a superfície do solo ou a uma profundidade de 1 cm.

**Palavras-chave:** pH, profundidade de semeadura, germinação de sementes, temperatura, planta daninha.

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<sup>2</sup> Department of Agronomy and Plant Breeding, University of Guilan, Iran; <sup>3</sup> Department of Agronomy, University of Agricultural Sciences and Natural Resources Gorgan, Iran; <sup>4</sup> Corresponding author, <sadeghloo87@yahoo.com >.



## INTRODUCTION

Weeds have the ability to adapt to unfavorable environmental conditions. Plants growing in unpredictable environments evolve and make adaptations associated with seed physiology and morphology, such as seed size variability, dormancy and development of special structures for seed dispersal to cope with uncertain conditions. Seed traits are determined both by parental environment and by seed genotype (Galloway, 2001a, b).

Velvetleaf is an annual weed from the Malvaceae family. For a long time this species has been infesting crops of maize, cotton, sunflower and potato. Barnyardgrass is a Poaceae species that grows well in wet soil and can continue to grow when partially submerged. Barnyardgrass is a major weed that affects rice production (Holm et al., 1977), and it is a problem weed in 42 countries (Holm et al., 1979). Young Barnyardgrass plants have similar morphology to rice plants and can be hard to differentiate.

Koger et al. (2004) reported that seed germination is a key event that determines the success of a weed in an agro ecosystem. Seed germination in weeds is influenced by many environmental factors, such as temperature, pH, light, salinity, and moisture (Chauhan & Johnson, 2008a,b). Temperature plays a major role in determining the periodicity of seed germination and the distributions of species (Guan et al., 2009). Alvarado & Bradford. (2002) stated that germination rate usually increases with temperature linearly, at least within a well-defined range, and then declines sharply at higher temperatures.

Other major factors that affect seed germination are drought and salinity. Zhou & Deckard (2005) reported that osmotic and salt stress can reduce, delay or prevent germination. A reduction in water availability, associated with the toxic effect of salts, interferes in the process of water absorption by seeds. This may influence germination and seedling vigor and consequently, normal plant development (Yamashita et al., 2009; Ghaderi-Far et al., 2010). Germination is also affected by pH (Norsworthy & Oliveira, 2006). Weed seedling emergence may also be affected by

seed depth and by tillage, among other factors (Chauhan et al., 2006).

An understanding of weed growth and development is needed to design effective weed management programs. For example, it has been well documented that knowing the relative time for weed emergence of a crop is an important factor to consider in weed management decisions (Knezevic et al., 2001; Bensch et al., 2003). A better understanding of the biology of seed germination in these species could contribute to the development of weed management technologies to help counter those undesirable shifts in weed populations. It could also enable sustainable and intensified cultivation of rice cropping systems and promote changes to crop management practices (Chauhan & Johnson, 2008c). The main objective of this study was, therefore, to determine the effects of temperature, light, pH, osmotic water potential, salt stress and burial depth on the germination and emergence patterns of Velvetleaf and Barnyardgrass in order to optimize timing in weed management strategy.

## MATERIAL AND METHODS

### Seed samples

Velvetleaf and Barnyardgrass seeds were collected from an infested soybean and rice field in the Iranian province of Golestan in late September 2009. Immediately after harvest, seeds were dried and kept in a refrigerator at  $5 \pm 2$  °C for later use. Seed dormancy was interrupted in Velvetleaf seeds by soaking them in boiling water for 5, 10, 30 and 60 seconds; the seeds treated for 5 seconds achieved 92% germination. Barnyardgrass seeds were also dipped in concentrated sulfuric acid (98%) for 3, 5, 7, 9, 10, 15, 20, 25 and 30 minutes; then they were washed with distilled water. 95% germination was achieved from soaking seeds in concentrated sulfuric acid for 15 min.

### The effect of temperature

Seed germination was tested on 4 replicates of 50 seeds in moist paper towels placed in incubators at continuous temperatures of 0, 5, 10, 20, 25, 30, 35, 40, 45

and 50 °C in darkness. The distilled water used for moisture replenishment was kept in the incubator to ensure that the temperature of the water was consistent with that of the incubator. Germinated seeds were checked three times a day for a period of 20 days and were considered germinated when their radicle reached 2 mm (Soltani et al., 2002; Ghaderi-Far et al., 2010). Water was added as needed.

The germination progress curve versus time was drawn for each replication and the time needed for cumulative germination to reach to 50% at each replication ( $D_{50}$ ) was estimated by interpolation. Germination rate ( $R_{50} \text{ h}^{-1}$ ) was calculated as follows (Soltani et al., 2002):

$$R_{50} = 1/D_{50} \quad (\text{eq. 1})$$

### The effect of salinity stress

In order to examine the effect of salinity stress on seed germination, 4 replicates of 50 seeds were incubated in moist paper towels in prepared aqueous solutions of NaCl in concentrations of 0, 45, 90, 135, 180 and 220 mM for Velvetleaf seeds and 0, 45, 90, 135, 180, 225, 265, 310, 355 and 400 mM for Barnyardgrass seeds. Seeds were incubated at 32 °C and 30 °C in darkness for Velvetleaf and Barnyardgrass seeds, respectively.

### The effect of drought stress

To study the effect of drought stress on seed germination, 4 replicates of 50 seeds were incubated in moist paper towels saturated with seven osmotic potentials of 0, -0.2, -0.4, -0.6, -0.8, -1.0 and -1.2 MPa. Solutions were prepared by dissolving the appropriate amount of polyethylene glycol 6000 (PEG) in deionized water. The amount of PEG 6000 for each concentration was obtained using the equation proposed by Michel & Kaufmann (1973). Seeds were incubated at 32 °C and 30 °C in darkness for Velvetleaf and Barnyardgrass, respectively.

### The effect of pH

To investigate the effect of pH on seed germination, 4 replicates of 50 seeds were

incubated in moist paper towels and a buffer solution with pH = 4, 5, 6, 7, 8 and 9. Buffer solutions were comprised of potassium hydrogen phthalate and 0.1 M HCl or 0.1 M NaOH to adjust the pH solutions to 4, 5, and 6. A 25 mM sodium tetraborate decahydrate solution was used in combination with 0.1 M HCl or 0.1 M NaOH to obtain pH solutions of 7, 8 and 9 (Susko et al., 1999). Seeds were incubated at 32 and 30 °C in darkness for Velvetleaf and Barnyardgrass, respectively.

### The effect of planting depth

Seed planting depths were tested in 4 replicates of 50 seeds. Seeds from each species were planted in plastic pots on the surface of the soil and at depths of 1, 2, 4, 6, 8, 10, 12 and 14 cm and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 9 and 11 cm for Velvetleaf and Barnyardgrass seeds, respectively. The soil used for the experiment had a silty clay loam texture. The bottom and uppermost layers of the soil in all of the treatments were leveled and then pressured before and after seed placement at each depth to increase the contact between the soil and the seeds. Pots were placed in a greenhouse with temperatures from 32 and 30 °C in darkness for Velvetleaf and Barnyardgrass seeds, respectively. Seedling emergence was monitored daily for 20 d. Seedling were counted at the appearance of the cotyledon and removed by cutting them at the soil surface

### Statistical analyses

A completely randomized experimental design with four replications was used for all the studies. A segmented model (Equation 2) was applied to study the reaction of germination rate versus temperature and to determine cardinal temperatures (Soltani et al., 2006).

$$f(T) = (T - T_b) / (T_o - T_b) \quad \text{if } T_b < T \leq T_o$$

$$f(T) = (T_c - T) / (T_c - T_o) \quad \text{if } T_o < T < T_c \quad (\text{eq. 2})$$

$$f(T) = 0 \quad \text{if } T \leq T_b \text{ or } T \geq T_c$$

where T is temperature, and  $T_b$ ,  $T_o$  and  $T_c$  are base, optimum and ceiling temperatures, respectively. Osmotic stress data were fitted



to a linear model and salt stress data showed a three-parameter logistic model for both weeds (Equation 3).

$$G(\%) = G_{max} / (1 + (x/x_{50})^{Grate}) \quad (\text{eq. 3})$$

where  $G$  denotes the total germination (%) at concentration  $x$ ,  $G_{max}$  is the maximum germination (%),  $x_{50}$  is the NaCl potential required for 50% inhibition of the maximum germination and  $Grate$  indicates the slope of the curve in  $x_{50}$ .

Data for planting depth were fitted to a three-parameter logistic (Equation 3) and exponential model for Velvetleaf and Barnyardgrass, respectively. All tests were analyzed using SAS software.

## RESULTS AND DISCUSSION

### The effect of temperature on germination

Maximum germination for Velvetleaf was 73-85% and occurred between 10 and 40 °C, and then declined at 45 °C (Figure 1A). Maximum germination for Barnyardgrass was 78-94% and occurred between 15 and 35 °C and then declined at 10 and 40 °C (Figure 1B).

A Segmented model was used to describe the influence of temperature on germination rate (Figure 2A). No germination occurred at constant temperatures of 0 and 45 °C for Barnyardgrass and 5 and 50 °C for Velvetleaf. The rate of germination increases from the base temperature to the optimum temperature and then decreases to a ceiling temperature.

Base, optimum and ceiling temperatures were estimated as 5, 34.84 and 48.16 °C, and 5.014, 38.12 and 45 °C for Velvetleaf and Barnyardgrass seeds, respectively (Figure 2).

Seeds germinated over a temperature range of 10-40 °C for Velvetleaf and range of 5-40 °C for Barnyardgrass, which could allow for germination throughout the spring and summer months in many parts of the world.

### The effect of salinity and drought stress on germination

A three-parameter logistic model (Figure 3) was fitted to obtain the germination percentage at different concentrations of NaCl for Velvetleaf and Barnyardgrass. (Figure 3A) shows that there was no change in seed germination up to 45 mM NaCl concentration (74-89%), but germination decreased sharply with increased NaCl concentration, while no germination occurred at 225 mM NaCl. The concentration of NaCl required for 50% inhibition of the maximum germination ( $x_{50}$ ), estimated from the fitted model, was 110 mM (Figure 3A). These data suggest that only a few seeds of Velvetleaf may germinate in high-salinity soil.

Figure 3B shows that Barnyardgrass germination was greater than 80% up to a concentration of 150 mM NaCl; 68% germination occurred at 225 mM NaCl; germination was completely inhibited at 400 mM NaCl. The concentration for 50% inhibition of maximum germination was

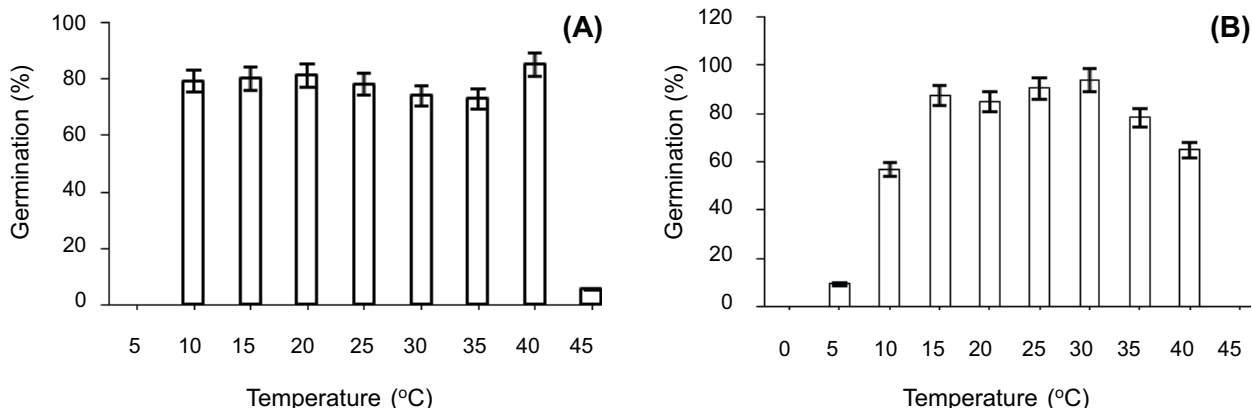


Figure 1 - Effect of constant temperature on germination percentage of Velvetleaf (A) and Barnyardgrass (B).

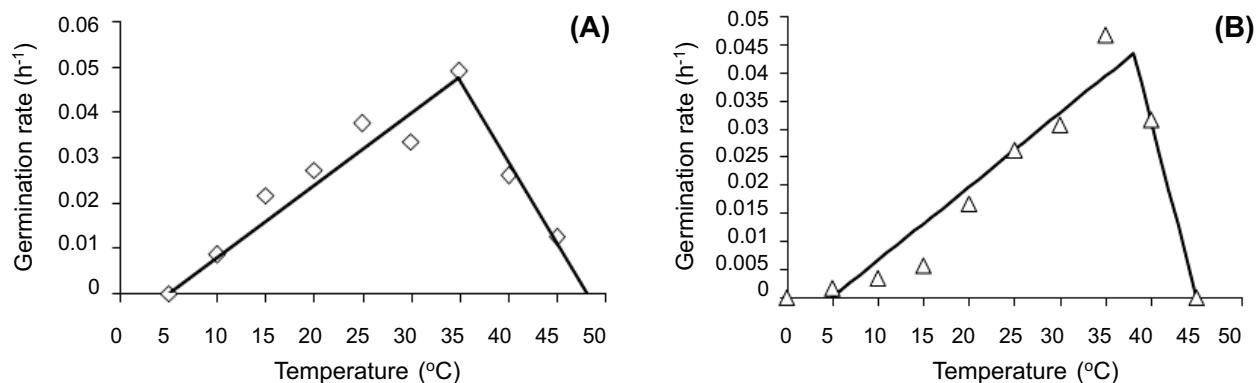


Figure 2 - Effect of constant temperature on germination rate of Velvetleaf (A) and Barnyardgrass (B).

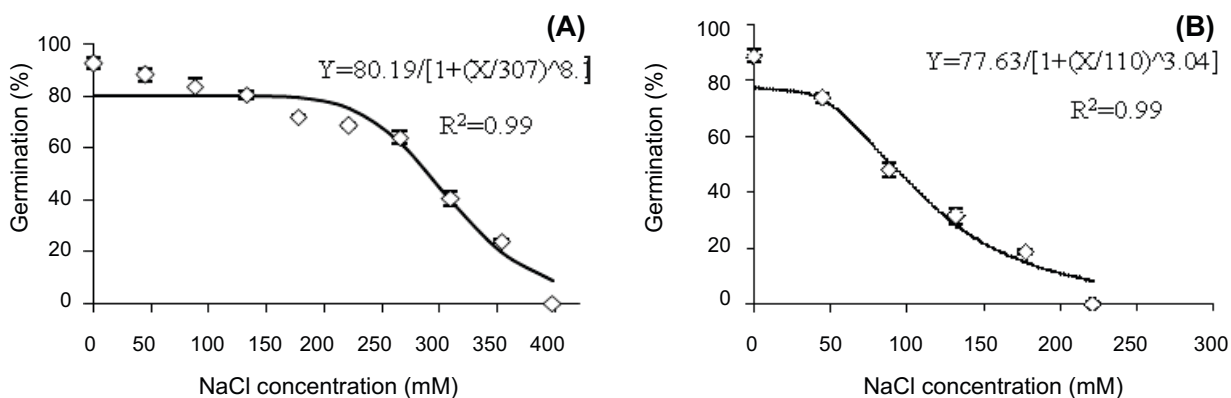


Figure 3 - Effect of NaCl concentration on germination of Velvetleaf (A) and Barnyardgrass (B).

307 mM NaCl. Chauhan & Johnson (2008b) reported that *Eclipta prostrata* had a high tolerance to salt at the germination phase and subsequent stages of growth. It has also been determined that germination was greater than 60% up to a concentration of -50 mM NaCl, while it was completely inhibited at 200 mM NaCl for Junglerice (Chauhan & Johnson, 2009).

A linear model ( $y = -14.1x + 85.55$ ,  $R^2 = 0.99$ ) was fitted to germination values (%) obtained at different osmotic potentials for Velvetleaf (Figure 4A). The maximum germination estimated from the fitted model was 86.5%, and germination decreased sharply as osmotic potential increased from 0 to -0.6 MPa. Germination was completely inhibited at an osmotic potential of -0.6 MPa or lower. Osmotic potential for 50% inhibition

of the maximum germination ( $x_{50}$ ), estimated from the fitted model, was -0.3 MPa (Figure 4A). Germination of Barnyardgrass seeds decreased linearly ( $y = -9.0571x + 94.119$ ,  $r^2 = 0.97$ ) as the osmotic potential of the germination medium increased from 0 to -1.0 MPa (Figure 4B). However, even at an osmotic potential of -0.8 MPa, approximately 20% of the seeds germinated. In general, germination of Barnyardgrass seeds is inhibited by water stress condition.

The results of this study suggest that Velvetleaf is not very tolerant to salinity and is sensitive to water stress, while Barnyardgrass is tolerant to both salinity and water stress. Salinity can negatively affect physiological processes and is a major abiotic constraint for plants (Greenway & Munns, 1980; DiTommaso, 2004).



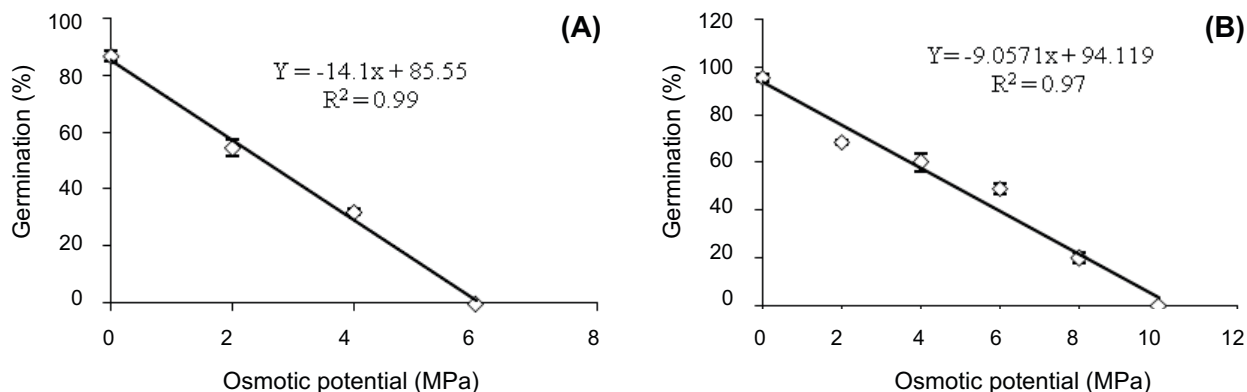


Figure 4 - Effect of osmotic potential on germination of Velvetleaf (A) and Barnyardgrass (B).

### Effect of pH on germination

Velvetleaf germination varied between 81-85% over a pH range of 4 to 9 (Figure 5A). Similarly, seeds of *Mimosa pudica* and *Ipomoea asarifolia* (Souza filho et al., 2001), Swallowwort (Pahlevani et al., 2008) and Trumpet creeper *Campsis radicans* (Chachalis & Reddy, 2000) have been reported to germinate over a wide pH range. Seed germination of Velvetleaf over a broad pH range indicates that pH is not a limiting factor for germination of this species. The optimum pH range for Barnyardgrass germination (> 61%) was between 4 and 6 (Figure 5B) and germination decreased at pH levels between 6 and 9 and there was only 11% germination at pH 9. Seed germination of Barnyardgrass and Velvetleaf over a broad pH range indicates that pH is not likely to be a limiting factor for germination in most soils. However, greater germination may be expected in acidic soil than in alkaline soil for Barnyardgrass.

### The effect of planting depth on seedling emergence

Seedling emergence of Velvetleaf was influenced by planting depth (Figure 6A) and maximum emergence (69%) occurred at a planting depth of 1 cm. Seeds placed on the surface of the soil had lower emergence (65%) compared with seeds planted at the depth of 1 cm. Seedling emergence slightly decreased as planting depth increased from 1 to 4 cm but decreased sharply when planted

deeper than 4 cm. Only 24% of seedlings emerged at a depth of 10 cm. No seedlings emerged from seeds buried at a depth of 12 cm. Limited contact between soil and seed, as well as reduced water availability are some environmental conditions that may limit seed germination on the surface of the soil (Clewis et al., 2007). This research suggests that light is not a requirement for Velvetleaf seed germination because 41% of the seeds germinated at a depth of 6 cm. Also, lower seedling emergence of seeds from deeper planting depths may be associated with limited seed reserves (Mennan & Ngouajio, 2006). Baskin & Baskin (1998) determined that larger seeds often have greater reserves and can emerge from a greater planting depth. Burcucumber (*Sicyos angulatus*) germinated from a depth of 16 cm (Mann et al., 1981). A possible management option for farmers may be deep tillage, which will bury seeds below 10 cm (maximum depths of emergence) because seeds of Velvetleaf cannot emerge from depths deeper than 10 cm.

Barnyardgrass seedling emergence was the greatest (31.5%) for seeds placed on the soil and decreased sharply with increased planting depth (Figure 6B). No seedlings emerged from seeds buried at a depth of 10 cm. Chauhan & Jahnson, (2009) reported that germination of Junglerice seed was 76% for seeds planted at a depth of 0.2 cm, and was the greatest for seeds placed on the surface of the soil (97%). However, the optimum depth of seedling emergence for Barnyardgrass was only 2 cm.

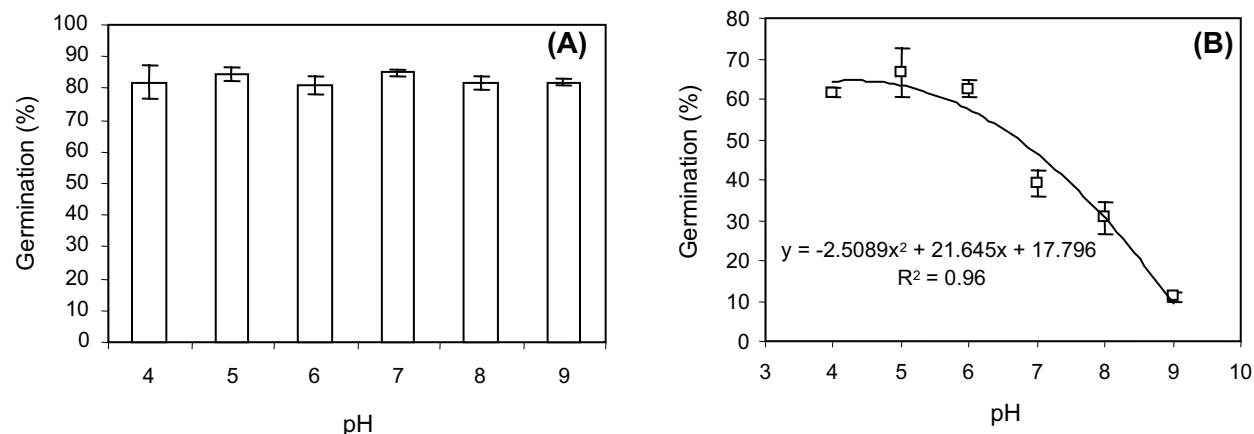


Figure 5 - Effect of pH on germination of Velvetleaf (A) and Barnyardgrass (B).

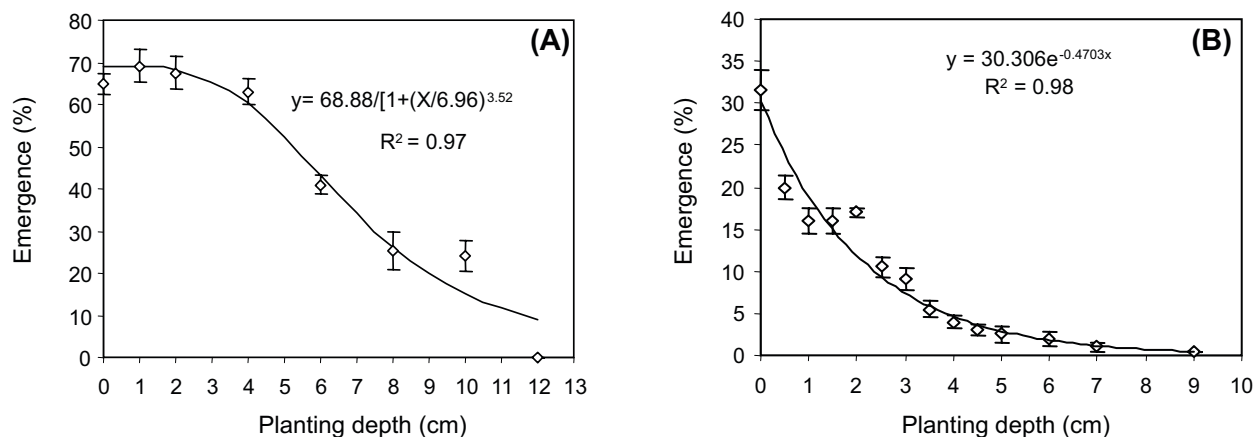


Figure 6 - Effect of planting depth on germination of Velvetleaf (A) and Barnyardgrass (B).

The results of this study indicated that seeds of Velvetleaf and Barnyardgrass can germinate under various environmental conditions. Germination of Velvetleaf and Barnyardgrass are influenced by temperature. For Velvetleaf, the base, optimum and ceiling germination temperatures were 5, 35 and 48 °C, respectively, and for Barnyardgrass they were 5, 38 and 45 °C, respectively. Velvetleaf is not very tolerant to salinity and is sensitive to water stress, but Barnyardgrass is tolerant to both salinity and water stress. Seed germination of Barnyardgrass and Velvetleaf over a broad pH range indicates that pH is not a limiting factor for germination of these species. However, greater germination may be expected in acidic soil than in alkaline soil for Barnyardgrass. These results suggested that under field condition, seeds of Velvetleaf

and Barnyardgrass would germinate only if near the soil surface.

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