

Influence of environmental factors, flooding period and seeding depth on germination and emergence of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.]

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Abstract: Agriculture would be far better served if weed scientists learned how to control weed seed dormancy and its germination so that weed problems could be prevented, rather than controlled as they appeared. Barnyardgrass has a problem weed plant and more abundant weed in 36 economic crops and in 61 countries. Laboratory and greenhouse experiments were conducted to determine the effect of photoperiod, salt stress, osmotic stress, solution pH, flooding period and burial depth on germination and emergence of annual barnyardgrass seeds. The results indicated that optimum germination was at solution pH 6, but further increases in pH had a more negative effect on germination. Barnyardgrass germination was 38% at pH 4, but was 0% at pH 9, which indicates that barnyardgrass germination was more tolerant of acidic than basic solutions. A significantly higher germination occurred when the seeds were planted at 6 cm depth; increasing burial depth delayed and significantly reduced the germination and no emergence occurred when seeding depth reached 15 cm. Similarly, the germination of the seeds was significantly reduced with simulated flooding. Germination decreased with decreasing solution osmotic potential, and no germination occurred when osmotic potentials were less than -0.9 MPa during 7 d incubation. Barnyardgrass was germinated in light as well as dark; however, light enhances the germination percentage. Germination was 100% at 50 mM NaCl, and some germination occurred even at 250 mM NaCl (33.3%). However germination was completely inhibited at 300 mM NaCl. Knowledge gained from this research will be instrumental in developing a better understanding of the requirements for barnyardgrass germination and emergence, allowing further development and improvement of integrated weed management strategies specific to this troublesome weed.

Keywords: photoperiod, osmotic potential, pH, burial depth, flooding period, weed biology, barnyardgrass, germination.

1. Introduction

Seeds of annual weeds germinate under a narrow range of environmental conditions. Temperature and moisture are perhaps the most important factors that affect germination. Light have a promoting or an inhibitory affect on the germination of many weed seeds.

An understanding the ecology and biology of weeds is needed for designing effective weed management programs. For example, it has been well documented that knowing the relative time of weed

emergence to the crop is an important factor in making weed management decisions^{1,2,3}. They add that developing a reasonable understanding of weed growth and development requires weed biology studies that are largely dependent on the successful seed germination and subsequent stand.

Agriculture would be far better served if weed scientists learned how to control weed seed dormancy and its germination so that weed problems could be prevented, rather than controlled as they appeared⁴.

Echinochloa crus-galli, a C₄ grass, is one of the world's most serious weeds⁵. Barnyardgrass is a problem weed in 61 countries and more abundant weed in 36 economic crops around the world⁶. All *Echinochloa* species have a C₄ photosynthetic cycle and show a great competitive advantage when they grow together with C₃ crops^{7,8}. The competition of 25 barnyardgrass plants/m² causes approximately 70% yield loss⁹. The barnyardgrass seedbank ranged from 0 to 11,100 seeds ft⁻² with a mean density of 500 seeds ft⁻² and the mean barnyardgrass seed production ranged from 20,000 plant⁻¹ (emerging with the crop) to 7,300 plant⁻¹ (35 days after crop emergence)¹⁰.

Rice panicle density was increased 14 panicles/m² and 750 kg/ha for each 10% increase in barnyardgrass control¹¹.

From the literature, the crop yield losses due to barnyardgrass competition have been reported as 8 to 82% in corn and 30 to 45% in cotton¹². At a density of 104 plants/m² of *Echinochloa crus-galli* with green mass of 18,052 kg/ha corn crop was fully compromised¹³.

Increased knowledge of germination biology of barnyardgrass would facilitate development of effective weed control programs.

Barnyardgrass (*Echinochloa crus-galli*) is the most problematic weed in rice-production systems in Arkansas and is gaining much attention due to the widespread occurrence of resistance to commonly used rice herbicides¹⁰. The success of this weed may be attributed to the production of large numbers of small, easily dispersed seeds per plant, possession of seed dormancy, rapid development and ability to flower under a wide range of photoperiods, and relative resistance of mature plants to herbicide sprays⁴.

The important factors that determine a weed's ecological interactions are light, temperature, water, wind, humidity; and seasonal aspects e.g. the climate, permeability of the seed coat and the time of weed emergence¹⁵.

Knowledge gained from this research will be instrumental in developing a better understanding of the requirements for barnyardgrass germination and emergence, allowing further development and improvement of integrated weed management strategies specific to this troublesome weed. An alternative of protecting crops against weeds is creating situations that force weed plants to start developing with fatal consequences¹⁶. This may be achieved at the stage of seed germination through agricultural practices that incite an untimely germination followed by death of the seedlings¹⁷.

The objective of this study was to determine the influence of some bio-ecological factors e.g. photoperiod, light exposure, osmotic pressure, pH, depth of planting and flooding period etc. on the germination of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.].

2. Materials and Methods

Seeds of barnyardgrass weed were collected in fields and stored at 5°C before tests under controlled environments. Laboratory and greenhouse experiments were conducted at Botany Department, National Research Centre, Dokki, Giza, Egypt in 2014 year (previously conducted also at Horticultural Sciences Department, Citrus Research and Education Centre, Institute of Food and Agriculture Sciences, University of Florida, USA) to determine the effect of photoperiod, osmotic stress, solution pH, flooding period and burial depth on barnyardgrass germination and emergence.

Only healthy seeds of barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] were surface sterilized by immersion in 0.5% sodium hypochlorite for 10 min. This sterilizing solution was filtered through a Whatman #4 filter paper in a Buchner funnel, and the seeds washed with several flushes of distilled water and allowed to dry at room temperature.

Unless stated otherwise, individual treatments for all experiments consisted of four replicates of 25 sterilized seeds placed on two sheets of filter paper in 9 cm Petri dishes. The filter paper was moistened initially with 5 ml of water or test solution and if necessary, 1 to 3 ml of the appropriate solution was added to maintain adequate moisture and Petri dishes sealed with parafilm to minimize desiccation. Germination studies were conducted in temperature- and light- controlled growth cabinets.

Unless stated otherwise a 12 h daily photoperiod was maintained in each chamber, with the light period extending from 1 h before to 1 h after exposure to the daily high temperature. Fluorescent lamps produced a photosynthetic photon flux density of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. Petri dishes assigned to dark treatments were kept wrapped in two layers of aluminum foil until the final day of experiment. Germination of seed was measured after 7 days (d) in each experiment. A seed was considered germinated when the radicle protruded 1 mm from the seed coat.

Experiments were conducted to examine the effects of:

2.1. Effect of Osmotic Stress

Fifty seeds were placed on three layers of filter paper in 9-cm diam petri dishes, were moistened with 8 ml of solutions of varying osmotic potential, and were held in a 28 °C incubator as described¹⁸.

Solutions with osmotic potentials of 0, -0.3, -0.4, -0.6, -0.9, and -1.3 mPa were prepared by dissolving 0, 154, 191, 230, 297, or 350 g of polyethylene glycol (PEG8000) in 1 L of deionised water¹⁹. Distilled water was used as a control

The effect of these osmotic stress conditions will be recorded on germination of weed seeds for 7 days.

2.2. Photoperiod on the germination

The experiments were conducted to determine the effect of photoperiod (light /dark regimes) on the germination of weed seed. Constant temperature of 30/25 °C was maintained and a range of light/Dark conditions were created in growth cabinets e.g. 0/24, 4/20, 8/16, 12/12, 16/8, 20/4 and 24/0 condition. Petri dishes assigned to dark treatments were kept wrapped in two layers of aluminum foil until the final day of experiment. The effect of these light and dark conditions was recorded on germination of weed seeds for 7 days.

2.3. pH

The solutions of pH 4, 5, and 6 were prepared using 0.1 M potassium hydrogen phthalate, while solutions with pH 7, 8, and 9 were prepared with 25mM borax¹⁹. Buffer solutions were adjusted to the appropriate pH using 0.5 M NaOH or 1 N HCL. Distilled water was used as a control.

2.4. Planting depth

Fifteen seeds were planted in Candler fine sand in styrofoam cups (16 cm in diameter x 20cm in height) at depths of 0, 0.5, 1, 2, 4, 6, 10 or 15 cm below the soil surface and were kept in a greenhouse maintained at 25/20°C ($\pm 2^\circ\text{C}$) and 70 % ($\pm 5\%$) relative humidity under normal daylight. All cups were surface irrigated daily to field capacity. Seedling emergence was recorded weekly for three weeks.

2.5. Simulated flooding

Fifteen seeds were planted in Candler fine sand as above at 1 cm depth. To simulate flooding, water was maintained approximately 2 cm above the soil surface for 0, 4, 7, 14, and 21 d after planting. After exposure to a given period of flooding, surface water was drained off, and thereafter watered as needed to maintain adequate moisture. Seedling emergence was recorded weekly for three weeks.

2.6. Effect of Salt Stress

Salt effects on germination were studied with the use of solutions of 0, 50, 100, 150, 200, 250, and 300 mM sodium chloride. Distilled water was used as a control. The effect of these variable salt stress conditions will be recorded on germination of weed seeds for 7 days.

Greenhouse trials were performed at temperatures of 30/24 C day/night and 16 h of light. Germination percentage was calculated as the total number of seeds germinated divided by the total number of seeds in each replication.

Experiments were repeated twice and the data from two studies were combined for homogeneity. The data collected consisted of cumulative germination percent. Prior to analysis, final percent values were arcsine square root transformed but are presented in original form. Factorial ANOVA was performed to assess the effect of light/dark condition; planting depth and time of emergence; and simulated flooding and time of emergence on the germination, and one-way ANOVA to assess the effect of other factors on emergence i.e. photoperiod and pH using Agriculture Research Manager.

Analysis of variance and mean separations were performed after the percent control data were normalized by arcsine square root conversion²⁰ but are presented in the figures in original form. Significant differences among treatments were identified using Fishers LSD test ($P \leq 0.05$).

3. Results and Discussion

3.1. Effect of Osmotic Stress

Complete germination of barnyardgrass was recorded at -0.3 MPa then decreased gradually with increasing the osmotic stress from -0.4 to -0.9 MPa and was completely inhibited at osmotic potential at -1.3 MPa. However 10% of seeds were germinated at -0.9 MPa (Fig. 1). This means that barnyardgrass seeds have some capacity to germinate under moisture stress conditions. Similar finding was reported on rigid ryegrass (*Lolium rigidum*)²¹ and on barnyardgrass^{3,22} who reported that seed germination of barnyardgrass was tolerant to drought stress and completely inhibited by a potential of -1.0 MPa.

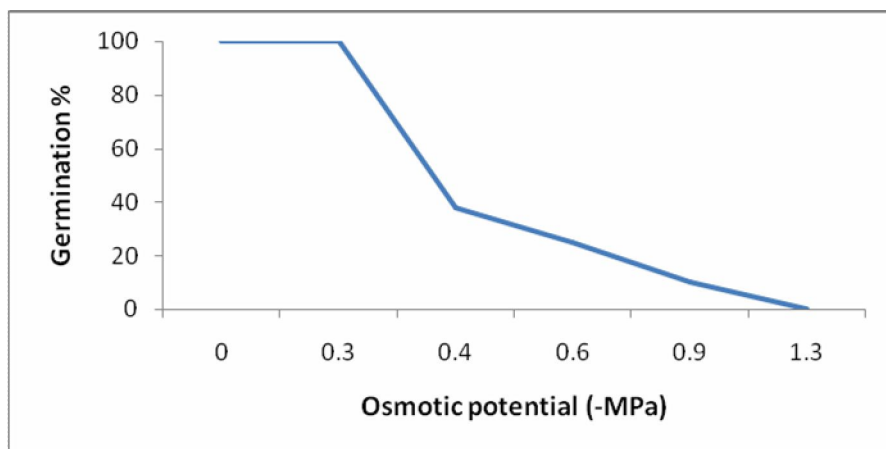


Figure 1. Effect of osmotic potential on germination of *Echinochloa crus-galli* seeds (LSD at 0.01 and 0.5: 2.20 and 1.41, resp.)

Echinochloa crus-galli considered as one species of mesophita, mesohygrophita, hygrophita and hygrohelophita with a large requirement to water being able to behave. On the other hand, the differences in water regime treatments did not significantly reduce the viability of weed seeds in the soil²³. Only a few or no seedlings of *E. crus-galli* emerged when the soil was flooded, but at field capacity or saturated soil, it grew readily²⁴.

3.2. Effect of photoperiod

Seed germination was favored by light; however, some germination (69%) occurred in the dark as well. Seed germination of barnyard grass was affected by photoperiod (Fig. 1).

The germination pattern indicated that barnyardgrass could germinate in light as well as dark (Fig. 2). Light stimulated seed germination but it was not an absolute requirement for germination⁵. However light is required for the germination of many weed species seeds²⁵, barnyardgrass seeds was germinated even at no light (Fig.2), maximum germination (99%) of barnyardgrass seeds occurred at 4/24 h (light/darkens h) with no difference between 0/24, 16/20, 20/24 and 24/0h.

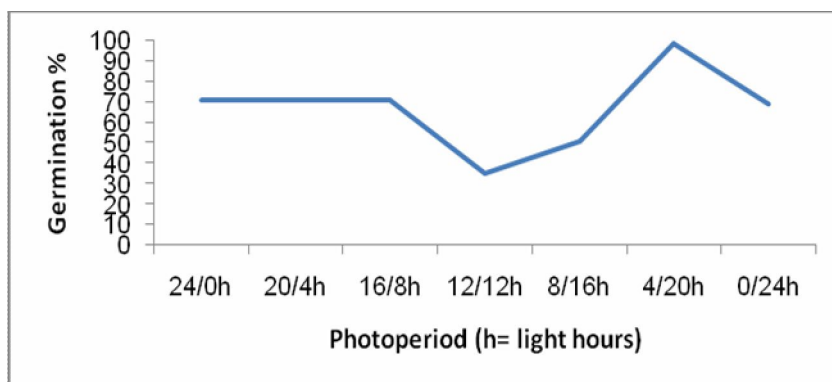


Figure 2. Effect of photoperiod's duration on germination of *Echinochloa crus-galli* seeds. LSD at 0.01 and 0.5: 2.58 and 1.71, resp.).

These results indicate that *Echinochloa crus-galli* could be well adapted to grow under plant canopies as well as open, well exposed areas such as between tree rows or in waste places.

3.3. Effect of pH

Optimum germination was at solution pH 6, but further increases in pH had a more negative effect on germination (Fig. 3). Barnyardgrass germination was 38% at pH 4, reached to 76% at pH 6 and no germination occurred at solution pH 9 (Fig. 3), which indicates that barnyardgrass germination was more tolerant of acidic than basic solutions.

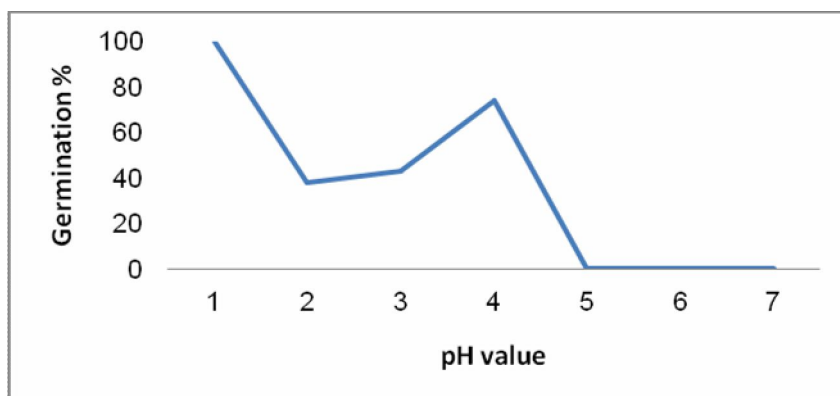


Figure 3. Effect of buffered pH solution on germination of *Echinochloa crus-galli* seeds. LSD at 0.01 and 0.5: 1.71 and 1.14, resp.)

Previous report indicated that the optimum pH range for barnyardgrass germination (> 61%) was between 4 and 6 and germination decreased at pH levels between 6 and 9 and there was only 11% germination at pH 9²². However, barnyardgrass seeds may germinate at a rather wide soil pH range of 4.7-8.3²⁶.

3.4. Effect of burial depth

Barnyardgrass emergence was affected by burial depth. Barnyardgrass could germinate without burying. Seedling emergence was 40% for seeds present on the soil surface and 0.5 cm, but increased with depth reached 63% at 1 cm depth, with insignificant differences between 1, 2 and 4cm depth.

Hundred germination percent was occurred at 6 cm depth, after that increasing burial depth delayed and significantly reduced the germination with no germination at 15 cm depth (Fig. 4). It will be expected that few seedlings will merge from seeds present on the soil surface and up to 0.5 cm deep, so low seeds will be germinated under no-till systems where most of the seed bank remains on the soil surface after crop sowing. Therefore, mechanical manipulation, e.g. burying seed by deep plowing and cross plowing might effectively reduce the emergence of weed seeds. Emergence of *Echinochloa crus-galli* seeds buried between 0-10cm with the highest emergence occurring at depths between 1-2 cm and no emergence occurring for seeds buried more than 10 cm deep^{14, 16}. These results are in agreement with report indicated that the proportion of germinated seeds decreased with burial depth to 0–20% at ≥ 0.1 m depth which is fatal for seedling establishment¹⁷. Also,

another researchers²⁷ reported that the total seedling emergence were 62.3% and 26.8% for seeds placed at soil surface, increased in seeds buried at 2 cm depth to 87 % and 29%. While, emergence reached to its minimum percentages (9.3 %, and 1.8 %) at the depth of 12 cm for the region 1 and 2, respectively²⁷.

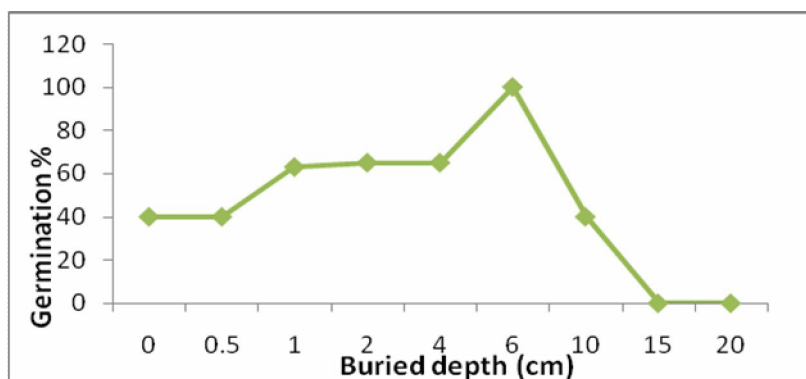


Figure 4. Effect of planting depth on germination of *Echinochloa crus-galli* seeds. LSD at 0.01 and 0.5: 1.55 and 1.05, resp.)

The reasons of zero germination at 15 cm depth (in these trials) may be attributed to that the seedlings germinated at ≥ 0.1 m depths are unlikely to penetrate the soil and their germination has a fatal end as mentioned¹⁷. Our results contradict with the researchers^{5, 22} whom reported that the proportion of seeds germinating was greatest for seeds placed on the soil surface (92%) but after that they confirmed our results where they mentioned that the emergence declined with increasing burial depth in soil; no seedlings emerged from the depth of 8cm and a burial depth of only 0.4cm reduced seedling emergence by 50%^{5, 22}.

For controlling this grass, it can bury seeds in up to 0.3-m depths, where they may suffer “fatal germination”, i.e. germination when seedling has no chance to reach the ground surface. Fatal germination could efficiently support seed mortality thus helping the regulation of barnyardgrass abundance¹⁷. They added that a tillage that brings the seeds at ≥ 0.1 m depths could cause approximately 10–20% seed mortality by fatal germination. While, from our results we could conclude that a tillage that brings the seeds at ≥ 15 cm depths could cause completely seed mortality by fatal germination. Limited soil-to-seed contact and water availability are some environmental conditions that may limit germination of seeds on the soil surface^{22, 28}. Seedling emergence was much lower from 5 cm (10%), whereas no seedlings emerged from seeds placed at a depth of 10 cm²¹. On the contrary, a general trend of decreasing emergence of barnyard grass (*Echinochloa colona* (L.) Link with increasing soil burial depth was found³⁰. They added that barnyard grass emerged primarily at the soil surface (0–2 cm), while little emergence was found at the burial depth of 10 cm.

Low seed germination at 2cm deep may be attributed to that the seeds buried more than 2 mm below the soil surface receive less than 1% of incident light and with increasing burial depth could be physical limitations of the seedling, i.e., insufficient seed reserves to enable it to reach the soil surface and larger seeds with more carbohydrate reserves are more able to emerge from greater burial depths compared with seeds that have lower reserves³.

3.5. Effect of flooding period

Higher plants are aerobic organisms which suffer from the oxygen deficiency imposed by partial or total submergence. However, some plant species have developed strategies to avoid or withstand severe oxygen shortage and, in some cases, the complete absence of oxygen (tissue anoxia) for considerable periods of time³¹.

Germination of the barnyardgrass seeds was significantly reduced with simulated flooding period. Where higher percentage was showed at in no flooding treatment, while with 4 days flooding delayed the germination and the highest germination was 63% recorded after 2 weeks. After 1week flooding delayed and reduced the germination rate (Fig.5). Increasing the flooding period to 21 days delayed the germination to 50% and the rest seeds may be decayed. Early and deep flooding significantly suppressed the growth of *E. crus-galli* seedlings⁵.

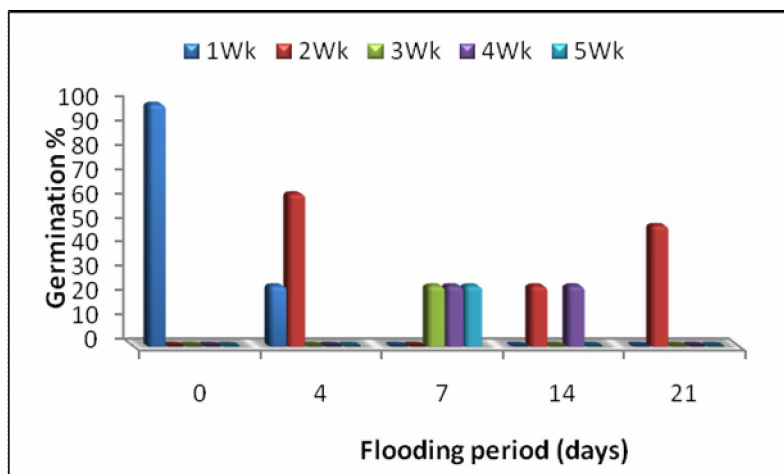


Figure 5. Effect of flooding period (Days) on germination of *Echinochloa crus-galli* seeds. LSD at 0.01 and 0.5: 0.68 and 0.44, 2.05 and 1.32, 0.68 and 0.44, 1.53 and 0.98, 0.68 and 0.44 for the 1st, 2nd, 3rd, 4th and 5th week, resp.)

3.6. Effect of salt stress

Hundred percent of seed germination was recorded at a low level of salinity (50 mM NaCl), and decreased to 64% at 100 mM NaCl, less than 50% germination inhibition was occurred at 150 mM NaCl and complet inhibition at 300 mM NaCl (Fig. 6). Previous studies indicated that germination of *E. colona* was completely inhibited at 200 mM NaCl³. No *E. crusgalli* seed germinating was found at 2% NaCl³². Another reports indicated that salinity stress up to 250 mM had no effect on barnyardgrass seed germination²²; germination was greater than 80% up to a concentration of 150 mM NaCl; 68% germination occurred at 225 mM NaCl and germination was completely inhibited at 400 mM NaCl. The concentration for 50%inhibition of maximum germination was 307 mM NaCl²². They added that salinity level for 50% inhibition of maximum germination was 307 mM, respectively. Germination of rigid ryegrass was completely inhibited by 320 mM NaCl²¹. No *E. crusgalli* seed germinating was found at 2% NaCl³².

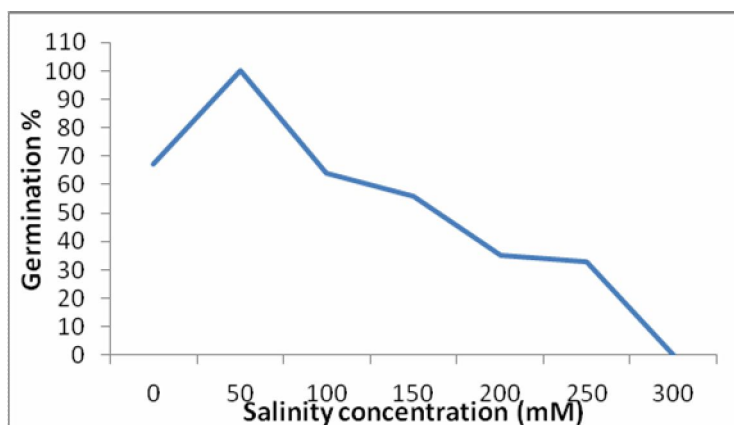


Figure 6. Effect of salt stress (mM) on germination of *Echinochloa crus-galli* seeds. LSD at 0.01 and 0.5: 1.20 and 0.80, resp.)

The harmful effect of salinity on seed germination may be attributed to a reduction in water availability, associated with the toxic effect of salts, interferes in the process of water absorption by seeds²². Another explanation of the harmful effect of salinity on plants may be attributed to the negative effect on the important physiological processes and sodium ions can alter soil structure and fertility by replacing calcium and magnesium in the anion-exchange process and this leads to nutrient and water stress³³.

4. Conclusion

Results agreed that pH, osmotic potential, burial depth or flooding duration will play an effective role in the germination of any seed. Burying them deeper, simulating flooding or tillage operation by deep plowing and cross plowing might effectively reduce emergence of weed³⁴.

5. References

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