



Interactive Effects of Salinity and Water Stress on Germination of Sunflower (*Helianthus annuus* L.)

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Abstract

Salinity and water deficit are major abiotic stresses limiting seed germination in semi-arid regions. This reduces agricultural productivity in the region and the world at large. Plant's response to salinity and water stress vary in physiological and biochemical processes. The study investigated the effects of salt and water stress on the germination of sunflower. This was carried out in Maiduguri a Sudano-Sahelian Savannah vegetation of Nigeria. Maiduguri has both rainy (June-October) and dry seasons (November-May) of a tropical climate. The experimental design was a Completely Randomized Block Design (CRBD), A total of fifteen (15) ($S_1(2.2\text{dS/m})$ S_{4I_1} - S_{4I_3} , S_{2I_1} , $S_2(2.8\text{dS/m})$ S_{2I_1} , S_{2I_2} , S_{2I_3} $S_3(3.5\text{dS/m})$ S_{3I_1} , S_{3I_2} , S_{3I_3} , $S_4(4.6\text{dS/m})$ S_{4I_1} , S_{4I_2} , S_{4I_3} ; $S_5(8.2\text{dS/m})$ S_{5I_1} , S_{5I_2} , S_{5I_3}) treatments were used in the experiment. Seeds at 8.2dS/m salinity did not germinate ($P>0.05$) significantly. The result showed that control treatment had the highest germination percentage of 93.33% and treatments S_{4I_1} - S_{4I_3} had the lowest germination percentage of 53.33%. The result showed a decrease in germination percentage with increase in salt stress and no change with increase in water stress. The result indicated that sunflower has a lower water demand for germination as seeds germinated under three (3) water stress levels. The result further showed that salt stress has a more negative effect on germination of sunflower seeds, as no germination was recorded in the highest salinity level of 8.2dS/m. The results also indicated that germination was significantly ($P > 0.05$) reduced by salt stress. The plants under the highest salt and water stress level accumulated more proline (12.01 $\mu\text{g/ml}$), compared to the control (2.18 $\mu\text{g/ml}$), at $P > 0.05$. Generally, salt and water stress negatively affect the germination of Sunflower.

Keywords: salinity, water stress, germination inhibition and sunflower.

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Introduction

Salinity and water deficit are major abiotic stresses, other stress conditions such as extreme temperature, heavy metals and flooding among others are also critical; limiting seed germination and early crop establishment in semi-arid regions. These reduces agricultural productivity in the region and the world over. It has been estimated that worldwide food demand will increase by 70% until the end of 2050 (FAO, 2013) due to 2.3

billion population growth of people (FAO, 2013). In this context, developing crop plants with high germination rate and better tolerance to harsh environmental conditions becomes an urgent need to meet future food demand for the next generations (FAO, 2013). When sensing salinity or water stress, plants have the capability to combine a range of responses in order to avoid stress injuries and complete their life cycle. By the activation of various defence mechanisms, plants can store energy

reserves in their organs and use them later for germination processes (Mantri *et al.*, 2021). Plant-associated organisms play an important role in improving the adaptation strategies of plants to environmental stresses. In this context, microorganisms, for example, can rescue plants from the deleterious effects of drought and salinity through their activity, such as nutrient solubilization, induced systemic tolerance (IST) and production of phytohormones (Indole-3-acetic acid, Cytokinin and Gibberellic acid), extracellular polymeric substances and 1-aminocyclopropane-1-carboxylic acid deaminase. The inoculation of plants with arbuscular mycorrhizal fungus can also increase plants' tolerance to short term salinity exposures (Scagel & Jungmin, 2020; Pedranzani *et al.*, 2016).

In general, plant's response to salinity and water stress may vary in physiological and biochemical processes. Most of the effects induced by salinity and drought are negative, however to some extent they can have positive effects as well (Raza *et al.*, 2019). Salt-stress resistance represents the ability of a plant to prevent, reduce or overcome the possible damaging effects caused directly or indirectly by the presence of excessive soluble salts (accumulation of toxic ions) in its surrounding's zone. A 50% reduction in germination can be considered a measure of salt stress (Boyer, 1982).

Sunflower (*Helianthus annuus*) is an annual plant native to Americas. It possesses a large inflorescence (flowering head). Sunflower (*H. annuus*) is an oilseed crop valued for its seeds and oil, with health benefits linked to digestion and cardiovascular health condition (Harsh *et al.*, 2014). The health benefits of sunflower are in two different ways; one from sunflower seeds and the other from sunflower oil. Sunflower seeds improve brain power (Harsh *et al.*, 2014).

The study was, therefore, conducted to determine whether the interactive effects of salt and water stress has significant impact on sunflower seed germination. Salt and water stress are significant abiotic factors that can have detrimental effects on seed germination and thus reducing agricultural productivity

worldwide. However, there is limited study on the combined effects of these conditions on germination of sunflower seeds. Most studies focused on the individual effects of these factors. In this context, understanding the interactive effects of salt and water stress on germination of sunflower seed becomes an urgent need. This work intends to investigate the combine effects of salt and water stress on the germination of sunflower.

The flexibility of normal metabolism allows the development of responses to environmental changes, which fluctuate regularly and predictably over daily and seasonal cycles (Shao *et al.*, 2008). Thus, whether stress plays a pivotal role in determining interaction outcomes because it is likely to strongly influence the strength of underlying positive or negative interactions. Boyer, (1982), presented how some environmental factors, such as air and temperature, can become stressful in just a few minutes for seed germination; others, such as soil water content, may take days to weeks, and factors such as soil mineral deficiencies can take months to become stressful. In arid regions, there may be periods of no net downward percolation and hence no effective leaching, so salts can accumulate in the soil. According to Hillel *et al.*, (2005), the combined effect of meagre rainfall, high evaporation, the presence of salt-bearing sediments, and – in many places, particularly river valleys and other low-lying areas – the occurrence of shallow, brackish groundwater gives rise to saline soils.

In Arid and semi-arid regions of the world such as North-eastern Nigeria, plant mostly faces inadequate, irregular and erratic nature of rainfall. Moreover, according to Verslues (2006), in the field, drought can cause a number of plant stresses including temperature, light and nutrient stresses. However, the stress component that defines drought is a decrease in the availability of soil water.

Pinus seed germination was evaluated in different conditions: water stress simulated by polyethylene glycols (PEG) agent experiments, salt stress using sodium chloride (NaCl) and diluted sea water, associated to

different temperatures (heat stress). Seed germination and the activities of the main enzymes involved in Pinus seed reserve utilization (glyoxylate cycle enzymes) decreased with increasing PEG, NaCl and seawater concentrations. In addition, the seawater treatment was the less severe on seed germination. As regard to heat treatments, the maximum germination percentage (80%) was obtained at 80°C and short exposure time (3 min) (Silva *et al.*, 2021). Salinity stress could delay germination, although most plants are tolerant and there may be no difference in the percentage of germinated seeds.

Helianthus annuus seed germination was evaluated using treatment to overcome salt and drought stress during germination. Läuchli and Grattan (2007), in their study verified that seeds subjected to salinity and water stress showed reduced germination in both conditions. Germination was higher in NaCl than PEG conditions, but mean germination time and abnormal germination percentage were lower in these conditions as revealed by the authors (Silva *et al.*, 2021; Läuchli & Grattan 2007). Another study by Kayaa *et al.* (2006) with germination approached to salt and water stress on seed germination and early embryo growth in two cultivars of sweet sorghum observed that salt stress prolonged the germination time. This study evaluated the individual and interactive effects of salt and water stress on the germination of sunflower.

Materials and Methods

Study Area

The study was conducted in a screen house at the Faculty of Agriculture, University of Maiduguri, Borno State from January to May, 2024. Maiduguri is located at latitude 11° 50' 42''N and 13° 9' 36''E within the Sudano-Sahelian Savannah vegetation of Nigeria. Maiduguri has both rainy (June-October) and dry seasons (November-May) of a tropical climate. The climate is hot and dry for most of the year (Nwogboso & Uyanga, 1999). The mean total annual rainfall of Maiduguri is 519.34 mm (Abatcha *et al.*, 2024). The mean monthly temperature of Maiduguri is above 20°C, but the daily extremes vary in a wide

range reaching up to 47°C in April (Bello *et al.*, 2023).

Study species

The plant (*Helianthus annuus*) has a rough, hairy stem, broad, coarsely toothed, rough leaves and circular heads of flowers. The heads consist of many individual flowers which mature into seeds, often in hundreds, on a receptacle base. What is usually called the "flower" on a mature sunflower is actually a "flower head" (also known as a "composite flower") of numerous florets (small flowers) crowded together. The outer petal-bearing florets are the sterile florets and can be yellow, red, orange, or other colours. The florets inside the circular head are called disc florets, which mature into seeds. Sunflower has abundant health benefits that ensure a healthy living in the long run.

Planting Material

Mature seeds of sunflower (*H. annuus*) were obtained from Lake Chad Research Institute, Maiduguri, Borno State. A total of fifteen (15), (S₁(2.2dS/m) S₄I₁-S₄I₃, S₂I₁, S₂(2.8dS/m) S₂I₁, S₂I₂, S₂I₃ S₃(3.5dS/m) S₃I₁, S₃I₂, S₃I₃, S₄(4.6dS/m) S₄I₁, S₄I₂, S₄I₃; S₅(8.2dSs/m) S₅I₁, S₅I₂, S₅I₃) treatments were used in this experiment.

Soil Collection and Sterilization

Sandy-loam soil was collected at a depth of 0-20 cm from the agricultural farm of the Faculty of Agriculture, University of Maiduguri, transported to the experimental site using a wheelbarrow. The soil was sterilized in an autoclave at 121°C to kill any pathogens or insects that may infect or eat up seedlings from the soil.

Potting Preparation and Seed Sowing

The sterilized soil was air-dried in open air and subsequently sieved through a 2mm sieve. Then 12kg of the soil was filled in 5L plastic pots with perforated bottoms. Sunflower seeds were washed several times with running tap water for surface cleaning, and then sown in the soil medium. Five (5) seeds were sown in each pot at a depth of 1cm, which were thinned out to two seedlings per pot after germination as advocated by Khan *et al.* (2016).

The experiment was a factorial design with two factors laid in Completely Randomized Block Design (CRBD). The first factor was

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salt stress at five electrical conductivity (EC) levels arranged as $S_1(2.2\text{dS/m})$, $S_2(2.8\text{dS/m})$, $S_3(3.5\text{dS/m})$, $S_4(4.6\text{dS/m})$ and $S_5(8.2\text{dS/m})$, the second factor was water stress at three irrigation interval regimes arranged as I_1 (2days), I_2 (4days) and I_3 (6days). And a control with zero salinity and daily irrigation replicated four times. One liter of tap water

was used for the irrigation. Salt treatments were prepared using sodium chloride (NaCl) and 500ml of distilled water (Sedaghatthoor & Abbasnia Zare, 2019). A total of fifteen (15) treatments were used in the experiment. The treatments combination is shown in table 1: below shows randomization of the treatments.

Table 1: the five salt concentration treatments

Treatments for salt stress at five electrical conductivity				
S1	S2	S3	S4	S5
S2	S3	S4	S5	S1
S3	S4	S5	S1	S2
S4	S5	S1	S2	S3
S5	S1	S2	S3	S4

A total of fifteen (15) treatments were used in the experiment. The treatments combination is shown in table 2.

Table 2: Treatments combination

Salt (S) (dS/m)	Irrigation interval regime = $I_1(2\text{days})$ $I_2(4\text{days})$ $I_3(6\text{days})$		
$S_1(2.2\text{dS/m})$	S_1I_1	S_1I_2	S_1I_3
$S_2(2.8\text{dS/m})$	S_2I_1	S_2I_2	S_2I_3
$S_3(3.5\text{dS/m})$	S_3I_1	S_3I_2	S_3I_3
$S_4(4.6\text{dS/m})$	S_4I_1	S_4I_2	S_4I_3
$S_5(8.2\text{dS/m})$	S_5I_1	S_5I_2	S_5I_3
Control(S_0I_0)			

Key: S= Salt stress, I= Irrigation interval regime and dS/m= decisiemens/meter

Table 3: Showing Randomization of treatments

S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C
S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1
S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2
S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3
S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1
S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2
S_3I_1	S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3
S_3I_2	S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1
S_3I_3	S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2
S_4I_1	S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3
S_4I_2	S_4I_3	S_5I_1	S_5I_2	S_5I_3	C	S_1I_1	S_1I_2	S_1I_3	S_2I_1	S_2I_2	S_2I_3	S_3I_1	S_3I_2	S_3I_3	S_4I_1

S ₄ I ₃	S ₅ I ₁	S ₅ I ₂	S ₅ I ₃	C	S ₁ I ₁	S ₁ I ₂	S ₁ I ₃	S ₂ I ₁	S ₂ I ₂	S ₂ I ₃	S ₃ I ₁	S ₃ I ₂	S ₃ I ₃	S ₄ I ₁	S ₄ I ₂
S ₅ I ₁	S ₅ I ₂	S ₅ I ₃	C	S ₁ I ₁	S ₁ I ₂	S ₁ I ₃	S ₂ I ₁	S ₂ I ₂	S ₂ I ₃	S ₃ I ₁	S ₃ I ₂	S ₃ I ₃	S ₄ I ₁	S ₄ I ₂	S ₄ I ₃
S ₅ I ₂	S ₅ I ₃	C	S ₁ I ₁	S ₁ I ₂	S ₁ I ₃	S ₂ I ₁	S ₂ I ₂	S ₂ I ₃	S ₃ I ₁	S ₃ I ₂	S ₃ I ₃	S ₄ I ₁	S ₄ I ₂	S ₄ I ₃	S ₅ I ₁
S ₅ I ₃	C	S ₁ I ₁	S ₁ I ₂	S ₁ I ₃	S ₂ I ₁	S ₂ I ₂	S ₂ I ₃	S ₃ I ₁	S ₃ I ₂	S ₃ I ₃	S ₄ I ₁	S ₄ I ₂	S ₄ I ₃	S ₅ I ₁	S ₅ I ₂
C	S ₁ I ₁	S ₁ I ₂	S ₁ I ₃	S ₂ I ₁	S ₂ I ₂	S ₂ I ₃	S ₃ I ₁	S ₃ I ₂	S ₃ I ₃	S ₄ I ₁	S ₄ I ₂	S ₄ I ₃	S ₅ I ₁	S ₅ I ₂	S ₅ I ₃

S = salt stress, I= Irrigation interval regime

KEY: C= Control S= Salt stress I= Irrigation interval regime

Germination Percentage (%)

Seed germination was recorded on daily basis. This was continued up to two weeks. Total number of germinated seeds were counted from each treatment and germination percentage was calculated using the following formula;

Seed germination % = Total number of germinated seeds/Total number of seeds sown × 100 (Matoor *et al.*, 2019), for 14 weeks.

Statistical Analysis

Data obtained were subjected to two-way Analysis of Variance (ANOVA) using STATISTIX 10.0 analytical software. Means

were separated using Tukey's HSD at 5% significance level.

Results

Germination Percentage (%)

The seeds under 8.2dS/m salinity level did not germinate, therefore their record was not taken. The result showed the control treatment having the highest germination percentage of 93.33 % and treatments S₄I₁-S₄I₃ had the lowest germination percentage of 53.33%. The result showed a decrease in germination percentage with increase in salt stress and no change with increase in water stress, as indicated in figure 1.

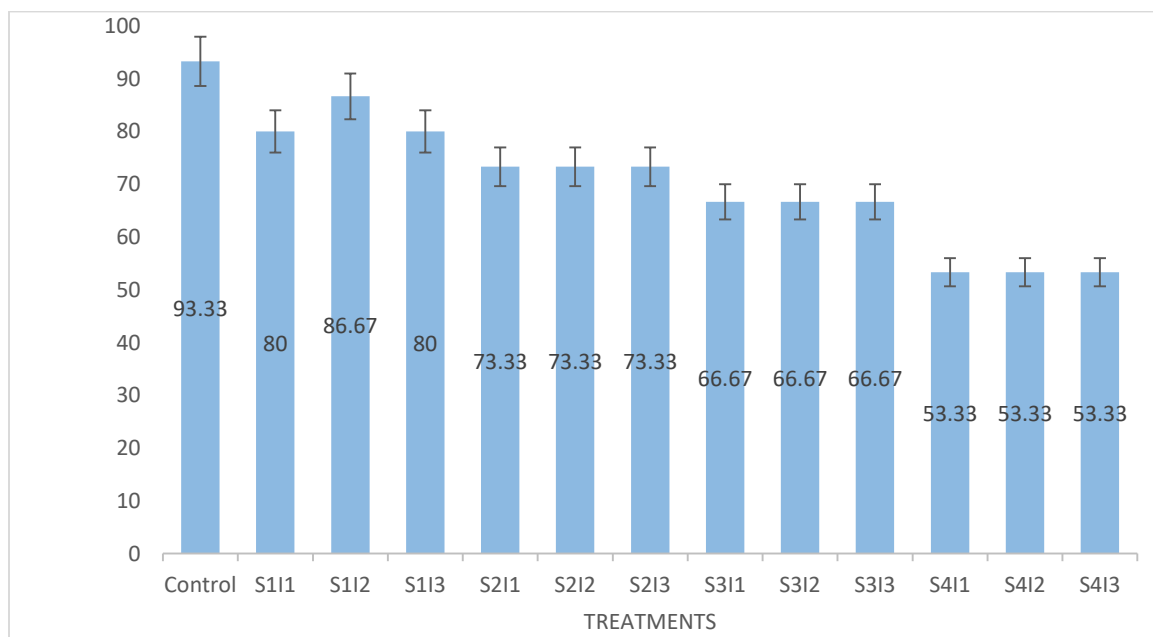


Figure 1: Effects of salt and water stress on germination percentage

S= Salt stress I= Irrigation interval regime

Discussion

Salt stress has been reported by many authors to inhibit seed germination. In this study, increase in salt stress significantly ($P>0.05$) reduced germination percentage. This agrees with Welbaum *et al.* (1990) who reported that high soil salinity inhibits seed germination due to the low osmotic potential created around the seed, which prevents water uptake. This reduction in germination is also in agreement with the work of Wu *et al.* (2015) who recorded a reduction in the germination rate of sunflower. Similar result was also observed in crops such as wheat (*Triticum aestivum*) by Akbarimoghaddam *et al.* (2011), rice (*Oryza sativa*) by Xu *et al.* (2011) and maize (*Zea mays*) by Khodarahmpor *et al.* (2012). The findings from this study revealed that water stress had no significant effect on germination percentage, this disagrees with the observation made by Haiyan *et al.* (2013), who reported a marked decrease in the germination percentage of *Eremosparton songoricum* with increase in Polyethylene glycols (PEG) in their work on The Effect of Drought Stress on Seed Germination and early Seedling Growth of the Endemic Desert Plant, *E. songoricum*. The result indicated that sunflower has a lower water demand for germination as the seeds germinated under the three (3) water stress levels. The result further showed that salt stress has a more negative effect on sunflower germination, as no germination was recorded in the highest salinity level of 8.2dS/m.

Salt stress has been reported by many authors to inhibit seed germination (Akbarimoghaddam *et al.* 2011; Wu *et al.*, 2015). In this study, increase in salt stress significantly reduced germination percentage. The results showed seed germination was reduced by salt stress only. Base on the findings from this study, it can be concluded that combine salt and water stress negatively affect the germination of sunflower seeds. Water stress showed no additive effect on germination percentage. No germination was recorded under 8.2dS/m salinity level, which suggests that the threshold is 4.6dS/m. Further studies should be conducted on the effects of salt and water stress under field condition.

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