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Response of tree species to saline stress during germination and early seedling growth phases

Resposta de espécies arbóreas ao estresse salino nas fases de germinação e crescimento inicial de plântula

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ABSTRACT
Given the constant environmental impacts that the vegetation of the Semi-Arid Region of Minas Gerais is currently experiencing, it is necessary to select native species that are tolerant to water and salt stress, aiming at the recovery of these degraded areas. The present study aimed to evaluate the influence of salt stress caused by the presence of salts at different concentrations on the germination of seeds

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of three tree species from the Fabaceae family. A completely randomized experimental design was adopted with four repetitions of 25 seeds, in a 3 x 5 factorial scheme, studying the response of seeds of three tree species from the Fabaceae family [mulungu (*Erythrina velutina* Willd.), goat's eye (*Ormosia arborea* (Vell.) Harms) and anglerfish (*Enterolobium contortisiliquum* (Vell.) Morong.)] at five levels of osmotic potentials (0.0; -0.3; -0.6; -1.2 and -1.8 MPa) through the use of sodium chloride (NaCl) to simulate salt stress. At 28 days after sowing, the following parameters were evaluated: germination percentage (%); germination speed index (GSI); mean germination time (MGT); length of the root system; and dry mass of the root system. As the osmotic potential of the medium became more negative, a drastic reduction was observed in germination percentage, GSI, embryo axis elongation, and root system dry mass of the seedlings evaluated.
KEYWORDS: *Enterolobium contortisiliquum* (Vell.) Morong.; *Erythrina velutina* Willd.; *Ormosia arborea* (Vell.) Harms; NaCl; Osmotic potential.

RESUMO

Diante dos constantes impactos ambientais que a vegetação da Região Semiárida Mineira vem sofrendo atualmente, se faz necessária a seleção de espécies nativas tolerantes ao estresse hídrico e salino, visando a recuperação dessas áreas degradadas. O presente trabalho teve como objetivo avaliar a influência do estresse salino provocado pela presença de sais em diferentes concentrações na germinação de sementes de três espécies arbóreas da família Fabaceae. Adotou-se o delineamento experimental inteiramente casualizado com quatro repetições de 25 sementes, no esquema fatorial 3 x 5, sendo estudada a resposta de sementes de três espécies arbóreas da família Fabaceae [mulungu (*Erythrina velutina* Willd.), olho de cabra (*Ormosia arborea* (Vell.) Harms) e tamboril (*Enterolobium contortisiliquum* (Vell.) Morong.)] a cinco níveis de potenciais osmóticos (0,0; -0,3; -0,6; -1,2 e -1,8 MPa) por meio do uso de cloreto de sódio (NaCl) para simulação de estresse salino. Aos 28 dias após a semeadura foram avaliados os seguintes parâmetros: porcentagem de germinação (%); índice de velocidade de germinação (IVG); tempo médio de germinação (TMG); comprimento do sistema radicular e massa seca do sistema radicular. À medida que o potencial osmótico do meio se tornou mais negativo, pode-se verificar uma redução drástica na porcentagem de germinação, no IVG, no alongamento do eixo embrionário e na produção de massa seca do sistema radicular das plântulas avaliadas.
PALAVRAS-CHAVE: *Enterolobium contortisiliquum* (Vell.) Morong.; *Erythrina velutina* Willd.; *Ormosia arborea* (Vell.) Harms; NaCl; Potencial osmótico.

INTRODUCTION

Arid and semi-arid regions cover about 33% of the planet's areas, and most of their soils are characterized by the accumulation of salts, which occurs due to factors such as climatic conditions where evapotranspiration rates exceed precipitation, natural deficiencies in internal drainage, richness of the parent material, subsoil waters rich in soluble salts, and excessive use of fertilizers (Freire et al., 2014).

In Brazil, saline soils are mainly found in the Northeast region and in the North of the state of Minas Gerais, or more specifically in irrigated perimeters, which occupy approximately 57% of the total area of the semi-arid region (Ragagnin, 2013). In these areas, salinity has become one of the main limiting factors for agricultural and forestry production, as the excess of salts affects plant growth and development, primarily due to the decrease in the osmotic potential of the soil solution and toxicity caused by the high concentration of specific ions such as sodium and chloride (Sousa et al., 2012).

Salinity constitutes a major obstacle to seed germination and species propagation, as it can interfere with plant development in two ways: By increasing the osmotic potential of the soil, which raises the energy required to absorb water and with it the other vital elements; and through the toxicity of certain elements (sodium, boron, bicarbonates, and chlorides), which at high concentrations cause physiological disorders in plants (Pedrotti et al., 2015).

The action of excessive sodium chloride (NaCl) can go beyond a simple decrease in the soil water potential, potentially causing osmotic and ionic imbalance in many species and, as a consequence of these primary effects, high salt concentrations in the soil can also cause toxic and oxidative effects on plants (Lima, 2013). The increase in saline concentration can negatively affect the manifestation of physiological potential through germination, seed vigor, cause an increase in the percentage of abnormal seedlings, and reduce the development of the aerial part and roots of the seedlings in proportion to the reduction of osmotic potential (Kaiser et al., 2016).

Depending on their genotype, plants can exhibit various responses when exposed to environments with high salt levels. According to Willadino and Camara (2010), these are classified into two major groups based on their tolerance to salinity: Glycophytes and halophytes, with the first group being less resistant and the second more resistant. The authors also state that 99% of the world's flora can be classified as glycophytes, these being plants incapable of developing and completing their life cycle in environments with high salt concentrations.

Studies that investigate the germination response of seeds from forest tree species subjected to saline stress conditions are of great technical and scientific relevance; however, they can still be considered insufficient.

One of the most commonly used methods for determining plants' tolerance to salt stress is observing the seeds' germination capacity under these conditions, as this process corresponds to one of the most critical stages in the plants' life cycle (Monteiro, 2013).

In view of the above, the present study aimed to verify the effect of saline stress caused by the use of sodium chloride (NaCl) on seed germination and the early development of seedlings of three native tree species from the Fabaceae family: mulungu (*Erythrina velutina* Wilid.), goat's eye (*Ormosia arborea* (Vell.) Harms) and anglerfish (*Enterolobium contortisiliquum* (Vell.) Morong.).

MATERIALS AND METHODS

The work was conducted at the "Seed and Propagation of Forest Species Laboratory" of the Federal Institute of Education, Science, and Technology of Northern Minas Gerais (*Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais* - IFNMG), Salinas Campus.

A completely randomized experimental design (CRD) was adopted with four replications of 25 seeds, in a 3 x 5 factorial scheme, evaluating the response of seeds from three tree species of the Fabaceae family [mulungu (*Erythrina velutina* Willd.), goat's eye (*Ormosia arborea* (Vell.) Harms) and anglerfish (*Enterolobium contortisiliquum* (Vell.) Morong.)] at five levels of osmotic potentials (0,0; -0,3; -0,6; -1,2 and -1,8 MPa) through the use of NaCl for salt stress simulation.

The mulungu and goat's eye seeds were collected at Fazenda Pedra Redonda, in the municipality of Santa Cruz - Minas Gerais, while the anglerfish seeds were collected from mother trees located at IFNMG - Salinas Campus.

Saline solutions of NaCl (0,0; -0,3; -0,6; -1,2 and -1,8 MPa) were prepared according to Vant´Hoff's formula, cited by Salysbury and Ross (1992):

$$\Psi_{osm} = -RTC$$

Where:
Ψ_{osm} - osmotic potential (atmosphere);
R - universal gas constant = 0.082 atm L/mol/°k;
T - temperature (Ok);
C - molar concentration (moles of solute/1,000 g of water).

To overcome the seed coat dormancy of mulungu, anglerfish, and olho de cabra seeds, mechanical scarification was performed using a No. 60 sandpaper (Fowler and Bianchetti, 2000).

Next, the seeds were sanitized in 2% sodium hypochlorite for three minutes, and subsequently sown, maintaining equal spacing, on three sheets of Germitest® paper, with two as the base and one to cover, moistened with an amount equivalent to 2.5 times the dry weight of the paper using the NaCl solutions described previously. Then, the papers were rolled up and packed in transparent plastic bags, which were sealed to reduce moisture loss, and kept in a BOD-type incubator at a temperature of 25°C ± 1°C and a photoperiod of 8 hours for 28 days.

At 28 days after sowing, the following were evaluated: germination percentage (%); germination speed index (GSI); mean germination time (MGT); root system length (cm) and dry mass of the root system (MSR, g plant^{-1}).

The number of germinated seeds was evaluated daily, always at the same time, adopting as a criterion for germination the seeds that had emitted a primary root (Brasil, 2009). The germination speed index (GSI) was determined according to the methodology proposed by Maguire (1962), while the mean germination time (MGT) was calculated according to the formula proposed by Laboriau (1983), with the result expressed in days after sowing. The length of the seedlings' root system was determined with the aid of a millimeter ruler.

After measuring the length of the seedlings' root system, they were harvested and separated into aerial parts and root system, and taken to a drying oven with forced air circulation at approximately 65ºC until a constant weight was reached, assessing the dry mass of the root (DMR).

The obtained data were subjected to analysis of variance according to the design previously proposed, and when the effect of the forest species was significant, the means were compared using Tukey's test ($p < 0.05$). Each level of osmotic potential was analyzed through regressions, with the F value being corrected, and equations whose highest-degree coefficients were significant ($p < 0.05$) were presented. The statistical analyses were carried out using the ExpDes.pt package version 1.2.2 (Ferreira et al., 2021) in the free R software version 4.1.2 (R Core Team, 2021), with support from the RStudio platform version 1.1.463 (Rstudio Team, 2019).

RESULTS AND DISCUSSION

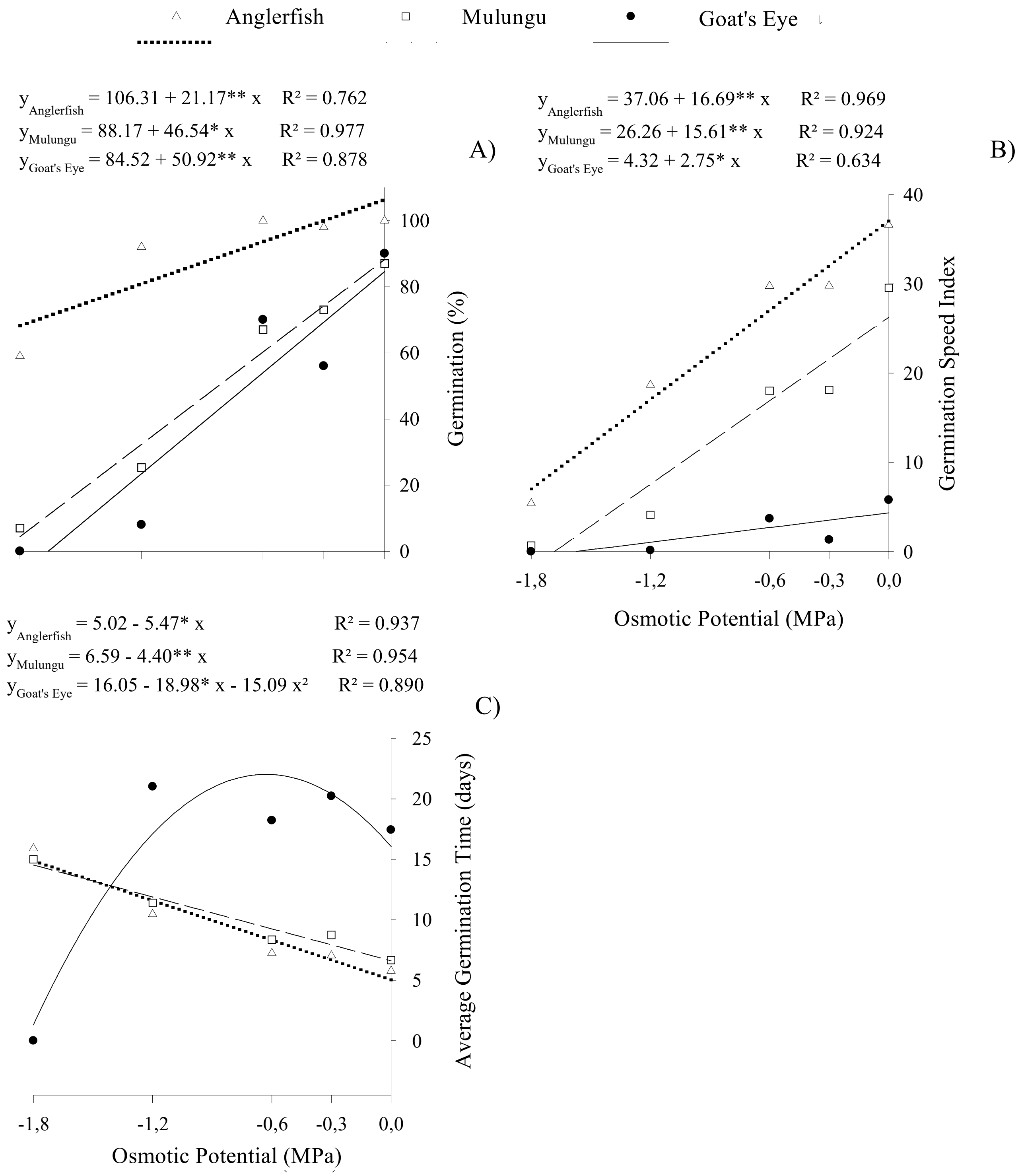
A significant effect was observed for the interaction between the factors evaluated (forest species and osmotic potentials) for all variables analyzed (Table 1 and Figure 1).

Table 1 - Germination behavior, germination speed index, mean germination time, length of the longest root, and root dry mass of seeds and seedlings of mulungu, goat's eye, and anglerfish in individual response to five different osmotic potentials.

Species	Osmotic Potential (MPa)				
	0.0	**- 0.3**	**- 0.6**	**- 1.2**	**- 1.8**
			Germination (%)		
Anglerfish	100.0 A	98.0 A	100.0 A	92.0 A	59.0 A
Mulungu	87.0 B	73.0 B	67.0 B	25.3 B	7.0 B
Goat's Eye	90.0 B	56.0 B	70.0 B	8.0 C	0.0 B
			Germination Speed Index		
Anglerfish	36.6 A	29.8 A	29.8 A	18.7 A	5.4 A
Mulungu	29.5 B	18.1 B	18.0 B	4.1 B	0.6 B
Goat's Eye	5.8 C	1.3 C	3.7 C	0.1 C	0.0 B
			Average Germination Time (days)		
Anglerfish	5.8 B	7.0 C	7.2 B	10.5 B	15.9
Mulungu	6.7 B	8.7 B	8.3 B	11.4 B	15.0
Goat's Eye	17.4 A	20.2 A	18.2 A	21.0 A	0.0*
			Greatest Root Length (cm)		
Anglerfish	14.4 A	7.6 A	9.1 A	3.0 A	1.4 A
Mulungu	6.1 B	4.5 B	3.7 B	1.2 B	0.6 AB
Goat's Eye	2.8 B	0.9 C	1.5 B	0.4 B	0.0 B
			Dry Root Mass (g plant^{-1})		
Anglerfish	2.224 A	1.176A	1.559 A	0.496 A	0.188 A
Mulungu	1.264 B	0.531 B	0.532 B	0.044 B	0.012 B
Goat's Eye	0.257 C	0.077 C	0.124 B	0.015 B	0.000 B

Means followed by the same letter in the column do not differ from each other according to the Tukey test at a 5% probability level. *It was not possible to perform the mean test.

Figure 1 - Germination response curves (%) (A), Germination rate index (GRI) (B), Mean germination time (MGT) (C) of seeds and seedlings of mulungu, goat's eye, and anglerfish subjected to five levels of osmotic potentials.



The germination of anglerfish seeds was statistically higher at all tested NaCl concentrations, showing little effect from the increased salt concentration, reaching rates above 90% up to an osmotic potential of -1.2 MPa (Table 1). This behavior was also observed by Amorim (2015) in seedling production of *Cucurbita pepo* L.

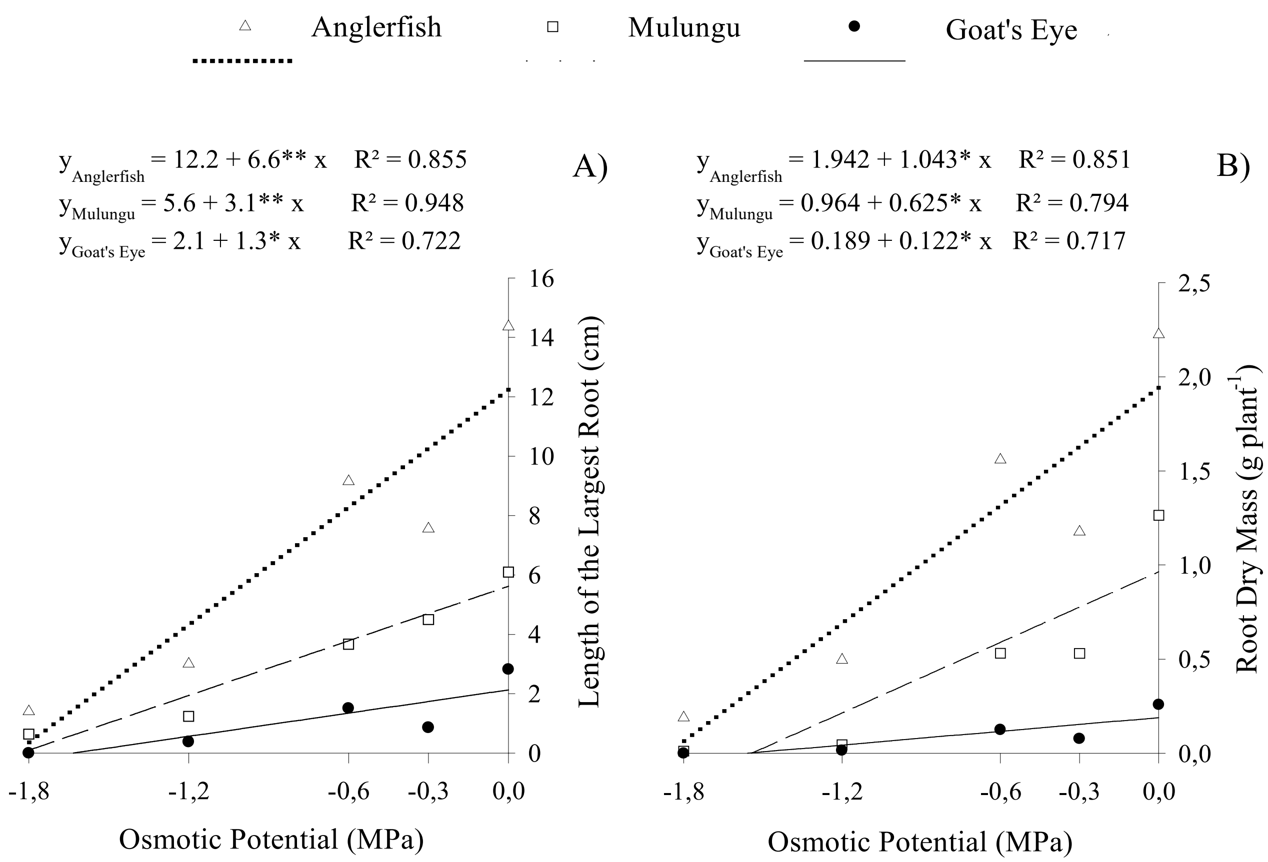
For the IVG variables, length of the largest root, and dry root mass yields, the drumstick was also the one that statistically showed the best values at all osmotic potentials evaluated among the species studied (Table 1).

The negative highlight of the analysis of the behavior results of forest species within each level of NaCl concentration was the goat's eye, which was statistically lower than the other species, obtaining the highest values of RGR, the shortest average root system length, and a zero germination rate at the -1.8 MPa concentration (Table 1).

Evaluating the effect of salt stress on mulungu seeds (*Erythryna mulungu* Mart.) and jatobá (*Hymenaea courbaril* L.), Pereira et al. (2022) also observed quite distinct behavior among the species. The authors report that the different doses studied did not interfere with the germination of mulungu, however, jatobá was negatively affected, with a decline in its germination rate as the salt concentration in the growth medium increased, reaching 0% at the highest dose tested.

In general, it can be observed that the behavior of the NaCl doses evaluated in each of the three species showed a decreasing linear response, that is, as the osmotic potential of the medium decreased, there were significant drops in germination values (Figure 1A), GVI (Figure 1B), length of the longest root (Figure 2A), and RDM (Figure 2B).

Figure 2 - Length of the longest root (A), and Root dry mass (B) of seeds and seedlings of mulungu, goat's eye, and anglerfish subjected to five levels of osmotic potentials.



According to Betoni et al. (2011), the decrease in germination percentage associated with increased salt stress may be related to the physiological drought it produces, because when the concentration of salts in the germination medium increases, there is a decrease in osmotic potential and, consequently, a reduction in water potential. This reduction can directly affect the process of water absorption by the seeds (osmotic effect), raising the concentration of ions in the embryo to toxic levels (toxic effect).

Significant reductions in germination and seedling development due to higher salt concentrations in the medium were also observed by Silva Júnior et al. (2013), Masetto et al. (2014), and Nogueira et al.

(2012) for the species herbaceous cotton, *Dimorphandra mollis* Benth., and *Delonix regia*, respectively. It is worth highlighting that even though anglerfish showed the highest average values of germination (Figure 1A), GV (Figure 1B), length of the longest root (Figure 2A), and RML (Figure 2B), as well as mulungu and olho de cabra, it is noticeable that there were reductions in their values from the first tested osmotic potential. A decreasing linear response to increasing NaCl concentration was observed in the germination and initial growth of seedlings of *Ochroma pyramidale* and *Toona ciliata* var. australis, were also observed, respectively, by Cruz et al. (2020) and Sá et al. (2023).

The low production of MSR (Figure 2B) and the decrease in the length of the longest root (Figure 2A), observed in the three species studied as the NaCl concentration increased, can be explained by the reduced water absorption by the seeds, which, according to Taiz and Zeiger (2004), would result in a slowdown in the rate of physiological and biochemical processes. Consequently, seedlings grown in this environment, with lower moisture levels, show reduced development, characterized by decreased seed germinability and lower accumulation of dry matter weight.

The average germination time (AGT) was the only variable that showed an increase in its values in the presence of higher doses of NaCl (Figure 1C), indicating a delay in the development process.

The monkfish showed the lowest mean germination time (Figure 1C), starting its germination process around the fifth day in the 0.0 MPa treatment, but with the increase in salinity a significant delay in germination was observed, resulting in the mean time being five times longer (15 days) at the osmotic potential -1,8 MPa.

According to Pelegrini et al. (2013), faster germination can be a strategy of the species to establish itself in the environment as quickly as possible, taking advantage of favorable conditions for development. In light of this statement, it is possible to notice that the anglerfish, like the mulungu and the goat's eye, showed great fragility to salt stress caused by the high presence of NaCl.

Given the results, it can be concluded that although the monkfish stood out, salinity negatively affected the germination and development of all three species studied, as an increase in the concentration of NaCl in the growth medium caused a significant impairment in all evaluated characteristics.

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