

Improving the germination performance of kalmegh (*Andrographis paniculata* wall. ex Nees) through hydropriming, halo-priming and osmopriming under salinity condition

Abstract

Salinity is a significant global issue that adversely affects seed germination and seedling vigor across a wide range of crops. Priming, however, is considered a basic method to enhance seed performance (germination and vigor) under saline conditions. The aim of this study is to evaluate the effects of different priming treatments on early growth parameters of 14-day-old seedlings under varying salinity levels (0, 4, 6, 8, and 10 dS m⁻¹) in Kalmegh, a traditionally valuable medicinal plant. Seeds were primed through four methods *viz.*, T₀- Non-primed (control), T₁- Hydro primed (distilled water), T₂- halo priming with NaCl (0.8%), T₃- halo priming with CaCl₂ (0.8%) and T₄-osmopriming with KNO₃ (1%) for 6 hours at 25°C in the dark. This experiment was arranged in a factorial design based on a CRD (Completely Randomized Design) with three replications. The results demonstrated that various priming methods effectively mitigated the adverse effects of salinity stress on the early germination stage of Kalmegh. All priming treatments significantly improved germination percentage, seedling length (cm), seedling fresh weight (mg), seedling dry weight (mg), and vigor indices. Notably, seeds primed with KNO₃ showed the highest germination performance at all salinity levels. Even at the highest salinity level, they achieved a germination percentage of 94% and a Seedling Vigor Index I of 505.72, compared to the control group, which exhibited a germination percentage of 88% and a Seedling Vigor Index I of 297.56. KNO₃ priming at 0 dS/m produced the highest seedling length (8.5 cm), root length (2.7 cm), shoot length (6.4 cm), fresh weight (26.32 mg), and dry weight (10.3 mg). Following KNO₃, CaCl₂ and NaCl priming also effectively improved germination performance and early seed growth parameters across all salinity levels.

Key words: Germination percentage, medicinal plant, priming, salinity, seed vigour indices

1. Introduction

Salinity is a broad term that refers to the existence of increased concentrations of various salts like sodium chloride, magnesium, calcium sulfates, and bicarbonates in both soil and water (Huang *et al.*, 2014). Soil salinity is considered a global concern that affects more or less 20%

of irrigated land and reduces crop productivity significantly (Qadir *et al.*, 2014). Elevated salt levels have adverse impacts on both the quantity and quality of crops, including slowing down plant growth and development, hindering enzymatic functions, and diminishing the rate of photosynthesis (Lee *et al.*, 2013).

Numerous research findings suggested that elevated stress leads to a delay in seed germination and a decrease in germination rates (Shruti and Jangde, 2023).

Andrographis paniculata, also referred to as "King of Bitters," is known as 'kalmegh' in India and "kariatu" as in local name in the Gujarat region. This herb is a highly esteemed medicinal plant and is prevalent in various Asian nations, including Malaysia, Indonesia, Pakistan, India, and Sri Lanka. It is extensively grown in regions such as Thailand, China, Mauritius, and the East and West Indies (Niranjan *et al.*, 2010). This medicinal plant contains active compounds like andrographolide, DDAG, DAG, and 14-deoxy-11, with potential for treating serious illnesses like cancer, hepatitis, and HIV (Liu *et al.*, 2015).

A. paniculata typically reproduces naturally through seeds, but seed dormancy poses a challenge, causing low germination rates due to physical dormancy and the seeds' innate characteristics (Conrath *et al.*, 2015). The ability of seeds to endure salinity during germination and the initial stages of seedling growth is essential for the successful establishment of plant growth in saline soils (Vicente *et al.*, 2007). Salt concentration impacts seed germination, delaying it in both low and high concentrations. Low salt (below optimum) induces dormancy, while high salt hinders sprouting and reduces germination due to water loss around roots and increased transpiration (Cavalcanti *et al.*, 2007).

Globally, numerous research efforts have been undertaken to address salinity stress, and among these, seed priming stands out as a promising strategy with the potential to mitigate the adverse effects of salinity and enhance both crop yield and quality (Johnson and Puthur, 2021). Seed priming is an inexpensive and straightforward method that improves germination and the establishment of seedlings by stimulating a range of physiological and metabolic processes (Conrath *et al.*, 2015). This method is employed to enhance the speed of germination, seed vigor, seed establishment and crop yield (Khajeh-Hosseini *et al.*, 2003).

Hydro priming, an eco-friendly method, involves soaking seeds in water and allowing them to re-dry to their original moisture level before planting. This process enhances water absorption and seed hydration under such conditions (McDonald, 2000). Cis-priming refers to a type of priming in which the stimulus and stress are identical., Halopriming is the prevalent

form of cis-priming and helps plants develop tolerance to salinity stress. The process of priming with salt solutions is commonly known as "halopriming." Priming seeds with inorganic salts enhances the activity of many enzymes engaged in seed germination and modifies the distribution of organic substances within the embryo (Farhoudiet *al.*, 2011). Priming of seeds through some agents like KNO_3 or PEG 8000 or 6000 known to be "osmopriming".

The aim of this research was to evaluate the effects of different priming methods like, hydopriming, halopriming and osmopriming on the germination performance of kalmegh (*Andrographis paniculata*) seedlings under salinity condition.

2. Material and Methods

Andrographis paniculata seeds were collected from the Medicinal and Aromatic Plants Research Station, Anand Agricultural University, Anand, Gujarat, India. The seeds were surface sterilized by immersing them in a 5% sodium hypochlorite (NaClO) solution for 2 minutes (Talei *et al.*, 2011), followed by thorough rinsing with distilled water. The seeds were then soaked in NaCl (0.8%), CaCl_2 (0.8%), KNO_3 (1%), or distilled water for 6 hours in a dark environment at room temperature (25°C). After soaking, the seeds were rinsed in sterilized distilled water for 2 minutes. They were subsequently air-dried in darkness at 25°C for 24 hours, until they regained their initial fresh weight and colour before sowing (Abdullahi *et al.*, 2021). The primed seeds were then sown in 15 cm diameter Petri dishes lined with 90 mm Whatman No. 1 filter paper, which was moistened with a consistent amount of sterile water. The experiment was conducted in a controlled growth chamber set to a temperature of $25\pm 4^\circ\text{C}$ and a relative humidity of 75% for 14 days. Germination was defined as the point when the radicle (embryonic root) reached a minimum length of 2 millimeters. All germination parameters were observed and recorded on the 14th day after sowing.

For the experiment five salinity levels consisted of Electrical conductivity (0, 4, 6, 8 and 10). Salinity was introduced by the addition of salts *viz.*, Calcium chloride (CaCl_2), Magnesium chloride (MgCl_2), Magnesium sulphate (MgSO_4) and Sodium chloride (NaCl) in the ratio of 5:8:2:25 respectively (Iqbalet *al.*, 1998). A stock solution was prepared and then diluted to achieve the desired salinity concentrations.

Germination percentage, ratio of the number of seeds emerged over the total number of seeds sown ($\times 100\%$)

Seedling growth parameters like seedling length, seedling shoot and root length, seedling fresh and dry weight was recorded at 14th day of germination.

Seedling Vigour Index was calculated by the method of Abdul- Baki and Anderson(1973), was expressed in whole number:

Seedling Vigour Index-I = Germination (%) x Total seedling length (cm)

Seedling Vigour Index-II = Germination (%) x Seedling dry weight (mg)

3. Results and Discussion

According to the analysis of variance, all seedling growth parameters showed significant effects, except for seedling fresh and dry weight. Salinity impacted all seedling growth parameters, and the interaction between salinity and priming significantly influenced seedling morphological growth. The highest germination percentage was observed in KNO₃-primed seeds across all salinity levels, with a maximum of 88% at 0 dS/m and a minimum of 78% at 10 dS/m. Increasing salinity levels significantly reduced germination percentages across all priming treatments (Fig. 1), and the related data are presented in Table 1.

Seedling length significantly increased with KNO₃ treatment (8.2 cm), followed by CaCl₂ (7.6 cm), NaCl (6.36 cm), and the shortest length was observed in hydroprimed seeds (4.8 cm). Higher salinity levels also had a negative impact on seedling length. The interaction between priming and salinity showed significant differences in seedling length across all salinity levels (Fig. 2). KNO₃ treatment led to the highest shoot length (4.35 cm) and root length (2.2 cm) compared to the other treatments, followed by CaCl₂, NaCl, and hydropriming. Salinity stress significantly reduced both shoot and root lengths in growing seedlings (Figs. 3a and 3b). The control group (0 dS/m) exhibited the greatest shoot and root lengths.

Except for KNO₃, there were no significant differences among the various priming treatments in terms of their effects on seedling fresh and dry weight under Petri dish conditions. A higher percentage of dry matter was observed at lower salinity levels compared to higher salinity levels. The highest seedling fresh weight (25.5 mg) and dry weight (10.3 mg) were recorded in seeds primed with KNO₃, followed by CaCl₂. Increasing salinity levels had a negative impact on both seedling fresh and dry weight (Figs. 4a and 4b).

Seedling Vigor Index-I (SVI-I) and Seedling Vigor Index-II (SVI-II) significantly decreased with increasing salinity levels, with the highest values observed at 0 dS/m. At every salinity

level, KNO₃ priming resulted in higher SVI-I (805) and SVI-II (968) compared to the other treatments, while the lowest values were observed in hydropriming (656) (Figs. 5a and 5b).

Increasing salinity levels significantly affected the germination percentage of seedlings. Among the various priming techniques, KNO₃ consistently improved germination percentages across all salinity levels compared to other treatments. While NaCl and CaCl₂ had similar effects on seedling length, fresh weight, dry weight, shoot length, and root length, the highest values were recorded with KNO₃ priming, outperforming hydropriming (water primed seeds). Our findings on the superior germination percentage with KNO₃ are supported by studies from Abdullahi *et al.*, (2012), Lara *et al.*, (2014), Shim *et al.*, (2008) and Nawaz *et al.*, (2018). The decrease in germination percentage with increasing salt concentrations may be attributed to the accumulation of harmful ions, a reduction in the osmotic potential of the solution, or an imbalance in the remobilization of seed reserves. This decline in germination percentage due to higher salinity levels is consistent with the findings of Ashkan and Jalal, (2013).

Liu *et al.*, (2015) also observed that decreased germination was linked to reduced water potential and limited water availability to the seeds. Seedling shoot and root length showed significant similar result for NaCl and CaCl₂ priming treatment but the highest was observed at KNO₃ treatment and reduction in shoot and root length was observed at increasing salinity levels. This result was in accordance with Gholami *et al.*, 2015. Following KNO₃, CaCl₂ emerged as a more effective alternative priming agent for enhancing both the fresh and dry matter of seedlings. This trend is consistent with observations made by Basra *et al.*, (2006) in wheat. The positive effects of CaCl₂ may be attributed to calcium's role as a second messenger within plant cells, its protective function against unfavorable environmental stress, and its influence on hormonal balance, as suggested by Iqbal *et al.*, (2006).

The seedling vigor index of wheat showed a significant decline as salinity levels increased. Priming the seeds with KNO₃ resulted in the highest seedling vigor index values at the 0 dS/m level, but higher salinity levels led to a reduction in this index. Seedling vigor serves as an indicator of the cumulative damage incurred as seed viability decreases. This damage accumulates within the seeds, rendering them incapable of germination and ultimately leading to their demise (Marcos-Filho, 2005). The decline in seedling vigor index associated with increased salinity levels is due to salt stress hindering initial growth, particularly of the

shoots. The decrease in seedling vigor index under water-restricted conditions is a common observation reported in other studies (Singh *et al.*, 2015).

Ibrahim (2016), suggested that the negative impact of salinity on both seed germination and seedling growth can be mitigated through KNO₃ priming. This improvement occurs through various mechanisms like enhanced activity of germination enzymes, modification of the mobilization of organic molecules in different seed parts, elevation in the levels of proteins, free amino acids, and soluble sugars, increased activity of acid phosphatase and phytase in both seeds and seedlings, and a reduction in peroxidase activity.

4. Conclusion

The result of experiment showed that priming with NaCl, CaCl₂ and KNO₃ improved the germination performance of seedling growth as compared to only water primed seeds. Since, increased levels affected significantly to all all seedling growth parameters. Priming with KNO₃ (osmo priming) resulted in highest germination percentage. NaCl and CaCl₂ showed similar significant length of shoot and root of seedling growth, but the highest was observed in KNO₃. Higher level of salinity produced significant increased amount of seedling fresh and dry matter as compared to only hydro-primed seeds. Seedling Vigour Index (I and II) showed significant interaction of salinity and priming techniques with the highest observed index at first level of salinity and minimum value observed at fifth level of salinity. We may suggest this strategy to farmers so they can boost the percentage and homogeneity of medicinal plant emergence under environmental stress condition since priming with NaCl, CaCl₂ and KNO₃ in particular is easy and inexpensive.

8. References

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Table 1: Effect of priming treatments on the seedling germination and growth parameters under salinity condition

Treatment		Seedling growth parameters							
EC levels (dS/m)	Priming	Germination (%)	Length (cm)	Shoot length (cm)	Root length (cm)	Fresh weight (mg)	Dry weight (mg)	Vigour Index-I	Vigour Index-II
0	Control (water)	80	8.20	5.96	2.20	19.56	6.60	656	528
	NaCl	87	8.30	6.00	2.30	23.20	7.30	722.10	637.71
	CaCl ₂	90	8.40	6.20	2.40	24.10	8.80	756	797.40
	KNO ₃	94	8.50	6.40	2.70	25.50	10.30	805	968.20
4	Control (water)	78	7.50	4.90	2.60	15.13	6.10	585	475.80
	NaCl	83.60	7.60	4.80	2.60	17.63	7.03	633.80	587.70
	CaCl ₂	88.30	7.70	4.66	2.80	21.93	8.50	679.91	755.85
	KNO ₃	91.30	7.80	4.60	2.90	23.30	9.50	721.27	909.34
6	Control (water)	73.60	6.20	3.43	2.30	14.20	5.50	454.46	409.21
	NaCl	77	6.28	3.60	2.40	16.30	6.40	483.56	492.80
	CaCl ₂	87.60	6.36	3.73	2.50	18.66	8.06	557.13	706.05
	KNO ₃	91	6.50	3.93	2.63	21.06	5.20	591.50	839.93
8	Control (water)	69.30	4.50	2.73	1.30	13.30	4.90	311.98	341.64
	NaCl	76	4.60	2.98	1.40	15.50	6.10	349	463.60
	CaCl ₂	86.30	4.63	3.12	1.76	18.60	7.90	400	684.36
	KNO ₃	88.30	4.80	3.23	1.90	20.84	9.30	443	823.83
10	Control (water)	68	3.16	1.98	1.10	10.46	4.03	224.40	274.04
	NaCl	74	3.26	2.01	1.20	11.43	5.20	241.24	389.24
	CaCl ₂	85	3.30	2.10	1.26	13.30	7.70	280.50	657.05
	KNO ₃	86	3.46	2.20	1.50	14.50	8.90	297.56	767.98
Interaction Treatment A (EC) × Treatment B (Priming)									
C.D		3.047	0.192	0.236	0.194	0.877	0.289	4.021	3.257
S.E.(m)		1.062	0.067	0.082	0.067	0.306	0.101	1.402	1.135

Graph plate

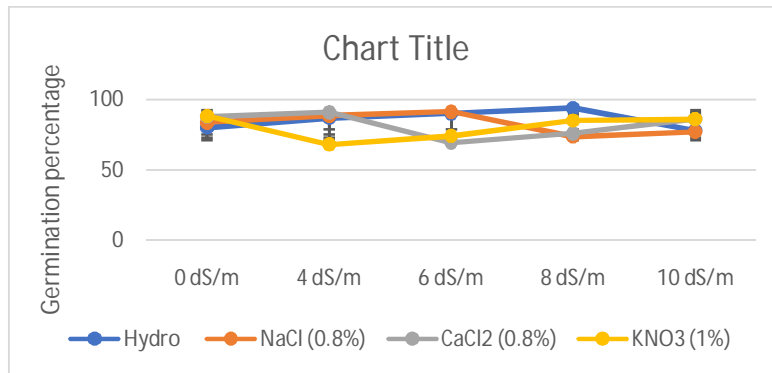


Fig 1. Effect of priming techniques and salinity levels on Germination percentage of kalmegh (*Andrographis paniculata*). Vertical bars represent means \pm SE.

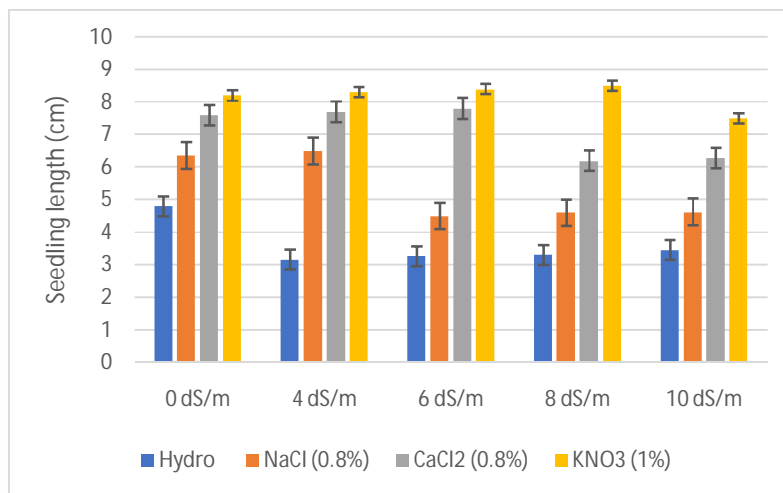
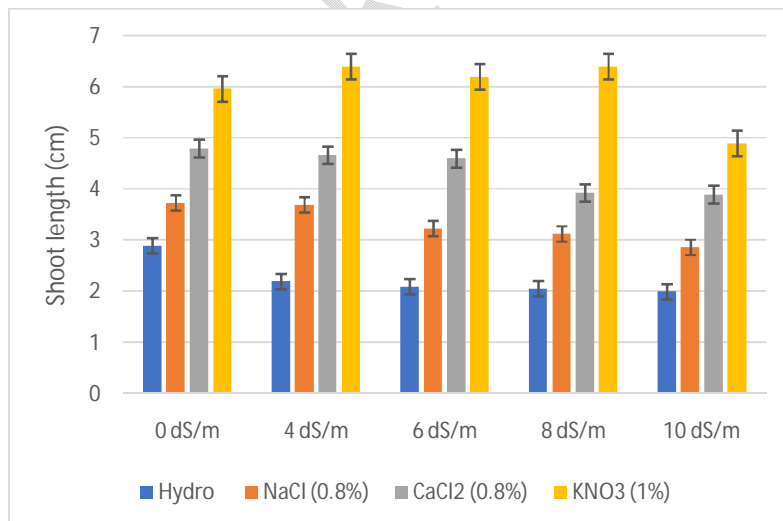


Fig 2. Effect of priming techniques and salinity levels on seedling length of kalmegh (*Andrographis paniculata*). Vertical bars represent means \pm SE.



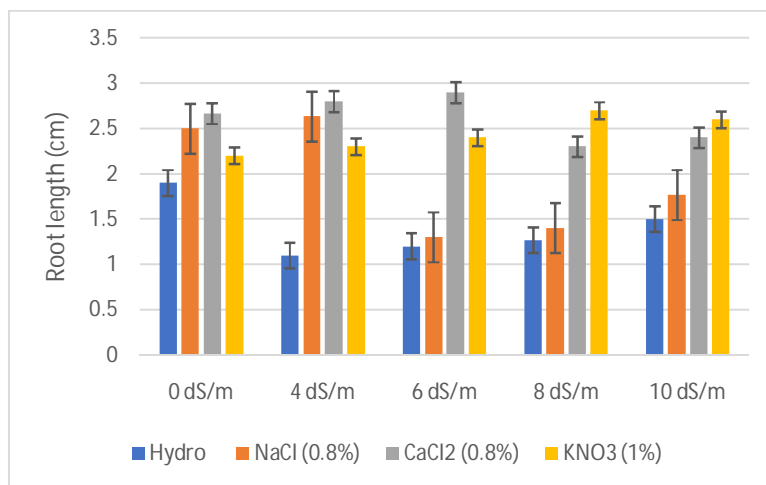


Fig 3. Effect of priming techniques and salinity levels on shoot length (a) and root length (b) of kalmegh (*Andrographis paniculata*). Vertical bars represent means ± SE.

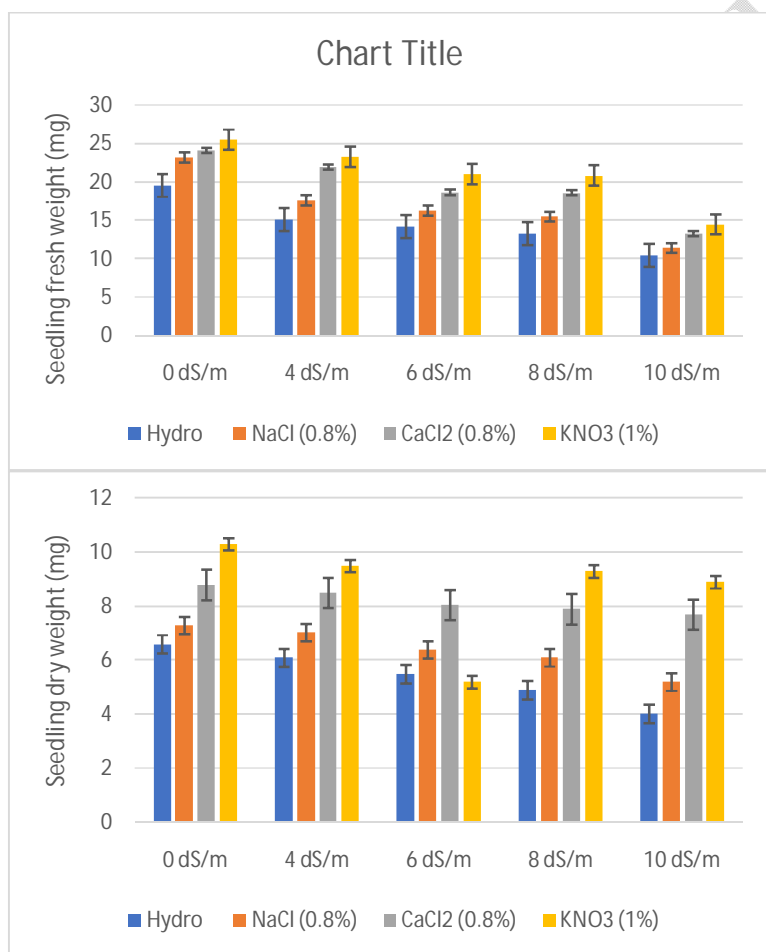


Fig 4. Effect of priming techniques and salinity levels on seedling fresh weight (a) and seedling dry weight (b) of kalmegh (*Andrographis paniculata*). Vertical bars represent means ± SE.

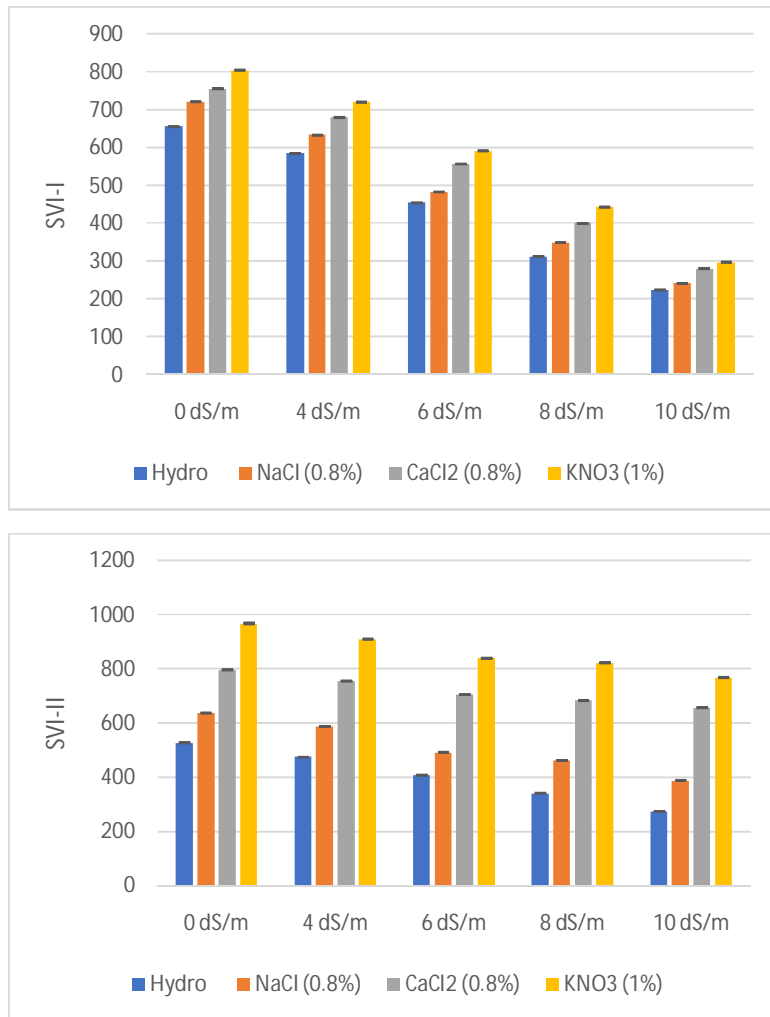


Fig 5. Effect of priming techniques and salinity levels on Seedling Vigour Index-I (a) and Seedling Vigour Index-II (b) of kalmegh (*Andrographis paniculata*). Vertical bars represent means \pm SE.