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## DECODING THE ROLE OF HALOPRIMING: ASSESSING THE SEED QUALITY AND HEALTH IN ONION SEEDS (*ALLIUM CEPA* L.)

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### ABSTRACT

Onion is an essential vegetable crop globally known for its nutritional and medicinal benefits but is challenged by poor seed quality, susceptibility to diseases, and suboptimal post-harvest performance. To advance sustainable crop production while minimizing chemical interventions, this study decodes the role of halopriming (seed treatment with inorganic salts) focusing on its influence on seed quality and health in onion seeds. Seeds of the 'cv. Palam Lohit' were primed with varying concentrations (0.5%, 1.0%, 1.5%) of calcium chloride (CaCl<sub>2</sub>) and sodium chloride (NaCl) at distinct priming durations (6, 12, 24 h). Seed quality was evaluated by germination percentage, speed of germination, seedling length, dry weight, seed vigor indices, and seed microflora incidence. Seed treatment with 1.0% CaCl<sub>2</sub> for 12 h exhibited the highest germination rate (82%), with marked enhancements in vigour and seedling growth when compared to control. The same treatment regime also resulted in a substantial reduction in seed-borne fungal contaminants, such as *Alternaria*, *Fusarium*, *Penicillium*, and *Aspergillus* spp., demonstrating its role in promoting seed health. These findings confirmed that halopriming optimally modulates seed germination processes during early seed development, bolstering germination, seedling vigour, and promotes resistance to microbial contamination.

**Keywords:** Halopriming, Onion, CaCl<sub>2</sub>, Seed health, Diseases.

### Introduction

Onion (*Allium cepa* L.), an important member of the Amaryllidaceae family, is a globally cultivated vegetable known for its wide range of culinary and medicinal uses. It holds significant nutritional and medicinal value due to higher contents of vitamins B and C, nicotinic acid, flavonoids, and polyphenols, which impart antioxidant, anti-inflammatory, anti-cancer, and cholesterol-lowering properties (Chakraborty *et al.*, 2022). Grown as an annual for bulb production and biennial for seed production (Dhotre *et al.*, 2025), onion can be grown in diverse agro-climatic zones. However, its yield and quality are often hampered by several biotic and abiotic stresses. Major challenges include poor seed quality, higher temperature and rainfall, soil nutrient deficiencies, pest infestations, and diseases, all of which affect

germination, seedling vigor, crop yield, and post-harvest quality (Yeshiwas *et al.*, 2023).

Onion is reported to be susceptible to around 66 diseases (Schwartz and Mohan, 2008), and the pathogens pose serious threats to commercial onion cultivation, reducing both yield and storage potential. While chemical pesticides remain a commonly used control measure due to their quick action, their excessive use has raised significant concerns about environmental and health hazards (Pandey *et al.*, 2023), highlighting the urgent need for sustainable and eco-friendly alternatives. Among such alternatives, seed priming using inorganic salt solutions like calcium chloride (CaCl<sub>2</sub>) and sodium chloride (NaCl) has emerged as a simple, cost-effective, and environmentally compatible technique. Seed priming involves controlled hydration of seeds before sowing,

which activates metabolic processes necessary for germination without allowing radicle emergence (Diya *et al.*, 2024). This approach has shown promising results in improving seed performance, stress tolerance, and seedling establishment. In particular, halopriming has been recognized for its dual ability to enhance seed quality parameters and suppress seed-borne pathogens. Calcium ( $\text{Ca}^{2+}$ ), an essential macronutrient, plays a critical role in maintaining stability, permeability, and structure of the cell membrane. It aids the major physiological processes such as cell division, elongation, enzyme activation, and nitrogen metabolism (Khare *et al.*, 2025). During seed imbibition, calcium helps in maintaining osmotic balance, water uptake, and activation of antioxidants, leading to improved and uniform germination (Chen *et al.*, 2022). Similarly, sodium ( $\text{Na}^+$ ), when applied at moderate levels through NaCl priming, assists in maintaining osmotic balance during seed imbibition and improves water uptake, leading to better seedling establishment, particularly under stress conditions. However, higher concentrations or prolonged exposure can lead to sodium toxicity, resulting in osmotic stress, ion imbalance, and reduced seed vigor and viability (Paul *et al.*, 2023).

Altogether, halopriming with  $\text{CaCl}_2$  and NaCl represents a promising approach to boost seed germination, enhance seedling vigor, and manage seed-borne infections in onion and other crops. By modulating major physiological and biochemical processes during early seed development, these salts contribute to the sustainable cultivation of healthy and high-yielding crops in stress-prone environments. Therefore, present study was designed to explore the impact of non-expensive ordinary salts at different concentrations and durations on seed quality and seed health characteristics. The investigation will open up more economic and impactful alternatives to the growers as well as seed businesses for better production and productivity of onions.

### Materials and Methods

The present study was conducted under laboratory conditions for two consecutive years (2021-2023) in the Department of Seed Science and Technology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). Seeds of onion cultivar 'Palam Lohit' were used for the experiment. Salts including calcium chloride ( $\text{CaCl}_2$ , Life Sciences Pvt. Ltd.) and sodium chloride (NaCl, Life Sciences Pvt. Ltd.) were used for priming treatment. Other chemicals including ethanol ( $\text{C}_2\text{H}_5\text{OH}$ , EMD Millipore Corporation, USA), sodium hypochlorite ( $\text{NaOCl}$ ,

Central Drug House Pvt. Ltd., New Delhi) and Potato Dextrose Agar, granulated (Himedia Laboratories Pvt. Ltd. Maharashtra, India, GM096-500G) were of analytical grade.

### Laboratory trials

For priming, solutions of calcium chloride and sodium chloride were prepared by mixing the weighed quantities *i.e.* 0.5 g, 1 g and 1.5 g separately of both the salts in small amount of distilled water in a beaker and stirred with glass rod until dissolved completely. More distilled water was added to make the final volume of the solution to 100 ml (Farooq *et al.*, 2019; Mohammadi *et al.*, 2023). Seeds were primed in the prepared solutions by putting the seeds in the beakers containing the solutions five times the quantity of the seeds and were soaked for the required time period at room temperature ( $25^\circ\text{C}$ ). After completion of the priming duration, the seeds were rinsed with distilled water and dried in shade for 24 h before sowing (Shatpathy *et al.*, 2018).

In the experiment, the standardization of doses and time durations for halopriming was done under *in vitro* conditions with 4 replications under Completely Randomised Design (CRD). The seeds were primed with salt solutions at 3 concentrations of 0.50, 1.00 and 1.50% each at 6, 12 and 24 h. Standard methods recommended by International Seed Testing Association (ISTA) for the seed quality and health testing were followed (Anonymous, 1996). Three laboratory methods were employed to study onion seed quality and microflora association (all the pathogens were identified based on their morphological and cultural characteristics). The rolled paper towel method (Anonymous, 1996) was used to evaluate seed germination and vigor, in which 100 treated seeds were placed between moist germination papers, rolled, and incubated at  $25^\circ\text{C}$  for 12 days, and then evaluated. The blotter paper test or Standard Petri Plate method (ISTA, 2005) was followed for microflora association in which 25 seeds were placed on moist blotter papers in Petri plates, and these plates with seeds were incubated at  $25^\circ\text{C}$  temperatures for 12 days, and examined microscopically for pathogen presence. The agar plate method involved placing seeds on sterilized potato dextrose agar in Petri plates, incubating them at  $28\pm 2^\circ\text{C}$ , and identifying fungal colonies microscopically based on growth characteristics.

The parameters like per cent germination, seedling length, seedling dry weight, seed vigor index-I and II, speed of germination and seed microflora were observed, based on which the best performing concentration and time combination was selected for

further field trials. The germination was tested using the paper towel method and counts were taken on the 6<sup>th</sup> and 12<sup>th</sup> days. Germination per cent was formulated as:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds used}} \times 100$$

For speed of germination, the number of seedlings emerged were recorded daily and the speed was calculated as:

$$\text{Speed of gemination} = n1/d1 + n2/d2 + n3/d3 + \dots + n12/d12$$

(where, n = number of germinated seeds, d = number of days)

Seedling length was measured from the shoot tip to the root tip of 10 randomly selected seedlings after 12 days, with a scale and mean values were expressed in centimetres (cm). The same seedlings were oven dried at 60° C for 48 h, and the mean weight was recorded in milli gram (mg). Seed vigour index-I and II were calculated as per the formulas given by Abdul-Baki and Anderson (1973).

Seed vigour index-I = Germination (%) x Seedling length (cm)

Seed vigour index-II = Germination (%) x Seedling dry weight (mg)

The seed microflora was observed using the Standard Petri Plate method and the number of infected seeds were recorded daily and per cent incidence was calculated by the following method:

$$\text{Seed microflora (\%)} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100$$

### Statistical analysis

The experiment was designed using CRD. The statistical analysis was conducted using R software (version 4.2.1) to Analysis of Variance (ANOVA) using the "agricolae" package in R to determine the significance of differences among treatments ( $p \leq 0.05$ ). For the graphical representation, treatment effects on various seed quality and health parameters were analysed and visualized using the "ggplot2" package.

## Results

### Effect of CaCl<sub>2</sub> and NaCl Seed Priming on Seed Quality Parameters

Assessment of seed quality parameters is necessary because they are the principal determinants of plant growth, yield and overall crop quality as it is the initial seed quality which responsible for the early establishment of the seedling, its strength and uniformity which eventually decides whether a plant will have proper growth, vegetative and reproductive phases, flowering, fruiting and seed production, be able

to tolerate stress, and will reach its genetic potential or not (Sundareswaran *et al.*, 2023). In this regard, the seed quality parameters of onion seed primed with different concentrations of CaCl<sub>2</sub> and NaCl were assessed before sowing in the field. The obtained and analysed results are presented in the Table 1, depict that the foremost seed quality parameter, *i.e.*, seed germination was significantly improved ( $p \leq 0.05$ ) on priming seeds with 0.50 and 1.00% CaCl<sub>2</sub>, and 1.00% NaCl for 12 h, however the highest germination (82.00%) was achieved with 1.00% CaCl<sub>2</sub> for 12 h and the lowest of 72.00% was recorded in the seeds primed with 1.50% NaCl for 24 h. The data obtained for speed of germination ranged from 17.99 (1.50% NaCl for 24 h) to 23.20 (1.00% CaCl<sub>2</sub> for 12 h). Seedling length and seedling dry weight were also significantly enhanced on priming the seeds with 1.00% CaCl<sub>2</sub> for 12 h exhibiting an average seedling length of 12.26 cm and an average dry weight of 2.68 mg per seedling, however, the untreated seeds showed comparatively poor growth and remained shorter having average 9.73 cm long seedling with a dry weight of 2.16 mg. Besides, the effect of seed priming on seedling length and dry weight eventually influenced the SVI-I and II, being the highest at 1005.73 and 220.03, respectively in seeds primed with 1.00% CaCl<sub>2</sub> for 12 h and the lowest at 700.17 and 156.06, respectively in untreated seeds. Moreover, the ranking analysis revealed that all the dependent variables under study were mainly influenced by the priming duration (Factor C), followed by the salt concentration (Factor B) and were least affected by the type of salt used (Factor A), elucidating those priming seeds for 12 h with both the salts enhanced the seed quality parameters. It is evident from these results that priming of onion seeds with 1.00% CaCl<sub>2</sub> for 12 h performed best among all the treatments and significantly enhanced the seed quality parameters as the Ca<sup>2+</sup> ions have a major function in maintenance of permeability, stability and integrity of plant cell membrane through formation of cell turgor during water imbibition contributing to improved ion balance and osmotic signalling for proper uptake of water, which ultimately leads to better and faster germination of the seeds (Khare *et al.*, 2025; Chen *et al.*, 2022). Ca<sup>2+</sup> is known to be a principal messenger in activation of enzymes and proteins required for metabolic processes for initiation of germination, and further activation of antioxidants for sequestering the oxidative damage caused during those processes (Ravi *et al.*, 2023). Moreover, due to major role of Ca<sup>2+</sup> as well as Na<sup>+</sup> ions in maintenance of ion homeostasis, there exogenous supply to seeds via priming leads to better establishment of seedlings, especially under stress conditions, making them more vigorous

(Mamedí *et al.*, 2022). Several other studies have also recorded similar findings in crops like mung bean, sorghum and rice, where seed priming with  $\text{CaCl}_2$  or  $\text{NaCl}$  resulted in better germination, seedling growth and vigour, owing to similar physiological mechanisms (Mohanrao *et al.*, 2023; Chen *et al.*, 2022 and Wang *et al.*, 2022).  $\text{CaCl}_2$  when applied at 1.00% might have attained the optimum balance but the lower and higher concentrations or priming durations may not have been adequate for necessary physiological alterations, resulting in slower germination processes. The better

performance of seeds primed with 1.00%  $\text{NaCl}$  for 12 h could be attributed to the fact that  $\text{Na}^+$  ions aid seeds in the regulation of internal osmotic balance for proper water absorption eventually resulting in faster and better germination, however, only at controlled levels. If the  $\text{Na}^+$  ions are in excess or have been supplied exogenously for longer durations, could lead to sodium toxicity causing ion imbalance and salt stress in the seeds hindering proper uptake of water (Paul *et al.*, 2023), which could explain the poor germination parameters in seeds primed with 1.50%  $\text{NaCl}$  for 24 h.

**Table 1:** Effect of halopriming on seed quality parameters in onion under laboratory conditions

Salt	Concentration (%)	Priming duration (h)	Germination (%)**	Speed of germination	Seedling length (cm)	Seedling dry wt. (mg)	SVI – I (Length)	SVI – II (Mass)
CaCl <sub>2</sub>	0.5	6	77.00±3.19 <sup>bcd</sup> <sub>e</sub>	20.13±0.25 <sup>fg</sup>	11.12±0.20 <sup>def</sup>	2.40±0.04 <sup>efghi</sup>	856.70±16.99 <sup>fg</sup>	184.78±3.33 <sup>def</sup>
		12	80.00±1.8 <sup>ab</sup>	23.10±0.52 <sup>ab</sup>	12.02±0.29 <sup>ab</sup>	2.64±0.03 <sup>ab</sup>	976.63±43.14 <sup>ab</sup>	214.1±8.68 <sup>a</sup>
		24	76.50±0.62 <sup>cdef</sup>	19.99±0.27 <sup>gh</sup>	11.31±0.50 <sup>cde</sup>	2.48±0.08 <sup>cdef</sup>	865.43±28.08 <sup>efg</sup>	189.45±2.05 <sup>de</sup>
	1	6	79.25±0.07 <sup>abc</sup>	22.90±0.72 <sup>bc</sup>	11.91±0.32 <sup>ab</sup>	2.58±0.05 <sup>abc</sup>	944.31±6.81 <sup>bc</sup>	204.7±7.64 <sup>b</sup>
		12	82.00±1.26 <sup>a</sup>	23.85±0.99 <sup>a</sup>	12.26±0.46 <sup>a</sup>	2.68±0.11 <sup>a</sup>	1005.73±38.98 <sup>a</sup>	220.03±6.35 <sup>a</sup>
		24	77.25±3.27 <sup>bcd</sup> <sub>e</sub>	20.95±0.07 <sup>ef</sup>	10.83±0.11 <sup>fgh</sup>	2.37±0.08 <sup>fghij</sup>	836.99±5.28 <sup>gh</sup>	182.99±2.64 <sup>efg</sup>
	1.5	6	76.25±2.54 <sup>cdef</sup>	19.85±0.27 <sup>ghi</sup>	11.03±0.04 <sup>efg</sup>	2.35±0.01 <sup>ghij</sup>	841.62±18.97 <sup>g</sup>	179.22±6.62 <sup>fgh</sup>
		12	75.50±1.91 <sup>defg</sup>	19.03±0.30 <sup>ij</sup>	10.61±0.10 <sup>ghi</sup>	2.32±0.10 <sup>ijk</sup>	800.43±12.27 <sup>hi</sup>	175.32±7.59 <sup>gh</sup>
		24	73.50±2.12 <sup>fgh</sup>	18.22±0.54 <sup>jk</sup>	10.22±0.09 <sup>ij</sup>	2.26±0.06 <sup>kl</sup>	751.76±13.55 <sup>k</sup>	166.32±3.60 <sup>ij</sup>
NaCl	0.5	6	78.75±0.78 <sup>abcd</sup>	21.52±0.19 <sup>de</sup>	11.38±0.18 <sup>cde</sup>	2.45±0.04 <sup>defg</sup>	895.83±0.81 <sup>de</sup>	192.95±6.26 <sup>cd</sup>
		12	79.50±1.50 <sup>abc</sup>	22.01±0.28 <sup>cd</sup>	11.59±0.15 <sup>bcd</sup>	2.51±0.08 <sup>cde</sup>	921.18±33.13 <sup>cd</sup>	199.73±0.36 <sup>b</sup>
		24	77.25±0.21 <sup>bcd</sup> <sub>e</sub>	20.97±0.93 <sup>ef</sup>	10.94±0.33 <sup>efgh</sup>	2.37±0.06 <sup>fghij</sup>	845.13±14.47 <sup>g</sup>	183.09±6.44 <sup>efg</sup>
	1	6	80.00±2.47 <sup>ab</sup>	22.81±0.21 <sup>bc</sup>	11.78±0.04 <sup>bc</sup>	2.56±0.02 <sup>bcd</sup>	942.41±10.19 <sup>bc</sup>	204.82±8.68 <sup>b</sup>
		12	81.50±3.45 <sup>a</sup>	23.20±0.92 <sup>ab</sup>	11.87±0.18 <sup>ab</sup>	2.63±0.10 <sup>ab</sup>	967.59±40.12 <sup>b</sup>	214.14±0.58 <sup>a</sup>
		24	78.00±0.70 <sup>bcd</sup>	21.13±0.67 <sup>de</sup>	11.34±0.36 <sup>cde</sup>	2.44±0.04 <sup>efgh</sup>	884.56±23.92 <sup>def</sup>	190.53±6.35 <sup>de</sup>
	1.5	6	76.50±1.93 <sup>cdef</sup>	20.07±0.38 <sup>fg</sup>	11.28±0.43 <sup>def</sup>	2.41±0.09 <sup>efghi</sup>	862.96±10.89 <sup>efg</sup>	184.2±2.99 <sup>def</sup>
		12	74.50±0.07 <sup>efgh</sup>	19.12±0.21 <sup>hij</sup>	10.50±0.44 <sup>hi</sup>	2.33±0.02 <sup>hijk</sup>	781.95±8.46 <sup>ij</sup>	173.14±0.37 <sup>hi</sup>
		24	72.00±0.97 <sup>h</sup>	17.99±0.71 <sup>k</sup>	9.86±0.25 <sup>jk</sup>	2.23±0.08 <sup>kl</sup>	709.41±20.46 <sup>k</sup>	160.56±4.49 <sup>jk</sup>
Untreated (Control)			72.25±3.00 <sup>gh</sup>	18.29±0.64 <sup>jk</sup>	72.25±3.00 <sup>gh</sup>	18.29±0.64 <sup>jk</sup>	9.73±0.33 <sup>k</sup>	2.16±0.04 <sup>l</sup>
Factor A		K <sub>a1</sub>	77.48	20.9	11.26	2.46	875.52	190.77
		K <sub>a2</sub>	77.56	20.98	11.18	2.44	867.9	189.24
		K <sub>A</sub>	0.09	0.09	0.09	0.02	7.63	1.53
Factor B		K <sub>b1</sub>	78.17	21.29	11.4	2.48	893.49	194.02
		K <sub>b2</sub>	79.84	22.38	11.67	2.55	931.52	203.17
		K <sub>b3</sub>	74.71	19.05	10.59	2.32	791.36	173.13
		K <sub>B</sub>	5.13	3.34	1.08	0.23	140.17	30.04
Factor C		K <sub>c1</sub>	77.96	21.22	11.42	2.46	890.64	191.78
		K <sub>c2</sub>	78.84	21.72	11.48	2.52	908.92	199.41
		K <sub>c3</sub>	64.93	17.04	9.22	2.03	699.04	153.28
		K <sub>C</sub>	13.91	4.69	2.27	0.5	209.88	46.14

Factor A, B, and C represents the effect of salt, concentration, and priming duration, respectively

The values are presented as the mean±SD of (n=3) replications.

### Effect of $\text{CaCl}_2$ and $\text{NaCl}$ Seed Priming on Seed Health Parameters

Seed health is also the major component of seed quality and is necessary for ensuring good quality seed which can be assessed at laboratory level to detect seed borne pathogens or contaminants associated with seeds (Elias, 2024). This evaluation helps in determining the extent of association or infection along with detection of potential pathogen or contaminant at seed level

before sowing, so that seed health can be managed and seed quality can be enhanced. In the present study, seed health assessment revealed a significant effect of priming on microbial contamination as presented in the Table 2. The results showed that priming onion seeds with 1.00%  $\text{CaCl}_2$  for 12 h significantly reduced the contamination of *Alternaria*, *Fusarium*, *Penicillium* and *Aspergillus* spp. to an extent of 1.00, 1.25, 2.00 and 1.50% respectively.

**Table 2 :** Effect of haloprimering on seed health (%) in onion under laboratory conditions

Salt	Concentration (%)	Priming duration (h)	<i>Alternaria</i> spp. (%)	<i>Fusarium</i> spp. (%)	<i>Penicillium</i> spp. (%)	<i>Aspergillus</i> spp. (%)
CaCl <sub>2</sub>	0.5	6	2.00±0.07 <sup>g</sup>	2.75±0.11 <sup>f</sup>	2.75±0.10 <sup>h</sup>	2.50±0.03 <sup>i</sup>
		12	1.50±0.05 <sup>h</sup>	1.75±0.05 <sup>j</sup>	2.25±0.01 <sup>j</sup>	1.75±0.08 <sup>l</sup>
		24	2.50±0.07 <sup>e</sup>	2.25±0.01 <sup>h</sup>	3.25±0.07 <sup>f</sup>	3.00±0.09 <sup>g</sup>
	1	6	1.50±0.06 <sup>h</sup>	2.00±0.07 <sup>i</sup>	2.50±0.09 <sup>i</sup>	2.00±0.05 <sup>k</sup>
		12	1.00±0.03 <sup>i</sup>	1.25±0.03 <sup>j</sup>	2.00±0.08 <sup>k</sup>	1.50±0.06 <sup>m</sup>
		24	2.00±0.06 <sup>g</sup>	2.50±0.03 <sup>g</sup>	3.00±0.05 <sup>g</sup>	3.25±0.14 <sup>f</sup>
	1.5	6	2.75±0.09 <sup>d</sup>	3.25±0.07 <sup>d</sup>	3.50±0.07 <sup>e</sup>	3.50±0.12 <sup>e</sup>
		12	2.50±0.09 <sup>e</sup>	2.75±0.06 <sup>f</sup>	3.50±0.12 <sup>e</sup>	3.00±0.05 <sup>g</sup>
		24	3.00±0.13 <sup>c</sup>	3.75±0.07 <sup>b</sup>	4.50±0.01 <sup>c</sup>	4.00±0.15 <sup>c</sup>
NaCl	0.5	6	2.25±0.03 <sup>f</sup>	3.00±0.13 <sup>e</sup>	3.25±0.07 <sup>f</sup>	3.00±0.04 <sup>g</sup>
		12	2.00±0.04 <sup>g</sup>	2.25±0.02 <sup>h</sup>	2.75±0.09 <sup>h</sup>	2.50±0.01 <sup>i</sup>
		24	3.00±0.01 <sup>c</sup>	3.50±0.01 <sup>c</sup>	3.50±0.11 <sup>e</sup>	3.50±0.04 <sup>e</sup>
	1	6	2.00±0.01 <sup>g</sup>	1.75±0.02 <sup>j</sup>	2.50±0.06 <sup>i</sup>	2.25±0.04 <sup>j</sup>
		12	1.50±0.02 <sup>h</sup>	1.50±0.05 <sup>k</sup>	2.00±0.04 <sup>k</sup>	1.75±0.07 <sup>l</sup>
		24	2.75±0.02 <sup>d</sup>	2.00±0.07 <sup>i</sup>	3.00±0.10 <sup>g</sup>	2.75±0.07 <sup>h</sup>
	1.5	6	2.00±0.07 <sup>g</sup>	2.25±0.02 <sup>h</sup>	3.50±0.14 <sup>e</sup>	3.75±0.12 <sup>d</sup>
		12	3.00±0.10 <sup>c</sup>	3.00±0.08 <sup>e</sup>	4.25±0.01 <sup>d</sup>	4.00±0.08 <sup>c</sup>
		24	3.50±0.11 <sup>b</sup>	3.75±0.13 <sup>b</sup>	5.25±0.10 <sup>b</sup>	4.75±0.21 <sup>b</sup>
Untreated (Control)			5.25±0.22 <sup>a</sup>	5.50±0.07 <sup>a</sup>	6.75±0.21 <sup>a</sup>	6.50±0.06 <sup>a</sup>
Factor A		K <sub>a1</sub>	2.09	2.48	3.03	2.73
		K <sub>a2</sub>	2.45	2.56	3.34	3.14
		K <sub>A</sub>	0.37	0.09	0.31	0.42
Factor B		K <sub>b1</sub>	2.21	2.59	2.96	2.71
		K <sub>b2</sub>	2.09	1.75	2.5	2.25
		K <sub>b3</sub>	2.8	3.13	4.09	3.84
		K <sub>B</sub>	0.71	1.38	1.59	1.59
Factor C		K <sub>c1</sub>	2.09	2.5	3	2.84
		K <sub>c2</sub>	1.92	2.09	2.8	2.42
		K <sub>c3</sub>	2.4	2.54	3.22	3.04
		K <sub>C</sub>	0.48	0.46	0.43	0.62

Factor A, B, and C represents the effect of salt, concentration, and priming duration, respectively

The values are presented as the mean±SD of (n=3) replications.

The seeds primed with 0.50% CaCl<sub>2</sub> and 1.00% NaCl for 12 h followed these results closely and reduced the contamination of respective fungi to an extent ranging from 1.50-2.25%. The untreated seeds were not able to protect themselves and recorded the highest contamination among all the treatments to an extent of 5.25% *Alternaria*, 5.50% *Fusarium*, 6.75% *Penicillium* and 6.50% *Aspergillus* spp. The obtained results were also confirmed by the ranking analysis of the obtained data which revealed that the concentration of the salt mainly influenced the fungus infection and the salt used had the least effect on the results obtained, showing that Ca<sup>2+</sup> and Na<sup>+</sup> salts work equally effectively on the seed microbiota, it's the concentration of 1.00% in both the salts that gave optimum results. The better performances of seeds primed with 1.00% CaCl<sub>2</sub> and NaCl for 12 h could be attributed to the role of Ca<sup>2+</sup> and Na<sup>+</sup> ions that create saline conditions on and around the seed making it

difficult for fungi to associate and multiply on or in the seed resulting in subsequent reduction in the infection (El-Shafey and Elamawi, 2010). Since the Ca<sup>2+</sup> ions are known to cause alterations in the fungal cell walls by either disruption of the signalling pathways especially the calcium-calcinerium pathway responsible for chitin synthesis and cell wall integration (Liu *et al.*, 2015) or binding to cell wall components like mannans and glucans, weakening the cell wall organisation, making fungi susceptible for structural damages and more vulnerable in unfavourable conditions (Hasim and Coleman, 2019). Moreover, on attack of pathogens, seeds and plants release toxic compounds against them containing antifungal proteins like defensins which interact with fungal cell walls and degrade them (Van Der Weerden *et al.*, 2010). These interactions are regulated by the Ca<sup>2+</sup> ions, so when Ca<sup>2+</sup> activity is increased by exogenous application of CaCl<sub>2</sub>, the activity of proteins



is enhanced leading to reduced infection. Na also creates osmotic stress causing cellular dehydration in the fungi which damages their cell walls and make them more prone to invasion by antifungal compounds and proteins. Similar results have been reported in crops such as lettuce, wheat, mungbean and maize, where seed priming with  $\text{CaCl}_2$  or  $\text{NaCl}$  resulted in enhanced resistance to seed-borne fungal pathogens, due to comparable underlying physiological mechanisms (Adhikari *et al.*, 2024; El-Shazoly *et al.*, 2024; Mohanrao *et al.*, 2023 and Khan *et al.*, 2022).

### Conclusion

This study clearly proves the efficacy of halopriming specifically with 1.00% calcium chloride ( $\text{CaCl}_2$ ) and sodium chloride ( $\text{NaCl}$ ) for 12 h and significantly enhanced both the quality and health of onion seeds. Primed seeds exhibited higher germination rates, improved seedling vigor, and increased seedling biomass compared to untreated controls, confirming the positive physiological impact of halopriming on early plant development. Moreover, these treatments effectively reduced seed-borne fungal contamination, notably from *Alternaria*, *Fusarium*, *Penicillium*, and *Aspergillus* spp., indicating a strong protective role against major seed pathogens. The findings reinforce that halopriming, by optimizing critical physiological and biochemical pathways, not only strengthens seed performance and establishment but also provides an environmentally friendly alternative to conventional chemical seed treatments.

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