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Effects of Sodium Bicarbonate Stress on Seed Germination and Seedling Growth of Mustard (*Brassica campestris* L.)

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Abstract: ***Numerous stresses cause negative impacts on crops and salinity-alkalinity plays a significant role in these stresses that harm the crops and their production. Sodium bicarbonate is an alkaline salt that also affects crops. The present experiment was performed to study the impacts of seed germination and early seedling growth of mustard under various $NaHCO_3$ salt treatments. This experiment was a completely randomized design, and seeds of mustard were sown in petridishes lined with Whatman No. 1 filter paper in triplicates. Different concentrations (0, 2.5, 5.0, 7.5, and 10.0 mM) of $NaHCO_3$ salt solution were used to moisten the filter papers. The control (0 mM) treatment was applied using distilled water. The seedlings were measured on the fourteenth day of sowing. The outcomes demonstrated that the germination percentage declined significantly at sodium bicarbonate salt treatments (5.0, 7.5, and 10.0 mM), while the % DFC for germination was found to be increased with increasing salt levels. In various salt (2.5, 5.0, 7.5, and 10.0 mM) concentrations, there was a significant reduction got in seedling growth, as assessed by the seedling vigor index, length of seedlings, and fresh weight of seedlings. The percentage of phytotoxicity increased while the tolerance index dropped with salt treatments. The dry weight of shoot and whole seedlings was also inhibited significantly by salt treatments (2.5, 5.0, 7.5, and 10.0 mM), and root dry weight got a significant reduction at (5.0, 7.5, and 10.0 mM) levels of salt treatments.***

Keywords: ***Sodium bicarbonate; Brassica campestris L.; Germination Percentage; Seedling Vigor Index.***

I. INTRODUCTION

Seed germination is a vital process to successfully survive the plants' life. Various atmospheric factors affect seed germination and plant growth. Salinity-alkalinity also limits seed germination and seedling establishment (Guan et al., 2009, [1]; Li et al., 2010, [2]). Increasing concentrations of alkaline salts in soil and water causes deleterious effects on the seed germination, seedling growth and yield of the crop (Wang et al., 2011, [3]; Zhang et al., 2012, [4]). Due to the increasing human population, industrialization, and urbanization; the soil, and water is contaminated by different contaminant, and the quality of irrigation water is also impaired. This caused a reduction in seed germination and seedling growth, and finally, crop yield. Na^+, Ca^{++}, Mg^{++}, and K^+ are cations mainly present in saline and alkaline soil, and their anions are Cl^-, SO_4^{--}, HCO_3^-, CO_3^{--} and NO_3^- react with each other and change into neutral and alkaline salt. Salinity and alkalinity also affect seed germination, as well as plant growth. Salinity is caused by common salts such as NaCl, Na_2SO_4, and alkalinity is caused by alkaline salts like Na_2CO_3 and $NaHCO_3$. An increasing amount of bicarbonate salts ($NaHCO_3$) reduces nutrient ion intake and causes chlorosis in plants due to an imbalance of ions and high pH (Carter et al., 2005, [5]; Mohsenian et al., 2012, [6]). Salt stress causes osmotic stress and ionic imbalance and alkaline stress causes osmotic stresses, ion toxicity, and high pH value therefore more harmful than neutral salt stress. The physiological functions and cellular structure can also be affected by alkaline stress due to high pH. Alkalinity increases the pH value of soil which disturbs general physiological functions performed by the root (Munns 2002, [7]; Yang et al., 2008, [8]). Higher amounts of salt affect the plants negatively. It causes osmotic stress and limits the water-holding capacity of roots. Ionic stress creates free radicals and reactive oxygen species (Munns and Tester 2008, [9]; Johnson et al., 2022, [10]). The oilseed crop *Brassica* species, *B. napus*, *B. campestris* and *B. juncea* have the third rank now in the world to providing the edible oil (Downey, 1990, [11]). Genus *Brassica* has many species, in which some are amphitetraploids like *Brassica napus*, *B. carinata*, and *B. juncea*, some are amphidiploids, and some species are diploids such as *B. campestris*, *B. nigra*, *and B. oleracea*. Amphitetraploids species of the genus *Brassica* have more tolerance to salinity and alkalinity than diploid species (Kumar, 1995, [12]).

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II. MATERIAL AND METHOD

A laboratory experiment was conducted in the Department of Botany, Agra College, Agra. By using distilled water as control (0 mM) and four concentrations (2.5, 5.0, 7.5, and 10.0 mM) of salt ($NaHCO_3$) solutions. Seeds of mustard (*Brassica campestris* cv. T-9) bought from the local market and were sanitized by 0.1% $HgCl_2$ (mercuric chloride) for 1 minute then washed 5 to 6 times with distilled water. After this seeds were dried between two filter papers. Sterilized petridishes were bedded with Whatman number 1 filter paper. Twenty equal-sized seeds of mustard (*Brassica campestris* cv T-9) were kept in each petridish in triplicates and the experiment was completely randomized design.

The filter papers were wetted with four different concentrations of $NaHCO_3$ solution consisting of 2.5, 5.0, 7.5, and 10.0 mM. Distilled water was used as a control (0 mM concentration). The seeds were allowed to germinate for 5 to 7 days in a dark growth chamber with a temperature of 15 °C ± 2°C (figure 1). Seeds were considered germinated when the radical attained a 2 mm length (Mohammadi, 2009, [13]). Growth parameters were recorded on the 14^{th} day of sowing.

2.1 Growth parameters:

There are several growth parameters which are listed below:

- **Germination percentage:** Germination percentage was computed by using this formula Germination % = [Number of germinated seeds / Total number of seeds] × 100
- **Percentage difference from control (% DFC) for germination:** % DFC was computed by using formula given by (Mhatre and Chaphekar's, 1982, [14]). % DFC = [Germination % of control - Germination % of test solution / Germination % of control] × 100
- **Seedling vigor index (I):** SVI (I) was calculated by using formula proposed by (Abdul-Baki and Anderson, 1973, [15]). SVI (I) = Germination percentage × [root length (cm) + shoot length (cm)].
- **Seedling vigour index (II):** It was calculated by using formula given by (Hossein and Kasra, 2011, [16]).
  SVI (II) = Germination Percentage × Dry Weight of Seedling (mg)
- **Percentage of phytotoxicity:** PP for shoot and root was calculated according to the formula of Chou *et al*., 1978, [17]).
  % Phytotoxicity = [root or shoot length of control – root or shoot length of test solution / root or shoot length of control] × 100
- **Tolerance index:** TI of shoot and root was determined according to the formula of (Turner and Marshal, 1972, [18]) as follows- TI = [mean length of longest root or shoot in test solution / mean length of longest root or shoot in control] × 100
- Length and weight (fresh and dry) of root shoot and total seedlings were measured fourteenth day of seed sowing.

2.2 Statistical analysis

Data collected from germination and seedling growth characters were subjected to statistical analysis to assess the extent of individual variation by mean and standard error, and analyzed by one - way ANOVA by using SPSS software, and Least Significance Difference (LSD) at 0.05 probability to compare the means between different of salt treatments.

III. RESULTS AND DISCUSSION

To observe the effects of salt ($NaHCO_3$) on mustard (*Brassica campestris* L. cv. T-9) elucidated its consequences on seeds germination, physiological characteristics such as germination percentage, seedling vigor index I (cm) and seedling vigor index II (mg), root and shoot length and fresh and dry weight of root and shoot of mustard.

3.1 Germination percentage:

When mustard seeds were exposed to various salt concentrations (0, 2.5, 5.0, 7.5, and 10.0 mM), the salt harmed the seed germination (figure 1). Table 1 shows that salt reduced the percentage of seeds germination. At a lower concentration (2.5 mM), the reduction was insignificant. However, at higher concentrations (5.0, 7.5, and 10.0 mM), the germination percentage decreased significantly. Various studies show confirmation with a given result such as (Zhang and Mu, 2009, [19]; Li et al., 2010, [2]; Ali, et al., 2017, [20]; Wang et al., 2022, [21]).

3.2 Length of seedling:

Additionally, Table 1 shows that progressively increased salt concentrations reduced seedling lengths. Various salt concentrations significantly reduced root, shoot, and whole seedling length. These findings are supported by the study of various researchers (Ahmad et al., 2014, [22]; Yu et al., 2019, [23]).

3.3 Weight of seedlings (fresh and dry):

According to Table 2, the fresh weight of root, shoot, and whole seedlings was affected negatively by various concentrations of salts. The Root fresh weight, the shoot fresh weight, and the fresh weight of the whole seedling reduced significantly under different salt treatments.

The dry weight of root, shoot, and whole seedlings was declined by $NaHCO_3$ salt (Table 3). A significant reduction was measured in root dry weight at various salt treatments (5.0, 7.5, and 10.0 mM), but it was insignificant at a least concentration (2.5 mM). The dry weight of shoot and whole seedlings were decreased significantly by various salt treatments. Similar results were reported by many studies (Gao et al., 2014, [24]; Zhang et al., 2018, [25]).

3.4 Percentage difference from control for germination (% DFC) and seedling vigor index (SVI):

The percentage DFC was also affected by the given salt stress. It increased with salt concentrations increasing. figure 2 indicates the highest value (19.24) of % DFC for germination was reported at the highest concentration (10.0 mM) of salt and it was the minimum (8.6) at low salt treatment.

Figure 3 illustrates when salt concentrations increased, seedling vigor index (cm) decreased significantly. The range of seedling vigor index (cm) was 673.5 to 1515.36; the minimum SVI (cm) was recorded at the highest treatment of $NaHCO_3$ and the maximum at control.

A significant reduction was recorded in seedling vigor index II (mg) with increments in salt concentrations (Figure 4). The highest value (3582.33) of seedling vigor index II (mg) was reported at the control treatment, and the lowest value (2615.58) was observed at the highest salt concentration (10.0 mM). Similar outcomes were reported by (Ali et al., 2017 [20]).

Table 1. Effects of different concentrations of $NaHCO_3$ on the seeds germination, and length of root, shoot and whole seedlings (cm) of mustard.

$NaHCO_3$ concentrations (mM)	Germination percentage	Root length (cm)	Shoot length (cm)	Whole seedling length (cm)
0	95.00 ± 2.88	6.30 ± 0.19	9.66 ± 0.09	15.96 ± 0.29
2.5	86.66 ± 3.33^{ns}	5.08 ± 0.08^{a}	8.06 ± 0.13^{a}	13.14 ± 0.20^{a}
5	83.33 ± 4.40^{a}	4.27 ± 0.12^{ab}	6.93 ± 0.07^{ab}	11.2 ± 0.20^{ab}
7.5	80 ± 5.00^{a}	4.05 ± 0.07^{ab}	5.77 ± 0.09^{abc}	9.82 ± 0.16^{abc}
10	76.66 ± 1.66^{a}	3.92 ± 0.10^{ab}	5.07 ± 0.03^{abc}	8.99 ± 0. 13^{abc}

Data is mean of 3 replicates, Mean± SE (standard Error); 'ns' for non significant at $p<0.05$; 'a' for significant at $p<0.05$ and compare with control; 'b' for significant at $p<0.05$ and compare with 2.5 mM; 'c' for significant at $p<0.05$ and compare with 5.0 mM.

Table 2. Effects of different concentrations of $NaHCO_3$ on the fresh weight of root and shoot and whole seedlings (mg) of mustard.

$NaHCO_3$ concentrations (mM)	Root fresh weight (mg)	Shoot fresh weight (mg)	Whole seedling fresh weight (mg)
0	42.8 ± 0.4	233.4 ± 3.4	276.2 ± 3.6
2.5	40 ± 0.4^{a}	222.11 ± 3.1^{a}	262.11 ± 2.8^{a}
5.0	37.44 ± 0.5^{ab}	211.44 ± 1.3^{ab}	248.88 ± 0.8^{ab}
7.5	31.11 ± 0.6^{abc}	196.66 ± 1.7^{abc}	227.77 ± 1.1^{abc}
10.0	26.44 ± 0.3^{abc}	182.44 ± 1.3^{abc}	208.88 ± 1.0^{abc}

Data is mean of 3 replicates, Mean± SE (standard Error); 'ns' for non significant at $p<0.05$; 'a' for significant at $p<0.05$ and compare with control; 'b' for significant at $p<0.05$ and compare with 2.5 mM; 'c' for significant at $p<0.05$ and compare with 5.0 mM.

Table 3. Effects of different concentrations of $NaHCO_3$ on the dry weight of root and shoot and whole seedlings (mg) of mustard.

$NaHCO_3$ concentrations (mM)	Root dry weight (mg)	Shoot dry weight (mg)	Whole seedling dry weight (mg)
0	10.2 ± 0.1	27.5 ± 0.3	37.7 ± 0.3
2.5	10 ± 0.1^{ns}	26.1 ± 0.2^{a}	36.1 ± 0.2^{a}
5.0	9.2± 0.3^{a}	24.2 ± 0.1^{ab}	33.4 ± 0.4^{ab}
7.5	8.2 ± 0.1^{abc}	23.2 ± 0.2^{abc}	31.4 ±0.1^{abc}
10.0	7.1 ± 0.1^{abc}	21.7 ± 0.1^{abc}	28.8 ± 0.1^{abc}

Data is mean of 3 replicates, Mean± SE (standard Error); 'ns' for non significant at $p<0.05$; 'a' for significant at $p<0.05$ and compare with control; 'b' for significant at $p<0.05$ and compare with 2.5 mM; 'c' for significant at $p<0.05$ and compare with 5.0 mM.

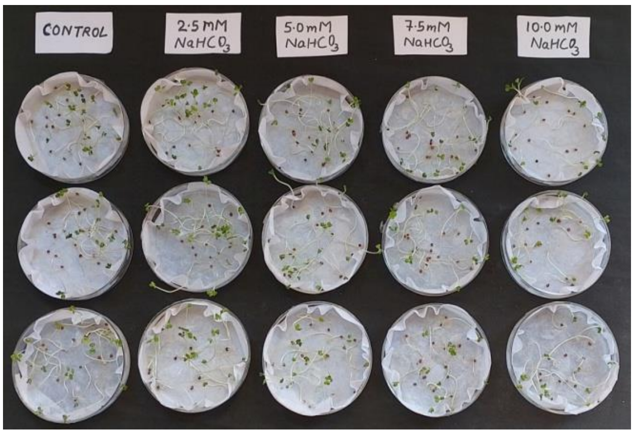

Figure 1. Effect of $NaHCO_3$ on seed germination of *Brassica campestris* L. cv T-9. on the 14th Day.

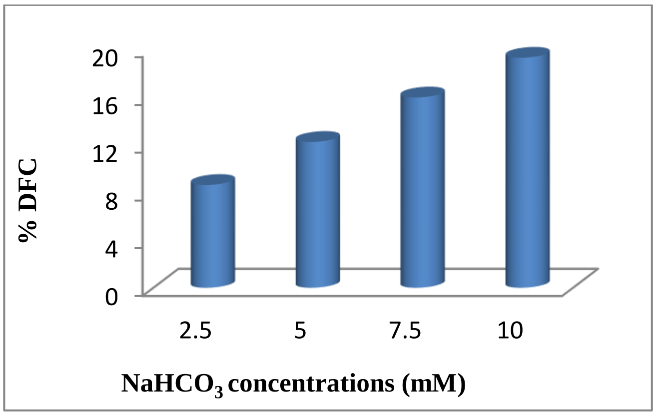

Figure 2. Effects of different concentrations of $NaHCO_3$ on % difference from control for germination of *Brassica campestris* L. cv T-9.

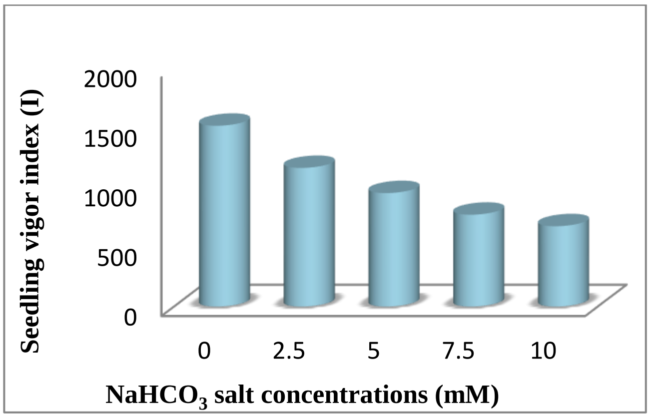

Figure 3. Impacts of different concentrations of $NaHCO_3$ on seedling vigor index (cm) of *Brassica campestris* L. cv T-9.

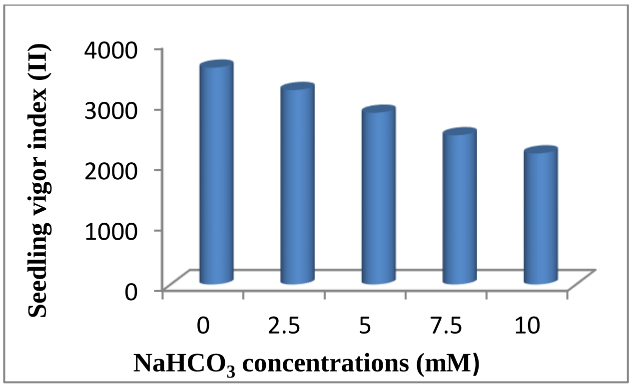

Figure 4. Impacts of different concentrations of $NaHCO_3$ on seedling vigor index (mg) of Brassica campestris L. cv T-9.

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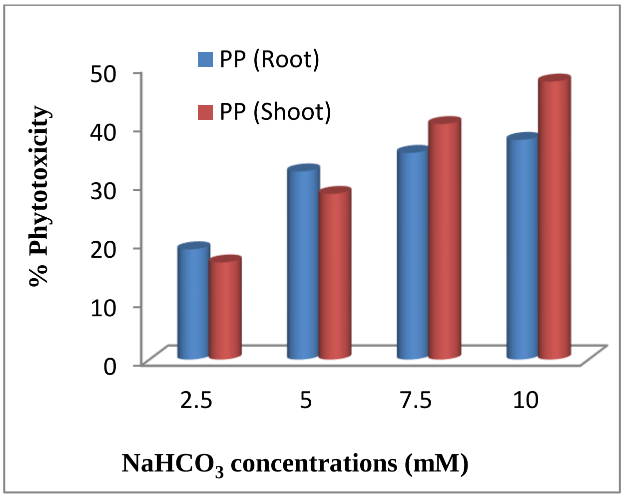

Figure 5. Effects of different concentrations of $NaHCO_3$ on % of phytotoxicity of Brassica campestris L. cv T-9.

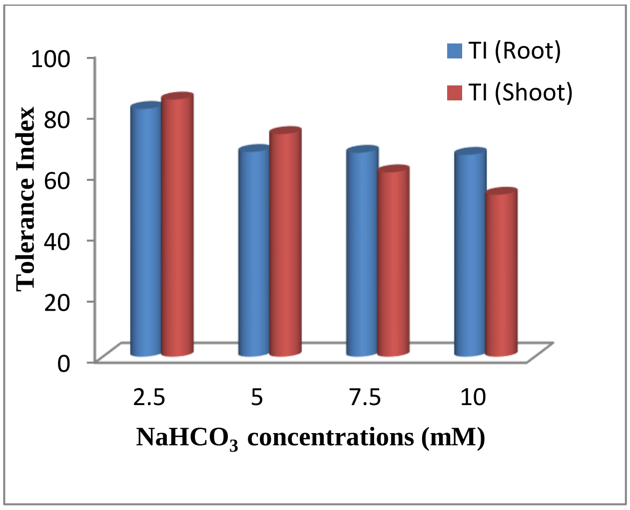

Figure 6. Effects of different concentrations of $NaHCO_3$ on Tolerance Index of Brassica campestris L. cv T-9.

3.5 Percentage of phytotoxicity and tolerance index figure 5 shows that the percentage of phytotoxicity (PP) increased with increasing salt treatments. The maximum % of phytotoxicity for root and shoot was 37.49 and 47.44 respectively, at the highest salt treatment. The minimum value of root phytotoxicity and shoot phytotoxicity were 18.81 and 16.57 respectively, under the least salt treatment. The tolerance index value was reduced with increments in salt treatments of mustard seedlings (figure 6). The greatest TI value for the roots was 81.17, and for the shoots was 84.24, recorded under a low salt treatment. The smallest TI value was 66.13 and 53.07 for the root and shoot respectively found at the highest salt level. (Ali, et al., 2017 [20]) also reported that percentage of phytotoxicity was increased and tolerance index was decreased due to $NaHCO_3$ salt.

IV. CONCLUSIONS

Salts cause harmful effects on crop plants. Sodium bicarbonate salt-induced stress caused inhibition in the seed germination and increased percentage difference from control for germination of mustard. Seedling growth and seedling vigor index were also reduced by salt treatments. Biomass (fresh and dry) was also suppressed significantly by $NaHCO_3$ salt. The percentage of phytotoxicity increased, and the tolerance index decreased with increasing salt treatments. Previous studies also concluded that the yield of crops was also reduced due to sodium bicarbonate salt stress. This study is also helpful to produce salt-tolerant and alkaline-tolerant varieties of mustard.

DECLARATION

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Ethical Approval and Consent to Participate	No, the article does not require ethical approval and consent to participate with evidence.
Availability of Data and Material/ Data Access Statement	Not relevant.
Authors Contributions	First author prepared overall paper such as choosing topic, collecting data and calculating results. Second author reviewed this article and provide sufficient comments.

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