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AMELIORATION OF SALINITY STRESS BY EXOGENOUS APPLICATION OF KH_2PO_4 ON *BRASSICA CAMPESTRIS* L.

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Abstract

This investigation was conducted to assess the possible impact of exogenously applied potassium dihydrogen phosphate (KH_2PO_4) in alleviating salinity-induced stress in mustard plants (*Brassica campestris* L.). A pot experiment was conducted under field conditions with one plant/pot. After 30 days, plants were divided into eight groups *i.e.*, A: Control (without salt and KH_2PO_4) B: Plants irrigated with 60 mM salt and without KH_2PO_4, C: Plants irrigated with 80 mM salt and without KH_2PO_4, D: Plants irrigated with 100 mM salt and without KH_2PO_4, E: Plant treated with 0.5 mM KH_2PO_4 alone, F: with 0.5 M KH_2PO_4 and 60 mM salt, G: with 0.5 M KH_2PO_4 and 80 mM salt, H: with 0.5 M KH_2PO_4 and 100 mM salt. According to the study's findings, salt significantly decreased all developmental and metabolic indicators. Nevertheless, the application of KH_2PO_4, at a concentration of 0.5 mM demonstrated its remarkable utility in enhancing growth and mitigating the negative consequences of salinity stress. The enhancement in growth could potentially be attributed to its comprehensive ameliorative properties, given that KH_2PO_4 is recognized for triggering seed germination, facilitating water absorption, and potentially functioning as a signaling molecule in saline environments. This study suggests that there is a possibility that suitable concentrations of KH_2PO_4 may be quite helpful in boosting plant growth and yield under stressful environments.

Key words: *Brassica campestris* L. Potassium dihydrogen phosphate. Salinity.

Introduction

There are many abiotic factors as wind, extreme temperature, soil salinity, flood and drought which adversely affect the vegetation. The high amount of water-soluble salts in soil is termed as soil salinity. Salinity refers to the one of the most harsh environmental factors decreasing the crop production as mostly plants are sensitive to high salt concentrations. Among these environmental pressures, salinity is the most deadly and has a negative impact on crop and land output (Shahzad *et al*., 2019). Salinity in the soil is a significant issue that negatively affects physiological and metabolic functions, ultimately reducing growth and yield (Safdar *et al*., 2019). Salinity is typically characterized using the term "electrical conductivity (EC)," and an alternative expression, osmotic potential, is also employed. In general, soil is deemed saline when its EC in saturated extract surpasses 4 dS m^{-1} (approximately equivalent to 40 mM NaCl) at a temperature of 25°C, and it possesses exchangeable sodium at a level of 15%. The crop production of many plants is decreased at this EC_e, though many more sensitive crops show reduction in productivity at comparatively low EC_e (Zaman *et al*., 2018). Around the world, 33% of irrigated fields and 20% of all cultivated land have been damaged by salinity which is the base of financial loss of 12 billion US dollars of agribusiness (Ayaz *et al*., 2019). Salinity induces drought conditions as it decreases the water potential by affecting the soil porosity. Reduced crop productivity is brought on by a significant ecological issue called soil salinity (Majeed & Muhammad, 2019). Plants cannot absorb phosphorus from salinized soil because phosphate ions precipitate with calcium ions (Bhatla *et al*., 2018). Plant growth and yield are diminished as a result of reduced leaf area, chlorophyll content, stomatal conductance, and, to some extent, a decline in photosystem II efficiency (Shoukat *et al*., 2019). Salinity has caused severe degradation to a significant portion of Pakistan's land, 6.68 million hectares out of the entire 20.1 million hectares are permanently ruined (Woldu, 2014; Sajid, 2023).

Salinity is not only an environmental problem it is also a chemical stress factor for plants (Idikut *et al*., 2012). Abiotic stress decreased the plant growth and production (Jamil *et al*., 2010). Salinity altered the porosity of soil and due to presence of high salts in soil it is difficult for plants to attain water and nutrients supplements by lowering water potential. High salinity likewise created decrease in production, for example, leaf area, leaf length, root and shoot length, root and shoot fresh as well as dry weights (Farooq *et al*., 2010). Salinity, a prominent abiotic stres, exerts detrimental effects on crops by disrupting homeostasis, altering water potential and ion distribution, ultimately imposing constraints on growth and inducing oxidative modifications as an alternative form of stress. In plants, reactive oxygen species were created as a result of salt (Zhang *et al*., 2016). Enhanced tolerance to salt stress was investigated in wheat, maize (Khodary, 2004; Hussein *et al*., 2007), mungbean (Nazar *et al*., 2011), and tomato (Shahba *et al*., 2010; Stevens *et al*., 2006) through the application of various biomolecules like salicylic acid (SA), ascorbic acid etc.

Brassica campestris L. is cultivated mainly for edible oil and its leaves are also used as vegetable. In Pakistan, following cotton, rapeseed considered as the second most important oil source. This crop covers an extensive area of 307,000 hectares, yielding approximately 233,000 tons annually and contributing roughly 17% to the nation's edible oil production (Ijaz *et al*., 2019). According to physiological research on plants grown in salty environments, the damaging effects on plants is the osmotic imbalance between the soil and the plants (Rasool *et al*., 2013). Salt stress relates with factors that limit the plant production. Further, in response to different stresses, reactive oxygen species for example superoxide, H_2O_2 and hydroxyl radicals are produced abundantly (Meloni *et al*., 2009; Gull *et al*., 2023). Plants have developed diverse strategies for survival in saline environments including the preferential uptake of ions, synthesis of compatible solutes, compartmentalization of ions, activation of antioxidant

enzymes, and the adaptive control of plant hormones like ethylene (ET), abscisic acid (ABA) and Jasmonates (JAs) (Pervaiz *et al*., 2023). To avoid salinity-induced oxidative stress, plant has established an antioxidant defense system. Many crop plants have been found to have salt resistance in a relationship with a more effective antioxidant system both enzymatic and non-enzymatic. The antioxidant system aids in the survival of plants under stress by working to eliminate the damaging radicals generated during oxidative stress (Raid *et al*., 2022; Sajid & Aftab, 2022).

Salt-induced stress had detrimental effects on the plant development, crop output, photosynthetic ability, and mineral nutrient accumulation in plants. To cope with this salinity stress, potassium dihydrogen phosphate (KH_2PO_4) in different concentrations as foliar-spray was applied in previous studies and it was observed to be successful in enhancing growth and yield. The stimulation of sunflower growth by KH_2PO_4 was correlated with improved photosynthetic capacity, efficiency in water use, and relative water content (Akram & Ashraf, 2011). Similarly, in rice small quantity of KH_2PO_4 was also prove effective which notably boost the grain production of rice crop in hot weather conditions during the heading-flowering phase (Belov *et al*., 2021). In another study, the composite mixture of granular potassium dihydrogen phosphate and microcrystalline cellulose, which was formulated and employed, exhibits superior physical characteristics, heightened agricultural efficacy, and lessened adverse impacts on soil microorganisms when differentiated with potassium dihydrogen phosphate (Jančaitienė, *et al*., 2023). Looking at above mentioned literature, present research aimed to investigate the role of KH_2PO_4 in altering morphology, biochemistry and physiology in response to saline stress in Mustard plant (*Brassica campestris* L.). Additionally, it sought to assess the potential of KH_2PO_4 in alleviating salinity-induced stress in mustard. Obtaining ideas from the relation between KH_2PO_4 treatments against salinity stress in mustard (*Brassica campestris* L.) and then suggesting similar kind of trials on other crops under stress. It was hypothesized that exogenous application of KH_2PO_4 enhanced growth and biochemical attributes in (*Brassica campestris* L.) under salinity stress condition.

Material and Methods

Plant material: The current research was conducted to study the amelioration of salinity stress resistance in *Brassica campestris* L. by exogenous use of KH_2PO_4 through foliar application. Two experiment was installed, one to check the germination % in petri dishes and 2nd pot experiment to see the ameliorative effect of KH_2PO_4. For this, authenticated Meteor variety of *Brassica* seeds (Pakola variety) precured from the market located in Allama Iqbal Town, Lahore, Pakistan. The seeds were selected with uniform size and color, devoid of wrinkles, and showed no signs of disease. Petri-dishes were autoclaved and well dried in oven. Afterwards a layer of sterilized cotton was placed inside each dish and filled with 4 ml of distilled water as control and distilled water containing various concentrations of salts (60, 80 and 100 mM) with or without KH_2PO_4 (0.5 mM) as experimental ones. KH_2PO_4 was applied as seed priming before sowing in petri dishes. The petri dishes were categorized into various sets according to the treatments and three seeds were planted in each of the eight petri dishes.

Pot experiment: The pot experiment was installed during October - December 2022 in the green house of University of Education, Township Campus, Lahore, Pakistan. Experimental conditions were 20 ± 2°C temperature, 64% relative humidity and 16/8 hours light and dark period. For pot experiments, clay pots measuring 45 cm in diameter and 55 cm in height with 3 holes at the bottom were used. The well mixed garden soil with an equal weight of farmyard manure was used to fill the pots. The potting mix was sieved before filling the pots. Subsequently, each pot was filled with four kilograms of soil. Each container contained five seeds, which were then allowed to germinate and grow. Overall, the experiment was consisted of eight pots, each with three replicates at varying concentrations. The pots were randomly arranged to maintain uniformity. All experimental pots were provided with tap water for duration of 15 days. Following this, the plants received respective treatment through irrigation with varying concentrations of NaCl and foliar application of KH_2PO_4. Tween-20 was utilized as a surfactant to facilitate the absorption of KH_2PO_4 into the leaf tissues. Within each block, eight groups were formed, each containing three potted plants for different concentrations. Treatments of foliar spray was done after every seven days for month however salt treatment were given twice after 15 days interval. The plants were divided into eight groups on the basis of various treatment of salt and foliar spray of KH_2PO_4 *i.e*., **T1:** Control (without salt and KH_2PO_4) **T2:** with 60 mM salt and without KH_2PO_4 **T3:** with 80 mM salt and without KH_2PO_4 **T4:** with 100 mM salt and without KH_2PO_4 **T5:** with 0.5 mM KH_2PO_4 and without salt **T6:** with 0.5 mM KH_2PO_4 and 60 mM salt, **T7:** with 0.5 mM KH_2PO_4 and 80 mM salt **T8:** with 0.5 mM KH_2PO_4 and 100 mM salt (Fig. 1).

Physical properties of potting mixture: The potting mixture's physical properties such as pH, Total dissolved solids (TDS), and electrical conductivity (EC), were measured using a pH/EC meter. A sample of soil (5 grams) was collected from all the pots of each treatment group. The potting mix was taken and mixed up with tap water and left for setting for ten minutes. The filtrate was taken to measure pH, EC and TDS (Table 1).

Harvesting of plants and data collection: After sixty (60) days following the application of salt and KH_2PO_4 treatments, the plants were harvested and various morphological attributes were assessed for instance, shoot length, root length, leaf area were determined. Leaf samples were also collected for biochemical analysis. The plants after harvesting were cleaned using tap water for the removal of dust particles, dried with blotting paper and separated into roots and shoots to determine the fresh as well as dry weights. The leaves were wrapped in aluminum foil, dried in oven at 65°C for the period of five days and then were weighed to find out the dry weights.

Fig. 1. Selected photographs showing of *Brassica campestris* plants at various concentation of salt and KH_2PO_4. **T1:** Control plants without salt and KH_2PO_4, **T2:** Plants with 60 mM salt, **T3:** Plants with 80 mM salt, **T4:** Plants with 100 mM salt, **T5:** Plants with 0.5 mM KH_2PO_4, **T6:** Plants with 60 mM salt + 0.5 mM KH_2PO_4, **T7:** Plants with 80 mM salt + 0.5 mM KH_2PO_4, **T8:** Plants with 100 mM salt + 0.5 mM KH_2PO_4.

Table 1. The physical properties of the potting mixture.

Treatment	pH	EC	TDS
Control	6.5 ± 0.2	1.46 ± 0.6	646
Salt 60 mM	7.2 ± 0.1	2.65 ± 0.9	694
Salt 80 mM	7.4 ± 0.2	2.79 ± 0.1	732
Salt 100 mM	7.7 ± 0.7	2.86 ± 0.2	755
0.5mM KH_2PO_4 without salt	7.4 ± 0.4	2.87 ± 0.4	737
0.5mM KH_2PO_4 + 60 mM salt	7.2 ± 0.2	2.49 ± 0.1	756
0.5mM KH_2PO_4 + 80 mM salt	7.5 ± 0.1	2.64 ± 0.2	761
0.5mM KH_2PO_4 + 100 mM salt	7.6 ± 0.3	2.85 ± 0.8	765

Measurement of Morphological Parameters

Shoot length and diameter: The shoot length of individual plants was measured by using scale, while Vernier calipers were utilized for determining plant diameters.

Plant biomass: The root and shoot were isolated and subsequently rinsed with tap water to remove any soil

particles. Following this, they were dried using blotting paper, and their fresh weights were measured using a digital balance. To determine the dry mass, the plants were enveloped in aluminum foil and subjected to oven dried at 65°C, after 72 hours their weights were recorded.

Leaf area: The Image J program (developed by W. S. Rosband) was used to calculate the leaf area for each plant (U.S. National Institute of Health http://rsb.info.nih.gov/IJ/).

Biochemical analysis: Biochemical attributes were also recorded and analyzed, such as antioxidant enzymes, proline and total soluble protein contents.

Quantitative assay of proteins: Fresh leaf tissue (0.5 grams) was painstakingly pulverized in an ice-cold mortar and pestle with 0.1 grams of polyvinyl polypyrollidone (PVP), 0.5% Tritone X-100 (v/v), and 1 ml of a 0.1 M phosphate buffer at pH 7.2. After homogenizing the leaf tissue and buffer at a ratio of 1:2 (w/v), the homogenate was centrifuged at 14,000 rpm for 30 minutes at 4°C. The resultant supernatant was kept at 0°C and employed for protein measurement. The Biuret method, as outlined by Racusen and Johnstone in 1961, was employed to determine the total soluble protein content. Biuret reagent was prepared. The samples prepared for experiment and control were as under.

Constituents	Sample	Blank
Proteins extract	0.1 ml	-
Biuret reagent	2.0 ml	2.0 ml
Distilled water	-	0.1 ml

The samples were left to stand for 5 minutes to allow the reaction to finish. Afterward, optical density at 545 nm was measured using a spectrophotometer (U-1100). The standard curve, which was created using bovine serum albumin, was used to calculate the protein contents. The total protein contents were calculated using the following formula:

$$\text{Protein contents (mg/g)} = \frac{\text{CV x TE}}{\text{EU x Wt x 1000}}$$

CV stands for Curve value, TE represents Total extract, EU signifies Extract used, and Wt. stands for Weight of tissue.

Quantitative assay of enzymes

Extraction of antioxidant enzymes: A 0.5-gram segment of fresh leaf material was meticulously mixed in a compound consisting of 1.0 ml of 0.1 M phosphate buffer (with a pH of 7.2), 0.1 grams of polyvinyl polypyrrolidone (PVP), and 0.5% Triton X-100 (*v/v*). The resulting mixture was then subjected to centrifugation at 14,000 rpm for 30 minutes at 4°C. The supernatant obtained was carefully stored at 0°C and used subsequently for the quantitative assessment of enzymes. To measure peroxidase activity, a modified method of Racusen and Foote (1965) was employed. To facilitate this, solutions of Guaiacol (1%), H_2O_2 (0.3%), and Tris. HCl (0.1 M) were prepared and enzyme activity was determined as below:

Constituents	Sample	Blank
Enzyme extract	10 µl	10 µl
Tris HCl buffer (0.1M)	2.5 ml	2.5 ml
Guaiacol (1 %)	0.2 ml	-
Distilled water	-	0.2 ml

Both tubes were well shaken before being let to stand for five minutes to allow the reaction to finish. The two tubes were then each given 0.2 ml of H_2O_2. A spectrophotometer set to the wavelength of 470 nm was used to measure the reaction solution's absorbance. The POD enzyme activity was then calculated using the following formula:

$$\text{Peroxidase activity} = \frac{\text{A x df}}{\text{EU x Wt x 1000}}$$

whereas A stands for Absorbance, df Dilution factor, EU Extract used, Wt. weight of tissue.

The assessment of superoxide dismutase activity was conducted following the procedure outlined by Maral *et al*., (1977), which relies on gauging the enzyme's capacity to impede the photochemical reduction of nitroblue tetrazolium (NBT). For the estimation of SOD activity, the sample and blank were prepared as follows:

Constituents	Sample	Blank
Enzyme extract	10 µl	10 µl
Reaction mixture	2.0 ml	2.0 ml

Test tubes were covered with black paper, and both were subsequently exposed to light (provided by 60 W fluorescent tubes) for duration of 10 minutes. The absorbance of the solution after irradiation at 560 nm was quantified using a spectrophotometer. The definition of one unit of SOD activity was based on the quantity of enzyme that induced a 50% reduction in the photochemical reduction of NBT. The ensuing equation was applied for the calculation of SOD activity.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of experimental sample} - \text{Absorbance of control sample}}{\text{Absorbance of control sample}} \text{ x } 100$$

Estimation of proline: The approach described by Bates *et al*., (1973) was employed to assess proline levels. Solutions of sulfosalicylic acid (3%) and acid ninhydrin were prepared, and proline concentrations were determined. A homogenized sample of leaf tissue weighing 0.5g was combined with 10 ml of 3% sulfosalicylic acid before being centrifuged at 13000 rpm for ten minutes at 4°C. After that, a test tube containing 2.0 ml of the supernatant, 2.0 ml of acid ninhydrin, and 2.0 ml of glacial acetic acid was incubated at 100°C for an hour before being cooled to room temperature. After that, the solution was well mixed with 4.0 ml of toluene

before being added to the test tube. The resulting sample was then allowed to sit undisturbed for 10 minutes to help the toluene and aqueous phases separate. With toluene serving as the blank, the absorbance of the top toluene layer was carefully removed and measured at 520 nm. Proline concentrations were computed utilizing a standard curve, and the results were documented based on the fresh weight using the following formula:

$$\mu m \text{ Proline / g fresh weight} = \frac{(\mu m \text{ Proline / ml x ml of toluene /115.5})}{(\text{g of sample})} \text{ x } 100$$

Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) using SPSS (version 22.0.0). Duncan's multiple range tests was used with a significance level of 0.05% to compare the mean values.

Results

Effect of exogenous application of KH_2PO_4 on percentage germination: The results about the effect of KH_2PO_4 under salt stress in *Brassica campestris* seeds is exhibited in table 2 regarding germination rate, shoot and root length of seedlings. The application of KH_2PO_4 resulted in a positive impact on the percentage of germination when the seeds were treated with it exogenously. Under control conditions, rate of germination was 96.43%. Under high concentrations of NaCl (100 mM) 50.23% seeds were sprouted and rates of germination were recorded as 58.30, 53.68, and 50.23% at 60, 80 and100 mM salt. When seeds were pretreated with KH_2PO_4 and then germinated on various salt concentrations, germination was increase significantly and highest rate (72.04%) was observed at 60 mM salt + 0.5 mM KH_2PO_4 followed by (67.14%) 80 mM salt + 0.5 mM KH_2PO_4 and 61.29% at 100 mM salt + 0.5 mM KH_2PO_4. Maximum growth (96.4%) was seen when seeds were pretreated with 0.5 mM KH_2PO_4 alone without any salt treatment.

Effect of exogenous application of KH_2PO_4 on shoot length: A noticeable increase in shoot length was observed with the pre-treatment of seeds using KH_2PO_4. The findings indicated a substantial impact of salt stress on *Brassica* seedlings, as their shoot length was enhanced to 1.3 cm from 0.5 cm under 100 mM salt treatment. The antagonistic impacts of salinity were decreased when seeds were pretreated with KH_2PO_4 (Table 2; Fig. 1).

Effect of exogenous application of KH_2PO_4 on root length: The application of KH_2PO_4 under salt stress conditions resulted in an augmentation of root length. The most notable enhancement (1.7 cm) in root length was observed at a concentration of 0.5 mM KH_2PO_4 in the absence of salt. An increase in salt concentration led to a marked reduction in root length.

Results are presented as the mean ± SE of three replicates for each treatment. Duncan's multiple range tests indicates by different alphabetic letters in each column differ significantly at $p \leq 0.05$.

Impact of foliar spray of KH_2PO_4 on shoot length, diameter and leaf area of mustard: The data regarding shoot diameter, leaf area and shoot length of Mustard plant is represented in tables 3 that suggests exogenous use of KH_2PO_4, reduced the deleterious effects of salinity significantly.

Leaf area: An improvement in leaf area was seen with the use of KH_2PO_4, under saline conditions. At 100 mM NaCl concentration, the leaf area recorded was 132 cm^2. After foliar use of KH_2PO_4, (0.5 mM) an increasing trend in leaf area was observed under different concentrations of NaCl. Although maximum leaf area (179 cm^2) was found in treatment having 0.5 mM KH_2PO_4 without NaCl.

Shoot length and diameter: A constant rise in shoot length and diameter was observed with an increased concentration of salt when KH_2PO_4 was applied as a foliar spray. The highest increase was recorded at 0.5 mM KH_2PO_4 without salt. Shoot length of the salt treated plants was 126.65 cm under 100 mM NaCl which increased to 134.19 cm when 0.5 mM KH_2PO_4, treatment was given. Shoot diameter also shown positive effect to KH_2PO_4 (Table 3).

Impact of exogenous application of KH_2PO on shoot fresh and dry weights: Foliar spray of KH_2PO_4 effectively mitigated the growth inhibition caused by salinity, particularly in terms of biomass production. Across all three salinity stress levels (60, 80, 100 mM NaCl), the application of KH_2PO_4 (0.5 mM) proved effective in enhancing growth parameters, as evidenced by the increase in shoot fresh weight of plants. Notably, the application of KH_2PO_4 coupled with 60 mM NaCl resulted in the greatest improvement in shoot dry weight (Table 4).

Impact of exogenous application of KH_2PO on root fresh and dry weights: As indicated in table 4, it was evident that foliar application of KH_2PO_4 mitigated the adverse effects of salinity on both root fresh and dry weights, resulting in an overall increase across all tested concentrations.

Impact of exogenous application of KH_2PO_4 on protein contents of *Brassica* plants: The impact of KH_2PO_4 applied via foliar spray on soluble protein levels is given in table 5. It was evident that treating plants with KH_2PO_4 affect significantly on soluble protein contents. The plants' soluble protein content increase in comparison to the control group after being irrigated with 100 mM NaCl. When salt stress was introduced, the amount of soluble protein increased, however when KH_2PO_4 was applied, the amount of soluble protein decreased in mustard plants. Different salt and KH_2PO_4 treatments resulted changes in soluble protein levels as 0.32, 0.29 and 0.31 mg/g of tissue at 60, 80 and 100 mM NaCl, respectively. As it is obvious from the data that maximum reduction was seen at 0.5 mM of KH_2PO_4 without salt.

Table 2. Effect of KH_2PO_4's on the lengths of the shoots and roots and seed germination.

Treatments	Germination (%)	Shoot length(cm)	Root length (cm)
Control	96.43 ± 0.03^{a}	1.3 ± 0.42^{b}	1.6 ± 0.28^{a}
Salt 60 mM	58.32 ± 0.18^{c}	0.8 ± 0.01^{e}	1.0 ± 0.16^{b}
Salt 80 mM	53.68 ± 0.24^{c}	0.7 ± 0.13^{d}	0.9 ± 0.32^{c}
Salt 100 mM	50.23 ± 0.02^{cd}	0.5 ± 0.01^{d}	0.8 ± 0.13^{c}
0.5 mM KH_2PO_4 and without salt	96.58 ± 0.03^{a}	1.4 ± 0.81^{a}	1.7 ± 0.31^{a}
0.5 mM KH_2PO_4 + 60 mM salt	72.04 ± 0.24^{b}	1.1 ± 0.02^{b}	1.3 ± 0.22^{b}
0.5 mM KH_2PO_4 + 80 mM salt	67.14 ± 0.05^{c}	1.0 ± 0.15^{d}	1.1 ± 0.13^{b}
0.5 mM KH_2PO_4 + 100 mM salt	61.29 ± 0.13^{c}	0.9 ± 0.11^{c}	0.9 ± 0.34^{c}
Significance with df 4 and 49	*	*	*

Table 3. Application of KH_2PO_4 impacts on the length, diameter and leaf area of the shoots of plants growing under salt stress.

Treatments	Shoot length (cm)	Shoot diameter (cm)	Leaf area (cm^2)
Control	135.33± 4.40^{b}	5.57 ± 0.97^{b}	174 ± 8.62^{a}
Salt (60 mM)	129.45 ± 1.25^{c}	4.22 ± 0.25^{de}	151 ± 2.53^{d}
Salt (80 mM)	128.79 ± 3.75^{c}	3.78 ± 0.40^{e}	138 ± 3.51^{d}
Salt (100 mM)	126.65 ± 1.29^{d}	3.23 ± 0.28^{f}	132 ± 5.28^{d}
0.5 mM KH_2PO_4	149.49 ± 3.21^{a}	6.28 ± 0.32^{a}	179 ± 4.26^{a}
Salt (60 mM) + 0.5 mM KH_2PO_4	139.20 ± 2.57^{b}	5.28 ± 0.52^{c}	170 ± 2.13^{b}
Salt (80 mM) + 0.5 mM KH_2PO_4	136.44 ± 2.23^{b}	5.03 ± 0.34^{c}	168 ± 2.61^{c}
Salt (100 mM) + 0.5 mM KH_2PO_4	134.19 ± 3.48^{c}	4.84 ± 0.22^{d}	153 ± 3.21^{cd}
Significance with df 4 and 49	*	*	*

For each treatment, ± SE represent the mean of three replicates. Duncan's multiple range test reveals a significant difference by different alphabetic letters in each column at $p \leq 0.05$

Table 4. Effect of foliar spray of KH_2PO_4 on plant biomass growing under salt-stress.

Treatments	Shoot weight (g)		Root weight (g)	
	Fresh wt. (g)	Dry wt. (g)	Fresh wt. (g)	Dry wt. (g)
Control	245.53 ± 1.93^{b}	75.05 ± 1.48^{b}	14.19 ± 2.58^{a}	5.08 ± 0.43^{a}
Salt (60 mM)	209.63 ± 1.92^{c}	63.70 ± 0.24^{d}	11.14 ± 1.24^{d}	3.94 ± 0.32^{c}
Salt (80 mM)	187.86 ± 0.83^{d}	56.54 ± 0.16^{de}	10.85 ± 1.17^{d}	2.37 ± 0.22^{d}
Salt (100 mM)	134.15 ± 1.59^{e}	48.01 ± 0.34^{e}	10.17 ± 0.79^{d}	2.21 ± 0.15^{d}
0.5 mM KH_2PO_4	263.94 ± 0.14^{a}	86.88 ± 0.22^{a}	14.64 ±0.65^{a}	5.52 ± 0.22^{ab}
Salt (60 mM) + 0.5 mM KH_2PO_4	241.42 ± 0.52^{b}	74.90 ± 0.23^{b}	12.43 ± 2.17^{c}	4.04 ± 1.24^{c}
Salt (80 mM) + 0.5 mM KH_2PO_4	210.89 ± 0.36^{c}	68.92 ± 0.17^{c}	12.04 ± 1.24^{c}	3.94 ± 1.13^{c}
Salt (100 mM) + 0.5 mM KH_2PO_4	189.94 ± 0.94^{d}	63.70 ± 0.23^{d}	11.21 ± 0.54^{d}	2.95 ± 0.31^{d}
Significance with df 4 and 49	*	*	*	*

For each treatment ± SE represent the mean of three replicates. Duncan's multiple range test reveals a significant difference with different alphabetic letters in each column at $p \leq 0.05$

Table 5. The influence of foliar application of KH_2PO_4 on protein contents, antioxidant enzyme activities and proline contents of salt-stressed plants.

Treatments	Soluble protein contents (mg/g tissue)	Peroxidase activity (mg/g tissue)	Superoxide dismutase activity (U/mg protein)	Proline Contents (µ mol/g FW)
Control	0.38 ± 0.004^{a}	0.16 ± 0.008^{a}	40.57 ± 1.36^{b}	13.98 ± 0.639^{e}
Salt (60 mM)	0.35 ± 0.012^{a}	0.12 ± 0.006^{b}	37.26 ± 0.143^{c}	22.08 ± 2.676^{d}
Salt (80 mM)	0.32 ± 0.011^{b}	0.11 ± 0.008^{b}	34.97 ± 0.25^{c}	21.35 ± 1.008^{d}
Salt (100 mM)	0.31 ± 0.05^{c}	0.09 ± 0.06^{b}	30.68 ± 1.467^{d}	19.97 ± 1.719^{b}
0.5mM KH_2PO_4	0.30 ± 0.008^{c}	0.17 ± 0.007^{a}	46.49 ± 1.137^{a}	37.88 ± 0.822^{a}
Salt (60 mM) +0.5 mM KH_2PO_4	0.37 ± 0.06^{a}	0.15 ± 0.05^{a}	43.14 ± 1.133^{b}	33.02 ± 2.153^{b}
Salt (80 mM) + 0.5 mM KH_2PO_4	0.34 ± 0.014^{b}	0.15 ± 0.008^{a}	42.12 ± 0.25^{b}	31.23 ± 0.003^{b}
Salt (100 mM) + 0.5 mM KH_2PO_4	0.32 ± 0.03^{b}	0.15 ± 0.09^{a}	40.38 ± 2.667^{b}	29.97 ± 1.752^{c}
Significance with df 4 and 49	*	*	*	*

Values are the means ± SE three replicates. Different alphabetic letters in each column represent the significant difference at $p \leq 0.05$ according to Duncan's multiple range tests

Effect of exogenous application of KH_2PO_4 on peroxidase activity of rapeseed plants: The peroxidase enzyme activity in plants subjected to foliar spraying with a 0.5 mM KH_2PO_4 solution remained consistent at approximately 0.15 mg/g of tissue at various salt concentrations. This indicated a decline from 0.12 to 0.11 mg/g of tissue and 0.09 mg/g of tissue at NaCl concentrations of 60, 80, and 100 mM, respectively. It was observed that at 0.5 mM KH_2PO_4 without NaCl concentration proved most effective in mitigating negative impacts of salinity.

Effect of exogenous application of KH_2PO_4 on SOD activity of rapeseed plants: Table 4 illustrates that the application of 0.5 mM KH_2PO_4 notably enhances superoxide dismutase (SOD) activity in comparison to the control. This increase in SOD activity is well evident in plants treated with KH_2PO_4.

Effect of exogenous application of KH_2PO_4 on proline contents of rapeseed plants: The foliar treatment of KH_2PO_4 caused considerable changes in the proline contents of mustard plants. The proline concentration was calculated to be 13.89 mol/g FW (fresh weight) under control conditions. However, under salinity stress, mustard plants showed an increase 22.04 mol/g FW in proline concentration. This level was further elevated to 29.15 µmol/g fresh weight when exogenous 0.5 mM KH_2PO_4 was applied via foliar spray. The data presented in table 5 indicated that proline content increased further with decreasing salt concentration and highest level was recorded at 0.5 mM KH_2PO_4 without salt (38.88 µmol/g).

Discussion

This study reveals that how KH_2PO_4 impacts several developmental and metabolic processes in mustard plants growing under salt stress. As high levels of salt stress disrupt ion balance and cause osmotic stress in a variety of crop species. It has been discovered in earlier studies that salt stress has a significant inhibitory influence on plant growth and development (Munns & Tester, 2008; Rauf *et al*., 2022). These effects can lead to various forms of stress, including oxidative damage, ultimately resulting in reduced plant growth (Zorb *et al*., 2004). Similar outcomes were observed in our study, where the application of higher salt levels (100 mM NaCl) had a detrimental impact on numerous growth parameters as well as on biochemical parameters. Comparable results were also reported in various pea varieties subjected to different salt concentrations (Ben-Ahmed *et al*., 2010) and similar trends were observed in other plant cultivars (Rabhi *et al*., 2007; Rui *et al*., 2009; Reman *et al*., 2022).

Seed and seedling germination is important phase in plants particularly grown under stressful environment. Scientists employ a variety of techniques in different plant species to mitigate the hostile properties of salinity. It is well known that seed priming is a highly beneficial strategy for improving many crops' tolerance to salt stress. A significant reduction in all growth parameters like shoots and root fresh and dry weight, particularly reduction in seed germination percentage. Akram (2006) tested the hypothesis that salinity has detrimental effects on sunflower plants might be mitigated by foliar application of potassium + phosphorus (K+P) in the form of KH_2PO_4. In his study, 18 day old non-stressed and salt stressed (0 and 150 mM of NaCl) sunflower seedlings were used and spray with different quantities of KH_2PO_4 (0, 5 + 4, 10 + 8 and 20 + 16 mgg^{-1} K+P). The effects of salt stress on growth, photosynthetic ability, mineral nutrient buildup, and crop output were recorded at conclusion of experiment. A significant positive effect of spray was observed in enhancing growth under stress. Similarly in another study, varied concentrations of foliar KH_2PO_4 application were successful in boosting sunflower when salt stress was present (Arafa *et al*., 2009).

The shoot and root length were also reduced under saline conditions. The harm full impacts of salinity were ameliorated with the foliar application of KH_2PO_4 as evident from the data. Similar findings were observed in a pot experiment, where the application of KH_2PO_4 through foliar spray showed a positive influence on shoot length in the presence of saline conditions. In the present investigation, 0.5 mM KH_2PO_4 was proved effective in mitigating the detrimental impacts of salinity. Kaya *et al*., (2001) established an experiment to observe the ameliorative impact of foliar potassium phosphate (4 mM KH_2PO_4) spray on strawberry plants grown under high NaCl concentration (35 mM). It is found that the plants of both varieties grown under high salt had less dry mass, chlorophyll content and fruit production as compared to control. Foliarly applied KH_2PO_4 decreased the negative effects of salinity on plant growth and fruit production. High level of NaCl increased the membrane permeability and it was reduced by KH_2PO_4 sprays (Barus *et al*., 2018). Elevated salinity significantly diminishes various parameters in spinach, including seedling growth, vegetative growth, chlorophyll concentration, relative water content, and water use efficiency. However, the treatment of KH_2PO_4 exhibited improvements in fresh weight, relative water content, water use efficiency, and chlorophyll concentration. It was also found that membrane permeability decreased by salinity was also improved by foliar application of 5 mM KH_2PO_4 by decreasing electrolyte leakage. Results showed that foliar spraying of KH_2PO_4 rectified the plant's deficits in both P and K. Phosphorus and potassium concentrations in leaves were decreased by high salinity, but were increased by additional potassium (K) and phosphorus (P). These findings suggested that additional P and K can lessen the negative effects of excessive salinity on plant growth and physiological development. Studies shows that P and K shortages in the leaves were caused by excessive NaCl levels.

It is well evident that plants utilize biochemical stratigies to counteract the effects of salt stress. These strategies include osmotic regulation by accumulating compatible inorganic and organic solutes. Additionally, plants employ an effective enzymatic and non-enzymatic antioxidant system. In this study, the investigation focused on the changes in protein content in salt-treated plants when spray with KH_2PO_4. Sajid (2023) studied some of the

biochemical aspects (protein and proline contents and peroxidase and SOD) under salinity stress and suggested that salinity severely inhibited all the biochemical parameters. Similar findings from earlier studies have also been reported, showing that the amount of total soluble proteins increases at lower salt levels and decreases as salinity levels rise. Devkota & Jha (2010) investigated the combined influence of foliar application of non-enzymatic antioxidants along with different levels of salt stress on proline content in *Triticum aestivum* L. A noticeable increase in proline content was observed following the foliar application of salicylic acid. These finding are consistent with our study and also in line with the Hussein *et al*., (2004) and Ejaz *et al*., (2012) who reported similar results in *Zea mays* and *Saccharum* hybrid, where increased levels of proline, antioxidant enzymes and protein contents were observed under salt stress conditions through the foliar application of salicylic acid and ascorbic acid.

Conclusion

The results of the present study indicated that salt significantly decreased all developmental and metabolic attributes. Whereas the application of 0.5 mM KH_2PO_4 concentration, enhanced the growth while significantly reduced the saline stress in *Brassica*. The enhancement in growth could potentially be attributed to its comprehensive ameliorative properties, given that KH_2PO_4 is recognized for triggering seed germination, facilitating water absorption and potentially functioning as a signaling molecule under saline environments. So, the present study suggests that a specific but a mild concentration of KH_2PO_4 may be quite helpful in boosting plant growth as well as the yield of plants under salt stress.

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