Impact of ZnO NPs, Pi Availability and the Interaction of Pi x NaCl in Reducing the Negative Effect of Salinity and Pi Deficiency on Growth of Arabidopsis Plant

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Abstract: ***Most crop production is decreased by salinity in agriculture, which is one of the main abiotic stressors that lowers crop production. One of the detrimental effects of salinity on plant growth and productivity is known as nutritional imbalance such as phosphate (Pi). Approximately 54% of the cultivated area of Saudi Arabia suffers from salinization. While its soils characterized by low available Pi. Such regions in Saudi Arabia, the factors of soil salinity and Pi deficiency pose major challenge to agricultural production. Nowadays, zinc oxide nanoparticles (ZnO NPs) are widely used in agriculture, and many investigations were carried out to determine the impact of ZnO NPs on plants under abiotic stress. So far, few research investigated into the potential impact of ZnO NPs on the growth of Arabidopsis plants under salt and Pi deficiency, and their interaction. This study aimed to investigate the effect of ZnO NPs application (10 mg/L), Pi availability and the interaction of Pi x NaCl in reducing the negative effect of the salinity and Pi deficiency on growth of Arabidopsis. Arabidopsis plants were grown hydroponically under different treatments of Pi and NaCl, and the interaction of Pi x NaCl with/without ZnO NPs and ZnO BPs. The results indicated that salinized plants had a reduction in plant growth traits, but, the values of these traits increased with high Pi (100 and 250 µM). Pi at 10 µM is more detrimental than NaCl at 100 mM for plant growth. Salt-treated plants (50, 100 mM) exposed to high Pi (100 and 250 µM) showed higher salt tolerance. In contrast, plants subjected to NaCl (150 mM) and Pi (250 µM) exhibited reduce in the values of growth parameter. Results also showed that ZnO NPs and ZnO BPs was sufficient to protect plant against salinity and Pi deficiency, however ZnO NPs was more efficient than ZnO BPs. The Pi availability, the combined treatment of Pi x NaCl, and ZnO NPs can be a promising application to alleviate the negative impacts of salinity and Pi deficiency on plant growth.***

Keywords: Arabidopsis thaliana, ZnO-NPs application, salinity stress, deficiency and availability of Pi, Interaction of Pi x NaCl

1. Introduction

The sustainability of agriculture is seriously threatened by both the loss of arable land and the growth of the human population (Shokat and Grosskinsky, 2019). One of the primary abiotic stresses restricting crop production is high salt content in soils, which is a significant environmental issue for agriculture globally (Srivastava, 2020). By the entry of both Na and Cl into the cells result in severe ion imbalance and excessive uptake may result in significant physiological disorder. Where High levels of Na prevent K uptake which is a necessary component for growth and development of plant (James *et al*,. 2011).

It is known that only 10-30% of the applied Pi fertilizer can be recovered by the crop grown (Syers *et* al., 2008), while above 80% of the Pi remains immobile and is not taken up by plants (Kapri and Tewari 2010). The world reliance on fertiliser of Pi for production of food results in decreasing resources for rock Pi and increasing in expenses of the production of Pi fertiliser in the next decade. All indicate that a crisis is about to occur which will have disastrous effects for the world's food security (Cordell *et al*., 2009). Soil nutrients must be managed carefully, and feasible strategies must be in place for enhancing agricultural productivity in low Pi soils. One of these strategies is to utilise Pi fertiliser more wisely, efficiently, and in an adequate quantity without causing any environmental effect (Mahapatra *et al*., 2022).

The salinity factor, which is rising in many agricultural soils, particularly dry and semiarid, is well recognized to increase the loss of phosphorus (P) availability (Tchakounté *et al*., 2020). Thus, in these types of soils, plants experience both salinity and Pi deficiency (Dey *et al*., 2021). Thus, nutrient supplementation in the form of chemical fertilizer is needed for optimum plant growth and high yields since nutrients are crucial for development and growth of plant (Usman *et al*., 2020) [5]. However, the excessive use of chemical fertilizers in agricultural activities causes several environmental problems. Nowadays, the important issue is to find proper and sustainable technique to help plants adapt more rapidly to environmental factors without harming ecosystems (Vermeulen *et al*., 2012). Using nanoparticles (NPs) application for agricultural purposes is a new and promising approach to enhance agricultural practices. By optimising the administration of nutrients, the use of NPs aims to minimise nutrient losses during fertilisation, lower the quantity of plant protection chemicals use, and boost yields (Das *et al*., 2015). One of the most widely applied nanoparticles in agriculture is zinc oxide nanoparticles (ZnO NPs) (Lang *et al*., 2021). ZnO NPs as nano-fertilizer has a favourable effect on plants and it can be effectively applied in

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agriculture without damaging the ecology or releasing nutrients into the environment (Thounaojam *et al.*, 2021). We evaluated the effect of ZnO NPs application, appropriate amount of Pi and the interaction of Pi and NaCl in reducing the negative effect of the salinity stress and Pi deficiency on Arabidopsis growth.

2. Material and Methods

2.1 Model plant

In the experiments of this project, we used *Arabidopsis thaliana* Colombia-0 plant. The seeds were obtained from King Abdullah University of Science and Technology (KAUST), in Saudi Arabia. The growth was carried out in cabinet room by using an ideal hydroponic growth system we designed to adapt to *Arabidopsis* growth habit.

2.2 General growth conditions for Arabidopsis

Arabidopsis thaliana plant was used in all experiments involving: (1) 4 different levels of NaCl (0,50, 100 and 150 mM), (2) 4 different levels of Pi (0, 10, 100 and 250 µM) and (3) and their interaction Pi x NaCl (4x NaCl and 4xPi, 16 processing) with/without applications of ZnO NPs and ZnO BPs (10 mg/l). All experiments were performed in the hydroponic system containing Gibeaut's solution which consisted of macronutrients and micronutrients (Table1.1), all solutions were autoclaved (Gibeaut *et al.*, 1997). The controlled hydroponic experiments were carried out in growth rooms at 24 °C with a 16/8 hr day/night cycle.

2.3 Hydroponic growth

2.3.1 Preparation of hydroponic growth

The purpose of this hydroponic system was to minimize physical limitations in the hydroponic system and Pi effectively became a mobile nutrient. Also, this system is faster and more accurate for analyzing a range of growth trait *Arabidopsis thaliana*. Seed holders were prepared by cutting off the bottom and gluing on a piece of grey mesh (1 cm) to avoid the loss of agar. The lids were made of a rectangular plastic sheet. 56 holes (1.3 cm diameter) were drilled in these lids to carry the seed-holders during growth. Black plastic containers) filled up with Gibeaut's solution. In such designs for hydroponic growth, the growth of algae is prevented as well as the media solution remains clear, because the media containers are made of black plastic (Figure 1).

Table 1: Chemical composition of Gibeaut's solution was used in the experiment of hydroponic growth system. Gibeaut's solution consisted of Gibeaut's Macronutrients, and Gibeaut's Micronutrients

Gibeaut's Macronutrients						
Molecule	MW	Stock [M]	[g]/ [L]	[g]/250 [ml]	Final conc. [mM]	Volume of stock in media [ml/10l]
$Ca(NO_3)_2$ x $4H_20$	236.15	1	236.15	59.0375	1.5	15
KNO_3	101.11	1	101.11	25.2775	1.25	12.5
$MgSO_4$	120.37	1	120.37	30.0925	0.75	7.5
$Na_2SiO_4 \cdot 9H_2O$	284.2	0.1	28.42	7.105	0.1	10
Fe-EDDHA	435.2	0.036	15.6672	3.917	0.036	10
Gibeaut's Micronutrients						
Molecule	MW	Stock [mM]	[g]/ [L]	[g]/250 [ml]	Final conc. [µM]	Volume of stock in media [ml/10l]
KCl	74.56	50	3.728	0.932	50	10
$MnSO_4 \cdot H_2O$	169.01	10	1.6901	0.4225	10	
$CuSO_4 \cdot 5H_2O$	249.68	1.5	0.37452	0.0936	1.5	
$ZnSO_4 \cdot 7H_2O$	287.54	2	0.57508	0.1437	2	
H_3BO_3	61.83	50	3.0915	0.7728	50	
$(NH_4)_6Mo_7O_{24}$	1235.9	0.075	0.092693	0.0231	0.075	

2.3.2 Preparation of germination

Before germination, seeds of *Arabidopsis thaliana* were imbibed in 0.1% agar at 4 °C for 5 days before transferring to growth room where the hydroponic system will be placed. Around 1ml of melted 0.4% agar (germination medium) was poured into each seed-holders and left to solidify for 20 mins. Once it cooled down, the seed-holders were placed on the lid hole of the containers. Stratified seeds were placed on the top once the agar was solid. To enhance humidity, the containers covered with cling film for a few days (4 days) until the seed germinate.

2.3.3 Stage of seedling pre-growth

When plants were directly germinated and grown in different treatments, we found that the results were unreliable due to the very large differences in size. Thus, we decided that in order to obtain reliable results for this study, uniform growth was of great importance and should be taken into account for the experimental design. Thus, we used the pre-growth stage before treating our plants with different levels of treatments in Gibeaut's solution. 56 seeds of *Arabidopsis* plant were used for the early growth process, where the seeds grown for 10 days in hydroponic solution containing 20 µM Pi (control) with the aim of obtaining plants showing uniform morphology. Also, to achieve a large enough set of plants for further growths under different treatments (Figure 1).

2.3.4 Stage of Treatments

In this stage, 12 uniform 10-d-old of *Arabidopsis* plants were transferred into different hydroponic systems containing various treatments: (1) 4 levels of NaCl (0,50, 100 and 150 mM), (2) 4 levels of Pi (0, 10, 100 and 250 µM) and (3) and their interaction Pi x NaCl (4x NaCl and 4xPi, 16 processing) with or without applications of ZnO NPs and ZnO BPs (10 mg/L) in Gibeaut's solution as shown in (Table. 2). Growth parameters (root lengths, fresh and dry weight per plant and leaf area) were measured to as the role of ZnO NPs application and the role of combined effect of Pi x NaCl in reducing the negative effect of the salinity stress and Pi deficiency in *Arabidopsis* plant. To maintain constant Pi, NaCl and ZnO concentrations, if necessary, media was added every 3-4 days except on the day of nutrient application. Approximately 2 weeks after growing in Gibeaut's solution, the mature plants were harvested for the following growth parameters.

2.4 Plant growth measurements

These measurements include root lengths (cm), leaf area square centimetre (cm^2) and weights of fresh and dry per plant (g) per plant, with 3 replicates for each treatment.

Table 2: Different treatments were used in the experiments of plant growth for *Arabidopsis thaliana* Colombia-0 in hydroponic system containing Gibeaut's solution.

Treatments	
Pi treatments	
Control - Pi (0, 10, 100 and 250 µM)	
Pi (0, 10, 100 and 250 µM) + ZnO BPs (10 mg/L).	
Pi (0, 10, 100 and 250 µM) + ZnO NPs (10 mg/L).	
NaCl treatments	
Control - NaCl (0, 50, 100 and 150 mM).	
NaCl (0, 50, 100 and 150 mM) + + ZnO BPs (10 mg/L).	
NaCl (0, 50, 100 and 150 mM) + ZnO NPs (10 mg/L).	
Interaction between NaCl x Pi	
NaCl (50 mM)	Control - Pi (0, 10, 100 and 250 µM)
	NaCl (50 mM) with Pi (0, 10, 100 and 250 µM) + ZnO BPs (10 mg/L).
	NaCl (50 mM) with Pi (0, 10, 100 and 250 µM) + ZnO NPs (10 mgL).
NaCl (100 mM)	Control - Pi (0, 10, 100 and 250 µM)
	NaCl (100 mM) with Pi (0, 10, 100 and 250 µM) + ZnO BPs (10 mg/L).
	NaCl (100 mM) with Pi (0, 10, 100 and 250 µM) + ZnO NPs (10 mg/L).
NaCl (150 mM)	Control - Pi (0, 10, 100 and 250 µM)
	NaCl (150 mM) Pi with (0, 10, 100 and 250 µM) + ZnO BPs (10 mg/L).
	NaCl (150 mM) with Pi (0, 10, 100 and 250 µM) + ZnO NPs (10 mg/L).

2.4.1 Determination of root lengths

A metric scale was used to measure the plants lengths, and the results were represented in centimetres (cm).

2.4.2 Leaf area measurements

The measurement of the total rosette (leaf) surface was made using ImageJ analysis in accordance with Schnaubelt *et al.*, (2013)[18]. ImageJ is open-source software for processing and analysing scientific images.

2.4.3 Determination of fresh and dry weight per plant

From the hydroponic system, plants were harvested together, and the root tissues were carefully separated from the shoot tissues and washed 3 times with distilled water to remove media residue and the fresh weight was immediately determined by using analytical balance [HR-200] and expressed in grams (g). To dry shoot and root tissues, samples of dry shoots and roots were moved to a crucible that had been previously weighed and heated to 85°C in the oven for 18 hr.

2.5 Statistical analyses

Statistical Package for the Social Sciences (SPSS) version 25 was used to enter and analyse our data. All data presented are the mean values, and the standard error of the mean (± SE) of 3 replicates, t-test and Two-way ANOVA (Analysis of Variance) were applied to compare the average values between treatments and to analyse the effect and interaction between Pi and NaCl, with statistical significance shown at a confidence level of significant differences in $P < 0.05$.

3. Results

3.1 Root length

3.1.1 Effect of different levels of NaCl (±ZnO NPs-BPs) on Root length

Figure 2a shows the impact of different NaCl levels (0, 50, 100, and 150 mM) with or without 10 mg/L of ZnO NPs and ZnO BPs on the length of the roots of *Arabidopsis thaliana*. The measured values of root length significantly decreased as the NaCl concentration increased in Gibeaut's solution. A considerable reduction was found at high NaCl levels (100 and 150 mM) (5.4073 and 5.4073 cm), respectively. In comparison to the controls, the addition of ZnO BPs and ZnO NPs (10 mg/L) led to highly significant increase in root length. The root length of the plant given ZnO NPs treatment reached (5.4653 cm) at the high level of NaCl (150 mM), whereas the root length of the ZnO BPs -treated plants reached (3.9287 cm) in comparison to the control (3.3223 cm).

3.1.2 Effect of different levels of Pi (±ZnO NPs-BPs) on root length

At varying Pi concentrations (0, 100, and 250 µM), *Arabidopsis thaliana* roots grew longer (Figure 2b). After 2 weeks of treatment, the root length was seen to be suppressed as Pi concentrations decreased below 10 µM, whereas root length increased significantly at 100 and 250 µM. Using application of ZnO BPs helped to lengthen the roots, however, ZnO NPs considerably lengthened the roots at 0, 10, and 250 µM Pi in contrast to the control group (Figure 2b).

3.1.3 Effect of NaCl-Pi Interaction (±ZnO NPs-BPs) on root length

Different amounts of Pi, NaCl, and their combination had a significant impact on root length, according to a two-way ANOVA (Figure 3). Root length was significantly decreased by low Pi and salt, with salinity having a stronger impact on root growth. Salinity significantly decreased the length of *Arabidopsis thaliana*'s roots regardless of the Pi supply,

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however at low Pi (10 µM) supply than at control, 100, and 250 µM Pi supply, the loss in root length was more pronounced. It is interesting to note that increasing the Pi dose in Gibeaut's solution prevents the average root height from decreasing under saline conditions. The maximum average root length values were found at the saline concentration levels of 100 and 250 µM for each saline concentration (50 and 100 mM). wile, the lowest values were found at the saline concentration levels of 10 µM for each saline concentration (50, 100, and 150 mM). When ZnO BPs were added to a Gibeaut's solution that had various amounts of NaCl and Pi, the lengths of the roots significantly increased. In comparison to control, the increase was 7.9533 cm at 100 µM Pi in 50 M NaCl and 5.8170 cm at 250 µM Pi in 100 mM NaCl, respectively (Table 2). In the Gibeaut's solution containing ZnO NPs, the maximal root length growth rates were 8.6513 cm and 5.8300 cm at 250 µM Pi in 50 and 100 mM NaCl, respectively.

3.2 Leaf area measurements

3.2.1 Effect of different levels of NaCl (±ZnO NPs-BPs) on Leaf area

The values of leaf area has decreased with all treatments of NaCl (50, 100, and 150 mM) compared to the control in treated plants (Figure 4a), indicating the negative impacts of salt stress. We observed an increase in leaf area after adding ZnO BPs and ZnO NPs at a level of 10 mg/L of each. In all NaCl concentrations, added ZnO BPs increased leaf area, however, addition of ZnO NPs significantly increased leaf area. In comparison to the control leaf area (6.4757 cm^2), ZnO BPs and ZnO NPs treatments at (100 mM NaCl) increased leaf areas to be recorded at 7.0863 cm^2 and 8.8023 cm^2, respectively.

3.2.2 Effect of different levels of Pi (±ZnO NPs-BPs) on leaf area

The findings in Figure 4b demonstrate that *Arabidopsis* plants treated with various concentrations of Pi (10, 100, and 250 µM) tend to increase significantly in (100 and 250 µM) Pi reaching (10.3320 and 12.1080 cm^2). Whereas, the decrease was highly significant at concentrations (10 µM) Pi reaching (5.4680 cm^2), respectively. Except for the control condition, where the increase in leaf area was highly significant, the leaf area rose non-significantly for plants treated with different levels of Pi (10, 100, and 250 µM) and ZnO BPs (Figure 4b).

However, with ZnO NPs, the increase in leaf area at all concentrations was high significant, with the exception of the lowest concentration of Pi (10 µM).

3.2.3 Effect of NaCl-Pi Interaction (±ZnO NPs-BPs) on leaf area

At the 50, 100, and 150 mM NaCl levels, respectively, the leaf area rose by (9.1960, 7.0057, and 5.2693 cm^2) at Pi level (100 µM) as compared to the lowest Pi level (10 µM) (Figure 5). At the 50 and 100 mM NaCl levels, the highest Pi level (250 µM) enhanced the leaf area of plants, but not to the same extent as the 100 µM Pi did with 150 mM of NaCl. Our findings show that the leaf area decreased, when NaCl (150 mM) was applied with the highest Pi level (250 µM). We observed that the addition of ZnO BPs and ZnO NPs (10 mg/L) markedly decreased the detrimental effects of NaCl stress and Pi deficiency (10 µM) on *Arabidopsis* plants, resulting in a notable rise in leaf area. However, plants treated with ZnO NPs showed the greatest significant increase in leaf aera (Figure 5).

3.3 Fresh and dry weight per plant

3.3.1 Effect of different levels of NaCl (±ZnO NPs-BPs) on Fresh and dry weight

The fresh weight per plant was significantly decreased in all NaCl treatments after two weeks of treatment in hydroponic system (Figure 6a). In comparison to control plants (0.2637 g), plants under 150 mM of NaCl saw more losses of for fresh weight (0.0626 g). During the experiment, we observed that ZnO BPs) raised the fresh weight of the shoot non-significantly in all NaCl concentrations. Whereas ZnO NPs frequently enhanced this measure by a considerable margin of significance in comparison to their respective controls (Figure 6a).

In NaCl stressed plants (0, 50, 100, and 150 mM), Figure 6b demonstrates a significant decrease in the dry weight per plant in response to salt treatments as compared to untreated plants. The reduction in the dry weight at 50 mM was significant, while it was highly significant at 100 and 150 mM. The NaCl stressed plants and treated plants with ZnO NPs or ZnO BPs significantly varied from those without both ZnO where the dry weight increased in the treated plant tissues after application of ZnO BPs and ZnO NPs. However, ZnO NP had the higher values of dry weight than ZnO BP application,

3.3.2 Effect of different levels of Pi (±ZnO NPs-BPs) on Fresh and dry weight

Figure 7a shows the impact of different Pi concentrations (0, 10, 100, and 250 µM) on the fresh weight of *Arabidopsis* plants in the presence or absence of ZnO BPs and ZnO NPs. The findings in Figure 7a demonstrate that the fresh weight per plant increased with increasing Pi level to be recorded at (0.1143, 2043, and 0.2887 g) at (0, 100, and 250 µM Pi), respectively, and subsequently dropped to (0.1060 g) at (10 µM), this value was increased to be at (0.1597 g) by applying ZnO NPs. Comparing fresh weights of *Arabidopsis* plants grown in Gibeaut's solution containing ZnO NPs and ZnO BPs (10 mg/L) to those grown in ZnO-free media, the plants grown in media with ZnO NPs and ZnO BPs showed higher fresh weights. However, plants exposed to ZnO NPs had the greatest fresh weights (0.4890 g).

The dry weight per plant significantly decreased at the lower dose (10 µM), where it was recorded at (0.01153 g). then, it significantly increased at the higher doses (100 and 250 µM), where it was recorded at (0.03190 g and 0.03553 g, respectively, in accordance with values shown in (Figure 7b). When ZnO BPs had been added to Gibeaut's solution, the dry weight increased significantly over the corresponding controls, whereas ZnO NPs significantly raised these values above the controls. At (100 and 250 µM Pi), the dry weight of plants treated with ZnO BPs reached (0.03843 and 0.04707 g) and plants treated with ZnO NPs reached (0.04433 and 0.05277 g).

3.3.3 Effect of NaCl-Pi Interaction (±ZnO NPs-BPs) on Fresh and dry weight

With increasing Pi-addition treatments at all NaCl treatments, *Arabidopsis* plant fresh weight obviously increased (Figure 8). The interaction of NaCl (150 mM) and Pi (250 µM) resulted in a fresh weight drop of (0.0470 g). while the interaction between NaCl (150 mM) and 100 µM Pi resulted in increasing fresh weight to reach at (and 0.1057 g). Low Pi (10 µM) significantly decreased fresh weight compared to high Pi (250 µM) at all NaCl treatments. Applications of 10 mg/L ZnO NPs and ZnO BPs with NaCl and Pi in Gibeaut's solution had significant effects on fresh weight (Figure 8). The findings showed that the treated plants with ZnO BPs had increased fresh weight. However, plants exposed to ZnO NPs showed the greatest improvement in fresh weight (2.4 times).

With the exception of 250 µM Pi, the dry weight per plant increased at all NaCl levels as the treatments of Pi increased (Figure 9). The interaction between NaCl (150 mM) and Pi (100 µM) led to increase the dry weight by (0.0130 g). Whereas treatments of NaCl (150 mM) and Pi (250 µM) decreased the dry weight by (0.0090 g). The applications of ZnO BPs and ZnO NPs along with treatments of NaCl and Pi in Gibeaut's solution had significant effects on the dry weight per plant. The results showed that plants given 10 mg/L of ZnO BP had higher dry weight values than plants who weren't given ZnO BP treatments. Obviously, plants treated with ZnO NPs and exposed to NaCl-Pi interaction had the largest improvement in dry weight per plant (Figure 9).

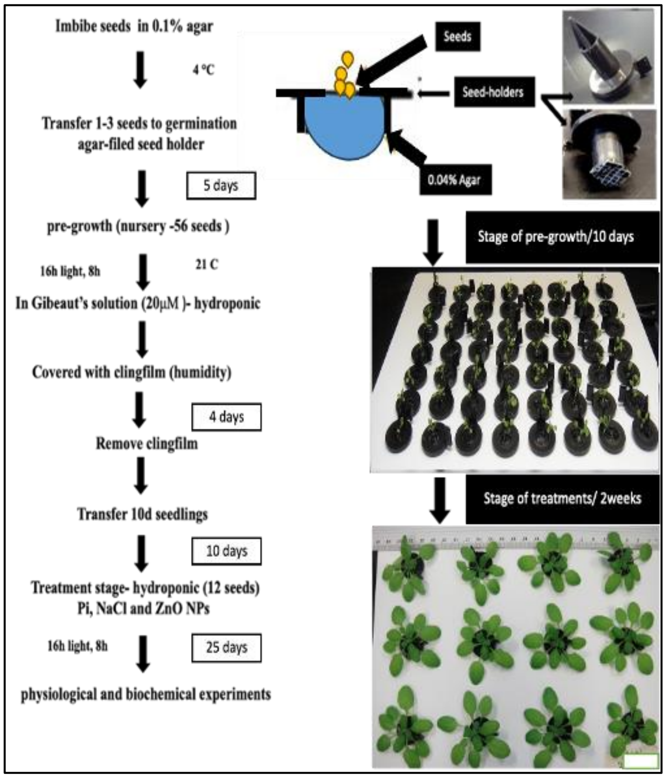

Figure 1: Method for hydroponically growing Arabidopsis. A flowchart detailing the process's major phases and timing. Images in the right-hand side depict the seed germination process in action as well as typical shots of seedlings and mature plants.

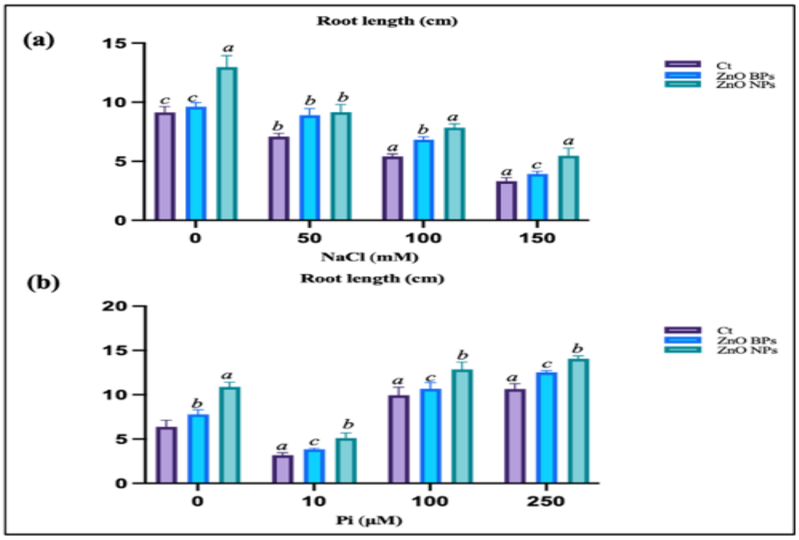

Figure 2: Effect of different concentrations of (a) NaCl (mM) and (b) Pi (µM) +/- (ZnO NPs - ZnO BPs) on Root length (cm) of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, *$P \leq 0.05$*), *a= (highly significant), b= (significant), c= (not significant).*

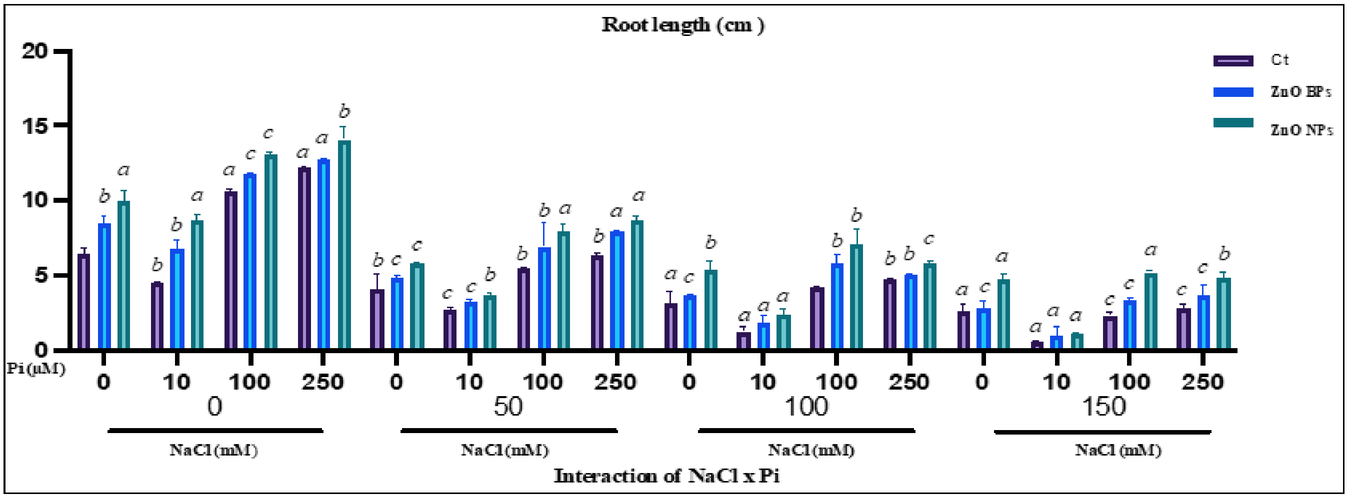

Figure 3: Effect of NaCl x Pi Interaction +/- ZnO NPs and ZnO BPs on Root length (cm) of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, P ≤ 0.05), *a= (highly significant), b= (significant), c= (not significant)*

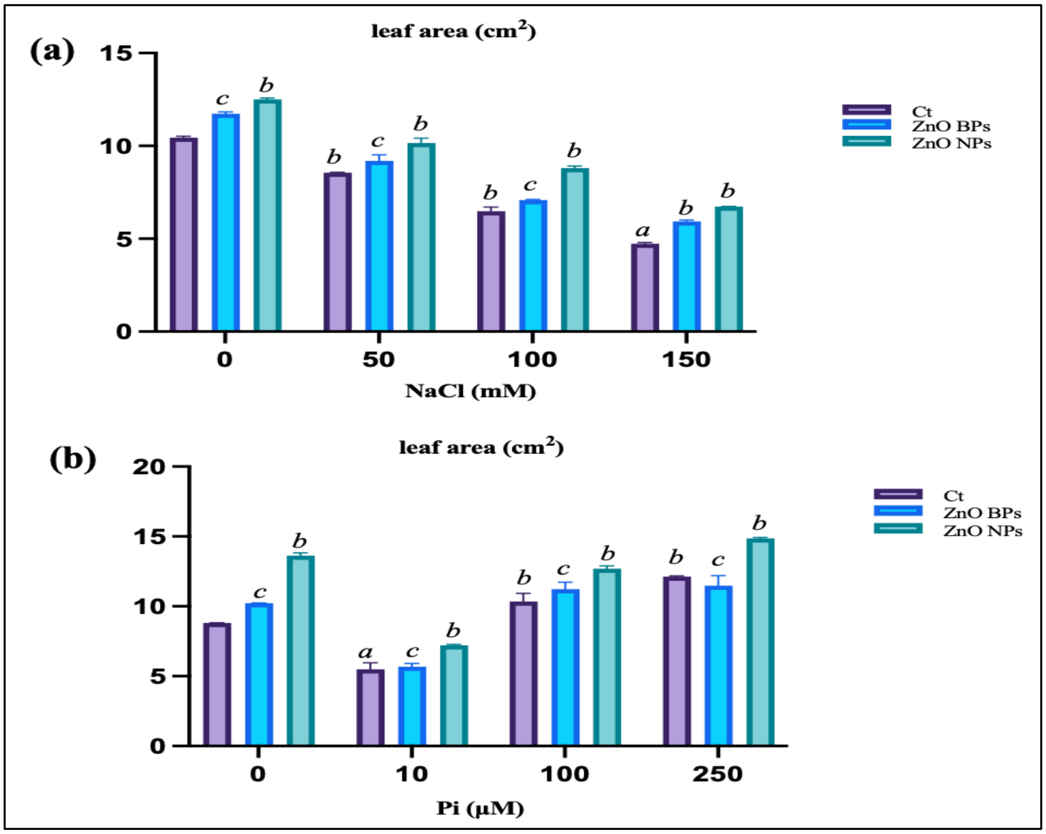

Figure 4: Effect of different concentrations of (a) NaCl (mM) and (b) Pi (µM) +/- (ZnO NPs - ZnO BPs) on Leaf area (cm^2) of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, *P ≤ 0.05*)), *a= (highly significant), b= (significant), c= (not significant)*.

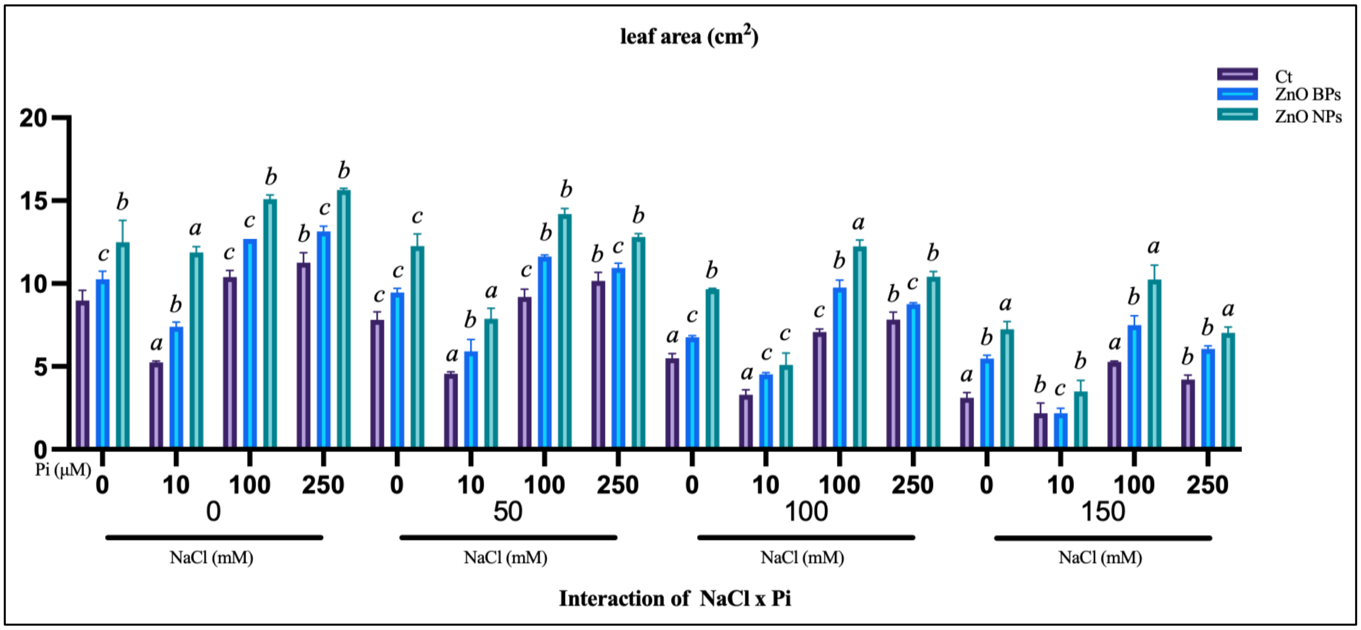

Figure 5: Effect of NaCl x Pi Interaction +/- ZnO NPs and ZnO BPs on Leaf area (cm^2) of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, $P \leq 0.05$)), *a= (highly significant), b= (significant), c= (not significant)*.

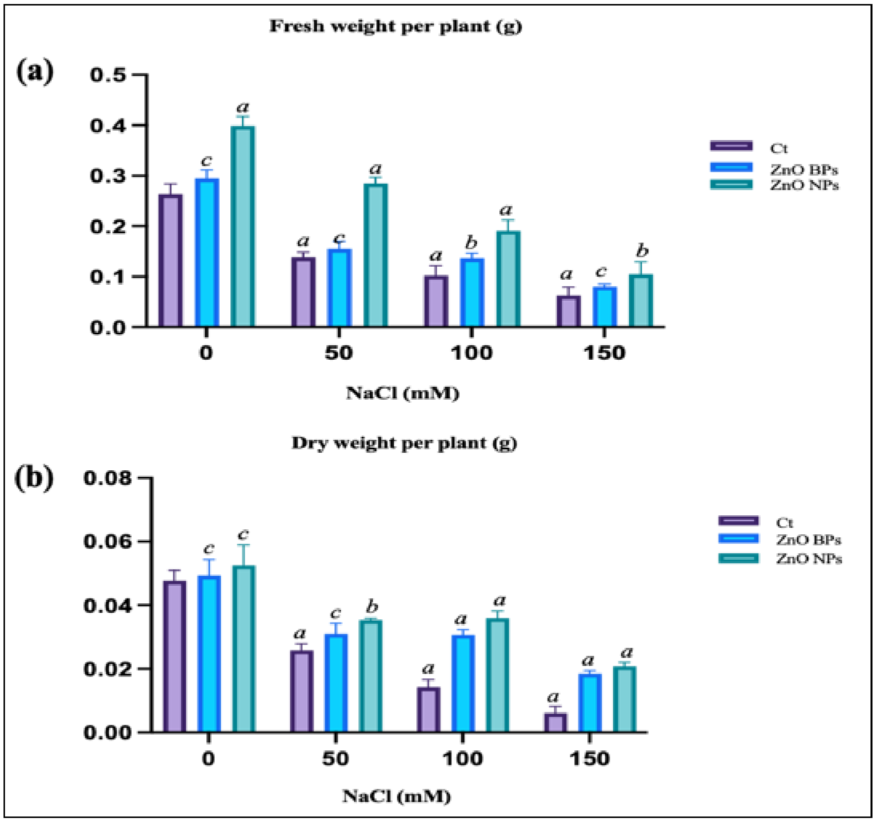

Figure 6: Effect of different concentrations of NaCl (mM) +/- (ZnO NPs) and (ZnO BPs) on (a) Fresh weight (g) and (b) Dry weight of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, $P \leq 0.05$), *a= (highly significant), b= (significant), c= (not significant)*.

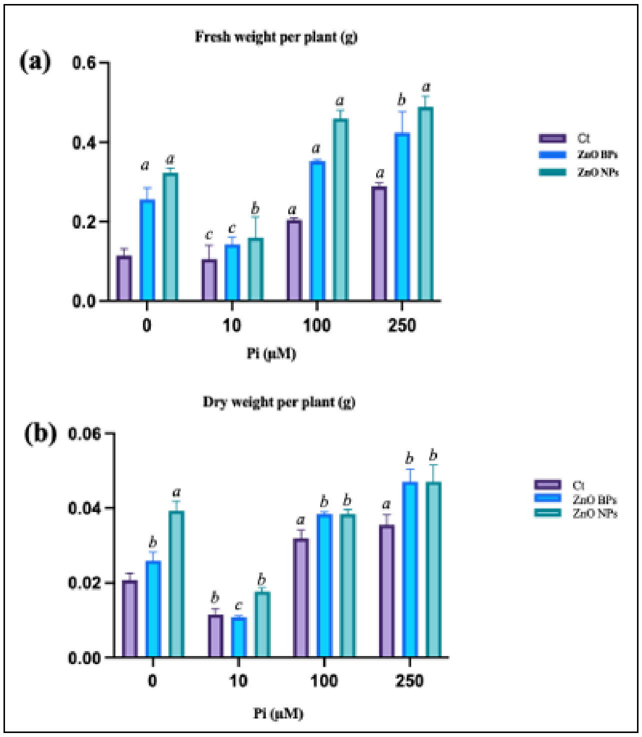

Figure 7: Effect of different concentrations of Pi (µM +/- (ZnO NPs) and (ZnO BPs) on (a) Fresh weight (g) and (b) Dry weight of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, $P \leq 0.05$), *a= (highly significant), b= (significant), c= (not significant)*.

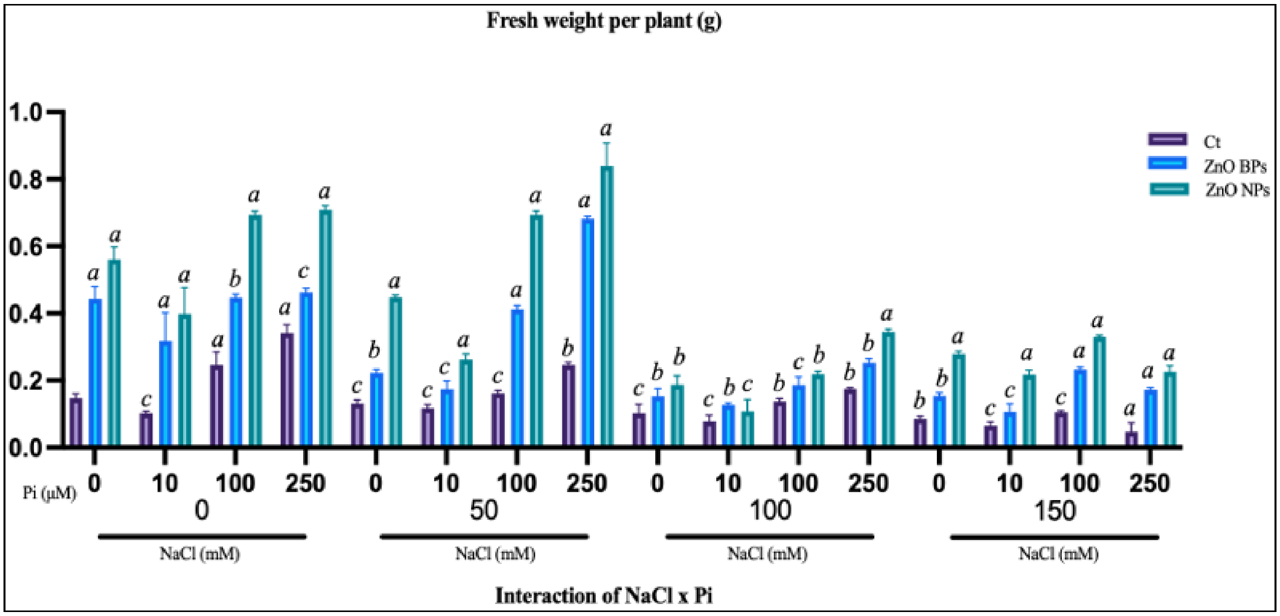

Figure 8: Effect of NaCl x Pi Interaction +/- ZnO NPs and ZnO BPs on Fresh weight (g) of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, $P \leq 0.05$), *a= (highly significant), b= (significant), c= (not significant)*.

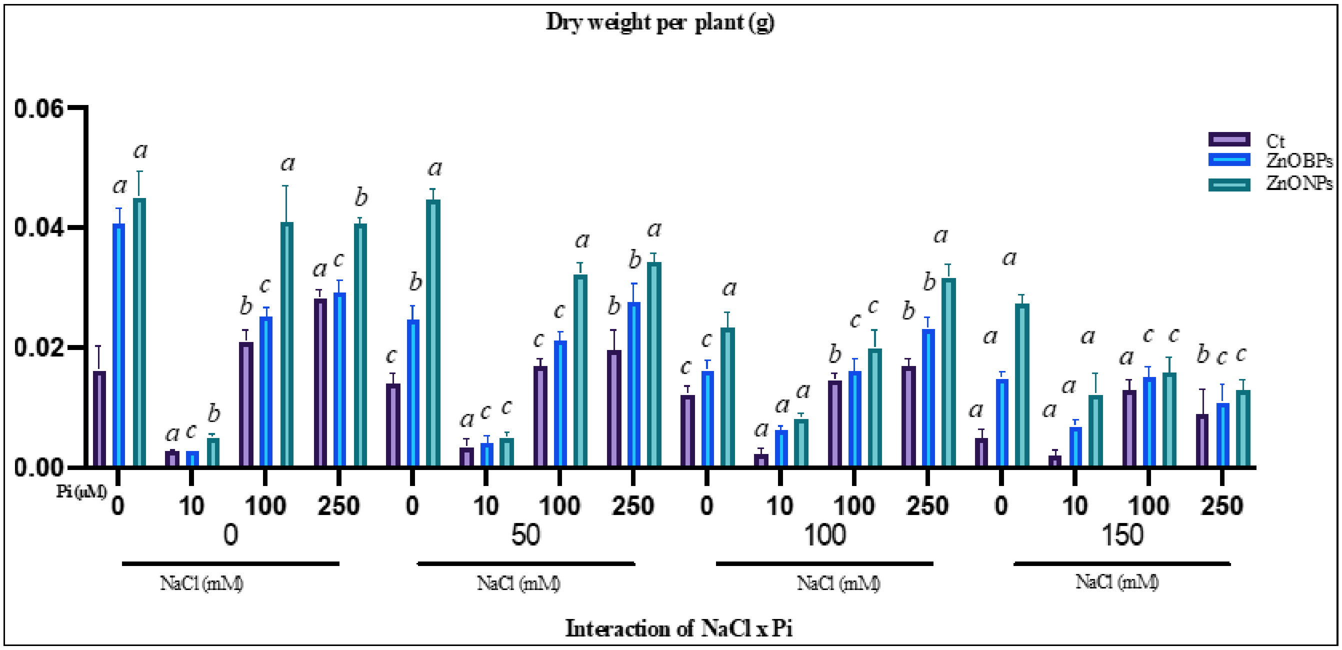

Figure 9: Effect of NaCl x Pi Interaction +/- ZnO NPs and ZnO BPs on Dry weight (g) of *Arabidopsis thaliana* (Col) plants grown in Gibeaut's solution in hydroponic system (means of 3 replicates ± SE, P ≤ 0.05), *a= (highly significant), b= (significant), c= (not significant)*

4. Discussion

Morphological traits in plants include lengths of root and shoot, weights of shoot and root and leaf area which are indicators of plant health (Rizwan *et al.*, 2017). The findings of this study indicated that different concentrations of NaCl (50, 100, and 150 mM) inhibited the development of *Arabidopsis thaliana* plants in hydroponic culture in terms of the parameters of root lengths, fresh and dry weight per plant, and leaf area. The current results aligns with some previous studies such as (Mahmoud *et al.*,2020) on barley, (Panwar *et al.*, 2016) on chickpea, (Lai *et al.*, 2014) on alfalfa and (Abd El-Mageed *et al.*, 2020) on hot pepper. Moreover, the application of different saline water concentrations (0, 1000, 2000, and 3000 ppm) resulted in a decrease in dry weight and plant height of the herb, and root in sweet basil (Ibrahim *et al.*, 2019). In accordance with García-Caparrós and Lao, (2018), a decline in plant height and number of leaves result in a decrease in fresh or dry weight. A reduction in the total leaf area is one of the most common reactions to salt stress. As reported by Munns and Tester, (2008), changes in both cell wall properties and a decrease in photosynthetic rate are all reasons for a reduction in leaf area.

In our study, *Arabidopsis* plants cultivated hydroponically under the low Pi (10 µM) condition failed to show much growth. However, it was noticed that the plants treated with high Pi (100 and 250 µM) had a larger proportion of biomass in the shoot and roots. This reaction has been documented for many plants with Pi deficit as a method of overcoming the depleted zone and to obtain more P (YAO *et al.*,2007). From the cellular to the whole plant level, Pi is a crucial component influencing the growth of plants. As stated by Assuero *et al.* (2004), Pi is essential for cell growth and division. It discovered that P-deficient leaf cells are smaller than P-sufficient leaf cells. Because certain plants utilise Pi more effectively than others, the amount that different plants needs for growth and development vary (Vickers, 2017 ; Reich, *et al.*, 2009). Similar statistics for several species have been published by GUO *et al*, (2012). Also, Yoneyama *et al.* (2012) reported that low levels of Pi considerably reduced the dry weight of several crops. Likewise, barley (Nadira *et al.*, 2014), Chinese fir (Xianhua *et al.*, 2018), strawberry (Valentinuzzi *et al.*, 2015), Stylosanthes (Luo *et al.*, 2020), maize (Li *et al.*, 2012), and tomato (Schausberger, 2018) all had decreased growth in their shoots due to a Pi deficiency.

Plants are frequently forced to choose between two stressors when they are simultaneously subjected to salt and deficiency in nutrients, and their growth and development are governed by the most limiting factor of growth (Van der Ploeg, 1999). The results of our study support this argument since growth of *Arabidopsis thaliana* was significantly reduced under high salinity compared to 10 µM of Pi deficiency, indicating that salinity stress affects *Arabidopsis* growth more than Pi shortage. Our findings are corresponding with earlier research in *Hordeum vulgare* (Talbi Zribi *et al.*, 2011; Zribi *et al.*,2015) and in *Lycopersicon esculentum* (Mohammad *et al.*,1998). However, our findings do not coincide with those reported in previous published research by Rogers *et al.*, (2003) which revealed that organs of shoots are the more negatively impacted by deficiency of nutrient than salinity stress. On other side, our findings indicated that the growth parameters values of *Arabidopsis* plant was higher when it was treated with high Pi (100 µM) at all NaCl concentrations, these results are in line with those of Kaya *et al.* (2003), who found that adding additional Pi to soil at a concentration of 272 mg might partially alleviate the detrimental effects of excessive salinity on pepper and cucumber. According to Erdal *et al.* (2011), the decreased in fresh and dry weight, leaf area and root length at high NaCl concentration may be the result of changes in plant metabolism in reaction to salt. Consequently, numerous researchers (Kaya *et al.*, 2001) ; Gulmezoglu and Daghan, 2017) have reported on the impact of Pi treatment in reducing the negative effects of salinity on plants. Aslam *et al.* (1996) stated that Pi had positive impact on rice development when salt is present in the media. Likewise, Kaya *et al.* (2001) insured that Pi treatment reduced the detrimental impacts of salinity stress on growth of plants. These researchers |observed that the growth of rice at (50 mM NaCl) increased by increasing exogenous P, level up to 100 (mM). Application of 18 kg of P to pots with saline soil also led to a notable increase in paddy production (Singh, 1988). Any further addition of P, led to a decrease in growth plant (paddy and straw). In the findings of Roberts *et al*, (1984), this is a consequence of the build-up of toxic amounts of P in plant tissues, which is considered to be an evidence of P toxicity caused by salt. These outcomes partly support the conclusions we reached in our investigation. In contrast, as stated by Abbas *et al*, (2018), that low P (10 M) is more damaging to shoot and root development than 100 mM NaCl (medium salinity). This conclusion validates the results we obtained from treating our plants under the same conditions. According to (Singh *et al.*, 2018), plants with P amendments in greenhouse circumstances exhibited better growth metrics than plants under salt stress without P fertilization. Therefore, it is necessary to increase the availability of Pi to plants under salinity conditions for osmotic adjustment and growth. Sadji-Ait Kaci *et al*, (2017) indicated that applying Pi to salinized soil may improve the field's situation by restoring ionic and osmotic balance in the rhizosphere. Also, they stated that applying P to salinized soil may improve the field's situation by restoring ionic and osmotic balance in the rhizosphere, which may have lessened and/or inhibited the salinity's severe impacts.

In our findings, the treatment of ZnO NPs and ZnO BPs under salt stress enhanced the measured growth parameters in Arabidopsis plants. However, application of ZnO NPs performed more effective than ZnO BPs which are consistent with conclusion of study caried out by Torabian *et al*, (2016). They noticed that the use of ZnO NPs boosted the leaf area and shoot dry weight in salt-stressed sunflower plants and produced greater results than the normal ZnO application approach. Also, our findings concur with those reported in other studies that have been published, including those on strawberries (Luksiene *et al.*, 2020), and maize (Shinde *et al.*, 2020). As previously stated by Sturikova *et al.* (2018)[87], ZnO NPs may be able to reduce the harmful impacts of abiotic stress on development and growth of plants.

This increased effect of application is attributed to the essential function of zinc in biological and metabolic processes, including enzyme activity, nitrogen metabolism, photosynthetic pigments and accumulation of phospholipids (Mehrabani, *et al*., 2018). Regarding interaction between Pi and ZnONPs, our findings are at contrast with those of Dimkpa *et al.* (2012); Stewart *et al.* (2015), and Watson *et al.* (2015), who reported that *T. aestivum's* growth decreased when it was treated with Pi and exposed to ZnONPs. Furthermore, studies by Zafar *et al.* (2016) and Mukherjee *et al.* (2016) demonstrated similar growth inhibition in *Pisum sativum* and Brassica species treated to ZnONPs. However, in accordance with the combination of P and Zn caused a significant increase in the dry matter of chia due to the treatment of P at 100 mg kg $^{-1}$ soil coupled with Zn at 10 mg kg $^{-1}$ soil, which is consistent with our findings. Similar findings indicated that when Zn and P were applied together rather than separately, growth and yield characteristics in maize genotypes were enhanced (Imran *et al.*, 2016). These results are consistent with the findings of Ova *et al.* (2015), who demonstrated that P and Zn had beneficial interactions in wheat yield. They also demonstrated that Zn application did not increase dry matter at low P levels, but plants generated greater yield when exposed to increased Zn at high P levels. Similar findings were found in other crops as well, including maize (Amanullah *et al.*, 2016), rice (Amanullah and Inamullah, 2016), bean (Gianquinto *et al.*, 2000) and cowpea (Kumar *et al.*, 2016), showing that combined Zn and Pi enhanced dry biomass production.

5. Conclusion

Proper amount of Pi improved the salt tolerance of *Arabidopsis* plants by enhancing exclusion of Na. Also, the results showed that in the combined treatment of Pi and NaCl was more effective than in the single treatment. ZnO-NPs application in low doses (10 mg/L) is an eco-friendly, cheap cost, and can be considered as a promising application to alleviate the harmful impacts of salt and stress of mineral on plants. In sum, our result provides new alternative strategies for increasing crop productivity which are hydroponic growth allowing for more effective mineral absorption, interaction NaCl x Pi and Pi treatments as the biochemical fertilizers and ZnO NPs as nanofertilizers, all can be considered as powerful tools to boost plant growth under abiotic stress. This study is promising to open a new path for nanotechnology particularly in Saudi Arabian context as per the kingdom's vision 2030 and Saudi green initiative.

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