

Effect of Nano Chitosan on Growth, Physiological and Biochemical Parameters of *Phaseolus vulgaris* under Salt Stress

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ABSTRACT

Salt stress is one of the abiotic stresses; it is a major factor which reduces crop productivity. Nano particles have become a pioneer material in agriculture research nowadays because they have unique physicochemical properties. This study aimed to investigate the effects of chitosan nanoparticles on germination and seedling growth parameters under salt stress condition for bean plant (*Phaseolus vulgaris* L). Moreover the effect of nano chitosan combined with selected concentration of salinity (100mM NaCl) on germination and vegetative growth was also examined. Different concentrations of salinity (50 mM, 100 mM and 150 mM NaCl) was used. Data indicated that salinity decreased salt tolerance index S.T.I of germination percentage as well as seedling growth. The bad effect was higher in 150 mM concentration; so the second experiment was continued with the 100 mM NaCl concentration. The treatment of bean plant with 100 mM of salt combined with different concentration of nano chitosan (0.1%, 0.2% and 0.3%) indicated that nano chitosan in all concentrations significantly promoted seed germination and radical length under salt stress. The best treatment on germination was 0.3 % of nano chitosan. Growth variables (plant height, leaf area, fresh and dry weights of the shoot and root) were increased significantly. High significant increase in both M.S.I and Chl.a, similarly, catalase, proline, RWC, Chl.b, carotenoids and antioxidant enzymes showed positive effect at 0.1% concentration.

Keywords: Nano chitosan, antioxidant enzymes, *Phaseolus vulgaris*, physiological traits, germination.

INTRODUCTION

Salinity is environmental stress which limits the growth and productivity of plant. It has been estimated that more than 20% of all cultivated lands around the world contain salt levels high enough to cause salt stress to crop plants (Moud and Maghsoudi, 2008). One of the major abiotic stresses is salinity which affects crop production especially in arid and semi-arid regions. Osmotic and ionic stresses are two physiological mechanisms which salinity affect in plant (Murphy and Durako, 2003). Salinity reduces the ability of plants to utilize water and causes changes in plant metabolic processes and it causes reduction in growth rate (Munns, 2002). Increasing salinity significantly reduced seed germination, plant height and shoot dry weight in cowpea plant (Amador *et al.*, 2006) also reduction in length and weight of root due to salinity (Ibrahim *et al.*, 2007). Plant height, leaves area and dry weight of root, stem and leaves decreased as the salt concentration increased. This means that there is a negative relationship between salt stress degree and plant growth characters (Hussein and Abou-Baker, 2014). High salt contents can affect various major plant processes like protein synthesis, photosynthesis and also energy and lipid metabolisms (Li *et al.*, 2008).

Chitosan extracted from natural chitin which is the structural component in the exoskeleton of crustaceans by N-deacetylation, it is containing D-glucosamine and N-acetyl-D-glucosamine molecules in a linear polymer form linked by β -1,4-glycosidic bond, it has been used in agriculture as plant growth promoter (Katiyar *et al.*, 2014). Chitosan increased root and shoot length, shoot dry weight also relative water content under salinity stress (Mahdavi and Rahimi, 2013). Also, Ma *et al.* (2011) reported that wheat seeds which treated with chitosan showed higher growth than control under salinity stress. Chitosan have many special properties through amine and -OH groups which making it applicable in many areas and easily available for

chemical reactions. Chitosan is non-toxic material which interacts with poly anions to form complexes and gels (Se and Niranjana, 2005).

Nanotechnology is a promising technology in many fields including agriculture (Dimetry and Hany, 2016). Also, it changes material at the nuclear, atomic or macromolecular level to make objects on the nanometre scale with novel properties in view of their little size, (Leiderer and Dekorsy, 2008). Using different levels of nano-silver has significant effect on the height of thyme plants (Aghajani, *et al.* 2013). Numerous studies have demonstrated that TiO₂ nanoparticles promoted photosynthesis and nitrogen metabolism and thus greatly improved growth of spinach (Hong, *et al.* 2005). Silica nanoparticles had significant increases on *Zea mays* growth (Javad *et al.*, 2014) nanomaterials are leading to significant improvement in plant through enhancing the growth and hence dry weight, leaf area and growth rate (Hasaneen *et al.*, 2016). Eshghi *et al.* (2014) studied the physicochemical and bioactive components of strawberry when coated with nano chitosan. Heba *et al.*, (2015) reported that direct exposure of wheat plants to specific type of nanoparticles caused significant increase in all growth parameters determined at optimum concentrations of nano solution.

One of the most favorable legumes valued for its nutritional value is *Phaseolus vulgaris* L. (Ogbonnaya, *et al.*, 2003). It is a warm season annual legume crop which is cultivated primarily for its protein and energy-rich dry seeds. Addition to this crop have the ability to maintain soil fertility through its excellent capacity to fix atmospheric nitrogen, so bean (*Phaseolus vulgaris*) does not require very fertile land for growth (Lobato *et al.*, 2006). *Phaseolus vulgaris* is classified as a salt-sensitive plant (Maas and Hoffman, 1977).

The present investigative aimed to study the effect of nanoparticles of chitosan on reducing the negative effects of salinity on bean plant.

MATERIALS AND METHODS

The experiments were carried out in the period elapsed from June, 2015 to March 2016, at the laboratory of Plant physiology, depart. of Agric. Botany, Fac. of Agric., Al-Azhar Univ., Cairo, Egypt, and department of Biological and Environmental Sciences, Fac. of Home Economics, Al-Azhar Univ. Seeds of bean (*Phaseolus vulgaris* L); were obtained from Food Legumes Research Section, Sakha Agric. Research Station (SARS), Kafr El-Sheikh, Egypt. Chitosan was purchased from Cornell Lap Company. Chitosan (C₆H₁₁NO₄)_n molecular weight :100.000 - 300.000.

1- Preparation of nano chitosan

For preparing nano chitosan particles, the method of Hu, *et al.* (2002) was used with some modifications; 0.1% (w/v) chitosan dissolved in 1%

(v/v) acetic acid solution and left under magnetic stirring for 30 minutes. Twenty ml of an aqueous tripolyphosphate (TPP) solution (0.25% w/v) were added to 100 ml of chitosan solution under magnetic stirring. Nanoparticles were collected by centrifugation at 6000 rpm for 30 min. at 4°C. The pellets were taken and freeze-dried before further use or analysis. Particle size distribution and the zeta potential of chitosan nanoparticles were determined using Zetasizer Nano-ZS90 (Malvern Instruments Co., UK). The analysis was performed at a scattering angle of 90° at a temperature of 24.9°C using samples diluted to different intensity concentration with de-ionized distilled water. The size distribution profile, as shown in Figure 1, represents a typical batch of nanoparticles with a mean diameter of 46.32 nm and a narrow size distribution (polydispersity index <1).

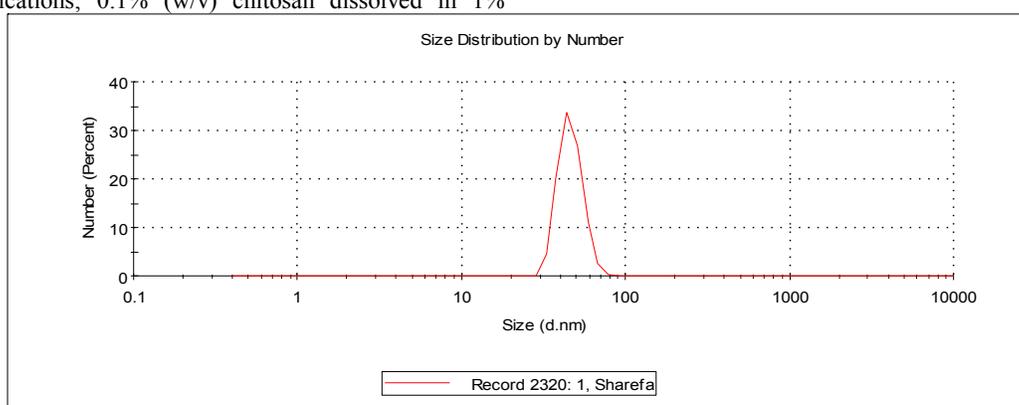


Fig 1. The size distribution profile for nano chitosan particles by using the Zetasizer analysis

2-Salinity experiments:

Germination: Bean seeds were tested with four levels of NaCl salt stress (0, 50, 100 and 150 mM NaCl) in the germination stage. Seeds were planted in black plastic bags filled with washed sand on the half strength of Hoagland's nutrient solution with different treatments of salt. Sub-irrigation was used. They were put in trays with nutrient solution. The pH of the standard nutrient solution was adjusted to (5.8 - 6.0) before adding salt and renewed weekly with continuously aerated, each treatment was replicated thrice (20 seeds per replicate). After 48 and 96 hours the germination percentage and radical length were calculated. The shoot length, fresh and dry weight of roots and shoots were taken after 7 days.

Vegetative stage:

Phaseolus vulgaris seedlings were treated with the four concentrations of salt stress (0, 50, 100 and 150 mM NaCl) for 10 days. Seedlings were harvested and the data was recorded for growth and physiological traits.

3.Nano chitosan combined with salt NaCl experiments:

In this experiment seeds of bean were soaked for 3 hours in different concentrations of nano chitosan (0.1%, 0.2% and 0.3% dissolved in 0.1% HCl) combined with salt stress at one concentration (100 mM NaCl). The seeds were sowed in black plastic bags filled with washed sand (six seeds per bag) and were put in

trays with the half strength of Hoagland's nutrient solution.

Germination %:

The germination percentage and the radical length were taken after 24, 48 and 72 h.

Vegetative:

After 10 days of planting, the seeds treated with 100 mM NaCl and plants were harvested after 10 days from salt treatment. The data were recorded as the growth and physiological traits.

4. Growth traits:

The growth traits which were recorded on five seedlings from each treatment were: plant height, leaf area, root fresh weight (g), root dry weight (g), shoot fresh weight (g) and shoot dry weight (g). Salt tolerance trait index (STTI) was calculated (Ali, *et al.* 2007) using the equation:

$$STTI = \frac{(\text{Value of trait under stress condition})}{(\text{Value of trait under control condition})} \times 100$$

5. Physiological and biochemical traits:

To test the plant tolerance to salinity stress, the physiological and biochemical traits were recorded on five fresh seedlings from each treatment.

Chlorophyll and carotenoids content:

Chlorophylls were estimated by the spectrophotometric method according to Lichtenthaler and Wellburn (1985), chlorophyll was expressed as µg/ml methanol.

Membrane stability index (MSI).

Electrical conductivity (EC) was used to determine MSI for leaf of *Phaseolus vulgaris* according to Sairam *et al.*, 2002.

Relative water content (RWC):-

The relative water content was done by the method of Weatherley, 1950 according to the equation;

$RWC = (\text{fresh weight} - \text{dry weight}) / (\text{turgence weight} - \text{dry weight}) \times 100$.

Antioxidants enzymes activity assay:

Assay of Catalase activity (CAT).

The absorbance was recorded on spectrophotometer (Jenway 6305 UV/Visible) at 240 nm for 60s. The enzyme action was accounted by calculating the quantity of decomposed H₂O₂ according to the method of (Aebi, 1984).

Assay of peroxidase activity (POD):

POD activity was measured at 420 nm by the method of Chance and Maehly (1955).

Assay of polyphenol oxidase activity (PPO):

The determination of PPO activity was done according to (Duckworth and Coleman, 1970) at 420 nm and 25°C.

6 - Assay of proline:

Proline content was determined spectrophotometrically at 520 nm. Proline concentration expressed as mg/1g fresh weight (Bates, 1973).

7- Data Analysis

Obtained data were expressed as means \pm standard deviation (SD). The data were subjected to One-Way Analysis of Variance; statistical package for social sciences (SPSS) software for windows version 20, followed by Duncan test to compare the significance of differences between means. The results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of salt stress on seed germination:

Table 1 shows that germination of seeds after 48 h and 96 h was significantly decreased with increasing salt stress and there was significant differences ($P > 5\%$) between treatments compared with control. The emergence of radical delayed with increasing salt stress compared to control. Moreover, the results indicated that increasing salt concentration caused decrease in STI values of radical length which were 63.59 % and 33.08% at salinity concentration of 150 mM NaCl compared with lower concentration (50 mM) which showed 86.58% and 74.64 % after 48 and 96 h respectively. Similarity, shoot length was decreased after 96 h, and showed a decrease at the highest concentration (150 mM) which recorded 17.85% compared with the lower concentration (69.53%). These results are in agreement with many researchers who refer to the bad effects of salinity on the germination of seeds and consequently the vigor of seedling. The reduction in seed germination may be due to an inhibition in water absorption (Munns, 2002). Rahman *et al.*, (2008) found that the rate and percentage of germination were significantly reduced by salinity, which in turn may lead to uneven stand establishment.

Also the bad effects of high salinity may be due to ion toxicity on seed germination, as a consequence of a coincident increase in cation and anion (Panuccio *et al.*, 2014).

The vigor of seedlings was significantly decreased with increasing salt stress, the increase in salinity caused a gradual decrease in STI values of shoot length after seven days and were recorded the lowest ratio in 150 mM NaCl which was 5.87 % compared with 50 mM 37.31%. Similar results were recorded by Cokkizgin (2012) who reported significant reduction in seed germination and plant height with increasing salinity in cowpea. Ibrahim *et al.* (2007) concluded that length and weight of root were reduced due to salinity. The same Table (Table 1) shows that fresh and dry weight was also decreased as they recorded 96.50% in salinity 50 mM while it was 12.33% in salinity 150 mM in fresh weight. In relation to dry weight of root the results showed the lowest percentage in the concentration of 150 mM (19.25%) compared with 50 mM (85.00 %). These inhibition effects may be due to an external osmotic potential that prevents uptake of water or due to the toxic effects of Cl⁻ and Na⁺ ions or both on the germinating seed (Murillo-Amador *et al.*, 2002). On the other hand it was found that, there was a significant decrease in shoot length and fresh weight. Whereas an increase in dry weight (216.11%) with increased salinity (150 mM) compared with 50 mM (133.45%). This may be due to the inhabiting effect of salt stress on food stored in the cotyledons and therefore does not weighting less. So added an increase in shoot weight with increasing salt concentration. Therefore the shoot was more affected than the root and a clear inhibition of the shoots growth was showed with 150 mM NaCl salinity concentration. Similar results were found with Zeng *et al.* (2002) who reported that under imposed stress, shoot growth was inhibited more than root growth. Salinity affected shoot growth due to the inhibitory effect of salt on cell division also enlargement in the growing point (McCue and Hanson, 1990). Salinity had adverse effects on plant height, number of leaves, root length and shoot/root ratio in several legumes, such as faba bean (Zahran and Sprent, 1986) soybean, and bean (*Phaseolus vulgaris*) (Wignarajah, 1992). Also results were in agreement with (Parida and Das, 2005) who reported that salinity can reduce fresh and dry weight, seed germination and seedling growth.

Effect of salinity on vegetative growth:

There was highly significant decreases in fresh and dry weight of shoot and root due to salinity regarding shoot growth and dry matters. Increasing concentrations of NaCl lead to significantly decrease in all growth measurements studied (Table 2) as indicated by STI values for leaf area, shoot fresh and dry weight and root fresh and dry weight compared with control. The decreasing percentage on plant height recorded 87.21% in 50 mM NaCl and reached to 59.27% in 150 mM NaCl. In relation to leaf area, it was reduced with increasing NaCl concentrations and recorded 84.31% reached maximum reduction in 150 mM (54.33%), Table 2.

Reduction in fresh and dry weight recorded 46.19% and 67.27% respectively. Also fresh and dry weight of root was with increasing salinity levels reached to 87.53% and 86.93% in 150 mM in fresh and dry weight respectively. These results are in agreement with Ibrahim *et al.*, (2007) who reported significant reduction in shoot dry weight, plant height and weight of root when salinity increased. Mohsen *et al.* (2014) found that fresh and dry weight of *Vicia faba* have been decreased with salt treatments. According to Cha-um *et al.* (2009) the fresh and dry weights of shoot and root and the shoot height as well as leaf area were significantly decreased. Hussein and Abou-Baker (2014) found negative relationship between plant growth characters and salt stress degree. The inhibitory

effect of salinity may be due to its effects on cell division, also enlargement in the growing point (Mccue and Hanson, 1990). These results in accordance with those of Khodarahmpour *et al.* 2012, who showed that the shoot was more affected by salinity than the root. Furthermore, Albacete *et al.* (2008) attributed the effects of salinity to the hormonal equilibrium (cytokinins/auxins) which in turn causes trouble in the growth of shoot and changes in biomass partitioning. The decrease in dry biomass maybe caused by the increase of Cl ion concentration in the tissue of plant (Tavakkoli *et al.* 2011). Decrease in leaf area could be explained by the bad effect of salt on photosynthesis that leads to the reduction of leaf and plant growth (Abdul Qados, 2011).

Table 1. % inhibition of germination, radical and shoot length, fresh and dry weight of shoot and root of *Phaseolus vulgaris* after 48h, 96 h and 7 days as affected by salinity.

| Treatments | Germination % (48 h) | Germination % (96h) | Radical length % (48h) | Radical length % (96h) | Shoot length % (96h) | Shoot Length % (7 days) | Shoot fresh weight % (7 days) | Shoot dry weight % (7 days) | Root fresh weight % (7 days) | Root dry weight % (7 days) |
|------------|--------------------------|-------------------------|---------------------------|-------------------------|--------------------------|-------------------------|-------------------------------|-----------------------------|------------------------------|----------------------------|
| 50Mm | 97.12±12.04 ^a | 92.80±0.84 ^a | 86.58±9.04 ^{ab} | 74.64±8.83 ^a | 69.53±22.99 ^a | 37.31±7.36 ^a | 74.12±5.04 ^a | 133.45±9.73 ^b | 96.50±15.73 ^a | 85.00±6.00 ^a |
| 100Mm | 80.50±0.58 ^b | 90.04±1.82 ^b | 73.09±11.01 ^{bc} | 63.06±8.48 ^a | 37.43±2.64 ^b | 13.19±0.63 ^b | 42.34±3.02 ^b | 197.77±21.19 ^a | 32.08±1.41 ^b | 45.18±8.98 ^b |
| 150mM | 69.80±7.05 ^b | 77.07±1.41 ^c | 63.59±11.66 ^c | 33.08±2.73 ^b | 17.85±6.80 ^b | 5.87±1.25 ^c | 35.99±5.55 ^b | 216.11±19.77 ^a | 12.33±5.39 ^c | 19.25±9.70 ^c |
| Sig | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Table 2. % inhibition of S.T.I growth parameters of *Phaseolus vulgaris* after vegetative stage as affected by salinity

| Treatments | Plant height % | leaf area % | Shoot fresh weight % | shoot dry weight % | root fresh weight % | Root dry weight % | TSTI % |
|-------------|-------------------------|--------------------------|--------------------------|-------------------------|-------------------------|-------------------------|--------|
| 50 mM NaCl | 87.21±6.58 ^a | 84.31±12.30 ^a | 84.63 ±3.43 ^a | 88.70±1.40 ^a | 87.53±.76 ^a | 86.93±.98 ^a | 86.55 |
| 100 mM NaCl | 70.54±7.51 ^b | 69.84±5.14 ^{ab} | 71.50 ±3.59 ^b | 82.28±1.81 ^b | 63.51±1.32 ^b | 82.10±0.93 ^b | 73.29 |
| 150 mM NaCl | 59.27±1.58 | 54.33±2.13 ^b | 46.19±1.0 ^c | 67.27±1.88 ^c | 56.29±3.59 ^c | 72.48±.98 ^c | 59.30 |
| | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | |

Effect of salinity on physiological and biochemical traits :

Results in Table (3- a) show a significant decrease in S.T.I. values for relative water content (RWC) in all different concentration of NaCl when compared with control. It recorded the lowest decline in 150 mM concentration where it reached 84.93% compared to control. Similar decrease in RWC was found in the leaves of various plants which were affected by the salinity (Wróbel and Mikiciuk, 2010). This reduction may be associated with a decrease in plant vigor (Halder and Burrage, 2003). Results in the same Table revealed that salinity had a significant effect on membrane stability index (MSI), compare with the control. It was recorded in 50mM treatment 95.56% while the maximum decrease of MSI was recorded in 150mM treatment which was 81.46%. These results are in agreement with Katsuhara *et al.*, (2005) who reported that under salt stress conditions, peroxidation of membrane lipids is an indication of membrane leakage and damage. Jain *et al.* (2001) suggested that decrease in membrane stability reflects the extent of lipid peroxidation caused by reactive oxygen species. Mittler,

(2002) indicated that salt stress induced reactive oxygen species (ROS) which in turn caused membrane damage in plants

Photosynthesis is the most important process affected in plants under saline conditions. Results in the present study show an increase in chlorophyll (a , b) and carotenoids contents of leaves in response to salt stress. The maximum increase in chlorophyll a , b and carotenoid was recorded in 150mM treatment which were 183.50%, 216.47% and 195.16% respectively. The results are in agreement with Wang *et al.* (2013) who reported that under mild saline-alkali stress, the content of chlorophyll in cultivars are higher than control. Also Wang and Nil (2000) observed an increase in pigment content in *Amaranthus tricolor*. Similarity Beeflink *et al.* (1985) showed an increase in chlorophyll in onion under drought stress. The results disagree with Ahmad (2009) who concluded that chlorophyll a , b and total chlorophyll and carotenoid content decreased in response to salinity stress. Normal cellular activities such as photorespiration and oxidation of fatty acids are usually generated reactive oxygen species

(ROS), When plants were exposed to biotic or abiotic stress conditions the levels of ROS increased.

The antioxidant compounds such as PPO, POD and CAT eliminated ROS, Antioxidant capacity can prevent damages due to ROS formation which increased under salinity stress conditions (Harinasut *et al.*, 2003). Changes in activities of various antioxidant enzymes under salinity stress have been reported by Koskeroglu and levent (2008). Table (3- b) illustrates the stress tolerance index (STI) values for PPO activity. A significant increase with increasing salt concentration was detected with PPO activity in leaves which increase by 244.02%, 402.51% and 559.72% at 50, 100, and 150 mM NaCl respectively. POD activity was significantly enhanced when the plants were grown under salinity, POD activity in leaves increasing with increase salt concentration by 266.21 % and 300.00 % at 100 and 150 mM NaCl respectively. With regard to CAT activity, the results refer to decrease in low concentration of salt (50 mM) which was 94.11 and gradually increased with increasing salinity concentration. It recorded in concentration of 100 mM NaCl 111.76% and 168.62% in concentration 150 mM when compared with control. Again, there is an increase in TSTI with increasing salinity. These results agree with Weisany *et al.* (2012) who clarify the activity of catalase which increased in leaf tissues of soybean under salinity stress. Similarity the increase in antioxidant enzymes shown in Table (3-b). Agreement with Agarwal and Pandey (2004) who reported the role of these enzymes

which offer protection against oxidative damage also introduce adaptive mechanism to reduce H₂O₂. The combined activity of CAT and PPO are induced by accumulation of the ROS and H₂O₂ which in turn are induced by various environmental stresses (Nagesh and Devaraj,2008).

Proline accumulation occurs normally in cytosol in response to drought and salinity stress. There are a positive relationship between proline content and salt level (Mansour, 2000). The relationship between proline accumulation in the shoots of bean plants and salinity is shown in Table 3- b. The results indicated that proline content significantly increased with increasing salt concentration. The free proline content was significantly enhanced in the stressed plants over control plants. A more highly increase was observed in 150 mM NaCl treatment which was 358.11% in comparison with the other concentration. These results are in agreement with Akça and Samsunlu (2012) they stated that from the most commonly solutes that accumulate to high concentrations in certain species at saline condition are proline and sucrose. It was suggested that increased proteolysis or decreased protein synthesis are the reason for proline accumulation. Proline can participate to osmotic potential of leaf and thus to osmotic adjustment, so the higher concentration of proline under salt stress is favorable to plants. There are many functions for proline besides the role of osmolyte, it can confer enzyme protection and increase membrane stability under various condition (Nagesh and Devaraj, 2008).

Table 3 a and b. Percentage decrease of S.T.I of physiological and biochemical traits of *Phaseolus vulgaris* after vegetative stage as affected by salinity.

| Table 3a. physiological parameters | | | | | |
|---|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| Treatment | R.W.C% | M.S.I% | Chl a % | Chl b % | Caroteniods % |
| 50 mM Nacl | 94.59±.44 ^a | 5.56±1.22 ^a | 112.14±0.20 ^c | 132.55±0.52 ^c | 125.53±0.53 ^c |
| 100 mM Nacl | 91.13±2.77 ^b | 85.74±0.67 ^b | 122.34±0.31 ^b | 158.25±0.26 ^b | 129.06±0.08 ^b |
| 150 mM Nacl | 84.93±1.04 ^c | 81.46±1.04 ^c | 183.50±0.51 ^a | 216.47±0.54 ^a | 195.16±0.15 ^a |
| Sig | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |

| Table 3b. biochemical parameters | | | | | |
|---|----------------------------|----------------------------|--------------------------|----------------------------|---------------|
| Treatment | PPO % | POD % | CAT % | Proline % | TSTTI% |
| 50 mM Nacl | 244.02 ± 9.30 ^a | 179.72. ±8.19 ^a | 94.11±.000 ^a | 167.58 ± 2.84 ^a | 126.42 |
| 100 mM Nacl | 402.51 ±28.82 ^b | 266.21 ±3.09 ^b | 111.76±5.88 ^b | 343.65 ±1.84 ^b | 281.03 |
| 150 mM Nacl | 559.74 ±72.56 ^c | 300.00 ±4.05 ^c | 168.62±2.44 ^c | 358.11 ± 1.02 ^c | 346.61 |
| Sig | 0.00 | 0.00 | 0.00 | 0.00 | |

Effect of nano chitosan on S.T.I of germination percentage and growth measurements under salt stress condition:

Seed germination results indicated that nano chitosan in all its concentrations promoted seed germination and radical length under salt stress. The differences value of S.T.I for germination percentage and radical length were significant between treatments and also when compared with control (Table 4).

The highest percentage value of S.T.I for germination after 24, 48 and 72 h was achieved by using 0.3% concentration of nano chitosan. It was recorded 250%, 130.35% and 123.94% respectively. Also the highest values of S.T.I for radical length after 24, 48

and 72 h in 0.3% concentration and it was recorded 170.83%, 241.15 % and 231.96% respectively. These results agree with Haghghi *et al.* (2012) who found that nano augments increased seed germination in tomato under abiotic stress. Similarity Siddiqui *et al.* (2014) reported that nano SiO₂ enhanced seed germination and stimulated the antioxidant system under NaCl stress. Nano-particles ameliorate different defence mechanisms of plants against salt toxicity (Sabaghnia and Janmohammad, 2015). These results agree with Helaly *et al.* (2014) who reported that nano ZnO supplemented with MS media improving tolerance to biotic stress and induced proline synthesis activity of superoxide dismutase, catalase, and peroxidase. Very few report

about nano chitosan and application with salt in agriculture, this study may be consider the first one in this field.

Table 5 show that treatment of *phaseolus vulgaris* plants with nano chitosan under salt stress led to high significant increase in value of S.T.I for all growth variables (plant height, leaf area and fresh and dry weight of shoot and root). The results indicated significant increase in value of S.T.I for plant height compared to control. There is a significant increase in leaf area when it is treated with nano chitosan under 100 mM salt stress and it was recorded the maximum value at 0.3% of nano chitosan which recorded (187.44%). Moreover, there is a significant increase in value of S.T.I for fresh and dry weight of shoot and root with increasing nano concentration, It recorded the maximum value in the nano treatment at 0.3% in fresh and dry weight of shoot and root which recorded 151.85% , 142.02% , 161.69% and 185.05% respectively. These results agree with Kalteh *et al.* (2014) and Siddiqui *et al.* (2014) as they reported that under salinity stress, nano particles improved leaf fresh and dry weight which improving the tolerance of plants to abiotic stress. Also, Jaberzadeh *et al.* (2013) reported that TiO₂ nanoparticles augmented wheat growth and yielded components under water deficit stress condition. Almutairi (2016) found that germination rate, root length and seedling fresh and dry weight of tomato were improved after exposure to Ag nanoparticles under NaCl stress. The good effects of nanoparticles may be due to decrease of Na⁺ ion toxicity which reduce the crop growth and yield. So nanoparticles are leading to enhance crop growth and yield and help crop improvement under adverse conditions by reducing Na⁺ ion absorption by plant tissues (Haghighi *et al.*, 2012). The results in the study also agree with Sabaghnia and Janmohammad (2015) who reported that SiO₂ nanoparticles ameliorate different defence mechanisms of plants against salt toxicity.

Effect of nano chitosan on S.T. I for physiological and biochemical traits under salt stress condition:

Application of nanofertilizers are among the most promising method which can potentially enhance plant resource use efficiency and reduce environmental toxicity due to accumulation of unused chemical fertilizers and pesticides in the soil. The results in Table 6 indicated significant increase in value of S.T.I for physiological and biochemical traits studied. The treatment of 0.3% nano chitosan concentration with salt had the highest percentage in the following traits;

relative water index (131.42%), percentage of Chl. b (154.27%), percentage of carotenoid (129.67%), PPO (224.83%) and POD (218.94%). Meantime, treatment of 0.2% nano chitosan with salt had the highest percentage in membrane stability index (136.54%) and percentage of Chl. a (121.51%). In relation to CAT the highest percentage was recorded in treatment 0.1% nano with salt (427.77%). The results agree with Javad, *et al.* (2014) who reported the positive effects of silica nanoparticles (nanoSiO₂) on developmental stages of *Zea mays* L. especially on seed germination, rate of root and stem elongation, relative water content, photosynthetic pigment, chlorophyll content, leaf fresh weight , leaf dry weight, proline accumulation and upregulated antioxidant enzymes activity under salinity stress.

Similarity Ashkavand *et al* (2015) found a positive effect pre-treatment of silicon nano particles on photosynthesis parameters, malondialdehyde, relative water content, membrane electrolyte leakage as well as chlorophyll, carotenoid, carbohydrate and proline contents. The exact mechanism is need to be understood, but may be involvement of silicon nano particles in maintaining critical physiological and biochemical attributes in order to induce drought tolerance in hawthorn seedlings under drought stress. Also Li *et al.* (2012); Siddiqui *et al.* (2014) reported that under salinity stress, nano particles improves leaf chlorophyll content and proline accumulation. They added that the accumulation of proline, free amino acids, content of nutrients, antioxidant enzymes activity were increased due to the nano-SiO₂. Thereby improving the tolerance of plants to abiotic stress. Plants are generally protected against this oxidative stresses by a wide range of radical scavenging systems such as antioxidative enzymes like superoxide dismutase (SOD), (POD), ascorbate peroxidase (APX), and (CAT), as well as non-enzymatic compounds like carotenoids (Zimmermann and Zentgraf, 2005). Similarly, Wang *et al.* (2011) observed a decrease in salt stress due to application of silicon NPs in alfalfa plant and they attributed this decreased salt stress to elevated activities of SOD, POD and CAT. The NPs might have reduced ROS stress in treated onion seedling by reducing H₂O₂, superoxide radicals, and lipid peroxidation products (malonyldialdehyde content) and increasing activities of SOD, APX, guaiacol peroxidase, and CAT enzymes as observed in spinach (Lei *et al.*, 2008).

Table 4. Effect of nano chitosan (N. Chs) on S.T.I of germination percentage and radical length after 24, 48, 72 hours of planting *Phaseolus vulgaris* seeds under salt stress condition.

| Treatment + Salt 100 mM) | Germination (%) (24h) | Germination (%) (48h) | Germination (%) (72h) | Radical length (%) 4h) | Radical length (%) (48h) | Radical length (%) (72h) |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 0.1% N. Chs | 222.07±1.78 ^c | 113.34±0.46 ^c | 119.42±0.98 ^b | 130.95±2.06 ^c | 188.11±1.72 ^c | 204.31±1.35 ^c |
| N. Chs 0.2% | 234.05±4.41 ^b | 126.55±0.32 ^b | 120.12±0.07 ^b | 158.45±3.50 ^b | 235.13±1.72 ^b | 220.58±1.76 ^b |
| 0.3% N. Chs | 250.36±6.18 ^a | 130.35±0.89 ^a | 123.94±0.00 ^a | 170.83±1.03 ^a | 241.15±2.60 ^a | 231.96±5.00 ^a |
| Sig | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Table 5. Effect of nano chitosan (N. Chs) treatments on S.T.I of growth measurements of *Phaseolus vulgaris* vegetative under salt stress condition.

| Treatment with NaCl | Plant height % | leaf area % | Shoot fresh weight % | Shoot dry weight % | Root fresh weight% | Root dry weight % | TSTTI % |
|---------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|----------|
| 0.1% N. Chs | 120.88±3.40 ^c | 166.56±1.47 ^c | 151.85±2.81 ^b | 142.02±2.51 ^c | 161.69±1.72 ^b | 185.05±1.99 ^c | 154.675 |
| N.Chs 0.2% | 135.54±1.2 ^b | 181.87±0.91 ^b | 149.07±0.46 ^b | 156.52±4.34 ^b | 169.14±1.49 ^a | 220.68±3.44 ^b | 168.8033 |
| 0.3% N.Chs | 140.66±2.55 ^a | 187.44±1.27 ^a | 185.49±0.26 ^a | 195.65±8.69 ^a | 170.65±4.30 ^a | 239.08±1.99 ^a | 186.495 |
| Sig | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | |

Table 6 . Effect of nano chitosan (N.Chs) treatments on S.T. I of physiological and biochemical triats of *Phaseolus vulgaris* vegetative under salt stress condition.

| Treatment+ NaCl | M.S.I % | RWC % | Value of Chl. a % | Value of Chl. b % | Value of carotenoids % | CAT % | PPO % | POD % | Proline % |
|-----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 0.1% N. Chs | 111.52±0.52 ^c | 126.34±0.31 ^c | 104.33±0.70 ^c | 116.44±0.16 ^c | 102.03±0.83 ^c | 427.77±9.6 ^a | 134.83±0.26 ^b | 121.76±0.86 ^c | 287.63±0.56 ^a |
| N. Chs 0.2% | 136.54±0.08 ^a | 129.37±0.35 ^b | 121.51±0.43 ^a | 152.16±0.15 ^b | 106.23±0.35 ^b | 231.66±1.66 ^b | 124.66±0.28 ^c | 180.48±0.42 ^b | 117.34±0.57 ^c |
| 0.3% N.Chs | 132.03±0.03 ^b | 131.42±0.50 ^a | 119.93±0.43 ^b | 154.27±0.21 ^a | 129.67±0.18 ^a | 105.00±0.00 ^c | 224.83±0.27 ^a | 118.94±0.48 ^a | 158.46±0.06 ^b |
| Sig | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

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تأثير النانو كيتوزان علي مؤشرات النمو والبيوكيميائية لنبات الفاصوليا *Phaseolus vulgaris* تحت الاجهاد الملحي

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يعتبر الاجهاد الملحي واحد من الاجهادات اللا احيائية كما أنه من العوامل الرئيسية التي تقلل من إنتاجية المحصول. أصبحت جزيئات النانو من المواد الرائدة في أبحاث الزراعة في الوقت الحاضر لأنها تمتلك خصائص فيزيائية وكيميائية فريدة. لذلك كان الهدف هو دراسة تأثير جزيئات النانو كيتوزان علي نباتات الفاصوليا في مرحلة الانبات و مؤشرات النمو الخضري تحت الظروف الطبيعية وظروف الاجهاد الملحي. لذلك قمنا أولاً بدراسة تأثير الاجهاد الملحي ثم دراسة تأثير النانو كيتوزان علي نباتات الفاصوليا تحت ظروف الاجهاد الملحي بتركيز مختار من الملح خلال مرحلتي الانبات والنمو الخضري. عند دراسة الاجهاد الملحي قمنا باختيار ثلاثة تركيزات من الملح (٥٠ و ١٠٠ و ١٥٠ ملي مول من كلوريد الصوديوم). ولقد أظهرت نتائج الاجهاد الملحي علي النباتات في مرحلة الانبات والنمو الخضري أنه بزيادة تركيز الملوحة يؤدي ذلك إلي انخفاض دليل تحمل الملوحة في نسبة الانبات وجميع مؤشرات النمو و المؤشرات الفسيولوجية والبيوكيميائية مقارنة بالكنترول وقد أظهر التركيز ٥٠ ملي مول تأثيراً سلباً للغاية لذلك أختير التركيز ١٠٠ ملي مول من كلوريد الصوديوم لإجراء التجارب الأخرى. وأدت معاملة نباتات الفاصوليا بتركيزات مختلفة من النانو كيتوزان مع تركيز (١٠٠ ملي مول من كلوريد الصوديوم) أن جميع تركيزات النانو كيتوزان عززت بشكل كبير إنبات البذور وطول الجذير تحت ظروف الاجهاد الملحي. وكان التركيز ٠.٣% أفضل معاملة في مرحلة الانبات، وقد أعطي أيضاً أعلى قيمة معنوية في دليل تحمل الملوحة مقارنة بالتركيزات الأخرى لجميع المؤشرات (طول النبات ومساحة الورقة والوزن الرطب والجاف للمجموع الخضري والجذري) ومن ناحية أخرى بالنسبة للمؤشرات الفسيولوجية والبيوكيميائية أعطي التركيز ٠.٢% أعلى قيمة معنوية في ثبات الاغشية ومحتوي كلوروفيل أ، بينما أعطي التركيز ٠.١% أعلى قيمة معنوية في نشاط إنزيم الكتاليز ومحتوي البرولين في حين ان التركيز ٠.٣% أعطي أعلى قيمة معنوية في محتوى الماء النسبي وكلوروفيل ب والكاروتنويدات ونشاط إنزيمي بولي فينول إوكسيديز والبيروكسيديز.