Influence of Engineered Nanoparticles on Growth Parameters and Photosynthetic Pigments of Wheat (Triticum Durum L.) Under Salt Stress Levels

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Abstract

Salinity stress is a major limitation to global crop production. Wheat is one of the world's crops has salt sensitive characteristics than other crops. Meanwhile, rapid advancement of nanotechnology is introducing more and more engineered nanoparticles (ENPs) in agricultural soil. While some negative effects of ENPs on plant health at very high concentrations have been reported, more beneficial effects at low levels are increasingly noticed. Calcium phosphate nanoparticles (CPNPs) are one of the most widely used nanoparticles to interact with ecosystem and of great interest in agricultural sciences. The current study evaluates the effect of Calcium phosphate nanoparticles on germination percentage and early growth rate of wheat germinated under sodium chloride stress. As compared to the control, the results clearly showed that germination percentage not affected after NPs treatments whereas growth, fresh mass and dry mass of seedlings were highly increased at low levels of CPNPs. As salt concentration increased, chlorophyll a, b and carotenoids contents decreased. The results revealed that CPNPs interacted with salt stressed seedlings caused an increasing in photosynthetic pigments as compared to the control.

Keywords: CPNPs, salt stress, plant growth, pigments.

Introduction

Nanotechnology is a very promising field of science and technology possesses exceptional physical and chemical properties that has the potential to open up new applications in the field of agriculture and biotechnology Stamploulis et al [1]. Ultrafine particles are between one and 100nm in size. Nanoparticles widely used as antimicrobial agent and safety associated with human and environmental use Jampilek and Kralova [2]. In agriculture, fertilizers are very important for plant growth and development, most of the applied fertilizers are unavailable to the plants due to many factors such as hydrolysis, degradation, photolysis and leaching. Hence it is necessary to increase the crop yield through new application with the help of nanomaterial Siddiqui and Al-Whaibi [3]. Nanoparticles have unique physiochemical properties and the potential to boost the plant metabolism Giraldo et al [4]. The use of nanoparticles in the germination and growth of plants and for the control of stress plants is a recent practice Mahajan et al ; Ahmed and Awwad [5,6]. Most biotic and abiotic stresses have harmful effect on plant growth and considered an unresolved issue. Various studies had revealed good attempt to understand the impact of nanoparticles on the growth of plants Lutts et al ; Knight [7,8]. Lieu and Lal [9] reported positive impact of nanoparticles on plant with its potential to be used as future nanofertilizer. Calcium phosphate nanoparticles (CPNPs) exhibit synergistic growth promotion, root proliferation and vitality improvement of Zea mays Rane [10]. Moreover, upadhyaya et al. [11] showed that Calcium phosphate nanoparticles may help in the formulation of new nano growth promoter and nanofertilizers for agricultural use. Therefore, it could potentially help in reduction of the quantity of fertilizer applied to crops. Salt stress induced decreases of shoot, root dry weight, leaf area and leaf gas exchange, chlorophyll a, b and total chlorophyll content compared to control Fathi et al [12]. To our knowledge, there is little experimental evidence on the interaction between nanoparticles and salt-stressed plant species, thus the proposed research is to understand the interaction between Calcium phosphate nanoparticles and wheat plant under salt stress conditions

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Materials and Methods

Plant material

Wheat variety were collected from Agriculture Research Center, King Saud university, Riyad, Kingdom of Saudi Arabia.

Size and Shape of Nanomaterial

Calcium Phosphate nanoparticles (CPNPs) used were purchased from Nanotech Egypt for Photo Electronics, Cairo, Egypt. Transmission Electron Microscope (TEM) was performed on JEOL JEM-2100 high resolution Transmission Electron Microscope at an accelerating voltage of 200 KV Nanotech Company for Electronics. The average size of nanoparticles (TEM) is less than 50 nm. The shape (TEM) of used nanoparticles is spherical. To investigate the response of wheat plant to CPNPs, different levels of nanomaterial (0 , 50 and 100 mg/L.) were prepared. The normal form (Bulk) of Calcium Phosphate was represented by (0-mg/L.). Saline solution preparation Sodium chloride was used in this investigation for salt stress experiments. Salinity levels (0, 75, and 150 mM) were investigated in this work. No salt solution was represented by control (0- mM). Statically design and randomized method of experiment. On May (2018), equal sizes of viable wheat grains were collected, sterilized with 10% Sodium Hypochlorite for 10 minutes and washed thoroughly with distilled water. Sterilized seeds were placed in equal size petri-plates containing moister filter paper. Distilled water was used for the control (untreated) experiments whereas saline solution (NaCl) was used for treatments. The saline solution was only added at the beginning of the experiment. Two groups of treatments were prepared, the first group represented salinity levels (0, 75 and 150 mM NaCl), whereas mixtures of prepared nanoparticles (0, 50 and 100 mg/L) with each of salinity treatments represented the second group. All experiments (control and treatments) were kept and allow for germination at 23°C. A grain was considered to have germinated when radical emerged from the seed coat. After two days, germination percentages (%) were calculated as the proportion of the grains that germinated to total number of grains multiplied by 100. On the third day of germination, seedlings were transferred into plastic pots of small diameter (8 cm) containing 5.0 ml distilled water. One- week experimental duration was sufficient for various analysis.

Seedling growth

Seedlings length (s) were measured in all treatments and respective controls using centimeter ruler and expressed in centimeter (cm). Fresh mass of seedling was taken and then oven dried at 80°C for 48hrs to estimate the dry mass. Both fresh and dry mass was expressed in gram (gm).

Photosynthetic pigments determination

Using pure acetone, pigments were extracted from excised foliage leaves of all treatments and related controls Fadeel [13]. The optical density of extracted pigments were measured using Spectrophotometer at 665, 649 and 445 for chlorophyll a, chlorophyll b and carotenoids respectively. The chlorophyll and carotenoids contents were referred as mg/g fresh weight and calculated according to Cherry [14].

Statically method

Each experiment was conducted three times and data presented are means of three independent repeats ± (n=3), where ± standard deviation of means.

Results and Discussion

Germination percentage

Salt stress is one of the main environmental factors that affect plant growth and morphology Crain et al [15]. In this investigation, salinity induced highly decreases of germination percentage at high level (150mM) whereas the effect was slightly at the other lower levels of the saline conditions (Table 1 & Fig.1). There were no apparent visual differences in seed germinated under salt stress treatments with nanoparticles (Table 2-4 & Fig.4,7 and 10), indicating that the engineered particles not pose any toxicological effects on the seeds during the germination process [6]. This trend was seen in other studies which suggested that NPs of (Ag, Zn and Al) had less or no effect on seed germination Zheng et al ; Galbraith; Mahajan et al ; Boonyanitipong et al ; Alzandi [16-20]. This may be explained by the protective effect of grain coats which can have selective permeability Wierzbicka and Obidzinska [21]. In addition, NPs may aggregate or make complex by ligands which can cause a decrease in toxicity and would lead to lower exposure to seeds Ziu et al [22].

Growth parameters

In particular, as salt concentration increased, degree of seedling growth arrest became obvious recording highly reduction (Table 1&Fig.2), in addition, fresh and dry mass were lower than that of control (Table 1 &Fig. 3). However, at higher level of (CPNPs), seedling growth showed toxicity (Tables2-4 &Figs.5,8&11)). The results revealed that seedling fresh and dry mass has increased with the increasing of (CPNPs) levels as compared to control (Fig.6,9&12). Thus, Calcium Phosphate nanoparticles may interact with plant and improves growth parameters including, seedling length as well as fresh and dry mass [10]. These results are consistent with those reported by previous many studies. Several studies have reported that nanoparticles have some negative effects on plant health at very high concentrations, while more beneficial effects were recorded at relatively low levels Rossi et al; Prasad et al; Wang et al; Das and Pandey [23-26]. Calcium is an essential plant nutrient, promotes proper plant cell elongation, and strengthen cell wall. High levels of calcium can alter both growth and Sodium exclusion of plant roots exposed to NaCl stress Alireza et al; Mohamad et al [27-28]. Phosphorus is also an essential element required for the energy storage and transfer within plants. It is a major component in ATP, the molecule that provides energy to the plant for different processes. Phosphorous has been observed to increase root growth and influence early maturity, crop quality and disease resistance. The use of nanoparticles in the growth of plants and for the control of plant diseases is a recent practice [27-28].

Photosynthetic pigments

As predicated, salt stress induced decreases of chlorophyll a, b and carotenoids as compared to control (0 NaCl) (Table 5) whereas application of normal form of Calcium Phosphate (Bulk) slightly enhanced the contents of chlorophyll a, b and carotenoids of wheat compared to control (Table 6&Fig.13). On the other hand, CPNPs treatments at (50mg/L.) highly increased chlorophyll a, b as well as carotenoids (Table 7&8 and Figs.14-16), whereas the high level of CPNPs (100mg/L.) treatment highly decreased the contents of pigments compared with normal form (bulk).Results of our investigation are consistent with previous studies on the interactive effect of salinity and nanoparticles on photosynthetic pigments of some plants such as maize [12], rape [23] peanut [24] and wheat [27&28].Such findings indicate that Calcium Phosphate NPs at low concentrations check mitochondrial ROS which may lead to growth promotive function of and enhancement of physiological performance of plant health .One possible explanation for these results may be related to ability of engineered nanoparticles (ENPs) to enhancement of plant photosynthesis , could modify plant anatomy and improve plant salt stress tolerance [23].

Table (1): Effect of salinity treatments on germination (%), seedling length (cm), fresh and dry mass (gm) of wheat compared to control (0 mM). Data presented are the mean ± SE (n=3).

Salinity(mM)	Germination (%)	Length (cm)	Fresh mass	Dry mass

			(gm)	(gm)
0	87. 6	6.4	3.0	1.33
75	73.4	4.2	1.8	O.91
150	22.3	0.93	0.63	0.33

Table (2): Interactive effect of CPNPs & (0mM) NaCl on germination (%), seedling length (cm), fresh and dry mass (gm) of wheat, Data presented are the mean ± SE (n=3).

CPNPs (mg/L.)	Germination (%)	Length(cm)	Fresh mass (gm)	Dry mass (gm)
0 (Bulk)	90.3	7.2	3.8	1.86
50	93.2	8.0	4.9	2.4
100	26.4	2.3	1.7	0.08

Table (3): Interactive effect of CPNPs & low salinity (75mM) NaCl on germination (%), seedling length (cm), fresh and dry mass (gm) of wheat. Data presented are the mean ± SE (n=3).

CPNPs (mg/L.)	Germination (%)	Length(cm)	Fresh mass (gm)	Dry mass (gm)
0(Bulk)	80.6	5.6	3.0	1.2
50	86.4	6.5	4.3	1.8
100	18.2	1.8	1.4	0.08

Table (4): Interactive effect of CPNPs & high salinity(150mM)NaCl on germination (%),seedling length (cm),fresh and dry mass (gm) of wheat., Data presented are the mean ± SE(n=3).

CPNPs (mg/L.)	Germination (%)	Length(cm)	Fresh mass (gm)	Dry mass (gm)
0(Bulk)	30.2	1.8	1.4	0.09
50	38.4	2.9	2.8	1.0
100	16.7	1.3	1.0	0.03

Table (5): Effect of salt stress on chlorophyll a, chlorophyll b and carotenoids of wheat compared to control (0 m M) Na Cl. Data presented are the mean ±SE (n=3) and refers to mg/g. Fresh Weight (F.W.)

Salinity (mM)	Chlorophyll a (mg/g) F.W.	Chlorophyll b (mg/g) F.W.	Carotenoids (mg/g) F.W.
0	6.8	4.9	2.4
75	4.3	2.8	1.7
150	1.3	0.9	0.6

Table (6): Interactive effect of CPNPs & no salt (0 mM) NaCl on chlorophyll a, chlorophyll b and carotenoids of wheat. Data presented are the mean ± SE (n=3) and refers to mg/g. Fresh Weight (F.W.)

CPNPs (mg/L.)	Chlorophyll a (mg/g)F.W.	Chlorophyll b (mg/g)F.W.	Carotenoids (mg/g)F.W.
0 (Bulk)	7.2	5.4	2.9
50	8.4	6.2	4.2
100	3.6	1.4	0.9

Table (7): Interactive effect of CPNPs & low salinity (75mM) NaCl on chlorophyll a, chlorophyll b and carotenoids of wheat. Data presented are the mean ± SE (n=3) and refers to mg/g. Fresh Weight (F.W.).

CPNPs (mg/L.)	Chlorophyll a (mg/g)F.W.	Chlorophyll b (mg/g) F.W.	Carotenoids (mg/g)F.W.
0 (BulK)	6.4	5.6	2.2
50	7.8	6.9	3.6
100	1.6	1.2	0.5

Table (8): Interactive effect of CPNPs &high salinity (150mM) NaCl on chlorophyll a , chlorophyll b and carotenoids of wheat. Data presented are the mean ± SE (n=3) and refers to mg/g. Fresh Weight (F.W.).

CPNPs (mg/L.)	Chlorophyll a (mg/g)F.W.	Chlorophyll b (mg/g)F.W.	Carotenoids (mg/g)F.W.
0 (Bulk)	2.7	2.2	1.7

50	3.3	2.9	2.1
100	1.2	1.0	0.2

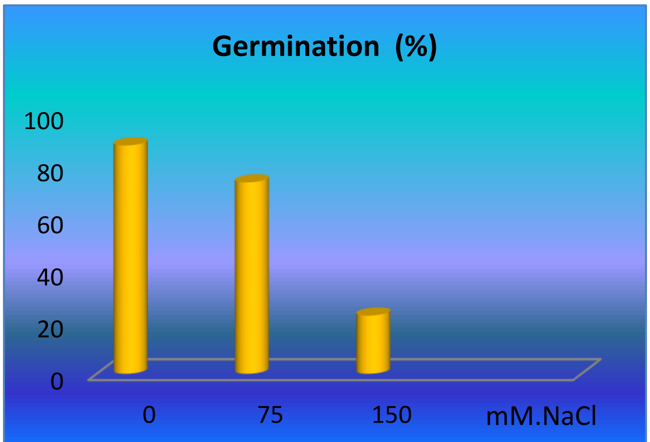

Fig (1):Effect of salinity on germination(%) of wheat.

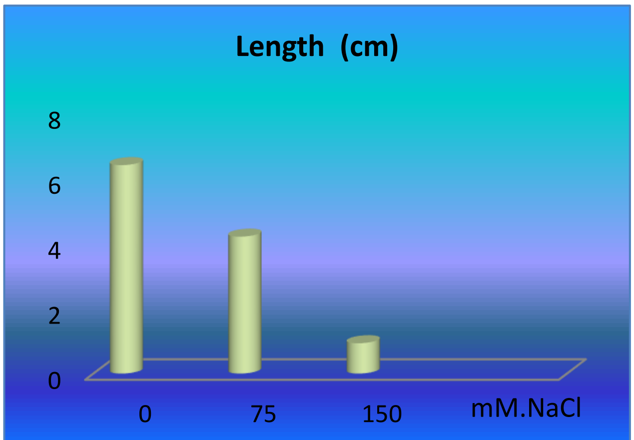

Fig (2):Effect of salinity on seedling length (cm) of wheat

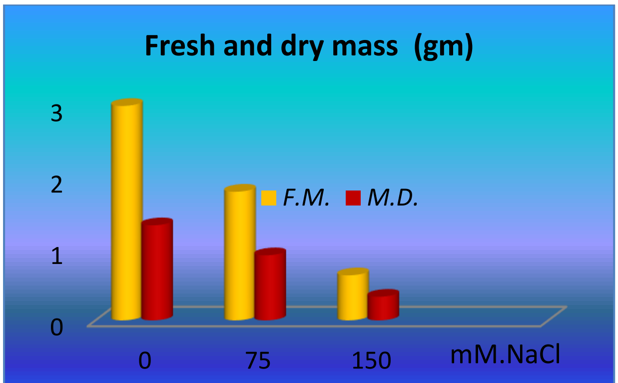

Fig (3):Effect of salinity on fresh and dry mass of wheat.

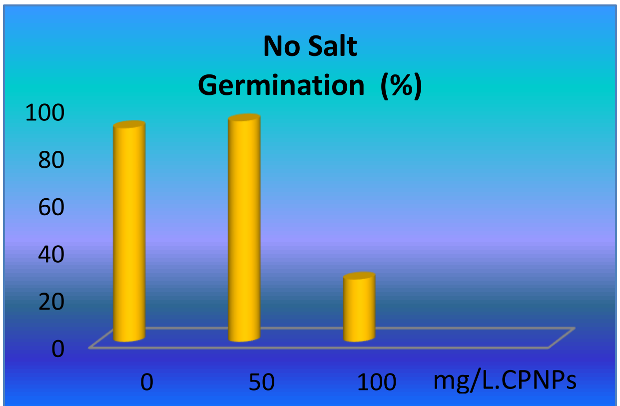

Fig (4):Interactive effect of CPNPs &(0 salt) on germination(%) of wheat.

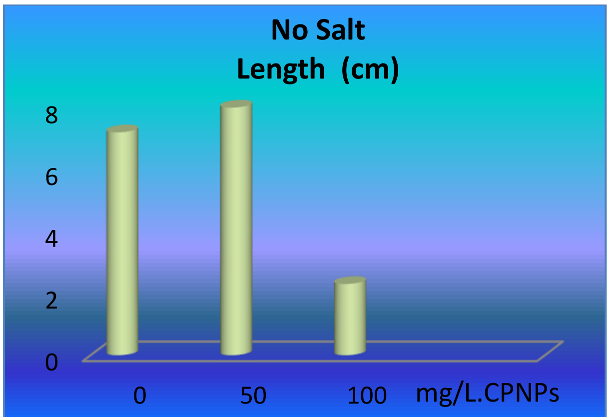

Fig (5):Interactive effect of CPNPs&(0 salt) on seedling length(cm) of wheat.

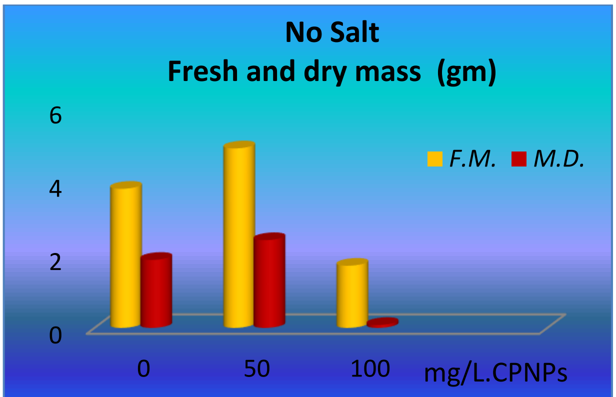

Fig (6):Interactive effect of CPNPs & (0 salt) on fresh and dry mass of wheat.

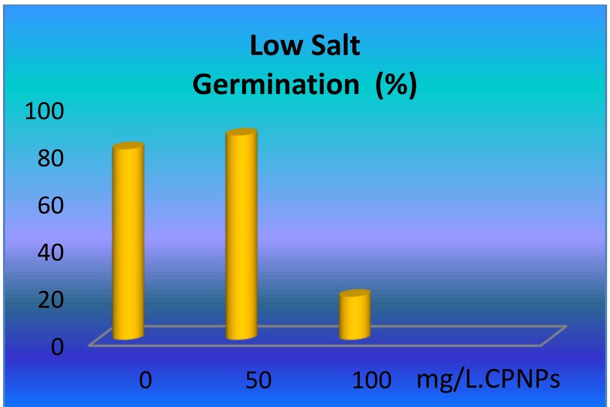

Fig (7):Interactive effect of CPNPs &(low salt) on germination (%) of wheat.

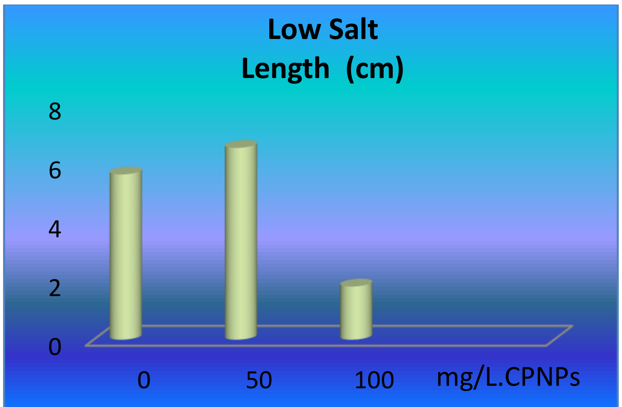

Fig (8):Interactive effect of CPNPs &(low salt) on length of wheat.

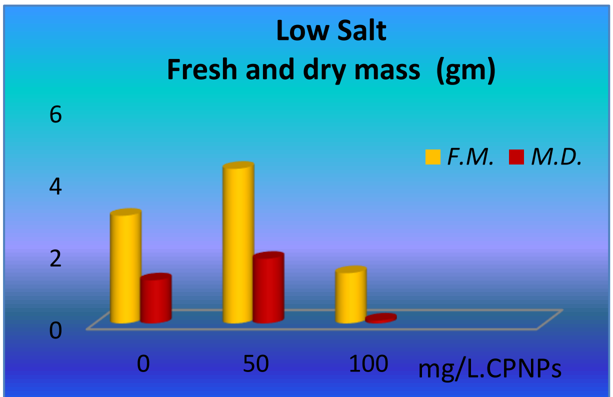

Fig (9):Interactive effect of CPNPs &(low salt) on fresh &dry mass of wheat.

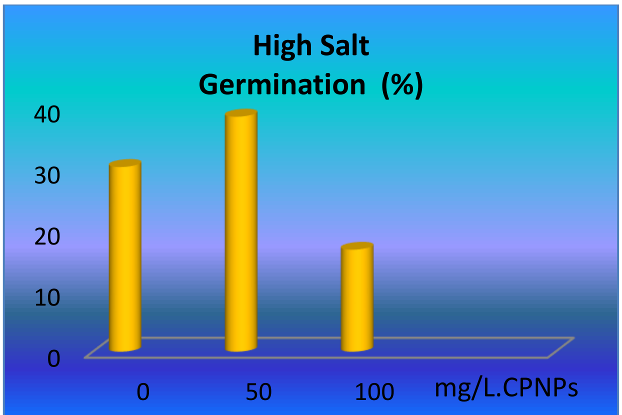

Fig (10):Interactive effect of CPNPs &high salt on germination (%) of wheat..

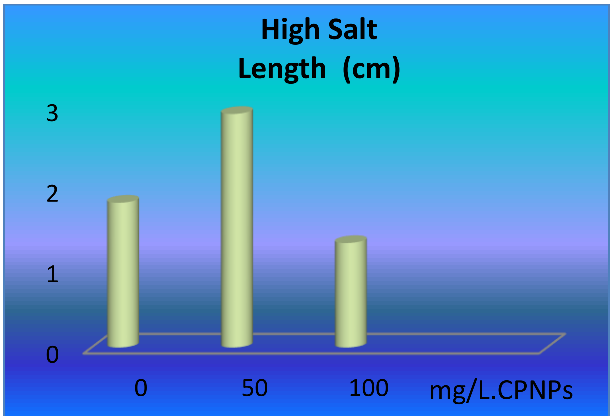

Fig (11):Interactive effect of CPNPs &high salt on length of wheat.

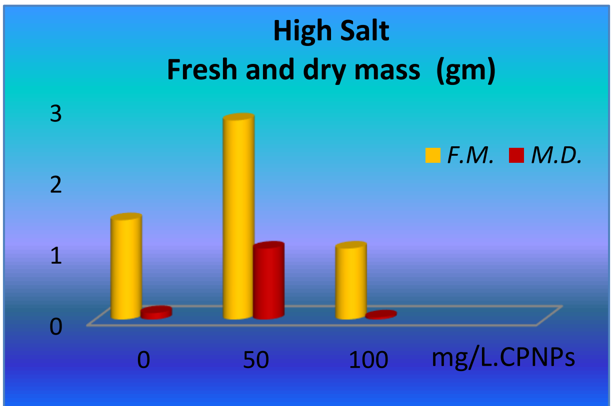

Fig (12):Interactive effect of CPNPs & high salt on fresh&dry weight of wheat.

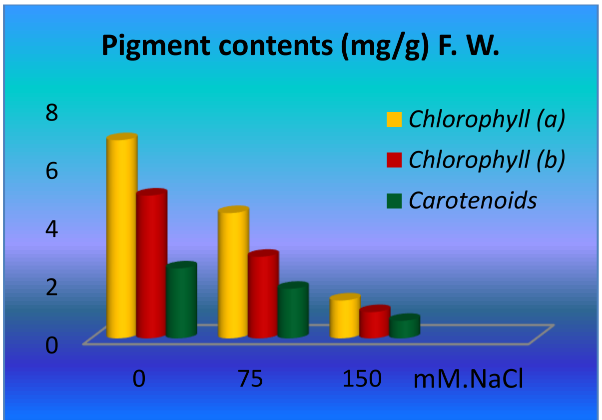

Fig (13):Effect of salinity on photosynthetic pigments of wheat.

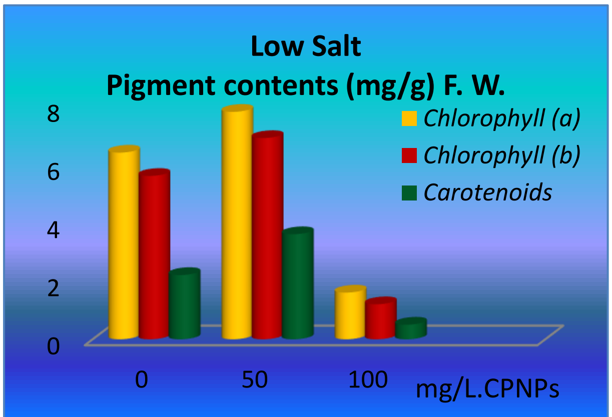

Fig (15):Interactve effect of CPNPs&(low salt)on pigments of wheat.

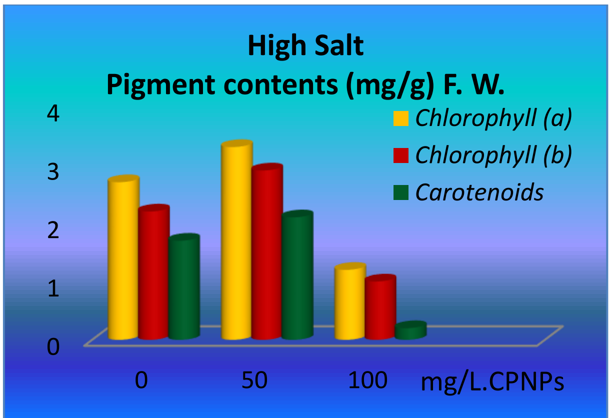

Fig (16):Interactive effect of CPNPs&(high salt) on pigments of wheat.

Conclusion

Calcium phosphate nanoparticles (CPNPs) may interact with plant and alter growth responses as well as physiological changes in dose dependent manner. Moreover, Calcium phosphate nanoparticles may help in formulation of new nano growth promoter and nanofertilizers for agricultural use. Therefore, it could reduce fertilizer wastage and in turn environmental pollution. In addition, NPs were more effective than bulk (normal form), which may be due to their shape, size, distribution and characteristics.

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