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Effect of Nano-Zirconium Oxide and Other Applications on Cowpea Seedlings Growth Under T Salt Stress

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Abstract

This study was carried out in the botanical garden / department of biology /college of science in Mustansiriyah University, Baghdad. During spring 2017 under controlled environmental conditions in greenhouse to study the role of Nano ZrO_2 activity in decrease negative effect of salinity under two levels from NaCl . The treatments included (Salinity levels : natural soil , 3000 ppm and 6000 ppm NaCl) and (Applications : control , 100 , 300 Zirconium oxide , 5 ppm of liquorice root extract and 10 ppm liquorice root extract and 300 ppm GA3) , The collected data were analyzed statistically using factorial completely randomized design. The results appeared that 300 ppm nano zirconium gave the highest values in all morphological traits (except leaves number) than other treatments. In addition to the highest values in fresh weight, dry weight, Chlorophyll a, Chlorophyll b and carbohydrate contain. Also 300 ppm nano zrconium oxide reduced the effect of salinity stress (3000 and 6000 ppm NaCl) by giving the highest values in morphological traits and in chemical contains (chlorophyll and carbohydrate) in Cowpea seedling growth.

Keywords: nano zrconium oxide, NaCl, Cowpea seedling.

تاثير اوكسيد الزركونيوم النانوي مع تطبيقات اخرى على نمو بادرات اللوبياء تحت الاجهاد الملحي

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الخلاصة

نفذت هذه التجربة في الحديقة النباتية التابعة لقسم علوم الحياة\ كلية العلوم \ الجامعة المستنصرية، بغداد. في الموسم الربيعي 2017 في البيت البلاستيكي بهدف دراسة تقييم فعالية مادة اوكسيد الزركونيوم النانوية في اختزال التأثير السلبي للملوحة لمادة كلوريد الصوديوم بتركيز 3000 و 6000 جزء بالمليون في نمو بادرات نبات اللوبياء. تم تحليل البيانات التي تم جمعها إحصائيا باستخدام تصميم تام العشوائية . كشفت النتائج عن التاثير الايجابي لمادة اوكسيد الزركونيوم النانوية (بشكل واضح في التركيز 300 جزء بالمليون) في تحسين نمو بادرات اللوبياء من حيث الصفات المظهرية (عدا عدد الاوراق) ومن المحتوى الكيميائي كلوروفيل ا وب ، والكربوهيدرات بدون وجود ملح الطعام. بالإضافة الى ذلك اختزل من تاثير التراكيز المختلفة من ملح الطعام على بادرات نبات اللوبياء ،واعطى اعلى النتائج من حيث المظهر الخارجي والمحتوى الكيميائي من حيث كمية كلوروفيل ا وب والكربوهيدرات مقارنة مع باقي المعاملات

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Introduction

Cowpea (*Vigna unguiculata* L.) is one of the important legume crops, which primarily its seed used as a food for human [1]. The roots protect the soil from erosion and have the ability to fix nitrogen into the soil [2] . In Baghdad the production and area planted with the crop was 37% by about 29296 tons, and the area about 28% by 14443 dunums [3]. The economic resources and their productivity outputs represented the cornerstone in the agricultural development economics, and the economic use of those resources represented one of the main objectives of economic growth in the agricultural sector [3]. The seeds are an important source of protein [4]. The total vegetative after harvesting has contained a high nutritional value and desirable by livestock. Roots of this legume plant are protecting the soil from erosion and the ability to supply nitrogen to the soil [4]. On a large scale , there are 20% of total cultivated and 33% of irrigated agricultural lands are exacerbated by high salinity , high salinity levels generate osmotic stress on plants, and causes the ionic toxic[5]. In addition, salinity conditions cause nutrient imbalances and disruptions in plant nutrient. Thus, the role of proper nutrition is important in contributing of nutrient balance and plant growth [6].

Several studies indicated that some substances such as nano particles enhanced the salt tolerance in many field crops [7 & 8]. Nano materials are into group of substances which is very small size (The dimensions are between 1–100 nm) , the nanoparticles are atomic or molecular aggregates with three dimensions. (8). Nano materials can change physico-chemical properties compared to bulk materials. It has a great surface area because its larger surface areas give solubility and high surface reactivity [9].

The modern studies are focused to study the Zirconium oxide (ZrO_2) as nanoparticles, GA3 (as phytohormones which is regulated plant growth and development) and liquorice root extract as plant growth promoter under salinity conditions , So it's possible improvement of plants ability to salinity tolerance by nanopractical ,GA3 and liquorice root extract on morphological trait such as root and stem growth, fresh and dry weight, and biochemical changes including chlorophyll and carbohydrate content in Cowpea.

Material and methods:

The experiment was carried out in the greenhouse of botanical garden in biology department in college of science, Mustansiriyah University Baghdad, Iraq, in spring season 2017. Seeds were sterilized with sodium hypochlorite solution (10%) for 10 minutes, and then washed three times with distilled water. The seeds were then planted in pots 12 cm diameter. Each pot was filled with loamy sand soil with 10 seeds per pot. Thinning was performed after 1 week of germination by leaving three plants per pot. Completely randomized design, was used with three replications: first level contained loamy sand soil (control), the second level was 3000 ($mg.L^{-1}$) of NaCl and the third level was 6000 ($mg.L^{-1}$) of NaCl . Each level included these treatments:

1. control
2. 100 Zirconium oxide (dissolved 0.1g in one liter distal water)
3. 300 zirconium oxide (dissolved 0.3g in one liter distal water)
   The supplier data were cooler white shape tetragonal shape size 20 – 40 nanometer
4. (5 g\ l) of liquorice root extract and (10 g\ l) liquorice root extract
5. 300 ppm GA3.

After week of thinning, the pots irrigated with water. 3000 ppm and 6000 ppm of NaCl, The electrical conductivity of each set was measured after the application of NaCl.

Table 1-The electrical conductivity in different concentrations of NaCl.

Treatment	Electrical Conductivity (EC) $ds.m^{-1}$
Control	2.25
3000 ($mg.L^{-1}$)	4.11
6000 ($mg.L^{-1}$)	7.98

After two weeks, the sub treatments were applied as foliar in twice times, the first time was after 20 days from planted , the second time was after 30 days from planted . After 40 days the samples were collected for analysis, to determine the fresh and dry weight of plant, plant height, leaves number, long of root and root nods. Chlorophyll a, b were prepared from fresh leaves of plant. The contents of chlorophyll a, b were determined by using the spectrophotometer method of [10]. The soluble carbohydrate was determined by [11], the dates were analyzed by using less significant difference test 5%.

Results and Discussion

The results in Table-2 showed that 300 ppm nano zrconium oxide in general gave the highest results in all morphological traits (except leaves number) compared to control treatment.

Table -3. Showed that 300 ppm nano zrconium oxide had the highest values than other treatments, in fresh weight (1.85), dry weight (0.48), Chlorophyll a (1.73), Chlorophyll b (0.99) and carbohydrate (0.47) compared to control treatment. The results obtains there is no significant differences between gibberellin treatment and 300 ppm nano zrconium oxide.

The results in Tables-(2, 3) indicated that 300 ppm of nano zirconium oxide treatment act as a promoting seedling growth and It excel than gibberellin in the results of morphological trait and the chemical content (chlorophyll a,b and carbohydrate) in normal condition (without the addition of NaCl), that mean nano zirconium oxide in 300ppm concentration improved seedling growth also induced the synthesis of chlorophyll (a, b) and accumulation of carbohydrate contain. This result was agree with many researchers [12-14], they indicated that the interaction of nano practicle with plan promoting of the plant growth and development, such as increased the root length, plant height the number of leaves, leaf area, sugar and chlorophyll content. Also induced synthesis of protein and carbohydrate with decreased the total phenol content [15].

Table 2-Effect of different treatments on morphological traits without NaCl addition

Treatments	Root length (mm)	Stem length (cm)	Leaves number	root nods number
Control	**18**	**24.5**	**3**	**2**
nano zrconium oxide 100 ppm	**22**	**24**	**3**	**3**
nano zrconium oxide 300 ppm	**24.5**	**27.2**	**3**	**4**
liquorice root extract 5gm./l.	**19**	**24**	**3**	**3**
liquorice root extract 10gm./l.	**21**	**24.5**	**3**	**4**
Gibberellin 300 ppm.	**24**	**25.1**	**3**	**3**
$L.S.D_{0.05}$	**1.78**	**1.57**	**N.S**	**1.11**

Table 3-Effects of different treatments on fresh and dry weight, Chlorophyll (A&B) and carbohydrate in Cowpea seedlings without NaCl addition

Treatments	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A Con.	Chlorophyll B Con.	Carbohydrate Con.
Control	**1.43**	**0.21**	**1.49**	**0.84**	**0.30**
Nano zrconium oxide 100 ppm	**1.59**	**0.37**	**1.23**	**0.81**	**0.36**
Nano zrconium oxide 300 ppm	**1.85**	**0.48**	**1.73**	**0.99**	**0.47**
liquorice root extract 5gm./l.	**1.56**	**0.30**	**1.39**	**0.85**	**0.39**
liquorice root extract 10gm./l.	**1.62**	**0.35**	**1.48**	**0.91**	**0.43**
Gibberellin 300 ppm.	**1.80**	**0.40**	**1.55**	**0. 88**	**0.40**
$L.S.D_{0.05}$	**0.10**	**0.06**	**0.04**	**0.08**	**0.13**

Many studies on the effects of metal oxide nano practicle on growth of plants improved biomass, root and shoot length, chlorophyll and protein synthesis and other growth parameters [16]. also increasing photosynthetic rate, transpiration rate, electron transport rate and other physiological parameters [17& 18].

The results in Table-4 in the treatment 3000 ppm NaCl showed that the same observation with treatment 300 ppm nano zrconium oxide gave the highest values in root length was 23 mm in compared to control (14 mm), stem length 30 cm compared to control 26 cm and in root nods was 3 compared to control (0).

Table 4- Effect of different treatments on morphological traits with 3000 ppm NaCl addition

Treatments	Root length (mm)	Stem length (cm)	Leaves number	Root nods number
Control	**14**	**26**	**3**	**0**
nano zrconium oxide 100 ppm	**18**	**27**	**3**	**3**
nano zrconium oxide 300 ppm	**24**	**30**	**3**	**3**
liquorice root extract 5gm./l.	**22**	**25**	**3**	**3**
liquorice root extract 10gm./l.	**20**	**23**	**3**	**4**
Gibberellin 300 ppm.	**22**	**24**	**3**	**3**
$L.S.D_{0.05}$	**1.77**	**2.50**	**N.S**	**1.4**

Table-6 indicated that 300 ppm nano zrconium oxide gave the highest values in chemical analysis contents in fresh weight, dry weight, Chlorophyll b and carbohydrate.

The Tables-(6, 7) indicated that the nano zrconium oxide in concentration 300 ppm had the highest values in chemical analysis compared to other treatments under 6000 ppm NaCl stress.

In Tables-(4, 5, 6 and 7) in the treatments 3000 and 6000 ppm NaCl, nano zirconium oxide in 300 ppm concentration gave the highest values that mean this practical act as a reducing agent for the salinity effect on plant growth. These results agree with many studies, which were indicated that nano practicle improve plant salt tolerant by attributing in uptake and transport of potassium and sodium in plants, also the nano practicle application led to balance growth reduction by salinity in several pathways through the changes in stomatal behavior, drop of leaf, increase K^+ tissues content [19], or by increase the anti-oxidative enzymes activity which might be balance salinity damages [20] & [21].

Table 5-Effect of different treatments on weight, Chlorophyll and carbohydrate in Cowpea seedlings with 3000 ppm NaCl addition

Treatments	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A Con.	Chlorophyll B Con.	Carbohydr ate Con.
Control	**1.90**	**0.30**	**0.91**	**0.48**	**0.22**
Nano zrconium oxide 100 ppm	**2.63**	**0.44**	**1.48**	**0.85**	**0.40**
Nano zrconium oxide 300 ppm	**3.27**	**0.54**	**1.75**	**0.97**	**0.46**
liquorice root extract 5gm./l.	**1.89**	**0.33**	**1.39**	**0. 68**	**0.31**
liquorice root extract 10gm./l.	**1.95**	**0.34**	**1.40**	**0. 72**	**0.35**
Gibberellin 300 ppm.	**1.96**	**0.38**	**1.63**	**0. 79**	**0.38**
$L.S.D_{0.05}$	**0.04**	**0.03**	**N.S**	**0.36**	**0.03**

The application of NPs at the appropriate dose rate significantly enhanced growth traits in salt-stressed plants, the plantlets adapt to salt stress by increasing the activities of antioxidant enzymes, and increase in the number of protein bands which associated with salt stress responses [22].

Table 6-Effect of different treatments on morphological traits With 6000 ppm NaCl addition

Treatments	Root length (mm)	Stem length in (cm)	Leaves number	Root nods number
Control	10.5	19	2	0
nano zrconium oxide 100 ppm	16.0	22	4	2
nano zrconium oxide 300 ppm	20	28	4	3
liquorice root extract 5gm./l.	13.5	26	2	4
liquorice root extract 10gm./l.	16	25	2	2
Gibberellin 300 ppm.	17	28	3	2
$L.S.D_{0.05}$	0.19	1.72	0.94	0.73

Table 7-Effects of different treatments on weight, Chlorophyll and carbohydrate in Cowpea seedlings with 6000 ppm NaCl addition

Treatments	Fresh weight (gm)	Dry weight (gm)	Chlorophyll A Con.	Chlorophyll B Con.	Carbohydrate Con.
Control	1.24	0.20	0.70	0.40	0.20
Nano zrconium oxide 100 ppm	1.67	0. 30	0.78	0.60	0.29
Nano zrconium oxide 300 ppm	3.58	0.65	1. 70	0.71	0.38
liquorice root extract 5gm./l.	1.48	0.37	0.75	0.54	0.23
liquorice root extract 10gm./l.	1.89	0.40	0.60	0.44	0.28
Gibberellin 300 ppm.	2.95	0.48	1. 60	0.66	0.34
$L.S.D_{0.05}$	0.04	0.03	0.31	0.02	0.02

Conclusion

In this study, we can conclude that nano zrconium oxides in 300 ppm concentration can enhancement seedling growth (root and stem length, number of root node in Cowpea seedling in addition to improve chlorophyll and carbohydrate contains in normal condition (without salinity stress).

Also 300 ppm nano zrconium oxide can decrease the effect of salinity stress (3000 and 6000 ppm NaCl) on the morphological traits and in chemical contains (chlorophyll and carbohydrate) in Cowpea seedling growth.

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