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Biochemical changes in plant growth in response to Zn nanoparticles

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Abstract
Wheat (*Triticum aestivum* L.) is one of the most important food crops in Egypt and worldwide, the most important winter cereal, is the staple for millions around the world. So the the present study was conducted to investigate whether application of ZnSo4 (Zn) and zinc ONP could regulate the growth of plant the. Addition of Zn or ZnONP markedly increased the morphology (shoot length (cm), root length (cm) and number of leaves / plant, fresh and dry weights of shoots and roots, No. of tillers, No. of spikes, No. of grains weight of grains and weight 1000 of grains.

Key words: zinc nanoparticles, wheat , zinc , grains, morphology

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops in Egypt and worldwide, the most important winter cereal, is the staple for millions around the world.

Wheat is the “big” cereal crops worldwide with paramount impact on human nutrition and, consequently, human health **(Shewry, 2009**). According to FAO reports, wheat plays a particular role in covering daily caloric requirements of humans in worldwide. According to a report published by **Hotz and Brown (2004).**

Zinc (Zn) is an essential nutrient required by all living organisms and represents the 23rd most abundant element on earth (**Broadley et al., 2007**) and the 2nd most abundant transition metal, subsequent to iron (**Jain et al., 2010).** It is the required in six different classes of enzyme, which include oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (**Auld, 2001**).

Zinc has been considered as an essential micronutrient for metabolic activities in plants and animals including humans. Although it is required in trace amounts in plants but, if it is not available in required amount, it creates physiological imbalances and affects enzyme activities and other metabolic processes (**Baybordi, 2006**).

Zinc has important functions in the synthesis of auxin or indoleacetic acid (IAA) from tryptophan as well as in biochemical reactions required for formation of chlorophyll and carbohydrates. It also regulates the functions of stomata by retaining potassium content of protective cells. The crop yield and quality of produce can be affected by deficiency of Zn (**Jamali et al., 2011**). It was found that zinc has an important role in management of reactive oxygen species and protection of plant cells against oxidative stresses (**Sheikh et al., 2009).**

Researchers like **Camp and Fudge (1945), Chapman (1966), Anderson (1972), Mengel and Kirkby (1978), Marschner (1993), Brown et al. (1993) and Fageria et al. (2002)** have demonstrated essentiality and role of zinc in plant growth, reproduction and yield. It has been indicated that the retention time of Zn in the plant system is low and hence, the bioavailability of Zn for long period is not sure with the use of ZnSO4 fertilizer. Farmers are using both zinc sulfate and EDTA-Zn chelate for soil and foliar applications; however, the efficacy is low.

Nanoparticles (NPs) with small size and large surface area are expected to be the ideal material for use as a Zn fertilizer in plants. Currently use of nanomaterials has been expanded in every fields of science including agriculture. It has been stated that application of micronutrient fertilizers in the form of NPs is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of fertilizer pollutions (**Naderi and Abedi, 2012**). It is because of that when materials are transformed to a nanoscale, they change their physical chemical and biological characteristics as well as catalytic properties and even more increase the chemical and biological activities (**Mazaherinia et al., 2010**).

Reynolds, (2002) demonstrated that micronutrients in the form of NPs can be used in crop production to increase yield.

Prasad et al. (2012) studied the effect of nanoscale zinc oxide on the germination, growth and yield of peanut and observed significantly more growth and yield.

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Materials and Methods

These experiments were carried out Kafr EL-sheikh, Cairo, Egypt on the date 6.4.2015. The grains of wheat (*Triticum aestivum* ssp.) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

A pot experiment was designed as follows: A homogenous wheat grains (Giza 168) were sown in pots (30 cm in diameter) containing 8.0 K.g. of clay soil. The pots were divided into nine groups representing the following treatments,

Treatments

- Tap water (control)
- Zn (100 ppm as zinc sulphat)
- ZnONP (25 ppm as zinc oxide nanoparticles)

The plants of wheat were treated twice with the above mentioned treatments (as foliage spraying). The first treatment was made when the age of plants was 30 days, while the second treatment was made when the age of plants was 75 days of sowing. The plant samples were collected for analysis when the plants were 85 (Stage II) days old. At the end of the growth season (140 days), analysis of the grains yielded from the different treatments as well as the control were done and the irrigation of water level occur at 15 days throughout the ages of plant.

Growth Measurements:

1. 1. Shoot Height:

The heights of eight shoots were taken at random from each line and the measurements were carried out from above soil surface till the end of growing tip of the plants and recorded in meters.

2. 2. Leaves Number:

Eight plants were taken from each line of every treatment to account the number of leaves.

3.3. Fresh and Dry Weight of Both Shoot and Root:

The plants in some line of every treatment were weighted immediately after clipping and estimation of the fresh weight of both shoot and root. The fresh materials were dried at 70ºC to constant dry 50 weights. The fresh and dry weight of both shoot and root were calculated as g/plant.

4.4. Root Length:

The measurements were carried out from the soil surface to the end of root tip and recorded in centimeters.

Statistical analysis:

We calculate sample size according to Raosoft and All statistical calculations were done using SPSS (statistica package for the social science version 25.00) statistical program. at 0.05 level of probability (**Snedecor and Cochran, 1982**). Quantitative data with parametric distribution were done using Analysis of variance the One-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non significant (NS) at the level of > 0.05, significant at the level of < 0.05, 0.01 and highly significant at the level of < 0.001.

RESULTS AND DISCUSSION:

Characterization of the Nanoparticle

Figure 1 shows an TEM image of the nanoparticle sample. The image shows ZnO nanoparticles with mean particle diameter of 25 nm and they looked slightly aggregated as there were no protecting ligands on the surface. The particles are crystalline as revealed by the high magnification image and the lattice of ZnO is clearly seen. Nanoparticles showed lattice spacing of 0.26 nm and 0.28 nm corresponding to (0002) and (10¯I0) planes of ZnONP (**Lin et al., 2009, Zhu et al., 2009**).

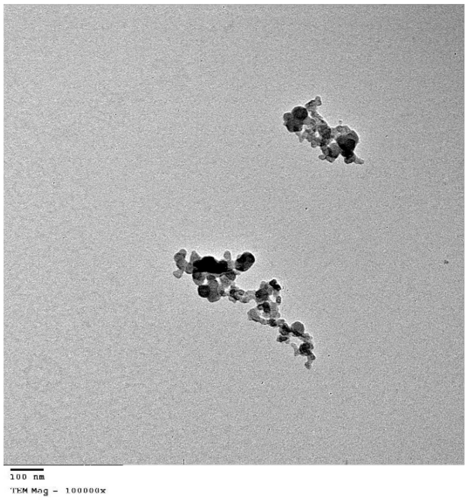


FIGURE 1 Large area TEM image of ZnO nanoparticles. Inset shows the high resolution image of a single particle.

The obtained results (Tables 1-3; figs 2-8) revealed also that, treated wheat plants with Zn or ZnONP resulted in significant increases in shoot length, fresh and dry weight of both shoots and roots and the number of leaves / plant as being compared with plants grown in normalcondition.

Praba et al 2009 demonstrated that In wheat there was a significant decrease in leaf fresh weight, leaf area, dry weight, plant height and shoot dry weight after vegetative stage drought. Greater plant height may be an important adaptive mechanism in environments characterized by major pre anthesis moisture stress (**Singh et al. 2001**).

The positive effects of foliar spray of Zn on vegetative growth of wheat under interval irrigation were in harmony with the results observed by some investigators (**Aldesuquy et al. 2012; Azimi et al. 2013**). In one study it was found that Zn application significantly increased the vegetative growth (**Monjezi et al. 2013**).

Statistically significant enhancement on shoot and root elongation. Thus zinc nanoparticles can be reported with minimal toxicity on the tested plants, this is a good evidence for demonstrating that wheat plants respond to add Zn NPs in a limited range, above which toxic levels are reached causing subsequent declines in growth. These results agree with **Mahajan et al. (2011); Mihaela and Dorina (2007) and Seif et al. (2011).**

Yield and yield components

Table (4) figs. (9-13)revealed that, both number tillers and spikes per plant, number & weight of 1000 grains per plant were highly significantly increased in response to the treatments with Zn or ZnONP with normal condition. Except interactive effects, irrigation water at (7 days) with Zn revealed insignificant increase in number spikes per plant. These outcomes are in agreement with **Farooq *et al.* (2009) and Monjezi et al. (2013).**

At high number of grain per spike leads to higher grain yield in plants **Sharafizad *et al.* (2013**), (**Zamaninejad *et al.* 2013**).

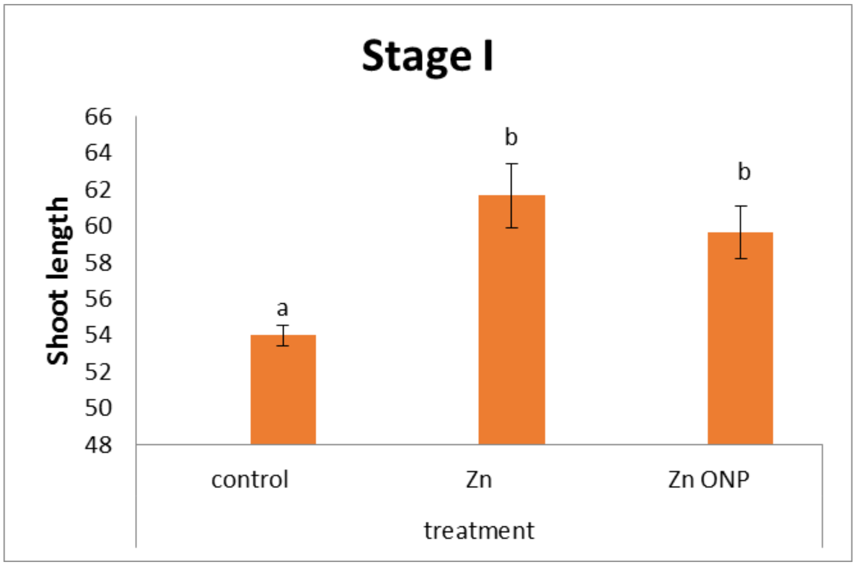
Maleki *et al.* (2014) also stated that zinc sulfate increase s on maize grain yield and yield components. A similar protective effect of zinc sulfate was also observed by **Malek- Mohammadi *et al.* (2013).**

The fresh and dry weights were both significantly higher than those of the untreated plants (controls). The best results were found at 60 ppm Zn nanoparticles, the fresh weight and dry weight per plant and yield content were higher than those of the control **Lin and Xing (2008).**

Table (1): Effect of Zn, ZnONP on shoot length (cm), root length (cm) and number of leaves / plant of wheat. Each value is mean of 10 replicates ± standard error of means.

Treatments	Shoot length (cm)		Root length (cm)		Number of leaves	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	54.00±58	71.33±0.88 a	6.06±0.03 a	11.83±0.17a	11.67±0.33 a	23.00±2.00 a
Zn	61.67±1.77 b	79.67±1.45 b	8.15±0.18 b	14.67±0.33 b	16.67±0.33 b	30.00±1.00 b

Zn ONP	59.67±1.45 b	77.33±0.88 b	7.73±0.12 b	14.33±0.44 b	15.67±0.33 b	30.33±0.88 b
F ratio	8.50	**15.121**	77.083	21.583	63.000	8.904
P value	**0.018** S	**0.005** S	**0.000** HS	**0.002** HS	**0.000** HS	**0.016** S



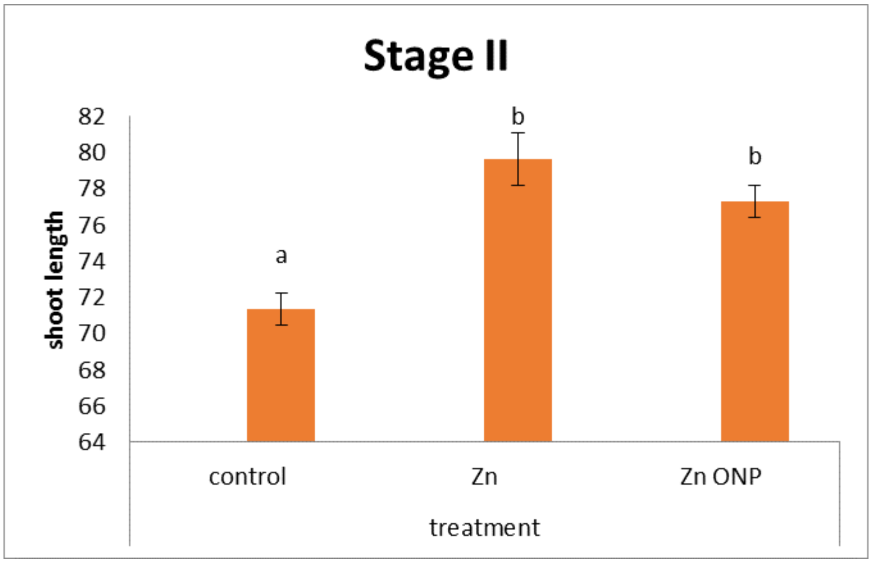


Figure (2): Effect of Zn, Zn ONP on shoot length of wheat.

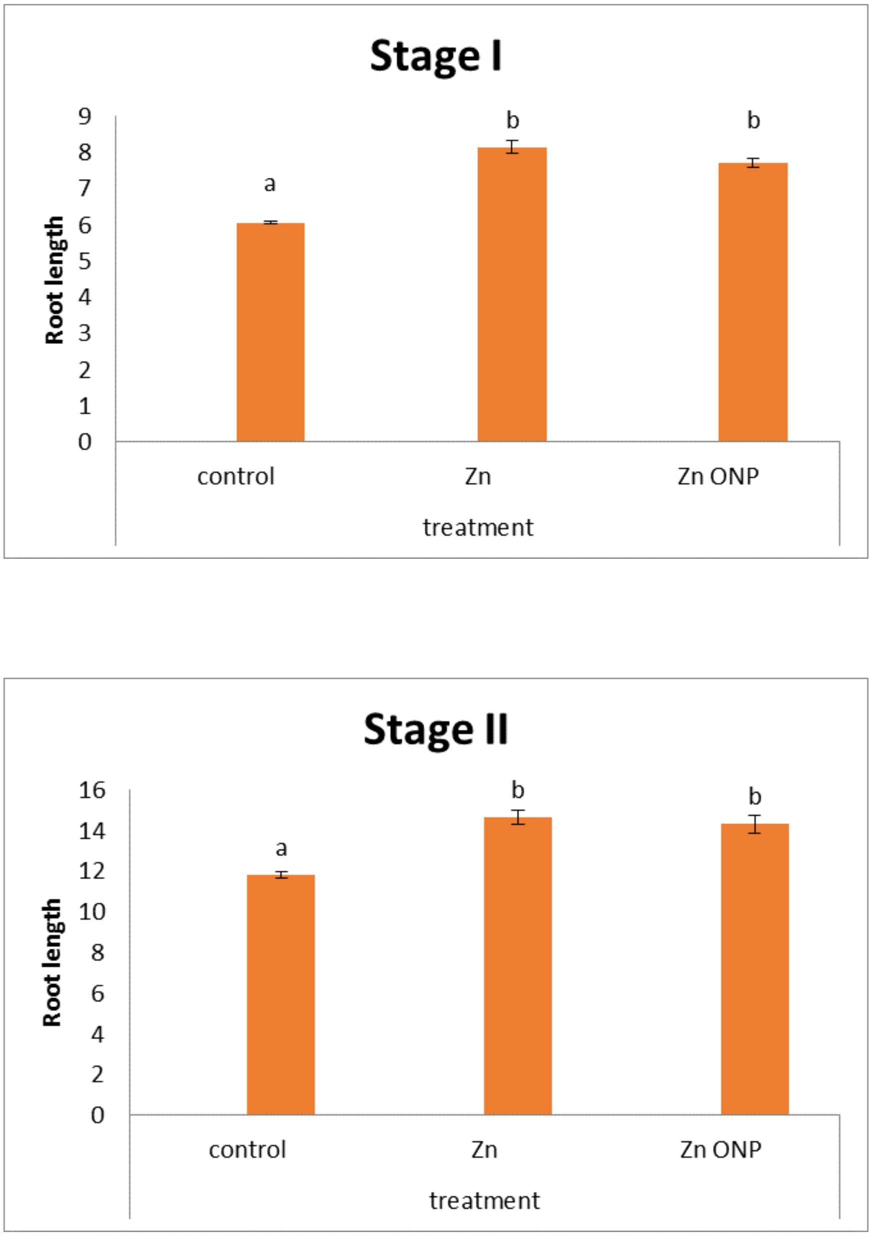
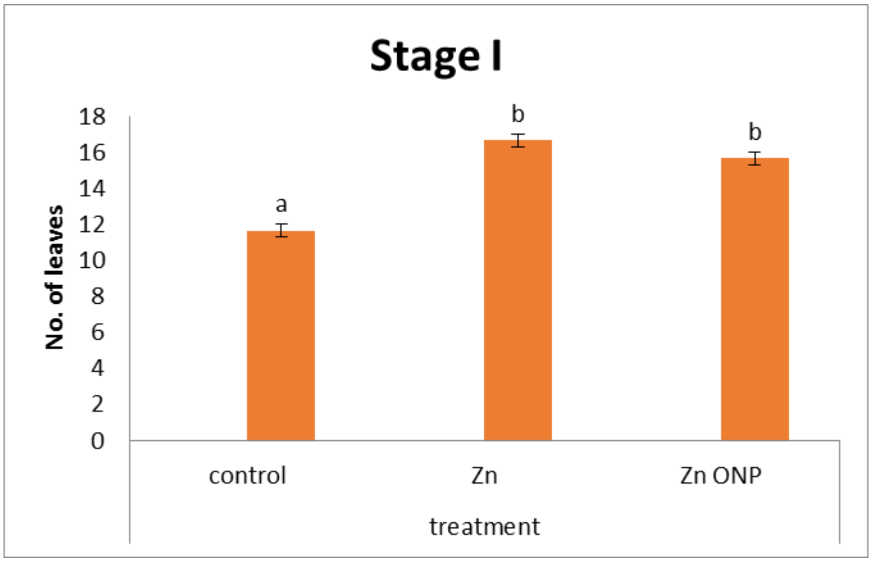


Figure (3): Effect of Zn, Zn ONP on root length of wheat



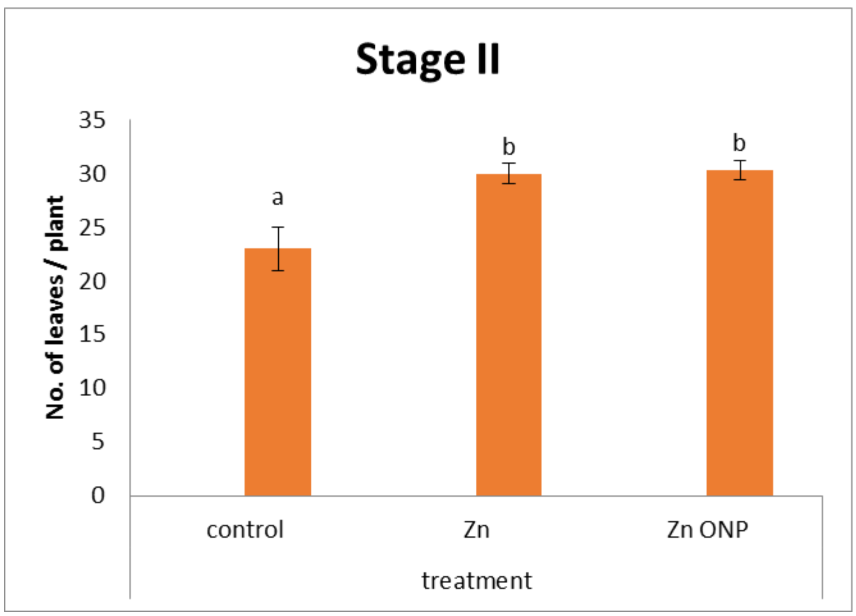


Figure (4): Effect of Zn, Zn ONP on number of leaves of wheat

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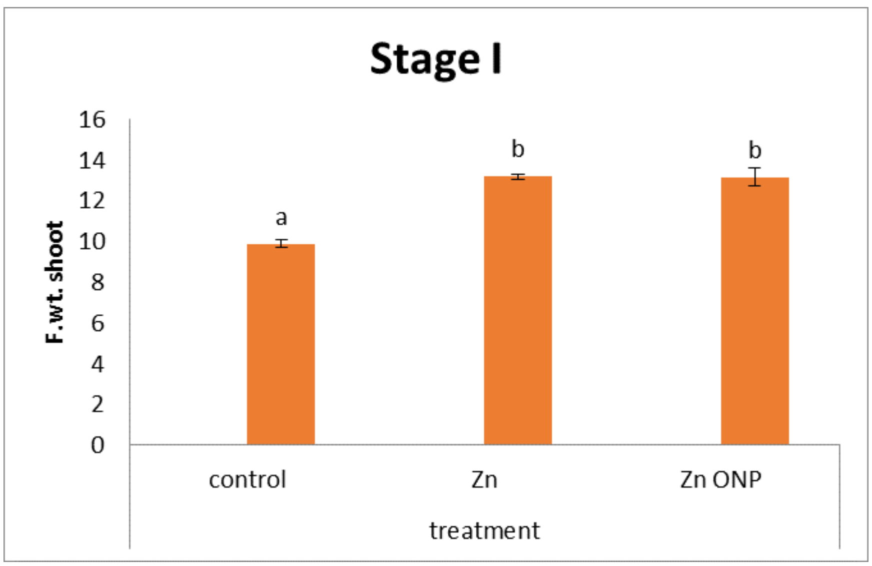
Table (2): Effect of Zn, Zn ONP on fresh and dry weights of shoots of wheat .Each value is mean of 10 replicates ± standard error of means, NS = Non significant, S = Significant and HS = Highly significant.

Treatments	F.wt.Shoot (gm)		D.wt.Shoot (gm)	
	Stage I	**Stage II**	**Stage I**	**Stage II**
Control	9.90±0.21 a	16.07±0.39 a	1.10±0.06 a	2.01±0.05 a
Zn	13.20±0.12 b	19. 33±0.30 b	1.60±0.06 b	3.04±0.03 b
Zn ONP	13.17±0.44 b	16.81±0.50 a	1.37±0.09 ab	2.70±0.06 c
F ratio	42.934	11.876	13.00	121.241
P value	**0.000** ^{HS}	**0.008** ^{S}	**0.007** ^{HS}	**0.000** ^{HS}



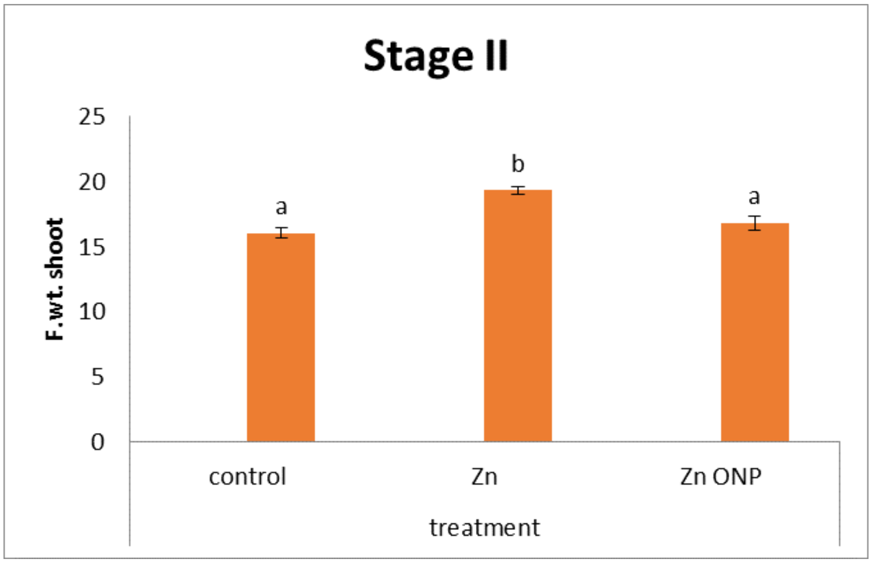
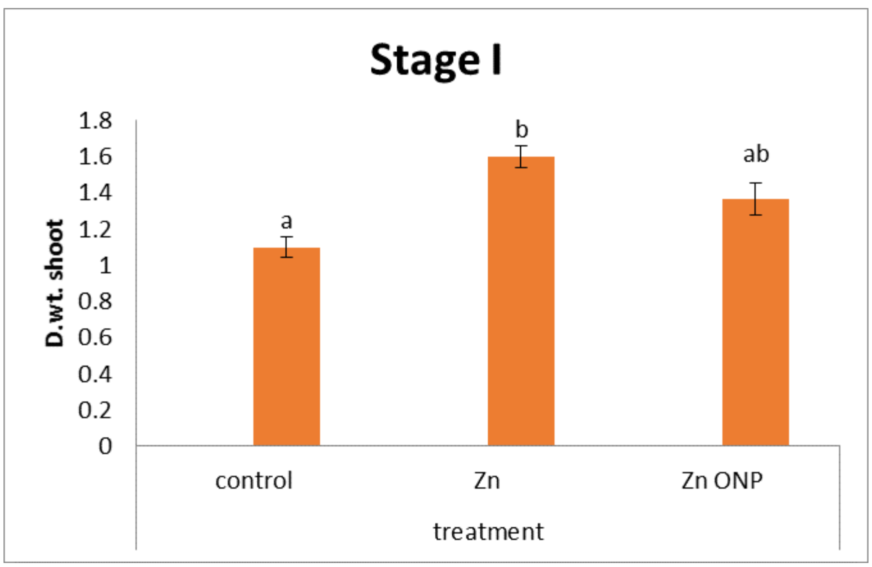


Figure (5): Effect of Zn, ZnONP on fresh weights of shoots of wheat.



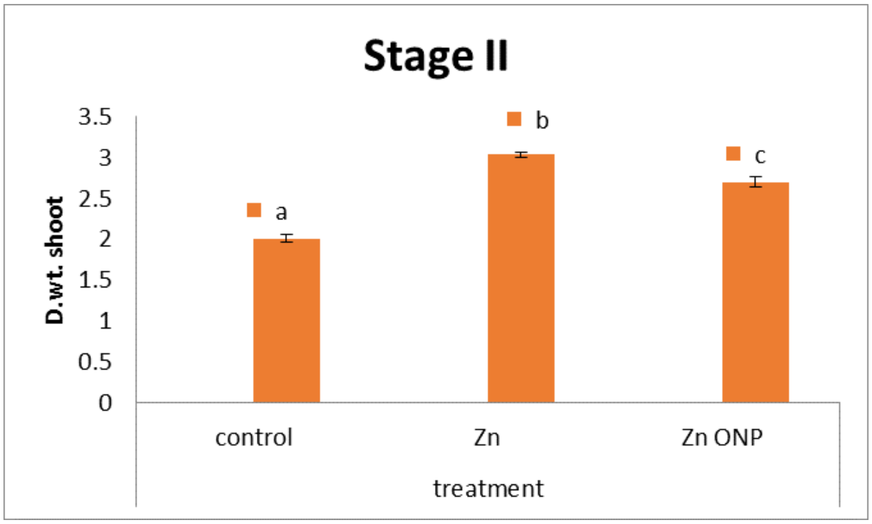


Figure (6): Effect of Zn, Zn ONP on dry weights of shoots of wheat.

Table (3): Effect of Zn, Zn ONP on fresh and dry weights of roots of wheat .Each value is mean of 10 replicates ± standard error of means, NS = Non significant, S = Significant and HS = Highly significant.

Treatments	F.wt. Root (gm)		D.wt. Root (gm)	
	Stage I	Stage II	Stage I	Stage II
Control	5.70±0.12 a	11.11±0.06 a	1.03±0.03 a	1.97±0.09a
Zn	8.31±0.09 b	17.00±0.53 b	1.37±0.09 b	2.84±0.08 b
Zn ONP	7.13±0.32 c	15.43±0.50 c	1.20±0.06 ab	2.29±0.30 ab
F ratio	41.676	52.485	6.818	5.479
P value	0.000 ^{HS}	0.000 ^{HS}	0.029 ^{S}	0.044 ^{S}

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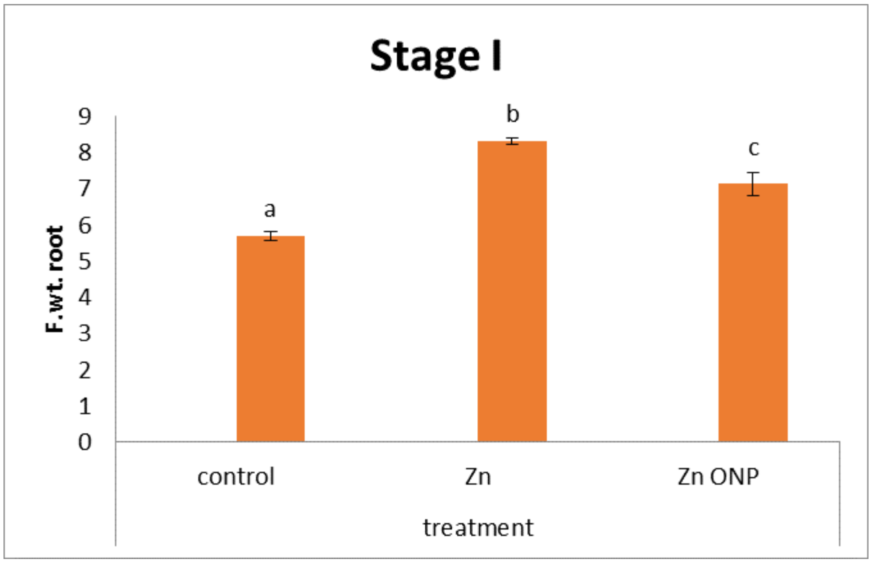


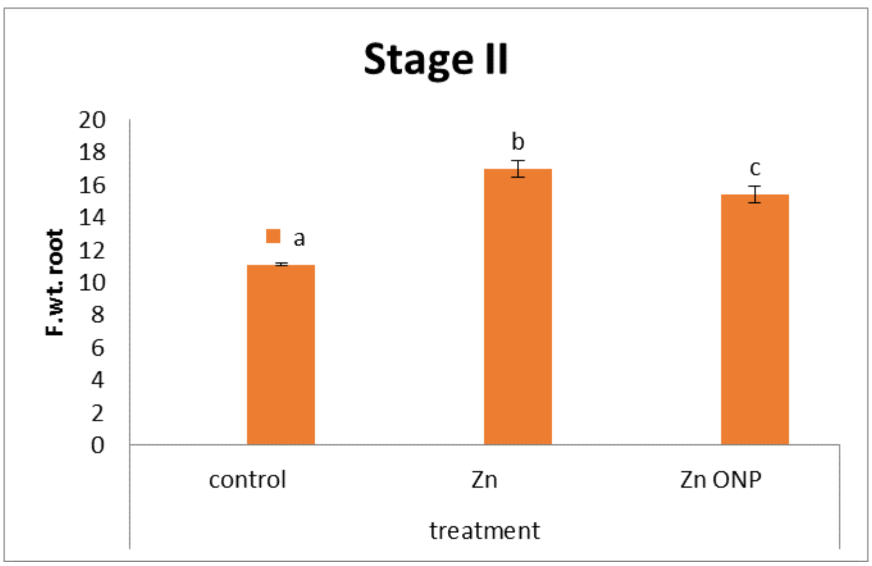
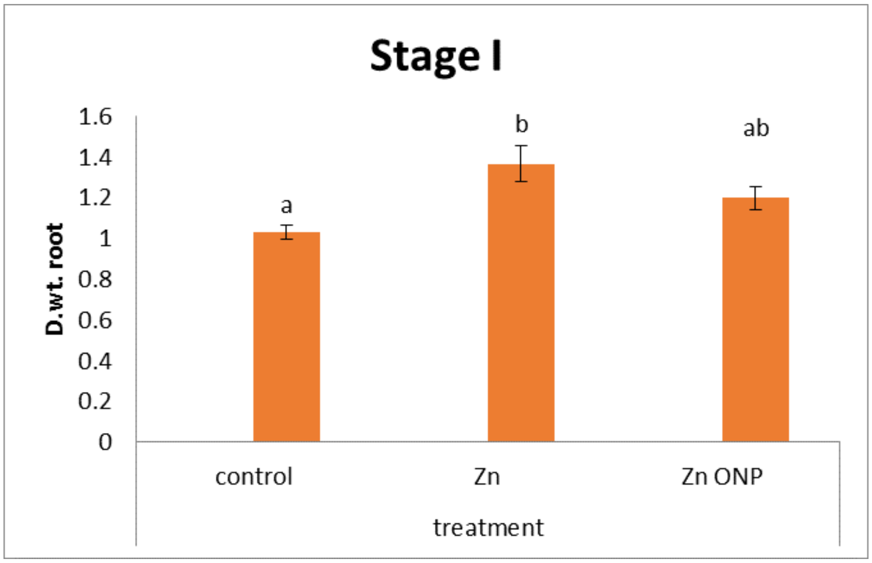


Figure (7): Effect of of Zn, Zn ONP on fresh weights of roots of wheat.



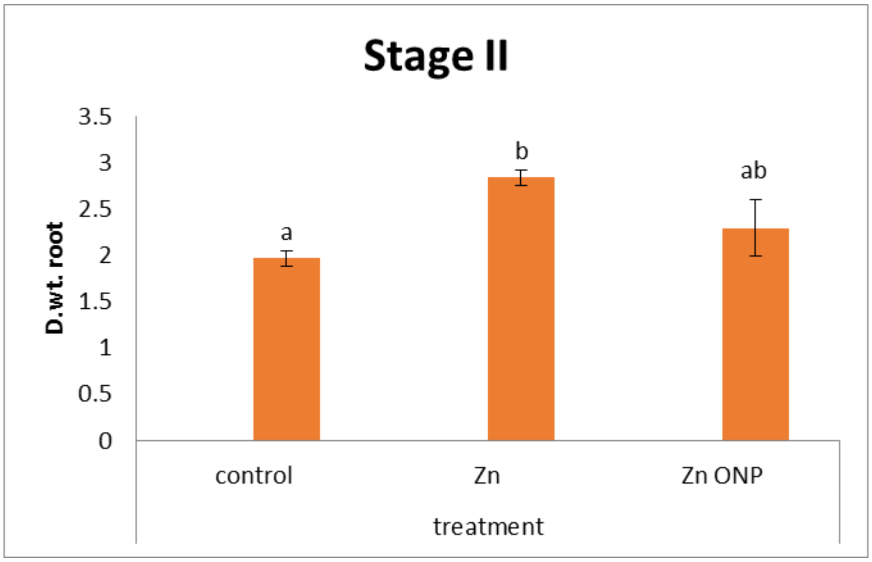


Figure (8): Effect of Zn, Zn ONP on dry weights of roots of wheat.

Table (4): Effect of Zn, Zn ONP on No. of tiller, No. of spikes, No. of grains, weight of grains and weight of 1000 grains of wheat .Each value is mean of 10 replicates ± standard error of means, NS = Non significant, S = Significant and HS = Highly significant.

Treatments	No. of tillers	No. of spikes	No. of grains	weight of grains (gm)	weight of 1000 grains (gm)
Control	5.67±0.33 a	4.10±0.06 a	129.35±0.87 a	5.11±0.06 a	40.79±0.61 a
Zn	6.33±0.33 ab	4.59±0.51 a	168.58±0.97 b	7.55±0.27 b	43.36±0.86 b
Zn ONP	7.33±0.33 b	5.14±0.38 a	159.00±4.36 c	6.87±0.24 b	45.00±0.58 b
F ratio	6.333	1.981	60.644	35.012	9.264
P value	**0.033** ^{S}	**0.219** ^{NS}	**0.000** ^{HS}	**0.000** ^{HS}	**0.015** ^{S}

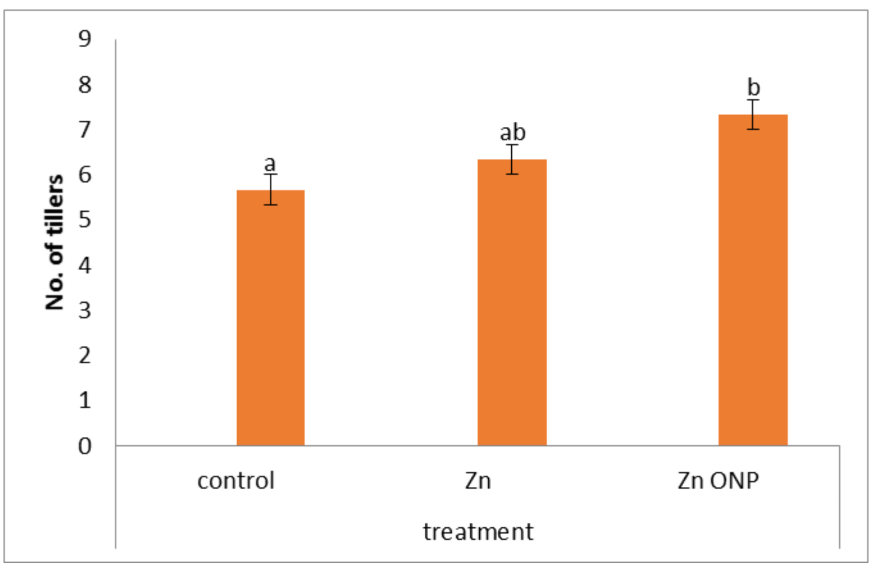


Figure (9): Effect of Zn, Zn ONP No. of tillers of wheat.

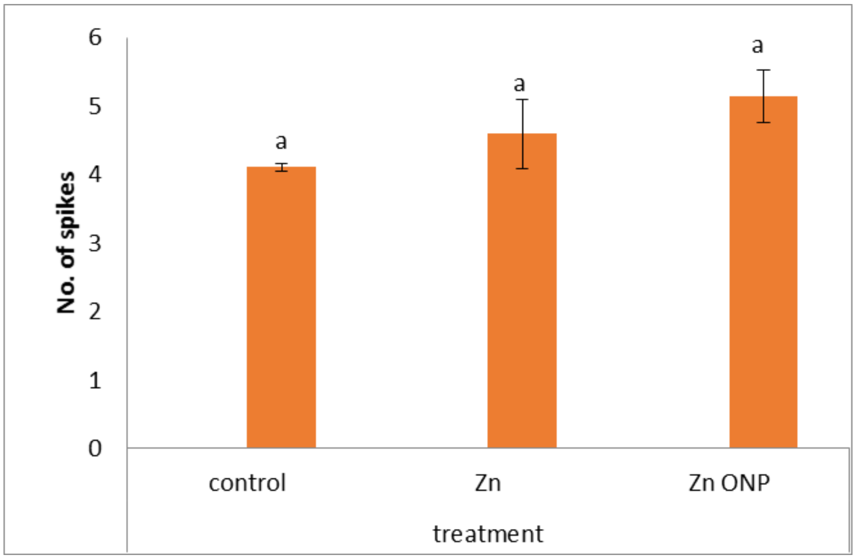


Figure (10): Effect of Zn, Zn ONP on No. of spikes of wheat.

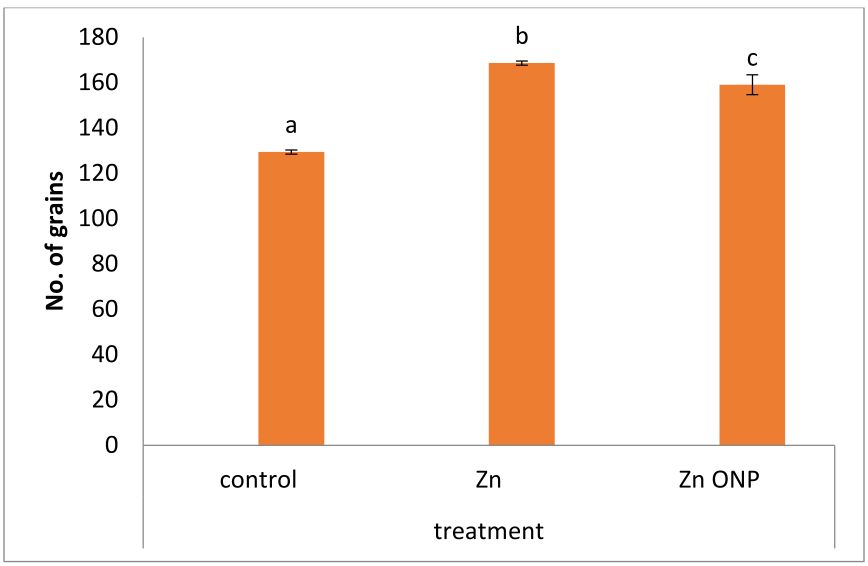


Figure (11): Effect of Zn, Zn ONP on No. of grains of wheat.

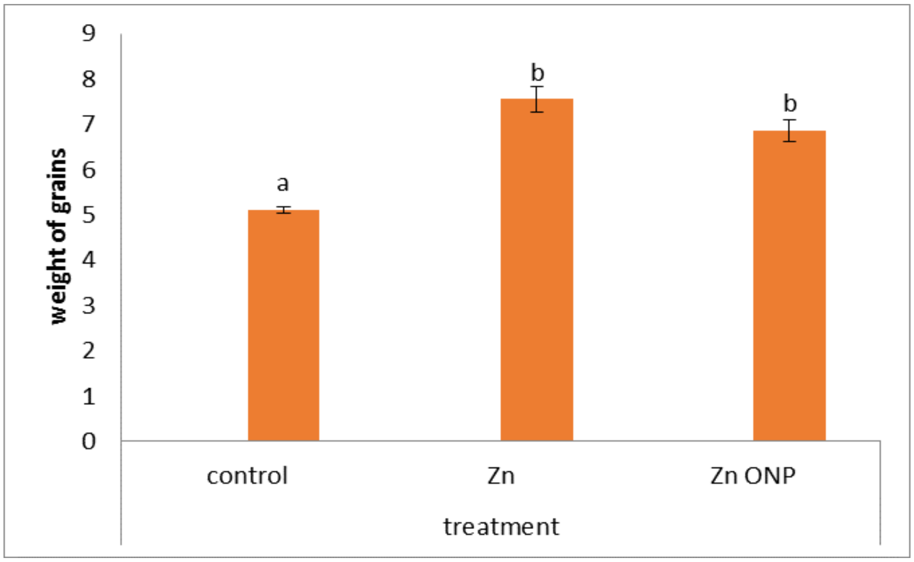


Figure (12): Effect of Zn, Zn ONP on weight of grains of wheat.

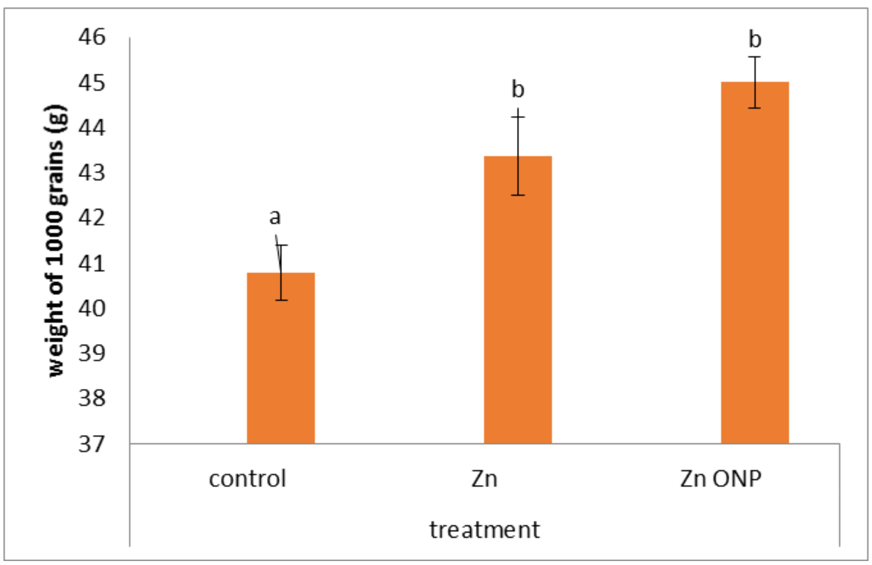


Figure (13): Effect of Zn, Zn ONP on dry weights of 1000 grains of wheat.

CONCLUSION

In conclusion, Zn or ZnONP application improved quality of plant. In general, it is concluded that by using Zinc can vegetative better as compared to with or without using this ZnONP fig (14).

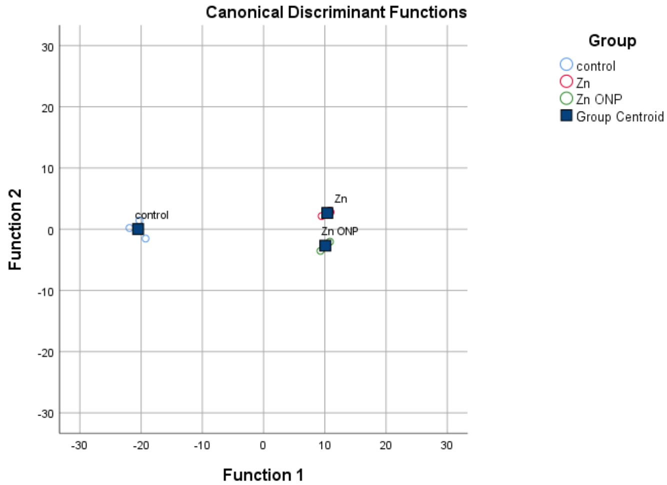


Figure 14. Discriminant of the vegetative of the wheat in response to Zn and ZnONP

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