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Metabolic Changes In Common Bean Plants In Response to Zinc Nanoparticles and Zinc Sulfate

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ABSTRACT

A field experiment was conducted to evaluate the effects of biologically-synthesized zinc oxide nanoparticles (ZnONPs), zinc sulfate ($ZnSO_4$) and their interactions on carbohydrates, proteins, endogenous acidic phytohormones and minerals contents, as well as the activities of some hydrolytic enzymes of common bean (*Phaseolus vulgaris* L.) plants. The results observed that carbohydrate and protein contents in common bean plants were enhanced when the plants treated with ZnONPs (25, 50, 100 and 200 ppm), $ZnSO_4$ (50 and 100 ppm) and their interactions. The tested treatments showed different responses in amylase and protease activities in addition to the stimulation which found in phytohormones (Gibberellic acid, Indole acetic acid and Abscisic acid). These treatments markedly increased nitrogen (N), phosphorus (P) and potassium (K) contents and these increases reached to 45.7 %, 55.6 % and 23.8 %, respectively. Our results obtained that biologically-synthesized zinc oxide nanoparticles have positive effects on carbohydrate, protein, amylase, protease, gibberellic acid (GA_3), indole acetic acid (IAA), abscisic acid (ABA), nitrogen, phosphorus and potassium contents of common bean plants.

Keywords: *Zinc oxide nanoparticles - Zinc sulfate - Phaseolus vulgaris – metabolic activities.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most valuable leguminous crops in many regions of the world, especially in Egypt due to its high nutritive value in both energy and protein contents. It is a primary source of protein in the diet for both local utilization and exportation. Beans are one of the most important seed-pod plants, which have the major contribution in the human diet and provide an important part of human protein requirement. The amount of seed protein in legumes is about 2-4 times of cereal and 10-20 times of tuber plants. It is rich in protein, dietary fibers, nutritional minerals and vitamins with 22-37 % protein and high amino acids **(Kaya *et al.*, 2005)**.

It is necessary to discover new techniques for increasing the crop production. Fertilization including micronutrients is one of these new techniques that are considered more effective on growth, yield and protein content.

Nowadays nanotechnology has expanded horizons in all fields of science. Nanotechnology can be used in crop production to increase yield **(Reynolds, 2002).** Replacing traditional methods of fertilizer application with nano-fertilizers is a way to release nutrients into the plant gradually and in a controlled way **(Naderi and Abedi, 2012).** Transforming materials to a nano scale changes their physical, chemical and biological characteristics as well as affecting catalytic properties. Nanoparticles are atomic aggregations with at least one dimension between 1 to 100 nanometer **(Ball, 2002)**. Nanoparticles exhibit unique chemical and physical features that differ from their bulk forms **(Lin and Xing, 2008)**.

One of the most important usages of nanotechnology in agriculture is using nano-fertilizers for plant nutrition. Nano-agriculture involves the usage of nanoparticles in agriculture with the particles that have specific beneficial effects to the crops **(Morla *et al.*, 2011).** The success of nanoparticles may be due to its great density at reactive areas or increased reactivity of these areas on the particle surfaces. Nano-fertilizers have been developed and have provided a new efficient alternative to normal regular fertilizers. The properties of nanoparticles may help in increasing the reactive points of these particles and hence increase the reactivity of these nanoparticles. This leads to changes in the chemical and physical properties of these nanoparticles, which help in the absorption of fertilizers in plants **(Anonymous, 2009).** Supplying of chemical fertilizers in the form of nanoparticles has recently received a great attention. The results of several studies indicate different responses of plants to particles in the form of nano **(Zhu *et al.*, 2008).** Because of the use of nano fertilizers, the time and speed of the release of elements synchronize and regulate plant nutritional requirements, thus the plant can uptake a large amount of nutritional elements and thus the product yield will increase as well.

Nanoparticles synthesis can be run using a number of routinely used physical and chemical methods. However, altogether, these methods are energy and capital intensive, and they employ toxic chemicals and non polar solvents in the synthesis procedure, thus avoiding their applications in clinical, biomedical and agricultural fields. Therefore, the need for the advancement a clean, reliable, biocompatible and eco-friendly methods for synthesis of nanoparticles leads to turning scientists toward biological methods **(Jain *et al.*, 2011).**

Zinc is an important micronutrient needed in small amounts by crop plants. Zinc plays important roles in various metabolic and physiological processes in the plant, where it activates some enzymes, regulate metabolism of carbohydrates and proteins, which are essential for various processes, critical to development and differentiation of plant cells **(Farahat *et al.*, 2007).** It has been reported that nearly 300 proteins in humans and over 500 proteins in the plant contain zinc as a prosthetic group **(Graham, 2008).** Zinc itself is involved in the formation of IAA hormone **(Choudhary *et al.*, 2015)**. It is well known that zinc acts as a co-factor (activator) of different enzymes (nearly 300) and affects some biological processes **(Baghdady *et al.*, 2014).**

Keeping in mind the above importance, the aim of this study is to assess some metabolic activities and biochemical constituents of common bean (*Phaseolus vulgaris* L.) plants to biologically-synthesized zinc oxide nanoparticles and zinc sulfate.

MATERIALS AND METHODS

I-Materials:

1. Seeds

Seeds of common bean plants (*Phaseolus vulgaris* L. var. *Valentino*) which used for planting, belongs to family Fabaceae (Leguminosae), were obtained from Legume Crops Research Department, Field Crop Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

2. Biologically-synthesized zinc oxide nanoparticles

Zinc oxide nanoparticles are used as nano-fertilizer at a concentration of 25, 50, 100 and 200 mg/l **(Farrag, 2015; Jayarambabu *et al.*, 2015** and **Sanjay *et al.*, 2015)**. Suspension of zinc

oxide nanoparticles was prepared by regular dispersion using ultrasonic vibrator for 30 min. for avoiding aggregation.

3. Zinc

Zinc is used as zinc sulfate ($ZnSO_4$) solution (a common zinc supplement) with the concentration of 50 and 100 mg/l **(Mousa *et al*., 2001; Gamal El-Din, 2005; Kumar *et al*., 2014; Shokr *et al*., 2014** and **Ibrahim and Ramadan, 2015)**.

II-Methods:

1. Green synthesis of ZnONPs from *Ocimum basilicum* leaves:

This method prepared according to **Raut *et al*. (2015)** with slight modifications.

1.1. Preparation of leaves extract of *Ocimum basilicum*:

The leaves of *Ocimum basilicum* plant were collected form Botanical Garden of Al-Azhar University, Nasr city, Cairo, Egypt. For the preparation of leaves extract of *Ocimum basilicum*, leaves were washed many times with water and dry it in sunlight. Then 10 g of dried leaves were taken with 200 ml distilled water in beaker (500 ml). The mixture of leaves and distilled water was boiled for 10 min. until the color of the solution turns into reddish color. The mixture solution was cooled at room temperature. The leaves extract was filtered using filter paper and then stored in refrigerator for the synthesis of ZnO nanoparticles as shown in figure (1).

1.2. Green synthesis of ZnO nanoparticles using a leaf extract of *Ocimum basilicum* plant:

For synthesis of ZnO nanoparticles, 50 ml of *Ocimum basilicum* leaf extract was boiled to 60-80 ℃ using a magnetic stirring heater. Then 2 g of zinc sulfate was added to the leaf extract of *Ocimum basilicum* plant when the temperature reaches to 70 ℃ then boiled it until the reduced deep reddish paste formed. This paste was dried in the dryer at temperature 100-130 ℃ for 40-45 min. Zinc oxide nanoparticles obtained in the form of light yellow colored powder. This powder was mashed in a ceramic mortar pestle to get the finer matter for characterization purpose as shown in figure (2).

1.3. Characterization of zinc oxide nanoparticles

1.3.1. X-ray Diffraction (XRD) Analysis:

The crystalline nature of prepared zinc oxide nanoparticles was observed using X- ray diffractometer (Shimadzu XRD-6000) using Cu Kα radiation, at 40 keV, Current 30 milliampere, Scan Speed 0.02 degree/min.

1.3.2. Transmission Electron Microscopy (TEM):

The size and morphology of the synthesized nanoparticles were recorded by using Transmission Electron Microscopy (TEM), model JEOL electron microscopy JEM-100 CX. TEM studies were prepared by drop coating zinc oxide nanoparticles onto carbon-coated TEM grids. The film on the TEM grids were allowed to dry, the extra solution was removed using a blotting paper.

(a) **(b)** **(c)**

Figure (1): the sample (a) and extract (b & c) of leaves of *Ocimum basilicum* plant

Figure (2): the synthesized-sample of zinc oxide nanoparticles.

2. Experimental design:

Uniform ***"(Phaseolus vulgaris* L. var. *Valentino)"*** seeds were planted in Botanical Garden, Botany and Microbiology Dept., Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt in a plot (4 m. width and 8 m. length) containing 15 ridges (45 cm. width and 3 m. length), representing the different treatments of zinc oxide nanoparticles (25, 50, 100 and 200 ppm), zinc sulfate (50 and 100 ppm) and their interactions. The developed plants were irrigated whenever required. The plants of common bean were treated twice with the above mentioned treatments as the following:-

(1) The first treatment was foliage spraying at 33 days after sowing.

(2) The second treatment was foliage spraying at 44 days after sowing.

The plant samples were collected for analysis when the plants were 41 days after sowing (Stage I), 53 days after sowing (Stage II) and 86 days after sowing (Harvesting or Yield).

3. Metabolic and biochemical tests:

Contents of soluble carbohydrates were extracted according to method of **Said *et al.* (1964)** and determined using anthrone technique according to **Umbriet *et al.* (1969)**. Contents of soluble proteins were estimated according to the methods of **Lowery *et al.* (1951).** Extraction of enzymes was according to **MuKherjee and Choudhuri (1983).** Amylase activity was estimated using a method modified from that described by **Afifi *et al.* (1986)** Protease activity was determined using the method of **Ong and Gauchier (1973).** The method of extraction of endogenous acidic phytohormones was essentially similar to that adopted by **Shindy and Smith (1975)** and described by **Hashem (2006).** To estimate the amounts of acidic phytohormones GA_3, IAA and ABA, the plant hormone fractions and standard ones were methylated according to **Vogel (1975)** to be ready for GC analysis.Plant samples were digested according to a method described by **Chapman and Pratt (1961)**. The determined macro-nutrients were expressed as percentages for N, P and K. Nitrogen was determined by the micro-kjeldahl method, Phosphorus was determined using U.V-visible spectrophotometer (Spectronic 21D) and Potassium was determined by using a flame photometer (Jenway PFP7) according to a method described by **Jackson (1973).** All statistical calculations were done using SPSS (statistical package for the social science version 18) statistical program at the 0.05 level of probability **(Snedecor and Cochran, 1982).** Comparison of percentage was done using the One-way ANOVA and Post hoc-LSD tests.

RESULTS AND DISCUSSION

1- Characterization of ZnONPs.

1-1- X-ray diffraction

The X-ray diffraction (XRD) pattern of the synthesized ZnO nanoparticles was demonstrated in figure (3). The X-ray diffraction data were recorded by using Cu Kα radiation. X-ray diffraction was done to confirm the phase of zinc oxide nanoparticles. The peaks at 2θ values of 31.70°, 34.52°, 36.21°, 47.43°, 57.6°, 62.81°, 67.88° and 69.07° corresponded to the crystal planes of (100), (002), (101), (102), (110), (103), (112) and (202) of zinc oxide nanoparticles and all the diffraction peaks agreed with the reported JCPDS data. The mean grain size (D) of the particles was determined from the XRD line broadening measurement using Scherrer equation:

$$D_p = \frac{K\lambda}{\beta \cos\theta},$$

Where k is the crystalline factor (it was considered as 0.94), λ is the incident X-ray wavelength (Cu K_α= 1.5406 °A), β is full width at half maximum (FWHM) of the peak corresponding to maximum intensity and θ represents the diffraction angle of the most intense peak in degrees. The synthesized materials are in the nanometer range and around 18 nm. The strong and narrow peak denotes that the product has the well crystalline nature of zinc oxide nanoparticles according to standard card (JCPDS 36-1451). These results were coincide with **Raut *et al.* (2015); Senthilkumar (2014)** and **Devi and Gayathri (2014).**

1-2- TEM analysis:

The typical transmission electron micrograph of the phyto-mediated zinc oxide nanoparticles are shown in figure (4). Transmission electron microscope image reported the shape of zinc oxide nanoparticles is spherical particles in nano range (13.4 nm - 24.1 nm), which is in good agreement with the particle size calculated from XRD analysis.

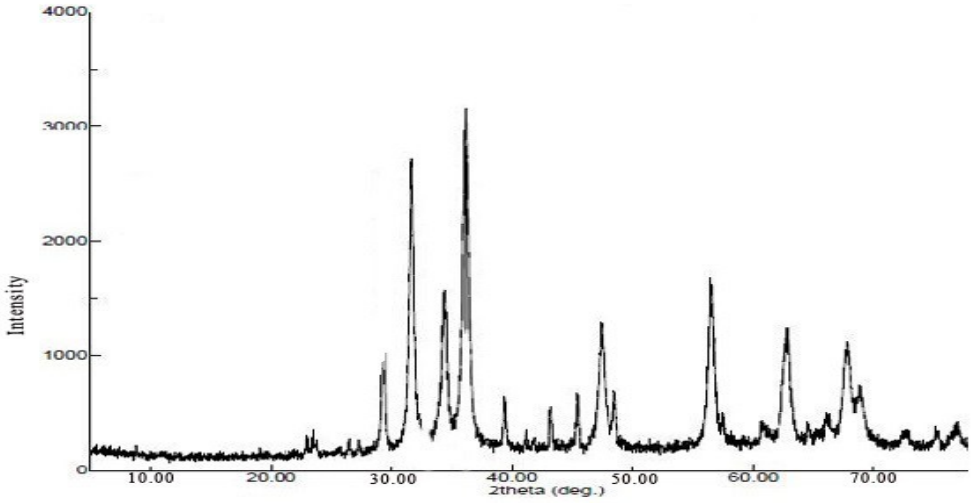

Figure (3): XRD pattern for zinc oxide nanoparticles.

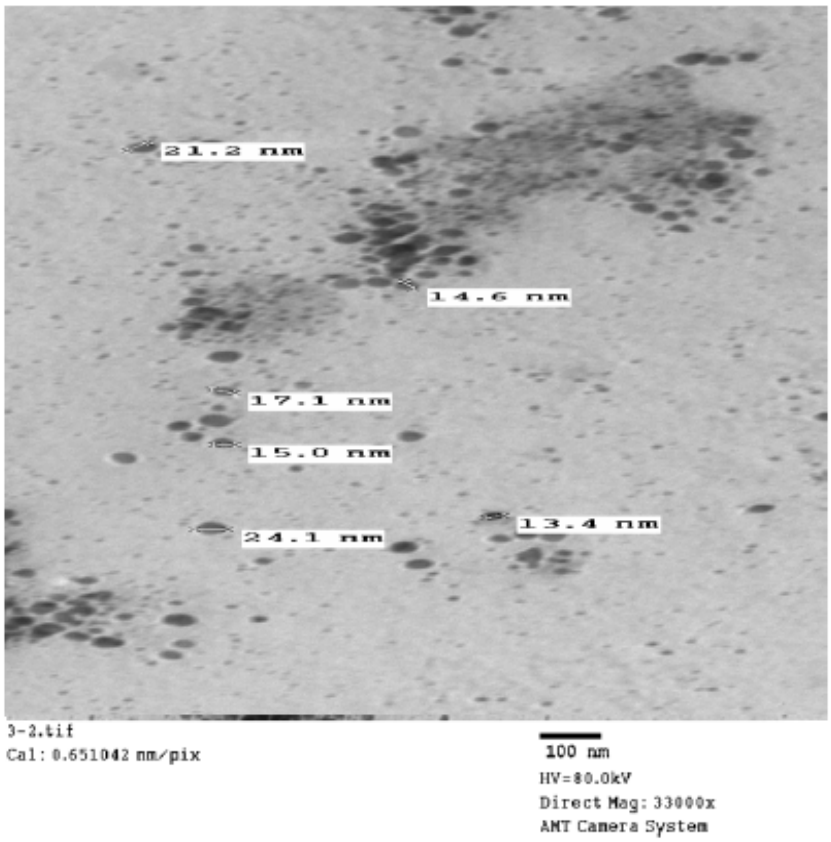

Figure (4): TEM image for zinc oxide nanoparticles.

2- Effect of zinc oxide nanoparticles (ZnONPs), zinc sulfate ($ZnSO_4$) and their interactions on certain metabolic activities of common bean plants

2-1- Soluble carbohydrates:

Contents of soluble carbohydrates in common bean plants were significantly increased (in most cases) as a result of foliar application of different concentrations of ZnONPs (25, 50, 100 or 200 ppm), $ZnSO_4$ (50 or 100 ppm) and their interactions. These results were shown in table (1). In case of shoot carbohydrates, the most significant treatment was ZnONPs 100 ppm + Zn 50 ppm at stage I and ZnONPs 50 ppm + Zn 100 ppm at stage II. In case of carbohydrates contents in root, the most significant treatment was Zn 50 ppm at stage I and ZnONPs 100 ppm + Zn 100 ppm at stage II. With respect to the contents of carbohydrate in seeds, the most effective and significant treatment was ZnONPs 200 ppm.

Our results are agreement with **Soliman *et al.* (2015)** who showed that foliar applications of the moringa *(Moringa peregrina*) plants with nano-zinc at different concentrations (30, 60 and 90 mg/l) led to enhancement in carbohydrates percentage more than those recorded in normal plant conditions (control). Also, results of **Narendhran *et al.* (2016)** indicated that higher carbohydrate contents of *Sesamum indicum* (L.) plants were noted significantly at different concentrations of biologically-synthesized ZnONPs (0.1, 0.25, 0.5, 1 and 2 g/l). Moreover, Results of **Reda *et al.* (2014)** indicated that carbohydrates content of faba bean (*Vicia faba* L.) plants significantly increased in the leaves and the seeds of plants treated with foliar spray of zinc. Also, **Yadavi *et al.* (2014)** found that foliar application of Zn increased soluble carbohydrates of bean (*Phaseolus vulgaris* L.) about 7.9 %. The stimulation in the contents of carbohydrates in response to zinc fertilizer is probably due to the role of zinc in activation of the enzymes that responsible for photosynthesis, biosynthesis and transformation of carbohydrates.

Table (1): Effects of different concentrations of two fertilizers (ZnONPs & $ZnSO_4$) and their interactions on soluble carbohydrate contents (mg/g dry weight) of common bean plants. Each value is a mean of 3 replicates ± standard error of means.

Treatments (ppm)	Shoot carbohydrate		Root carbohydrate		Seeds carbohydrate
	stage I	stage II	stage I	stage II	
Control	27.43 ± 0.86 k	94.02 ± 3.78 d	18.24 ± 0.5 i	62.91 ± 0.2 j	125.75 ± 2.22 f
ZnONPs 25	37.09 ± 0.28 ef	107.36 ± 0.35 b	23.37 ± 0.2 de	92.34 ± 0.33 gh	135.4 ± 5.3 def
ZnONPs 50	32.34 ± 0.08 j	108.66 ± 0.67 b	22.45 ± 0.33 ef	96.25 ± 0.65 ef	138.97 ± 6.18 cde
ZnONPs 100	33.26 ± 0.08 ij	110.19 ± 0.88 ab	23.37 ± 0.28 de	113.03 ± 1.51 b	148.39 ± 2.63 bc
ZnONPs 200	27.74 ± 0.55 k	96.63 ± 0.73 d	22.53 ± 0.27 ef	99.39 ± 1.2 e	163.68 ± 1.74 a
Zn 50	47.74 ± 1.08 b	95.56 ± 1.64 d	29.58 ± 0.2 a	64.6 ± 1.46 j	129.19 ± 2.41 ef
Zn 100	39.31 ± 0.35 d	96.02 ± 1.9 d	28.2 ± 0.08 b	68.35 ± 0.63 i	132.99 ± 2.99 def
ZnONPs 25 + Zn 50	38.62 ± 0.96 de	101.38 ± 1.06 c	19.54 ± 0.13 h	106.82 ± 1.33 c	146.55 ± 1.74 bc
ZnONPs 25 + Zn 100	48.05 ± 0.48 b	96.25 ± 0.28 d	24.37 ± 0.48 cd	90.35 ± 1.13 h	127.24 ± 3.97 f
ZnONPs 50 + Zn 50	36.25 ± 0.6 fg	107.66 ± 1.54 b	21 ± 0.2 g	103.3 ± 0.8 d	151.84 ± 2.27 b
ZnONPs 50 + Zn 100	46.44 ± 0.46 b	114.41 ± 2.26 a	18.77 ± 1 hi	110.88 ± 1.26 b	129.2 ± 4.01 ef
ZnONPs 100 + Zn 50	55.4 ± 0.13 a	96.55 ± 0.53 d	23.07 ± 0.2 e	94.1 ± 0.86 fg	140.34 ± 3.65 cd
ZnONPs 100 + Zn 100	35.02 ± 0.54 gh	94.1 ± 1.14 d	21.53 ± 0.33 fg	117.39 ± 1.16 a	128.73 ± 5.09 ef
ZnONPs 200 + Zn 50	41.46 ± 0.54 c	110.8 ± 0.81 ab	19 ± 0.5 hi	71.34 ± 0.08 i	125.63 ± 1.8 f
ZnONPs 200 + Zn 100	34.41 ± 0.31 hi	102.07 ± 0.35 c	25.13 ± 0.08 c	93.72 ± 1.99 fg	127.59 ± 3.54 f
L.S.D at 0.05	1.64	4.32	1.13	3.18	10.3

Similar symbols have no significant value, but different symbols have significant value.

2-2- Soluble proteins:

Results in table (2) revealed that soluble protein contents in common bean plants were significantly increased by foliar application of ZnONPs, $ZnSO_4$ and their interactions throughout growth stages. In case of shoot protein, the most significant treatment was Zn 50 ppm at stage I and ZnONPs 50 ppm + Zn 50 ppm at stage II. Concerning root protein, the most significant treatment was Zn 50 ppm at stage I and Zn 100 ppm at stage II. The highest value of protein content in seeds were recorded in response to the treatment with ZnONPs 200 + Zn 50 ppm.

These finding are supported by the study of **Baybordy and Mamedov (2010)** who explained that zinc is an important element in enzymes structure involved in amino acid biosynthesis and because amino acids are the base of protein synthesis, protein content increases in the case of using this micronutrient. The experimental results of **Soliman *et al.* (2015)** indicated that foliar applications of the moringa (*Moringa peregrina*) plants with nano-zinc at different concentrations (30, 60 and 90 mg/l) lead to increased crude protein percentage more than those recorded in normal plant conditions. Also, Results of **Narendhran *et al.* (2016)** showed that, *Sesamum indicum* (L.) plants treated with biologically-synthesized ZnO nanoparticles at 0.1, 0.25 and 0.5 g/l showed highest protein content. Also, **Kumar *et al.* (2014)** observed that protein contents was significantly increased by 21% and 69% in the leaves of wheat plants grown in soil amended with Zn at 50 and 100 mg/kg. In addition, **Reda *et al.* (2014)** found significant increases in protein percent in the seeds of faba bean (*Vicia faba* L.) plants obtained at treatment Zn. Recently, **Kobraee and Shamsi, 2015,** reported that application of Zn on soybean (*Glycine max*) plants had the most impact on seed protein contents.

Table (2): Effects of different concentrations of two fertilizers (ZnONPs & $ZnSO_4$) and their interactions on soluble protein contents (mg/g dry weight) of common bean plants. Each value is a mean of 3 replicates ± standard error of means.

Treatments (ppm)	Shoot protein		Root protein		Seeds protein
	stage I	stage II	stage I	stage II	
Control	28.84 ± 0.84 h	51.55 ± 1.59 e	20.7 ± 1.15 d	29.88 ± 1.34 fg	141.86 ± 4.31 g
ZnONPs 25	37.4 ± 0.74 bc	67.64 ± 1.27 ab	26.05 ± 0.31 ab	35.33 ± 0.44 abc	148.18 ± 0.75 de
ZnONPs 50	36 ± 1.28 cd	62.98 ± 1.36 abcd	23.84 ± 0.71 bc	32.65 ± 1.26 cdef	157.25 ± 1.97 ab
ZnONPs 100	35.72 ± 1.01 cde	67.64 ± 2.16 ab	24.19 ± 0.51 bc	30.64 ± 0.77 efg	153.76 ± 1.48 bc
ZnONPs 200	31.26 ± 1.12 fgh	60.27 ± 1.59 cd	23.49 ± 0.65 c	34.49 ± 0.44 bcd	151.39 ± 1.12 cd
Zn 50	41.02 ± 1.28 a	59.69 ± 1.91 cd	27.79 ± 1.11 a	36.33 ± 0.44 ab	145.08 ± 0.65 efg
Zn 100	39.91 ± 1.01 ab	62.6 ± 1.72 abcd	27.44 ± 0.65 a	37.84 ± 0.89 a	143.37 ± 1.26 fg
ZnONPs 25 + Zn 50	31.81 ± 0.97 fgh	62.02 ± 3.45 bcd	21.98 ± 0.4 cd	33.32 ± 0.44 cde	146.66 ± 1.21 ef
ZnONPs 25 + Zn 100	31.81 ± 1.28 fgh	63.95 ± 1.78 abc	23.49 ± 0.31 c	30.31 ± 0.6 fg	153.95 ± 0.78 abc
ZnONPs 50 + Zn 50	32.65 ± 0.48 efg	68.02 ± 1.54 a	22.67 ± 1.76 cd	32.15 ± 0.77 def	143.37 ± 0.88 fg
ZnONPs 50 + Zn 100	31.26 ± 0.74 fgh	57.56 ± 0.89 d	24.42 ± 1.01 bc	26.62 ± 1.26 h	157.64 ± 0.81 ab
ZnONPs 100 + Zn 50	33.49 ± 1.28 def	62.6 ± 2.47 abcd	22.44 ± 0.71 cd	31.81 ± 1.1 def	154.38 ± 0.77 abc
ZnONPs 100 + Zn 100	29.58 ± 1.48 gh	63.95 ± 3.2 abc	22.79 ± 0.81 cd	30.98 ± 1.02 ef	153.95 ± 0.84 abc
ZnONPs 200 + Zn 50	32.65 ± 0.48 efg	66.67 ± 1.36 ab	22.33 ± 0.92 cd	30.81 ± 1.77 ef	158.33 ± 1.23 a
ZnONPs 200 + Zn 100	28.86 ± 1.91 h	63.37 ± 1.46 abc	22.21 ± 0.42 cd	27.96 ± 0.73 gh	146.32 ± 1.04 efg
L.S.D at 0.05	3.24	5.69	2.45	2.79	4.47

Similar symbols have no significant value, but different symbols have significant value.

2-3- Enzyme activities (Amylase and Protease):

Regarding the activity of amylase, results in table (3) showed that amylase activities in common bean plants were increased significantly (with some exceptions) at stage I and stage II of common bean plants due to the treatment with the different concentrations of ZnONPs and $ZnSO_4$ and the most effective treatment was ZnONPs 200 ppm + Zn 100 ppm at stage I and ZnONPs 100 ppm + Zn 100 ppm at stage II.

With respect to activity of protease, it was observed in table (3) that protease activities in common bean plant were influenced in response to the application of ZnONPs (25, 50, 100 or 200 ppm), $ZnSO_4$ (50 or 100 ppm) and their interactions during growth stages. In case of stage I the most significant increase was recorded with the treated plants with ZnONPs 50 ppm but the most significant decrease was recorded with ZnONPs 25 ppm + Zn 100 ppm. In case of stage II the different concentrations of the tested treatments (singly or in interactions) significantly decreased the activities of protease. Many studies have shown the stimulatory effects of zinc application regarding the activity of enzymes among of them; **Tobbal (2006)** found that the activities of amylases and proteases in Celosia plants were significantly increased in response to the application of zinc. Also, **Tajlil *et al.* (2014)** found that zinc application was significantly increased the activities of amylase in chickpea (Cicer ariegtinum) plants. In this respect, it is known that zinc is structurally linked to some important plant enzymes, the activities of which are negatively influenced under zinc-deficient conditions **(Hacisalihoglu *et al.*, 2003)**. It is the needed in the different six classes of enzyme, which include oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases **(Auld, 2001).**

Table (3): Effects of different concentrations of two fertilizers (ZnONPs & $ZnSO_4$) and their interactions on amylase and protease activities (mg/g fresh weight) of common bean plants. Each value is a mean of 3 replicates ± standard error of means.

Treatments (ppm)	amylase		protease	
	stage I	stage II	stage I	stage II
Control	0.86 ± 0.22 i	2.53 ± 0.04 def	0.52 ± 0.11 bcd	0.163 ± 0.006 a
ZnONPs 25	1.66 ± 0.05 defg	2.46 ± 0.046 ef	0.54 ± 0.05 bc	0.145 ± 0.003 b
ZnONPs 50	2.01 ± 0.09 abc	2.92 ± 0.065 ab	0.82 ± 0.06 a	0.151 ± 0.008 ab
ZnONPs 100	1.49 ± 0.02 g	2.74 ± 0.094 bcd	0.74 ± 0.1 ab	0.145 ± 0.004 b
ZnONPs 200	1.88 ± 0.09 bcde	2.96 ± 0.054 a	0.71 ± 0.04 ab	0.146 ± 0.005 b
Zn 50	1.14 ± 0.07 h	2.83 ± 0.037 abc	0.38 ± 0.05 cde	0.152 ± 0.004 ab
Zn 100	1.87 ± 0.07 bcde	2.91 ± 0.074 ab	0.43 ± 0.06 cde	0.145 ± 0.006 b
ZnONPs 25 + Zn 50	1.78 ± 0.14 cdef	2.87 ± 0.057 ab	0.8 ± 0.05 a	0.143 ± 0.005 b
ZnONPs 25 + Zn 100	1.61 ± 0.05 efg	2.64 ± 0.074 cde	0.2 ± 0.05 e	0.138 ± 0.009 b
ZnONPs 50 + Zn 50	2.13 ± 0.04 ab	2.98 ± 0.031 a	0.29 ± 0.09 de	0.14 ± 0.003 b
ZnONPs 50 + Zn 100	1.95 ± 0.01 abc	2.4 ± 0.063 f	0.44 ± 0.13 cd	0.139 ± 0.005 b
ZnONPs 100 + Zn 50	1.58 ± 0.12 fg	2.58 ± 0.064 def	0.47 ± 0.02 cd	0.139 ± 0.009 b
ZnONPs 100 + Zn 100	1.92 ± 0.05 bcd	3.02 ± 0.011 a	0.52 ± 0.11 bcd	0.138 ± 0.003 b
ZnONPs 200 + Zn 50	1.85 ± 0.13 cdef	2.52 ± 0.194 def	0.3 ± 0.03 de	0.142 ± 0.007 b
ZnONPs 200 + Zn 100	2.21 ± 0.08 a	2.53 ± 0.078 def	0.43 ± 0.13 cde	0.14 ± 0.003 b
L.S.D at 0.05	0.28	0.221	0.23	0.64

Similar symbols have no significant value, but different symbols have significant value.

2-4- Endogenous acidic phytohormones:

Results in figures (5) showed the effect of different concentrations of ZnONPs and $ZnSO_4$ and their interactions on the contents of endogenous acidic phytohormones (GA_3, IAA and ABA) in terminal buds of shoots of common bean (*Phaseolus vulgaris*) plants during stage II of plant growth. It was observed that the contents of endogenous acidic phytohormones (GA_3, IAA as well as ABA) were markedly stimulated due to the different concentrations of ZnONPs and $ZnSO_4$ (singly or in interaction) and the highest result was recorded when the plants treated with ZnONPs 200 ppm.

The higher promoting phytohormones content in response to the application of zinc may be due to the fact that zinc has an effect on building up and biosynthesis the natural auxin (indole acetic acid). It is obvious that when a plant will get sufficient nutrient to promote its growth, phytohormone concentration will increase i.e. nano ZnO at particular concentration helps in the secretion of growth hormones (**Pandey *et al.*, 2010**). Zinc itself is involved in the formation of indol acetic acid hormone **(Choudhary *et al.*, 2015)**. Exposure of *Lemna gibba* (L.) plants to ZnONPs at 25, 50 and 100 mg/l resulted in general increase of gibberellins (GA_s) and indole-3-acetic acid (IAA) contents as compared to control samples **(Farrag, 2015). Agamy *et al.* (2004)** worked on marigold (*Calendula officinalis* L.) plants. They found that the contents of auxins in plant were significantly increased when plants treated with Zn (50, 100 and 200 mg/l). Also, **Tobbal (2006)** reported that zinc treatments greatly increased contents of indol-3-acetic acid and gibberellic acid in both Celosia and Zinnia plants while contents of abscisic acid were decreased especially at the lower dose of zinc (200 ppm). Zinc treatments significantly increased IAA and GA_3 contents of snap bean (*Phaseolus vulgaris* L.) plants (**El-Tohamy and El-Greadly 2007**).

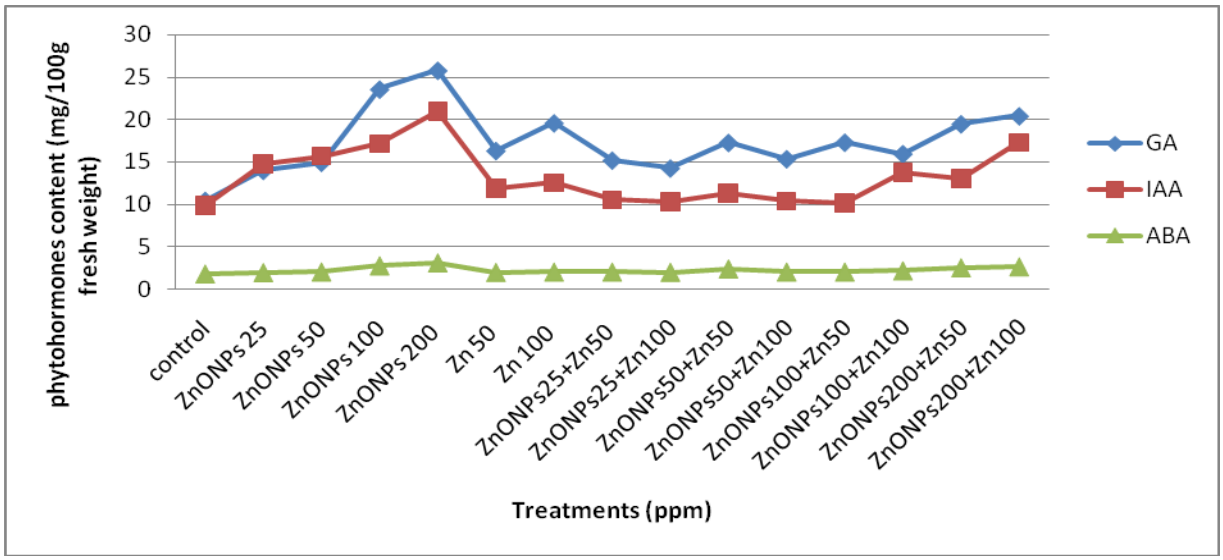

Figure (5): Effect of zinc oxide nanoparticles (ZnONPs), zinc sulfate ($ZnSO_4$) and their interactions on Gibberellic acid (GA_3), Indol acetic acid (IAA) and Abscisic acid (ABA) (mg/100g fresh weight) activity of common bean plants at stage II.

2-5- Nitrogen (N), Phosphorus (P) and Potassium (K) content:

Treatment of common bean plants with the different concentrations of ZnONPs or $ZnSO_4$ (individually or in interaction) observed increases in nitrogen, phosphorus and potassium percentage in the yielded-seeds (Figure 6). The results showed that the application of the tested

treatments enhanced nitrogen contents in the seeds of common bean plants and the treatment ZnONPs 200 ppm recorded the highest percentage (45.7 %). In case of phosphorus, the highest value (55.6 %) was recorded in response to the treatment with ZnONPs 25 ppm + Zn 100 ppm. With respect to potassium, the treatment ZnONPs 25 ppm + Zn 50 ppm recorded the highest percentage (23.8 %).

In this concern, **El-Tohamy and El-Greadly (2007)** indicated that zinc treatments improved quality and nutritional values of yield of snap bean (*Phaseolus vulgaris* L.) plants. Also, Application of Zn on groundnut (*Arachis hypogaea* L.) plants significantly increased N and K content in the seed **(El-Habbasha *et al.*, 2013)**. Moreover, Nitrogen (N), phosphorus (P) and potassium (K) concentrations in dry bean (*Phaseolus vulgaris* L.) were increased by zinc treatments (at 100 ppm, as zinc sulfate) as compared with the control treatment **(Ibrahim and Ramadan, 2015)**. Recently, Results of **El-Habbasha (2015)** illustrated that nitrogen, phosphorus and potassium seed content were affected when plants of groundnut (*Arachis hypogaea* L.) treated with Zn. Furthermore, Results of **Vafa *et al.* (2015)** indicated that effect of Zn nano-fertilizer (50, 100, 200 mg/l) on phosphorus content of savory (*Satureja hortensis* L.) plants was significant.

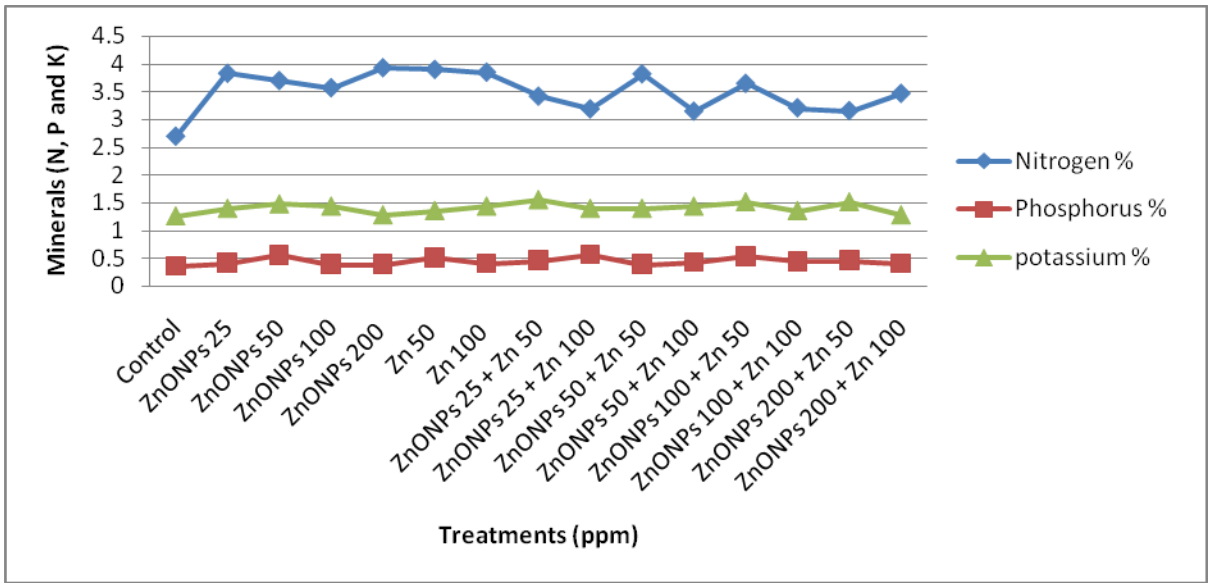

Figure (6): Effect of zinc oxide nanoparticles (ZnONPs), zinc sulfate ($ZnSO_4$) and their interactions on nitrogen (N), phosphorus (P), and potassium (K) contents (%) of seeds common bean plants.

CONCLUSION

The present study exhibited green method for the preparation of zinc oxide nanoparticles by using leaves extract of *Ocimum basilicum* (L.). Green synthesis methods are simple, clean, non toxic, rapid, inexpensive and eco-friendly method. The crystalline nature of nanoparticles was confirmed with X-Ray diffraction technique. The transmission electron microscope image showed spherical shape of zinc oxide nanoparticles with diameter range 13.4 - 24.1 nm. It was established that application of zinc oxide nanoparticles or zinc sulfate have great impact on metabolic mechanism for improving nutritional value of common bean plants. Application of zinc oxide nanoparticles or zinc sulfate (singly or in interaction) on common bean (*Phaseolus*

vulgaris L. var. *Valentino*) plants observed significantly increases in carbohydrate and protein contents. Amylase activity was increased significantly during growth stages while protease activity was increased at stage I and decreased at stage II. The different concentrations of the tested treatments observed markedly stimulation and enhancement in the phytohormonal contents {gibberellic acid (GA_3), indole acetic acid (IAA), abscisic acid (ABA)}. The results also showed different increases in nitrogen, phosphorus and potassium contents. Finally, biologically-synthesized zinc oxide nanoparticles had significant effects and positive applications in biological fields.

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REFERENCES

Afifi, W.M.; Ahmed, M.I.; Moussa, Z.A. and Abd El-Hamid, M.F. (1986): Effect of gamma irradiation and GA_3 on amylase activity of pea seedlings. Ann. Agric. Sci., Moshtohor, Vol. 24(4): 2047-2057.

Agamy, R.A.; Medani, R.A.; El-Yazal, M.A. and Mohamed, S.A. (2004): Effect of some micronutrients on botanical characters and chemical constituents of marigold plant (*Calendula officinalis* L.) Egypt. J. Appl. Sci.; 19(3B): 497-518.

Anonymous (2009): Nano technology in agriculture. J. Agric. Technol., 114: 54-65.

Auld, D.S. (2001): Zinc coordination sphere in biochemical zinc sites. Biometals, 14: 271-313.

Baghdady, G.A., Abdelrazik, A.M., Abdrabboh, G.A., Abo-Elghit, A.A. (2014): Effect of foliar application of GA3 and some nutrients on yield and fruit quality of Valencia orange trees. Nature and Science, 12(4): 93-100.

Ball, P. (2002): Natural strategies for the molecular engineer. Nanotechnology 13: 15-28.

Baybordy, A. and Mamedov, G. (2010): Evaluation of application methods efficiency of zinc and iron for canola (*Brassica napus* L.). Notulae Scientia Biologicae, 2(1): 94-103.

Chapman, H.D. and Pratt, R.E. (1961): Methods of Analysis for Soil, Plants and Water. Dept. of Soil, Plant Nutrition, Univ. of California, U.S.A.

Choudhary, H.D.; Sharma S.R.; Jat R.S. and Jat G. (2015): Effect of soil and foliar application of zinc and iron on yield, quality and economics of fennel. Annals of Plant and Soil Research, 17(2): 200-203.

Devi, R.S. and Gayathri R. (2014): Green synthesis of zinc oxide nanoparticles by using *Hibiscus rosa-sinensis*. Intern. J. of Current Engineering and Technology, 4(4): 2444-2446

El-Habbasha, S.F.; Taha, M.H. and Jafar, N.A. (2013): Effect of nitrogen fertilizer levels and zinc foliar application on yield, yield attributes and some chemical traits of groundnut. Res. J. Agric. & Biol. Sci., 9(1): 1-7.

El-Habbasha, S.F. (2015): Impact of nitrogen fertilizer and zinc foliar application on growth, yield, yield attributes and some chemical constituents of groundnut. International journal of plant & soil science. 4(3): 259-264.

El-Tohamy, W.A. and El-Greadly, N.H.M. (2007): Physiological responses, growth, yield and quality of snap beans in response to foliar application of yeast, vitamin E and zinc under sandy soil conditions. Australian Journal of Basic and Applied Sciences, 1(3): 294-299.

Farahat, M.M.; Ibrahim, S.; Taha, L.S. and El-Quesni, F.E.M. (2007): Response of vegetative growth and some chemical constituents of *Cupressus sempervirens* L. to foliar application of ascorbic acid and zinc at Nubaria. World J. Agric. Sci., 3(4): 496-502.

Farrag, H.F. (2015): Evaluation of the growth responses of *Lemna gibba* L. (Duckweed) exposed to silver and zinc oxide nanoparticles. World Applied Sciences Journal, 33 (2): 190-202.

Gamal El-Din, K.M. (2005): Physiological response of fenugreek plant to heat hardening and zinc. Egypt. J. of Appl. Sci., 20(6B): 400-411.

Graham, R.D. (2008): Micronutrient deficiencies in crops and their global significance. In: micronutrient deficiencies in Global Crop Production (Ed Alloway, B.J). Springer. pp. 41-61.

Hacisalihoglu, G.; Hart, J.J.; Wang, Y.H.; Cakmak, I. and Kochian, L.V. (2003): Zinc efficiency is correlated with enhanced expression and activity of zinc-requiring enzymes in wheat. Plant Physiol., 131(2): 595–602.

Hashem, H.A. (2006): Physiological and molecular actions of jasmonic acid on soybean plant. Ph.D Thesis, Fac. of Sci., Ain Shams Univ., Cairo, Egypt.

Ibrahim, E.A. and Ramadan W.A. (2015): Effect of zinc foliar spray alone and combined with humic acid or/and chitosan on growth, nutrient elements content and yield of dry bean (*Phaseolus vulgaris* L.) plants sown at different dates. Scientia Horticulturae, 184: 101-105.

Jackson, M.L. (1973): Soil chemical Analysis. Prentice Hall of Englewood cliffs, New Jersey, USA.

Jain, N.; Bhargava, A.; Majumdar, S. and Panwar, J. (2011): Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: A mechanism prospective. Nanoscale, 3:2, 635−641.

Jayarambabu, N.; Kumari, B.S.; Rao, K.V. and Prabhu, Y.T. (2015): Beneficial role of zinc oxide nanoparticles on green crop production. International journal of multidisciplinary advanced research trends. 2(1): 273-282.

Kaya, M.; Atak, M.; Khawar, K.M.; Çiftçi, C.Y. and Özcan S. (2005): Effect of pre-sowing seed treatment with zinc and foliar spray of humic acids on yield of common bean (*Phaseolus vulgaris* L.). Int. J. Agri. Biol., 7(6): 875-878.

Kobraee S. and Shamsi K. (2015): Relationships between oil, protein and dry matter in soybean seed with some of micronutrients fertilization. Res. J. Soil Biol., 7(2): 56-63.

Kumar, R.; Giri, P.; Singh, S. and Sharma, Y. (2014): Effect of Zn and Cu application on growth, yield and biochemical responses of wheat plants (*Triticum aestivum* L. cv-PBW-343). J. Biol. Chem. Research. Vol. 31(2): 953-961.

Lin, D. and Xing, B. (2008): Root uptake and phytoxoxicity of ZnO nanoparticles. *Environmental Science & Technology* 42: 5580-5585.

Lowery, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with the folin reagent. J. Biol. Chem., 193: 265-275.

Morla, S.; Rao, C.S.V.R. and Chakrapani, R. (2011): Factors affecting seed germination and seedling growth of tomato plants cultured in vitro conditions. J. Chem. Bio. Phy. Sci 2011, 1 (2), 328-334.

Mousa, G.T.; I.H. El-Sallami and E.F. Ali, (2001): Response of *Nigella sativa* L. to foliar application of gibberellic acid, benzyladenine, iron and zinc. Assiut J. Agric. Sci., 32(2): 141 156.

Mukherjee, S.P. and Choudhuri, M.A. (1983): Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant., 58(2):166-170.

Naderi, M.R. and Abedi, A. (2012): Application of nanotechnology in agriculture and refinement of environmental pollutants. Journal of Nanotechnology, 11(1): 18-26.

Narendhran, S.; Rajiv, P. and Sivaraj, R. (2016): Influence of zinc oxide nanoparticles on growth of *Sesamum indicum* L. in zinc deficient soil. International Journal of Pharmacy and Pharmaceutical Sciences, 8(3): 365-371.

Ong, P.S. and Gauchier, G.M. (1973): Protease production by thermophilic fungi. Can. J. Microbiol., 19: 129-133.

Pandey, A.C.; Sanjay, S.S. and Yadav, R.S. (2010): Application of ZnO nanoparticles in influencing the growth rate of *Cicer arietinum*. J Exp Nanoscience, 5(6): 488–497

Raut, S.; Thorat, P.V. and Thakre, R. (2015): Green synthesis of zinc oxide (ZnO) nanoparticles using *Ocimum tenuiflorum* leaves. International Journal of Science and Research, 4(5): 1225-1228.

Reda, F.; Abdelhamid, M.T. and El-Lethy, S.R. (2014): The role of Zn and B for improving *Vicia faba* L. tolerance to salinity stress. Middle East Journal of Agriculture Research, 3(4): 707-714.

Reynolds, G.H. (2002): Forward to the future nanotechnology and regulatory policy. Pacific Research Institute, 24: 1-23.

Said, A.; Naguib, M.I. and Ramzy, M.A. (1964): Sucrose determination as a mean of estimations of the "Drow Back Tax" on exported Halawa Tehinia., Bull. Fac. Sci., Cairo Univ., 39: 209.

Sanjay, S.S.; Pandey, A.C.; Singh, M. and Prasad, S.M. (2015): Effects of functionalized Zno nanoparticles on the phytohormones: growth and development of *Solanum melongena* L. (brinjal) plant .World Journal of Pharmaceutical Research. 4(5): 1990-2009

Senthilkumar, S.R. (2014): Green synthesis of silver (ag) and zinc oxide (Zno) nanoparticles and studies on phytochemicals, antioxidant and antimicrobial potentials of indian green teas (C*amellia sinensis* L. Kuntze). Ph.D. Thesis, Department of Botany, Annamalai University, Annamalai nagar, Tamilnadu, India.

Shindy, W.W. and Smith, O. (1975): Identification of plant hormones from cotton ovules. Plant Physiol., 55: 550-554.

Shokr, M.M.B.; Elsaid, M.E. and Shafeek, M.R. (2014): Effect of some stimulative substances as foliar applications on snap bean (*Phaseolus vulgaris* L.) productivity under milder thermo-stress of local summer season. Middle East Journal of Applied Sciences, 4(2): 175-180.

Snedecor, G.M. and Cochran, W.G. (1982): Statistical methods. 7th edition, lowa state Univ., Press, Ames., lowa U.S.A., pp. 325-330.

Soliman, A.S.; El-feky, S.A. and Darwish, E. (2015): Alleviation of salt stress on *Moringa peregrina* using foliar application of nanofertilizers. Journal of Horticulture and Forestry, 7(2): 36-47.

Tajlil, A.H.; Pazoki, A. and Asli, D.E. (2014): Effects of seed priming by mannitol and zinc sulfate on biochemical parameters and seed germination of chickpea. International Journal of Farming and Allied Sciences (IJFAS), 3(3): 294-298.

Tobbal, Y.F.M. (2006): Physiological studies on the effect of some nutrients and growth regulators on plant growth and metabolism. Ph.D. Thesis, Fac. Sci. Al-Azhar Univ.

Umbriet, W.W.; Burris, R.H.; Stauffer, J.F.; Cohen, P.P.; Johsen, W.J.; Lee page, G.A.; Patter, V.R. and Schneicter, W.C. (1969): Manometric techniques, manual describing methods applicable to the studs of tissue metabolism. Burgess publishing co., U.S.A; pp. 239.

Vafa, Z.N.; Sirousmehr, A.R.; Ghanbari, A.; Khammari, I. and Falahi, N. (2015): Effects of nano zinc and humic acid on quantitative and qualitative characteristics of savory (*Satureja hortensis* L.). International Journal of Biosciences, Vol. 6(3): 124-136.

Vogel, A.J. (1975): A text book of practical organic chemistry, 3rd ed., English Language Book Society and Longman Group Ltd., pp: 843-845.

Yadavi, A.; Aboueshaghi, R.S.; Dehnavi, M.M. and Balouchi, H. (2014): Effect of micronutrients foliar application on grain qualitative characteristics and some physiological traits of bean (*Phaseolus vulgaris* L.) under drought stress. Indian Journal of Fundamental and Applied Life Sciences, 4(4): 124-131.

Zhu, H.; Han, J.; Xiao, J.Q. and Jin, Y. (2008): Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J. Environ. Monit., 10: 713–717.