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Effect of Different Concentrations from Zinc Oxide Nanoparticles Prepared in Date Pits Extract on Pea (*Pisum sativum* L.) Plant

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Abstract

A field experiments were conducted to evaluate the effects of green synthesis of zinc oxide nanoparticles (ZnO NPs), that prepared in the presence of date palm pits extract (DPPE) through two successive seasons 2018-2019 and 2019-2020 at Agricultural Experimental Research Station of Tag El-Ezz, Agricultural Research Center, Dakahlia Governorate, Egypt, to assay the response of pea plant (*Pisum sativum* L.) cv. Master-B cultivars to foliar spray by different zinc oxide nanoparticles concentrations (20, 40, 60, 80, 100, 200, 400, 500, 1000 mgl^{-1}) compared with spraying with zinc sulfate 1000 mgl^{-1}as control treatment. The field experiment was carried out in a randomized complete block design with three replicates. The results showed that plants spraying with nano zinc oxide with different concentrations gave various responses compared with those spraying with zinc sulfate. Spraying with 100 mgl^{-1} was significantly improved the growth attributes of pea plant through two seasons. While treating pea plants with 200 mgl^{-1} ZnO NPs improved weight of green pods per hectare and weight of 100 seeds during two seasons. Additionally, their positive stimulation for mineral contents, carbohydrates and protein of pea seeds.

Keywords: Nano zinc oxide; Date pits extract; *Pisum sativum*; Pod yield

1. Introduction

After the common bean, the pea (*Pisum sativum* L.) is the second most important legume crop [1]. It is one of Egypt's most important winter crops, both for domestic and export markets. Pea seeds, whether dried or fresh, have significant nutritional value due to their high protein, carbohydrate, and vitamin and mineral content [2] which make it an important for human, animal nutrition and in industrial use [3]. In Egypt, The total cultivated area that grown with peas gradually increased to 20592 hectare in 2018 produced about 9.846 tons ha^{-1} [4].

Nutrient elements play an essential role through plant growth and for adaptation to the environment [5-6]. Similar to macroelements, microelements are also needed for plant growth [7]. They are the components and prosthetic groups of numerous enzymes and would lead to increase plant yield and chemical constituents (such as carbohydrates protein and oil) [8, 9]. In the absence of microelements, young leaves and stems will grow slowly, with yellowing leaves and causing falling off leaves and mottled patches on the leaves [5, 10]. Zinc is fundamental micronutrient for plants, animals and human. After iron, zinc (Zn) is the second most prevalent transition metal in organisms, and it is the only metal found in all six enzyme groups (oxidoreductases, hydrolases, lyases, transferases, isomerases and ligases) [11]. Zinc is responsible in protecting and keeping structural stability of cell membranes [12, 13]. It has an effective role in protein synthesis, cell elongation and membrane function [13]. Also, Zinc is required for chlorophyll production and has vital role in biomass production [14]. Tolerance to environmental stresses like drought stress [15]. One pathway of direct micronutrient (Zn) application is foliar fertilization. Deficiency of zinc causes: stunting (reduced height), interveinal chlorosis (yellowing of the leaves between the veins), bronzing of chlorotic leaves, small and abnormally shaped leaves and/or stunting of leaves. Moreover, plant yields can be reduced by 20% or more without visible

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symptoms. This is called hidden, latent or subclinical deficiency. Zinc-deficient soils causing hidden deficiency may not discover for many years unless soil or plant samples are analysed [16].

Agricultural scientists are facing various challenges like decreasing crop yields, low nutrient use efficiency, decreasing soil organic matter, multi-nutrient deficiencies, climate change and water availability. So many researchers in agriculture field attend to face these challenges by using new technology. Present days nano-fertilizers consider as alternatives to traditional fertilizers for enhancing yield, nutrients in soils and minimizing utilization of chemical fertilizers [15, 14]

The usage of nano fertilizers improves crop efficiency, reduces soil toxicity and reduces the negative effects of overdosing with traditional fertilizers. Hence, nanotechnology holds a lot of promise for attaining sustainable agriculture, particularly in developing countries [17]. The foliar application of Zn is an effective way to promote the absorption of Zn in plants. However, the soluble salts of Zn might damage the leaf and its cost is very high [18]. While ZnO NPs are regarded a safe substance for biological species because of their efficiency in promoting seed germination and plant growth, as well as in disease suppression and plant protection due to their antimicrobial activity [19]. Also, foliar nanofertilizers can be more effective than classical foliar fertilizers, since their release slower and more gradual [20]. However, it is not specified whether this effect is related to the nanoparticle's absorption or the dissolution of its products [21]. Biosynthesis of nanoparticles is a kind of bottom up approach in which the main reaction occurring is reduction/oxidation. The reduction of metal compounds into nanoparticles is mainly caused by plant extracts with anti-oxidant or reducing properties. Chemical methods for preparation of nano particles lead to the presence of some toxic chemicals that adsorbed on the surface and had bad effects in medical applications [22]. But, in case of green synthesis of metal oxide using extract of date palm pits which works as a capping agent that prevents nano zinc oxide coagulation and eco-friendly method which consider as alternative method than chemical [23].

Date palm is one of the oldest tree that grown in the world. All parts of the date palm are used for a purpose that suitable for human being. Besides fruit, date palm contains a lot of products which used in our life such as date pits which represented about (10–15%) of the total date fruit weight. Total production of date fruit produced each year is 10 million tons, resulting approximately 1 million ton of date pits. This amount is very large, low cost, non-toxic, biodegradable family. So we should pay attention towards it [24, 25].

The current study assayed the effect of foliar fertilizers of green synthesis of Zinc Oxide Nanoparticles (ZnO NPs) with different concentration on growth, yield and chemical constituents of Pea (*Pisum sativum* L.)

Materials and Methods

2.1. Materials

Date palm pits were collected from local markets in Cairo, Egypt. Zinc nitrate was purchased from Sigma –Aldrich Co. USA. Other chemicals were used without further purification. Zinc oxide nanoparticles are used as nano-fertilizer at a concentration of (20, 40, 60, 80, 100, 200, 300, 500 and 1000 mgl^{-1}) [26-28]. Zn is used as zinc sulfate ($ZnSO_4$) solution (a common zinc supplement) with the concentration of 1000 mg/l [26, 27]. Seeds of Pea (*Pisum sativum* L.) cv. Master-B were obtained from Legume Crops Research Department, Field Crop Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

2.2. Methods

2.2.1. Green synthesis of ZnO NPs from date palm pits extract

Date palm pits washed, dried at 105 ^oC after that grinding. Extraction process was prepared according the method described by Maha *et al*. [29]. The extract was prepared by placing 10 g of ground pits in 500 mL of distilled water, then stirring till complete homogeneity at room temperature overnight. After that 1g of zinc nitrate was dissolved in 50 mL of date pits extract, placed in a 250 mL beaker, a few drops of NH_4OH were added dropwise till precipitation occurred of $Zn(OH)_2$, then filter, washing, drying and firing the samples at 550 ^oC for 3 h to form nano zinc oxide particles.

2.2.2. Site of experiment:

Two field experiments were conducted at the Experimental Station of the Agricultural Research Center in Tag El- Ezz, Dakahlia Governorate, Egypt (31°31' 47.64" N latitude and 30°56' 12.88" E longitude) during the winter seasons of 2018/2019 and 2019/2020 to evaluate the influence of nano-zinc foliar spray on vegetative growth parameters, yield and chemical composition of pea plant. The experiment of this study includes eleven treatments with three replicates. The soil of the experimental site described in a Table (1)

Experiment layout and Treatments:

The study experiments consists of eleven treatments were arranged in a complete randomized block design with three replicates; thus, the total numbers of experiment were 33 experimental units. The plot area was 10.5 m^2, which contained three rows, 5m long and 0.7 m wide. Seeds were planted at 10 cm apart between each other and on one side of ridges.

1) Zinc sulfate 1000 mgl^{-1} (control); 2) nano zinc 20 mgl^{-1}**;** 3) nano zinc 40 mgl^{-1}**;** 4) nano zinc 60 mgl^{-1}**;** 5)

nano zinc 80 mgl^{-1}; 6) nano zinc 100 mgl^{-1}; 7) nano zinc 200 mgl^{-1}; 8) nano zinc 300 mgl^{-1}; 9) nano zinc 400 mgl^{-1}; 10) nano zinc 500 mgl^{-1} and 11) nano zinc 1000 mgl^{-1}.

$ZnSO_4$ and ZnO NPs suspensions were prepared just application. Pea plants were foliar sprayed three times, first one was after 45 days after sowing. The spraying repeated twice (14 days among them).

Table 1. Average physico-chemical properties of the experimental soil during two seasons.

Properties	Values	properties	Values
Clay (%)	29.50	**Available macro and micronutrients ($mg\ kg^{-1}$ soil)**	
Sand (%)	33.50	N	37.15
Silt (%)	37.00	P	5.75
Texture	Sand clay loam	K	197
Organic matter (%)	1.15	Zn	0.43
E.C. dsm^{-1}	2.13	Fe	1.23
pH soil suspension (1:2.5)	7.93	Mn	1.10

Table 2. Chemical analysis of poultry manure as average of two seasons

Properties	Value
pH (1:10)	5.91
EC (1:10)(dSm^{-1})	3.44
Organic matter (%)	35.89
Nitrogen (%)	2.17
Phosphorous (%)	0.68
Potassium (%)	0.94
Fe ($mg\ kg^{-1}$)	42.73
Zn ($mg\ kg^{-1}$)	23.50

2.2.3. Cultivation process: Seeds of pea (*Pisum sativum* L.) cv. Master-B were planted in second week of October for two seasons. The organic manure, i. e. poultry manure was added during preparing the soil to sowing at one portion (10 m^3fed^{-1}), Fertilization with calcium super phosphate (15% P_2O_5) at rate of 100 kg fed^{-1} was applied during soil preparation. Ammonium sulphate (20.6%N) at rate 100 kg.fed^{-1} and potassium sulphate (48% K_2O) at the rate of 50 kg fed^{-1} were added during the growth seasons.

2.3. Data Recorded:

2.3.1. Vegetative growth criteria: Samples were randomly taken from plots at 70 days after planting to determine vegetative growth (Plant height (cm), branches $plant^{-1}$ and fresh and dry weight (g).

2.3.2. Yield attributes: The plants were harvested and weighed. Pods were separated manually for seed yield and chemical analysis. Determine the number of pods $plant^{-1}$, weight of pods $plant^{-1}$ (g), seeds $plant^{-1}$, 100 seed weight and weight of pods ha^{-1} (ton) were calculated.

2.3.3. Chemical analysis:

Estimation of photosynthetic pigment content: three samples were taken from each plot for determination chlorophyll a, chlorophyll b and carotenoid. Pigment content was estimated according to Gavrilenko and Zigalova [30]

Determination of mineral in pea seeds: N, P and K % were determined according to the methods mentioned by Mertens [31]. Zn ($mg\ kg^{-1}$) were determined according to the methods mentioned by Khazaei *et al.* [32]

Protein estimation (%) and total carbohydrates (%) were estimated according to Srivastava and Prasad [33]

2.4. Statistical analysis: The eleven treatments of both seasons each were arranged in a complete randomized block design with three replicates. Data were analyzed with statistical analysis software; CoState [34]. All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using Duncan's multiple range test at a P level of 0.05.

3. Results and discussion

3.1. Characterization of date palm pits extract and nano zinc oxide

Date palm pits extract and nano zinc oxide were characterizes by GCMs, IR, EDX, TEM, DLS, Zeta potential and XRD as discussed in our published article by Maha *et al*. [29], which confirmed the preparation of nano zinc oxide in presence of date palm pits extract as capping agent and PDI of prepared nano zinc is 0.493 which means the particles is monodispersed in nature and calculated average particle size was 45.5 nm as mentioned by [29] and shown in figure 1.

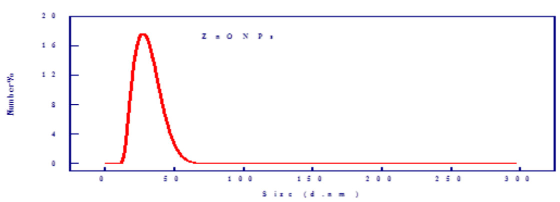

Figure 1. Particle size distribution of prepared nano zinc oxide

3.2. Vegetative growth criteria

The results show that plants spraying with nano zinc oxide with different concentrations gave various response compared with those spraying with zinc sulfate. Spraying with 100 mgl^{-1} was significantly improved the growth attributes of pea plant through two seasons. Where, the plants height increased by (12.82, 13.36%), number of branches $plant^{-1}$ by (44.44, 55.56%), no. leaves per plant by (59.32, 57.58%), fresh weight of plant by (37.74, 29.60%) and dry weight of plant by (56.57, 52.20%) compared with control plants during two seasons 2018-2019 and 2019-2020, respectively, followed by spraying with ZnO NPs 200 mgl^{-1}. on other hand, 1000 mgl^{-1} ZnO NPs gave opposite results (Table 3)**.** This effect may be related to the efficiency of nanoparticles on improved expression of some genes that code for proteins [35]. This stimulates some metabolic processes, resulting in higher plant growth criteria (Table 3). Or it could be owing to the impacts of nanoparticles on cell division and growth, particularly in meristematic sites, the production of more tryptophan and auxin as a hormone supporting longitudinal growth and therefore an increase in internode length and height [36]. The low concentration of ZnO NPs promotes plant shoot development and plant growth hormone [27]. The results were in harmony with Sultan *et al*. [37] who pointed that ZnO NPs at conc 100 mgl^{-1} increase growth of *Pisum sativum* L. Also, Tag El-Din [38] on sorghum.

3.3. Yield attributes

The yield and yield components were significantly increased with ZnO NPs concentration (Table 4). In contrast, 1000 mgl^{-1}ZnO NPs gave the lowest yield attributes. There is no significant difference between ZnO NPs concentration at 100 and 200 mgl^{-1} for pods number per plant and pods length (cm). While treating pea plants with 200 mgl^{-1}ZnO NPs improved weight of green pods per plant by (22.97, 22.69%) and per ha. by (22.95, 22.69%). Also, weight of 100 seeds by (20.75, 25.13%) during two seasons respectively, this effect demonstrated that the 200 $mgl^{-1}$of ZnO NPs was sufficient as co-enzyme to cell differentiation for stimulation pods and seeds development in pea plant. These results could be attributed to enhancing the plant growth of pea plants with a concentration of 100 and 200 mgl^{-1}ZnO NPs Previously, similar results have been discovered with Elizabath *et al*. [39] who showed that the foliar application with 150 $mgl^{-1}$of ZnO NPs increased the yield of carrot plant (ton ha^{-1}). Also, Sultan *et al*. [37] who showed that ZnO NPs at conc. 100 mgl^{-1} increase yield criteria of *Pisum sativum* L. Also, Tag El-Din [38] recorded similar results on sorghum yield.

3.4. Chemical analysis

3.4.1. Photosynthetic pigments

Data in (Table 5) represented that the pigments in leaves were significantly increased with 100 and 200 mgl^{-1} ZnO NPs application. Where, chlorophyll a and carotenoid significantly improved with 100 mgl^{-1} ZnO NPs as a foliar application by (22.38, 17.39 & 55.56, 86.67%) compared with control plants in 1st and 2nd season respectively. While the chlorophyll b increased with 200 mgl^{-1} by (87.78, 63.64%) comparing with those treated with zinc sulfate through two seasons, respectively. While there were no significant between values of total chlorophyll that spraying with 100 or 200 mgl^{-1} ZnO NPs as a foliar application. This impact could be owing to the presence of zinc, which is required for protochlorophyllide formation. As a result, zinc is the most essential elements to synthesis chlorophyll. Furthermore, plants sprayed with ZnO NPs had a higher ratio of glutamic to glycine [35]. These amino acids are responsible for the formation of plant tissues and the synthesis of chlorophyll. This could also be because metal nanoparticles are involved in photosynthetic efficiency, causing light absorption by chlorophyll and energy transfer from chlorophyll to nanoparticles [40, 41]. Beside this, Zn is essential for the activity of carbonic anhydrase that mediate hydration of CO_2 to bicarbonate to the chloroplasts [42]. Carbonic anhydrase is involved in the control of stomata [43]. So, optimum Zn supply helps plants improve photosynthesis [28]. These results agreed with Sultan *et al*. [37] on pea plant and Srivastav *et al*. [28] on both maize and wheat plants.

Table (3): Effect of ZnO NPs concentrations on vegetative growth criteria of pea plants during two successive seasons (2018-2019/2019-2020).

Treatments	Plant height (cm)		Branch (No.)		Leaves (No.)		F.wt shoot (g $plant^{-1}$)		D. wt shoot (g $plant^{-1}$)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control ($ZnSo_4$)	54.75	56.68	4.50	4.50	14.75	16.50	26.50	28.55	3.66	3.87
ZnO NPs 20 mgl^{-1}	53.72	57.15	4.44	4.75	15.50	18.00	27.00	29.75	4.07	4.22
ZnO NPs 40 mgl^{-1}	54.50	58.70	4.66	4.75	16.50	18.95	28.30	31.50	4.19	4.38
ZnO NPs 60 mgl^{-1}	56.00	59.35	5.00	5.15	18.00	20.00	29.66	32.00	4.43	4.53
ZnO NPs 80 mgl^{-1}	59.50	61.50	5.75	6.00	19.50	22.50	32.50	34.50	4.93	5.27
ZnO NPs 100 mgl^{-1}	61.77	64.25	6.50	7.00	23.50	26.00	36.50	37.00	5.73	5.89
ZnO NPs 200 mgl^{-1}	60.00	62.50	6.00	6.50	22.00	25.50	34.75	35.85	5.30	5.44
ZnO NPs 300 mgl^{-1}	58.15	60.00	4.95	5.50	19.00	21.00	31.00	31.50	4.77	5.02

ZnO NPs 400 mgl^{-1}	56.25	58.25	4.50	4.85	15.50	19.50	30.15	30.77	4.51	4.84
ZnO NPs 500 mgl^{-1}	55.00	57.50	4.50	4.75	15.00	18.75	27.5	27.73	4.12	4.21
ZnO NPs 1000 mgl^{-1}	52.50	55.57	4.20	4.50	13.50	16.00	25.00	24.46	3.47	3.67
LSD at 5%	1.27	1.30	0.33	0.29	0.59	0.55	0.50	0.48	0.16	0.14

Table (4): Effect of ZnO NPs concentrations on yield attributes of pea plants during two successive seasons (2018-2019/2019-2020).

Treatments	**No of pods plant^{-1}**		**Weight of pods plant^{-1} (gm)**		**No of seeds pod^{-1}**		**Pod length (cm)**		**Weight of green pods ha^{-1} (ton)**		**Weight of 100 seeds(g)**	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control ($ZnSo_4$)	9.80	9.50	49.37	49.68	6.50	6.00	6.23	6.40	7.32	7.36	19.37	19.70
ZnO NPs 20 mgl^{-1}	9.25	9.13	50.12	51.17	6.25	6.00	6.10	6.15	7.43	7.58	19.00	19.86
ZnO NPs 40 mgl^{-1}	10.00	10.05	51.66	52.91	6.85	6.75	6.13	6.22	7.66	7.84	19.50	20.00
ZnO NPs 60 mgl^{-1}	10.75	10.57	54.64	54.83	7.15	7.50	6.50	6.50	8.10	8.13	20.43	21.00
ZnO NPs 80 mgl^{-1}	11.50	11.15	56.00	56.27	7.47	7.85	6.50	6.93	8.30	8.34	21.37	21.25
ZnO NPs 100 mgl^{-1}	12.15	12.00	58.46	58.77	7.75	8.25	7.12	7.48	8.66	8.71	22.55	23.07
ZnO NPs 200 mgl^{-1}	12.00	11.75	60.71	60.95	7.60	8.00	7.23	7.43	9.00	9.03	23.39	24.65
ZnO NPs 300 mgl^{-1}	10.75	11.00	57.00	56.07	7.45	7.45	6.77	7.00	8.45	8.31	21.67	21.15
ZnO NPs 400 mgl^{-1}	10.00	10.50	51.12	52.4	7.00	7.15	6.50	6.71	7.58	7.77	20.73	20.20
ZnO NPs 500 mgl^{-1}	9.50	10.00	49.55	50.15	6.50	6.75	6.00	6.50	7.34	7.43	19.44	19.69
ZnO NPs 1000 mgl^{-1}	9.00	9.00	47.32	47.44	6.00	6.20	5.87	6.00	7.01	7.03	17.44	17.89
LSD at 5%	0.29	0.28	0.38	0.31	0.23	0.19	0.24	0.19	0.06	0.05	0.14	0.16

Table (5): Effect of ZnO NPs concentrations on photosynthetic pigments of pea leaves during two successive seasons (2018-2019/2019-2020).

Treatments	**chlorophyll a (mg g^{-1} f. wt)**		**chlorophyll b (mg g^{-1} f. wt)**		**Total chlorophyll (mg g^{-1} f. wt)**		**Carotenoids content (mg g^{-1} f. wt)**	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control ($ZnSo_4$)	0.210	0.230	0.090	0.110	0.300	0.340	0.117	0.135
ZnO NPs 20 mgl^{-1}	0.203	0.229	0.093	0.101	0.296	0.33	0.131	0.146
ZnO NPs 40 mgl^{-1}	0.21	0.233	0.099	0.114	0.309	0.347	0.133	0.153
ZnO NPs 60 mgl^{-1}	0.224	0.246	0.11	0.123	0.334	0.369	0.144	0.182
ZnO NPs 80 mgl^{-1}	0.23	0.252	0.136	0.149	0.366	0.401	0.164	0.207
ZnO NPs 100 mgl^{-1}	0.257	0.27	0.143	0.165	0.400	0.435	0.182	0.252

ZnO NPs 200 mgl^{-1}	0.242	0.259	0.169	0.18	0.411	0.439	0.173	0.227
ZnO NPs 300 mgl^{-1}	0.229	0.247	0.13	0.147	0.359	0.394	0.142	0.198
ZnO NPs 400 mgl^{-1}	0.218	0.226	0.099	0.119	0.317	0.345	0.133	0.160
ZnO NPs 500 mgl^{-1}	0.210	0.211	0.088	0.100	0.298	0.311	0.110	0.133
ZnO NPs 1000 mgl^{-1}	0.186	0.197	0.07	0.093	0.256	0.29	0.097	0.117
LSD at 5%	0.007	0.009	0.009	0.009	0.011	0.011	0.014	0.017

3.4.2. Chemical constituents

The different concentrations spray of ZnO NPs caused a significant effect on chemical constituents in seeds of pea plants during two seasons 2018-2019 and 2019-2020 (Table 6) in contrast 1000 mgl^{-1}ZnO NPs showed lowest contents. Foliar application of ZnO NPs concentration on pea plants showed that there was no significant difference between 100 and 200 mgl^{-1} ZnO NPs in nitrogen and zinc of seeds through two seasons. The phosphorous content in the seeds were increased by increasing the concentration of ZnO NPs up to 200 mgl^{-1} by (32.17, 28.22%) compared with control through 1st and 2nd season, respectively, Also, the increment of potassium in seeds by (20.41, 28.72%) at two seasons, respectively. These findings could be attributable to the effect of ZnO NPs concentration. Since, the nutrients in nano-size were more available to nanoscale plant pores resulting in increased efficiency [44]

Concerning with total protein, application of ZnO NPs up to 200 mgl^{-1}showed increased in total protein in seeds by (13.26, 13.42%) compared with those spraying with zinc sulfate for 1st and 2nd season respectively. These results are supported by the study of Baybordy and Mamedov [45] who explained that zinc plays a crucial role in the structure of enzymes involved in amino acid production, and that amino acids are the building blocks of proteins..

Also, results recorded there was no significant difference among 100 and 200 mgl^{-1} ZnO NPs for carbohydrate content of seeds through two seasons. The stimulation in the contents of carbohydrates is probably due to the role of zinc in activation of the enzymes that responsible for photosynthesis, biosynthesis and transformation of carbohydrates [46]. The results matched with Amin and Badawy [46] who explained that carbohydrate and protein contents in common bean plants were enhanced when the plants treated with ZnO NPs (25, 50, 100 and 200 mgl^{-1}). Also, Salama *et al*. [35] found that ZnO NPs foliar application has positive effect in mineral content and carbohydrate percentage of pea seed.

4.Conclusions

Foliar nano fertilizers can be more effective than conventional fertilizers. Where, the application of green synthesis of ZnO NPs as foliar spray significantly increased growth and development of pea plants. At a concentration of 100 mgl^{-1} promoted plant vegetative growth. While, at 200 mgl^{-1} ZnO NPs had positive effect on pea yield with best quality. In contrast, at 1000 mgl^{-1}ZnO NPs had lower and minor effects on pea growth and yield. Therefore, the ZnO NPs effect depends on the concentration applied. Where, low concentration of ZnO NPs gave better results than higher one. On other hand, the application of 1000 mgl^{-1} $ZnSO_4$ had little effects compared with ZnO NPs.

Table (6): Effect of ZnO NPs concentrations on chemical constitutes of pea seeds during two successive seasons (2018-2019/2019-2020).

Treatments	Nitrogen (%)		Phosphorous (%)		Potassium (%)		Zinc (mg kg^{-1})		Total protein (%)		Carbohydrate (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control ($ZnSo_4$)	3.38	3.38	0.230	0.241	0.98	0.94	19.09	18.95	18.33	18.41	45.75	45.67
ZnO NPs 20 mgl^{-1}	3.31	3.37	0.239	0.239	0.90	0.92	18.23	18.61	17.93	17.87	45.31	45.39
ZnO NPs 40 mgl^{-1}	3.45	3.45	0.241	0.255	0.95	0.96	19.37	19.08	18.39	18.50	46.04	46.19
ZnO NPs 60 mgl^{-1}	3.54	3.51	0.256	0.271	1.02	1.06	20.44	21.78	19.44	19.19	46.33	46.45

ZnO NPs 80 mgl^{-1}	3.65	3.68	0.269	0.283	1.10	1.14	22.15	23.50	20.36	20.44	47.00	46.91
ZnO NPs 100 mgl^{-1}	3.71	3.76	0.287	0.290	1.15	1.17	23.68	24.91	20.54	20.61	47.35	47.46
ZnO NPs 200 mgl^{-1}	3.77	3.79	0.304	0.309	1.18	1.21	23.77	24.96	20.76	20.88	47.26	47.41
ZnO NPs 300 mgl^{-1}	3.59	3.55	0.276	0.277	1.03	1.11	21.49	23.81	19.77	19.61	46.59	46.77
ZnO NPs 400 mgl^{-1}	3.45	3.43	0.255	0.260	0.99	1.04	19.11	19.90	18.98	19.07	46.05	46.09
ZnO NPs 500 mgl^{-1}	3.39	3.30	0.231	0.240	0.96	0.98	18.47	19.00	18.53	18.63	45.77	45.68
ZnO NPs 1000 mgl^{-1}	3.13	3.17	0.210	0.220	0.91	0.92	17.18	18.49	17.62	17.77	45.00	45.11
LSD at 5%	0.068	0.075	0.008	0.009	0.070	0.068	0.41	0.31	0.25	0.20	0.21	0.20

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