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IMPACT OF ZINC OXIDE NANOPARTICLES ON *ASPARAGUS OFFICINALIS* PLANT

S.F. Desoukey[1], ZeinabK. Taha[1], Hattem M. El-Shabrawi[2] and Atef Z. Sabh[1]

[1]Agricultural Botany Dept., Faculty of Agriculture, Cairo University, Giza, Egypt.
[2]Plant Biotechnology Dept., National Research Center, Giza, Egypt.

Abstract

The current experiment was performed on *Asparagus officinalis* L. (Mary Washington 500 W cultivar) of the family Asparagaceae, during the two successive growing seasons 2017 and 2018 to display the effect of ZnO nanoparticles on *Asparagus officinalis* L. plant by using some morphological, histological and biochemical parameters. Seeds were soaked in different concentrations of ZnO nanoparticles (0.0, 0.5, 1, 3, 5, 10, 30, 50, 100 and 500 ppm) for 48h, in addition to Zn acetate Bulk (40 ppm) treatment and untreated control for comparison. The obtained results indicated that ZnO NPs treatment at the concentration of 500 ppm showed the highest values of all studied morphological characters of Asparagus plant with the exception of root length. In addition, a significant increase in catalase enzyme activity was observed when using a concentration of 500 ppm compared to the other treatments. The concentration of 3 ppm led to the highest stimulation effect for both types of flavonoids (Rutin and Myricetin) and the highest Naringenincontent and total flavonoids were detected when 5 ppm of ZnO NPs was used. With regard to the effect of Nano-Zinc on the anatomical features of the asparagus plant, it was found that there was a clear effect on the anatomical characteristics of the plant (root and stem) treated with a concentration of 500 ppm of ZnO NPs compared to plant treated with Zinc acetate at 40 ppm and untreated control plant.

***Key words*:** Asparagus, Zinc Oxide nanoparticles, growth parameters, anatomy, flavonoids, catalase activity.

Introduction

Asparagus (*Asparagus officinalis* L.)plant belongs to the Aspargaceae family. It is a perennial plant, with a rhizome, bulb, or corm, rarely shrub by or tree-like (Chenxinqand Xujiemei, 2000). Asparagus reproduces by rhizomes and seeds, but the seed germination rate is very low. Asparagus is one of the promising horticultural crops in Egypt (Hassan, 2001). *Asparagus officinalis* L. is a highly prized economically important vegetable crop. Markets in some Asian, African, European and American countries demand a large quantity from fresh green, purple and white spears. Its young shoots are used as a spring vegetable. Asparagus is one of the most nutritionally well-balanced vegetables in existence, which is high in folic acid, thiamin, vitamin B6 and a good source of potassium. It represents sources of rutin, a drug that strengthens the capillary wall and an excellent source of folacin, vitamin B, which helps in the duplication of cells for growth and repair of the body and in blood cell reproduction in the bone marrow. It contains glutathione which is one of the most potent anticarcinogens and antioxidants found within the body. Asparagus has no fat, no cholesterol and low in sodium (Hassan, 2001). *Asparagus officinalis* L. is the most economically important asparagus which is a highly prized dioeciously vegetable crop (Stajner *et al.*, 2002). One novel (Sarsasapogenin O) and seven known steroids were isolated from the roots of *Asparagus officinalis*. All the compounds were evaluated for their *in vitro* cytotoxic activity against variety types of cancer cell lines (Huang *et al.*, 2008). Wang *et al.*, (2003) reported that the distribution of rutin and protodioscin within the shoots was found to vary by location, with the tissue closest to the rhizome found to be a rich source of protodioscin, at an average level of 0.025% tissue fresh weight in the three tested lines, while the upper youngest shoot tissue contained the highest amount of rutin at levels of 0.03-0.06% tissue fresh weight. The analysis of individual flavonoids by HPLC-DAD-MS has allowed the determination of eight naturally occurring flavonol derivatives in several genotypes of Triguero Asparagus. Those compounds included mono-, di- and tri-glycosides of three flavonols, that is, quercetin, isorhamnetin and kaempferol. The detailed analysis of the flavonoid profiles

revealed significant differences among the distinct genotypes. Rutin is a citrus flavonoid glycoside, which is a low molecular weight polyphenolic compound. There are various physiological functions of rutin and related flavonoids in the human body and other species, including plants. Rutin is one of the best natural antioxidants and is also called vitamin P, which is widely distributed in vegetables, fruits and medicinal herbs such as Asparagus. Rutin has various pharmacological activities such as antibacterial, antiprotozoal, antitumor, anti-inflammatory, antiallergic, antiviral, cytoprotective, vasoactive, hypolipidaemic, antiplatelet, antispasmodic and antihypertensive as mentioned by Pateland Patel (2019).

There are large numbers of problems face agricultural researchers such as stagnation in crop yields, low nutrient use efficiency, declining soil organic matter, multi-nutrient deficiencies, climate change, shrinking arable land and water availability so they need a novel technology to found anew and cheap approach to solve these problems such as nanotechnology to detect and deliver the correct quantity of nutrients and pesticides that promote productivity while ensuring environmental safety and higher use efficiency as pointed by Pradhan *et al.*, (2017).

There are many types of nanoparticles such as ZnO nanoparticles and Nano carbon, Nano cobalt and Nano copper have a stimulatory effect. ZnO nanoparticles (NPs) have a positive role in seedling treated by 1.5 ppm compared with control and biomass accumulation increased in the ZnO nanoparticles treated seedlings as in chickpea (*Cicerarietinum* L. *var.* HC-1), Burman *et al.*, 2013. Also, ZnO NPs treatment caused a significant increase in shoot and root growth, biomass accumulation with higher values of height (16.8%), leaf area (30.3%), total biomass production (59.5%), root dry biomass (112.5%), stem dry biomass (76%) and root length (24.4%). Zn acts as a cofactor for auxin production and improves cell division and elongation, influence on the reactivity of indole acetic acid in pepper plant. ZnO NPs might be involved in the biosynthesis of cytokinins and gibberellins; as well as on the induction of the greater activity of antioxidant enzymes (Méndez-Argüello *et al.*, 2016). There were significant increases in superoxide dismutase (SOD, 267.8%) and peroxidase (POX, 174.5%) enzymes activity, whereas decreased catalase (CAT, 83.2%) activity compared with control of *Gossypiumhirsutum* L. plant (Priyanka and Venkatachalam, 2016). ZnO nanoparticles cause some anatomical alteration. ZnO nanoparticles cause some anatomical alteration like the cortical cells were highly vacuolated and collapsed, while the vascular cylinder was shrunk. The main structure of roots damaged at higher concentrations in Mungbean (*Vignaradiata*) and Gram (*Cicerarietinum*) plants (Mahajan *et al.*, 2011).

The target of this study is to show the effect of ZnO nanoparticles on *Asparagus officinalis* L. plant by using some morphological, histological and biochemical parameters.

Materials and Methods

Plant material

The current experiment was performed on *Asparagus officinalis* L. (Mary Washington 500 W cultivar) of the Asparagaceae family. Seeds were procured from Agricultural Research Center (Vegetable Research Institute), Dokki, Giza, Egypt.

Pot experiment

A pot experiment was carried out in the Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive growing seasons 2017 and 2018. Seeds were soaked in different concentrations of ZnO nanoparticles (0.0, 0.5, 1, 3, 5, 10, 30, 50, 100 and 500 ppm) in addition to Zn acetate Bulk (40.0 ppm) for 48 h. Treated seeds were sown in pots (40 cm diameter) filled with light loamy soil.

Germination rate in each treatment was determined 30 days after cultivation according to ISTA, 1999.

Germination rate = No. of germinated seeds/No. of planted seeds

Seedling length (cm) measured from the top of the shoot let to the top of the root 45 days after cultivation

Seed Vigor Index (SVI) = Germination % × Seedling length

The following measurements were recorded five months after the start of the experiment:

1. Plant height (cm) measured from the base of the root system up to the uppermost point of the plant.

2. Number of main stem internodes.

3. Number of lateral branches/plant.

4. Root length/plant (cm).

5. Fresh and dry weights of vegetative growth/plant (g) were measured at the end of the experiment. Fresh weights were recorded and then dry weights were carried out by packing the samples in a perforated paper – bags and dried in an oven at 70°C for 48 hours till constant weights were reached.

6. Dry weight / Fresh weight ratio

Statistical analysis

The Completely Randomized Block Design (CRBD)

was followed. The experiment included three replicates each one represented by 3pots, each one contained 5 seeds.Data were subjected to appropriate statistical and conventional methods of analysis of variance according to Snedecor and Cochran, 1989. Designed computer program (Microsoft Excel 2007) was used. The mean differences were compared by a least significant difference test (LSD) at $P \leq 0.05$.

Anatomical study

The anatomical structure of Asparagus plant root and stem for each of ZnO NPs at 500 ppm, Zn acetate at 40 ppm and untreated control treatments were studied. Micro technique practices were carried out at the laboratory of Agric. Bot. Dept. Faculty of Agric., Cairo University, during the second season. Materials were killed and fixed for at least 48 hours in F.A.A. solution, dehydrated and then embedded in paraffin wax (Sass, 1951). Sections that were cut on a rotary microtome at a thickness of 15-20 microns were stained with crystal violet/ erythrosine before mounting in Canada balsam. Slides were examined microscopically and photomicrographed.

Catalase enzyme activity determination

The method includes the measured decrease in absorbance of the test sample by the induced decomposition of H_2O_2 in the presence of the catalase enzyme. This rate is recorded by measuring the reduction in absorbance during 3 minutes at 240 nm in 1.5 ml of reaction mixture consisting of 13.2 mμ H_2O_2 in 50 mμ phosphate buffer pH 7 and 0.1 ml of the sample as control mixture containing the same buffer solution (Aebi, 1984).

Flavonoids determination by HPLC for *in vivo* plants

One gram of Asparagus plant has been extracted with 10 ml ethanol 80%, soaked in brown bottle for 2hours at room temperature, then sonicated using a KQ-200VDE ultrasonic bath (Kunshan Ultrasonic Instrument Co. Ltd., Kunshan City, Jiangsu Province, China) with the output power 200 W for 10 min, the volume was adjusted up to 50 ml with 80% ethanol, then filtered through Whatman filter paper 42 (125 mm).

HPLC conditions

Aglient 1260 Infinity HPLC series (Aglient, USA) equipped with quaternary pump, a Zorbax Eclipse Plus C18 column 150 mm × 4.6 mm i.d., (Aglient Technology, USA), operated at 30°C, Eluent methanol, H_2O with 0.5% H_3PO_4, 50:50 with flow rate 1 ml/min, the injected volume was 20 µl. Detection: UV. Detector set at 210 nm, (Schneider, 2014).

Results and Discussion

Seed germination rate

Data presented in table 1 showed the germination rate after soaking Asparagus seeds in different concentrations of Zinc Oxide nanoparticles in addition to Zinc acetate Bulk (40 ppm) and untreated control. Different survival ratios were observed by applying different concentrations of ZnO nanoparticles (0.0 to 500 ppm) as well as Zinc acetate Bulkat 40 ppm in comparison with untreated control treatment.

Table 1: Seed germination rate and seedling growth parameters of *Asparagus officinalis* L. plant treated with different concentrations of ZnO NPs.

ZnO NPs Conc. ppm	Germination rate(%)		Seedling length (cm)(%)		Seedling Vigor Index (SVI)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S
0.0	15.5	14.5	3.2	3.0	49.6	43.5
0.5	17.7	16.8	3.5	3.2	62.0	53.8
1	20.0	18.5	3.5	3.3	70.0	61.1
3	20.0	19.2	3.6	3.3	72.0	63.4
5	22.2	21.5	3.8	3.7	84.4	79.6
10	28.8	27.2	4.0	3.9	115.2	106.1
30	31.1	29.6	4.1	3.9	127.5	115.4
50	31.1	30.4	4.5	4.3	140.0	130.7
100	33.3	31.8	5.3	4.6	176.5	146.3
500	37.8	35.3	5.5	5.2	207.9	183.6
Zn acetate	37.7	34.5	3.8	3.5	143.3	120.8
LSD (0.05)	2.45	1.81	0.21	0.23	10.48	5.99

The germination rate results recorded 30-days after planting revealed that there were significant differences between most treatments under study. ZnO NPs treatment at the concentration of 500 ppm showed the highest germination rate (143.9 and 143.4% over the control) in the 1st and 2nd seasons, respectively, without significant differences with Zinc acetate Bulkat 40 ppm (143.2 and 137.9% over the control) compared to the other treatments, then 100 ppm ranked the second (114.8 and 119.3% over the control) and 30 and 50 ppm ranked the third (100.6, 100.6% and 104.1, 109.7% over the control), in both seasons, respectively, while the germination rate of 0.5ppm was the lowest one of ZnO NPs treatments (14.2 and 15.9% over the control). There were significant differences in germination rate between all Zn treatments and untreated control except for ZnO NPs at 0.5 ppm in the 1st season. These concluded results were in harmony with Afrayeem and Chaurasia (2017) in chilli; Awasthi *et al*., (2017) in wheat; Hajraand Mondal (2017) in *Cicerarietinum*; Solankiand Laura (2018) in wheat. Raskarand Laware (2014) used Zinc Oxide nanoparticles as photocatalyst and water decompos-

erandlowering Zinc Oxide concentration improved cell division in onion.

However, the effect of Zinc Oxide nanoparticles on Asparagus germination could be due to the generation of active anions that had an indirect role in accumulating water, increasing oxygen consumption and accelerating electron transport which essential for rapid germination. Shankramma *et al.,* 2016 mentioned that Fe_2O_3 NPs in the range of 50-200 mgL^{-1} increased the germination percentage up to 97 % at 200 mgL^{-1} in tomato. (Munir *et al.,* 2018) found Zinc Oxide nanoparticles have an effective transfer and penetration rate of wheat plant cells.

Several workers have reported that zinc seed treatment induces a set of biochemical changes in the seeds, required to start the germination process, such as dormancy, hydrolysis or inhibitory metabolism, syrup and enzyme activation as Harris *et al.,* 2007 and Kantabathini *et al.,* 2018 were indicated. It was also observed that *Vignamungo* L. seeds soaked in ZnO nanoparticles for 8 hours at concentrations 5 to 25 mg/100 ml, resulted in a significantly improved germination ratio of 111.3%. Moreover, Madbouly, 2018 observed that the low concentration of biosynthesized NPs acts as a new ecofriendly plant growth regulator. On the other hand, Adhikari *et al*., (2015) mentioned that Zinc Oxide nanoparticles don't lead to any change in seed osmotic potential during the growing season. Dogarogluand Köleli (2019) mentioned that barley (*Hordeumvulgare* L.) seeds treated by different concentrations of TiO_2 and ZnO Nanoparticles (0, 5, 10, 20, 40 and 80 mg/kg showed non-significant effect on germination at all the concentrations of ZnO and TiO_2 due to ZnO gave higher toxicity against barley that led to generate ROS as stress mechanism in plant cells, so that seedling gave different behaviours according to the concentration and that required defense mechanism through antioxidant; *i.e*. the retardation in growth in some concentrations.

Seedling growth parameters

Seedling length (cm)

Data in table 1 clarified seedling length 45-days after cultivation of Asparagus seeds treated with ZnO NPs in addition to Zn acetate Bulk and untreated control treatments. The results indicated that ZnO NPs at lower concentrations reduced seedling growth, while at higher concentrations promoted seedling growth. The differences were significant between all treatments and untreated control with the exception of ZnO NPs at 0.5 ppm in the 2nd season. The use of highest Nano-Zinc concentration (500 ppm) yielded the longest seedlings (5.5 and 5.2 cm) with an increase percentage of 71.9 and 73.3 % more than the control in the first and second seasons, respectively. While the use of lowest Nano-Zinc concentration (0.5 ppm) resulted in the shortest seedling length of 3.5 and 3.2 cm with an increase percentage of 9.4 and 6.7% related to the control in both seasons, respectively. By comparing the length of seedlings obtained as a result of ZnO NPs treatment at the highest concentration (500 ppm) with the treatment of Zn acetate Bulk, it was found that the first treatment exceeded the second by 44.7 and 48.6% in both seasons, respectively. These results are consistent with Afrayeem and Chaurasia (2017) in chili who indicated that ZnO NPs at lower concentration reduced seedling growth, but at higher concentration promoted seedling growth. Also, Solanki and Laura (2018) in wheat stated that the highest shoot and root length was observed with the application of ZnO NPs at a concentration of 500 ppm and the lowest was observed with the control. Hao *et al.,* 2016 mentioned that Fe_2O_3NPs at a concentration of 5 mg/Lto 50 mg/ Lpromoted the elongation of rice seedlings at low concentration because it acted as a co catalytic for oxidation and bioavailability of iron molecules to the seed radicals. Al-Harbi *et al*., (2019) mentioned that ZnO NPs at concentrations of 500 and 2000 mg/L in *Viciafaba* (Fabeaceae) had significant effect on mitotic division of meristematic root tips.

Contrary to these results, Prasad *et al*., (2012) in groundnut reported that ZnO NPs at lower concentration increased the root and shoot length and confirmed his point of view by Zn as an only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases), in additional ZnO NPs hadalarge surface make them more effective in the reaction. Such increase in the growth of corn plant at lower concentration could be ascribed to higher precursor activity of Zn, especially ZnO NPs in auxin production (Kobayashi and Mizutani, 2013). Burman *et al.,* (2013) indicated that chickpea (*Cicerarietinum* L. *var.* HC-1) seedlings treated by foliar application of ZnO nanoparticles (1.5 ppm aqueous solution) hada positive effect on biomass accumulation compared with control.

Seedling Vigor Index (SVI)

The seedling vigor index gives a clear picture of the effectiveness of the treatment in affecting the strength of seedlings' growth and, consequently, on the strength of growth of the resulting plants. Results in table 1 stated that there were clear differences between all Zn treatments and Zn- free treatment. SVI value was the highest (207.9 and 183.6) by using ZnO NPs at 500 ppm

in comparison with the control (49.6 and 43.5) and Zn acetate Bulk (143.3 and 120.8) in both seasons, respectively. Increasing the concentration of Nano-Zn increased the SVI value. Nano-Zinc treatment at the lowest concentration (0.5 ppm) gave the lowest SVI value (62.0 and 53.8). These results were corresponding with the results of Yilmaz (1997), Solanki and Laura, (2018) and Younes *et al*., (2020) because of accelerating vital metabolic system which is required for seedling growth. Abdel Latef *et al.*, (2020) and Itroutwar *et al.*, (2020) mentioned that ZnONPs concentrations (5, 10, 25, 50, 100 and 200 mg/L) improved Seedling Vigor Index (SVI) of Rice (*Oryza sativa* L.) due toZnONPs increased the level of indole acetic acid in roots, which led to increase in the growth rate and also refer to high concentrations have vital roles in physiological process during seed germination and early seedling growth. Otherwise, Gowayed and Kadasa (2015) found that increasing of ZnONPs dose decreased Seedling Vigor Index (SVI) in Faba bean due to the mitotic inhibition of seedling cells.

Plant growth parameters

Data in table 2 clarified the plant growth parameters represented in plant height (cm), No. of main stem in ternodes, No. of branches/plant, plant fresh weight (g), plant dry weight (g), dry weight/fresh weight % and average root length (cm) of Asparagus plant grown at different concentrations of ZnO NPs.

Plant height (cm)

Table 2 showed plant height (cm) at different concentrations of ZnO NPs. The use of ZnO NPs in its different concentrations led to a gradual increase in plant height compared to the non-treated control. All the concentrations of ZnO NPs results were significant related to the untreated control except for Zn-Nano at 0.5 ppm in the 1st season. The treatment of ZnO NPs at a concentration of 500 ppm resulted in the maximum height of the plant (118.8 and 99.0 cm) with an increase of 71.4 and 63.6% compared to the untreated control plants in both seasons, respectively. While, the use of ZnO NPs with a concentration of 0.5 ppm resulted in the lowest plant height (72.1 and 63.3 cm), with an increase of 4.0 and 4.6% compared to the control in both seasons, respectively. On the other hand, the use of Zn acetate Bulk with a concentration of 40 ppm resulted in a significant increase in plant height of 58.2 and 48.8% compared to the untreated control plants in both seasons, respectively, although this increase was insignificant compared to the highest concentration (500 ppm) of ZnONPs. Zinc oxide (100 ppm) causes an increase in growth parameters of carrot such as plant height (cm)

Table 2: Effect of different concentrations of (ZnO) nanoparticles on plant growth parameters of *Asparagus officinalis L.* plant after the fifth month of 2017 and 2018 seasons

ZnO NPs Conc. ppm	Plant height (cm)		No. of main stem internodes/plant		No. of branches/plant		Plant fresh weight (g)		Plant dry weight (g)		DW/FW %		Root length (cm)	
	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S	1st S	2nd S
Control	69.3	60.5	26.6	23.3	16.3	13.3	19.09±0.80	16.35±0.55	4.64±0.83	3.81±0.52	24.3	23.3	4.6	4.3
0.5	72.1	63.3	28.3	26.6	19.5	15.3	25.82±0.20	20.73±0.33	6.66±1.39	5.16±1.22	25.8	24.9	6.8	6.2
1	78.6	65.6	31.6	30.0	21.3	17.6	28.09±3.35	23.51±1.45	7.70±1.14	5.97±1.17	27.4	25.3	7.5	6.8
3	83.3	70.6	33.3	32.3	23.6	19.0	33.80±3.69	25.32±1.71	9.43±1.45	6.99±1.35	27.9	27.4	9.1	8.3
5	89.3	74.3	35.3	34.5	24.3	19.7	39.31±1.70	27.37±1.25	11.12±0.59	7.69±0.47	28.3	28.1	11.3	9.7
10	95.0	79.0	37.6	35.0	24.6	21.3	42.29±1.64	31.77±1.35	12.52±1.55	9.66±1.41	29.6	30.3	8.5	7.7
30	99.0	84.6	38.5	36.3	24.9	21.6	47.81±1.39	38.11±1.52	14.58±0.45	11.74±0.55	30.5	30.8	8.3	7.4
50	105.6	88.7	42.3	37.6	26.6	22.8	51.66±1.13	41.33±1.02	16.32±1.60	12.98±1.44	31.6	31.4	8.0	7.1
100	112.3	92.0	44.6	39.6	29.0	23.5	54.80±2.29	44.54±1.85	18.41±1.08	14.92±0.95	33.6	33.5	7.5	6.6
500	118.8	99.0	48.8	41.3	33.3	26.3	57.20±1.28	47.12±0.98	20.02±1.63	16.12±1.51	35.0	34.2	7.0	6.4
Zn Bulk	109.6	90.0	39.0	38.3	25.0	22.0	39.90±2.32	33.53±1.62	11.93±0.80	8.72±0.92	29.9	26.0	6.6	6.0
LSD (0.05)	3.62	2.36	1.72	1.87	1.92	1.88	2.88	1.85	1.69	1.56	2.98	3.34	1.01	0.92

as mentioned by Elizabeth *et al.*, (2017) and Mahdieh *et al.*, (2018) in pinto bean.

No. of main stem internodes

Table 2 showed the number of main stem internodes at different concentrations of ZnO NPs. These results clarified that the more concentrations the more number of main stem internodes. Most treatments were significant when compared with Zn-free treatment. The highest concentration (500 ppm) of ZnO NPs showed the highest significant No. of main stem internodes (83.5 and 77.3%) over untreated control plants in both seasons, respectively. There was a significant difference in No. of main stem internodes between the highest concentration (500 ppm) of ZnO NPs and Zn acetate Bulk as the latter recorded 46.6 and 64.4% over untreated control plant in both seasons, respectively. Similar results mentioned by Mahdieh *et al*., (2018) ZnO nanoparticles at concentration of 0.10% and 0.15% improved vegetative characteristics in pinto bean (such as internode numbers).

No. of branches/plant

The same behavior for number of branches/plant table, 2 was followed, as the use of high concentrations of ZnO NPs resulted in the highest number of branches per plant compared to the low concentrations. The use of ZnO NPs at a concentration of 500 ppm doubled the number of branches per plant as measured by untreated control in both seasons. The increase percentage in number of branches was the lowest (19.6 and 15.0%) when the plants treated with the minimum concentration (0.5 ppm) of ZnO NPs compared to untreated control in both seasons, respectively. Branches No depends on type of nanoparticles that conformed by Younes and Nassef (2015) who observed that Silver nanoparticles (20-40 ppm) reduced branches no. per plant in tomato (*Solanumlycopersicom*, Mill.).

Fresh and dry weights/plant (g)

Regarding fresh and dry weights of Asparagus plant table 2, both increased with increasing concentration of ZnO NPs. ZnO NPs at 500 ppm had the highest fresh and dry weights of (57.20, 47.12g) and (20.02, 16.12 g), as the increase percentages were (199.6, 188.2%) and (331.5, 323.1%), compared with untreated control in both seasons, respectively. However, the lowest fresh and dry weights were observed in the control plant (19.09, 16.35g) and (4.64, 3.81g) in both seasons, respectively. All Nano-Zn concentrations significantly increased than untreated control except for plant dry weight at 0.5 ppm of Nano-Zn in the 2nd season. Fresh and dry weights of plants treated with ZnO NPs at 500 ppm was (43.4, 40.5%) and (67.8, 84.9%) higher than Zn acetate Bulk (40 ppm). Similar results have found by Dhoke *et al.*, (2013) in *Vigna radiate* and Solanki and Laura (2018) in*Triticumaestivum*.Pérez Velasco *et al.*, (2020) noticed that Tomato plants treated with ZnO-NP significantly increased dry weight. Moreover, the dry and fresh weights of *Anthemisgilanica* seedlings' root at concentrations of 4 and 6 g L^{-1} of SiO_2 NPs significantly increased as mentioned by Ahmadi *et al.*, (2020). This result may be due to enhancement the accumulation of water in fresh biomass under such level of Nano metal oxide application. Moreover, ZnO NPs have different rate of permeability through cell wall.

Dry weight/Fresh weight ratio

Data on dry weight/fresh weight ratio of Asparagus plant are shown in table 2. This ratio is greatly influenced by ZnO NPs at higher doses than Zn acetate Bulk. However, at the highest dose (500 ppm), ZnO NPs hada pronounced effect on plant DW/FW ratio as the increase percentages reached 17.1 and 31.5% in ZnO NPs over Zn acetate Bulk in the 1st and 2nd season, respectively. Moreover, the increase percentages reached 44.0 and 46.8% in ZnO NPs over untreated control plant in the 1st and 2nd season, respectively. On the other hand, the lowest dose of ZnO NPs (0.5 ppm) obtained 6.2 and 6.9% of DW/FW ratio over the control in both seasons, respectively. Most ratios were significant related to the untreated control.

Root length (cm)

Root length data table 2 clearly revealed that ZnO NPs have an adverse effect on root elongation of Asparagus plant. In comparison with control, the average root length was correlated with the concentration of ZnO NPs, as the root length increased with increasing Nano-Zn dose up to the concentration of 5 ppm (the highest root length) after that the values gradually decreased up to the concentration of 500 ppm, similar to Hendel *et al.*, (2019) results who observed that AuNPs nanoparticle atcerta in dose caused inhibition at root tip meristematic zone division and that led to decrease in root elongation. The highest increase percentages were 145.7 and 125.6% when the plants were treated with 5 ppm of ZnO NPs related to untreated control plants in both seasons, respectively. As well as, ZnO NPs at the concentration of 5ppm surpassed Zn acetate Bulk by the values of 71.2 and 61.7% in both seasons, respectively. An almost a similar result was reported by Yang and Watts (2005) who stated that ZnO nanoparticles have an inducer effect on plant growth in tomato at concentrations ranged

between (0.0 to 1000 mg/kg) due to increase the soil enzymes activity such as phytase, acid phosphatase and alkaline phosphatase that enhanced solubility and native phosphorous nutrientmobilization in the rhizosphere as mentioned by Raliya *et al.,* (2015). Zinc oxide (100 ppm) causes an increase in growth parameters of carrot such as root length (cm) and increase in cell permeability as mentioned by Elizabeth *et al.,* (2017).

ZnONPs (25 ppm) cause a marked increase in root length (cm) (Sofy *et al.,* 2017in wheat) and (Dhoke *et al.,* 2013in *Vignaradiata*).

On the other hand, there is a negative correlation between copper nanoparticles and seedling root elongation as reported by Adhikari *et al.,* (2015) in *Zea mays* L.

Wang *et al.,* (2018) recorded that the ZnO NP treatments significantly inhibited tomato root growth and Rajput *et al.,* (2018) mentioned that CuO NPs reduced the growth of onion root tip and showed that root treated with 80 mg L^{-1} CuO NPs inhibited growth and stopped growth completely after 72 h exposure and also indicated that, when nanoparticles began to accumulate led to reduction in plant growth parameters as feedback inhibition. Mushinskiy and Aminov (2019) described that effect of Fe, Cu and Mo NPs for the *Solánumtube-rósum* mode on plant varies depending on their sensitivity, as well as physicochemical features of the nanomaterials under study. Mahmoud *et al.,* (2019) in Red Radish and Pérez Velasco *et al.,* (2020) in Tomato concluded that the previous increasing percentages in the mentioned morphological traits led to suppose that there is a positive correlation between agronomic traits as plant height and dry weight.

Anatomical study

In light of the positive impact of zinc nanoparticles on the above-mentioned vegetative growth characteristics of Asparagus plant, it was found worthy to conduct an anatomical study on the plant in order to clarify the effect of zinc nanoparticles on the anatomical structure of the plant, which was directly the reason for improving the vegetative growth attributes of the plant.

Root anatomy

Table 3 and Fig. 1A, B, C show the anatomical parameters of cross-sections in the Asparagus plant root, 3 months old, in plants treated with ZnO NPs at a concentration of 500 ppm compared to the plant root treated with Zinc acetate at a concentration of 40 ppm, as well as untreated control plant.

By comparing the roots of Asparagus plants treated with Zinc acetate (40 ppm) with untreated control plants Fig. 1, A and B, it had been found an increase in the diameter of root in the first treatment from the second one and this increase is attributed also to the increase in the thickness of the different tissues of the treated plant roots than the untreated ones where the increase rates reached 50, 63 and 56% for each of the epidermis thickness, cortex thickness and diameter of the vascular cylinder on Straight. The increase in the thickness of the cortex in the Zinc acetate-treated plant roots is attributed to the increase in the thickness of both the exodermis (150%), the number of its layers (50%), the average thickness of the exoderms cells (67%), the mesodermis (50%), the average thickness of the mesodermis cells (108%) and the endodermis (67%), compared to untreated control. The increase in the diameter of the vascular cylinder in the treated plants is attributed to the increase in the metaxylem diameter (283%) and pith diameter (40%), compared to the untreated control.

On the other hand, by comparing the roots of Asparagus plants treated with ZnO NPs at a concentration of 500 ppm with untreated control plants Fig. 1, A and C, the results showed an increase in the diameter of the treated roots by 110% over the untreated control, due to the increase

Table 3: Anatomical parametersof *Asparagus officinalis*root affectedby different concentrationsof ZnO nanoparticles.

Root anatomical parameters	Con-trol (Untre-ated)	Zn ace-tate (40 ppm)	± % to Con-trol	Zn O NPs (500 ppm)	± % to Con-trol	± % to Zn ace-tate
Epidermis thickness (µm)	10	15	50	20	100	33
Cortex thickness (µm):	270	440	63	610	126	39
Exodermis (suberified cells) thick.	30	75	150	75	150	0
No. of exodermis layers	2	3	50	4	100	33
Average exodermis cell thickness	15	25	67	25	67	0
Oval Mesodermis thickness	210	315	50	480	129	52
No. of mesodermis layers	18	12	- 33	15	- 17	25
Average mesodermis cell thickness	12	25	108	30	150	20
Endodermis thickness	30	50	67	55	83	10
Vascular cylinder diameter (µm)	135	210	56	285	111	36
No. of xylem arms	10	10	0	14	40	40
Average metaxylem diameter (µm)	6	23	283	30	400	30
Pith diameter (µm)	75	105	40	150	100	43
Root diameter (µm)	720	1145	59	1515	110	32

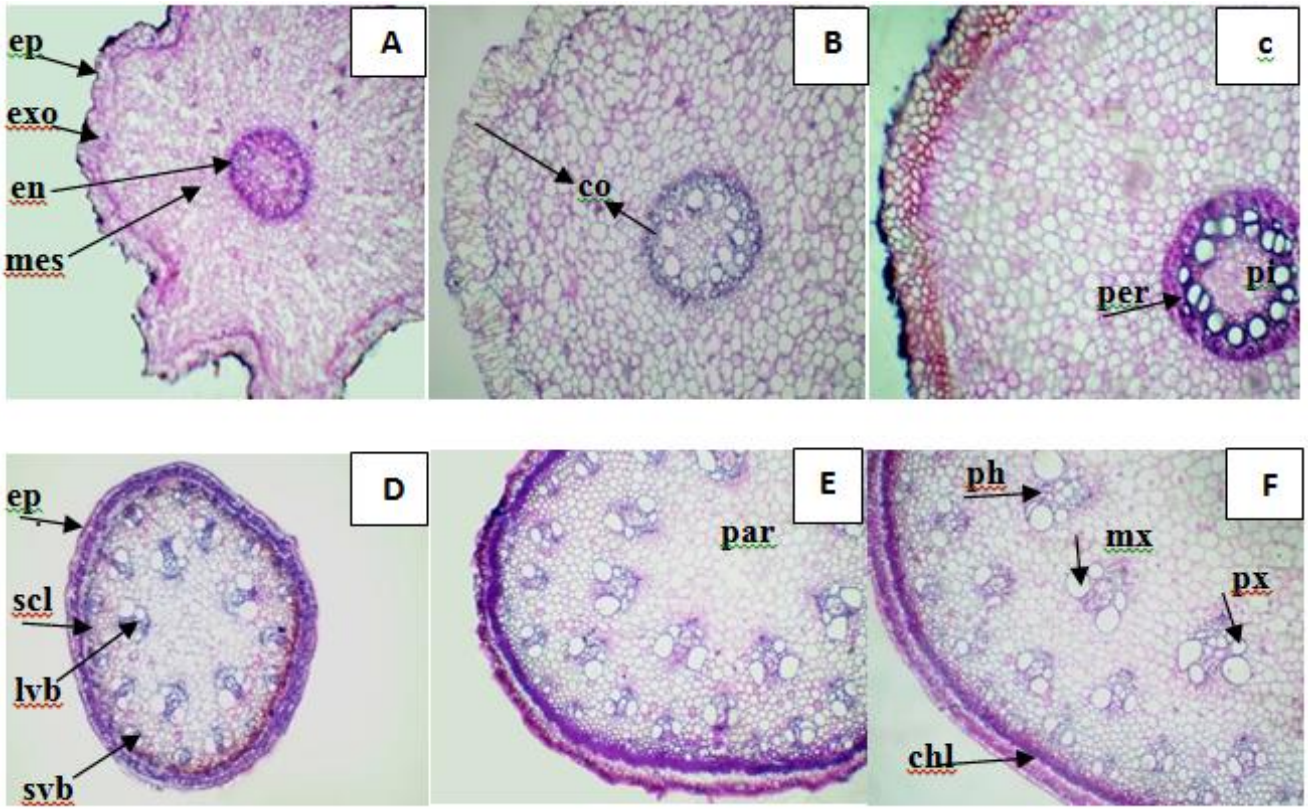

Figure (1): Transverse sections of the median portion of *Asparagus officinalis*root (A-C) and stem (D-F) affected by different concentrations of ZnO nanoparticles show the different anatomical parameters. (A and D) Control, (B and E) Zn acetate at 40 ppm, and (C and F) ZnO NPs at 500 ppm *Details*: Epidermis (ep), Cortex (co), Pith (pi), Large vascular bundle (lvb), Small vascular bundle (svb), Metaxylem (mx), Protoxylem (px), Sclerenchyma (scl), Parenchyma (par), Chlorenchyma (chl), Phloem (ph), Endodermis (en), Pericycle (per), Exodermis (exo), Mesodermis (mes).

in the thickness of the different tissues of the treated plant roots, where the increase rate reached 100, 126 and 111% for each of the thicknesses epidermis, cortex and diameter of the vascular cylinder, respectively.

The increase in the thickness of the cortex in the plants treated with ZnONPs is due to the increase in the thickness of each of the exodermis (150%), the number of its layers (100%), the average thickness of the exodermis cells (67%), the mesodermis (129%), the average thickness of the mesodermis cells (150%) and the endodermis (83%), compared to the untreated control. Also, the increase in the diameter of the vascular cylinder in plants treated with ZnONPs is due to the increase in both the number of xylem arms (40%), the average diameter of metaxylem (400%) and the diameter of the pith (100%), compared to untreated control plants.

When comparing the roots of plants treated with ZnO NPs with the roots of plants treated with Zinc acetate Fig. 1 B and C, it had been found an increase in the root diameter in the first treatment by 32% over the second one and this increase is attributed to the increase in the thickness of the different tissues of the root in the first case from the second one, where the increase rate reached 33, 39 and 36% for each of the thicknesses of epidermis, cortex and diameter of the vascular cylinder, respectively. The increase in the diameter of the cortex in the roots of plants treated with ZnO NPs from related to Zinc acetate treatment is due to the increase in thickness of both the mesodermis (52%), the average thickness of the mesodermis cells (20%) and the endodermis (10%). As for the increase in the diameter of the vascular cylinder, it is attributed to the increase in the number of xylem arms (40%), average metaxylem diameter (30%) and pith diameter (43%) in the roots of the first treatment from the second ones.

Stem anatomy

Table 4 and Fig. 1D, E, F illustrate measurements and numbers of the anatomical parameters of cross-sections of Asparagus plant stem, 3-month-old treated with ZnO NPs at a concentration of 500 ppm compared to Zinc acetate at a concentration of 40 ppm as well as the root of untreated control plant.

With regard to the treatment of Zinc acetate at a concentration of 40 ppm compared to the untreated control Fig. 1D and E, an increase in the root diameter of the first treatment was observed by 119% compared to the

second treatment. This increase is due to a clear increase in the thickness of different tissues, the average thickness of their cells and the number of their layers. This increase was represented by the thickness of the epidermis (100%), the cortex (33%), the thickness of the fibrous sheath (67%), the diameter of the ground tissue (133%), the number of its layers (40%) and the average thickness of the ground tissue cells (67%). It was also noted an increase in the number of vascular bundles for the stems of plants treated with Zinc acetate amounted to 82% higher than untreated control. Similarly, the dimensions of vascular bundles increased as the percentage of the increase reached 20 and 40% in the length and width of large bundles and 33 and 44% in the length and width of small bundles on straight. This increase is due to the increase in the thickness of the vascular tissues, where the increase reached 8 and 67% in xylem and phloem for large bundles, respectively and 27% in xylem for small bundles without an increase in the thickness of the phloem. Also, the increase in the mean diameters of metaxylem vessels reached 25 and 67% in the large and small bundles, respectively, in the stems of plants treated with Zinc acetate compared to the stems of untreated control plants.

By comparing the diameter of Asparagus plant stem treated with ZnO NPs with the untreated control stem diameter Fig. 1D and F, it was found that the first treatment increased the diameter of the stem of the plant by 226% compared to the second treatment (untreated control) and this was attributed to the increase in the thickness of both the epidermis (200%) and the cortex (100%) and the number of its layers (100%), the thickness of the fibrous sheath (100%) and the number of its layers (33%), the diameter of the ground tissue (251%), the number of its layers (50%) and the average thickness of the ground tissue cells (133%). It was also noticed that there is an increase in the number of vascular bundles by 136% in the stems of plants treated with ZnO NPs than the untreated control. Vascular tissues were also affected positively in Nano treated-plants than untreated ones, as the length and width of large vascular bundles increased by 60 and 80%, respectively, as a result of an increase in the thickness of xylem and phloem by 50 and 100%, respectively and the average diameter of the metaxylem vessels by 50%. In small vascular bundles, the length and width of the bundles increased by 33 and 56%, respectively, as a result of an increase in the thickness of the tissues of xylem and phloem by 50 and 47%, respectively and the average diameter of the metaxylem vessels by 100%.

Table 4: Anatomical parameters of *Asparagus officinalis* stem affected by different cocentrations of ZnO nanoparticles.

Stem anatomical parameters	Control (Untreated)	Zn acetate (40 ppm)	± % to Control	ZnO NPs (500 ppm)	± % to Control	± % to Zn acetate
Epidermis thickness (µm)	10	20	100	30	200	50
Cortex thickness (µm)	30	40	33	60	100	50
No. of cortex layers	2	2	0	4	100	100
Fibrous sheath thickness (µm)	30	50	67	60	100	20
No. of fibrous sheath layers	3	3	0	4	33	33
Ground tissue diameter (µm)	450	1050	133	1580	251	51
No. of ground tissue layers	30	42	40	45	50	7
Average ground tissue cell thick. (µm)	15	25	67	35	133	40
No. of vascular bundles	22	40	82	52	136	30
Large vascular bundle length (µm)	75	90	20	120	60	33
Large vascular bundle width (µm)	75	105	40	135	80	29
Xylem tissue thickness (µm)	60	65	8	90	50	39
Phloem tissue thickness (µm)	15	25	67	30	100	20
Average metaxylem diameter (µm)	40	50	25	60	50	20
Small vascular bundle length (µm)	45	60	33	60	33	0
Small vascular bundle width(µm)	45	65	44	70	56	8
Xylem tissue thickness (µm)	30	38	27	45	50	18
Phloem tissue thickness (µm)	15	15	0	22	47	47
Average metaxylem diameter (µm)	15	25	67	30	100	20
Stem diameter (µm)	565	1235	119	1840	226	49

By comparing the effect of each of the ZnO NPs at a concentration of 500 ppm and Zinc acetate at a concentration of 40 ppm on the anatomical composition of the stems of Asparagus plant Fig. 1E and F, it was found that there was a noticeable increase in the diameter of the stem of the Asparagus plant in the first treatment, which was 49% higher than the second treatment. This increase in diameter is due to the increase in both the thickness of the epidermis (50%), the cortex (50%), the number of its layers (100%), the thickness of the fibrous sheath (20%), the number of its layers (33%), the ground tissue diameter (51%) and the number of its layers (7%), the average cells thickness of the ground tissue (40%) in the first treatment compared to the second one. As for the effect of ZnO NPs on the vascular tissues of Asparagus stem compared to Zinc acetate, it was found that there was an increase in the number of vascular bundles amounting to 30% and the dimensions of vascular bundles

amounted to 33 and 29% in the length and width of large bundles, respectively and 8% in the width of small bundles. An increase in the thickness of xylemand phloem tissues of 39 and 20% in the largebundles and 18 and 47% in the small bundles, was obtained respectively. The treatment of ZnO NPs increased the average diameter of the metaxylem vessels by 20% in both large and small bundles. The above mentioned results that given by Rajput *et al.,* 2018 on spring barley clarified similar effect of copper oxide nanoparticles, It caused an increase in metaxylem area. Also, Tirani *et al.,* (2019) observed the vascular expansion and cell wall thickening of the collenchyma and parenchyma cells in root of tobacco plant. Mahajan *et al.,* (2011) in Mung (*Vignaradiata*) and Gram (*Cicerarietinum*) observed that the low concentration of ZnONPs caused a degree of stimulation for different tissues as histological parameters. On the other hand, the high concentration (2000 ppm) caused an inhibition in root and shoot as the cortical cells were highly vacuolated and collapsed, while vascular cylinder was shrunk. In longitudinal section of cucumber root, the vascular cylinder also shrank at high ZnO NPs concentration (1000 mg L^{-1}), that observed by Moghaddasi *et al.,* (2017) and confirmed by Debnath *et al.,* (2020) that ZnO nanoparticles at a higher concentration caused a reduction in mitotic index of *Allium cepa* root tip and was similar with Hendel *et al.,* (2019) who explained that AuNPs nanoparticle treatments in *Arabidopsis thaliana* L. caused a change in root histological zone such as a decrease in root meristematic zone, enhancement of root hairs formation and an increase in radial cell dimension of cortex cell as well as CuO nanoparticles that stimulated a hairy root cell proliferation through affecting on IAA distribution as found in wheat by Adams *et al.,* (2017).

Menesy *et al.,* (2018) indicated that the histological effect depends on the type and concentration of nanoparticles for example; Silica nanoparticles (80 ppm) had effects on the root anatomy of *Pimpinellaanisum* L. causing an increase of root epidermis thickness and cortex thickness nevertheless, caused a decrease in vascular cylinder thickness compared with the control.

Silica nanoparticles are absorbed into the plants and then interacted with polyphenols in cell walls of xylem and affected lignin deposition and biosynthesis. In Tobacco plant treated with (Fe_3O_4) nanoparticles (5 nm), all concentrations, especially 30 mgL^{-1} led to damage epidermal cells, thickened cell walls in the vascular tissues (mainly xylem), the impaired shape of the cells, reduced number of cortical cell layers, vacuolated root cortex and have an advantage for osmolalities storage in stress condition like salinity and draught (Alkhatib *et al.,* 2019). Hajraand Mondal (2017) stated that *Cicerarietinum* treated with ZnO nanoparticles showed interruption in root formation and never grew more than 0.93 cm. Caldelas *et al.,* (2020) reported that ZnO caused damage in the epidermis due to loss of turgor, protoplasm shrinkage, accumulation of electrondense granules in the vacuoles and increased aerenchyma formation. Fe_2O_3 (6 mgL^{-1}) and MgO nanoparticles concentrations led to are markable increase in all measurements and counts recorded on the histological features of the *Pobulus alba* L. stem; thickness of the epidermis, cortex, vascular tissues and pith as well as xylem vessels diameters. The recorded increase percentages in stem diameter and cortex were 30.62 and 51.73 %by (Fathy *et al.,* 2019). (Moghanloo *et al.,* 2019) pointed that silicon nanoparticle give different effect in *Astragalusfridae*led to decrease xylem diameter in root and stem diameter consider as growth suppressor at high dose. There are obvious alteration in vascular tissue especially xylem root and basal stem in *Capsicumannuum* treated by Selenium nanoparticles 30 ppm cause inhibition in xylem differentiation as described by Korani *et al.,* (2020). SiO_2 NPs cause increase in Stele diameter, xylem vessels, phloem area and metaxylem number in *Anthemisgilanica* root at concentration 2, 4 and 6 g L^{-1} as found by Ahmadi *et al.,* (2020).

Catalase activity of *Asparagus officinalis* plant under the influence of different concentrations of ZnO NPs (U/g)

Data in table 5 elucidates catalase activity (U/g) of Asparagus plant as affected by different concentrations of ZnO NPs. Significant increases were found in the activity of catalase enzyme in all treatments under study

Table 5: Catalase activity (U/g) inAsparagus plants treated with different concentrations of ZnO NPs.

ZnO NPs Conc. (ppm)	Catalase activity (U/g)	
	1st Season	2nd Season
Control	0.82 ± 0.01	0.77 ± 0.02
0.5	1.37 ± 0.02	1.31 ± 0.01
1	1.51 ± 0.01	1.43 ± 0.02
3	1.60 ± 0.02	1.53 ± 0.01
5	1.74 ± 0.03	1.69 ± 0.03
10	1.74 ± 0.04	1.69 ± 0.02
30	1.80 ± 0.01	1.76 ± 0.01
50	1.94 ± 0.02	1.88 ± 0.02
100	1.94 ± 0.03	1.90 ± 0.03
500	4.74 ± 0.01	4.21 ± 0.03
Zn acetate	1.44 ± 0.06	1.38± 0.04
LSD (0.05)	0.04	0.03

in relation to untreated control treatment. The highest significant increases were at 500 ppm (4.74 and 4.21 U/g) of ZnO NPs in comparison with Zn free -control treatment (0.82 and 0.77 U/g) in the 1st and 2nd season, respectively. The increase percentages were 478.0 and 446.8% related to the control in both seasons, respectively.

On the other hand, it was found that the increase values of catalase enzyme activity amounted to 229.2 and 205.1% in both seasons, respectively when the highest concentration of Nano-zinc (500 ppm) compared with Zn acetate Bulk at a concentration of 40 ppm. Also, there were increases of 75.6 and 79.2% higher than untreated control when using Zn acetate Bulk at a concentration of 40 ppm in both seasons, respectively. The results showed that there were significant differences among most Nano-Zinc concentrations in both seasons.

Previous mentioned results refer to Zinc Oxide nanoparticles had a direct role in increasing enzymatic antioxidants such as catalase as observed by Lee *et al.,* (2013) who evaluated catalase activity after treatments of 1, 000 and 2, 000 mg/L of ZnO NPs. CAT enzyme activity was increased at all treatment concentrations in *Fagopyrumesculentum* L. as a defense mechanism against nanoparticles stress due to the formation of reactive oxygen species as noticed by Moharrami *et al.,* (2017); Josué *et al.,* (2018); Abbasi *et al.,* (2019) *and Mosavat et al.,* (2019). ZnO nanoparticles used as growth stimulator led to highly significant increase in catalase activity at 5 ppm reach to 478% similar to its significant effect in increasing superoxide dismutase (267.8%) and peroxidase (174.5%) in *Gossypiumhirsutum* L. plant at concentrations (0, 25, 50, 75, 100 and 200 mg l^{-1}) as described by Priyanka and Venkatachalam (2016) and Wang *et al.,* (2018) who stated that high concentration of ZnO NPs led to an increase in catalase activity because of enhancement the transcription of genes related to antioxidant capacity and suggesting that ZnO NPs could enhance the defense response by increasing activities of antioxidant enzymes. López-Vargas *et al.,* (2018) mentioned that Cu nanoparticles (50, 125, 250, 500 mg L^{-1}, diameter 50 nm) applied in tomato caused an increase in catalase activity.

ROS at low or moderate concentrations can act as secondary signal in many biomolecular processes in cells, tolerance stimulation to both biotic and abiotic stresses in plants and led to an increase level of enzymatic antioxidant system response to ROS as pointed by Adisa *et al.,* (2019). Also, *Samart et al.,* (2017) referred to antioxidant enzymes involved in the defense mechanism against the toxicity of ZnO nanoparticles and decrease the harmful effects on photosynthetic pigments in plant cells. Faizan *et al*., (2020) concluded that reducing the toxic effect of Cd by ZnONPs (50 mg L^{-1}) in tomato led to oxidative stress by increasing hydrogen peroxide (H_2O_2) levels resulted in a decline in cell viability through stimulating activity of the antioxidant system.

HPLC results for different flavonoids in Asparagus plants treated with different concentrations of ZnO NPs

HPLC analysis of Asparagus plant revealed that there are six components of flavonoids (Rutin, Myricetin, Quercetin, Naringenin, Kaempferol and Apigenin). They have been identified according to retention times as an additional reference. Data in table 6 gave an idea about different flavonoids content in Asparagus plants treated by different concentrations of ZnO NPs as well as Zn acetate Bulk and untreated control Fig. 2a, b, c, d. The highest content of Rutin (188.56 mg/100g FW) was recorded at the concentration of 3 ppm ZnO NPs followed by Zn acetate Bulkat 40 ppm (140.60 mg/100g FW), then Nano-Zn at 0.5 ppm (118.76 mg/100g FW) and 5 ppm (109.60 mg/100g FW) while, 1 ppm of ZnO NPs gave the lowest content of Rutin (31.3 mg/100g FW) compared to Zn-free treatment (53.20 mg/100g FW) and all the other treatments. Regarding Myricetin content, the highest one was recorded at 3 ppm of Nano-Zinc (151.15 mg/100g FW), followed by the two concentrations 30 and Zn acetate Bulk at 40 ppm of 108.67 and 94.10mg/100g FW, respectively, then 5 ppm of Nano-Zn (69.52mg/100g FW). The concentration, 1 ppm, had also the last

Table 6: HPLC results for different flavonoids inAsparagus plants treated by different concentrations of ZnO NPs.

Flavonoids ZnO NPs Conc. (ppm)	Rutin mg/ 100g FW	Myri-cetinn mg 100g FW	Quer-cetin mg/ 100g FW	Narin-genin mg/ 100g FW	Kaem-pferol mg/ 100g FW	Api-genin mg/ 100g FW	Total flavonoids mg/ 100g FW
Control	53.20	14.94	ND	ND	53.38	ND	121.52
0.5	118.76	42.04	ND	1.50	81.60	ND	243.90
1	31.30	7.63	ND	ND	49.75	ND	88.68
3	188.56	151.15	ND	ND	ND	ND	339.71
5	109.60	69.52	ND	155.00	118.11	ND	452.23
10	70.20	45.28	ND	56.81	58.13	ND	230.42
30	97.90	108.67	ND	ND	0.70	ND	207.27
50	61.90	ND	ND	ND	1.43	197.67	261.00
100	78.50	61.98	ND	57.32	ND	ND	197.80
500	79.22	44.63	ND	ND	ND	ND	123.85
Zn acetate	140.60	94.10	ND	ND	119.42	ND	354.12

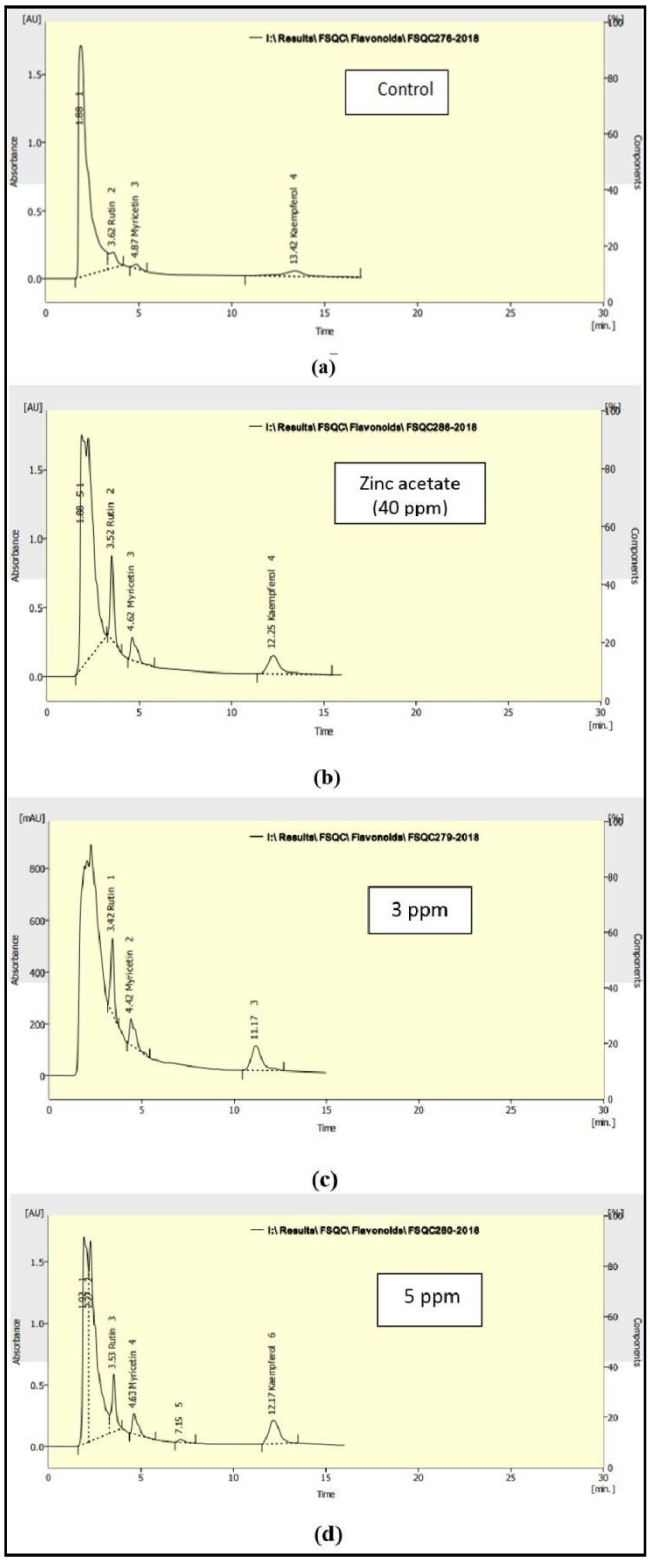

Fig. 2: HPLC chromatograph results for different flavonoids in Asparagus plants.

(a) Untreated control (b) Treated by Zn acetate (40ppm) (c) Treated by ZnO NPs (3ppm) (d) Treated by ZnO NPs (5ppm)

place in Myricetin content (7.63 mg/100g FW) when compared with untreated control (14.94 mg/100g FW) and the other Nano-Zn concentrations. The other flavonoids types did not appear in most ZnO NPs concentrations.

From previous results it could be concluded that the concentration of 1ppm ZnO NPs had the lowest stimulation effect for Rutin and Myricetin, but 3 ppm led to the highest stimulation effect for both types of flavonoids. In general, the concentration of 3 ppm ZnO NPs ranked first in Rutin (188.56 mg/100g FW) and Myricetin content (151.15 mg/100g FW), while ranked third in total flavonoids content (339.71 mg/100g FW) and the concentration 5 ppm came in the fourth rank in Rutin (109.60 mg/100g FW) and Myricetin content (69.52 mg/100g FW), while ranked first in Naringenin (155.00 mg/100g FW) as well as total flavonoids content (452.23 mg/100g FW) and second in Kaempferol content (118.11 mg/100g FW). Moreover, the concentration of 50 ppm ZnO NPs ranked first in Apigenin content (197.67 mg/100g FW) and fourth in total flavonoids content (261.00 mg/100g FW), while the concentration of 0.5 ppm ZnO NPs ranked third in the content of Rutin (118.76 mg/100g FW) and Kaempferol content (81.60 mg/100g FW) and fifth in total flavonoids content (243.90 mg/100g FW). On the other hand, Zn acetate Bulk ranked second in Rutin (140.60 mg/100g FW) as well as total flavonoids content (354.12 mg/100g FW) while ranked third in Myricetin (94.10 mg/100g FW) and first in Kaempferol content (119.42 mg/100g FW).

The previous observation indicated that ZnONP plays as a flavonoid elicitor at certain concentrations (Wei and Guo, 2014) who mentioned that Zn-binding at a flavonoid structure makes zinc has a stimulator effect at a certain physiological condition. Cobalt NPs cause an increase in total flavonoids at high concentrations (2000 and 4000 ppm) as pointed by Jahani *et al.*, (2019) so, the current study led to explain the effect of nanoparticles on metabolite depending on the type due to its different pathways. The type of eliciting flavonoids differs according to the type of used nanoparticle for example; Singh *et al.*, (2018) mentioned that CuO nanoparticles significantly enhanced flavonoids content in *Withaniasomnifera* L. Dunal in shoot and root after 20 days of treatment (23.076mg/gQuercetin).

The recorded results refer to Rutin, the most important flavonoid fraction in Asparagus; it represented 0.015 to 0.45% of fresh weight of 12 hybrid lines of Asparagus according to the genotype as described by Al-Snafi (2015) and Fan *et al.*, (2015). The total flavonoids content in *A. gilanica* seedlings that were treated with 2, 4 and 6 g L^{-1} of SiO_2 NPs were 1.10, 1.21 and 1.69-fold greater than that of the control seedlings because

the stimulation effect of polyphenols biosynthesis pathway in spite of its reduction at a high concentration as pointed out by Ahmadi *et al.,* (2020).

Conclusion

It could be concluded from the current study that, ZnO NPs at 500 ppm achieved the best results of morphological characters of *Asparagus officinalis* plants such as; germination rate, seedlings length, SVI value, plant height, number of main stem inter nodes, No. of branches, plant fresh weight, plant dry weight and DW/FW over the untreated control. The histological parameters of root and stem resulted from treating the plants with the concentration of ZnO NPs (500 ppm) supported the morphological characters. The biochemical parameters clarified an increase in catalase activity at the highest concentration of Nano-Zn (500 ppm) as an antioxidant defense mechanism led to increasing secondary metabolite production. The highest total flavonoid content as well as Naringenincontent was attained at a concentration of 5 ppm. The highest contents of Rutin and Myricetin were recorded at a concentration of 3 ppm Nano-Zinc. ZnO NPs are used as ecofriendly growth stimulator and secondary metabolite elicitor at 3 and 5 ppm, especially Rutin as a type of flavonoids.

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