Effect of Zinc Nano-particle as Foliar Spray on Growth, Seed Yield and Active Constituents of *Silybum marianum* L. Plants

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Abstract: The experimental work was conducted during the two successive seasons of 2018/2019 and 2019/2020 at the Experimental Farm of Ismailia Agriculture Research Station, Ismailia Governorate Egypt, to study the effect of zinc nanoparticles as foliar spray (0.25, 0.50 and 1.0 g/l, beside unsprayed (control treatment)) on growth, seed yield and active constituents of *Silybum marianum* L. The experiment was designed as randomized complete blocks design with three replicates. As for the response of *Silybum marianum* L. plants to foliar application with zinc nanoparticles, results indicated that, treating *Silybum marianum* L. with ZnO-NPs at 1 g/l as foliar spray increased plant height, number of leaves/plant, number of inflorescences/plant, stem diameter, root length, number of roots/plant, dry weight of roots, herb and inflorescences/plant, chlorophyll a, chlorophyll b and total chlorophyll (a+b), seed yield /feddan., N, P and K contents in leaves and Zn concentrations in fruits, TXF, SDN, SBN, SCN, ISBN and total silymarin in fruits, in both seasons.

Keywords*:* *Silybum marianum* L., ZnO-NPs, Vegetative growth, seed yield, TXF, SDN, SBN, SCN, ISBN and silymarin

INTRODUCTION

An annual plant of the Asteraceae family, milk thistle (*Silybum marianum* L.) is native to the Mediterranean region and is common throughout the world, including Iran. Milk thistle extracts have been used in medicine since the 4th century B.C., and in the 16th century they became a popular treatment for hepatobiliary illnesses (Sedghi *et al*., 2010).

Milk thistle, (*Silybum marianum* L.) produces fruits that are rich in isomeric flavonolignans known as silymarin, which includes silydianin, silychristin, isosilybin and silybin (Kurkin *et al*., 2001).

Almost all fields, from basic to applied sciences, have been significantly touched by nanotechnology. Different kinds of nanomaterials have emerged during the past ten years, revolutionising many fields of endeavour with amazing applications. Nanomaterials are manufactured substances with sizes between 1 and 100 nm. Due to the structural changes that have occurred at the molecular level, these materials acquire certain distinct characteristics at the micro scale in terms of quicker activity. Engineered nanoparticles with sizes ranging from 1 to 100 nm, such as silver, carbon, zinc, gold, magnesium, copper, etc., have demonstrated considerable advantages in the fields of medicine, food, cosmetics, materials science, agriculture and environmental research. Among metallic nanoparticles (NPs), zinc oxide nanoparticles (ZnONPs) have garnered more interest due to their distinct catalytic capabilities and wide range of uses in metallurgy, medicine, agriculture and engineering (Shehzad *et al*., 2021).

In order to establish a technical strategy for the agricultural use of affordable nano materials, the current study's objective is to investigate the effects of zinc oxide (ZnO) nanoparticles (NPs) on the plant growth and active components of *Silybum marianum* L. plants.

MATERIALS AND METHODS

The study examined the effects of zinc oxide (ZnO) nanoparticles (NPs) on the active components, growth, and seed yield of the *Silybum marianum* L. plant over two consecutive seasons, 2018–2019 and 2019–2020, at the Experimental Farm of Ismailia Agriculture Research Station, Ismailia Governorate, Egypt.

The experimental soil in the two seasons was sandy soil and its characteristics are shown in Table (1).

The irrigation water during the two seasons was taken from the same source and had low salts contents as presented in Table (2).

This experiment was included three concentrations of ZnO oxide nanoparticles as foliar spray (0.25, 0.50 and 1.0 g/l), beside unsprayed (control plants).

Table (1): Physical and chemical characteristics of the experimental soil

Characteristics	2018/2019 season	2019/2020 season
Physical properties		
Sand%	96.70	96.60
Silt%	1.60	1.60
Clay%	1.70	1.80
Textural class	Sand	Sand
EC, dSm^{-1}	0.28	0.27
pH	7.91	7.89
Chemical properties		
Cations, meq /100g soil		
Ca^{2+}	0.31	0.31
Mg^{2+}	0.22	0.21
Na^{+}	0.82	0.78
K^{+}	0.04	0.04
Anions, meq/100g soil		
Cl^{-}	0.85	0.79
HCO_3^{-}	0.18	0.19
SO_4^{2-}	0.36	0.36
CO_3^{2-}	--	--
Available N mg Kg^{-1}	8.63	8.69
Available P mg Kg^{-1}	8.88	8.92
Available K mg Kg^{-1}	30.08	30.06
Total N mg Kg^{-1}	9.40	9.60
Organic Matter g Kg^{-1}	1.80	1.90

Table (2): Chemical characteristics of the water used for irrigation of *Silybum marianum* L.

EC, dSm	pH	Cations, meq /L				Anions, meq /L			
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	HCO_3^{-}	$SO^{2}_{4}{}^{-}$	CO^{2-}_{3}
0.36	7.10	0.50	0.40	2.60	0.10	2.70	0.40	0.50	---

Preparation and characterization of ZnO-NPs:

From Sigma Aldrich (99.9%) NaOH, zinc acetate and isopropyl alcohol (2-propanol) 99.5% were purchased. At 60oC, 0.073 mmol of zinc acetate was dissolved with stirring in 50 ml of 2-propanol. In a second flask, at 60°C, 25 ml of 2-propanol was vigorously stirred to dissolve 1.5 mmol of NaOH. The acetate solution was stirred while drops of NaOH solution were added. At 60 degrees Celsius, the product was swirled for an hour before being cooled to room temperature. The precipitate was centrifuged at 4500 rpm for 30 minutes after being washed twice with 2-propanol (Khater *et al.,* 2013).

TEM imaging was used to study the physicochemical parameters of Zn nanoparticles Fig (1). The images of the nanoparticles show a spherical form with an average particle size ranging from 4 nm to 100. Plot area was 8.4 m^2 and contained two rows, each was (3.5 m) in length and (1.2 m) in width and planting distance was (0.5 m) apart, since the numbers of plants were seven in each row which counts (14) plants per plot. Seeds were sown in 17th and 18th October for 2018/2019, 2019/2020 seasons.

Silybum marianum L. Seeds were achieved from Medicinal and Aromatic Plants Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The plants were treated two times by foliar spraying with the different concentrations. The first time before flowering stage in 9th of February, the second time after flowering stage in 9th of March in the two seasons. A hand sprayer was used to apply the foliar spray. (capacity 2 L) at 9 o'clock. In 15th October of both seasons, the soil was divided into plots (2.4 × 3.5 m). During the preparation process, (10 m^3/fed.) compost was added and well mixed with the soil three weeks before planting. Chemical fertilizer used was NPK at "24:12:18 kg/fed." (Urea 46 % N), (potassium sulfate 48.5 % K2O) and (calcium super phosphate 15.5 % P2O5).

Three repetitions of a randomised complete blocks design were used for the experiment. All plants were exposed to standard agricultural techniques as needed.

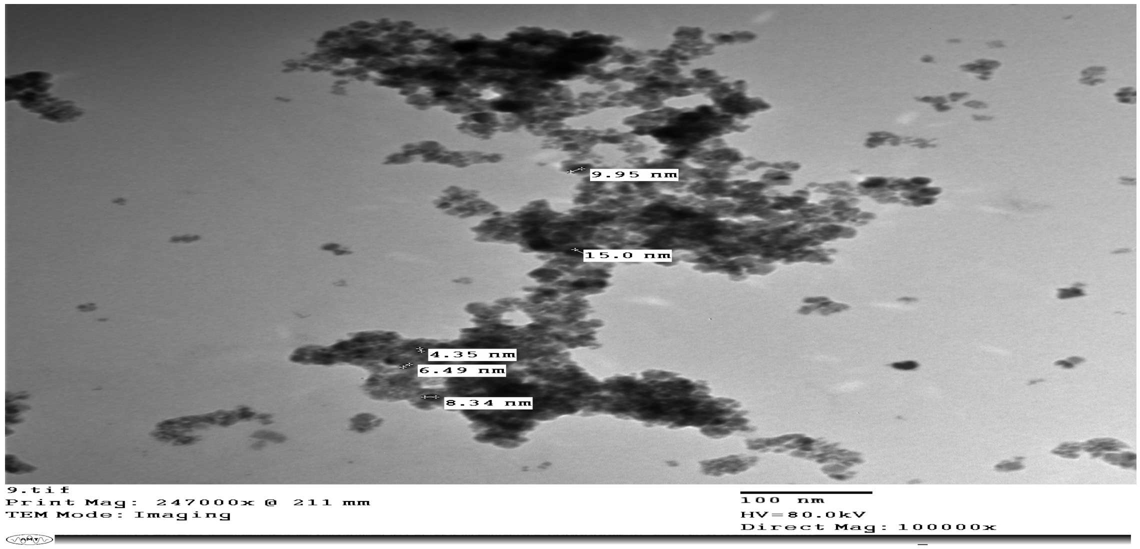

Fig. (1): TEM imaging of the prepared Zn nanoparticles revealed a spherical shape of the particles, with an average size of 4 nm to 100 nm (inset shows electron diffraction pattern)

Recorded data:

1-Vegetative growth parameters:

Each plot's five plants were randomly selected 150 days following sowing for both seasons and the following parameters were determined:, number of leaves per plant, stem diameter (mm), plant height (cm), root length (cm), and number of roots/plant are all factors to consider. Plant parts such as, roots, herb and inflorescences were dried at 70°C until they reached a constant weight, and the following data were collected: herbs, dry weight (gm) of roots, total dry weight (gm) per plant and inflorescences per plant (roots + herbs + inflorescences).

2-Estimation of Chlorophylls:

A known fresh weight of leaves at 150 days after sowing in both seasons was homogenised in 85% aqueous acetone for five minutes after sowing in both seasons. After centrifuging the homogenate, the supernatant was diluted with 85% acetone by volume. The photosynthetic pigments were identified using the spectrophotometric method. (chlorophyll-a, chlorophyll-b, and total chlorophyll) as recommended by Metzner *et al.* (1965). Using a spectrophotometer VEB Carl Zeiss, Three wavelengths, A452.5, A644, and A663 (nm), were used to test the absorbance against a blank of pure 85% aqueous acetone while accounting for dilution. The concentrations of the pigment components could be found. (Chlorophyll-a, chlorophyll-b and total chlorophyll) as mg/ml using the following equations:

Chlorophyll-a = 10.3 A_{663} – 0.918 A_{644} mg/100 g fresh weight

Chlorophyll-b = 19.7 A_{644} – 3.870 A_{663} mg /100g fresh weight

Total Chlorophyll = Chlorophyll-a + Chlorophyll-b mg /100g fresh weight

3-Analysis of mineral elements:

Air-dried leaves and fruits were collected 150 days after sowing and oven dried at 70 ºC till constant weight. 0.5 gm from dried samples were acid digested with a mixture of hydrogen peroxide and sulfuric acid before being brought to a final volume of (50 ml) with distilled water., to determine the following: -

1. Total nitrogen percentage in the leaves was determined using micro-kjeldahl method as described by (Mazumdar and Majumder, 2003).

2. Total Phosphorus percentage in the leaves was determined color metrically at 640 nm wave length according to (Mazumdar and Majumder, 2003).

3. Potassium percentage in the leaves was determined by using flame photometer according to (Mazumdar and Majumder, 2003).

4. Concentration of Zn was determined in the air-dried fruits digest solutions by Inductively Coupled Plasma Mass Spectrometry (ICP MS) according to (Ammann , 2007).

4- Crud's yield:

In both seasons, the harvesting process took place on the first of April until the first of May. The following data regarding the fruit yield were recorded as follows: Weight of seeds (g/plant), weight of seeds per plot and seed yield/fed. (Kg).

5- Determination of silymarin percentage in the dry fruits:

1. The active ingredients:

Total silymarin percentage was determined in the powdered fruits of *Silybum marianum* L. plants during the two seasons after harvesting according to the method described by (Martinelli *et al.*,1991):

2. Extraction procedure:

(1.0 g) grounded finely powdered was extracted with petroleum ether for (4 hours) in extraction apparatus. The extraction solution discarded and the apparatus extracted by methanol for (5 hours). The methanol evaporated and vacuum dried in about (50^{o}C).

3. HPLC conditions:

The extracted material solubilized with (100 ml) methanol. Filtered with Millipore (0.3 um) for three times and (50 ul) of extraction injected into HPLC apparatus with flow rate 1 ml/min. Standard silymarin mixture (Taxifolin TXF, Silychristin SCN, Silydianin SDN, Silybin SBN and Iso silybin ISBN) used as total amount of Silymarin and injected into column in gradual series standards: (0.05, 0.2, 0.5, 1.0, 1.5, 3.0 and 6 mg/ml).

Statistical analysis:

Two experiments were arranged in randomized complete blocks design and collected data were subjected to proper statistical analysis of variance according to (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Effect of zinc oxide nanoparticles (ZnO-NPs) as foliar application on growth, photosynthetic pigments, seed yield and active ingredients of *Silybum marianum* L.:

Morphological characters:

Data in Table (3) illustrate that spraying *Silybum marianum* L. with zinc oxide nanoparticles (ZnO-NPs) had significant effect on plant height, number of leaves/plant, number of inflorescences/plant and stem diameter at 150 days after sowing compared to unsprayed (control plant) in both seasons. Morphological characters increased with the increasing of ZnO NPs up to 1 g/l in both seasons.

Table (3): Effect of foliar spray with zinc oxide nanoparticles concentrations on vegetative growth characters of *Silybum marianum* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	Plant height (cm)	Number of leaves/plant	Number of inflorescences /plant	Stem diameter (mm)
	2018/2019 season			
Control	78.80	7.60	7.80	15.80
0.25 g/liter	82.33	8.66	8.20	16.46
0.5 g/liter	83.06	9.33	9.50	16.66
1.0 g/liter	91.73	10.46	10.36	18.40
LSD at 0.05 level	**3.45**	**0.27**	**0.44**	**1.09**
	2019/2020 season			
Control	68.83	7.66	7.30	13.73
0.25 g/liter	70.13	8.73	7.43	14.00
0.5 g/liter	81.66	9.60	9.33	16.40
1.0 g/liter	83.76	10.73	10.40	16.80
LSD at 0.05 level	**2.88**	**0.48**	**0.71**	**0.79**

Silybum marianum's increased vegetative development may be related to Zn's crucial role in preserving and protecting the structural stability of cell membranes (Welch *et al*., 1982), as well as its use in protein synthesis, membrane function, cell elongation, and tolerance to environmental stresses (Cakmak, 2000).

Similar findings were reported by (Khater *et al.,* 2013) on *Ocimum basilicum*, (Laware and Raskar, 2014) on onion plants, (Elizabath *et al*., 2017) on carrot.

Root system:

Data in Table (4) indicate that treating with ZnO-NPs as foliar application increased root system of *Silybum marianum* L. compared to control (unsprayed plant). ZnO-NPs at 1 g /l significantly increased root length and number of roots/plant in both seasons.

The achieved outcomes are comparable to those reported by (Méndez-Argüello *et al*., 2016) on pepper demonstrated that, when compared to control plants, foliar application of ZnO-NPs to plants produced the highest values of root dry biomass and root length. (Sadak and Bakry, 2020) indicated that spraying flax plants with ZnO-NPs at 40 mg/l significantly increased dry weight, root length and root fresh.

Table (4): Effect of foliar spray with zinc oxide nanoparticles concentrations on root parameters of *Silybum marianum* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	Root length (cm)		Number of roots/plant	
	2018/2019 Season	2019/2020 Season	2018/2019 season	2019/2020 season
Control	19.40	17.06	6.00	4.66
0.25 g/liter	20.53	17.66	6.06	5.40
0.5 g/liter	20.80	20.33	6.53	5.93
1.0 g/liter	23.00	21.13	7.06	6.33
LSD at 0.05 level	**1.61**	**1.58**	**0.82**	**0.64**

Dry weight:

The obtained results in Table (5) indicated that ZnO-NPs reflect a significant effect on dry weight of plant parts of *Silybum marianum* L. compared to control in both seasons. Treating with ZnO-NPs at 1 g/l increased dry weight of roots, herb, inflorescences/plant and total dry weight/plant, followed by ZnO-NPs at 0.5 g/l.

The develops in total dry weight/plant were about 44.2 and 93.9 % for spraying with ZnO-NPs at 1 g/l 30.6 and 78.9% for spraying with the ZnO-NPs at 0.5 g/l and 18.0 and 10.5% for spraying with the ZnO-NPs at 0.25 g/l over unsprayed plants in the 1st and 2nd seasons, respectively.

The metabolism of other micronutrients as well as the creation and breakdown of nucleic acids, proteins, lipids and carbohydrates depend on the presence of zinc, which is an essential component of various enzymes. It also contributes to the production of biomass. It also plays a role in biomass generation (Marschner, 1995). Obtained results are similar to those reported by (Vafa *et al*., 2015) concluded that on savory (*Satureja hortensis*), (Torabian *et al.,* 2016) on sunflower, (Hassanpouraghdam *et al.,* 2019) on *Rosmarinus officinalis* and (El-Khateeb *et al.,* 2020) on marjoram plant.

Table (5): Effect of foliar spray with zinc oxide nanoparticles concentrations on fresh weight of *Silybum marianum organs* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	DW of roots/plant (g)	DW of herb/plant (g)	DW of inflorescences/ plant (g)	Total DW / plant (g)	Relative increases in total DW over control
			2018/2019 season		
Control	93.33	173.33	26.42	293.08	00.0
0.25 g/liter	109.67	203.67	32.61	345.95	18.0
0.5 g/liter	122.50	227.50	32.85	382.85	30.6
1.0 g/liter	134.17	249.17	39.28	422.62	44.2
LSD at 0.05 level	**5.04**	**4.06**	**1.49**	**21.97**	**--**
			2019/2020 season		
Control	67.33	118.33	21.05	210.71	00.0
0.25 g/liter	72.33	134.33	26.19	232.85	10.5
0.5 g/liter	121.33	225.33	30.23	376.89	78.9
1.0 g/liter	131.83	244.83	31.90	408.56	93.9
LSD at 0.05 level	**4.55**	**5.68**	**1.55**	**31.96**	**--**

DW=dry weight.

Leaf pigments

Leaf pigments in leaf tissues of *Silybum marianum* increased with increasing ZnO-NPs up to 1 g/l. This means that ZnO-NPs at 1 g/l increased the concentrations of chlorophyll a, chlorophyll b and total chlorophylls (a+b) in leaf tissues Table (6).

Zn is necessary for plant growth because it affects the synthesis of indole acetic acid (IAA), a phytohormone that regulates plant growth. It is also essential for glucose biosynthesis and chlorophyll synthesis (Burt, 2004) Furthermore, Zn impacts the performance of important enzymes such as carbonic anhydrase, which includes a Zn atom and catalyses CO2 hydration, allowing carbon dioxide to flow to carboxylation sites in plants (Pullagurala *et al.,* 2018).

These findings are similar to those reported by (Hassanpouraghdam *et al*., 2019) indicated that spraying *Rosmarinus officinalis* with nano-Zn foliar applications at 3 mg/l increased total chlorophyll in herb than unsprayed plants.

Table (6): Effect of foliar spray with zinc oxide nanoparticles concentrations on leaf pigments (mg/100g FW) of *Silybum marianum* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	Chlorophyll a		Chlorophyll b		Total chlorophyll (a+b)	
	2018/2019 Season	2019/2020 Season	2018/2019 Season	2019/2020 season	2018/2019 season	2019/2020 season
Control	147.2	151.5	97.9	96.1	245.1	247.6
0.25 g/liter	157.0	167.2	103.9	103.9	260.9	271.1
0.5 g/liter	181.5	177.6	109.7	112.5	291.2	290.1
1.0 g/liter	191.3	189.4	131.3	123.9	322.6	313.4
LSD at 0.05 level	**19.40**	**11.30**	**13.83**	**12.82**	**18.01**	**21.55**

Seed yield:

Data in Table (7) indicated that foliar spray of spraying *Silybum marianum* L. with ZnO-NPs at 1 g/l significantly increased seed weight/plant, seed weight/plot and seed weight/feddan in both seasons followed by ZnO-NPs at 0.5 g/l.

The increases in total seed weight/feddan were about 62.6 and 48.9 % for spraying with ZnO-NPs at 1 g/l, 20.0 and 35.6% for spraying with ZnO-NPs at 0.5 g/l and 19.1 and 11.1% for spraying with ZnO-NPs at 0.25 g/l over unsprayed plants in the 1st and 2nd seasons, respectively.

The stimulative effect of ZnO-NPs at 1 g/l on seed yield may be due to that spraying with ZnO-NPs at 1 g/l increased number of leaves/plant Table (3), root length Table (4), dry weight of inflorescences and total dry weight/plant Table (5) and leaf pigments Table (6).

Table (7): Effect of foliar spray with zinc oxide nanoparticles concentrations on yield of seeds of *Silybum marianum* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	Weight of seeds/plant (g)	Weight of seeds/plot (g)	Weight of seeds/fed. (kg)	Relative increases in total seeds yield /fed. over control
	2018/2019 season			
Control	12.46	174.42	87.208	00.0
0.25 g/liter	14.84	207.78	103.892	19.1
0.5 g/liter	14.95	209.30	104.650	20.0
1.0 g/liter	20.25	283.58	141.792	62.6
LSD at 0.05 level	**0.49**	**6.99**	**3.49**	**--**
	2019/2020 season			
Control	9.75	136.50	68.250	00.0
0.25 g/liter	10.83	151.67	75.833	11.1
0.5 g/liter	13.21	185.03	92.517	35.6
1.0 g/liter	14.51	203.23	101.617	48.9
LSD at 0.05 level	**0.54**	**7.61**	**3.80**	**--**

Auxin synthesis, cell division, and the maintenance of membrane shape and function all depend on zinc, a micronutrient. Zinc deficiency has an impact on plant development, flowering, pollen viability, and the production of seeds and fruits. (Marschner, 1995).

These results agreement with those reported by (Pirzad, and Barin, 2018) on anise plants, (Rezaei-Chiyaneh *et al*., 2018) on black cumin and (García-López *et al*., 2019) on Habanero Peppers.

Chemical composition:

Leaf chemical composition, i.e., N, P and K contents in leaves and Zn concentration in fruits increased with treating *Silybum marianum* L. by ZnO-NPs at 1 g/l followed by ZnO-NPs at 0.5 g/l in both seasons Table (8). Control plants (unsprayed) gave the lowest values of N, P and K contents in leaves and Zn concentration in fruits followed by spraying with ZnO-NPs at 0.25 g/l.

These findings were nearly identical to those of Alloway (2008). They demonstrated that zinc therapy increased the total amount of nitrogen consumed, supporting the idea that it primarily affects the major physiological systems involved in nutrient uptake. Greater absorption from the Zn source and less translocation to other plant parts may be responsible for the increase in Zn concentration in the leaves (Mitra and Sadhu, 2006). Zn stimulates plant enzymes that are involved in glucose metabolism, cellular membrane integrity, protein synthesis, and auxin synthesis (Marschner, 1995).

Table (8): Effect of foliar spray with Zn oxide nanoparticles concentrations on N, P and K contents in leaves as well as Zn concentrations in fruits of *Silybum marianum* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	Leaves (%)			Fruits (ppm)
	N	P	K	Zn
	2018/2019 season			
Control	1.03	0.097	1.11	0.63
0.25 g/liter	1.65	0.149	1.37	0.83
0.5 g/liter	2.17	0.162	1.50	1.2
1.0 g/liter	2.42	0.201	1.89	1.9
LSD at 0.05 level	**0.17**	**0.018**	**0.19**	**0.11**
	2019/2020 season			
Control	1.02	0.093	1.11	0.74
0.25 g/liter	1.69	0.120	1.35	0.95
0.5 g/liter	2.43	0.149	1.60	1.5
1.0 g/liter	3.12	0.208	1.83	2.4
LSD at 0.05 level	**0.35**	**0.020**	**0.16**	**0.14**

These outcomes are consistent with those mentioned by (Khater *et al*., 2013) stated that spraying with 20 ml/l were the best for increasing N, K, Fe, Zn and Cu %, while spraying with 30 mg/l was the best for increasing P contents in shoots than other concentrations of *Ocimum basilicum.* Also, (Torabian *et al.,* 2016) found that foliar treatment of ZnO-NPs resulted in higher K content and Zn concentration in sunflower leaves than unsprayed plants. Likewise, (Hassanpouraghdam *et al*., 2019) indicated that spraying *Rosmarinus officinalis* with nano- Zn foliar applications at 3 mg/l recorded the highest concentration of Zn and Fe in herb than unsprayed plants. In the same time, (Mohammad *et al.,* 2019) The maximum Ca and Fe content, protein percentage, â-carotene, percentage of total carbohydrate and vitamin C content in leaves were obtained from spraying moringa with zinc oxide nano particles at a concentration of 50 mg/l.

Active ingredients in fruits:

Treating *Silybum marianum* L. with ZnO-NPs at different concentrations increased silymarin components in fruits compared to control Table (9). Silymarin components increased with increasing ZnO-NPs up to 1 g/l.

This means that ZnO-NPs at 1 g/l increased TXF, SDN, SBN, SCN, ISBN and total silymarin in fruits in both seasons.

Table (9): Effect of foliar spray with zinc oxide nanoparticles concentrations on active constituents of *Silybum marianum* during 2018/2019 and 2019/2020 seasons under sandy soil conditions

Treatments with ZnO-NPs	TXF (%)	SDN (%)	SBN (%)	SCN (%)	ISBN (%)	Total Silymarin (%)
	2018/2019 season					
Control	0.13	80.33	3.39	0.012	0.053	0.80
0.25 g/liter	1.90	80.54	4.11	0.025	0.098	1.84
0.5 g/liter	3.60	80.78	5.10	0.035	0.155	2.72
1.0 g/liter	5.34	81.07	7.56	0.050	0.217	2.93
	2019/2020 season					
Control	0.13	80.33	3.23	0.012	0.052	0.80
0.25 g/liter	1.80	80.54	4.15	0.022	0.100	2.04
0.5 g/liter	3.50	80.78	5.28	0.047	0.144	2.56
1.0 g/liter	5.14	81.07	6.76	0.048	0.211	2.78

TXF= Taxifolin, SDN = Silydianin, SBN= Silybin, SCN =Silychristin and ISBN= Iso silybin all used as total amount of silymarin.

These findings are similar to those reported by (Abdel Wahab and Taha, 2018) on *Eruca sativa*, (Mohammad *et al*., 2019) on moringa spraying with zinc oxide nano particles at concentration of 50 mg/l of gave the highest values of niaziridin, chlorogenic acid, gallic acid in leaves. However, (El-Khateeb *et al.,* 2020) showed that spraying plants with nano- Zn at 100 ppm recorded the highest values of main constituents of oil were: sabinene, y-terpinene, a-terpinene, terpinolene, pcymene, linalool, cis-sabinene hydrate, terpinen-4-ol, linalyl acetate and y-terpineol than 50 ppm or untreated plants.

From the foregoing results, it could be concluded that, treating *Silybum marianum* L. with ZnO-NPs at 1 g/l as foliar spray increased plant height, number of leaves/plant, number of inflorescences/plant, stem diameter, root length, number of roots/plant, dry weight of roots, herb and inflorescences/plant, chlorophyll a, chlorophyll b and total chlorophyll (a+b), seed yield /feddan., N, P and K contents in leaves and Zn concentrations in fruits, TXF, SDN, SBN, SCN, ISBN and total silymarin in fruits, in both seasons.

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تأثير الرش الورقى بجزيئات الزنك متناهية الصغر على نمو ومحصول البذور والمواد الفعالة لنبات شوكة مريم

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تم إجراء العمل البحثي بالمزرعة التجريبية بمحطة البحوث الزراعية بالإسماعيلية بمحافظة الإسماعيلية مصر، لدراسة تأثير الزنك بتركيز (صفر، ٠.٢٥، ٠.٥٠، ١.٠ جم/لتر) على صورة أكسيد الزنك النانوي على النمو، محصول البذور والمواد الفعالة لنبات شوكة مريم خلال موسمي ٢٠١٨/٢٠١٩ و ٢٠١٩/٢٠٢٠ م. وقد صممت التجربة في قطاعات كاملة العشوائية وفي ثلاث مكررات. وقد أدت معاملة نباتات شوكة مريم باستخدام جزيئات أكسيد الزنك النانوية عند ١جم/لتر في صورة رش ورقي إلى زيادة طول النبات، عدد الأوراق/نبات، عدد النورات/نبات، قطر الساق، طول الجذر، عدد الجذور/نبات، الوزن الجاف للجذور والعرش والنورات/نبات، الكلوروفيل أ، الكلوروفيل ب والكلوروفيل الكلي أ + ب، محصول البذور/فدان، محتويات الأوراق من النيتروجين والفوسفور والبوتاسيوم وكذلك الزنك في ثمار نبات شوكة مريم، التاكسيفولين، السيليديانين، السيليبين، السيليكريستين، الأيزوسيليبين و السيليمارين الكلي في الثمار في كلا الموسمين.

الكلمات الدالة: نبات شوكة مريم – أكسيد الزنك النانوي – النمو الخضري – محصول البذور –التاكسيفولين – السيليديانين – السيليبين – السيليكريستين – الأيزوسيليبين – السيليمارين.