Ann. For. Res. 66(1): 1014-1021, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

EFFECT OF NANO-SPRAYING ZINC AND NANO-CALCIUM AND THEIR INTERACTIONS ON SOME PHYSIOLOGICAL INDICATORS OF PLANT *OCIMUM BASILICM*

Ahmed Adnan Hussein & Samah Salih AL-Shybany
Al -Qadisiyah University / College of Science / Department of Life Sciences
Email: samah.saleh20@qu.edu.iq, E-mail:ahmediq260@gmil.com

Abstract: The experiment was designed with randomized complete block. It consisted of two factors and with three replications. The first factor consisted of three concentrations (1.5gm - 3gm - 4gm .liter^{-1}) in addition to the comparison factor. The second factor consisted of two concentrations (2gm - 4gm .liter^{-1}) in addition to the comparison factor. **Aim of the research:** To verify the effect of two types of Nano-fertilizers on the growth and the active substance of the *Ocimum basilicum* plant and to know the increasing and different concentrations of the Nano-fertilizers in the vegetative and physiological indicators and the production of the active substance of the *Ocimum basilicum* plant. Determining the optimal concentrations for each factor, as well as the combination resulting from their interaction in giving the highest vegetative and physiological indicators and the production of the active substance.

Methods of work:

Content of chlorophyll in the leaves (mg.gm^{-1} fresh weight)

The content of chlorophyll a, chlorophyll b, and total chlorophyll in *Ocimum basilicum* leaves was estimated according to the Mackinney (1941) method, by taking the weight of 1 fresh plant leaf within one treatment, cutting it into small pieces, and then crushing it in a ceramic mortar with 10 ml of acetone (80%). Then, the filtrate was separated from the precipitate using a centrifuge (Type 35 EBA Hettich, Germany).

origin) at a speed of 300 cycles. 1 minute for 15. The process of separating the leachate from the precipitate was repeated several times until the precipitate was gone from the green dye, after which the optical density of the leachate was measured by a spectrophotometer (type 2005 UK-S22 Bichrom-Libra) at two wavelengths of 663 nanometers for corophyll a and 645 for corophyll b, and by applying the following equations according to the chlorophyll in the leaves:

Chlorophyll – a(mg. g tissues^{-1})= $\frac{[12.7(D66\ \ )+2.69(D\ 645)]xV}{1000*W}$

Chlorophyll – b (mg. g tissues^{-1})= $\frac{[22.9(D645)+4.68(D663)]xV}{1000*W}$

Total Chlorophyll (mg. g tissues^{-1})= $\frac{[20.2(D645)+8.02(D663)]xV}{1000*W}$

2-nitrogen concentration(%)

According to the method of Cressre and Parsons (1979), the nitrogen content was determined by adding 10 ml of NaOH (35%) and 10 ml of the previously digested sample solution to a distillation flask containing 50 ml of H3BO3 (4%) in a nitrogen distillation apparatus (Macrokjeldhal, German origin) to collect ammonia NH4 for a period ranging between 30-40 minutes, after which the acid containing ammonia is pulverized with sulfuric acid (0.05 M), and then the volume ofThe acid consumed (in the leaching process.

3-Phosphorous concentration (%) According to the Spectrophotometric Vanadium Phosphomolybdate Method reported in Cressrer and Paesons (1979), the phosphorus concentration was estimated by a spectrophotometer at a wavelength of 882 nm.

4-potassium concentration(%)

According to the method of Cresser and Parsons (1979) and A.O.A.C. (2000) Potassium concentration was estimated by Atomic Absorption Spectrometry (AAS) (Perkin Elmer, USA 5000 type origin). **A- Content of chlorophyll a- in leaves (mg.100g^{-1} fresh weight)**

The results of table(1) indicated significant differences for the content of chlorophyll a- in plant leaves, as a concentration of 1.5 g.l^{-1} Nano-zinc when sprayed gave the highest average effect of Nano-zinc amounted to 35.21 mg.100 g^{-1} fresh weight.
.

As for the effect of spraying with calcium nanoparticles, the concentration of 4 gm.l^{-1} gave the highest average of chlorophyll a-content amounted to 39.26 mg.100 gm^{-1} fresh weight.
.

The interaction between the concentration of 3 g.l^{-1} Nano-zinc and the concentration of 4 g.l^{-1} Nano-calcium gave the highest mean of interaction which reached 42.94 mg.100 g^{-1} fresh weight compared to the untreated plants which recorded 29.63 mg.100 gm fresh weight.

Table (1) Effect of spraying different concentrations of zinc Nano-fertilizer and calcium Nano-fertilizer and their interactions on the average content of chlorophyll-a in leaves (100 mg $^{-1}$ fresh weight) of plant *Ocimum basilicum*

Average effect of zinc Nanoparticles	**) Nano Calcium Concentrations (gm.L^{-1}**			**Zinc concentrations Nanoparticles (g m .L $^{-1}$)**
	4	**2**	**0**	
31.53	**36.85**	**28.12**	**29.63**	**0**
35.21	**41.29**	**35.84**	**28.5**	**1.5**
34.90	**42.94**	**40.02**	**21.73**	**3**
32.13	**35.96**	**36.05**	**24.37**	**4**
	39.26	**35.01**	**26.06**	**Average effect of calcium Nanoparticles**

to overlap= 8.095	**for calcium Nanoparticles =4.047**	**for zinc Nanoparticles =4.673**	**LSD$_{(P<0.05)}$**

B-

Chlorophyll b-content in the leaves (mg.100gm^{-1}fresh weight) The results of Table (2) showed that the content of chlorophyll b- in the leaves of the *Ocimum basilicum* plant increased significantly as a result of for spraying with Nano-zinc compared to control plants, as the concentration of 4 g.l^{-1} achieved the highest average of chlorophyll b-content amounted to 32.46 mg.100 gm^{-1} fresh weight compared with 19.41 mg.l^{-1} fresh weight for control plants as for the significant effect of calcium Nano-fertilizer, it was given by the highest concentration used, of which 4 gm.l^{-1} had the highest average of chlorophyll b- content in basil leaves amounting to 33.51 mg.100 gm^{-1} fresh weight, compared with control plants that recorded 14.06 mg.100 gm^{-1}. soft weight with regard to the interaction between the two study factors, spraying with Nano-zinc concentration of 4 g.l^{-1} and 4 g.l^{-1} of Nano-calcium gave the highest significant average of chlorophyll b- content in the leaves of the basil plant, which amounted to 50.42 mg.100 gm^{-1} fresh weight, compared with 27.11 mg.100 gm^{-1} fresh weight of comparison plants. **Table (2)** Effect of spraying different concentrations of zinc Nano-fertilizer and calcium Nano-fertilizer and their interactions on the average chlorophyll-b content in the leaves (mg. 100 gm $^{-1}$ fresh weight) of *Ocimum basilicum* plant

Average effect of zinc Nanoparticles	**) Nano Calcium Concentrations (gm.L^{-1}**			**Zinc concentrations Nanoparticles (g m .L $^{-1}$)**
	4	**2**	**0**	
19.41	**23.36**	**7.76**	**27.11**	**0**
14.96	**22.94**	**7.58**	**14.36**	**1.5**
20.20	**37.3**	**15.2**	**8.11**	**3**
32.46	**50.42**	**40.3**	**6.67**	**4**
	33.51	**17.71**	**14.06**	**Average effect of calcium Nanoparticles**
to overlap= 23.61		**for calcium Nanoparticles =11.81**	**for zinc Nanoparticles =13.63**	**LSD$_{(P<0.05)}$**

The total chlorophyll content of the leaves (mg.100g^{-1} fresh weight) -C

It was observed from the results of Table (3) the significant effect of spraying with Nano-zinc in recording the highest average of the total chlorophyll content in the leaves of the basil plant, which amounted to 64.59 mg.100 gm^{-1} fresh weight, compared to the untreated plants, which averaged 44.36 mg.100 gm^{-1} fresh weight. as for the effect of Nano-calcium, its addition led to a significant increase in the average total chlorophyll content over the content of control plants when treated with 2 and 4 g.l^{-1}, which recorded 52.71 and 72.76 mg.100 g.l^{-1} fresh weight, respectively, compared to 35.19 mg.100 g.$^{-1}$ Fresh weight of comparison plants. The significant interaction between the concentration of 4 g.l^{-1} Nano-zinc and 4 g.l^{-1} Nano-calcium gave the highest mean of total chlorophyll content in basil leaves, which amounted to 86.36 mg.100 gm^{-1} fresh weight, compared to 37 mg.l^{-1} fresh weight. for comparison plants.

Table (3) Effect of spraying different concentrations of Nano-zinc fertilizer and Nano-calcium fertilizer and their interactions on the average total chlorophyll content in the leaves (mg. 100 gm $^{-1}$ fresh weight) of plant *Ocimum basilicum*

Average effect of zinc Nanoparticles	**) Nano Calcium Concentrations (gm.L^{-1}**			**Zinc concentrations Nanoparticles (g m .L $^{-1}$)**
	4	**2**	**0**	
44.36	**60.21**	**35.88**	**37**	**0**
50.17	**64.23**	**43.42**	**42.85**	**1.5**
55.09	**80.22**	**55.2**	**29.84**	**3**
64.59	**86.36**	**76.35**	**31.05**	**4**
	72.76	**52.71**	**35.19**	**Average effect of calcium Nanoparticles**
to overlap= 22.16		**for calcium Nanoparticles =11.08**	**for zinc Nanoparticles =12.79**	**LSD$_{(P<0.05)}$**

(%) The concentration of nitrogen in the leaves D-

The results of Table (4) indicated that there were significant differences in the concentration of nitrogen by the effect of spraying with zinc nanoparticles, as the highest significant mean was 1.76% when spraying with a concentration of 3 g.l^{-1}, compared with 1.23% for comparison plants. as for the calcium Nano-fertilizer, it had a significant effect on the nitrogen concentration, as it recorded in the concentration of 4 g.l^{-1} the highest concentration of nitrogen, amounting to 1.83%, compared to the comparison plants, which had a significantly lower average of 1.26% for the trait. The interaction between the two factors of the study showed that there were significant differences in the average nitrogen concentration, which reached its highest value of 2.42% when a combination of zinc Nano-fertilizer with a concentration of 3 g.l^{-1} and calcium Nano-fertilizer with a concentration of 4 g.l^{-1} compared to untreated plants recorded 1.26% . **Table (4)** Effect of spraying different concentrations of zinc Nano-fertilizer and calcium Nano-fertilizer and their interactions on the average nitrogen concentration (%) in the leaves of *Ocimum basilicum* plant

Average effect of zinc Nanoparticles	**)Nano Calcium Concentrations (gm.L^{-1}**			**Zinc concentrations**
	4	**2**	**0**	

				Nanoparticles (g m .L $^{-1}$)
1.23	**1.38**	**1.3**	**1.003**	**0**
1.65	**1.73**	**1.9**	**1.33**	**1.5**
1.76	**2.42**	**1.64**	**1.21**	**3**
1.74	**1.8**	**1.91**	**1.5**	**4**
	1.83	**1.69**	**1.26**	**Average effect of calcium Nanoparticles**
to overlap= 0.362		**for calcium Nanoparticles =0.181**	**for zinc Nanoparticles =0.209**	**LSD $_{(P<0.05)}$**

E- Phosphorus concentration in leaves
The results of table (5) showed that the average concentration of phosphorus in the leaves of basil plants had significant differences with the effect of different concentrations of zinc Nano-fertilizers, as the highest mean of the trait was 0.39% in the plants that were sprayed with a concentration of 3 g.l^{-1} compared to the comparison plants, which amounted to 0.28% Calcium Nano-fertilizer treatments gave a direct significant increase in the average concentration of phosphorus for basil plants with an increase in the concentration used, as it reached 0.52% at a concentration of 4 g.l^{-1} for calcium Nano-fertilizer compared to comparison plants with an average of 0.15% As for the significant bilateral interaction between the two study factors, it was significant on the concentration of phosphorus in the leaves, as it reached an average of 0.620% when spraying the plant with 1.5 gm.l^{-1} Nano-zinc fertilizer with 4 g.l^{-1} Nano-calcium fertilizer compared to untreated plants. Which recorded 0.062% **Table (5)** Effect of spraying different concentrations of zinc Nano-fertilizer and Nano-calcium fertilizer and their interactions on the average phosphorus concentration (%) in

the leaves of basil plant *Ocimum basilicum*

Average effect of zinc Nanoparticles	**) Nano Calcium Concentrations (gm.L^{-1}**			**Zinc concentrations Nanoparticles (g m .L $^{-1}$)**
	4	**2**	**0**	
0.28	**0.493**	**0.270**	**0.062**	**0**
0.37	**0.620**	**0.350**	**0.139**	**1.5**
0.39	**0.518**	**0.402**	**0.255**	**3**
0.33	**0.454**	**0.404**	**0.142**	**4**
	0.52	**0.36**	**0.15**	**Average effect of calcium Nanoparticles**

to overlap= 0.103	for calcium Nanoparticles =0.051	for zinc Nanoparticles =0.059	LSD(P<0.05)

F- Potassium concentration in leaves(%)
The results of table (6) showed that the concentration of potassium in the leaves of the *Ocimum basilicum* plant increased significantly with the effect of spraying different concentrations of zinc Nano-fertilizer, as the concentration of 4 g.l^{-1} Nano-zinc achieved the highest average potassium concentration in the leaves of 1.93% compared to untreated plants, which recorded 1.27%. The calcium Nano-fertilizer showed a significant effect on the concentration of potassium in the leaves, as indicated by the recording of the plants treated with the highest concentration of 4 g.l^{-1}. The highest mean for the studied trait was 1.85% compared to the comparison plants, which recorded 1.06%. As for the interaction between the zinc Nano-fertilizer and the calcium Nano-fertilizer, it gave the highest mean for the concentration Potassium in leaves when spraying plants with Nano-zinc fertilizer at a concentration of 4 g.L^{-1} with calcium Nano-fertilizer at a concentration of 4 g. L^{-1}, reaching 2.39% compared to 0.614% for comparison plants . **Table (6)** Effect of spraying different concentrations of zinc Nano-fertilizer and calcium Nano-fertilizer and their interactions on the average potassium concentration (%) in the leaves of the *Ocimum basilicum*

Average effect of zinc Nanoparticles	) Nano Calcium Concentrations (gm.L^{-1}			Zinc concentrations Nanoparticles (g m .L $^{-1}$)
	4	2	0	
1.27	1.86	1.33	0.614	0
1.44	1.52	1.56	1.23	1.5
1.53	1.62	1.76	1.22	3
1.93	2.39	2.23	1.17	4
	1.85	1.72	1.06	Average effect of calcium Nanoparticles
to overlap= 0.728		for calcium Nanoparticles =0.364	for zinc Nanoparticles =0.420	LSD(P<0.05)

Discussion
Chlorophyll is essential in the process of photosynthesis and is responsible for the process of making food in plant leaves (Havlin et al., 2014).

As the effect increases with the increase of the level of Nano-fertilizer added, and since the addition of Nano-fertilizers leads to an increase in the availability of nutrients ready for the plant for a longer period and with liberalization commensurate with the stages of plant growth, which leads to an increase in the formation of chlorophyll and thus an increase in the average photosynthesis, and an improvement in the growth of the total plant, and this is consistent with what has been reached. (Al-Shami, 2019) and (Al-Mamouri, 2020).

In view of the importance of Nano-calcium fertilizer and its calcium content, it helps the plant to grow, which in turn works for the plant to grow well, which increases the chlorophyll content in the leaves, as increasing the leaf content of chlorophyll pigment is important in activating the photosynthesis process as a result of absorbing the largest possible amount of energy photosynthesis and converting it into bioenergy within the plant in the photosynthetic system (Al Sahhaf, 1989).

As N has an important role in building the chlorophyll molecule by entering the composition of amino acids and proteins important in building chloroplasts.

The leaf is one of the most important parts of the plant in which all physiological processes take place and one of the most suitable parts of the plant for judging the status of nutrients because it is the plant part in which nutrients are mixed with plant food and associated with the products of photosynthesis (Idris, 2007).

Therefore, the increase in the content of the NPK elements in the leaves (Tables 4, 5, 6) to the relatively high levels of these elements is due to the role of spraying calcium Nano-fertilizer in appropriate quantities that contribute to increasing the efficiency of its absorption and its transfer to the vegetative system and its accumulation inside the plant, which leads to Increasing its concentration in the leaves (Al Sahhaf, 1989).

In addition, calcium Nano-fertilizer is characterized by its rapid decomposition and plant readiness with the elements (Ali and Al-juthery, 2018).

Or the increase in some elements, such as phosphorus, table (5), is attributed to the significant effect of Nano-zinc on growth regulators, and then stimulating the plant to actively perform its structural and vital activities, which requires the withdrawal of more quantities Phosphorous to meet the plant's need for it, as it is an important element in the formation of nucleic acids, proteins, cell membranes, and energy compounds (Moore, 1979).

Also, the small size of the nanoparticles makes it easier for them to penetrate the pores of the cell wall easily, reaching the vascular bundles. Ma et al. (2010).

The use of Nano-fertilizers of zinc and calcium has provided sufficient nutrients necessary for plant growth, especially (N, P, K), in addition to its content of nutrients that have the ability to stimulate vegetative cells to divide and elongate, so the increase in N in the leaves led to an increase in the protoplasm mass And then increase the vegetative growth of the plant, and on the other hand its effect on the process of photosynthesis through chlorophyll pigment and increase the area surface and then increase the surface area and then increase food processing in the plant (Hamza et al., 2012) and (Unlu et al., 2013).

References

A.O.A.C. (Association of Offcial Analytical Chemists) (2000). Official of Analysis of the Assocation of official Analytical Chemists 17th Ed.Washington, D.C.;USA.

Al-Juthery, H. W. A. and S. F Saadoun.2018. Impact of Foliar application of some micronutrients Nano-fertilizer on growth and yield of Jerusalem artichoke.

The Iraqi Journal of Agricultural Science, *49*(4) P:577-585.
Al-Sahhaf, Fadel Hussein Reda (1989). Applied plant nutrition. Baghdad University. Ministry of Higher Education and Scientific Research - Iraq.
Al-Sham i, Muhammad Saeed and Akram Muhammad Al-Balkhi.2010. Soil fertility and fertilization. Al-Rawda Press. Damascus University Publications. Faculty of Agriculture. Syria.
Cresser, M.S. and Parsons, J.W. (1979). Sulphuric-Perchloeic acid digestion of plant material for determination of nitrogen, phosphorus, potassium, calcium and magnesium. Analytica Chimica Acta, 109 (2): 431-436.
Hamza, Ali Hussein, Zahraa Hamid Muhammad, Iyad Jassim Jaber, Haider Safaa Ibrahim. 2012. Response of tomato plants to spraying with Cycosil and the nutrient N P K grown in desert soils. Kufa Journal of Agricultural Sciences, Volume (4) Issue (2): 7 3 1 - 6 5 1.
Havlin,J., L., J.D., Beaton, S.L. Tisdale, and **W.L. Nelson.2014.** Soil Fertility and Fertilizers "An Introduction to Nutrient Management 11th Ed Prentice Hall. New J.
Idris, Mohamed Hamed. 2009. Plant Physiology. Suzanne Mubarak Center for Scientific Exploration. The Egyptian Arabic Republic .
Ma X.; Geiser-Lee, J. ; Deng , Y. and Kolmakov , A.(2012). Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicty , uptake and accumulation. Science of the Total Enviornment, 408(16): 3053-3061.
Mackinney, G. (1941). Absorption of light by chlorophyll solutions. Journal of Bioiogical Chemistry,140 (2) : 315-322.
Moor, T.C. (1979) . Biochemistry and Physiology of Plant Hormones. New York, Springer – Verlag, USA.
Unlu, H.O. H, Unlu, Y. Karakurt, and H. Padem. 2013. Changes in fruit and yield and quality in response to foliar and soil humic acidapplication in a cucumber. Scientific Research and Essays. Vol. 6 (13). PP. 2800-2803.