ORIGINAL ARTICLE



INTERACTED EFFECT OF HUMIC ACID AND SPRAYING DIFFERENT CONCENTRATIONS OF NANO ZINC OXIDE AND ZINC OXIDE ON THE GROWTH AND YIELD OF ONION (ALLIUM CEPA L.)

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Abstract : This factorial field experiment had been conducted in 2019 at Al-Jazeera township in Al-Garma district (Anbar province, Iraq) according to Randomized complete block design to investigate the influence of foliar application of Humic Acid ($H_0 = 0$ mg HA L^{-1} and $H_1 = 500$ mg HA L^{-1}), the formulas and concentrations of sprayed Zinc which are ($Zn_0 = 0$ mg Zn L^{-1} ; $Zn_N = 20$ mg Zn L^{-1} as Nano-particles zinc oxide; $Zn_{NC} = 20$ mg Zn L^{-1} as Nano-particles zinc oxide with Chelated material (EDTA); $Zn_T = 100$ mg Zn $Zn_N = 100$ mg Zn Z

Key words: Foliar application, Humic acid, Zinc oxide, Nano zinc oxide, Onion.

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1. Introduction

Onions, which belong to the Alliaceae family are one of the important crops for the large deities importance of nutrient content as fresh onions contain 1.2 mg of proteins, 11 mg of carbohydrates and 2 mg of fat, as well as their medicinal benefits [Anonymous (2014)]. It is considered a crop of a high nutrient requirements, including zinc element, which is one of the necessary micronutrients and essential for plant growth, as it enters in many life processes within the plant, including the formation of several enzymes and plant hormones, including IAA responsible for cells' elongation, also included in the stimulation of oxidation processes in the plant cells, this element is involved in many physiological processes such as photosynthesis,

increased metabolism outputs, chlorophyll formation and enzymatic reactions, thereby influencing plant growth and development [Marschner (2012)]. However, zinc's availability to the plant is affected by several factors, the most important of which is the soil content of calcium carbonate, the degree of soil reaction (pH) and its organic matter content that decrease most of micronutrients including zinc [Havlin *et al.* (2004)]. In order to overcome the problems of ground application from stabilization and sedimentation, it is resorted to the use of several alternatives and strategies, including the use of leaf nutrition and to make maximum use of these nutrients, therefore, they was provided with substances that increase their absorption, including Humic Acid (HA), which is one of the organic bio

stimulators that affect the development of plant growth and increase the yield. In his work, he behaves similarly to that of hormones [Canellas and Olivares (2014)]. Recently, the use of Nano fertilizers is an effective alternative to traditional fertilizers for their use in small quantities and high stability under different environmental conditions, as the fertilizers used by the size of Nano-particles (NP) have a great role in reducing waste by applying them in very small quantities and reducing environmental risks and increasing the efficient utility of micronutrients use due to their quickly absorption by the plant, penetration in the cells, transportation and metabolism within the tissues of the plant [Morales-Diaz et al. (2017)]. Several researchers had appointed the positive effects of Humic acid spraying on the most of measured growth indicators [Selladurai and Purakayastha (2016)] or to spray zinc in all formulas, whether Nano-particles or Traditional [Laware and Raskar (2014)] so the goal of this study is to investigate the role played by in zinc oxide Nanoparticles (ZnO NPs) and zinc oxide (ZnO) in some characteristics of growth and yield of onion plant with the presence of and the absence of Humic acid.

2. Particles and Methods

2.1 Study area and the installation of drip irrigation system

The study was carried out in one of the agricultural fields of Al-Garma district in Anbar province, in the agricultural season 2018-2019 by planting Texas grano bulbs cultivar on September 1, 2019 in loamy sand soil, the soil chemical traits were analyzed according to Page *et al.* (1982) whereas physical properties according to Black (1965) as illustrated in Table 1. The T-Tape system of drip irrigation was used to irrigate the crop as the system is supplied with water from an aquabasin and all treatments are processed in equal quantities of irrigation water depending on the water requirements of onions.

2.2 Experimental design and crop management

A factorial experiment had been executed using RCBD design of three replications. The first factor consisted of three treatments; non-sprayed HA ($\rm H_0$) and sprayed HA with 500 mg concentration ($\rm H_1$), as well as using soluble HA of 90% HA. The second factor consisted of different concentrations and formulas of Zn foliar applications treatments; non-spraying only water ($\rm Zn_0$), Nano particles Zinc oxide 20 mg.L⁻¹

Table 1: Soil physical and chemical properties before planting.

Characte	r	Unit	Value	
pH1:1		_	7.80	
(EC)		ds.m ⁻¹	3.60	
CaCO ₃		gm. Kg ⁻¹	9.00	
O.M			1.12	
Available N			35.26	
Available P			13.44	
Available K			165.97	
Available Zn		mg. Kg ⁻¹	1.72	
Available Fe			2.79	
Available Mn			2.67	
Soil separates	sand		645	
	silt	gm. Kg ⁻¹	230	
	clay		125	
Soil texture		Loamy sand		
Bulk density		Mg m ⁻³	1.25	

(Zn_N), Nano particles Zinc oxide 20 mg.L⁻¹ with chelating agent (Zn_{NC}) and Zinc oxide (Zn_T) of 100 mg.L⁻¹, it had produced 24 experimental units of treatments and interactions. The HA acid was dissolved using water, but zinc formulas were dissolved with a diluted acid solution and then the required concentrations had prepared, the preparation of zinc chelated by using EDTA as chelating material, the method described in Sylvie et al. (2015) was adopted. The experimental unit consisted of three lines of 2.1 m line length, 0.75 m distance among lines and the distance between one plant and another was 0.15 m, so the number of plants for each experimental unit was 42 plants and left a distance of 1 meter between the experimental units to ensure that the splash of the spray solution does not transfer from one unit to another. The treatments were sprayed three times as the first spray was 60 days after the beginning of cultivation and the second spray was at 15 days after the first one and the third spray after 15 days of the second spray whereas control treatment was sprayed with only water. All treatments had received the same quantity of fertilizers of which the ground application were used according to the recommendation of 185 kg N.ha⁻¹, 52 kg P.ha⁻¹ and 100 kg K.ha⁻¹. These fertilizers were added in the feedin method at the beginning of the cultivation for phosphorus and potassium, while nitrogen was added

in two batches, the first at the beginning of the cultivation and the second after 40 days of cultivation. Crop service is done whenever it required.

2.3 The harvesting and estimated indicators

The plants were harvested on February 1, 2020 where they showed features of maturity and measurements of vegetative growth and the yield of bulbs were measured as follows:

The indicators of vegetative and radical growth

To calculate vegetative growth indicators, a random sample of 10 plants from the middle row was taken after leaving a number of guard plants surrounding the sample during the growing season and the measurements included the following: The height of the plant (PLH), the number of leaves (NOL), the weight of the bulb (BW), the length of the root (RL), the bulbs yield (BY), the leaf area (LA) and fertilization efficiency (FE)

2.4 Analysis of elements

The samples were dried in an oven at 65°C temperature and included both leaves and bulbs and then grinded and 0.2 g of each sample was taken and

digested. The concentrations of zinc in the leaves and bulbs were measured by using the atomic absorption device while the concentration of nitrogen in the bulbs was estimated using the Kjeldahl device in accordance with the method used by Page *et al.* (1982).

3. Results and Discussion

The results of Table 2 shows the existence of significant effects of sprayed HA (500 mg.L⁻¹) in most of the characteristics of morphological growth measured and this can be due to the role of HA in increasing cell elongation and increasing cellular division, Humic acids rise the rate of plant growth and make the best conditions for cell division [Canellas and Olivares (2014)]. The effect was significant of all the formulas and concentrations of the sprayed foliar applied zinc element on the plant compared to the non-spraying treatment, which can be explained by the role of zinc in composition of (IAA) which has an effect on increased cell division, which enhances cell activity and divisions, It also may be attributed to the big role of zinc in the synthesis and growth of plants through participating as co-agent in stimulating about 300 of various enzymes [Marschner (2012)]. The treatment of sprayed foliar

Table 2: Effect of Humic acid and Spraying zinc formulas and concentrations on some morphological characters of onion.

Treatment		Some Morphological characters plant ¹						
Humic Acid	Znic Form	Shoot hight (cm)	Leaf number Leaf. plant ¹	Leaf area (dcm²)	Root length (cm)	Bulb thick (mm)		
$\mathbf{H}_{_{0}}$	Zn_0	38.30	11.40	28.65	19.70	42.57		
	$Zn_{_{\mathrm{N}}}$	48.43	17.83	34.25	26.50	54.77		
	Zn _{Nc}	48.20	16.87	33.14	26.13	55.03		
	Zn _T	45.90	13.53	32.51	25.67	48.33		
H ₁	Zn_0	48.83	15.97	34.25	27.87	55.53		
	Zn _N	50.57	20.13	46.15	29.63	63.67		
	Zn _{Nc}	51.33	21.00	48.60	30.20	64.37		
	Zn _T	46.80	18.57	39.29	27.07	60.50		
	LSD _{0.05}	2.144	4.421	0.588	3.002	3.618		
H mean	HA0	45.21	14.91	32.14	24.5	50.18		
	HA1	49.38	18.92	42.07	28.69	61.02		
	LSD _{0.05}	1.342	0.959	1.977	0.930	1.618		
ZN mean	Zn_0	43.57	13.68	31.45	43.57	49.05		
	Zn _N	49.50	18.98	40.20	49.50	59.22		
	Zn _{Nc}	49.77	18.93	40.87	49.77	59.70		
	Zn _T	46.35	16.05	35.90	46.35	54.42		
	LSD _{0.05}	2.122	1.516	3.126	2.122	2.558		

Nano particles chelated zinc of 20 mg.L⁻¹ had exceeded all other formulas of zinc due to the fine molecular size of Zn that increase the specific area of contact between plant and fertilizer which cause improvement of absorption, these nano-particles or their clusters are less than the path size in the cell wall can easily enter through that wall and reach the plasma membrane. It is also noticed the insignificancy of differences between non chelated nano particles zinc (Zn_{NC}) treatments whereas the interaction treatment of H_1 Zn_{NC} had the highest values of PLH, NOL, LA, RL and BT with an increase of 34.02%, 84.21%, 69.63%, 53.30% and 51.21% respectively compared to the H_0 Zn_0 treatment, which indicate the least values to the above properties.

The results of statistical analysis in Table 3 indicated the presence of significant effects of HA addition in the characteristic of zinc concentration in the leaves and bulbs as well as an increase in protein concentration in addition to a noticeable increase in the BY and in the fertilization efficiency (FE). The concentration of 500 mg.L⁻¹ indicated the highest significant values in comparison with control treatment because of the direct

effect of HA on the cellular membranes, where it increases their permeability and facilitate transformation of nutrients to the active locations that require them. This effectiveness is related to the function of active clusters of carboxyl and hydroxyl in HA and this leads to an increase in absorption of added nutrients. The results of the same table also showed the significant effect of all zinc formulas added to the plant as a result of the increased concentration of this nutrient in the leaves and bulbs, which may be due to the process of spraying and the resulting absorption of this nutrient, but the increase of protein may be due to the large and important role of zinc in stimulating the absorption of essential elements, especially the nitrogen element responsible for high protein content [Laware and Raskar (2014)]. Zinc is also an important component in plants because of its essential role in stimulating enzymes and the formation of proteins in the plant [Havlin et al. (2004)] in addition to the effects of zinc, which is achieved through its important role in the physiological functions in all living systems and the maintenance of the safety of biological membranes, which caused an increase in most of the measurement indicators, including the BY, thus raising the FE, the formula of

Table 3:Effect of Humic acid and spraying zinc formulas and concentrations on some element concentration and yield of onion.

Treatment		Some element concentration and growth characters					
Humic acid	Zinc Form	Zn (%) leaf	Zn (%) bulb	Protein (%)	Yield (Ton.H ⁻¹)	Fertilization efficiency (%)	
H ₀	Zn_0	15.1	15.67	6.44	10.39	24.19	
	Zn _N	26.6	27.17	8.21	12.91	23.62	
	Zn _{Nc}	23.5	28.40	8.90	12.85	15.92	
	Zn _T	23.4	25.97	7.11	12.05	32.82	
	Zn_0	21.7	20.63	12.65	13.81	61.98	
	Zn _N	34.4	31.17	15.40	16.84	66.41	
$\mathbf{H}_{_{1}}$	Zn _{Nc}	33.9	31.93	14.60	17.30	32.49	
	Zn _T	26.5	28.52	13.08	13.77	1.500	
	LSD _{0.05}	10.54	3.284	1.185	0.219	15.93	
H mean	HA0	22.1	24.30	7.67	12.02	48.53	
	HA1	29.3	28.06	13.93	15.23	0.949	
	LSD _{0.05}	4.71	2.077	0.530	0.098	16.41	
Zn mean	Zn_0	18.4	20.63	9.54	12.10	43.08	
	Zn _N	30.5	31.17	11.80	14.87	45.02	
	Zn _{Nc}	28.7	31.93	11.75	15.07	24.20	
	Zn _T	25.0	28.52	10.10	12.91	2.121	
	LSD _{0.05}	7.45	4.644	0.838	0.154		

chelated nano-particles $(Zn_{_{NC}})$ has the highest values compared to the rest of the formulas and concentrations of zinc sprayed on the plants. This can be attributed to the high area of fertilizer's contact with plants, which leads to improved absorption, and the transfer of nano particles into the plant cell through the stomata in leaves of plants [Derosa et al. (2010)] the NP is also transform faster to the plant cells than non-Nano particles. They have rapid penetrability to plant cells, in addition to the possibility and activity of ZnO NPs in increasing agricultural production and rising zinc absorption by plants [Hajira et al. (2017)]. The ability of NP to penetrate the layer of leaves is determined by the distribution of elements in plant tissues through their ability to spread and transport within the plant [Morales-Diaz et al. (2017)]. These NP facilitate and expand the size of the pathway and encourage cells to increase their absorption and the use of zinc in particles form leads to activation of cell elongation and increased cell wall elasticity and thus an increase in most growth indicators of onions [Laware and Raskar (2014)]. The results of the table also show a significant effect of the interaction between study factors, the treatment of interaction of spraying HA acid and Zn_{NC} achieved the highest values and also shows the insignificance of differences between the treatments (Zn_N, Zn_{NC}) . The H₁Zn_{NC} interaction treatment achieved the highest values in zinc concentration in the bulbs and in BY, with increase ratios of 134.20% and 39.91 successively compared to H_0Zn_0 , the same treatment H_1Zn_{NC} achieved the highest value for FE (66.41%) Compared to the H₁Zn_T treatment that recorded the least value of 15.92%. H₁Zn_N interaction treatment achieved the highest values in zinc concentration in the leaves in protein concentration with an increase of 127.8%, 15.40%, respectively compared to H₀Zn₀ treatment.

4. Conclusion

The results of this study clearly indicate that the spraying of HA was positive and significant in the traits studied, as well as the spraying of all formulas and concentrations of applied zinc had achieved a significant increase in most of the measured properties. The ZnO NPs with either presence chelated material or without it had greatest effect in the above properties while the differences between the two Nano particles formulas of zinc molecules were not significant, and the treatment of the interactions between spraying with HA at the concentration of 500 mg.L⁻¹ and ZnO NPs sprays

with a concentration of 20 mg.L⁻¹ in both formulas had the best values for the morphological properties as well as for the concentration of zinc in both leaves and bulbs as well as in the EF and in the BY where the rate of increase was 39.91% compared to the control treatment.

Recommendations

- Spraying with HA on the onion plant with a concentration of 500 mg. L-1 associated with 20 mg.L-1 of ZnO NPs spray without adding a chelated under the same conditions as the study area.
- Sprayed ZnO NPs can be applied on the vegetative part without the need to add chelated material due to the insignificancy of the differences between the chelated and nonchelated zinc formulas.

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