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Impact of Spraying Chelated Zinc with Humic Acid Extracted from Compost on the Growth, Yield and Nutritional Status of Japanese Cabbage Plants Grown in Sandy Soil

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Abstract: Two field experiments were conducted during the two successive winter seasons of 2020 and 2021 in the Research and Production Station, National Research Centre, El-Nubaria location, Beheara Governorate, Egypt, to investigate the response of cabbage plant to the foliar application of chelated zinc and humic acid (extracted from compost), focusing on the effects on growth, yield and N, P, K and Zn content. The experiment included 6 treatments representing the interaction of two humic acid concentrations (2 and 3 ml l^{-1}) with three chelated zinc concentrations (0, 150 and 300 mg l^{-1}). The main results remarked that the combination of humic acid and chelated zinc enhanced the growth characteristics, quantity and quality of the crop, as well as improved the content of nitrogen, potassium and zinc in cabbage leaves. The remarkable results was obtained by the interaction of spraying of chelated zinc at a concentration of 300 mg l^{-1} with humic acid at both rates (2 and 3 ml l^{-1}) during the two successive growing seasons. The data also implied the importance of increasing the rate of P fertilizer with increasing the applied chelated-Zn in order to assure the nutrients balance and eliminate the antagonistic effect between them. The study revealed the efficiency of using humic acid extracted from compost as a natural organic liquid fertilizer in presence of a vital nutrient (Zn) in stimulating growth and yield quality of cabbage plants grown in sandy soil.

Key words: Humic acid · Chelated Zn · Japanese cabbage · Growth · Yield · Nutrients content

INTRODUCTION

Cabbage (*Brassica oleracea*) is an important leafy vegetable grown in Egypt. It is rich in minerals and vitamins such as A, B1, B2 and C [1], it is source of essential amino acids and some anti-oxidant compounds because it is rich in certain substances with high antioxidant capacity like carotenoids and polyphenols [2].

Humic acid is a substantial component of humic substances that includes many functional groups located at the carbon series. They could be acidic (e.g., carboxylic acid and phenol), alkaline (e.g., amine, imines), or neutral collection (e.g., alcohol, aldehyde, ketone, ether, ester and amide) [3]. Several utilization of this material in agricultural field have been mentiond in literature as soil and plant applications. Humic acid enhanced soil health and its characteristics attributes as well as improved plant growth [4, 5]. Abdel-Razz *et al.* [6] found that excellent effect for improving vegetables output by sprayed with humic acid for two times. Shehata *et al.* [7] reported that when spraying cucumber plants with 1.5g l^{-1} of humic acid solution led to increase plant growth, total yield compared with control treatment. Shafeek *et al.* [8] reported that foliar spraying by 4g/l from humic acid on bean plants achieved the high values of growth parameters like number of leaves and branches, fresh weight of plant, as well as total yield such as pod length and number of pod/plant.

Vegetables have a high zinc requirement due to its many functions in plant growth. Zinc utilization can give remarkable profits than other nutrients and it is less expensive [9]. As a plant micronutrient, it has the pronounced impact on yield and quality of vegetable crops. It also plays a main role in the activation of enzymes, nucleic acids and involved in the forming of biotin and thiamine. Recently, zinc deficiency has been

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recognized frequently on crops and it became a factor limiting yield and quality of crops. The deficiency of zinc may occurred due to high crop yield, consequently, higher rate of zinc is removed by crops and lesser zinc fertilizers use [10]. This case is commonly occurred in arid and semi arid areas, which reflect on yield productivity and quality, hence the field profitability.

Working on this issue, the paper aimed to study effect of foliar application of chelated Zn combined with different rates of humic acid (extracted from compost) on growth, yield and nutritional status of Japanese cabbage plants grown in sandy soil.

MATERIALS AND METHODS

Experimental Site and Soil: Two field experiments were carried out during the growing season of 2020 and 2021 in the Research and production Station, National Research Centre, El-Nubaria location, Beheara Governorate, Delta Egypt, to study the effect of foliar application of Zn and humic acid (HA) extracted from the mature compost made from the residues of medicinal and aromatic plants on cabbage plants grown in sandy soils.

The physical and chemical properties of El-Nubaria soil were determined for each growing season; the particle size distributions and soil moisture content were measured as described by Blackmore [11]. Soil pH, EC, cations and anions, $CaCO_3$ percentage and the available N, P, K, Fe, Zn and Mn were run according to Black *et al*. [12]. Soil of the experiment was newly reclaimed sandy in texture, the soil properties and the characteristics of irrigation water are described in Tables 1 and 2, respectively.

The monthly average climatic factors, i.e., average air temperatures, relative humidity, rain precipitation, wind speed and solar radiation (MJ/m^2/day) for the studied seasons of 2020 and 2021 were also obtained from the Central Laboratory of Meteorology, Ministry of Agriculture and Land Reclamation, Egypt, for this area of study and presented in Table 3.

Table 1: Some physical and chemical properties of the experimental soil at the beginning of the experiment

Soil properties		Values: First season	Values: Second season
Particle size distribution (%)	Sand	93.32	94.0
	Silt	4.68	3.56
	Clay	2.00	2.44
	Texture	Sandy soil	Sandy soil
$CaCO_3$ (%)		2.21	2.24
pH$_{(1:2.5\ soil\ suspension)}$		8.21	8.20
EC (dS m^{-1})		1.98	1.80
Soluble cations (mmol L^{-1})	Ca^{++}	8.02	7.96
	Mg^{++}	2.88	3.16
	Na^{+}	5.95	5.20
	K^{+}	2.95	1.68
Soluble anions (mmol L^{-1})	CO_3^{--}	-	-
	HCO_3^{-}	1.52	1.52
	Cl^{-}	8.30	7.82
	SO_4^{--}	9.98	8.66
Available nutrients (mg kg^{-1})	N	24.2	26.5
	P	2.05	2.00
	K	68.6	59.5
	Fe	4.11	3.95
	Mn	0.97	1.01
	Zn	1.20	0.99

Table 2: Some chemical characteristics of irrigation water

		Soluble cation (meq. L^{-1})				Soluble anions (meq. L^{-1})			
pH	EC dS m^{-1}	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	SO_4^{-}
7.10	2.69	7.45	3.81	15.2	0.44	nd.	5.20	14.6	7.10

Table 3: Climatic data of the experimental site during (2020/2021) seasons

	Temperature Average (°C)	Relative Humidity Average (%)	Rain Precipitation	Wind Speed (m/s)	Solar Radiation (MJ/m^2/day)
			2020		
January	13.80	71.90	68.00	3.74	13.28
February	14.27	72.06	24.40	3.09	16.33
March	15.89	68.93	70.00	3.57	20.61
April	18.22	67.27	92.70	3.23	25.81
May	22.14	64.21	0.00	3.40	28.69
			2021		
January	15.48	70.01	15.10	3.28	13.53
February	15.02	69.10	44.70	2.95	16.99
March	15.67	67.85	192.00	3.47	20.98
April	18.70	62.60	1.00	3.38	26.61
May	24.44	56.47	0.00	3.02	29.05

Table 4: Elemental composition (as %) of humic acid (HA)

Elemental composition	C	H	N	O	S
(%)	50.2	4.3	2.2	42.8	0.30

Experimental Procedures

Extraction of Humic Acid: One hundred grams of compost was mixed with 500 ml of 0.25 M KOH using an orbital shaker for 24 h to extract humic acid (HA). The solid residue in the solution was separated by centrifuging and filtering. The recovered solid was further subjected to two more successive KOH treatments. The combined supernatants from the extraction were acidified with 6 M hydrochloric acid (HCl) to pH 1.0 to precipitate HA in the solution. The humic acid was allowed to coagulate for 24 h before centrifuging to recover HA. The recovered HA was further subjected to two repeated 0.25 M KOH solutions and precipitations by acidification processes to produce relatively pure HA. Table (4) shows the elemental composition (C, H, N, S, O) of the extracted humic acid was analyzed using a Vario Elementar apparatus.

Two concentrations of humic acid (2 ml l^{-1} and 3 ml l^{-1}) were spraying with three concentrations of Zn (source, Zn-EDTA) 0, 150 and 300 mgl^{-1}. The seedlings (at the third truly leaves) were spraying with humic acid and Zn, this was repeated every two weeks and it was stopped until about a month before the harvest. All agronomic practices and fertilization for raising Japanese cabbage plants were performed following the conventional and recommended farmer agricultural practices.

Measurement of Vegetative Growth and Yield: All growth parameters of cabbage plants such as plant height, plant diameter number of leaves per plant as well as fresh weight per plant were recorded according to FAO [13]. Cabbage yield parameters such as head height, head diameter, head weight and marketable yield were carried out according to Gabal *et al.* [14].

Measurement of Nutritional Status and Chemical Constituents: Macronutrients (N, P and K) and micronutrient (Zn) were measured in the cabbage leaves followed the procedures of Cottenie *et al.* [15]. The chemical constituents such as total protein, total carbohydrate, total soluble sugars and vitamin "C" of cabbage leaves were estimated according to A.O.A.C [16].

The treatments were replicated three times and all the obtained data were subjected to statistical analysis of variance according to Snedecor and Cochran [17] and means separation were done by L.S.D.at 0.05 level of probability.

RESULTS AND DISCUSSIONS

The result in Table (5) shows the effect of spraying different concentrations of chelated zinc with two spray concentrations of humic acid on cabbage plants grown in sandy soil during the two successive growing seasons. In the case of the treatment in which chelated zinc was not applied, increasing the concentration of sprayed humic acid from 2 to 3 ml l^{-1} improved growth and different yield traits, as well as the resulting total yield. In the experimental treatments, in which the spraying of chelated zinc began, this greatly enhanced the growth and yield characteristics of cabbage plants, compared to the treatment with no chelated zinc spray, implying the importance and great role of zinc in influencing the growth

Table 5: Effect of chelated Zn and humic acid concentrations on growth and yield parameters of Japanese cabbage

Humic acid ml l^{-1}	Zn mg l^{-1}	No. of leaves	Stem weight g	Stem height (cm)	Stem diameter (cm)	Plant height (cm)	Head diameter (cm)	Head weight kg	Total yield ton fed^{-1}
First season									
2	0	67.0	68.4	4.52	3.23	15.1	38.5	3.00	24.6
	150	82.6	86.6	6.71	4.16	18.3	44.3	4.13	33.9
	300	95.2	98.9	7.33	4.53	20.0	45.7	4.58	37.6
3	0	71.6	76.6	5.38	4.10	17.2	41.8	3.58	29.4
	150	88.5	94.4	7.50	4.80	19.0	45.2	4.43	36.3
	300	105.1	117.2	8.42	5.00	21.6	46.5	5.06	41.5
$LSD_{0.05}$		14.2	13.1	2.60	0.25	1.30	1.15	0.50	4.71
Second season									
2	0	71.1	70.0	4.51	3.11	15.0	38.4	3.10	25.4
	150	84.5	88.5	6.80	4.20	18.5	44.5	4.16	34.1
	300	94.7	97.1	7.21	4.45	20.2	45.6	4.60	37.7
3	0	73.6	78.5	5.42	4.19	17.5	41.3	3.60	29.4
	150	89.2	93.5	7.38	4.68	19.4	45.5	4.45	36.5
	300	103.2	114.1	8.10	5.00	22.0	46.8	5.05	41.5
$LSD_{0.05}$		13.8	12.9	2.54	0.24	1.26	1.10	0.49	4.70

Table 6: Effect of chelated Zn and humic acid concentrations on yield quality of Japanese cabbage

Humic acid ml l^{-1}	Zn mg l^{-1}	Total protein (%)	Total carbohydrate (%)	Total soluble sugars (%)	Vitamin C mg/100 g FW
First season					
2	0	4.90	13.3	7.69	35.6
	150	5.38	14.6	8.47	38.2
	300	5.87	15.2	8.66	38.4
3	0	5.65	13.9	7.77	36.2
	150	6.40	16.1	8.56	38.5
	300	6.92	16.2	8.70	38.6
$LSD_{\cdot 0.05}$		0.23	1.22	0.60	2.30
Second season					
2	0	4.85	13.2	7.71	34.7
	150	5.40	14.1	8.39	37.9
	300	5.79	14.9	8.60	38.0
3	0	5.54	13.5	7.74	35.9
	150	6.32	15.9	8.51	38.0
	300	6.71	16.0	8.68	38.1
$LSD_{\cdot 0.05}$		0.20	1.19	0.56	2.25

and the coordinates of cabbage yield. The obtained enhancements were almost similar during the two growing seasons, thus confirming the implication of zinc in plant life to gain a high yield. Further, increasing the concentration of chelated zinc spraying from zero to 300 mg l^{-1} led to a significant increase in growth and yield parameters of Japanese cabbage; the trend was congruent at both sprayed concentrations of humic acid (2 and 3 ml l^{-1}). The best treatment that was gave the highest values in terms of growth and yield was spraying a concentration of 300 mg l^{-1} of chelated zinc with 3 ml l^{-1} of humic acid followed by the ones with 2 ml l^{-1} humic acid, this was happen at two successive growing seasons.

Lguirati *et al*. [18] reported that humic acids isolated from compost present higher N and H content than the other sources, probably due to the incorporation of N-containing groups and polysaccharides-like structures, which may be decomposed by microbial activities when the process takes more time, or even, the type and the characteristics of the processes used for the stabilization of the organic residues. El-Sharkawy and Abdel-Razzak [19] demonstrated that applying humic acid increased plant weight and stem diameter of cabbage plants compared with the control treatment, which might be related to its role in the stimulation of plant growth by the assimilation of enzymes, changes in membrane permeability, along with the protein synthesis and finally the activation of biomass production. Foliar application of humic acid (4 %) increased vegetative growth, yield and fruit quality of tomato plants [20]. Abd El-Rheem *et al*. [21] investigated the response of potato plants grown in sandy soil to the utilization of humic acid extracted from compost. They illustrated that utilization of the extracted humic acid promoted plant growth and yield of potato, since it contain growth promoter compounds, in addition to high amount of necessary nutrients for plant. They also added that application of humic acid reduced the used mineral fertilizers, hence decreased the production costs and enhanced the profit.

Vegetable crops need zinc, due to its extreme importance in the growth of plants due to its essential role in many functions within the plant and compared to other elements [9]. Zinc is a micronutrient which wants with scarce quantities play a necessary role in plant growth and development [22]. Singh and Singh [23] reported that increasing levels of zinc significantly increased dry matter production in cabbage plants; because zinc addition remarkably stimulated the growth and the translocation of more photosynthates towards sink and consequently accumulated more dry matter in edible heads.

Data presented in Table (6) outline the response of Japanese cabbage yield quality to different spraying concentrations of chelated Zn and humic acid. Increasing the concentration of chelated zinc spraying from zero to 300 mg l^{-1} induced the quality of the cabbage yield, especially with the high spraying concentration of humic acid. Therefore, the best values for each of total protein, carbohydrate and soluble sugars as well as vitamin C were obtained when spraying humic acid extracted from compost at a working rate 3 ml per liter accompanied by spraying chelated zinc at a concentration of 300 mg l^{-1}, during the two successive growing seasons.

The positive influence of humic acid was also confirmed by Shafeek *et al*. [8]. They mentioned that the foliar application of liquid humic acid increased percentage of protein and N contents in bean seeds. The obtained results are also in harmony with that stated by Mubarak *et al*. [24], who pointed out the efficacy of a foliar application of Zn fertilizer as chelated and nano-particles forms on improving the growth of safflower plants. Where the enhancements reached 109 and 57.6 % for the carbohydrate and protein content in the plant leaves by spraying Zn-EDTA at 150 mg l^{-1}. Zinc is a significant factor in the synthesis of proteins, carbohydrates and enzymes inside the plant [25]. Concerning the central role of Zn in stability of biomembranes and proteins, Zn deficiency can affect the photochemical processes in the thylakoids and thus inhibits biophysical processes of photosynthesis [26]. Zinc assist in formation of carbohydrate and chlorophyll. Additionally it helps in tryptophan syntheses which finally produce indole acetic acid [27].

It was clear from Table (7) that nutritional status of cabbage leaves i.e. nitrogen, potassium and zinc, except for phosphorus, were significantly enhanced by zinc and humic acid foliar application in both seasons. Among all treatments, the lowest values of the above mentioned nutrients were resulted from the application of humic acid alone. Whereas, the highest values were obtained for plants that treated with 2 ml l^{-1} of humic acid + 300 mg l^{-1} of Zn and those sprayed with 3 ml l^{-1} of humic acid + 300 mg l^{-1} of Zn. The current study was conducted on sandy soil, which is characterized usually by low fertility, as evidenced by the low nutrients availability (Table 1), the spraying of HA alone was insufficient to enhance the nutritional status of plants. However the combination between humic acid and the micronutrient (chelated-Zn) was an urge to obtain a healthy plant. Concurrence with the current results, Khaled and Fawy, [28] reported that foliar application of liquid humic acid at 2 % under sandy soil increased the uptake of N, P and K by corn plants. Total nutrients content in cucumber leaves increased with elevating of humic acid rate to 3 g L^{-1} [29]. Recent study demonstrated that foliar zinc enhanced the uptake of K, Ca, Mg, Fe, Zn, Mn and Cu in plant leaves of safflower plants compared to control treatment [24]. Other study reported increasing the nitrogen, phosphorus and potassium content in cabbage plants with rising concentrations of applied zinc fertilizer [23].

Increasing the content of nutrients resulting by the combined application of humic acid and Zn treatments might be interpreted by the impact of both sprayed materials on nutrients absorption. Since humic acid has intrinsic role on improving the cell membrane permeability [30], in addition to the inducing influence of Zn on the translocation and accumulation of nutrients in the plant organs and improving the metabolic activities [31], thus reflecting an elevation in nutrients uptake. Moreover, other interpretation could be related to the extraction processes of humic acid, since it was extracted from a green compost containing many nutrients, whether macro or micro nutrients, which enhanced their presence in the leaves of plants after the spraying treatments.

Table 7: Effect of chelated Zn and humic acid concentrations on N, P, K and Zn content in the leaves of Japanese cabbage

Humic acid ml l^{-1}	Zn mg l^{-1}	N	P	K	Zn
		-------------	%	-----------	mg kg^{-1}
			First season		
2	0	1.13	0.38	2.53	26.2
	150	1.94	0.35	2.56	37.5
	300	2.02	0.33	2.58	48.1
3	0	1.12	0.39	2.62	25.4
	150	1.98	0.36	2.71	36.1
	300	2.06	0.34	2.77	50.1
$LSD_{\cdot 0.05}$		0.01	0.02	0.61	12.2
			Second season		
2	0	1.18	0.40	2.57	27.8
	150	1.95	0.36	2.60	34.4
	300	2.01	0.32	2.61	45.1
3	0	1.21	0.39	2.66	26.8
	150	2.01	0.35	2.74	33.5
	300	2.08	0.31	2.77	48.5
$LSD_{\cdot 0.05}$		0.01	0.02	0.59	13.1

Zinc is an active element in biochemical processes and has chemical and biological interactions with each other items. Phosphorus is the most important element that interferes with the absorption of zinc by plants and there is an inverse relationship between phosphorus and zinc within the plant, as the increase in zinc concentration decreases the plant's phosphorus content and vice versa (denoted as antagonistic effect). Barben *et al*. [32] reported that phosphorus concentrations in the top leaves and middle leaves and stems (middle) are depressed with increasing Zn activity in solution. Therefore, it is necessary to take into consideration increasing phosphate fertilization during the change of the applied zinc concentration, in order to maintain the balance between these elements and eventually get a valued and high quality crop production.

CONCLUSIONS

This paper emphasized the importance of zinc in the life of the cabbage plant, in order to obtain good growth and a high yield of excellent quality. It also noted the importance of the possibility of converting compost the medicinal plants into organic acids such as humic acid, which in turn was effective on the growth and yield of cabbage plant as a source of nutrients and a natural growth promoter material. Aforementioned results confirmed the positive influence of the interaction of humic acid and chelated zinc on growth parameters, nutritional status and yield of cabbage plants. The best treatments were exhibited by spraying chelated zinc at a rate of 300 mg l^{-1} with humic acid at a concentration of 2 and 3 ml l^{-1}.

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