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Applying Two Types of Humic Acid to Improve the Pliability to Salinity Stress of Drip-Irrigated Cabbage Plants Grown in Sandy Soil

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Abstract: Under saline water irrigation conditions, two consecutive experiments were conducted in the winter seasons of 2022 and 2023 at the Research and Production Station affiliated with the National Research Centre in the Nubaria region - Beheira Governorate. The aim of the study was to study the response of cabbage plants to spraying with two types of humic acid, which were extracted from the mature compost made from the residues of medicinal and aromatic plants. Two types of humic acid were used, where the first type was humic acid resulting from a normal extraction process, while the second type was activated using nitric acid to define the acid produced as nitro-humic acid. Humic acid (normal and activated) was sprayed at two concentrations of 2 and 3 ml per liter of water. Spraying activated humic acid gives relatively better results than spraying conventional extracted humic acid. The importance of spraying humic acid, especially at a concentration of 3 ml/l, and comparing it with the control (no spraying humic acid) is also evident. Spraying cabbage plants with humic acid, whether extracted by the traditional method or activated by nitric acid, improved a lot and more significantly compared to the control treatment, which shows the importance of spraying with humic acid and its activating effect on increasing the productivity of cabbage plants grown in sandy soil. It can be said that the use of new extraction, which involve activation using nitric acid, produces humic acid that is more effective and influential on the growth and yield of cabbage compared to the traditional method. Therefore, it is recommended to use this method to extract humic acid from compost and produce a high-quality organic compound.

Key words: Compost · Humic acid · Nitro humic acid · Cabbage plants · Growth · Yield · Nutrients content

INTRODUCTION

Composting of organic wastes such as crop residues and animal's wastes garbage in agriculture may have a role in diminishing the massive consumption in using mineral fertilizers in Egypt [1]. The process of composting is the biochemical decomposition of organic wastes and converting it into what is known as humus. Therefore, aerobic decomposition was used to produce organic materials that can be used as balanced organic fertilizer [2].

Humic acid is found in many materials found in nature, such as clay soil, compost, peat and various types of charcoal. Humic acid refers to the biomass of organic materials, which is formed in a form different from the materials from which it is derived, as it appears in an amorphous form [3]. Humic acid can be known as a heterogeneous organic substance found in nature. It is characterized by having a very large molecular weight, and its color ranges from dark yellow to black [4].

The oxidation of coal and various organic materials using different oxidizing agents leads to the production of what is known as nitro-humic. Nitro-humic acid is characterized by containing nitro($-NO_2$) and nitroso (-NO) groups, which are formed during the nitration process, and therefore its composition differs from the composition of humic acid extracted by normal methods [5]. Humic acids could be converted into nitro humic acids

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through the nitration process, in which nitro groups (NO_2) are located on the aromatic rings. The nitration process leads to an increase in the nitrogen content of nitro-humic acid [6].

Both humic acid and nitrohumic acid play an important role in increasing plant growth and thus increasing yield, by increasing the absorption of various nutrients and improving cell division. They also have a role in increasing plants' tolerance to salinity and increasing the activity of microorganisms in the soil [7]. Additionally, humic acids improve plant growth and photosynthesis processes under abiotic stresses such as salinity and increases antioxidant enzyme activity [8].

Cabbage (*Brassica oleracea*) is grown in large areas in Egypt, where it is considered one of the most important leafy vegetables because it contains many various minerals and vitamins [9], in addition to containing some amino acids rich in sulfur, as well as some antioxidants such as vitamin C, carotenoids and polyphenols [10].

The aim of the paper was to extract two types of humic acid from compost using two different methods, and to know their effect on the growth, yield and nutritional status of cabbage plants under water salinity stress.

MATERIALS AND METHODS

Two field experiments were carried out in consecutive winter season of 2022 and 2023 at the experimental research station of the National Research Centre in the Nubaria region - Beheira governorate. The aim of this study was to investigate the response of cabbage plants to spraying with two types of humic acid, which were extracted from the mature compost made from the residues of medicinal and aromatic plants. Two types of humic acid were used, where the first type was humic acid (HA) resulting from a normal extraction process, while the second type was activated using nitric acid to define the acid produced as nitro-humic acid (NHA). Humic acid (normal and activated) was sprayed at two concentrations of 2 and 3 ml per liter of water.

Physical and chemical properties of El-Nubaria soil sample were determined well as particle size distributions and soil moisture were determined as described by [11]. Soil pH, EC, cations and anions, organic matter, $CaCO_3$, total nitrogen and available P, K, Fe and Mn were run according to [12]. Soil of the experiment was newly reclaimed sandy in texture, physical and chemical analysis of the experiment soil is described in (Table 1) and analysis of irrigation water (Table 2).

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

Soil properties		Values
Particle size distribution (%)	Sand	95.52
	Silt	3.48
	Clay	1.00
	Texture	Sandy soil
$CaCO_3$ (%)		1.65
$pH_{(1:2.5\ soil\ suspension)}$		8.11
EC (dS m^{-1})		1.19
Soluble caions (mmol L^{-1})	Ca^{++}	5.54
	Mg^{++}	1.19
	Na^{+}	4.44
	K^{+}	0.73
Soluble anions (mmol L^{-1})	CO_3^{--}	-
	HCO_3^{-}	1.52
	Cl^{-}	5.44
	SO_4^{--}	4.94
Available nutrients mg kg^{-1}	N	14.3
	P	1.01
	K	41.6
	Fe	3.21
	Mn	0.77
	Zn	1.21

Extraction of Humic Acid (HA) and Nitro-Humic Acid (NHA): One hundred grams of compost was mixed with 500 ml of 0.25 M KOH in an orbiter shaker for 24 h to extract humic acid. 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 humic acid in the solution. The humic acid was allowed to coagulate for 24 h before centrifuging to recover humic acid. The recovered HA was further subjected to two repeated 0.25 M KOH solutions and precipitations by acidification processes to produce relatively pure humic acid.

For nitro-humic acid extraction, 100 g of compost were treated with of 1000 ml of 3 M HNO_3 at for 50 min. Heating was accompanied by continuous stirring to prevent foaming. The oxidized material was recovered and dried in oven for 24 h at 105°C. Nitro humic acid was then

Table 2: Some chemical analyses of irrigation water.

		Soluble cations (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

extracted from the 'oxidized material' using 0.25 M KOH following the humic acid extraction procedure as outlined above. The extraction methods are described by [13].

Chemical and FTIR Spectra Analysis of HA and NHA: Elemental composition (C, H, N, S, O) of HA and NHA were analyzed using a Vario Elementar apparatus. Percent Oxygen (O) was calculated by subtracting the sum of C, H, N, and S from 100%. The FTIR spectra of pelleted samples were recorded over a spectral range of 4,000 to 400 cm^{-1} on a FTIR spectrometer (FTIR-NEXUS 670, Thermo Nicolet Corporation, America).

Fertilization of Cabbage Plants: Superphosphate at 100 kg per fadden; farmyard manure at 8 ton per feddan and ammonium sulphate at 100 kg per fedden were added during soil preparation before sowing. The seedlings (at the third truly leaves) were spraying with two types of humic acid; and this was repeated every two weeks, and it was stopped until about a month before the harvest. New Argal (produced by Shura Company) was added as a diffuser and adhesive when spraying humic acids.

Measurement of Vegetative Growth: All growth parameters of cabbage plants such as plant height, plant diameter, number of leaves per plant and plant weight were recorded according to [14].

Measurement of Yield: Cabbage yield parameters such as head height, head diameter, head weight and marketable yield were recorded according to [15].

Measurement of Nutritional Status: In cabbage leaves, macronutrients (N, P, K), micronutrient (Fe, Mn, Zn and Cu) were determined according to [16].

Measurement of Chemical Constituents: Total proteins, total carbohydrates, total soluble sugars as well as vitamin "C" of cabbage leaves were determined according to [17].

Statistical Analysis: Data of the two seasons were arranged and statistically analyzed using Mstatic (M.S.) software. The comparison among means of the different treatments was determined, as illustrated by [18].

RESULTS AND DISCUSSION

The elementary composition of NHA differed from HA derived from the compost. The C content of NHA decreased while H, O and N increased relative to the HA (Fig. 1). The increase in the nitrogen element in nitro-humic acid was twice the content of that element in humic acid, as this is due to the nitro-humic acid extraction method in which nitric acid was used.

FTIR spectra of HA and NHA derived from a compost (Fig. 2) were obtained to study the structural changes brought about by HNO_3 oxidation. Adsorption bands that were common to HA and NHA were broad peaks between 3,386-3,410 cm^{-1} for hydrogen-bonded

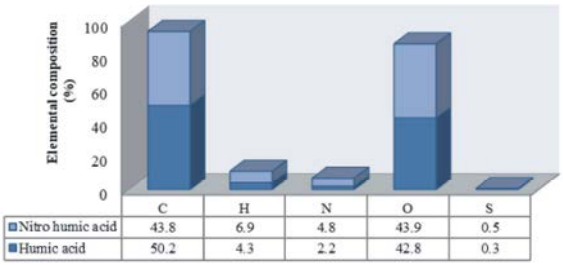

Fig. 1: The elementary composition of humic acid and nitro humic acid derived from the compost

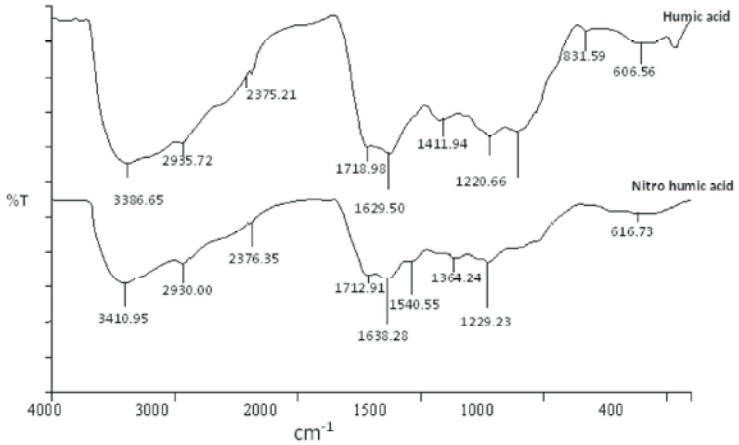

Fig. 2: FTIR spectra of humic acid and nitro-humic acid derived from compost

Table 3: Effect of two types of humic acid on growth of cabbage plants.

Humic acid type	Concentration (ml L^{-1})	Plant weight (kg)	Plant diameter (cm)	Plant height (cm)	No. of leaves
		2022			
Humic acid	2	4.69	44.30	18.30	93.73
	3	5.00	45.70	19.00	100.10
Nitro-humic acid	2	4.73	45.20	19.30	98.50
	3	5.26	46.50	19.80	105.10
Control		3.35	38.50	15.20	78.00
$LSD_{0.05}$		0.15	1.12	0.03	14.20
		2023			
Humic acid	2	4.71	44.50	18.50	93.66
	3	5.05	45.90	19.20	100.00
Nitro-humic acid	2	4.81	45.60	19.40	99.45
	3	5.37	46.90	19.90	106.00
Control		3.34	37.90	15.00	79.10
$LSD_{0.05}$		0.15	1.12	0.02	13.90

Table 4: Effect of two types of humic acid on yield parameters of cabbage plants.

Humic acid type	Concentration (ml L^{-1})	Head weight (g)	Head diameter (cm)	Head height (cm)	Yield (ton fed^{-1})
		2022			
Humic acid	2	91.80	3.98	6.22	15.50
	3	99.00	4.57	6.31	17.60
Nitro-humic acid	2	94.40	4.65	6.60	15.90
	3	104.80	4.81	7.44	18.30
Control		70.00	3.81	5.31	12.90
$LSD_{0.05}$		4.40	0.30	0.11	1.20
		2023			
Humic acid	2	93.10	4.01	6.23	15.40
	3	100.10	4.60	6.32	17.70
Nitro-humic acid	2	97.50	4.71	6.56	15.90
	3	109.20	4.90	7.43	18.20
Control		69.50	3.82	5.30	12.00
$LSD_{0.05}$		3.96	0.41	0.12	1.42

O-H stretch, strong clear peaks at 1,718-1,720 cm^{-1} for C=O stretch from ketone + ether + COOH, and distinct bands at 1,220-1,229 cm^{-1} for C=O stretch of COOH. Broad bands around 1,4111 cm^{-1} appeared on HA spectra and were attributed to CH_2 or CH_3 stretching. NHA spectra from compost showed moderate two peaks at 1,540 cm^{-1} and 1,364 cm^{-1}. The 1,540 cm^{-1} peak was attributed to nitro (-NO_2) stretch (19) & [20] while the 1,364 cm^{-1} peak was however ascribed to possibly tertiary aliphatic nitroso (NO) group [21]. The FTIR spectra provided evidence that oxidation by HNO_3 introduced nitro (NO_2) and nitroso (NO) into the NHA molecule.

Table 3 showed to the effect of the two types of humic acid on the growth of cabbage plants under saline water stress. It was found that spraying with activated humic acid gives relatively higher results compared to spraying with humic acid extracted in the normal method. It is also clear the importance of spraying with humic acid, especially at a concentration of 3 ml per liter, and comparing that with the control (not spraying with humic acid).

As a result of humic acid containing many nutrients, this leads to improving soil fertility as a result of the availability of nutrients in a form that is easy for plants, and thus plant growth and productivity increase [22]. A strong relationship was found between reducing the effect of salinity on plant growth and humic acid, as humic acid works to reduce the significant effect of salt on plants [23]. Humic acid enhances the growth of plants by improving cell division and increasing the absorption of elements, humic acid also has an effective role in reducing soil salinity [24]. Nitro-humic acid has been used as a source of nitrogen fertilization, as well as a soil improver. It also has a role in the biological revitalization of plants and as a chelating agent for nutrients [25].

The data in Table (4) showed to the effect of spraying two types of humic acid with different concentrations and extracted from compost on the yield characteristics of cabbage plants and comparing that with the treatment in which no type of humic acid was sprayed. Spraying cabbage plants with humic acid, whether extracted by the traditional method or activated by nitric acid, improved a lot and more significantly compared to the control treatment, which shows the importance of spraying with humic acid and its activating effect on increasing the productivity of cabbage plants grown in

Table 5: Effect of two types of humic acid on yield quality of cabbage plants.

Humic acid type	Concentration (ml L^{-1})	Total protein (%)	Total carbohydrate (%)	Total soluble sugars (%)	Vitamin C mg/100 g FW
			2022		
Humic acid	2	5.38	14.60	8.47	36.80
	3	5.88	15.10	8.66	38.20
Nitro-humic acid	2	6.38	16.10	9.25	38.40
	3	6.93	16.50	9.60	38.50
Control		4.80	13.30	7.69	35.60
$LSD_{0.05}$		0.23	1.15	0.77	1.23
			2023		
Humic acid	2	5.36	14.60	8.46	36.70
	3	5.79	15.00	8.61	38.20
Nitro-humic acid	2	6.37	16.10	9.22	38.40
	3	6.92	16.40	9.52	38.50
Control		4.82	13.10	7.63	35.50
$LSD_{0.05}$		0.25	1.14	0.76	1.23

Table 6: Effect of two types of humic acid on nutrients content of leaves cabbage plants.

		(%)			(ppm)			
Humic acid type	Concentration (ml L^{-1})	N	P	K	Fe	Mn	Zn	Cu
				2022				
Humic acid	2	0.86	0.35	0.53	188	38.7	26.2	42.3
	3	0.94	0.36	0.56	190	40.2	27.1	45.6
Nitro-humic acid	2	1.02	0.37	0.62	191	41.1	28.1	44.1
	3	1.06	0.37	0.66	191	42.0	29.0	45.8
Control		0.78	0.32	0.49	178	36.3	23.5	40.5
$LSD_{0.05}$		0.11	0.01	0.11	9.20	2.32	2.24	1.35
				2023				
Humic acid	2	0.83	0.34	0.52	186	38.6	26.1	42.1
	3	0.93	0.35	0.55	188	40.0	27.2	45.4
Nitro-humic acid	2	1.00	0.37	0.61	189	41.0	28.0	43.2
	3	1.04	0.37	0.65	190	41.8	28.9	45.7
Control		0.76	0.33	0.48	177	36.3	22.8	40.3
$LSD_{0.05}$		0.11	0.01	0.11	8.12	2.33	3.11	1.62

sandy soil. When comparing the two types of humic acid on the yield of cabbage, it was found that spraying with activated humic acid is superior to humic acid extracted by the traditional method, especially when spraying with a concentration of 3 ml per liter.

The structure of nitrohumic acid differs from humic acid in the presence of nitro and nitroso groups that are present during the nitration process, while they are similar in many other structures [20]. Recent studies indicated that nitro-humic acid could also be used to improve soil structure, retain plant nutrients, stimulate soil microorganism activity and improve buffering capacity of the soil [21]. Mazumdar *et al*. [26] indicated that application of Nitro-humic acid with nitrogen fertilizers increased wheat yield. Under salinity conditions, spraying nitro-humic acid at a rate of 6 liter per hectare significantly improved the rice yield and its components compared to the control treatment [27]. Humic acids play an important role in lands affected by salinity, as it reduces the degree of soil salinity and improves its natural and chemical properties [28].

Tables (5) and (6) showed to the effect of normal and activated humic acid on the cabbage leaves content of total protein, total carbohydrates, total soluble sugars and vitamin C, as well as their content of macronutrients (N, P and K) and micronutrients (Fe, Mn, Zn and Cu). The results showed no difference from the above in terms of the importance of spraying with humic acid, and it's activating effect on increasing the quality of the cabbage yield. It also showed the superiority of spraying activated humic acid at a concentration of 3 ml per liter over the rest of the experimental treatments.

Adding humic acid at a rate of 3 g per liter increased the content of nitrogen, phosphorus and potassium in cucumber leaves [29]. Nitro-humic acid enhanced chemical activities relative to humic acid in terms of total acidity and CEC. In addition, the N content of nitro-humic acid is about two times higher than that in the humic acid due to its corporation of nitro ($-NO_2$) and nitroso (-NO) functional groups in the oxidized compost. This means that nitro-humic acid can be a source of nitrogen supply to the plants when nitro-humic acid is spraying on plants

[30]. Many researchers have emphasized the importance of spraying humic acid on plants irrigated with salt water, through improving the absorption of macro- and microelements within the plants, such as [31] on black cumin, and [32] on *Matricaria recutita.* The superiority of using nitro humic acid compared to humic acid in improving the absorption of nitrogen, phosphorus and potassium for Savory plant [33].

CONCLUSION

Activated humic acid was more effective and had an effect on growth and yield characteristics, and the content of leaves cabbage of protein, carbohydrates and vitamin C, as well as their content of various nutrients. The N content of nitrohumic acid is about two times higher than that in the humic acid due to its corporation of nitro (-NO_2) and nitroso (-NO) functional groups in the oxidized compost. This means that nitro-humic acid can be a source of N supply to the plant. Spraying humic acid or nitro humic acid improves the growth and yield of cabbage plants irrigated with salt water and grown in sandy soil.

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