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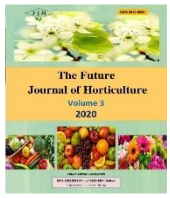

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IMPACT OF ENRICHED HUMIC ACID WITH BENEFICIAL MICROORGANISM IN REDUCING MINERAL FERTILIZERS ON JERUSALEM ARTICHOKE PLANTS UNDER SANDY SOIL CONDITION

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ABSTRACT: A field experiment was carried out at experimental farm at El-Kassasin, Horticulture Station, Agricultural Research Center (ARC), Ismallia Governorate, Egypt during summer season of 2016 and 2017 to evaluate the potentiality effect of humic acid and/or biofertilizers combined with different rates from mineral fertilization to reduce dependence on mineral fertilizers,and improve growth and productivity of Jerusalem artichoke plants (*Helianthus tuberosus L.* cv. Fuseau). The highest values of growth parameters i.e., plant height, number of main stems, number of branches, fresh and dry weight of shoots were exhibited with humic acid + biofertilizers treatment combined with 75% NPK in both seasons. This treatment also indicated the maximum NPK percentage values and uptake of these elements in shoots in both seasons. Still this treatment 75% NPK + H + BF was superior in tubers yield as it gave 25.001 and 25.429 ton/fed and the relative increase over control reached to 2.57 and 1.69% in both seasons, respectively. Concerning the tubers quality represented by total carbohydrates and inulin, the application of humic acid with biological fertilization combined with 75% NPK increased the tubers 'content of inulin, which reached 2.77 and 2.798 ton/ fed in both seasons, respectively, the relutive increase over the control by 4.33 and 4.47 %, respectively. While the tubers contentof carbohydrate was not affected by addition H and BF with 75% NPK compared with control. The obtained results revealed that the total microbial count and yeast count in the rhizospheric area were higher with the treatment 75% NPK + H + BF compared to control, while the maximum count of *Azotobacter* sp. was attained with treatment 50% NPK + H + BF in both seasons. The mixture treatment (75% NPK + H + BF) was still the superior one where it gave the highest enzymes activity represented in nitrogenase (µmole C2H4/g rhizosphere/h), Dehydrogenase (µg TPF/g dry soil/day) and acid and alkaline phosphatase (µg/g dry soil). Generally, these results undoubtedly confirm that humic acid dual with bio-fertilizers could saving the use of chemical fertilizers and consequently improve the quality and quantity of plants and produce more save foods at the same time.

Key words: Humic acid, biofertilizers, mineral fertilizers, Jerusalem artichoke, growth, yield, nitrogenase

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus* L.) is an important tuberous plant for healthy food, many of these health effects can be attributed to the ability of inulin to stimulate the growth of bifidobacterium, whereas, it is one of the nontraditional crops accumulates substantial amounts of inulin for costeffective extraction **(Kays and Nottingham, 2008)**. In this regard, it is suitable for diabetics, enhancing immunity, reducing blood cholesterol and improving calcium absorption **(Orafti, 2005 and Panchev *et al.*, 2011)**, using its stalks for feeding animals **(Zaky, 2009)**. Recently, it's being an important source of renewable energy **(Juśko *et al.*, 2012)**. Jerusalem artichoke is a folk remedy for diabetes, digestive, rheumatisand and mdiuretic.

Excessive application of chemical fertilizers has led to health and environmental hazards. Therefore, sustainable ecological agriculture requires agricultural practices that are healthy to the environment and maintain the long-term balance of the soil ecosystem. In this context, use of organic fertilizers and microbial inoculants (biofertilizers) in agriculture represents an environmentally safely alternative to further applications of mineral fertilizers **(Khan *et al.*, 2007 and Ajallie and Salehi, 2012).**

In general, organic fertilizers and humic acid contains components that can increase many elements which improve the soil fertility, increase the availability of nutrients, enhancing roots, plant growth, plant tolerance against both biotic and a biotic stress, moreover, environmental stresses such as heat, cold and drought as well as root system development **(Abd El-Al *et al.*, 2005 and Ajallie and Salehi, 2012)**. Hence, the application of humic acid to soil produces beneficial effects on the chemical, biochemical and physical quality of soil, increased soil microbial population and its plant nutrition capacity **(Spaccini and Piccolo, 2009)**. Moreover, humic acid can potentially stimulate crop growth and development through the actions of plant growth - promoting hormone like substances, including cytokinins, auxins and gibberellins **(Tejada *et al.*, 2006)**.

The use of beneficial soil microorganism inoculant (biofertilizers) in agriculture represents on environmentally safely alteration to further applications whereas the beneficial soil microorganisms could increase soil fertility and accelerating certain microbial processes. The beneficial effects of plant growth promoting rhizobacteria (PGPR) have been attributed to biological nitrogen fixation **(Keyeo *et al.*, 2011)**, production of phytohormones **(Ashrafuzzaman *et al.*, 2009)**, root development and proliferation resulting in more efficient uptake of water and nutrients. Moreover, beneficial microorganism reduces pathogen infection, improved fertilizers use efficiency, improved resistance such as drought, mineral deficiency and salinity **(Kim *et.al.*, 2011 and Amprayn *et.al.*, 2012)**. In addition, production of phytohormons, siderophores and vitamins that acts as plant growth regulators **(Pan *et.al.*, 2002)**.

A diverse range of yeasts exhibit plant growth promoting characteristics including pathogen inhibition, phytohormone production and phosphate solubilization, production of vitamin B and amino acids **(Amprayn *et.al.*, 2012)**. Furthermore, some researchers stated that the yeast application could enhance its role in cell division, cell elongation producing more leaf area and thus increasing photosynthesis, producing bioactive substances such as phyto-hormones and enzymes (phosphatase and dehydrogenase) **(Hussain *et al.*, 2002)**.

Therefore, the present investigation was under taken to evaluate the effect of humic acid and / or biofertilizers to reduce mineral fertilizers and its impact on growth and productivity of Jerusalem artichoke under sandy soil conditions.

MATERIALS AND METHODS

A field experiment was carried out during the summer seasons of 2016 and 2017 at the Experimental Farm at El-Kassasin, Hort. Station , Ismallia Governorate, Egypt, to study the effect of different rates of mineral fertilizers combined with humic acid and beneficial microorganisms (*Azotobacter chroococcum* + *Bacillus megaterium* + *Bacillus circulans* + *Saccharomyces cerevisiae*) on growth and productivity of Jerusalem artichoke plants (*Helianthus tuberosus* L. cv. Fuseau) under sandy soil conditions.

Soil Experiment

The soil texture was sandy having the following characteristics: sand 95.0%, silt 2%, clay 3%, pH 7.75, EC 1.15 dS/m, organic matter 0.08%, available N 6.9ppm, available P 6.2 ppm, available K 64 ppm, $CaCO_3$ 0.26%, and water holding capacity 14.5%.

Experimental design

The experiment included seven treatments were arranged in a complete randomized block design with three replicates as follows:

1- 100% NPK (recommended rates)

2- 75% NPK + H (humic acid),

3- 75% NPK + H (humic acid) + BF (*Az. chroococcum* + *B. megaterium* + *B. circulans* + *S. cerevisiae*),

4- 75% NPK + BF (*Az. chroococcum* + *B. megaterium* + *B. circulans* + *S. cerevisiae*),

5- 50% NPK + H (humic acid),

6- 50% NPK + H (humic acid) + BF (*Az. chroococcum* + *B. megaterium* + *B. circulans* + *S. cerevisiae*),

7- 50% NPK + BF (*Az. chroococcum* + *B. megaterium* + *B. circulans* + *S. cerevisiae*).

Microbial inoculants

Active strains of N_2-fixers (*Azotobacter chroococcum*), phosphate solubilizers (*Bacillus megaterium*), potassium release (*Bacillus circulans*) and yeast strain (*Saccharomyces cerevisiae*) were kindly obtained from Microbiology Department, Soils, Water and Environment Research Institute (SWERI), ARC, Giza, Egypt.

The bacterial strains were individually enriched on nutrient broth medium **(Difco, 1985)**, whereas *Saccharomyces cerevisiae* was grown on glucose peptone and yeast extract medium (GPY) **(Difco, 1985)**. Bacterial and yeast cultures containing 1×10^8 cells.ml^{-1} were used. Bacterial and yeast strains were mixed just before applied to the soil at a rate of 10 l/fed. according to the treatments.

Extraction and purification of humic acid (H)

Extraction of humic acid was estimated according to the method described by **Sanchez- Monedero *et al*. (2002).** Purification of humic acid was done as described by **Kononova (1966)**. The chemical properties of the used compost are shown in Table (1). Total phosphorus was determined as described by **(Murphy and Riley, 1962)**. Total potassium was determined by using flame photometer **(Chapman and Pratt, 1961)**.

Table 1. Physical and chemical analysis of the used compost

Macronutrient (%)			Organic carbon (%)	Organic Mater (%)	C/N Ratio	EC (ds/m)	pH	Parasite
N	P	K						
1.35	0.52	0.55	25	43	18.5/1	3.21	7.6	Not detected

Elemental analysis (C, H, N, S and O_2) of the purified humic acid was performed by gas microanalyser as described by Goh and Stevenson (1971). Total acidity of humic acid and Carboxyl groups were determined as described by **Dragunova (1958) and Schnitzer and Gupta (1965)**, respectively. However, phenolic groups were determined as described by **Kononova (1966)** in Table (2).

Table 2. Characteristic of humic acid (H) extracted from compost

C %	N %	H %	S %	O_2 %	Total acidity	Carboxyl groups	Phenolic groups
					(m mole/ 100g)	(m mole/ 100g)	(m mole/ 100g)
50.0	4.1	5.0	1.0	39.9	425	195	230

Fertilization: The recommended rates (300 kg/fed.) of ammonium sulphate (20.5%N) and 150 kg/fed. potassium sulphate (48% K_2O) were applied in two equal doses after 45 and 75 days after planting. Whereas, phosphorus was added as calcium superphosphate (15.5% P_2O_5) at a rate of 250 kg / fed. at soil preparation. The inorganic NPK was applied at a rate of 50 and 75% of recommended rates.

Field trials

Tuber seeds of Jerusalem artichoke cultural were planted on April 30th at 40 cm apart, during the 2016 and 2017 summer seasons on sandy soil. The experiments were conducted in a complete randomized block design. The experiment plot area was 12.6 m^2, it contains three dripper lines with 6m length each 70 cm distance between each two dripper lines. One line was used to measure the vegetative growth parameters and the other two lines were for yield determination. Humic acid was added to plots with water irrigation (fertigation) as 3 l/fed. in three equal doses at 45, 75 and 105 days from planting. The liquid cultures from different biofertilizers were added with the water irrigation (fertigation) at a rate of 10 l/fed. in three equal dose above mentioned.

Measurements

1. Vegetative Growth characters

A random sample of three plants from each experimental plot was randomly taken at flower initiation stage (at 120 days after planting) to calculate plant height (cm), number of main stems/ plant, number of branches / plant and fresh and dry weight of shoot (leaves + branches)/ plant.

2. N, P and K contents and up take

Total nitrogen, phosphorus and potassium were determined according to the methods described by **Bremner and Mulvaney (1982), Olsen and Sommers (1982) and Jackson (1973)**, respectively, then uptake of N, P and K by shoots was calculated.

3. Yield and its components

At harvest stage the yield parameters i.e. number of tuber/plant, average tuber weight (g), total yield / plant (kg), and total/yield /fed.(ton) were determined. One hundred grams of the grated tubers mixture were dried at 105^{O}C till constant weight and DM (%) was recorded. Total carbohydrates (%) was determined calorimetrically in dry tubers as the methods described by **Michel *et al*., (1956)** while, inulin content (%) was determined in tubers according to the method of **Winton and Winton (1958)**.

4- Biological activities in Jerusalem artichoke rhizosphere

The population dynamics of total microbial count and yeast count were determined in the rhizosphere area of Jerusalem artichoke roots were determined by the plate count technique according to **(Reinhold *et.al*., 1985)**. While the *Azotobacter* sp. population counts in the rhizospheric zone were determined using the most probable number (CFU/g dry rhizosphere) method described by **Cochran (1950)**. The activities of nitrogenase (µmole C_2H_4.g dry rhizosphere^{-1} / **h**$^{-1}$), dehydrogenase (µg TPF.g dry rhizosphere^{-1} / day^{-1}) and alkaline and acid phosphatases (mg/g dry rhizosphere^{-1} / day^{-1}) were determined according to the methods described by **Somasegaran and Hoben, (1994), Skujins (1976) and Tabatabai (1982)**, respectively.

Statistical analysis

The collected data were subjected to statistical analysis of variance using the normal (F) test and the means separation were compared by using least significant difference (L.S.D.) at 5% level according to **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Plant Growth

Under filed conditions, the combination with different rates of NPK (75 and 50%) from recommended rate, organic stimulation (humic acid) and biofertilizers (*Az.* chroococcum, *B. megaterium*, *B. circulans* and *S. cerevisiae*) showed significant increment in plant growth parameters (plant height, number of main stems, number of branches, fresh and dry weight of shoots (leaves+ branches) more than control (recommended dose of N P K alone) Table, 3.

Data present that the plant height in 2016 and 2017 seasons increased concomitantly and significantly with the application of 75% NPK + H+ BF up to 219 and 220 cm/plant, respectively. Moreover, the maximum values of number of main branches were detected by the treatments of 75% NPK + H and 75% NPK + H+ BF which gave 4.0 and 3.33 in both seasons, respectively. While the number of shoots/ plant, the treatment 75% NPK + H + BF was recorded the highest value (32 and 34) in both season, respectively. Regarding fresh and dry weight of plant shoots, the highest significant of values were detected by the above treatment in both seasons which gave 1088.3 and 1100 g/plant fresh weight while 302.5 and 305.9 g/plant dry weight in both seasons, respectively.

Table 3. Effect of different rates of mineral fertilization, humic acid and biofertilizers on growth parameters of Jerusalem artichoke during 2016 and 2017 seasons

Treatments	Plant height (cm)		No. of main stems /plant		Number of branches/ plant		Fresh weight/ shoot (g)		Dry weight/ shoot (g)		Relative ±in dry weight of shoot than control (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK 100% (control)	214.0	213.0	3.67	3.00	27.33	29.33	1071.2	1070.1	298.70	297.30	0.00	0.00
NPK 75% + H	213.0	215.0	4.00	3.33	29.33	31.67	1080.1	1075.2	300.20	298.80	0.50	0.50
NPK 75% + H + BF	219.0	220.0	4.00	3.33	32.00	34.00	1088.3	1100.3	302.50	305.90	1.27	2.89
NPK 75% + BF	215.0	217.0	3.67	3.00	30.33	32.00	1070.2	1080.2	299.90	300.20	0.40	0.98
NPK 50% + H	186.0	190.0	3.33	2.67	20.33	20.00	965.0	972.0	254.00	259.60	-14.96	-12.68
NPK 50% +H+BF	193.0	197.0	3.67	2.67	25.00	24.33	980.0	985.9	271.20	271.50	-9.21	-8.68
NPK 50% + BF	190.0	194.0	3.67	2.67	23.00	22.00c	970.0	980.0	266.50	269.60	-10.78	-9.32
LSD at 5 % level	**10.80**	**13.44**	**0.22**	**0.22**	**1.56**	**2.68**	**67.24**	**53.79**	**16.13**	**14.12**	**--**	**--**

** BF: biofertilizers *(Az. chroococcum + B. megaterium + B. circulans + S. cerevisiae*)

These results enhance the role of growth promoting biofertilizers and humic acid in activating the secretion of hormones as well as its role in improving the soil structure, which reflected positively on moisture retention and provide water for plant growth. Results are in arrangement with **Abou-Aly and Mady (2009)** who stated that the combined application of humic acid with the biofertilizers is a good tool for growth promotion and yield as well as improving soil texture. The humic substances significantly also enhanced plant growth through increasing cell membrane permeability, respiration, photosynthesis, oxygen and minerals uptake and enhancing root growth **(Pizzeghello *et al.*, 2013 and Morsy *et al.*, 2016)**.

N, P and K contents and up take

The translocation and uptake of macroelements directed naturally from roots to shoots then to seeds, grains and fruits of almost plants. N, P and K percentages in shoot dry matter were increased in Jerusalem artichoke plants inoculated with humic acid and biofertilizers combined with different levels from mineral NPK in both seasons (Table, 4).

The results proved that N content (%) was significantly increased in Jerusalem artichoke plants especially that inoculated plant with the treatment 75% NPK + H in first season which gave 3.18% whereas the highest values was recorded with the treatment 75% NPK + H + BF it gave 3.33% in the second seasons. In this context, the analysis of phosphorus and potassium content in shoot dry weight of Jerusalem artichoke plants during the two seasons were presented in Table (4). It was observed that the phosphorus and potassium contents were significantly higher in treatment (75% NPK + H + BF) than other treatments and control where the values were (0.364 and 2.65 %) in first season and (0.378 and 2.72%) in second season, respectively.

In concern, the uptake of nitrogen, phosphorus and potassium in Jerusalem artichoke shoots at two seasons illustrated in (Table, 4) which confirmed the role of humic acid and biofertilizers for enhancing growth and increasing yield. At first season, the treatment (75% NPK + H) postulated the highest significant values of nitrogen uptake it gave 9546 mg while phosphorus and potassium uptake detected the highest values with the treatment 75% NPH + H + BF (1101 and 8016 mg, respectively). Whereas the second season, the highest values in the uptake of N, P and K were detected by the treatment (75% NPK + H + BF) which recorded 10186, 1156 and 8320 mg, respectively. This may be attributed to the promoting effect of humic acid and different biofertilizers on soil physical properties to release nutrients in the rhizosphere which supply a power of available nutrients to plants. The obtained data were in agreement with **Waili (2010) and (Morsy *et.al.*, 2016)** who reported the beneficial effect of combination with mineral, organic and biofertilizers on nutrients uptake by the plants.

Table 4. Effect of different rates of mineral fertilization, humic acid, biofertilizers on N, P and K contents and its uptake of Jerusalem artichoke during 2016 and 2017seasons

Treatments	Contents (%)						Uptake (mg/ plant)					
	N		P		K		N		P		K	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK 100% (control)	3.12	3.24	0.360	0.363	2.56	2.690	9319.0	9633	1075.0	1079. 0	7647.0	7997.0
NPK 75% + H	3.18	3.22	0.355	0.358	2.54	2.650	9546.3	9621	1066.0	1070. 0	7625.0	7918.0
NPK 75% + H + BF	2.85	3.33	0.364	0.378	2.65	2.720	8621.0	10186	1101.0	1156.0	8016.0	8320.0
NPK 75% + BF	2.75	3.23	0.358	0.362	2.57	2.680	8247.0	9696	1074.0	1087.0	7707.0	8045.0
NPK 50% + H	2.85	2.85	0.328	0.335	2.41	2.400	7239.0	7399	835.3	870.0	6121.3	6230.0
NPK 50% +H+ BF	2.76	2.92	0.339	0.349	2.45	2.470	7485.0	7928	919.0	947.7	6644.0	6730.0
NPK 50% + BF	2.76	2.88	0.330	0.340	2.43	2.440	7355.0	7764	879.0	917.0	6476.0	6578.0
LSD at 5 % level	**0.12**	**0.14**	**0.018**	**0.008**	**0.15**	**0.11**	**561.0**	**551.0**	**84.6**	**51.8**	**542.0**	**659.0**

** BF: biofertilizers *(Az. chroococcum + B. megaterium + B. circulans + S. cerevisiae*)

Yield and its components

Data summarized in Table (5) showed the beneficial effect of humic acid and biofertilizers with mineral fertilizers on number of tubers/plant, average tuber weight, yield of tubers/plant and total yield of tubers/fed in this experiment in both seasons.

The data showed that there was a significant effect between treatments on the average of tubers number / plant as a result of using humic acid and biofertilizers with different levels of mineral fertilizers compared to control which the average of tubers number/plant ranged from 37 to 45 in the first season and 37 to 42 in the second season, respectively. While the beneficial effect of humic acid and biofertilizers was appeared on the average weight of tuber, which the treatment 75% NPK + H + BF gave the highest significant among the treatments and control in the first season (39.77 g), while in the second season, there was no significant effect among different 75% N P K treatments and control, where the same previous treatment (75% N P K+H+BF) recorded the highest value (42.38 g). In relation to the crop productivity, data showed no significant effect between the treatment of 75% mineral fertilizer and the control on tubers yield /plant (kg) and total tubers yield (ton/fed.) in the experiment in both seasons. While, the positive impact of humic acid and biofertilizers with 75% NPK, its increased the total yield (ton/fed.) compared with control and other treatments in both seasons which values of total tubers yield 25.001 and 25.429 (ton/fed.) in both seasons, respectively, whereas the control recorded 24.358 and 25.0 (ton/fed.) in both seasons, respectively. Furthermore, the application of humic acid and biofertilizers with 75% N P K increased the total tubers yield by 2.57 and 1.69 % than control in both seasons, respectively. The obtained results are in a harmony with many investigators **(Pizzeghello *et al.*, 2013 and Morsy *et.al.* 2016)** who mentioned that the humic substances and biofertilizers significantly enhanced plant growth due to the increase of cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake and supplying root cell growth which will be reflect positively on crop productivity.

Table 5. Effect of different rates of mineral fertilization, humic acid and biofertilizers on yield and its components of Jerusalem artichoke during 2016 and 2017 seasons

Treatments	Number of tubers/ plant		Average tuber weight (g)		Tuber/plant (kg)		Total yield (ton/fed.)		Relative ±in total yield than control (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK 100% (control)	44.00	42.00	38.75	41.67	1.705	1.750	24.358	25.000	0.00	0.00
NPK 75% + H	44.00	42.00	37.34	41.00	1.687	1.724	24.100	24.629	-1.10	-1.48
NPK 75% + H + BF	45.00	42.00	39.77	42.38	1.750	1.780	25.001	25.429	2.64	1.72
NPK 75% + BF	41.00	42.00	38.70	41.52	1.703	1.744	24.329	24.915	-0.12	-0.34
NPK 50%+ H	40.00	37.00	31.25	31.08	1.250	1.150	17.858	16.429	-26.69	-34.28
NPK 50%+H+BF	41.00	41.00	35.37	37.39	1.450	1.533	20.715	21.900	-14.96	-12.40
NPK 50%+ BF	37.00	40.00	28.38	32.50	1.050	1.300	15.000	18.572	-38.42	-25.71
LSD at 5 % level	**2.68**	**2.01**	**1.57**	**1.68**	**67.24**	**96.82**	**1.412**	**1.277**	**--**	**--**

** BF: biofertilizers *((Az. chroococcum + B. megaterium + B. circulans + S. cerevisiae*) *B*),

The quality and marketing of the Jerusalem artichoke tubers are governed by their relative content of carbohydrates and inulin. Data in (Table, 6) show the effect of organic matter (humic acid) and biofertilizers combined with different N P K levels from recommended dose on the quality of Jerusalem artichoke tuber as a nutritive value for humans., it was found that dry matter (%) and total carbohydrates content of tubers (%) in Jerusalem artichoke tubers have not affected by the addition of humic acid and biofertilizers pooled with 75% NPK compared with control, while the negative effect was obtained with the same additions pooled with 50% NPK. Therefore, the treatment 75%NPK + H + BF treatment which recorded (22.17 & 21.83 %) for dry matter and (17.05 % & 16.79%) for tubers content of total carbohydrates in both seasons, respectively. Where, the percentage of inulin was recorded the significant increase with the treatment (75%NPK + H + BF) which 11.08 and 10.99 % in two seasons, respectively. Moreover, this treatment gives 2.77 and 2.795 ton/fed from total yield of inulin in both seasons, respectively. In the same context was observed as mentioned above the content of Jerusalem artichoke tubers from total yield of inulin increased than control as a result to applicable of humic acid and biofertilizers by 4.33 and 4.47 % in both seasons.

Table 6. Effect of different rates of mineral fertilization, humic acid and biofertilizers on tuber quality of Jerusalem artichoke during 2016 and 2017 seasons

Treatments	DM (%)		Total carbohydrates (%)		Inulin (%)		Total yield of inulin (ton/fed.)		Relative ±in total yield of inulin than control (%)	
	1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
NPK 100% (control)	22.10	21.80	17.00	16.77	10.88	10.68	2.650	2.670	0.00	0.00
NPK 75% + H	21.98	21.70	16.91	16.69	10.69	10.61	2.576	2.613	-2.79	-2.13
NPK 75% + H + BF	22.17	21.83	17.05	16.79	11.08	10.99	2.770	2.795	4.53	4.68
NPK 75% + BF	22.07	21.78	16.98	16.75	10.82	10.66	2.632	2.656	-0.68	-0.52
NPK 50%+ H	21.14	21.20	16.26	16.33	10.41	10.50	1.859	1.725	-29.85	-35.39
NPK 50%+H+BF	21.55	21.42	16.60	16.46	10.59	10.55	2.194	2.310	-17.21	-13.48
NPK 50%+ BF	21.07	21.32	16.32	16.40	10.46	10.43	1.569	1.937	-40.79	-27.45
LSD at 5 % level	**0.76**	**0.53**	**0.40**	**0.30**	**0.27**	**0.33**	**0.150**	**0.136**	**--**	**-**

** BF: biofertilizers *((Az. chroococcum + B. megaterium + B. circulans + S. cerevisiae*).

Most of the recent literature concerning the percentage of carbohydrates and inulin show the ability of inoculants as biofertilizers represented in some plant growth promoting rhizobacteria and the presence of humic acid increase the filling of tubers and consequently reflected on the healthy state of the crop **(Morsy *et al.,* 2014)**. Also, soil application of humic acid to tuber crops enhanced plant growth characteristics, total and marketable yield, and tuber root quality, as well as, reduced the weight loss and decay percentages **(El Sayed *et al.,* 2011)**.

Microbial count

Microbial populations in both diversity as well as numbers in soil are influenced by the amount and type of various compounds entering soil through plant litter, root exudates and management factors like mineral and organic fertilizers. This in turn affects crop production and sustainability of soil health

All treatments showed increase in the dynamics of total microbial populations, total yeast counts and *Azotobacter* count in the rhizosphere comparison with control of Jerusalem artichoke plants during two seasons (Table, 7). Where, the treatment (75% NPK + H + BF) was recorded higher increases of total microbial populations and total yeast count than all treatments in rhizospheric plants, during the two experiment seasons. At first season, the total microbial and yeast count gave 22.8×10^5 and 12.3×10^4 CFU/g dry rhizosphere, respectively. Whereas, the enhancement in total microbial population and yeast count increase in the second season reach to 25.1×10^5 and 14.6×10^4 CFU/g dry rhizosphere, respectively. Regarding the population of *Azotobacter*, the treatment 50% NPK + H + BF altered the maximum count reach to 9.1×10^5 and 11.5×10^5 CFU/ g rhizosphere in both seasons, respectively.

It is reasonable expect that the use of humic acid and biofertilizers clearly reflected the positive effect on plants by increasing nutrient availability and production of some growth regulators like auxins and vital enzymes, atmospheric nitrogen fixation and converting the unavailable forms of nutrient elements to available ones. moreover, soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or enhance the decomposition of plant residues to release vital nutrients and increase soil humic content, will been vironmentally begin approach for nutrient management and ecosystem function **(Wu *et al*., 2005 and Morsy *et.al.*, 2016)**.

Table 7. Effect of different rates of mineral fertilization, humic acid and biofertilizers on microbial populations in rhizospher of Jerusalem artichoke during 2016 and 2017 seasons

Treatments	Total bacterial count (CFU×10^5)		Total yeast count (CFU×10^4)		*Azotobactr* count (CFU×10^5)	
	1st	2nd	1st	2nd	1st	2nd
NPK 100%	3.2	3.9	2.5	4	5.1	5.5
NPK 75% + H	15	17.3	5.4	6.7	5.71	6
NPK 75% + H + NF	22.8	25.1	12.3	14.6	6.33	6.4
NPK 75% + NF	20	23	10	13.5	6.1	6.21
NPK 50% + H	15.9	17	4.4	5.1	7.8	8.2
NPK 50% + H + NF	18.4	20.6	10	13.5	9.1	11.5
NPK 50% + Nf	16.5	17.7	9.1	11.2	8.5	10.1

** BF: biofertilizers *(Az. chroococcum* + *B. megaterium* + *B. circulans* + *S. cerevisiae*)

Enzymes activities

The activities of, nitrogenase, dehydrogenase, both acid and alkaline phosphatases have been used as a general biochemical indicator in measurements of biological activity in soil and plant rhizosphere (Table 8).

Nitrogenase activity depended on the viability of both native N_2- fixing bacteria and the diazotorophic groups which supplemented with treatments. Data presented in Table (8) reveal that the N_2-ase enzyme activity values increased with the treatment 50% NPK + H + BF more than 75% NPK and control which recorded 3.79 and 5.13 µmole C_2H_4 during the growth in the two seasons, respectively. The increase of nitrogenas enzymes depends on the ability of N_2-fixing bacteria to fix more atmospheric nitrogen in the presence of low dose of nitrogen fertilizer. The combination of different biofertilizers and humic acid enhanced the plant growth where biofertilizers produced some plant growth regulators that enhanced plant growth and this created synergistic effect between different biofertilizers and humic acid which used in this experiment. The lower activity of soil N_2-ase with control (100% NPK) as compared to combined treatments can be attributed to the lack of sufficient substrate i.e. organic carbon acts as energy source for proliferating the microbial population. The activity of N_2ase enzyme is controlled by the lack of mineral nitrogen as the ability to fix atmospheric nitrogen increased and decreased with the increase dose of mineral nitrogen **(Massoud *et al.*, 2014)**.

The dehydrogenase enzyme activity is considered as a true indicator of microbial activity in soil as this oxidative enzyme depended mainly upon the activity of only living microbial cells in the rhizospere of plants. Therefore, the maximum activity of this enzyme was recorded 40.15 µg TPF /g dry rhizosphere **/ day** with treatment (50% NPK + H + BF) at first season whereas, at second season the highest activity was recorded by treatment (75% NPK + H + BF) reached to 52.37 µg TPF / g rhizosphere **/ day** than control and other treatments (Table 8). Dehydrogenase enzyme activity was higher in the second season than the first one. These increases may be due to the addition of humic acid and biofertilizers which promote the growth and activity of native microorganisms. The microbial activity in the rhizospheric area is limited by the ability of beneficial microorganisms to exist in large populations and consequently the increase of its enzymes activity particularly, dehydrogenase and nitrogenase. This activity has been considered as a sensitive indicator of soil quality **(Caravaca *et.al.* 2003).**

Phosphatases enzymes group are of great agronomic value because they catalyze the hydrolysis of organic phosphorus compounds and transform them into an inorganic form of available phosphorus, which assimilated by plants and microorganisms. Therefore, data in Table (8) showed the highest significant of acid phosphatase activity recorded by the treatment 75% NPK + H + BF (91.41 and 96.18 mg /g **dry** rhizosphere **/ day**) in both seasons, respectively compared to other treatment and control. In the same context the highest values from alkaline phosphatase were obtained with the same treatment (12.67 and 13.25 mg/g dry rhizosphere **/ day**) in both seasons, respectively. The greater effectiveness of the NPK fertilizers may be attributed to higher rates of decomposability and mineralization of organic matter as evidenced by the relatively high microbial biomass turnover rate in the NPK than in other treatments. Alkaline phosphatase is an enzyme of great agronomic value because it hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus that are assimilated by plants **(Maestre *et.al.*, 2011)**. Several studies have been observed inverse relationships between inorganic P availability and phosphatase activity although this depends on initial bio-available P **(DeForest *et al.*, 2012)**.

Table 8. Effect of different levels of mineral fertilization, humic acid and biofertilizers on the activity of nitrogenase, dehydrogenase, acid and alkaline phosphatase in rhizosphere of Jerusalem artichoke during 2016 and 2017 seasons

Treatments	Nitrogenase µmole C_2H_4/g dry rhizosphere /h		Dehydrogease µg TPF /g dry rhizosphere / day		Acid phosphatase mg/g dry rhizosphere / day		Alkaline phosphatase mg/g dry rhizosphere / day	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
NPK 100% (control)	0.79	0.94	31.34	35.06	60.23	61.75	5.65	6.25
NPK 75% + H	1.66	1.72	28.51	37.38	72.29	77.28	6.87	6.27
NPK 75% + H + BF	3.53	4.45	37.74	52.37	91.41	96.18	12.67	13.25
NPK 75% + BF	3.42	3.23	32.24	48.42	88.20	90.40	11.81	12.07
NPK 50% + H	1.63	1.70	26.15	41.30	69.48	74.20	5.10	7.58
NPK 50% +H+BF	3.79	5.13	40.15	46.52	87.79	94.11	10.28	11.67
NPK 50% + BF	2.93	3.65	35.67	43.75	83.20	88.33	11.14	12.59
LSD at 5 % level	**0.95**	**0.95**	**1.50**	**0.87**	**2.15**	**2.76**	**0.76**	**0.84**

** BF: biofertilizers *(Az. chroococcum* + *B. megaterium* + *B. circulans* + *S. cerevisiae*)

Conclusion

Obtained results indicated the possibility of saving NPK fertilizers by 25% of the recommended dose without affecting the yield., the biofertilizers which producing plant growth promoting substances in addition to soil amendment with humic acid can be a true success story in sustainable agriculture through their numerous direct or indirect mechanisms of action It is essential to adopt a strategy of introducing organic and bio fertilizers along with mineral fertilizers in various crops. Accordingly, it can be concluded to reduce of mineral fertilization with the addition of organic matter and beneficial microorganisms as a biofertilizers to produce crops free of diseases and more safe for either environment or humans as a basic goal of sustainable agriculture.

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