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Maximizing Use Efficiency of Mineral Fertilizers Using K Fulvate and *Azotobacter chroococcum* DSM 2286 and Their Effect on Wheat Production and Nutrients Uptake

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DURING two successive growing winter seasons (2016/2017and 2017/2018) at the experimental farm of Meet Ghamr, Dakahlia Governorate, Egypt. A field experiment was carried out to maximizing use efficiency of mineral fertilizers (N, P and K) by application of potassium fulvate (KF) and bio-fertilization (*Azotobacter chroococcum* DSM 2286) on growth, yield and some chemical compositionsof wheat plant (*Triticum aestivum* L.) variety Giza 171. The layout of the experiment was a split- plot design, with the main plots arranged in a randomized complete blocks design, with three replicates. This experiment includes three main treatments and four sub-main treatments. Where the three levels of mineral fertilization "NPK" (60, 80 and 100% of the recommended mineral fertilization) were assigned in the main plots. On the other hand, the application of potassium fulvate and biofertilization were assigned in the sub-main plots. After 75 days from sowing, fresh and dry matter yields, chlorophyll (a, b and a+b), carotenoids in leaves, dehydrogenase activity in rhizospheric soil and the N, P, K, Fe, Mn and Zn in shoots of wheat plants were determined. As well as at harvest stage, weight of 1000 grain (g), yields of straw and grains (ton ha^{-1}), and their content of N, P, K, Fe, Mn and Zn were determined. The obtained results indicated that, the application of KF and *Azotobacter chroococcum* alone or in a combination resulted in a significant increase in all studied parameters compared to the control. Under 80 % of the recommended mineral fertilization (N, P and K), the dual application of KF + *Azotobacter chroococcum* showed a significant augmentation in all studied parameters compared to the control under 60, 80 and 100 % of the recommended mineral fertilization. Therefore, it considered as a more beneficial treatment in the cultivation of wheat plants (Giza 171 variety) at Nile Delta due to it resulted in a high yield, quality and reducing the environmental pollution as a result from reducing the additions of the mineral fertilizer with the organic and the biofertilization one.

Keywords: Mineral fertilizers, Potassium fulvate, Biofertilizer, Wheat

Introduction

Increasing the yield of wheat (*Triticum aestivum* L.) is an important national goal to meet the ever-increasing food needs of the Egyptian population. Wheat is one of the three major kinds of cereal crops (along with maize and rice). Also, it is a main source of energy, renewable resources for food, feed and industrial raw materials, the source of protein and fiber in the human diet, and staple food crops for more than a third of the world's population (Ramadoss et al., 2013) grown as a spring and winter crop. Zaman et al. (2010) found that, the use of synthetic fertilizers is closely related to crop yield. It is evident that only half of the applied nitrogen fertilizers are taken by the crops and the second half are lostfrom the agricultural field through surface runoff and leaching of nitrate as well as volatilization of ammonia and emission of NO_x gases (Gogoi and Baruah, 2012). For these reasons, great attention needs to be paid to finding a new type of fertilizer that, ideally, can be low cost with high nitrogen utilization efficiency (Chander, 2012).

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Humic acid (HA) and fulvic acid (FA) are two important fractions of soil humic substances (HS) and have a relevant role in the elemental recycling in the environment and in soil ecological functions. Humic acid and fulvic acid have an important role in the primary recycling in the environment and in the environmental functions of soil. Fulvic acid plays an important role in the retention, release, bioavailability and mobility of macro- and micronutrients and organic chemicals in the soil (Hu et al., 2019).

Fulvic acid has a significant effect on chemical reactions due to the presence of more electronegative oxygen atoms than any other humate materials, which enhances the permeability of the membrane (Mosa et al., 2020). Fulvic substances can affect the physiological processes of plant growth directly or indirectly (Yang et al., 2013 and Mohamed, 2020) and can improve plant growth under soil conditions by enhancing the absorption of nutrients and reducing the absorption of some toxic elements (Kulikova et al., 2005). Anjum et al. (2011) reported that fulvic acid increases the chlorophyll and water content of the leaves. It also increased photosynthesis, reduced stomata opening, and transpiration, which stimulated growth and reduced water loss. Several beneficial effects have been attributed to the application of potassium fulvate to leaves or soil, including stimulation of plant metabolism, increased enzyme activity, increased bioavailability and absorption of nutrients and increased crop growth and productivity (Abd EL-Kader, 2016 and Hamad and Tantawy, 2018).

Biofertilizers have been identified as an alternative to mineral fertilizers to increase soil fertility and crop production in a sustainable manner (Sakara and Badour, 2020). Also, biofertilizers have emerged as an important component of the integrated nutrient management programs and hold a great promise to improve crop yield with minimum environmental degradation. Strains of *Azotobacter, Rhizobium, Bradyrhizobium, Azospirillum, Pseudomonas, Bacillus* and *Acetobacter* etc. have been developed as biofertilizers for cereals, pulses, vegetables, oil seeds, cotton, sugarcane, wheat, etc. (Swarnalakshmi et al., 2013).

Therefore, the present study aims to investigate the potential of potassium fulvate as "organic fertilization" and *Azotobacter chroococcum* DSM 2286 "as bio-fertilization" individually and in combination on reduces the requirement of mineral fertilization, enhanced nutrient uptake and productivity of wheat plants under clayey loam soil conditions.

Materials and Methods

This study was performed at private farm of Meet Ghamr, Dakahlia Governorate, Egypt (Latitude 30° 71' N and the Longitude 31° 25'E), during two successive growing winter seasons of 2016/2017and 2017/2018. This experiment was conducted to study the ability of potassium fulvate (KF) and *Azotobacter chroococcum* DSM2286 (biofertilization) to maximizing use efficiency of mineral fertilization which applied at 60, 80 and 100 % of recommended doses of N, P and K according to Egyptian Ministry of Agriculture (75 kg N fed^{-1}, 15 kg P_2O_5 fed^{-1} and 24 kg K_2O fed^{-1}) on growth, yield and some chemical compositionsof wheat plantsvariety Giza (171).

This experiment was conducted in a split-plot design, includes twelve treatments in three replicates. Therefore this study including 36 experimental plots, where the area of each plot was 10.5 m^2 (3.5m × 3 m). These plots were divided into three main groups (12 plots/the main group) representing the application of mineral fertilizers (N, P and K) as a percent (60, 80 and 100%) of its recommended doses*, i.e.* urea 163" kg fed^{-1}", mono calcium phosphate 100" kg fed^{-1}" and potassium sulfate 50" kg fed^{-1}". The plots of each main group were divided into four sub-main groups (3 plots/the sub-group) representing the sub-main study factor by the following four treatments, *i.e.*control (without any applications of KF or biofertilizer), potassium fulvate (KF) alone at an application rate of 2 kg fed^{-1} as a soil application, biofertilizer alone (*Azotobacter chroococcum* DSM 2286) at an application rate of 20 L fed^{-1} "feddan=4200m^2 "as a soil application and combined application of KF and biofertilizer.

Random disturbed soil samples from the surface layer (0-30 cm) of the tested soil were collected before planting. Soil samples were air - dried, ground, mixed well,sieved through a 2 mm sieve. The samples then were analyzed for determination of some physical and chemical properties, also, the content of some available macro- and micronutrients according to the methods described by Page et al. (1982) and Klute (1986). Also, the used potassium fulvate "KF" was purchased from the agriculture commercial market, where it's analyzed for some physical and chemical properties according to Page et al. (1982). The obtained data were recorded in Table 1.

Azotobacter chrococoum DSM 2286 was grown in King's medium (Atlas, 1995). Cultures were incubated at 28 C^o for three days on a rotary shaker until the early log phase to ensure a population density of 10^9 cfu/ml culture.

This strain *(Azotobacter chroococcum* DSM 2286), was obtained from bank strains of Laboratory Soil Microbiology, Department of Soil Science, Faculty of Agriculture, Menoufia University, The strain was previously defined by genotypic identification which performed by amplification and partial nucleotide sequencing of the 16s ribosomal DNA (16s rDNA) (El Zemrany et al., 2015).

Wheat (*Triticum aestivum* L.) grains were obtained from Crops Research Insatiate, ARC, Giza, Egypt. The sowing date was at 15 November and harvesting date was 29 April, of each season. The addition of N and K fertilizers at three application rates of recommended dose were divided into two equal portions after 30 and 60 days of sowing in both study seasons. The amount of P-fertilizer was added to the soil during soil preparation. Potassium fulvate "KF" and biofertilization treatments were applied in a liquid formulation as a soil application at 0, 30 and 60 days after sowing.

In both two seasons, at 75 days of sowing, plant samples were successively taken randomly from three replicates of every treatment at single to determine fresh and dry matter yields (g/m^2), chlorophyll a, b, a+b and carotenoids (mg g^{-1} FW) in leaves according to the methods described by Moran (1982). Dehydrogenase activity in rhizospheric soil "DHA" µ formazan g^{-1} hour^{-1} was determined according to Page et al. (1982). Also, at the same time, the plant samples contents of N, P, K, Fe, Mn and Zn were determined Cottenie et al. (1982).

In both two seasons, at harvesting time, the plants of each plot were harvested separately. The grains were separated from straw to measure: weight of 1000 grains (g), grains and straw yield as ton ha^{-1} and % protein in grains. Grain and straw samples were air-dried then, oven-dried at 70 C^o for 48 hr., weighed, ground and digested for chemical determination according to the method described by Chapman and Pratt (1961). N, P and K content in the digests were determined according to the methods described by A.O.A.C. (1980) and Cottenie et al. (1982). Crude protein percentage was estimated in the different parts by multiplying N % values by 5.75 as described by A.O.A.C. (1985). The atomic absorption spectrophotometer was used to determine Fe, Mn and Zn concentrations in the prior parts according to the methods recommended by Cottenie *et al.* (1982).

Statistical analysis

Results from identical experiments of the 2 years were combined for analysis. Significant differences among treatments means were determined at $P \leq 0.05$ by using LSD test and Duncan's Multiple Comparisons Test. Data of the present study were statistically analyzed using CoSTATE Computer Software, according to Gomez and Gomez (1984).

Results and Discussion

Plant measurements of vegetative growth period

Fresh and dry matter yields (g/m^2)

The present data in Fig. 1 showed that fresh and dry matter yields (g/m^2) of wheat (Giza 171 variety) plants shoots as affected by the studied treatments of mineral fertilizers (N, P and K) at a rate of 60, 80 and 100 % of recommended doses, at 75 days after sowing. There is a significant increase of both fresh and dry matter yields consequently applied separately and in a combination of K fulvate +*A. chroococcum*, in compared to the treatments received only mineral fertilizers. The maximum values of the dry matter yields were obtained with the treatment of "KF+Bio" which received 100 % of recommended mineral fertilization compared to unamended control treatments with the same percentage of recommended mineral fertilization (100 % NPK).

As regards the effect of KF + *A. chroococcum* application in combination with 80 % of the recommended mineral showed a significant increase of the fresh and dry matter yields of wheat plants shoots over the treatments of 60, 80 and 100 % of the control treatments. These results are confirmed with that obtained by Hamouda et al. (2015) and Abou El Hassan and Husein (2016).

Data in Fig. 1 indicated that, the *A. chroococcum* individually or with KF have a beneficial effects on fresh and dry matter yields of wheat plant shoots. These results agreed with those of Lopez-Gomez et al. (2014a and b) and Kang et al. (2015).These findings are due to the high potency of *Azotobacter chroococcum* DSM 2286, as Co-inoculation of nitrogenase activity, P-solubilization capacity, salt tolerance, and IAA production rate (El Zemrany et al., 2015). These explanations are in coincidence with Farid et al., (2018) and El Zemrany et al. (2019) who reported that the application of biofertilizers and humic, fulvic and K-humate had a positive effect on the growth and enhanced NPK mineral uptake of faba bean and peanut plants.

TABLE 1. Initial properties of the soil used and applied potassium fulvate (KF), during the two growing seasons (2016/2017 and 2017/2018) (combined seasons)

a. Soil physical properties

Particle size distribution (%)				Textural class
C. sand	F. sand	Silt	Clay	
3.2	22.5	37.5	36.8	Clay loam

b. Soil chemical properties

O.M.	$CaCO_3$	SP	pH (1:2.5, soil: water Susp.)	EC, soil paste, (dSm^{-1})	Soluble ions (mmole L^{-1})							
					Cations(mmole L^{-1})				Anions(mmole L^{-1})			
gkg^{-1}		%			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
0.16	0.12	75.0	7.91	2.28	0.52	0.46	1.10	0.20	–	0.35	1.31	0.62

c. Soil nutrient contents

Macronutrients (Available) mgkg^{-1}			Micronutrients (DTPA extractable) mgkg^{-1}			
N	P	K	Cu	Fe	Mn	Zn
42.60	6.40	200.50	2.46	6.45	1.50	1.46

Potassium fulvate properties

Appearance	Moisture (%)	Solubility (%)	Fulvic acid (%)	pH	N (%)	P (%)	K (%)
Dark brown powder	5.75	100	65	5.61	0.55	2.97	8.0

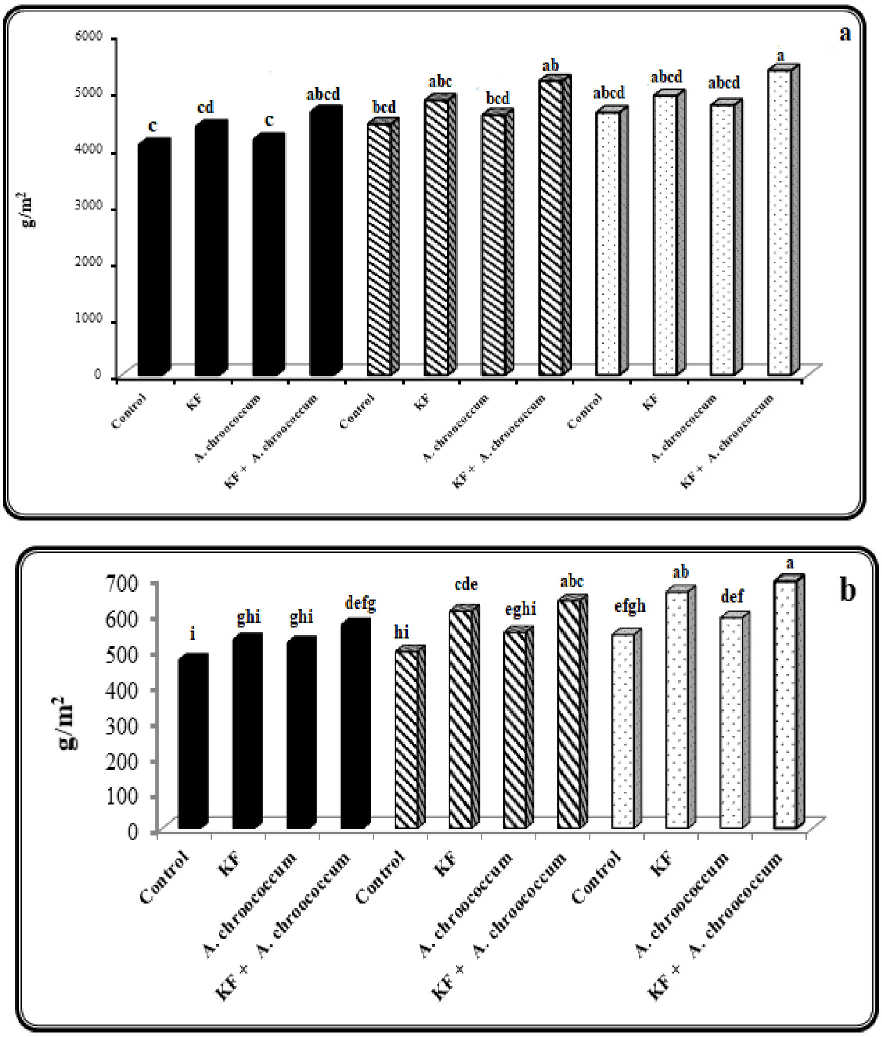

Fig. 1. Mean values (2016/2017 and 2017/2018) of fresh "a" and dry matter yields "b" (g/m^2) of wheat (Giza 171 variety) plants shoots at 75 days after sowing, as affected by bio and organic fertilizers (A. chroococcum + K Fulvate) under 60 (black), 80 (shadow) and 100 % (dotted) of recommended dose mineral fertilization (N, P and K).KF: Potassium fulvate. a to i Differences between values having the same high script in each column are not significant at P ≤ 0.05

Nutrients content of the wheat plant shoots

Macronutrients content

Data listed in Table 2 revealed that, in general there are a significant increases in the concentration (%)of N, P and K and their uptake (mg plant^{-1}) in the shoots of wheat plants due to the applications of KF and biofertilization with *A. chroococcum* individually or in combination compared to the control treatments. These findings were found with the three levels of mineral fertilization.

In addition, the combination treatments of "KF+ *A. chroococcum* "with 100% of mineral fertilization, gives the maximum values of N, P and K concentrations (%) which were: 2.46, 0.32 and 3.71%, compared to those found in the control, which were: 2.28, 0.26 and 3.51 % for N, P and K, respectively, whereas, the same treatments resulted in uptake values of 68.04, 8.72 and 102,31, while in their control treatments these values, were: 49.24, 5.57 and 75.89 mg plant^{-1} for N, P and K, respectively.

Micronutrients content

Data in Table 2 also show that KF and *A. chroococcum* individually or in combination increased Fe, Mn and Zn (mg kg^{-1}) and their uptake (mg plant^{-1}) as compared with control treatment under the three levels of mineral fertilization (60, 80 and 100% of recommended dose). The maximum values of Fe, Mn, and Zn concentration (mg kg^{-1}) were: 337.50, 52.50, and 19.00 mg kg^{-1}, where these values with their control, were: 289.00, 47.00 and 15.50 mg kg^{-1}, respectively. Also, their main uptake of Fe, Mn and Zn (mg plant^{-1}) were: 0.93, 0.145 and 0.052, while, in their control, were: 0.63, 0.102 and 0.034 mg plant^{-1},respectively,where these maximum values were obtained with the combination treatments of "KF+ *A. chroococcum*" along 100 % of mineral fertilization.

The treatment of KF+ *A. chroococcum* with 80% at the recommended dose of mineral fertilization gained macro- and micronutrient concentration and its uptake over application rates at 60, 80 and 100 % of the recommended mineral fertilization does. Similar results were obtained by Nowick (2014) and Farid et al. (2018).

On general view over the results of the increases effect of the interaction of the dual application of "KF × biofertilization" ,"biofertilization × mineral fertilization" and " KF × mineral fertilization" on N, P, K, Fe, Mn and Zn concentration and uptake in wheat plants were highly significant.

Chlorophyll and carotenoids contents

Determination of chlorophyll content as an indirect method of estimating the productivity of vegetation represents a good way to gain an understanding of the photosynthetic regime of plants. The content of chlorophyll a, chlorophyll b, chlorophyll (a+b), and carotenoids contents were measured in all experimental treatments as a function of mineral nutrition at the outset of flowering. Data in Table 3 declared that , the contents of chlorophyll a, chlorophyll b, chlorophyll (a+b) and carotenoids in wheat plants affected by individual KF, biofertilization and combination "KF +biofertilization" treatments were significantly greater higher than that found in the control treatments. Indeed, the found chlorophyll (a+b) content recorded were 0.930, 0.938, 0.946 and 0.949 mg g^{-1} FW with the treatments of control, "KF","*A. chroococcum*" and "KF + biofertilization" at 100 % of recommended dose of mineral fertilization, respectively. Whereas, the carotenoids contents were 0.352, 0.359, 0.361 and 0.364 mg g^{-1}FW with the same abovementioned treatments. The obtained results show the importance of inoculation by *A. chroococcum* with KF under mineral fertilization in enhancing the chlorophyll and carotenoids contents, consequently its beneficial effect on promoting the productivity of wheat plants. The higher values of chlorophyll and carotenoids content in "KF+ *A. chroococcum*" treatments of the wheat plants have contributed to the higher grain and straw productivity in the plants grown with the 100 % recommended dose of mineral fertilization (Tables, 3 to 5).Anjum et al. (2011) reported that fulvic acid increased chlorophyll and water content of leaves. It also increased photosynthesis, reduced stomata opening status, and transpiration thus led to growth stimulation and water loss reduction (Li *et al.,* 2005). Also, they found that fulvic acid and humic acid have been used to regulate plant growth under well-water.

Dehydrogenase activity "DHA"

Dehydrogenase activity (DHA) is frequently used as a measurement of the overall microbial activity in the soil. Data reported in Table 3 display the values of dehydrogenase activity (µg formazan g^{-1} soil hour^{-1}) in rhizospheric soil of wheat plants (Giza 171 variety) at 75 days after sowing as affected by individual or combined application of KF or/and *A. chroococcum* treatments. Results pointed out that dehydrogenase activity in the rhizospheric soil of wheat plants significantly increased with all organic and biofertilizers treatments more than the untreated one.

TABLE 2. Mean values (2016/2017 and 2017/2018) of N, P and K concentration (%) and Fe, Mn and Zn (mg kg-1) and its uptake (mg plant-1) in wheat plants shoots at 75 days after sowing, as affected by organic and biofertilization under three rates of recommended dose of mineral fertilization (N, P and K).

Mineral fertilization levels	Organic and biofertilization treatments	Macronutrients						Micronutrients					
		N		P		K		Fe		Mn		Zn	
		Conc. (%)	Uptake (mg plant-1)	Conc. (%)	Uptake (mg plant-1)	Conc. (%)	Uptake (mg plant-1)	Con. (mg kg-1)	Uptake (mg plant-1)	Con. (mg kg-1)	Uptake (mg plant-1)	Con. (mg kg-1)	Uptake (mg plant-1)
60 %	Control	2.04 j	38.42i	0.211	4.01h	2.92i	54.92h	245.5i	0.46g	38.50f	0.073g	13.50c	0.025f
	KF	2.12i	43.02ghi	0.23j	4.67gh	3.15gh	63.84fg	280.0f	0.57ef	40.00f	0.081fg	17.50ab	0.036def
	A. chroococcum	2.16h	44.75fgh	0.22k	4.60gh	3.06h	63.37g	260.0h	0.54f	39.00f	0.081fg	16.00abc	0.033ef
	KF + *A. chroococcum*	2.19fg	49.90de	0.24i	5.50ef	3.23g	73.54de	291.5de	0.66cd	40.50f	0.092ef	18.00ab	0.041bcde
Mean		2.13c	44.02c	0.23c	4.70c	3.09c	63.92c	269.3c	0.56c	39.50c	0.082c	16.25c	0.034c
80 %	Control	2.17gh	42.74hi	0.25h	4.89fg	3.35f	66.07efg	271.0g	0.54f	44.00e	0.087fg	15.50bc	0.031ef
	KF	2.21f	53.53cd	0.29d	7.03cd	3.50de	85.04c	297.5cd	0.72c	45.00de	0.109cd	18.00ab	0.044abcd
	A. chroococcum	2.24e	47.26fgh	0.27f	5.65e	3.42ef	72.22def	279.5f	0.59ef	44.50e	0.094ef	17.50ab	0.037cde
	KF + *A. chroococcum*	2.29d	58.34bc	0.30c	7.60bc	3.61abc	91.76bc	318.5b	0.81b	47.50c	0.121bc	18.50ab	0.047abc
Mean		2.23b	50.47b	0.28b	6.29ab	3.47b	78.77b	291.6b	0.66b	45.25b	0.103b	17.38b	0.040ab
100 %	Control	2.28d	49.24def	0.26g	5.57ef	3.51cde	75.89d	289.0e	0.63de	47.00cd	0.102de	15.50bc	0.034def
	KF	2.33c	61.66b	0.31b	8.13ab	3.63ab	95.96ab	317.5b	0.84b	50.00b	0.132b	19.00ab	0.049ab
	A. chroococcum	2.39b	56.27bc	0.28e	6.56d	3.58bcd	84.20c	303.5c	0.71c	49.00bc	0.115c	18.50ab	0.046abc
	KF + *A. chroococcum*	2.46a	68.04a	0.32a	8.72a	3.71a	102.3a	337.5a	0.93a	52.50a	0.145a	19.50a	0.052a
Mean		2.37a	58.80a	0.29a	7.25a	3.61a	89.59a	311.9a	0.78a	49.63a	0.124a	18.13a	0.045a

KF: Potassium fulvate.

a to l Differences between values having the same high script in each column are not significant at P 0.05.

TABLE 3. Mean values (2016/2017 and 2017/2018) of chlorophyll a, b, (a+b) and carotenoids (mgg-1FW) and dehydrogenase activity in rhizospheric soil of wheat plants at 75 days after sowing, as affected by organic and biofertilization under three rates of recommended dose of mineral fertilization (N, P and K).

Mineral fertilization levels	Organic and biofertilization treatments	Chlorophyll a	Chlorophyll b	Chlorophyll (a+b)	Carotenoids	Dehydrogenase activity
		mg g-1 FW				µg formazan g-1 soil h-1
60 %	Control	0.568i	0.257i	0.825i	0.348i	48.91i
	KF	0.588g	0.258g	0.846g	0.353g	60.76g
	A. chroococcum	0.596e	0.262e	0.858e	0.355e	85.85e
	KF + A. chroococcum	0.608b	0.265b	0.873b	0.358b	94.93bc
Mean		0.590c	0.261c	0.851c	0.354bc	72.613b
80 %	Control	0.617h	0.259h	0.876h	0.350h	52.15f
	KF	0.632f	0.267f	0.899f	0.355f	66.09f
	A. chroococcum	0.639d	0.269d	0.908d	0.356d	89.83d
	KF + A. chroococcum	0.668b	0.270b	0.938b	0.361b	96.24b
Mean		0.639b	0.266b	0.905b	0.356bc	76.078a
100 %	Control	0.656h	0.274h	0.930h	0.352h	53.18h
	KF	0.662f	0.276f	0.938f	0.359f	67.47f
	A. chroococcum	0.669c	0.277c	0.946c	0.361c	93.91c
	KF + A. chroococcum	0.670a	0.279a	0.949a	0.364a	98.40a
Mean		0.664a	0.277a	0.941a	0.359a	78.240a

KF: Potassium fulvate.

a to i Differences between values having the same high script in each column are not significant at $P \leq 0.05$.

This trend was recorded with the three levels of recommended dose of mineral fertilization (NPK) but there resulted in a significantly one higher increase of the “DHA” than those of individual KF and *A. chroococcum* treatments. The 100 % of recommended mineral fertilization (NPK) treatments appeared the most positive effect on the increase of DHA in comparison to the other treatments (60 and 80%), under the individual or in a combination of KF and *A. chroococcum* treatments. The application of KF and *A. chroococcum* along with NPK fertilization resulted in an increase in microbial activities over other treatments(Bharali et al., 2017).

Yield and yield attributes at harvest stage

Weight of 1000 grain

Data in Table 4 denoted that weight of 1000 grain (g) of wheat plants (Giza 171 variety) was significantly increased by the studied treatments in compared to the un-treated treatments (control). Moreover, the data show that the combination treatments of KF + *A. chroococcum* resulted in a significant increases in 1000 grain weight (g) as compared to the otherapplications, under the three levels (60, 80 and 100%) of recommended mineral fertilization (NPK).The higher number of filled grain percent influenced the 1000 grain weight (g) which has contributed to the higher grain yield due to the application of KF and *A. chroococcum*.

Grain and straw yields (ton ha-1)

Data presented in Table 4 implied that grain and straw yields (ton ha^{-1}) of wheat plants (Giza 171 variety) were affected by the applied organic and biofertilizers, under the three levels of recommended dose of mineral fertilization (NPK). The grain and straw yields of the wheat revealed wide variations among the experimental treatments. There was an increment in grain and straw yields due to the treatments with the order of KF + *A. chroococcum* (in combination treatment)> KF (alone)>*A. chroococcum* (alone). In addition, the treatments of levels of recommended mineral fertilization (NPK) had significant differences among them.The combined applications of KF+ *A. chroococcum* raised wheat grain yields up to 7.880, 9.249 and 9.965 ton ha^{-1}compared to the untreated plants (control) recorded yield of 6.759, 7.737 and 8.806 ton ha^{-1} with 60, 80 and 100 % of recommended mineral fertilization, respectively. Whereas the straw yields with the same above mentioned treatments reached to: 10.160, 11.119 and 12.152 ton ha-1 compared 9.211, 9.879 and 10.710 ton ha^{-1} for the control treatments under 60, 80 and 100% of recommended mineral fertilization, respectively. These results are in agreement with those obtained by Kamel et al. (2014), they revealed that, the foliar application of fulvic acid improved plant growth and yield quantity and quality of cucumber plants.

In a general view, the results of the all studied parameters with the combination treatments (KF + *A. chroococcum*) that received 80% of the recommended mineral fertilization were high and significant in compared to the results of control that received 60, 80 and 100 % of the recommended mineral fertilization.

Macro-and micronutrients concentrations in grains and straw

Macronutrients

Nitrogen, phosphorus and potassium contents (%) in grains and straw (at harvest stage) of wheat plants (Giza 171 variety) as affected by organic and biofertilizers with 60, 80 and 100 % of recommended mineral fertilization, are listed in Table 5. The data demonstrated that all KF and/or *A. chroococcum* treatments significantly increased NPK content (%) in both grains and straw of wheat plants under the three recommended mineral fertilization levels, compared to the control treatments.

The maximum values of NPK contents of grains and straw were obtained with the combined treatments of KF + *A. chroococcum* under 100 % of recommended mineral fertilization, where they found contents were 2.89, 0.26 and 0.44 % in grains and were: 0.86, 0.12 and 2.60 % in straw, respectively, while these contents were 2.35, 0.19 and 0.37% in grains, and 0.71, 0.09 and 2.33 in straw for the control treatments with the same level of recommended mineral fertilization.

TABLE 4. Mean values (2016/2017 and 2017/2018) of grain and straw yields (ton ha-1), 1000 grain weight (g) and protein (%) in grains, of wheat plants after harvest, as affected by organic and biofertilization under three rates of recommended dose of mineral fertilization (N, P and K)

Mineral fertilization levels	Organic and biofertilization treatments	Grain yield	Straw yield	1000 grain weight	Protein in grains
		ton ha^{-1}		g	%
60%	Control	**6.759^{h}**	**9.211^{h}**	**59.42^{e}**	**10.12^{g}**
	KF	**7.426^{g}**	**9.891^{fg}**	**61.90^{cd}**	**11.21^{fg}**
	A. chroococcum	**7.119^{gh}**	**9.513^{g}**	**61.59^{cde}**	**11.85^{ef}**
	KF + *A. chroococcum*	**7.880^{f}**	**10.160^{e}**	**62.14^{cd}**	**12.36^{def}**
Mean		**7.296^{c}**	**9.694^{c}**	**61.26^{c}**	**11.39^{b}**
80%	Control	**7.737^{f}**	**9.879^{fg}**	**61.20^{de}**	**13.00^{cde}**
	KF	**8.663^{d}**	**10.670^{d}**	**62.55^{cd}**	**13.97^{bcd}**
	A. chroococcum	**8.323^{e}**	**10.251^{e}**	**61.70^{cde}**	**15.01^{abc}**
	KF + *A. chroococcum*	**9.249^{c}**	**11.119^{c}**	**65.09^{ab}**	**15.58^{ab}**
Mean		**8.493^{b}**	**10.480^{b}**	**62.64^{b}**	**14.39^{a}**
100%	Control	**8.806^{d}**	**10.710^{d}**	**61.55^{cde}**	**13.51^{def}**
	KF	**9.696^{b}**	**11.726^{b}**	**64.00^{abc}**	**15.41^{cde}**
	A. chroococcum	**9.346^{c}**	**11.210^{c}**	**63.20^{bcd}**	**16.04^{a}**
	KF + *A. chroococcum*	**9.965^{a}**	**12.152^{a}**	**66.08^{a}**	**16.62^{a}**
Mean		**9.453^{a}**	**11.449^{a}**	**63.71^{a}**	**15.40^{a}**

KF: Potassium fulvate.

a to h Differences between values having the same high script in each column are not significant at P 0.05.

TABLE 5. Mean values (2016/2017 and 2017/2018) of NPK (%), Fe, Mn and Zn (mg kg-1) concentration in grains and straw of wheat plants at harvest, as affected by organic and biofertilization under three rates of recommended dose of mineral fertilization (N, P and K)

Mineral fertilization levels	Organic and biofertilization treatments	Grains						Straw					
		N	P	K	Fe	Mn	Zn	N	P	K	Fe	Mn	Zn
		%			mg kg-1			%			mg kg-1		
60%	Control	1.76g	0.12l	0.30i	161.00h	27.50g	15.00h	0.35i	0.060i	2.05h	54.50g	7.00g	9.50e
	KF	1.95fg	0.15j	0.32gh	175.50g	30.00ef	16.50fg	0.46h	0.070h	2.21fg	59.00g	9.00def	13.00cd
	A. chroococcum	2.06ef	0.14k	0.31hi	173.00g	29.00fg	16.00gh	0.52g	0.067hi	2.18g	56.50g	8.83ef	12.00d
	KF + A. chroococcum	2.15def	0.17i	0.34f	179.50fg	32.00de	17.50ef	0.56f	0.073gh	2.23f	64.50f	10.83bcd	14.00cd
Mean		1.98c	0.15c	0.32c	172.25c	29.63c	16.25c	0.47c	0.068c	2.17c	58.63c	8.92c	12.13c
80%	Control	2.26de	0.18h	0.33fg	179.50fg	30.50ef	18.00de	0.58f	0.080fg	2.19fg	65.00f	8.00fg	12.50cd
	KF	2.43bcd	0.23d	0.37d	191.00e	33.00cd	19.00d	0.64e	0.103cd	2.33d	75.50de	10.50cdef	13.50cd
	A. chroococcum	2.61abc	0.20f	0.35e	186.50ef	32.00de	18.50de	0.70d	0.093e	2.28e	71.50e	10.00def	13.00cd
	KF + A. chroococcum	2.71ab	0.24c	0.40c	207.50cd	34.50bc	21.50c	0.74c	0.110bc	2.36d	82.50c	12.50b	18.00b
Mean		2.50b	0.21b	0.36b	191.13b	32.50b	19.25b	0.67b	0.965b	2.29b	73.63b	10.25b	14.25b
100%	Control	2.35cde	0.19g	0.37d	201.00d	33.50bcd	20.50c	0.71d	0.083f	2.33d	79.00cd	9.67bcde	15.00c
	KF	2.68def	0.25b	0.42b	218.00b	35.50b	23.00b	0.80b	0.113ab	2.51b	90.50b	11.00b	18.00b
	A. chroococcum	2.79a	0.21e	0.39c	210.00c	34.50bc	21.00c	0.82b	0.100de	2.44c	88.00b	10.67bc	17.50b
	KF + A. chroococcum	2.89a	0.26a	0.44a	225.50a	37.50a	24.50a	0.86a	0.120a	2.60a	101.50a	14.25a	21.00a
Mean		2.68a	0.23a	0.41a	213.63a	35.25a	22.25a	0.80a	0.104a	2.47a	89.75a	11.40a	17.88a

KF: Potassium fulvate.

a to k Differences between values having the same high script in each column are not significant at $P \leq 0.05$.

Micronutrients contents

Data in Table 5 manifested the contents (mg kg^{-1}) of the determined micronutrients (Fe, Mn, and Zn) in grains and straw of wheat plants (Giza 171 variety). The applications of KF or *A. chroococcum* alone or in combination led to significant increases of Fe, Mn and Zn contents in grains and straw of wheat plants compared to the control treatments under all levels of recommended mineral fertilization. The highest values of Fe, Mn and Zn contents were recorded with the combined application of KF and *A. chroococcum,* where these contents in grains were: 225.50, 37.50 and 24.50 mg kg^{-1}, respectively, but in straw were: 101.50, 14.25 and 21.00 mg kg^{-1}, respectively with 100 % of recommended mineral fertilization treatments. These results are in lines with Bocanegra et al. (2006) and Yang et al. (2013) whose they concluded that, the fulvic acids had the capacity to chelate nutrients and move through membranes, consequently had suggested that the fulvic acids may play similar roles as natural chelators in the mobilization and transport micronutrients.

Grain protein content

Data recorded in Table 4 demonstrated that grains protein content of wheat plants (Giza 171 variety) was increased significantly by the aforesaid treatments with a comparison with the unfertilized plants. Also, treatments of KF + *A. chroococcum* as in combination treatment significantly augmented grain protein content more than that individually treatments by KF or *A. chroococcum* under 60, 80 and 100 % of recommended mineral fertilization. In addition, the addition of 100% recommended mineral fertilization (NPK) gave a high grain protein content and surpassed the other mineral fertilization treatments for all treatments of KF and *A. chroococcum*.

The obtained results in the present study indicate that, the dual application of KF and *Azotobacter chroococcum* had a high capacity to promote the fresh and dry matter yields, contents of both macro- and micronutrients (N, P and K) and (Fe, Mn and Zn) in shoots, grains and straw of wheat plants, chlorophyll and carotenoids pigments and dehydrogenase activities, at all experimental treatments. Those findings might be due to the high potential of such isolates for IAA production, nitrogenase activity and solubilization P (El Zemrany *et al.,* 2015). Also, these treatments produced the highest fresh and dry matter yields of wheat plants as well as the contents of macro- and micronutrients*i.e.* (N, P and K) and (Fe, Mn and Zn) (Fig. 1 and Tables 2 to 5). This could be referred to as local changes in root morphology and biomass, *i.e.* larger numbers of tips extending surface within the rhizosphere and augmentation of the polysaccharide enrichment of the inoculated roots, as compared to the unamended controls (El Zemrany *et al.*, 2006 and 2007). These promotive effects of potassium fulvate and *A. chroococcum* could stimulate plant growth, absorption of nutrients and their efficiency, as well as the metabolism of photosynthates. These results stand in accordance with those obtained by Verma *et al.* (2010). Silva *et al.* (2016) observed that, fulvic acid easily binds or chelate minerals such as iron, calcium, copper, zinc and magnesium, as it can deliver these elements to plant directly. It is also important to mention that the*A. chroococcum* DSM 2286 may help the plants to save their requirements from nitrogen at the beginning of the plant life until the first addition of mineral nitrogen fertilizer. These in turn reflect an increase in dry matter accumulation, chlorophyll pigments and dehydrogenase activities, grain and straw yields. The application of KF and *A. chroococcum* amendments along with the mineral fertilization provided a favorable environment for rapid microbial growth which caused a greater increase in nutrient availability of these soils and results are well corroborated with some previous findings. These results are in conformity with the findings of Bharali *et al*. (2017). Taha *et al.* (2016), revealed that, the addition of humic substances (humic and fulvic acid) significantly increase the plant growth and mineral contents of a lettuce plant especially when increasing the application dose.

Conclusion

According to the obtained results, it can be concluded that under 80% of the recommended mineral fertilization (NPK), the dual application of KF + Azotobacter chroococcum as in combination treatment is considered a recommended treatment in the cultivation of wheat plants (Giza 171 variety) at Nile Delta due to it resulted in high yield, quality and reducing the environmental pollution as a result from reducing the additions of mineral fertilizer with organic and biofertilization one.

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تعظيم كفاءة استخدام الأسمدة المعدنية باستخدام فولفات البوتاسيوم والأزوتوباكتر كروكوكم وتأثيرهما على إنتاجية القمح وامتصاص العناصر الغذائية

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تم إجراء تجربة حقلية خلال موسمين نمو شتويين متتاليين (٢٠١٦/٢٠١٧ و٢٠١٧/٢٠١٨) في مزرعة تجريبية في ميت غمر - محافظة الدقهلية – مصر و ذلك لتقييم تعظيم كفاءة استخدام الأسمدة المعدنية (النيتروجين ، الفوسفور و البوتاسيوم) باستخدام فولفات البوتاسيوم (KF) والتسميد الحيوي (*Azotobacter chroococcum* DSM 2286) وتأثير ذلك على النمو و المحصول و بعض المكونات الكيميائية لنباتات القمح (صنف جيزة ١٧١). حيث تم تصميم تجربة قطع منشقة في تصميم كامل العشوائية في ثلاث مكررات.و قذ تضمنت هذه التجربة ثلاث م□عاملات رئيسية وهي إضافة الأسمدة المعدنية (النيتروجين ، الفوسفور و البوتاسيوم) بثلاث مستويات إضافة ٦٠، ٨٠ و١٠٠٪ من الموصي به من قبل وزارة الزراعة المصرية، و أربعة م□عاملات تحت الرئيسية وهي عبارة عن تسميد عضوي و حيوي. وقد تم توزيع إضافة الأسمدة العضوية و الحيوية داخل المعاملات الرئيسية الثلاث كالتالي: كنترول (بدون أي إضافات من فولفات البوتاسيوم أو الأسمدة الحيوية)، إضافة فولفات البوتاسيوم منفردة، إضافة سماد حيوي (*Azotobacter chroococcum* DSM 2286) منفرد وإضافة مشتركة من فولفات البوتاسيوم + *Azotobacter chroococcum*.وعند ٧٥ يوم من الزراعة، تم تقديروزن المادة الطازجة والجافة للنباتات، و في أوراق النباتات تم تقدير الكاروتين و الكلوروفيل (أ ، ب و أ + ب)، نشاط إنزيم الديهيدروجينيز في التربة المحيطة بجذور النباتات (الريزوسفير). وأيضا□ تم تقدير المغذيات الكبرى (النيتروجين ، الفوسفور و البوتاسيوم) و المغذيات الصغرى (الحديد، المنجنيز و الزنك) في كل□ من العينات النباتية عند ٧٥ يوم من الزراعة وفي الحبوب والقش للقمح بعد الحصاد.وكذلك تم حساب وزن ١٠٠٠ حبة (جم) وأيضا□ وزن الحبوب والقش (طن / هكتار).أوضحت النتائج الم□تحصل عليها: أن إضافة فولفات البوتاسيوم و الأزوتوباكتر كروكوكم بمفردهما أو مجتمعة أدت إلى زيادة معنوية في جميع القياسات المدروسة في هذا البحث مقارنة بمعاملة الكنترول تحت المستويات المختلفة من التسميد المعدني. كما وجد أنه تحت ٨٠٪ من الموصي به من التسميد المعدني أظهرت الإضافة المشتركة من فولفات البوتاسيوم + الأزوتوباكتر كروكوكم زيادة معنوية في جميع معاملات الدراسة مقارنة بمعاملة الكنترول تحت ٦٠ , ٨٠ و ١٠٠٪ من الموصي به من التسميد المعدني. و تعتبر هذه المعاملة موصى بها عند زراعة نباتات القمح (صنف جيزة ١٧١) في دلتا النيل وذلك لأنها أدت الي إنتاج محصول وجودة عالية مع تقليل التلوث البيئي عن طريق الاستعاضة الجزئية بالأسمدة العضوية والحيوية عن التسميد المعدني.