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The Combined Effect of Different Types of Fertilizers and Antioxidants as a Strategy to Improve Wheat Yield

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ABSTRACT

Two field experiments in two locations were conducted during the winter season of 2014/2015 on two types of soils, clay at Kafr El-Shiekh and calcareous at Borg Al-Arab, Alexandria, Egypt. The experiments were conducted to study the combined effects of different types of fertilizers (mineral, organic and bio fertilizers) as well as two promoting substances or antioxidants (Humic and Ascorbic acids) on grain yield and its components of wheat cultivar Gemmiza 11. The experiment design was split-plot with three replications. Main plots were assigned to the different fertilizers sources and the sub-plots were occupied by the promoting substances. The results indicated thatthere were a significant differences betweenthe two locations inthe measured characters where Kafr El-Shiekh surpassed Borg Al-Arabin chlorophyll content in leaves, leaf area index, dry matter at 60 and 102 days after sowing, plant height (cm), number of tillers/m^2, grain yield/fed and harvest index, while Borg Al-Arabexceeded Kafr El-Shiekh in1000 grains weight (g) and flag leaf area. Also, the results revealed thatthere were a significant difference among the different fertilizers sources where, application of mineral, organic and bio-fertilizers especially the treatment 50%NP+O+Bio significantly increased grain yield andits components. Concerning the foliar spraying with the promoting substances, the results illustrated thatthere were a significant effect on all the studied growth traits at all sampling dates. Finally, the interaction between the two locations, the different fertilizers sources, and promoting substances showed a significant effect on grain yield.

Keywords: bio-fertilizer, grain yield, organic fertilizer, wheat, promoting substances

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INTRODUCTION

Not only in Egypt but also in the world, wheat (*Triticum aestivum* L.) is known as the important cereal crop. Its rank is the first among cereal crops. Wheat represents 30% of all cereal food worldwide and considered the major food for at least one third of the people all over the world and supplies about 20% of the total food calories. Wheat is considered the most strategic crop in Egypt where it is cultivated in 3 million feddan with a production of about 8.3 million tons per year. The gap between production and consumption of wheat in Egypt represents about 40% of the country's demand which imported annually, this gap is continuously increased due to the increase in population and the limited agricultural lands. After the aforementioned facts, attention must be taken to increase wheat yield via improving the productivity of unit area and increasing the cultivated areas and consequently reducing the gap between production and consumption. To increase the productivity of wheat per unit area, soil fertility must be maintained and plant nutrients must be applied in balanced amounts sufficiently [1]. Egypt suffers a remarkable reduction in fertile agricultural lands in both Delta and Nile Valley which represents 3-4% of the total area of the country. So, Egypt gives great interesting to new lands as a resource of agricultural extension to face the continuous demands of population such as calcareous soils which have many problems that affects the availability of the nutrients. To encounter such a problem a special agricultural practices must be applied like adding organic and bio fertilizers, foliar application of micronutrients. Nitrogen is the most important macronutrients to all crops because its presence in protein and nucleic acid which are the building substances of all cells. Also, its presence in chlorophyll make crops able to convert sunlight energy via photosynthesis. Moreover, nitrogen affects leaf area, cell size, and photosynthesis rate [1-2]. Adding organic materials to new lands enhances seed germination, growth, root penetration, yield, and uptake of nutrients because it provides carbon as a source of energy [3-4]. The combined application of organic and inorganic nitrogen fertilizers helps enhancing grain and biological yield, number of grains per spike, and 1000 grains weight of wheat when applied at 25:75 poultry manure and chemical nitrogen [5]. Also, adding bio fertilizers to new lands improve the growth of crops, reduce the cost of chemical fertilizers, and decrease the harmful effect on the environment. Bio fertilizers can play an important role in fixing atmospheric nitrogen and producing growth promoting substances where it contains microorganisms like Azospirillum*sp*. and Azotobacter*sp.* which are used as fertilizers [6]. Ascorbic acid is an organic compound applied in trace quantities to maintain normal growth of the plants [7]. Application of 200 ppm ascorbic acid produced the best flowering parameters while 100 ppm produced highest soluble sugars. Total indols, total phenols, carotenoids and NPK contents [8]. Humic acid improve yield and plant growth [9], indirectly through its effects on soil fertility where it increases soil microbial population, improve soil structure, enhancing cation exchange capacity, and directly via its biochemical effects on photosynthesis [10]. The aim of the current study is to investigate the effect of humic and ascorbic acids and the combined effect of mineral, organic, and bio fertilizers on yield, yield components, and chemical composition of wheat grown in calcareous and clay soils for improving wheat productivity and minimizing costs and pollution of mineral fertilizers.

MATERIALS AND METHODS

Two field experiments in two locations were conducted during 2014/2015 winter season on two types of soils, clay and calcareous soils at Kafr El-Shiekh and Experimental Farm of City of Scientific Researches and Technological Application in Borg Al-Arab, Alexandria, Egypt, respectively. The preceding crop was rice in Kafr El-Shiekh location, while there was no preceding summer crop in Borg Al-Arab location. The chemical analyses of the organic fertilizers (poultry manure (PM)are presented in Table 1 while the chemical and physical properties of the soil of the experimental sites at the depth of 0-30 cm are presented in Table 2.

Table 1: Chemical characteristics of poultry manure (PM)

Organic matter (%)	Organic carbon (%)	C/N ratio	pH	E.C. (dS/m)	N (%)	P (ppm)	K (ppm)
51.50	29.40	14.00	7.75	2.2	2.15	124	115

The experiment was laid out in split-plot design with three replications in the two locations. Main plots were assigned to fertilizer sources while the sub-plots were assigned to promoting substances. All the

experimental plots were fertilized with 24 kg K_2O / feddan in the form of potassium sulphate (48 % K_2O) during seedbed preparation.

Table 2: Physical and chemical properties of the experimental soil before sowing at the two locations.

Soil properties	Kafr EL-Shiekh	Borg El- Arab
Mechanical		
Clay %	56.00	20.64
Silt %	32.00	16.31
Sand %	12.00	63.05
Texture	Clayey	Sandy clay loam
Chemical		
Organic Matter (O.M)%	1.50	0.93
pH(1:2.5 soil suspension)	8.44	8.55
Ec ($dS.m^{-1}$)	3.34	1.08
Total N (ppm)	430.50	220
Available P (ppm)	12.00	5.10
Available K (ppm)	432	420
Soluble anions, $meq.L^{-1}$		
HCO^-_3	6.20	4.40
Cl^-	9.10	0.40
Soluble Cations, $meq.L^{-1}$		
Ca^{2+}	10.70	3.00
Mg^{2+}	5.00	0.10
Na^+	2.00	11.00
K^+	15.60	1.30
Total carbonate %	14.00	32.50

The treatments were as follow:

A. Fertilizer sources (Main plots):

Five combinations among mineral fertilizers (NP), organic fertilizer (poultry manure) and mixed of two bio-fertilizers (*Azotobacter sp. and mycorrhizae fungi) inoculation:*

1. 100% NP, the recommended doses of NP fertilizer.
2. 50% NP + organic fertilizer.
3. 67% N + mixed of bio-fertilizers (Azotobacter and mycorrhizae).
4. 67% N+50% P+ mixed of bio-fertilizers.
5. 50% NP + organic + mixed of bio-fertilizers.

B. Promoting substances (Sub-plots): The solutions of promoting substances (antioxidant) were used as foliar spraying:

1. Ascorbic acid (1g/L)
2. Humic acid (4 g/L)
3. Water (control)

Wheat grains were sown by hand drilled in rows 20 cm apart at the rate of 70 kg seed fed^{-1} on November 22 and 25 in Kafr El-Shiekh and Borg Al-Arab locations, respectively. The plot size was 6 m^2 (2 X 3 m). Each plot included 10 rows. The recommended rate of nitrogen fertilizer was 75 and 100 kg N/feddan and phosphorus was 15.5 and 22 kg P_2O_5/feddan at Kafr El-Shiekh and Borg Al-Arab locations, respectively. Nitrogen fertilizer in the form of ammonium nitrate (33.5% N) was applied in three doses, 20% at sowing, 40% at the first irrigation and the last 40% applied at the second irrigation in the Kafr El-Shiekh. Whereas, applied in three equal doses in Borg Al-Arab location. Poultry manure (PM) as organic fertilizer was obtained from Animal Production Research Station, Borg Al-Arab, Agricultural Research Center, Egypt.

Organic fertilizer applied at the rate of 20 m^3/fed. Poultry manure (PM) and phosphorus fertilizer in the form of calcium superphosphate (15.5 % P_2O_5) were added and well mixed well with the soil two weeks before sowing. Bio fertilizers (*Azotobacter* and *Mycorrhiza*) were obtained from the Agricultural Research Center, Egypt. Before using, the activity of bio fertilizer was examined in the Agronomy Department, Faculty of Agriculture, Alexandria University. Humic acid 85% is a commercial product supplied from Biotech for Bio-acids and Fertilizers Company, Al Sadat City, Egypt. Ascorbic acid is a commercial product supplied from Sigma Chemical Company, USA. The solutions of humic and ascorbic acids were foliar sprayed on wheat plants at 30, 45 and 60 DAS**.**

Statistical analysis:

Data of the two experiments were subjected to analysis of variance according to [11]. Treatment means were compared using Duncan Multiple Range Test [12]. All statistical analysis performed using "MSTAT-C" Statistical Software Package 1990.

Studied characteristics:

A. Growth attributes:

Plant samples from 1 m long were taken at random from the second row of each sub-plot at 60, 81 and 102(DAS) to determine the following traits.

1. Plant height (cm)
2. Number of tillers / m^2
3. Chlorophyll content (uE= uMol m^{-2} s^{-1})
4. Leaf area index (LAI)
5. Flag leaf area (cm^2)
6. Dry matter accumulation (DMA) (g/m^2)

B. Yield and yield components:

1. Number of spikes/m^2
2. Spike length (cm)
3. Number of grains/spike
4. Weight of grains/spike (g)
5. 1000-grain weight (g)
6. Biological yield (tons/fed.)
7. Grain yield (tons/Fed.)
8. Harvest index % (HI)

RESULTS AND DISCUSSIONS

Growth:

Effect of locations:

The results in Tables 4 and 5 show that there was a significant difference between the two locations in chlorophyll content in leaves, leaf area index and dry matter accumulation (g/m^2) at 60, 81 and 102 days after sowing (DAS), flag leaf area at 110 DAS, plant height and number of tillers /m^2 at harvest. Wheat plants grown at Kafr El-Shiekh significantly surpassed those grown at Borg El-Arab in all the aforementioned traits, except for flag leaf area. Whereas, the inverse was the case. These differences between the two locations may probably related to differences in weather conditions and soil properties.

Effect of fertilizers sources:

The combined effect of different fertilizers sources had a significant effect on all the studied growth traits, except for leaf area index at 81 DAS where application of 50% NP combined with organic and bio fertilizers resulted in a significant increase in chlorophyll content in leaves, leaf area index, flag leaf area, dry matter accumulation, plant height and number of tillers/m^2 compared with all the other treatments of fertilizers sources at all sampling dates. The results indicated that the organic fertilizer was more effective than bio-fertilizer on growth characters. Inoculation with bacteria (*Azotobacter*) and fungi (*mycorrhiza*) and application of 67% N with or without P fertilizer did not differ significantly from application of 100% NP alone in most growth traits. Since the application of organic fertilizers enhances the availability of nutrients in the soil solution [13-14], the available nutrients might have helped in the stimulation of various physiological processes resulting in more tillers, leaf numbers and leaf area, which resulted in more photosynthesis activity and consequently increased dry matter accumulation. The enhanced growth characters as a result of bio fertilizer application may be attributed to their role in enhancing plant growth due to secretion of growth

hormones [15] and the facilitated uptake of nutrients. These results are in agreement with those achieved by [16-17].

Effect of promoting substances:

Foliar spraying with the promoting substances showed significant effect on all studied growth traits at all sampling dates. Plants sprayed with ascorbic or humic acids significantly exceeded those sprayed with water (control) in leaf area index, dry matter (g m^{-2}), and number of tillers/m^2. On one hand, application of ascorbic acid significantly increased dry matter (g m^{-2}) and number of tillers /m^2 compared with humic acid at all sampling dates, on the other hand, humic acid significantly surpassed ascorbic acid in chlorophyll content at 81 DAS. There was no significant difference between ascorbic acid and humic acid in chlorophyll at 60 and 102 DAS, leaf area index at all sampling dates, flag leaf area, and plant height. These results are in accordance with those reported by [10-18].

Effect of interaction:

All the first order interactions had a significant effect on all the studied growth traits. However, the interaction among location, fertilizers sources and promoting substances showed a significant effect on dry matter (g m^{-2}) and leaf area index at 102 DAS only.

Table 4: Chlorophyll content in leaves (uE), Leaf area index and Flag leaf area (cm^2) of wheat cv. Gemmiza 11 as affected by locations, fertilizers sources and antioxidant substances

	Chlorophyll (uE)			Leaf area Index			Flag leaf area (cm^2)
	Days after sowing						
	60	81	102	60	81	102	110
	Locations (L)						
Kafr El-Sheikh	37.14a	32.9a	30.53a	6.43a	8.16a	9.10a	90.33 b
Borg El-Arab	31.94b	29.6b	26.75b	3.66b	4.84b	5.43b	105.38 a
	Fertilizers (F)						
100% NP	30.61c	27.71d	25.63cd	4.98bc	5.87 a	6.85cd	95.39b
50%NP+O	38.22b	33.79b	30.53b	6.12a	7.11 a	7.65b	101.67a
67%N+ Bio	30.69c	28.03cd	23.82d	4.26c	6.05 a	6.66d	93.22b
67%N+50%P+Bio	32.67c	28.37c	26.98bc	4.17c	5.86 a	7.15c	94.28b
50%NP+O+Bio	40.51a	38.34a	36.25a	5.68ab	7.59 a	8.00a	104.72a
	Antioxidant (A)						
Ascorbic acid	34.52a	31.45b	28.75ab	5.39a	6.71a	7.53a	101.33a
Humic acid	36.34a	32.59a	30.60a	5.19a	6.74a	7.53a	99.37ab
Control	32.76b	29.71b	26.58b	4.54b	6.04b	6.73b	92.87b

Means designated by the same letter are not significantly different at 5% level using Duncan's multiple range test

Table 5: Dry matter (g/ m^2), plant height(cm) and number of tillers/m^2 of wheat cv. Gemmiza 11 as affected by locations, fertilizers sources and antioxidant substances.

	Dry matter (g/m^2)			plant height (cm)	No. of tillers /m^2
	Days after sowing				
	60	81	102	At harvest	
	Locations (L)				
Kafr El-Sheikh	435.11 a	1055.31 a	1697.29 a	104.39 a	301.00 a
Borg El-Arab	165.09 b	304.07 b	971.38 b	90.09 b	284.67 b
	Fertilizers (F)				

100% NP	263.12b	605.39b	1105.73b	96.37b	274.22b
50%NP+O	411.44a	789.06a	1539.61a	98.26a	317.17a
67%N+ Bio	213.78c	653.61b	1149.45b	97.81a	264.17b
67%N+50%P+Bio	221.83c	567.06b	1187.61b	95.02c	275.17b
50%NP+O+Bio	390.33a	783.33a	1689.28a	98.75a	333.45a
	Antioxidant (A)				
Ascorbic acid	324.97a	724.1a	1418.87a	97.99ab	309.53a
Humic acid	306.93b	696.53b	1366.63b	98.3a	294.83b
Control	268.4c	618.43c	1217.5c	95.43b	274.14c

Means designated by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Yield and yield components:

Effect of locations:

The results in Table 6 show that number of spikes/m^2, spike length, number of grains/spike, weight of grains/spike, biological yield, grain yield and harvest index were significantly different at Kafr El-Shiekh from Borg El-Arab. The inverse was the case in 1000 grain weight. The increase in grain yield at Kafr El-Shiekh over Borg El-Arab may be due to the difference in soil properties.

Effect of fertilizers sources:

Fertilizer sources had a significant effect on all the studied yield traits. The combined effect of mineral, organic and bio-fertilizers (50% NP + O and 50% NP + O + Bio) resulted in a significant increase in number of spikes/m^2, number of grains/spike, 1000 grain weight, biological yield and grain yield compared with the recommended mineral NP rate (100%) alone. There was no significant difference between the treatments of 50% NP+O and 50%NP+O+Bioin number of spikes/m^2, weight of grains/spike, 1000 grains weight, biological yield, and grain yield, these results suggests that the addition of organic fertilizer may save 50% of the applied mineral fertilizers without the need of bio fertilizers. Also, the results showed that the treatments of recommended rate of mineral NP (100%), 67% N + Bio, and 67% N+ 50%P+ Bio had no significant differences among them in grain yield and consequently may save 33% of inorganic N fertilizer and 100% or 50% P. These results are in harmony with those reported by [16-19].

Effect of promoting substances:

The results in Table 6 show that foliar application with the promoting substances showed a significant effect on all yield components, biological yield, grain yield, and harvest index. Foliar application of ascorbic acid or humic acid significantly exceeded control treatment or the foliar spraying with water in number of spikes/m^2 and grain weight/spike. However, foliar application of humic acid only significantly exceeded the control in spike length, number of grains/spike and 1000 grains weight. On one hand, humic acid recorded none significantly higher values of biological and grain yields compared to the control. On the other hand, ascorbic acid recorded significantly higher values. A positive correlation between foliar application of promoting substances and grain yield has been reported by [18-20-21] on wheat and rice.

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Table 6: Number of spikes/m^2, spike length (cm), number of grains/spike(g), grain weight/spike(g), 1000 grain weight(g), biological yield (tons/fed), grain yield (tons/fed) and Harvest Index (%) of wheat cv. Gemmiza 11 as affected by locations, fertilizers sources and antioxidant substances.

	No. of spikes/ m^2	Spike length (cm)	No. of grains / spike	weight of grains / spike (g)	1000 grains weight (g)	Biological yield (tons/fed)	Grain yield (tons/fed)	Harvest Index (%)
	Locations (L)							
Kafr El-Sheikh	296.64 a	19.92 a	66.68 a	3.54 a	55.55 b	7.60 a	2.56 a	33.68 a
Borg El-Arab	278.78 b	15.64 b	26.60 b	1.78 b	72.81 a	4.64 b	1.10 b	23.71 b
	Fertilizers (F)							
100% NP	271.78b	17.21d	44.12c	2.67bc	63.77b	5.13b	1.62b	31.58 a
50%NP+O	314.17a	18.86a	53.14a	2.94a	65.16a	7.45a	2.06a	27.65 b
67%N+ Bio	260.83b	17.71c	45.43c	2.50cd	61.78c	5.00b	1.58b	31.60 a
67%N+50%P+Bio	269.17b	16.59e	40.33d	2.41d	65.01ab	5.32b	1.73b	32.52 a
50%NP+O+Bio	322.61a	18.51b	50.18b	2.77ab	65.18a	7.72a	2.16a	27.98 b
	Antioxidant (A)							
Ascorbic acid	304.3a	17.88ab	47.48ab	2.73a	64.73ab	6.81a	1.97a	28.93 b
Humic acid	291.0b	18.14a	48.17a	2.74a	65.66a	6.08ab	1.87ab	30.76 a
Control	267.84c	17.31b	44.26b	2.51b	62.15b	5.48b	1.65b	30.11 ab

Means designated by the same letter are not significantly different at 5% level using Duncan's multiple range test.

Effect of interactions:

Grain and biological yields, yield components, and harvest index of wheat were significantly affected by the first and second order interactions as shown in Table 7.

The interaction between Locations and fertilizers:

Grain yield was significantly different at Kafr El-Shiekh from Borg El Arab location at any treatment of fertilizers sources. No significant difference in grain yield was found among fertilizer sources at Kafr El-Shiekh location, while it was significant at Borg El Arab location. Whereas, organic and bio-fertilizers (50% NP + O and 50% NP + O + Bio) resulted in a significant increase in grain yield compared with the recommended NP rate (100%) alone or with the other treatments, which did not differ significantly at Borg El Arab location.

The interaction between Locations and antioxidant:

Kafr El-Shiekh out yielded Borg El Arab in grain yield at the same antioxidant treatment. Plants sprayed with ascorbic acid or humic acid produced significantly higher grain yield than those sprayed with water at the same location. There was no significant difference between ascorbic acid and humic acid in grain yield at either Kafr El-Shiekh or Borg El Arab. The highest grain yield was obtained from plants sprayed with ascorbic acid or humic acid at Kafr El-Shiekh, while the lowest one was obtained from those sprayed with water at Borg El Arab.

The interaction between Fertilizers and antioxidant:

Application of 50%NP+O or 50%NP+O+bio along with foliar spraying with ascorbic acid or humic acid were among those treatments having high grain yield compared with application of 100%NP with foliar spraying with water.

The interaction among Locations, fertilizers, and antioxidant:

Kafr El-Shiekh had significant higher grain yield in comparison to Borg El Arab at all combinations between fertilizers sources and antioxidants. At Kafr El-Shiekh location, grain yield was increased by foliar application of ascorbic acid or humic acid at any treatment of the fertilizers sources. At Borg El Arab location, the combination containing organic fertilizer significantly increased grain yield compared with the recommended rate of mineral NP with or without foliar spraying of promoting substances. Plants inoculated with bio fertilizers and fertilized with organic fertilizer and foliar spraying with water significantly surpassed those fertilized by the recommended rate of mineral NP (100%) alone at the same location. The increased grain yield as a result of inoculation with bio fertilizer may be attributed to their enhancing role of crop growth via secretion of growth hormones [15].

Table 7: Grain yield (tons/feddan) of wheat cv. Gemmiza 11 as affected by the first and second order interactions

Locations (L)	Fertilizers (F)	Antioxidants (A)			L×F
		Ascorbic acid	Humic acid	Control	
		L×F×A*			
Kafr El-Sheikh	100% NP	2.98	2.50	2.34	2.61a
	50%NP+O	2.53	2.35	2.30	2.39a
	67%N+ Bio	2.76	2.56	2.22	2.51a
	67%N+50%P+Bio	2.65	2.77	2.50	2.64a
	50%NP+O+Bio	2.88	2.66	2.46	2.67a
Borg El-Arab	100% NP	0.69	0.67	0.53	0.63c
	50%NP+O	1.97	1.88	1.31	1.72b
	67%N+ Bio	0.64	0.79	0.51	0.65c
	67%N+50%P+Bio	0.81	0.88	0.79	0.83c
	50%NP+O+Bio	1.77	1.63	1.57	1.66b
	L×A				
Kafr El-Sheikh		2.76a	2.57a	2.36b	
Borg El-Arab		1.18c	1.17c	0.94d	
	F×A*				
	100% NP	1.84	1.59	1.44	
	50%NP+O	2.25	2.12	1.81	
	67%N+ Bio	1.70	1.68	1.37	
	67%N+50%P+Bio	1.73	1.83	1.65	
	50%NP+O+Bio	2.33	2.15	2.02	

Means designated by the same letter are not significantly different at 5% level using Duncan's multiple range test.
*Least Significant Range (LSR) was ranged between 0.85 and 1.04.

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