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EFFECT OF PLANT GROWTH PROMOTING RHIZOSPHERIC MICROORGANISMS AND HUMIC ACID APPLICATION ON PRODUCTIVITY OF WHEAT CROP

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ABSTRACT

In quest of eco-friendly methods to promote plant growth and improve crop productivity is a priority for sustainable agriculture approach. To address this, a field experiment was conducted during 2020/2021 in winter season at Desert Research Center experimental station, Baloza district, North Sinai Governorate to study the effect of Plant Growth Promoting Rhizospheric microorganisms (PGPR) and humic acid on wheat productivity. Treatments included two separate nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*), *Saccharomyces cerevisiae*, *Arbuscular mycorrhizae* (AM) and humic acid individually or in combination.

The results showed a significant variations between all wheat treatments on plant height, number of spikes/plant, weight of thousand grain and grain yield, along with grain contents of N, P, K and protein. The combination of N-fixing bacteria, yeast, and (AM) and humic acid significantly increased counts of Azotobacter, Azospirillum, yeast, VAM spores and the total microbial counts in the wheat rhizosphere. Consequently, growth parameters, grain yield and protein content markedly increased.

Plant Growth Promoting Rhizospheric microorganisms (PGPR) and Humic acid (HA) were among the most effective methods that improve wheat growth and productivity.

Key Words: *Azotobacter chroococcum, Azospirillum brasilense, Saccharomyces cerevisiae, Arbuscular mycorrhizae* (*AM*), humic acid and wheat.

INTRODUCTION

Plant growth promoting rhizospher (PGPR) is important for agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers. It can affect plant growth directly by the synthesis of phytohormones & vitamins, inhibiting plant ethylene synthesis, enhancing stress resistance, improving nutrient uptake, fixing atmospheric nitrogen, solubilizing inorganic phosphate and mineralizing organic phosphate **(Adedayo *et al*., 2022; Bhanse *et al*., 2022).** Nitrogen fixing biofertilizers provide only a modest increase in

crop nitrogen uptake. Reports proved that application of *Azotobacter* and *Azospirillum* improves the yield of both annual and perennial grasses **El-Said *et al*., (2014).** *Azotobacter* strains could affect seed germination and seedling growth **(Ahmed *et al*., 2021 ; Ibrahim and El-Sawah 2022)** in a plant. It has been shown that wheat yield increased up to 30% with *Azotobacter* inoculation **(El-Sorady *et al*., (2022).** Although *Azospirillum* was first isolated from cereals and most of its initial, inoculation has been done on the main cereal crops and, there are more non-cereal species successfully inoculated with *Azospirillum* than cereals **(Ladha *et al*., 2022).** *Saccharomyces cerevisiae* is considered a new promising plant growth promoting yeast for different crops. It became in the last few decade a positive alternative to chemical fertilizers safely used for human, animal and environment **Fernandez-San Millan *et al*., 2020; Abd-Elbaky *et al*., (2021).** The enhancing effect of yeast on germination rate and on the vegetative growth parameters were strongly supported by **Thalooth *et al*., (2019).** They stated increased activity regulating catabolic productions in eukaryotic cells treated with *S. cerevisiae*. *Arbuscular mycorrhizae* (AM) are symbiotic associations formed between plants and soil fungi that benefit both partners. The role of AM in acquisition and sorption of nutrients from the soil has been recognized. Pronounced response had been obtained in the solubility of micronutrients in newly reclaimed soil when mycorrhiza was accompanied with organic substrates **(Khan *et al.*, 2022; Rehman *et al*., 2022).** Soil organic contents are one of the most important parts that they directly affected the soil fertility and textures as well as increasing the microbial activities in the soil **(Ahmed and Al-Mutairi 2022).** In recent years, humic substances can be added to the soil for improvement the crop yield. From the point of view of producers, these chemical preparations have been perceived and accepted as a kind of hormone promoting the growth rather than improving the chemical and physical conditions of the soil **(Fatima *et al.* 2022).** Also, humic acid may form an enzymatically active complex which can carry on reactions that are usually assigned to the metabolic activity of living microorganisms **(Fouda 2021 ; Cudowski *et al*., 2022).**

Wheat (*Triticum aestivum* L.) is the most important grain crop not only in Egypt but also all over the world. Its production in many regions of the world is below average because of adverse environmental conditions **(El-Sabagh *et al.*, 2021).** Hence, under the prevailing circumstances, restoration and maintenance of soil fertility is a basic and critical problem, particularly in the newly reclaimed soil. This can be accomplished by adding organic material, biological active substances and plant growth promoting microorganisms and in addition to other field practices **Ruiz and Salas Sanjuan (2022).**

Therefore, the aim of this study was to investigate the impact of soil amendment with *Azotobacter chroochoccum*, *Azospirillum brasilense*, *Saccharomyces cerevisiae* and *Arbuscular mycorrhizae* (*AM*) as biofertilizers and humic acid as organic fertilizer on the growth parameters and productivity of Wheat (*Triticum aestivum L.)*

MATERIALS AND METHODS

Microbial culture preparation:

The fresh liquid cultures were prepared from pure strains of *Azotobacter chrococcum* and *Azospirillum brasilense* which previously isolated, purified and identified.The biofertilizers strains were added in the form of individual and mixed inoculations at the rate of 10^8 cfu /ml as soil treatment. For yeast culture used in this study, it was prepared by inoculating 1L of nutrient broth with 10g of active dry yeast and incubated for 48h, after that one litre inoculum commercial dry yeast added to 10L nutrient broth for yeast treatment. The spores of AM were collected from different Egyptian Governorates by the wet sieving technique, described by **Gerdemann and Nicolson (1963).** The collected VA Mycorrbizal spores were propagated in soil and the roots of barley plants were infected with VAM. These collected spores can be used as inoculum with rate (500 spores per plant).

Humic acid: Humic acid (85%) which contain 56% C, 4.5% H, 31% O and 4.5% N was obtained from Sphinx for International Trade Company, Cairo, Egypt. Humic acid was added at the rate of 5 kg/fed. after 30 and 60 days from sowing in two equal doses.

Field experiments: The present investigation was carried out during the season of 2020 / 2021 in the Agricultural Experimental Station of the Desert Research Center at Baloza station, North Sinai Governorate.

Grains of wheat (Sakha 93) were successfully washed with water and air-dried. Then, grains were soaked in solution of humic acid (2g /L) for two hrs and/or cell suspension of *A. chroococcum*, *Azospirillum brasilense*, *Saccharomyces cerevisiae* and *VAM*. The experiments were arranged in randomized complete block design with three replicates. The plot area was 10.5 m^2 (3x 3.5m). For bacterial treatments, wheat seeds were moistened in CMC solution (1%) before application of inoculum to get a thin, uniform coating of bacterial inoculum on seeds. Inoculated seeds were dried in shade before sowing **(Samasegaran *et al.*, 1982)**. While untreated control seeds were maintained. The physical and chemical analysis of soil, as well as, irrigation water were presented in Tables (1,2). phosphatic fertilizer as calcium super phosphate (15.5% P_2O_5) was added at a rate of 150 kg /fed. during seed bed preparation, 100 Kg of potassium sulphate (47% K_2O) was added at flowering stage, nitrogen fertilizer was applied as ammonium sulfate (20.5% N) at rate of 60 kg/fed.(half of recommended dose in sandy soil) where 1/3 of the amount

was incorporated in dry soil before sowing, 1/3 was added one month after sowing and the rest was added one week pre flowering stage. Mycorrhiza was added just before sowing. The other required culture practices for growing wheat were followed as recommended. This experiment included the following treatments:

1- Control (A1)
2- *A. chroococcum* (A2)
3- *Azospirillum brasilense* (A3)
4- *Saccharomyces cerevisiae* (A4)
5- *Arbiscular mycorrhiza* (AM) (A5)
6- Humic acid (A6)
7- A2+A3+A4+A5+A6 (A7)

Table (1): Mechanical and chemical analysis of the experimental soil (water extract 1:2.5)

Depth	pH	E.C. (µmohs/ cm)	CaCo 3%	Cations (PPm)				Anions(PPm)				Particle size distribution			
				Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}	Sand	Silt	Clay	Textural class
0-30	7.9	1080	1	156	31.2	63.25	19.5	-	24.41	106.5	381.6	91.12	2	6.88	sand

Table (2):Aalysis of the irrigation water

pH	E.C.	Soluble anions (mgl^{-1})				Soluble cations (mgl^{-1})			
	(mmohs/cm	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}	Ca^{++}	Mg^{++}	Na^{+}	K^{+}
8	2.8	Nil	73.22	815.60	206.73	80.16	72.95	407.08	31.37

The soil and irrigation water were analyzed at the laboratories of Desert Research Center, as shown in Tables 1 and 2. El-Salam Canal irrigation water was used and chemical analysis of the irrigation water was presented in Table 2.

Microbiological analysis of wheat rhizosphere:

Total bacterial, *Azotobacter spp.*, *Azospirillum spp.* and yeast conuts in the rhizosphere samples were counted on Bunt and Rovira medium **(Bunt and Rovira, 1955)**, nitrogen deficient medium **(Abd El-Malek and Ishac, 1968)**, semi-solid malate medium **(Dobereiner, 1978)** and malt extract media **(USFDA. 2001),** respectively. Also, CO_2 evolution (µg/g dry soil/ hr.) in the rhizosphere were determined according to **Pramer and Schmidt (1964).** Soil dehydrogenase activity (µg TPF/g dry soil/24 h) was analyzed by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by **Friedel *et al*., (1994).** The spores AM fungi from lower sieve were then washed on to a 9 girded filter paper disc. The filter paper was transferred to clean Petri dish lids and enumerated. AM fungal spore enumeration included both dead and viable spores, although every attempt was made to count only healthy-looking spores. Spores were recorded as representatives of AM fungal species present in 100 g of sample **(Smith and Dickson, 1997).** This was done using a dissecting microscope (Leica S4E).

Measurements of growth characters, yield and yield components:

At harvest, the following traits were carried out plant height, number of spikes, weight of thousand grains, grains weight /plant(gr) and grains weight /fed (kg)

Chemical analysis of wheat seed:

It was carried out after harvest to determine potassium, phosphorus and total nitrogen according to **Jackson, (1958) ; Watanabe & Olsen, (1965) ; Bremner and Mulvaney, (1982),** respectively. The protein content was calculated by multiplying the total nitrogen by 6.25 **(Tripath *et al*., 1971).**

Statistical analysis:

The results of this study were statistically examined using Statistix version 9 computer software, and differences between treatment alternatives were declared significant when they were more than the least significant differences (LSD) at the 5% stage. **(Analytical software, 2008).**

RESULTS AND DISCUSSION

Data in Tables (3,4) show that plant height, number of spikes, weight of thousand grains, grains weight /plant and grains weight /fed as well as chemical composition of wheat grains significantly increased in response to any of the tested biofertilizer compared to control. Also, humic acid had positive effect on the same parameters. Moreover, humic acid application triggered and increased the positive effects of mixure treatments of plant growth promoter. These results are in agreement with **Hafez *et al*. (2021)** and **Hafez *et al*. (2022),** who stated that inoculation with PGPR and humic acid application increased grain yield status, N, P, and K in plants, plant growth rate, and plant productivity under growing wheat plant in arid regions.

Table (3) Effect of Plant Growth Promoting Rhizospheric microorganisms (PGPR) and humic acid application on growth parameters of wheat at the harvest stage.

Treatment	Growth parameter				
	plant height(cm)	No. of spikes/ plant	1000 grain weight(g)	Grains weight /plant (g)	Grains weight /fed (kg)
A1	70d	7e	44.23g	17.54f	910.560g
A2	73bc	12bc	52.20c	20.00c	1092.462c
A3	72c	11cd	51.21d	19.83d	1029.42e
A4	72c	10d	49.63f	18.22e	1020.18f
A5	74b	12bc	50.83e	19.93d	1051.302d
A6	78a	13ab	53.44b	20.47b	1119.216b
A7	77a	14a	60.01a	23.86a	1189.44a

A1: Control, A2: *Azotobacter*, A3: *Azospirillum* , A4:*Saccharomyces*, A5: *Arbuscular mycorrhizae* (*AM*), A6:Humic acid, A7:(A2+A3+A4+A5+A6)

Table (4) Effect of Plant Growth Promoting Rhizospheric microorganisms (PGPR) and Humic acid application on chemical components of wheat grains.

Treatment	Chemical analysis of seeds(%)			
	Nitrogen	Phosphorous	Potassium	Protein
A1	1.92f	0.22c	0.53e	12d
A2	2.12cd	0.27c	0.69c	13.25bc
A3	2.60b	0.30bc	0.76b	16.25a
A4	2.03e	0.26c	0.58d	12.69c
A5	2.07de	0.40a	0.75b	12.94c
A6	2.18c	0.39ab	0.76b	13.63b
A7	2.69a	0.43a	0.81a	16.81a

A1: Control, A2: *Azotobacter*, A3: *Azospirillum* , A4:*Saccharomyces*, A5: *Arbuscular mycorrhizae* (*AM*), A6:Humic acid, A7:(A2+A3+A4+A5+A6)

Table (5) Effect of Plant Growth Promoting Rhizospheric microorganisms (PGPR) and Humic acid application on microbiological analysis in wheat rhizosphere at the harvest.

Microbiological analysis							
Treatment	Total bacterial counts (Counts x 10^5 CFU/g dry soil)	*Azotobacter* counts (Counts x 10^3 CFU/g dry soil))	*Azospirillum* counts (Counts x 10^3 CFU/g dry soil)	Yeast counts (Counts x 10^3 CFU/g dry soil))	CO_2 evoluted (µg/g dry soil/ hr.)	Dehaydrogenase (µg TPF/g dry soil/24 h	VAM count (Spores /100 g dry soil)
A1	8.2e	3.1d	2.9e	10.6g	13.2f	0.221g	104g
A2	16.4d	3.2d	3.0e	21.3f	14.5e	0.352f	186d
A3	20.1b	4.6bc	3.8d	23.4e	15.11d	0.451e	190c
A4	19.00c	4.7b	3.9cd	26.8a	16.31c	0.511d	177e
A5	20.45b	4.5bc	4.1bc	24.2d	16.43bc	o.691c	1420b
A6	21.9a	4.4c	4.3b	24.6c	16.52b	0.705b	135f
A7	22.7a	7.3a	5.4a	25.3b	17.23a	0.780a	1720a

A1: Control, A2: *Azotobacter*, A3: *Azospirillum* , A4:*Saccharomyces*, A5: *Arbuscular mycorrhizae* (*AM*), A6:Humic acid, A7:(A2+A3+A4+A5+A6)

Initial total microbial counts in soil were 3.6 $\times 10^5$ cfu/g dry soil. It is indicated from Table (5), that total microbial counts in the rhizosphere of wheat samples increased in all treatments compared to the control at harvesting stages of plant growth. These results are in agreement with **Yasmin *et al*. (2020); Xie *et al*. (2022)** who stated that inoculation with the plant growth promoting rhizobacteria had stimulation effect on the population of rhizosphere microorganism and increased their numbers by more than 50% at the end of the experiment comparing with the number recorded before planting.

For *Azotobacter* and *Azospirillum* counts, inoculated plants gave the highest increase in the counts and the highest increase compaire to control **(Shoeip *et al*. (2022).** The diazotroph bacterial inoculation significantly increases the grain maize yield, plant height and microbial population in soil **(Jalal *et al*. (2022).** It is worthy to notice that the initial count of *Azotobacter chrococcum* and *Azospirillum lipoferum* bacteria in soil were 2.1×10^2 and 1.3×10^2 cfu/g of dry soil, respectively. For yeast counts, inoculation treatment with mixed inoculation gave the highest counts. The initial yeast count in soil was 3.4×10^2 cfu/g of dry soil. Concerning to CO_2 evolution, Bacterial inoculation showed significant increase in CO_2 values for all treatments compared to control ones. Also, both the results of microbial counts in the rhizoshere samples and CO_2 evoluted were compatible to each other. Mixed inoculation with the three biofertilizers followed by dual inoculation recorded the highest values while single inoculation with *Azotobacter chrococcum* or *Azospirillum lipoferum* recorded the lowest ones comparing to control ones . Data of CO_2 evolution were almost in harmony with those of total microbial counts **(Aileni 2022).** For mycorrhizal spore counts shown in Table (5) exhibited a gradual increase with inoculation by *AM* fungi, while it showed significant increase with individual application of diazotroph bacteria, yeast and humic acid comparing to the control treatment. VAM spores counts were significantly increased by application of humic acid in combination with other treatments. The results are in agreement with those obtained by **Wang *et al*. (2022)** who reported that organic compounds significantly increased colonization of mycorrhiza. It was also noticed from Table (5) that individual application of humic acid or biofertilization significantly increased dehydrogenase activity in wheat rhizosphere as compared to the control treatment. The mixed inoculation with biofertilizers and humic acids increased enzymes activity more than the individual inoculation. Also, the highest values of enzymes activity were recorded in rhizosphere of the plants that treated with humic acid in the presence of biofertilizer especially the dual inoculation. This may be due to the mechanisms of diazotroph bacteria, yeast and AM on soil properties. Addition of humic acid may be of special importance in restoring optimal levels or organic matter for plant growth and for microbial activity which associated with enzymes activity **Ennan *et al*. (2022).** These results showed a good agreement with **Phour and Sindhu (2022).** Who reported an increase in enzymes activity with application of humic acid. They also reported that the microbial population and soil enzymes in the rhizosphere could be built up for the efficient utilization of nutrients.

CONCLUSION

The combined application of plant growth promoting rhizospheric microorganisms and humic acid is a good tool for growth and yield promotion as well as improving soil health, particularly in newly soil. Finally, the combination of natural materials and environmental friendly by products has become one of the most important practices concerning soil enhancement and yield increase.

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تأثير ميكروبات الريزوسفير المحفزه للنمو واضافة حمض الهيوميك على انتاجية محصول القمح

سماح محمد عبد العزيز يوسف

قسم خصوبة و ميكروبيولوجيا الاراضي – مركز بحوث الصحراء – المطرية – القاهرة – مصر

اصبح البحث عن طرق صديقة للبيئة لتحسين نمو النبات وتحسين إنتاجية المحاصيل الزراعية من أولويات الزراعة المستدامة. وفي إطار هذا التوجه أقيمت تجربة حقلية خلال موسم زراعة 2021/2020 بمحطة تجارب مركز بحوث الصحراء بمنطقة بالوظه (محافظة شمال سيناء) لدراسة تأثير الميكروبات المحفزة للنمو وحمض الهيوميك على انتاجية محصول القمح. وكانت المعاملات كالاتي: التلقيح ببكتريا الأزوتوباكتر وبكتريا الأزوسبيريلليم كمثبتات للنتروجين والخميرة (خميرة الخباز) و فطر الميكروهيزا والهيوميك اسيد منفردين أو مجتمعين. وأظهرت النتائج وجود اختلافات معنوية بين جميع معاملات القمح وان لها أثر واضح على جميع القياسات المحصولية محل الدراسة مثل (طول النبات– عدد السنابل/ نبات– وزن ال 1000 حبة –وزن الحبوب/نبات– وزن الحبوب/فدان) وكذلك محتوى الحبوب من البروتينات والنيتروجين والبوتاسيوم والفسفور.

وجد ان التلقيح بخليط من اللقاحات مع الهيوميك اسيد أعطى زيادة كبيره فى الأعداد الكلية للميكروبات وبناء عليه زيادة كل من القياسات المحصولية ومحتوى الحبوب من البروتين والنيتروجين والبوتاسيوم والفسفور.

وبالتالي نجد ان استخدام الميكروبات المحفزة للنمو مع حمض الهيويميك من بين أكثر الطرق فعالية في تحسين نمو وانتاجية نبات القمح.