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# The efficiency of application of bacterial and humic preparations to enhance of wheat (*Triticum aestivum* L.) plant productivity in the arid regions of Egypt

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ABSTRACT

Lower nitrogen use efficiency is major crop productivity limiting factor in semi-arid regions due to its poor organic contents in the soil; our research work is considered to optimize crop productivity by soil and plant chem-biological correction. This study designed and aimed to use soil inoculation with plant growth-promoting rhizobacteria of the genus *Azospirillum* and foiler application by humic preparations ALCRI-CropHelp and ALCRI-CropHelp-M compared with control and NPK fertilizer under wheat plants in arid regions of Egypt. Experimental data showed that biological correction contributed to a significant increase in wheat yield in the arid regions. The specific results for *Azospirillum*, ALCRI-CropHelp, and ALCRI-CropHelp-M were significantly increased wheat grain yield on 256, 267, and 278 % relative to the control, respectively. Simultaneously, water use efficiency has increased by more than 2.5 times, compared with control and NPK treatments. This effect was achieved due to multiple effects on the metabolism of agricultural plants, and applications of ALCRI-CropHelp and ALCRI-CropHelp-M treatments revealed an increase in the biological yield, spike length, harvest index compared with control and NPK treatments. In conclusion, this study has shown that the ALCRI-CropHelp Az and ALCRI-CropHelp-M foliar applications significantly ($P < 0.05$) increased grain yield status, N, P, and K in plants, plant growth rate, and plant productivity under growing wheat plant in arid regions. We recommended these treatments for the enhancement of wheat plant productivity in arid regions.


## 1. Introduction

The production process of crops, especially on lands subject to degradation, can be controlled using several types of correction: physical, chemical, and biological [13]. Physical correction is understood as an agro-technical system, agro-meliorative and irrigation, and drainage measures to create and maintain clear water, thermal, and air regimes for cultivated plants and soil biological activity. This type of correction is the first important component of regulating the plant production process; historically, it is the second evolutionary path of crop production [7].

Chemical correction of crop productivity is a system of measures associated with chemical fertilizers, such as: replenishing the reserves of ash elements and nitrogen in the soil, foliar feeding of plants with compounds of macro-and microelements, regulation of acid and salt regimes of soils, as well as the use of chemical plant protection products. It can be stated that the path of chemical correction is fully implemented in industrial agriculture. This type of correction is mainly focused on obtaining gross crop production (as a rule, without taking into account its quality). It ignores the natural laws, due to which, in natural conditions, plants, together with the soil, form an interconnected and interdependent trophic system (Popov, 2002; [3]; Hafez et al., 2019).

To obtain a high economically viable yield with a simultaneous improvement for crop production quality and create favorable conditions for the growth and development of crops through chemical farming and various reclamation measures, it is necessary to influence plant biology [13] directly. Such effective and economically justified methods of influencing the production process of plants are soil inoculation with rhizosphere nitrogen-fixing bacteria of the genus

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*Azospirillum* [4,10] and foliar treatment of crops with solutions of humic substances (HS) (Popov, 2002; [3]).

One of the effective and economically justified biological correction methods of productivity, which makes it possible to compensate for the lack of humus in agricultural soils, is the foliar treatment of crops with solutions of humic substances and microbial applications [19]. Humic substances (HS) are the key components of soil, water, and sediment organic matter. They can be chemically represented as a series of complex, relatively low molecular mass components of organic molecules forming dynamic associations stabilized by nanometer-scale hydrophobic interactions and hydrogen bonds [12]. The root system plays a central role in acquiring water and nutrients in a natural heterogeneous soil environment and how plants promote changes in their rhizosphere for defense, improve nutrient mineralization, and select microbial community. HS could modulate these processes. It is not a surprise that HS can modify plant root growth and architecture since the genesis and soil use history is profoundly marked by HS for- mation [8].

This study aimed to demonstrate the effectiveness of *Azospirillum brasilense* and two humic preparations: ALCRI-CropHelp and ALCRI-CropHelp-M, on wheat grain yield and plant growth characterization in the arid regions of Egypt.

## 2. Materials & methods

### 2.1. Description of experimental

Field trials of the effectiveness of the use of bacterial and two humic preparations: ALCRI-CropHelp and ALCRI-CropHelp-M, were carried out from December 2019 to April 2020 on irrigated old arable calcareous saline soils located (30°53′33.17″ N, 29°22′46.43″ E) Alexandria, Egypt. Object – wheat (*Triticum aestivum* L.). The area of one plot is 500 $m^2$. The repetition is three-fold. Five treatments were carried out in the order: control (without amendments); NPK (mineral fertilizers: urea (46 %) with 38 $g/m^2$, $P_2O_5$ (45 %) with 10 $g/m^2$, $K_2O$ (50 %) with 12 $g/m^2$); *Azospirillum brasilense* (Sp245) was isolated from soil and cultivated; as outlined in [11], growth and inoculation were done (Az) bacteria was introduced into the soil around the plant as 0.7 $ml/m^2$); neutral HS solution foliar application 0.5 ml / $m^2$ (ALCRI-CropHelp); and humic substances + micronutrients 1.0 ml /$m^2$ for soil inoculation (ALCRI-CropHelp-M). All treatments were foliar for wheat plants at 31 ± 1 °C for the first two months from seed sowing.

A special saline solution isolated humic substances from vermicompost, produced from spent grain organic waste of brewing. We examined these solutions by colorimetric analysis; they did not contain chlorides, carbonate ions, polyaromatic hydrocarbons, lipids, and radio-nuclides.

Five days after our second foliar spray (ALCRI-CropHelp, and ALCRI-CropHelp-M) application, we harvested and analyzed six plants per plot. These plants had been subjected to five treatments: control, NPK, Az, ALCRI-CropHelp, and ALCRI-CropHelp-M with or without foliar spray. The foliar application was performed 15 and 30 days after transplantation with humic substances. We used a randomized block design with three replications.

#### 2.1.1. Soluble humic substance extraction and micronutrients solutions preparation

Soluble-humic substances were extracted from vermicomposted spent grain with 0.1 M NaOH in a 1:20 solid-liquid ratio; the procedure involved mechanical shaking and left it for 12 h. The suspension was centrifuged at 5000×g and filtered through a Whatman no. 42 filter paper to provide the humate. The humate was dialyzed against water using a 1000-Da cut-off membrane [15]. Relevant humate characteristics were as follows: 39.3 ± 1.78 g $kg^{-1}$ of organic matter (dry weight basis), 26.8 ± 1.26 g $kg^{-1}$ of total humic substances, 15.3 ± 1.18 g $kg^{-1}$ of humic acids, 14.6 ± 1.43 g $kg^{-1}$ of fulvic acids, pH 8.25, electrical conductivity 10.4 mS $cm^{-1}$, total nitrogen content 1.7 g $kg^{-1}$, total P content (as $P_2O_5$) 14.54 g $kg^{-1}$, and 2.5 % ash content. The humic substances extraction from vermicompost and Humates solution were diluted 20-fold in deionized water before the application.

Micronutrients solution (2.0 mL), 1.64 % (Fe-EDTA) solution (4.0 mL), and KOH (4.5 g). One-hundred milliliters of vitamin solution contained biotin (10.0 mg) and pyridoxol-HCl (20.0 mg); 1.0 L of the micronutrient solution contained $CuSO_4$ (0.4 g), $ZnSO_4 \cdot 7H_2O$ (0.12 g), $H_3BO_3$ (1.4 g), $Na_2MoO_4 \cdot 2H_2O$ (1.0 g) and $MnSO_4 \cdot H_2O$ (1.5 g). The pH of the medium was adjusted to 5.8 [13]. We determined the bacterial population associated with the plant after substrate inoculation in soil or after foliar spraying under field conditions by the most probable number technique (MPN) using Popov and Sukhanov [17] tables (with three replicates per dilution). The humic substances solution and *Azospirillum* were mixed with microelements solution then saved in the refrigerator at 4 °C until application in the field experiments.

### 2.2. Analysis of wheat plants

In each fertilizer treatment, the leaves of 10 plants located in the central area of each plot were selected. Leaf samples were collected in two stages of growth: (1) after three months of seed sowing to determine total nitrogen (TN), total phosphorus (TP), and total potassium (TK), occurring on January 20th, 2020. (2) at harvest after six months, which took place on April 16th, 2020, selecting the spike leaves for plant height measurements, grain yield, biological yield, number of plants/$m^2$, number of tillers/$m^2$, spike length, and harvest index described in [12]. For each period and fertilizer treatment, all the wheat located in each experimental

**Table 1**
Influence of *A. brasilense* soil inoculation and foliar treatment of crops with ALCRI-CropHelp and ALCRI-CropHelp-M preparations on wheat grain yield.

| Treatments/Parameters | Grain yield, Ton/ha | Increase in yield | | Water consumption efficiency, kg grain/$m^3$ water $ha^{-1}$ |
|---|---|---|---|---|
| | | Ton/ha | % | |
| Control | $3.45 \pm 0.123^a$ | 0 | 0 | $0.48 \pm 0.017^a$ |
| NPK | $4.77 \pm 0.301^b$ | 1.32 | 138 | $0.67 \pm 0.042^b$ |
| *A. brasilense* | $8.84 \pm 0.580^c$ | 5.39 | 256 | $1.24 \pm 0.081^c$ |
| ALCRI-CropHelp | $9.22 \pm 0.228^{cd}$ | 5.77 | 267 | $1.29 \pm 0.032^{cd}$ |
| ALCRI-CropHelp-M | $9.58 \pm 0.480^d$ | 6.13 | 278 | $1.34 \pm 0.067^d$ |
| $F_{05}$ | 3.48 | – | – | 3.48 |
| $F_{c.}$ | 16.751 | – | – | 167.51 |
| $LSD_{05}$ | 0.693 | – | – | 0.097 |

*Note*: $F_{05}$ – theoretical Fisher's criterion; $F_{C.}$ – Fisher's criterion actual; $LSD_{05}$ – the lower significant difference at P = 95 %. Data correspond to the means of three replicates ± standard deviation. Different letters indicate statistically significant differences ($p \leq 0.05$) among treatments.

plot was collected. The number of grains per wheat and crop yield (kg $ha^{-1}$) was determined in samples collected from each plot in January and April 2020, respectively.

### 2.3. Statistical analysis

Statistical analysis was carried out using the SPSS v.16 software (Visauta, 2007). Data were submitted to a normality test before the analysis of variance. When statistical significance was found ($P \leq 0.05$), a comparison of the means was carried out using the Tukey test. Furthermore, a Pearson correlation analysis was carried out to observe the degree of association between some of the studied variables.

## 3. Results and discussion

### 3.1. Macro-nutrients concentration after plant treatments

The data obtained in the course of field trials of the bacterial preparation (*A. brasilense*) and the humic preparations ALCRI-CropHelp and ALCRI-CropHelp-M indicated these preparations allowed obtaining a significant increase in wheat grain yield relative to the control (Table 1). Soil inoculation with *A. brasilense* and foliar treatment of crops with humic preparations contributed to an almost 2-fold increase in wheat grain yield than the variant with NPK. Also, as a result, the efficiency of water consumption has significantly increased.

As is known [4], associative nitrogen-fixing rhizosphere bacteria of the genus *Azospirillum* have a beneficial effect on plant growth and development due to nitrogen fixation, biosynthesis of phytohormones and ionophore organic compounds, reducing the effect of stressors, controlling numerous phytopathogens, as well as mobilizing phosphates, improving water and N, P, K nutritional regimes shown (Fig. 1).

Humic substances, getting inside plants, are capable of: accelerating the circulation of nutrients in plants, causing induction of gene expression, enriching energetically, optimizing: respiration, photosynthesis, biosynthesis, the ratio of organic and inorganic anions, synthesizing phytoncides and phytoalexins [14,16], to increase the resistance of plants to the action of ionizing radiation and pesticides [7]. Management of crop productivity using modern biotechnology achievements refers to the biological correction of plant growth and development. The methodology of biological correction is based on the following key provisions [13]: 1)- green vascular plants can absorb and assimilate organic compounds; 2)- in green vascular plants, one of the pathways that ensure the transport of substances in the system of protoplasts of plant cells, united into one whole by numerous plasmodesmata, which allows plants to absorb nutrients not only with the help of roots but also with leaves; 3)- the production process of plants is largely determined by the rate of movement of nutrients both from the root to the leaves and from the leaves to the root. In this case, soil fertility is considered as a consequence of the biological cycle of biophilic elements in ecosystems [15].

### 3.2. Number of plants and tillers ($m^{-2}$)

These are considered the most imperative among all yield-determining parameters, as Tahir et al. [18] endorsed that wheat yield mainly depends on productive/fertile tillers in the field. Foliar application and soil treatments of organic amendments significantly affected the number of plants and fertile tillers per $m^{-2}$. The data noted are given in (Fig. 2). Significantly, the highest number of plants for productive tillers (725.3 $m^{-2}$) was found in ALCRI-CropHelp treatment. An increase in productive tillers (693.3 $m^{-2}$ ) was observed in productive tillers with ALCRI-CropHelp-M compared to control. Simultaneously, the number of plants was significantly increased in ALCRI-CropHelp treatment and ALCRI-CropHelp-M foliar applications compared to control and NPK fertilizers.

Similarly, Hafez et al. [7] reported improved plants and productive tillers with foliar application of humic substances and *Azspirillum* treatments. Ahmad and Irshad [16] advocated an increase in productive tillers $m^{-2}$ with the ALCRI-CropHelp-M application. Those tillers that do not bear grains in their spikes and some do not even bear spikes are considered non-productive. Non-productive tillers are not as important as productive tillers. Still, the transformation of non-productive to productive tillers is important as productive tillers are directly related to wheat yield. Az and ALCRI-CropHelp treatments significantly reduced non-productive tillers $m^{-2}$ and enhanced the number of plants per square meter. The reduction in unfertile tillers is due to ALCRI-CropHelp and ALCRI-CropHelp-M with microelements foliar applications played a key role in grain formation. Humic substances with boron reduced the sterility of wheat and produced grains in each spike, increasing productive tillers by decreasing unfertile tillers in the field. These findings are similar to Tahir et al. [18], as they documented a significant B in a wheat grain setting. Boron spray at the booting stage did not affect the total tillers as tillering is an early stage, while B's foliar application was made at the lateral stage.

### 3.3. Spike length (cm)

It is an important parameter to consider. Data recorded for spike length is provided in (Fig. 3). The highest spike length (12.16 cm) and (11.96 cm) were observed with ALCRI-CropHelp-M treatment compared to control. The lowest spike length (9.36 cm) and

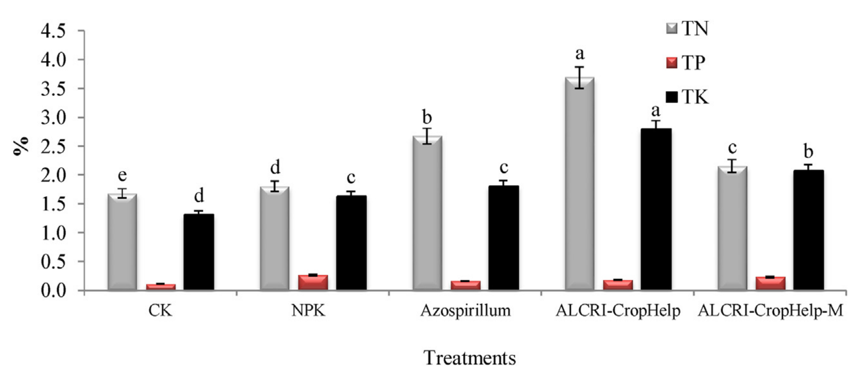


**Fig. 1.** Influence of *A. brasilense* soil inoculation and ALCRI-CropHelp and ALCRI-CropHelp-M foliar applications on NPK concentration in the wheat plant. Data correspond to means of three replicates ± standard error. Different letters indicate statistically significant differences ($P \leq 0.05$) among treatments.

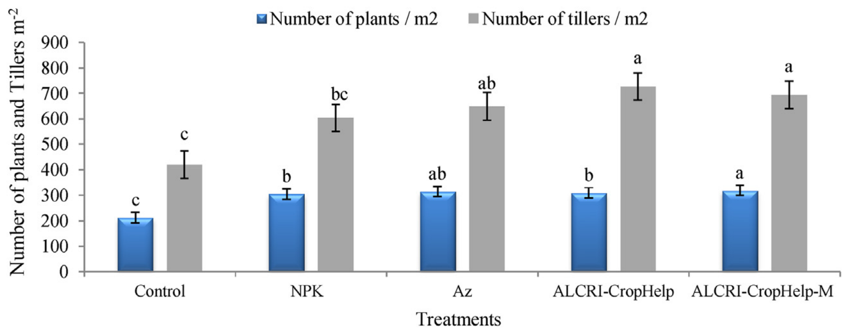


**Fig. 2.** Influence of A. brasilense soil inoculation and ALCRI-CropHelp and ALCRI-CropHelp-M foliar applications on Number of plants and tillers ($m^{-2}$) in the wheat plant. Data correspond to means of three replicates ± standard error. Different letters indicate statistically significant differences ($P \leq 0.05$) among treatments.

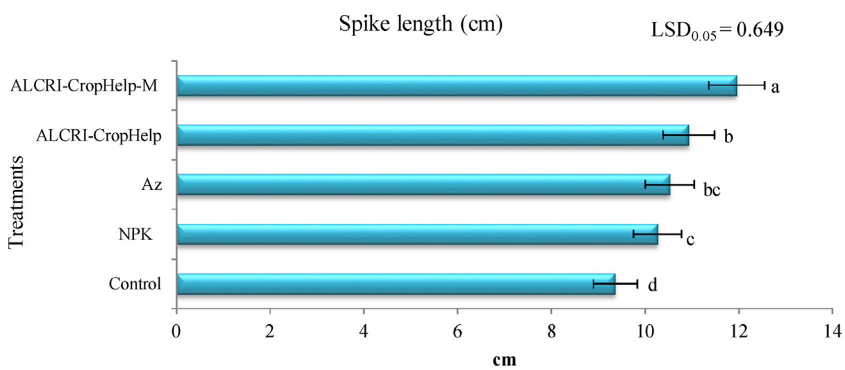


**Fig. 3.** Influence of *A. brasilense* soil inoculation and ALCRI-CropHelp and ALCRI-CropHelp-M foliar applications on spike length (cm) in the wheat plant. Data correspond to means of three replicates ± standard error. Different letters indicate statistically significant differences ($P \leq 0.05$) among treatments.

(10.26 cm) was observed with the control and NPK treatments. Fakir et al. [17] have also observed and interpreted increased spike length by microelements folier applications. Leghari et al. [9] also documented an increase in the spike length of wheat with microelements application.

### 3.4. Biological yield

It is important in measuring the photosynthetic activity of crop. Data recorded is given in (Fig. 4). ALCRI-CropHelp-M treatments spray significantly affected biological yield. Statistically significant and the highest biological yield (16.89 t $ha^{-1}$) was obtained with ALCRI-CropHelp-M foliar at the booting stage. It was increased by 377.85 % compared to control treatments. An increase in grain weight is the possible reason to increase the biological yield as grains are a biological yield component. Current outcomes are similar to Khan et al. [8], as they observed an increase in biological yield with the application of micronutrients at different growth stages of wheat. Similarly, Hafez et al. [7] also reported a significant plant growth and yield improvement when applied to humic substances and *Azospirillum* treatments.

### 3.5. Harvest index (H.I)

It is the measurement of the efficiency of the crop to translate assimilates into grain yield. All treatments enhanced the harvest index (H.I) significantly of the wheat crop. The foliar application of HS ALCRI-CropHelp-M and ALCRI-CropHelp showed significant results as compared with control and NPK treatments. (Fig. 5) revealed that significantly higher H.I (61.3 %) was calculated in ALCRI-CropHelp treatment. Increase in H.I was 133.3 % and 121.7 % with ALCRI-CropHelp and ALCRI-CropHelp-M in comparison to control treatment, respectively. An increase in harvest index with ALCRI-CropHelp-M and ALCRI-CropHelp application has also been

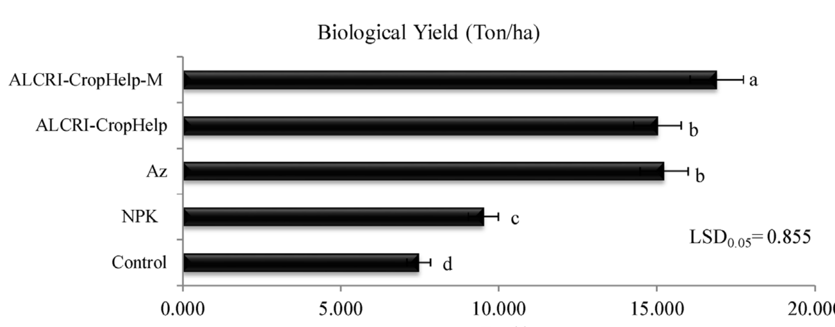


**Fig. 4.** Influence of *A. brasilense* soil inoculation and foliar ALCRI-CropHelp and ALCRI-CropHelp-M foliar applications on biological yield (ton/ha) in the wheat plant. Data correspond to means of three replicates ± standard error. Different letters indicate statistically significant differences ($P \leq 0.05$) among treatments.

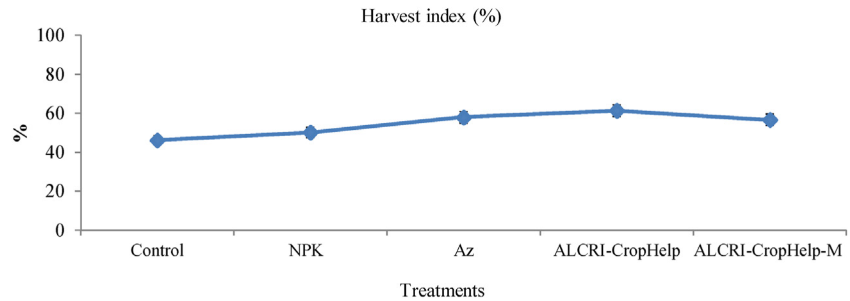


**Fig. 5.** Influence of *A. brasilense* soil inoculation and ALCRI-CropHelp and ALCRI-CropHelp-M foliar applications on harvest index (%) of the wheat plant. Data correspond to means of three replicates ± standard error.

suggested by Khan et al. [8]. Also, Tahir et al. [18] have also indicated an uprise in harvest index by microelements foiler applications on wheat.

## 4. Conclusions

Inoculation of the soil with *Azospirillum brasilense* and foliar treatment of crops with humic preparations ALCRI-CropHelp and ALCRI-CropHelp-M contributed pronounced increases in wheat grain yield, number of plants and tillers, and biological yield. Such biotechnologies are based on the principle of biological conformity. Finally, the combination of natural materials and environmental friendly byproducts has become one of the most important practices concerning soil enhancement and yield increase. The whole ALCRI-CropHelp-M was better than the NPK fertilizer. At the same time, the ALCRI-CropHelp and Az significantly ($p < 0.05$) improved their impacts on the productivity of wheat plants in arid regions.

## Author contributions

All authors have contributed equally to this study.

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## Declaration of Competing Interest

The authors report no declarations of interest.

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