1874-3315/22 Send Orders for Reprints to reprints@benthamscience.net

The Open Agriculture Journal

Content list available at: https://openagriculturejournal.com

RESEARCH ARTICLE

The Biological Correction Using Humic Substances, Vermicompost, and *Azospirillum* as an Optimum Way of Optimizing Plant Production and Enhancing Soil Micronutrients in Arid Regions

Mohamed Hafez[1,2,*], Alexander I. Popov[1] and Mohamed Rashad[2]

[1]Department of Soil Science and Soil Ecology, Institute of Earth Sciences, Saint Petersburg State University, Embankment, 7/9, Saint Petersburg, 199034, Russia

[2]Department of Land and Water Technologies, Arid Lands Cultivation Research Institute, the City of Scientific Research and Technological Applications, Alexandria, Egypt

Abstract:

Aims:

This field experiment aimed to investigate the effect of using foliar treatments and applying different fertilizers on wheat (*TriticumaestivumL.*) growth, productivity, and soil fertility under arid conditions.

Background:

Agriculture is a critical socio-economic sector in Egypt, generating approximately 10 to 15% of the national gross domestic product. Abiotic stresses arising from climate change negate crop growth and yield, leading to food insecurity.

Methods:

Six treatments were carried out in the order: CK (control without amendment); NPK (mineral fertilizer: mineral fertilizers, 38 g/m^2 of urea (46% of N), 10 g/m^2 of calcium phosphate (45% of P_2O_5), 12 g/m^2 of potassium sulfate (50% of K_2O)); *Azospirillum brasilense* (Az), bacteria were soaked with the seeds for 4 hours before sowing and introduced three-time into the soil with irrigation water at 0.7 ml/m^2; vermicompost (VC) applied to the soil (2.5 kg/m^2); humic substances (0.5 ml/m^2) for foliar inoculation (HS); HS + micronutrients (HS-M) soil application (1 ml/m^2) with irrigation water. All treatments were applied as soil additives and foliar spray to wheat plants at 31±1 °C for the first two months from seed sowing in calcareous soil. Analysis of variance (ANOVA) of experimental data showed the significance of positive effects of all treatments on soil fertility and plant growth compared to the control and NPK treatments.

Results:

The HS and VC treatments had positive effects on the development of plant nutrition, wheat growth, soil pH, and plant availability of micronutrients, which was also reflected in the wheat grain yield and water use efficiency. It was also evident that treating the plants and soil with HS-M increased wheat yield from 3.45 to 8.97 t ha^{-1} (260%) compared to the control. On the other hand, VC and Az treatments increased the grain yield by 278% and 267%, respectively, compared to control and NPK treatments. The water use efficiency (kg grain / m^3) values increased by 279, 268, 262, 258, and 139% for HS-M, HS, VC, Az, and NPK treatments, respectively, compared to CK. The plants' total N, P, and K content were higher with all organic and biological treatments than NPK and control.

Conclusion:

This study has shown that the HS, Az, VC, and HS-M soil additives and foliar applications significantly ($p \leq 0.05$) increased the status of Fe, Zn, Mn, Cu, and B in soil, plant growth rate, grain yield, 1000 grain weight, water use efficiency, and soil macro-nutrient of wheat plants growing on calcareous soil.

Keywords: Plant productivity, Biological correction, Soil degradation, Humic substances, Wheat, Technologies.

Article History	Received: December 15, 2021	Revised: January 24, 2022	Accepted: February 17, 2022

1. INTRODUCTION

The world's population is growing alarming, implying that food supplies must be raised through sustainable agriculture to satisfy the rising demand. At about 7 billion, the world population will grow to 10 billion or more during the next 50 years. The intensive input of agrochemicals and mineral fertilizers is used to help degenerate soil organic matter and increase land degradation. These detrimental effects can be at least to some extent reduced by a range of physical, chemical, and biological remedies (Scheme **1**) [1, 2]. Physical correction is understood as a system of agrotechnical, agroameliorative, irrigation, and drainage measures to create and maintain clear water, thermal, and air regimes for cultivated plants and the biological activity of soils. This type of correction is the most important component of regulating the plant production process; historically, it is the second evolutionary path of crop production [1].

Chemical corrections to improve crop productivity are a set of measures associated with chemical materials, such as replenishing nitrogen and other mineral nutrient reserves in the soil, foliar feeding of plants with macro-and microelement compounds, regulating acid and salt regimes of soils, and using chemical plant protection products (Scheme **1**). Chemical correction can be said to be fully applied in industrial agriculture. This correction method is mainly concerned with gaining gross crop production (often without regard for quality). It disregards natural laws, resulting in plants and soil-forming an integrated and interdependent trophic system under natural conditions. It should be emphasized that plants are adequately resistant to different detrimental impacts in natural soil-plant systems [2].

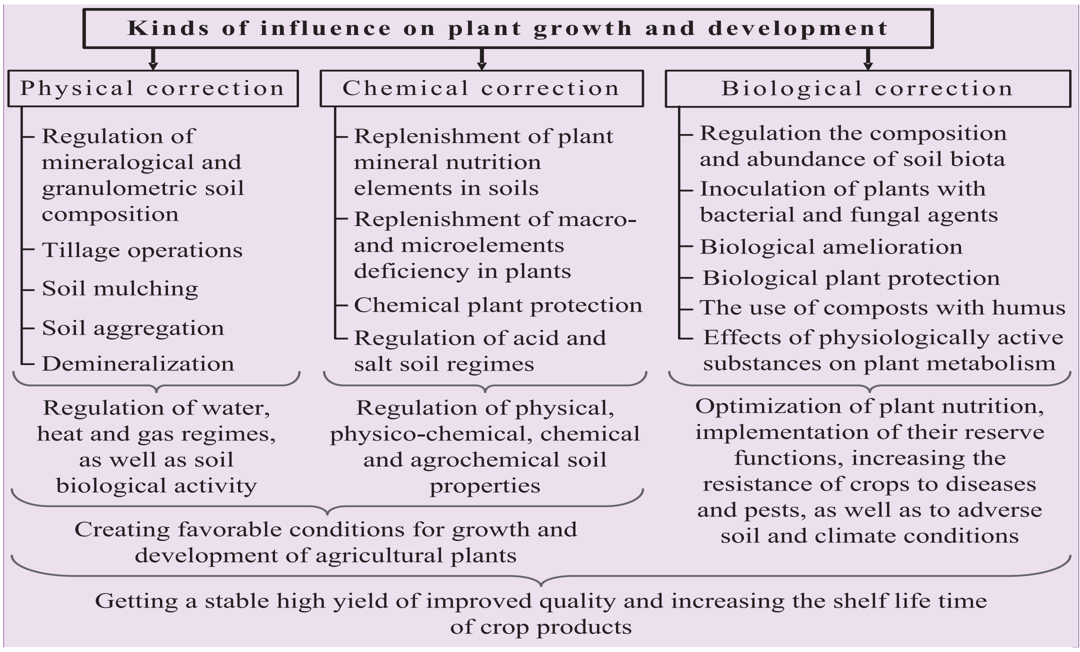
Agricultural practices, like precision farming, focus on precise machinery movement within and between site-specific crop management in the field, such as assisted by global position systems. The correct agricultural systems can also reduce the overlapping of agricultural applications, thus helping to lower the input of energy, water, organic matter, and agro-chemicals in intensive crop production systems [3, 4]. The imbalance of trophic elements in industrialized agroecosystems, such as produced by the systematic use of mineral fertilizers, is one of the main reasons for various disturbances in the agroecosystems and the biosphere [5, 6]. The ongoing chemical impact of agriculture combined with repeated soil treatment, without respect to the nature of soil as a living, organic part of the ecosystem, practically follows the inert substrate paradigm of mineral fertigation systems. Therefore, this way of managing the production process of cultivated plants is a dead end [7]. One of the effective approaches to managing the production process and plant protection is seen in biological correction (Scheme **1**). This is because the combination of methods of directed influence on plant biology further increases the yield of cultivated plants with an improvement in the quality of the products obtained and increases plants' tolerance to biotic and abiotic stresses [7, 8]. This type of influence on plant growth and development is the third evolutionary path of plant production.

In the present study, we investigated the applications of plant growth-promoting rhizobacteria (PGPR) and humic substances (HS) as a natural additive to plant and soil systems.

The application of PGPR has improved growth and yield parameters with extensive root growth, facilitating water and mineral nutrients [9]. Similar effects of *Azospirillum* were recorded against salinity in wheat seedlings with relatively higher water content [10, 11], which could be due to various physiological changes induced by the colonizing bacteria. *Azospirillum* bacteria are regarded as plant growth-promoting rhizobacteria, which strongly enhance nitrogen fixation, plant growth, and the fertility of soils [12]. Therefore, organic and biological ameliorants can improve soil properties in salt-affected soils, and they are crucial to ensure farmers' income, particularly in arid regions. They are regarded as applicable practices, which may help to obtain greater sustainability of the agroecosystems.

One of the effective and economically justified biological correction methods, which makes it possible to compensate to some extent for humus in agricultural soils, is the foliar treatment with humic compounds that may also have beneficial effects on plant growth. Nevertheless, soil microorganisms and humic compounds have functions in soils, which foliar treatment cannot replace with humic compounds [7, 13, 14]. HS are the key components of soil, water, and sediment organic matter. They can be chemically represented as a series of complex organic molecules of relatively low molecular mass forming dynamic associations stabilized by nanometer-scale hydrophobic interactions and hydrogen bonds [15]. Once inside plants, humic substances can accelerate the turnover of nutrients in plants, causing induction of gene expression, optimizing respiration, photosynthesis, the ratio of organic and inorganic anions, and biosynthesis, and synthesizing phytoncides and phytoalexins in plants [16, 17]. They increase plant resistance to biotic and abiotic stress [1], improving seeds germination and germination energy [18]. As a result, foliar treatment with humic preparations and *Azospirillum* contributes to ensuring the high biological productivity of crops and increasing their resistance to adverse climatic influences [19]. The objectives of the present study include (1)- Investigating the effect of using foliar treatments and applying different soil-plant fertilizers on wheat growth, productivity, and soil fertility under arid conditions, (2)- Increasing the wheat grain yield in arid regions, (3)- Improving micronutrient availability in calcareous soil, and (4)- More efficient use and decreased input of chemical fertilizers by using biological correction systems.

* Address correspondence to this author at the Department of Land and Water Technologies, Arid Lands Cultivation Research Institute, the City of Scientific Research and Technological Applications, New Borg El-Arab, 21934, Alexandria, Egypt; E-mail: mhafez290@yahoo.com



Scheme (1). Physical, chemical, and biological effects on plant growth and development.

2. MATERIALS AND METHODS

2.1. Location and soil properties

The study area was located in the arid regions (30° 53' 33.17 “N 29° 22' 46.43”E), the North-Western part of Egypt. The study site has a dry climate, hot summers, and semi-cool and semi-wet winters. The mean annual temperature was 19.3 °C from 2019 to 2020, ranging from 14.0 °C to 24.6 °C, and the mean annual precipitation was 128.2 mm [20]. The soil has a sandy clay loam texture and can be classified as calcareous soil [21]. The soil samples were sieved with a 2 mm mesh size before analyses were undertaken. The physical, chemical, and biological properties of the main soil (0- 20 cm depth) are summarized in the following Table **1**.

Table 1. The physical, chemical, and biological properties of the soil before organic and biological treatments.

Soil characterization	**Calcareous Soil**
pH (soil: water, 1:2.5 w:v)	8.34± 0.057
EC (dS/m, 1:5 w:v)	3.86± 0.12
Total N (g kg^{-1})	0. 30± 0.05
NO_3–N (mg kg^{-1})	24.5± 0.73
NH_4–N (mg kg^{-1})	1.20± 0.17
Available P (mg kg^{-1})	4.20± 0.28
Available K (mg kg^{-1})	320.2± 50.1
Sand (%)	64.1
Silt (%)	15.2
Clay (%)	20.7
Texture	Sandy Clay Loam
Micronutrients, DTPA- Extractable (mg kg^{-1})	
Fe	4.10± 0.18
Zn	1.43± 0.09
Mn	3.49± 0.22
Cu	0.61± 0.05
B	0.30± 0.02
Dehydrogenase (µg TPF ml^{-1})	234±29.2
Urease (mgNH_3-H$g^{-1}h^{-1}$)	66±4.21

2.2. Preparation and Description of Amendments

2.2.1. Biological Amendments

As a microbial treatment, *Azospirillum brasilense* (Az) inoculum formulation was applied to work as a plant growth-promoting rhizobacterium. Az was grown in LB medium (10g L^{-1}triptone, 5g L^{-1}NaCl, 10g L^{-1} yeast extract) for 4 days under continuous shaking at 30°C [22]. The bacteria were then centrifuged for 15 minutes at 180 rpm and washed four times with a sterile solution (NaCl 0.85% w/v). After that, the *Azospirillum* bacteria were ready for injection into the seeds and soil. The inoculant was prepared by diluting 200 ml of bacterial suspension in 800 ml of distilled water at pH 7.0 to produce a final concentration of 20 mg C L^{-1} and a final bacterial concentration of 5 × 10^8 cells ml^{-1}.

2.2.2. Vermicomposting

Vermicompost was obtained from a red wiggler /ENC mix worm farm in Alexandria, Egypt. It was produced as a by-product from the beer industry residues (spent grain) by brandling worm red wiggler (1500 worms m^2). The chemical properties of the spent grain and vermicompost are shown in Table **2**.

2.2.3. Soluble Humic Substance Extraction

Soluble-humic substances were extracted from vermicompost, produced as described in 2.2.2 with 0.1 M NaOH in a 1:20 solid-liquid (w:v). The extraction samples were shaken at 2000 rpm for 4 hours and left overnight. The supernatant was centrifuged at 5000 rpm and filtered through a Whatman no. 42 filter paper to show the Az production's humate. The HS characteristics were characterized as indicated in Table **2**.

2.2.4. Micronutrient's Preparation

The micronutrients were prepared with 1.64% (Fe-EDTA) solution (4.0 mL), and KOH (4.5 g) was mixed with one liter of the micronutrient solution containing $CuSO_4$ (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 solution was adjusted to 5.8.

2.3. Field Experimental Setup

2.3.1. Plant-soil Treatment and Application Rates

The field experiment included six treatments comprising CK (control without amendment); mineral fertilizers (NPK), (38 g/m^2 of urea (46%); 10 g/m^2 of calcium phosphate (45%); 12 g/m^2 of potassium sulfate (50%)); *Azospirillum sp.* (Az), a cultivated suspension of plant growth-promoting rhizobacteria seeds, soaked in the suspension for 4 hours before sowing and introduced three times into the soil with irrigation water at 0.7 ml/m^2; vermicompost (VC) was applied to the soil at a rate of 2.5 kg/m^2; humic substances + micronutrients (HS-M), neutral HS with micronutrients solution 1.0 ml / m^2 soil inoculation; and humic substances (HS) 0.5 ml /m^2 for foliar spray application on plants and soil. All treated plots were sown in November 2020 with wheat (*TriticumaestivumL.*). The wheat plants were grown under experimental field conditions for 6 months. The treatment descriptions and application rates are shown in Table **3**.

Table 2. Physical and chemical characteristics of soil organic additives before application.

Parameter	Spent grain	Vermicompost	HS
pH in water (1:5 w:v)	4.16± 0.03	6.89± 0.03	6.91 ± 0.34
EC (dS/m, 1:5 w:v)	1.45± 0.21	3.31 ± 0.27	12.41 ± 0.54
Organic Matter (g kg^{-1})	750 ± 0.57	890 ± 1.3	39.3 ± 1.78
Total N (%)	3.12 ± 0.68	3.88 ±0.77	1.10 ± 0.07
Total P (%)	1.86 ± 0.54	2.60 ± 0.1.1	0.87 ± 0.00
Total K (%)	1.74 ± 0.63	1.93 ± 0.43	0.61 ± 0.01
C: N ratio	13.9 ± 0.12	13.30 ± 0.15	20.72 ± 0.11
Organic carbon (%)	43.5 ± 0.94	51.62 ± 1.30	22.79 ± 1.4
Fe (mg kg^{-1})	1130 ± 3.87	2631 ± 3.23	5602 ± 4.3
Zn (mg kg^{-1})	368 ± 2.34	473 ± 2.00	356 ± 2.1
Mn (mg kg^{-1})	210 ± 1.98	290 ± 1.21	----
Cu (mg kg^{-1})	98 ±1.54	122 ± 1.34	----
Polyphenol (%)	1.28 ± 0.24	0.53 ± 0.00	----
Lignin (%)	28.26 ± 0.66	17.89 ± 0.32	----
Cellulose (%)	57.63 ± 1.1	37.55 ± 0.44	----

Table 3. Organic and bio-fertilizer application rates.

Treatments	Field experimental and application rates	
	Description	**Application rates (m^2)**
CK	Control	without soil amendments
NPK	Mineral fertilizer	38 g/m^2 of urea (46%); 10 g/m^2 of calcium phosphate (45%); 12 g/m^2 of potassium sulfate (50%).
Az	*Azospirillum*	Seed soaking before sowing and soil inoculation with irrigation water (0.7 ml/ m^2).
VC	Vermicompost	2.5 kg/ m^2 mixed with soil
HS	Humic substances	0.5 ml/ m^2 mixed with 100 ml of water to foliar spray fertilizer.
HS+M	Humic + Micro-elements	1 ml/ m^2 with irrigation water.

The total trial area had a size of 500 m^2 comprising 18 plots, each of the six treatment variants was tested in three replicate plots; the plot size was (5.5m x 5m) distributed in a randomized complete block design. The mass seeds (weight) were 10.7 kg/ 500 m^2, about 22 g (500 seeds) per m^2. The cultivation method included scattering seeds in the soil before irrigation water was supplied. To maintain adequate seed germination moisture, all plots were watered from the Nile River to the soil saturation capacity. The water source was the limited source, and on the research farm, we stored water every 2-3 months for research experiments. Therefore, there were restrictions on saving water constantly. The vermicompost and HS characteristics are characterized as indicated in Table **2**.

2.4. Plant and Soil Sampling and Analytical Determinations

2.4.1. Plant Sampling and Analysis

In each fertilizer treatment, the leaves of 10 plants located in the central area of each plot were selected. Leaf samples were collected three months after seed sowing on January 20th, 2020, to determine N, P, and K concentrations. Moreover, the grain yield was collected at harvest six months after sowing, which occurred on April 16th, 2020, by selecting ears.

The measurements of plant parameters included the weight of 1000 seeds (g), grain yield (ton/ha), biological yield (ton/ha), and N, P, and K (%) contents [37]. All the wheat in each experimental plot was collected; each plot's grain yield of wheat ton ha^{-1} was calculated.

2.4.2. Soil Chemical Analysis

The electrical conductivity (EC) was measured in soil extract in water (1:5) using an EC meter; the pH was measured in a soil: water suspension (1:2.5 w/v) [28]. The total nitrogen (N) was determined using the Micro-Kjeldahl method; extractable phosphorus (P) was extracted with 0.5N $NaHCO_3$ and measured using a spectrophotometer at 670 nm wavelength. Available potassium (K) was extracted with 1 N ammonium acetate solution and measured by flame photometry. N, P, and K were measured as explained previously [23], [24], and [25]. Micronutrients (Fe, Zn, Mn, Cu, and B) were extracted from DTPA solution as explained in a previous study [30], then measured with inductively coupled plasma (ICP) atomic emission spectroscopy

2.4.3. Soil Biological Analysis

The soil dehydrogenase activity was determined by tri-phenyl-tetra-zolium chloride (TTC) following a method described previously [26]. Soil urease (S-UE) was determined using previously published methods [27], involving: 5 g of soil was placed in 100-ml Erlenmeyer flasks and wetted with 2.5 ml of a 0.08 M aqueous urea solution. The flasks were stoppered and placed in an incubator at 37 °C. After 1 hr., the stoppers were removed, 50 ml 1*N* of KC1 to 0.01*N* of HC1 was added and the mixtures were shaken on a shaker for 30 min. The resulting suspensions were filtered, and ammonia in the filtrates was determined calorimetrically. 1 ml of filtrate was diluted to 10 ml with distilled water, and 5 ml of sodium salicylate and 2 ml of 0.1% sodium dichloroisocyanurate were successively added. The sodium salicylate solution was prepared for use by mixing 100 ml of 0.12% sodium nitroprusside, 100 ml of 17% sodium salicylate, and 100 ml of distilled water. Optical density was determined at 690 nm after a 30 min incubation at room temperature.

2.5. Water use Efficiency Calculation

The overall agronomic water use efficiency (WUE) was evaluated [28, 29] as follows:

$$WUE = \frac{CP}{V}$$

Where: CP is crop production, and V is the irrigation water amount.

2.6. Statistical Analysis

Statistical analyses were carried out using the Tukey test ($p \leq 0.05$) to compare means. Furthermore, the Pearson correlation analysis was performed to determine the degree of a statistical association between some studied variables.

3. RESULTS AND DISCUSSION

3.1. Effect of soil and plant treatment on plant productivity after 6 months of seed sown

3.1.1. Grain Productivity with Water use Efficiency Relationship

Data (Fig. **1**) show the effect of soil treatment and foliar fertilizer on the wheat yield, which increased significantly during the season 2019/2020. When studying the impact of each factor, it was found that the soil treatments with HS-M, HS, and VC before sowing resulted in the highest yield records of wheat of 9.58, 9.22, and 8.84 t ha^{-1}, respectively, after six months of seed sowing compared to the CK and NPK treatments. The water use efficiency (kg grain / m^3) values increased by 279, 268, 262, 258, and 139% for HS-M, HS, VC, Az, and NPK treatments, respectively, compared to the control treatment. The water use efficiency calculated was more significant in the higher grain yield than in the HS-M, HS, and VC treatments. The wheat productivity increased when using a preparation supplemented with micronutrients (Fig. **1**). On the other hand, there were differences between the effects of fertilizers. Compared to the control treatment, the plant productivity increased by 260% and 138% with *Azospirillum* and NPK treatments. These results were in agreement with the previous findings [30].

Fig. (**1**) shows soil treatment and HS foliar fertilizers on the water use efficiency for wheat plants during the research season. The inoculation of *Azospirillum* with seeds or HS, HS-M, and CV treatments and application of VC increased the plant growth and grain yield during the growing season. The VC as an organic treatment increased soil water retention, increasing water use efficiency during the research season. The positive effect of organic fertilizers on the water use efficiency was noticed in plant growth during the research season [31]. The treatment of wheat plants with NPK, VC, Az, HS, and HS-M increased the water use efficiency by 139%, 262%, 258%, 269%, and 279%, respectively, compared to the control, respectively. On the other hand, the foliar fertilizing effect of HS-M and HS increased by 1.34 and 1.29 kg m^{-3}, respectively, compared to the control. While the VC and Az treatments increased 1.26 and 1.24 kg m^{-3} compared to the control and NPK treatments, these results were in agreement with the findings of other studies [32, 34].

3.1.2. Weight of 1000 Grains

Fig. (**2**) shows the effect of soil treatment and HS foliar fertilizer on the 1000 grain weight of wheat. Data showed a positive impact on the weight of 1000 grains for the wheat plant when using the HS, HS-M, VC, and az treatments compared to control and NPK treatments. The maximum average 1000 grain weight increased by 149.53, 135.39, 152.30, 120.65, 99.61% for HS-M, HS, VC, Az, and NPK treatments, respectively, compared to CK treatments. The VC treatment significantly ($p \leq 0.05$) increased the weight of 1000 grains of the wheat plant. The direct relationship between the weight of 1000 grains and grain yield (tons/ ha) is an indicator of HS-M and VC treatment applications that can increase wheat plant productivity in arid regions. Thousand-grain weight is regarded as one of the most important factors concerning wheat quality (Table **3**). It has been generally accepted that the plant seeds should have as large a 1000-grain weight as possible because such grain has larger amounts of storage substances and more developed embryos. This enables plants to develop and grow faster, which is important in arid and semi-arid regions. The weight of 1000 grains as a final component of grain yield depends on many parts developed during the previous phases of ontogenesis. As there is a high production of all plant organs in each stage of wheat plant growth, it is possible to influence the weight of 1000 grains [35, 36].

3.1.3. Effect of Soil Treatment and Foliar Application on Plant Nutrition

3.1.3.1. N, P, and K Concentrations at Three Months after Sowing

Fig. (**3**) shows the nitrogen (N), phosphorus (P), and potassium (K) concentrations in plants three months after sowing when the weather conditions improved relatively, especially (rainfall and air temperature) by using HS treatment. The concentrations of N, P, and K in plants increased in the order HS > Az > HS-M > VC > NPK > CK, respectively, compared to the control, but no significant differences between the VC and HS treated plants were found. When studying the effect of different organic treatments on the highest productivity of wheat, plants were treated with HS and VC fertilization, which resulted in an increase of N by 221% and 177%, respectively, compared to the control treatment.

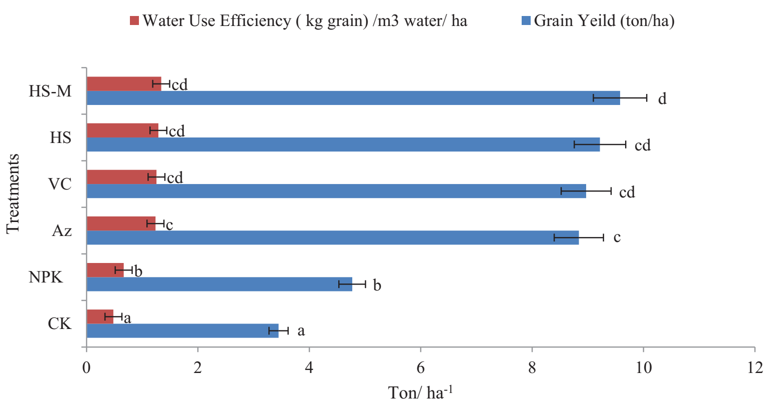


Fig. (1). Influence of soil and foliar spray treatments on grain yield and water use efficiency of the wheat plant. Columns and error bars represent mean values ± standard error of the mean (n = 3). Data were tested using a one-way ANOVA followed by the least significant difference (LSD) test. Values not sharing the same letters in postscripts are significantly different ($p \leq 0.05$).

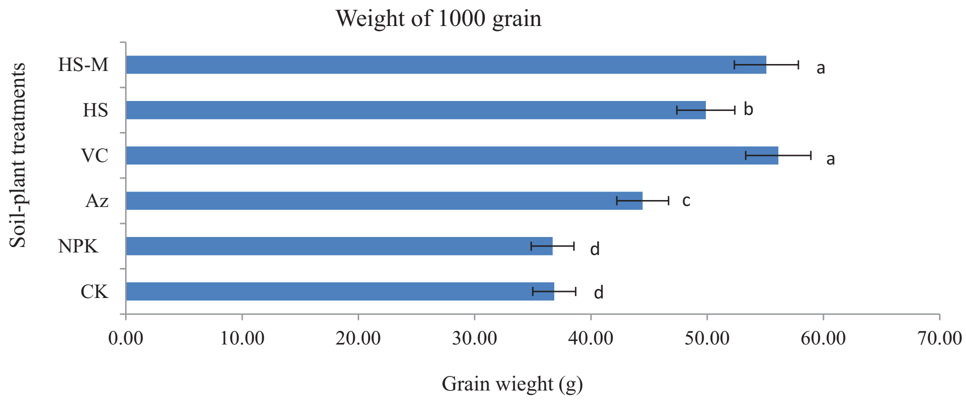


Fig. (2). Influence of soil and foliar spray treatment on the weight of 1000 grains of the wheat plant. Columns and error bars represent mean values ± standard error of the mean (n = 3).

During the research period, the positive effect after plant and soil were treated with HS and VC treatments on macronutrient concentrations compared to control and mineral fertilizers confirms similar findings [37 - 39]. Our findings concerning the effect of VC and HA on nutrient contents are in line with the results reported in a study [40] that found that the use of HA and organic sources significantly increased the concentration of P, K in the leaves of the Calendula plant. The application of ALCRI-Help and ALCRI-Help-M as a humic substance foliar treatment enhanced the N, P, and K concentrations, spike length, harvest index, and biological yield of the wheat plant [19, 41].

3.2. Effect of Soil Amendment on Soil Fertility After Plant Harvest

3.2.1. Effects on pH Values

Soil pH is a critical factor that affects the solubility and availability of plant nutrients. Increasing the availability of certain plant nutrients, such as P, can be achieved by decreasing soil pH in calcareous soils. The soil analysis showed that soil pH was significantly reduced relative to the control (Fig. **4**). The calcareous soil pH values treated with organic and bioorganic ameliorants decreased by 2.84, 3.19, 5.91, 7.90, and 12.77% for HS, HS-M, NPK, Az, and VC treatments, respectively, compared to CK treatment. The VC and Az treatments resulted in the lowest pH levels in calcareous soil. No significant ($p \leq 0.05$) difference was observed in CK, HS, and HS-M treatments compared to VC and Az applications. In the VC treatment, decreased soil pH was enhanced by organic matter content in the initial vermicompost source. The type of amendment influenced the reduction of pH values. The increasing application of Az and VC enhanced pH reduction. However, soil pH increase may induce the Ca^{2+} to become more alkaline and, therefore, more sodic as calcium solubility is suppressed; this was also proposed in a study conducted previously [41]. In arid and semi-arid climates, the solubility of Ca^{2+} and Mg^{2+} becomes low; Na^{+} and K^{+} ions accumulate in soil solution when CO_3^{-2} and HCO_3^{-} increase [42]. It has been found that the applications of organic amendments, such as spent grain and *Azospirillum,* might reduce pH in calcareous soil [38]. A long-term soil incubation experiment was conducted to examine the effect of different organic amendments on the chemical properties of soil, which positively impacted soil pH in calcareous soil [43]. Found applications of organic amendments to soil decreased the soil pH and enhanced phosphorus availability in sandy soil.

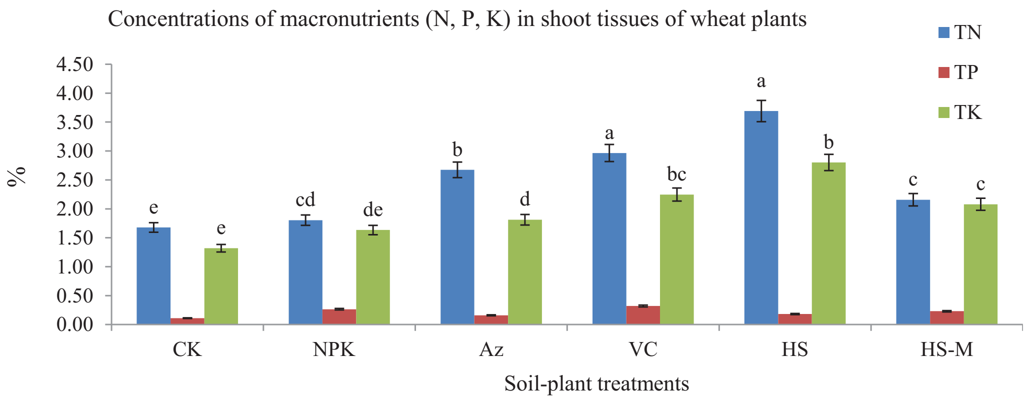


Fig. (3). Influence of soil and foliar spray treatment on the plant macronutrients concentrations of the wheat plant after three months of seed sown. Columns and error bars represent mean values ± standard error of the mean (n = 3).

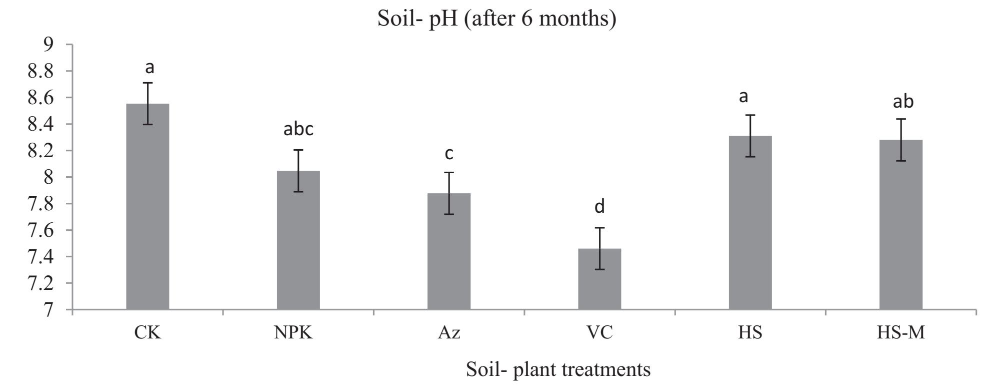


Fig. (4). Influence of soil and plant foliar spray treatment on soil pH in the calcareous soil under wheat plant growth after 6 months of seed sown. Columns and error bars represent mean values ± standard error of the mean (n = 3).

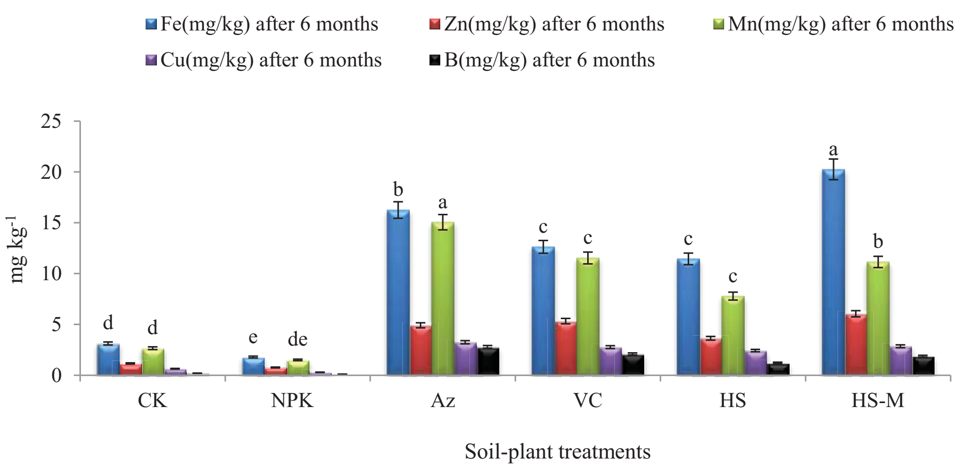


Fig. (5). Influence of soil and foliar spray treatment on micronutrient concentration (mg kg^{-1}) of calcareous soil under wheat plant growth after 6 months of seed sown. Columns and error bars represent mean values ± standard error of the mean (n = 3).

3.2.2. Effect of Soil Amendment on Extractable Micronutrients

The soil additives were found to have a significant effect on the extractability of micronutrients. Fig. (**5**) shows Fe, Zn, Mn, Cu, and B concentrations six months after sowing. The Fe, Zn, Mn, Cu, and B concentrations in the soil after wheat harvest followed the order HS-M > Az > VC > HS > CK > NPK, respectively. The soil's higher concentrations of micronutrients were observed in variants with HS-M and Az treatments, while the lowest increases were observed with NPK and CK treatments after six months of seed sowing. The Az and HS-M application rates were superior to soil fertility than HS and NPK treatments for micronutrient status (Fig. **5**). The superiority of HS-M and Az was due to their effect on lowering soil pH and solubility and microelement chelating the soil treatments.

Accordingly, Az application to enrich the soil with micronutrients is highly recommended in calcareous soils [43]. The Az mineralization of organic matter leads to a decrease in soil pH, which increases micronutrient availability in calcareous soil. The presented results agree with the findings presented in a study [41] that reported that the available micronutrients improve using plant growth-promoting bacteria and organic source fertilization. The organic amendments, especially HS-M, increased the concentrations of most of the essential elements. One-way ANOVA showed a significant ($p \leq 0.05$) effect of microbial amendments and humic substances on micronutrient status in calcareous soil. The Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+} significantly ($p \leq 0.05$) increased the wheat plant growth in Az, HS-M, and HS amended to soil and plant. The applications of NPK fertilizer had lower Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+} concentrations than most microbial and organic treatments [41].

CONCLUSION

The soil-plant additives with *Azospirillum*, vermicompost, and foliar spray treatment of wheat plants with HS and HS-M induced pronounced plant growth and increased grain yield.

Such biotechnologies are based on the principle of biological conformity. *Azospirillum* with vermicompost and whole HS with and without micronutrient treatment improved the extractability of micronutrients in the soil significantly and had higher uptake and transfer factors of the microelements in comparison to the NPK fertilizer. The whole HS treatment was better than the NPK treatment, while HS-M and VC significantly ($p \leq 0.05$) improved their soil fertility and wheat plant productivity. The results revealed that the HS, VC, and Az treatments significantly increased the N, P, and K plant concentrations compared to the control treatment. Therefore, HS and VC applications are ideal for soil alkaline amelioration and effectively increase nutrient availability in calcareous soil. Finally, applying humic substances and *Azospirillum* treatment as a natural material has become one of the most critical practices concerning soil-plant enhancement and yield increase. It is also a suitable means of efficiently utilizing both products as sustainable soil conditioners /fertilizers. It is an equally environmentally friendly alternative to inorganic fertilizers for sustainable agriculture and food security.

LIST OF ABBREVIATIONS

PGPB	=	Plant-growth promoting Rhizobacteria
SOM	=	Soil organic matter
TN	=	Total nitrogen
TP	=	Total phosphorus
TK	=	Total potassium
Az	=	*Azospirillum* bacteria
EDTPA	=	Ethylene diamine tetra-acetic acid

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The author declares there are no actual or potential competing interests, including any financial, personal, or other relationships with other people or organizations.

ACKNOWLEDGEMENTS

The author would like to thank the colleagues of the Department of Soil Science and Soil Ecology, Institute of Earth Sciences of St. Petersburg State University, Saint Petersburg, Russia, colleagues of the Department of Land and Water Technologies, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt, and Cultural Affairs and Missions Sector, Ministry of Higher Education and Scientific Research.

REFERENCES

[1] Popov AI. Biological correction of the productivity of agro-phytocenoses Bulletin of St Petersburg 2006; (1): 136-47.

[2] Hafez M, Rashad M, Popov AI. The biological correction of agro-photosynthesis of soil plant productivity. Plant Nutraion 2020; 43(19): 2929-80. [http://dx.doi.org/10.1080/01904167.2020.1799008]

[3] Yakushev VP. Towards precision farming SPb: Publishing house of Petersburg. Institute of Nuclear Physics 2002; p. 458.

[4] Sarantis M. Europe Entering the Era of Precision Agriculture 2017.https://www.euractiv. com/section/science-policymaking/news/europe-entering-the-era-of-precision-agriculture/

[5] Kudeyarov VN, Semenov VM. Agrochemical research at the Institute of Physicochemical and Biological Problems of Soil Science of the Russian Academy of Sciences. Prob Agro-Chem Ecol 2013; (4): 16-24.

[6] Mineev VG. Chemicalization of agriculture and the natural environment. M. Agropromizdat 1990; 287.

[7] (a) Ermakov EI, Popov AI. Foliar treatment of plants with humic substances as an ecologically harmonious adjustment of the productivity and sustainability of agroecosystems. Bull Russian Agr Acad 2003; (4): 7-11. (b) Gorovaya AI, Orlov DS, Shcherbenko OV. Humic substances: Structure, function, mechanism of action, protector, properties, ecological role. Kiev: NaukovaDumka 1995; 12: 303-3015.

[8] Popov AI, Panina EP, Ergenova SE. Mechanisms of action of humic substances on plants Humus and soil formation. SPb. SPb GAU 2015; 19-26.

[9] Dobbelaere S, Croonenborghs A, Thys A, *et al.* Responses of agronomically important crops to inoculation with *Azospirillum*. Funct Plant Biol 2001; 28(9): 871. [http://dx.doi.org/10.1071/PP01074]

[10] Abdelraouf RE. El-Habbasha, Hozayn, M., Hoballah. Water stress mitigation on growth, yield and quality traits of wheat (*Triticumaestivum L.*) using biofertilizer inoculation. J Appl Sci Res 2013; 9(3): 2135-45.

[11] Güneş A, Turan M, Güllüce M, Şahin F. Nutritional content analysis of plant growth-promoting rhizobacteria species. Eur J Soil Biol 2014; 60: 88-97. [http://dx.doi.org/10.1016/j.ejsobi.2013.10.010]

[12] Hamdia MAE, Shaddad MAK, Doaa MM. Mechanisms of salt tolerance and interactive effects of Azospirillum brasilense inoculation on maize cultivars grown under salt stress conditions. Boyer 1982; pp. 165-74.

[13] Popov AI, Sukhanov PA. Humic preparations are an effective means of biological correction of mineral nutrition of crops, their growth, and development Agro-Pilot. Information-analytical bulletin of the Committee on Agriculture of the Government of the Leningrad Region. SPb 2002; (18-19): 23-41.

[14] Zhou G, Qiu X, Chen L, Zhang C, Ma D, Zhang J. Succession of organics metabolic function of bacterial community in response to addition of earthworm casts and zeolite in maize straw composting. Bioresour Technol 2019; 280: 229-38. [http://dx.doi.org/10.1016/j.biortech.2019.02.015] [PMID: 30772635]

[15] Piccolo A. The supramolecular structure of humic substances: A novel understanding of humus chemistry and implications in soil science. Adv Agron 2002; 75: 57-134. [http://dx.doi.org/10.1016/S0065-2113(02)75003-7]

[16] Popov AI. Humic substances: properties, structure, education / Ed EI Ermakova SPb: Publishing house of St. Petersburg University 2004; p. 248.

[17] Popov AI. Soil fertility and the production process of agricultural crops. Humus and soil formation Coll. scientific Works of St Petersburg State agrarian SPb 1999; 58-62.

[18] Stefanova NA, Popov AI. Influence of foliar treatment with humic substances on the sowing quality of barley seeds Agro-Pilot Information-analytical. bul Committee for rural households of the government of Leningrad area SPb 2002; (18-19): 63-4.

[19] Hafez M, Mohamed AE, Rashad M, Popov AI. The efficiency of application of bacterial and humic preparations to enhance of wheat

(*Triticum aestivum* L.) plant productivity in the arid regions of Egypt. Biotechnol Rep (Amst) 2020; 29: e00584. [http://dx.doi.org/10.1016/j.btre.2020.e00584] [PMID: 33489786]

[20] Climate-Data.org. climate-dataorg 2020. Available from: www.data.org/

[21] FAO. FAO Soils Portal: Management of Calcareous Soils 2016. Available from: http://www.fao.org/soils-portal/soil-management/management of-some-problem-soils/calcareous-soils/ar/

[22] Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biol Fertil Soils 2015; 51(4): 402-15. Available from: http://link.springer.com/10.1007/s00374-015-0996-1 [http://dx.doi.org/10.1007/s00374-015-0996-1]

[23] Bremner JM, Mulvaney CS. Nitrogen total.Method of soil analysis, chemical and microbiological properties second Wisconsin USA. Madison 1982; pp. 575-624.

[24] Page AL, Miller RHDRK. Second Edition // Methods Of Soil Analysis / ed A L Page. Miller R.H.D.R.K 1982.

[25] Anderson JPE, Page AL, Miller RH, Keeney DR. Methods of Soil Analysis and Soil Respiration. 1982.

[26] Casida LE, Klein DA, Santoro T. Soil dehydrogenase activity. Soil Sci 1964; 98: 371-6. [http://dx.doi.org/10.1097/00010694-196412000-00004]

[27] Kandeler E, Gerber H. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol Fertil Soils 1988; 6(1): 68-72. [http://dx.doi.org/10.1007/BF00257924]

[28] French RJ, Schultz JE. Water use efficiency of wheat in a Mediterranean-type environment. 1. The relation between yield, water use and climate. Aust J Agric Res 1984; 35(6): 743-64. a [http://dx.doi.org/10.1071/AR9840743]

[29] Siddique KHM, Tennant D, Perry MW, Belford RK. Water use and water use efficiency of old and modern wheat cultivars in a Mediterranean-type environment. Aust J Agric Res 1990; 41(3): 431-47. b [http://dx.doi.org/10.1071/AR9900431]

[30] Hossain MZ. von Fragstein und Niemsdorff P, Heß J. Effect of different organic wastes on soil properties and plant growth and yield: A review. Sci Agric Bohem 2017; 48(4): 224-37.

[31] Subhan A, Khan QU, Mansoor M, Khan MJ. Effect of organic and inorganic fertilizer on the water use efficiency and yield attributes of wheat under heavy textured soil. Sarhad J Agric 2017; 33(4): 582-90. [http://dx.doi.org/10.17582/journal.sja/2017/33.4.582.590]

[32] Olivares FL, Busato JG, Paula AM, Lima LS, Aguiar NO, Canellas LP. Plant growth promoting bacteria and humic substances: Crop promotion and mechanisms of action. Chem Biol Technol Agric 2017; 4(1): 30. [http://dx.doi.org/10.1186/s40538-017-0112-x]

[33] Canellas LP, Balmori DM, Medici LO, *et al.* A combination of humic substances and Herb *Azospirillum seropedicae* inoculation enhances the growth of maize (Zea mays L.). Plant Soil 2013; 366(1-2): 119-32. [http://dx.doi.org/10.1007/s11104-012-1382-5]

[34] Abdelhamid SEA, Bugaev P D. The role of biological preparations in improving the quality of seeds and increasing the productivity of spring barley. Fertility Journal 2019; 291: 614-8.

[35] Hafez M, Popov AI, Rashad M. The response of saline-sodic soils to reclamation using biological and organic amendments under arid regions of Egypt. E3S Web of Conferences 2021. [http://dx.doi.org/10.1051/e3sconf/202124701047]

[36] Sarkar AK, Gulati JML, Misra B. Path coefficient, and correlation study in wheat. Environ Ecol 1988; 6: 774-5.

[37] Association of Official Seed Analysts (AOSA). Seed vigor testing handbook Contribution to the handbook on seed testing. Ithaca, NY, USA: AOSA 1983; 32.

[38] Hafez M, Popov AI, Rashad M. Influence of agro-industrial wastes and *azospirillum* on nutrients status and grain yield under corn plant growth in arid regions. Biosci Res 2019; 16: 2119-30.https://doi.org/www.isisn.org

[39] Abdul-Ratha AL, ALmamori HA, Abdul-ratha H. Effect of addition of vermicompost, bio and mineral fertilizer on the availability of some nutrients in soil and potato yield. Iraqi J Agric Sci 2020; 51(2): 644-56. [http://dx.doi.org/10.36103/ijas.v51i2.992]

[40] Ehsan K, Mostafa Sh, Maryam DA, Jalal G, Mohsen Z. The Effect of Humic Acid and Biochar on Growth and Nutrients Uptake of Calendula (*Calendula officinalis L*.). Commun Soil Sci Plant Anal 2020; 51(12): 1658-69. [http://dx.doi.org/10.1080/00103624.2020.1791157]

[41] Hafez M, Popov AI, Rashad M. Integrated use of bio-organic fertilizers for enhancing soil fertility–plant nutrition, germination status and initial growth of corn (Zea Mays L.). Environ Technol Innov. Elsevier BV 2021; 21101329 [http://dx.doi.org/10.1016/j.eti.2020.101329]

[42] Hafez M, Popov AI, Rashad M. A novel environmental additives to decrease nitrate level in agriculture wastewater and enhancement nutrient status under. Plant Arch 2020; 20: 3165-72.

[43] Rashad M. Hafez. M, Emran. M, Aboukila. E, N. I. Influence of environment-friendly organic wastes on the properties of sandy soil under growing zea mays L. in arid regions. World Acad Sci Eng Technol 2016; 10(9): 588-94. [http://dx.doi.org/10.5281/zenodo.1126479]