



The combined effect of mineral n, organic and bio-fertilizers on wheat productivity

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ABSTRACT

Two field experiments were carried out at The Experimental Farm, faculty of Agricultural at Dar El Ramed, Fayoum University, Egypt, during two winter seasons of 2015/2016 and 2016/2017. The objectives of experiments were to assess the effect of replacing part of chemical N fertilizers through the organic, bio, and foliar application of organic stimulants on growth parameters (fresh weight of shoots per plant (g), fresh weight of roots per plant (g), dry weight of shoots per plant (g), dry weight of roots per plant (g), Plant height (cm) and chemical composition (plant pigments), macro elements content and enzymatic activities (dehydrogenase activity ($\mu\text{g TPF/ g dry soil/ day}$) & Nitrogenase activity ($\mu\text{ mole C}_2\text{H}_4 / \text{g rhizosphere /h}$ }), of wheat plants (*Triticum aestivum* L.) cv. Sids12. The experiment comprising eleven treatments in three replications was laid out in a randomized block design. Results indicated that, wheat growth criteria (fresh weight of shoots per plant (g), fresh weight of roots per plant (g), dry weight of shoots per plant (g), dry weight of roots per plant (g), Plant height (cm), chlorophyll content of leaves, N, P, and K uptakes in the flag leaf of wheat plants with all treatments receiving bio- fertilizer + 50% N- compost + 50% N-mineral treatment showed the highest significant increase in growth criteria followed by 50% N-mineral +50%N- compost + foliar application of mix organic acids. Combined inoculation of mixture bio-fertilizers with 50% N-mineral +50%N- compost (T9) gave the highest nitrogenase and dehydrogenase activities in the rhizosphere as well as the macronutrient uptakes in the flag leaf of wheat plants. The highest significant increases for all growth characters and yield components of wheat plants were also recorded by the same treatment (T9). It could be concluded that there is a possibility of using microbial inoculations (*Azotobacter* and *Azospirillum*) with the addition of organic manure to soil in order to reduce the amount of mineral fertilizers and to produce a good yield and quality of wheat in addition to improve the biological properties of the soils.

KEY WORDS: bio-fertilizers, growth, nutrient uptake, organic stimulants, productivity, Wheat.

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown in the world. It is used as a staple food grain for urban and rural societies. Despite the significant increase in production, it is not enough to meet the needs of the consumer, which led to increased reliance on import from foreign markets to fill the food gap and thus, led to the formation of a significant burden on the balance of payments and the exposure of food security of Egypt to many risks. Therefore, increasing the productivity of wheat is one of the main goals of the Egyptian agricultural policy. This can be achieved through horizontal expansion of the cultivation of newly reclaimed land and vertical expansion through the use of best agricultural transactions, including the foliar application of organic compounds as Ascorbic acid, potassium humate, bio-fertilizer, and organic manure.

Maintaining soil fertility and the use of plant nutrients in sufficient and balanced amounts is one of the key factors in increasing crop yield. Nitrogen (N) is the most important nutrient supplied to most non-legume crops, including wheat. The most important role of nitrogen in plants is its presence in the structure of the protein and nucleic acids, which are the most important building and information substances of every cell. In addition, nitrogen is also found in chlorophyll that enables the plant to transfer energy from sunlight by photosynthesis. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm, and chlorophyll formed. Moreover, it influences the cell size, leaf

area, and photosynthetic activity (**Diacono et al., 2013 and Piccinin et al., 2013**).

Also, compost is a rich source of organic matter which plays an important role in sustaining soil fertility and hence in sustainable agricultural production. In addition to being a source of plant nutrients, it improves the physicochemical and biological properties of the soil. As a result of these improvements, the soil: (i) becomes more resistant to stresses such as drought, diseases, and toxicity; (ii) helps the crop in improved uptake of plant nutrients; and (iii) possesses an active nutrient cycling capacity because of vigorous microbial activity.

Bio-fertilizers are considered as a promising alternative approach for wheat and other crop species production. These bio-fertilizers are mainly based on beneficial microorganisms in a viable state applied to seed or soil aiming to increase soil fertility and plant growth by increasing the number and biological activities of desired microorganisms in the rhizosphere. As soil is a complex system that can be affected by several factors. Improving such beneficial microbial communities in the soil is an important factor in the biogeochemical cycling of both inorganic and organic nutrients, specifically, in the rhizosphere zone which can increase the availability of nutrients to plants and also improve the soil quality. *Azotobacter* sp. and *Azospirillum* sp. are used as bio-fertilizers in the cultivation of many agricultural crops. The estimated contribution of these free-living N fixing prokaryotes to the N input of soil ranges from 0–60 kg/ha per year (**Vessey, 2003**).

The existence of microbial communities like *Azotobacter* sp. and *Azospirillum* sp. in the rhizosphere promotes the growth of the plant through the cycling and availability of nutrients, increasing the health of roots during the growth stage by competing with root pathogens and increasing the absorption of nutrients and water (Zorita and Canigia, 2009 and Daneshmand *et al.*, 2012).

Recently, great attention has been focused on the possibility of using natural and safe substances in order to improve plant growth. In this concern, antioxidants have synergistic effects on the growth, yield, and yield quality of many plant species. These compounds have beneficial effects on catching the free radicals or the active oxygen that were produced during photosynthesis and respiration processes (Ali *et al.*, 2006). Leaving these free radicals without chelating or catching leads to lipids oxidation and the loss of plasma membrane permeability and the death of cells within the plant tissues. One of the most familiar antioxidants is ascorbic acid (vitamin C) which is synthesized in higher plants and affects plant growth and development.

Ascorbic acid plays an important role in plant growth such as regulation of cell division, photosynthesis, flowering, cell wall expansion, and other developmental processes (Davey *et al.*, 2000 and Barth *et al.*, 2006). The effect of ascorbic acid on plant growth may be due to the fundamental role of ascorbic acid in many metabolic and physiological processes. Ascorbic acid acts as an important cofactor in the biosynthesis of many plant hormones such as gibberellin and abscisic acids (De Tullio & Arrigoni, 2003). Also, ascorbic acid is considered as

one of no enzymatic antioxidants that caused an increment in growth characters of wheat plants, compared with untreated plants (Abd El-Al, 2009).

Humic (HA) acids as a foliar spray to plant as a vital part of their fertilizer program. Humic substances are a vital soil component because they constitute a stable fraction of carbon and improve water holding capacity, pH buffering, and thermal insulation (McDonnell *et al.*, 2001). Humic substances have also, a positive effect on plant growth through their importance of optimum mineral supply, independent of nutrition (Yildirim, 2007). Stimulation of plant growth through increased cell division as well as optimized uptake of nutrients and water, besides, humic acid stimulated the soil microorganisms (Chen *et al.*, 2004). Humic acid plays a prominent role in various physiological and biochemical processes related to environmental stresses. Ghorbani *et al.*, (2010) reported that foliar spray with humic acid has remarkable effects on the vegetative growth of the plant and increases photosynthetic activity and leaf area index of corn.

The objectives of experimentation were to assess the effect of replacing part of chemical N fertilizers through the organic, bio, and foliar application of organic stimulants on growth, productivity, nutrient uptake, and quality of wheat plants (*Triticum aestivum* L.) cv. Sids12 and secondly for improving the biological properties of these soil.

2. MATERIALS AND METHODS

Two field experiments were carried out at El- Fayoum Agricultural Research Station, El- Fayoum Governorate, Egypt, during two winters seasons of 2015/2016 and 2016/2017. The objectives of experimentation were to assess the effect of replacing part of chemical N fertilizers through organic, bio and foliar application of organic stimulants on growth, productivity, nutrient uptake and quality of wheat plants (*Triticum aestivum* L.) cv. Sids 12 and secondly for improving the biological properties of these soils. Randomized samples were taken from the experimental soils before sowing and compost to determine the physical and chemical properties according to **Page *et al.*, (1982) and Klute (1986)** as shown in Tables (1 & 2). The organic manure (compost) was thoroughly mixed with 0-20 cm soil surface layer two weeks before sowing.

Table 1. Physical and chemical properties of the experimental soil (0-20cm) before planting (Average of two years)

Soil properties	Value
	Coarse sand
	07.13
	Fine sand
	20.56
Particle size distribution %	Silt
	23.34
	Clay
	48.97
	Textural class
	Clay
	Chemical analysis
EC (dSm⁻¹) (soil paste extract)	2.7
pH(1:2.5, soil suspension)	8.1
Organic matter (%)	1.7
Total CaCO₃ (%)	2.8
	CO₃⁼
	0.00
Soluble anions (meq/L)	HCO₃⁻
	2.65
	Cl⁻¹
	10.92
	SO₄⁼
	13.56
	Ca⁺⁺
	7.58
Soluble cations (meq/L)	Mg⁺⁺
	3.43
	Na⁺
	15.70
	K⁺
	0.42
	N
	40.2
	P
	9.57
Available macro and micronutrients (mg/kg)	K
	286.6
	Fe
	3.74
	Mn
	0.76
	Zn
	0.37

Table 2. Physical and chemical properties of the compost (Average of two years)

Parameter	Values
EC dS/m (1:10)	7.90
pH (1:10)	6.70
Moisture content (%)	28.00
Organic matter (%)	44.48
Organic carbon (%)	25.80
Total nitrogen (%)	1.42
C/N ratio	18.20
Soluble ammonia-N (ppm)	615.00
Soluble nitrate-N (ppm)	362.00
P (%)	0.57
K (%)	0.82
Total Fe (ppm)	1012
Total Mn (ppm)	116
Total Zn (ppm)	28

The experiment was laid out in Randomized Block Design (RBD) with three replications in plots of 3 m × 3.5 m size in both the years.

Experimental treatments

T1: 100% recommended dose of N-fertilize (Full rec. N dose) =75kgN/fed (as ammonium sulphate 20.6%N)

T2: 100% O.N.F (8.4 ton/fed organic N fertilizer)

T3: 50%M.N.F+ 50 % O.N.F. (4.2 ton/fed organic N fertilizer as compost)

T4: 50 % O.N.F. + 50%M.N.F + (Foliar spray with Ascorbic acid at 400 mg/L) (A1)

T5: 50 % O.N.F. + 50%M.N.F + (Foliar spray with Potassium humate at 400 mg/L) (A2).

T6: 50 % O.N.F. + 50%M.N.F +Mixture of (A1 + A2)

T7: 50 % O.N.F. +50%M.N.F + *Azotobacter* (B1)

T8: 50 % O.N.F. +50%M.N.F + *Azospirillum* (B2)

T9: 50 % O.N.F. + 50%M.N.F + Mixture of (B1+ B2)

T10: 75 % O.N.F +Mixture of (B1 + B2)

T11: 75%M.N.F +Mixture of (B1 + B2)

Agricultural practice

Wheat grains (*Triticum aestivum* L.) cv. Sids 12 were supplied by Wheat Research Dept., Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. The organic manure was thoroughly mixed with 0-20 cm soil surface layer two weeks before sowing. The superphosphate fertilizer (15% P₂O₅) was added as one dose at soil preparation at a rate of 30 kg P₂O₅ / fed. before planting. Mineral N was added as (ammonium sulfate 20.6%N), nitrogen rate was split into four doses: the first (20%) at sowing, the second (50%) at first irrigation (25 days from sowing), the third (20%) at following irrigation (55days from sowing) and the rest of N(100%) was added at the third irrigation (75 days from sowing). 50 kg fed⁻¹ of potassium sulfate fertilizer (48% K₂O) at sowing. The

recommended agronomic practices were followed throughout the experiment for raising the crop.

Grains inoculation

As for bio fertilization, Wheat grains were inoculated with bio-nitrogen fertilizer *Azotobacter* or *Azospirillum* and mixture of N-free fixing bacteria *Azotobacter* and *Azospirillum* before sowing (containing 10^7 cfu ml⁻¹ from each bacterium) were kindly obtained from Microbio. Dept., Soils, Water, and Environ. Res. Instit. Agric. Res. Center, Giza, Egypt. Bacterial inoculation was coated with wheat grains by using gum before planting directly.

Organic compounds applications

Wheat plants were foliar sprayed twice with potassium humate and/or ascorbic acid at the rates of (400 mg/ L) for each compound. The plants were sprayed with potassium humate and/or ascorbic acid at the tillering stage (after 45 days) and at booting stage (65 days) from sowing at the rate of 400 L fed⁻¹.

Determination of plant growth parameters

- Plant samples were taken randomly from each plot at 80 and 100 day from sowing to determine the following: -
- Plant height (80, 100 day from sowing and at harvesting)
- Root fresh and dry weight (g/plant)
- Shoots fresh and dry weight (g/plant)

Chemical analysis of plants

- Contents of N, P and K of leaves were determined

Leaf samples were taken at 80 days from sowing in each season wash by distilled water and dried in an oven at 70 C⁰ for 48 hrs, ground and wet digested and the digested product was used for

determining N with micro-Kjeldahl **Chapman and Pratek, (1961)**. Phosphorus was determined colorimetrically according to **Ryan et al., (1996)** and potassium using flame photometer according to **AOAC, (2005)**.

• The chlorophylls a, b and total chlorophyll were determined according to the method of **Arnon (1949)**, and calculated by the following formula:

$$\text{Chl a} = [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] \times V/1000 \times W$$

$$\text{Chl b} = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times V/1000 \times W$$

V = volume of the extract (mL)

W = weight of the fresh leaf tissue (g)

Assay of enzymes activities:

After 80 days from sowing, five plants were uprooted with rhizosphere soil to evaluate enzyme activities i.e. N₂-ase and dehydrogenase activities. The N₂-fixers bacteria were undertaken to determine their Nitrogenase enzyme activity (μ mole C₂H₄ / g rhizosphere /h) was measured in wheat rhizospheric roots as acetylene reduction assay (ARA) by GC analysis using a 5880 HP chromatograph (Hewlett Packard Inc Palo Alto, CA, USA) with an ionization flame detector at 135°C according to (**Somasegaran and Hoben, 1994**) and the Dehydrogenase enzyme activity (μ g TPF/ g dry soil/ day) in the rhizosphere of wheat plants was determined according to the method described by (**Skujins, 1976**).

Statistical Analysis

All data were subjected to the analysis of variance (ANOVA) using least significant difference (LSD) at 0.05 according to **Snedecor and Cochran, (1990)**.

3. RESULTS AND DISCUSSION

Growth parameters of wheat

Plant height (cm):

The data on plant height at 80, 100 DAS and at harvest as influenced by the application of different treatments of N-sources fertilizers, bio, and foliar application of organic stimulants are presented in Fig. (1). The data indicated that the highest mean plant height at three growth stages was significantly affected by inoculation of wheat grains with bio-fertilizers treatments in combination with 50% N- mineral +50% N- organic followed by application of a mixture of potassium humate and ascorbic acid as a foliar application with 50% N- mineral +50% N- organic while the lowest mean plant height was obtained by T₂. The highest increases in plant height were obtained by T₉ (111.55cm), while the lowest values were obtained by T₂ (92.77cm).

These results were true at the three growth stages. The increased plant height at 50% N-mineral +50%N- compost + mixture bio could be attributed to the increasingly adequate supply of nitrogen in this treatment which includes N-mineral, N- organic and bio- fertilizers since nitrogen has an important role in encouraging cell elongation, cell division and consequently increasing vegetative growth and activation of photosynthesis process which enhance the amount of metabolites necessary for building plant organs.

Moreover, the application of 50% N-mineral plus 50%N- compost may provide balanced micro and macronutrients as well as enhanced availability of plant nutrients, which would help to enhance the metabolic activity of microorganisms and improvement of plant growth. These results are in agreement with those obtained by Vanlauwe et al., (2002).

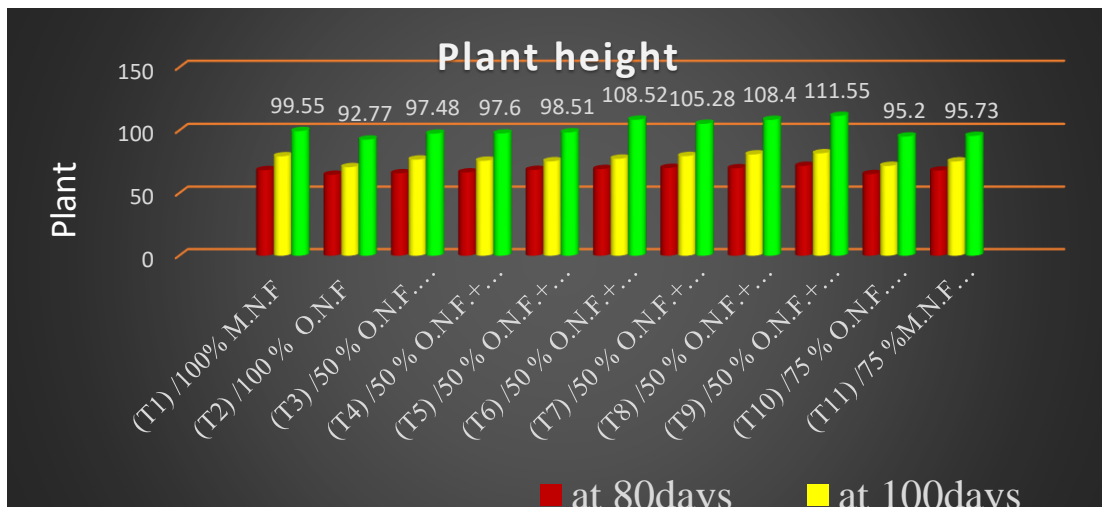


Fig. 1. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on plant height (cm) at different intervals

On the other hand, the enhancing effect of using organic fertilizer on plant growth may be due to that such organic manures play a role as a soil amendment which improves water holding capacity and increase macro and micro elements availability in the rhizosphere around roots system which in turns increased plant growth. These results are in agreement with those obtained by (Shoman, *et al.*, 2006 and Hosam El-Din, 2007). This promoting effect could be related to the N supplementary effect of N fixing bacteria (used as bio N-fertilizer) to plants due to their ability to fix free molecular atmospheric nitrogen as well as the role of these bacteria in improving the availability of soil elements through secreting chelating substances (such as organic acids) which are important for solubilizing sparingly soluble inorganic compounds to more available forms for plants uptake **Kandil *et al.*, (2011)**.

It was clearly evident that treatments that received 50% N- mineral +50% N- organic or 75%N- mineral in combination with a mixture of N- fixers recorded higher values of plant height when compared with 50% N- mineral +50% N- organic or 100% as organic in the form of compost . Mixed inoculums, generally had a more favorable effect on the majority of studied parameters than single inoculants. These results indicated that mixed inoculants promote greater beneficial effects than single strain inoculate and this depends on selecting the most compatible efficient strains.

Foliar spray of growth-promoting substances i.e. K- humate, ascorbic acid (AA) as well as a mixture of K- humate and ascorbic acid as a foliar application had a significant effect on plant height. The

highest value of plant height (108.52cm) was scored from plants sprayed by the application of a mixture of K- humate and ascorbic acid (AA) compared to control (spraying by water). These results were in harmony with those obtained by **Bolkhina *et al.*, (2003)**, who stated that ascorbic acid is the most abundant antioxidant which protects the cell, ascorbic acid is presently measured to be a regulator on plant growth and development owing to their effects on cell division, and differentiation and added that ascorbic acid is involved in a wide range of important function as antioxidant defense, photo-protection, and regulation of photosynthesis processes and growth.

Moreover, the positive effect of ascorbic acid(AA) on growth parameters may be due to that, ascorbic acid has a stimulatory effect of many physiological processes, such as respiration activities, cell division and many enzymes activities as reported by **Zewail (2007) and Bakry *et al.*, (2012)**. It also plays an important role in the regulation of photosynthetic carbon reduction. Also, **Yang *et al.*, (2004)** reported that the humic materials can affect direct and indirect physiological processes of plant growth. Their direct effects including an increase in cell membrane permeability, respiration, nucleic acid biosynthesis, ion uptake, enzyme activity, and sub-enzyme activity. Humic acid (HA) reduces the amount of fertilizer consumption and makes plant tolerant against heat stress, drought stress, cold, diseases, insects, and other environmental and agricultural pressures. Also, the production of total plant weight and increases yield.

Root weight per plant

Data of Fig. (2) showed that root fresh and dry weights were substantially

improved by the application of N-mineral+ N-organic in combination with either some bio or foliar organic stimulants. Maximum fresh weight of root (6.46g) was observed at 100 days after sowing for treatment T9 (50% N-mineral +50%N- compost + mixture bio) which was statistically at par with treatment T6 (50% N-mineral +50%N- compost + foliar application of mix organic stimulants) having a fresh root weight of (6.43 g). Treatment T6 was followed by treatment T7 which was having fresh root weight of (5.97g). The plant samples from treatment T2 had a minimum of root fresh weight (3.17g) at 100 days after sowing.

The dry weight of roots increased from 80 to 100 days after planting. The maximum dry weight of root (3.88g) was observed in plant samples at 100 days with treatment T9 (50% N-mineral +50%N-compost+ mixture of N- fixers) followed

by treatment T6 which was having a dry root weight of (3.64g). These results may be due to the free-living nitrogen-fixing bacteria such as *Azotobacter* and *Azospirillum* have the ability not only to fix nitrogen, but also to produce adequate amounts of certain phytohormones of Gibberellic Acid (GA3), Indol Acetic Acid (IAA), and cytokinins ,which could stimulate plant growth With increasing the surface area per unit, root length and enhanced the root hair branching with an eventual increase on the uptake of nutrients from the soil.In addition, bacteria have a beneficial effects on reducing soil pH by secreting organic acids (e.g., acetic, propionic, fumaric, and succinic) which leading to change nutrients to available forms ready for uptake by plants. These results were in harmony with those obtained by **Vacheron et al., (2013)**.

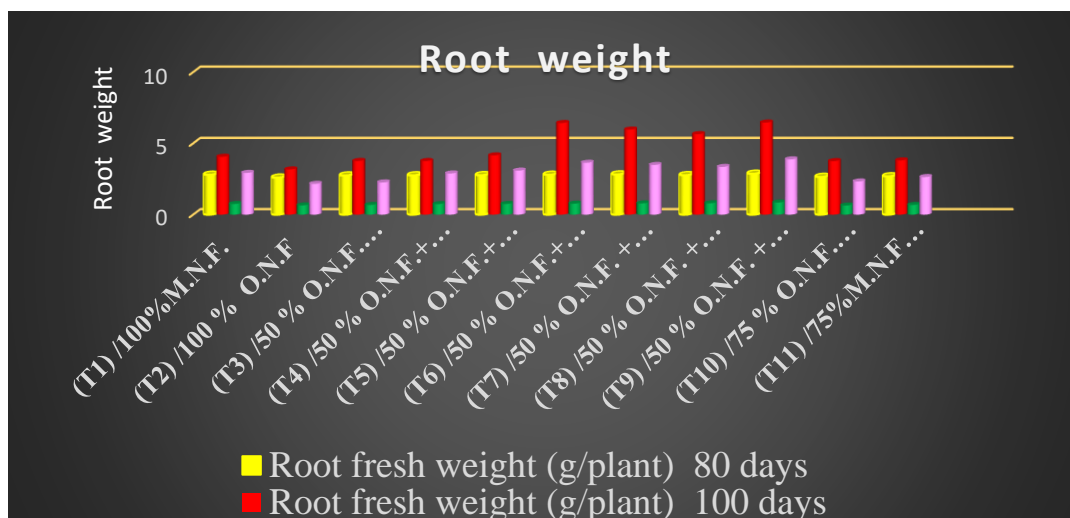


Fig. 2. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on root weight per plant at different intervals

It is also clear from Fig.(2), data indicate that foliar application with a mixture of organic stimulants exhibited a

superior effect on root dry and fresh weights than those un-sprayed. The beneficial effect of organic stimulants on

the growth parameter of wheat plants may be due to that organic stimulants as a natural source for cytokines had stimulatory effects on cell division and enlargement, protein, and nucleic acid synthesis and chlorophylls formation (Abdel-Hameed *et al.*, 2004). These results were in harmony with those obtained by Delfine *et al.*, (2005) indicated that K- humate was in general beneficial to shoot and root growth of corn plants.

In addition, the presence of humic molecules raised the effect on plants of the fertilization based on N, phosphorus, and potassium. K- humate influences respiration and photosynthesis, the formation of a complex with mineral ions, catalysis to enzymes, and stimulation of nucleic acid metabolism. Also, Xu *et al.*, (2015) indicated that ascorbic acid (AA) plays role in plant growth and development, cell division, cell wall metabolism, and cell expansion shoot apical meristem formation, root development, photosynthesis, regulation of fluorescence, and regulation of leaf senescence. Also, it is a cofactor for enzyme activity, and effects on plant anti-oxidation capacity, heavy metal evacuation and detoxification, and stress defense.

Shoot weight per plant

According to the data in Fig. (3), fresh and dry weights of shoot/plant of wheat plants were significantly increased as a result of applying different treatments. Results indicated that fresh and dry weights of shoot / plant were higher in treatments receiving bio -fertilizer supplemented with 50 % O.N.F. + 50% M.N.F. It is obvious that, T9 treatment showed the highest significant increase in fresh and dry weights of shoot / plant followed by T8 and T7. The maximum fresh weight of shoot was recorded at 100 DAS (44.37g) for treatment T9 which was significantly higher than that of all other treatments, while the lowest fresh weight of shoot (24.59g) was in the T10. The favorable effect of nitrogen fertilizer may be due to N stimulation of plant growth, which would increase the amount of light energy intercepted by leaves and increase photosynthetic pigments and photosynthesis, and in turn, increase synthesized metabolites and consequently fresh and dry weights of shoot/plant. Also, increased fresh and dry weights of shoot/plant due to the application of bio fertilizer as well as producing growth-regulating hormones.

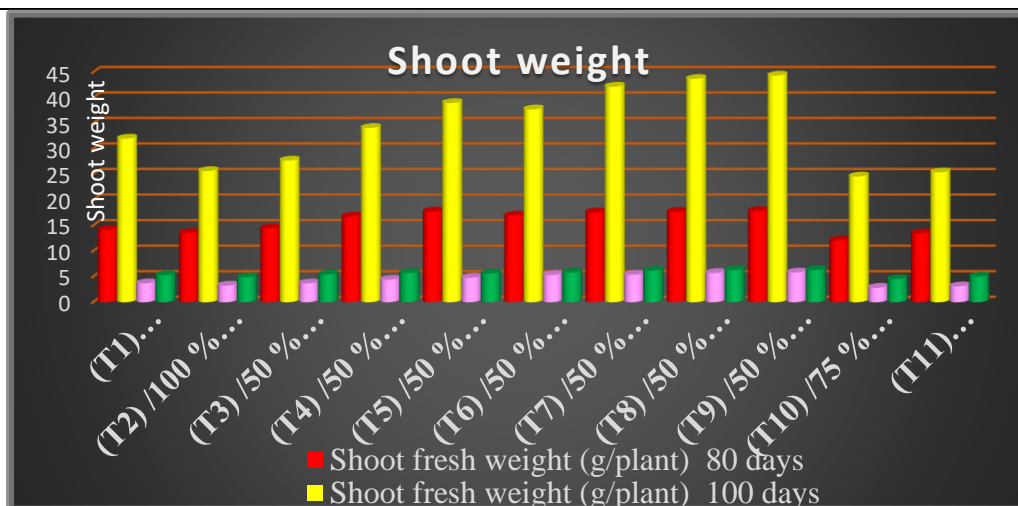


Fig. 3. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on shoot weight per plant at different interval

Amin et al., (2008) found that K-humate or ascorbic acid (AA) and their combinations increased fresh weights of the wheat plant and that might be attributed to an increase in the number of tillers and spikes as well as leaf area, leading to increased photosynthetic activity. The increase of shoot dry weight might be due to the essential role of ascorbic acid in the regulation of different physiological processes including plant growth and development that were reflected in improving vegetative growth such as stomata behavior and regulation is a very important factor for photosynthetic ability.

Nutrient Uptake

The results in Figs. (4, 5 and 6) showed clearly that all treatments caused a significant increase in N, P, and K uptakes in the flag leaf of wheat plants at 100 DAS. Results indicated that N, P, and K uptakes in the flag leaf of wheat plants were higher in treatments receiving bio-fertilizer supplemented with 50 % O.N.F. + 50%M.N.F followed by the application of a mixture of organic stimulants with 50 %

O.N.F. +50%M.N.F. It is obvious that T9 treatment showed the highest significant increase in N, P, and K uptakes followed by and T8 and T7 may be referred to as the increase in microorganisms activity and increasing the adsorbing capacity of essential nutrients against leaching. Moreover, adding mineral + organic fertilizer together improves the mineralization of organic-N. These results might be due to that *Azotobacter* and *Azospirillum* species increased the solubility of soil nutrients as mentioned before. Furthermore, *Azotobacter* and *Azospirillum* could produce IAA and cytokinins which increase the surface area per unit root length and were responsible for root hair branching with an eventual increase in the acquisition of nutrients from the soil. This might be also attributed to the increased activity and efficiency of bacteria in the reduction of soil pH by secreting organic acid, i.e. acetic, propionic, fumaric, and succinic, and consequently more solubility and availability of nutrients for plants. These

results were in harmony with those obtained by **El-Kouny et al., (2004)**.

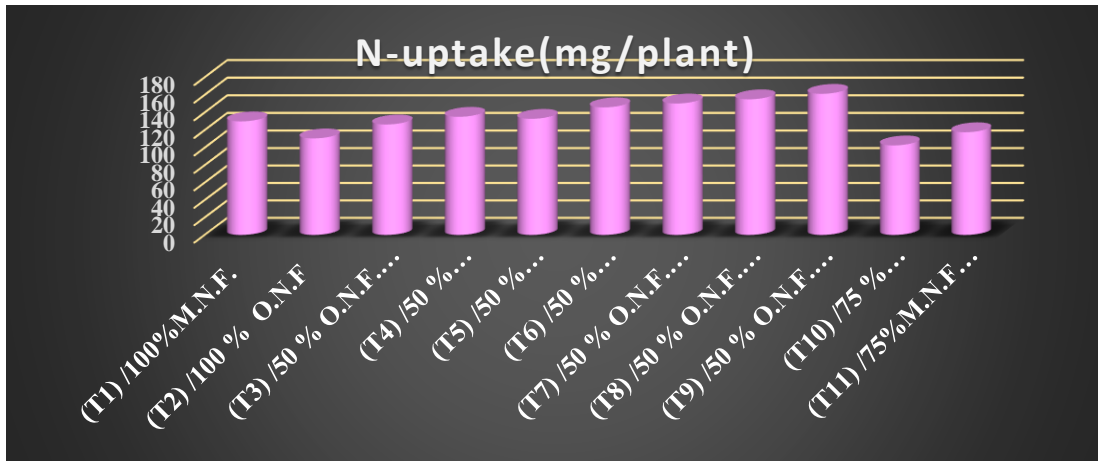


Fig. 4. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on N-uptake in the flag leaf of wheat plants at 100 DAS

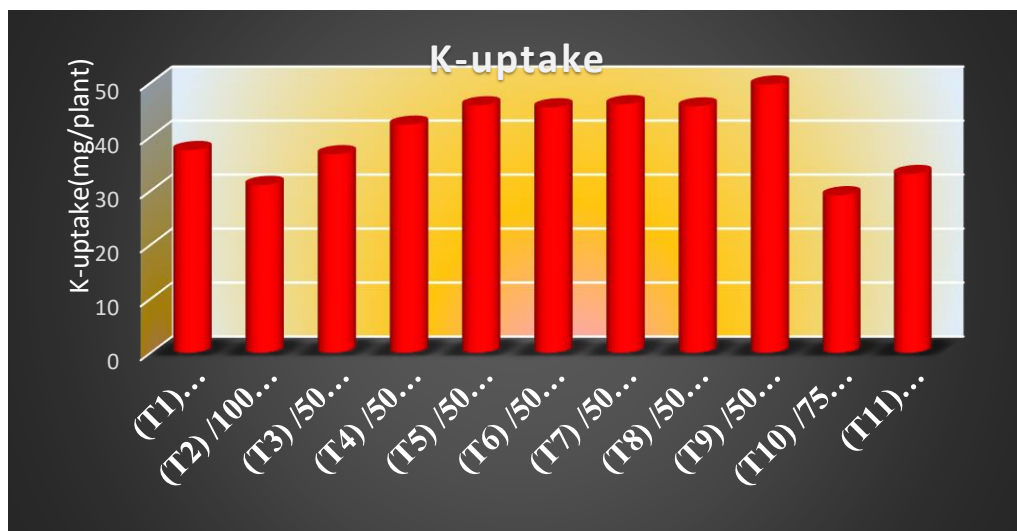


Fig. 5. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on P-uptake in the flag leaf of wheat plants at 100 DAS

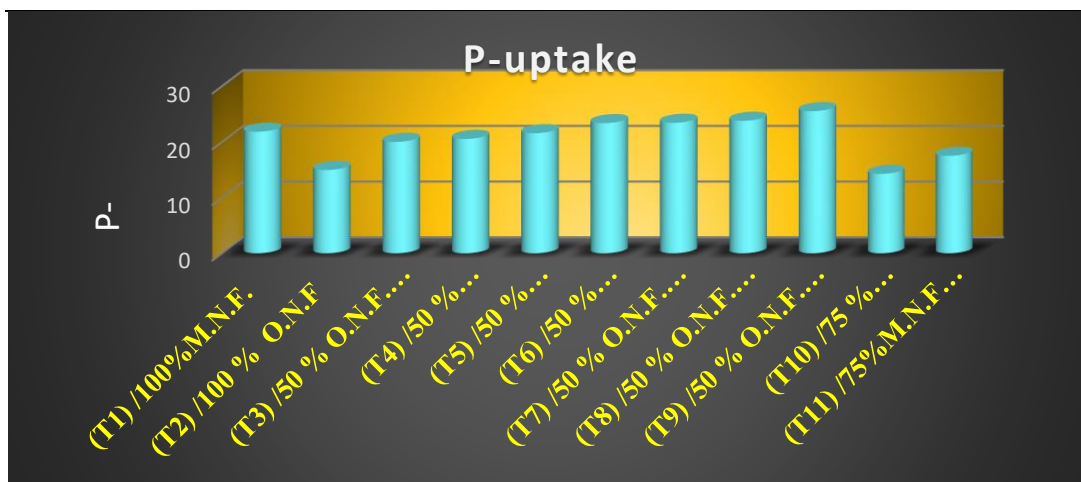


Fig. 6. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on K-uptake in the flag leaf of wheat plants at 100 DAS

The beneficial effects of using organic fertilizers along with mineral -N fertilizer on increasing nutrients uptake of the wheat plant could be due to their effect on providing plants with their requirements from different nutrients at a longer time as well as their effect on increasing the availability of nutrients in the soil for uptake by plants and enhancing the nutritional status of the plants in favors of yield and quality. Moreover, **El-Kouny et al., (2004)** reported that the increasing of N, P and contents and uptake in wheat plants with organic manure application, may be attributed to the mineralization of organic matter and slow releasing of minerals in an available form from organic manure or may be due to the effect of several organic acids, produced during manure decomposition, which solubilize the native P of the soil and partly due to the formation of a coating on CaCO_3 which did not allow to react with soil P, and thus P availability increased and consequently the content in plant increased. Also, this may be due to the decomposition of organic manure supplying more available

nutrients as well as the formation of organic and inorganic acids during decomposition which slightly reduce the soil pH which in turn enhanced the solubility and availability of N, P, and K. These results were in harmony with those obtained by **El-Kouny et al., (2004)**.

Mohamed et al., (2015) reported that the addition of organic matter plays a major role in soil fertility through different functions: (1) the storage of nutrients like P, Ca, K, and Mg. They have released during organic matter decomposition and their dynamics are thus dependent on that of organic matter, (2) the increase in CEC (cation exchange capacity). This function is linked to the surface properties of soil organic and organic mineral components: Cation and anion exchange capacity, physical and chemical adsorption, and desorption properties. These properties define the availability of some nutrients, cation equilibrium, and the efficiency of fertilizers and xenobiotic molecules, (3) the improvement of soil structural stability, and (4) the stimulation of faunal, microbial, and enzymatic activities that

determines carbon, nitrogen, and phosphorus and sulfur cycles.

Regarding the effect of foliar applications (potassium humate and/or ascorbic acid) on N, P, and K uptake in leaves data showed that different foliar applications caused a significant increase in N, P, and K uptake as compared with the control treatment. The highest values were obtained from plants sprayed with potassium humate and ascorbic acid followed by potassium humate. The incremental effect of organic stimulants might attribute to the influence on metabolism and their stimulating effect on photosynthetic pigments and enzyme activity that in turn increased the vegetative growth of the plant.

Data also indicated that, increasing in N, P, and K uptake response to K-humate could be attributed to increasing the efficiency of the photosynthesis process, which leads to the accumulation of nutrients in the plant and stimulation the plant to increase the number of tillers, and it has a role in increasing the cytokines that have an obvious effect in promoting the growth of plant **El-Bassiony et al., (2014)**. Also, humic substances (HS) have been known to have an enhancing effect on plant shoot and root growth, through effects on cell membranes by solubilization of macro and micro mineral nutrients, promoting photosynthesis, regulating enzyme activity, behaving like plant hormones, reducing toxic elements, and increasing microbial populations **Delfine et al., (2005)**.

The enhancement effect of foliar spray of ascorbic acid on N, P and K may be attributed to increases in leaf area and photosynthetic pigments thus increase the photosynthesis process, and hence more

photosynthesis being created as well as enhancement of mineral translocation from roots to leaves. These results were in harmony with those obtained by **Shawky (2003)**.

Regarding the effect of ascorbic acid, **Dawa et al., (2014)** showed that foliar spray of ascorbic acid, enhanced plant growth, yield, and quality in a number of plant species due to its action on different physiological and metabolic processes and involved in the regulation of photosynthesis, cell expansion, root elongation, and trans-membrane electron transport and in several important enzyme reactions, such as violaxanthin deep oxidase these led to increasing macro and microelement uptake.

Photosynthetic Pigments

The results in Fig. (7) Indicated that the addition of different treatments significantly increased pigment concentrations in leaves. Chlorophyll a, b, and total chlorophyll (a + b) were substantially improved by the application of N- mineral+ N-organic in combination with either some bio or foliar organic stimulants. Maximum chlorophyll a, b, and total chlorophyll (a+ b) were observed for treatment T9 followed by T6. The increase of chlorophyll a, b, and total chlorophyll (a+ b) may be due to more photosynthetic activities of the plant on the account of an adequate supply of nitrogen in this treatment which include N-mineral, N-compost, and bio fertilizers since nitrogen has an important role in encouraging cell elongation, cell division and consequently increasing vegetative growth and activation of photosynthesis process which enhance the amount of metabolites necessary for building plant organs.

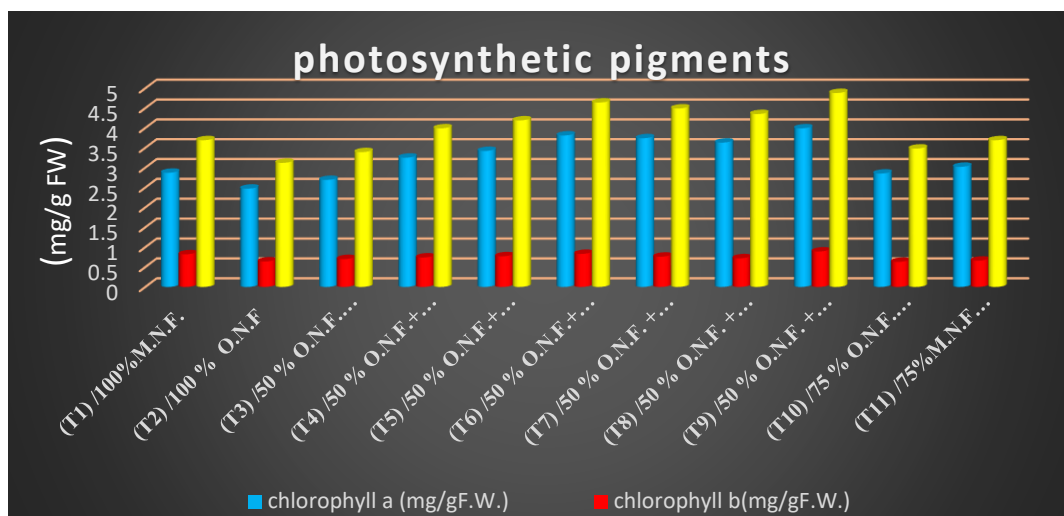


Fig. 7. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on chlorophyll a, b and total chlorophyll (a+ b) in the flag leaf of wheat plants at 80 DAS

The obtained results agree with those observed by a number of investigators, for example, **Theago et al., (2014)** found a close correlation between chlorophyll content in the leaves and the total nitrogen concentration in the plants, which is independent of the conditions of the growth of the plants. It is explained by nitrogen, which is a structural element of chlorophyll and protein molecules and has an impact on the formation of chloroplasts and chlorophyll accumulation in them. Concerning the effect of bio-fertilizer on chlorophyll a, b and total chlorophyll (a+ b) data showed that chlorophyll a, b, and total chlorophyll (a+ b) in leaves were significantly increased with inoculation of grains pre-sowing compared to the control treatments (without inoculation). The positive effect of bio-fertilizer treatments on chlorophyll a, b, and total chlorophyll (a+ b) may be attributed to their N-fixing activity and the production of plant growth-promoting substances such as IAA, gibberellins, and

cytokinins-like substances (**Zaki et al., 2007**).

Data also indicated that foliar spraying of organic stimulants significantly increased the mean values of chlorophyll a, b, and total chlorophyll (a+ b). The promoting effect of using mix organic stimulants as a foliar application could be due to the role of ascorbic acid (AA) and/ or K- humate on the growth parameter of wheat plants may be due to that organic stimulant as a natural source for cytokines had stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis and chlorophylls formation (**Abdel-Hameed et al., 2004**). K- humate might be caused by an enhancement in the synthesis of the chlorophyll and/or delayed chlorophyll degradation even under different stress as reported by **Nardi et al., (2002)**.

The synergistic effect of ascorbic acid (AA) on leaf total chlorophyll content may be attributed to its major role in increasing its endogenous concentration

which regulates and protect photosynthetic processes and in turn probably led to more synthesis of pigments including total chlorophylls content. Our results are supported by the results of **Shao et al., (2008)** who revealed that the application of ascorbic acid increased the chlorophyll content of the plant.

Nitrogenase and dehydrogenase activities

Effect of N- sources fertilizers, bio, and foliar application of organic stimulants on soil biological activities after 100 days of sowing were showed in Fig. (8 & 9). Data obtained revealed that N- sources fertilizers and bio-fertilizers resulted in more enhancements on nitrogenase (μ mole C_2H_4/g rhizosphere /h.) and dehydrogenase activities (μg TPF/g dry soil/ 24 hrs) in wheat (*Triticum aestivum* L.) rhizosphere at 100 days. These results were in harmony with those obtained by **Habib et al., (2010)** who reported that N-fixing bacteria combined with organic manure have more response and enhanced the soil's biological activity in terms of increasing dehydrogenase and nitrogenase activity.

In this respect, **Al-Haddad et al., (2014)** showed that the highest increase in percentages of enzyme activity (dehydrogenase) was recorded in the treatment inoculated with the mixed microbial treatment (*Azotobacter* and *Azospirillum*) rather than that of individual treatments. It was also reported that bacteria can be incorporated into the soil as organic matter and also as a source of enzymes as they produce extracellular acid and alkaline phosphatases that are active in solution or located in the per plasmatic space of the cell wall.

Both biomass exopolysaccharides incorporated into the soil induced a growth promotion of other microorganisms and increased the activity of soil enzymes that participate in the liberation of nutrients required by plants (**Cairea et al., 2000**). Regarding, dehydrogenase enzyme activity, this enzyme is considered as an indicator of the abundance of microbial groups in the rhizospheric area. Treatment T9 that involved a mixture of N₂- fixers + 50% N-mineral +50%N- compost obtained the highest activity of this enzyme that reflected The increase of microbial activity in the soil and the symbiotic relations among the microorganisms.

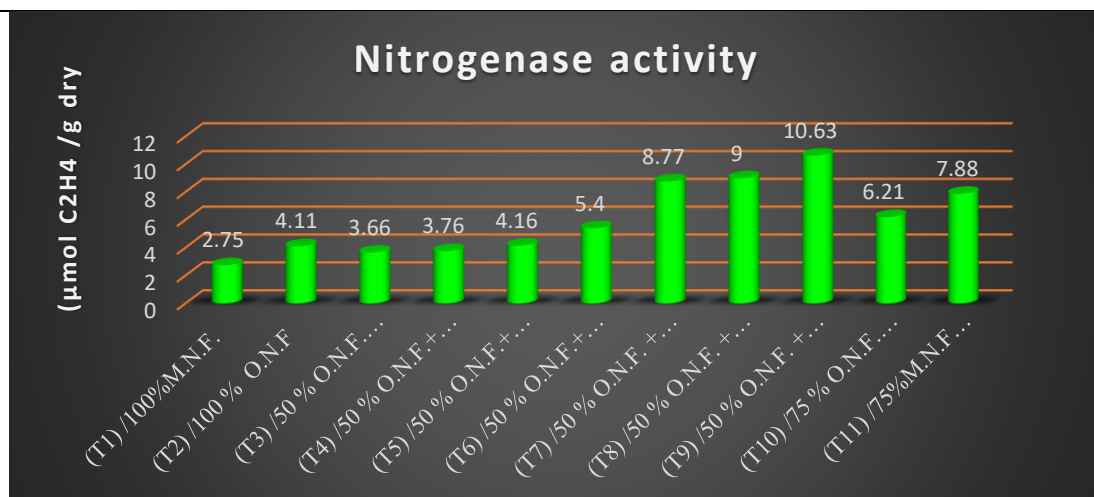


Fig 8. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on nitrogenase activity (μmol C₂H₄ /g dry soil/h)

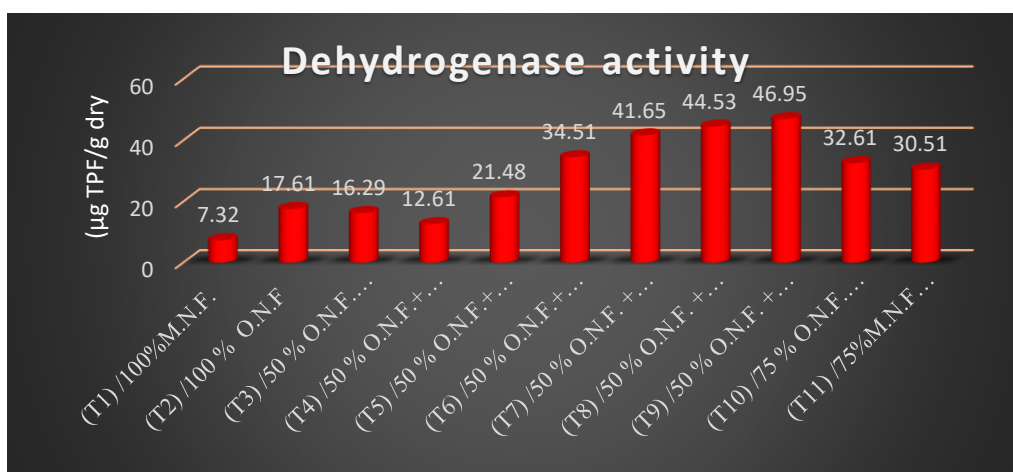


Fig 9. Effect of N- sources fertilizers, bio and foliar application of organic stimulants on Dehydrogenase activity (μg TPF/g dry soil /day)

The activities of nitrogenase and dehydrogenase enzymes (Figs. 8 and 9) markedly increased with treatment T9 and T8 where the highest activity of N₂-ase enzyme being 10.63 and 9 μmole C₂H₄ / gm soil were recorded after 100 days, respectively. The corresponding figures for dehydrogenase activity were 46.95 and 44.53 μg TPF/ gm soil with T9 and T8 after 100 days of sowing, respectively. The least activities of both enzymes were recorded

with control treatment. The microbial activity in the rhizospheric area is limited by the ability of beneficial microorganisms to exist in a large population and consequently increase their enzyme activity particularly, dehydrogenase and nitrogenase enzymes.

Abou-Zeid and Bakry, (2011) concluded that bacterial inoculation, generally, enhanced the soil's biological activity in terms of increasing

dehydrogenase and nitrogenase enzyme activities. This increase in the soil's biological activity increased the soil fertility, in turn, that is reflected positively in crop production. These increases may attribute to the N-fixing bacteria inoculation promote the microbial activity of all other microbes in the rhizosphere zone and consequently increased biological activity in soil (**Tantawi, 2006**).

Inoculation of wheat grains with N₂-fixing bacteria improved nitrogenase (N₂-ase) activity of wheat roots and enhanced dehydrogenase (DHA) enzyme activity in rhizosphere soil of wheat after 100 days from planting compared to the untreated control. Also, the joint application of T9 that involved a mixture of N- fixers + 50% N-mineral +50%N-compost resulted in maximum values of soil biological parameters followed by T8, respectively. The enhancements of dehydrogenase and nitrogenase over control were relatively higher in all treatments, especially that of a mixture of N₂- fixers inoculation. These effects could be attributed to the improvement in soil physical, chemical, and biological properties resulting in more release of available nutrient elements to be absorbed by plant roots (**Metin et al ., 2010**). This can affect the physiological process such as photosynthesis activity as well as the utilization of carbohydrates in addition to water use efficiency by different plants (**Massoud, 2005**). Consequently, when wheat cultivated soil treated with organic manure and bio-fertilizers, it led to the positive response in all growth parameters besides the activity of nitrogenase and dehydrogenase enzymes where the increase of both enzymes at 100 days of sowing due to the favorable effects of the

combination between humic acid (HA) and bio-fertilizers (*Azotobacter* and *Azospirillum*). Bio-fertilizers are also living or biologically active products or microbial inoculants of bacteria, algae, and fungi (separately or in combination) which are able to enrich the soil with nitrogen, phosphorus, organic matter, etc. Bio fertilizers act as a compound that enriches the nutrient quality of the soil by using microorganisms that establish symbiotic relationships with the plants. Bio fertilizers are low-cost renewable sources of plant nutrients that supplement chemical fertilizers. Bio-fertilizers generate plant nutrients like nitrogen and phosphorous through their activities in the soil or rhizosphere and make them available to the plants on the soil.

The use of bio fertilizers is gaining importance because of the proper maintenance of soil health, the minimization of environmental pollution, and the cut-down in the use of chemicals. Bio-fertilizers are one of the important components of integrated nutrient management, as they are a cost-effective and renewable source of plant nutrients to supplement and/or replace the chemical fertilizers for sustainable agriculture. These are preparations containing living cells or latent cells of efficient strains of microorganisms that help the uptake of nutrients in crop plants by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants. This behavior could be explained on the basis of the beneficial effects of bacteria and organic manure on nutrient availability and consequently,

increasing the production of some growth regulators (auxins and vital enzymes) involving nitrogenase, where the nitrogenase efficiency increases with increasing the efficiency of N- fixing bacteria. (Metin *et al.*, 2010).

The organic manure and humic acid association with plant roots usually increase the growth of plants especially in the presence of N – fixing bacteria, which enhances water and nutrient uptake especially phosphorus. Therefore, organic manure and N – fixers have the potential to be considered as a major component of the sustainable agroecosystem (Mardukhi *et al.*, 2011). Humic acid (HA) had a direct effect on soil microbiology by increasing plant exudates, which enhanced the activity of microorganisms. Humic acid showed the highest increase in dehydrogenase and nitrogenase activity. This study revealed that the foliar application of HA improved plant growth and yield quantity and quality of wheat

plants. Additionally, HA has the advantage as an effective and environmentally friendly agent.

Conclusion

Organic and bio fertilizer have their advantages in terms of nutrient supply, soil quality, and crop growth. As well, biological fertilization with N₂ fixing bacteria is of great importance in increasing crop production and saving mineral fertilizers. It could be concluded that there is a possibility of using microbial inoculations (*Azotobacter* and *Azospirillum*) with the addition of organic manure to soil to reduce the amount of mineral fertilizers to produce a good yield and quality of wheat grains, where the microbial inoculations are benefited at nitrogen fixation, mobilizing phosphate, potassium, and micronutrients in soil, besides it, can produce plant growth-promoting that led to improving wheat yield.

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الملخص العربي

التأثير المشترك للأسمدة النيتروجينية المعدنية والعضوية والحيوية على إنتاجية القمح

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أقيمت تجربتان حقليتان بمحطة البحوث الزراعية لكلية الزراعة- جامعة الفيوم بناحية دار الرماد بمحافظة الفيوم- مصر ، خلال موسمي الشتاء في ٢٠١٥/٢٠١٦ و ٢٠١٦/٢٠١٧ ، لدراسة تأثير استبدال جزء من التسميد النيتروجيني المعدنى باستخدام التسميد الحيوي و العضوي و رش الأوراق باستخدام بعض المنشطات العضوية (هيوما توتاسيوم وحمض الاسكوربيك) على بعض صفات النمو الخضرى و على محتوى النبات من بعض المركبات الكيميائية (الصبغات النباتية – بعض العناصر الكبرى (النيتروجين ، الفسفور والبوتاسيوم)- و على نشاط بعض الإنزيمات (النيتروجيناز و الدهيدروجيناز) على نباتات القمح (*Triticum aestivum* L) صنف. سدس ١٢ وثنائياً لتحسين الخواص البيولوجية لهذه التربة.

تم وضع التجربة التي تضمنت أحد عشر معاملة في ثلاث مكررات في تصميم قطاعات كاملة العشوائية.

ويمكن تلخيص اهم النتائج المتحصل عليها كالآتي:

- تم تسجيل زيادة معنوية على طول النبات عند عمر ٨٠ و ١٠٠ يوم وعند الحصاد والوزن الطازج للجذر لكل نبات (جم) ، الوزن الجاف للجذور لكل نبات (جم) ، الوزن الطازج للمجموع الخضري لكل نبات (جم)، الوزن الجاف للمجموع الخضري لكل نبات (جم)، ارتفاع النبات (سم) ، ومحتوى الكلوروفيل في الأوراق ، وامتصاص النترجين والفسفور والبوتاسيوم في ورقة العلم مع تلقح حبوب القمح بمخلوط الأسمدة الحيوية بالاشتراك مع ٥٠% N- معدنى + ٥٠% N- عضوي يليها ٥٠% N- معدنى + ٥٠% N- عضوي مع الرش الورقي بالمنشطات العضوية.
- هناك زيادة معنوية في محتوى الأوراق من صبغات البناء الضوئي (كلوروفيل أ- كلوروفيل ب و الكلوروفيل الكلى) وكانت افضل النتائج مع معاملة (٩) 50% من النيتروجين المعدنى + 50% من النيتروجين العضوى + مخلوط التلقح الحيوى البكتيرى، يليها معاملة (٦) ٥٠% من النيتروجين المعدنى + 50% من النيتروجين العضوى + رش الأوراق باستخدام خليط من (حمض الاسكوربيك + هيوما توتاسيوم) حيث أدى الرش الورقي بالمنشطات تحت نفس مستويات النيتروجين إلى زيادة معنوية في محتوى الكلوروفيل.
- فيما يتعلق بتأثير المعاملات المختلفة على نشاط إنزيم النيتروجيناز و نشاط إنزيم الدهيدروجيناز في منطقة جذور نباتات القمح أعطت معاملة ٩ افضل القيم على نشاط إنزيم النيتروجيناز و نشاط إنزيم الدهيدروجيناز.
- توصي الدراسة بالاستخدام المشترك للأسمدة الحيوية مع التسميد العضوى والمعدنى حيث كانت أكثر فعالية فى تحسين النمو و لتعزيز خصوبة التربة و إنتاجيتها تحت نظم الزراعة المستدامة.