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# Effect of Bio-Organic Amendments on Growth, Yield, Nodulation Status, and Microbial Activity in the Rhizosphere Soil of Peanut Plants under Sandy Soil Conditions

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## ABSTRACT

In a field experiment, plant growth, nodulation status, yield, mineral content, and the rhizosphere enzyme activities of peanut (*Arachis hypogaea* L), cultivated in sandy loam soil were studied under the effect of the amendment with humic acid and the inoculation with plant growth promoting rhizobacteria (PGPR) that applied as foliar and\or as seed coating. *Bradyrhizobium* was used as a single inoculum and\or accompanied with PGPR (*Azospirillum* sp.+ *Paeniacillus polymyxa*+ *Serratia marcescens*) with or without humic acid. Results revealed that inoculation with *Bradyrhizobium* as a single inoculum or accompanied with PGPR, increased significantly shoot dry weight, and yield of peanut under coating and foliar applications. Incorporation of humic acid at two rates of 3L/fed and 5L/fed as foliar and seed coating gave the highest significant values for all tested parameters. inoculation with *Bradyrhizobium* as single inoculum or accompanied with PGPR enhanced the nodulation status and nitrogenase activity. Also, the values of nodules and nitrogenase activity achieved by coating application method were higher than those recorded by foliar application. In addition, the combined application of coinoculation *Bradyrhizobium* and PGPR accompanied with humic acid led to significant increases in the content of N, P, and K in shoot plant and in soil. Likewise, humic acid amendment can stimulate the microbial activities in the rhizosphere soil. Higher values of dehydrogenase activity in the rhizosphere soil and carbon dioxide were found with coinoculation and foliar application of humic acid.

**Keywords:** Plant growth promoting Rhizobacteria, nodulation, Humic acid and soil respiration.

## INTRODUCTION

Nitrogen has an important role in several processes for convenient plant development and reproduction because it is a component of chlorophyll, amino acids, proteins, and enzymes. A proper nitrogen supply is required for efficient plant metabolism and increasing plant photosynthesis and yield (Erisman *et al.* 2010). Introduction of nitrogen is directly revealing a direct positive impact and most limiting factor on the overall yield of most crops. In this concern, Barik *et al.* 1998 recorded increments in the yield and yield components of peanut plants terms pod or seed yield due to increasing the nitrogen fertilizer. However, a considerable loss in mineral nitrogen fertilizers when overdoses were applied leading to serious risks depending on many factors such as the site and climate conditions as well as nitrogen forms. On other hand, nitrogenous fertilizers economically represent the largest component in the overall cost of the cultivated crops which ranged from 20 to 30% of the variable production cost in cereals crops. Recently, an application of plant growth-promoting rhizobacteria (PGPR) in order to improve the growth and production of leguminous plants has been represented as an effective practice for overcome the negative effects due to excessive use of chemical fertilizers (Badawi *et al.,* 2011). Enhancement of nodulation and biological nitrogen fixation of legumes by co-inoculation with PGPR microorganisms is becoming a practical way to improve nitrogen use efficiency in sustainable agricultural production systems (Bai *et al.,* 2002). They also added that, PGPR can stimulate plant growth and increasing crop yields. El-Sayed *et al.* (2017) found that peanut co-inoculated with *Bradyrhizobium* and PGPR recorded improvement in the nodulation status, straw and grain yield as compared to single inoculation.

Peanut (*Arachis hypogaea* L.) is a leguminous crop grown in Egypt that produce an edible seed with high nutritive value for human and a green leafy hay for livestock feeding. Furthermore, the peanut seeds contain a considerable amount of oil, protein, carbohydrates and fibers which reached to 50, 25-30, 20 and 5%, respectively (Fageria *et al*., 1997).

The application of mineral N fertilizers have increased in the world agriculture, but too much of such fertilizer can lead to a terrestrial and aquatic pollution and needs to be reduced (Mengel *et al*., 2001). Biofertilizers are alternatives to increase soil productivity and improve plant growth in a sustainable agriculture regime. Biological nitrogen fixation (BNF) is the most important biochemical reaction for life on earth (Bohlool *et al*., 1992).

PGPR can enhance plant growth either directly, by phytohormons production, $N_2$-fixation and siderophores production…etc., or indirectly, through biological control of pathogens or induction of host defense mechanisms (Verma *et al.,* 2010).

Enhancement of nodulation and biological nitrogen fixation by co-inoculation of legumes with PGPR are becoming a practical way to improve nitrogen availability in sustainable agricultural production system (Bai and Smith, 2002) and Abdel-Wahab *et al.,* (2008). The most commonly implicated mode to stimulate legume-*Rhizobium* symbiosis is phytohormones inducing stimulation of root growth, to provide more sites for rhizobial infection and nodulation (Vessey, 2003).

Humic acid constitute the most component of humic substances which widely used as organic fertilizer and represent one of the vital and most used tools in crop production. The humified matter produced as a result of biotransformation of organic matter, including plant and/or animal residues, under action of microbial activities (Anonymous, 2010). The foliar application of humic molecules stimulate leaf water retention, photosynthesis and antioxidant metabolism under water stress (Fu Jiu *et al.,* 1995). A promising mechanisms of humic acid for improve the plant growth have been proposed in many studies including the nutrient uptake enhancements, reduction of toxic materials uptake, increase permeability of cell membrane, oxygen uptake, respiration, photosynthesis and root cell elongation (Russo and Berlyn, 1990 and Masciandaro *et al.,* 2002). On other hand, humic acid is also play an important role for cultivated soils through an improvement of physiochemical properties and nutrient availability as well as biological properties (Khaled and Fawy, 2011).

Therefore, current work aims to investigate the influence of bioorganic fertilization on growth, nodulation status, productivity and microbial activities in the rhizosphere soil of peanut, under field conditions.

## MATERIALS AND METHODS

**Microorganisms used**

*Bradyrhizobium* sp. (strain USDA 3456) and mixture of PGPR (*Azospirillum brasilense* + *Paenibacillus polymyxa* + *Serratia marcescens*) were obtained from the Biofertilizers Production Unit, Agriculture Microbiology. Dept., Soils, Water and Environ. Research Institute (SWERI), Agriculture Research Center (ARC), Giza, Egypt.

**Preparation of bacterial inocula**

*Bradyrhizobium* was cultured in yeast extract mannitol broth medium (Vincent, 1970). *Azospirillum*, *Paeniacillus polymyxa* and *Serratia marcescens* were grown on a Kings medium B (Atlas, 2010). Each bacterial culture was incubated at 28º C for 3 days on a rotary shaker. Powdered vermiculite was supplemented with 10% Irish peat packed in polyethylene bags (300g carrier), then sealed and sterilized by gamma irradiation (5.0 x$10^6$ rads). Bacterial culture was injected into sterilized carrier to reach 60 % of the water holding capacity.

**Humic acid (HA)**

Humic acid is purchased from the local market ( Humizone ( HumiStrong 18% Humic Acid) )

Humic acid was applied at two rates of 3L and 5L/Fed. before sowing, seeds were socked for 3 h. to be coated and as foliar application at three equal doses applied at 30, 45and 60 days from sowing.

**Field experiment**

A field experiment was conducted in a private farm at Meat Fares, Perket El-Sabaa, Menoufia Governorate, Egypt. Physical and chemical properties of soil are shown in Table (1) and determined following the methods described by (Cottenie *et al.,* 1982).

Peanut seeds cv. Ismaeilia 1 were cultivated during summer season 2016 to study the effect of inoculation with *Bradyrhizobium* individually or mixed with (PGPR) and/or humic acid applied by two methods, coating and foliar applications, on growth, nodulation status, and productivity of peanut, as well as mineral content in plant tissue.

**Table 1. Physical and chemical analyses of the experimental soil**

| $CaCO_3$ % | | Organic matter, % | | Particle size distr., % Sand | Silt | Clay | Texture class | | |
|---|---|---|---|---|---|---|---|---|---|
| 1.90 | | 0.03 | | 88.59 | 4.8 | 6.61 | Sandy | | |
| $PH^*$ | EC** m mohs/cm | Soluble cations (meq/L) $Ca^{++}$ | $Mg^{++}$ | $K^+$ | $Na^+$ | Soluble anions (meq/L) $CO_3^{--}$ | $HCO_3^-$ | $Cl^-$ | $SO_4^{--}$ |
| 7.63 | 1.82 | 0.36 | 0.32 | 0.13 | 0.56 | - | 0.41 | 0.36 | 0.61 |

*In the 1:2.5 Soil: water suspension. **In the 1:5 Soil ; water then extract.

**Fertilization**

T1: Uninoculated plants without any mineral fertilizer (control).

T2: Mineral fertilizers (at recommended dose) (RD).

T3: Inoculation with *Bradyrhizobium* sp.+ (50 % RD)

T4: Inoculation with *Bradyrhizobium* sp and HA 3L/ fed.+ (50 % RD).

T5: Inoculation with *Bradyrhizobium* sp and HA 5L/ fed.+(50 % RD).

T6: Inoculation with *Bradyrhizobium* sp and PGPR+(50 % RD).

T7: Inoculation with *Bradyrhizobium* sp + HA 5L/ fed.+ PGPR+(50 % RD).

All experimental plots received half of the recommended dose of mineral fertilizers (superphosphate “15.5 % $P_2O_5$ “at a rate of 200Kg fed, potassium sulfate “48% $K_2O$” at a rate of 50 Kg/fed and ammonium sulfate “20.5%N” at a rate of 60KgN /fed. Except for the control treatment where plants did not receive any mineral fertilizer and the plants in the second treatment received the recommended dose of mineral fertilizer. Peanut seeds were sown into hills at 50cm between ridges and 10cm between hills. After 20 day from sowing, plants were thinned to secure one plant per hill. After45 days from sowing, five plants were uprooted at random from the second row of each plot to determine the plant dry weight, then after 75 days from sowing , five plants were uprooted with rhizosphere soil to evaluate the plant dry weight, nodulation status and enzyme activities i.e. $N_2$-ase and dehydrogenase activities. At harvest, ten guarded plants were randomly collected from the second inner two rows of each experimental unit to evaluate peanut seed yield. The experimental treatments were randomly arranged in complete randomization design with three replicates.

**Assay of enzymes activities:**

Dehydrogenase activity was determined calorimetrically, using the 2,3,5- triphenyl formazan (TPF) produced from the reduction of 2,3,5- triphenyl tetrazolium chloride (TTC), was addition of acetone for extraction (Thalmann., 1967).

Nitrogenase activity was determined by means of gas liquid chromatography for ethylene produced from the reduction of acetylene (Hardy *et al*., 1973).

$CO_2$ evaluation: $CO_2$ was determined as mg /kg dry soil according to modified closed technique of Pramer and Schmidt (1964).

**Statistical Analysis.**

All obtained results were statistically analyzed by analyses of Variance (ANOVA) method according to Gomez and Gomez (1984) using the least significant difference at 0.05 level to compare the differences among means.

## RESULTS AND DISCUSSION

### Effect of microbial inoculation on peanut shoot dry weight

Data in Table (2) reveal that inoculation with *Bradyrhizobium* as single inoculation and or combined with PGPR (*Azospirillum* sp.+ *Paenibacillus polymyxa*+ *Serratia marcescens)* as mixed inoculum, combination with humic acid at 3L/fed and 5L/fed. significantly increased shoot dry weight of plant under coating and foliar applications. Coating application was higher than foliar application giving a mean of 18.87 g compared foliar which gave 17.23 g after 45 day of planting. Data in Table (2) showed that, incorporation of humic acid improved the growth of shoot and increased shoot dry weight at 45and 75 day after sowing.

**Table 2. Effect of microbial inoculation and humic acid on peanut shoot dry weight per plant (gram plant $^{-1}$)**

| Treatment | After 45 days from sowing | | | After 75 days from sowing | | |
|---|---|---|---|---|---|---|
| | Coating | Foliar | Mean | Coating | Foliar | Mean |
| T1 | 13.59 | 12.45 | 13.02 | 15.40 | 14.15 | 14.77 |
| T2 | 19.56 | 18.20 | 18.88 | 21.90 | 20.08 | 20.99 |
| T3 | 17.48 | 15.80 | 16.64 | 18.10 | 17.55 | 17.82 |
| T4 | 16.48 | 14.83 | 15.65 | 19.30 | 17.97 | 18.63 |
| T5 | 18.61 | 17.05 | 17.83 | 23.00 | 21.18 | 22.09 |
| T6 | 21.87 | 20.05 | 20.96 | 25.90 | 23.56 | 24.73 |
| T7 | 24.47 | 22.25 | 23.36 | 28.40 | 26.09 | 27.24 |
| Mean | 18.87 | 17.23 | - | 21.73 | 20.08 | - |
| L.S.D. at 5% | 0.278 | 0.792 | - | 1.45 | 1.13 | - |

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Brady rhizobium*, $T_4$:Br.+HA3L, $T_5$: Br,+ HA5L,$T_6$: Br+ PGPR and $T_7$: Mixed of all.**

It was also observed that, the mixed of all treatment ($T_7$) gave extra increases in shoot dry weights under two methods applications at 45and 75 days from sowing when gave 24.47, 22.25, 28.40 and 26.09 g plant $^{1}$, respectively. These increases in shoot dry weights might be due to increase in vegetative growth, which resulted in improvement of the nutrient uptake with adding microbial inoculation and humic acid. Hence, the inoculation of *Bradyrhizobium* and PGPR save $N_2$-Fixation, P solubilization, plant growth regulators such as phytohormones and vitamins, and this growth regulators influence plant growth, i. e. increase root surface area, respiration rate and metabolism, and improving mineral and water uptake. These results led to increase in shoot dry weights of plant. The results agree with those recorded by El-Howeity (2004). Also, Stockwell and Stack (2007) and Sindhu *et al*. (2009) mentioned that the microbial colonization of legumes rhizosphere acts to enhance the plants growth through improvement the nutrition status of plants, production of plant growth promoters such as vitamins, amino acids and phytohormones, bioremediation of toxic agents in contaminated soils and biological control of plant pathogens via production of antibiotics, siderophores, and/or hydrolytic enzymes.

### Nodulation status

Data in Table (3) demonstrated that inoculation with *Bradyrhizobium* increased significantly the number and dry weight of nodules/plant in both application methods, increases in number of nodules were 124.66 % and 144.15% above control, respectively, while Also, increases in dry weight of nodules were 115.82% and 119.42%, respectively, above control, respectively. However, Coating method was more effective than foliar application method with number and dry weight of nodules, being 66.23, 313.59, 55.90- and 304.16 mg plant $^{-1}$, respectively. Likewise, coinoculation with *Bradyrhizobium* and PGPR was more effective than single inoculation or humic acid addition. So, application of combined treatments ($T_7$) gave the highest increases and thus was more effective compared to the other treatments. Humic and PGPR application stimulating legume – rhizobia symbiosis. Since, rhizobium infection take place by the formation of infection threads in the root hair (Gage and Margolin, 2000), the stimulation of a greater infectable root hair cells, may provide more sites for infection and nodulation.

**Table 3. Effect of microbial inoculation and humic acid application on nodulation status**

| Treatment | Number of nodules plant $^{1}$ | | | Dry weight of nodules (mg plant $^{1}$) | | |
|---|---|---|---|---|---|---|
| | Coating | Foliar | Mean | Coating | Foliar | Mean |
| T1 | 13.66 | 16.00 | 14.83 | 144.33 | 138.20 | 141.26 |
| T2 | 27.00 | 19.66 | 23.33 | 153.26 | 151.20 | 152.23 |
| T3 | 60.66 | 48.00 | 54.33 | 326.46 | 333.76 | 330.11 |
| T4 | 70.33 | 64.00 | 67.16 | 361.50 | 337.97 | 349.73 |
| T5 | 82.33 | 75.00 | 78.66 | 373.86 | 372.20 | 373.03 |
| T6 | 93.33 | 84.33 | 88.83 | 406.06 | 393.50 | 399.78 |
| T7 | 116.33 | 84.32 | 100.32 | 429.63 | 407.16 | 418.39 |
| Mean | 66.23 | 55.90 | - | 313.59 | 304.16 | - |
| L.S.D. at 5% | 5.49 | 9.51 | - | 33.66 | 13.72 | - |

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, $T_4$:Br.+HA3L, $T_5$: Br,+ HA5L,$T_6$: Br+ PGPR and $T_7$: Mixed of all.**

**Nitrogenase activity of peanut nodules**

Nitrogen fixation by bacterial inoculums is evidenced by the rate of acetylene reduction by root nodules, as well as the quantities of N assimilation by the seedling shoots. Nitrogenase activity of peanut root nodules is affected by co-inoculation with *Bradrhizobium* and PGPR-microorganisms, giving the highest rate of $N_2$-ase activity. Also, adding humic acid as coating application stimulated $N_2$-fixation.

Results in Fig. (1) show that, there were various values of nitrogenase activity among different inoculation treatments under study. The un-inoculated plants (T1) showed the lowest $N_2$-ase activity, i.e., 9.25 and 12.22 µmol $C_2H_4$ g dwt of nodules$^{-1}$ plant$^{-1}$ hr$^{-1}$, respectively. Bradyrhizobium inoculation increased the rate of acetylene reduction by root nodules as they increased by 20.34 µmol $C_2H_4$ g dwt of nodules$^{-1}$ plant$^{-1}$ hr$^{-1}$ (119. 89%) compared by those recorded by the control treatment. Also, addition of humic acid with *Bradrhizobium* led to increases in $N_2$-ase activity with both methods applied foliar and coating.

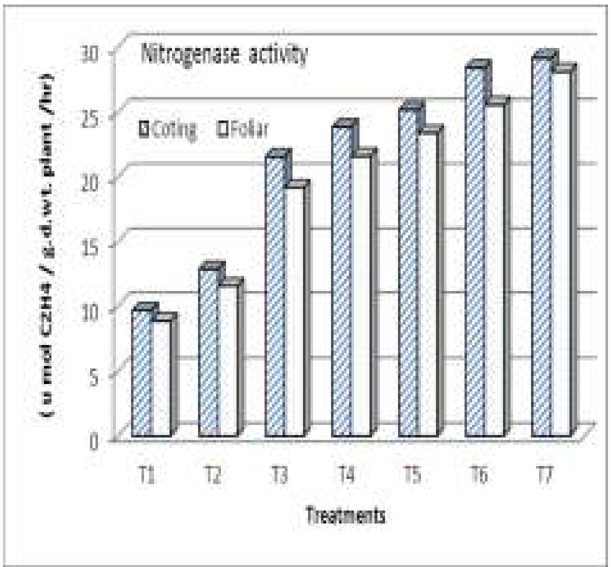


**Figure 1. Effect of microbial and humic acid on nitrogenase activity in root nodules of peanut plants.**

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, $T_4$:Br.+HA3L, $T_5$: Br,+ HA5L,$T_6$: Br+ PGPR and $T_7$: Mixed of all.**

**Yield productivity**

Table (4) show the effect of the incorporation of biofertilizers and humic acid as coating or foliar application on peanut yield. Results indicated that, foliar application was more effective than coating on peanut yield and the weight of 100 seeds, but the increases were slight. Irrespective of biofertilizers, inoculation with *Bradyrhizobium* plus half doses of nitrogen fertilizer led to significant increases in yield and weight of 100 seed yields, being 33.30 and 6.48%, respectively compared to control. Increases were more pronounced when using coating application than using foliar application, giving increases 33.59 & 7.53% while foliar gave (32.99& 5.44 %) over control for yield and weight of 100 seed yield, respectively.

Also, incorporation of humic acid at rate 3and 5l/fed. led to more increases in yield and weight of 100 seed yield over control and that of single *Bradyrhizobium*, increases with adding humic acid combined with Bradyrhizobium reached to (43.48 &12.90%) and ( 74.88 & 12.50% ) compared to control for yield and weight of 100 seed yield with adding 3 and 5L fed$^{-1}$ , respectively.

**Total contents of N, P and K in peanut shoot:**

Data in Tables (5and 6) show that the total contents of N, P and K in peanut shoots at 45 and 75 days from sowing greatly affected by inoculation with *Bradyrhizobium,* Plant growth promoting rhizobacteria (PGPR) and humic acid applied as foliar or coating. Results showed that the treatments combination ( (Br +50%PK +PGPR +5LHA) (T7) was superior to control and all the other treatments in nitrogen , phosphorus and potassium contents in shoot after 45 and 75 days from sowing the highest mean values 825.3, 133.81 and 735.44 mg plant $^{-1}$(Table 4). Likewise, it gave 963.75, 157.12 and 769.7 for N, P, and K at 45 day, respectively, for N, P and K at 75 day after sowing, respectively. Also, coating application was more effective with N and K compared to foliar application, but foliar application was more for phosphorus. As well , using full doses from mineral fertilizer increased the content of N,P and K contents in peanut shoot at 45 and 75 days , when gave the mean values of (669.8, 110.55 and 599.43 and 748.70, 125.63 and 654.3 mg plant $^{-1}$) For N,P and K at 45 and 75 days after sowing , respectively.

**Table 4. Effect of microbial inoculation and humic acid on peanut yield**

| Treatment | Yield (Ardb fed$^{-1}$) | | | Weight of 100 seeds (g) | | | Shelling % | | |
|---|---|---|---|---|---|---|---|---|---|
| | Coating | Foliar | Mean | Coating | Foliar | Mean | Coating | Foliar | Mean |
| T1 | 10.33 | 10.70 | 10.51 | 69.93 | 71.60 | 70.76 | 66.40 | 67.20 | 66.80 |
| T2 | 12.30 | 12.43 | 12.36 | 72.76 | 73.26 | 73.01 | 67.04 | 67.29 | 67.16 |
| T3 | 13.80 | 14.23 | 14.01 | 75.20 | 75.50 | 75.35 | 67.05 | 67.61 | 67.33 |
| T4 | 15.06 | 15.10 | 15.08 | 78.43 | 81.53 | 79.98 | 68.26 | 68.93 | 68.59 |
| T5 | 18.10 | 18.66 | 18.38 | 82.36 | 80.67 | 79.61 | 69.13 | 69.12 | 69.12 |
| T6 | 19.23 | 20.33 | 19.78 | 78.56 | 83.13 | 82.74 | 70.23 | 69.91 | 70.00 |
| T7 | 18.20 | 18.90 | 18.55 | 78.05 | 78.91 | 78.48 | 72.98 | 72.97 | 72.90 |
| Mean | 15.29 | 15.80 | - | 76.47 | 77.80 | - | 68.73 | 69.07 | - |
| L.S.D. at 5% | 0.566 | 0.374 | - | 1.27 | 0.52 | - | 0.3 | 0.169 | - |

**$T_1$: Control,$T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, $T_4$: Br.+HA3L, $T_5$: Br, + HA5L,$T_6$: Br+ PGPR and $T_7$: A mixture of all.**

**Table 5. Effect of microbial inoculation and humic acid on mineral contents of peanut shoots at 45 day from sowing.**

| Treatment | N- content (mg/plant) | | | P - content (mg/plant) | | | K - content (mg/plant) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Coating | Foliar | Mean | Coating | Foliar | Mean | Coating | Foliar | Mean |
| T1 | 402.4 | 362.2 | 382.3 | 53.95 | 58.94 | 56.44 | 330.14 | 311.23 | 320.68 |
| T2 | 696.0 | 643.7 | 669.8 | 107.60 | 113.50 | 110.55 | 618.45 | 580.42 | 599.43 |
| T3 | 545.7 | 497.9 | 521.8 | 76.28 | 81.89 | 79.08 | 493.98 | 488.23 | 491.10 |
| T4 | 527.8 | 474.3 | 501.3 | 74.68 | 79.65 | 77.16 | 476.79 | 466.95 | 471.87 |
| T5 | 622.9 | 559.1 | 591.0 | 91.19 | 92.42 | 91.80 | 546.61 | 537.30 | 541.95 |
| T6 | 755.5 | 686.2 | 720.8 | 106.27 | 117.39 | 111.83 | 645.40 | 636.76 | 641.08 |
| T7 | 870.5 | 780.2 | 825.3 | 129.22 | 137.65 | 133.81 | 753.80 | 717.09 | 735.44 |
| Mean | 631.6 | 571.9 | - | 91.39 | 97.85 | - | 552.03 | 534.00 | - |
| L.S.D. at 5% | 20.69 | 25.34 | - | 3.05 | 2.28 | | 11.18 | 24.99 | - |

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, $T_4$:Br.+HA3L, $T_5$: Br,+ HA5L, $T_6$: Br+ PGPR and $T_7$: A mixture of all.**

**Table 6. Effect of microbial inoculation and humic acid on mineral contents of shoots at 75 day from sowing.**

| Treatment | N - content (mg $plant^{-1}$) | | | P - content (mg $plant^{-1}$) | | | K - content (mg $plant^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Coating | Foliar | Mean | Coating | Foliar | Mean | Coating | Foliar | Mean |
| T1 | 456.5 | 411.0 | 433.75 | 61.22 | 66.72 | 63.97 | 374.98 | 353.8 | 364.3 |
| T2 | 787.2 | 710.2 | 748.70 | 120.70 | 130.57 | 125.63 | 689.54 | 619.19 | 654.3 |
| T3 | 569.2 | 576.9 | 573.05 | 79.56 | 85.06 | 82.31 | 515.32 | 509.34 | 512.3 |
| T4 | 625.1 | 582.2 | 603.65 | 87.91 | 93.09 | 90.50 | 561.11 | 549.46 | 555. |
| T5 | 764.4 | 694.8 | 729.60 | 112.72 | 114.28 | 113.50 | 675.42 | 664.19 | 669. |
| T6 | 934.1 | 806.1 | 870.10 | 131.05 | 137.87 | 134.46 | 757.99 | 781.52 | 769.7 |
| T7 | 1013.2 | 914.3 | 963.75 | 153.81 | 160.43 | 157.12 | 876.69 | 834.45 | |
| Mean | 735.69 | 670.8 | - | 106.71 | 112.57 | - | 635.91 | 611.18 | |
| L.S.D. at 5% | 21.18 | 30.44 | - | 5.58 | 3.77 | - | 8.76 | 38.61 | |

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, $T_4$:Br.+HA3L, T5: Br,+ HA5L, $T_6$: Br+ PGPR and $T_7$: A mixture of all.**

Furthermore, inoculation with Brady rhizobium plus half dose from mineral fertilizer did not replace full doses, but humic acid with microbial inoculation as mixture led to higher increases in N, P, K contents in peanut shoot at 45 and 75day after sowing. The improvement in nutrient uptake by using plant growth promoting rhizobacteria may be attributed to several mechanisms such as fixing atmospheric nitrogen, producing siderophores that chelate iron and make it available to the plant root, solubilizing minerals such as phosphorus and producing hormones and synthesizing some compounds or enzymes that can develop plant growth.

Indirect growth stimulation of plants could also be a result of protecting them against the effects of phytopathogens. PGPR bacteria are also capable of the production of secondary metabolites with antibiotic properties or are antifungal substances, insecticides, and immune suppressants (Glick, 2005). The PGPR include free-living soil bacteria that occur in the root zone and endophytic bacteria, colonizing the root cells. The largest group of PGPR bacteria are *Pseudomonas, Bacillus, Enterobacter*, and *Erwinia*. Indoleacetic acid (IAA) that is produced by the bacteria may enhance the effects of plant auxin and can directly affect root growth by stimulating cell division and elongation of the plant (Kalitkiewicz and Kepczy nska, 2008). Islam *et al.* (2012) revealed that, the biofertilizer *Azospirillum* strains " BM9" and "BM11" positively affected on N,P, and k uptake in rice grain and straw yields as single inoculation , they also, found that the interaction of two strains significantly influenced affected on N,P and K uptake .

**Microbial activity in the rhizosphere soil**

**Soil respiration (evolution of $CO_2$):**

Soil respiration ($CO_2$ evolution) is used as an indicator for the overall microbial activity in soil. Rates of $CO_2$ evolution from the rhizosphere soil of peanut plants treated with the bio-organics application, namely Brady rhizobium PGPR, and humic acid are illustrated in Figure (2).

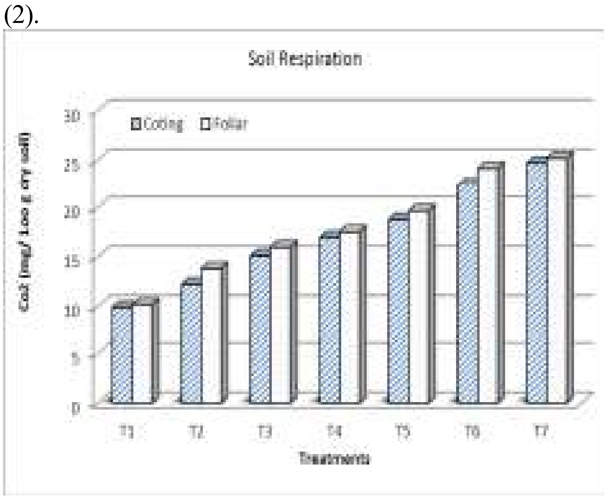


**Figure 2. Effect of microbial and humic acid on ($CO_2$-evoluation) in rhizosphere soil of peanut plants.**

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, T4: Br.+HA3L, $T_5$: Br,+ HA5L, $T_6$: Br+ PGPR and $T_7$: A mixture of all**

Addition of full dose of mineral fertilizer improved soil microbial respiration when giving 29.91% over control. Moreover, incorporation of microbial inoculations encourage soil microbial respiration and gave higher increases over control 54.93%. Likewise, the highest increases in soil respiration were 132.10 and 148.55% compared to the control for humic acid (T6 and T7), respectively. These increases may enhance the root exudates, e.g., organic acids and simple sugar that encourage microbial activity. Allam *et al*. (2009) found that dehydrogenase activity and respiration in the rhizosphere soil under Citrus seedlings were increased by adding biofertilizer and Aminokem as organic fertilizer. Also, Shalaby *el al.,* (2010) found increases in microbial activities in the rhizosphere soil of wheat plants by adding potassium humate and *Azospirillum* sp. as foliar application. Likewise, El-Howeity (2012) found that the addition of yeast as a biofertilizers led to stimulation of microbial activity in the rhizosphere soil of citrus seedlings.

**Dehydrogenase activity (DHA):**

DHA is frequently used as a measurement of the overall microbial activity in soil. The results illustrated in Figure (3) showed that, all applied treatments improved DHA in the rhizosphere soil of peanut plants. However, addition of full dose of mineral fertilizer (T2) increased the DHA activity by 97.7 %. As well as, using microbial inoculation led to more increases especially co-inoculation between *Bradyrhizobium* + PGPR + humic acid (T7 and T6), they gave higher increases compared to control and single inoculation, increases reached to 265.33 and 309.45% over control. Also, Foliar application gave higher increases in DHA compared to coating application, when gave the mean of 28.28, but the coating method gave the mean of 22.67 mg TPF 10 g dry soil$^{-1}$day$^{-1}$. These increases in DHA reflect the enhancement of the biological activity in the soil, with incorporation microbial amendment and humic acid.

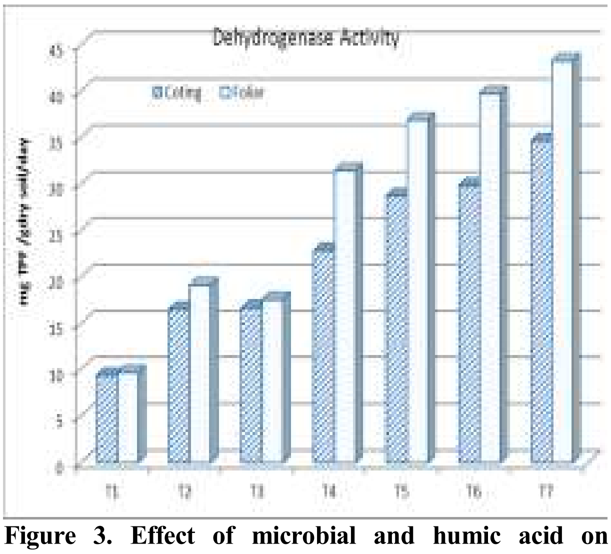


**Figure 3. Effect of microbial and humic acid on dehydrogenase activity in rhizosphere soil of peanut plants.**

**$T_1$: Control, $T_2$: Recommended dose of mineral fertilizers, $T_3$: *Bradyrhizobium*, $T_4$: Br.+HA3L, $T_5$: Br, + HA5L, $T_6$: Br+ PGPR and $T_7$: A mixture of all.**

Siczek and lipiec (2016) reported that dehydrogenase activity was higher in the soil with inoculation of faba bean by *Rhizobium leguminosarum* compared to un- inoculated plants. Also, Innangi *et al.* (2017) showed that, using organic matter amendment can enhancement soil enzymes.

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## تأثير محسنات التربة الحيوية والعضوية على النموالمحصول وحالة التعقيد والنشاط الميكروبى فى منطقة الريزوسفيرلنبات الفول السودانى تحت ظروف التربة الرملية

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تهدف الدراسة الى تقييم تأثير الاضافات العضوية المتمثلة فى حمض الهيوميك بمعدلين 5,3 لتر /فدان والتسميد الحيوى بالريزوبيا المتخصصة (البرادى ريزوبيم ) ومنشطات النمو البكتيرى( *Azospirillum sp.+ Penibacillus polymyxa+ Serratia marcescens* ) وأثر ذلك على نمو نبات الفول السودانى وحالة التعقيد والمحصول الكلى وكذلك حالة النبات الغذائية من النيتروجين والفوسفور والبوتاسيوم وكانت الاضافات اما رشا على النباتات او تغليف مع البذرة , وأيضا تم قياس النشاط الميكروبى لمنطقة الريزوسفير وتمثل قياس النشاط الميكروبى فى قياس انزيم الديهيدروجينيز ومعدل انطلاق ثانى اكسيد الكربون من التربة. أظهرت النتائج أن استخدام التلقيح بالريزوبيا المتخصصة منفردا او مشتركا مع البكتيريا المنشطة للنمو أدى الى زيادة معنوية فى النمو الخضرى والانتاجية للفول السودانى . كذلك أوضحت النتائج ان اضافة حمض الهيوميك بمعدل 3و 5 لتر/فدان ادى إلى زيادة معنوية فى كل من الوزن الجاف و الانتاجية وكذلك محتوى العناصر الغذائية , وكذلك أدى اضافة منشطات النمو الى زيادة معنوية فى النمو وحالة التعقيد ومحتوى العناصر الغذائية لنبات الفول السودانى , وكانت أفضل المعاملات فى الانتاجية هى معاملة الخليط بين التلقيح البكتيرى وحمض الهيوميك , وتميزت معاملة الرش عن الاضافة الأرضية فى زياده حالة التعقيد ونشاط وانزيم النيتروجينيز . وكان النشاط الميكروبى فى أعلى معدلاتة أيضا مع استخدام معاملة التلقيح االمشترك لكل من الريزوبيا المتخصصة ومنشطات النمو واضافة حمض الهيوميك بالمستوى الأعلى 5لتر /فدان وكانت أفضل معاملة للتنشيط مع معاملة الرش مقارنة بالتغليف .