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# RESPONSE OF SORGHUM TO INOCULATION WITH AZOSPIRILLUM, ORGANIC AND INORGANIC FERTILIZATION IN THE PRESENCE OF PHOSPHATE SOLUBILIZING MICROORGANISMS

**BY**

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## ABSTRACT

Response of sorghum (*Sorghum bicolor*) to inoculation with effective strain of *Azospirillum lipoferum*, biogas manure and/or ammonium sulphate fertilization in the presence of either *B. megatherium* var. *phosphaticum* or *Glomus mosseae* was studied. Results show that counts of ammonifiers and nitrifiers were the highest in case of biogas manure + ammonium sulphate application at a rate of 50 kg N/fed. from each of them combined with mycorrhizal inoculation. Inoculation with *Az. lipoferum* in the presence of half dose of inorganic N-fertilizer combined with VAM fungus increased the counts of *Azospirilla*. Mycorrhizal inoculation treatments gave higher values of total nitrogen, NH4-N, NO3-N and $CO_2$ evolution in soil than phosphate solubilizing bacteria treatments. Inoculation with *G. mosseae* gave higher values of total and available phosphorus and total and soluble potassium in soil than inoculation with *B. megatherium* var. *phosphaticum* regardless the type of nitrogenous fertilization. The treatments included mycorrhizal inoculation also gave higher values of plant height, leaves area/plant, fresh and dry weights of root and shoot system/plant than parallel treatments which included phosphate solubilizing bacteria. *Azospirillum* inoculum + ammonium sulphate (50 kg N/fed.) combined with *Glomus mosseae* gave the highest values of NPK and crude protein in shoot of sorghum plants. The treatments included *G. mosseae* gave higher values of chlorophyll pigments and carotenoids than parallel treatments which included phosphate solubilizing bacteria.

## INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is considered one of the most important cereal crops in Egypt. It ranks the fourth after maize, wheat and rice. Sorghum is well adapted to wide range of ecological conditions and plantation of sorghum in new reclaimed soil is recommended. Biofertilizers, either solely or in combination with certain chemical additives proved to be an efficient tool in increasing available nutrients in soil as well as crop yields (Kabesh *et al.*, 1975). Ahmed and Jha (1978) found that mixed inoculation with N2-fixers and phosphate solubilizing bacteria increased dry matter content, grain yield and phosphorus uptake in cereal crops. Monib *et al.*, (1982) reported that inoculation with N2-fixing *Azospirillum lipoferum* improved yield components of sorghum. The importance of interaction between mycorrhiza and non-symbiotic N2-fixing bacteria has been reported by Daft *et al.*, (1985) and their study show that the interaction improves plant growth. El-Haddad *et al.*, (1986a) reported that grains inoculation with non-symbiotic N2-fixing bacteria resulted higher yields and reduced N-requirements fertilization to 50%. Sheoran *et al.*, (1991) reported that inoculation of sorghum with mycorrhiza (*Glomus mosseae*) increased forage dry matter yield. Mohansingh and Tilak (1992) found that inoculation with *Glomus versiforme* increased sorghum growth and phosphorus uptake. Ishac *et al.*, (1993) mentioned that wheat inoculation with N2-fixing bacteria resulted considerable improvement of plant growth and N-uptake. Also, mixed inoculation with *Azotobacter* and VAM fungus increased growth parameters, N-uptake and grain yield of wheat. Abbas *et al.*, (1993) and Hussein *et al.*, (1993) mentioned that inoculation of wheat grains with associative N2-fixers (*Azospirillum brasilense*) increased dry matter, grain yield, protein content and nitrogen uptake. Zaghloul *et al.*, (1996b) found that organic carbon, NH4-N, NO3-N, total and available phosphorus were higher in soil when wheat grains were inoculated with VAM fungus compared with its inoculation with phosphate solubilizing bacteria. Also, inoculation with VAM fungus gave higher values of N, P and K in wheat plants as well as all measured growth characters were increased.

With regard to the effect of organic manuring on plant growth and microbial activity, Makawi (1982) and Vidyorthy and Nisra (1982) show that addition of organic manures to the soil encouraged proliferation of soil microorganisms, increased microbial populations and activity of microbial enzymes i.e. dehydrogenase and urease.

Mahmoud *et al.*, (1982) found that biogas manure had a significant stimulation effect on growth characters of maize plants when compared with application of the same level of nitrogen from urea. Imam *et al.*, (1984) reported that significant increases in dry matter yield and mineral content of the plant were obtained from biogas manure treatments compared with farm yard manure and urea. Zaghloul *et al.*, (1996a) show that N, P and K concentrations in wheat plants were higher in case of biofertilization with *Az. brasilense* compared with the organic manuring. Also, growth characters and grain yield/plant were significantly increased with wheat grains bacterization compared with the organic manures and inorganic nitrogen fertilization.

This investigation aims to study the efficiency of sorghum inoculation with *Azospirillum*, and fertilized with biogas manure and/or ammonium sulphate combined with phosphate solubilizers and study their effects on sorghum growth and soil fertility.

## MATERIALS AND METHODS

A pot experiment was conducted under greenhouse condition at the Fac. of Agric., Moshtohor to evaluate the response of sorghum to inoculation with *Azospirillum lipoferum* and fertilization with either biogas manure or ammonium sulphate in the presence of phosphate solubilizers i.e. *B. megatherium var. phosphaticum* or mycorrhizal strain (*Glomus mosseae*). Chemical and mechanical analyses of the experimental soil are presented in Table (1). It was obtained from cultivated soil from El-Nubaria city, El-Behira Governorate and the soil samples were collected from 0- 15 cm layer, air dried and thoroughly mixed. Thirty cm diameter pots were filled with the soil (6 kg/ pot). Biogas manure was added before sowing at a rate of 100 kg N/feddan, while the inorganic nitrogen fertilizer (Ammonium sulphate) was added in two equal doses at 30 and 60 days at the rates mentioned thereafter. All pots were supplemented with calcium super phosphate (15.5 % $P_2O_5$) and potassium sulphate (48% $K_2O$) at a rate of 30 kg $P_2O_5$ and $K_2O$/ fed. in two equal doses at 30 and 60 days. Biogas manure analysis is presented in Table (2).

Table (1): Chemical analysis of experimental soil.

| **Organic matter (%)** | **pH** | **E.C. mmhos/ cm** | **Total Nitrogen %** | **Total Phosphorus %** |
|---|---|---|---|---|
| 0.03 | 8.62 | 0.274 | 0.0063 | 0.0015 |

| Cations (meq/l.) | | | | Anions (meq/l.) | | | |
|---|---|---|---|---|---|---|---|
| $K^+$ | $Na^+$ | $Ca^{++}$ | $Mg^{++}$ | $Cl^-$ | $HCO_3^-$ | $CO_3^=$ | $SO_4^=$ |
| 0.28 | 0.6 | 0.35 | 0.95 | 0.73 | 0.14 | 0.28 | 1.03 |

Mechanical analysis of experimental soil

| Sand (%) | Silt (%) | Clay (%) | Textural class |
|---|---|---|---|
| 98.25 | 0.55 | 1.2 | Sandy |

Chemical analysis was estimated in saturated soil paste according to Black *et al.*, (1965). Whereas, mechanical analysis was estimated according to Piper (1950).

*Azospirillum lipoferum* and *B. megatherium* strains were kindly supplied from Microbiology Dept. Soils and Water Res. Inst., Agric. Res. Center, Giza, Egypt. Mycorrhizal strain (*Glomus mosseae*) was obtained from Tropical Inst., Goettingen Univ., Federal Republic of Germany by El-Deepah (1981).

Table (2): Analysis of biogas manure.

| Parameters | Unit | Value | Parameters | Unit | Value |
|---|---|---|---|---|---|
| Organic matter | % | 68.40 | Iron | ppm | 18.50 |
| Organic carbon | % | 39.67 | Zinc | ppm | 7.80 |
| Total nitrogen | % | 1.60 | Manganese | ppm | 6.00 |
| Total phosphorus | % | 0.92 | Copper | ppm | 3.50 |
| Total potassium | % | 1.12 | Sodium chloride | % | 0.18 |
| C : N ratio | | 24.79 | Density | kg/m3 | 280 |

For preparation of *Azospirillum* inoculum, Dobereiner medium (1978) was inoculated with effective strain of *Azospirillum lipoferum* and incubated at 32°C for 7 days till the viable count reached 108 cell/ ml. For preparation of *B. megatherium* var. *phosphaticum* inoculum, Bunt and Rovira medium(1955) modified by Abdel-Hafez, (1966) was inoculated by the above-mentioned strain and incubated at 35°C for 7 days till the viable count reached 108 cell/ ml. Sorghum grains were inoculated with *Azospirillum lipoferum* and/or B. *megatherium* var. *phosphaticum* at sowing time.

The VAM fungus (*Glomus mosseae*) was propagated on onion plants using the propagation technique described by Al-Fassi *et al.*, (1990). After three months from onion cultivation the mycorrhizal roots of onion bulbs together with its adjacent soil were collected and used for mycorrhizal inoculation. The mycorrhizal inoculum composed of infected onion roots and its rhizospheric soil were added just before sowing at a rate of 10 g/pot.

**This experiment included the following treatments :**

1- *Azospirillum* inoculum + Ammonium sulphate (50 kg N/fed.) + *B. megatherium* var. *phosphaticum*.
2- *Azospirillum* inoculum + Ammonium sulphate (50 kg N/fed.) + *Glomus mosseae*.
3- Biogas manure (100 kg N/fed.) + *B. megatherium* var. *phosphaticum*.
4- Biogas manure (100 kg N/fed) + *Glomus mosseae*.
5- Biogas manure (50 kg N/fed. ) + Ammonium sulphate (50 kg N/fed.) + *B. megatherium* var. *phosphaticum*.
6- Biogas manure + (50 kg N/fed) + Ammonium sulphate (50 kg N/fed.) + *Glomus mosseae*.
7- Ammonium sulphate (100 kg N/fed.) + *B. megatherium* var. *phosphaticum*.
8- Ammonium sulphate (100 kg N/fed.) + *Glomus mosseae*.

Six pots were used as replicates for each treatment in a randomized complete block design. Cultivation was performed by sowing ten inoculated or un-inoculated grains of sorghum (*Sorghum bicolor* var. *dorado*) in each pot and thinned later to five plants.

**Sampling and determinations :**

After 30, 60 and 90 days from sowing, rhizosphere soi samples of the developed plants were taken. Soil samples were microbiologically and chemically analyzed.

**1- Microbiological analyses :**

**1.1 Rate of carbon dioxide evolution:**

Determination of $CO_2$ evoluted by soil microorganisms was carried out using the method described by Antoun and Jensen (1979).

1.2- Ammonifying bacteria were counted by using the medium described by Allen (1953).

1.3- Nitrifying bacteria were counted by using the medium described by Black *et al.*, (1965).

1.4- Azospirilla counts were counted by using the medium described by Dobereiner (1978). The numbers of above-mentioned groups of bacteria were estimated from MPN statistical tables (Cochran, 1950).

**2- Chemical analyses :**

2.1- Total nitrogen was determined in soil using Kjeldahl digestion method described by Jackson (1973).

2.2- Ammoniacal and nitrate nitrogen were estimated in soil according to Bremner and Keeny (1965).

2.3- Total phosphorus was determined in soil colourimetrically according to American Public Health Association (APHA, 1989).

2.4- Available phosphorus was extracted from soil according to Olsen *et al.*, (1954) and colourimetrically determined according to APHA., (1989).

2.5- Total potassium was estimated in soil by using flame photometer apparatus (Brown and Lilliland, 1946).

2.6- Soluble potassium in soil was extracted by ammonium acetate according to the method described by Chapman and Pratt (1961) and determined by flame photometer apparatus.

In addition, total nitrogen, crude protein, total phosphorus and total potassium were estimated in the digested plant material at 90 days. Chlorophyll a, b and carotenoids were determined in the 3rd leaf of the plant after 60 days according to the method described by Wettstein (1957).

**Growth characters :**

After 60 days from sowing; plant height, leaves area/plant were measured, while, fresh and dry weights of root and shoot/plant were estimated at the end of the experiment.

**Statistical analysis :**

Analysis of variance (ANOVA) of data obtained from chemical analysis and growth characters were carried out and significant differences among the means of various treatments were distinguished by L.S.D. (Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

**Effect of different soil applications on microbial populations :**

Data in Table (3) show that microbial counts increased with the increasing of growth period to reach their maximum values at 60 days and decreased thereafter. and this was obvious in all treatments. The highest ammonifying and nitrifying bacteria counts were in case of biogas manure + ammonium sulphate application at a rate of 50 kg N/ fed. from each of them combined with mycorrhizal inoculation and this trend was observed at all growth periods. On the other hand, ammonifying and nitrifying bacteria showed the lowest counts in case of biogas manure + ammonium sulphate application at a rate of 50 kg N/ fed. from each of them combined with phosphate solubilizing bacteria.

As regard to *Azospirilla* counts, data in Table (3) clearly indicate that *Azospirilla* counts increased with the increasing of growth period and this increase was observed in all treatments. Sorghum grains when inoculated with *Az. lipoferum* and receiving 50 kg N/fed of ammonium sulphate with mycorrhizal inoculation gave the highest counts of *Azospirilla*. Whereas, *Azospirilla* counts were the lowest in case of ammonium sulphate application only at a rate of 100 kg N/fed. combined with phosphate solubilizing bacteria (PSB). Generally, it is worthy to notice that mycorrhizal inoculation treatments gave higher microbial counts i.e. ammonifiers, nitrifiers and *Azospirilla* than phosphate solublizing bacteria treatments. This result could be attributed to mycorrhiza fungi produced growth promoting substances and increased the availability of most nutrient elements especially phosphorus and micro-nutrients which encourage the proliferation of different soil microorganisms (Bellone and de Bellone, 1993). Alagawadi and Gaur (1992) found that populations of *Azospirilla* and phosphate solublizing bacteria in rhizosphere of sorghum plants were higher in the respective inoculation with symbiotic N2-fixers than in uninoculated ones. Also, Zaghloul *et al.*, (1996a) found that inoculation of wheat grains with *Azospirillum brasilense* led to increase of *Azospirilla* and inorganic phosphate solubilizer's counts compared with using either organic manures or inorganic nitrogen fertilization.

**Effect of different soil applications on nitrogen forms and $CO_2$ evolution in soil :**

Data presented in Table (4) show that total nitrogen in soil increased with the increasing of growth period to reach its maximum values at 60 days and decreased thereafter. This trend of results was obtained in all treatments. Also, ammoniacal and nitrate nitrogen concentrations in soil show the same trend of results except for the treatment of ammonium sulphate at a rate of 100 kg N/fed.

**Table (3):** Ammonifiers, Nitrifiers and Azospirilla counts in rhizosphere soil of sorghum plants at 30, 60 and 90 days of growth (counts x $10^2$/g dry weight of soil).

| Bacterial groups / Treatments | Ammonifiers | | | Nitrifiers | | | Azospirilla | | |
|---|---|---|---|---|---|---|---|---|---|
| | 30 days | 60 days | 90 days | 30 days | 60 days | 90 days | 30 days | 60 days | 90 days |
| Az. inoculum + A.S. (50 kg N) + P.S.B. | 35.20 | 43.0 | 27.45 | 8.49 | 12.20 | 6.88 | 8.3 | 12.2 | 15.20 |
| Az. inoculum + A.S. (50 kg N) + *G. mosseae* | 49.45 | 69.0 | 36.90 | 11.28 | 14.10 | 9.37 | 18.14 | 24.5 | 28.75 |
| Bio. (100 kg N) + P.S.B. | 28.60 | 34.0 | 26.40 | 8.26 | 11.40 | 7.45 | 7.73 | 13.0 | 18.10 |
| Bio. (100 kg N) + *G. mosseae* | 50.75 | 60.0 | 42.20 | 11.49 | 14.20 | 8.64 | 13.11 | 20.0 | 23.83 |
| Bio. (50 kg N) + A.S. (50 kg N) + P.S.B. | 25.30 | 30.0 | 18.86 | 4.60 | 9.08 | 3.88 | 9.21 | 11.0 | 14.70 |
| Bio. (50 kg N) + A.S. (50 kg N) + *G. mosseae* | 84.00 | 94.0 | 60.24 | 16.46 | 24.11 | 12.75 | 12.55 | 16.4 | 20.78 |
| A.S. (100 kg N) + P.S.B. | 30.50 | 40.0 | 35.00 | 6.49 | 8.30 | 5.62 | 3.22 | 4.2 | 6.37 |
| A.S. (100 kg N) + *G. mosseae* | 38.60 | 45.1 | 40.00 | 6.60 | 11.48 | 6.00 | 4.12 | 6.6 | 8.70 |

**Bio** : Biogas manure
**P.S.B.** : Phosphate solubilizing bacteria (*B. megatherium* var. *phosphaticum*)
**Az.** : *Azospirillum lipoferum*
**A.S.** : Ammonium sulphate

**Table (4):** Nitrogen forms and $CO_2$ evolved in rhizosphere soil of sorghum plants at 30, 60 and 90 days of growth.

| Periods (day) / Treatments | 30 | | | | 60 | | | | 90 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total nitrogen (ppm) | $NH_4$-N (ppm) | $NO_3$-N (ppm) | $CO_2$ µg/g soil/hr. | Total nitrogen (ppm) | $NH_4$-N (ppm) | $NO_3$-N (ppm) | $CO_2$ µg/g soil/hr. | Total nitrogen (ppm) | $NH_4$-N (ppm) | $NO_3$-N (ppm) | $CO_2$ µg/g soil/hr. |
| Az. inoculum + A.S. (50 kg N) + P.S.B. | 253 | 37.8 | 37.4 | 126.13 | 613 | 45.0 | 71.0 | 135.8 | 396.0 | 38.0 | 65.0 | 108.7 |
| Az. inoculum + A.S. (50 kg N) + *G. mosseae* | 412 | 51.0 | 78.8 | 153.28 | 643 | 56.0 | 83.0 | 190.8 | 520.0 | 46.0 | 76.5 | 164.1 |
| Bio. (100 kg N) + P.S.B. | 420 | 52.4 | 48.4 | 140.41 | 837 | 68.4 | 55.4 | 178.6 | 421.7 | 44.0 | 45.0 | 133.0 |
| Bio. (100 kg N) + *G. mosseae* | 462 | 70.0 | 52.0 | 168.16 | 857 | 82.0 | 68.0 | 200.1 | 556.3 | 52.6 | 60.0 | 165.2 |
| Bio. (50 kg N) + A.S. (50 kg N) + P.S.B. | 246 | 63.0 | 50.0 | 100.70 | 390 | 73.0 | 66.0 | 108.5 | 368.7 | 65.5 | 48.2 | 72.3 |
| Bio. (50 kg N) + A.S. (50 kg N) + *G. mosseae* | 288 | 73.0 | 65.4 | 147.49 | 636 | 80.0 | 78.4 | 195.2 | 454.3 | 73.5 | 62.8 | 154.1 |
| A.S. (100 kg N) + P.S.B. | 220 | 113.4 | 68.0 | 58.81 | 380 | 65.0 | 60.4 | 95.6 | 296.7 | 48.5 | 38.5 | 81.1 |
| A.S. (100 kg N) + *G. mosseae* | 246 | 120.5 | 70.2 | 120.60 | 603 | 75.6 | 63.1 | 172.0 | 307.7 | 52.8 | 42.6 | 99.8 |
| L.S.D. at 5% | 205.7 | 21.8 | 16.8 | 18.81 | 249.2 | 14.61 | 14.32 | 20.52 | 177.89 | 9.25 | 15.8 | 16.42 |

**Bio** : Biogas manure
**P.S.B.** : Phosphate solubilizing bacteria (*B. megatherium* var. *phosphaticum*)
**Az.** : *Azospirillum lipoferum*
**A.S.** : Ammonium sulphate

combined with either mycorrhizal inoculation or phosphate solubilizing bacteria. In this treatment, ammoniacal and nitrate nitrogen decreased in the soil cultivated with sorghum with the increasing of growth period.

The rate of $CO_2$ evoluted from the activity of soil microorganisms. increased with the increasing of growth period and gave the highest values at 60 days and decreased thereafter. The same trend of results was obtained in all treatments.

Total nitrogen and $CO_2$ evolution rates reached the highest values in case of biogas manure application at a rate of 100 kg N/fed. combined with VAM fungus (*G. mosseae*).

The lowest values of total nitrogen and $CO_2$ evolution resulted in case of sorghum plants fertilized with ammonium sulphate and inoculation of grains with *B. megatherium* var. *phosphaticum*. The same trend of results was observed at all growth periods. Results in Table (4) also showed that the rate of CO2 evolution was significantly enhanced with mycorrhizal inoculation since these treatments show significant increase in $CO_2$ evolution.

Data in Table (4) also show that the highest values of NH4-N were resulted from the application of ammonium sulphate at a rate of 100 kg N/fed., biogas manure at a rate of 100 kg N/fed., and biogas manure (50 kg N/fed.) + ammonium sulphate (50 kg N/ fed.) in the presence of *G. mosseae* at 30, 60 and 90 days, respectively. On the other hand. the lowest values of NH4-N were observed in the treatment in which grains were inoculated with *Az. lipoferum* combined with addition of ammonium sulphate (50 kg N/fed.) in the presence of PSB (*B. megatherium* var *phosphaticum*). This result was generally observed at all growth periods.

The highest values of $NO_3^-N$ were resulted when sorghum grains were inoculated with *Az. lipoferum* combined with addition of ammonium sulphate (50 kg N/ fed.) in the presence of VAM fungus (*G. mosseae*). This result also was obtained at all growth periods. Whereas, values of $NO_3^-N$ were relatively lower when sorghum grains were inoculated with *Az. lipoferum* combined with addition of ammonium sulphate (50 kg N./fed.), application of biogas manure (100 kg N/fed.), and ammonium sulphate (100 kg N/fed.) in the presence of PSB (*B. megatherium* var. *phosphaticum*) at 30, 60 and 90 days, respectively. (Table. 4).

These results are in harmony with Zaghloul *et al.*, (1996b) who found that organic carbon, ammoniacal and nitrate nitrogen were relatively higher in the tested soil when wheat grains were inoculated with VAM fungi compared with its inoculation with phosphate solubilizing bacteria.

**Effect of different soil applications on total and available phosphorus and potassium in soil :**

Data in Table (5) indicate that total phosphorus in soil decreased with the increasing of growth period in case of biogas manure treatments. On the contrary, available phosphorus increased with the increasing of growth period under the same conditions. While, total phosphorus increased with the increasing of growth period and reach their maximum values at 60 days and decreased thereafter in case of *Azospirillum* inoculum and ammonium sulphate application at a rate of 100 kg N/fed. in the presence of either phosphate solubilizing bacteria or VAM fungus (*G. mosseae*) inoculation. Also, in the treatments which included *Azospirillum* inoculum and ammonium sulphate application at a rate of 100 kg N/fed., available phosphorus in soil increased with the increasing of growth period.

The application of biogas manure at a rate of 100 kg N/ fed. combined with either *G. mosseae* or *B. megatherium* var. *phosphaticum* gave the highest values of total phosphorus at all growth periods

As regard to available phosphorus, data presented in Table (5) clearly indicate that application of biogas manure and ammonium sulphate (50 kg N/fed. from each of them) combined with mycorrhizal inoculation gave the highest values of available phosphorus at all growth periods. Available phosphorus obtained from application of ammonium sulphate at a rate of 100 kg N/fed. combined with PSB was relatively lower. The same trend of results was observed at all growth periods. These results are in accordance with the findings of Anonymous (1979) who found that the application of biogas manure increased total and available phosphorus in soil.

Data in Table (5) also show that values of total potassium in soil were lower at 60 days than either 30 or 90 days in case of biogas manure included treatments. Soil potassium content increased with the increasing of growth period in case of *Azospirillum* inoculum and ammonium sulphate (50 kg N/fed.) application in the presence of phosphate solubilizers. The highest values of total potassium were in the treatment of biogas manure (100 kg N/fed.) combined with either *G. mosseae* or *B. megatherium* var. *phosphaticum* at all growth periods of sorghum plants. On the other hand, the application of ammonium sulphate at a rate of 100 kg N/fed. combined with either *G. mosseae* or *B. megatherium* var. *phosphaticum* gave the lowest values of total potassium at all growth periods of sorghum plants.

The effect of different applications on soluble potassium in soil is shown in Table (5). It is clearly indicate that all treatments gave the highest values of soluble potassium at 90 days compared with other growth periods of sorghum plants. The application of biogas manure at a rate of 100 kg N/fed. combined with either mycorrhizal fungus or PSB inoculation gave the highest

**Table (5):** Total and available phosphorus and total and soluble potassium in rhizosphere soil of sorghum plants at 30, 60 and 90 days of growth

| Periods (day) / Treatments | 30 | | | | 60 | | | | 90 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T.P (ppm) | A.P (ppm) | T.K (ppm) | S.K (ppm) | T.P (ppm) | A.P (ppm) | T.K (ppm) | S.K (ppm) | T.P (ppm) | A.P (ppm) | T.K (ppm) | S.K (ppm) |
| Az. inoculum + A.S. (50 kg N) + P.S.B. | 61.0 | 11.40 | 26 | 20 | 92.0 | 14.40 | 20.0 | 17.0 | 38.3 | 16.70 | 30 | 26 |
| Az. inoculum + A.S. (50 kg N) + *G. mosseae* | 81.7 | 16.50 | 28 | 24 | 96.2 | 18.13 | 21.0 | 19.0 | 52.3 | 20.07 | 28 | 24 |
| Bio. (100 kg N) + P.S.B. | 84.7 | 12.23 | 72 | 60 | 61.0 | 15.00 | 65.0 | 45.0 | 44.3 | 18.30 | 66 | 47 |
| Bio. (100 kg N) + *G. mosseae* | 104.7 | 15.90 | 73 | 61 | 100.3 | 16.30 | 64.0 | 43.0 | 61.3 | 19.33 | 67 | 49 |
| Bio. (50 kg N) + A.S. (50 kg N) + P.S.B. | 63.3 | 12.07 | 49 | 43 | 60.3 | 14.30 | 40.0 | 38.0 | 44.0 | 16.73 | 45 | 42 |
| Bio. (50 kg N) + A.S. (50 kg N) + *G. mosseae* | 83.0 | 17.70 | 50 | 46 | 80.3 | 19.50 | 38.0 | 35.0 | 48.3 | 21.80 | 48 | 44 |
| A.S. (100 kg N) + P.S.B. | 46.7 | 10.90 | 25 | 18 | 52.0 | 13.67 | 18.0 | 16.5 | 34.7 | 15.23 | 26 | 23 |
| A.S. (100 kg N) + *G. mosseae* | 48.0 | 15.00 | 24 | 22 | 64.0 | 17.33 | 17.0 | 15.0 | 35.0 | 20.00 | 25 | 22 |
| L.S.D. at 5% | 24.19 | 2.74 | 9.56 | 0.06 | 15.92 | 3.58 | 6.81 | 0.07 | 12.9 | 4.09 | 8.31 | 1.34 |

**T.P** : Total phosphorus
**A.P** : Available phosphorus
**S.K** : Soluble potassium
**A.S** : Ammonium sulphate
**T.K** : Total potassium
**Bio** : Biogas manure
**P.S.B.** : Phosphate solubilizing bacteria (*B. megatherium* var. *phosphaticum*)
**Az.** : *Azospirillum lipoferum*

values of soluble potassium in soil and the same trend of results was observed in all treatments. While, the lowest values of soluble potassium were obtained from ammonium sulphate application at a rate of 100 kg N/fed. combined with either *G. mosseae* or PSB. The same trend of results was observed at all growth periods of sorghum plants.

These results are in accordance with the findings of Kabesh *et al.*, (1975) who found that the bioferilizers either solely or in combination with certain chemical additives increased the availability of nutrients in soil. Blaszkowski (1993) stated that when wheat grains inoculated with mycorrhizal fungi, soil content of nitrogen, phosphorus and potassium were significantly increased.

**Effect of different soil applications on growth characters of Sorghum plants:**

Data presented in Table (6) show that all studied growth characters were significantly increased in case of the treatment in which *Azospirillum* inoculum + ammonium sulphate (50 kg N/fed.) were combined with *G. mosseae*.

Generally, treatments included mycorrhiza fungus (*G. mosseae*) gave high results of studied growth characters. These results are in harmony with those obtained by Sheoran *et al.*, (1991), Mohansingh and Tilak (1992) and Blaszkowski (1993) who reported that sorghum or wheat plants inoculated with mycorrhizal fungi were significantly taller than uninoculated as well as roots and shoots dry weights were significantly increased.

Also, Zaghloul *et al.*, (1996b) reported that inoculation of wheat grains with VAM fungus (*G. mosseae*) gave high values of dry weight of roots and shoots, spike weight and grains weight/spike. Azospirillum inoculation also show good results of the studied growth parameters. This is in harmony with the findings of Glatiz and Martin (1979) who found that inoculation with *Azospirillum* increased root/shoot ratio of grasses and this result support the view that these bacteria improved plant growth by hormonal stimulation beside N2-fixation. Also, Hegazi *et al.*, (1982), Eid *et al.*, (1984) and El-Haddad *et al.*, (1986b) reported that inoculation with *Azospirilla* increased amounts of plant growth, dry matter and the highest records were obtained from Azospirilla inoculated plants receiving simultaneously a low dose of 50 kg N/ha.

**Effect of different soil applications on some chemical constituents of sorghum plants :**

Data in Table (7) show that total nitrogen, phosphorus and potassium in shoots of sorghum plants were significantly increased in the treatment of *Azospirillum* inoculum + A.S. (50 kg N/fed.) combined with mycorrhizal inoculation with *G. mosseae* compared with other investigated treatments. This result is in agreement with those obtained by El- Haddad *et al.*, (1986b) and Abbas *et al.*, (1993) who mentioned that the inoculation with associative $N_2$-

**Table (6):** Effect of different soil application on some growth characters of sorghum plants.

| Parameters / Treatments | Plant height (cm) | Leaves area cm²/ plant | Shoot system | | Root system | |
|---|---|---|---|---|---|---|
| | | | Fresh weight g/ plant | Dry weight g/ plant | Fresh weight g/ plant | Dry weight g/ plant |
| Az. inoculum + A.S. (50 kg N) + P.S.B. | 79.5 | 250.7 | 22.57 | 7.59 | 18.70 | 6.29 |
| Az. inoculum + A.S. (50 kg N) + *G. mosseae* | 94.5 | 490.3 | 40.01 | 12.31 | 25.37 | 13.37 |
| Bio. (100 kg N) + P.S.B. | 50.0 | 230.7 | 11.24 | 3.06 | 5.37 | 1.46 |
| Bio. (100 kg N) + *G. mosseae* | 60.0 | 270.0 | 12.73 | 3.86 | 8.37 | 2.84 |
| Bio. (50 kg N) + A.S. (50 kg N) + P.S.B. | 61.5 | 300.0 | 29.93 | 9.37 | 15.77 | 3.92 |
| Bio. (50 kg N) + A.S. (50 kg N) + *G. mosseae* | 90.8 | 440.0 | 31.85 | 10.22 | 17.90 | 8.19 |
| A.S. (100 kg N) + P.S.B. | 70.0 | 280.2 | 22.88 | 6.14 | 8.53 | 1.58 |
| A.S. (100 kg N) + *G. mosseae* | 77.0 | 290.3 | 25.10 | 6.53 | 8.87 | 1.73 |
| L.S.D. at 5% | 17.93 | 110.88 | 6.23 | 4.57 | 3.44 | 1.55 |

**Bio** : Biogas manure
**P.S.B.** : Phosphate solubilizing bacteria (*B. megatherium* var. *phosphaticum*)
**Az.** : *Azospirillum lipoferum*
**A.S** : Ammonium sulphate

**Table (7):** Effect of different soil application on some chemical constituents of sorghum plants.

| Parameters / Treatments | T.N % | T.P ppm | T.K ppm | Chlorophyll (a) mg/g F.W | Chlorophyll (b) mg/g F.W | Carotenoids mg/g F.W | Crude protein (%) |
|---|---|---|---|---|---|---|---|
| Az. inoculum + A.S. (50 kg N) + P.S.B. | 1.14 | 118.8 | 310.0 | 1.59 | 0.87 | 1.29 | 7.13 |
| Az. inoculum + A.S. (50 kg N) + *G. mosseae* | 1.51 | 209.8 | 393.0 | 1.89 | 0.91 | 1.16 | 9.44 |
| Bio. (100 kg N) + P.S.B. | 1.10 | 150.5 | 300.0 | 1.26 | 0.90 | 1.09 | 6.88 |
| Bio. (100 kg N) + *G. mosseae* | 1.30 | 158.8 | 330.0 | 1.76 | 1.03 | 1.34 | 8.13 |
| Bio. (50 kg N) + A.S. (50 kg N) + P.S.B. | 1.07 | 109.0 | 346.5 | 2.35 | 1.30 | 1.55 | 6.69 |
| Bio. (50 kg N) + A.S. (50 kg N) + *G. mosseae* | 1.38 | 129.0 | 370.0 | 2.78 | 1.48 | 1.66 | 8.63 |
| A.S. (100 kg N) + P.S.B. | 1.01 | 136.0 | 243.0 | 2.24 | 1.15 | 1.48 | 6.31 |
| A.S. (100 kg N) + *G. mosseae* | 1.26 | 185.3 | 273.0 | 2.31 | 1.21 | 1.49 | 7.87 |
| L.S.D at 5% | 0.81 | 25.29 | 42.12 | 0.14 | 0.16 | 0.12 | 0.94 |

**Bio** Biogas manure
**P.S.B.** Phosphate solubilizing bacteria (*B. megatherium* var. *phosphaticum*)
**Az.** : *Azospirillum lipoferum*
**A.S** : Ammonium sulphate
**F.W** : Fresh weight

fixers (*Az. lipoferum* or *Az. brasilense*) had increased nitrogen uptake by maize or wheat plants, respectively, over the uninoculated treatments. Also, Zaghloul *et al.* (1996a) found that inoculation of wheat grains with *Az. brasilense* led to increase of N, P, and K content of wheat plants compared with the organic manuring. While, the lowest values of total nitrogen, phosphorus and potassium resulted from the application of ammonium sulphate (100 kg N/fed.) + PSB., biogas manure + ammonium sulphate (50 kg N/fed. from each of them) + PSB, and ammonium sulphate at a rate of 100 kg N/fed. combined with either VAM fungus or PSB, respectively.

As regard to the effect of phosphate solubilizers Mohansingh and Tilak (1992) found that inoculation with *Glomus versiforme* increased the level of phosphorus uptake (about 15 - 47 %) by sorghum plants compared with uninoculated treatment. Zaghloul *et al.*, (1996b) found that inoculation with VAM fungus (*G. mosseae*) gave higher values of N, P and K in wheat plants. Data in Table (7) also emphasized that the highest value of crude protein was in case of the treatment of *Azospirillum* inoculum + A.S. application (50 kg N/fed.) combined with *G. mosseae*. While, lower values of crude protein were observed in the treatments of application of ammonium sulphate at a rate of 100 kg N/fed. combined with phosphate solubilizing bacteria, biogas 100 kg N/fed. + PSB and biogas 50 kg + A.s. 50 kg N/fed. + PSB.

As regard to the effect of Azospirilla inoculation, Abbas *et al.*, (1993) and Hussein *et al.*, (1993) mentioned that the inoculation of grains with associative N2- fixers (*Az. brasilense*) had increased protein yield and nitrogen uptake by wheat over the uninoculated treatment.

Regarding the chlorophyll pigments and carotenoids, obtained data show that the highest values of chlorophyll a, b and carotenoids were resulted from the application of biogas manure and ammonium sulphate (50 kg N/fed. from each of them) combined with *G. mosseae*. Whereas, lower values of chlorophyll a, b and carotenoids were obtained in case of the applications of biogas manure (100 kg N/fed.) + PSB, *Azospirillum* inoculum + A.S. (50 kg N/fed.) + PSB and biogas manure (100 kg N/fed.) + PSB, respectively.

## CONCLUSION

The obtained results clearly show that : Biogas manure application gave higher counts of ammonifiers, nitrifiers and Azospirilla than the application of inorganic N-fertilizer.

Sorghum grains bacterization with associative N2-fixing bacteria (*Az. lipoferum*) and mycorrhiza fungus (*G. mosseae*) addition relatively improved the growth characters and chemical components of sorghum plants compared with grains bacterization with both *Az. lipoferum* and *B. megatherium* var. *phosphaticum*.

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## استجابة نبات الذرة الرفيعة (السورجم) للتلقيح بالآزوسبيريللام والتسميد العضوي والمعدني في وجود الميكروبات المذيبة للفوسفات

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في هذا البحث تم دراسة استجابة نبات الذرة الرفيعة (السورجم) للتلقيح بسلالة فعالة من الـ *Azospirillum lipoferum* والتسميد بسماد البيوجاز وسلفات الأمونيوم في وجود إما بكتيريا الـ *B. megatherium* var. *phosphaticum* أوفطر الميكورهيزا *Glomus mosseae* وتأثير ذلك على نمو السورجم وخصوبة التربة.

ولقد أوضحت النتائج أن أعداد بكتيريا النشدرة والتأزت في منطقة الريزوسفير قد ازدادت في حالة التسميد بسماد البيوجاز وسماد سلفات الأمونيوم بمعدل ٥٠ كجم ن/فدان من كل منهما وذلك في وجود التلقيح بفطر الميكورهيزا. عند التلقيح ببكتيريا الـ *Az. lipoferum* مع إضافة نصف جرعة سماد معدني من سلفات الأمونيوم في وجود التلقيح بفطر الميكورهيزا أدى ذلك إلى الحصول على أعلى أعداد للأزوسبيريللا في منطقة الريزوسفير. كذلك أدى التلقيح بفطر الميكورهيزا *G. mosseae* إلى زيادة مستوى النيتروجين الكلي ، النيتروجين الأمونيومي والنتراتي ومعدل تصاعد ك أ٢ في التربة بالمقارنة بالتلقيح بالبكتيريا المذيبة للفوسفات *B. megatherium* var. *phosphaticum* . عند التلقيح بفطر الميكورهيزا وجد أن مستوى الفوسفور الكلي والميسر وكذلك البوتاسيوم الكلي والذائب في التربة كان عاليا نسبيا بالمقارنة بالتلقيح بالبكتيريا المذيبة للفوسفات بغض النظر عن نوع التسميد النيتروجيني.

وقد أوضحت النتائج أن المعاملات التي لقحت بفطر *G. mosseae* أعطت أعلى قيم من أطوال النباتات ، مساحة الأوراق/نبات ، الأوزان الطازجة والجافة لكل من المجموع الجذري والخضري مقارنة بالمعاملات التي لقحت ببكتيريا *B. megatherium* var. *phosphaticum* وعند التلقيح ببكتيريا الـ *Az. lipoferum* مع إضافة نصف جرعة من سماد سلفات الأمونيوم في وجود التلقيح بفطر الميكورهيزا تم الحصول على أعلى قيم للنيتروجين والفوسفور والبوتاسيوم الكلي وكذلك البروتين الخام في نباتات السورجم.

بخصوص مستوى الكلوروفيلات والكاروتينيدات في النبات فإن النتائج أوضحت أن المعاملات التي لقحت بفطر الميكورهيزا أدت إلى زيادة محتوى نباتات السورجم من صبغات التمثيل الضوئي السابقة بالمقارنة بالمعاملات التي لقحت بالبكتيريا المذيبة للفوسفات.