# Zea mays L. Response to Humic Acid and Vaccination with Bacteria Azotobacter and Azospirillum

Jawad Abdul Kadhim Kamal <sup>1</sup>(College of Agriculture / University of Al-qadisiyah, Iraq) Email: jawad.alshabbany@qu.edu.iq

Received : **12/09/2018** Final Revision : **28/11/2018** 

*Abstract:* A field experiment was conducted in the agricultural season (2016 - 2017) in one of the fields of college of Agriculture - University of AL-qadisiyah on mays L. Zea to study the interrelated effect of both the acid and Azotobacter and Azospirillum. The experiment was applied according to RCBD design and three replicates. The treatment included 12 treatments resulting from the treatment of Control and Azotobacter , Azospirillum , organic fertilizer, The most important results reached:

- 1. Azotobacter + Azospirillum + organic fertilizer have the superior to the vegetative growth characteristics of mays by giving the highest average (number of leaves, dry weight of leaves, dry weight of root total) (24.67, 17.5 g, 8.17) g respectively, except for treatment (Azospirillum + organic fertilizer) was superior in plant height 92.0 cm.
- 2. Azotobacter + Azospirillum + organic fertilizer has the highest values for the chemical grade of mays in (potassium, calcium, sodium, respectively (0.243, 2.60 and 0.077) %.

Keyword: Zea mays L , Azotobacter , Azospirillum , humic acid .

#### I. INTRODUCTION

Azotobacter and Azospirillum bacteria were the most active free nitrogen-fixing bacteria. Research has shown that 100 bacterial strains of nitrogen could be isolated from the rhizosphere, but Azotobacter and Azospirillum are the most efficient in stabilization [1]. These two species are non-specialized bacteria that stabilize atmospheric nitrogen in varying amounts and improve plant growth through the release of certain hormones, enzymes and vitamins, which positively effect on plant growth and increase productivity [2]. The studies indicated that there is a positive overlap between nitrogen-free living bacteria [3]. This overlap improves the performance of the plant through several mechanisms including:

1 - Improve the relationship between the microbiology itself (Microb-Microb) by the secretion of some metabolic compounds that stimulated the growth and reproduction of these organisms.

2 - Improve the relationship between the biology and plant itself (Microb-Plant).

The idea of the use of Azotobacter as a biomass is mainly based on the production of large numbers of this organism, which is spread in the area of the rhizosphere and around the roots of the plants to provide primary energy and other nutrients to the plant [4]. The same researcher pointed out that the Azotobacter vaccine can be used in different ways, It is either mixed with seeds before planting or immersed in the roots of the initiatives or is added directly in the field near the roots of the growing plants, and the Azeotobacter from the differentiated nutrition and depends on the organic matter as a source of carbon, energy and the extent of its benefit from carbon resources is important for the diagnosis bacteria. The availability of energy sources was a determinant of the activity and rate of atmospheric nitrogen fixation. The addition of 1% glucose to the Azotobacter environment resulted in an increase in the amount of nitrogen installed relative to the non-glucose environment [5].

The inhalation with Azospirillum affects the nitrogen feedings of the plant through two ways: atmospheric nitrogen uptake and nitrogen uptake by plants [6]. Azospirillum also has other benefits, as it is one of the most active soil biotechnologies for effective plant hormones [7]. On the liquid farm, the most important hormones represented by Azospirillum are IAA and its presence depends on the presence of tryptophan on the farm. IAA can also be produced by Azospirillum in the absence of tryptophan in old farms. Pacovsky [8], Essofermal significant changes occur in the root system mainly due to IAA. These alterations have to do with improving the absorption of water and nutrients by the fertilized the plant. Bacteria also produce a number of gibberellins and cytokines. It also has the ability to secrete certain vitamins such as riboflavin, niacin and thiamine. Neilands [9] also noted that most nitrogen-fixing organisms, including isosperm, produce molecules with low molecular weights (Siderophores), which are efficient complexes to ferment iron.

The Humic acids are part of the humic substances (complex organic substances produced by the degradation of plants and animals materials in the process of doping), which play a key role in soil fertility and plant nutritions. The qualities of humic acids positively affect plant growth, such as increased permeability of cellular membranes, Cellular cell elongation will increase the production of plant enzymes such as transaminase, phosphatase, invertase and stimulate

intracellular vitamins such as vitamins A, D, E and K, [10]). Has given these acids a promising future to increased crop production as a source of low-cost natural fertilizers [11], [12].

The research goals were:

- 1. The effect of the interaction between organic fertilizer extract (humic acid), Azotobacter and Azospirillum bacteria in the growth of maize plant.
- 2. The effect of the interaction between the bio in the plant content of nutrients.

## II. Materials and Methods

#### 1. Executing the experiment :

The research was carried out in the green house of College of Agriculture / University of AL-qadisiyah during the agricultural season 2016/2017 to demonstrate the effect of adding organic and bio-fertilizer in the indices of vegetative and chemical growth indicators of mays plant. The seeds were grown and the addition of organic fertilizer which was added on 1/3/2016. the design of the random segments of the full RCBD and three sectors the narrator and behind God, (2000), and the combinations were as follows:

- Control
- Azotobacter
- Azospirillum
- organic fertilizer
- Azotobacter + Azospirillum
- Azotobacter + organic fertilizer
- Azospirillum + organic fertilizer
- Azotobacter + Azospirillum + organic fertilizer

The mean values were measured according to the Duncan test and at a probability level of 0.05%. All service operations were performed as required. The vegetative growth measurements were taken during two periods in the middle of the experiment and at the end of the experiment

- Indicators of vegetative growth in the first period
- Number of plants

The number of plants produced from the seeds was calculated in the experimental unit Length of plants (cm). The length of the plants was measured by the metric bar of each plant in the experimental unit. Number of leaves (leaf), the number of leaves per plant per plant was calculated in the experimental unit

- Vegetative growth indicators in the second period
- Number of plants

The number of plants produced from the seeds was calculated in the experimental unit

- Plant length (cm)

The length of the plants was measured by the metric bar of each plant in the experimental unit

- Number of leaves (leaf ) .

The number of leaves per plant was calculated in experimental unit.

- dry weight of the total vegetative (g)

The vegetative parts (leaves, vegetative stems and stalks) were placed in paper bags perforated after cutting them into small pieces and dried in an oven oven at a temperature of 48 m until proven weight [13] and weighing the dry weight of the seedlings.

- Dry weight of root total (g)

The dry weight of the root mass was estimated by drying the wet root samples in the same way as the total vegetation was dried.

- Securities content of nutrients (potassium, calcium and sodium)

The leaves samples were collected from the corn plants for each experimental unit where the mature leaves were taken and then washed and dried in an oven at 70 ° C for 48 hours until the weight stability was then grinded by the electric mill. 200 mg per experimental unit was taken and digested in Birex flasks by adding 3 ML of concentrated sulfuric acid for 24 hours in accordance with the method proposed by [14]. Add 1 ml of concentrated sulfuric acid and 1 ml of pyrochloric acid and heat flask digestion and vapors until a clear transparent solution was obtained. Then the liquid was cooled. The solution was filtered and the size was completed to 50 ml. The nutrients in the digestion solution were then evaluated according to the estimation method used.

The three elements above were estimated by flame-photometer according to the method proposed by Horneck and Hanson [15].

### 2. The statistical analysis :

After collecting the data on the studied traits, they were analyzed using the statistical program genstate and compared the differences between the mean and the least significant difference between LSD and the probability level of 0.05.

#### **III. Result and discussion :**

#### 1. Characteristics of vegetative growth

The results shown in table 1 indicate that Azotobacter and Azospirillum were superior with the addition of humic acid as organic fertilizer in all the treatments of the growth of the mays plant except for plant height. This treatment gave the highest values for the number of leaves, dry weight of leaves and roots, which reached 24.67 and 17.5 g. Plant -1 and 8.17 g. Plant -: respectively compared to the control treatment, which gave the lowest values for these traits, which reached 12.33 and 4.3 g. Plant -1 and 2.0 g. Plant -1. The values of this treatment did not differ from the treatment of the addition of organic manure with one of the bacteria. This increase in growth was due to the role of these bacterial species in the stabilization of nitrogen air and the increased processing of the plant, as well as these bacteria the ability to release some growth organizations such as alcohols and glyblins and important cytokines in the growth and the development of plants. Humic acid also plaied an important role in increasing nutrient availability in soil, which has increased plant growth. The results were agreed with [16], [17], [18].

Fertilizer transactions	Length of plant (cm)	Number of leaves	Dry weight of leaves (gm)	Dry weight of roots (gm)
Control	a52.7	c12.33	b4.3	b2.00
Azotobacter	a73.7	c15.67	ab12.6	b7.53
Azospirillum	a86.7	c13.00	ab15.0	b8.43
organic fertilizer	a75.0	bc12.33	ab12.4	b2.60
Azotobacter + Azospirillum	a66.0	bc20.67	ab12.5	ab4.73
Azotobacter + organic fertilizer	a70.3	abc15.00	ab10.3	a2.41
Azospirillum + organic fertilizer	a92.0	ab22.33	ab13.4	a3.63
Azotobacter + Azospirillum + Organic fertilizer	a77.3	a24.67	a17.5	a8.17

Table 1:Effect of organic and biological fertilization on vegetative growth characteristics of maize plant:

\* The rates that carry the same alphabet are not significantly different from each other and according to the Denkin polynomial test at a probability level of 0.05

The length of the plant was highest in the treatments of Azospirillum with compost according to the data of Table 1, which amounted to 92.0 cm compared to the comparison treatment, which reached the length of the plant 52.7 cm. We note from the same table that the effect of bacteria alone without the addition of humic acid had a weak effect " This may be due to the fact that acid could be an important for bacterial species.

#### 2. Chemical properties :

The effect of organic and bio-fertilization on chemical traits studied, which included potassium, calcium and sodium in maize plant (Table 2). The best treatment given the highest values of the studied chemical properties is the addition of Azotobacter + Azospirillum + organic fertilizer which amounted to 0.243 and 2.6 And 0.077% for potassium, calcium and sodium respectively, compared with the comparison treatment which gave the lowest values for the above characteristics, which reached ( 0.183, 1.51 and 0.012)% for the above elements respectively, knowing that calcium and sodium were not significantly affected by all the treatments. for all transactions and increased The percentage of plant fertilizer in the plant, especially when treated with Azotobacter + Azospirillum + organic fertilizer and this is due to the role and importance of organic manure in reducing the degree of soil interaction, which leads to increased readiness of the elements and thus increase absorption, and the role of bacteria in reducing the degree of soil interaction of through the secretion of many organic acids and also increase the concentration of carbon dioxide, which leads to the reduction of pH, this decrease increases the readiness of elements in the soil and increase absorption of the plant. These results were consistent with [19] , [20].

Fertilizer transactions	K%	Ca%	Na %
Control	d0.183	a1.51	a0.012
Azotobacter	cd0.220	a2.09	a0.015
Azospirillum	bc0.200	a2.38	a0.016
organic fertilizer	bc0.210	a1.68	a0.014
Azotobacter + Azospirillum	bc0.220	a1.72	a0.016
Azotobacter + organic fertilizer	b0.213	a1.90	a0.017
Azospirillum + organic fertilizer	b0.215	a2.15	a0.016
Azotobacter + Azospirillum + Organic fertilizer	a0.243	a2.60	a0.077

Table 2: Effect of organic and biological fertilization on some chemical properties of mays plant:

\* The rates that carryont the same alphabet are not significantly different from each other and according to the Dunkin polynomial test at a probability level of 0.05.

#### REFERENCES

- [1] Foriani, G., Pastorelli, R., Branzoni, M. and Favilli, F. (1995). Root colonization efficiency and potentially related properties in plant associated bacteria. J.Gent. Plant Breeding. 49(4): 343-431.
- [2] Barea,J.M. and Brown,M.E. (1974). Effect on plant growth produced by Azotobacter paspali related to synthesis of plant growth regulating substances. J.Appl. Bacteriol. 37: 583-593.
- [3] Ishac,Y.Z. (2000). Interaction of azotobacter and vesicular arbusicular mycorrhizas In : Azotobacter in sustainable Agriculture ch.(9) . ed. Neeru Narula., India.
- [4] Lakshmana,M. (2000). Azotobacter inoculation and crop productivity. In : Azotobacter in sustainable Agriculture. Ch.11 (ed.) Neeru Narula. India.
- [5] Alexander, M. (1977). Introduction to soil microbiology. John wiley and Sons . Inc , New York.
- [6] Chahal, P.P.K. and Chahal, V.P.S. (1988). Advances in plant nematology, Shamin Printing Press. Karachi: 257-263.
- [7] Fallik, E. and Okon, Y. (1996). Inoculants of Azospirillum brasilense : biomass production, survival and growth promotion of setaria italica and Zea mays. Soil Biol. Biochem., 28 : 123-126.
- [8] Pacovsky, R.S. (1990). Development and growth effect in the sorghum Azospirillum association. J. Appl. Bact. 68 : 555-563.
- [9] Neilands, J.B. (1984). Methodology of siderophores structure bonding. 58: 1-24.
- [10] Tan, H. Kim. 2004. Humic matter in soil and the environment principles and controversies. Library of congress. NY. USA.

- [11] Sherif, H. O; and M.A.Sherif.2007. Utilization of agriculture residues is an environmental and agricultural necessity: V-Production of K-humate from sugar cane bagasse compost.African Crop Science Socity . 8:1585-1587.
- [12] Ayuso, M; J. L. Moreno; and C. Garca.1997.Characterisation and evaluation of humic acid extracted from urban waste as liquid fertilizers. J.Sci .Food and Agric.(75):481-488.
- [13] Al-Sahaf, Fadel Hussein. (1989). Applied plant nutrition. Ministry of higher education and scientific research. University of Baghdad - House of Wisdom - Iraq.
- [14] Cresser, M.S. and J.W. Parsons(1979). Sulphuric perchloric acid digestion of plant material for the determination of nitrogen, phosphorus potassium, calcium and magnesium. Analytic Chem. Acta., 109: 431-436.
- [15] Horneck, D. A., and D. Hanson. 1998. Determination of potassium and sodium by flame emission spectrophotometry. pp. 153-155. In: Kalra, Y. P. (ed.). Handbook of Reference Methods for Plant Analysis. Soil and Plant Analysis Council, Inc. CRC Press. FL. USA. pp. 287.
- [16] Tarrand, J.J., Krieg, N.R. and Dobereiner, J. (1978). A taxanomic study of the Spirillum lipoferum group with descriptions of a new genus, Azospirillum gen. nov. and two species, Azospirillum lipoferum (Beijerink) comb. Nov. and Azospirillum brasilense sp. Nov. Can.J.Microbio. 24: 967-980.
- [17] Schloter,M., Krichhof,G., Heinmann,U., Dobereiner,J. and Hartmann,A. (1994). Immunological studies of the wheat-root colonization by Azospirillum brasilense strain SP7 and SP245 In: Hegazi,N., Fayez,M. and Monib,M. Nitrogen fixation with nonlegumes : 290.
- [18] Okon, Y. and Kapulnik, Y. (1986). Development and function of Azospirillum inoculated. roots. Plant and Soil. 90: 3-16.
- [19] Vancura, V. (1961). Detection of gibberellic acid in Azotobacter cultures. Nature. London. 192-198.
- [20] Dey,B.K. (1973). Bacterial inoculation in relation to root exudates and rhizosphere effect. 3 Effect of root exudates on the rhizosphere microflora of Azotobacter inoculated maize (Zea mays L.) and Rhizobium inoculated gram (C.arietinum). Ind. Agric., 17: 125-133.