



EFFECT OF USING DIFFERENT ISOLATES OF BACILLUS BACTERIA AND LEVELS OF ORGANIC MATTER ON SOME GROWTH CHARACTERISTICS OF MAIZE (*ZEAMAYS* L.)

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Abstract

A field experiment was carried out in Dhi-Qar province of Nasiriyah district (Al-Fraih) for 2019 in Silty Loam soil to study the effect of using Bacillus isolates and levels of organic fertilizer on some growth characteristics of maize (*Zea mays* L.) spring variety. Randomized Completely Block Design (RCBD) was used with three replicates. Experiment factors included four levels of bio-fertilizers B_0 , B_1 , B_2 and B_1+B_2 and organic fertilizer 0, 2, 4%, which were given symbols O_0 , O_1 , O_2 , respectively. The results showed a significant increase in $B_1 + B_2$ inoculation, increase in plant height, leaf area, chlorophyll, dry weight, 1000 grain weight and grain yield, treatment of organic fertilizer (O_2) achieved significant differences in leaf area, chlorophyll and dry weight.

Key words: Bacillus bacteria isolates, organic matter, growth characteristics, maize (*Zea mays* L.).

Introduction

Population growth was the most important providing food challenges facing, led to the search for new sources and modern technologies aimed at increasing, production, improving quality and reducing economic costs, avoiding the use of chemical fertilizers that reduce the pollution that gets to the soil and water, which negatively affects human life, bio-fertilizer was a modern technology used, microorganisms that were added as bio-fertilizers for the purpose of processing plants with nutrients are isolated and labeled (Al-Dulaimi, 2014). Soil microorganisms play an important role in agricultural systems, especially bacteria that stimulate plant growth by acting as biofertilizers such as nitrogen-fixing bacteria and phosphate-solvent bacteria such as *Bacillus* spp (Fadhel, 2018). Iraqi soils were characterized by high levels of carbonate minerals, high calcium ions, low organic matter, the higher the pH value, reduces the amount of ready phosphorus, as a result of exposure to the deposition processes that reduce the readiness, which negatively affects growth and production (Taban, 2014). Phosphorus is important in plant growth and productivity, an appropriate level of phosphorus ready for absorption should be provided, by adding bio-fertilizers, as well as the use of appropriate amounts of organic fertilizers, which

in turn increase the amount of nutrients needed by plants, in addition to improving soil properties (Dadhich *et al.*, 2011). This experiment aimed at the effect of inoculation with different isolates of *Bacillus* bacteria and levels of organic matter in some maize growth traits (*Zea mays* L.).

Material and Methods

The experiment was carried out in Al-Fraih area, Dhi-Qar Governorate during the spring season 2018-2019 in a silty loam soil, to study the effect of using *Bacillus* isolates and levels of organic fertilizer on some growth characteristics of maize (*Zea mays* L.) spring variety. Randomized Completely Block Design (RCBD) was used with three replicates. Experiment factors included four levels of bio-fertilizers B_0 , B_1 , B_2 and B_1+B_2 and organic fertilizer 0, 2, 4%, which were given symbols O_0 , O_1 , O_2 , respectively. It was mixed with soil, the site was chosen, which was not added by the bio and organic fertilizers. Soil samples were taken randomly from the experiment site with a depth of 0-30, was mixed to obtain a soil sample. A part of the sample was taken to find out the number of bacteria before planting, as for the rest of the sample, it was dried in the oven and ground, pass through a sieve 2 mm hole, to conducting laboratory tests, which included the physical and chemical properties shown in table 1.

Table 1: Some physical and chemical properties of soil before planting*.

Properties	unit	Value
Electrical Conductivity (EC)	ds.m ⁻¹	3.70
pH		7.10
Available nitrogen		23.10
Available phosphorus	mg. kg ⁻¹	8.10
Available potassium		186.30
Organic Matter		7.10
CaCO ₃	g/ kg ⁻¹	33.01
Dissolved calcium Ca ⁺²		11.01
Dissolved magnesium Mg		6.31
Dissolved sodium Na ⁺		3.71
Dissolved bicarbonate HCO ₃	meq/L	0.90
Melting chlorine Cl		30.12
Dissolved potassium K		1.01
Soil types		
Sandy soil		292.00
Silty soil	g/ kg ⁻¹	540.00
Clay soil		168.00
Soil Texture	Silt Clay mixed	
Total number of bacteria	cell.g-1 dry soil	0.21×10 ⁴

* Analysis was carried out in Soil Science Department Laboratory, Agriculture College, Al-Muthanna University.

Mineral fertilizers were added according to the recommended quantities mixed with the soil: Nitrogen was 240 N (kg/ha⁻¹) as urea fertilizer 46% N, Phosphate fertilizer was 80 P (kg/ ha⁻¹) as TSP, 44% P₂O₅ (120 kg / ha⁻¹) as potassium sulfate fertilizer (41.5% K), 20% of the fertilizer recommendation before planting mixed with soil, also used compost decomposed organic fertilizer at 25 tons e according to Al-Zubaidi (2010).

As for the experimental treatments, bio-fertilizers was added at four levels: B₀= without addition, B₁= isolation 1, B₂= isolation 2 and B₃=B₁+B₂, as for the organic fertilizer (bovine fertilizer), it is added in three levels: O₀= without adding, O₁= 2% and O₂= 4%.

As for the studied growth traits, they were plant height, leaf area, chlorophyll content (SPad), weight of 1000 grains (g. Kg), dry weight of the vegetative, total grain yield.

Statistical analysis

The data obtained during the study analyzed statistically according to randomized complete block design (RCBD).

Results and Discussions

Table 2, showed a significant difference in the plant height rate when pollinating with *P. Polymyxa* isolates alone. The treatment B₁+B₂, gave the highest average quality for this trait of 173.84 cm, there was no significant

Table 2: Effect of using different isolates of Bacillus bacteria and levels of organic matter on plant height (cm).

Organic Fertilizers	O ₀	O ₁ 2%	O ₂ 4%	Mean
	without adding			
Isolates				
B ₀ (without addition)	164.10	161.24	169.26	164.87
B ₁ (isolation 1)	171.28	172.68	171.56	171.84
B ₂ (isolation 2)	166.07	171.62	172.49	170.06
B ₃ (B ₁ +B ₂)	173.48	172.20	175.83	173.84
NPK	177.5			
Mean	168.73	169.44	172.28	
L.S.D _{0.05}	O=N.S	B=3.61	B×O=N.S	

difference from treatment B₁, which recorded an average of 171.84 cm, which increase over the comparison treatment, they reached 5.44 and 4.22%, the same treatments were overtook B₂, which averaged 170.06 cm, the reason for the increase can be attributed to the increased microbial activity as a result of bacterial inoculation, reduces soil pH in the rhizosphere, which facilitates the absorption of elements in general, especially rare ones, a positive impact on improving plant characteristics (Mansour, 2014). As for the effect of organic fertilizer and the bilateral interaction between organic and organic fertilizers, it was not significant.

Table 3, indicated that the addition of the biological fertilizer led to a significant increase in the average leaf area of the maize, the treatment B₁+B₂ achieved the highest value of the average leaf area which reached 4743 cm²/ plants⁻¹, with an increase of 25.67% compared to the control treatment that reached the lowest average of 3774 cm²/ plants⁻¹. The reason for the increase in leaf area of the plant may be attributed to the role of microorganisms that increase the surface of the roots exposed to the soil, leads to an increase in the absorption of nutrients and water by the plant, to improve the nutritional status, reflects positively on the growth and development of the plant, by improving the roots and vegetative group of the plant (Dahich, 2011).

Table 3, also indicated that adding organic fertilizer to the soil was a significant difference in the average leaf area, as the level achieved 4%, the highest value reached 4370 cm² plant⁻¹ and an increase of 8.30% compared to the non-addition treatment with a minimum average of 4035 cm² plants⁻¹. This may be due to the role of organic fertilizers added to the soil, which equip the plant with the necessary elements, especially nitrogen, phosphorous and potassium, the plant growth improves when these nutrients are available (Hayat *et al.*, 2010). There were no significant differences in the treatment of bilateral interference between biological fertilizers and organic fertilizers added to the soil.

Table 3: Effect of using different isolates of *Bacillus* bacteria and levels of organic matter on leaf area (cm²/ plants⁻¹).

Organic Fertilizers Isolates	O ₀ without adding	O ₁ 2%	O ₂ 4%	Mean
B ₀ (without addition)	3773	3738	3812	3774
B ₁ (isolation 1)	3824	3996	4100	3973
B ₂ (isolation 2)	4052	4355	4406	4271
B ₃ (B ₁ +B ₂)	4489	4580	5160	4743
NPK	4768			
Mean	4035	4167	4370	
L.S.D _{0.05}	O= 169.3	B=195.5	B×O=N.S	

Table 4, shows that the addition of organic fertilizer to the soil led to significant differences in the chlorophyll content for maize, achieving the highest value of the average chlorophyll content at level 4%, which reached the highest average of 47.38 Spad and a significant difference from the level 2%, which gave an average of 45.51 Spad, with an increase of 11.03 and 6.65% consecutively compared to the comparison treatment (without fertilization), which reached the lowest average of 42.67 Spad.

Treatments B₁+B₂O₁, B₂O₂ and B₁+B₂O₂ have the highest rates of 48.77, 49.17 and 49.57 Spad, with increases of 29.70, 30.77 and 31.83% consecutively compared to the comparison treatment which reached the lowest average of 37.60 Spad. The reason for the increase may be attributed to the common role between microorganisms and the organic matter added to the soil by providing a large amount of nutrients, especially N. K.P.K elements that work to build pigments during photosynthesis (Sarwar and Kremer, 2013).

Table 5, showed significant differences in the dry weight trait of the vegetate total of maize when using *Bacillus polymyxa* regardless of the addition of organic fertilizers, It gave the highest mean when treating B₁+B₂ and it reached 196.02 g/ plant⁻¹ and a significant difference

Table 4: Effect of using different isolates of *Bacillus* bacteria and levels of organic matter on chlorophyll content (Spad).

Organic Fertilizers Isolates	O ₀ without adding	O ₁ 2%	O ₂ 4%	Mean
B ₀ (without addition)	37.60	43.33	44.10	41.68
B ₁ (isolation 1)	41.37	42.30	47.47	43.71
B ₂ (isolation 2)	46.83	46.83	49.17	47.61
B ₃ (B ₁ +B ₂)	44.90	49.57	48.77	47.74
NPK	49.78			
Mean	42.67	45.51	42.67	
L.S.D _{0.05}	O= 1.034	B=1.190	B×O=2.067	

Table 5: Effect of using different isolates of *Bacillus* bacteria and levels of organic matter on Vegetate total (g/plant⁻¹).

Organic Fertilizers Isolates	O ₀ without adding	O ₁ 2%	O ₂ 4%	Mean
B ₀ (without addition)	162.25	159.15	159.35	160.25
B ₁ (isolation 1)	160.84	161.55	168.90	163.76
B ₂ (isolation 2)	185.35	181.87	192.71	186.64
B ₃ (B ₁ +B ₂)	191.54	199.00	197.51	196.02
NPK	207.39			
Mean	174.99	175.39	179.62	
L.S.D _{0.05}	O= 3.844	B=4.439	B×O=7.689	

from the B₁ and B₂ isolates that recorded 186.64, 163.76 g/plant⁻¹, the percentage increase in the superior treatment was 22.32% compared to the measurement treatment, which reached the lowest average of 160.25 g/plant⁻¹. The reason for the increase in the dry weight of the vegetative group when pollinating the plant with *Bacillus Polymyxa* isolates may be due to the role of these neighborhoods in dissolving and processing nutrients from their insoluble compounds in the soil, in particular providing phosphorous, which supplies the plant with energy and helps divide the cells in the plant, reflects positively on plant growth, including of the increase in the dry weight of the vegetative group in the plant (Sarwar *et al.*, 2012). As for adding organic fertilizers, the levels 4, 2% achieved separately a significant effect on the dry weight characteristic of the vegetative group, as they reached the highest average of 175.39, 179.62 g/plant⁻¹ respectively and with increases of 2.64 and 0.22% respectively, compared to the treatment Do not add (O₀) which was 174.99 g/plant⁻¹. The reason may be due to the role that organic fertilizers (cow fertilizer) added to the soil play in affecting the susceptibility of the soil to increasing biological activity and water retention, in addition to preparing the soil with the necessary nutrients in plant growth (Waniyo *et al.*, 2012). As for the bilateral interaction between the biological pollination and the fertilizer levels, the treatment was superior to B₁+B₂ O₁,

Table 6: Effect of using different isolates of *Bacillus* bacteria and levels of organic matter on Vegetate total (g).

Organic Fertilizers Isolates	O ₀ without adding	O ₁ 2%	O ₂ 4%	Mean
B ₀ (without addition)	263.9	273.13	276.80	271.28
B ₁ (isolation 1)	276.17	275.83	275.87	275.96
B ₂ (isolation 2)	278.37	280.77	281.17	280.10
B ₃ (B ₁ +B ₂)	282.90	282.80	284.57	283.43
NPK	289.11			
Mean	275.33	278.13	279.60	
L.S.D _{0.05}	O= 1.984	B=2.291	B×O=N.S	

Table 7: Effect of using different isolates of Bacillus bacteria and levels of organic matter on total yield (M.g/h⁻¹).

Organic Fertilizers Isolates	O ₀ without adding	O ₁ 2%	O ₂ 4%	Mean
B ₀ (without addition)	5.992	5.404	4.840	5.412
B ₁ (isolation 1)	6.812	6.564	6.386	6.588
B ₂ (isolation 2)	7.407	7.111	6.999	7.173
B ₃ (B ₁ +B ₂)	7.828	7.653	7.699	7.727
NPK	7.861			
Mean	6.48	6.68	7.01	
L.S.D. _{0.05}	O=0.247	B=0.285	B×O=N.S	

gave the highest value of 199.00 g/plant⁻¹, non-significant difference from the rest treatments compared to the control that gave the lowest value was 162.25 g/plant⁻¹. The reason for the increase in this characteristic may be attributed to the fact that the organic matter increases the number of leaves, the soft weight and the chlorophyll content, improving the nutritional status of the plant, which reflects positively on the increase in the characteristic of dry weight (Noni, 2016).

Table 6, indicated that there was a significant effect on the trait of the weight of 1000 grains of maize when using the bio-fertilizer for *Bacillus Polymyxa* B₁+B₂ isolation recorded the highest average of 283.42 g, with an increase of 4.47% compared to the measurement treatment, which gave less Average 271.28 g. The reason for these differences may be attributed to the positive effect of the organisms used in the process of bio-fertilizer and their effective role in providing growth regulators, especially the oxins that increase the weight of grains and this may be consistent with (Al-Ziyadi, 2018). Significant increase in the average weight of 1000 grains, achieving the highest value of the weight of 1000 grains when adding the levels 2, 4%, who did not differ significantly and the average of each of them was 278.13, 279.60 g in succession, with an increase of 1.01, 1.55% compared to the level of non-addition which reached the lowest average 275.33 g. The reason for this may be attributed to the positive role of organic fertilizer added to the soil in accelerating the seed germination process and the growth and development of the plant root system, which increases the absorption process of nutrients, reflects on the weight of the grains (Ortas, 2002). In the case of interaction between the bio-fertilizer pollination and the organic fertilizer added to the soil, there were no significant differences in the treatment of the bilateral interaction between the biological fertilizers and the organic fertilizers added to the soil.

Table 7, indicated a significant effect on the total yield characteristic of maize plants when using *B.*

Polymyxa isolates as a vital vaccine as treatment B₁+B₂ was superior, as it gave the highest average of 7.72 M.g/h⁻¹ with an increase of 50.19% compared to treatment. The measurement, which gave the lowest mean average of 42.69 M.g/h⁻¹, the reason for the increase may be attributed to the role of microorganisms in increasing the readiness of nutrients through microbial activity in the soil and at the rhizosphere, which leads to a decrease in the degree of soil interaction. The addition of organic fertilizer to the soil led to the superiority of the O₂ treatment, which was the addition rate of 4% of the fertilizer recommendation, as it gave the highest average of 7.01 M.g/h⁻¹, with an increase rate of 8.17% compared to the non-addition treatment (Measurement treatment), which gave the lowest average of 6.48 M.g/h⁻¹. The reason for the increase in the yield may be attributed to the role of the organic matter added in increasing the vital activity in the soil in addition to improving the physical properties of the soil through the effect on porosity, which enhances plant growth and thus positively reflects the increase in the total yield (Hassan, 2017).

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