Effect of animal organic manure type and Azotobacter bacteria type on some growth traits of Zea mays L. plant

Hussein Ahmed Abdul Ali 1* and Jawad Abdul Kadhim Kamal 2
) 1,2Department of Soil Science and Water Resources, Faculty of Agriculture, University of Al-Qadisiyah, Al-Qadisiyah, Iraq. (
1E-mail: hsynahmdbdly4@gmail.com
2E-mail: jawad.alshabbany@qu.edu.iq

Abstract

The research included isolation and identification of Azotobacter chroococcum bacteria and Azotobacter paspali bacteria, and studying the effect of these two types of bacteria with three types of animal organic manure (Sheep , Buffalo , Poultry) in some traits of Zea mays L. growth (Plant height, Chlorophyll content, Number of leaves , Leaf area , dry weight of roots and Stem diameter). The field experiment was carried out in Al-Muthanna Governorate, Juwaylan area, and designed using a completely randomized block design.

The results of isolation and diagnosis of these two types of bacteria showed that the morphological and microscopic characteristics of these two types matched the characteristics of Azotobacter spp. bacteria. The results of studying the biochemical characteristics of these two types of bacteria also showed that they gave a positive result for growth tests at 1%. NaCl , 0.1% phenol , catalase, reduce nitrate , glycerol , starch , motile and negative for benzoate test.

The effect of fertilization with Azotobacter bacteria and organic manure was significant in most of the studied plant traits. The bacterial type A. paspali gave the highest values in Plant height, Chlorophyll content and dry weight of roots which amounted to 193.96 cm/plant-1 and 52.66 spad and 16.78 gm/plant-1 respectively and with a significant increase compared to the control treatment 175.37 cm/plant-1 and 52.18 spad and 15.08 gm/plant-1 respectively . while the A. chroococcum type gave the highest values in number of leaves, leaf area and Stem diameter which amounted to 12.78 leaf. plant-1 and 633.66 cm2.plant-1 and 26.71 mm respectively and with a significant increase compared to the control treatment 12.42 leaf. plant-1 and 551.81 cm2.plant-1 and 25.85 mm respectively .

The Poultry manure treatment gave the highest values in all of the studied plant traits which amounted to 220.71 cm.plant-1, 56.82 spad , 13.93 leaf.plant-1 , 732.27 cm2.plant-1 , 23.00 g.plant-1 and 33.76 mm respectively and with a significant increase compared to the control treatment 175.91 cm.plant-1 , 51.50 spad , 11.74 leaf.plant-1 , 472.88 cm2.plant-1 , 12.73 g.plant-1 and 22.00 mm respectively.

The interaction treatment (A. paspali + Poultry manure) gave the highest values in Plant height, Chlorophyll content and dry weight of roots which amounted to 231.23 cm.plant-1 , 58.57 spad and 25.67 g.plant-1 respectively and with a significant increase compared to the control treatment 151.57 cm.plant-1 , 49.52 spad and 9.99 g.plant-1 respectively , while the interaction treatment (A. chroococcum + Poultry manure) gave the highest values in the number of leaves and Stem diameter 14.33 leaf.plant-1 and 34.72 mm respectively and with a significant increase compared to the control treatment 11.22 leaf.plant-1 and 20.91 mm respectively.

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Keywords: Organic manure, Azotobacter bacteria, Azotobacter chroococcum , Azotobacter paspali , Zea mays.

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Introduction

Organic matter in the soil is one of the basic components, and it forms with the mineral part of the soil what is called the solid phase, which consists mainly of plant and animal wastes and the remains of living organisms, as a result of the environmental and climatic conditions to which Iraqi soils are exposed, which lead to rapid destruction and decomposition of organic matter, with the lack of a permanent source of compensation in most soils, they suffer from a deficiency of this material, therefore, the need arises for the continuous addition of organic matter in order for it to work as an improver of some of the physical properties of the soil (1 .(
Inoculation with Azotobacter bacteria directly and indirectly affects the growth and development of the plant by biologically fixing atmospheric nitrogen and preparing it for the plant. It also supplies the plant with phosphorus and iron, secretes plant hormones that are beneficial to the plant, and protects plants from diseases caused by pathogenic microorganisms. It also reduces the effects of chemical fertilizers on the plant (2.(

(3) conducted a pot experiment to study the effect of inoculating yellow corn plants with A. chroococcum bacteria. The results showed that inoculation gave a significant increase in plant height, leaf area and dry weight, as the values reached 17.12 cm, 22.94 cm2 and 5.84 g for the above traits, respectively.

(4) studied the effect of adding four levels of poultry waste 0, 10, 20, 30 tons/ha-1 on some growth traits of maize plants. The results showed that adding organic waste significantly affected the percentage of potassium in the vegetative group, plant height, leaf area and dry weight of the vegetative group.

(5) studied the effect of combined use of Azotobacter bacteria with vermicompost and animal waste (FYM) on some yield characteristics of maize and found that the combined treatment (Azotobacter bacteria + 2 t/ha-1 vermicompost + 10 t/ha-1 animal waste + 50% chemical fertilizer recommendation) gave the highest value in grain yield (4223 kg/ha-1), straw yield (6266 kg/ha-1), harvest index (41.53%) and 1000-grain weight (366.86 g) respectively, with a significant increase compared to the control treatment which gave the lowest values.

This study aims to:
.1Isolate two types of Azotobacter bacteria .
.2Study the effect of two type of Azotobacter bacteria with three types of organic manure in some growth triats of Zea mays plant.

MATERIALS AND METHODS

• Isolation of Azotobacter chroococcum bacteria and Azotobacter paspali bacteria

Eight soil samples were collected from the rhizosphere of radish plant(this plant was selected randomly) and two soil samples from the rhizosphere of garden cress plant, Where the collection was carried out within the geographical area of Al-Muthanna Governorate, as shown in Table (1), then the samples were placed in plastic bags and kept in the refrigerator until used. Then the bacteria were isolated as follows:

The test tube method or streaking plate method mentioned in (6) was followed, which included adding 0.5 g of soil samples to test tubes containing 10 ml of the liquid Azotobacter chroococcum medium described by (7) Which is considered a specific medium for Azotobacter chroococcum bacteria and 10 ml of Azotobacter paspali liquid medium described by (7) Which is considered a specific medium for Azotobacter paspali bacteria, Then it was incubated in an incubator at a temperature of 30-37°C for a week until a yellow film for Azotobacter paspali bacteria and brown film for Azotobacter chroococcum bacteria appears on the surface of the medium with its color changed, and this indicates the presence of these two types. Then, part of the growth was streaked on the specialized solid medium for each type and incubated for 3-5 days for the purpose of purification and studying the culture characteristics of the colonies. The growth was re-streaked several times for the purpose of obtaining pure colonies of bacteria for studying the biochemical characteristics, The isolates were then preserved in the solid medium mentioned above .

Table (1) Isolation region and Bacteria type and Isolation symbol

Isolation region	Bacteria type	Isolation symbol
Samawah	*Azotobacter paspali*	A2, A4, A5, A6 ,A7 ,A8
Samawah	*Azotobacter chroococcum*	B1, B2

• Diagnosis of A. paspali bacteria and Azotobacter chroococcum bacteria

- Morphological properties: The phenotypic characteristics of the colonies growing on the specialized medium for each species were recorded, which included colony color, colony elevation, colony surface shape, colony opacity, colony consistency and Photochromic characteristics according to the method of [8.[

- Microscopic properties : Microscopic examination of the bacteria was carried out after staining them with Gram stain, and the shapes of the cells and their interaction with the dye were observed according to the method of [8.[

- Biochemical tests : To identify the vitality of bacteria, many biochemical tests were performed, including: Growth in 1% NaCl and in 0.1% phenol (9) Nitrate reduction [10].Growth on glycerol as the sole carbon source [11] , 1% sodium benzoate [9].Starch hydrolysis [12].Catalase test [13] and Movement of bacteria test [8.[

• Testing the efficiency of bacterial isolates in fixing atmospheric nitrogen:

100 ml of the liquid medium for each type of bacterial growth was inoculated, then the media were incubated for a week at a temperature of 30-37°C with shaking from time to time, then the amount of ammonia formed in the medium was estimated by taking 10 ml of it and estimating it with the Kjeldahl device according to the method of (14.(

• Field experiment site and land preparation:

The field experiment was conducted in a Sandy clay mixture soil to grow the Zea mays plant in the Al-Juilan area in the Samawah District - the center of Al-Muthanna Governorate. The soil was prepared for cultivation by plowing, smoothing, leveling, and dividing it into experimental units with dimensions of 2*3 m2 for each experimental

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unit, leaving a distance of one meter between the experimental units and one meter between one replicate and another. The treatments were distributed using a completely randomized block design (RCBD), with three replicates, and the number of experimental units in each replicate was 12. The manure was added on the basis of weight, by weighing the manure of each type separately, and it was added inside each plate and mixed with the soil. Soil samples were taken from more than one location in the field at a depth of 0-30 cm and mixed together to form a composite sample for the purpose of conducting some chemical, physical, and biological analyzes shown in Table 2.

Table 2. Some chemical and physical and biological properties of the study soil

Adjective	Azotobacter bacteria CFU.gm^{-1} soil	Total bacteria CFU.gm^{-1} soil	available potassium	available phosphorus %	available nitrogen %	EC (**ds**/ m^{-1}) (1:5)	PH (1:5)	texture
Measurement	5*10^{6}	8*10^{6}	9.3	0.017	0.05	1.31	8.26	Sandy clay

- Experimental design and factors distribution

The field experiment was designed using a completely randomized block design. The experiment included the following factors:

- The first factor: It is an Azotobacter bacteria inoculation which was added in two types and took the following symbols: B0 = without addition, B1= Azotobacter chroococcum, B2= Azotobacter paspali.
- The second factor: is the animal organic manure, which was added in three types and at a level of 35 tons/ha-1 and took the following symbols: A0 = comparison, A1 = Sheep manure, A2 = Buffalo manure, A3 = Poultry manure. Table 3. show some chemical analyzes of the studied organic manure.

Table 3. Chemical analysis of organic manure used in the study.

adjective / Type of organic manure	Total organic carbon%	K%	P%	N%	EC (ms.cm^{-1})	PH	C:N
Poultry manure	49.35	2.15	0.74	2.33	19.4	8.38	1 : 21
Buffalo manure	24.95	1.82	0.60	0.84	26.2	6.70	1 : 29
Sheep manure	19.17	1.51	0.21	0.36	26.5	8.32	1 : 53

- Preparation of Azotobacter inoculation

isolate A5 of the Azotobacter paspali bacteria and isolate B1 of the Azotobacter chroococcum bacteria were selected in inoculated Sterile glass bottles containing 500 ml of the medium for each type and then

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incubated for 9 days, the bacterial density for each type was estimated by the Most Probable Number count method according to the (15) method, It was 2,300 x 10-2 cells.ml-1 for A.paspali bacteria and 47 x 10-2 cells.ml-1 for A.chroococcum bacteria.

- Cultivation and service of the crop

The seeds were added to beakers containing 500 ml of the medium for each type of bacteria, then 100 ml of the gum arabic suspension prepared at a concentration of 10% was added in order to ensure increased adhesion of the inoculum to the seeds while leaving the seeds of the control treatment uncontaminated .Planting was done in lines, and the distance between one line and another was 0.75 m (16), Each experimental unit contained four lines.

Nitrogen fertilizer was added in the amount of 320 kg N.ha-1 in the form of urea in two batches: at planting and at branching. DAP fertilizer was added in the amount of 100 kg P. ha-1 in two batches: at planting and branching, where chemical fertilizers were added to some experimental treatments. hoeing and weeding operations were carried out whenever necessary. Irrigate the field with water as needed. Plant measurements were taken after 90 days, which included Plant height, Chlorophyll content, Number of leaves , Leaf area , Dry weight of roots and Stem diameter .

- Statistical analysis

Field experiments were carried out using a randomized complete block design (RCBD) and data were statistically analyzed according to the analysis of variance method using the SPSS program and the means were compared using Dunnett's test .

Results and discussion

- Study the cultural, microscopic and biochemical characteristics of A.chroococcum bacteria and A. paspali bacteria:

The results of the phenotypic and microscopic study of the studied bacterial isolates shown in Table 4. showed that the colonies growing on the surface of the solid medium for each type were characterized by being viscous, semi-opaque, raised, convex, glistening, and smooth.The surface shape of the colony for most isolates was smooth, and the color of the colony for the Azotobacter chroococcum type was brown, while the color of the colony for the Azotobacter paspali type was yellow or red. The cells of these types were characterized by being of multiple shapes, ranging from rod-like to spherical, and they were Gram-negative. The results of this study are consistent with the results of both (9) and other researchers.

Table 4. also shows some biochemical characteristics of the studied isolates. All of the bacterial isolates were motile, tested positive for the catalase enzyme, and were able to reduce nitrate to nitrite. Most of the isolates were also able to grow in the presence of salt (1% NaCl) and 0.1% phenol and glycerol medium as the only source of carbon, it was also able to decompose starch and was unable to grow in benzoate medium.

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Table (4) Culture and microscopic and Biochemical characteristics for Azotobacter chroococcum bacteria and Azotobacter paspali bacteria

Morphological and microscopic and Biochemical characteristics	Isolates number							
	B1	B2	A2	A4	A5	A6	A7	A8
Colony color	brown	brown	Yellow/red	red	Yellow/red	Yellow	Yellow	Yellow/red
Colony Elevation	Raised-convex	Raised-convex	Convex-Very convex	convex	Convex-Very convex	convex	convex	Convex-Very convex
Colony surface shape	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Photochromic characteristics	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny
opacity	Semi opaque	Semi opaque	Semi opaque	Semi opaque	Semi opaque	Semi opaque	Semi opaque	Semi opaque
Consistency	Viscous	Viscous	Viscous	Viscous	Viscous-Butyraceous	Viscous	Viscous	Viscous
Cell shape	Rod	Rod-spheroid	Rod	Rod	Rod	Rod	Rod	Rod
Gram stain	-	-	-	-	-	-	-	-
Growth in (1%) NaCl	±	±	+	+	+	+	+	+
Growth in (0.1%) phenol	±	±	+	+	+	+	+	+
Nitrate reductase	+	+	+	+	+	+	+	+
1% sodium benzoate	-	-	-	-	-	-	-	-
Growth on glycerol as the sole carbon source	-	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+
Movement of bacteria	+	+	+	+	+	+	+	+

- Fixation of atmospheric nitrogen by A. chroococcum bacteria and A. paspali bacteria

The results of measuring nitrogen fixation by the studied bacterial isolates (Table 5) showed differences in their ability to fix atmospheric nitrogen, as the values of nitrogen fixation by A.paspali bacteria isolates ranged between 0.0024-0.0042 mg. L-1, as isolate A2 gave the highest value of 0.0042 mg. L-1, while the fixation values by A.chroococcum type isolates reached 0.0025 and 0.0034 mg. L-1 for isolate B1 and B2, respectively.

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Table 5. Amount of nitrogen fixed for A. paspali bacteria and A.chroococcum bacteria

Isolate symbol	B1	B2	A8	A7	A6	A5	A4	A2
Amount of NH4 fixed ($mg.L^{-1}$)	0.0025	0.0034	0.0026	0.0033	0.0024	0.0025	0.0033	0.0042

• The effect of adding Azotobacter bacteria and organic manure in plant height, chlorophyll content and number of leaves traits

Table (6) shows the significant effect of Azotobacter bacteria inoculation on the traits of plant height, chlorophyll content and number of leaves, as the A. paspali type gave the highest values in the traits of plant height and chlorophyll content 193.96 cm.plant-1 and 52.66 spad, respectively, with a significant increase compared to the control treatment, which gave the values of 175.37 cm.plant-1 and 52.18 spad, respectively, while the A.chroococcum type gave the highest values in the trait of number of leaves 12.78 leaf.plant-1 and with a significant increase compared to the control treatment, which gave the lowest values of 12.42 leaf.plant-1. The reason for this may be attributed to the production of many growth regulators by Azotobacter bacteria, such as cytokinins, auxins, and gibberellic acid, which are the main components in controlling plant growth by increasing its nitrogen content (17), in addition to the known role of these bacteria in fixing atmospheric nitrogen, as this element plays an important role in increasing the growth of the parts Such as plant height, chlorophyll content, number of leaves . This result is consistent with the results of both (18) and (19.(

It is also noted from the same table the significant effect of adding most of the organic manure on all the above traits compared to the treatment of control treatment as the poultry manure treatment gave the highest values in all the above traits, reaching 220.71 cm.plant-1, 56.82 SPAD, and 13.93 leaf.plant-1, respectively, with a significant increase compared to the control treatment, 175.91 cm.plant-1, 51.50 Spad, and 11.74 leaf.plant-1, respectively. The reason for the high effect of poultry manure may be attributed to its high content of nutrients, especially the nitrogen element, as well as its high content of organic matter and organic carbon, (Table 3), and also the low ratio of carbon to nitrogen in this manure, which led to giving the highest effect on the growth and yield of the plant, as the lower the ratio of carbon to nitrogen in manure, the greater the ready amount of nutrients present in it for the plant and vice versa. This result is consistent with the results of both (20) and (21). The sheep manure treatment did not differ significantly from the control treatment in the plant height trait. This may be attributed to the high carbon to nitrogen ratio in this manure (Table 3), which reduced its value for the plant due to the long time it takes to decompose. The buffalo manure treatment did not differ significantly from the comparison treatment in the Chlorophyll content trait.

The interaction between the two types of Azotobacter bacteria and organic manure had a significant effect on the above traits. The treatment (A. paspali + pollutry manure) gave the highest value in Plant height and chlorophyll content traits 231.23 cm.plant-1 and 58.57 SPAD respectively, with a significant increase compared to the control treatment, which gave the values 151.57

cm.plant-1 and 49.52 SPAD respectively. The treatment (A.chroococcum + pollutry manure) also gave the highest value in the number of leaves trait 14.33 leaf .plant-1, with a significant increase compared to the control treatment, which gave the lowest 11.22 leaf .plant-1. The reason for the increase may be attributed to the high pollutry manure content of the nitrogen element in addition to the known role of Azotobacter bacteria in fixing atmospheric nitrogen and increasing its availability to the plant. Nitrogen plays an important role in increasing the growth and yield of the plant through its biological union with carbon, hydrogen, oxygen and sulfur to form amino acids, which are the basic building blocks for building proteins. These amino acids are also used in the formation of protoplasm, which is the site of cell division. Thus, increasing protoplasm leads to increased growth and development of the plant (22.(

Table 6. The effect of adding Azotobacter bacteria and organic manure in plant height, chlorophyll content and number of leaves

	type of OM	type of azotobacter bacteria			
		control	A.chroococcum	A. paspali	average
plant height (cm.plant^{-1})	control	151.57	185.79	190.38	175.91^{a}
	Sheep	159.17	163.68	179.41	167.42^{a}
	Poultry	206.08	224.81	231.23	220.71^{b}
	Buffalo	184.67	189.26	174.83	182.92^{c}
	average	175.37^{a}	190.88^{a}	193.96^{b}	
chlorophyll content (spad)	type of OM	type of azotobacter bacteria			
		control	A.chroococcum	A. paspali	average
	control	49.52	46.88	58.09	51.50^{a}
	Sheep	52.69	51.93	52.04	52.22^{b}
	Poultry	53.36	58.54	58.57	56.82^{c}
	Buffalo	53.13	46.32	41.96	47.14^{a}
	average	52.18^{a}	50.92^{b}	52.66^{c}	
number of leaves (leaf.plant^{-1})	type of OM	type of azotobacter bacteria			
		control	A.chroococcum	A. paspali	average
	control	11.22	11.78	12.22	11.74^{a}
	Sheep	12.00	12.22	12.00	12.07^{b}
	Poultry	13.89	14.33	13.56	13.93^{c}
	Buffalo	12.56	12.78	13.11	12.81^{d}
	average	12.42^{a}	12.78^{b}	12.72^{c}	

- The effect of adding Azotobacter bacteria and organic manure in the leaf area, dry weight of roots and stem diameter

Table (7) shows the significant effect of inoculation with Azotobacter bacteria on the leaf area, root dry weight and stem diameter traits, as the A.chroococcum type gave the highest values in the Leaf area and stem diameter traits 633.66 cm2.plant-1 and 26.71 mm respectively, with a significant increase compared to the control treatment, which gave

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the lowest value 551.81 cm2.plant-1 and 25.85 mm respectively, while the A. paspali type gave the highest value in the root dry weight trait 16.78 g.plant-1 and with a significant increase compared to the control treatment, which gave the value 15.08 g.plant-1. The reason for the increase may be attributed to the production of microorganisms, including Azotobacter, many growth regulators such as abscisic acid, which encourages the roots to absorb iron (23), thus increasing their growth and then increasing the Leaf area, root dry weight and stem diameter traits as a result of increasing the absorption of nutrients. It may also be attributed to these bacteria increasing the availability of nutrients such as boron, Which plays an important role in increasing root growth (22). This result is consistent with what was found by (18.(

It is also noted from the same table the significant effect of adding organic manure on the above traits compared to the control treatment, as the poultry manure treatment gave the highest values in the above traits, reaching 732.27 cm2.plant-1, 23.00 g.plant-1, and 33.76 mm, respectively, with a significant increase compared to the control treatment, which gave the values, 472.88 cm2.plant-1, 12.73 g.plant-1, and 22.00 mm, respectively. The reason for the high effect of poultry manure may be attributed to the positive effect of organic matter on the biological, physical, and chemical properties of the soil, in addition to its effect in increasing the availability of nutrients for the plant (24), as organic matter plays a very important role in the secretion of organic acids such as oxalic, which affects the reduction of the degree of soil reaction and thus increases the availability of nutrients and thus increases the plant’s absorption of them, and this was reflected in the increase in growth traits Such as dry weight, leaf area and stem diameter. This result is consistent with the results of (25.(

The interaction between the two types of Azotobacter bacteria and organic manure had a significant effect on the above traits (except for one trait). The treatment (poultry manure + without bacteria), the treatment (A.paspali + poultry manure) and the treatment (A.chroococcum + poultry manure) gave the highest values in the Leaf area, root dry weight and stem diameter traits, which reached 740.39 cm2.plant-1, 25.67g.plant-1 and 34.72 mm, respectively, with a significant increase compared to the control treatment, which gave the values of 333.58 cm2.plant-1, 9.99 g.plant-1 and 20.91 mm, respectively. The reason for the increase may be attributed to the organic manure content of the element phosphorus in addition to the important role of Azotobacter bacteria in increasing the availability of nutrients, including phosphorus. This element plays a fundamental role in the processes of photosynthesis and mitosis through its effective participation in storing and transferring energy in the form of ATP and ADP, as well as its entry into the construction of nucleic acids responsible for transferring genetic information, as well as its important role in increasing the growth of roots, seeds and fruits (22.(

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Table 7. The effect of adding Azotobacter bacteria and organic manure in the leaf area, dry weight of roots and stem diameter

	type of OM	type of azotobacter bacteria			
		control	A.chroococcum	A. paspali	average
leaf area (cm^2.$plant^{-1}$)	control	333.58	523.88	561.17	472.88[a]
	Sheep	604.96	635.85	581.13	607.31[b]
	Poultry	740.39	733.21	723.20	732.27[c]
	Buffalo	528.30	641.70	548.08	572.69[d]
	average	551.81[a]	633.66[b]	603.40[c]	
dry weight of roots (g.$plant^{-1}$)	type of OM	type of azotobacter bacteria			
		control	A.chroococcum	A. paspali	average
	control	9.99	11.77	16.43	12.73[a]
	Sheep	14.67	8.99	10.67	11.44[b]
	Poultry	21.00	22.33	25.67	23.00[c]
	Buffalo	14.67	16.67	14.33	15.22[d]
	average	15.08[a]	14.94[a]	16.78[b]	
stem diameter(mm)	type of OM	type of azotobacter bacteria			
		control	A.chroococcum	A. paspali	average
	control	20.91	22.03	23.06	22.00[a]
	Sheep	24.76	24.78	24.01	24.52[b]
	Poultry	34.55	34.72	32.01	33.76[c]
	Buffalo	23.17	25.31	26.25	24.91[d]
	average	25.85[a]	26.71[b]	26.33[a]	

abbreviated	meaning
A.chroococcum	*Azotobacter chroococcum* bacteria
A. paspali	*Azotobacter paspali* bacteria
CFU	Colony Forming unit
SPSS	Statistical Package for the Social Sciences
type of OM	type of organic manure

Conclusion

We isolated and identified of A. chroococcum bacteria and A. paspali bacteria by conventional methods and found that the morphological and microscopic characteristics of these two species matched the characteristics of Azotobacter spp. bacteria. We also studied the effect of adding these two type of bacteria with three types of animal organic manure (sheep, buffalo, pollutry) on some growth characteristics of the maize plant, and we found that this addition had a significant effect on most of the studied maize growth characteristics.

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