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THE EFFECT OF BACTERIAL BIOFERTILIZER (*AZOSPIRILLUM BRASILENSE*), COMPOST AND CHEMICAL FERTILIZER ON THE GROWTH AND YIELD OF POTATO PLANT (*SOLANUM TUBEROSUM* L.)

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Abstract

The nitrogen-fixing bacteria were isolated from the (10) root samples of different plants using the semi-solid culture medium (NFB), where the isolated bacterial isolates were identified based on the culture and microscopic traits and biochemical tests. The identification results showed the presence of two isolates belonging to the *Azospirillum brasilense* and was given a symbol A_2 and A_5, then their ability to nitrogen fixation was tested in the laboratory, and it was observed that the isolate A_5 was more able to nitrogen fixation by 11.78 mg $N.kg^{-1}$. The most efficient isolate of nitrogen fixation was grown in the liquid culture medium (Nutrient.broth) several times to use a biological inoculant. Potato tubers were inoculated with the biological inoculant and Arabic gum, then they were planted in rows after adding organic and chemical fertilizers before planting, then the irrigation was done by the drip irrigation system. Finally, The results showed that the highest concentration of nitrogen% in potato tubers was for treatment (B_1) *Azospirillum brasilense* with organic fertilizer by an average of 2.06 N% compared to the comparison treatment 0.40 N%. In addition, it achieved the highest concentration of available nitrogen in the soil by an average of 44.28 mg $N.kg^{-1}$ soil compared to the comparison treatment that achieved 16.15 mg $N.kg^{-1}$ soil. The treatment of *Azospirillum brasilense* with organic fertilizer (B_1O_1) also exceeded in the total yield of the potato plant and achieved an average of 33.03 Mg. Ha^{-1} compared to the comparative treatment of 10.71 Mg. Ha^{-1}. These results confirm that the bacterial biofertilizer with organic and chemical fertilizer achieved the best results for some potato growth parameters and yield.

Key words: Biofertilizer, Organic fertilizer, Potato, Nitrogen fixation.

Introduction

The importance of using microorganisms as biological fertilizers is to increase plant growth and yield, as it helps to reduce the use of chemical fertilizers by a percentage of approximately (40-50%) and has a role in preserving the environment, as well as improving the plant's ability to absorb nutrients and water from the soil. The bacteria *Azospirillum brasilense* is one of the biofertilizers that have the ability to nitrogen fixation, in addition to its production of hormones, Auxins and Abscisic acid that encourage root growth and thus increase the absorption of water and nutrients (Purwautisari *et al.,* 2019). The results of a study carried out by (Farage *et al.,* 2013) showed that adding bacterial biofertilizer to the potato plant had led to an increase in the total yield and the percentage of dry matter and starch compared to the comparison treatment. Furthermore, (Naqqash *et al.,* 2018) experimented with the effect of bacteria that stimulate plant growth represented by nitrogen-fixing and phosphate dissolving bacteria on the growth and yield of the potato plant and its nutrient content. The conclusion was that the interaction treatment between biofertilizer microorganisms exceeded the comparison treatment in all measured parameters, and resolved that adding biofertilizer in a combination is better than the individual addition of each bacterium. Moreover, (Pathak *et al.,* 2017) showed the superiority of the interaction treatments between *Azospirillum* spp. and the fertilizer recommendation for chemical fertilizer on the growth and yield of the potato plant compared to the comparison treatment and the chemical fertilizer treatment only. (Swamy and Avinash, 2018) experimented to isolate and identify bacteria *Azospirillum* from the plant rhizosphere, as 40 bacterial isolates were isolated, and among the tests that were based on it are the consumption of glucose, the requirements of biotin, the production of acids, and the possibility of nitrogen fixation. The results showed that 20 isolates needed to biotin in their growth and that 16 isolates were positive in nitrogen fixation. (Hassan and Abdel, 2003) noted that the potato is one of the most important crops and ranks the fourth in the world after wheat, corn and rice in terms of nutritional importance, as it accounts for 75-90% of the daily food of the world's population. Therefore, this research aimed to study the effect of *Azospirillum brasilense* as a biofertilizer on the growth and yield of the potato plant, as well as study the interaction between bio, organic and chemical fertilizers in some parameters of potato growth and yield.

Materials and Methods

The roots of plants were collected from different soils and were placed in sterile plastic boxes then brought to the laboratory. Parts of the roots were mashed in a test tube with distilled water, and a series of decimal dilutions was performed up to 10^{-8}. In addition, of 0.5 ml of the last dilution was transferred to the semisolid culture medium (NFb) Nitrogen Free according to the (Baldani and Dobereiner, 1980) method. After incubation, white annular growth was observed near the surface of the culture medium, and then a portion was transported by the loop to dishes contained on the root compartment Rc medium having the Red Congo dye, where the dishes were incubated at 30°C for 72 hours according to the (Rodrigues Caceres, 1982) method. The colonies were purified by re-streaking them in the same medium, then bacterial isolates were identified in terms of culture, microscopic and biological traits according to (Holt *et al.,* 1994). Once the identification of bacterial isolates complete, the efficacy of bacterial isolates belonging to the *Azospirillum brasilense* in nitrogen fixation was tested to select the most efficient and use a biological inoculant. The nitrogen fixation was measured using the Micro-Kjeldahl device according to (Bremner and Keeney, 1966) method, where the most efficient isolates in nitrogen fixation were grown in a 2-liter flask containing the Nutrient. Broth, and after incubation, the sporulation was estimated.

Field experiment

The experiment was conducted in one of the fields of the College of Agricultural Engineering Sciences, University of Baghdad and after the process of preparing the soil for cultivation. A soil sample was taken for analyzing and investigate some physical, chemical and biological traits, where the available EC, pH., OM, N, P, K, in soils, soil texture, and total bacterial and fungal count were measured. However, the experimental factors were the biofertilizer at two levels: without adding (B_0), and (B_1) adding biofertilizers, besides, organic fertilizers at two levels: without adding (O_0) and (O_1) adding 20 Mg. Ha^{-1}. As well as, chemical fertilizers at three levels without adding (S_0) and (S_1) adding 50% of the fertilizer recommendation, and (S_2) adding 100% of the fertilizer recommendation for the potato plant (N, P, K), thus, the number of experimental units became 36 experimental units with three replicates. Potato tubers were washed with distilled water and sterilized with sodium hypochlorite and then were contaminated with the biological inoculant and Arabic gum for adhesion bacterial cells on the surface of the tubers. The inoculated tubers were planted in the rows, as bio and chemical fertilizers were added before planting, then the rows were irrigated with a drip irrigation system. The experiment was factorial according to the randomized complete block design (RCBD), where the results were analyzed by statistical analysis and the averages were compared on significant difference 0.05.

Results and Discussion

Soil analyzed results

The results showed that the electrical conductivity EC is 1.82 $ds.m^{-1}$, the soil reaction pH is 7.75, the organic matter is 8.4 $g.kg^{-1}$ soil, available nitrogen is 28.01 $mg.kg^{-1}$ soil. Besides, the available phosphorous 6.53 $mg.kg^{-1}$, available potassium 148.0 $mg.kg^{-1}$, clay 188 $g.kg^{-1}$ soil, silt 396 $g.kg^{-1}$, sand 416 $g.kg^{-1}$, the texture was Loam, the total fungal count 2.5×10^4 C.F.U/g soil and the total bacterial count 4.2 C.F.U$\times10^6$ C.F.U/g soil.

Isolation and identification of bacteria

The isolation results showed that there were ten bacterial isolates belonged to the *Azospirillum* and that only two isolates belonged to the *Azospirillum brasilense,* which are A_5, A_2. The isolated bacterial isolates were subjected to biochemical, oxidase, and catalase tests, starch hydrolysis, motility, urease, and carbon source consumption, Mannitol, Glucose, and Sucrose, all of these tests had a positive result for the isolates A_2 and A_5. Furthermore, The results showed that the isolate A_5 is the most efficient in nitrogen fixation in the laboratory experiment as it achieved 11.78 mg $N.kg^{-1}$ compared to the isolate A_2 that achieved 9.53 8 mg Nkg^{-1}. Based on these results, the isolate A_5 was chosen to produce the biofertilizer that was used in the experiment and its density was 5×10^6 cells/ml.

The effect of bio, organic and chemical fertilizers

on tubers nitrogen%

The results of table 1 showed that the treatment (B_1) was significantly superior and achieved 1.42% N compared to the treatment (B_0) 1.04% N and the treatment (B_0O_1) was also superior to an average of 2.06% N compared to the comparison treatment 0.79 N%. The treatment (O_1) was significantly superior and achieved 1.57% N compared to the treatment (O_0) 1.03% N, as well as the treatment (S_2) was significantly superior and achieved 2.07% N compared to treatment (S_0) 0.90% N. The reason for this is the efficiency of the biofertilizer represented by *Azospirillum brasilense,* which contributes to the availability of nutrients necessary for plant growth, and the most important nitrogen, especially that bacteria have the ability to nitrogen fixation.

In addition to the ability of these microorganisms to secrete some growth regulators, such as gibberellin, which helped in plant growth and benefiting from the available nitrogen in the soil and its reflection on the content of potato tubers of nitrogen. These results are consistent with (Zeffa *et al.,* 2018), in terms of the effect of organic and chemical fertilizers by contributing to improving the plant's nitrogen content to carry out its biological efficacy and nitrogen concentration in tubers. Additionally, these results are consistent with (Kumari and Kumar 2018) indicated that the plant's yield has a high value of nitrogen when integrated fertilization is available; it helps the plant grow well.

Table 1: The effect of adding a bio, organic and chemical fertilizers on the nitrogen% in tubers after harvesting.

Bio fertiliza-tion	Organic Fertilization (Mg.ha^{-1})	Chemical fertilizer (%) S_0	S_1	S_2	Interaction between bio and organic fertilization
B_0	O_0	0.40	0.96	1.56	0.97
	O_1	0.69	1.36	1.83	1.29
B_1	O_0	0.73	1.52	1.92	1.39
	O_1	1.37	2.26	2.57	2.06
L.S.D for triple interaction		0.39			L.S.D= 0.20
Chemical fertilizers averages		0.90	1.68	2.07	
L.S.D for chemical fertilizer		0.30			
Bio fertilization X Chemical fertilization					
Biofertilization		S_0	S_1	S_2	Bio fertilization averages
B_0		0.45	1.00	1.69	1.04
B_1		0.78	1.49	2.90	1.42
L.S.D		0.27			L.S.D= 0.19
Organic Fertilization X Chemical Fertilization					
Organic Fertilization (Mg.ha^{-1})		S_0	S_1	S_2	Organic fertilization averages
O_0		0.38	1.06	1.67	1.03
O_1		1.03	1.69	2.00	1.57
L.S.D		0.36			L.S.D= 0.22

B_0: Biofertilizer without addition, B_1: Adding biofertilizer
O_0: Organic fertilizer without addition, O_1: Organic fertilizer with the addition of 20 Mg.ha^{-1}
S_0: Chemical fertilizer without addition S_1: Chemical fertilizer, adding 50% of the fertilizer recommendation
S_2: Chemical fertilizer, adding 100% of the fertilizer recommendation.

The effect of bio, organic and chemical fertilizers on available nitrogen in the soil (mg.kg^{-1})

The results of table 2 showed that the treatment (B_1) was significantly superior to an average of 39.52 mg.kg^{-1} soil compared to the comparison treatment (B_0) 27.86 mg.kg^{-1} soil. Besides, the interaction treatment (B_1O_1) achieved an average of 44.28 mg.kg^{-1} compared to the treatment (B_0O_1) that achieved an average of 36.43 mg.kg^{-1}, compared to the comparison treatment that achieved an average of 26.55 mg.kg^{-1}.

The results showed that chemical fertilization affected the concentration of nitrogen in the soil, especially at the level of (S_2), as ($B_1O_1S_2$) achieved 50.65 mg.kg^{-1} compared to the treatment (S_0) 16.15 mg.kg^{-1}. This may be due to the activity of the added bacteria as a biofertilizer, which is characterized by its ability to nitrogen fixation and increasing the nitrogen concentration in the soil. As well as, the nitrogen-fixing bacteria that stimulate plant growth with the presence of the enzyme nitrogen, which leads to an increase in the amount of nitrogen in the soil (Dubey *et al.,* 2020). The addition of nitrogen fertilizers (urea), which is one of the chemical fertilizers, will increase the concentration of nitrogen in the soil, coinciding with the addition of bio and organic fertilizers and its role in adding nitrogen to the soil, as these results were consistent with (Turan, 2006) pointed out.

The effect of bio, organic and chemical fertilizers on the total yield of potato plant (Mg.ha^{-1})

The treatment (B_1) was significantly superior to the comparison treatment as shown in table 3, which achieved an average of 21.37 Mg.ha^{-1} and the comparison treatment achieved 19.98 Mg.ha^{-1}. Also, (B_1O_1) achieved an average of 33.03 Mg.ha^{-1} compared to the comparison treatment was 19.35 Mg.ha^{-1}, while the treatment ($B_1O_1S_2$) achieved a significant superiority over the treatment ($B_0O_0S_0$) and achieved 42.14 and 10.71 Mg.ha^{-1}, respectively. These results

Table 2: The effect of adding a bio, organic and chemical fertilizers on available nitrogen in the soil after harvesting (mg.kg^{-1}).

Bio fertilization	Organic Fertilization (Mg.ha^{-1})	Chemical fertilizer (%)			Interaction between bio and organic fertilization
		$_0S$	S_1	S_2	
B_0	O_0	16.15	25.15	38.35	26.55
	O_1	29.95	33.50	40.00	34.48
B_1	O_0	24.85	39.35	45.10	36.43
	O_1	34.45	47.75	50.65	44.28
L.S.D for triple interaction		3.64			L.S.D= 1.12
Chemical fertilizers averages		25.75	37.61	43.94	
L.S.D for chemical fertilizer		5.07			
Bio fertilization X Chemical fertilization					
Biofertilization		$_0S$	S_1	S_2	Bio fertilization averages
B_0		18.11	26.32	39.17	27.86
B_1		27.65	40.65	47.37	39.52
L.S.D		6.76			L.S.D= 1.24
Organic Fertilization X Chemical Fertilization					
Organic Fertilization (Mg.ha^{-1})		$_0S$	S_1	S_2	Organic fertilization averages
O_0		20.86	30.06	41.16	30.69
O_1		31.65	35.97	46.25	37.95
L.S.D		4.97			L.S.D= 2.07

Table 3: The effect of adding organic, bio and mineral fertilizers on the total yield of potato plants (Mg.ha^{-1}).

Bio fertilization	Organic Fertilization (Mg.ha^{-1})	Mineral fertilizer (%)			Interaction between bio and organic fertilization
		$_0S$	S_1	S_2	
B_0	O_0	10.71	19.37	28.51	19.53
	O_1	15.82	22.60	33.18	23.86
B_1	O_0	13.78	20.97	29.03	21.26
	O_1	19.48	37.47	42.14	33.03
L.S.D for triple interaction		5.15			L.S.D= 4.21
Chemical fertilizers averages		15.61	27.79	35.75	
L.S.D for chemical fertilizer		7.22			
Bio fertilization X Mineral fertilization					
Bio fertilization		$_0S$	S_1	S_2	Bio fertilization averages
B_0		11.04	18.79	27.11	18.98
B_1		12.38	21.57	30.17	21.37
L.S.D		5.12			L.S.D= 2.73
Organic Fertilization X Mineral Fertilization					
Organic Fertilization (Mg.ha^{-1})		$_0S$	S_1	S_2	Organic fertilization averages
O_0		11.81	21.25	27.57	20.21
O_1		17.15	27.88	32.55	25.86
L.S.D		4.76			L.S.D= 5.06

showed the role of the biological inoculant in the total yield of the potato plant. However, the reason for this possibly is the biofertilizer and its significant effect on the number of tubers that are reflected from the total yield of the plant through the ability of the biofertilizer to provide the nutritional needs of the plant (Singh *et al.,* 2017). The interaction between bio and organic fertilizers has led to an increase in the total yield of what organic fertilizer characterized by adding some nutrients suitable for plant growth and microorganisms, which in turn secrete hormones, growth regulators, and acids (Allison, 2010). Finally, the interaction between bio and chemical fertilizers has led to a significant increase in the total yield of the potato plant. The reason for this is to increase the effectiveness of microorganisms and increase the readiness of some important nutrients for the plant by increasing the chemical fertilizer concentration represented by the (NPK) elements that provide energy sources for the microorganisms. As well as their absorption by the plant, because it is ready for absorption (Oswald *et al.,* 2010; Tahir and Sarwar, 2013).

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