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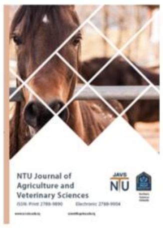

Effect of biofertilization on some nutrients availability and pH of strawberry (Fragaria x ananassa Duch.) Camarosa cultivar soil.

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A B S T R A C T

The study was conducted from 1/10/2023 to 1/5/2024 on strawberry Camarosa cv seedlings . planted in one of the plastic houses belonging to Horticulture and Landscape Design Dep. / Agriculture and Forestry college / mosul University aim of determining the effect of adding Bio fertilizers, namely Azotobacter, which were added at three levels (0, 2 and 4 g plant^{-1}), and Azospirillum at three levels as well (0, 2and 4 g plant^{-1}), and two levels of mycorrhizal fungi (0 and5 g plant^{-1}). All fertilizers from bacteria and fungi were added at once and distributed randomly. The experment was executed a Randomized Complete Block Design (RCBD) with three replications and 8 plants per experimental unit. The results were statistically analyzed according to the design used, and the means were compared using Duncan's multiple range test at a probability level of 0.05. The results confirmed that adding Azotobacter, Azospirillum at levels of 4 g plant $^{-1}$ for each, and mycorrhizal fungi at level of 5g plant^{-1} individually, as well as their triple interaction, significantly increased available concentration of nitrogen, phosphorus, and potassium in the soil ,meanwile soil pH unsignificantly effected with the application of bio-fertilizers.

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Introduction

Strawberry (*Fragaria x ananassa* Duch) is one of the economically important perennial herbaceous fruits, widely distributed around the world, belonging to the order Rosales, subfamily Rosaideae, and family Rosaceae [1], [2] It is believed that the strawberry's native habitat is North America, but it is also cultivated in Europe, Asia, Africa, and the Americas [3] Some strawberry varieties have the ability to grow in subtropical regions and even up to latitude 70˚ north of the equator, but they are predominantly found between the latitudes of 15-55°N [4] Globally, strawberries rank fourth in consumption and preference among consumers, following apples, oranges, and bananas [1],[5] The area cultivated with strawberries worldwide is estimated to be approximately 389,665 hectares, with a production of 9,175,384 ton, China is the largest country in terms of cultivated area and production of strawberries, with a production of about 3,380,478 tons and a cultivated area of 111,132 hectares. The United States follows, with a production of approximately 1,211,090 tons and a cultivated area of 19,919 hectares [9]. As for Iraq, there are no statistics available regarding the cultivated areas and production, the use of bio fertilizers containing microorganisms (beneficial bacteria and fungi) has increased. Among the most important bio fertilizers are those containing Azotobacter bacteria, which are among the most effective free-living nitrogen fixers. This genus includes several species, the most important and widespread of which is Azotobacter Chroococcum, This bacterium promotes plant growth and production by converting nitrogen from its elemental form (N2) to ammonium (NH4+) with the help of the enzyme Nitrogenase, It also decomposes organic matter, produces chelating compounds, and reduces ethylene, and is used in biological control. This bacterium contributes in the root zone by providing protection to the plant from various pathogenic agents present in the soil through its direct and indirect effects by competing with pathogenic organisms for space and nutrients and preventing the pathogen from reaching the infection sites. Additionally, *A. Chroococcum* induces significant changes in the root system, including promoting the formation of lateral roots and increasing the root surface area, which is mainly attributed to its secretion of the auxin IAA, These changes are related to improving water and nutrient absorption by the inoculated plant [7],[8]

Azospirillum bacteria are also used in bio-fertilization, which are considered microorganisms that can live in a symbiotic manner with plant roots [9]. They can be used as a bacterial inoculant that fixes atmospheric nitrogen for plant's and secretes certain plant hormones that enhance the growth and development of the root system, thereby increasing the plants' capacity to absorb nutrients such as phosphorus and potassium from the soil [10] additionally, mycorrhizal fungi live in mutualistic relationship with the roots of many plants and play an important role in dissolving insoluble phosphates present in the soil, transferring it through the fungal hyphae to the plant. it also have the capability to increase the absorption of several other nutrients besides phosphorus, along with improving soil structure and increasing plants' resilience to drought, soil salinity, and diseases. Fungi taken from soils infected with these fungi are directly utilized in the inoculation process [11]

This study aimed to improve the vegetative and fruiting growth of Camarosa strawberry plants, and to determine the suitable concentrations of Azotobacter bacteria, Azospirillum bacteria, and mycorrhizal fungi that should be added to the soil of these plants to achieve this, due to the lack of similar studies on this variety in the city of Mosul.

Materials and Methods

the study was conducted on Camarosa strawberry plants grown in one of the unheated greenhouses belonging to the Department of Horticulture and Landscape Design / College of Agriculture and Forestry / University of Mosul during the agricultural season 2023 – 2024, where some physical and chemical properties of the soil some the estimated before planting, Table (1).

Table 1. Some chemical and physical properties of plastic house soil.

Value	Unit	Parameter
2.71	(dsm. m-1)	EC
7.44	………	pH
6.31	gm kg^{-1}	Organic matter
617.9	gm kg^{-1}	Sand
143.1	gm kg^{-1}	Clay
240.8	gm kg^{-1}	Silt
Silty		Soil texture
42	mg kg^{-1}	Available nitrogen
25	mg kg^{-1}	Available phosphorus
124	mg kg^{-1}	Available potassium

The analysis was conducted in the central laboratory of the College of Agriculture and Forestry / University of Mosul.

The Camarosa strawberry seedlings were selected from the nurseries affiliated with the College of Agriculture and Forestry at the University of Mosul, which are almost homogeneous in growth. They were uprooted directly from the soil, and the damaged roots and large leaves were removed while keeping two new leaves. The NPK fertilizer was added before planting (50 % of fertilization recommendations), by spreading it on the upper surface of the soil, mixed well with the soil, and watered immediately. The soil was covered with black polyethylene, holes on a distance of 25 cm between each were made on the cover top for, which took place on the first of October 2023. The plants were planted in three rows, with a distance of 25 cm between each. Fertilization treatments were carried out using Azotobacter bacteria at three levels (0, 2, and 4 g per plant) and Azospirillum bacteria at three levels (0, 2, and 4 g per plant), and mycorrhizal fungi at two levels (0 and 5 g per plant) were added before planting in the soil on 27/9/2023, The study utilized a complete randomized block design (RCBD) with three factors and three replications, with 8 plants per experimental unit, resulting in a total of 432 plants used in the study. The following traits were estimated:

1) soil pH using pH meter.

2-) available nitrogen in the soil (mg kg $^{-1}$) using a micro-Kjeldahl device according to the Bremner method (1965) as cited by Black (1965).

3) available phosphorus in the soil (mg kg $^{-1}$) according to the method described by Page et al. (1982).

4) available potassium in the soil (mg kg $^{-1}$) according to the Black (1965) [5].The results were analyzed using the SAS program [15], and the means were compared using the Duncan multiple range test at a probability level of 0.05.

Results and Discussion

Effect of Azotobacter: The results Tables (2-5) indicated that fertilization with Azotobacter at 4 g plant^{-1}, led to a significant increase in the studied traits, which yielded the highest averages for available nitrogen phosphorus and potassium in the soil, reaching (46.97,24.10and349.8 mg kg-1), compared to the control treatment, which provided the lowest values for these traits 37.23 mg kg^{-1}, 20.81 mg kg^{-1}, and 349.8 mg kg^{-1}, respectively. Meanwhile, the soil pH was not significantly affected by the fertilization with Azotobacter .

Table 2. Effect of bio-fertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on soil pH *.

Azotobacter Fertilizer Levels (gm plant-1)	Mycorrhiza fertilizer levels (gm plant-1)	Azospirilum Fertilizer Levels (gm plant-1)			Interaction between Azotobacter And Mycorrhiza	Mycorrhiza Fertilizer means
		0	2	4		
0	0	16.33 k	29.40 ij	39.20 gh	28.31 e	30.39 b
2		19.67 k	29.40 ij	39.20 gh	29.42 e	
4		26.20 j	34.30 hi	39.80 gh	33.43 d	
0	5	50.60 de	29.40 ij	58.43 c	46.14 c	53.70 a
2		44.10 fg	52.27 d	66.97 b	54.44 b	
4		46.07 ef	58.70 c	76.77 a	60.51 a	
Interaction between Azospirilum and Mycorrhiza	0	20.73 e	31.03 d	39.40 c	Azotobacter Fertilizer means	
	5	46.92 b	46.79 b	67.39 a		
Interaction between Azotobacter and Azospirilum	0	33.47 ef	29.40 g	48.82 c	37.23 c	
	2	31.88 ef	40.83 d	53.08 b	41.93 b	
	4	36.13 e-	46.50 c	58.28 a	46.97 a	
Azospirilum Fertilizer means		33.83 c	38.91 b	53.39 a		

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 3. Effect of bio-fertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi and their interactions on the concentration of available nitrogen in the soil (mg kg^{-1} soil)*

Azotobacter Fertilizer Levels (gm $plant^{-1}$)	Mycorrhiza fertilizer levels (gm $plant^{-1}$)	Azospirilum Fertilizer Levels (gm plant-1)			Interaction between Azotobacter and Mycorrhiza	Mycorrhiza Fertilizer means
		0	2	4		
0	0	16.33 k	29.40 ij	39.20 gh	28.31 e	30.39 b
2		19.67 k	29.40 ij	39.20 gh	29.42 e	
4		26.20 j	34.30 hi	39.80 gh	33.43 d	
0	5	50.60 de	29.40 ij	58.43 c	46.14 c	53.70 a
2		44.10 fg	52.27 d	66.97 b	54.44 b	
4		46.07 ef	58.70 c	76.77 a	60.51 a	
Interaction between Azospirilum and Mycorrhiza	0	20.73 e	31.03 d	39.40 c	Azotobacter Fertilizer means	
	5	46.92 b	46.79 b	67.39 a		
Interaction between Azotobacter and Azospirilum	0	33.47 ef	29.40 g	48.82 c	37.23 c	
	2	31.88 ef	40.83 d	53.08 b	41.93 b	
	4	36.13 e-	46.50 c	58.28 a	46.97 a	
Azospirilum Fertilizer means		33.83 c	38.91 b	53.39 a		

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 4. The effect of bio-fertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi, and the interactions among them, on the concentration of available phosphorus in the soil (mg kg^{-1} soil)*.

Azotobacter Fertilizer Levels (gm plant-1)	Mycorrhiza fertilizer levels (gm plant-1)	Azospirilum Fertilizer Levels (gm plant-1)			Interaction between Azotobacter and Mycorrhiza	Mycorrhiza Fertilizer means
		0	2	4		
0	0	11.29 k	17.63 j	19.42 h - j	16.11 e	17.57 b
2		13.40 k	18.05 ij	20.67 g - i	17.37 e	
4		17.26 j	18.70 ij	21.69 f - h	19.22 d	
0	5	22.06 f-h	23.76 ef	30.70 c	25.51 c	27.20 a
2		22.50 fg	25.36 de	33.43 b	27.09 b	
4		23.15 e-g	27.42 d	36.41 a	28.99 a	
Interaction between Azospirilum and Mycorrhiza	0	13.98 f	18.13 e	20.59 d	Azotobacter Fertilizer means	
	5	22.57 c	25.51 b	33.51 a		
Interaction between Azotobacter and Azospirilum	0	16.67 f	20.70 e	25.06 c	20.81 c	
	2	17.95 f	21.71 de	27.05 b	22.23 b	
	4	20.20 e	23.06 d	29.05 a	24.10 a	
Azospirilum Fertilizer means		18.27 c	21.82 b	27.05 a		

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

Table 5. Effect of bio-fertilization with Azotobacter and Azospirillum bacteria and mycorrhizal fungi on the available potassium in the soil (mg kg^{-1} soil)*.

Azotobacter Fertilizer Levels (gm $plant^{-1}$)	Mycorrhiza fertilizer levels (gm $plant^{-1}$)	Azospirilum Fertilizer Levels (gm plant-1)			Interaction between Azotobacter and Mycorrhiza	Mycorrhiza Fertilizer means
		0	2	4		
0	0	172.0 l	276.7 Ij	310.7 hi	253.1 f	279.2 B
2		232.0 k	283.3 h-j	324.0 gh	279.8 e	
4		250.0 jk	304.0 Hi	360.0 fg	304.7 d	
0	5	372.0 f	431.3 De	536.0 b	446.4 c	473.6 A
2		384.0 f	469.3 Cd	567.3 ab	473.6 b	
4		424.0 e	478.7 C	600.0 a	500.9 a	
Interaction between Azospirilum and Mycorrhiza	0	218.0 f	288.0 E	331.6 d	Azotobacter Fertilizer means	
	5	393.3 c	459.8 B	567.8 a		
Interaction between Azotobacter and Azospirilum	0	272.0 g	354.0 De	423.3 b	349.8 c	
	2	308.0 f	376.3 Cd	445.7 b	376.7 b	
	4	337.0 e	391.3 C	480.0 a	402.8 a	
Azospirilum Fertilizer means		305.7 c	373.9 B	449.7 a		

* The averages that share a common letter of the alphabet for each factor and each interaction do not show significant differences when analyzed using the Duncan multiple range test at a 0.05 significance level.

The effect of Azospirillum: The results mentioned in Tables (2 – 5) showed that fertilization with Azospirillum at 4 g plant $^{-1}$ led to a significant increase in the available nitrogen in the soil (53.39 mg kg $^{-1}$), available phosphorus in the soil (27.05 mg kg $^{-1}$), and the available potassium in the soil (449.7 mg kg $^{-1}$) compared to the control treatment, which gave the lowest values for these properties, measuring 33.83 mg kg $^{-1}$, 18.27 mg kg $^{-1}$, and 305.7 mg kg $^{-1}$, respectively, Meanwhile, the soil pH was not significantly affected by the fertilization with Azospirillum.

The effect of mycorrhizal fungi: It is observed from the results shown in tables (2 – 5) that fertilization with mycorrhizal at 5 g plant -1 led to a significant increase in the available nitrogen in the soil (53.70 mg kg $^{-1}$) and the available phosphorus in the soil (27.20 mg kg $^{-1}$) and the available potassium in the soil (473.6 mg kg $^{-1}$) compared to the control treatment, which yielded the lowest values for these traits,which were 30.39 mg kg $^{-1}$,17.57 mg kg $^{-1}$, and 279.2 mg kg $^{-1}$, respectively. Meanwhile, the soil pH was not significantly affected by fertilization with mycorrhizal fungi.

The effect of interactions among the studied factors: The results of (Tables 2-5) indicate that all binary interactions and the triple interaction among Azotobacter, Azospirillum, and mycorrhizal fungi significantly affected available N,P and K in the soi As. the highest levels of these fertilizers (4 g plant $^{-1}$, 4 g plant $^{-1}$, and 5 g plant $^{-1}$ respectively) yielded the highest values for the studied traits, especially the triple interaction, where the values of available nitrogen in the soil reached (76.77 mg kg^{-1}), available phosphorus in the soil (36.41 mg kg^{-1}), and available potassium in the soil (600.0 mg kg^{-1}) compared to the control treatment, which provided the lowest values for these traits, measuring 16.33 mg kg^{-1}, 11.29 mg kg^{-1}, and 172.0 mg kg^{-1}, respectively. This is attributed to the synergistic effect of both Azotobacter and Azospirillum, along with mycorrhizal fungi, in enhancing the vegetative and root growth traits.

The results mentioned in tables (2-5) indicate that bio-fertilization with both Azotobacter and Azospirillum, especially at 4 g $plant^{-1}$ for each, and the Mycorrhizae fungus, either separately or together, led to a significant increase in the availability of nitrogen, phosphorus, and potassium elements in the soil compared to the control treatment. This may be attributed to the mineralization of complex organic materials present in the soil, which reached a rate of 6.31g kg^{-1} soil (Table 1) through both types of bacteria as well as fungi, converting them into inorganic (mineral) ions such as ammonia and nitrates through the process of nitrogen mineralization, as well as phosphorus and potassium [16], [11] stated that the complete mineralization of organic matter in the soil by microorganisms results in water, carbon dioxide, mineral elements, and energy, in addition to the formation of phosphor- humic compounds. These compounds prevent the precipitation of phosphorus, making it available for absorption by plants [17]. Furthermore, these organisms play a significant role in increasing the availability of nutrients in the soil by dissolving complex compounds containing these elements, as they secrete some organic acids that enhance the release of nutrients, making them ready for absorption by plants [18], [19] [11] The reason may also be attributed to the ability of the bacteria and fungi used in the study to stimulate the secretion of chelating compounds known as Siderophores to chelate some nutrients present in the soil, such as phosphorus, iron, and other nutrient elements, especially micronutrients. This prevents their interaction with the chemical components of the soil, thereby increasing their availability for absorption by plants and subsequently increasing their concentrations in plant tissues [20] [21]. The reason may also be due to the role of both types of bacteria in fixing atmospheric nitrogen [22]. Additionally, Azospirillum bacteria work to degrade pectin and dissolve certain compounds containing potassium and calcium, increasing their availability [11]. Furthermore, mycorrhizal fungi play a role in dissolving complex compounds containing phosphorus and potassium, enhancing their availability [23] [24].

Conclusions:

We conclude from the study that fertilization with Azotobacter bacteria, Azospirillum bacteria, and mycorrhizal fungi for the Camarosa strawberry plant, particularly at high levels (4 g $plant^{-1}$ Azotobacter, 4 g $plant^{-1}$ Azospirillum, and 5 g $plant^{-1}$ mycorrhiza), especially when added together, has increased the concentrations of available nutrients in the soil, which may positively affect the yield of the plants.

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