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USES *BACILLUS SUBTILIS*, *RHIZOBIUM* BACTERIA, AND *ARBUSCULAR MYCORRHIZAL* FUNGI TO ENHANCE SOME PHYSICAL AND CHEMICAL CHARACTERISTICS GROWTH ON APRICOT SAPLING (*PRUNUS ARMENIACA* L).

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Abstract

The current study assesses the effects of utilizing *Bacillus subtilis*, *Rhizobium* bacteria, and Arbuscular *mycorrhizal* fungus as a biofertilizer on apricot sapling cultivar Bayaa. Concurrently, the use of to promote plant growth was developed. Furthermore, three factors were used. The first component was added for soil apricot sapling by dilution suspension of *Bacillus subtilis* with three levels (0, 100, 200 ml.L^{-1}) while the second factor was added by dilution suspension of *Rhizobium* spp. with two levels (0, 200 ml.L^{-1}) and third factor was dilution suspension add *Arbuscular mycorrhizal* fungus with three levels (0, 10, 20 ml.L^{-1}). The results demonstrate that adding Bacillus subtilis bacteria to the soil of apricot saplings at both levels (100, 200 ml.L^{-1}) had a significant influence on the leaf area, total chlorophyll, N%,P%,Mg%, and reduced proline and peroxidase. The effects of *Rhizobium* bacteria at level (200 ml.L^{-1}) on all trails under study were comparable to non-addition of *Bacillus subtilis* .*Arbuscular mycorrhizal* fungus of the treated saplings at both levels (10 and 20 ml.L^{-1}) to the soil of apricot saplings had a significant effects on the leaf area, the increase in the heigh,total chlorophyll ,N%,P%,Mg% and reduce proline and peroxidase. The interaction among *Bacillus subtilis*, *Rhizobium* bacteria, and *Arbuscular mycorrhizal* fungi were effects on trails under study especially (20 ml.L^{-1}*Arbuscular mycorrhizal* +200 ml.L^{-1} *Rhizobium* + 200 ml.L^{-1} *Bacillus subtilis*) treatment, it was high significant in all trails.

Keywords: *Bacillus subtilis*, *Rhizobium* bacteria, *Arbuscular mycorrhizal,* apricot.

Introduction

The Rosaceae family's apricot (*Prunus armeniaca* L.), one of the most popular stone fruit crops, with an annual global production of 4.25 million tons. Spain is the second-largest producer in Europe and seventh overall, accounting for 23.42 percent of global output (FAO, 2020). Microbial activity is important in agriculture because it assures the availability and mobility of nutrients required for plant growth (Kennedy *et al*., 2004). Bio-fertilizer is gaining popularity because it boosts crop production, growth, and development by supplying and increasing the availability of nitrogen (N) and producing plant-growth-promoting compounds including auxin, cytokinin, and gibberellins (Hegazi *et al*., 1998). To stimulate plant growth and nitrogen fixation, biological nitrogen-fixing (BNF) bacteria such as *Rhizobium* and Brady*Rhizobium* species generate auxins, cytokinins, abscisic acid, vitamins, riboflavin, lipochito oligosaccharides, and

lumichrome (Hardarson ,1993). Environmentally friendly sustainable agriculture practices have lately gained favor. Bio-fertilization is critical in the development and application of sustainable agriculture methods to prevent the deterioration of natural and environmental contaminants (O'Connell, 1992). Numerous bacterial species from the genera Pseudomonas, Azospirillum, Burkholderia, Bacillus, and others can support plant growth. For many years, scientists have been studying the use of such bacteria in agriculture as biofertilizers or biocontrol agents (Sturz and Nowak, 2000). The development of organic fruits depends heavily on organic fertilization because the use of chemical fertilizers is impractical. Therefore, N2-fixing bacteria are often used in organic plant culture, including Bacillus species, Azotobacter species, Azospirillum species, Beijerinckia species, and Pseudomonas species (Dobereiner, 1997). In order to achieve high quality and high output, intensive agriculture practices require chemical fertilizers, but they are also costly and may have adverse environmental repercussions. Environmentally friendly sustainable agriculture practices have grown in favor recently (Orson, 1996). In order to reduce the deterioration of natural and environmental contaminants, biofertilization is essential in the creation and use of sustainable agriculture methods (O'Connell 1992). The capacity of numerous bacterial species, mostly those present in the plant rhizosphere, to positively affect plant growth is widely acknowledged. The use of such bacteria as biofertilizers or pest control agents in agriculture has been the subject of investigation for a while. The bacteria referred to as "plant growth promoting *Rhizobium* (PGPR)" include Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, *Rhizobium*, Erwinia, Serrotia, Alcaligenes, Arthrobacter, Acinetobacter, and Flavobacterium strains (Bloemberg and Lugtenberg 2001). PGPR bacteria, crops including mulberry, high bush blueberry, potato, radish, tomato, lettuce, apple, citrus, beans, and cucumber can all see greater growth and yield (Sudhakar *et al*. 2000). It is well known that by being infected with powerful rhizobia, legume crops may fix N2 in a symbiotic manner (Döbereiner 1997; Vance 1997). Asymbiotic N2-fixing bacteria found in the rhizosphere and/or endophytically attached to plants usually boost crop yields (Hecht-Buchholz 1998) Numerous bacterial species have the capacity to fix nitrogen dioxide (N2-fixing properties), including Bacillus sp., Azotobacter sp., Azospirillum sp., Beijerinckia sp., and Pseudomonas sp (Vance 1997). Nitrogen-fixing bacteria have been sprayed onto mulberry bushes as a treatment (Sudhakar *et al*. 2000).

The yield, growth, and nutrient element composition of apricot leaves are not known to be impacted by PGPR bacteria. According to recent studies (Sahin *et al*. 2000), Bacillus OSU 142 was able to fix N2 asymbiotically and boost plant growth and yield in barley, sugar beet, tomato, and pepper. In the past, OSU 142 was selected as a biological control agent to treat several plant diseases.

Fungi (AMF), which may raise the host plant's intake of nutrients, improve its capacity to survive drought, and improve its resistance to disease, have the potential to create symbiotic relationships with the majority of plant species. Mycorrhizal symbioses in agro-ecosystems are impacted by a variety of agricultural methods, including soil tillage, fertilizer, and plant protection techniques. All practical agronomical and plant protection techniques should be

considered and combined in integrated pest management in order to ensure food production and to decrease the spread of populations of dangerous organisms in a way that is sustainable from an ecological and integrated pest management (IPM). This strategy combines sustainable biological, physical, and nonchemical procedures with appropriate growth practices, balanced fertilization, and cultivar selection. Pesticides of low risk shouldn't do much harm to the environment, non-target animals, or humans. Fungi (AMF) work in symbiosis with plant roots to increase nutrient intake, plant development, and stress tolerance (Kareem *et al*. ,2022). Depending on the plant and AMF species, as well as environmental parameters such as soil nutrient availability, light intensity, and temperature, plant responses to AMF colonization might be favorable or negative (Johnson *et al*., 1997; Al-janabi and Al-hasnaawi,.2021). This difference has been seen in AMF isolates from the same and distinct species (Smith *et al*., 2004).

Almost majority of the knowledge on the various AMF functions was acquired in assays where plants were infected with single AMF isolates and plant growth or total phosphorus absorption were examined. Because numerous AMF species are typically found in a single root system in the field, our findings are not totally relevant in such situations (Al-janabi and Al-Rawi, 2018). AMF structures like as spores and hyphae may be exposed to active chemicals in the field when they are applied as a soil drench, seed treatment, foliar spray, or when they are present in leaf application run-off or drift to the soil. It's also feasible that changes in the host plant's physiology will have an indirect influence on the *Rhizobium*(AM) symbiosis(Rodrigues *et al*. ,2021).

Materials and Methods

Apricot sapling (*Prunus armeniaca* L.) were homogeneous age and size, selected at the Citrus Production Nursery, belonged to the General Directorate of Horticulture and Forests, Holy Karbala. Governorate Iraq. The aim of study was identify the apricot growth by adding two factors for soil sapling by two types of bacteria (*Bacillus subtilis* and *Rhizobium*) , the 112 CFU/ml of bacteria suspensions were used to treat apricot sapling and different dilution and a second factor was add fungus (*Arbuscular mycorrhizal*) as individual and overlaps. Three levels from *Bacillus subtilis* dilution (0, 25 ,50 ml.L^{-1}) and two levels of *Rhizobium* (0, 250 ml.L^{-1}) and three levels were add from *Arbuscular mycorrhizal* (0, 200, 400 ml.L^{-1}).add for soil apricot sapling by dilution bacteria or fungi suspension ,the control sapling were sprayed with sterile water. The potting medium, consisting of 2:2:1 topsoil, perlite, and peat moss, was infected with 20 mL of 1% endophyte inoculum in deionized water per pot. To guarantee the total removal of soil-borne illnesses, the potting mixture was heated to 100 °C for three weeks while covered with a tarp. After the potting medium had warmed up to the average greenhouse temperature of 25 °C, it was inoculated with an endophyte inoculum. A total of 216 pots were used three factors (3*2*3) , each treatment were have four apricot sapling for three blocks.An inquiry was carried out at a private nursery in Iraq during the 2020-2021 growing season. while the addition suspensions of *Bacillus subtilis* , *Rhizobium* and *Arbuscular mycorrhizal* were on six dates: 1May , 1 June, 1 Julay, 1 August, 1 September, and 1 November In 2020.

Apricot saplings with diameters of 3 cm and heights of 30 cm were grown in pots. Because the pots were only on one side of the greenhouse, a factorial experiment using a randomized complete block design (RCBD) was used to account for and/or correct for the light gradient. Data were analyzed using the Genestat program, and means were separated using Duncan's multiple range tests. The greenhouse was kept at a relative humidity range of 60 to 70%. Temperatures varied between 28 and 35 degrees Celsius during the day and night. The physical and chemical parameters of the experimental soil were as follows: Sand 41.2%, Clay 38.9%, Silt 19.9% (Texture Sandy Clay), Field Capacity 18.44%, pH 7.88, EC 0.78 (dS/m). The following apricot sapling development and gene expression parameters were investigated:

1- Mean Leaves Area (cm^2): Using the gravimetric method described by Edson *et al*., the mean of the area of the 6th to 10th leaves (Reisinauer, 1978) from the top corresponding to the transplants was calculated (1995). Each leaf had a disc with a surface area of 0.302 cm2 excised, which was then weighed. The leaves were dried until the weight remained consistent in an oven set at 70°C. The average leaf area was then calculated using the calculation shown below:

A.L.A = L.D.W x a.d/d.w.d (Where A.L.A stands for average leaf area in square meters (cm2), L.D.W for average dry leaf weight (g), a.d for average leaf disc area (0.302 cm2), and d.w.d for average dry weight of the discs made from leaves (g).

2-Increase in Mean Plant Length (cm): At the conclusion of the experiment, the length increase was measured using a measuring tape.

3-Total Chlorophyll (mg/g fresh weight): To measure total chlorophyll, the removed leaves were subjected to an 80% acetone treatment. Following a paper filtering step, the leaves were centrifuged for five minutes at 3000 rpm. The light absorption corresponding to 663 and 645 nm wavelengths was measured using a spectrophotometer. The following formula was used to get the total chlorophyll content.

-Total chlorophyll = 20.20 × A645 + 8.02 × A663 Where, A663 and A645 are the spectrophotometer readings corresponding to 663 and 645 nm, respectively .

4-Nitrogen %(Determination of the leaves content of Nitrogen %): The Micro Kjeldahl Apparatus was used to determine the nitrogen content of the leaves in percentage terms. by combining 10 ml of sodium hydroxide with 10 ml of each sample. 40 percent concentration was used, followed by distillation, and the ammonia that was produced was collected in a glass beaker with 20 milliliters of 2% boric acid and a combination of methyl red and bromocresol green. Total nitrogen was determined using the formula: Volume of acid used during grinding x Acidity Standard x 14 x Dilution Volume x 100/Volume of Sample collected during Distillation x Weight of Digested Sample x 1000

5-Phosphorus %(Determination of the leaves content of Phosphorus %): Phosphorus content of leaves (%) was determined using the Self-Davis technique of ammonium molybdate and ascorbic acid (Self-Davis *et al*., 2000).

6- Magnesium%:(Determination of leaves content of Magnesium %): Grinding was used to estimate the magnesium content of the leaves, and the digested sample was transferred to a volumetric flask of 100 ml capacity and filled to the mark with distilled water. 25 ml of the

digestion solution was taken and placed in a standard flask of 100 ml capacity, and 5 ml of 5% iron chloride was added. Standard sodium was added until it changed color and became turbid brown, with no precipitate observed, and 20 ml of standard acid solution (Standard Acetate pH 4.63) was added, and the sample was placed in a water bath for 15 minutes with shaking from time to time, then cooled and completed to the mark, and then filtered from the solution. Using regular filter paper, In a 300 ml conical flask, 25 ml of the final filtrate was added to 10 ml of NaOH with a little quantity of meroxide, then flattened with EDTA-Na2 (0.5 N) until its color changed to brown and the following equation was used (percentage magnesium=100 x (100 x 100 x 100 x h) / (1000 x 25 x 25 x y). The h=Mg(mg)for 25 ml of sample, and y=weight of sample.

7-Proline(Determination of proline in leaves µg.g^{-1} fresh weight) : Proline is measured using a light spectrum meter in the following manner: Add 1000 l of the reaction solution (nanhydrin 1% (w/v) in 60% (v/v) acetic acid and 20% (v/v) ethanol) to 1.5 mL hermetically sealed test tubes. To create the proline curve, add 500 l of plant extract (100 l of standard proline to include different dilutions of standard proline (0.2, 0.5, 1, 2.5 mmol of proline) and complete the volume to 400 l using Ethanol: water (40:60 v/v). Close the pipes, mix, and heat it in a water bath for 20 minutes at 95 ° C To remove the precipitate, use a centrifuge (1 minute, 10,000 rpm). Transfer the candidate to a 1.5 ml glass cell and measure at 520 nm. The quantity of proleen in extracts is calculated using the following equation: Proline in nmol.mg^{-1} FW = (Abstract - blank)/slope*Volextract/Volaliquot*1/FW. Keep in mind that FW stands for fresh weight. blank - abstract = excluded – blank).

8-Peroxidase activity:(Determination of the total activity of the peroxidase enzyme – POX IU. g^{-1} fresh weight): On November 1, 2021, fresh leaves were collected in the early morning after 6 months of the experiment and placed in transparent polyethylene bags, which were then kept in a cool cork box containing ice to preserve the samples from wilt and were transferred directly to the freezer at $^{-1}$8°C until the analyses were performed. Used materials and solutions: 1- Solution of guaicaol: Prepare by combining 1.36 mL of guaiacol in a volumetric flask, followed by 250 mL of distilled water. H2O2 hydrogen peroxide solution at 0.1% concentration: Make a volume of 0.4 ml of 30% H2O2 and fill it with 120 ml of pure water. The working method: 1 ml of H2O2 + 1 ml of Guaicaol were mixed together, and the samples were centrifuged at a rotation speed of 12000 rpm for 15 minutes. A spectrophotometer with a wavelength of 240 nm was used to measure the absorbance. The enzyme activity was determined by introducing 2 ml of the reaction mixture to the spectrophotometer cell, then 0.1 ml of the sample, and measuring the change in light absorption every 30 seconds for 3 minutes at 240 nm.

Results and Discussion

Table (1) shows that the addition of *Arbuscular mycorrhizal* fungus to the soil of apricot saplings had a significant effect on the leaf area of the treated saplings at both levels (10 and 20 ml.L^{-1}), which amounted to (3052 and 3530 cm^2), respectively, compared to the treatment of no

addition.

It appears from Table (1) that the soil addition of *Rhizobium* bacteria had a significant effect on the average leaf area of the treated apricot saplings, as the leaf area of the saplings treated with *Rhizobium* bacteria was (3873.6 cm^2) compared to the non-comparison treatment. Table (1) shows that the leaf area was significantly affected by the addition of *Bacillus subtilis*, for both concentrations (100 and 200 $ml.L^{-1}$), as it reached (3736 and 4435 cm^2) compared to the treatment of no addition. As for the overlap treatments, they were significant compared to the comparison treatment, especially the triple overlap treatment ($ml.L^{-1}$20 *Arbuscular mycorrhizal* +200 $ml.L^{-1}$ *Rhizobium* +200 $ml.L^{-1}$ *Bacillus subtilis*, which gave the highest significant values of (4919). cm2 compared to the control treatment that gave the lowest non-significant value of (622 cm2).

Table 1:Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on average Mean Leaf Area (cm^2) (%) in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 $ml.L^{-1}$	**10 $ml.L^{-1}$**	**20 $ml.L^{-1}$**		
Bacillus subtilis	**0 $ml.L^{-1}$**	**0 $ml.L^{-1}$**	622 d	2166 c	2081 C	**2308 c**	
		200 $ml.L^{-1}$	2937 c	3106 b	2937 C		
	100 $ml.L^{-1}$	**0 $ml.L^{-1}$**	3552 b	3394 b	3552 b	**3736 a**	
		200 $ml.L^{-1}$	6033 b	3624 b	4693 A		
	200 $ml.L^{-1}$	**0 $ml.L^{-1}$**	3827 b	3992 b	4827 a	**4435 a**	
		200 $ml.L^{-1}$	4144 a	4902 a	4919 A		
	Effect of *Arbuscular mycorrhizal*		**2476 b**	**3530 a**	**3052 a**	**Effect of *Rhizobium***	
						0 $ml.L^{-1}$	**$ml.L^{-1}$200**
						3112.3 b	3873.6 a

Table (2) shows that the addition of *Arbuscular mycorrhizal* fungus to the soil of apricot saplings had a significant effect on the increase in the height of the treated saplings for both levels (10 and 20 $ml.L^{-1}$), which amounted to (16.6 and 15.4 cm), respectively, compared to the treatment of no addition. Table (2) shows that the soil addition of *Rhizobium* bacteria had a

significant effect on the rate of increase in the height of the treated apricot saplings, as the leaf area of the saplings treated with *Rhizobium* bacteria was (16.5 cm) compared to the non-comparison treatment. Table (2) shows that the increase in sapling height was significantly affected by the addition of *Bacillus subtilis* bacteria, for both concentrations (100 and 200 ml.L^{-1}), reaching (15.25 and 16.65 cm) compared to the treatment of no addition. As for the interaction coefficients between the experimental factors, they were significant compared to the comparison treatment, especially the triple interference treatment (ml.L^{-1}20 *Arbuscular mycorrhizal* +200 ml.L^{-1} *Rhizobium* +200 ml.L^{-1} *Bacillus subtilis*, which gave the highest significant values of (20.7 cm).) compared to the comparison treatment, which gave the lowest non-significant value of (11.3 cm).

Table 2 **Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on** average Increase in Stem length (mm) in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	**11.3 d**	**13.5 c**	**14.3 c**	**13.7 c**	
		200 ml.L^{-1}	**13.1 c**	**14.5 bc**	**15.8 b**		
	100 ml.L^{-1}	**0 ml.L^{-1}**	**11.40 d**	**14.7 bc**	**14.9 bc**	**15.25 b**	
		200 ml.L^{-1}	**15.3 b**	**16.5 b**	**18.6 a**		
	200 ml.L^{-1}	**0 ml.L^{-1}**	**14.5 bc**	**14.6 bc**	**15.6 b**	**16.65 a**	
		200 ml.L^{-1}	**15.8 b**	**18.9 a**	**20.7 a**		
	Effect of *Arbuscular mycorrhizal*		**13.5 c**	**15.4 b**	**16.6 a**	**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
						13.8 b	16.5 a

Table (3) shows that the addition of *Arbuscular mycorrhizal* fungus to the soil of apricot saplings had a significant effect on the total chlorophyll of the treated saplings, especially the concentration (10 ml.l^{-1}), which gave the highest chlorophyll content in the leaves of (7.13 mg.g^{-1} fresh weight).) compared to the non-addition treatment. It appears from Table (3) that the soil addition of *Rhizobium* bacteria did not have a significant effect on the total chlorophyll rate in

the treated apricot saplings compared to the non-comparative treatment. Table (3) shows that the leaves content of total chlorophyll was significantly affected by the addition of *Bacillus subtilis*, for both concentrations (100 and 200 ml.L^{-1}), as it reached (7.22 and 6.64 mg.g^{-1} fresh weight) compared to the treatment of no addition. As for the interaction coefficients between the experimental factors, they were significant compared to the comparison treatment, especially the triple interference treatment (ml.L^{-1}20 *Arbuscular mycorrhizal* +200 ml.L^{-1} *Rhizobium* +200 ml.L^{-1} *Bacillus subtilis*, which gave the highest significant values of (8.74 mg) .g^{-1} fresh weight) compared to the control treatment that gave the lowest non-significant value of (3.49 mg.g^{-1} fresh weight).

Table (3) **Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on** average of **Total Chlorophyll (mg.g^{-1} fresh weight)** in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	3.49 c	5.75 b	5.49 b	**5.59 c**	
		200 ml.L^{-1}	5.62 b	6.61 b	6.62 b		
	100 ml.L^{-1}	**0 ml.L^{-1}**	5.85 b	7.11 ab	7.65 ab	**6.64 b**	
		200 ml.L^{-1}	5.02 b	7.09 ab	7.12 ab		
	200 ml.L^{-1}	**0 ml.L^{-1}**	6.08 b	6.89 ab	7.18 ab	**7.22 a**	
		200 ml.L^{-1}	6.74 ab	7.75 ab	8.74 a		
	Effect of *Arbuscular mycorrhizal*		5.46 C	6.86 b	7.13 A	**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
						6.16	6.81

Table (4) shows that the addition of *Arbuscular mycorrhizal* fungus to the soil of apricot saplings had a significant effect on the percentage of nitrogen in the treated apricot saplings at both levels (10 and 20 ml.L^{-1}), which gave the highest percentage of nitrogen in the leaves of (0.463). and 0406 %), respectively) compared to the no-additive treatment. Table (4) shows that the soil addition of *Rhizobium* bacteria had a significant effect on the percentage of nitrogen in the leaves

of apricot saplings treated, as the percentage of nitrogen in the saplings treated with *Rhizobium* bacteria was (0.454%) compared to the non-comparison treatment. Table (4) shows that the percentage of nitrogen in the leaves of apricot saplings treated was significantly affected by the addition of *Bacillus subtilis* and for both concentrations (100 and 200 ml.L^{-1}) as it reached (0.416 and 0.469%) compared to the treatment of no addition. As for the interaction coefficients between the experimental factors, they were significant compared to the comparison treatment, especially the triple interference treatment (ml.L^{-1}20 *Arbuscular mycorrhizal* +200 ml.L^{-1} *Rhizobium* +200 ml.L^{-1} *Bacillus subtilis*, which gave the highest significant values of 0.592%).) compared to the comparison treatment, which gave the lowest non-significant value of (0.269%).

Table4: **Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on** average percentage of Nitrogen(%) in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	0.269 c	0.318 c	0.369 c	**0.318 b**	
		200 ml.L^{-1}	0.409 b	0.355 c	0.409 b		
	100 ml.L^{-1}	**0 ml.L^{-1}**	0.376 c	0.406 b	0.376 c	**0.416 a**	
		200 ml.L^{-1}	0.378 c	0.383 c	0.578 a		
	200 ml.L^{-1}	**0 ml.L^{-1}**	0.354 c	0.436 b	0.454 b	**0.469 a**	
		200 ml.L^{-1}	0.442 b	0.541 a	0.592 a		
	Effect of *Arbuscular mycorrhizal*					**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
			0.371 B	0406 A	0.463 A	0.372667 b	0.454 a

Table (5) shows that the addition of the fungus *Arbuscular mycorrhizal* to the apricot sapling soil had a significant effect on the percentage of phosphorus of the treated apricot sapling leaves. compared to the non-addition treatment. Table (5) shows that the soil addition of *Rhizobium* bacteria had a significant effect on the percentage of phosphorus in the treated apricot saplings,

as it reached (0.645%) for the saplings treated with *Rhizobium* bacteria, compared to the non-comparison treatment. Table (5) shows that the percentage of phosphorus in the leaves of apricot saplings did not have a significant effect of adding *Bacillus subtilis* to both concentrations (100 and 200 ml.L^{-1}) compared to the treatment of no addition. As for the interaction coefficients, they were significant compared to the comparison treatment in the percentage of phosphorus for the leaves of the saplings, especially the treatment of the triple overlap (ml.L^{-1}20 *Arbuscular mycorrhizal* +200 ml.L^{-1} *Rhizobium* +200 ml.L^{-1} *Bacillus subtilis*, which gave the highest significant values of (0.942%) compared to the treatment of triple interference (ml.L^{-1}20 *Arbuscular mycorrhizal* +0 ml.L^{-1} *Rhizobium* + 200 ml.L^{-1} *Bacillus subtilis*, which gave the lowest non-significant value as the percentage of phosphorus reached (0.354%).

Table 5: **Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on** average percentage of phosphorous(%)in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	**0.369 d**	**0.418 d**	**0.669 b**	**0.534 a**	
		200 ml.L^{-1}	**0.509 c**	**0.605 bc**	**0.639 bc**		
	100 ml.L^{-1}	**0 ml.L^{-1}**	**0.376 d**	**0.426 d**	**0.676 b**	**0.569 a**	
		200 ml.L^{-1}	**0.478 d**	**0.683 b**	**0.778 b**		
	200 ml.L^{-1}	**0 ml.L^{-1}**	**0.354 d**	**0.638 bc**	**0.794 b**	**0.650 a**	
		200 ml.L^{-1}	**0.442 d**	**0.731 b**	**0.942 a**		
	Effect of *Arbuscular mycorrhizal*		**0.421 C**	**0.583 b**	**0.749 A**	**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
						0.524 b	**0.645 a**

Table (6) shows that the addition of *Arbuscular mycorrhizal* fungus to the soil of apricot saplings had a significant effect on the percentage of magnesium in the treated apricot saplings. The higher the fungus concentration, the higher the percentage of magnesium in the leaves of the saplings, reaching at the level of 10 ml.L^{-1} 1.614. The highest significant percentages were followed by the level treatment of 20 ml.L^{-1}, at which the percentage of magnesium reached

1.575 compared to the treatment of no addition, which amounted to 1.251%.Table (6) shows that the ground addition of *Rhizobium* bacteria had a significant effect on the percentage of magnesium in the treated apricot sapling leaves, as the percentage of magnesium in the saplings treated with *Rhizobium* bacteria was (1.623%) compared to the non-comparison treatment. Table (6) shows that the percentage of magnesium in the treated apricot sapling leaves was significantly affected by the addition of *Bacillus subtilis* and for both concentrations (100 and 200 ml.L^{-1}) as it reached (1.76 and 1.55%) compared to the treatment of no addition. As for the interaction coefficients between the experimental factors, they were significant compared to the comparison treatment, especially the triple interference treatment (ml.L^{-1}20 *Arbuscular mycorrhizal* +200 ml.L^{-1} *Rhizobium* +200 ml.L^{-1} *Bacillus subtilis*, which gave the highest significant values of 1.970%).) compared to the comparison treatment that gave the lowest non-significant value of (0.519%).

Table 6: **Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on** average percentage of Magnesium (%) in leaves apricot sapling .

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	0.519 f	1.158 e	1.019 e	**1.11 c**	
		200 ml.L^{-1}	1.176 e	1.353 d	1.476 d		
	100 ml.L^{-1}	**0 ml.L^{-1}**	1.174 e	1.544 c	1.574 c	**1.55 b**	
		200 ml.L^{-1}	1.510 c	1.705 b	1.840 a		
	200 ml.L^{-1}	**0 ml.L^{-1}**	1.508 c	1.733 b	1.808 b	**1.76 a**	
		200 ml.L^{-1}	1.619 ab	1.958 a	1.970 a		
	Effect of *Arbuscular mycorrhizal*		1.251 c	1.575 b	1.614 a	**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
						1.337 b	1.623 a

It appears from Table (7) that the addition of *Arbuscular mycorrhizal* fungus to the soil of apricot saplings had a significant effect on the proline content in the treated apricot sapling leaves. In the leaves, it reached 82.9 µg.g^{-1} fresh weight, compared to the no-adding treatment, which

gave the highest proline content of 98.4 µg.g^{-1} fresh weight. It appears from Table (7) that the ground addition of *Rhizobium* bacteria reduced the proline content of the treated apricot sapling leaves, as it reached 86.9 µg.g^{-1} fresh weight in the saplings treated with *Rhizobium* bacteria compared to the no-additive treatment, which gave the highest values of 95.2 µg.g- 1 fresh weight. Table (7) shows that the proline content in the treated apricot sapling leaves was significantly affected by the addition of *Bacillus subtilis*, for both concentrations (100 and 200 ml.L^{-1}), as the proline content of the leaves decreased, reaching (87.3 and 90.3 µg.g^{-1}). fresh weight) compared to the no-add treatment. As for the interaction coefficients between the experimental factors, they were significant compared to the comparison treatment, as the bacteria and fungus treatments were a reducing agent for proline in the leaves, which indicates that the bacteria and fungi used in the experiment reduce the stress on the plant, whether it is biotic or abiotic.

Table 7: Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on average of Proline content in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	82.5 a1	115.3 b	92.5 c	**95.5 a**	
		200 ml.L^{-1}	101.6 b	98.2 c	83.6		
	100 ml.L^{-1}	**0 ml.L^{-1}**	105.8 b	92.0 c	91.8	**90.3 b**	
		200 ml.L^{-1}	87.5 c	85.8 c	79.5 d		
	200 ml.L^{-1}	**0 ml.L^{-1}**	118.7 b	80.7 d	77.8 d	**87.3 c**	
		200 ml.L^{-1}	94.5 c	79.9 d	72.5 d		
	Effect of *Arbuscular mycorrhizal*					**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
			98.4 A	91.9 b	82.9 C	95.2 a	86.9 b

Table (8) shows that the addition of *Arbuscular mycorrhizal* fungus to the apricot sapling soil had a significant effect on the activity of the peroxidase enzyme in the treated apricot sapling leaves. Compared to the treatment of no addition, which gave the highest activity of the peroxidase enzyme, reaching 10.78 IU.g^{-1} fresh weight. It appears from Table (8) that the ground

addition of *Rhizobium* bacteria reduced the content of the treated apricot sapling leaves from the activity of the peroxidase enzyme, as it reached IU.g^{-1} fresh weight 8.46 in the saplings treated with *Rhizobium* bacteria, compared to the no-additive treatment, which gave the highest values of 10.05 IU.g. $^{-1}$ fresh weight.

Table (8) shows that the activity of the peroxidase enzyme in the treated apricot sapling leaves was significantly affected by the addition of *Bacillus subtilis*, for both concentrations (100 and 200 ml.L^{-1}), as the proline content of the leaves decreased, reaching 9.02 and 8.43 IU.g^{-1}. fresh weight compared to the no-add treatment.

As for the coefficients of interaction between the experimental factors, they were significant compared to the comparison treatment, as the bacteria and fungus treatments were a reducing factor for the effectiveness of the peroxidase enzyme in the leaves, which indicates that the bacteria and fungi used in the experiment reduce the stress on the plant, whether it is biotic or abiotic.

Table(8) Effect of *Bacillus subtilis* , *Rhizobium* bacteria , and *Arbuscular mycorrhizal* fungi and interaction on peroxidase activity (IU.g^{-1} fresh weight) in leaves apricot sapling.

		Rhizobium	***Arbuscular mycorrhizal***			**Effect of *Bacillus subtilis***	
			0 ml.L^{-1}	**10 ml.L^{-1}**	**20 ml.L^{-1}**		
Bacillus subtilis	**0 ml.L^{-1}**	**0 ml.L^{-1}**	13.90 a	11.33 a	9.92 b	10.32 a	
		200 ml.L^{-1}	9.06 bc	9.71 b	8.06 c		
	100 ml.L^{-1}	**0 ml.L^{-1}**	10.73 b	9.69 b	8.73 c	9.02 b	
		200 ml.L^{-1}	9.67 b	7.77 cd	7.57 cd		
	200 ml.L^{-1}	**0 ml.L^{-1}**	10.47 b	8.28 c	7.49 cd	8.43 c	
		200 ml.L^{-1}	10.90 b	6.97 d	6.50 d		
	Effect of *Arbuscular mycorrhizal*		10.78 a	8.95 b	8.04 b	**Effect of *Rhizobium***	
						0 ml.L^{-1}	**ml.L^{-1}200**
						10.05 a	8.46 b

Conclusion :

Regular application of *Bacillus subtilis* and *Rhizobium* to the soil's surface underneath the drip emitters was successful in increasing the population of the beneficial fungus in the soil under the conditions of our experiment. Increasing resistance to rhizomorphic *A. mellea*. Our data suggest that using *Bacillus subtilis* and *Rhizobium* affected root form and survival in a similar way to using *Arbuscular mycorrhizal* biofertilizer. *Arbuscular mycorrhizal* fungi have been shown to boost secondary root branching and root length in plants (Berta *et al*. ., 2002). Similarly, Schellenbaum *et al*. (1991) demonstrated that the *Arbuscular mycorrhizal* fasciculatum enhanced the branching of Vitis vinifera roots, with a greater influence on higher order laterals with increased length. Furthermore, *Rhizobium* and a variety of Bacillus species have been shown to solubilize inaccessible P and convert it into accessible forms, enhancing AMF's ability to absorb P.(Rodrigues *et al*., 2021).

According to the study and the tables above, the fungi *Bacillus subtilis*, *Rhizobium*, and am all had a substantial influence on the majority of the examined features. *Bacillus subtilis* and *Rhizobium* were responsible for increasing atmospheric nitrogen fixation in the soil, as well as increasing systemic absorption of minerals such as phosphorus and microelements, and therefore improving nutritional value. To the plant through the organisms in the cart or the fungal vaccinations added (Kareem etal, 2022 ;Mohammad and Al-Janabi , 2022). The explanation might be that the fungus promotes increased release of growth hormones, particularly auxin, which enhances plant development. Furthermore, the *Arbuscular mycorrhizal* fungus increased the available phosphorus in the soil of the plants treated with the inoculum, which increased biological activities such as oxidation, reduction, the production of energy required for growth, and an increase in the gene expression of hormones, particularly cytokinin, which led to an increase in division and an increase in the number of cells, which reflected positively on the plan's growth. According to Hartman(2000), the soil microbial community transforms plant litter into organic and inorganic soil components. Microbes are essential components of the soil ecosystem, and their abundance is a sensitive indicator of soil fertility. At the conclusion of the study, plots treated with *Bacillus subtilis* plus *Rhizobium* had more obvious impacts as a consequence of the addition of the two bio - fertilizers, which enhanced the soil's microbial biomass. Similar results were observed in a tomato field, where the application of biofertilizers resulted in a 36-37% increase in soil microbial biomass. (Pirlak *et al*. , 2007 ; Baldi *et al*. ., 2020). An increase in soil biological activity has a positive influence on the nitrogen cycle because it enhances soil OM mineralization and nutrient availability for plants.

Biofertilizers are bacteria or fungi that can fix nitrogen, solubilize phosphate, oxidize sulfur, create plant hormones, or degrade organic substances (Verma *et al*., 2019). Bio-fertilizer functions as a soil conditioner by providing organic matter, which helps to bind soil particles together and reduce soil erosion, desertification, and erosion while improving the soil's water retention capacity (Swathi, 2007). According to recent studies, bio-fertilizers are becoming more popular due to their low cost, environmental friendliness, and good impacts on plant development. Bio-fertilizer manufacturing has expanded dramatically, and one such product is the bio health item, which

comprises Bacillus sublutus and Trichoderma harzianum. Bacillus bacteria have a great capacity to dissolve phosphorus, according to the researchers, since they release organic acids that reduce the pH of the soil, boosting the availability of certain micronutrients and phosphorous. (Sharma *et al*., 2012). It also influences the formation of a thick root system, which increases the surface accessible for absorbing water and nutrients. (Hartman,2000), enhancing pathogen resistance and availability of nutrients while lowering root absorption of radioactive elements and salt, degrading soil organic compounds, and reducing root absorption of radioactive elements and salt (Lee *et al*., 2013; Bhuvaneswari *et al*., 2014 and Oskiera *et al*., 2015). Several studies have discovered that *Bacillus subtilis*, *Rhizobium*, and *Trichoderma* fungus release a wide range of enzymes, the most important of which is cellulose enzyme, which breaks down plant leftovers in the soil. It also secretes B^{-1}03-Glucalas and Chitenas enzymes, which help in the elimination of the harmful fungus Pythium. Furthermore, it stops Pythium from secreting auxin and gibberellin-related chemicals, boosting iron availability, dissolving phosphorus, and improving its readiness. (Al-Samerria, 2017). (Porras *et al*., 2009) reported that Inoculating olive trees with mycorrhizal fungus increased plant growth and capacity to absorb nitrogen, phosphate, and potassium. . Ameen and Al-Hamdani (2022), discovered that applying biofertilizers to olive trees of the cultivar Sorani of *Olea europaea* L. significantly increased the studied properties at a rate of 40 gm L^{-1} (yield rate per tree, fruit weight, fruit volume, pulp thickness). After applying biofertilizer at a rate of 15g per tree to olive trees of the Manzanillo cultivar, all fruit output parameters improved significantly. Furthermore, feeding olive trees 2 liters of biostimulant enhanced the amount of mineral elements (N. P. K. Zn. Cu. Fe) in leaves. (Davies, 1982).

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