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Using Some Sources of Biofertilizers to Improve Growth, Productivity and Fruit Quality of Le-Conte Pear Trees

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Abstract: This experiment was conducted at the experimental farm of the Horticulture Research Institute during the 2020-2021 and 2021-2022 seasons on fifteen years old trees to evaluate the impact of individual and mixed biofertilizer treatments (Cyanobacteria, *Bacillus subtilis* and *Bacillus amyloliquefaciens*) compared to chemical fertilizer as a control on growth, fruit quality and Productivity "Le-Conte" pear trees. The highest enzyme DHA and hormone IAA were (0.168mg TPF g^{-1} dry rhizosphere $soil^{-1}$ day^{-1} and 3.12 mg g^{-1} $soil^{-1}$) respectively which reported by cyanobacteria + *B. amylo*+ *B. subtilis* treatment followed by (0.104 mg TPF g^{-1} dry rhizosphere soil-1 day-1 and 2.9 mg g^{-1} $soil^{-1}$) respectively cyanobacteria + *B. amylo* in the second season. The study results showed Cyanobacteria treatments significantly increased N P K levels in leaves due to their ability to fix nitrogen and dissolve phosphate more effectively than other treatments. In the second season, the same treatment yielded the highest fruit set percentage and diameter, moreover, maximum fruit fresh weight, size, TSS%, total sugar%, Vitamin C, total carbohydrates, C/N ratio and yield, while acidity decreased in the second season. While in the first season a significant difference between the treatments with the combination of the three biofertilizers resulted in the greatest fruit length and firmness. Soil microbial activity increased in all treatments due to biofertilizers' capability to enhance soil microorganisms and release indole acetic acid in soil with individual or combined applications. However, we advise adding the rates of previous biofertilizer, which gave better results in the terms of all growth measures, yield, fruit quality and soil properties for "Le-Conte" pear trees under this experiment conditions. However, we advise adding the rates of previous biofertilizer, which gave better results in the terms of all growth measures, yield, fruit quality and soil properties for "Le-Conte" pear trees under this experiment conditions.

Key words: Le-Conte pear trees · Biofertilizers · Cyanobacteria · *Bacillus subtilis* · *Bacillus amyloliquefaciens*

INTRODUCTION

Pear (*Pyrus spp.*) is a popular and economically useful rosaceous fruit crop grown in temperate zone climates. It originated in the mountains of south-western China and has since spread throughout [1, 2]. In warmer regions, it ranks as the third most important fruit crop after grapes and apples [3]. The most common pear cultivar grown in Egypt is the Le-Conte pear cultivar, across between of *Pyrus communis* and *P. Serotina*. Le-Conte pear, a hybrid of *Pyrus communis* and *P. Serotina*, is the principal pear cultivar farmed in Egypt [4]. Egypt Pear production differs by year and orchard. Factors such as fire blight, rootstock, insufficient chilling hours, flower pollination and fertilization can all contribute to limited fruit set [5].

Biofertilizers improve crop growth and productivity by supplying nutrients through organic matter decomposition, nitrogen fixation, salt mineralization and phosphorus solubilization [6]. Microorganisms are cultivated on a large scale in the lab and combined with the appropriate carrier to create biofertilizers [7].

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Biofertilizer, a liquid combination of microorganisms and nutrients, protects cells and forms cysts and resting spores to withstand harsh environmental conditions [8]. Modern biofertilizers contain microorganisms that benefit plants and contribute to environmental sustainability, along side organic manures [9]. Biofertilizers are both environmentally beneficial and cost-effective, as they use natural and innate bacteria found in plants and soil [10]. Biofertilizer bacteria stimulate plant growth hormone production, including auxin, cytokinin, gibberellic acid and ethylene, leading to increased productivity. Rhizobacteria with strong plant growth-promoting (PGPR) activity have received attention in this area [11, 12]. Many are directly engaged in nitrogen fixation, mobilization and solubilization of phosphates, potassium, sulfur and iron. They promote plant growth indirectly by eliminating pathogens by the secretion of siderophores, antibiotics, enzymes, or fungicides [13, 14]. Biofertilizer microbes affect plant growth and productivity in three ways: indirectly by suppressing disease, promoting nutrient absorption and secreting phytohormones and biostimulants [15].

Cyanobacteria are the most prevalent class of organisms on earth. They live in a variety of environments and are autotrophic, with a preference for fresh water and marine environments. The best source of nutrients for growing cyanobacteria is marine water. They frequently form large colonies, are small and are typically unicellular. Cyanobacteria are made up of a wide variety of bacteria in various sizes and shapes. Cyanobacteria have shown promise as a biofertilizer [16]. They can convert solar energy into biomass by utilizing CO_2, water and nutrients. Efficient applications of cyanobacteria in agricultural practices have been reported to reduce global warming by reducing CO_2 gas emissions. Cyanobacteria biomass can be used to improve soil physicochemical properties, control soil-borne diseases, add organic matter, release growth-promoting substances, solubilize insoluble phosphates, use as nutraceuticals. As a result, biofertilizers derived from cyanobacteria are both cost-effective and environmentally friendly [17].

Bacillus spp. live longer in soil and promote plant growth more effectively than other plant growth promoting bacteria (PGPB) that do not produce endospores [18]. *Bacillus* spp. act as biostimulants by producing phytohormones, auxin and cytokinin, as well as expansin, which aid in plant growth and development [19]. The use of *Bacillus* inoculants in farmlands reduces the release of nitrogen and ammonia gases [20]. It plays a crucial role in the regulation of the biogeochemical cycles providing soil with a proper aeration capacity to maintain its ecosystem [21]. Furthermore, it has been reported that *Bacillus* spp. improves plant photosynthetic capacity by producing siderophores that chelate iron and supply iron required for photosynthetic machinery. *Bacillus* spp. reduce iron deficiency in plants and reduces heavy metal-induced oxidative stress by protecting indole-3-acetic acid (IAA) from oxidative damage [22].

Bacillus subtilis is a common PGPR that can be used effectively to optimize plant growth and yield. It defends the plant against a variety of stressors through ISR, biofilm formation, lipopeptide, siderophore and exopolysaccharide secretion. It acts as an efficient denitrifying agent in the agroecosystem and promotes soil health through environmentally friendly remediation technologies. *Bacillus amyloliquefaciens*, a Gram-positive spore-forming bacterium found in soil, has the ability to colonise plant rhizospheres and grow under stressful conditions. It has been investigated as a non-toxic and environmentally friendly plant growth stimulant [23]. *B. amyloliquefaciens*, as a plant growth-promoting rhizobacteria (PGPR), is an excellent agent for researching biofertilizers and biocontrol in agriculture and it is used to improve plant tolerances to biotic and abiotic stresses [24]. *Bacillus amyloliquefaciens* is a promising plant growth promoting bacteria (PGP) that has no negative side effects. BA's PGP mechanisms have been extensively studied as an excellent agent for biofertilizer and biocontrol in agriculture [25].

This study aims to investigate the effect of applying cyanobacteria, *B. subtilis*, and *B. amyloliquefaciens* and their mixture, as biofertilizers for Le-Conte pear trees on growth, yield and fruit characteristics.

MATERIALS AND METHODS

The experiment was carried out on Le-Conte pear trees at Giza Station, Agriculture Research Centre, during the 2020-2021 and 2021-2022 seasons to investigate the effect of individual and mixed biofertilizers [Cyanobacteria, *Bacillus subtilis* (*B. subtilis*) and *Bacillus amyloliquefaciens* (*B. amylo.*)] , which obtained from the Microbiology Department of the Soils, Water and Environmental Institute, Agriculture Research Centre, on growth, fruit set, yield and fruit quality. The experiment was carried out on 24 trees with 7 treatments as follows:

- Cyanobacteria 25 ml ($5x10^6$ cfu\ml\tree).
- *B. subtilis* 50ml ($3x10^2$ cfu\ml\tree).
- *B. amylo* 50ml ($3x10^2$ cfu\ml\tree).

Table 1: Physical and chemical properties of experimental soil

		A Valuable Nutrients (ppm)					
pH (1:2.5) soil suspension	EC dSm^{-1} (Soil paste)	N	P	K	Fe	Zn	Mn
8.30	1.70	0.50	0.29	5.2	3.8	3.10	2.66
Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	Organic Matter (%)	Texture class	
19.44	18.20	23.50	58%	5.39	1.65	Clay	

- Cyanobacteria 12.5 ml ($5x10^6$ cfu\ml\tree) + B. subtilis 25ml ($3x10^2$ cfu\ml\tree).
- Cyanobacteria 12.5 ml ($5x10^6$ cfu\ml\tree) + B. amylo 25ml ($3x10^2$ cfu\ml\tree).
- *B. subtilis* 25ml ($3x10^2$ cfu\ml\tree + B. amylo 25ml ($3x10^2$ cfu\ml\tree).
- Cyanobacteria 8.3 ml ($5x10^6$ cfu\ml\tree) + B. subtilis 16.7 ml ($5x10^6$ cfu\ml\tree) + B. amylo 16.7 ml ($5x10^6$ cfu\ml\tree).
- Trees that did not receive any biofertilizers treatments and fertilized by chemical recommend program served as control.

Bio fertilizers were applied to the trees' rhizospheres (15 cm in depth) *via* soil drench treatment by at a dose of 0.5 liter for one tree during (March, April, May and June) and then after harvest, with three replicates of each using a completely randomized design.

Table (1) shows the physical and chemical parameters of the experimental farm soil.

Data Recorded: Twenty of well distributed branches of shoots were selected around each tree and their flowers at full bloom were counted during March, 29th 2021 and March, 30th 2022. Fruit yield was determined in maturity stage of the "Le-Conte "pear cultivar [26]. While, fruit number was recorded (after fruit drop) for fruit retention determination. Fruit set % was calculated on the basis of initial number of flowers as follows:

$$\text{Fruit set\%}=\frac{\text{Total No. of fruitlets}}{\text{Total No. of flowers}}X100$$

At harvest, ten fruits were randomly selected from each replicate to analyze the physical quality [Fruit weight, fruit size (cm^3), fruit firmness (cm^2/kg) and fruit diameter (cm)] and chemical quality [Total soluble solids percentage (TSS%), total sugar, acidity (mg/100 g F.W), vitamin C (mg/100 g F.W)] according to Sparks *et al.* [27] and Hernandez *et al.* [28], also, [total carbohydrates, C/N ratio of the fruit] according to AOAC [29] and Dewis and Freitas [30]. While, nitrogen, phosphorus and potassium contents were determined in leaves according to Cataldo *et al.* [31].

Furthermore, soil samples were obtained from each treatment to measure dehydrogenase activity using the Casida method [32] and to determine indole acetic acid using the Gordon and Weber [33].

Statistical Analysis: The experimental data were subjected to analysis of variability (ANOVA) using the procedures described by Snedecor and Cochran [34].

RESULTS AND DISCUSSION

Sustainable agriculture requires renewable inputs that optimize ecological benefits and minimize environmental risks. This study evaluates the effect of microbial fertilizer *B. subtilis* and *B. amylo* on Le-Conte pear fruit growth and productivity through soil inoculation, either alone or with cyanobacteria extracts.

Table (2) shows the influence of different biofertilizers as individual or combination treatments on the NPK leaf contents of "Le-Conte" pear over the 2021 and 2022 seasons. In respect of presence of cyanobacteria, a nitrogen fixer and phosphate dissolving biofertilizer and *Bacilli* sp., plant growth promoting rhizobacteria (PGPR), NPK content increased in all single and combined treatments. Overall, the second season reported an increase in all elements over the first season in all treatments when compared to control trees. The highest N P K content was recorded by the three combined biofertilizers (2.85, 0.93 and 1.6 %) respectively, followed by the mixture of cyanobacteria and one of the *Bacilli* strains was with a little difference between the mixing of two *Bacilli* strains, then single treatments and the control treatment had the lowest N P K concentration at all.

Our findings on the effectiveness of bio-fertilizers on improving leaf nutritional status in Le-Conte pear trees are consistent with early studies on apricot trees on N, P and K [35]. Biofertilizers in soil provide nutrients for plant growth, aiding in yield development and physiological processes. Furthermore, they play important activities in photosynthesis include capturing light energy and converting it into chemical energy [36]. Biofertilizers use microorganisms to boost soil production by promoting

Table 2: Effect of biofertilizers on nitrogen, phosphorous and potassium contents in leaves of "Le-Conte" pear trees during seasons 2021-2022

Treatments	Nitrogen %		Phosphorus %		Potassium%	
	2021	2022	2021	2022	2021	2022
Control	1.25 (e)	1.67 (e)	0.22 (d)	0.62 (d)	0.45 (f)	1.25 (f)
Cyanobacteria	1.57 (d)	1.93 (d)	0.29 (c)	0.58 (c)	0.64 (d)	1.44 (d)
B. subtilis	1, 67 (cd)	2.10 (cd)	0.33 (b)	0.83 (b)	0.57 (e)	1.38 (e)
B. amylo.	1.57 (d)	2.19 (bc)	0.32 (bc)	0.79 (bc)	0.55 (e)	1.35 (e)
Cyanobacteria + *B. subtilis*	1.79 (bc)	2.21 (bc)	0.40 (a)	0.85 (b)	0.58 (e)	1.39 (e)
Cyanobacteria + *B. amylo*	1.87 (b)	2.35 (b)	0.36 (b)	0.88 (b)	0.7 (c)	1.5 (c)
B. subtilis + *B. amylo*	1.85 (b)	2.34 (b)	0.35 (b)	0.85 (b)	0.75 (b)	1.55 (b)
Cyanobacteria + *B. subtilis* + *B. amylo*	2.1 (a)	2.85 (a)	0.43 (a)	0.93 (a)	0.81 (a)	1.6 (a)
L.S.D.(0.05)	0.13	0.22	0.03	0.04	0.05	0.05

essential nutrients [37]. Biofertilizer administration resulted in higher leaf N, P, K concentrations compared to control [38]. Biofertilizers were found to affect the nitrogen level of Eureka lemon leaves. Soil nutrient levels correlate positively with leaf nutritional condition [39]. Ibrahim *et al.* [40] reported that biofertilizer treatments treatment improved the mineral content of leaves and fruit production. Biofertilizer treatments may increase soil fertility through nitrogen fixation and the secretion of beneficial compounds. Cyanobacteria which can fix atmospheric nitrogen, increase phosphorus availability and improve element absorption by Eureka lemon trees, leading to increased growth and productivity [41].

Data in Table (3) showed the effect of biofertilization on total carbohydrates and C/N ratio of "Le-Conte" pear trees during seasons 2021-2022. Total carbohydrates measured increased significantly in inoculated treatments compared to controls in both seasons, following the same pattern as the study results. Cyanobacteria + *B. subtilis* + *B. amyl*o had the greatest carbohydrates content (26.03), followed by Cyanobacteria + *B. amylo* (25.5) in the second season, there was little variation between Cyanobacteria + *B. subtilis* and *B. subtilis* + *B. amylo*. treatments, however the individual treatments recorded lower carbohydrates contents than mixed applications but also more than control treatment in both seasons. Furthermore, the C/N ratio improved significantly in the presence of biofertilizers, resulting in enhanced fruit production per tree in both seasons due to the increased nitrogen content. The mixed three biofertilizers had the highest C/N ratio (14.8), with little variation compared to the double biofertilizer mix in the second season, however control treatment in the first season recorded the lowest C/ N ratio. These findings are with agreement with Mohammed *et al.* [42].

Data of fruit physical and chemical quality (Fruit weight, fruit size, fruit firmness, fruit diameter, TSS, acidity, total sugar, vitamin C and total carbohydrates) of the fruit as shown in Tables 3, 4, 5. Data revealed that mixes of three biofertilizer strains (cyanobacteria, *B. subtilis* and *B. amylo*.) generated the most significant outcomes from treatment in both seasons. These were followed by mixes of two biofertilizer strains (Cyanobacteria + *B. amylo*., Cyanobacteria + *B. subtilis* and *B. subtilis* + *B. amylo*) and finally single treatment compared to control trees that did not receive any biofertilizers. The highest fruit weight and size was 194.31g 218.89 cm^3 respectively recorded by the mixed of three biofertilizers in the second season and the lowest weight was 74.02 g 77.14 cm^3 respectively recorded by control treatment in the first season, fruit length, diameter and firmness also increased significantly in the presence of biofertilizers than in the control treatment (Table 4).Concerning the availability of biofertilizers, treated trees with three combined biofertilizers had the highest TSS%, total sugar, vitamin C.

Table (5) shows that inoculating with a combination of three biofertilizers resulted in the greatest percentage of total soluble solids (TSS%) and total sugar content, with just a little difference between the other mixed and single treatments and the control, which had the lowest TSS% and total sugar content at all in both studied seasons. Furthermore, vitamin C showed the same trend in both seasons, but all mixed and single biofertilizers increased Vit C and decreased acidity by up to 15% compared to the control treatment in both seasons, whereas the single B. amylo application had the highest Vit. C content (12.30 mg/100g F.W) in the second season and the lowest Vit. C content (8.39 mg/100g F.W) in the first. Also, the presence of biofertilizers led to an increase in fruit set % and fruit yield (Figs 1and 2), either in mixed or single applications, when compared to the control and the second season results were greater than the first seasons by up to 13.65%. The mix of three biofertilizers produced the maximum fruit set % and fruit yield (16.03 %, 50.94 kg/tree) respectively, followed by cycanobacteria + *B. subtilis* (14.87 , 43.28 kg/tree) in the second season and the control treatment produced the minimum fruit set %

Table 3: Effect of biofertilizers on total carbohydrates and C/N ratio, fruit yield and fruit set of "Le-Conte" pear trees during seasons 2021-2022

Treatments	Total Carbohydrates		C/N ratio		Fruit set %		Fruit yield (kg /tree)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	10.78 (c)	11.58(c)	6.91 (c)	8.5 (d)	7.64 (d)	8.84(e)	19.78 (h)	23.75 (f)
Cyanobacteria	23.11 (ab)	23.91(ab)	12.14(a)	13.72 (a)	1 2.88(b)	13.75(cd)	22.65(g)	26.52 (e)
B. subtilis	24.72 (a)	20.51(ab)	10.74(a)	10.65(cd)	11.80 (c)	13.08 (d)	31.19 (e)	36.32 (d)
B. amylo.	19.71 (b)	23.54(ab)	10.74 (a)	11.55(bc)	11.67 (c)	12.77(d)	29.78 (f)	36.32 (d)
Cyanobacteria + *B. subtilis*	25.23 (a)	25.52 (a)	11.07 (a)	13.12(ab)	13.71 (b)	14.87 (b)	35.96 (b)	43.28 (b)
Cyanobacteria + *B. amylo*	24.12(a)	25.37 (a)	11.27 (a)	13.488(ab)	13.53 (b)	14.55(bc)	34.04 (d)	41.76(bc)
B. subtilis + *B. amylo*	22.74 (ab)	25.5 (a)	12.14 (a)	14.45 (a)	13.49 (b)	14.94 (b)	35.03 (c)	39.9 (c)
Cyanobacteria + *B. subtilis* + *B. amylo*	24.57 (a)	26.03 (a)	12.38 (a)	14.8 (a)	14.87(a)	16.03 (a)	44.03 (a)	50.94 (a)
L.S.D. (0.05)	3.83	3.39	1.64	2.14	0.85	1.02	0.900	1.98

Table 4: Effect of biofertilizers on physical fruit quality of "Le-Conte" pear trees during seasons 2021-2022

Treatments	Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)		Fruit Size (cm^3)		Fruit firmness ($Lb/inch^2$)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
Control	74.02 (e)	80.54 (e)	6.17 (d)	6.3 (e)	4.73 (c)	5.43 (d)	77.14 (f	86.61 (d)	17.39 (c)	18.42 (c)
Cyanobacteria	154.62(bc)	171.08 (c)	8.00 (ab)	8.1 (ab)	6.4 (a)	6.83(ab)	138.1(de)	134.46 (c)	20.62 (b)	18.84 (c)
B. subtilis	146.19(c)	165.3 (d)	6.60 (cd)	7.7 (bc)	6.61 (a)	6.16(cd)	133.15 (e)	170. 19(b)	21.75(ab)	19.46 (bc)
B. amylo.	126.96 (d)	159.66(cd)	7.53 (bc)	7.8(bc)	5.57 (b)	6.8(abc)	128 (e)	174.21 (b)	22.29(ab)	18.92 (c)
Cyanobacteria + *B. subtilis*	178.22(a)	182.16 (b)	8.93(a)	8.2 (ab)	6.4 (a)	6.4(bc)	191.78(ab)	174.89 (b)	22.41(ab)	19.94 (bc)
Cyanobacteria + *B. amylo*	178.56 (a)	183.17.(b)	7.4 (bc)	8.00 (ab)	6.14(a)	7.3 (ab)	175.12(bc)	192.133 (b)	22.15(ab)	20.35(abc)
B. subtilis + *B. amylo*	170 (ab)	182.27(b)	6.67 (cd)	7.27(c)	5.53 (b)	6.12(cd)	160.92(cd)	192.03 (b)	22.99(ab)	21.55 (ab)
Cyanobacteria + *B. subtilis* + B. *amylo*	180 .59(a)	194.31	8.8 (a)	8.61 (a)	6.46 (a)	7.6(a)	210.98 (a)	218.89 (a)	23.43 (a)	22.71 (a)
L.S.D.(0.05)	14.42	8.79	1.21	0.64	0.36	0.95	23.23	22.20	2.64	2.37

Table 5: Effect of biofertilizers on chemical fruit quality of "Le-Conte" pear trees during seasons 2021-2022

Treatments	TSS %		Acidity %		Total Sugar (g/100g D.W)		Vit. C (mg/100g F.W.)	
	2021	2022	2021	2022	2021	2022	2021	2022
Control	14.4(c)	15.2 (e)	0.4 (a)	0.43 (a)	9.74 (c)	10.54 (c)	8.39 (e)	9.10 (e)
Cyanobacteria	15.3(b)	16.67(bcd)	0.186 (b)	0.18 (b)	17.36 (a)	18.16 (a)	9.82 (cd)	10.52 (cd)
B. subtilis	15.50 (b)	15.8 (de)	0.127 (c)	0.12 (cd)	15 (b)	15.8 (b)	9.13 (de)	9.83 (de)
B. amylo.	15.60 (b)	16.6 (bcd)	0.093 (cd)	0.08 (de)	14.7 (b)	15.52 (b)	11.60 (a)	12.30 (a)
Cyanobacteria + *B. subtilis*	15.50 (b)	15.8 (de)	0.120 (bc)	0.10 (cde)	15.33(ab)	16.13 (ab)	10/90 (ab)	11.60 (ab)
Cyanobacteria + *B. amylo*	15.97(b)	16.97 (abc)	0.143 (bc)	0.13 (bcd)	13.51 (b)	14.31 (b)	10.29 (bc)	11.00 (bc)
B. subtilis + *B. amylo*	17.03 (a)	17.3 (ab)	0.133 (bc)	0.14 (bc)	15.75(ab)	16.55 (ab)	11.14 (ab)	11.85 (ab)
Cyanobacteria + *B. subtilis* + *B. amylo*	17.20 (a)	17.9 (a)	0.06 (d)	0.057 (e)	17.54 (a)	18.34 (a)	11.62 (a)	12.32 (a)
L.S.D.(0.05)	0.784	0.970	0.055	0.059	2.24	2.25	1.023	1.023

and fruit yield (7.64, 19.78 kg/tree) respectively in the first season. These results agree with the result obtained by Abobatta and El-Azazy [43] who demonstrated that biofertilizer application increasing fruit yield in citrus trees. Biofertilizer treatments increase yield by enhancing nitrogen fixation and secretion of soil fertility. Organic matter also increases bacterial activity, which can fix atmospheric nitrogen, increase phosphorus availability in soil and enhance element absorption by plant [39].

On contrast to the control, all single and mixed treatments with biofertilizers resulted in a decrease in acidity. The combination of three biofertilizers in the second season reported the lowest acidity percentage (0.057 %), while the control treatment reported the largest acidity percentage in the same season (0.43 %). The obtained results were in agreement with those of Gashash *et al.* [44], who showed that all growth parameters were elevated in the presence of *Bacillus* sp. and cyanobacteria. According to Meena *et al.* [45], Cyanobacteria can fix nitrogen, produce plant growth regulators (auxins, gibberellins, cytokines), improve soil fertility by adding organic matter, nitrogen and phosphorus, degrade agrochemicals (pesticides and herbicides) and control pathogenic effects of other microorganisms and plants, all of which can benefit agriculture.

Table 6: Effect of biofertilizers on total chlorophyll and carotenoids in leaves of "Le-Conte" pear trees during seasons 2021-2022

Treatments	Total Chlorophyll (mg /100g)		Carotenoids (mg /100g)	
	2021	2022	2021	2022
Control	1.26 (c)	2.06 (c)	0.33 (d)	0.83 (d)
Cyanobacteria	1.47 (b)	2.28 (b)	0.49 (c)	0.99 (c)
B. subtilis	1.49 (b)	2.29 (b)	0.38 (b)	0.88 (d)
B. amylo.	1.59 (b)	2.38 (a)	0.36 (d)	0.86 (d)
Cyanobacteria + *B. subtilis*	1.51 (b)	2.031 (b)	0.55 (ab)	1.05 (ab)
Cyanobacteria + *B. amylo*	1.46 (b)	2.31 (b)	0.54 (abc)	1.04 (abc)
B. subtilis + *B. amylo*	1.45 ((b)	2.25 (b)	0.53 (bc)	1.03 (bc)
Cyanobacteria + *B. subtilis* + *B. amylo*	1.59 (a)	2.31 (b)	0.6 (a)	1.10 (a)
L.S.D.(0.05)	0.07	0.06	0.06	0.06

Data in Table (6) showed the effects of single and combination biofertilizers on total chlorophyll and carotenoids content in the leaves of Le-Conte pear trees. All treatments significantly increased total chlorophyll and carotenoids content after two seasons. However, the combination of cyanobacteria and *Bacilli* sp. reported the greatest substantial boost, followed by double mixed treatments. It was obvious that soil bio-fertilization produced the highest level of leaf total chlorophyll and carotenoids when compared to the other controls. The second season's combined treatment with three biofertilizers had the highest chlorophyll and carotenoids levels (2.31 and 1.10 mg/100g, respectively). The control treatment had the lowest chlorophyll and carotenoids levels (1.26 and 0.33 mg/100g) during the first season. Chlorophyll and carotenoids are pigments found in plant tissues that contribute to color differences ranging from dark green to yellow. Carotenoids offer vibrant colors, serve as antioxidants and may be a source of vitamin A activity [36]. Our results were similar to Pishchik *et al.* [46] and Gashash *et al.* [44] who reported that combining *B. subtilis with B. amylo*. and cyanobacteria significantly increased the total chlorophyll (a + b) and carotenoids concentration in tomato leaves, indicating increased photosynthetic activity. Vitale *et al.* [47] reported that microorganisms have increased photosynthetic capacity and plant growth. Cyanobacteria have demonstrated favorable effects on crop growth, nutritional status, yield and soil fertility [48]. The presence of cyanobacteria increased chlorophyll levels and plant development. It promotes plant growth through nitrogen fixation (diazotrophy), producing auxins and secreting nitrogenous/carbon-containing substances and secondary metabolites [49].

Table (7) presented the biological activity, dehydrogenase activity (DHA), which is an indicator of microbial activity in soil and represents energy transfer and indole acetic acid (IAA) content in the pear tree's rhizosphere soil over the seasons 2021-2022. Both parameters rose during the two seasons due to the presence of biofertilizers; however, treatments combination demonstrated greater activity than single treatments. The highest DHA and IAA were (0.168mg TPF g^{-1} dry rhizosphere $soil^{-1}$ $day^{-1}$and 3.12 mg $g^{-1}soil^{-1}$) respectively, which reported by cyanobacteria + *B. amylo*+ *B. subtilis* treatment followed by(0.104 mg TPF g^{-1} dry rhizosphere soil-1 day-1 and 2.9 mg g^{-1} $soil^{-1}$) respectively, by cyanobacteria + *B. amylo* in the second season These results were in agreement with Ghazal *et al.* [50] and Ashmawi *et al.* [51] who demonstrated that the presence of biofertilizers increased the microbial and biological activities in soil. Auxins, especially indole acetic acid (IAA), serve critical roles in regulating plant growth, such as cell elongation, vascular tissue formation, apical dominance and the presence of cyanobacteria and Bacilli sp. in individual and combined treatments Increased IAA concentration in soil due to they are PGPR. The second season reported higher IAA in soil then the first season and the combined use of three biofertilizers had been recorded the largest content (2.9 mg g^{-1} $soil^{-1}$) with little difference between the mixed of two biofertilizers and single biofertilizer recorded lower IAA concentration in soil but it was higher than control treatment.

Soil microorganisms play an important part in ecosystem activities such as nutrient cycling and organic matter decomposition. They also promote plant health and growth through bio-fertilization [52, 53]. Microbial populations play a crucial role in root health, nutrient uptake, environmental stress tolerance and crop responses. This has been widely acknowledged. Plant growth-promoting rhizobacteria (PGPRs) are bacteria found in the rhizosphere that have both direct and indirect benefits for plant growth [54]. Cyanobacterial was chosen for its multiple PGP properties, including biological nitrogen fixation, biofilm formation and indole

Table 7: Effect of biofertilizers on biological activities in the rhizosphere soil of "Le-Conte" pear trees during seasons 2021-2022

	DHA (mg TPF g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})		Indole Acetic Acid (mg g^{-1} soil $^{-1}$)	
Treatments	2021	2022	2021	2022
Control	0.057	0.068	0.77	0.83
Cyanobacteria	0.071	0.088	1.27	1.8 4
B. subtilis	0.061	0.082	1.26	1.79
B. amylo.	0.065	0.085	1.32	1.47
Cyanobacteria + *B. subtilis*	0.083	0.096	1.46	1.80
Cyanobacteria + *B. amylo*	0.078	0.104	1.84	2.6
B. subtilis + *B. amylo*	0.076	0.098	1.74	2.9
Cyanobacteria + *B. subtilis* + *B. amylo*	0.138	0.168	1.89	3.12

compounds, making it a potential inoculant for many trees [55]. The findings of our study are consistent with those of Ghazal and Salem [56], who found that IAA, which is produced by cyanobacteria, promotes the growth of plants.

B. subtilis promotes growth in several fruit trees. Inoculation with *B. subtilis* increased seed germination, seedling vigor and growth through direct and indirect processes. *B. subtilis* creates a biofilm on roots to colonize the rhizosphere over time. *B. subtilis* strains can positively impact plants, allowing for the development of new, safe and environmentally friendly seed treatments [57]. *B. amylo* genes produce various substances, such as phytohormones for plant growth, polysaccharides for biofilm formation, siderophores for iron solubilization, lytic enzymes and non-ribosomal synthesized polyketides and lipopeptides for pathogen inhibition [58]. *B. amylo* has been shown to enhance plant growth by producing hormones and siderophores, as well as enhancing soil nutrient availability and reducing pathogens [59].

As stated by Choudhary and Johri [60], *B. amylo* and *B. subtilis* strains have been observed to interact with plants, offering advantages like resistance, disease protection and growth stimulation through siderophore production. Our study found that mineral fertilization and bacterial inoculation increased microbial biomass (total cultural bacteria, PSB and fungal) and altered community structure based on the treatments applied. In comparison to the control group, *Bacillus* sp. and cyanobacteria enhanced plant development (measured in centimeters of plant height, number of leaves/plant and number of fruits/plot). Inoculants are critical to ensuring a long-term synergistic link since *B. amylo*. can survive in the soil. This is comparable to the findings of Jamal *et al.* [61]. Vitamin C (ascorbic acid) is required for plant physiological activities such as enzyme activity, gene expression, environmental signaling and transport. Furthermore, it is a chemical with known antioxidant properties that is widely present. Improving agricultural techniques is important as vitamin C cannot be manufactured by the body and must be received through diet [62]. Also, Zhou *et al.* [63] revealed that PGPR indirectly improves plant growth by producing a fungal cell wall disintegrating enzyme, which protects plants against infections.

CONCLUSION

Biofertilizers are environmentally friendly microorganisms that improve soil fertility and properties, as well as plant growth. In our study, we found that applying individual and mixed applications of cyanobacteria, *B. subtilis* and *B. amylo*, as biofertilizers to Le-Conte pear trees over the course of two successive seasons increased the trees' yield and the quality of their fruits.

However, we advise adding the rates of previous biofertilizer, which gave better results in the terms of all growth measures, yield, fruit quality and soil properties for "Le-Conte" pear trees under this experiment conditions.

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