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Original Article

The effect of biological fertilizer associated with chemical fertilizers on olive

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ABSTRACT

The experiment was conducted in a complete randomized design with three replications in Ilam in 2020. The treatments included bio-fertilizer treatment as mycorrhiza, *Bacillus subtilis* and *Pseudomonas* bacteria with amino acids, chemical fertilizer treatment based on soil test and olive nutrition recommendations, combined application of chemical and biological fertilizers and control. The evaluated characteristics included physical and chemical properties of the soil, leaf nutrient concentration, and quantitative characteristics of olive fruit. The aim of this study was to identify the effect of biological fertilizer associated with chemical fertilizers on olive. Duncan's multiple range test showed that fruit yield in the combined application of bio-fertilizer and chemical fertilizer treatment was in the superior statistical group. Fruit yield in the bi-ofertilizer and chemical fertilizer treatment alone also had significant differences compared to the control. So that after the superior treatment, the chemical fertilizer treatment and the bio-fertilizer treatment were respectively. The highest fruit yield with 3179 kg/ha was obtained due to the combined application of chemical fertilizer and bio-fertilizer and the lowest was obtained in the no fertilizer treatment with 2452 kg/ha. The concentration of nitrogen increased from 1.61 percent in the control to 1.89 percent with the combined application of bio- and chemical fertilizers. This increase in concentration in the case of phosphorus was from 0.11 in the control to 0.16 in the superior treatment and in the case of potassium from 1.06 in the control to 1.17 in the combined application treatment. The increase in the concentration of zinc, iron, manganese and boron elements in the combined application of biological and chemical fertilizers was 32.2, 14.2, 24.6 and 20.5 percent compared to the control, respectively. Overall, the use of mycorrhizal fungi and plant growth-promoting bacteria, especially in combination with chemical fertilizers, improved the nutritional status and increased the growth and yield of olive trees.

1. Introduction

Olive (*Olea europaea* L.) is an evergreen plant that belong to the family of Oleaceae growing in Iran. It's fruits and leaves has medicinal and economic concerns. The consumption of olive fruit and oil has gradually increased due to the increase of worldwide population. Olive groves are commonly established in the poor-fertility soils in western Iran (Zebardast *et al*., 2023). These soils have low biological index characteristics as well as a deficiency in nutrient elements (Javed *et al*., 2022). Consequently, crop production in poor-fertility soils faces minimum productivity. The deficiency of nutrient elements is compensated by chemical fertilization. Continuous application of chemical fertilizers leads to the increase in heavy metals in plant tissues (Jote *et al*., 2023). The use of biofertilizers in increasing the reproductive and vegetative performance of olives can be considered as a very important factor in the expansion and economic production of olive in the country. Achieving appropriate treatments to increase the yield of olive trees, given the biocompatibility of biological products, is the basis for the increasing development of olive. Given the development of olive cultivation in the country and the increasing demand for olive oil, it seems that the use of microorganisms, as well as organic stimulants and environmentally friendly compounds significantly reduces the problems related to chemical fertilizers and, as a result, increases the yield (Ansabayeva *et al*., 2025). In general, amino acids are fundamental elements in the initial stages of protein synthesis, and this can increase the efficiency of enzymes involved in the biosynthesis of fatty acids in olive trees and the transport of nutrients within the trees. Therefore, the use of some amino acids in the formulation of biofertilizer inoculants along with fulvic acid was effective in the proliferation and survival of microorganisms in the inoculants, and in this project, amino acid application was used for this purpose. Improving horticultural strategies is essential to increase productivity and prevent possible changes in the quantity and quality of fruit and oil (Al-Yasiry *et al.*, 2024). Nutritional management can be a very effective factor in improving flowering and yield of olive trees (Zipori *et al.*, 2020). Nutrient deficiency is very important, especially under drought stress conditions, due to the reduction of their absorption, transport and redistribution within the plant (Zhu *et al.*, 2024). Nowadays, the use of biofertilizers has increased significantly in increasing the yield of various crops. Khosravinejad *et al.* (2026) stated that foliar spraying of biofertilizers in olives improves the quantitative and qualitative traits of the fruit. The olive tree responds very well to the combined use of organic and biological fertilizers, and the simultaneous use of these two fertilizers has a much greater effect than chemical fertilizers on crop production (Sande *et al.*, 2024). The formulation and appropriate composition of bacteria and its additives are very effective factors in the efficiency of biological fertilizers, and there is a lot of diversity in the formulation, and additives can play a very important role in the durability and effectiveness of biological fertilizers, and amino acids and even fulvic and humic acids are also very effective in the composition of biological fertilizer formulations. Increasing the bioavailability of nutrients in the vicinity of the olive root increases the metabolism in this plant and, as a result, increases the quality of the produced product (Hammam *et al.*, 2024). Symbiotic bacteria that form colonies in the rhizosphere of plant roots in populations of millions are responsible for the production of many vital secondary metabolites in the soil that directly or indirectly affect plant growth. Identifying the best fertilizer treatments based on environmentally friendly materials is very important in increasing olive yield and reducing the use of chemical fertilizers in olive management. It has become need to find alternative eco-friendly sources of fertilizers to improve poor soils. Non-chemical fertilization depends on using natural materials such as microorganisms. Bio-fertilizers are promising approach to reducing the application of chemical fertilizers, particularly under drought conditions (Shahwar et al., 2023). Also, the use of a certain amount of amino acid in the formulation of biofertilizer inoculation medium is effective in the reproduction and survival of microorganisms in the inoculation medium, and in this project, a small amount of this type of amino acid was used for this purpose (Kanishka et al., 2025). Therefore, the aim of this research was to investigate the effect of combined nutrition of bio-fertilizers along with chemical fertilizers in increasing olive yield and to investigate the effectiveness of bio-fertilizers (environmentally friendly) in increasing the yield of olive trees.

2. Materials and Methods

2.1 Geographical location

Ilam province, with an area of 2020 square kilometers, is the twenty-second largest province in Iran. Ilam Province is located in the southwest of Iran in the Zagros mountains and Iraq border to the west, Khuzestan province to the south, Lorestan province to the east, and Kermanshah province to the north. The lands used for olive cultivation have poorly developed soils and there are problems in terms of the concentration of absorbable elements in them (Tables 1 and 2). About 60 percent of the olive trees produced in the province are oil and 40 percent are canned variety. The most cultivated olive varieties in Ilam province are Zard. Olive trees are cultivated in seven counties in the province such as Chavar county. Olive harvesting in Ilam province, which began in October with the harvesting of canned olives, will continue until the end of December.

2.2 Field operations

Soil and water sampling was carried out in October and leaf sampling in August, and based on soil analysis (Table 1), fertilizer recommendations were made. The dominant cultivar in these area was Zard. The experiment was conducted in a complete randomized block design (CRBD) with three replications and five stands for each plot. The four experimental treatments included 1- control treatment 2- chemical fertilizer treatment (based on soil test, Table 3) 3- biofertilizer treatment (a combination of mycorrhiza and *Bacillus subtilis* + *Pseudomonas* and amino acids applied to the soil and a single foliar spray without mycorrhiza) 4- combined treatment of chemical and biological fertilizers. Soil test showed that soil samples had high pH at both depths of 0-30 to 30-60 cm. At depth of 30-60 cm, the pH was higher than the surface soil. Organic carbon content was low at both depths and at a depth of 30-60 cm the organic carbon was lower than the surface soil. Irrigation, pruning (winter pruning and removal of suckers and dead branches), weed control (mechanical control at the tree edge and between rows with paraquat) and pesticides against pests (mites and olive psyllids) were applied uniformly in all treatments. Leaf samples from a total of four stands in each plot were randomly prepared from mature leaves of the current season's growth and complete analyses of nutrients were performed. At harvest time, the average product weight for stands was recorded by weighing the harvested fruits in each plot. The yield and fruit size as well as fertilizer indices (the concentration of micro- and macro-elements in the leaves of treated trees were evaluated and chemically analyzed. Data variance analysis was performed using SAS 9.2 software and the mean data were compared using Duncan's multiple range test (DMRT).

3. Results

The results of the analysis of variance (two-year composite) on the effect of bio-fertilizer management on yield and yield components are given in Table 2. Analysis of variance data showed that changes in fruit yield in different fertilizer treatments were significant at the five percent level (Table 2). Also, Duncan's multiple range test showed that fruit yield in the combined application of biofertilizer and chemical fertilizer treatment was in the superior statistical group. Fruit yield in the biofertilizer and chemical fertilizer treatment alone also had significant differences compared to the control. So that after the superior treatment, the chemical fertilizer treatment and the biofertilizer treatment were respectively. The highest fruit yield with 3179 kg/ha was obtained due to the combined application of chemical fertilizer and biofertilizer and the lowest was obtained in the no fertilizer treatment (control) with 2452 kg/ha. In the combined application of bio- and chemical fertilizers, the application of chemical fertilizers alone, and the application of bio-fertilizers alone, olive fruit yield increased by 29.7, 20.5, and 16.8 percent, respectively, compared to the control (Table 3).

3.1 Olive fruit length and diameter

Analysis of variance of the data showed that the changes in olive fruit length and diameter in different fertilizer treatments were significant at the five and one percent levels, respectively (Table 2). Duncan's multiple range test showed that the length and diameter of olive fruit in the combined application of bio- and chemical fertilizers were in the superior statistical group. The length and diameter of olive fruit were also significantly different in the chemical fertilizer application treatment and the bio-fertilizer application treatment compared to the control. The highest length and diameter of olive fruit were obtained with 2.82 and 1.93 centimeters, respectively, with the combined application of chemical fertilizers and bio-fertilizers, which represented an increase of 25.4 and 24.2 percent, respectively, compared to the control treatment (Table 3).

3.2 Fresh weight and dry weight of olive fruit

Analysis of variance of the data showed that the changes in the fresh weight of 50 fruits and the dry weight of 50 fruits in different fertilizer treatments were significant at the five and one percent levels, respectively (Table 2). Duncan's multiple range test also showed that the fresh weight of fruit yield in the combined application of biological and chemical fertilizers was in the superior statistical group. The yield of olive fruit in the treatments of biological and chemical fertilizers alone also had significant differences compared to the control. The highest fresh weight and dry weight of olive fruit were obtained with 169 and 64.6 grams, respectively, as a result of the combined application of chemical fertilizers and biological fertilizers. In the combined application of biological and chemical fertilizers, the application of chemical fertilizers alone and the application of biological fertilizers alone increased the dry weight of 50 fruits by 28.7, 20.1 and 15.9 percent, respectively, compared to the control (Table 3).

3.3 Changes in the concentration of nitrogen, phosphorus and potassium

Analysis of variance of the data showed that the changes in the concentration of nitrogen, phosphorus and potassium in olive leaves in different fertilizer treatments were significant at the five, one and five percent levels, respectively (Table 4). Duncan's multiple range test showed that the concentration of nitrogen, phosphorus and potassium elements in the combined application of bio- and chemical fertilizers were in the superior statistical group. The concentrations of these elements in the chemical fertilizer application treatment and the bio-fertilizer application treatment also had a significant difference compared to the control. The concentration of nitrogen increased from 1.61 percent in the control to 1.89 percent with the combined application of bio- and chemical fertilizers. This increase in concentration in the case of phosphorus was from 0.11 in the control to 0.16 in the superior treatment and in the case of potassium from 1.06 in the control to 1.17 in the combined application treatment (Table 5).

3.4 Changes in calcium and magnesium concentrations

Analysis of variance of the data showed that changes in calcium and magnesium concentrations in olive leaves in different fertilizer treatments were significant at the one and five percent levels, respectively (Table 4). Duncan's multiple range test showed that the concentrations of calcium and magnesium elements in the combined application of bio- and chemical fertilizers were in the superior statistical group. The concentrations of these elements in the chemical fertilizer application treatment and the bio-fertilizer application treatment also had a significant difference compared to the control. The calcium concentration increased from 1.46 percent in the control to 1.77 percent with and combined application of bio- and chemical fertilizers. This increase in the concentration of magnesium was from 0.17 in the control to 0.27 percent in the combined application of bio- and chemical fertilizers (Table 5).

Table 1. Soil characteristics in depths of 0 to 30 and 30 to 60 centimeters

Depth Cm	EC	OC	pH	TN	P	K	B	Zn	Fe	Mn	Cu
	ds m^{-1}	%		%	----------------------------mg kg^{-1}---------------------------------						
0-30	0.68	1.17	7.26	0.12	11.2	347	0.72	1.24	7.51	8.36	1.91
30-60	0.46	0.78	7.36	0.08	7.01	302	0.65	0.86	5.13	5.81	1.05

Table 2. Summary of variance analysis (mean square) of data

	Fruit Yield	Fruit length	Fruit diameter	Fresh weight	Dry weight
	835 ^{ns}	361 ^{ns}	293 ^{ns}	321 ^{ns}	110 ^{ns}
	535 ^{ns}	774 ^{ns}	614 ^{ns}	721 ^{ns}	535 ^{ns}
	40643 *				
F×Y	173 ^{ns}	793 ^{ns}	106 ^{ns}	112 ^{ns}	1763 ^{ns}

**Significant at the one percent level, *Significant at the five percent level, and ns not significant.

Table 3. Variation of yield and yield components of olive

Treatment	Yield	Fruit length	Fruit diameter	Fresh weight (50 fruit)	Dry weight (50 fruit)
	kg/ha	cm		gr	
Control	2452c	2/21c	1/49c	128 c	50.2c
Chemical Fertilizer(C)	2955b	2/76b	1/85b	157b	60.3b
Biological Fertilizer(B)	2865b	2/68b	1/82b	152b	58.2b
C+B	3179a	2/82a	1/93a	169a	64.6a

Table 4. Variance analysis (mean square) of macronutrient concentrations

	N	P	K	Ca	Mg
	214 ^{ns}	351 ^{ns}	293 ^{ns}	311 ^{ns}	257 ^{ns}
	118 ^{ns}	407 ^{ns}	428 ^{ns}	243 ^{ns}	124 ^{ns}
	4158*			9035**	4013*
F×Y	163 ^{ns}	293 ^{ns}	196 ^{ns}	203 ^{ns}	104 ^{ns}

Table 5. Concentration of macronutrient in the olive leaves

Treatment	N	P	K	Ca	Mg
	%				
Control	1.61c	0.11c	1.06c	1.46c	0.17c
Chemical Fertilizer(C)	1.82ab	0.14b	1.19b	1.67b	0.24ab
Biological Fertilizer(B)	1.79b	0.13b	1.16b	1.62b	0.22b
C+B	1.89a	0.16a	1.17a	1.77a	0.27a

- Similar letters indicate no significant difference between means according to Duncan's multiple range test (DMRT) at the 5% level.

Table 6. Variance analysis (mean square) of macronutrient concentrations

	df	Zn	Fe	Mn	Cu	B
		415 ^{ns}	351 ^{ns}	155 ^{ns}	501 ^{ns}	532 ^{ns}
		321 ^{ns}	507 ^{ns}	612 ^{ns}	315 ^{ns}	415 ^{ns}
		5156*	3	7	5517**	7032*
F×Y		263 ^{ns}	493 ^{ns}	167 ^{ns}	336 ^{ns}	127 ^{ns}

**Significant at the one percent level, *Significant at the five percent level, and ns not significant.

3.5 Changes in the concentration of zinc, iron, manganese, copper and boron

Analysis of variance of the data showed that the changes in the concentration of zinc, iron, manganese and boron in olive leaves in different fertilizer treatments were significant at the five, one, five and five percent levels, respectively, and in the case of copper, the changes in this element were not significant (Table 4) Duncan's multiple range test showed that the concentration of zinc, iron, manganese and boron elements in the combined application of biological and chemical fertilizers was in the superior statistical group. The concentration of these elements in the chemical fertilizer application treatment and the biological fertilizer application treatment also had a significant difference compared to the control. The increase in the concentration of zinc, iron, manganese and boron elements in the combined application of biological and chemical fertilizers was 32.2, 14.2, 24.6 and 20.5 percent compared to the control, respectively (Table 5).

Table 7. Concentration of macronutrient in the olive leaves

Treatment	Zn	Fe	Mn	Cu	B
	mg/kg				
Control	19.9c	107c	31.8c	2.86a	34.3c
Chemical Fertilizer(C)	23.4b	117b	43.6b	2.82a	39.9b
Biological Fertilizer(B)	22.1b	117b	42.8b	2.91a	39.6b
C+B	26.3a	123a	46.5a	2.89a	40.9a

Similar letters indicate no significant difference between means according to Duncan's multiple range test (DMRT) at the 5% level.

4. Discussion

Beneficial microorganisms that have a crucial role in mobilizing unavailable nutrient forms. Accordingly, these advantages ameliorate fruit productivity under poor-fertile soils. The results showed that both fertilizers; bio-fertilizers (360kg/ha) and chemical fertilizers (3815 kg/ha), significantly had increasing effects on olive trees. Also, these fertilizers significantly enhanced leaf nutrient element concentrations and fruit yield compared to control. The improvement of evaluated indexes was attributed to the enhanced nutrient availability. The present investigation showed that biological fertilizers associated with chemical fertilizers affected the concentration status of elements in the leaves compared to the control treatment. Some nutrient elements increased in the leaves (for example, nitrogen, phosphorus, zinc, manganese and boron) and in some cases no significant change was observed in the concentration of nutrient elements in the leaves (such as copper). In this context, Mohamed et al. (2026) depicted that applying bio-fertilizer provided a high fruit yield and protected olive trees from drought stress (Mohamed et al., 2026). Similarly, Hmmam et al. (2025) proved that bio-fertilizer enhanced fruit set, fruit yield, and quality of fruit olive in comparison with control. Some nutrients such as potassium and zinc are important in the relationship between water in the plant. Under environmental stress, these changes can lead to a relative improvement in the nutritional status of olive trees and increased tolerance to water stress. The use of bio-fertilizers is effective in increasing the concentration of leaf nutrients and allocating photosynthetic products to the reproductive organs due to its direct and indirect effects on increasing the source and reservoir power in olive trees. Therefore, the results of this study are consistent with previous findings that emphasize the importance of bio-fertilizers in increasing plant availability of nutrients. This can be attributed to the ability of phosphate-solubilizing bacteria (such as *Pseudomonas*) to release inorganic phosphorus from insoluble forms and make it available to the plant (Pang *et al*., 2024). This effect was enhanced in the presence of phosphorus fertilizer and increased phosphorus uptake in the biological fertilizers associated with chemical fertilizers treatment compared to BF. Calcium (Ca) and magnesium (Mg) in calcareous soils are usually less available to the plant due to precipitation in the form of insoluble carbonates, but mycorrhizal fungi play a role in improving the uptake of these elements by increasing the absorption surface and changing the pH of the rhizosphere (Wang *et al*., 2022). Micronutrients such as iron (Fe) and zinc (Zn) are less available to the plant in many calcareous soils with high pH due to precipitation and insolubility. The results of this study showed that the biological fertilizers associated with chemical fertilizers treatment had the greatest increase in the absorption of these elements. The presence of *Pseudomonas* and *Bacillus subtilis* bacteria in the biological fertilizers treatment probably increased the production of siderophores. These compounds chelate insoluble iron and make it available to the roots (Timofeeva *et al*., 2023). Also, *Pseudomonas* bacteria have the ability to solubilize zinc, which can justify the increased absorption of Zn in the biological fertilizers and biological fertilizers associated with chemical fertilizers treatments (Sethi *et al*., 2025). Fungi increase the absorption of less mobile elements such as iron, zinc and boron through their extensive hyphal networks. Also, the symbiotic relationship of mycorrhiza with roots, the secretion of organic acids and the increase in the activity of phosphatase enzymes can play an important role in improving the absorption of these elements (Pang *et al.,* 2025). The increase in nutrient concentrations resulting from the combined application of bio-chemical fertilizers can be attributed to several physiological and biochemical mechanisms that contribute to the improvement of nutrition and growth of olive trees. One of the most important of these mechanisms is the increase in the availability of nutrients in the soil. The symbiosis of mycorrhizal fungi with plant roots increases the level of root uptake and improves the transport of nutrients, especially phosphorus, calcium and magnesium (Bhupenchandra, *et al*., 2025). In addition, the *Bacillus subtilis* and *Pseudomonas*, through the production of siderophores, increase the absorption capacity of iron and make this element more available to the plant (Gu *et al*., 2026). These effects can directly contribute to increasing plant yield and improving product quality. Also, microbial activities resulting from the application of biofertilizers increase the organic matter content of the soil, which leads to an increase in cation exchange capacity (CEC) and improved absorption of macro and micronutrients (Liu *et al*., 2026). This improvement in the physical and chemical conditions of the soil, in addition to increasing the efficiency of nutrient concentration, leads to improved soil water status and better development of the root system, which can ultimately have a significant effect on the yield. The results also showed that in the second year, the uptake of elements increased in all treatments, which was probably due to the cumulative effects of biological treatments and the gradual improvement of soil conditions. Similar studies have shown that the effect of bio-fertilizers is limited in the early years, but in the long term, with increased microbial activity, the efficiency of these fertilizers increases (Ma *et al*., 20025). This is especially important in sustainable agricultural systems and integrated plant nutrition management. Other studies have also confirmed that the use of biofertilizers can reduce plant dependence on chemical fertilizers and improve environmental sustainability while increasing yield (Shahzad *et al*., 2025). This is especially important in sustainable agricultural systems and optimal fertilization management. Overall, these results indicate that the simultaneous application of bio- and chemical fertilizers can be considered as an efficient strategy to improve the nutritional status of olives and increase the productivity of olive. This method not only increases the concentration of nutrients and improves the quality of the fruit, but also promotes the sustainability of the agronomic system and reduces the negative environmental impacts caused by excessive use of chemical fertilizers. Overall, the use of mycorrhizal fungi and plant growth-promoting bacteria, especially in combination with chemical fertilizers, improved the nutritional status and increased the growth and yield of olive trees.

5. Conclusion

This investigation indicated the potential of bio-fertilizers in enhancing fruit length, fruit diameter, fruit yield and leaf nutrient elements concentration (N, P, K, Zn, Fe, Mn and Cu) of ‘Zard’ cultivar in comparison with control. Biological treatment alone also performed well and was even more effective than chemical treatment in some cases. In addition, the effects of crop year on fruit yield and quality were significant, such that in the second year of the experiment, fruit quality characteristics such as fresh and dry weight, dry matter and oil percentage improved, while tree yield was higher in the first year. The effects of crop year on nutrient concentrations and functional characteristics were significant, indicating the importance of environmental conditions in determining the effectiveness of treatments. In general, the results of this study showed that the use of a combination of bio-chemical fertilizers is recommended as the best method for feeding olive trees, because in addition to increasing yield and quality of the crop, it can lead to improved soil nutritional conditions and reduced dependence on chemical fertilizers. Conclusively, the co-application of bio-fertilizer and chemical fertilizer could be recommended in poor-fertile soils.

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