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The effect of Bio and Nano Fertilizer on Growth and Yield of Basil Plant and its Content of Active Ingredients

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Abstract

A factorial experiment was conducted at the College of Education for Pure Sciences Ibn al-Haitham / University of Baghdad for the 2023 growing season to study the effect of the biofertilizer represented by the mycorrhizae vaccine and the NPK Nano fertilizer on some indicators of vegetative and root growth, yield, and volatile oil production of the basil plant, *Ocimum basilicum* L., the experiment included two factors. The first factor was mycorrhizae inoculum at 2 levels: 0 (no addition) and an addition of 25 g plant^{-1}, as the seeds were inoculated upon planting. The second factor was spraying the plant's shoots with NPK Nano fertilizer at four levels (0, 0.5, 1, 1.5) g l $^{-1}$. The results showed a significant effect of the treatment of inoculating basil seeds with mycorrhizae at the level of 25 gm in giving them the highest values in plant height, which reached 51.17 cm plant^{-1}, the number of leaves was 44.83 leaves per plant^{-1}, the yield per hectare was 5.94 tons' ha^{-1}, and the percentage of volatile oil in the leaves was 1.319 %. In the interactions between the study factors, the treatment of mycorrhizae inoculum 25 g with Nano-fertilizer 1 gm l^{-1} was significantly superior, and the highest values were recorded in plant height, number of leaves, yield per hectare, and percentage of volatile oil in the leaves, as it reached 55.67 cm plant^{-1} and 49.33 leaves plant^{-1} and 6.83 tons' ha^{-1} and 1.660 %, respectively, compared to the control treatment.

Keywords: Basil plant, Mycorrhizae, Medical Plants**,** Nano fertilizer, Volatile oil.

Introduction

Basil, *Ocimum basilicum* L. belongs to the Lamiaceae family, which includes about 200 species spread throughout Asia and Africa, which are believed to be the homelands of the most important species of the genus Ocimum. Sweet basil, *Ocimum basilicum* L, is one of the most widespread species. It is characterized by its strong and branched growth, especially in the upper parts, and reaches a height of 60-100 cm. Its leaves are lanceolate or oval in color and dark green, while the flowers are white to purple. This is due to the variety[1]. This type is used in food and is used in the pharmaceutical and economic industries. Other varieties to which it belongs are red basil, purple *Ocimum basilicum* L. Lemon basil (*Ocimum basilicum* "citriodorum") and dark opal basil (*Ocimum basilicum* "purpurascens). Basil is grown as a leafy crop for fresh food consumption or as an

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aromatic plant to extract active compounds from it. Basil varieties contain many active compounds, including the volatile oil that is concentrated in the leaves and consists of 140 compound[2], the most important compounds are Linalool, α-Pimene, Nerol, Limonene and Eugenol, which are antioxidant and antibacterial compounds. It has a medical effect in treating cancer and preventing blood clots. It is used to preserve foods and nutrients such as meat and fish. It works as a treatment for asthma, headaches, fever, coughs, and colds, and stimulates the immune system[3]. The plant is classified within a group of Antiseptic Plants[4].

The process of inoculation with mycorrhizae known as Plant Growth Promoting Fungi (PGPF) is one of the most successful technologies for creating a significant effect and increasing the growth and content of active compounds in medicinal plants[5] because of its effective role in providing the plant with organic acids, nutrients, and plant hormones[6] , as well as improving the properties and texture of the soil and raising the efficiency of the plant through symbiotic living between fungus and Plants[7] . In recent decades, there has been excessive and frequent use of chemical fertilizers by farmers, which has increased yields. On the other hand, it causes an accumulation of elements harmful to human health, such as nitrates and nitrous amine compounds[8], in addition to Losing large quantities of them through washing or volatilization.

Since the middle of the last century, researchers have tended to find conditional, slow-release alternatives[9]. The nanotechnology revolution appeared, as the nanoparticles were so small that they penetrated the cell membrane of the plant easily and slowly with increasing surface area. Thus, the high consumption of traditional fertilizers was controlled through legalization of their quantities and the use of Nano-fertilizers to increase yields and improve quality[10].

Scientific studies have shown that there is a significant effect of mycorrhizae inoculum and Nano fertilizer in increasing growth and production of active compounds[3]. demonstrated that mycorrhizae inoculum achieved significant superiority in growth and vegetative traits and the production of active compounds in basil plants.

The results of a study on the peppermint plant conducted by[11] confirmed Treating the plant with mycorrhizae inoculum and Nano fertilizer showed a significant effect on growth characteristics and the production of active compounds. In a study on chamomile plants[12] confirmed that the treatment with mycorrhizae inoculum was significantly superior and recorded the highest values in indicators of vegetative and root growth and volatile oil production.

Due to the nutritional and medicinal importance of the basil plant, the study aimed to:

The possibility of increasing growth by finding the best combination of mycorrhizae vaccine and NPK Nano fertilizer to give the best results in vegetative growth, yield, and medically effective compounds in basil plants.

Materials and Methods

The experiment was carried out in the botanical garden belonging to the College of Education for Pure Sciences Ibn al-Haytham / University of Baghdad for the summer agricultural season 2023, in order to study the effect of mycorrhizae vaccine and NPK Nano fertilizer and the interaction between them on the growth, yield and production of volatile oil of the basil plant (Local). The field was divided into panels with dimensions of 1.5 x 1.5 m. The area of the experimental unit was 3 square meters, leaving a distance of 20 cm from the edges for isolation. Seeds were planted with 4 seeds in each hole. The planting distance was 20 cm and on the lines, the distance between one line and another was 20 cm. The experimental unit included 25 plants after the plants were thinned after germination and growth and the best seedlings were selected. They grew, using a wooden frame measuring 40X40 cm[13]. Samples of field soil were collected at different depths and analyzed to determine the physical and chemical characteristics according to the methods contained in Page *et al*. [14] (Table 1).

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Table 1. Some physical and chemical properties of field soil.

Properties	Value	Units
PH	7.43	
ECe	0.53	dS m^{-1}
O.M	0.89	gm kg^{-1}
N	11.50	mg kg^{-1}
P	3.95	mg kg^{-1}
K	25.10	mg kg^{-1}
Mg^{++}	0.49	mmol. L^{-1}
Na^{+}	2.23	
Ca^{++}	1.72	
HCO_3	0.64	
Cl	4.73	
CO_3	Nill	
Silt	260.0	g kg^{-1}
Clay	72.0	
Sand	664.0	
Texture		Loamy sand

The seeds were planted on 1/4/ 2023 in lines inside the panels. Seed germination began on 5/4/ 2023. The experiment was carried out according to Randomized Complete Block Design (RCBD). The 8 treatments were randomly distributed with three replicates, so that the number of experimental units was 24 units and were compared. Averages according to the least significant difference (LSD) test at the 5% probability level.

Study factors

The first factor used the mycorrhizae vaccine (*Glomus mosseae*) and included two levels of the measurement treatment, coded M_1 without any additives. Seed inoculation treatment. The vaccine treatments are coded M_2 when planting. 25 gm $plant^{-1}$ of mycorrhizae inoculum was added to the place prepared for planting according to the treatment and mixed well with the seeds. The mycorrhizae inoculum loaded on peat moss was obtained. The number of spores was 50 spores' gm^{-1} soil from the Department of Science and Technology. It was returned to the Ministry of Higher Education and Scientific Research, then it was watered immediately after planting with a manual sprinkler

The second factor, Nano fertilizer, included four concentrations (0, 0.5, 1, 2) g l^{-1}. The nutrient solutions were prepared according to the concentrations and were sprayed for three times, the first spraying after the first 4 leaves appeared, then after 14 days after the first treatment, then two weeks after the second treatment, until complete wetness. Adding half the fertilizer recommendation of NPK fertilizer (20,20,20) to all treatments before planting.

Measured characteristics

1- Plant height cm $plant^{-1}$

2- Number of leaves leaf $plant^{-1}$

3- Dry weight of one plant gm $plant^{-1}$

4- Estimating the percentage of chlorophyll in leaves (spad unite)

5- Estimating the percentage of roots infected with mycorrhizae (%)

6-Total yield per hectare (ton $hectare^{-1}$)

7-Oil percentage (%).

Percentage of volatile oil %:The oil was extracted using the Water distillation method in the way reported by Hcini et al. [15] The percentage of volatile oil was estimated according to the equation reported by Guenther [16]

Percentage of volatile oil

= weight of the resulting oil (g) / weight of plant sample (g) × 100

Results and Discussion

The effect of study factors on the height of basil plants

The results showed significant superiority of the plant height characteristic for the basil seed mycorrhizae bio inoculum treatment, 25 g $plant^{-1}$, as it recorded 51.17 compared to the control treatment, which recorded 36.83 cm $plant^{-1}$, and the 2 g l^{-1} Nano fertilizer treatment recorded 47.17, and did not differ significantly from the 1 g l^{-1} treatment,

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which recorded 46.67 compared to with the control treatment, which recorded 39.50 cm plant^{-1}

In the two-way interaction between the study factors, the treatment of mycorrhizae 25 g plant^{-1} with Nano-fertilizer 1 g l^{-1} recorded the highest average 55.67 cm plant $^{-1}$ compared to the control treatment, which recorded 32.00 cm plant $^{-1}$ (Table 2).

Table 2. The effect of Bio and Nano fertilizer on high of Basil Plant cm plant^{-1}

Mycorrhizae	Nano fertilizer(g l^{-1})				
	0	(0.5)	(1)	(2)	Average Mycorrhizae
0	32.00	35.00	37.67	42.67	36.83
(25g)	47.00	50.33	55.67	51.67	51.17
Average Nano	39.50	42.67	46.67	47.17	
L.S.D	4.382				3.099
	6.197				

The effect of study factors on the number of leaves

The results of the study showed that the mycorrhizae inoculum treatment for basil seeds, 25 g plant^{-1}, was superior, recording 44.83, compared to the control treatment, which recorded 35.00 leaves plant $^{-1}$. In the Nano-fertilizer treatments, the 2 and 1 gl^{-1} treatments were superior, recording 42.67 and 42.50, respectively, as they did not differ significantly between them in comparison. With the control treatment, which recorded 36.67 leaf per plant

In the two-way interaction between the study factors, the treatment of mycorrhizae 25 g with Nano-fertilizer 1g l^{-1} recorded the highest average number of leaves, reaching 49.33 compared to the control treatment, which recorded 31.33 leaf plant^{-1} (Table 3).

Table 3. The effect of Bio and Nano fertilizer on number of leaves of Basil Plant (leaf plant^{-1})"

Mycorrhizae	Nano fertilizer (g l^{-1})				
	0	(0.5)	(1)	(2)	Average Mycorrhizae
0	31.33	33.33	35.67	39.67	35.00
(25gm)	42.00	42.33	49.33	45.67	44.83
Average Nano	36.67	37.83	42.50	42.67	
L.S.D	1.851				1.851
	3.703				

The effect of study factors on dry weight (g plant^{-1})

The results of the study showed that the mycorrhizae inoculum treatment of 25 g was superior, as it recorded 11.04 compared to the control treatment, which recorded 7.03 g. As for the Nano-fertilizer treatments, there were no significant differences between the 1and 2 g 1^{-1} treatments, as they recorded 10.18 and 10.25, respectively, compared with the control treatment, which recorded 7.35 g plant^{-1}

The highest dry weight value was in the double-crossing treatment mycorrhizae 25 g with Nano-fertilizer 1g l^{-1}, which reached 12.70 g, compared to the lowest value in the control treatment, which amounted to 5.63 g plant^{-1} (Table 4).

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Table 4. The effect of Bio and Nano fertilizer on dry weight of Basil (g plant^{-1})

Mycorrhizae	Nano fertilizer (gl^{-1}) 0	(0.5)	(1)	(2)	Average Mycorrhizae
0	5.63	6.70	7.67	8.13	7.03
(25 g)	9.07	10.03	12.70	12.37	11.04
Average Nano	7.35	8.37	10.18	10.25	0.501
L.S.D	0.708 1.002				

The effect of study factors on the chlorophyll content of plant leaves. (SPAD) unite

Through the results of the study, it was found that the mycorrhizae inoculum treatment of 25 g excelled and recorded 39.27 compared to the control treatment, which recorded 30.94 SPAD, and in Nano-fertilizer treatments, the 2 and 1 g l^{-1} treatment excelled and recorded 37.37 and 37.63, respectively, which did not differ between them. Significantly, compared to the control treatment, which recorded 31.60 Spad.

The dual interaction treatment (mycorrhizae 25 g plant^{-1} with Nano-fertilizer 1 g l^{-1}) showed significant superiority and recorded the highest value, reaching 41.77, compared to the control treatment, which recorded the lowest value, amounting to 26.80 spad (Table 5).

Table 5. The effect of Bio and Nano fertilizer on content leaves of chlorophyll of Basil SPAD .Unit

Mycorrhizae	Nano fertilizer(g l^{-1}) 0	(0.5)	(1)	(2)	Average Mycorrhizae
0	26.80	29.00	32.97	35.00	30.94
(25gm)	36.40	38.63	41.77	40.27	39.27
Average Nano	31.60	33.82	37.37	37.63	
L.S.D	0.826 1.168				0.584

The effect of study factors on the percentage of roots infected with mycorrhizae (%)

The 25 gm plant^{-1} Mycorrhizae inoculum treatment excelled, recording the highest infection rate, reaching 44.83 %, compared to the control treatment, which did not record any infection. As for the Nano-fertilizer treatments, the 1 gm l^{-1} treatment excelled, recording 32.50 %, compared to the control treatment, which recorded 12.17 %. The results of the research were based on the superiority of the dual interaction treatment mycorrhizae 25 gm with Nano-fertilizer 1 g l^{-1} significantly in recording the highest infection rate, reaching 65.00 %, compared to the lowest value in the control treatment, which reached 0.00 (Table 6).

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Table 6. The effect of Bio and Nano fertilizer on Percentage of roots infected with mycorrhizae of Basil %

Mycorrhizae	Nano fertilizer(g l^{-1}) 0	(0.5)	(1)	(2)	Average Mycorrhizae
0	0.00	0.00	0.00	0.00	0.00
(25gm)	24.33	35.00	65.00	55.00	44.83
Average Nano	12.17	17.50	32.50	27.50	
L.S.D	3.488 4.933				2.466

The effect of study factors on the amount of yield (ton ha^{-1})

The results of the study showed that the mycorrhizae inoculum treatment of 25gm excelled and recorded 5.94 compared to the control treatment which recorded 3.25 tons' ha^{-1}. As for the Nano-fertilizer treatments, the 1 gm l^{-1} treatment excelled and recorded 5.21 compared to the control treatment which recorded 3.77-ton ha^{-1}, It was significantly superior to the dual interaction treatment (mycorrhizae 25 g $plant^{-1}$ with Nano-fertilizer 1 g l^{-1}) and recorded the highest value for the yield per hectare, which amounted to 6.83, compared to the lowest value recorded in the control treatment, which amounted to 2.55-ton ha^{-1} (Table 7).

Table7. The effect of Bio and Nano fertilizer on biological yield of Basil (ton ha^{-1})

Mycorrhizae	Nano fertilizer(g l^{-1}) 0	(0.5)	(1)	(2)	Average Mycorrhizae
0	2.55	3.14	3.58	3.74	3.25
(25gm)	4.99	5.82	6.83	6.12	5.94
Average Nano	3.77	4.48	5.21	4.93	
L.S.D	0.079 0.112				0.056

The effect of study factors on the percentage of volatile oil (%)

The results of the research showed that the mycorrhizae inoculum treatment of 25 gm was superior, recording 1.319 compared to the control treatment, which recorded 0.882 %. As for Nano-fertilizer treatments, the 1 g l^{-1} treatment was superior, recording 1.328 %, compared to the control treatment, which recorded 0.885%.

In the binary interaction treatments, the treatment (mycorrhizae 25 g $plant^{-1}$ with Nano-fertilizer 1 g l^{-1}) was significantly superior, and the highest percentage was recorded at 1.660 %, compared to the lowest percentage at 0.650 % in the control treatment (Table 8).

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Baghdad Science Journal

Table8. The effect of Bio and Nano fertilizer on Volatile oil percentage of Basil (%)

Mycorrhizae	Nano fertilizer(g l^{-1}) 0	(0.5)	(1)	(2)	Average Mycorrhizae
0	0.650	0.800	0.990	1.083	0.882
(25 gm)	1.120	1.180	1.660	1.316	1.319
Average Nano	0.885	0.990	1.328	1.200	
L.S.D	0.01483 0.02098				0.01049

From observing the results of the previous tables, we conclude that pollinating basil seeds with mycorrhizae vaccine achieved an increase in vegetative growth indicators represented in plant height (Table 2), the number of leaves (Table 3), dry weight (Table 4), and the percentage of chlorophyll in the leaves (Table 5), which may be attributed to their role. It is effective in increasing the readiness of macro- and micro-nutrients in the soil through its chelation of these elements, as it is one of the siderophores[17], that work to meet part of the plant's growth needs, in addition to its role in secreting many organic compounds and plant hormones such as gibberellins, auxins, and cytokinins Which contributes to cell division, elongation, and differentiation of meristematic cells[18]. The physiological and metabolic activity of the plant is affected by the activity of microscopic organisms in the soil, including mycorrhizae fungi, and their role in increasing the readiness of phosphorus in the soil solution and the production of organic acids such as lactic and oxalic acid[19] it provides a suitable environment for the growth of roots in the rhizosphere region and encourages their colonization by[12], which was evident in the increase in the infection rate (Table 6). In the roots of pollinated plants. Increasing the height of the plant, the number of leaves, and the percentage of chlorophyll in the leaves as a result of the role of mycorrhizae in providing nutrients and stimulating their absorption by the plant by facilitating their transfer to the root system and stimulating the process of photosynthesis[20], was reflected positively in increasing the dry weight of the plant and thus increasing the yield per hectare (Table 7). The active compounds represented by the volatile oil (Table 8) in plants inoculated with mycorrhizae fungi compared to those not inoculated between the success of the symbiotic relationship between the basil plant and mycorrhizae fungi. This may be attributed to the activity of the bio-vaccine and its mechanism of action in increasing the readiness of elements in the soil and improving carbon metabolism, the formation of organic compounds such as carbohydrates and filling the plant's need for nutrients, which contributed to the increase in the production of secondary compounds through the transfer of metabolic compounds from the source to the outfall[21].

It is noted from Tables (2, 3, 4, and 5) that there is a significant superiority in the interaction coefficients between the bio fertilizer and the Nano fertilizer. This may be attributed to the effectiveness of the nanoparticles in increasing the biological activity within the plant[22] , which reflects positively on the vegetative indicators and the yield, as the nanoparticles are so small that they penetrate easily and slowly into Cell membranes[23] , which contributed to increasing division and elongation[24] as the nitrogen element is involved in the synthesis of amino acids and the porphyrin ring[25] which is the basis for the formation of the chlorophyll molecule in the plant leaf, which works to increase the effectiveness of photosynthesis[24], and the reason for the increase in vegetative indicators may be attributed to the effect of the element phosphorus, which contributes to building cell membranes[26]. It is also involved in the synthesis of DNA and RNA and increases the efficiency of roots in actively absorbing water and nutrients and producing energy compounds[27] , while Potassium activates a number of synthetic enzymes, oxidation and reduction

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enzymes, hydrogen addition enzymes (hydrogenases), transferases[28] and energy release enzymes (kinases), in addition to its role in activating more than 120 enzymes within the plant[29], and it has an effective role in the mechanics of opening and closing stomata and regulating The osmotic potential of the plant, as well as its role in transporting the products of the photosynthesis process from the leaves as a source to their storage places, thus increasing growth indicators[30] The reason for the increase in volatile oil in the leaves of plants treated with nanoparticles may be due to its effect on increasing the percentage of chlorophyll in the leaves (Table 5), which may lead to an increase in carbohydrates that may control the construction, storage, and catabolism of monoterpenes[31]. The reason may be due to the role of the products of photosynthesis provide energy and the carbon skeleton to build terpene compounds thus increasing the percentage of volatile oil in the leaves.

Conclusion

Mycorrhizae inoculum at a level of 25 gm had a clear effect on improving vegetative growth characteristics and volatile oil production. The interaction treatment between mycorrhizae and Nano-fertilizer achieved the best results at a concentration of 1 g l^{-1}, which confirms the response of basil seeds to inoculation with mycorrhizae and Nano-fertilizers.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of write the name of the university or center of which you received the approval.

Authors' Contribution Statement

A.R.A.H., H .A.M.A.T. and S.D.A.A. T. Contributed to the design and implementation of the research, to the analysis of the results and to the writing and translation of the manuscript.

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تأثير التسميد الحيوي والنانوي في نمو وحاصل نبات الريحان ومحتواه من المركبات الفعالة

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الخلاصة

اجريت تجربة عاملية في كلية التربية للعلوم الصرفة ابن الهيثم / جامعة بغداد لموسم النمو 2023 بغية دراسة تأثير السماد الحيوي المتمثل بلقاح المايكورايزا والسماد النانوي NPK في بعض مؤشرات النمو الخضري والجذري والحاصل وانتاج الزيت الطيار لنبات الريحان *Ocimum basilicum L* ، اشتملت التجربة عاملين العامل الاول لقاح المايكورايزا بمستويين هما 0 (بدون اضافة) و اضافة 25 غم ، اذ تم تلقيح البذور عند الزراعة والعامل الثاني رش المجموع الخضري للنبات بالسماد النانوي NPK باربعة مستويات (0 ، 0.5 ، 1 ، 1.5) غم لتر $^{-1}$. اظهرت النتائج تاثيرا معنويا لمعاملة تلقيح بذور الريحان بالمايكورايزا عند مستوى 25 غم نبات$^{-1}$ في اعطائها اعلى القيم في ارتفاع النبات بلغت 51.17 سم نبات$^{-1}$ وعدد الاوراق 44.83 ورقة نبات$^{-1}$ وحاصل الهكتار 5.94 طن هكتار$^{-1}$ ونسبة الزيت الطيار في الاوراق 1.319 %. في التداخلات بين عوامل الدراسة تفوقت معاملة لقاح المايكورايزا 25 غم نبات$^{-1}$ مع السماد النانو 1غم لتر$^{-1}$ معنويا وسجلت اعلى القيم في ارتفاع النبات وعدد الاوراق وحاصل الهكتارونسبة الزيت الطيار في الاوراق اذ بلغت 55.67 سم نبات$^{-1}$ و 49.33 ورقة نبات$^{-1}$ و 6.83 طن هكتار$^{-1}$ و 1.660 % على التتابع مقارنة بمعاملة السيطرة .

الكلمات المفتاحية: نبات الريحان، مايكورايزا، نباتات طبية، سماد نانوي، الزيت الطيار.