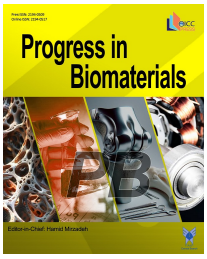

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Evaluation of Effects of Mycorrhizal fungi and Azotobacter Bacteria on Growth, Quantity, Quality, and Antimicrobial Properties of Essential Oil of Fennel plant *Foeniculum vulgare* Mill

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Original Research

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Abstract:

The combined effects of mycorrhizal fungi and Azotobacter bacteria alongside NPK fertilizer on growth, yield, essential oil quality, and antimicrobial properties of *Foeniculum vulgare* (fennel) were evaluated. Seed inoculation with biofertilizers and chemical fertilizer applications under controlled greenhouse conditions significantly enhanced plant height (112.5 ± 2.9 cm), umbels per plant (39 ± 3.6), thousand-seed weight (3.95 ± 0.21 g), biological yield (4245 ± 230 kg/ha), seed yield (1544 ± 228 kg/ha), and harvest index ($36.3 \pm 3.3\%$) compared to the control ($p < 0.01$). Essential oils extracted from treated plants demonstrated improved antimicrobial efficacy, with minimum inhibitory concentrations (MIC) against *Aspergillus niger* reduced by 50% to 62.5 µg/mL. Similar reductions in MIC values were observed for *Penicillium digitatum*, *Escherichia coli*, and *Staphylococcus aureus*. Minimum fungicidal and bactericidal concentrations (MFC/MBC) also decreased substantially with combined treatments. Results confirm that integrating biofertilizers enhances fennel growth, yield, and the antimicrobial potency of its essential oil, providing a sustainable alternative to chemical fertilizers in medicinal plant cultivation.

Keywords: Biofertilizers; Mycorrhiza; *Azotobacter chroococcum*; *Foeniculum vulgare*; NPK fertilizer

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1. Introduction

In recent decades, heavy reliance on chemical inputs in agriculture has caused significant environmental problems such as soil degradation, water pollution, and biodiversity loss [1]. Ecological agriculture offers a sustainable alternative by emphasizing long-term principles within cropping systems. This approach integrates crop rotation with legumes, use of plant residues, livestock manure, and the application of organic and biological fertilizers instead of synthetic chemicals. These practices not only improve soil nutrient reserves but also enhance weed and pest control, increase biodiversity, and stabilize crop yields [2–4].

A key strategy in ecological agriculture is the use of biofertilizers. These contain beneficial soil microorganisms that supply nutrients and promote plant growth, providing a viable alternative to chemical fertilizers [5]. Biofertilizers include organic materials like animal manure and plant residues, as well as bacteria and fungi involved in nitrogen fixation, phosphorus solubilization, and other nutrient cycling processes. Among the most important beneficial microbes are nitrogen-fixing bacteria, mycorrhizal fungi, and plant growth-promoting bacteria [6, 7].

Within the realm of medicinal plant cultivation, a global trend emphasizes ecological agriculture combined with microbial inoculation techniques, such as those using Azotobacter bacteria and mycorrhizal fungi [8]. Fennel (*Foeniculum vulgare*), a widely used medicinal plant, is notable for its seed essential oil, which is extensively applied in pharmaceutical, food, cosmetic, and hygiene industries [9–11]. The principal bioactive component, anethole, can constitute up to 80% of fennel essential oil, while estragole and other compounds like fenchone and limonene make up smaller but important proportions [12–14]. Essential oil yield from fennel seeds varies between 2% and 4% (v/w), influenced by cultivation and processing conditions [14, 15].

Traditionally, fennel has been used to treat more than 40 ailments, with its efficacy in alleviating digestive and respiratory disorders supported by clinical data showing symptom relief in up to 75% of cases [11, 14]. Fennel is a member of the Apiaceae family found in both wild and cultivated forms across various climates. Wild fennel is perennial, but cultivation usually results in a biennial lifecycle. Its tuberous, aromatic roots and distinct cylindrical, grooved stems support its adaptation to Mediterranean-origin environments. Optimal growth occurs at soil pH between 4.8 and 8 and temperatures of 20 to 22 °C, with irrigation and balanced nutrition being critical for yield and essential oil quality [12, 16, 17].

Mycorrhizal fungi form one of the most important mutualistic root associations in nature. They constitute a large portion of soil microorganisms and facilitate nutrient uptake by extending hyphal networks beyond the nutrient depletion zones around roots. Mycorrhizae are especially effective in enhancing phosphorus and micronutrient absorption. These fungi acquire carbohydrates from host plant photosynthesis, creating a reciprocal nutrient exchange [18, 19].

Mycorrhizal fungi also improve soil structure via glomalin production, which stabilizes aggregates and promotes root growth [18–21]. Depending on morphology and host specificity, mycorrhizal fungi are categorized into groups such as vesicular-arbuscular, ectomycorrhiza, and ericoid types [18]. Their widespread presence enhances plant resilience to abiotic stresses and diseases and contributes significant ecological and economic value by maintaining soil health [18, 19].

Azotobacter, a key free-living nitrogen-fixing bacterium, inhabits diverse ecosystems worldwide. *Azotobacter chroococcum* is notable for its pleomorphic, Gram-negative, and motile nature. This bacterium fixes atmospheric nitrogen non-symbiotically at rates between 20 and 40 kg N/ha/year, thereby enriching soil nitrogen directly [22]. Azotobacter also produces plant growth hormones, including indole-3-acetic acid (IAA) at concentrations of 1–10 µg/ml, gibberellin, cytokinin, and ethylene, stimulating root elongation, nutrient uptake, and enhanced plant growth [22]. Additionally, its exopolysaccharides improve soil aggregation and increase water retention, benefiting plants under drought stress [22, 23].

While beneficial, Azotobacter's nitrogen-fixation efficiency is generally lower than symbiotic bacteria such as Rhizobium. Moreover, its free-living growth depends on the availability of simple carbon sources and suitable soil organic matter, limiting its activity in some agricultural soils [22]. Given the environmental costs of chemical fertilizers and nitrogen losses through volatilization and leaching, Azotobacter represents an important biological alternative for sustainable nitrogen supply and plant growth promotion [24].

This study aims to investigate the combined effects of mycorrhizal fungi and Azotobacter bacteria on the growth, yield, quality, and antimicrobial properties of essential oil from *Foeniculum vulgare*. Specifically, it assesses how these biofertilizers influence essential oil composition and enhance biologically important traits, supporting the development of sustainable medicinal plant cultivation and production of high-value, antimicrobial natural products.

2. Materials and methods

Soil preparation

An equal mixture of leaf mold, clay, and sand was prepared and sterilized using an autoclave at 121 °C for 15 minutes. Soil texture and chemical properties were analyzed by the Soil and Water Research Institute. The sterilized soil was filled to a 20 cm depth in 24 fruit boxes ($50 \times 30 \times 30$ cm^3), each lined with plastic and equipped with drainage to prevent waterlogging and soil loss.

Preparation of materials

Fennel seeds with a purity of 93% and a germination rate of 80% were obtained from Pakan Bazr Isfahan Company. *Azotobacter chroococcum* (IBRC–M 10787) was sourced from the Iranian Biological Resource Center. Mycorrhizal fungus (inoculable liquid form) was acquired from the Soil and Water Research Institute. Commercial NPK fertilizer[1] (20-20-20) was purchased from a certified supplier.

Sample preparation

The Azotobacter sample was first cultured in a specific medium containing $CaCO_3$, NaCl, K_2HPO_4, $CaSO_4$, $MgSO_4 \cdot 7H_2O$, and mannitol. The culture was streaked, sealed with parafilm, and incubated at 30 °C for 48 hours. Subsequently, an Azotobacter suspension was prepared according to the 0.5 McFarland standard (1.5×10^8 CFU/mL) in two 500 mL Erlenmeyer flasks.

Planting method and greenhouse conditions

The experiment consisted of four treatment groups, each with three replicates ($n = 3$): Simple planting (control), planting with mycorrhiza, planting with Azotobacter, and planting with both mycorrhiza and Azotobacter. Seedlings were grown in a controlled greenhouse with ambient temperature maintained at 22 ± 2 °C, relative humidity around $60 - 70\%$, and light intensity approximately 400 µmol/m^2·s. Planting occurred on April 30th, with the following protocols:

1. Simple planting: Seeds sown thinly, covered with manure, and irrigated. Three replicates received NPK fertilization (1 g/L) at the three-leaf stage.

[1] Nitrogen-Phosphorus-Potassium fertilizer

2. With mycorrhiza: Soil inoculated with 70 mL of mycorrhizal liquid per box. Seeds were soaked in mycorrhizal solution until swollen, then sown and irrigated. Three replicates received NPK similarly.

3. With Azotobacter: Soil treated with 70 mL of Azotobacter suspension before sowing. Seeds sown and covered with manure, irrigated. Three replicates received NPK.

4. With both mycorrhiza and Azotobacter: Mycorrhiza-inoculated seeds sown in soil treated with 70 mL of Azotobacter suspension, covered with manure, and irrigated. Three replicates received NPK.

After sowing, irrigation was daily until germination, then every other day. Growth parameters (plant height, umbels per plant, thousand-seed weight, biological yield, grain yield, harvest index) were recorded upon full plant development.

Steam distillation extraction of essential oil

First, samples were taken from harvested fennel seeds. The samples were washed, dried in the shade, and then ground. To prevent contamination of the seeds, all procedures were carried out under sterile conditions inside a microbiological hood. For essential oil extraction, 100 grams of the ground sample were placed into a Clevenger apparatus, and 500 mL of distilled water was added. After 4 hours of distillation, the obtained essential oil was collected into microtubes and wrapped in aluminum foil to protect it from light exposure. The extracted oils were dried over anhydrous sodium sulfate and stored at 4 °C until further use [3].

Investigation of the Antimicrobial properties of essential oil

Microbial strains and culture conditions

The filamentous saprophytic fungi *Aspergillus niger* (PTCC 5224) and *Penicillium digitatum* (PTCC 5251) were obtained from the Iranian Industrial Microorganism Collection Center. The bacterial strains *Escherichia coli* (PTCC 1860) and *Staphylococcus aureus* (PTCC 1189) were procured from the Iranian Research Organization for Science and Technology. Fungal samples were cultured on Potato Dextrose Agar (PDA), while bacterial samples were cultured on Eosin Methylene Blue (EMB) agar and Mannitol Salt Agar, respectively.

Preparation of stock solutions

Essential oil stock preparation

Dimethyl sulfoxide (DMSO) was used as the solvent for the essential oil. To prepare the stock solution, 400 mg of the essential oil was dissolved in 1 mL of DMSO in a cryovial. Then, 1980 µL of RPMI medium was added to a Falcon tube, followed by 20 µL of the essential oil stock solution (400 mg/mL), resulting in a final concentration of 4000 µg/mL. This solution was subsequently diluted in the wells of a 96-well plate to achieve a working concentration of 2000 µg/mL.

Fungal stock preparation

To prepare the spore suspension, fungi were cultured in sterile tubes containing PDA supplemented with chloramphenicol and incubated at 28 °C for 7 days. Physiological saline was prepared by dissolving 0.9 g of sodium chloride in 1 liter of distilled water, with the addition of one drop of Tween 80 per 100 mL. After 5 days, once sporulation was complete, sterile physiological saline with Tween 80 was added to the fungal culture, and the surface of the medium was gently scraped. The spore suspension was collected by passing it through two layers of sterile gauze and then counted.
Under a laminar flow hood, after thoroughly mixing the suspension, one drop was placed between a slide and a cover slip and examined under a 40× objective to count spores per milliliter. Using the formula M1V1 = M2V2 and RPMI medium, fungal stocks were prepared. Here:
M1 = initial spore concentration (total conidia per ml of suspension), approximately 5×10^6
V1 = volume of spore suspension to be diluted
M2 = desired final concentration, 5×10^4 (as per CLSI standards)
V2 = final volume, calculated as number of wells ×100 µL, i.e., 9600 µL

Bacterial stock preparation

A loopful of bacterial colony was inoculated into the nutrient broth 24 hours before each assay to prepare fresh cultures. For each test, a fresh 24-hour culture was prepared similarly. Using a pipette, 1 mL of the 24-hour bacterial suspension was transferred into a sterile nutrient broth tube. The turbidity of the bacterial suspension was adjusted visually against a McFarland standard (equivalent to approximately 1.5×10^8 CFU/mL) using a spectrophotometer at 625 nm wavelength, with an absorbance range of $0.08 - 0.13$.

Preparation of Solvent Stocks and Drugs

To prepare the solvent stock, 200 µL of solvent was drawn using a sampler, followed by the addition of 1800 µL of RPMI medium. Standard drug stocks were prepared at specific concentrations: Amphotericin B at 16 µg/mL for fungi, Erythromycin at 1 µg/mL for *Staphylococcus aureus*, and Ciprofloxacin at 1 µg/mL for *Escherichia coli*. Additionally, 70% ethanol was used for bacterial resistance testing in the presence of ethanol.

Investigating the antimicrobial properties of essential oils

Determination of MIC[2] and MFC[3]/MBC[4] by microbroth dilution method
The assay was performed in 96-well microplates, with each plate dedicated to a single microbial strain. Control columns included: Positive control (microbe + culture medium), Solvent control (solvent + microbe + medium), Drug control

[2]Minimum Inhibitory Concentration
[3]Minimum Fungicidal Concentration
[4]Minimum Bactericidal Concentration

(standard drug + microbe + medium), and Negative control (culture medium only).
The fifth column contained the highest concentration of the test substance. Except for the negative control wells, 100 µL of microbial stock suspension was added to all wells. Plates were sealed with parafilm and incubated for 24 hours for bacteria and 48 hours for fungi at 37 °C and 35 °C, respectively. After incubation, absorbance readings were taken at 625 nm for bacteria and 530 nm for fungi using a microplate reader. The positive and negative controls were examined to ensure assay validity. The MIC was defined as the lowest concentration showing no visible growth (clear well) when moving from higher to lower concentrations.
To determine the MFC or MBC, aliquots from wells showing no growth (MIC and higher dilutions) were plated onto solid media, PDA for fungi and Mueller-Hinton agar for bacteria. After further incubation, the lowest concentration that prevented microbial growth was recorded as the MFC for fungi or MBC for bacteria [25].

Statistical methods

Results are presented as the mean ± standard deviation. Statistical analyses, including the one-way analysis of variance (ANOVA), were conducted using SPSS (Version 26). Significance was accepted at the 1% level. The number of biological replicates (n = 3) was included to ensure statistical validity.

3. Results

Physical and Chemical Properties of the Soil

The physical and chemical properties of the soil, which is a mixture of leaf mold, clay, and sand, were determined by the Soil and Water Research Institute and are presented in Table 1. These characteristics indicate that the soil used is alkaline, moderately light in texture, and suitable for cultivating fennel plants.

Plant height

Based on the results of the analysis of variance, it was determined that the experimental treatments had a statistically significant effect on this trait at the 1% level. According to the mean comparison results, the highest average plant height, measuring 112 centimeters, was observed in the treatment combining NPK with Azotobacter and mycorrhiza. This represents a 22% increase compared to the control group and is statistically grouped with the treatment of NPK combined with mycorrhiza. The individual application of NPK, mycorrhiza, and Azotobacter treatments resulted in increases of 13%, 11%, and 9%, respectively, compared to the control. A comparison of the effects of the treatments on average plant height is presented in figure 1 a.

Number of umbels per plant

Based on the results of the analysis of variance, the experimental treatments had a statistically significant effect on this trait at the 1% level. This trait was influenced by the treatments, with the highest average number of umbels per plant (39) observed in the treatment combining NPK fertilizer with Azotobacter and mycorrhiza. The lowest average number (22) was recorded in the control treatment. Application of NPK, mycorrhiza, and Azotobacter alone resulted in increases of 36%, 18%, and 13%, respectively, compared to the control. A comparison of the effects of treatments on the average number of umbels per plant is shown in figure 1 b.

Thousand-grain weight

The analysis of variance results indicated that the experimental treatments had a statistically significant effect on thousand-grain weight at the 1% level. Regarding the thousand-grain weight, the treatments NPK, mycorrhiza, Azotobacter, and the combined mycorrhiza + Azotobacter treatment grouped statistically with values of 1.3, 2.9, 2.9, and 1.3 grams, respectively. The highest average thousand-grain weight, 3.9 grams, was observed in the treatment combining NPK with Azotobacter and mycorrhiza. A comparison of the effects of treatments on the average thousand-grain weight is presented in figure 1 c.

Biological yield

Based on the results of the analysis of variance, it was determined that the experimental treatments had a statistically significant effect on this trait at the 1% level. The highest and lowest mean biological yields, measured as the total plant weight harvested per hectare, were 4,245 kg/ha and 2,786 kg/ha, respectively. These corresponded to the treatments of NPK combined with Azotobacter and mycorrhiza, and the control treatment. Application of NPK, mycorrhiza, and Azotobacter individually resulted in increases of 27%, 11%, and 14% in biological yield compared to the control. A comparison of the treatment effects on mean biological yield is presented in figure 1 d.

Grain yield

The results of the analysis of variance indicated that the experimental treatments had a statistically significant effect

Table 1. Physical and chemical properties of the soil.

Parameter	Value	Parameter	Value	Parameter	Value	Parameter	Value
pH	7.4	N (mg/kg)	8.9	Ca (mg/kg)	0	Mn (mg/kg)	9.9
EC (Ds/m)	0.92	P (mg/kg)	16	Mg (mg/kg)	0	Zn (mg/kg)	0.68
OC (%)	0.7	K (mg/kg)	726	Fe (mg/kg)	7.4	Cu (mg/kg)	2.6

*OC: Organic Content or Organic Carbon, EC: Electrical Conductivity

on grain yield at the 1% level. Application of different treatments significantly increased grain yield, measured as the number of grains harvested per hectare, compared to the control. Specifically, the treatments of NPK fertilizer, mycorrhiza, and Azotobacter alone resulted in yield increases of 65%, 34%, and 29%, respectively, relative to the control. The highest and lowest average grain yields were 1544 kg/ha and 561.7 kg/ha, respectively, corresponding to the combined treatment of NPK with Azotobacter and mycorrhiza, and the control treatment. A comparison of the treatment effects on average grain yield is presented in figure 1 e.

Harvest index

Based on the results of the analysis of variance, the experimental treatments were found to be significant at the 1% level for this trait. Regarding the harvest index, calculated as (grain yield/biological yield) × 100, all treatments except for Azotobacter showed a significant increase compared to the control. The highest and lowest average harvest index values, 36.26% and 20.04% respectively, were observed in the NPK treatment combined with Azotobacter + mycorrhiza and the control treatment. A comparison of the effects of the treatments on the average harvest index is presented in figure 1 f.

Statistical analyses

Based on the results of the analysis of variance, the experimental treatments showed significant effects at the 1% level for the traits examined. The analysis of variance for these traits is presented in Table 2. Additionally, a comparative overview of trait diversity and measures of central

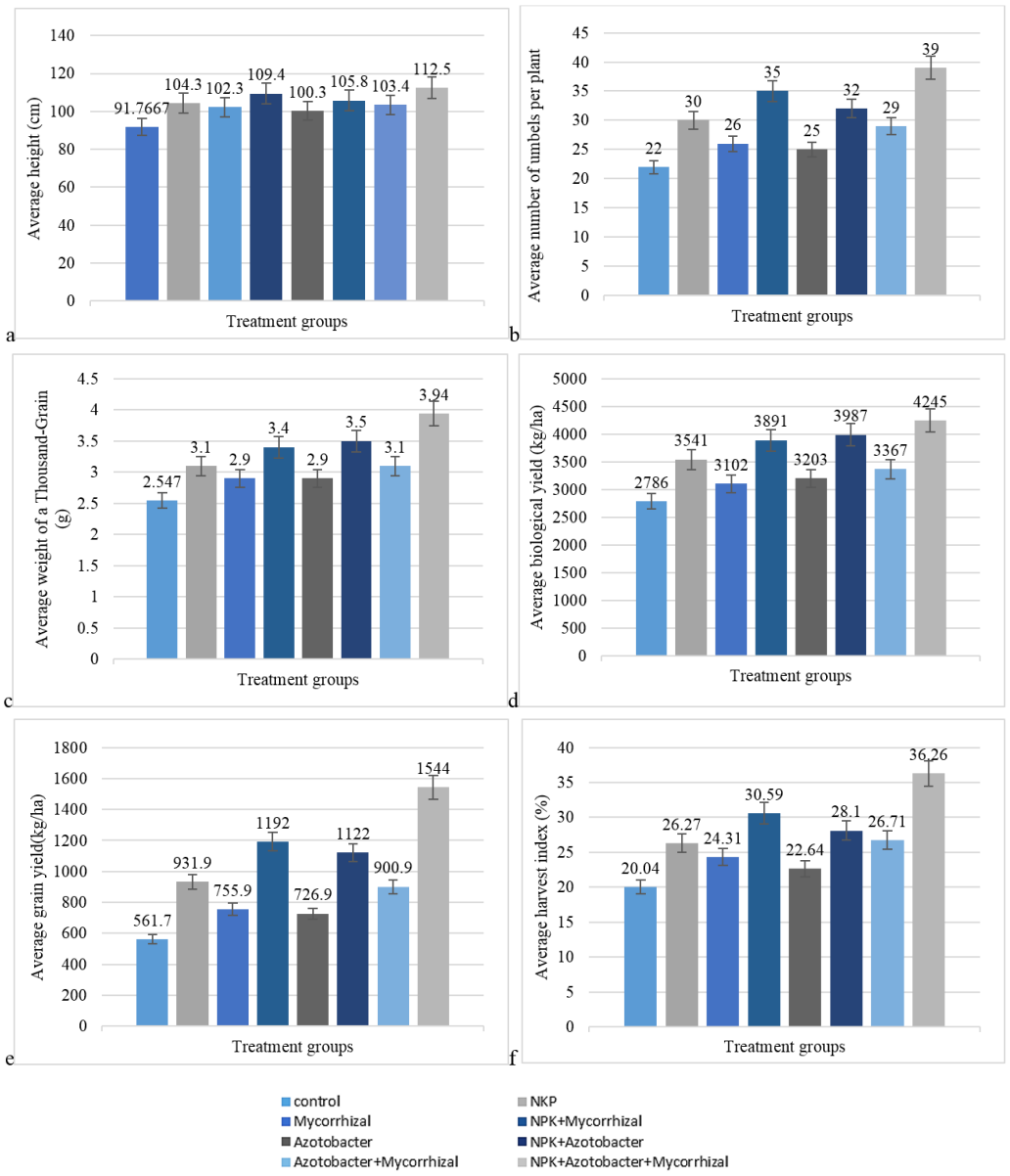

Figure 1. (a) Comparison of treatment means on average plant height, (b) Comparison of the effects of treatments on the average number of umbels per plant, (c) Comparison of the effects of treatments on the average thousand-grain weight, (d) Comparison of the treatment effects on the mean biological yield, (e) A comparison of the treatment effects on average grain yield, (f) A comparison of the effects of the treatments on the average harvest index ($n = 3$, $p < 0.01$).

tendency across all experimental treatments is also provided in Table 3.

Antimicrobial properties of essential oils

MIC Results

The results of the MIC values for the fungi *Aspergillus niger* and *Penicillium digitatum* are presented in figure 2 a and Table 4. The essential oils extracted from samples subjected to biological treatments demonstrated superior antifungal activity compared to the control. Specifically, the MIC for the control group was 125 µg/mL. The lowest MIC observed for *Aspergillus niger* was 62.5 µg/mL, corresponding to the treatment with the plant extract under the NPK + Myco + Azot regime. For *Penicillium digitatum*, the minimum MIC of 125 µg/mL was recorded under both the NPK + Myco + Azot and NPK + Myco treatments. Furthermore, the responses of the two fungi to the inhibitory treatments varied depending on the specific fertilizer treatments applied.

The results of the MIC values for *Escherichia coli* and *Staphylococcus aureus* are presented in figure 2 b and Table 4. At a concentration of 125 µg/mL, all fertilizer treatment groups effectively inhibited the growth of *Escherichia coli*. However, at this same concentration, bacterial growth of *Staphylococcus aureus* was observed in three treatment groups: The control, NPK, and Myco treatments. Overall, essential oils derived from samples subjected to biological treatments demonstrated superior antibacterial activity at 250 µg/mL against the tested bacterial strains. Moreover, the responses of the two bacteria to the inhibitory treatments varied depending on the type of fertilizer applied.

Minimum bactericidal/fungicidal concentration results

The results of the fungicidal concentration of the essential oils against *Aspergillus niger* and *Penicillium digitatum* are presented in figure 2 c and Table 5. As observed, the lowest fungicidal effect was recorded at a concentration of 1000 µg/mL, while the highest fungicidal activity was achieved with the NPK + Myco + Azot treatment against *Aspergillus niger* at a concentration of 125 µg/mL.

The results of the minimum bactericidal concentration of the essential oil against *Escherichia coli* and *Staphylococcus aureus* are presented in figure 2 d and Table 5. The maximum and minimum concentrations required were determined to be 1000 µg/mL and 250 µg/mL, respectively. Notably, the lowest fungicidal concentration of 250 µg/mL was observed for both *Escherichia coli* and *Staphylococcus aureus* in the treatment group NPK + Myco + Azot.

4. Discussion

In recent years, organic agriculture has received significant attention due to its focus on sustainability and reduced environmental impact. Organic farming, characterized by minimal use of chemical inputs, has emerged as a reliable and sustainable production method. Mycorrhizal fungi play a vital role in this context by enhancing plant growth and performance [8]. They increase phosphorus and other nutrient uptake, improve plant resistance to abiotic stresses, and help combat diseases. Plants with mycorrhizal associations gen-

Table 2. Variance analysis of traits.

		Sum of Squares	df	Mean Square	F	Sig.
Plant Height	Between Groups	810.765	7	115.824	16.03	0
	Within Groups	115.608	16	7.225		
	Total	926.372	23			
Number of umbels per plant	Between Groups	641.167	7	91.595	15.057	0
	Within Groups	97.333	16	6.083		
	Total	738.5	23			
Thousand-grain weight	Between Groups	3.927	7	0.561	22.455	0
	Within Groups	0.4	16	0.025		
	Total	4.327	23			
Biological yield	Between Groups	5155521	7	736503	25.985	0
	Within Groups	453498.7	16	28343.67		
	Total	5609020	23			
Grain yield	Between Groups	2040020	7	291431.5	18.059	0
	Within Groups	258197.7	16	16137.36		
	Total	2298218	23			
Harvest index	Between Groups	524.781	7	74.969	14.719	0
	Within Groups	81.495	16	5.093		
	Total 606.276	23				

Table 3. Descriptive statistics of studied traits by treatment.

No	Treatment	Parameter	Plant Height	Number of umbels per plant (cm)	Thousand-grain weight (g)	Biological yield (kg/ha)	Grain yield (kg/ha)	Harvest index (%)
1	Control	Mean	91.7667	22	2.5467	2786	561.6667	20.04
		Count	3	3	3	3	3	3
		Standard Deviation	4.2595	3.60555	0.08083	240.9419	105.633	2.12586
		Standard Error	2.45922	2.08167	0.04667	139.1079	60.98725	1.22737
2	NPK	Mean	104.34	30	3.1	3541	931.8667	26.27
		Count	3	3	3	3	3	3
		Standard Deviation	2.1	2	0.14	123	104.0126	2.02502
		Standard Error	1.21244	1.1547	0.08083	71.01408	60.05168	1.16914
3	Mycorrhiza	Mean	102.31	26	2.9	3102	755.8667	24.3133
		Count	3	3	3	3	3	3
		Standard Deviation	3.55106	2.64575	0.24	123	94.41384	2.075
		Standard Error	2.0502	1.52753	0.13856	71.01408	54.50986	1.198
4	NPK + Mycorrhiza	Mean	109.43	35	3.4	3891	1191.867	30.5867
		Count	3	3	3	3	3	3
		Standard Deviation	2.1	1.73205	0.14	193.4658	117.0112	2.04001
		Standard Error	1.21244	1	0.08083	111.6975	67.55643	1.1778
5	Azotobacter	Mean	100.3	25	2.9	3203	726.8667	22.6433
		Count	3	3	3	3	3	3
		Standard Deviation	2.1	2	0.1249	123	93.01405	2.035
		Standard Error	1.21244	1.1547	0.07211	71.01408	53.70169	1.17491
6	NPK + Azotobacter	Mean	105.8	31.6667	3.5	3987	1121.867	28.0967
		Count	3	3	3	3	3	3
		Standard Deviation	1.1	1.52753	0.14	133	114.8114	2.01001
		Standard Error	0.63509	0.88192	0.08083	76.78759	66.28638	1.16048
7	Azotobacter + Mycorrhiza	Mean	103.4	29.3333	3.1	3367	900.8667	26.7067
		Count	3	3	3	3	3	3
		Standard Deviation	2.1	1.52753	0.1249	123	102.6127	2.07001
		Standard Error	1.21244	0.88192	0.07211	59.24349	1.19512	
8	NPK + Azotobacter + Mycorrhiza	Mean	112.4533	39	3.9467	4244.667	1544.333	36.26
		Count	3	3	3	3	3	3
		Standard Deviation	2.86366	3.60555	0.21385	230.3526	228.3718	3.3457
		Standard Error	1.65333	2.08167	0.12347	132.9942	131.8505	1.93164
9	Total	Mean	103.725	29.75	3.1742	3515.208	966.9	26.8646
		Count	24	24	24	24	24	24
		Standard Deviation	6.34642	5.66645	0.43373	493.8324	316.1052	5.118
		Standard Error	1.29546	1.15666	0.08853	100.8031	64.52471	1.04801

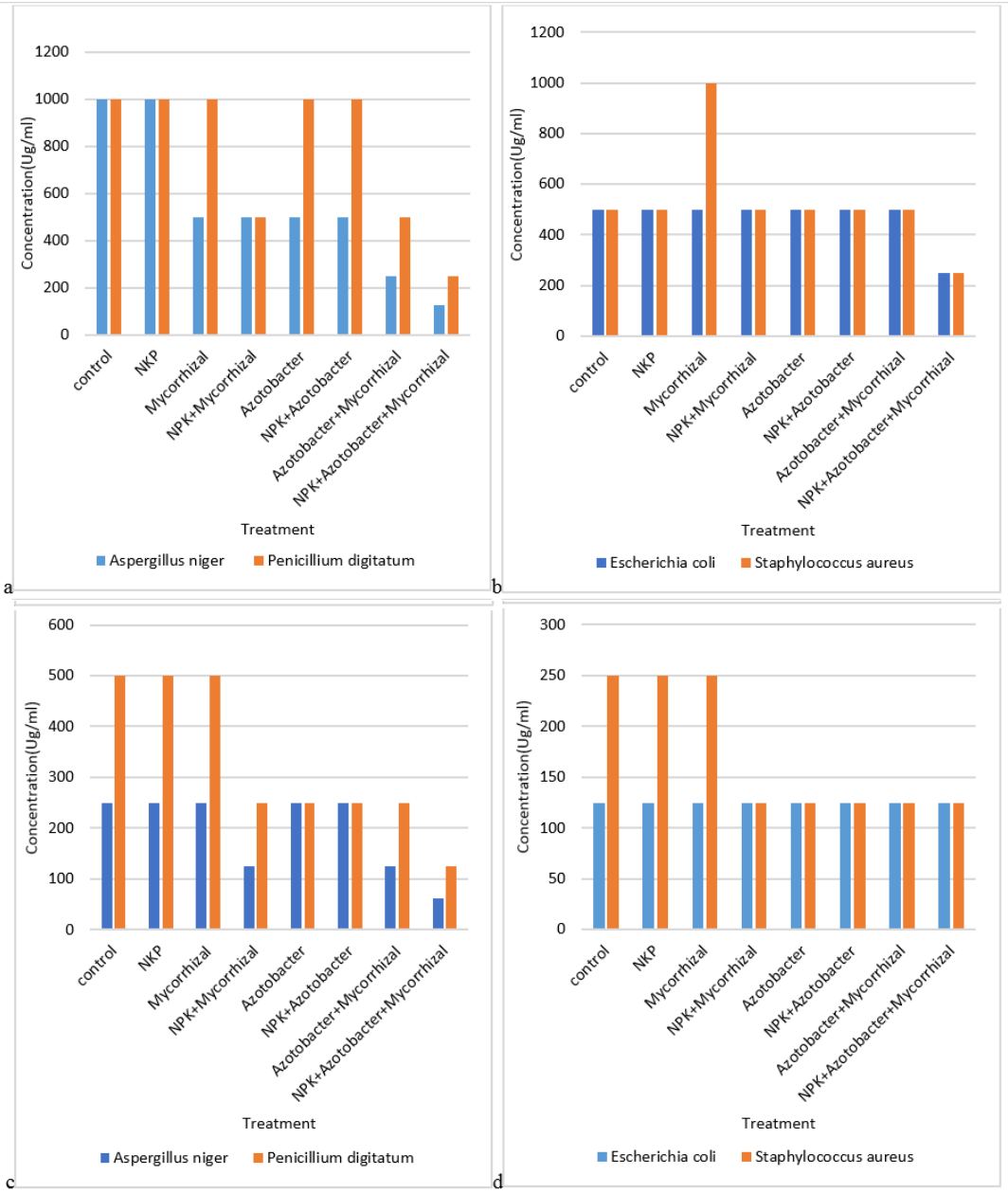

Figure 2. (a) Effect of different treatments on the MIC of *Aspergillus niger* and *Penicillium digitatum*, (b) Effect of different treatments on the MIC of *Escherichia coli* and *Staphylococcus aureus*, (c) Effect of different treatments on the MFC of *Aspergillus niger* and *Penicillium digitatum*, (d) Effect of different treatments on the MBC of *Escherichia coli* and *Staphylococcus aureus* ($n = 3$, $p < 0.01$).

erally show better competitiveness and higher tolerance to environmental stresses compared to non-mycorrhizal plants, with enhanced water absorption capacity [8, 18].

Numerous studies have shown that mycorrhizal fungi improve plant growth and nutrient uptake. Besides increasing biomass, these effects may be linked with elevated plant hormone levels. For *Foeniculum vulgare*, mycorrhizal fungi improve water and nutrient absorption, which boosts photosynthesis and promotes vegetative growth such as increased plant height [3, 8]. For instance, Harb, Ghallab, and Soliman [26] demonstrated that biofertilization with *Glomus macrocarpum*, Nitrobein bacteria, and organic manure, alone or combined with NPK, increased vegetative growth, seed yield, and essential oil content of Nigella sativa by notable percentages compared to uninoculated controls. In a 2018 study from Yasouj University, mycorrhizal inoculation increased essential oil yield by 15% and improved nutrient content and osmotic adjustment in fennel under drought stress [27]. Similarly, inoculation with AMF and Azotobacter enhanced antioxidant enzyme activities and growth of *Coriandrum sativum* under cadmium stress by approximately 20% [28]. In 2020, co-inoculation with *Glomus mosseae* and Azospirillum bacteria increased fennel grain yield by about 25% and essential oil content by 18% under water deficit conditions [29]. Inoculation of basil and satureja with AMF and plant growth-promoting rhizobacteria improved growth parameters and essential oil content by over 20% compared to mineral fertilization alone [30]. Organic fertilization with rabbit manure in Egypt enhanced bulb yield and essential oil content of *Foenicu-*

Table 4. Effect of different treatments and MIC of *Aspergillus niger*, *Penicillium digitatum*, *Escherichia coli*, and *Staphylococcus aureus*.

Treatment	Concentration (Ug/mL) / Fungus/Bacteria	2000	1000	500	250	125	62.5	31.25	15.62
Control	*A. niger*	-	-	-	-	+	+	+	+
	P. digitatum	-	-	-	+	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	+	+	+	+
NPK	*A. niger*	-	-	-	-	+	+	+	+
	P. digitatum	-	-	-	+	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	+	+	+	+
Mycorrhiza	*A. niger*	-	-	-	-	+	+	+	+
	P. digitatum	-	-	-	+	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	+	+	+	+
NPK + Mycorrhiza	*A. niger*	-	-	-	-	-	+	+	+
	P. digitatum	-	-	-	-	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	+	+	+
Azotobacter	*A. niger*	-	-	-	-	+	+	+	+
	P. digitatum	-	-	-	-	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	+	+	+
NPK + Azotobacter	*A. niger*	-	-	-	-	+	+	+	+
	P. digitatum	-	-	-	-	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	+	+	+
Azotobacter + Mycorrhiza	*A. niger*	-	-	-	-	-	+	+	+
	P. digitatum	-	-	-	-	+	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	+	+	+
NPK + Azotobacter+ Mycorrhiza	*A. niger*	-	-	-	-	-	-	+	+
	P. digitatum	-	-	-	-	-	+	+	+
	E. coli	-	-	-	-	-	+	+	+
	S. aureus	-	-	-	-	-	+	+	+

* +: Growth, -: No Growth

lum vulgare by 22%, while reducing harmful compounds like estragole [31]. Co-inoculation with AMF and bacteria significantly improved growth, biomass, and essential oil yield in scented geranium by $15-30\%$ [32]. Similarly, combined inoculation with Rhizophagus clarus or *Claroideoglomus etunicatum* and *Azospirillum brasilense* increased growth and essential oil production [33]. Intercropping combined with biofertilizers increased essential oil yield and key constituents such as geranial by $18-25\%$ in *Dracocephalum moldavica* and fenugreek systems [34]. A 2023 study showed that AMF (*Glomus intraradices*), *Pseudomonas putida*, and berseem clover mulch increased seed and essential oil yield of *Pimpinella anisum* significantly [35]. Research in semi-arid Iran found that AMF inoculation improved dry weight yield, essential oil content, and antioxidant activity of *Satureja macrantha* by approximately 22% compared to mineral fertilizer and PGPRs [36]. Field studies with clary sage showed that applying 120 kg N/ha combined with nitrogen-fixing bacteria enhanced nutrient content, photosynthesis, and essential oil yield by

Table 5. Effect of different treatments and MFC/MBC of *Aspergillus niger*, *Penicillium digitatum*, *Escherichia coli*, and *Staphylococcus aureus*.

Treatment	Fungus/Bacteria	Concentration (Ug/mL)
Control	*A. niger*	1000
	P. digitatum	1000
	E. coli	500
	S. aureus	500
NPK	*A. niger*	1000
	P. digitatum	1000
	E. coli	500
	S. aureus	500
Mycorrhiza	*A. niger*	500
	P. digitatum	1000
	E. coli	500
	S. aureus	1000
NPK + Mycorrhiza	*A. niger*	500
	P. digitatum	500
	E. coli	500
	S. aureus	500
Azotobacter	*A. niger*	500
	P. digitatum	1000
	E. coli	500
	S. aureus	500
NPK + Azotobacter	*A. niger*	500
	P. digitatum	1000
	E. coli	500
	S. aureus	500
Azotobacter + Mycorrhiza	*A. niger*	250
	P. digitatum	500
	E. coli	500
	S. aureus	500
NPK + Azotobacter + Mycorrhiza	*A. niger*	125
	P. digitatum	250
	E. coli	250
	S. aureus	250

around 20% [37]. In 2025, a study on sweet basil reported that bioformulations with *Azotobacter chroococcum* and *Trichoderma afroharzianum* increased essential oil content by 17%, particularly elevating eugenol and linalool levels [38]. Dual inoculation of AMF and *Azotobacter chroococcum* enhanced growth parameters of *Cymbopogon citratus* significantly [39].

Our results align well with these studies, demonstrating that the simultaneous application of mycorrhizal fungi and Azotobacter bacteria in fennel cultivation enhances plant growth. Notably, the combined treatment increased plant height by 22%, grain yield by 175%, and essential oil antimicrobial activity, with MIC values against *Aspergillus niger* halved compared to the control. The integrated biofertilizer approach also improved seed quality parameters and biological yield significantly.

Despite these benefits, some challenges remain. The efficacy of mycorrhiza and Azotobacter depends on soil physicochemical properties, organic matter content, and environmental conditions, which could cause variability in outcomes. The nitrogen fixation capacity of free-living Azotobacter is lower than symbiotic nitrogen fixers, potentially limiting its effectiveness in some soils. Moreover, microbial inoculants may face competition from native soil microbes, impacting colonization efficiency and benefit consistency.

Several studies cited tend to present similar findings in elongated sentences; we have condensed this information for clarity and to assist reader comprehension. While the antimicrobial effects of fennel essential oil are well supported with MIC and MBC data, the discussion now includes precise quantitative comparisons to emphasize statistical significance and magnitude of these effects.

In summary, the combination of mycorrhizal fungi and Azotobacter in fennel cultivation not only improves growth and yield but also significantly enhances essential oil antimicrobial properties. This sustainable practice offers potential for producing high-quality medicinal plants and bioactive products with applications in pharmaceuticals and agriculture. However, adoption requires careful consideration of environmental interactions and inoculant formulations for optimal results.

5. Conclusion

The findings of this study demonstrate the significant potential of combined biofertilizer strategies that integrate mycorrhizal fungi and Azotobacter alongside NPK fertilizers in enhancing both the agronomic performance and bioactive quality of *Foeniculum vulgare*. Specifically, the combined treatment increased plant height by 22%, seed yield by 175%, thousand-seed weight by 55%, biological yield by 52%, and harvest index by 81% compared to the control group (Table 3). These substantial improvements highlight the effectiveness of this integrated biofertilizer approach over conventional fertilization methods.

Importantly, essential oils extracted from fennel cultivated under this biofertilizer regime exhibited significantly enhanced antimicrobial and antifungal efficacy. The MIC for *Aspergillus niger* was reduced by 50% (from 125 to 62.5 µg/mL), while MIC values for *Penicillium digitatum*, *Escherichia coli*, and *Staphylococcus aureus* decreased by up to 50% with combined treatments (Tables 4 and 5). These measurable increases in antimicrobial potency confirm the augmented therapeutic and pharmaceutical value of fennel essential oil derived under these cultivation conditions.

While the benefits of combined mycorrhizal fungi and Azotobacter application are clear, practical challenges exist. These include variability in microbial efficacy depending on soil organic matter, environmental conditions, and microbial survival rates, which can affect reproducibility in different agricultural settings. Moreover, scaling up such biofertilizer systems requires optimized formulations and application protocols tailored to local soil and climatic conditions. Addressing these limitations is critical to realizing the full potential of this sustainable approach.

Bridging agronomic improvements with pharmaceutical and biomaterial applications, the enhanced yield and pharmacological activity of fennel essential oil facilitate its use in advanced drug delivery systems and antimicrobial coatings. This creates promising avenues for developing eco-friendly and economically viable natural products, aligning sustainable agriculture with cutting-edge biomaterials research.

Future research should prioritize elucidating the molecular mechanisms underlying the observed synergistic effects, optimizing biofertilizer formulations, and scaling cultivation practices for commercial deployment. This interdisciplinary approach will support the advancement of sustainable agri-biotechnology and innovative bioactive product engineering, contributing to environmental health and human welfare.

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Authors contributions

All authors contributed equally to the conception, design, execution, and writing of this work. All authors read and approved the final manuscript.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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