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Optimizing Nitrogen Fertilization to Maximize Yield and Bioactive Compounds in *Ziziphora clinopodioides*

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Abstract: *Ziziphora clinopodioides* L. is a valuable medicinal and aromatic plant; however, its special requirements for proper growth and enhanced secondary metabolite composition have limited its production and availability. The lack of appropriate fertilizer dosage recommendations is one of these limiting factors for ex situ conservation and large-scale cultivation. This study investigated the effects of nitrogen (N) fertilization on *Z. clinopodioides* in both open field and unheated greenhouse conditions. The determined research factor was N dosage (0, 40, 80, and 120 kg N ha^{-1} in the field) and (0, 200, 400, and 600 mg N pot^{-1} in the greenhouse). It was found that *Z. clinopodioides* plants could grow successfully outside their natural habitat with sufficient N fertilization yielding a satisfactory amount of metabolites per plantation area. Specifically, among the tested dosages, the 120 kg N ha^{-1} (which showed no significant difference from 80 kg N ha^{-1}) and 400 mg N pot^{-1} fertilizers increased the aboveground dry and fresh biomass, essential oil (EO) content, and yield. Nitrogen fertilization showed a direct correlation with menthone, neo-menthol, menthol, pulegone, *E*-ocimenone, and spathulenol, but a negative correlation with α-pinene, β-pinene, limonene, 1,8-cineol, *trans*-pulegol, and *iso*-menthone. This study is the first to evaluate the effects of N fertilization on the genus *Ziziphora*. The results indicate that utilizing N fertilizer at a moderate rate (80 kg N ha^{-1} in the field and 400 mg N pot^{-1} in the greenhouse) can improve the agronomic traits and EO of *Z. clinopodioides*.

Keywords: agronomic traits; kakuti; nutrient; essential oil; pulegone

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1. Introduction

In recent years, medicinal and aromatic plants have been considered as a main source for producing secondary metabolites [1], which are used in the health, perfume, cosmetics, and food industries [2–5]. These valuable compounds are influenced by various ecologically limiting factors, including temperature, carbon dioxide, light, ozone, soil water, soil salinity, and soil fertility [6–8]. The genus *Ziziphora* belongs to the *Lamiaceae* family and is native to regions such as Afghanistan, Anatolia, Armenia, Caucasus, Iraq, Central Asia, Pakistan, Syria, Turkey, Turkmenistan, and Western Siberia [9]. Four species, including *Z. clinopodioides*, *Z. persica*, *Z. tenuior*, and *Z. capitata*, are distributed in the Iranian flora, and in Persian, these species are collectively known as "kakuti" [10]. In traditional Iranian medicine, *Ziziphora* species are commonly used to treat a variety of illnesses, including infections, heart palpitations, digestive problems, stomachache, and insomnia [11,12]. Among all these

species, *Z. clinopodioides* is the most widely grown and is known for its antibacterial, antifungal, antioxidant, and insecticidal activities [13–15]. The EOs derived from *Z. clinopodioides* are rich sources of bioactive compounds, including sterols, fatty acids, flavonoids, and other components [16]. Mohammad-hosseini [9] found significant amounts of pulegone in the aerial parts of *Z. clinopodioides* EO. Thus, oxygenated monoterpenes are the major components of the relevant chemical profiles. Other oxygenated monoterpenes identified in the EO profiles include *iso*-mentone, *iso*-pulegone, thymol, menthon, piperitone, neo-menthol, menthol, carvacrol, and 1,8-cineole. Pulegone, an oxygenated monoterpene with a mint-like odor, is the major component of the essential oil of *Z. clinopodioides*, which is sometimes used in beverages and as a food additive for human consumption and occasionally in herbal medicine as an abortifacient drug [17]. The compound is genotoxic but not mutagenic, teratogenic, or a reproductive toxicant; however, it is carcinogenic, potentially causing cancer of the urinary bladder and liver at high doses [18,19]. Owing to the significance of the EOs of medicinal and aromatic plants, the domestication and cultivation of these plants in agricultural conditions are both environmentally and economically important. A limited number of species containing valuable compounds for human use are known; however, their scarcity in nature or difficulty in cultivation make them impractical for industrial use [20]. Therefore, it is essential to find effective and practical methods to increase the yield and quality of the compounds, especially for their cultivation in agricultural fields. Soil nutrient management, as one of the practical methods for successful production, is a critical factor for medicinal and aromatic plants, as well as for other plants [21,22].

Among all the nutritional elements, nitrogen (N) has the greatest effect on the growth and yield of all plants. Previous research in medicinal and aromatic plants showed that optimizing the N fertilization effectively improves both the total plant yield and the EO content, the latter being the most important characteristic for the industrial application [23]; specifically, in *Foeniculum vulgare* and *Thymus vulgaris*, applying 90 kg N ha^{-1} fertilizer increases yield and metabolite accumulation [24,25]. In a recent study by Liava et al. [26], N fertilization did not affect oil and silymarin accumulation in *Silybum marianum*, although it increased growth, yield, and the chlorophyll content. Conversely, in another study on *Thymus vulgaris*, when plants were treated with an appropriate amount of N fertilizer (0, 45, 90, and 135 kg N ha^{-1}), the EO yield was increased, although the effect was not statistically significant [27]. In a study conducted by Seif Sahandi [28], it was shown that the amount of soluble protein, antioxidant enzyme activity, and EO content of peppermint was significantly affected by N fertilizer. Considering various and sometimes controversial results in previous papers regarding the effects of N fertilizers in medicinal and aromatic plants, it is essential to examine the effect of N fertilizer on this specific plant. this examination can reveal how it helps to increase yield and secondary metabolite production while reducing N losses and regulating negative environmental impacts by increasing the uptake of applied N.

As medicinal and aromatic plants gain increasing economic and medicinal value, there has been a growing interest among researchers in collecting, domesticating, preserving, and cultivating these plants. To conserve, manage, and improve medicinal and aromatic plants in rangeland ecosystems and agricultural fields, we need to identify the most effective environmental factors, such as soil, topography, and climate conditions [29]. *Z. clinopodioides* is a widely used plant, with valuable bioactive compounds, and its production can be increased through cultivation in crop ecosystems by recognizing the ecological requirements, especially fertilizers such as N. However, to our knowledge, no studies have been conducted on the influence of N fertilizer on *Z. clinopodioides* yield and quality. This study aims to investigate the effect of N fertilizer on dry matter and the production of secondary metabolites of *Z. clinopodioides*. Moreover, the yield and secondary metabolite content of *Z. clinopodioides* cultivated in the field and greenhouse were compared to identify the best conditions for the successful production of this valuable medicinal and aromatic plant.

2. Materials and Methods

Two separate experiments were carried out to evaluate the effects of N fertilizer from a urea source on the growth, yield, and phytochemical composition of *Z. clinopodioides*. The concurrent experiments were conducted in the research field and greenhouse of the Faculty of Agriculture at Azarbaijan Shahid Madani University, Tabriz, Iran, located at 35°84′ N, 51°81′ E, and 1215 m above sea level. The experiments were carried out during the spring and summer of 2021. Light intensity and temperature data of two experimental conditions are shown in Figure 1. The experimental treatments included N fertilizer at four levels (0 (non-application), 40, 80, and 120 kg N ha^{-1} in the field, and 0 (non-application), 200, 400, and 600 mg N pot^{-1} in the greenhouse). The N source used was urea ($CO(NH_2)_2$), containing 46% N by weight as an organic compound. The amount of N used in the pots was calculated based on the number of plants ha^{-1}. To obtain a comparable result from the greenhouse and field experiments, we calculated and considered 200,000 plants ha^{-1} by determining the amount of N per plant in the field experiment (N per plant in the field = (kg N ha^{-1})/(200,000 plants ha^{-1})), where the N for each pot plant corresponded to the same level as the N for each field plant. The seeds used in this study were collected from the previous year's field-grown mother plants of *Z. clinopodioides* at the main research field of Azarbaijan Shahid Madani University. These seeds were first planted in peat moss (0.06% total N) trays, and approximately 45 days later, when the seedlings reached a height of 6 cm, they were transplanted to the field and into pots. The plants were transferred to the field on 20 June and planted in 2 m^2 plots with a space of 25 × 20 cm between two plants. The physico-chemical characteristics of the soil used in the experiment were assessed before applying the treatments (Table 1). Prior to the analysis, the soil samples were air-dried, grounded, passed through a 100-mesh sieve, and used for the analysis. The soil pH was determined at a soil-to-deionized water ratio of 1:2.5 (*w*/*v*) using a pH meter (PT-370, Boeco, Hamburg, Germany). Electrical conductivity (EC) was measured in a 1:5 (*w*/*v*) soil-to-deionized water mixture potentiometrically using a conductivity meter (AD330, Adwa Instruments, Szeged, Hungary). Soil organic carbon was determined using the Walkley–Black method, which involves potassium dichromate oxidation, followed by ferrous sulfate titration [30]. The cation exchange capacity was quantified titrimetrically, by distillation of ammonium displaced by sodium [31]. Total N concentration was measured using the Kjeldahl method [32] following sample digestion in H_2SO_4-salicylic acid-H_2O_2 [32]. The same digest was used to determine potassium (K) content by flame photometry [33] and phosphorus (P) concentration using the molybdenum blue method [34]. For the measurement of magnesium (Mg), iron (Fe), manganese (Mn), boron (B), zinc (Zn), and copper (Cu), samples were ashed at 500 °C for 4 h, then treated with 5 mL of 2 N hydrochloric acid and heated, and concentrations were determined by atomic absorption spectrophotometry [35]. The calcium carbonate content ($CaCO_3$%) was determined with a calcimeter, by treating soil samples with HCl and measuring the CO_2 release [36].

The N fertilizer was mixed with the soil twice during the growth period, at a depth of 2 cm. The first fertilization was performed on the 13th of July, and the second on the 27 August (early vegetative stage). In the greenhouse experiment, 72 pots were used, with six replicates per treatment, and in each replication, there were three pots for each treatment. Pots with a capacity of 7 L were filled with soil from the same field, and then *Z. clinopodioides* seedlings were planted. Similar to the field experiment, fertilization was performed twice in the greenhouse pots. A drip irrigation system was used in both the field and greenhouse experiments. Irrigation management was based on maintaining soil moisture at 70% of field capacity. Soil water status was continuously monitored using TDR (Time Domain Reflectometer) device (PMS-714, Lutron, Taipei, Taiwan) probes. Irrigation was triggered when soil water depletion reached 30% of field capacity, ensuring consistent soil moisture conditions throughout the study period, and the total number of irrigations during the experimental period was 11 in the field and 13 in the greenhouse. Throughout the experiment, the plants were closely monitored daily for any signs of pests, pathogens,

and weeds. No chemical treatments for pests and pathogens were necessary. However, weeds were hand-pulled during the growth season to reduce competition with the primary crop. At full flowering, morphological and yield measurements were collected. Plant height was measured from the base of the main stem to the top of the highest inflorescence. Plants were harvested by cutting the stems 7 cm above the ground. Fresh weight was determined immediately using a digital balance (BBI41, Boeco, Hamburg, Germany). To determine the dry weight, the harvested plant material was dried in an oven (BINDER D. 78532, Tuttlingen, Germany) at 60 °C for 48 h. Both fresh and dry weights were recorded, and then fresh and dry herb yields per hectare were calculated based on plot size and plant density in the field trial.

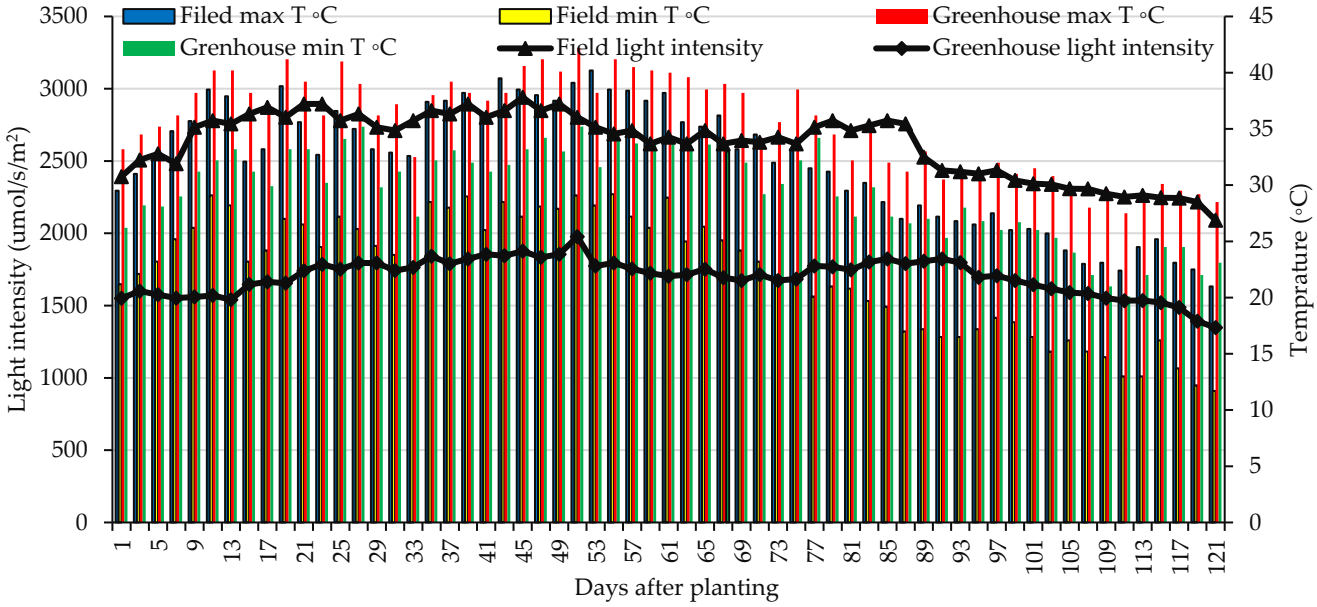

Figure 1. Light intensity, minimum and maximum field and greenhouse temperature (°C) during the study period.

Table 1. Properties of experimental soil in field and pot at the start of the experiment.

Item	Unit	Soil
Texture	-	Sandy loam
Organic carbon	(%)	0.51
EC	(ds/m)	1.36
pH	-	8.20
Total N	(%)	0.05
Available P	(mg kg^{-1})	7.80
Available K	(mg kg^{-1})	162
$CaCO_3$	(%)	4.50
Available Mg	(mg kg^{-1})	9.84
Available Fe	(mg kg^{-1})	8.10
Available Mn	(mg kg^{-1})	6.22
Available Zn	(mg kg^{-1})	1.12
Available Cu	(mg kg^{-1})	1.05
Available B	(mg kg^{-1})	0.48
Cation-exchange capacity (CEC)	(cmol kg^{-1})	9.5
Bulk density	(g cm^{-3})	1.35

The harvesting time for plants without application of N and those treated with 40 kg N ha^{-1} treatment was on 23 September, while the plants treated with 80 kg N ha^{-1}

and 120 kg N ha^{-1} were harvested on 6 October. The plots that had received the highest N rate showed delayed flowering time, and our aim was to harvest at the full flowering stage of the treatments. For plants in the greenhouse, the harvesting date was recorded on 27 October.

2.1. Shoot Nitrogen Content

After oven drying, the shoot samples (0.5 g) were finely ground using an electric mill. Total N was determined by the Kjeldahl method, as described by Novozamsky et al. [32]. This process involved digestion of the samples with sulfuric acid/hydrogen peroxide, followed by distillation with sodium hydroxide and, finally, titration of the ammonia-containing solution. The results were expressed as a percentage of total N on a dry weight basis.

2.2. Extraction of EO

In order to determine the EO content, the aerial parts of each sample were harvested at full flowering stage and dried at a temperature of 40 °C. The drying process continued until the residual moisture content of the plant material reached 10% of the initial fresh weight. The hydro-distillation method was used to isolate the EOs from the dried aerial parts of the plant. From each treatment, 50 g of dried samples were used and extracted three times using a Clevenger-type apparatus for three hours; this was made of heat resistant, low expansion, 3.3 borosilicate glass (upper grade). Two options included a 1000 mL round bottom flask (24/29) and a 300 mm Liebig condenser (24/29). For the rest, we followed the procedure described in the British Pharmacopoeia [37] for this extraction. The extracted EOs were dehydrated using Na_2SO_4 and stored in sealed, dark glass containers at 4 °C for further analysis. The weight EO obtained was measured, and the percentage of EO was calculated (*w*/*w* %). The EO yield was calculated by multiplying the percentage of EO by the dry yield (EO yield = % EO × dry yield).

2.3. Gas Chromatography (GC) and Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The GC analysis was conducted using an Agilent Technologies-7890A mass selective detector and an HP-5 fused silica capillary column (30 m × 0.32 mm (ID), 0.25 micron (FT)). The oven temperature was adjusted from 60 to 210 °C at an increasing rate of 3 °C min^{-1}, which was then increased up to 240 °C with an increasing rate of 20 °C min^{-1}. The temperature of 240 °C was then maintained constant for 8.5 min. Finally, the MS operating parameters were adjusted with an ionization voltage of 70 eV and an ion source temperature set to 200 °C.

GC-MS analysis was conducted using an Agilent Technologies-5975C MS and a 7890A-GC system fitted with a fused silica HP-5MS column (30 m × 0.25 mm (ID); coating thickness 0.25 µm). The oven temperature was adjusted from 60 to 210 °C with an increase rate of 3 °C min^{-1}, which was then increased to 240 °C with an increase rate of 20 °C min^{-1}. The final oven temperature was kept constant at 240 °C for 8.5 min. The total analysis time was set to 60 min. The electron ionization (EI) energy was 70 eV, and the ion-source temperature was set to 230 °C. We used an MS detector, where the interface line temperature was set to 280 °C, the injection port temperature was set to 280 °C, and a split ratio of 1:50 was used. Helium was used as the carrier gas, with a flow rate of 1 mL min^{-1}, and a mass range of 50–480 was applied to detect compounds. The compounds were identified by comparing their retention time and indices, as well as recorded mass spectra using the National Institute of Standards and Technology (NIST 11.0) mass-spectral library, the Wiley MS data system library (Wiley, Chichester, UK), and previous literature. To calculate the inhibition index, the C5-C24 n-alkanes series were used under similar thermal conditions. Further identification was made by matching the mass spectral fragmentation patterns of different compounds with corresponding data (Adams and Wiley 7.0 library) and other published mass spectra [38]. The percentage of each EO constituent was calculated by

determining the peak area of each compound relative to the total peak area of the entire chromatograph.

2.4. Data Analysis

The statistical analysis was conducted using SAS, version 9.2, and the results were compared using one-way ANOVA. Two separate experiments were considered: the field experiment was considered with three replications, in a randomized complete block design, and the greenhouse experiment was considered with six replications, in a randomized complete block design, and the data are presented as means ± standard errors. The PROC UNIVARIATE within SAS was used to test the assumptions of ANOVA, and the residuals were normally distributed, so the data did not need transformation. The Tukey test was used to determine whether there were significant differences between the treatments per experiment ($p < 0.05$).

3. Results

3.1. Plant Height in the Field and Greenhouse Settings

The results of this study revealed that the application of N fertilizer had a significant, positive effect on the height of *Z. clinopodioides* plants ($p < 0.001$). Plant height increased significantly with the N application in both cultivation conditions. In the field, the highest plant height was achieved when 80 kg N ha^{-1} was applied, compared to the non-application of N (25% higher). However, there was no significant difference between the treatments of 40 kg N ha^{-1} and 80 kg N ha^{-1}. Using 120 kg N ha^{-1}, the plant height decreased by 13% compared to 80 kg N ha^{-1} (Figure 2). In the greenhouse, plant height increased by 25% compared to the non-application of N when 400 and 600 mg N pot^{-1} were applied. However, there was no significant difference between the 200 mg N pot^{-1}, 400, and 600 mg N pot^{-1} treatments (Figure 2).

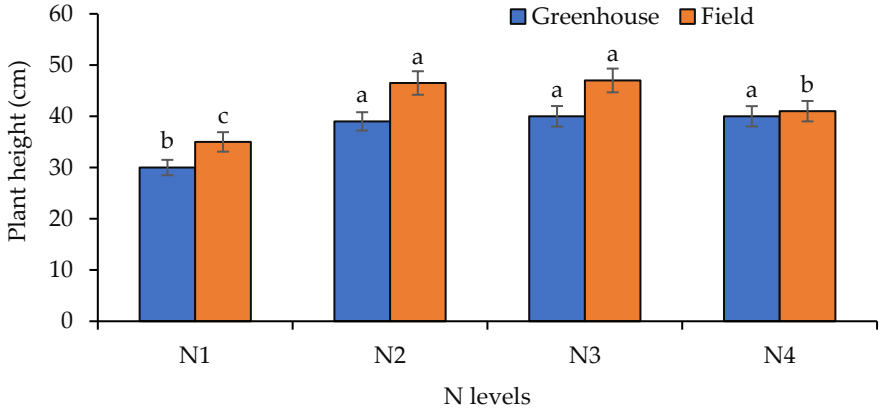

Figure 2. Effect of N fertilization on plant height in the field and greenhouse for *Z. clinopodioides*. N_1: 0, N_2: 40, N_3: 80, N_4: 120 kg N ha^{-1} in the field; N_1: 0, N_2: 200, N_3: 400, N_4: 600 mg N pot^{-1} in the greenhouse. Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within columns of the same color indicate statistically significant differences according to Tukey's test.

3.2. Fresh and Dry Biomass Production of Shoots

The fresh ($p < 0.001$) and dry ($p < 0.05$) biomass of the shoots was significantly affected by different amounts of N fertilizer under both field and greenhouse conditions. The fresh weight of the plants under field conditions varied from 42.20 to 75.60 g per plant, while the dry weight ranged from 15.56 to 27.78 g per plant. Both fresh and dry weights indicated that plants grown with 120 kg N ha^{-1} had the highest biomass compared to the non-application of N, which had the lowest value (Figures 3 and 4). Under greenhouse conditions, the dry weight ranged between 1.86 and 4.29 g per plant, while the fresh weight varied from 10.18 to 21.74 g per plant. The highest values were recorded for 400 mg N pot^{-1}, and the

non-application of N had the lowest amount. This study showed higher fresh and dry weights with the N application compared to the non-application of N under both field and greenhouse conditions. Under field conditions, 120 kg N ha^{-1} led to significant increases in fresh and dry weights, although these increases were not statistically different from those observed with 80 kg N ha^{-1}, whereas under greenhouse conditions, the 400 mg N pot^{-1} treatment performed better.

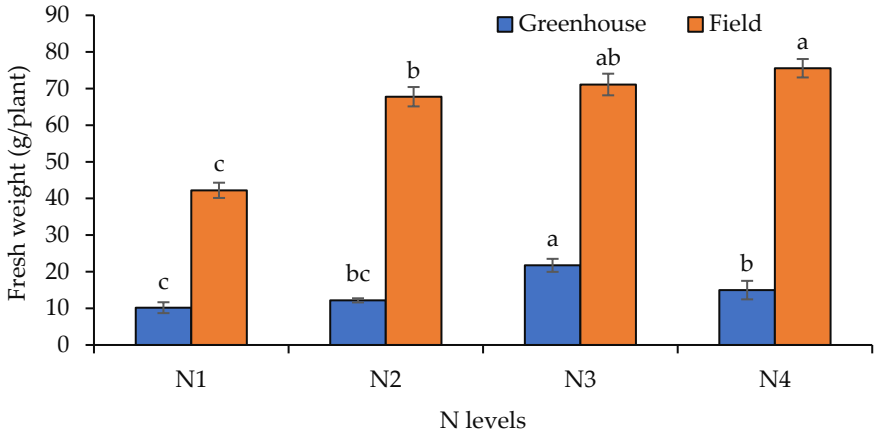

Figure 3. Effect of N fertilization on the plant fresh weight in the field and greenhouse for *Z. clinopodioides*. N1: 0, N2: 40, N3: 80, N4: 120 kg N ha^{-1} in the field; N1: 0, N2: 200, N3: 400, N4: 600 mg N pot^{-1} in the greenhouse. Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within columns of the same color indicate statistically significant differences according to Tukey's test.

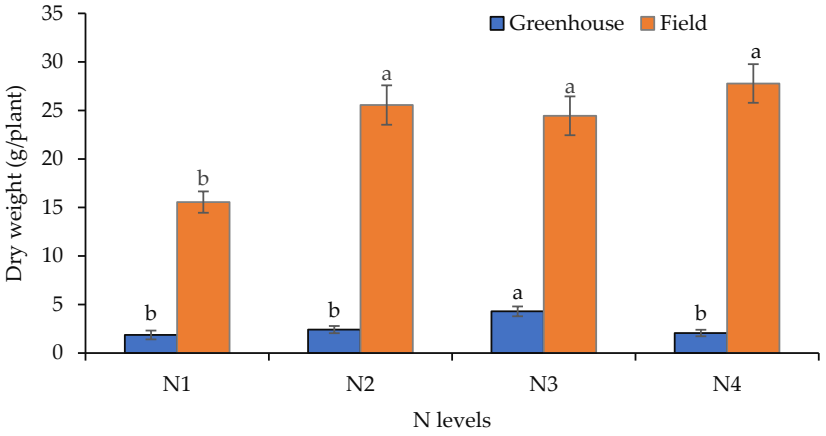

Figure 4. Effect of N fertilization on the plant dry weight in the field and greenhouse for *Z. clinopodioides*. N_1: 0, N_2: 40, N_3: 80, N_4: 120 kg N ha^{-1} in the field; N_1: 0, N_2: 200, N_3: 400, N_4: 600 mg N pot^{-1} in the greenhouse. Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within columns of the same color indicate statistically significant differences according to Tukey's test.

Figure 5 illustrates the effect of N fertilization on fresh and dry shoot yield of *Z. clinopodioides* under field conditions. N fertilization significantly affected both fresh and dry biomass production ($p < 0.001$), demonstrating the crucial role of N in plant growth and development. N fertilization progressively increased plant biomass, with the most pronounced effect observed at the highest application rate, of 120 kg N ha^{-1}, followed by 80 kg ha^{-1}, and the lowest value obtained from plants subjected to non-application of N. This treatment resulted in maximum fresh and dry yields of 10,577.8 kg ha^{-1} and 3888.89 kg ha^{-1}, respectively. Remarkably, these yields represent a 44% increase compared to the control treatment, where no nitrogen was applied, although these increases were

not statistically different from those observed with 80 kg N ha^{-1}. This substantial increase underlines the importance of an adequate N supply for optimal biomass production in *Z. clinopodioides*.

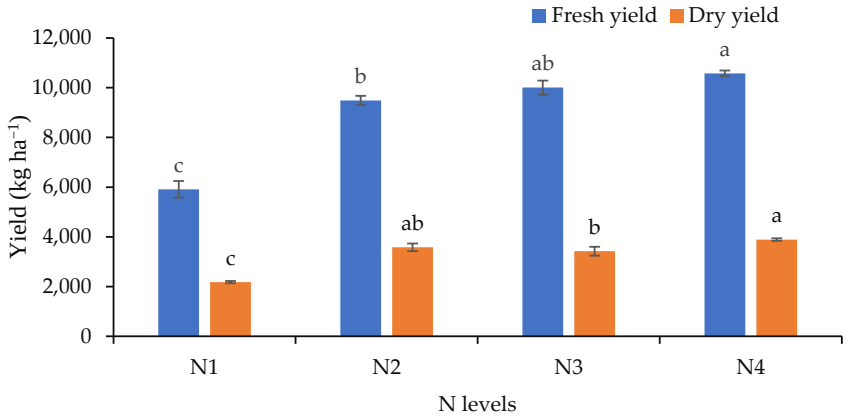

Figure 5. Effect of N fertilization on the shoot fresh and dry yields in the field of *Z. clinopodioides*. N_1: 0, N_2: 40, N_3: 80, N_4: 120 kg N ha^{-1} in the field. Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within columns of the same color indicate statistically significant differences according to Tukey's test.

3.3. Shoot Nitrogen Concentration

The shoot N content analysis provides an important physiological context for interpreting plant growth, yield, and EO production responses and serves as a quality control measure for fertilizer treatments. The results of this study revealed that both N application and growing conditions had a significant impact on leaf N concentration under field and greenhouse conditions ($p < 0.001$). The nitrogen concentration in the shoots was significantly higher in all treatments compared to the non-application of N in both conditions. In the field, the application of 120 kg N ha^{-1} resulted in a maximum leaf N content, representing a 43% increase compared to the non-application of N (Figure 6). In the greenhouse, the highest values were obtained for the application of 400 mg N pot^{-1}, while the lowest values were observed with the non-application of N (Figure 6). It is worth noting that the percentage of leaf N was significantly higher in the *Z. clinopodioides* plants grown in the field compared to those grown in the greenhouse.

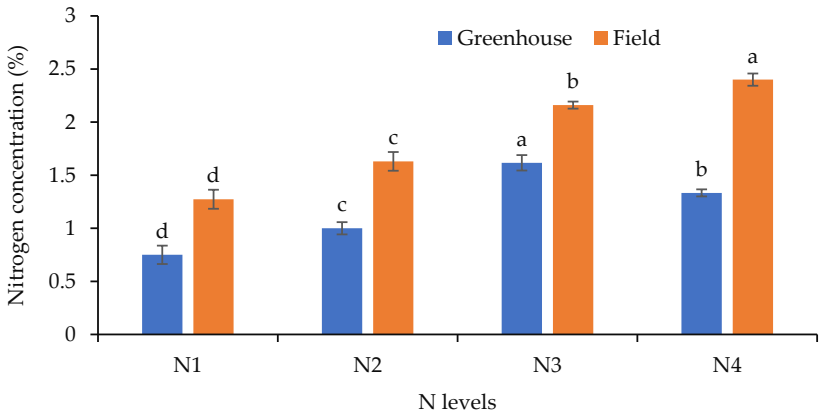

Figure 6. Effect of N fertilization on the shoot N concentration in the field and greenhouse for *Z. clinopodioides*. N_1: 0, N_2: 40, N_3: 80, N_4: 120 kg N ha^{-1} in the field; N_1: 0, N_2: 200, N_3: 400, N_4: 600 mg N pot^{-1} in the greenhouse. Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within columns of the same color indicate statistically significant differences according to Tukey's test.

3.4. Essential Oil Yield of Dry Matter in the Field

The EO yields were significantly affected by N fertilizers (Figure 7, $p < 0.001$). The results showed that the EO production under field conditions increased with the supply of 120 kg N ha^{-1}. The highest value of EO production in the field was recorded at 120 kg N ha^{-1} treatment, and the lowest was observed in the non-application of N. The EO yield increased from 44.07 kg ha^{-1} in the non-application of N treatment to 93.3 kg ha^{-1} in the 120 kg N ha^{-1} treatment, representing a 50.4% increase compared to the non-application of N. There was no significant difference in the EO yield values between the 80 kg N ha^{-1} and 40 kg N ha^{-1} treatments when compared to the 120 kg N ha^{-1} level (Figure 7).

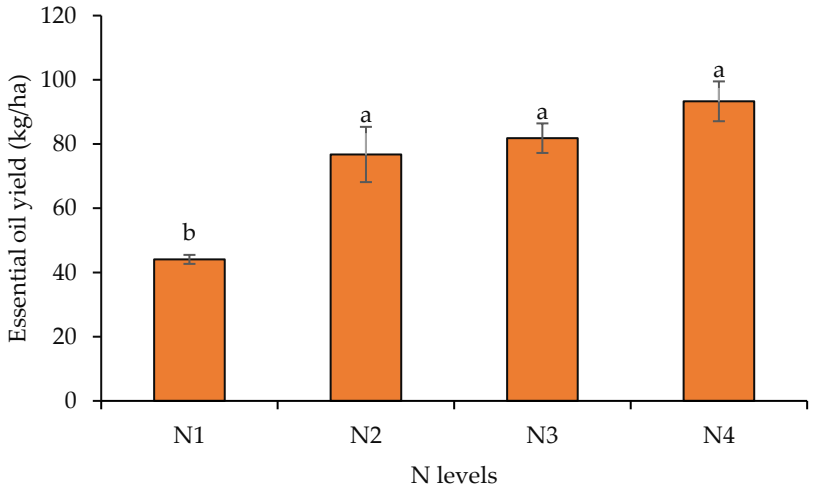

Figure 7. Effect of N fertilization on the shoot EO yields in the field for *Z. clinopodioides*. N_1: 0, N_2: 40, N_3: 80, N_4: 120 kg N ha^{-1}. A Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within each column indicate statistically significant differences according to the Tukey's test.

3.5. Essential Oils Content of Dry Matter in the Field and Greenhouse

Adding N fertilizer to plants enhanced the EO content of *Z. clinopodioides* aerial parts both in the field and greenhouse at the 5 and 1% probability levels, respectively. The content of EO in the field increased from 2.03% in non-application of N to 2.43% when N was applied at 120 kg N ha^{-1}, which is an increase of 17% over the non-application of N treatments (Figure 8). In greenhouse conditions, the percentage of EO increased from 0.42% in non-application of N to 0.98% after 600 mg N pot^{-1} was applied, representing a 56% increase compared to the non-application of N treatment (Figure 8). Although the percentage of EO was higher in the field than in the greenhouse, the beneficial effect of N fertilizer was more pronounced in the greenhouse, which was 56% compared to the non-application of N, while this increase was 17% in the field. The control registered the lowest value at both conditions. The ameliorative effect of basal application of N on the EO content could be ascribed to the pivotal roles played by N in plants.

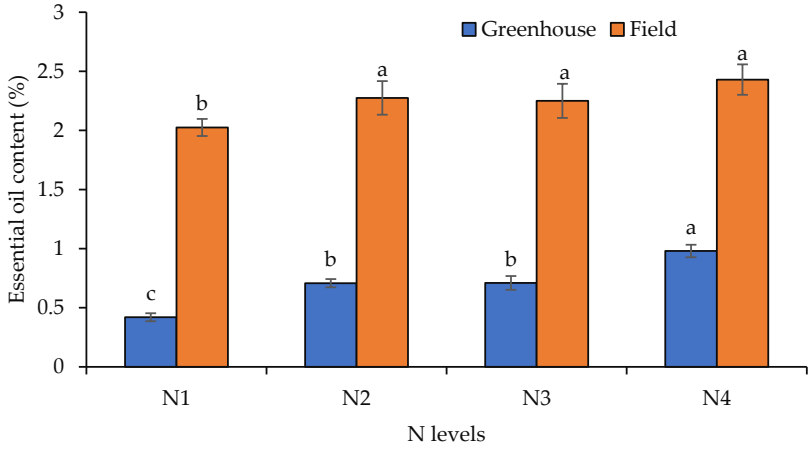

Figure 8. Effect of N fertilization on the content of plant EO in the field and greenhouse for *Z. clinopodioides*. N_1: 0, N_2: 40, N_3: 80, N_4: 120 kg N ha^{-1} in the field; N_1: 0, N_2: 200, N_3: 400, N_4: 600 mg N pot^{-1} in the greenhouse. Means (columns) and standard errors (vertical bars) of three replicates are depicted. Different letters within columns of the same color indicate statistically significant differences according to Tukey's test.

3.6. Essential Oil Compositions

Totally, 16 compounds were identified in the *Z. clinopodioides* plants grown under field and greenhouse conditions, and these compounds accounted for 96.57–99.25% of the total plant EO, among which oxygenated monoterpenes were the most abundant EO (Table 2). The mean relative abundance ± standard error of these compounds is shown in Table 2. Pulegone, menthone, neo-menthol, 1,8-cineole, and menthol were identified as the predominant EO compounds in plants (Figure 9).

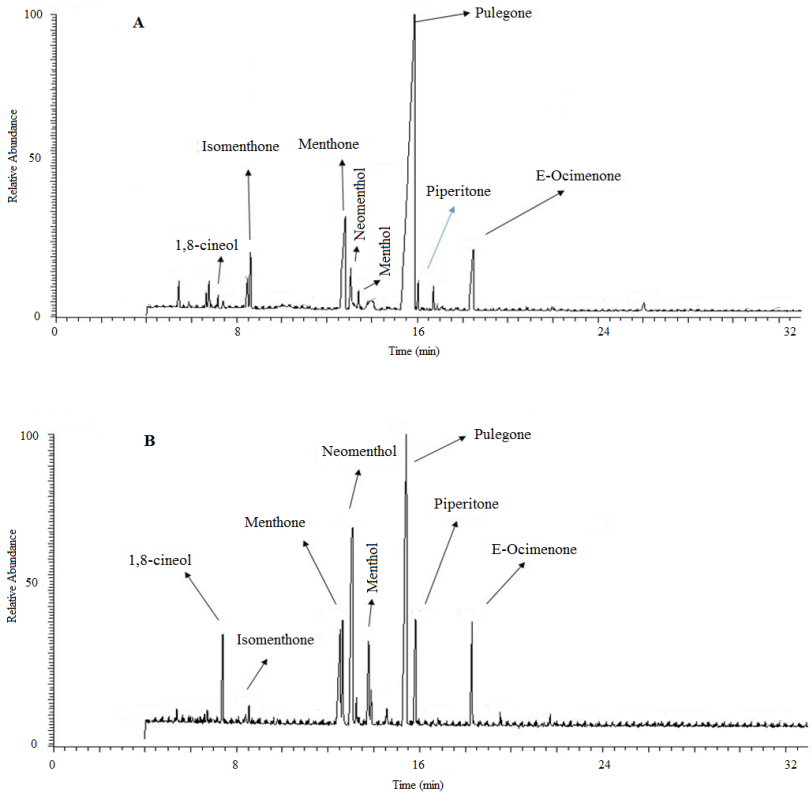

Figure 9. GC/MAS chromatogram of essential oil of *Z. clinopodioides* plant in the field (**A**) and greenhouse (**B**) conditions.

Table 2. Influence of N fertilization on the composition of essential oil of *Z. clinopodioides* under different conditions.

				kg N ha^{-1}						mg N pot^{-1}					
N.	**Compound Name**	**RI Calculated**	**RI Reference**	**0**	**40**	**80**	**120**	**Field Condition**	**Significance**	**0**	**200**	**400**	**600**	**Green House Condition**	**Significance**
1	α-Pinene	928	932	3.42 ± 0.02 a	3.66 ± 0.14 a	1.87 ± 0.27 b	0.3 ± 0.04 c	2.31	**	0.84 ± 0.06	1.10 ± 0.15	1.56 ± 0.26	0.94 ± 0.09	1.11	ns
2	Sabinene	967	969	2.54 ± 0.11 a	2.03 ± 0.03 b	1.63 ± 0.01 c	0.11 ± 0.01 d	1.58	***	00 ± 0.00 b	00 ± 0.00 b	1.51 ± 0.19 a	1.42 ± 0.07 a	0.73	**
3	β-pinene	972	974	3.84 ± 0.10 a	3.58 ± 0.02 a	2.48 ± 0.02 b	0.33 ± 0.03 c	2.56	***	1.20 ± 0.15	0.80 ± 0.01	0.82 ± 0.01	0.97 ± 0.07	0.95	ns
4	Limonene	1024	1024	1.78 ± 0.02 a	1.80 ± 0.10 a	1.29 ± 0.19 ab	1.01 ± 0.01 c	1.47	*	00 ± 0.00 b	00 ± 0.00 b	00 ± 0.00 b	0.60 ± 0.10 a	0.15	*
5	1,8-Cineol	1027	1026	6.91 ± 0.29 a	1.02 ± 0.01 c	1.33 ± 0.14 bc	1.87 ± 0.12 b	2.78	***	24.4 ± 1.90 a	8.51 ± 0.59 b	6.57 ± 0.45 b	5.98 ± 0.42 b	11.37	**
6	*iso*-menthone	1145	1158	9.32 ± 0.62 a	7.66 ± 0.40 a	7.56 ± 0.55 a	2.03 ± 0.03 b	6.64	*	2.75 ± 0.45	2.48 ± 0.48	2.64 ± 0.35	2.33 ± 0.22	2.55	ns
7	Menthone	1148	1148	10.84 ± 0.46 b	12.78 ± 0.78 b	12.36 ± 0.16 b	15.09 ± 0.60 a	12.77	**	8.56 ± 0.43 c	12.59 ± 1.41 b	15.44 ± 0.25 ab	16.62 ± 1.10 a	13.30	**
8	neo-Menthol	1160	1161	4.81 ± 0.49 c	5.11 ± 0.21 c	10.44 ± 0.56 b	15.84 ± 0.84 a	9.05	***	10.26 ± 0.74 c	16.26 ± 0.26 b	18.36 ± 0.86 a	15.41 ± 0.40 b	15.07	***
9	Menthol	1168	1167	2.53 ± 0.37 b	2.45 ± 0.25 b	4.04 ± 0.55 a	4.92 ± 0.18a	3.49	**	14.53 ± 1.27 a	9.15 ± 1.04 b	3.46 ± 0.54 c	8.48 ± 0.52 b	8.91	***
10	*trans*-pulegol	1215	1213	4.04 ± 0.16 a	3.93 ± 0.01 a	1.84 ± 0.06 b	0.35 ± 0.00 c	2.54	***	1.52 ± 0.08 a	1.62 ± 0.18 a	1.36 ± 0.16 a	0.44 ± 0.04 b	1.24	***
11	Pulegone	1227	1233	38.24 ± 0.78	40.55 ± 1.35	40.4 ± 1.95	40.01 ± 0.99	39.80	ns	14.06 ± 0.98 c	22.44 ± 0.65 b	25.11 ± 0.99 a	23.14 ± 1.35 ab	21.19	***
12	Piperitone	1249	1249	3.48 ± 0.8 b	3.74 ± 0.25 b	3.91 ± 0.19 b	5.16 ± 0.33 a	4.07	***	7.31 ± 0.41 c	9.26 ± 0.26 a	8.20 ± 0.57 ab	7.64 ± 0.37 b	8.10	**
13	*cis*- Piperitone Epoxide	1250	1250	2.87 ± 0.23 a	3.36 ± 0.04 a	1.26 ± 0.08 b	0.24 ± 0.04 c	1.93	***	0.21 ± 0.02	0.40 ± 0.10	0.27 ± 0.01	0.50 ± 0.10	0.35 a	ns
14	*E*-Ocimenone	1255	1235	1.78 ± 0.12 c	4.42 ± 0.22 b	5.42 ± 0.42 b	9.02 ± 0.97 a	5.16	***	8.40 ± 0.40	9.07 ± 0.06	9.95 ± 1.05	9.96 ± 0.96	9.35 a	ns
15	Caryophyllene	1378	1408	0.42 ± 0.02 a	0.34 ± 0.00 ab	0.27 ± 0.03 c	0.30 ± 0.05 b	0.33	*	2.45 ± 0.25 ab	2.84 ± 0.16 a	1.67 ± 0.02 b	1.60 ± 0.06 b	2.14	*
16	Spathulenol	1576	1577	0.20 ± 0.02 c	0.14 ± 0.02 c	1.41 ± 0.09 b	2.67 ± 0.17 a	1.10	**	1.52 ± 0.27	1.14 ± 0.06	1.33 ± 0.03	1.26 ± 0.16	1.31	ns
		Total	-	97.02	96.57	97.51	99.25	-	-	98.01	97.66	98.25	97.29	-	-

RI calculated: retention indices for each compound calculated on a DB-5 column using the retention times of n-alkanes (C8–C26). RI reference: retention indices that refer to NIST Chemical Web Book. ns, *, ** and *** indicate no significant, and significant at 0.05, 0.01 and 0.001% probability levels, respectively. Values are mean ± SE (n = 3); values marked in rows with different letters differ at $p < 0.05$ according to Tukey's test.

Adding N to the soil in the field and greenhouse conditions increased the amounts of menthone, neo-menthol, menthol, pulegone, *E*-ocimenone, and spathulenol. However, some important compounds, such as α-pinene, sabinene, β-pinene, limonene, 1,8-cineol, *trans*-pulegol, and *iso*-menthone were reduced when N fertilizer was applied to the plants (Table 2). The application of N in the field and greenhouse led to an increase in the amount of pulegone. Under field conditions, 40 kg N ha^{-1} produced the highest amount of pulegone, while the lowest amount was obtained in the non-application of N treatment, although there was no significant difference between the treatments (Table 2). However, this increase did not occur at high N levels in greenhouse conditions, where the highest amount of pulegone was observed in the treatment with 400 mg N pot^{-1}, and the lowest amount was observed in the non-application of N treatment. The amount of this compound increased by 18.6% when comparing field and greenhouse conditions (Table 2). Similarly, the percentage of menthone was higher with N treatment under both field and greenhouse conditions. In the field, the highest amount of menthone was obtained in the 120 kg N ha^{-1} treatment, which was 28% more than the lowest amount obtained in the non-application of N.

In greenhouse conditions, the highest percentage of menthone was observed in the treatment with 600 mg N pot^{-1}, which was 47% higher compared to the non-application of N. There was no difference in the menthol percentage between field and greenhouse conditions. The percentage of neo-menthol was also higher with the N application under both field and greenhouse conditions. In the field, the highest percentage of neo-menthol was obtained at 120 kg N ha^{-1} (69% more compared to the non-application of N). On the other hand, in the greenhouse, the highest amount of neo-menthol EO (18.36%) was obtained in the treatment with 400 mg N pot^{-1}, while the lowest amount of this compound (10.26%) was observed in the non-application of N. Additionally, the concentration of neo-menthol increased by 6.36% in the greenhouse compared to the field. Under both field and greenhouse conditions, the amount of 1,8-cineole decreased with the N application.

In the field, the highest and lowest concentrations of 1,8-cineole correlated with the non-application of N treatment (6.91%) and the 40 kg N ha^{-1} treatment (1.02%), respectively. Meanwhile, the reduction was from 24.4% in the non-application of N treatment to 5.98% when 600 mg N pot^{-1} was applied in the greenhouse. The percentage of this compound increased by 8.59% under greenhouse conditions compared to field conditions (Table 2). Furthermore, a significant reduction in *iso*-menthone was observed under both field and greenhouse conditions when N was added to the soil. In the field, the highest value (9.32%) was observed in the non-application of N, and this number was reduced by the N application and the 120 kg N ha^{-1} treatment, resulting in the highest reduction (78%).

In the field, the highest and lowest amounts of menthol were 4.92% and 2.45%, which were observed in the treatments with 120 kg N ha^{-1} and 40 kg N ha^{-1}, respectively. In the greenhouse, the highest menthol percentage was observed in the non-application of N (76% more than with 400 mg N pot^{-1}) condition, while the lowest percentage (3.46%) was associated with an application of 400 mg N pot^{-1}. This compound increased by 5.42% when the plants were grown in the greenhouse.

4. Discussion

Medicinal and aromatic plants are basically vital for farming since many of them are still collected from nature to meet the requirements of communities. However, due to increased industry demand, farmers have begun cultivating them on farms. Due to the limited knowledge of optimal soil conditions, including nutrient levels and other growth requirements, it is essential to carefully monitor and analyze management strategies in experimental fields and make the results accessible to the wider society. N is an essential nutrient for plant growth and development, and its demand increased significantly during the plant growth stages, as compared with other elements [39]. The role of N management in soil fertility, plant N content, growth, yield and phytochemical composition is of utmost importance [23,40]. Increased N availability through deposition or supplementation can enhance crop growth and productivity. Recognizing this, we were motivated to investigate

the optimal N application treatment for *Z. clinopodioides*, a valuable medicinal and aromatic plant in the Middle East. The results of the two separate experiments showed that adding N to *Z. clinopodioides* has a significant impact on plant growth and development. The N fertilizer rate had a significant impact on plant heights under both field and greenhouse conditions. Nitrogen promotes plant growth by stimulating a number of interrelated processes. It increases the number and length of internodes, as well as meristematic cell production, and facilitates stem cell enlargement. These combined effects lead to the formation of new shoots and taller, more robust plants, highlighting the critical role of nitrogen in plant development. The increase in plant height obtained with 80 kg N ha^{-1} in the field and 600 mg N pot^{-1} in the greenhouse can be a result of the N role in facilitating the assimilation of essential nutrients by the plant [41]. The increase in plant height under field conditions was significantly higher compared to the greenhouse, which can be due to the greater expansion of the roots and the absorption of more N from the soil. An increase in plant height can result in an increase in leaf number/plant [42], therefore leading to more photosynthesis capacity per plant.

Determining the ideal N application rate is an effective approach to achieve crop economic benefits while minimizing environmental risks. Crop yield or biomass is a commonly employed indicator for determining the optimal N application rate [43,44]. In this study, N fertilization positively influenced the biomass of *Z. clinopodioides* plants in both conditions of the experiment. When N fertilizers were applied to the soil, both the fresh and dry biomass weight increased. The plants treated with the highest N rates produced the greatest biomass, measured in fresh and dry weights, compared to the plants receiving non-application of N. In the field, higher rates of fertilizers were generally more effective, possibly due to the higher light intensity in the field compared to the greenhouse conditions. In addition, temperature rotation is crucial for plant growth, as the night temperature should be lower than the day temperature [45]. When the air temperature cooled at night, the shoot's growth declined, causing the roots to absorb more water and nutrients (particularly N), resulting in more growth during the day (Figure 1). Plant growth dynamics are significantly influenced by diurnal temperature variations, with cooler nighttime temperatures reducing transpiration and promoting nutrient uptake and root pressure. This nocturnal accumulation of resources facilitates a daytime growth surge when temperatures rise, allowing plants to optimize their growth by exploiting the distinct advantages of both nighttime and daytime conditions. Several studies have suggested that the quality, intensity, and photoperiod of light can significantly impact the way plants absorb and utilize nutrients [46–48]. The stimulation effects of applying N on vegetative growth may be attributed to the well-known functions of N in plant life. Higher N intake in the field has been shown to result in increased numbers of meristematic cells and their growth, as well as the growth of more shoots, leaves, and height [49]. Nitrogen is also an essential component of many metabolites, including amino acids, chlorophylls, coenzymes, enzymes, proteins, purines, and pyrimidines [50–52]. Furthermore, N applications increase the cytokinin levels within cells, leading to both an increase in the number of cells and their extensibility [41]. Therefore, it can be concluded that N may have directly or indirectly contributed to the proliferation of new cells and tissues, resulting in an increase in growth and yield characteristics [20]. Furthermore, plant protoplasm, which is the living matter found in cells and appears as a translucent substance, depends heavily on N as its primary building block. Nitrogen is vital for a range of plant functions, such as promoting flower differentiation, facilitating rapid shoot growth, maintaining the health of flower buds, enhancing the quality of the fruit set, and catalyzing the uptake of other essential minerals [53,54].

Although there is extensive research on the effects of N management in medicinal and aromatic plants in the field and greenhouse, information on *Z. clinopodioides* is limited. This research analyzed for the first time the cultivation of *Z. clinopodioides* under agronomically controlled conditions, examining the factors contributing to growth and productivity in an agricultural system. However, it is known that *Z. clinopodioides* is adaptable to harsh and

arid conditions, as well as infertile climates, thus finding the best agricultural management strategy is economically and environmentally beneficial.

Determining the optimal amount of N fertilizer for medicinal and aromatic plants has been a challenging task, as previous reports have shown that the best amount of N fertilizer varies among different medicinal and aromatic plants and does not follow a consistent pattern [55–57]. Reducing N sources in agricultural conditions, aimed at economic efficiency, can lead to the loss of a significant amount of yield from the field. This issue can be even more challenging for medicinal and aromatic plants such as *Z. clinopodioides*, which can have several stages of harvest each year. The present study's results showed a significant increase in both fresh and dry shoot weights when N was applied in field and greenhouse conditions. The higher biomass of plants grown in the field, as compared to those grown in the greenhouse, can be attributed to their more efficient uptake of N and higher N content in their shoots. This suggests that plants in the field can absorb N fertilizer more effectively. The best treatment under field conditions was 80 kg N ha^{-1}, while the best treatment in greenhouse conditions was 400 mg N pot^{-1}. The reason for the positive effect of N application on the N content compared to the control is obvious. As no N was applied to the control plants, they were completely dependent on the nutrient present in the soil. On the other hand, the treated plants received an adequate supply of this nutrient, ensuring ready availability and continuous uptake by the roots, followed by smooth translocation to the shoot and satisfactory distribution throughout the aerial parts, with effects on dry matter production and EO content and composition. Other studies on medicinal and aromatic plants, such as Azizi et al. [58], Bielski [59], Liava et al. [26], and Singh et al. [60], have shown similar results. Nitrogen plays a crucial role in increasing the number of meristematic cells, resulting in more leaves and branches [49]. Nitrogen is also a primary metabolic component involved in the synthesis of amino acids, nucleic acids, lipids, and enzymes, directly influencing cell division. Cell division plays an imperative role in enhancing the shoot length of plants [61]. Additionally, N fertilizer has been found to enhance plant height and biomass [62]. Previous studies on various crops, including tomato [63], soybean [64], cucumber [65,66], and wheat [67], have demonstrated that both the quantity and form of N significantly influence crop growth rates and yields. Research has consistently shown that the combined application of different N forms typically enhances crop growth and overall performance. N metabolism is a basic plant physiological process. The content of N among organs can reflect the nutrient status and physiological function of crop N [68]. Studies have shown a strong correlation between the applied dose of N fertilization in the soil and the N content in the shoot [69,70]. It was observed that increasing the N fertilizer dose led to a corresponding increase in the N content in the shoot (Figure 6). Among all the *Z. clinopodioides* plants examined, a dose of 120 kg N ha^{-1} in the field and 400 mg N pot-1 in the greenhouse significantly increased the N content in the shoot. Previous studies have shown that once the N application rates surpass a certain threshold, further increases in the N rate do not significantly enhance yield [43,71]. Our greenhouse study yielded similar results, supporting this observation.

Dry biomass production and EO content are crucial agronomic parameters for the commercial utilization of medicinal and aromatic plants. The EO yield (kg ha^{-1}) is directly correlated with biomass production. In this study, the EO content of the *Z. clinopodioides* crop exhibited an increase with the application of a higher N dosage. Consequently, the EO yield (kg ha^{-1}) also increased, due to the positive impact on EO content and dry biomass production. Similar positive effects of the N application on EO yield have also been documented for both field-grown [72] and greenhouse-grown [73] *Origanum* species. The EO content in medicinal and aromatic plants is the most important characteristic to consider. Based on the results of this research, the yield of EO increased with the application of N fertilizer, which is consistent with previous studies that found increased crop yield and EO production in response to higher levels of N fertilizer [74,75]. The non-application of N recorded the lowest value in both conditions. The beneficial effect of basal N application on EO levels could be attributed to the central role played by N in plants.

In *Z. clinopodioides*, leaves are the most important and productive site for the synthesis and accumulation of EO. Therefore, higher biomass, especially in leaves, can significantly improve the EO percentage and yield. Nitrogen fertilizer generally enhances leaf area development, biomass yields per unit area, leaf area development, the photosynthetic rate, and the photosynthesis rate in aromatic plants, thereby increasing the EO content and yield [60]. Previous studies on various crops under different cultivation conditions have demonstrated similar results regarding N fertilization. N fertilization positively impacted both yield and, consequently, EO content and production in several medicinal and aromatic plants, such as *Nigella sativa* [76], *Thymus vulgaris* [27], and *Oenothera biennis* [77], as well as in *Ocimum basilicum* [75] and *Mentha* spp. [60,74,78]. Higher levels of N have been shown to enlarge cells and induce succulence in plants, which could potentially explain the elongation of the EO glands and the higher EO content [79]. The highest EO content was recorded when 120 kg N ha^{-1} and 600 mg N pot^{-1} were applied. These results can be attributed to the direct involvement of N in the vegetative growth of the plant, resulting in an increased proportion of leafy parts in the herb. This led to a higher leaf-to-stem ratio compared to the non-application of N. Since EO is predominantly present in the leaves and flowers of *Z. clinopodioides*, plants treated with the N application and exhibiting a higher leaf + flower-to-stem ratio displayed an increased EO content compared to the control plants. These findings are consistent with the observations of Walia and Kumar [45] in *Tagetes.*

The optimal amount of N fertilizer can also affect the quality of EO in medicinal and aromatic plants, making it acceptable for international trade and industry usage. Ram et al. [80] observed that the major constituents of EO in *Pelargonium graveolens* (citronellol and geraniol) increased in response to the addition of N fertilizer (160 kg N ha^{-1}). In this study, in both the field and greenhouse conditions, the applications of 400 mg N pot^{-1} and 120 kg N ha^{-1} improved the quantity of EO (Table 2).

This study revealed that the utilization of N in both the field and greenhouse conditions significantly boosted the EO yields of *Z. clinopodioides*. This increase could possibly be attributed to the influence of N on enzymes that play a role in the production and metabolism of EOs. The findings also showed that N had a noteworthy impact on the EO compounds in both the field and greenhouse conditions, particularly for the dominant compounds. This effect could be attributed to the influence of N on enzymes that are involved in the biosynthesis of monoterpenes in the *Z. clinopodioides* [81]. In summary, the results suggest that the use of N can enhance the EO production of *Z. clinopodioides* and alter its EO composition, thus providing useful insights into improving the quality and quantity of EOs from this plant.

Significant differences were observed in the EO composition of *Z. clinopodioides* with different N doses. Our results showed that pulegone, menthone, neo-menthol, 1,8-cineole, and menthol were the major chemical components in *Z. clinopodioides* EO, consistent with previous studies conducted by Hazrati et al. [8] and Mohammad-hosseini [9]. The application of N fertilizers in our experiment led to significant alterations in the percentage composition of major components in *Z. clinopodioides* EO. Specifically, our results indicated that pulegone, as a major monoterpene found in the EO *Z. clinopodioides*, increased with adding N, with the maximum amount observed at 40 kg N ha^{-1} and 400 mg N pot^{-1} (Table 2). Pulegone is a precursor in the biosynthesis of menthol through menthone [82], and the most significant component of *Z. clinopodioides* EO. Developmental and environmental factors are known to greatly influence the yield and composition of *Z. clinopodioides* EO. For example, the EO content and the menthol content increase with leaf (and thus oil gland) maturity, and a range of stress conditions (related to light, temperature, and moisture status) tend to promote the accumulation of pulegone and menthofuran [83]. Our results showed that there was a significant increase in the pulegone content with the application of N and cultivation under field conditions, where the light intensity was higher and temperature fluctuations were more frequent. The application of N fertilizer may have enhanced the EO biosynthesis process by directly or indirectly influencing plant metabolism, resulting in the production of more plant metabolites, such as pulegone.

Nutrients such as N promote terpenoid emissions by promoting the electron transport rate and leaf photosynthesis, which provide the necessary ATP and carbon substrate for isoprene synthesis [84]. Since carbon-based secondary metabolites depend on CO_2 fixation, a relationship between nutrients and stored terpenoids can be explained [85]. According to the carbon nutrient balance hypothesis (CNBH), the availability of carbon and nutrients in the plant environment determines the production of metabolites. When nutrient resources are limited, plant growth is curtailed, resulting in an excess of carbohydrates. Under such conditions, the CNBH suggests that the surplus carbohydrates are not utilized for growth but rather serve as additional substrates for the synthesis of defense secondary metabolites [86]. As N becomes scarcer, differentiation becomes more prominent, leading to an increased accumulation of terpenoids at the expense of growth. This is because the plant allocates a higher proportion of the abundant resource, such as carbon, to acquire the scarce resource or to produce secondary metabolites, thereby promoting terpenoid synthesis [45,87].

Figure 10 depicts Geranyl diphosphate, an intermediate that can be converted by enzymes such as (−)-limonene synthase, linalool synthase, cineol synthase, (+)-*cis*-*iso*-pulegone isomerase, (−)-*iso*-piperitenone reductase, (+)-*cis*-*iso*-pulegone isomerase, cytochrome P450 (+)-MFS, (+)-PR (7), and (−)-menthone reductase into monoterpenes, such as (−)-limonene, linalool, 1,8-cineol, (−)-piperitone, (+)-*iso*-menthone, (+)-pulegone, and (−)-menthol. According to this study, the reduction in the levels of 1,8-cineole and *iso*-menthone may be due to a decrease in the enzyme activity involved in their biosynthesis, such as cineol synthase and (+)-menthone reductase. Moreover, the increased production of pulegone, menthol, neo-menthol, and other compounds due to the addition of N is a result of increased activity of the enzymes involved in their biosynthesis, such as (−)-*iso*-piperitenone reductase, (+)-*cis*-*iso*-pulegone isomerase, and (−)-menthone reductase.

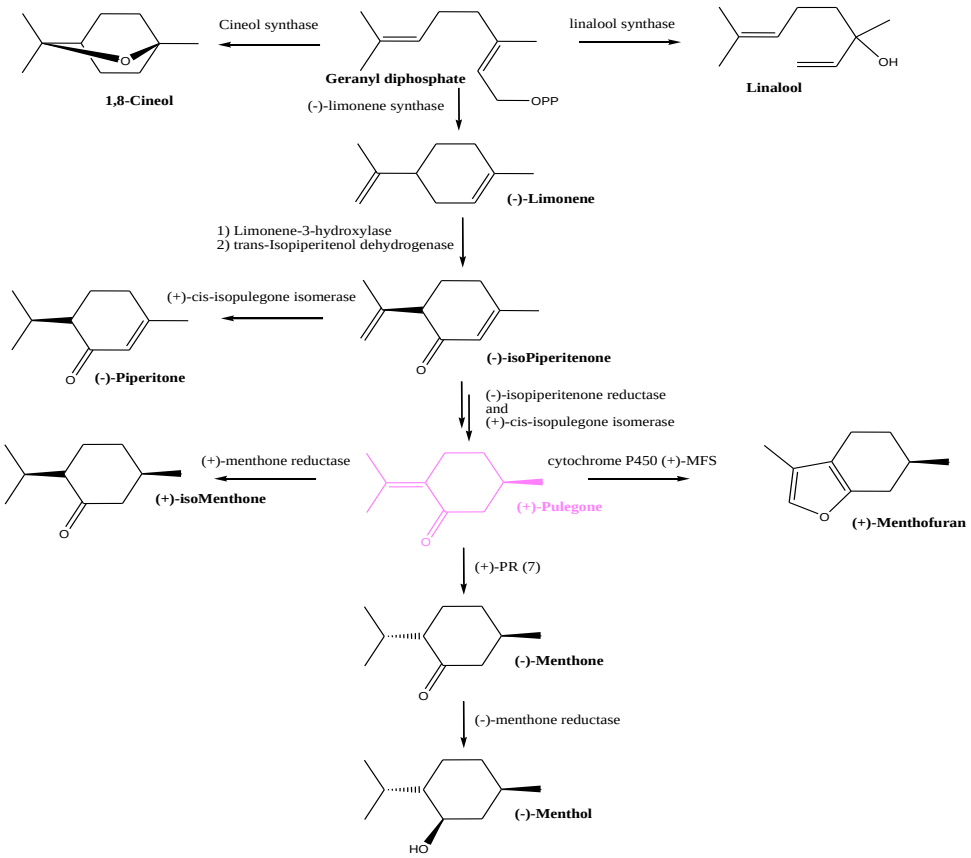

Figure 10. The principal pathways for monoterpene biosynthesis in *Z. clinopodioides*.

This study also found that oxygenated monoterpenes constitute the largest percentage of *Z. clinopodioides* EO, which was significantly enhanced by the addition of N. Nitrogen is an essential component of photosynthesis, providing a precursor for terpenoids [85] and increasing ATP and NADPH production, thereby increasing the synthesis of terpenoids [28]. Moreover, the addition of N fertilizer increases the monoterpene levels, particularly the oxygenated monoterpene levels [88].

5. Conclusions

Z. clinopodioides is a valuable medicinal and aromatic plant with numerous potential industrial applications. However, there is a scarcity of information regarding the ideal growth conditions for this plant. In our study, we found that N fertilization enhanced the fresh and dry biomass production and the EO content of *Z. clinopodioides*. These findings confirm that *Z. clinopodioides* can be cultivated in greenhouses and agricultural fields, even though its natural habitat is mostly in mountain areas. In the present study, *Z. clinopodioides* showed a positive response to N fertilization in both greenhouse and field cultivation. Moreover, higher N fertilization under field and greenhouse conditions significantly affected the production of pulegone, menthone, neo-menthol, and menthol. Comparing field and greenhouse conditions, we observed that *Z. clinopodioides* plants grown in fields were more influenced by their agronomic and phytochemical parameters, productivity, and EO accumulation. However, further research is needed to develop comprehensive management strategies for Z. *clinopodioides*. Future studies should investigate higher plant densities, the optimal harvesting times, and the nutritional requirements of the species, particularly in terms of different forms of N and other micro- and macronutrients. As the first study to evaluate the effects of N fertilization on the genus *Ziziphora*, these results provide valuable insights into the optimization of cultivation practices. This research addresses both conservation efforts and large-scale production challenges of this important medicinal and aromatic plant species, paving the way for its wider use in various industries.

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References

1. Selwal, N.; Rahayu, F.; Herwati, A.; Latifah, E.; Suhara, C.; Suastika, I.B.K.; Mahayu, W.M.; Wani, A.K. Enhancing secondary metabolite production in plants: Exploring traditional and modern strategies. *J. Agric. Food Res.* **2023**, *14*, 100702. [CrossRef]
2. Li, Y.; Kong, D.; Fu, Y.; Sussman, M.R.; Wu, H. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiol. Biochem.* **2020**, *148*, 80–89. [CrossRef] [PubMed]
3. Twaij, B.M.; Hasan, M.N. Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. *Int. J. Plant Biol.* **2022**, *13*, 4–14. [CrossRef]
4. Abdul, R.B. Medicinal plants (Importance and uses). *Pharm. Anal. Acta* **2012**, *3*, e139.
5. Ochatt, S.; Alan, A.R.; Bhattacharya, A.; Hano, C.; Kiselev, K.V.; Marconi, P.L.; Otoni, W.C.; Park, S.Y.; Tang, K.X.; Weathers, P.J. Secondary metabolites: A boon from plants, the best chemist in nature: Preface from the editors. *Plant Cell Tissue Organ Cult.* **2022**, *149*, 1–6. [CrossRef]

6. Jan, R.; Asaf, S.; Numan, M.; Kim, K.M. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy* **2021**, *11*, 968. [CrossRef]
7. Lombardo, S.; Restuccia, C.; Muratore, G.; Barbagallo, R.N.; Licciardello, F.; Pandino, G.; Scifò, G.O.; Mazzaglia, A.; Ragonese, F.; Mauromicale, G.O. Effect of nitrogen fertilisation on the overall quality of minimally processed globe artichoke heads. *J. Sci. Food Agric.* **2017**, *97*, 650–658. [CrossRef]
8. Hazrati, S.; Govahi, M.; Sedaghat, M.; Kashkooli, A.B. A comparative study of essential oil profile, antibacterial and antioxidant activities of two cultivated *Ziziphora* species (*Z. clinopodioides* and *Z. tenuior*). *Ind. Crops Prod.* **2020**, *157*, 112942. [CrossRef]
9. Mohammad-hosseini, M. The ethnobotanical, phytochemical, and pharmacological properties and medicinal applications of essential oils and extracts of different *Ziziphora* species. *Ind. Crops Prod.* **2017**, *105*, 164–192. [CrossRef]
10. Salehi, P.; Sonboli, A.; Eftekhar, F.; Nejad-Ebrahimi, S.; Yousefzadi, M. Essential oil composition, antibacterial and antioxidant activity of the oil and various extracts of *Ziziphora clinopodioides* subsp. rigida (BOISS.) RECH. f. from Iran. *Biol. Pharm. Bull.* **2005**, *28*, 1892–1896. [CrossRef]
11. Ozturk, S.; Ercisli, S. Antibacterial activity and chemical constitutions of *Ziziphora clinopodioides*. *Food Control* **2007**, *18*, 535–540. [CrossRef]
12. Šmejkal, K.; Malaník, M.; Zhaparkulova, K.; Sakipova, Z.; Ibragimova, L.; Ibadullaeva, G.; Žemlička, M. Kazakh *Ziziphora* species as sources of bioactive substances. *Molecules* **2016**, *21*, 826. [CrossRef]
13. Ahmadi, A.; Gandomi, H.; Derakhshandeh, A.; Misaghi, A.; Noori, N. Phytochemical composition and in vitro safety evaluation of *Ziziphora clinopodioides* Lam. ethanolic extract: Cytotoxicity, genotoxicity and mutagenicity assessment. *J. Ethnopharmacol.* **2021**, *266*, 113428. [CrossRef]
14. Asadi, A.; Cheniany, M. Enhancing effect of titanium dioxide nanoparticles on growth, phenolic metabolites production, and antioxidant potential of *Ziziphora clinopodioides* Lam. Russ. *J. Plant Physiol.* **2022**, *69*, 74.
15. Feizi, H.; Tahan, V.; Kariman, K. In vitro, antibacterial activity of essential oils from *Carum copticum* and *Ziziphora clinopodioides* plants against the phytopathogen *Pseudomonas syringae pv. syringae*. *Plant Biosyst.* **2023**, *157*, 487–492. [CrossRef]
16. Senejoux, F.; Demougeot, C.; Kerram, P.; Aisa, H.A.; Berthelot, A.; Bévalot, F.; Girard-Thernier, C. Bioassay-guided isolation of vasorelaxant compounds from *Ziziphora clinopodioides* Lam. (Lamiaceae). *Fitoterapia* **2012**, *83*, 377–382. [CrossRef]
17. Dhingra, A.K.; Chopra, B. Pulegone: An emerging oxygenated cyclic monoterpene ketone scaffold delineating synthesis, chemical reactivity, and biological potential. *Recent Adv. Anti-Infect. Drug Discov.* **2023**, *18*, 16–28.
18. da Silveira, N.S.; De Oliveira-Silva, G.L.; Lamanes, B.F.; Prado, L.C.; Bispo-da-Silva, L.B. The aversive, anxiolytic-like, and verapamil-sensitive psychostimulant effects of pulegone. *Biol. Pharm. Bull.* **2014**, *37*, 771–778. [CrossRef]
19. Hilfiger, L.; Triaux, Z.; Marcic, C.; Héberlé, E.; Emhemmed, F.; Darbon, P.; Marchioni, E.; Petitjean, H.; Charlet, A. Anti-hyperalgesic properties of menthol and pulegone. *Front. Pharmacol.* **2021**, *12*, 753873. [CrossRef]
20. Strzemski, M.; Dzida, K.; Dresler, S.; Sowa, I.; Kurzepa, J.; Szymczak, G.; Wójciak, M. Nitrogen fertilisation decreases the yield of bioactive compounds in *Carlina acaulis* L. grown in the field. *Ind. Crops Prod.* **2021**, *170*, 113698. [CrossRef]
21. Lombardo, S.; Pandino, G.; Mauromicale, G. Optimizing nitrogen fertilization to improve qualitative performances and physiological and yield responses of potato (*Solanum tuberosum* L.). *Agronomy* **2020**, *10*, 352. [CrossRef]
22. Pahalvi, H.N.; Rafiya, L.; Rashid, S.; Nisar, B.; Kamili, A.N. Chemical fertilizers and their impact on soil health. In *Microbiota and Biofertilizers*; Ecofriendly Tools for Reclamation of Degraded; Springer: Berlin/Heidelberg, Germany, 2021; Volume 2, pp. 1–20.
23. Muhammad, I.; Lv, J.Z.; Yang, L.; Ahmad, S.; Farooq, S.; Zeeshan, M.; Zhou, X.B. Low irrigation water minimizes the nitrate nitrogen losses without compromising the soil fertility, enzymatic activities and maize growth. *BMC Plant Biol.* **2022**, *22*, 159. [CrossRef]
24. Ayub, M.; Naeem, M.; Nadeem, M.A.; Tanveer, A.; Tahir, M.; Alam, R. Effect of nitrogen application on growth, yield, and oil contents of Fennel (*Foenoculum vulgare* Mill.). *J. Med. Plant Res.* **2011**, *5*, 2274–2277.
25. Pavela, R.; Žabka, M.; Vrchotová, N.; Tříska, J. Effect of foliar nutrition on the essential oil yield of Thyme (*Thymus vulgaris* L.). *Ind. Crops Prod.* **2018**, *112*, 762–765. [CrossRef]
26. Liava, V.; Karkanis, A.; Tsiropoulos, N. Yield and silymarin content in milk thistle (*Silybum marianum* (L.) Gaertn.) fruits affected by the nitrogen fertilizers. *Ind. Crops Prod.* **2021**, *171*, 113955. [CrossRef]
27. Baranauskienė, R.; Venskutonis, P.R.; Viškelis, P.; Dambrauskienė, E. Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). *J. Agric. Food Chem.* **2003**, *51*, 7751–7758. [CrossRef]
28. Seif Sahandi, M.; Naghdi Badi, H.; Mehrafarin, A.; Khalighi-Sigaroodi, F.; Sharifi, M. Changes in essential oil content and composition of peppermint (*Mentha piperita* L.) in responses to nitrogen application. *J. Med. Plants* **2019**, *18*, 81–97. [CrossRef]
29. Amiri, F.; Gholipouri, A.; Kheirkhah, M.; Mirjalili, M.H. Eco-physiology of *Ziziphora clinopodioides* Lam. (Lamiaceae) from Iran. *Ann. Bot.* **2019**, *9*, 63–72.
30. Nelson, D.W.; Sommers, L.E. Dry combustion method using medium temperature resistance furnace. *Chem. Microbiol. Properties* **1982**, *2*, 539–579.
31. Chapman, H.D. Cation-exchange capacity. In *Methods of Soil Analysis—Chemical and Microbiological Properties*; Black, C.A., Ed.; American Society of Agronomy: Madison, WI, USA, 1965; Volume 9, pp. 891–901.

32. Novamsky, I.; Van Eck, R.; Van Schouwenburg, J.C.H.; Walinga, I. Total nitrogen determination in plant material by means of the indophenol blue method. *Neth. J. Agri. Sci.* **1974**, *22*, 3–5. [CrossRef]
33. Knudsen, D.; Peterson, G.A.; Pratt, P.F. Lithium, sodium, and potassium. In *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties*; Page, A.L., Ed.; American Society of Agronomy: Madison, WI, USA, 1982; Volume 1, pp. 225–246.
34. Røtset, O. Determination of phosphate species in nutrient solutions and phosphorus in plant material as phosphovanadomolybdic acid by flow injection analysis. *Anal. Chim. Acta* **1985**, *178*, 217–221. [CrossRef]
35. Chapman, H.D.; Pratt, P.F. Methods of analysis for soils, plants and waters. *Soil. Sci.* **1962**, *93*, 68. [CrossRef]
36. Horváth, B.; Opara-Nadi, O.; Beese, F. A simple method for measuring the carbonate content of soils. *Soil. Sci. Soc. Am. J.* **2005**, *69*, 1066–1068. [CrossRef]
37. British Pharmacopoeia. *Published on the Recommendation of the Medicines Commission Pursuant to the Medicines Act*; Her Majesty's Stationary Office: London, UK, 1968.
38. Adams, P.R. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured Publishing Corporation, Carol Stream: DuPage, IL, USA, 2007.
39. Toor, M.D.; Adnan, M.; Rehman, F.U.; Tahir, R.; Saeed, M.S.; Khan, A.U.; Pareek, V. Nutrients and their importance in agriculture crop production; A review. *Ind. J. Pure App. Biosci.* **2021**, *9*, 1–6. [CrossRef]
40. He, X.; Wang, Y. Effects of regulated deficit irrigation on soil nutrient, microorganism and enzyme activity of isatis indigotica. In Proceedings of the Asia Conference on Geological Research and Environmental Technology, Kamakura City, Japan, 10–11 October 2020; IOP Publishing: Bristol, UK, 2021.
41. Gu, J.; Li, Z.; Mao, Y.; Struik, P.C.; Zhang, H.; Liu, L.; Yang, J. Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. *Plant Sci.* **2018**, *274*, 320–331. [CrossRef]
42. Torres-Bazurto, J.; Magnitskiy, S.; Sánchez, J.D. Effect of fertilization with N on height, number of leaves, and leaf area in banana (*Musa AAA Simmonds*, cv. Williams). *Rev. Colomb. Cienc. Hortic.* **2019**, *13*, 9–17. [CrossRef]
43. Ahmadi, F.; Samadi, A.; Rahimi, A. Improving growth properties and phytochemical compounds of *Echinacea purpurea* (L.) medicinal plant using novel nitrogen slow release fertilizer under greenhouse conditions. *Sci. Rep.* **2020**, *10*, 13842. [CrossRef]
44. Yao, Z.; Zhang, W.; Wang, X.; Zhang, L.; Zhang, W.; Liu, D.; Chen, X. Agronomic, environmental, and ecosystem economic benefits of controlled-release nitrogen fertilizers for maize production in Southwest China. *J. Clean. Prod.* **2021**, *312*, 127611. [CrossRef]
45. Walia, S.; Kumar, R. Nitrogen and sulfur fertilization modulates the yield, essential oil and quality traits of wild marigold (*Tagetes minuta* L.) in the Western Himalaya. *Front. Plant Sci.* **2021**, *11*, 631154. [CrossRef]
46. Liang, Y.; Cossani, C.M.; Sadras, V.O.; Yang, Q.; Wang, Z. The interaction between nitrogen supply and light quality modulates plant growth and resource allocation. *Front. Plant Sci.* **2022**, *13*, 864090. [CrossRef]
47. Sharma, P.K.; Kumar, S. Soil Temperature and Plant Growth. In *Soil Physical Environment and Plant Growth*; Springer: Cham, Switzerland, 2023.
48. Hatfield, J.L.; Prueger, J.H. Temperature extremes: Effect on plant growth and development. *Weather. Clim. Extrem.* **2015**, *10*, 4–10. [CrossRef]
49. Lawlor, D.W. Carbon and nitrogen assimilation in relation to yield: Mechanisms are the key to understanding production systems. *J. Exp. Bot.* **2002**, *53*, 773–787. [CrossRef]
50. Zayed, O.; Hewedy, O.A.; Abdelmoteleb, A.; Ali, M.; Youssef, M.S.; Roumia, A.F.; Seymour, D.; Yuan, Z.-C. Nitrogen Journey in Plants: From Uptake to Metabolism, Stress Response, and Microbe Interaction. *Biomolecules* **2023**, *13*, 1443. [CrossRef]
51. Ohyama, T. Nitrogen as a major essential element of plants. *Nitrogen Assim. Plants* **2010**, *37*, 1–17.
52. Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press. Inc.: New York, NY, USA, 2002.
53. Lombardo, S.; Pandino, G.; Mauromicale, G. Minerals profile of two globe artichoke cultivars as affected by NPK fertilizer regimes. *Food Res. Int.* **2017**, *100*, 95–99. [CrossRef]
54. Tehulie, N.S.; Eskezia, H. Effects of nitrogen fertilizer rates on growth, yield components and yield of food Barley (*Hordeum vulgare* L.): A Review. *J. Plant Sci. Agric. Res.* **2021**, *5*, 46.
55. Wang, L.; Yang, L.; Xiong, F.; Nie, X.; Li, C.; Xiao, Y.; Zhou, G. Nitrogen fertilizer levels affect the growth and quality parameters of *Astragalus mongolica*. *Molecules* **2020**, *25*, 381. [CrossRef]
56. Daneshian, A.; Gurbuz, B.; Cosge, B.; Ipek, A. Chemical Components of Essential Oils from Basil (*Ocimum basilicum* L.) Grown at Different Nitrogen Levels. *Int. J. Nat. Eng. Sci.* **2009**, *3*, 9–13.
57. Rioba, N.B.; Itulya, F.M.; Saidi, M.; Dudai, N.; Bernstein, N. Effects of nitrogen, phosphorus and irrigation frequency on essential oil content and composition of sage (*Salvia officinalis* L.). *J. Appl. Res. Med. Aromat. Plants* **2015**, *2*, 21–29. [CrossRef]
58. Azizi, A.; Yan, F.; Honermeier, B. Herbage yield, essential oil content, and composition of three Oregano (*Origanum vulgare* L.) populations as affected by soil moisture regimes and nitrogen supply. *Ind. Crops Prod.* **2009**, *29*, 554–561.
59. Bielski, S. Milk thistle (*Silybum marianum* L. Gaertn.) achene yield had a positive response to nitrogen fertilization, row spacing, sowing date, and weed control methods. *Ind. Crops Prod.* **2021**, *160*, 113104. [CrossRef]
60. Singh, M.; Khan, M.M.A.; Naeem, M. Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield and quality attributes in *Zingiber officinale* Rosc. *J. Saudi Soc. Agric.* **2016**, *15*, 171–178. [CrossRef]

61. Kishorekumar, R.; Bulle, M.; Wany, A.; Gupta, K.J. An overview of important enzymes involved in nitrogen assimilation of plants. *Methods Mol. Biol.* **2020**, *2057*, 1–13.
62. Xin, C.; Yan, Q.-W.; Sun, J.-L.; Xiao, S.; Xie, F.-C.; Chen, Y.-J. Research progress on nitrogen use and plant growth. *J. Northeast Agric. Univ.* **2014**, *21*, 68–74. [CrossRef]
63. Sun, J.; Jin, L.; Li, R.; Meng, X.; Jin, N.; Wang, S.; Xu, Z.; Liu, Z.; Lyu, J.; Yu, J. Effects of Different Forms and Proportions of Nitrogen on the Growth, Photosynthetic Characteristics, and Carbon and Nitrogen Metabolism in Tomato. *Plants* **2023**, *12*, 4175. [CrossRef]
64. Yamashita, N.; Tanabata, S.; Ohtake, N.; Sueyoshi, K.; Sato, T.; Higuchi, K.; Saito, A.; Ohyama, T. Effects of Different Chem-ical Forms of Nitrogen on the Quick and Reversible Inhibition of Soybean Nodule Growth and Nitrogen Fixation Activity. *Front. Plant Sci.* **2019**, *10*, 131. [CrossRef]
65. Mi, W.; Hong, Y.; Gao, F.; Ma, Y.; Sun, T.; Wu, L.; Wang, G.; Chen, S. Effect of Different Form of N Fertilization on Yield Sustainability and Soil Quality in Double Cropped Rice System in a Long-Term Experiment. *J. Soil. Sci. Plant Nutr.* **2024**, *24*, 2815–2824. [CrossRef]
66. Ma, C.; Ban, T.T.; Gao, Y.N.; Yu, H.J.; Li, Q.; Li, X.H.; Deng, Y.; Jiang, W.J. Different nitrogen forms on the growth and nitrogen utilization of cucumber. *J. Agric. Crop Res* **2020**, *8*, 90–97. [CrossRef]
67. Cui, H.; Luo, Y.; Li, C.; Chang, Y.; Jin, M.; Li, Y.; Wang, Z. Effects of nitrogen forms on nitrogen utilization, yield, and quality of two wheat varieties with different gluten characteristics. *Eur. J. Agron.* **2023**, *149*, 126919. [CrossRef]
68. Shen, N.S. Effects of different nitrogen forms and water stress on water absorption, photosynthesis and growth of *Oryza sativa* seedlings. *Chin. Bull. Bot.* **2007**, *24*, 477.
69. Ali, A.M.; Saudi, A.M.; El-Sadek, A.N. Developing a nitrogen fertilizer management model for wheat in calcareous soils using the critical nitrogen dilution curve. *Nutr. Cycl. Agroecosyst.* **2023**, *125*, 379–392. [CrossRef]
70. Geisseler, D.; Ortiz, R.S.; Diaz, J. Nitrogen nutrition and fertilization of onions (*Allium cepa* L.)–A literature review. *Sci. Hortic.* **2022**, *291*, 110591. [CrossRef]
71. Li, S.; Lei, Y.; Zhang, Y.; Liu, J.; Shi, X.; Jia, H.; Wang, C.; Chen, F.; Chu, Q. Rational trade-offs between yield increase and fertilizer inputs are essential for sustainable intensification: A case study in wheat–maize cropping systems in China. *Sci. Total Environ.* **2019**, *679*, 328–336. [CrossRef]
72. Ninou, E.; Cook, C.M.; Papathanasiou, F.; Aschonitis, V.; Avdikos, I.; Tsivelikas, A.L.; Stefanou, S.; Ralli, P.; Mylonas, I. Nitrogen effects on the essential oil and biomass production of field grown Greek oregano (*Origanum vulgare* subsp. hirtum) populations. *Agronomy* **2021**, *11*, 1722. [CrossRef]
73. Ninou, E.G.; Paschalidis, K.A.; Mylonas, I.G.; Vasilikiotis, C.; Mavromatis, A.G. The effect of genetic variation and nitrogen fertilization on productive characters of Greek oregano. *Acta Agric. Scand B Soil Plant Sci.* **2017**, *67*, 372–379. [CrossRef]
74. Abbaszadeh, B.; Farahani, H.A.; Valadabadi, S.A.; Moaveni, P. Investigation of variations of the morphological values and flowering shoot yield in different mint species at Iran. *J. Hortic. For.* **2009**, *1*, 109–112.
75. Arabaci, O.; Bayram, E. The effect of nitrogen fertilization and different plant densities on some agronomic and technologic characteristic of *Ocimum basilicum* L. (Basil). *J. Agron.* **2004**, *3*, 255–262. [CrossRef]
76. Ashraf, M.; Ali, Q.; Iqbal, Z. Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. *J. Sci. Food Agric.* **2006**, *86*, 871–876. [CrossRef]
77. Şekeroğlu, N.; Özgüven, M. Effects of different nitrogen doses and row spacing applications on yield and quality of *Oenothera biennis* L. grown in irrigated lowland and unirrigated dryland conditions. *Turk. J. Agric. For.* **2006**, *30*, 125–135.
78. Zheljazkov, V.; Margina, A. Effect of increasing doses of fertilizer application on quantitative and qualitative characters of mint. *Int. Symp. Med. Aromat. Plants* **1995**, *426*, 579–592. [CrossRef]
79. Said-Al Ahl, H.A.; Hussien, M.S. Effect of nitrogen and phosphorus application on herb and essential oil composition of *Satureja montana* L. 'carvacrol'chemotype. *J. Chem. Pharm. Res.* **2016**, *8*, 119–128.
80. Ram, M.; Ram, D.; Roy, S.K. Influence of an organic mulching on fertilizer nitrogen use efficiency and herb and essential oil yields in geranium (*Pelargonium graveolens*). *Bioresour. Technol.* **2003**, *87*, 273–278. [CrossRef]
81. Koeduka, T.; Fridman, E.; Gang, D.R.; Vassão, D.G.; Jackson, B.L.; Kish, C.M.; Pichersky, E. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10128–10133. [CrossRef]
82. Li, Y.; Liu, Y.; Ma, A.; Bao, Y.; Wang, M.; Sun, Z. In Vitro antiviral, anti-inflammatory, and antioxidant activities of the ethanol extract of *Mentha piperita* L. *Food Sci. Biotechnol.* **2017**, *26*, 1675–1683. [CrossRef]
83. Mahmoud, S.S.; Croteau, R.B. Menthofuran regulates essential oil biosynthesis in peppermint by controlling a downstream monoterpene reductase. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14481–14486. [CrossRef]
84. Ormeño, E.; Baldy, V.; Ballini, C.; Fernandez, C. Production and diversity of volatile terpenes from plants on calcareous and siliceous soils: Effect of soil nutrients. *J. Chem. Ecol.* **2008**, *34*, 1219–1229. [CrossRef]
85. Ormeno, E.; Fernandez, C. Effect of soil nutrient on production and diversity of volatile terpenoids from plants. *Curr. Bioact. Compd.* **2012**, *8*, 71–79.
86. Bustamante, M.Á.; Michelozzi, M.; Barra Caracciolo, A.; Grenni, P.; Verbokkem, J.; Geerdink, P.; Safi, C.; Nogues, I. Effects of soil fertilization on terpenoids and other carbon-based secondary metabolites in Rosmarinus officinalis Plants: A Comparative Study. *Plants* **2020**, *9*, 830. [CrossRef]

87. Lorio, P.L. Growth-differentiation balance—A basis for understanding southern pine-beetle tree interactions. *For. Ecol. Manag.* **1986**, *14*, 259–273. [CrossRef]
88. Burney, O.T.; Davis, A.S.; Jacobs, D.F. Phenology of foliar and volatile terpenoid production for *Thuja plicata* families under differential nutrient availability. *Environ. Exp. Bot.* **2012**, *77*, 44–52. [CrossRef]