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# Optimizing Hyssop (*Hyssopus officinalis* L.) Cultivation: Effects of Different Manures on Plant Growth and Essential Oil Yield

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**Abstract:** Using animal manure in organic systems can improve the quality of agricultural products, especially medicinal plants. In this study, the impact of different types and levels of animal manures on hyssop plant biomass and essential oil yield and profile was assessed. Three supply levels (Low, Medium, and High) were tested for poultry (Np), sheep (Ns), and cattle (Nc) manures. Through GC-MS and GC-FID analysis, 24 chemical constituents were identified in the hyssop essential oil, accounting for 93.7–97.8% of the total composition. The Medium-Nc and High-Np treatments had essential oil content ranging from 0.98% to 1.45%, significantly different from the control treatment at 1.17%. Essential oil yield in Low-Np, Medium-Np, and High-Np was 47.5, 53.8, and 49.2 kg ha$^{-1}$, respectively, showing increases of 42.5%, 61.6%, and 47.7% compared to the control. Medium-Nc and High-Nc treatments had the most potent antioxidant properties compared to the control. Different amounts of poultry, sheep, and cattle manures led to distinct differences in essential oil compounds, categorizing the manure treatments into three groups. Medium-Np had 44% more air-dried biomass than the control, while no significant difference was found in air-dried herbal product levels among sheep and cattle manures. Taken together, farmers focusing on biomass and essential oil should opt for Medium poultry manure. The pharmaceutical industry should explore other fertilizer options based on secondary metabolite needs.

**Keywords:** antioxidant capacity; volatile oil; *Hyssopus officinalis*; yield

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## 1. Introduction

Medicinal plant growers rely heavily on fertilization to meet the demands of a rapidly growing worldwide population [1]. Extensive research has been conducted to explore the most different aspects of this practice, evaluating the effect of fertilization on the yield and qualitative features of many medicinal plants.

Medicinal plants are increasingly cultivated with organic methods. Besides allowing their entrance into a particular market sector, enabling farmers to earn a higher income, organic cultivation is considered the best option to exploit herbal products. As a result, it is strongly advised that natural fertilizers be used to improve the excellence and efficiency of medicinal plants.

Hence, organic fertilization of medicinal plants is an expanding research sector. Many papers have addressed this topic [2–5], and in many cases, studies have documented a rise in the productivity of medicinal plants by utilizing organic fertilizers.

Besides plant productivity, also the quality aspects of production are crucial in medicinal plants. Medicinal plants are highly valued in industries worldwide, and those with elevated levels of secondary metabolites are especially prized [6].

Among the suggested organic fertilizers, manure is one of the most widely used.

Throughout history, manure has played a vital role in supplying crucial nutrients for plant growth, and it is widely recognized that its unique chemical and physical features make it an invaluable resource for improving soil conditions [7]. Animal manure from sources like chicken, sheep, and cow provides vital nutrients for plants and enhances soil quality by positively impacting its physical, chemical, and biological aspects. This creates ideal conditions for the growth of plants [1,8], ultimately leading to higher productivity while minimizing adverse effects on human well-being and the ecosystem.

In the cultivation of medicinal plants, animal manure was found not only to promote plant growth and productivity, but also to enhance the quality of the end products like essential oils [1,9].

Hyssop (*Hyssopus officinalis* L.), a member of the *Lamiaceae* family, is indigenous to Southern Europe, the Middle East, and the region encompassing the Caspian Sea. It is extensively cultivated in several European nations, including Italy, Spain, Russia, and France [10]. Despite its strong peculiar flavor, hyssop is employed as a culinary enhancer and is incorporated into a variety of sauces [11]. This plant holds numerous applications in traditional and modern medicine, serving as an expectorant, diuretic, and appetizer. Moreover, it exhibits beneficial effects in treating bronchitis, herpes simplex, asthma, and HIV [12]. In Iran, hyssop stands as one of the most significant medicinal plants, with its aerial parts being employed for the treatment of asthma, ulcers, wounds, bronchitis, and cough, as well as possessing antiseptic, carminative, and antimicrobial properties [13].

According to the existing literature, the essential oil profile of hyssop primarily consists of monoterpenes, with over 80% being composed of either oxygenated or hydrocarbon types. The dominant class of the essential oil profile is the oxygenated monoterpenes, accounting for a range of 43.6% to 63%. Among these, pinocamphone (both cis- and trans-isomers), iso-pinocamphone, 1,8-cineole, and β-pinene make up more than 70% of the essential oil profiles of hyssop. Another significant component is elemol, which ranges from 1.9% to 11.3% and is the main sesquiterpene in the plant's essential oils [14–16].

The effect of organic and chemical fertilization on hyssop yield and phytochemical traits has been targeted by only a few studies. According to Naderi et al. [17], the application on hyssop of a conventional NPK fertilizer (50:40:50) resulted in an essential oil percentage of 1.7%, with no noteworthy variation compared to the control. Otherwise, the use of cow manure resulted in the highest oil yield of 5.4 kg $ha^{-1}$, surpassing the control, which yielded 3.7 kg $ha^{-1}$ essential oil. Moreover, supplying the NPK fertilizer resulted in the highest iso-pinocamphone value (43.3%) in plants, compared to the control treatment, which had a value of 39.3%. The control treatment exhibited the highest value of pinocamphone (14.1%), whereas the cattle manure treatment had a value of 11.7%. Honorato et al. [18] demonstrated that the application of organic fertilizers significantly improved both the yield and chemical profile of essential oils in thyme (*Thymus vulgaris* L.). Specifically, plants treated with 9 kg $m^{-2}$ of cattle manure in conjunction with 3 kg $m^{-2}$ of green manure achieved the highest concentration of thymol, recorded at 65.4%. Moreover, the use of these organic amendments not only enhanced the production of essential oils but also increased the antioxidant properties of thyme. In a related study, Mwithiga et al. [19] found that the essential oil yield from rosemary (*Rosmarinus officinalis* L.) did not show significant variation when cultivated under conditions of cattle manure alone, cattle manure combined with fertilizer, or fertilizer alone. Nonetheless, the quality of rosemary oil was notably improved with the application of cattle manure compared to other soil treatments. Additionally, Fallah et al. [20] reported that during the first harvest of *Dracocephalum kotschyi*, the highest levels of neral (28.2%) and geranial (26.8%) were recorded in plants treated with sheep manure and chemical fertilizer, respectively. The untreated plants also produced a significant amount of α-pinene (15.5%), which was comparable to the levels found in the poultry manure treatment.

The scarcity of water resources in Iranian agriculture has led to alterations in cultivation practices and a focus on drought-resistant plants. Consequently, the hyssop plant has emerged as a viable choice due to its ability to withstand drought. However, many issues have to be addressed to explore the effects of usual agricultural practices on hyssop yield and chemical patterns. Among these practices, fertilization plays a crucial role, particularly when non-chemical inputs are considered. Hence, this research aims to assess the impact of various types and levels of organic fertilizers on the biomass yield of hyssop, as well as on the chemical profile and antioxidant activity of its essential oil.

## 2. Materials and Methods

### *2.1. Site Description*

The field experiment was conducted in Koohrang (50°16′ E; 32°29′ N), Chaharmahal and Bakhtiari Province, Iran, in 2018–2019. The study site, located at an elevation of 2234 m a.s.l., experiences a cold semi-arid climate, characterized by mild winters and relatively humid and hot summers. Details regarding the monthly average temperature and total monthly precipitation have been previously documented by our research team [1].

### *2.2. Field Experiment, Growth Conditions, and Treatments*

The tested animal manures were obtained from livestock farms in the vicinity of the research location in the Koohrang region.

Three supply levels (Low, Medium, and High) were tested for poultry (Np), sheep (Ns), and cattle (Nc) manures (Table 1). The determination of fertilizer levels was based on the nitrogen needs of the plant, the nitrogen content in animal manure, and the nitrogen mineralization range. The hyssop plant's nitrogen requirement was established at 100 kg per hectare in accordance with regional practices. The nitrogen content of each fertilizer was determined through analysis. Additionally, the nitrogen mineralization of manures was categorized into three levels: low (40%), medium (70%), and high (100%). Therefore, the amount of each manure was calculated from the following equation.

$$\text{Manure level}\left(\text{Mgha}^{-1}\right) = \frac{100}{\text{N}_{\text{manure}}(\%) \times \text{N}_{\text{mineralization}}(\%)} \tag{1}$$

**Table 1.** Types and doses of animal manures tested on hyssop in 2019.

| Treatment | Dose (Mg ha$^{-1}$) | | |
|---|---|---|---|
| | **Low** | **Medium** | **High** |
| Poultry (Np) | 2.2 | 3.2 | 5.6 |
| Sheep (Ns) | 3.8 | 5.4 | 9.5 |
| Cattle (Nc) | 4.3 | 6.2 | 10.8 |
| Control (C) | | No manure | |

This study was implemented using a randomized complete block design with three replications. Each elementary plot had an area of 3 × 3 m = 9 m$^2$. The spacing between rows and between plants within rows was consistently maintained at 50 cm. The plots were positioned 1 m apart, while replications (blocks) were spaced 2 m apart.

The transplants selected for the experiment were developed by growing the seeds sourced from the local hyssop genotype in a greenhouse for a span of 45 days. Upon completion of the nursery period, the height of the transplants was 10 cm. On 23 April 2018, hyssop transplants were planted into the experimental plots, followed by adding animal manures near the plant roots on 24 April 2019. After manuring, drip tape irrigation was promptly implemented, and weekly irrigation sessions were carried out until the harvest, with the volume of water applied being dependent on the capacity of the field crop. Manual weeding activities were carried out multiple times throughout the growth period. Additional treatments were deliberately omitted from the experimental field.

Soil samples were collected randomly to analyze the physical and chemical properties of the field before seedling transplantation.

The soil used for the trial exhibited a clayey texture, with a pH of 7.75; some salient characteristics of the soil, as well as the supplied types of animal manure, are reported in Table 2. The animal manures utilized were not subject to salinity limitations, as the quantity of manure incorporated was minimal in relation to the soil volume within the plant's rhizosphere area. Poultry manure contained double the nitrogen content compared to cattle and sheep manures, while sheep manure had a higher potassium content than cattle and poultry manures.

**Table 2.** Main traits of soil and animal manures tested on hyssop in 2019.

| Source | Electrical Conductivity (μS cm$^{-1}$) | Organic Carbon (g kg$^{-1}$) | N (%) | P (%) | K (%) |
|---|---|---|---|---|---|
| Soil | 760 | 7.6 | 0.08 | $8 \times 10^{-4}$ | $328 \times 10^{-4}$ |
| Np | 4750 | 312 | 4.5 | 1.70 | 9.8 |
| Ns | 4380 | 175 | 2.6 | 0.59 | 12.5 |
| Nc | 1980 | 195 | 2.3 | 0.56 | 6.2 |

Np: poultry manure; Ns: sheep manure; Nc: cow manure.

### *2.3. Field Measurements on Plants*

#### 2.3.1. Air-Dried Biomass

The shoots of hyssop plants were harvested at the early flowering stage on 17 June 2019 (54 days after manuring). Harvesting was carried out manually by using a sickle to cut all the plants in each plot from a height of 5 cm above the soil surface. The plants were dried in a shaded environment post-cutting to ensure the moisture content reached a stabilized level of 8%. Subsequently, the above-ground, air-dried herbal product was quantified in kilograms per hectare.

#### 2.3.2. Isolation of Essential Oil

The essential oil (EO) was extracted using the hydro-distillation technique. Fifty grams of crushed samples were soaked in 500 milliliters of distilled water and subjected to hydro-distillation for 3.5 h using a Clevenger apparatus, following the guidelines outlined in the British Pharmacopoeia [21]. The obtained EO was dehydrated with anhydrous sodium sulfate and stored in opaque vials at 4 °C until further analysis. The determination of the EO yield was achieved by multiplying the above-ground dry matter (in kg ha$^{-1}$) by the EO content (in g kg$^{-1}$), and the result was expressed in kilograms per hectare.

#### 2.3.3. Analysis of EO Samples: Qualitative and Semi-Quantitative Approaches

The EO samples were analyzed by means of gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). For the GC analysis, a Thermo-UFM gas chromatography system equipped with a flame ionization detector (FID) and a Ph-5 column (10 m × 0.10 mm i.d., film thickness 0.25 μm) was utilized. The EO samples from each treatment were injected into the GC/FID system. Helium was used as the carrier gas, flowing at a rate of 0.5 mL/min. The oven temperature varied from 60 to 285 °C, while both the injector and FID detector were maintained at a constant temperature of 280 °C. On the other hand, the GC/MS analysis was performed using an Agilent 7890A/5975C GC-MS system with a DB-5 fused silica column. The EO samples from different treatments were injected into the GC/FID system for quantification of compounds, while one sample was injected into GC/MS for identification of compositions. The oven temperature and other parameters were set according to the established protocol. To identify the chemical constituents of the EO samples, their mass spectra were compared with those stored in the computer library or obtained from authentic components. Additionally, retention indices (RI) were determined by comparing the retention times of n-alkanes (C8–C24) injected under the same conditions. The identities of the chemical components were confirmed using relative retention indices and references from the literature [22–24]. The sample's component mass fraction was established through the utilization of the percentage normalization technique [22].

#### 2.3.4. Antioxidant Capacity

The antioxidant capacity of the samples was assessed using the DDPH assay, following the procedure outlined by Fallah et al. [20].

In detail, 20 µL of EO from each treatment were mixed with 100 µL of 0.5 mM 2,2-diphenyl-1-picrylhydrazil (DPPH solution) in methanol. The resulting mixture was then adjusted to a final volume of 200 µL through microdilution. Subsequently, the mixture was vigorously agitated and placed in a dark environment at room temperature for 15 min. To determine the absorbance of the solutions, which included a blank (lacking sample) and various concentrations, an Awareness Technology, Inc., Elisa reader was used at a wavelength of 517 nm. The calculation of the inhibition percentage for the samples was carried out following the formula proposed by Xiao et al. [25]:

$$\text{Inhibition} = ((\text{AB} - \text{AA})/\text{AB}) \times 100 \quad (2)$$

where AB and AA denote the absorbance values of the DPPH radical in the absence and presence of the EO sample, respectively. Resulting inhibition percentages were then graphed against the sample concentrations. To ascertain the IC50 value, indicating the concentration at which 50% inhibition occurs, linear regression analysis was applied. The IC50 value is employed for assessing the antioxidant capability, where lower values are indicative of stronger antioxidant power [26].

### *2.4. Statistical Analysis*

The Statistical Analysis System software (SAS, Version 9.1) was utilized for data analysis. The means were compared, and significant variances were identified using the LSD test at a significance level of $p < 0.05$. Various parameters, such as biomass production, antioxidant capacity, EO content, EO yield, and EO compositions, were evaluated with three replicates in each treatment. Hierarchical clustering dendrogram and heat map for some essential oil compounds of hyssop under different levels of animal manures were done by Heatmapper (web-enabled heat mapping for all) [27] that were significantly different in percentage ($p < 0.05$ after LSD post-hoc testing).

## 3. Results

### *3.1. Biomass Production*

The amount of air-dried biomass in the medium-Np was 1.44-times higher than the control treatment ($p < 0.05$; Figure 1). The amount of air-dried biomass in the lower and upper levels of poultry manure was 15.8–26.6% higher than the control treatment, but statistically, there was no significant difference with the control treatment ($p > 0.05$; Figure 1). No significant difference was observed among different levels of sheep and cattle manures in terms of air-dried herbal product. Also, the biomass production at varying levels of sheep and cattle manures was similar ($p > 0.01$; Figure 1).

### *3.2. Essential Oil Content*

The content of essential oil in different manure treatments, except for High-Np and Medium-Nc, was similar to the control treatment ($p > 0.05$; Figure 2). The content of essential oil in poultry manure at low and medium levels (1.30–1.31%) was comparable to that of sheep manure at various levels (1.20–1.22%), as well as the low and high levels of cattle manure (1.10–1.26%) ($p > 0.05$; Figure 2). The essential oil content ranged from 0.98% to 1.45% in the High-Np and Medium-Nc treatments, respectively, which were significantly different from the control treatment (1.17%) ($p < 0.05$; Figure 2).

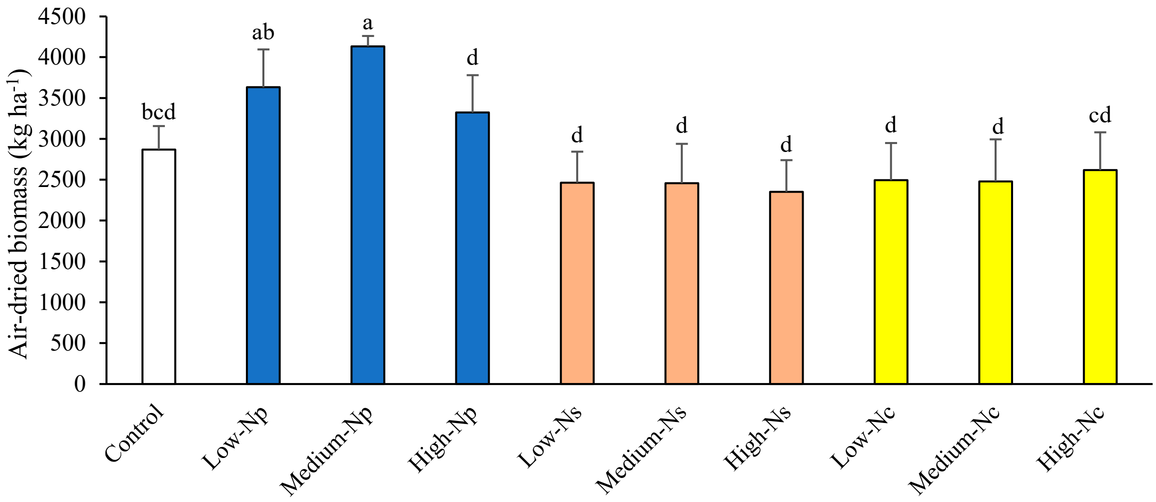


**Figure 1.** Air-dried biomass production (kg ha$^{-1}$) in hyssop exposed to different types and levels of animal manures. Means accompanied by the same letter are not significantly different, according to the LSD test ($p < 0.05$). Low-Np, Medium-Np, and High-Np: 2.2, 3.2, and 5.6 Mg ha$^{-1}$ of poultry manure, respectively; Low-Ns, Medium-Ns, and High-Ns: 3.8, 5.4, and 9.5 Mg ha$^{-1}$ of sheep manure, respectively; Low-Nc, Medium-Nc, and High-Nc: 4.3, 6.2, and 10.8 Mg ha$^{-1}$ of cattle manure, respectively; Control: unfertilized treatment (no manure). Error bars represent the standard deviation.

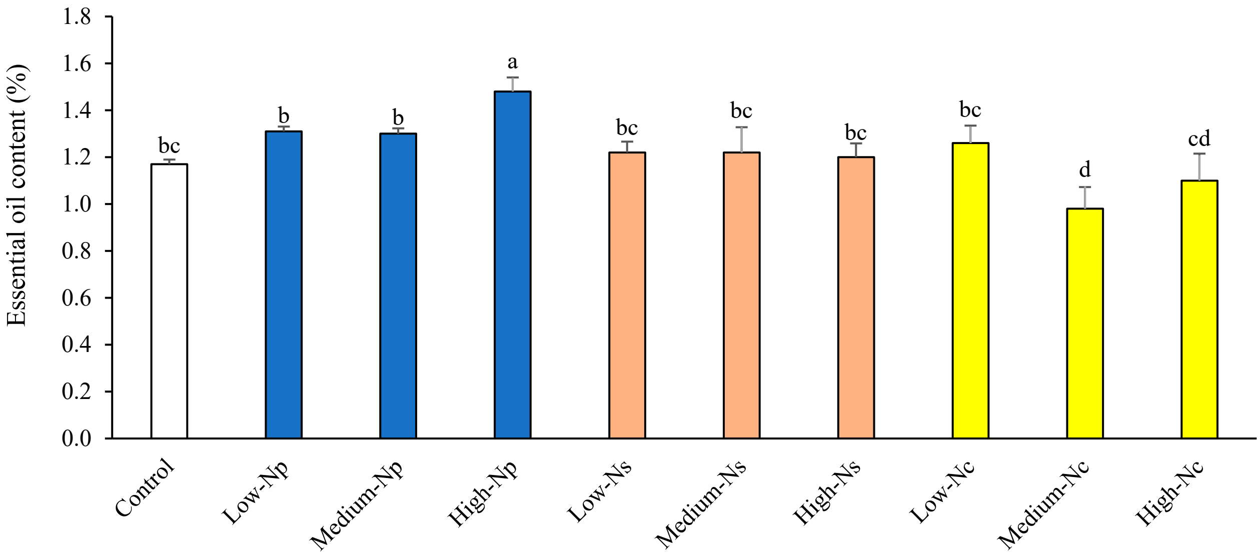


**Figure 2.** Essential oil content (%) in hyssop exposed to different types and levels of animal manures. Means accompanied by the same letter are not significantly different, according to the LSD test ($p < 0.05$). Low-Np, Medium-Np, and High-Np: 2.2, 3.2, and 5.6 Mg ha$^{-1}$ of poultry manure, respectively; Low-Ns, Medium-Ns, and High-Ns: 3.8, 5.4, and 9.5 Mg ha$^{-1}$ of sheep manure, respectively; Low-Nc, Medium-Nc, and High-Nc: 4.3, 6.2, and 10.8 Mg ha$^{-1}$ of cattle manure, respectively; Control: unfertilized treatment (no manure). Error bars represent the standard deviation.

### *3.3. Essential Oil Yield*

Essential oil yield in Low-Np, Medium-Np, and High-Np was 47.5, 53.8, and 49.2 kg ha$^{-1}$, respectively, which was higher in 42.5%, 61.6%, and 47.7%, compared to the control, respectively ($p < 0.05$; Figure 3). Different levels of sheep manure and cattle manure, except for Medium-Nc, did not cause significant changes in the yield of essential oil, and there was no significant difference with the control treatment ($p > 0.05$; Figure 3). The lowest yield of essential oil was obtained in Medium-Nc, which had a significant difference with the control treatment ($p < 0.05$; Figure 3).

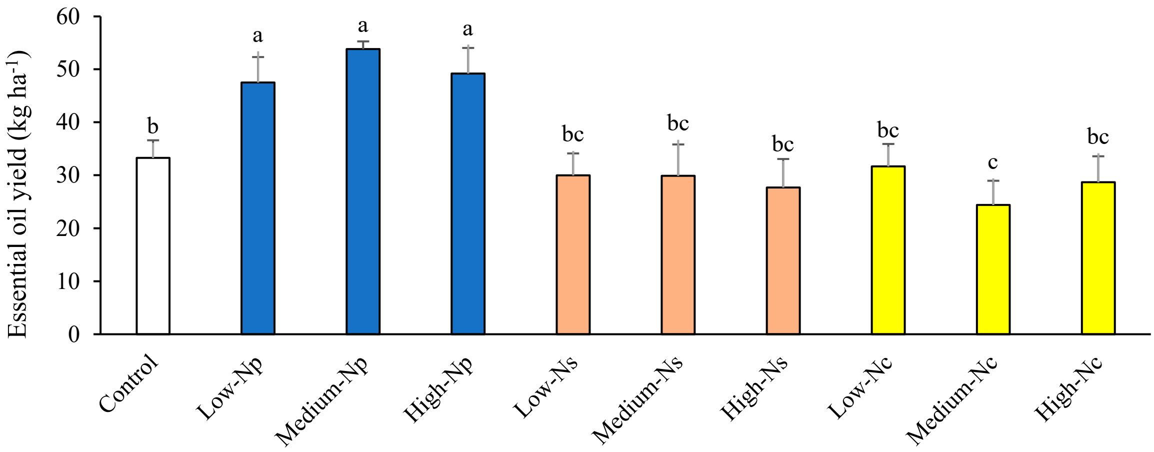


**Figure 3.** Essential oil yield (kg $ha^{-1}$) in hyssop exposed to different types and levels of animal manures. Means accompanied with the same letter are not significantly different, according to the LSD test ($p < 0.05$). Low-Np, Medium-Np, and High-Np: 2.2, 3.2, and 5.6 Mg $ha^{-1}$ of poultry manure, respectively; Low-Ns, Medium-Ns, and High-Ns: 3.8, 5.4, and 9.5 Mg $ha^{-1}$ of sheep manure, respectively; Low-Nc, Medium-Nc, and High-Nc: 4.3, 6.2, and 10.8 Mg $ha^{-1}$ of cattle manure, respectively; Control: unfertilized treatment (no manure). Error bars represent the standard deviation.

### *3.4. Essential Oil Composition*

Twenty-four components, comprising 93.7–97.8% of the overall hyssop oil makeup, were recognized (Table 3). Major constituents of hyssop oils include cis-pinocamphone (45.4–63.0%), trans-pinocamphone (7.3–20.9%), β-pinene (10.3–15.3%), 1,8-cineole (0.1–4.4%), pinocarvone (0.0– 4.4), myrtenol (1.1–3.6%), elemol (1.0– 3.2%), myrcene (0.0–2.3%), germacrene D (0.8–1.6%), (E)-caryophyllene (0.4–1.5%), bicyclogermacrene (0.5–1.3%), and β-bourbonene (0.4–1.3%) (Table 3).

Table 3 illustrates the impact of different levels of poultry, sheep, and cattle manure on the relative content of all detected essential oil compounds. Compounds such as elemol and cis-sabinene hydrate increased solely in the presence of poultry manure compared to the control. On the other hand, limonene and (E)-caryophyllene experienced an increase when exposed to cattle manure compared with the control ($p < 0.05$; Table 3). Moreover, five chemical compounds, such as α-pinene, camphene, β-pinene, β-bourbonene, and allo-aromadendrene, were observed to be impacted by Low to Medium levels of sheep and cattle manures when contrasted with the control. On the other hand, trans-pinocamphone, myrtenol, and (Z)-β-ocimene exhibited higher levels in the majority of manure applications in comparison to the control (Table 3; Figure 4).

According to the results of the hierarchical clustering (Figure 4), all manure treatments can be sorted into three groups based on the essential oil compounds they contain. The first group consists of Low and High levels of poultry manure, along with the control. Within this group, there was a high concentration of compounds such as cis-sabinene hydrate, cis-pinocamphone, elemol, β-eudesmol, γ-eudesmol, germacrene D, bicyclogermacrene, and (Z)-β-ocimene.

The second group comprises the Medium level of poultry manure, the Medium level of sheep manure, and the High levels of cattle and sheep manure. In this group, the levels of sabinene, linalool and, to some extent, α-thujene were notably elevated compared to the other groups. The variations in the levels of other compounds were relatively lower. Lastly, the third group encompasses the Medium level of cattle manure, as well as Low levels of cattle and sheep manure. β-eudesmol, myrtenol, limonene, β-bourbonene, allo-aromadendrene, (E)-caryophyllene, α-pinene, camphene, and β-pinene had a higher percentage in this group.

**Table 3.** Chemical composition of essential oils in hyssop exposed to different types and levels of animal manures.

| Compounds (%) | RI | Pr > F | Manure Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control | Low-Np | Medium-Np | High-Np | Low-Ns | Medium-Ns | High-Ns | Low-Nc | Medium-Nc | High-Nc |
| α-thujene (MT) | 937 | 0.023 | 0.30 abc | 0.25 cd | 0.27 bcd | 0.20 d | 0.34 ab | 0.36 a | 0.30 abc | 0.30 abc | 0.28 bc | 0.26 bcd |
| α-pinene (MT) | 949 | < 0.000 | 0.62 c | 0.57 c | 0.62 c | 0.65 bc | 0.78 b | 0.69 bc | 0.67 bc | 1.03 a | 0.78 b | 0.66 bc |
| Camphene (MT) | 966 | < 0.000 | 0.04 de | 0.04 de | 0.09 cd | 0.00 e | 0.09 cd | 0.15 bc | 0.10 cd | 0.28 a | 0.18 b | 0.08 cd |
| Sabinene (MT) | 978 | 0.044 | 1.46 ab | 1.29 bc | 1.54 ab | 1.35 bc | 1.67 a | 1.49 ab | 1.56 ab | 1.40 abc | 1.14 c | 1.47 ab |
| β-pinene (MT) | 1010 | 0.007 | 11.97 cde | 11.39 de | 12.03 cde | 10.85 e | 14.77 a | 12.70 bcd | 13.24 abc | 14.23 ab | 12.75 bcd | 13.42 abc |
| Myrcene (MT) | 1043 | 0.244 | 0.18 | 0.14 | 0.00 | 0.12 | 0.11 | 0.92 | 0.17 | 0.12 | 0.14 | 0.12 |
| Limonene (MT) | 1055 | 0.002 | 0.59 bc | 0.51 cd | 0.56 bc | 0.39 d | 0.61 bc | 0.67 ab | 0.56 bc | 0.74 a | 0.61 bc | 0.56 bc |
| 1,8-cineole (MT) | 1060 | 0.234 | 1.98 | 0.69 | 0.63 | 0.26 | 0.55 | 0.66 | 0.56 | 0.53 | 0.52 | 0.47 |
| (Z)-β-ocimene (MT) | 1063 | < 0.000 | 0.00 e | 0.52 a | 0.22 d | 0.46 ab | 0.44 abc | 0.23 cd | 0.29 bcd | 0.46 ab | 0.18 de | 0.56 a |
| γ-terpinene (MT) | 1084 | 0.458 | 0.36 | 0.28 | 0.25 | 0.26 | 0.35 | 0.32 | 0.33 | 0.53 | 0.27 | 0.41 |
| cis-sabinene hydrate (MT) | 1100 | < 0.000 | 0.32 bc | 0.14 cd | 0.38 bc | 0.72 a | 0.30 c | 0.55 ab | 0.31 bc | 0.00 d | 0.00 d | 0.00 d |
| Linalool (OMT) | 1113 | 0.004 | 0.85 a | 0.74 ab | 0.62 b | 0.42 c | 0.83 a | 0.81 a | 0.77 ab | 0.84 a | 0.72 ab | 0.82 a |
| trans-pinocamphone (OMT) | 1214 | 0.006 | 9.81 e | 12.58 b-e | 18.17 a | 10.05 de | 14.42 a–d | 11.85 cde | 15.58 abc | 17.19 ab | 16.64 abc | 9.89 de |
| Pinocarvone (OMT) | 1228 | 0.173 | 0.56 | 0.43 | 0.42 | 0.40 | 1.71 | 0.00 | 0.56 | 0.45 | 0.40 | 0.00 |
| cis-pinocamphone (OMT) | 1239 | 0.002 | 58.9 ab | 54.8 a–d | 50.0 de | 60.6 a | 50.8 cde | 56.5 a-c | 53.4 b-e | 47.4 e | 49.1 de | 58.8 ab |
| Myrtenol (OMT) | 1247 | 0.006 | 1.46 d | 2.19 bc | 2.30 bc | 2.33 bc | 2.13 bcd | 2.21 bc | 1.77 cd | 2.74 ab | 3.10 a | 2.64 ab |
| β-bourbonene (ST) | 1434 | < 0.000 | 0.77 bcd | 0.96 ab | 0.55 d | 0.69 cd | 1.06 a | 1.06 a | 0.67 cd | 1.07 a | 1.10 a | 0.88 abc |
| (*E*)-caryophyllene (ST) | 1476 | < 0.000 | 1.08 cd | 0.61 e | 1.10 bcd | 1.16 abc | 1.20 abc | 0.86 d | 1.22 abc | 1.38 a | 1.33 ab | 1.10 bcd |
| allo-aromadendrene (ST) | 1520 | 0.004 | 0.52 bcd | 0.61 abc | 0.39 de | 0.43 cde | 0.57 a–d | 0.67 ab | 0.53 bcd | 0.75 a | 0.58 a–d | 0.30 e |
| Germacrene D (ST) | 1534 | < 0.000 | 1.35 ab | 1.43 a | 0.96 d | 1.40 a | 1.38 ab | 1.37 ab | 1.39 ab | 1.20 bc | 1.26 ab | 1.04 cd |

**Table 3.** *Cont.*

| Compounds (%) | RI | Pr > F | Manure Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Control** | **Low-Np** | **Medium-Np** | **High-Np** | **Low-Ns** | **Medium-Ns** | **High-Ns** | **Low-Nc** | **Medium-Nc** | **High-Nc** |
| Bicyclogermacrene (ST) | 1548 | < 0.000 | 1.10 a | 1.16 a | 0.71 b | 1.18 a | 1.15 a | 1.11 a | 1.02 a | 1.00 a | 1.02 a | 0.73 b |
| Elemol (OST) | 1586 | 0.002 | 2.05 bc | 2.82 a | 1.76 bcd | 2.28 ab | 2.02 bc | 1.54 cd | 1.52 cd | 2.00 cd | 2.00 cd | 1.32 d |
| γ-eudesmol (OST) | 1696 | 0.010 | 0.30 ab | 0.29 b | 0.05 c | 0.53 a | 0.14 bc | 0.20 bc | 0.20 bc | 0.15 bc | 0.31 ab | 0.00 c |
| β-eudesmol (OST) | 1728 | <0.000 | 0.58 ab | 0.77 a | 0.23 cd | 0.40 bc | 0.34 cd | 0.34 cd | 0.14 d | 0.68 a | 0.62 ab | 0.12 d |
| Total (%) | | | 96.2 | 95.2 | 93.6 | 97.1 | 97.8 | 97.3 | 96.9 | 96.5 | 95.0 | 95.7 |

RI: Kovats index on the DB-5 column. MT: Monoterpene hydrocarbons; OMT: Oxygenated monoterpenes; ST: Sesquiterpene hydrocarbons; OST: Oxygenated sesquiterpenes; Low-Np, Medium-Np, and High-Np: 2.2, 3.2, and 5.6 Mg $ha^{-1}$ of poultry manure, respectively; Low-Ns, Medium-Ns, and High-Ns: 3.8, 5.4, and 9.5 Mg $ha^{-1}$ of sheep manure, respectively; Low-Nc, Medium-Nc, and High-Nc: 4.3, 6.2, and 10.8 Mg $ha^{-1}$ of cattle manure, respectively; Control: unfertilized treatment (no manure). For each compound, means accompanied by the same letter are not significantly different, according to the LSD test ($p < 0.05$).

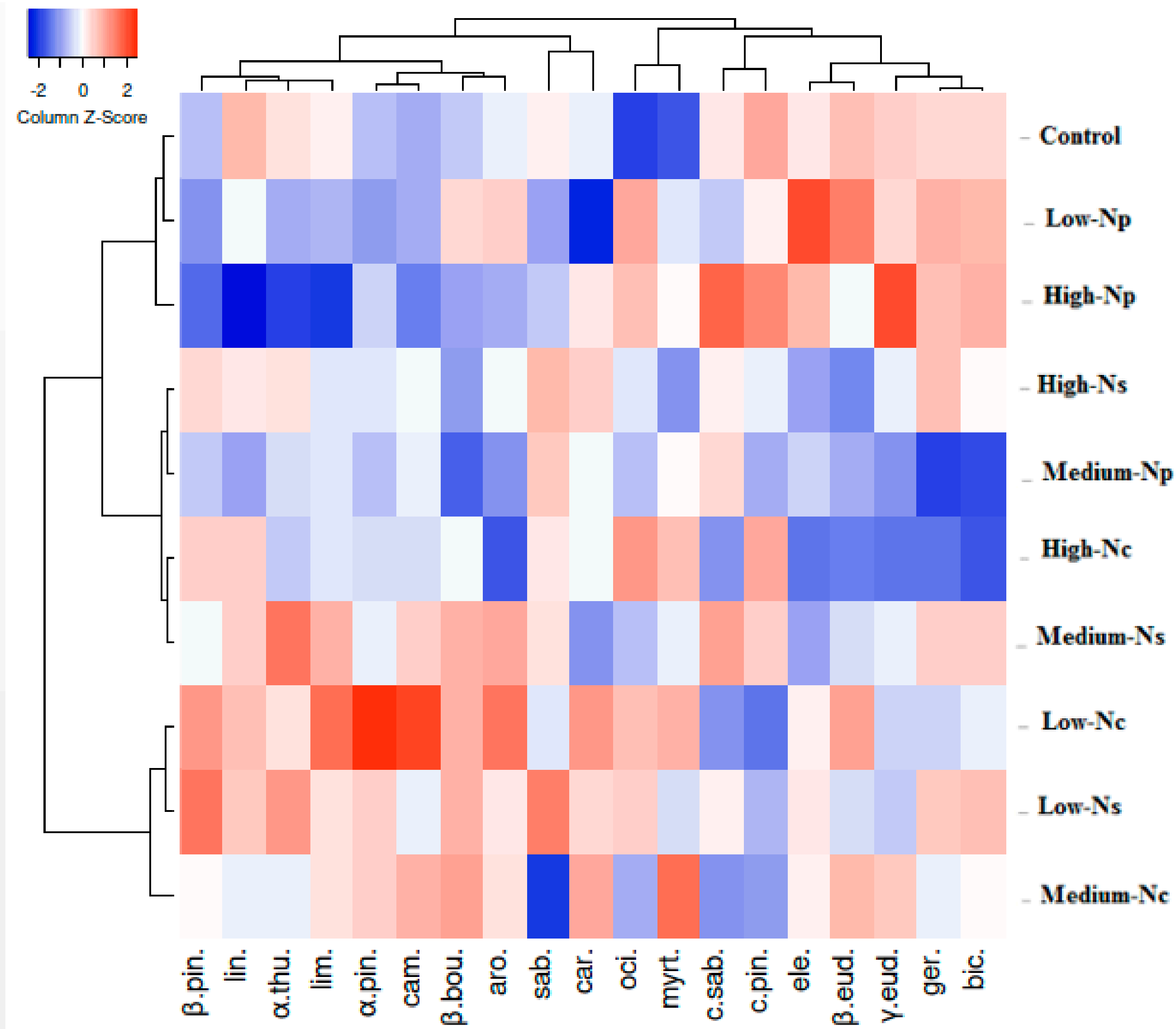


**Figure 4.** Hierarchical clustering dendrogram and heat map for some essential oil compounds of hyssop under different levels of animal manures that were significantly different in percentage ($p < 0.05$ after LSD post-hoc testing). Colors are representative of a relative scale (−2 to +2). The darker blue indicates lower values, while the darker red indicates higher values. Low-Np, Medium-Np, and High-Np: 2.2, 3.2, and 5.6 Mg $ha^{-1}$ of poultry manure, respectively; Low-Ns, Medium-Ns, and High-Ns: 3.8, 5.4, and 9.5 Mg $ha^{-1}$ of sheep manure, respectively; Low-Nc, Medium-Nc, and High-Nc: 4.3, 6.2, and 10.8 Mg $ha^{-1}$ of cattle manure, respectively; Control: unfertilized treatment (no manure). c.sab: cis-sabinene hydrate; c.pin: cis-pinocamphone; ele: elemol; β-eud: β-eudesmol; γ-eud: γ-eudesmol; ger: germacrene D; bic: bicyclogermacrene; oci: (Z)-β-ocimene; myrt: myrtenol; sab: sabinene; lin: linalool; α-th: α-thujene; lim: limonene; β-bou: β-bourbonene, aro: allo-aromadendrene; car: (E)-caryophyllene; α-pin: α-pinene; cam: camphene; β.pin: β-pinene.

### 3.5. Antioxidant Capacity of Essential Oil

Lower values of antioxidant capacity show that the antioxidant power of the essential oil is greater. As shown in Figure 5, the Medium-Nc and High-Nc treatments have the most potent antioxidant activity compared to the control ($p < 0.05$). Then, High-Ns and Medium-Np are placed in the following ranks, respectively. The difference in the antioxidant capacity of Medium-Nc, High-Nc, High-Ns, and Medium-Np treatments with the control treatment was significant ($p < 0.05$; Figure 5). The antioxidant capacity in Low-Np, Low-Ns, and Medium-Ns treatments was similar to the control ($p > 0.05$; Figure 5). In the High-

Np treatment, the antioxidant capacity was weaker than the control treatment ($p < 0.05$; Figure 5).

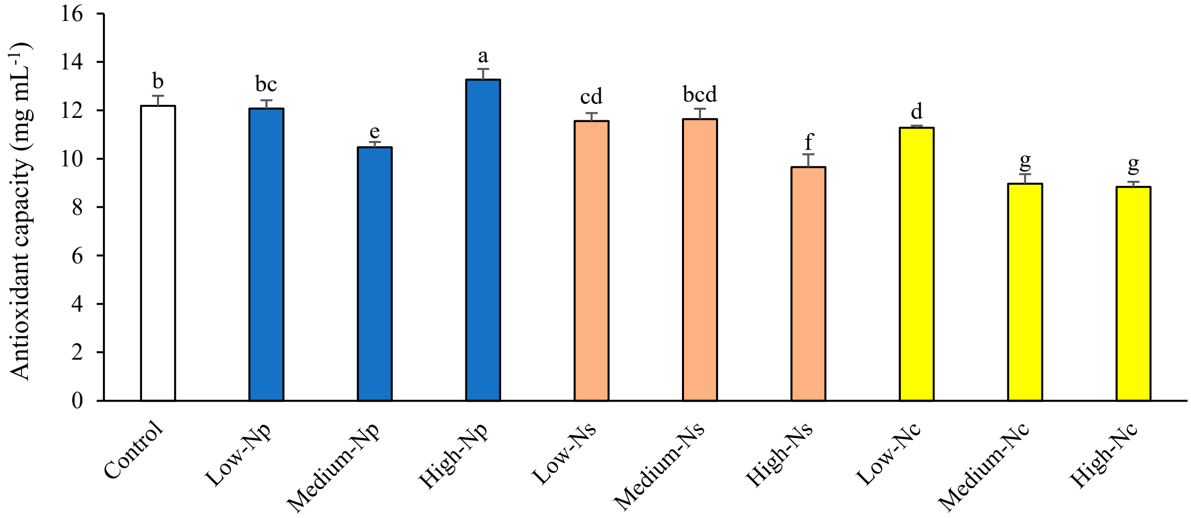


**Figure 5.** Antioxidant capacity (mg $mL^{-1}$) of essential oil in hyssop exposed to different types and levels of animal manures. Means accompanied by the same letter are not significantly different, according to the LSD test ($p < 0.05$). Low-Np, Medium-Np, and High-Np: 2.2, 3.2, and 5.6 Mg $ha^{-1}$ of poultry manure, respectively; Low-Ns, Medium-Ns, and High-Ns: 3.8, 5.4, and 9.5 Mg $ha^{-1}$ of sheep manure, respectively; Low-Nc, Medium-Nc, and High-Nc: 4.3, 6.2, and 10.8 Mg $ha^{-1}$ of cattle manure, respectively; Control: unfertilized treatment (no manure). Error bars represent the standard deviation.

## 4. Discussion

### *4.1. Herbal Biomass*

Manure application resulted in significant improvements in various soil properties and biological activities [28]. Potential enhancements may involve greater stability in water and soil organic carbon, increased availability of nutrients, and heightened enzymatic and microbiological activity. These findings suggest that the application of manure enhances the nutrient pool and decompositional capacity of the soil, which in turn positively impacts crop yield. In the current study, it was observed that the production of dry matter was significantly higher when using a Medium level of poultry manure compared to other manure treatments. When using a High level of poultry manure, no significant difference was observed compared to the other treatments. This suggests that the average level of manure adequately fulfilled the nutrient requirements of the hyssop plants, and any excess had no additional impact on the dry matter production. Previous research has indicated that organic nitrogen in poultry manure undergoes mineralization at a higher rate than other animal manures [29]. Additionally, Fallah et al. [1] reported that the concentration patterns of phosphorus and nitrogen observed in three harvests align with the peak mineralization of poultry and manure treatments in *Mentha piperita* (L.). However, in the case of cattle manure, it is likely that the mineralization process of lignin materials decreases after the rapid decomposition of substances. Consequently, the release of nitrogen and phosphorus becomes restricted [30].

### *4.2. Essential Oil Content*

The production of the greatest content of essential oils in the highest level of poultry manure suggests that nutrients absorbed by plants during the production of essential oil play a direct role in bioactive accumulation. Consequently, in the case of other animal manures, it is likely that the restricted availability of nutrients to hyssop has resulted in a reduction in the proportion of essential oils. Plant nutrient availability, particularly nitrogen, enhances the development of young leaves, resulting in a higher content of essential oil compared to mature leaves. Tripathi and Hazarika [31] demonstrated that the concentration of essential oil in immature leaves (4.5%) of *Pogostemon cablin* (Benth.) was greater than that in vegetative and fully mature leaves (4.0% and 3.8%, respectively). The increased concentration in immature leaves can be attributed to their higher biogenetic activity. Immature leaves that are still growing exhibit greater biogenetic activity compared to mature leaves, leading to a higher rate of essential oil synthesis and accumulation [32]. It appears that the sufficient amount of available nitrogen in High-Np treatment has contributed to the prolonged growth of the leaves, potentially resulting in a higher proportion of young leaves in this particular condition. In the current investigation, the highest concentration of essential oil identified was 1.48%, which closely resembled the essential oil content documented in prior research involving cattle manure treatment (1.59%) [17].

### 4.3. Essential Oil Yield

The performance of essential oil is crucial in cultivating medicinal plants, as it directly impacts the farmer's income. In addition to essential oil production, the cultivation of medicinal plants also focuses on improving the essential oil content. A study by Fallah et al. [30] found that different levels of poultry manure had a significant positive effect on essential oil production in *Dracocephalum kotschyi*. This increase in production was attributed to the improvement in biomass and essential oil content. The study also revealed that the biomass had a greater influence on essential oil yield, indicating no significant nutrient stress or hindered plant growth in the experiment. In a separate study conducted by Fallah et al. [1], it was observed that the essential oil yield of peppermint was similar in treatments using cattle manure and chemical fertilizer during the first harvest. However, plants treated with poultry manure exhibited the highest essential oil yield. In contrast, those treated with cattle manure had the lowest yield, which was not significantly different from the yield obtained with chemical fertilizer.

### 4.4. Essential Oil Components

Nutrient availability has a significant impact on the chemical compositions of essential oils. Nitrogen and phosphorus are crucial elements that enhance plant photosynthesis and the synthesis of primary and secondary metabolites in plants. This, in turn, contributes to the maintenance of terpenoid production [33,34]. Terpenoids are synthesized through the mevalonic pathway and methylerythritol phosphate pathways, which do not directly compete with nitrogen resources required for plant growth [33]. Moreover, terpenoid precursors, such as isopentenyl diphosphate, dimethylallyl pyrophosphate, geranyl diphosphate, and farnesyl diphosphate, contain phosphorus as a structural component. Therefore, increasing phosphorus availability could potentially stimulate these pathways and subsequently enhance terpene production [33,35]. The composition of cis-pinocamphone, trans-pinocamphone, and β-pinene was 79%, a finding that aligns with the findings of Ahmadi et al. [14]. Their study indicated that these compounds, along with their precursor β-pinene, collectively constitute over 70% of the essential oil composition of hyssop. Different manure types, such as poultry, cattle, and sheep, can elicit varying responses in essential oil compounds. The resemblance between poultry manure treatments and the control group was higher than cattle and sheep manure treatments. The specific compounds targeted for the pharmaceutical sector can yield diverse outcomes when evaluated with different manure levels. The composition of the essential oil in the present study was consistent with the report of Pandey et al. [16].

### 4.5. Antioxidant Capacity

The chemical composition of essential oils can play a role in the antioxidant properties of plants, with key compounds being particularly influential [36]. It is essential to recognize that this attribute is not solely determined by the concentration of a single compound [37]. Both major and minor components can act synergistically within the essential oil, boosting its overall antioxidant capabilities [38]. The results presented in Figure 5 indicate that higher levels of cattle and sheep manure lead to increased antioxidant activity. Additionally, there is a clear relationship between the content of essential oils and antioxidant capacity (r = 0.67, $p < 0.001$). Therefore, treatments rich in nutritional elements tend to exhibit higher antioxidant capacities. The increase in the antioxidant efficacy of essential oil is likely attributed to an elevation in the production of secondary metabolites possessing antioxidant characteristics [11]. In the present study, it is noteworthy to highlight substances like β-pinene, linalool, and (E)-caryophyllene, which exhibited higher levels in treatments characterized by strong antioxidant capabilities. Ozliman et al. [39] have shown that using different doses of farmyard manure not only enhances the content and components of essential oils but also boosts the antioxidant activity of dill (*Anethum graveolens* L.).

## 5. Conclusions

The current research findings indicate that hyssop plants, when nourished with High levels of animal manure, can produce essential oil rich in myrtenol, cis-sabinene hydrate, and (Z)-β-ocimene, and endowed with a strong antioxidant capacity. When comparing different types of animal manure, varying levels of cattle manure led to a broader range of essential oil compounds compared to poultry and sheep manure. However, it was found that the optimal content and yield of essential oil can be achieved by utilizing a Medium level of poultry manure. In conclusion, in soils with low organic carbon and phosphate, like our experimental field, farmers who prioritize both biomass and essential oil content should use a Medium level of poultry manure. However, for the pharmaceutical industry, alternative fertilizer treatments should also be considered, taking into account the desired secondary metabolite.

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