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Impacts of Nitrogen and Phosphorus Fertilization on Biomass, Polyphenol Contents, and Essential Oil Yield and Composition of *Vitex negundo* Linn

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Abstract: Nutrient management has increasingly become important in producing quality medicinal plant materials. *Vitex negundo* is an important perennial medicinal plant widely distributed in tropical Asia and Africa. This study aimed to examine the effects of nitrogen (N) and phosphorus (P) fertilization on the biomass, polyphenol contents, and essential oil yield and composition in field cultivated *V. negundo*. Two field experiments were conducted; one was performed on three different rates of N fertilizer (50, 100, and 200 kg-N ha^{-1}), and the other was on different P fertilizer rates (50, 100, and 200 kg-P ha^{-1}), with their respective control groups receiving no fertilization under field conditions. The results showed that at 200 kg-P ha^{-1}, *V. negundo* had the highest biomass and essential oil yield, the highest number of volatile components (45 compounds), and the content of bioactive ingredients (β-caryophyllene and eremophilene). Polyphenol contents were not significantly different between treatments. This study indicates that 200 kg-P ha^{-1} (NPK ratio of 1:2:1) treatment positively affects the yield of biomass, essential oils, and bioactive compounds in field cultivated *V. negundo*.

Keywords: medicinal plant; nutrient management; bioactive compounds; β-caryophyllene; eremophilene

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1. Introduction

Medicinal plants collected from the wild for medicinal, food, and industrial applications have long been used since ancient times. However, with the increasing demand in the international market and the cause of over-harvesting, these plant resources are rapidly decreasing and have become scarce in the wild [1]. Therefore, the domestication and cultivation of medicinal plant species with commercial applications have become a top research priority in many countries.

The quality of medicinal plant materials depends on the presence of quality and quantity of bioactive secondary metabolites, of which the rate of biosynthesis is susceptible to environmental conditions, such as climate, rainfall, soil, nutrients, and others [2,3]. Studies have shown that proper fertilization management can effectively increase the yield and quality of medicinal plants [3,4]. Nitrogen (N) and phosphorus (P) are essential structural components of proteins, phospholipids, and coenzymes and also participate in various physiological metabolisms of plants; their absorption and utilization can affect plant growth and biosynthesis of secondary metabolites. Increasing N fertilization was reported to enhance curcumin production in *Curcuma aromatica* [5], while decreasing the betaine content in *Lycium barbarum* [6]. Application of P fertilizer was shown to improve the accumulation of phenolic compounds but not the essential oil yield of *Mentha spicata* [7]. However, numerous studies have reported that increasing P fertilization increased essential oil production, such as in basil [8], lavender [9], marjoram [10], and sage [11]. These results suggest that the amount and method of fertilization need to be adjusted to promote the biosynthesis of secondary metabolites in different medicinal plant species, thereby increasing the content of the target bioactive ingredients.

Vitex negundo Linn., an important perennial medicinal plant from the Verbenaceae family, is mainly distributed in India, Pakistan, Malaysia, East Asia, and tropical Africa [12]. The whole plant, including fruits of *V. negundo* can be used as medicine, and its decoction is traditionally used to treat stomachache, headache, eye diseases, skin ulcers, rheumatoid arthritis, and other diseases [13,14]. Essential oil of *V. negundo* is popularly used in aromatherapy for relieving joint pain, fifty shoulders, and gout. Extracts of *V. negundo* shoots possess anti-inflammatory [15], antioxidant [16], anti-bacterial [17,18], cardioprotective [19], hepatoprotective [20,21], hypoglycemic [22] and anti-melanogenesis [23] activities. *V. negundo* extract was effective in reducing oxidative stress in rats treated with anti-tuberculosis drugs such as isoniazid, rifampin, and pyrazinamide [21]. Methanol extract of *V. negundo* leaves can inhibit the pathogenicity of *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Shigella* spp., and *Aeromonas* spp. [17]. The essential oil of *V. negundo* effectively inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia* coli, and *Pseudomonas aeruginosa* [18]. In a toxicity study, using 2000 mg kg^{-1} body weight of *V. negundo* essential oil for 90 days did not cause acute skin toxicity in rats [24].

At present, at least 35 compounds have been identified in the essential oil of *V. negundo* shoots, namely viridiflorol, β-caryophyllene, sabinene, 4-terpineol, γ-terpinene, caryophyllene oxide, 1-oceten-3-ol and globulol [25], δ-guaiene, ethylhexadecenoate, α-selinene, germacren-4-ol, caryophyllene epoxide, (E)-nerolidol, β-selinene, α-cedrene, β-eudesmol, germacrene D, hexadecanoic acid, p-cymene and valencene [18], eremophilene, eucalyptol, menthone and humulene [23], and others [13,14]. Among them, the main bioactive compounds are β-caryophyllene and eremophilene [23,26]. β-caryophyllene possesses anti-inflammatory, anti-depression [27], and anti-anxiety activities, while eremophilene has anti-microbial, anti-cancer, and immunomodulatory properties [28].

In Taiwan, *V. negundo* is mainly distributed in the southern part of low-altitude mountainous and coastal areas [29]; currently, it is commercially cultivated in Taitung. Previous studies have been conducted primarily to examine the pharmacological and chemical properties of *V. negundo*, and the separation and identification of its essential oil components; however, its agronomic information remains limited. Therefore, this study aimed to examine the effects of applying different N and P fertilizer rates on the biomass, polyphenol contents, and essential oil yield and composition in field cultivated *V. negundo*.

2. Materials and Methods

2.1. Experimental Materials

The experimental plant (*Vitex negundo* Linn.) seedlings were provided by Yuyuantang Biotechnology Company (Taitung, Taiwan). They were first cultivated in the greenhouse of the National Taiwan University for eight weeks. After growing up to 10 cm high, healthy plants were selected for field trials. Chemical fertilizers such as urea, superphosphate, and potassium chloride were used as N, P, and K fertilizers, respectively.

2.2. Time and Location of Experiments

All experiments were conducted at the Agricultural Experimental Farm of National Taiwan University. The experimental period was from 1 June 2018 to 29 December 2018, and the duration were about 180 days.

2.3. Experimental Design

The seedlings of *V. negundo* were moved to the field for planting on 1 June 2018. A randomized complete block design was used to assign the plants for different treatments. The row spacing was 1.5 m, the plant spacing was 2 m, and six plants were planted per row. The experiment was a single factor treatment, and a fixed amount of K fertilizer was used in both N and P treatments. The N fertilizer treatments comprised three different levels of N fertilizer (i.e., 50, 100, and 200 kg-N ha^{-1}), and they received the same amount of P (100 kg ha^{-1}) and K (100 kg ha^{-1}) fertilizers. In the P fertilizer treatments, three different levels of P fertilizer (i.e., 50, 100, and 200 kg-P ha^{-1}) were used, and each of these treatments

received the same amount of N (100 kg ha^{-1}) and K (100 kg ha^{-1}) fertilizers (Table S1). The control group in both N (NCK) and P (PCK) treatments received no fertilization, and each treatment group had five replicates.

Urea, superphosphate, and potassium chloride were applied in the form of basal fertilizer and top dressing. The application time of basal fertilizer was on 31 May 2018, and the first time of top dressing application was on 31 July 2018 (two months after planting). The second top dressing application time was on 29 September 2018 (four months after planting). The detailed design of fertilization treatments in the field is presented in Table S1. In addition to the natural rainfall, tap water was used to irrigate the experimental field if required.

The monthly average temperature and mean rainfall during the experimental period from June 2018 to December 2018 are shown in Figure S1.

2.4. Soil Sampling and Chemical Analysis

Before cultivation and after harvesting, soil samples at depths 0–30 cm were collected from all treatment sites. After drying, the ground soils were sieved through 20 mesh (0.85 mm) and then subjected to the analyses of pH, electric conductivity of saturation extract, and contents of total N, Mehlich III extractable P and K, and organic matter according to methods described previously [6].

2.5. Harvesting and Processing of Plant Materials

The aboveground parts (shoots) of *V. negundo* were harvested, weighed, and then divided into plant materials for chemical content and essential oil analyses.

The plant samples used for chemical analysis were taken and placed in a paper bag, and dried at 40 °C to constant weight. After taking the dry weight, the plant materials were ground to powdered form, collected, and kept in an air-tight plastic bag at 4 °C until analysis. The analyses were comprised of contents of total N, total P, total K [6], total phenols and total flavonoids [30].

For essential oil analysis, the freshly harvested plant materials were subjected to essential oil extraction after drying under shade at room temperature for two days. The essential oil collected was analyzed by gas chromatography-mass spectrometry (GC-MS).

2.6. Essential Oil Extraction

The plant materials (about 300 g) were subjected to hydrodistillation (8 h) in a Clevenger-type device. After estimating the yield, the essential oil was treated with anhydrous sodium sulphate to remove the residue moisture, filtered through a 0.45 µm membrane filter, then collected in an air-tight brown bottle and stored at 4 °C until analysis.

2.7. Essential Oil Composition Analysis

The GC-MS analysis of essential oil composition was carried out according to the method described previously with minor modifications [25,31]. In brief, separation and quantitation were performed using an Agilent 7890 gas chromatography coupled with a 5975 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and a DB-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The flow rate of helium carrier gas was kept at 1.0 mL min^{-1}. The initial temperature of the column was 50 °C, held for 5 min, then increased to 200 °C at a rate of 4 °C min^{-1} (kept constant for 1 min), followed by raising the temperature to 250 °C at a rate of 5 °C min^{-1}. A sample of 1 µL (0.5 mL of oil dissolved in 9.5 mL of dichloromethane) was injected in a split mode at a ratio of 1:15. The injector port and detector temperatures were 250 °C and 300 °C, respectively. The GC/MS interface was held at 230 °C, and mass spectra were obtained at 70 eV. The mass analyzer was set to scan from 50 to 550 amu every 0.7 s. The total running time for a sample was about 50 min. Essential oil constituents were identified by comparing their mass spectra with those stored in the Wiley (Wiley Registry of Mass Spectral Data, 11th ed.) and NIST

2017 mass spectral libraries. In addition, the peak area of the gas chromatogram was used to estimate the relative percentage of each essential oil component.

2.8. Statistical Analysis

All values are presented as mean ± standard deviation (SD), obtained from five independent analyses. The Student's *t*-test was used to assess the difference between the chemical properties of NCK and PCK soils before and after experiments. To determine whether the differences between group means were statistically significant, the data were analyzed by one-way ANOVA followed by post hoc Duncan's multiple range test. The *p*-value of less than 0.05 was regarded as statistically significant.

3. Results

3.1. Soil Chemical Properties

The experimental field soil has a silty loam texture, and its pH before the experiment was 4.6, which was slightly acidic. At the end of the experiment, an upward trend of pH values was noted with increasing fertilization rates of N or P (Table 1). Compared with the control group, both N and P fertilization led to increased EC values; however, there were no differences among the different rates of fertilizer treatment except P20 (Table 1).

Table 1. Effects of different fertilizer treatments on soil pH, electrical conductivity, organic matter, total nitrogen, phosphorus, and potassium contents.

Treatments	pH	EC	OM	TN	P	K
Before	4.6 ± 0.3	0.3 ± 0.1	31.9 ± 1.3	1.1 ± 0.8	19.4 ± 3.1	130.1 ± 24.3
			After nitrogen fertilization			
NCK (Control)	4.4 ± 0.2 b	0.3 ± 0.1 b	45.6 ± 2.1 a *	1.2 ± 0.2 c	45.0 ± 1.2 c *	149.1 ± 21.9 b
N5	4.7 ± 0.2 b	0.7 ± 0.2 a	49.3 ± 9.8 a	1.6 ± 0.1 b	91.9 ± 3.2 b	378.4 ± 39.7 a
N10	5.2 ± 0.2 a	0.7 ± 0.3 a	44.0 ± 4.4 a	1.7 ± 0.2 b	102.6 ± 7.8 a	319.2 ± 41.3 a
N20	5.4 ± 0.5 a	0.8 ± 0.4 a	49.6 ± 7.4 a	2.6 ± 0.6 a	111.5 ± 5.7 a	397.9 ± 72.2 a
			After phosphorus fertilization			
PCK (Control)	4.3 ± 0.1 b	0.5 ± 0.2 b	43.1 ± 4.4 a *	1.4 ± 0.2 c	33.5 ± 1.4 d *	202.5 ± 46.2 b
P5	4.7 ± 0.3 b	0.6 ± 0.2 b	52.4 ± 5.7 a	2.1 ± 0.3 a	51.8 ± 5.7 c	410.9 ± 73.8 a
P10	5.2 ± 0.2 a	0.6 ± 0.1 b	53.8 ± 12.1 a	1.9 ± 0.2 a	73.8 ± 9.8 b	338.2 ± 66.0 a
P20	5.1 ± 0.3 a	1.8 ± 0.2 a	55.2 ± 6.8 a	1.8 ± 0.2 a	143.5 ± 2.7 a	335.1 ± 35.3 a

Values are expressed as mean ± standard deviation (n = 5). * Indicates a significant difference between control after and before experiments as analyzed by Student's *t*-tests. Means in the same row followed by the same letter are not significantly different between treatments ($p < 0.05$, Duncan's multiple range tests). EC: Electrical conductivity (dS m^{-1}); OM: Organic matter (g kg^{-1}); TN: Total nitrogen (g kg^{-1}); P: Mehlich III extractable phosphorous (mg kg^{-1}); K: Mehlich III extractable potassium (mg kg^{-1}).

The total N and Mehlich III extractable P and K contents in soils before the experiment were 1.1 g kg^{-1}, 19.4 mg kg^{-1}, and 130.1 mg kg^{-1}, respectively; after the experiment, these values were significantly lower than those treated with fertilizers. The total N content in N200 was markedly higher than in other treatments. Compared with the control group, the extractable P and K contents in soils of both N and P fertilizer treatments were significantly increased, and P20 was noted to have the highest P content (Table 1). Although the soil organic matter contents in fertilizer treated groups were significantly higher than before the experiment, they were not statistically significant between the different N or P treatment rates.

3.2. Plant Biomass Production

Compared with the control group, a trend of increased biomass was noted with rising N fertilization rates (Figure 1g), whereas, among P fertilizer treatments, P10 and P20 appear to produce significantly higher biomass than the control (Figure 1h). Although increasing rates of P fertilization favor the growth and development of *V. negundo* shoots, the optimal yield was obtained with 100 kg-N ha^{-1} and 100 kg-P ha^{-1} fertilization.

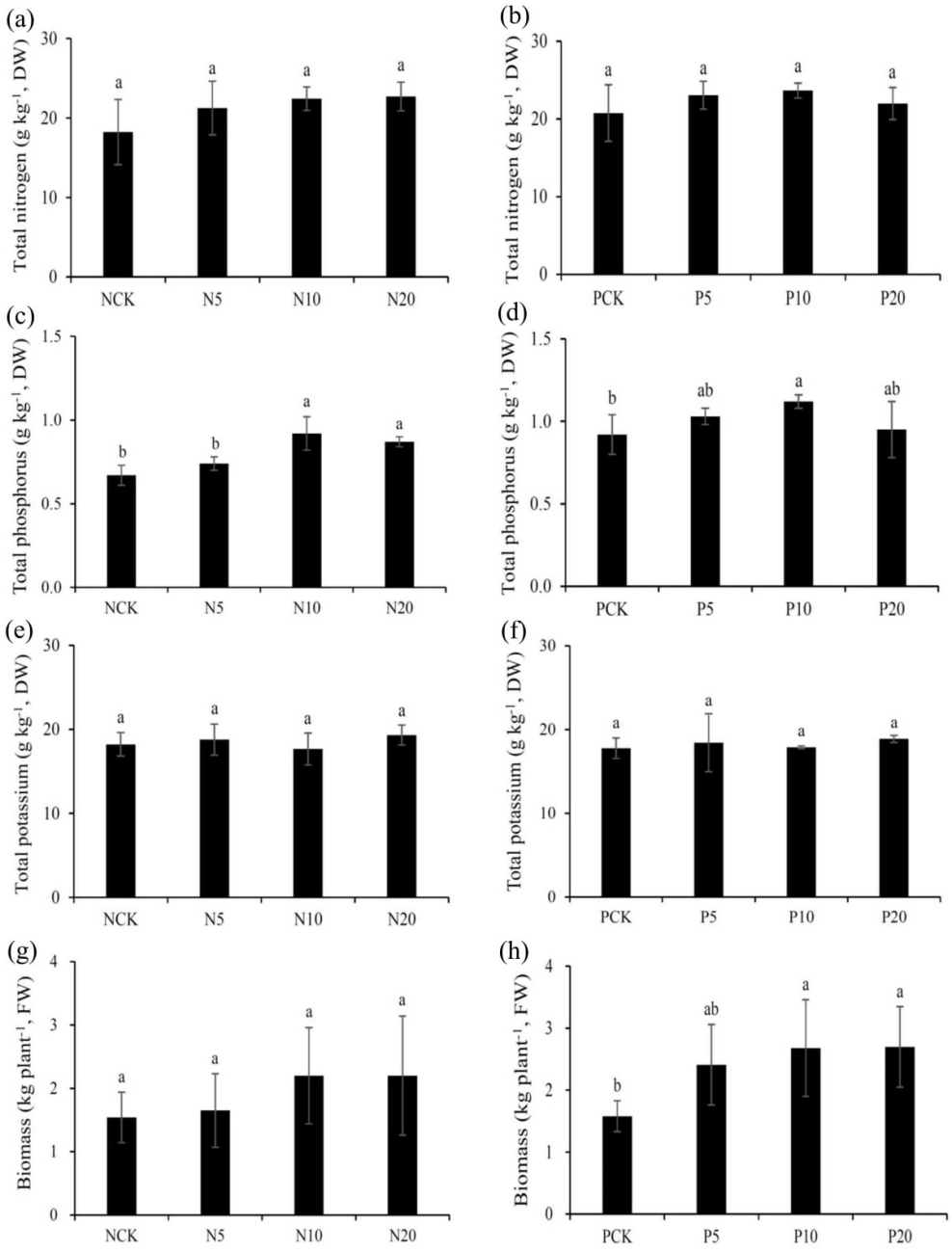

Figure 1. Effects of different fertilizer treatments on contents of total nitrogen, total phosphorous, total potassium, and biomass in shoots of *V. negundo*. (**a**,**b**) Total nitrogen; (**c**,**d**) total phosphorus; (**e**,**f**) total potassium; and (**g**,**h**) biomass. Error bars represent standard deviation (n = 5), and bars with the same letter are not significantly different between treatments ($p < 0.05$, Duncan's multiple range tests). FW: fresh weight; DW: dry weight.

3.3. Nutrient (N, P, and K) Contents in Plant Parts

Results showed that the N absorption was not significantly different among N or P treatments, but there was an increasing trend of N or P content in plant parts with increasing fertilization rates (Figure 1a,b). Compared with the control group, there was a trend of increased total P content in groups treated with increasing rates of N (Figure 1c). In P fertilizer treatments, although the total P content appears to increase and then decrease with increasing P application rates, there was no significant difference between treatments (Figure 1d). Similarly, there was no difference in the total K content of the plants among N

or P treatments (Figure 1e,f), indicating that the additional application of K fertilizer has minimal effect on the total K content in the plant tissues.

3.4. Total Phenolic and Total Flavonoid Contents

Results showed that the total phenolic content in the N-treated groups was not significantly different from the control group (Figure 2a). Similar results were also observed in the P treatments (Figure 2b). Compared with the control group, the N-treated groups showed an enhanced total flavonoid content at low N fertilization, but its content decreased with increasing N fertilization (Figure 2c). In the P treatments, it was noted that increasing P fertilization tends to reduce and then increase the total flavonoid content (Figure 2d).

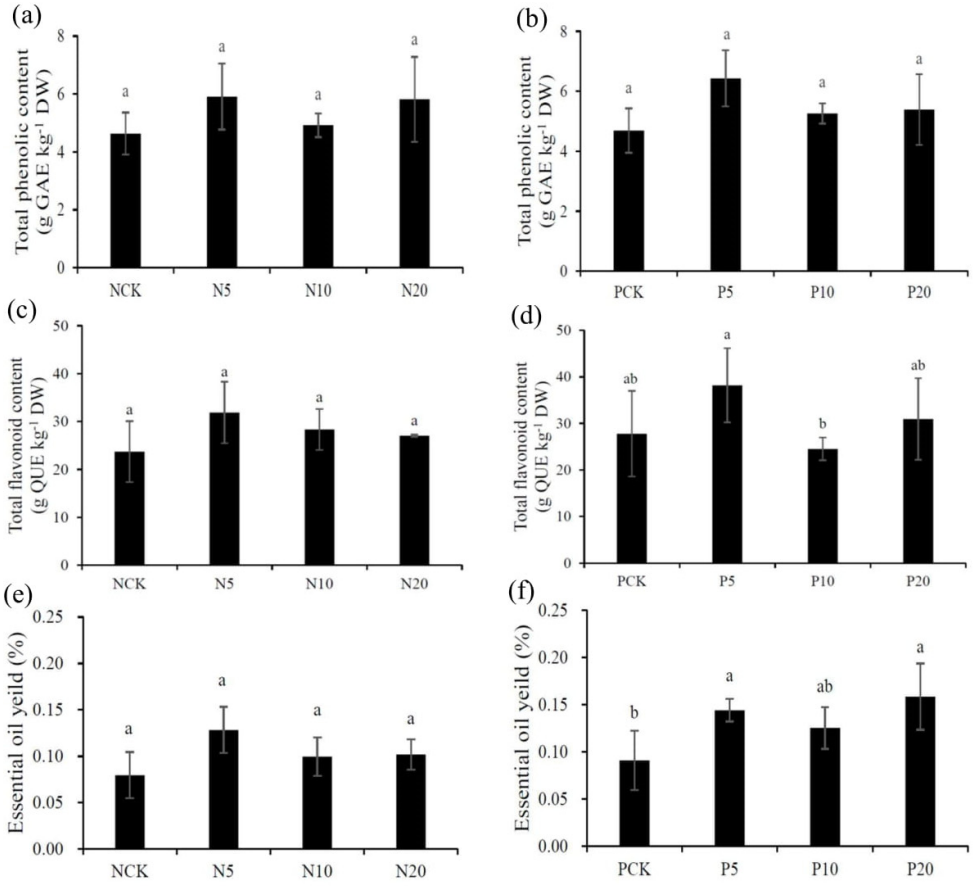

Figure 2. Effects of different fertilizer treatments on total phenolic and flavonoid contents, and essential oil yield in shoots of *V. negundo*. (**a**,**b**) Total phenolic content; (**c**,**d**) Total flavonoid content; (**e**,**f**) Essential oil yield. Error bars represent standard deviation (n = 5), and bars with the same letter are not significantly different between treatments ($p < 0.05$, Duncan's multiple range tests).

3.5. Essential Oil Yields

Compared with the control group, an increasing trend of essential oil yield was noted with increasing N application rates, but its yield among treatments was not significantly different (Figure 2e). An increase in the essential oil production was also noted in the P-treated group; among them, the P20 had the highest yield (Figure 2f), indicating that under a fixed amount of N and K fertilizer application, increasing the amount of P fertilizer can increase the essential oil production in *V. negundo*.

3.6. Essential Oil Composition

Results showed that P20 has the most diverse types of essential oil components (45 compounds), followed by P10 (42 compounds) and N20 and P5 (40 compounds), while N5 has the least essential oil components (33 compounds) (Table 2). Regardless of treat-

ments, the sesquiterpene-type β-caryophyllene and eremophilene were the major components of *V. negundo* essential oil (Table 2, Figure 3). This study showed that P20 has the highest relative content of eremophilene (23.44%), followed by the P5 treatment (22.61%). P10 exhibited the highest relative content of β-caryophyllene (38.92%), followed by the P20 (37.66%).

Table 2. Effects of different fertilizer treatments on essential oil components of *V. negundo*.

No	Components	RT	NCK	N5	N10	N20	PCK	P5	P10	P20
1	α-Thujene	7.24	0.37	0.50	0.49	0.92	0.62	1.23	0.86	0.39
2	α-Pinene	7.47	3.12	1.30	1.18	1.36	2.32	1.49	0.88	0.67
3	β-Phellandrene	8.81	5.97	11.01	9.43	10.96	4.90	8.91	4.78	6.44
4	β-Pinene	8.97	0.75	0.92	9.43	0.93	0.64	0.92	0.56	0.48
5	1-Octen-3-ol	9.05	-	-	-	0.06	-	-	-	-
6	β-Myrcene	9.38	0.67	0.37	0.25	0.30	0.49	0.48	0.23	-
7	β-Thujene	9.39	-	-	-	-	-	-	-	0.27
8	α-Phellandrene	9.98	0.12	-	-	0.06	0.13	0.09	-	-
9	δ-Carene	10.37	0.96	1.42	1.31	1.40	0.92	1.58	0.65	0.45
10	p-Cymene	10.64	0.22	0.16	0.20	0.22	0.20	0.30	0.22	0.08
11	D-Limonene	10.83	-	0.23	0.21	0.48	-	0.99	0.60	0.25
12	Sabinene	10.88	-	-	-	-	-	-	-	0.18
13	Eucalyptol	10.95	14.52	0.54	0.52	4.92	9.30	13.76	7.53	3.73
14	β-Ocimene	11.48	0.07	0.08	0.08	0.25	-	0.06	-	-
15	γ-Terpinene	11.91	1.52	2.30	2.21	2.24	1.52	2.48	1.04	0.74
16	(Z)-β-Terpineol	11.92	-	-	-	0.06	-	-	-	-
17	2-Carene	12.92	3.23	0.07	0.09	-	0.37	-	0.10	0.17
18	Methyl benzoate	13.21	-	-	-	-	-	-	0.14	-
19	Linalool	13.48	0.73	0.16	0.76	1.10	1.14	1.55	0.76	1.13
20	Fenchene	14.39	0.09	-	-	-	-	-	0.06	-
21	p-Menth-2-en-1-ol	14.40	-	0.11	0.11	0.13	0.09	0.09	-	-
22	Lavandulol	15.90	-	1.29	0.35	0.19	0.24	0.12	0.30	0.60
23	Myrcenol	16.12	-	-	-	0.07	-	-	-	0.11
24	Terpinen-4-ol	16.53	2.41	3.52	3.69	3.79	2.54	4.02	1.43	1.70
25	α-Terpineol	17.05	0.85	0.17	0.19	0.40	0.97	1.01	0.47	0.39
26	(R)-Lavandulyl acetate	20.31	0.15	-	0.33	-	1.18	0.33	0.81	1.83
27	Lavandulyl acetate	20.32	-	1.42	-	0.63	-	-	-	-
28	Pseudolimonen	21.36	-	-	-	-	-	0.06	0.06	-
29	(E)-β-Terpinolene	22.04	0.16	-	-	-	-	-	-	-
30	α-Terpinene	22.05	-	-	-	0.12	-	-	-	0.14
31	δ-Elemene	22.17	-	-	1.07	1.51	2.14	1.37	1.24	2.02
32	α-Terpinyl acetate	22.54	-	-	0.13	1.80	2.45	4.14	4.21	2.11
33	β-Bourbonene	23.85	-	-	-	-	-	0.09	-	-
34	β-Elemene	24.05	0.18	0.10	0.39	0.34	-	0.34	0.27	0.44
35	β-Caryophyllene	25.15	24.41	28.52	33.91	34.48	28.48	17.91	38.92	37.66
36	Artemisia triene	25.38	0.14	-	-	-	-	-	-	-
37	γ-Elemene	25.39	-	0.15	0.50	0.86	1.18	1.03	0.91	1.14
38	α-Funebrene	25.77	-	-	0.08	-	-	-	-	-
39	α-Muurolene	25.78	-	-	-	-	0.26	-	-	-
40	α-Ylangene	25.79	-	-	-	0.17	0.23	0.29	0.17	-
41	β-Patchoulene	25.80	-	-	-	-	-	-	-	0.24
42	α-Cubebene	26.04	-	-	-	-	-	0.08	-	-
43	Humulene	26.26	1.05	1.15	1.44	1.47	1.27	0.81	1.78	1.61
44	4,5-Di-epi-aristolochene	26.73	-	-	-	-	0.06	0.08	-	0.06
45	cis-Muurola-3,5-diene	26.95	-	-	-	-	-	-	0.24	-
46	Isoledene	26.96	-	-	-	-	-	-	-	0.27
47	Cyclosativene	26.98	-	-	-	0.16	-	-	-	-

Table 2. *Cont.*

No	Components	RT	NCK	N5	N10	N20	PCK	P5	P10	P20
48	β-Copaene	27.09	0.14	0.59	0.22	0.40	0.53	0.50	-	-
49	D-Germacrene	27.10	-	-	-	-	-	-	0.39	0.54
50	Eremophilene	27.35	9.20	11.50	17.41	18.51	20.93	22.61	12.68	23.44
51	Selinene	27.59	-	-	0.13	-	0.34	0.31	0.20	-
52	Guaia-9,11-diene	27.60	-	-	-	0.18	-	-	-	0.33
53	α-Longipinene	27.84	-	-	-	-	-	0.26	-	-
54	δ-Cadinene	28.28	-	-	-	-	0.12	0.07	0.14	0.09
55	Elixene	29.57	-	-	-	-	0.35	-	-	0.36
56	cis-3-Hexenyl benzoate	29.90	0.13	0.13	0.17	0.11	0.11	-	0.29	0.15
57	Caryophyllene oxide	30.30	1.41	0.86	1.68	3.01	3.49	2.35	6.90	3.15
58	Isocamphane	31.13	-	-	-	-	-	-	-	0.10
59	Humulene-1,2-epoxide	31.14	-	-	-	-	-	-	0.24	-
60	α-Patchoulene	31.36	-	-	-	-	-	-	-	0.18
61	Bulnesol	31.37	-	-	-	-	-	0.19	-	-
62	Longifolene	31.47	-	-	-	-	-	-	0.06	-
63	Valencene	31.53	0.19	-	-	-	0.13	-	0.14	-
64	β-Nootkatol	31.67	-	-	0.41	-	-	-	-	-
65	β-Spathulenol	31.68	-	-	-	1.54	1.93	2.05	2.17	1.81
66	Aromandendrene	31.85	-	-	0.10	0.07	0.08	2.05	-	0.11
67	Caryophylla-4(12),8(13)-diene-5β-ol	31.95	0.51	-	-	-	-	-	-	-
68	Isoaromadendrene epoxide	31.96	-	-	-	-	0.09	-	0.42	0.12
69	β-Gurjurene	32.03	-	-	-	-		-	-	0.17
70	α-Guaiene	32.04	0.46	-	-	-	-	0.28	-	-
71	α-Cadinol	32.48	-	1.14	-	-	3.47	-	-	-
72	β-Eudesmol	32.49	5.05	-	2.83	1.89	-	2.66	3.53	2.61
73	cis-Meta-mentha-2,8-diene	32.92	-	-	-	0.23	-	-	-	-
74	α-Selinene	38.84	0.95	1.33	1.23	-	-	-	-	-
75	4-Quinolinol, 2,7,8-trimethyl	40.08	1.94	-	-	-	-	-	-	-
76	2-Methyl-1-tripropylsilyloxypropan	40.09	-	-	0.79	-	-	-	-	-
77	1-Indanone, 3,3,4,5,7-pentamethyl	40.10	-	1.54	-	-	-	-	-	-
78	2,3-Dihydrothieno(2,3-b)quinoline	40.18	0.93	-	-	-	-	-	-	-
79	(E)-Farnesene epoxide	41.03	-	-	-	-	-	-	-	0.08
80	Pyridine,2-methyl-3-(2-propenyloxy)	41.05	-	1.83	0.46	-	-	-	0.10	-
81	Epimanool	41.52	-	2.19	-	-	-	-	-	-
82	Epimanoyl oxide	41.92	-	-	0.13	-	-	-	-	-
83	Isopimaradiene	42.02	-	-	-	0.27	-	-	-	-
84	7-Methyl-1,2,3,5,8,8a-hexahydro-naphthalene	42.65	2.04	12.19	0.99	-	-	-	1.02	0.23
85	Abitatriene	43.41	0.97	1.90	0.40	-	-	-	0.46	-
86	p-Toluic acid,2-adamantyl ester	44.08	-	-	-	-	-	-	-	0.29
87	1,8-Nonadiene-2,7-dimethyl-5-(methylethenyl)	44.77	0.42	-	-	-	-	-	-	-
	Total		86.03	90.69	95.30	97.59	95.21	98.94	97.96	99.06
	No. of compounds		36	33	39	40	37	40	42	45

Percentage values are means of five analyses. RT: Retention time; -: Not detected.

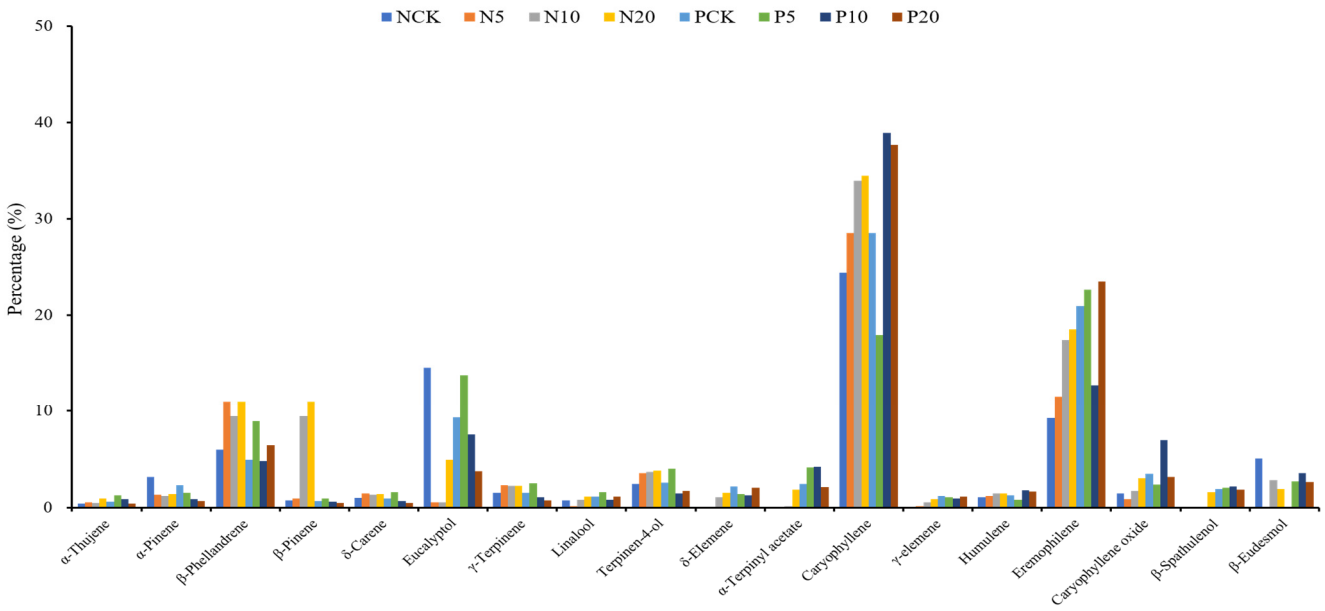

Figure 3. Effects of different fertilizer treatments on main essential oil components in shoots of *V. negundo*. Percentage values are the means of five analyses.

4. Discussion

This study demonstrates that appropriate N and P fertilizations can improve the yield of biomass, essential oils and bioactive components in field cultivated *V. negundo*. In soil chemical properties, the soil pH values were noted to increase with increasing N or P fertilization rates, which could be resulted from the hydrolysis of urea by urease to produce carbon dioxide, ammonia, and ammonium carbonate or ammonium bicarbonate, and the combined application of potassium chloride and urea may also reduce the ammonia losses [26]; these eventually may lead to the increase in soil pH. With the exception of P20, both N, and P fertilization led to an increase in EC values, this may explain by the fact that urea, superphosphate, and potassium chloride are water-soluble fertilizers, and the increasing applications of these fertilizers may lead to an increase in soil soluble salt contents, and hence causing the increase in soil EC values.

Although the differences in soil organic matter contents between before and after experiments in fertilizer treated groups were not statistically significant, indicating that the application of chemical fertilizers had a minimal effect on the soil organic matter content, and they were mainly derived from plant residues such as leaves and weeds dropped during the experimental period. In this study, the total N, and extractable P and K contents in soils before the experiment were significantly lower than those after the experiment, especially those treated with fertilizers; this indicates that when N, P, and K fertilizers were fertilized in the plants, the content of these available nutrients in soil increased significantly. At the end of the experiment, N5 and N10 were noted to have a lower soil total N content than the control group; this may explain that the combined application of medium and low amounts of N, P, and K fertilizers could promote the absorption of N nutrient by plants, and thus decreased this nutrient content in the soil; however, excessive N application increased its content in soils [32].

The extractable P and K contents in soils of both N and P fertilizer treatments were significantly increased compared with the control group; this observation indicates that the application of superphosphate and potassium chloride can substantially increase the content of extractable P and K in the soil. These results also show that the optimum amount of P application is around 100 mg ha^{-1}, and exceeding this would reduce the absorption of P nutrients by plants, as demonstrated by a high soil extractable P content in P20 treatment.

A trend of increased biomass was noted with increasing N fertilization rates, whereas P10 and P20 appear to produce significantly higher biomass than the control. Although

increasing rates of P fertilization favor the growth and development of *V. negundo* shoots, the optimal yield was obtained with 100 kg-N ha^{-1} and 100 kg-P ha^{-1} fertilization. Increasing P fertilization was also shown to significantly increase the biomass of sage [11], lavender grass [11,33], and calendula [34], but not the biomass of green mint [7]. These results indicate that the proportion of N and P fertilizer application rates may play an essential role in affecting the biomass production of different plant species.

Nitrogen is essential for plants to synthesize nucleic acids, proteins, phytohormones, coenzymes, and chlorophyll; it can promote plant growth and increase plant branches and green leaves [35]. Therefore, a sufficient N source is essential for normal plant growth and metabolism. Under the condition of fixed application rates of N or P and K fertilizers, increasing application rates of either N or P fertilizer did not lead to a statistically significant increase in the total N content in plant tissues.

Phosphorus is the main component of the plant nucleus and protoplast, and is an essential component of nucleic acid, phosphoester, and adenosine triphosphate; it can assist plant root development, and promote flower bud differentiation and flowering [35]. There was a trend of increased total P content in groups treated with increasing rates of N, indicating that the enhancement of P uptake after N application can probably be attributed to acidification of the rhizosphere [36] or the effects of mobilizing soil P or increasing P uptake efficiency by roots [37]. In P fertilizer treatments, although the total P content appears to increase and then decrease with increasing P application rates, there was no significant difference between treatments. Similar results were also observed in the study of spearmint plants [7]. The possible reason is that after P treatments, the plants absorb the available P as the basic requirement for growth and metabolism. The excess P is used in the biosynthesis of secondary metabolites such as monoterpenes, sesquiterpenes, and diterpenes, components of essential oils.

Potassium is a cofactor for protein synthesis in plants, which can regulate the opening and closing of leaf stomata, maintain electrical neutrality in plant cells, and promote the healthy development of branches [35]. There was no difference in the total K content of the plants among N or P treatments; the possible reason is that there is sufficient K in the soil to provide for the plant's normal growth. Hence, the additional application of K fertilizer has a minimal effect on the total K content in the plant tissues.

Phenols and flavonoids are common and important secondary metabolites of plants, which can help plants to adapt to the environment, resist adversity and reproduce offspring [38]. The effects of N fertilization on the biosynthesis of polyphenols have been reported to be dependent on the plant species [39]. In general, increasing N fertilization increases biomass production, but may cause a reduction in the concentration and yield of secondary metabolites. In this study, the total phenolic contents in both N- or P-treated groups were not significantly different from the control group. Although the N-treated groups appear to have an increased total flavonoid content at low N fertilization, their content decreased with increasing N fertilization. Studies have demonstrated that a higher phenolic content was generally observed in plants receiving less N or without fertilization [40]; this is because plant growth is heavily dependent on protein synthesis for the manufacture of photosynthetic, biosynthetic, and regulatory enzymes, and for structural protein, and hence the phenolic biosynthesis has to compete with the growth for the common substrate. As a result, vegetative growth generally receives resource priority over secondary metabolism and storage. This observation was consistent with the results reported on the cultivation of *Hypericum perforatum* [41], *Cyclocarya paliurus* [42], and *Origanum vulgare* ssp. *Hirtum* [43].

Increasing P fertilization tends to decrease and then increase the total flavonoid content; this result is consistent with the study of Chrysargyris et al. [7], where under the condition of fixed N fertilizer application, the increase in P applications led to a reduction and then increase in the total flavonoid contents in spearmint. Based on these results, the changes in the total flavonoid content, but not the total phenolic contents in the *V. negundo* plant may be related to the application rates of N and P fertilizers [44].

Compared with the control group, an increasing trend in essential oil yield was noted with increasing N or P application rates, but the difference in the yield of essential oil among N treatments was not statistically significant; however, under a fixed amount of N and K fertilizer application, increasing P fertilization can significantly increase the essential oil production in *V. negundo*. This observation was consistent with previous findings that increasing P fertilization can increase the essential oil production in basil, lavender, marjoram, and sage [8–11]. The possible explanation for this observation is that plant terpenoids are produced via the biosynthetic pathway, which utilizes acetyl-coenzyme A as the key substrate [45,46], and P is a constituent element of acetyl-coenzyme A, thus increasing the amount of P fertilizer can promote the biosynthesis of plant terpenoids, which in turn increases the yield of essential oil in *V. negundo* plant.

Terpenes are compounds synthesized with isoprene as the main molecular structure. According to the numbers of carbon atoms in the structure, they can be divided into hemiterpenes (5C) and monoterpenes (10C), sesquiterpenes (15C), diterpenes (20C), sesquiterpenes (25C), triterpenes (30C) and tetraterpenes (40C) and others [47]. The essential oil components of plants are mainly comprised of monoterpenes and sesquiterpenes. This study demonstrates that fertilization may affect the profile of essential oil composition, as shown by the differences in the number of essential oil components in different N or P treatments. Regardless of treatments, the sesquiterpene-type β-caryophyllene and eremophilene were the major components of *V. negundo* essential oil. Studies have shown that β-caryophyllene possesses anti-inflammatory, anti-anxiety, anti-depression and other effects [27], while eremophilene has antibacterial, anti-cancer, and immunomodulatory activities [28]. This study showed that P20 has the highest relative content of eremophilene (23.44%), followed by the P5 treatment (22.61%). P10 exhibited the highest relative content of β-caryophyllene (38.92%), followed by the P20 (37.66%). These results conclude that, under the condition of 100 kg ha^{-1} N fertilizer application, increasing P fertilization rates can increase the content of the main bioactive constituents (i.e., β-caryophyllene and eremophilene) in *V. negundo* materials.

The present findings have highlighted that the development and polyphenol and essential oil production of *V. negundo* may be affected positively or negatively by the ratio and amount of mineral nutrients. Previous studies have pointed out differences in essential oil compositions in *V. negundo* growing in different regions. For example, the main chemical constituents of *V. negundo* essential oil grown in India are viridiflorol and β-caryophyllene [48], and in the Philippines is α-pinene and β-caryophyllene [25], while the essential oil of *V. negundo* grown in southern Taiwan contained mainly β-caryophyllene and eremophilene [23]. These results suggest that the identification criteria for the origin of *V. negundo* could be established according to the difference in the composition of its essential oil.

5. Conclusions

This study demonstrates that under the condition of fixed P and K fertilization, increasing N application rates can increase the biomass of *V. negundo* plant, but not the yield of essential oil. However, under the fixed amount of N and K fertilizers, increasing P application rates can effectively promote biomass and essential oil production; furthermore, the application of 200 kg-P ha^{-1} has the optimal yield of *V. negundo* essential oil, and contents of bioactive ingredients (β-caryophyllene and eremophilene). This study also found that the contents of total flavonoids but not total phenolic in *V. negundo* shoots are related to N and P fertilization rates. Considering the effects of fertilizer on yield, quality, and potential environmental pollution, the optimum fertilizer application for *V. negundo* was 100 kg-N ha^{-1}, 200 kg-P ha^{-1} and 100 kg-K ha^{-1} (NPK ratio of 1:2:1).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12060859/s1, Figure S1: Average monthly field temperature and rainfall during the period of *V. negundo* cultivation (June 2018 to December 2018); Table S1: Design of different fertilizer treatments in field experiments.

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References

1. Chen, S.L.; Yu, H.; Luo, H.M.; Wu, Q.; Li, C.F.; Steinmetz, A. Conservation and sustainable use of medicinal plants: Problems, progress, and prospects. *Chin. Med.* **2016**, *11*, 37. [CrossRef] [PubMed]
2. Arsenijević, J.; Drobac, M.; Šoštarić, I.; Jevđović, R.; Živković, J.; Ražić, S.; Moravčević, D.; Maksimović, Z. Comparison of essential oils and hydromethanol extracts of cultivated and wild growing *Thymus pannonicus* All. *Ind. Crops Prod.* **2019**, *130*, 162–169. [CrossRef]
3. Pandey, V.; Patel, A.; Patra, D.D. Integrated nutrient regimes ameliorate crop productivity, nutritive value, antioxidant activity and volatiles in basil (*Ocimum basilicum* L.). *Ind. Crops Prod.* **2016**, *87*, 124–131. [CrossRef]
4. Bistgani, Z.E.; Ataollah Siadat, S.; Bakhshandeh, A.; Ghasemi Pirbalouti, A.; Hashemi, M.; Maggi, F.; Reza Morshedloo, M. Application of combined fertilizers improves biomass, essential oil yield, aroma profile, and antioxidant properties of *Thymus daenensis* Celak. *Ind. Crops Prod.* **2018**, *121*, 434–440. [CrossRef]
5. Chiu, S.M.; Lu, H.Y.; Liu, H.I. The growth and development of turmeric (*Curcuma aromatica* Salisb.) plants. II. Effects of N and K fertilization on plant growth and rhizome yield and quality. *J. Agric. Res. China* **1993**, *42*, 370–379.
6. Chung, R.S.; Chen, C.C.; Ng, L.T. Nitrogen fertilization affects the growth performance, betaine and polysaccharide concentrations of *Lycium barbarum*. *Ind. Crops Prod.* **2010**, *32*, 650–655. [CrossRef]
7. Chrysargyris, A.; Petropoulos, S.A.; Fernandes, Â.; Barros, L.; Tzortzakis, N.; Ferreira, I.C.F.R. Effect of phosphorus application rate on *Mentha spicata* L. grown in deep flow technique (DFT). *Food Chem.* **2019**, *276*, 84–92. [CrossRef]
8. Ramezani, S.; Reza Rezaei, M.; Sotoudehnia, P. Improved growth, yield and essential oil content of basil grown under different levels of phosphorus sprays in the field. *J. Appl. Biol. Sci.* **2009**, *3*, 96–101.
9. Erbaş, S.; Kucukyumuk, Z.; Baydar, H.; Erdal, I.; Şanli, A. Effects of different phosphorus doses on nutrient concentrations as well as yield and quality characteristics of lavandin (*Lavandula* × *intermedia* Emeric ex Loisel. var. *Super*). *Turk. J. Field Crops* **2017**, *22*, 32–38. [CrossRef]
10. Trivino, M.G.; Johnson, C.B. Season has a major effect on the essential oil yield response to nutrient supply in *Origanum majorana*. *J. Hortic. Sci. Biotechnol.* **2000**, *75*, 520–527. [CrossRef]
11. Nell, N.; Vötsch, M.; Vierheilig, H.; Steinkellner, S.; Zitterl-Eglseer, K.; Franz, C.; Novak, J. Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). *J. Sci. Food Agric.* **2009**, *89*, 1090–1096. [CrossRef]
12. Bansod, M.; Harle, U.N. *Vitex negundo* L: Phytochemical constituents, traditional uses and pharmacological properties: Comprehensive review. *Pharmacologyonline* **2009**, *1*, 286–302.
13. Gill, B.S.; Mehra, R.; Navgeet; Kumar, S. *Vitex negundo* and its medicinal value. *Mol. Biol. Rep.* **2018**, *45*, 2925–2934. [CrossRef] [PubMed]
14. Vishwanathan, A.S.; Basavaraju, R. A Review on *Vitex negundo* L.—A medicinally important plant. *Eur. J. Biol. Sci.* **2010**, *3*, 30–42.
15. Khan, A.; Naz, S.; Farooq, U.; Shahid, M.; Ullah, I.; Ali, I.; Rauf, A.; Mabkhot, Y.N. Bioactive chromone constituents from *Vitex negundo* alleviate pain and inflammation. *J. Pain Res.* **2018**, *11*, 95–102. [CrossRef]
16. Tiwari, O.P.; Tripathi, Y.B. Antioxidant properties of different fractions of *Vitex negundo* Linn. *Food Chem.* **2007**, *100*, 1170–1176. [CrossRef]
17. Kamruzzaman, M.; Bari, S.M.N.; Faruque, S.M. In vitro and in vivo bactericidal activity of *Vitex negundo* leaf extract against diverse multidrug resistant enteric bacterial pathogens. *Asian Pac. J. Trop. Med.* **2013**, *6*, 352–359. [CrossRef]
18. Khokra, S.L.; Prakash, O.; Jain, S.; Aneja, K.R.; Dhingra, Y. Essential oil composition and antibacterial studies of *Vitex negundo* Linn. extracts. *Indian J. Pharm. Sci.* **2008**, *70*, 522–526. [CrossRef]
19. Prasad, E.M.; Mopuri, R.; Islam, M.S.; Kodidhela, L.D. Cardioprotective effect of *Vitex negundo* on isoproterenol-induced myocardial necrosis in wistar rats: A dual approach study. *Biomed. Pharmacother.* **2017**, *85*, 601–610. [CrossRef]
20. Kadir, F.A.; Kassim, N.M.; Abdulla, M.A.; Yehye, W.A. Hepatoprotective role of ethanolic extract of *Vitex negundo* in thioacetamide-induced liver fibrosis in male rats. *Evid. Based Complement. Alternat. Med.* **2013**, *2013*, 739850. [CrossRef]
21. Tandon, V.R.; Khajuria, V.; Kapoor, B.; Kour, D.; Gupta, S. Hepatoprotective activity of *Vitex negundo* leaf extract against anti-tubercular drugs induced hepatotoxicity. *Fitoterapia* **2008**, *79*, 533–538. [CrossRef] [PubMed]

22. Villaseñor, I.M.; Lamadrid, M.R.A. Comparative anti-hyperglycemic potentials of medicinal plants. *J. Ethnopharmacol.* **2006**, *104*, 129–131. [CrossRef] [PubMed]
23. Huang, H.C.; Chang, T.Y.; Chang, L.Z.; Wang, H.F.; Yih, K.H.; Hsieh, W.Y.; Chang, T.M. Inhibition of melanogenesis versus antioxidant properties of essential oil extracted from leaves of *Vitex negundo* Linn and chemical composition analysis by GC-MS. *Molecules* **2012**, *17*, 3902–3916. [CrossRef] [PubMed]
24. Chattopadhyay, P.; Banerjee, S.; Pathak, M.P.; Agnihotri, A.; Karmakar, S.; Goyary, D.; Dhiman, S.; Veer, V. Acute and subchronic dermal toxicity of *Vitex negundo* essential oil. *Cutan. Ocul. Toxicol.* **2014**, *33*, 16–21. [CrossRef] [PubMed]
25. Singh, V.; Dayal, R.; Bartley, J.P. Volatile constituents of *Vitex negundo* leaves. *Planta Med.* **1999**, *65*, 580–582. [CrossRef] [PubMed]
26. Fidyt, K.; Fiedorowicz, A.; Strzadala, L.; Szumny, A. Beta-caryophyllene and beta-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Med.* **2016**, *5*, 3007–3017. [CrossRef]
27. Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J.Z.; Xie, X.Q.; Altmann, K.H.; Karsak, M.; Zimmer, A. Beta-caryophyllene is a dietary cannabinoid. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9099–9104. [CrossRef]
28. Yuyama, K.T.; Fortkamp, D.; Abraham, W.R. Eremophilane-type sesquiterpenes from fungi and their medicinal potential. *Biol. Chem.* **2017**, *399*, 13–28. [CrossRef]
29. Chou, C.H.; Yao, C. Phytochemical adaptation of coastal vegetation in Taiwan I. isolation, identification, and biological activities of compounds in *Vitex negundo* L. *Bot. Bull. Acad. Sin.* **1983**, *24*, 155–168.
30. Huang, S.H.; Ng, L.T. Quantification of polyphenolic content and bioactive constituents of some commercial rice varieties in Taiwan. *J. Food Compos. Anal.* **2012**, *26*, 122–127. [CrossRef]
31. Singh, A.; Sharma, P.; Garg, V.; Sharad, V. Extraction and analysis of essential oil of Nirgundi (*Vitex negundo* L.). *Der. Pharm. Sin.* **2011**, *2*, 262–266.
32. Li, Z.G.; Zhang, R.H.; Xia, S.J.; Wang, L.; Liu, C.; Zhang, R.Q.; Fan, Z.H.; Chen, F.; Liu, Y. Interactions between N, P and K fertilizers affect the environment and the yield and quality of satsumas. *Glob. Ecol. Conserv.* **2019**, *19*, e00663. [CrossRef]
33. Chrysargyris, A.; Panayiotou, C.; Tzortzakis, N. Nitrogen and phosphorus levels affected plant growth, essential oil composition and antioxidant status of lavender plant (*Lavandula angustifolia* Mill.). *Ind. Crops Prod.* **2016**, *83*, 577–586. [CrossRef]
34. Ahmad, I.; Jabeen, N.; Ziaf, K.; Dole, J.M.; Khan, M.A.S.; Bakhtavar, M.A. Macronutrient application affects morphological, physiological, and seed yield attributes of *Calendula officinalis* L. *Can. J. Plant Sci.* **2017**, *97*, 906–916. [CrossRef]
35. Lawlor, D.W.; Mengel, K.; Kirkby, E.A. Principles of plant nutrition. *Ann. Bot.* **2004**, *93*, 479–480. [CrossRef]
36. Thomson, C.; Marschner, H.; Römheld, V. Effect of nitrogen fertilizer form on pH of the bulk soil and rhizosphere, and on the growth, phosphorus, and micronutrient uptake of bean. *J. Plant Nutr.* **1993**, *16*, 493–506. [CrossRef]
37. Ruan, J.; Zhang, F.; Wong, M.H. Effect of nitrogen form and phosphorus source on the growth, nutrient uptake and rhizosphere soil property of *Camellia sinensis* L. *Plant Soil* **2000**, *223*, 65–73. [CrossRef]
38. Kougan, G.B.; Tabopda, T.; Kuete, V.; Verpoorte, V. Simple phenols, phenolic acids, and related esters from the medicinal plants of africa. In *Medicinal Plant Research in Africa*; Kuete, V., Ed.; Elsevier: Oxford, UK, 2013; pp. 225–249.
39. Olesińska, K.; Sugier, D.; Kaczmarski, Z. Yield and chemical composition of raw material from meadow Arnica (*Arnica chamissonis* Less.) depending on soil conditions and nitrogen fertilization. *Agriculture* **2021**, *11*, 810. [CrossRef]
40. Heimler, D.; Romani, A.; Ieri, F. Plant polyphenol content, soil fertilization and agricultural management: A review. *Eur. Food Res. Technol.* **2017**, *243*, 1107–1115. [CrossRef]
41. Radušienė, J.; Marksa, M.; Ivanauskas, L.; Jakštas, V.; Çalişkan, Ö.; Kurt, D.; Odabaş, M.S.; Çirak, C. Effect of nitrogen on herb production, secondary metabolites and antioxidant activities of *Hypericum pruinatum* under nitrogen application. *Ind. Crop. Prod.* **2019**, *139*, 111519. [CrossRef]
42. Deng, B.; Li, Y.; Xu, D.; Ye, Q.; Liu, G. Nitrogen availability alters flavonoid accumulation in *Cyclocarya paliurus* via the effects on the internal carbon/nitrogen balance. *Sci. Rep.* **2019**, *9*, 2370. [CrossRef] [PubMed]
43. Król, B.; Sęczyk, Ł.; Kołodziej, B.; Paszko, T. Biomass production, active substance content, and bioaccessibility of Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) following the application of nitrogen. *Ind. Crop. Prod.* **2020**, *148*, 112271. [CrossRef]
44. Sun, J.; Luo, H.; Jiang, Y.; Wang, L.; Xiao, C.; Weng, L. Influence of nutrient (NPK) factors on growth, and pharmacodynamic component biosynthesis of *Atractylodes chinensis*: An insight on acetyl-CoA carboxylase (ACC), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), and farnesyl pyrophosphate synthase (FPPS) signaling responses. *Front. Plant Sci.* **2022**, *13*, 799201. [PubMed]
45. Gershenzon, J. Metabolic costs of terpenoid accumulation in higher plants. *J. Chem. Ecol.* **1994**, *20*, 1281–1328. [CrossRef] [PubMed]
46. Yang, D.; Du, X.; Liang, X.; Han, R.; Liang, Z.; Liu, Y.; Liu, F.; Zhao, J. Different roles of the mevalonate and methylerythritol phosphate pathways in cell growth and tanshinone production of *Salvia miltiorrhiza* hairy roots. *PLoS ONE* **2012**, *7*, e46797. [CrossRef]
47. Roberts, S.C. Production and engineering of terpenoids in plant cell culture. *Nat. Chem. Biol.* **2007**, *3*, 387–395. [CrossRef]
48. Padalia, R.C.; Verma, R.S.; Chauhan, A.; Chanotiya, C.S.; Thul, S. Phytochemical diversity in essential oil of *Vitex negundo* L. populations from India. *Rec. Nat. Prod.* **2016**, *10*, 452–464.