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CROP SCIENCE

Green manure (*Crotalaria juncea* L.) enhances *Origanum vulgare* L. biomass accumulation, essential oil yield, and phytochemical properties

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Abstract: Green manure (GM) may reduce the use of chemical fertilizers, been an ecologically appropriate strategy to cultivation of medicinal plants. *Crotalaria juncea*, is one of the most used because it adapts to different climatic and high nitrogen content. *Origanum vulgare*. is widely used in cooking, pharmaceutical, cosmetic industries and food products. The objectives of this study were to evaluate the GM on biomass, essential oil (EO), phenolic and antioxidant. The experiment consisted: control; 150, 300, 450, and 600 g (Sh= leaves+steam) more 200 g roots (R); 600 g aerial part; 200 g roots; and soil with 300 g cattle manure per pot. The highest dry weights were observed in the presence of GM and cattle manure (90 days). The control had an EO production 75% lower in relation to the dose of 450 g GM (Sh+R). Principal component analysis showed that GM and cattle manure positively influenced the dry weight, content, yield, and EO constituents, and total flavonoids. The GM contributed to the accumulation of the major EO compounds (*trans*-sabinene hydrate, thymol, terpinen-4-ol). The GM management may be beneficial for cultivating, because it can increase the production of biomass and the active components, in addition to being an inexpensive resource.

Key words: organic cultivation, *trans*-sabinene hydrate, secondary metabolite, sunn hemp, terpinen-4-ol, thymol.

INTRODUCTION

Plants used for medicinal, aromatic, and seasoning purposes are widely used and consumed worldwide; among the most consumed species is oregano (*Origanum vulgare* L.), which is known mainly for its use in cooking, often in tomato sauce, pizzas, salads, and meat (De Oliveira et al. 2017). In addition, the constituents of this spice are used in the agricultural, pharmaceutical, and cosmetic industries as a flavoring substance in food products, alcoholic beverages, and perfumery due to its spicy fragrance (Coqueiro et al. 2012, Pereira & Dos Santos 2013).

Of the various essential oils produced worldwide, oregano oil is valued and important to several industrial segments. Its essential oil is used for the production of biodegradable films (Medeiros 2017); as an antimicrobial for bacteria, fungi, and yeasts treatments (Jan et al. 2020); as an antioxidant (Tapiero et al. 2019); in animal feed (Migliorini et al. 2019), in medicine as a therapy for chronic rhinosinusitis (Alagawany et al. 2020, Kamaneh et al. 2020); and increase the shelf-life of juices and other foods not containing synthetic preservatives (Lee et al. 2020). In addition, despite the extensive literature available on the use of essential oil and the utilization of oregano in the industry, there are few reports on the cultivation of this species (Skoufogianni et al. 2019).

According to Marques et al. (2018), techniques such as organic fertilization, minimum cultivation, and all organic agricultural practices are recommended for the cultivation and management of medicinal plants. Within these management recommendations, the preservation of active ingredients is encouraged by developing plants that are more resistant to and free of chemical pesticides that can compromise the chemical composition of a plant, changing it or even making it unusable as a medicinal plant (Marques et al. 2018).

Several studies show that over the long term, in comparison with using mineral fertilizers, using organic fertilizers (animal manure, plant residues, among others) is less expensive; improves soil structure, texture, and aeration; and increases the water holding capacity and organic matter (OM) content of soil. Mineral fertilizers in the short term bring benefits (rapid availability of nutrients) but in the long term causes serious side effects such as soil compaction, erosion, soil toxicity, and deterioration in total fertility (Li et al. 2018, Assefa & Tadesse 2019, Ma et al. 2021).

Among the various forms of organic fertilization with good costs/benefits and broad technical results for soils and plants, green fertilization is the easiest to apply and least costly (Rocha et al. 2009). A green fertilization system is characterized by the use of plants in succession, rotation, or intercropping with crops of economic interest. Such plants can be incorporated into the soil or removed and maintained on the surface, providing a general improvement in the physical, chemical, and biological characteristics of soil (Alcântara 2016, Espíndola et al. 1997, Meena et al. 2020).

Eiras & Coelho (2011) reported that plants in the legume family (Fabaceae) are the most commonly used as green manure (GM). One of the main reasons for their use is related to the fact that the species in this family have the ability to perform the symbiosis process with atmospheric N_2-fixing bacteria, and that they are hardy, high dry matter producers, and have deep root systems (Meena et al. 2020, Silva & Menezes 2007). In Brazil among the species used in GM, *Crotalaria juncea* L., commonly known as shunn hemp, is one of the most used because it adapts to different climatic conditions and soil types and stands out for its large biomass production capacity (21-60 t ha^{-1}), nutrient accumulation (150-450 kg of N ha^{-1}), and high residue quality (Araújo et al. 2010, Xavier et al. 2017).

The management of GM was tested and applied to *Ocimum selloi* Benth. and *Echinacea purpurea* (L.) Moench, and no increases in dry matter and chemical compounds were observed (Morais & Barbosa 2012, Susanti et al. 2017). However, other studies have shown that this type of management can provide higher dry matter yields, essential oil production, bioactive constituents, and enzymatic activity (Adeniji & Kumoye 2020, He et al. 2018, Massey et al. 2021, Honorato et al. 2022), showing that the production of medicinal plants is influenced by the management of GM as well as by the quality of the organic waste used.

To the authors knowledge, to date, there is a lack of studies relating the use of GM in the cultivation of the species *Origanum vulgare* L. Thus, proposing new management systems to improve its biomass and essential oil production, as well as its active constituent, is of great importance for providing a quality material to the market, as it is widely used. Given the above information, the objective of this study was to evaluate the effect of GM (*C. juncea* L.) on the biomass production, chemical composition of essential oil, accumulation of phenolic compounds, and antioxidant activity of *O. vulgare* L.

List of abbreviations:

Dimethyl sulfoxide (DMSO)
Equivalent Ascorbic Acid (EAA)
Equivalent Gallic Acid (EGA)
Equivalent Trolox (ET)
Flame Ionization Detector (FID)
Gas Chromatography (GC)
Green Manure (GM)
Leaf Area Ratio (LAR)
Leaf Dry Weight (LDW)
Organic Matter (OM)
Principal Component Analysis (PCA)
Quercetin Equivalent (EQ)
Randomized Block Design (RBD)
Root Dry Weight (RDW)
Shoot Dry Weight (ShDW)
Root-to-Shoot Ratio (R/Sh)
Specific Leaf Area (SLA)
Stem Dry Weight (SDW)
Total Antioxidant Capacity (TAC)
Total Dry Weight (TDW)
Total Leaf Area (TLA)
2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)
2,2-diphenyl-1-picrylhydrazyl (DPPH)

MATERIALS AND METHODS

Vegetal material and experimental conditions

Preliminary experiments were carried out in two consecutive years in the same location with several species to test the effect of green manure on plants. Experiments were conducted in pots of 10 L and was carried out in the Medicinal Plant Garden of the Federal University of Lavras, Brazil, located at the geographic coordinates 21º 14'S and 45º 00W, at 918 m altitude. The mean climatic conditions during the growing seasons were 18.57° C (minimum) and 28.95° C (maximum) as average daily temperature and 71.83% as mean relative humidity.

The exsiccate of the species is deposited in the ESAL Herbarium of the Department of Biology (number 22.156) in Federal University of Lavras. The oregano seedlings were produced using apical cuttings (approximately 4 to 5 cm in length). The plants were kept in a greenhouse with automated irrigation for 30 days and then the plants were transplanted into 10 L pots.

Six sunn hemp seeds were sown in 10 L pots containing soil and sand (2:1). At the beginning of flowering, the shoots (Sh = leaf+stem) was removed and ground, and the soil present in the pots was broken up so that the roots (R) were incorporated together with the aerial part according to each treatment.

Sunn hemp was chopped in a forage chipper, and then, the biomass was incorporated into the soil. The cultivation was performed 30 days after the incorporation of GM into the pots. The experiment was in a randomized block design (RBD) with eight treatments: control (soil); 200 g pot^{-1} (sunn hemp root); 150, 300, 450, and 600 g pot^{-1} (sunn hemp shoot) + 200 g pot^{-1} (sunn hemp root); 600 g pot^{-1} (sunn hemp shoot); and positive control (cattle manure 300 g pot^{-1}). Each treatment consisted of 4 replicates (five plants per replicate), totaling 160 experimental units. Irrigation was performed two/three times a week according to the needs of the plants. A soil sample from each treatment was collected for chemical analysis conducted according to Teixeira et al. 2017. The chemical characteristics of the substrate are described in Table I. Table II shows the nutritional characterization of sunn hemp and the amount of nutrients added to the soil according to each dose. The analysis of green manure (leaf and stem) was carried out according to Malavolta et al. (1997).

Table I. Soil chemical characterization after 90 days according to the doses of green manure (*Crotalaria juncae* L.) used in the cultivation of *Origanum vulgare* L.

Soil parameters	Control soil	Cattle manure	200	150	300	450	600	600
			g GM (R)	.... g GM (Sh+R)				g GM (Sh)
pH (H_2O)	6.30	5.60	5.50	5.50	5.70	5.80	6.00	6.10
K (mg/dm^3)	47.77	96.50	103.63	100.80	78.21	88.80	96.90	72.34
P (mg/dm^3)	0.84	12.52	0.00	0.00	0.00	0.00	0.00	0.00
Ca (cmolc/dm^3)	1.76	2.18	1.37	2.55	2.85	2.47	1.56	1.93
Mg (cmolc/dm^3)	0.39	0.62	0.30	0.50	0.51	0.52	0.38	0.50
Al (cmolc/dm^3)	0.10	0.00	0.00	0.10	0.10	0.10	0.00	0.00
H+Al (cmolc/dm^3)	1.40	1.90	1.60	1.50	1.50	1.60	1.60	1.60
EBS (cmolc/dm^3)	2.27	3.05	1.94	3.31	3.56	3.22	2.19	2.62
t (cmolc/dm^3)	2.37	3.05	1.94	3.41	3.66	3.32	2.19	2.62
T (cmolc/dm^3)	3.67	4.95	3.54	4.81	5.06	4.82	3.79	4.22
V (%)	61.92	61.56	54.80	68.80	70.40	66.80	57.74	61.98
m (%)	4.22	0.00	0.00	2.93	2.73	3.01	0.00	0.00
OM (dag/kg)	1.18	2.03	1.37	1.66	1.94	1.69	1.71	1.81
P-Rem (mg/L)	12.50	21.80	15.70	18.20	19.80	18.40	16.30	15.50
Zn (mg/dm^3)	1.00	2.60	0.90	2.30	2.40	2.40	1.00	1.50
Fe (mg/dm^3)	41.70	34.30	24.70	25.70	25.20	25.00	24.90	25.90
Mn (mg/dm^3)	15.60	14.90	9.60	14.00	14.00	13.80	11.50	12.20
Cu (mg/dm^3)	1.48	1.27	1.14	1.20	1.10	1.18	1.15	1.21
B (mg/dm^3)	0.01	0.13	0.05	0.04	0.11	0.13	0.03	0.04
S (mg/dm^3)	2.20	7.60	7.90	6.40	6.80	7.10	8.00	6.40

pH in water; EBS – exchangeable base sums; CEC (t) – effective cation exchange capacity; CEC (T) – cation exchange capacity pH 7.0; V – base saturation index; m – aluminum saturation index; OM – Organic matter; P-Rem – remaining phosphorus. GM (Green Manure); R (Root); Sh (leaf + stem).

Plant growth

At 90 days after transplanting, the following characteristics were evaluated: leaf dry weight (LDW- g plant^{-1}); stem dry weight (SDW- g plant^{-1}); root dry weight (RDW- g plant^{-1}); shoot dry weight (ShDW- g plant^{-1} = LDW+SDW); total dry weight (TDW- g plant^{-1} = LDW+SDW+RDW); and the root-to-shoot ratio (R/Sh). The total leaf area (TLA) was measured using *ImageJ* ® *software*. The following relationships were also observed: leaf area ratio (LAR = TLA/TDW) and specific leaf area (SLA= TLA/LDW).

Photosynthetic pigments and nutritional analysis

The photosynthetic content was extracted and analyzed according to De Assis et al. (2020). The samples were prepared in quintuplicate, and three aliquots (3 mL) of each replicate were transferred to a quartz cuvette; the optical density values were read in a spectrophotometer Tecan Infinity M200 PRO, 480, 649 and 665 nm, operated in the I -Control® data processing system (version 3.37), against blank DMSO (Dimethyl sulfoxide – Sigma - Aldrich®). The

Table II. Nutritional characterization of green manure (*Crotalaria juncea* L.) and the amount of macro- and micronutrients incorporated in the soil according to each dose used in the cultivation of *Origanum vulgare* L.

Treatments		Macronutrients						Micronutrients				
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Shoots (leaf+stem) g/pot	Roots g/pot	.... g/kg						.... mg/Kg				
Green Manure		34.5	1.9	9.9	11.2	3.2	2.1	24.9	6.3	655.1	50.5	34.3
Root GM		9.7	0.6	13.4	3.1	0.9	0.7	12.7	8.8	881	18.8	12.7
150	200	7.1	0.4	4.2	2.3	0.7	0.45	6.2	2.7	274.6	11.4	7.6
300	200	12.3	0.7	5.7	4.0	1.2	0.8	10.0	3.7	372.8	18.9	12.8
450	200	17.4	1.0	7.2	5.6	1.6	1.1	13.7	4.6	471.1	26.5	17.9
600	200	22.6	1.2	8.6	7.3	2.1	1.4	17.4	5.5	569.3	34.0	23.1
600	-	20.7	1.1	5.9	6.	2.0	1.26	14.9	3.8	393.0	30.3	20.6
-	200	1.9	0.1	2.7	0.62	0.18	0.1	2.5	1.8	176.3	3.8	2.5
Control soil (-)		-	0.0	0.03	0.14	0.006	0.002	0.02	1.4	43.0	15.3	0.46
Cattle manure (+)		11.0	5.0	12.0	7.0	4.0	2.0	140.0	57.0	33811	920.0	72.0

wavelengths and equations adopted for these calculations were based on the methodology of Wellburn (1994): Chlorophyll *a* 649 = (12.47 x A665) - (3.62 x A649); Chlorophyll *b* 665 = (25.06 x A649) - (6.5 x A665); Carotenoids 480= (1000 x A480 – 1.29 x C*a* – 53.78 x C*b*)/ 220; Total chlorophyll = chlorophyll *a* + chlorophyll *b*.

To determine the accumulation of N, P, K, Ca, Mg, S, B, Cu, Mn, Zn, and Fe in the dried leaves of *Origanum vulgare* L., 2 g of each treatment was sampled for analysis. Macronutrients were expressed in g per kg and micronutrients in mg per kg of dry leaf weight.

Extraction of essential oil

The essential oil was extracted from 20 g of dried leaves placed in a 2,000 mL volumetric flask filled with 500 mL of distilled water by hydrodistillation in a modified Clevenger apparatus. The extraction time was fixed at 90 min from the boiling point. The extraction was performed in quadruplicate for each treatment. The essential oil was weighed, stored in an airtight amber bottle, and kept in a refrigerator at 4 °C. The essential oil content was expressed in g 100 g^{-1} of leaf dry weight, and the yield was expressed in g $plant^{1}$ (De Assis et al. 2020).

Analysis of essential oil

The essential oil was analyzed according to De Assis et al. (2020). Briefly, the quantitative and qualitative chemical analyses were performed in triplicate in an Agilent® 7890A Gas Chromatography (GC) system operated in an MSD CHEM Station Ver. E.02.02.1431 data processing system equipped with injector/autosampler CombiPAL Autosampler System (CTC Analytic AG, Switzerland) and Flame Ionization Detector (FID). Retention index was calculated using the equation of Van Den Dool & Dec Kratz (1963); the evaluations of the retention index described in the literature were consulted (Adams 2007).

Preparation of extracts

The extracts were prepared according to the methodology of Baranauskaité et al. (2016) with adaptations, namely, 3 g of leaves ground and weighed on an analytical balance and added to a 500 mL volumetric flask for extraction in 300 mL of ethanol – Sigma - Aldrich® (92.8°) by thermal reflux. The extraction occurred for 360 min, and then, the extracts were placed in a rotary evaporator under reduced pressure for solvent evaporation to obtain the crude extracts.

Subsequently, 10 mg of the crude extracts was weighed in microtubes (1.5 mL), 1 mL of ethanol (92.8°) was added, and the samples were sonicated for 10 min. Next, the samples were vortexed and centrifuged for 10 min at 10,000 rpm, and the supernatant was collected to obtain the ethanolic extract at a concentration of 10 mg mL^{-1}.

Total phenols

The total phenol content was determined in the Folin-Ciocalteu reagent (Sigma - Aldrich®) based on the method of Slinkard & Singleton (1977). In total, 150 µL of ethanolic leaf extract was placed in microtubes at a concentration of 10 mg mL^{-1} and added to 300 µL of Folin-Ciocalteu reagent (10% v/v) and 375 µL of sodium carbonate (7% w/v). The samples were homogenized by vortexing and then incubated at room temperature in the dark for 120 min. After the incubation period, the tubes were centrifuged for 10 min at 10,000 rpm. Subsequently, the supernatant (275 µL) was added to a microplate and the absorbance was measured at 760 nm. The calibration curve was generated from a standard of gallic acid (Sigma - Aldrich®) in the concentration of distilled water (y= 0.0997 + 13.184x r^2 = 0.9989) ranging from 0.0078 to 0.25 mg mL^{-1}. The results were expressed in mg Equivalent Gallic Acid (EGA) per g dry leaf.

Total flavonoids

The flavonoid contents were quantified as described by Woisky & Salatino (1998) with slight modifications. An aliquot of 100 µL of the ethanolic extract of the leaf was placed on microplates at a concentration of 10 mg mL^{-1}, and then, 100 µL of aluminum chloride (Sigma - Aldrich®) solution (10% w/v) was added. The calibration curve was generated from the quercetin standard (Sigma - Aldrich®), with 70% ethanol (y = 0.0962 + 19.302 x r^2 = 0.9951) at a concentration ranging from 0.0078 to 0.125 mg mL^{-1}. The tests were performed in quintuplicates and the results were expressed in mg Quercetin Equivalent (EQ) per g dry leaf.

Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) was measured based on the ammonium molybdate reduction method described by Prieto et al. (1999). Two hundred microliters of the extracts (10 mg mL^{-1}) was mixed with 1500 µL of the reagent solution (0.6 M sulfuric acid, 28 mM monobasic sodium phosphate, and 4 mM ammonium molybdate – Sigma - Aldrich®). After 90 minutes of incubation at 95°C, the samples were cooled to room temperature, and their absorbances were measured at 695 nm. The calibration curve of aqueous ascorbic acid (Sigma - Aldrich®) solution was constructed from the absorbances recorded (y = 0.053 + 14.261x r^2 = 0.9997) at a concentration ranging from 0.0078 to 0.25 mg mL^{-1}. The tests were performed in quintuplicate and the results expressed in mg Equivalent of Ascorbic Acid (EAA) per g dry leaf.

Free radical scavenging activity (DPPH)

The scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (0.2 mM DPPH) radicals was determined by the method proposed by Brand-Williams et al. (1995). In microplates, 20 µL of the sample was added to 260 µL of a methanolic solution of DPPH (Sigma - Aldrich®). The mixture was incubated for 60 minutes in the dark at room temperature. After this period, the absorbance was measured at 517 nm. BHT (Sigma - Aldrich®) was used as a standard (positive control). The free radical scavenging activity was performed in quintuplicate. Subsequently, the IC_{50} was calculated, corresponding to the concentration of extract capable of inhibiting 50% of DPPH radicals, according to the equation:

$$\% IC_{50} = \frac{Ac - A_{Sample}}{Ac} \times 100 \text{ where:}$$

Ac: control absorbance (DPPH and methanol absorbance); As: absorbance Sample.

The results of the DPPH free radical scavenging assay were expressed in the Antioxidant Activity Index (AAI) proposed by Scherer & Godoy (2009), where the plant extract is considered to have low antioxidant activity (AAI ≤ 0.5); moderate antioxidant activity (0.5 ≤ AAI ≤ 1.0); strong antioxidant activity (1.0 ≤ AAI ≤ 2.0); and, very strong antioxidant activity (AAI ≥ 2.0).

Ability to absorb oxygen radicals (ORAC)

The analysis was based on the method of Ou et al. (2001). Onto 96-well black microplates, 30 µL of the sample and 150 µL of fluorescein – Sigma - Aldrich® (70 mM) prepared in phosphate buffer – Sigma - Aldrich® (75 mM and pH 7.4) were added. The microplate was preincubated for 10 minutes at 37°C. After this period, 30 µL of the 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH – Sigma - Aldrich®) radical (12 mM) was added, and the fluorescence was recorded every minute for 150 minutes. As a control, a Trolox standard (Sigma - Aldrich®) was used at concentrations from 0.250 to 0.016 mg mL^{-1}. As a blank, 30 µL of phosphate buffer (70 mM and pH 7.4) was used instead of AAPH. All treatments were evaluated in triplicate.

Results were calculated using a regression equation between Trolox concentration and net AUC (area under the curve). Relative ORAC value = $[(AUC_{Sample} - AUC_{Blank})/(AUC_{Trolox} - AUC_{Blank})]$ x (molarity of Trolox/molarity of sample), with ORAC values expressed as µmol equivalent of Trolox per gram of leaf dry weight (µmol ET per g of LDW).

Statistical analysis

The data were subjected to variance analysis. The means were compared using the Scott–Knott test ($p < 0.05$) with the statistical program SISVAR® (Ferreira 2019). Principal component analysis (PCA) was performed using Statistica® software, version 10.0 (StatSoft - Tulsa, USA).

RESULTS AND DISCUSSION

Biomass production and essential oil content and yield

The influence of GM doses on leaf dry weight (LDW), stem (SDW), root (RDW), shoot (ShDW), total (TDW), root/shoot ratio (R:Sh) and content and essential oil yield are shown in Table III. There was a significant difference in the doses of GM for all variables of dry weight and essential oil of oregano. The greatest differences were observed for the doses of 300 g GM (Sh+R), 450 g GM (Sh+R), 600 g GM (Sh+R), 600 g GM (Sh), and cattle manure, as they resulted in the highest means for LDW, SDW, RDW, ShDW, and TDW compared to those for the control, 150 g GM (Sh+R) and 200 g GM (R). Thus, the presence of GM and cattle manure were important for oregano dry weight gain, especially in relation to the control treatment and the dose containing only sunn hemp roots (200 g GM (R)) as it obtained a lower mean dry weight. For LDW, the highest means were 8.8, 8.71, 8.56, and 8.38 g for the doses 300 g GM (Sh+R), 600 g GM (Sh), 450 g GM (Sh+R), and cattle manure (300 g per pot), respectively. Compared to the control, which had the lowest mean LDW (2.82 g) and SDW (1.30 g), these same doses of GM and cattle manure increased the production of dry weight 3-fold for LDW and

Table III. **Doses of green manure (*Crotalaria juncea* L.) on dry weight production and essential oil of *Origanum vulgare* L. Leaf (LDW), stem (SDW), root (RDW), shoot (ShDW), total dry weight (TDW) and essential oil (EO).**

Treatments Shoots (leaf+stem) g/pot	Roots g/pot	LDW	SDW	RDW	ShDW	TDW	Ratio R/ShDW	EO Content %	Yield g EO/plant
		g/plant							
150	200	5.58c	3.1c	6.03c	8.69d	14.72c	0.70c	0.74b	0.04d
300	200	8.8a	5.91a	11.0b	14.72a	24.93a	0.75c	0.79b	0.07b
450	200	8.56a	6.24a	10.58b	14,8a	25.36a	0.72c	0.91a	0.08a
600	200	7.84b	5.31b	9.82b	13.15c	21.65b	0.72c	0.91a	0.07b
600	-	8.71a	5.92a	12.15a	14.62a	26.77a	0.83b	0.82a	0.07b
-	200	3.24d	1.51d	4.22d	4.75e	8.97d	1.0a	0.67b	0.02e
Control soil (-)		2.82d	1.30d	4.16d	4.12e	8.1d	0.94a	0.91a	0.02e
Soil + Cattle manure (+)		8.38a	5.98a	10.58b	14.36a	24.23a	0.76c	0.71b	0.06c
CV%		4.98	4.50	8.76	4.76	6.53	6.12	4.98	5.41

Means followed by the same letter in the column are not significantly different according to the Scott-Knott test ($p < 0.05$). CV: Coefficient of variation.

4-fold for SDW. This result demonstrates that the management of GM can result in significant gains in the production of oregano leaves and, therefore, can be adopted as an agricultural strategy to meet industrial demands since the leaves concentrate greater amounts of essential oil, which has high market value. Doses of 300 g GM (Sh+R), 450 g GM (Sh+R), 600 g GM (Sh), and cattle manure increased ShDW and TDW more than 3-fold compared to that produced in the treatment without GM (control soil) (Table III).

The results of this study were in agreement with the results of several studies that have shown GM treatment increased the plants dry weight. The doses of *C. juncea* L. (0, 3, 6, and 9 t/ha) provided higher dry weight in *Brassica oleracea* L. (Diniz et al. 2017). Another study reported that sources of GM in succession, improved dry weight gains (11.4%) in *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson (Marques et al. 2018). In addition, the doses of GM (*Merremia aegyptia* (L.) Urb.) also increased agronomic parameters (shoot dry weight, number of stems per plant and height) of the species *Coriandrum sativum* L. (Linhares et al. 2012). The significant plant growth and consequently higher plant dry weight with use GM are notorious. This confirms the importance of management technique in medicinal plants. Therefore, research shows the positive effects of using GM, as well as the combination of other organic fertilizers or low doses of mineral fertilizers (Rothé et al. 2019, Watthier et al. 2020, Peralta-Antonio et al. 2021, Massey et al. 2021, Honorato et al. 2022).

It is possible to observe that in comparison to the control and 200 g GM (R) treatments, the treatments with GM and cattle manure had higher shoot growth, which consequently influenced the dry weight (Figure 1). The higher mean dry weight of oregano can be explained by the greater availability of nutrients (K, Ca^{+2}, Mg^{+2}, Zn, B, and S) and organic matter (OM) (Table I and Table II) due to the increase in the doses of GM when compared to the treatment without GM. According to Meena et al. (2020), GM can fix atmospheric nitrogen and provide biomass that stimulates microbial diversity, consequently

Figure 1. *Origanum vulgare* L. plants cultivated under the influence of different doses of green manure (*Crotalaria juncea* L.). 1) 150 g shoot + 200 g root/pot, 2) 300 g shoot + 200 g root/pot, 3) 450 g shoot + 200 g root/pot, 4) 600 g shoot + 200 g root/pot, 5) 600 g shoot/pot, 6) soil + 200 g root, 7) soil (control -) and 8) soil + cattle manure 300 g/pot (control +).

improving soil fertility and releasing nutrients throughout the crop cycle.

Rosa et al. (2017) studied the quality of the soil cultivated with cover crops, and among the species studied, *C. juncea* L. experienced increases in carbon in the fulvic acid fraction, which contributed to aspects of soil fertility. According to Vakeesan & Nishanthan (2008), this increase in organic carbon and consequently in soil OM may have been related to the plant tissues that partially decomposed or decomposed green fertilizers, which fed the beneficial soil organisms. In addition, other research has already shown that the management of green manure can improve the structure, fertility and organic carbon of the soil, as well as reduce the use of chemical fertilizers (Li et al. 2020b, Kamran et al. 2021, Xu et al. 2023).

The R:Sh indicates the preferential allocation of dry mass to the root system. In comparison to the control treatment and 200 g GM (R), the plants managed with GM and the treatment with cattle manure resulted in a lower R:Sh ratio; i.e., there was a greater allocation of dry biomass to the shoots (Figure 1). The highest averages of R:Sh in the treatments without GM and the 200 g GM (R) dose may have occurred due to the lower levels of nutrients in the soil; thus, the lower growth of the shoots may have occurred due to the plants attempting to allocate more mass to the roots to increase absorption efficiency.

The content (%) and yield (g EO per plant) of oregano essential oil showed that the doses of GM resulted in a significant difference (Table III). The doses of 450 and 600 g GM (Sh+R) and the control resulted in the highest mean essential oil content, both at 0.91%, followed by the 600 g GM (Sh) dose at 0.82%. For essential oil production (g EO per plant), the lowest means were observed in the control and at the dose of 200 g GM (R), both resulting in 0.02 g EO per plant. Although the control had a high essential oil content (0.91%), the production of essential oil decreased by 75% compared to that resulting from the dose of 450 g GM (Sh+R) and by approximately 71% compared to that resulting from the doses of 300, 600 g GM (Sh+R), and 600 g GM (Sh). In comparison to the control, the treatment with cattle manure also showed an increase of 67% more essential oil, demonstrating the importance of organic fertilization for oregano.

The higher production of essential oil influenced by the doses of GM may be related to the better chemical characteristics of the soil and greater availability of nutrients provided by *C. juncea* L. (Table I and Table II). Silva et al. (2021) state that the species of the genus *Crotalaria* stand out within the Fabaceae family because they contribute to the accumulation of OM and nutrients, in addition to protecting the soil against erosion. Different sources of GM in succession, including sunn hemp, increased the production of essential oil in *L. alba* L. by approximately 14% compared to the control (Marques et al. 2018).

Cymbopogon flexuosus (Nees ex Steud.) W.Watson and *Mentha piperita* L. cultivated with GM and organic manure significantly increased the content and yield of essential oil, as well as oil composition (Massey et al. 2021, Javanmard et al. 2022). Nitrogen doses combined with GMs (*Medicago sativa* and *Secale montanum*) resulted in higher oil content and yield in *Mentha piperita* L. (Bidgoli & Mahdavi 2018). Also, sunn hemp doses (0, 3, 6 and 9 kg m^2) increased biomass, essential oil content and yield in *Thymus vulgare* L. (Honorato et al. 2022).

Photosynthetic pigments and leaf area

The mean values of photosynthetic pigments and oregano leaf area indices grown under different doses of GM are shown in Table IV. There was no significant difference in GM doses for the following variables: carotenoids, specific leaf area (SLA), and leaf area ratio (LAR). However, for the photosynthetic pigments chlorophyll *a* and *b* and total leaf area (TLA), there was a significant difference between the doses of GM (Table IV). It was observed that the highest means of chlorophyll *a* occurred in the presence of GM (150, 300, 450, and 600 g GM). However, the cattle manure and the control, both with 0.67 mg per g FW, and the 200 g GM (R) treatment with 0.64 mg per g FW, recorded the lowest means of chlorophyll *a*. This higher accumulation of chlorophyll *a* may be related to the higher concentrations of nitrogen and magnesium (Table II) in the soil from the

Table IV. Effect of green manure (*Crotalaria juncea* L.) doses on photosynthetic pigments and total leaf area (TLA), leaf area ratio (LAR) and specific leaf area (SLA) of *Origanum vulgare* L.

Treatments: Shoots (leaf+stem) g/pot	Treatments: Roots g/pot	Photosynthetic Pigments .... Chlorophlly a	Chlorophlly b	Chlorophlly Total	Carotenoids	Growth analysis........... TLA	LAR	SLA
		.... mg/g FW			....	cm²	.... cm²/g	
150	200	0.73a	0.73a	1.46a	0.25a	516.8b	59.1a	92.4a
300	200	0.71a	0.67a	1.31b	0.23a	680.9a	49.1a	80.9a
450	200	0.72a	0.58b	1.37b	0.23a	813.1a	53.7a	90.9a
600	200	0.72a	0.70a	1.35b	0.24a	744.9a	54.4a	88.6a
600	-	0.72a	0.70a	1.39b	0.24a	833.0a	56.1a	87.9a
-	200	0.64b	0.71a	1.48a	0.24a	240.8c	60.5a	87.5a
Control soil (-)		0.67b	0.71a	1.34b	0.25a	275.2c	53.8a	80.3a
Soil + Cattle manure (+)		0.67b	0.58b	1.36b	0.24a	793.1a	54.7a	94.3a
CV%		3.7	5.7	4.9	4.1	15.3	13.2	11.46

Means followed by the same letter in the column are not significantly different according to the Scott-Knott test ($p < 0.05$). FW: fresh weight, CV: Coefficient of variation.

doses of GM, as these nutrients are part of the chlorophyll molecule.

In comparison to the other treatments, the cattle manure and the 450 g GM (Sh+R) dose resulted the lowest chlorophyll *b* means, both with 0.58 mg per g FW. For total chlorophyll, the highest means were observed in the 150 g GM (Sh+R) and 200 g GM (R) treatments, at 1.46 and 1.48 mg per g FW, respectively.

Photosynthetic pigments play a direct role in photosynthesis because they absorb solar energy for the subsequent synthesis of complex carbon compounds (carbohydrates and the release of oxygen from carbon dioxide and water). The energy stored in these molecules can be used to boost cellular processes in a plant and serve as an energy source (Taiz et al. 2017). In addition, most of the total green pigments found in plants correspond to chlorophyll *a*, and chlorophyll *b* is a supplementary pigment (Borrmann 2009). Similar results were also found by Mutisya et al. (2014), who observed that in comparison to the treatment without GM, the treatments with doses of GM (*Sesbania sesban* (L.) Merr.) increased the photosynthetic pigments of chlorophyll *a* in *Solanum nigrum* L. *Origanum vulgare* L. cultivated with organic manure increased the levels of photosynthetic pigments compared to the control (Corrêa et al. 2009).

The highest mean TLA occurred in the presence of GM (150, 300, 450, and 600 g GM), as well as in the presence cattle manure. By relating these data to the dry weight data (Table III), it can be inferred that the larger leaf area, combined with the higher chlorophyll *a* content (Table IV), contributed to a greater accumulation of LDW, RDW, ShDW, and TDW in relation to the control and 200 g GM (R), which had the lowest mean leaf area, at 275.2 and 240.8 cm^{-2} per g, respectively. This smaller leaf area may be related to a possible stress condition caused by the low content of nutrients and OM in the soil in these treatments.

According to Taiz et al. (2017), plants have plasticity and develop the ability to modify leaf morphology to allow them to avoid or mitigate the effects of abiotic extremes. Such mechanisms include changes in leaf area, leaf orientation, leaf folding, trichomes, and waxy cuticles. In addition, large and flat leaves provide optimal surfaces for the production of photosynthesis. However, they can be harmful to the growth and survival of agricultural crops under stressful conditions because they expose a large surface area to water evaporation, which can lead to rapid depletion of soil water (Taiz et al. 2017).

Leaf nutrient accumulation

Table V shows the accumulation of macro- and micronutrients in dry leaves of *O. vulgare* L. grown under the influence of doses of *C. juncea* L. In general, in relation to macronutrients, the treatment without GM had higher accumulations of Ca, Mg, and S at 24.5, 2, and 4.9 g per kg, respectively. Similar to other studies, it was observed in the species *Justicia pectoralis* Jacq. higher accumulation of macronutrients (K, Ca, and Mg) in the treatment that did not receive fertilization compared to the fertilization treatments (Vieira et al. 2019). The higher levels of Ca, Mg, and S in the control in comparison to those in the treatments with GM may be related to plant growth since the plants without GM had less growth (Table III and Figure 1) than the fertilized plants. Chemical and environmental factors that cause changes in growth rates and nutrient absorption will affect the concentrations of the nutrients in plant tissue (Fontes 2001). If a plant has a low growth rate and nutrients continue to be absorbed, then concentration of nutrients will occur; however, if a plant grows rapidly, then nutrient dilution

Table V. Accumulation of macro and micronutrients in dry leaves of *Origanum vulgare* L. cultivated under the influence of doses of green manure (*Crotalaria juncea* L.).

Treatments Shoots (leaf+stem) g/pot	Treatments Roots g/pot	Macronutrients						Micronutrients				
		N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
		.... g/kg						.... mg/Kg				
150	200	14.7	2.1	22.8	23.3	1.8	3.6	51.8	11.7	2.300	65.8	66.2
300	200	14.7	2.1	19.0	23.4	1.7	3.0	52.2	10.8	1.135	57.2	61.5
450	200	15.2	2.2	19.9	22.9	1.7	3.0	52.2	11.0	2.697	56.1	65.8
600	200	18.6	2.0	21.4	22.9	1.9	3.4	48.8	10.3	1.576	72.8	57.5
600	-	14.1	1.9	19.4	22.1	1.8	3.0	47.9	10.1	3.302	70.8	54.5
-	200	14.6	2.0	24.4	23.1	1.9	4.6	49.1	11.1	2.087	122.1	68.3
Control soil (-)		15.6	2.1	23.8	24.5	2.0	4.9	47.4	11.0	1.514	97.2	68.9
Soil+Cattle manure (+)		15.1	2.4	20.8	22.0	2.3	3.3	44.7	11.3	2.023	61.6	77.8

will occur (Maia et al. 2005). For N, P and K, the highest accumulations (18.6, 2.2, and 24.4 g per kg, respectively) were observed in the presence of GM at 600, 450 g GM (Sh+R), and 200 g GM (R), respectively. The results of this study were in agreement with the higher accumulation of N, P, and K provided by GM, because both cattle manure and GM resulted in higher accumulation of leaf macronutrients in the species *Acmella oleracea* (L.) R.K.Jansen and *Bactris gasipaes* Kunth (Magalhes et al. 2020, Nordi 2021).

Regarding micronutrients, it was also observed that doses of GM, 150, 300, 450 g GM (Sh+R) and 600 g (Sh) influenced a greater accumulation of B, Cu, and Fe. According to Batista et al. (2018), the higher levels of macro- and micronutrients in oregano leaves may be related to the increase in OM in the soil provided by GM because OM is a controlling component of nutrient availability in the soil. Thus, nutrient availability can occur through mineralization or through the formation of organometallic complexes (soluble or insoluble), which prevent micronutrients from interacting with soil minerals or other dissolved ions that are easily absorbed by the plant (Batista et al. 2018).

Chemical composition of the essential oil

Table VI shows the chemical constituents of the essential oil of *O. vulgare* L. grown under the influence of different doses of *C. juncea* L. It was observed that the doses of GM influenced the chemical composition of the essential oil of oregano expressed in relative area percentage. The five main classes of compounds found were monoterpene hydrocarbons, oxygenated monoterpenes, phenolic monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. De Mastro et al. (2017) corroborated the results found in this study because the authors found the same classes of constituents in the chemical composition of the essential oil of oregano. Regarding the qualitative aspect of the essential oil of oregano, the presence of the constituent δ-cadinene was observed in the 200 g of GM (R), 150 g of GM (Sh+R), and cattle manure treatments.

Oxygenated monoterpenes were the main class present in the essential oil of oregano, recorded at levels between 46.63 and 52.77% relative area percentage, where the cattle manure treatment showed the highest average (52.77%), followed by doses of 300, 450, and 600 g of GM (Sh+R), presented the highest

Table VI. Chemical constituents of the essential oil of *Origanum vulgare* cultivated under the influence of doses of green manure (*Crotalaria juncea* L).

Compound	RI	Control	Cattle manure	200	150	300	450	600	600	CV%
				g GM (R)	.... g GM (Sh+200g R)				g GM (Sh)	
Alkenyl alcohol		2.6a	0.96f	2.1b	2.1b	1.7e	1.8d	1.9c	2.0c	
1-Octen-3-ol	971	2.6a	0.96f	2.1b	2.1b	1.7e	1.8d	1.9c	2c	2.1
Monoterpenes Hydrocarbons		25.21a	18.85e	23.48c	24.49b	21.73d	23.24c	23.57c	24.42b	
α-Thujene	925	0.47a	0.11g	0.32b	0.33b	0.23f	0.25e	0.27d	0.29c	2.8
α-Pinene	931	0.27a	nd	0.17c	0.18b	0.12f	0.11f	0.14e	0.16d	3.6
Myrcene	990	1.65a	0.87f	1.44b	1.48b	1.22e	1.29d	1.36c	1.43b	2.1
α-Phellandrene	1005	0.16b	0.12d	0.17b	0.17b	0.15c	0.19a	0.19a	0.17c	3.3
α-Terpinene	1015	1.82a	1.35e	1.53c	1.72b	1.42d	1.68b	1.66b	1.83a	1.8
p-Cymene	1023	5.7a	3.63e	5.6a	5.3b	4.86d	4.98c	5.0c	4.85d	1.6
β-phellandrene	1027	1.75a	1.18d	1.67b	1.62b	1.47c	1.63b	1.65b	1.63b	1.8
1,8-Cineole	1030	0.49b	0.46d	0.49b	0.49b	0.51a	0.52a	0.50a	0.47c	1.7
cis-β-Ocimene	1035	4.13a	3.31e	3.92b	4.16a	3.64d	3.78c	3.99b	4.21a	1.7
(trans)-β-Ocimene	1045	0.66a	0.53e	0.63b	0.68a	0.58d	0.61c	0.64b	0.67a	1.7
γ- Terpinene	1056	7.52b	6.64d	7.01c	7.74b	7.0c	7.56b	7.55b	8.03a	1.5
Terpinolene	1087	0.59d	0.65b	0.53e	0.62c	0.53e	0.64b	0.62c	0.68a	1.7
Oxygenated monoterpenes		47.49e	52.77a	46.73f	47.22f	51.39b	50.87c	49.79d	46.63f	
cis-Sabinene hydrate	1066	3.56d	3.69b	3.43e	3.40e	3.77a	3.62c	3.58d	3.18f	0.63
trans-Sabinene hydrate	1101	32.14e	32.77d	31.52f	31.16g	35.16a	33.85b	33.33c	29.58h	0.3
Terpinen-4-ol	1176	6.13f	9.87a	5.44h	6.87d	6.05g	7.2c	6.75e	7.71b	0.3
α-Terpineol	1190	2.88f	3.49a	2.87f	3.02e	3.06d	3.13b	3.02e	3.08c	0.5
Linalool acetate	1257	2.78e	2.95d	3.47a	2.77e	3.35b	3.07c	3.11c	3.08c	0.8
Phenolic monoterpenes		15.58c	17.48a	17.48a	16.91b	16.84b	17.01b	16.55b	17.83a	
Thymol methyl ether	1234	1.26b	1.17c	1.38a	1.08d	1.0e	1.0e	0.98e	1.1d	1.2
Carvacrol methyl ether	1243	0.95f	1.04b	1.11a	1.0d	0.97e	0.98e	0.95f	1.02c	0.6
Thymol	1294	13.17c	15.06a	14.75b	14.59b	14.64b	14.82b	14.42b	15.49a	2.0
Carvacrol	1302	0.20c	0.21c	0.24a	0.24a	0.23b	0.21c	0.20c	0.22b	2.5
Sesquiterpene hydrocarbons		5.83c	6.38a	6.5a	5.99b	5.39d	4.45e	5.61d	6.05b	
β-Bourbonene	1382	0.28b	0.29b	0.32a	0.27c	0.25d	0.19g	0.23f	0.24e	2.3
trnas-Caryophyllene	1416	1.99b	2.11a	2.13a	1.94c	1.74d	1.47e	1.75d	1.99b	1.2
α -Humulene	1450	0.22b	0.22b	0.28a	0.21b	0.20c	0.16d	0.19c	0.22b	3.0
Germacrene D	1478	1.75c	1.96a	1.92a	1.83b	1.68d	1.41e	1.70d	1.94a	1.8
Bicyclogermacrene	1493	1.59c	1.67b	1.72a	1.62c	1.52d	1.22f	1.74e	1.66b	1.8
δ-Cadinene*	1521	nd	0.13	0.13	0.12	nd	nd	nd	nd	
Oxygenated sesquiterpenes		2.08b	2.02b	2.45a	1.96c	1.92c	1.66e	1.72e	1.84d	
Spathulenol	1574	1.16b	1.13b	1.35a	1.09b	1.09b	0.97c	1.0c	1.08b	3.4
Caryophyllene oxide	1580	0.92b	0.89b	1.10a	0.87b	0.83c	0.69e	0.72e	0.76d	3.2
Total % of compounds		98.79	98.46	98.74	98.67	98.97	99.03	99.14	98.77	
Number of compounds		29	29	30	30	29	29	29	29	

RI: retention index relative to alkane series (C8 – C20) on HP-5MS column. Means followed by the same letter within the same line belong to the same group according to the Scott-Knott test ($p < 0.05$). GM (Green Manure); R (Root); Sh (Leaf + stem). *Compound was not identified in all treatments, CV: Coefficient of variation.

means, at 51.39, 50.87, and 49.79%, respectively. Marculescu et al. (2002) stated that the use of organic fertilizers plays an essential role in the development of plants and in the biosynthesis of organic substances at all levels because when using organic management (manure), they noted that the amount of active ingredient was high in the species *Chrysanthemum balsamita* L. Organic management provided better bioactivity of the essential oil of oregano because it resulted in antibacterial and antifungal activity, whereas conventional cultivation (NPK) showed no activity (Pereira et al. 2020).

The three main chemical constituents found were *trans*-sabinene hydrate, thymol and terpinen-4-ol. In the case of the main constituent with the highest relative area percentage, *trans*-sabinene hydrate (Figure 2), it was found that the doses 300 and 450 g of GM (Sh+R) provided the highest mean relative area, with 35.16 and 33.85%, respectively, that is, a production of 8.6 and 5.0% higher compared to the treatment without green fertilization (control soil), which presented 32.14% relative area. Similar results were found by Quiroga et al. (2011), who observed the same major compounds in the chemical composition of the essential oil of several oregano species. The presence of organic fertilizer doses (manure) obtained more of the main constituents (*trans*-sabinene hydrate, thymol, and terpinen-4-ol) of the oil of *O. vulgare* L. than the control (zero dose), demonstrating the importance of organic management for bioactive production (Corrêa et al. 2010).

Regarding thymol (Figure 2), it was observed that the highest content occurred in the presence of GM rather than in the control treatment, with emphasis on the dose of 600 g of GM (Sh+R) and cattle manure, which provided the highest content of this constituent, at 15.49

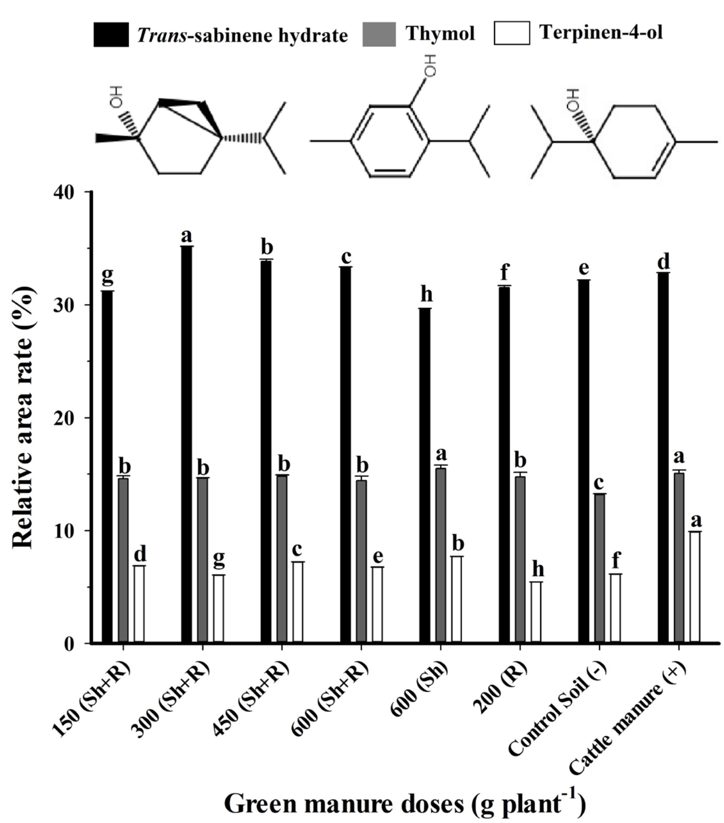

Figure 2. Major chemical constituents present in the essential oil of *Origanum vulgare* L. cultivated under the influence of doses of green manure (*Crotalaria juncea* L.). 150 g shoot + 200 g root/pot, 300 g shoot + 200 g root/pot, 450 g shoot + 200 g root/pot, 600 g shoot + 200 g root/pot, 600 g shoot/pot, soil + 200 g root, soil (control -) and soil + cattle manure 300 g/pot (control +).

and 15.06%, respectively. These doses produced approximately 15 and 12.5% more thymol than the control, which produced 13.17%. Thymol has several applications, acting in several industries, as an antispasmodic agent, antioxidant, antimicrobial, immunomodulatory, anticancer agent and anti-inflammatory agent, suppressing harmful compounds/free radicals, with the ability to change the intestinal microbiota and increase digestion, absorption, and metabolism of nutrients (Ezzat Abd El-Hack et al. 2016). The same authors stated that this constituent is also used in dietary supplementation as a food additive in the production of animals, fish, and birds because it improves productive and reproductive performance, nutrient bioavailability, and immunity. In addition, recent studies have shown that thymol may be a potential natural selective herbicide in monocotyledonous plant species (Gruľová et al. 2020).

Terpinen-4-ol (Figure 2) had the highest mean relative areas (9.87, 7.71, and 7.2%) in the cattle manure, 600 g GM (Sh), and 450 g GM (Sh+R) treatments, respectively. This constituent also has several applications because it is used to increase resistance of fruits against pathogens through its antibacterial and anticancer properties and used for the production of antimicrobial nanofibers (Cordeiro et al. 2020, Li et al. 2020a, Nakayama et al. 2017, Nepomuceno et al. 2018, Zhang et al. 2018, Zhao et al. 2021).

Analysis of phenols, total flavonoids, and antioxidant activity of oregano extracts

The amount of phenolic constituents and flavonoids, as well as the antioxidant activity of the extracts of dried leaves of *O. vulgare* L. grown under the influence of doses of *C. juncea* L., are shown in Table VII. It was observed that for all variables (total phenols, total flavonoids, TAC, ORAC and DPPH), there were significant differences due to the doses of GM. For the

Table VII. Total phenols, total flavonoids and antioxidant activity of extracts from dry leaves of *Origanum vulgare* L. cultivated under the influence of doses of green manure (*Crotalaria juncea* L.).

Treatments Shoots (leaf+stem) g/pot	Roots g/pot	Total phenols	Total flavonoids	TAC	ORAC	DPPH (AAI)
		mg of EGA/g	mg of EQ/g	mg of EAA/g	µmol ET/g	
			(dry leaf)			
150	200	214b	9.20c	27.7d	531a	3.2a
300	200	181c	8.20d	31.9a	525a	2.8b
450	200	191c	12.2a	27.5d	498b	3.1a
600	200	192c	12.1a	32.4a	501b	2.9b
600	-	179c	10.6b	27.8d	451c	2.9b
-	200	216b	7.90e	29.6c	419d	3.0b
Control soil (-)		482a	6.90e	30.9b	522a	2.7c
Soil + Cattle manure (+)		209b	7.60e	27.6d	534a	2.5d
CV%		8.6	6.6	1.6	3.9	3.6

Means followed by the same letter in the column are not significantly different according to the Scott-Knott test ($p < 0.05$). CV: Coefficient of variation. GM (Green Manure); R (Root); Sh (Shoot = leaf+stem). Total antioxidant capacity (TAC), ability to absorb oxygen radicals (ORAC), free radical scavenging activity (DPPH). Equivalent gallic acid (EGA); quercetin equivalent (EQ); equivalent in ascorbic acid (EAA); equivalent of Trolox (ET).

total phenol content, the treatment without fertilization (control soil) had the highest mean at 482 mg of EGA per g dry leaf. The lowest means occurred with doses of 300, 450, 600 g GM (Sh+R), and 600 g GM (Sh). However, the highest levels of total flavonoids occurred with 450 and 600 g GM (Sh+R) at 12.2 and 12.1 mg of EQ per g dry leaf, respectively. The lowest mean occurred in the control treatment at 6.9 mg of EQ per g dry leaf.

The influence of GM doses on the accumulation of active chemical constituents (flavonoids) and antioxidant activity may be related to the nutrition of oregano plants. The availability of nutrients should be taken into account in the cultivation of medicinal plants because in addition to influencing primary metabolism, nutrient availability can alter the production of secondary metabolites (Gobbo-Neto & Lopes 2007). Studies with *O. vulgare* L. have already shown that the concentrations of bioactive compounds in plants are significantly affected by some chemical properties of the soil (pH, N, Ca, P, and Mg) (Klimienė et al. 2021).

According to Choi et al. (2002), due to the complexity of the chemical substances present in the extracts, it is necessary to evaluate the antioxidant capacity of the plant with at least two methods. Thus, it was observed that for the three types of antioxidant analyses performed (TAC, ORAC, and DPPH), in comparison to the other treatments, the doses of GM provided greater activity. For the TAC, which was performed based on the reduction of Mo^{+6} to Mo^{+5}, the highest means were observed with doses of 300 and 600 g GM (R +Sh) at 31.9 and 32.4 mg of EAA per g dry leaf, respectively. For the ORAC, which consisted of the decrease in fluorescence caused by the AAPH radical, the cattle manure and the GM doses of 150 and 300 g GM (Sh+R) resulted in the highest means at 534, 531 and 525 µmol of ET per g dry leaf, respectively, followed by the that in the control at 522 µmol of ET per g dry leaf. For the free radical scavenging activity (DPPH), all extracts showed very strong antioxidant activity because they had an AAI above 2. The highest means were recorded with doses of 150 and 450 g of GM (Sh+R) at 3.2 and 3.1, respectively.

Similar to other studies, the antioxidant activity and phenol content were higher in the species *Matricaria chamomilla* L. when the management of GM (*Anabaena azollae* Strasb.) combined with organic compost was used (Kawthar et al. 2017). Another factor that may have contributed to the higher content of chemical constituents and antioxidant activity was the use of organic fertilizer. Oregano cultivated with organic fertilizers displayed higher bioactive potential than mineral fertilizer (Matłok et al. 2020). Organic system provided higher production of phenolic acids and total flavonoids in the species *Mentha piperita* L., *Salvia officinalis* L., *Melissa officinalis* L., and *Rosmarinus officinalis* L., in comparison to a conventional system (Kazimierczak et al. 2015). The results of this study confirm the importance of organic management for the production of secondary metabolites in medicinal plants, especially in the species *O. vulgare* L.

Principal Components Analysis (PCA)

The PCA results showed an overview of the doses of GM applied to oregano, as they explained 82.08% of the total variance (Figure 3). There was 66.87% variation associated with principal component 1, while principal component 2 explained 15.21% of the variation.

Four distinct groups were observed: first, control; second, 200 g GM (R) and 150 g GM (Sh+R); third, 300, 450, and 600 g GM (Sh+R); and fourth, 600 g GM (Sh) and cattle manure. The load analysis showed that the doses of GM (300, 450, and 600 g GM – Sh+R) positively influenced the dry weight (LDW, SDW, RDW, ShDW, TDW), TLA, and oil content and yield, as well as the

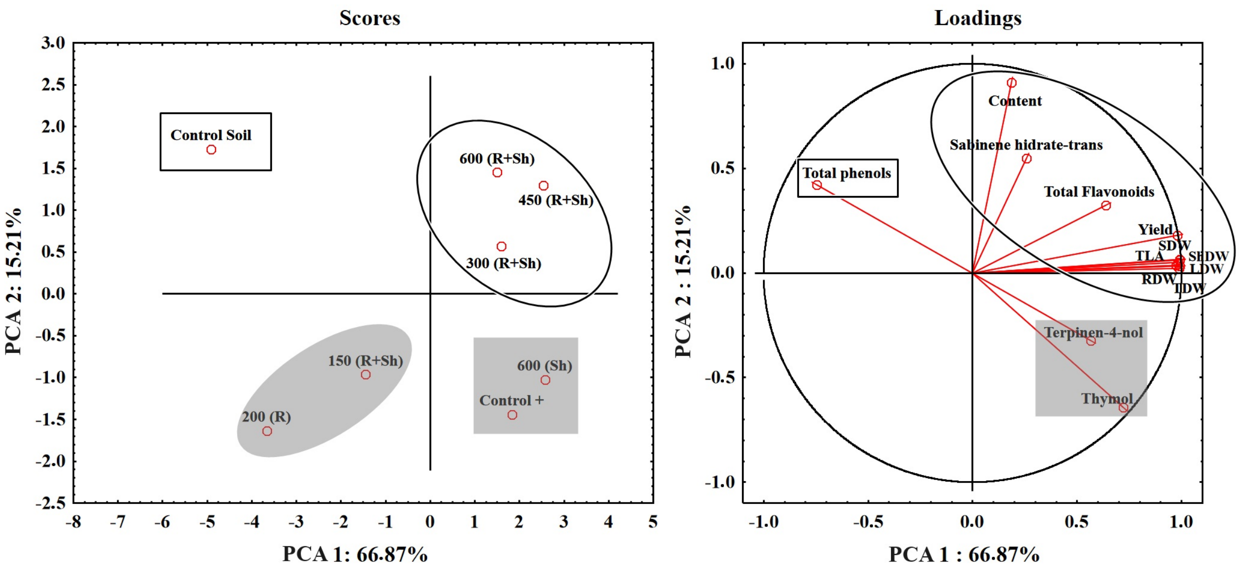

Figure 3. Principal component analysis (CPA) on composite matrix correlation using data for leaf dry weight (LDW), stem dry weight (SDW), root dry weight (RDW), total dry weight (TDW), total leaf area (TLA), content, yield, essential oil major compounds, phenols and total flavonoids.

accumulation of total flavonoids in oregano. Conversely, the cattle manure and the 600 g GM (Sh) dose contributed to the production of thymol and terpinen-4-ol. It was also found that the 200 g (R) and 150 g GM (Sh+R) dose treatments did not influence the observed variables of oregano production.

The present study showed that the management of GM and cattle manure significantly influenced the production and quality of essential oil of *O. vulgare* L., in addition to resulting in a greater accumulation of bioactive constituents.

CONCLUSION

The doses of *C. juncea* L. significantly enhanced chlorophyll a, total leaf area, and dry weight in *O. vulgare* L., leading to higher essential oil yields. This resulted in a significant accumulation of key chemical constituents, including *trans*-sabinene hydrate, thymol, and terpinen-4-ol, which hold considerable value for diverse industrial sectors. In addition, the presence of GM increased the total flavonoid content, causing greater total antioxidant activity and greater DPPH radical stabilization. Principal component analysis (PCA) showed that green manure and cattle manure positively influenced the dry weight, content, yield, and essential oil constituents, and total flavonoids. These findings suggest that organic management through the use of the GM *C. juncea* L. may be beneficial for cultivating *O. vulgare* L. because it can increase the production of biomass and the active principles of this species. Some benefits of this technique include the optimization of the quality and quantity of dry weight, secondary metabolites, improvement in vegetative growth and productivity, and the potential to reduce fertilizer usage. It is hoped that this study can contribute to improving plant management, reducing environmental impact without implying significant costs for the producer.

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