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Research article

Rosemary (*Rosmarinus officinalis* L.) growth rate, oil yield and oil quality under differing soil amendments

Gikuru Mwithiga[a], Samuel Maina[a,b,*], Josiah Gitari[a], Phyllis Muturi[a]

[a] *Department of Water and Agricultural Resource Management, University of Embu, P.O Box 6-60100, Embu, Kenya*
[b] *Department of Biological Sciences, University of Embu, P.O Box 6-60100, Embu, Kenya*

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ABSTRACT

In this work, we examined the influence of different soil amendments namely cow manure alone (Cm), cow manure plus fertilizer (Cm + F), fertilizer alone (F) and unamended check, control on rosemary growth rate, fat content, oil yield and oil quality. Plant height (PLH), number of primary branches per plant (NOPB) and survival rate were assessed at 0, 90, 180, 270 and 360 days after planting during the 2020–2021 growing season. Rosemary plant essential oil and fat content were extracted using steam distillation and Soxhlet methods, respectively, and determined for quality using the gas chromatography with mass spectrometry (GC-MS) method. Plants grown under Cm had a higher PLH and NOPB, whereas the F treatment largely affected the survival rate of rosemary plants than the control. A total of 26 constituents were identified from the obtained rosemary oil grown under different treatments by use of GC-MS analysis with Cm treatment containing the highest number of oil compounds. The main rosemary oil compounds in the present work were alpha-pinene (29.80%–34.34%), eucalyptol (27.15%–30.26%), verbenone (7.63%–8.14%) and geraniol (4.47%–5.22%). The oil yield from the steam distillation method ranged from 0.45% (v/w) to 0.59% (v/w) while the fat content as measured using the Soxhlet method ranged between 11.22% and 13.36% across various treatments. The essential oil yield and fat content from rosemary grown under Cm, Cm + F, or F conditions were not significantly different. This study shows that Cm markedly influenced rosemary oil quality when compared to other soil amendments.

1. Introduction

Rosemary (*Rosmarinus officinalis* L.), a member of the Lamiaceae family, is an evergreen perennial shrub, characterized by a unique aromatic odor (Ban et al., 2016). Among the three species of genus *Rosmarinus* used for essential oil production, *Rosmarinus officinalis* is one of the most productive species. Native to Mediterranean environments (Porte et al., 2000), this plant is now widely grown globally due to its medicinal, aromatic and ornamental properties (Evans, 2002; Derwich et al., 2011). Currently, the three largest producers and exporters of rosemary essential oils worldwide are Spain, Tunisia and Morocco.

Rosemary contains essential oils that are associated with interesting pharmacological properties including anti-inflammatory, anti-oxidant, anti-bacterial, anti-nociceptive, anti-fungal, anti-diabetic and anti-thrombotic (González-Trujano et al., 2007; Derwich et al., 2011; Rafie et al., 2017). The rosemary extracts are also used in culinary purposes, pest control products, folk medicine and cosmetic products (Isman, 2000; Koul et al., 2008; González-Minero et al., 2020). The highest quality of rosemary essential oils is contained in the leaves compared to other parts of the plant (Lo Presti et al., 2005). In addition, the main compounds of Iran rosemary essential oil were reported to be camphor (1.66–24.82%), alpha-pinene (14.69–20.81%) and 1,8-cineole (5.63–26.89%) (Bajalan et al., 2017) while that of Egypt contained camphor (38.6–44.8%), eucalyptol (13.1–15.5%) and alpha-pinene (14.5–21.1%) (Omer et al., 2020). Sharma et al. (2020) recently showed that the Italian rosemary variety contained higher levels of 1,8-cineole (23.39%), alpha-pinene (13.14%), camphor (13.02%) and camphene (6.54%) whereas that of French type revealed alpha-pinene (37.5%), 1,8-cineole (15.69%), verbenone (6.61%) and camphene (4.64%) as the highest compounds.

Due to the wide range of benefits of rosemary essential oils, various studies have been conducted to examine how the wide array of components of rosemary oil vary in response to external factors including plant spacing and soil management (Singh and Guleria, 2013; Tawfeeq et al., 2016). Other similar studies on lemongrass have been executed by Shahi and Singh (2013). Following these studies, it has been concluded that the components and yields of essential oils of plants differ in many ways.

* Corresponding author.
E-mail address: samuelthaithi92@gmail.com (S. Maina).

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However, the cause of variations remains elusive, as the composition of oil might also be affected by other factors such as pollution, climate and attack by pests (Figueiredo et al., 2008).

There have been recent attempts to evaluate the effects of organic and synthetic fertilizers on the growth, oil yield and oil quality of rosemary (Cáceres et al., 2017; Mostafa, 2019). Notably, organic-based fertilizers have been shown to exhibit a significant difference in the composition and yield of rosemary essential oils (Tawfeeq et al., 2016). For instance, seaweed liquid fertilizer has been demonstrated to induce a large amount of oil and leaf area than both inorganic fertilizers and the control (Tawfeeq et al., 2016). Abdelaziz et al. (2007) used a mixture of compost and microorganisms resulting in increased production of essential oils as well as vegetative growth of rosemary plants. A study by Valiki and Ghanbari (2015) revealed that agronomic traits of rosemary for example plant height, fresh weight and dry matter yield were markedly improved by the use of organic manure (sheep) than the use of inorganic fertilizers or the control. However, studies have also recorded insignificant impact of organic and synthetic fertilizers on rosemary essential oil quantity and quality (Singh and Wasnik, 2013; Singh, 2013).

The use of inorganic fertilizers, especially nitrogenous fertilizer has been found to strongly influence constituents, yield and biosynthesis of essential oils of oregano plants (Karamanos and Sotiropoulou, 2013). Importantly, it has been highlighted that the effects of nitrogenous fertilizers at the level of essential oil compounds are reliant on specific plants and constituents. Moghaddam and Mehdizadeh (2017) observed no substantial effects of inorganic fertilizers on various medicinal and aromatic plants, an observation that is also pointed out by Barreyro and Ringuelet (2005) in oregano plants. In another study, a combination of vermicompost (10 t ha-1) and nitrogen (N), phosphorus (P) and potassium (K) fertilizer (NPK) (100:25:25 kg/ha) markedly raised the amount of oil and herbage of rosemary relative to the control (no amendment) (Singh and Guleria, 2013). Multiple papers investigating different medicinal plants have indicated a positive effect of inorganic fertilizers on composition and essential oil yield (Ashraf et al., 2006; Kumar et al., 2009; Castro et al., 2010). However, Azizi et al. (2009) associated lower essential oil yields with inorganic fertilizers. At present, integrated supply of nutrients to plants using combinations of inorganic and organic sources is becoming an increasingly significant aspect of environmentally sound agriculture. Despite these studies, it is important to note that there is still limited information on the impacts of organic fertilizer on constituents and yields of rosemary oil (Tawfeeq et al., 2016).

While there have been some studies regarding the effect of organic and inorganic fertilizer on rosemary essential oil yields and composition, there is no conclusive agreement on their effect and more research needs to be carried out. This study therefore purposed to evaluate the influence of cow manure, cow manure plus fertilizer (NPK) and fertilizer alone on the rate of growth, fat content, oil yield and oil quality in rosemary plants.

2. Materials and methods

2.1. Study site

The present work was performed at the Research Station of the University of Embu, Embu County, Kenya (longitude, 0° 35′ 25″ S and latitude, 37° 25′ 31″ E; 1494 m above sea level) during the 2020–2021 growing season (March 2020–March 2021). The area lies in the Upper Midland (UM) 2 agro-ecological zone. The mean annual rainfall is 1230 mm which falls in a bimodal pattern, whereby the long rains occur from March to June while the short rains occur from October to December (Jaetzold, 2007). The mean temperature is 19.5 °C, with 25 °C and 14 °C as maximum and minimum temperatures, respectively. The dominant soil type in the area is *humic* Nitisols, characterized by deep, moderately to high inherent fertility, and highly weathered with friable clay (Iuss Working Group Wrb, 2015). The mean annual rainfall and temperature in the site during the study period were 1449 mm and 19.2 °C, respectively. The soil chemical properties of the study site composed of soil pH (5.94), total nitrogen (0.19%), phosphorus (0.98 mg kg-1) and potassium (30 mg kg-1).

2.2. Field evaluation and design

For field trial establishment, rosemary cuttings (*Rosmarinus officinalis* L.) of homogeneous size (ranging 35–40 cm) and that were propagated under phytosanitary conditions were selected. The experiment consisted of four treatment groups namely (i) application of cow manure, (ii) cow manure plus fertilizer (NPK), (iii) fertilizer alone and (iv) control plots (without any amendment). Cow manure used in this work was obtained from an intensive dairy cattle farm with similar physical chemical properties as described by Lekasi et al. (2003). While the recommended rate for NPK fertilizer (N:P:K ratio 17:17:17) application was used (IFDC, 2018). The rosemary cuttings were transplanted in field plots set in a randomized completed block design (RCBD), whereby each treatment was replicated four times making a total of 16 plots. Each plot, measuring 5 m by 5 m, comprised of 7 rows with 8 plants within each row, and the planting hole was approximately 20 cm in depth by 13 cm width. Plants were spaced at 50 cm and 60 cm within a row and between rows, respectively. At planting, cow manure was incorporated at the rate of 4640 kg/ha (Tanner et al., 2000) and synthetic fertilizer (NPK 17:17:17) was incorporated at the rate of 300 kg/ha (IFDC, 2018). Cow manure was applied 2–3 inches of the top layer soil and turned in using a hand hoe. For NPK, a proper amount was uniformly applied around the base of the rosemary plants using the ring method (Shahena et al., 2021). After six months, an additional dose of the same treatments was applied to the corresponding plots. The first irrigation was done immediately after transplanting by spot watering (flooding of the basin around the plant), followed by irrigating plots twice per week depending upon weather conditions (Singh et al., 2007). Weed management was implemented uniformly across the plots by use of a hand-hoe once a month or whenever necessary.

2.3. Collection of agronomic data

Data on PLH, NOPB and survival rate were collected from 15 randomly selected plants per plot. The data was collected at planting (0 days), 90, 180, 270 and 360 days after planting (Miguel et al., 2007). Plant height (cm) was measured using a tape from the soil surface to the tip of the highest stem (Omer et al., 2020). The NOPB (counts) was examined by a visual count of the functional branches as described by Omer et al. (2020). Survival rate (%) was computed by counting the number of surviving rosemary plants, divided by the number of rosemary plants originally planted per plot, and then multiplied by 100 as given in Eq. (1) according to Christophe et al. (2019).

$$\text{Survival rate }(\%) = \frac{\text{Number of remaining plants}}{\text{Number of plants originally planted}} \times 100 \tag{1}$$

2.4. Fat content, essential oil yield and composition of rosemary

2.4.1. Determination of fat content

To determine rosemary fat content using the conventional Soxhlet extraction method (CSEM), fresh herbage consisting of leaves plus twigs about 20–30 cm long were carefully obtained from 15 separate randomly selected bushes of rosemary plants in each treatment group at harvest (360 days after planting) and placed in well-labeled plastic bags. After harvesting, leaves plus twigs were washed with tap water in the laboratory. Samples were then pulverized into fine particles using a lab grinder model FW80–1 High-speed universal disintegrator for 1 min. A 10 g mass of pulverized rosemary was transferred in a 30 mm (internal diameter) × 80 mm (external length) cellulose thimble and placed in Soxhlet apparatus with a flask containing 200 ml of solvent (petroleum ether and hexane of polarity 0.1 and 0.00, respectively). All reagents were of analytical grade. The extraction was carried out for 6 h according to the modified AOCS Method (Am 5-04) as described by Keshun (2021).

Upon extraction, the extract was concentrated using a rotary evaporator model (ROVA-2L, MRC, Israel) connected to a vacuum pump model (VP-19, MRC, Israel) at 40 °C at a rotation speed of 100 rpm. The fat content (%) of fresh rosemary was quantified using Eq. (2) below as described by Keshun (2021):

$$\%\text{Fat content} = \frac{\text{Weight of extracted fat(g)}}{\text{Weight of fresh sample(g)}} \times 100 \quad (2)$$

All extractions from the four treatment groups were repeated three times.

2.4.2. Determination of oil yield and composition

Rosemary essential oil for composition analysis was extracted using the steam distillation method. A 2.5 kg of fresh leaves and twigs of rosemary were steamed with 7 L of water for 2 h at about 100 °C or until when no more oil was coming from the condenser using a steam distiller (model 10-15-20 gallon capacity Propane or Electric stills). After extraction, oil yield (%) was determined immediately as volume (ml) of essential oil per g of fresh plant material using Eq. (3) below as elucidated by Verma et al. (2020).

$$\text{Essential oil yield } (\%, \text{v}/\text{w}) = \frac{\text{Volume of extracted oil(ml)}}{\text{Weight of fresh sample(g)}} \times 100 \quad (3)$$

Subsequently, the oil was stored in amber flasks at -20 °C awaiting further testing (Thanh et al., 2017). The extraction of oil from the four treatment groups was repeated three times.

Moreover, the identification of rosemary oil constituents from the four treatments was evaluated using the gas chromatography with mass spectrometry model (GC-MS, QP2010SE, Shimadzu corporation) and an SLB - 5MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 μm). A 25 μL of rosemary oil was mixed with 1 mL n-hexane. The GC-MS system was operated under the following conditions: carrier gas (helium), flow rate (1.0 mL/min), split ratio (1:50), injection temperature was 200 °C and the injection volume was 1.0 μL. The oven temperature was maintained at 60 °C for 1 min, followed by 60–250 °C at a rate of 10 ℃/min for 25 min. The GC-MS solution software version 4.45 (Shimadzu, Kyoto, Japan) was used to acquire data, while bidimensional visualization was generated using Chromsquare software version 2.3. The PubChem (https://pubchem.ncbi.nlm.nih.gov/) and NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/#) databases were utilized for the characterization of components of rosemary essential oil.

2.5. Data analysis

Prior to statistical analysis, data on rosemary essential oil compounds were transformed by arcsine transformation as necessary to meet the assumptions of normality. In order to explore the influence of treatments on PLH, NOPB, survival rate, fat content, essential oil yield and composition, the data were evaluated using analysis of variance (ANOVA) implemented with the R vegan package (Oksanen et al., 2016). To account for variation between these factors, the Turkey HSD test was performed by use of the R agricolae package (De Mendiburu, 2015). Findings are presented as means ± standard error as well as graphs of at least three independent experiments. Differences were regarded statistically different at $P < 0.05$.

3. Results and discussion

3.1. Plant growth

The plant height of rosemary plants grown under different soil amendments over time ranged between 36.7 cm and 71.8 cm (Table 1). These values are comparable to those reported by Singh and Wasnik (2013) and by Mostafa (2019). In particular, plant height was higher in plots amended with F alone at 0 days and 90 days compared to the plants growing in control plots. However, at 180 days and 270 days, plants were significantly taller in plots treated with Cm + F and Cm alone, respectively. During the final sampling (360 days), plant height remained higher in Cm plots relative to the control. Notably, plant height at 90, 180, 270 and 360 days after planting exhibited a statistical difference at $P < 0.05$. These findings vary compared to those of Omer et al. (2020) who recorded no significant difference in rosemary plant height cultivated under various soil conditioners. A major reason for this observation could be attributed to the fact that fertilizer is taken up rapidly by the plant within a very short time (days) compared to manure (Han et al., 2016), thus manure inducing a higher plant height in the long run.

The lowest mean of NOPB was measured on day 0 at planting and ranged between 5.8 and 6.6 branches, and there was no significant

Table 1. Mean values of plant height, number of primary branches per plant and survival rate at different sampling times during the growth of rosemary plants under different soil amendments (Control (C), cow manure (Cm), cow manure plus fertilizer (Cm + F) and fertilizer (F)).

Treatment	C	Cm	Cm + F	F	*P* - value
Days after planting	Plant height (cm)				
0	39.0a±1.50	36.7a±1.24	38.2a±1.31	39.4a±1.34	0.508
90	48.7b ± 1.56	51.6ab±1.30	53.9ab±1.56	55.3a±1.59	0.014*
180	52.1b ± 1.92	60.0a±1.55	60.2a±1.70	58.8a±1.98	0.004**
270	58.0b ± 1.79	65.6a±1.82	63.7ab±1.94	63.3ab±2.24	0.041*
360	62.8b±2.12	71.8a±2.41	71.2ab±2.32	66.8ab±2.79	0.031*
	Number of primary branches per plant				
0	6.2a±0.40	6.4a±0.50	5.8a±0.44	6.6a±0.59	0.710
90	6.9a±0.44	6.9a±0.44	6.4a±0.65	6.4a±0.39	0.567
180	27.3ab±1.59	33.1a±1.83	30.8ab±1.75	26.2b ± 1.58	0.016*
270	42.1b ± 2.68	48.9ab±2.41	50.4a±2.79	42.1b ± 2.41	0.036*
360	44.4b ± 2.54	55.8a±3.24	53.0ab±2.90	46.9ab±2.92	0.021*
	Survival rate (%)				
0	100	100	100	100	-
90	94.6ab±3.18	94.6ab±1.63	85.7b ± 3.34	96.9a±0.86	0.040*
180	92.4ab±3.95	92.4ab±0.86	81.7b ± 4.34	96.9a±0.86	0.022*
270	92.4ab±3.95	92.0ab±0.89	81.7b ± 4.34	96.9a±0.86	0.023*
360	92.4ab±3.95	91.5ab±1.12	80.8b ± 4.57	96.9a±0.86	0.020*

Means followed by the same letters within the same row are not statistically different. *$P < 0.05$ and **$P < 0.01$.

difference between materials planted in each plot with respect to NOPB (Table 1). There was little increase in the NOPB in the first 90 days of cuttings development. However, at 180 days after planting, plants growing under Cm conditions had more branches than those growing under other amendments and they had significantly more branches than those growing in the fertilizer alone plots. By the time the plants were 270 days old, plants growing in the Cm + F plots had significantly more branches than those growing under control and those under F alone. By the time they reached the 360^{th} day, the Cm only plots had plants with the largest NOPB when compared to that of the control plots. There was a considerable increase in the number of primary branches per plant between samplings on the 180, 270 and 360 days at $P < 0.05$ after planting contrary to the study by Omer et al. (2020). As mentioned previously, a plausible explanation for this can be ascribed to the fact that fertilizer is usually absorbed quickly by the plant over a short period compared to manure, which tends to be taken up by plants more slowly (Diacono and Montemurro, 2010; Han et al., 2016).

The survival rate of rosemary plants revealed a substantial difference ($P < 0.05$) across the treatments at 90, 180, 270 and 360 days after planting (Table 1). In particular, it was higher in plots treated with F alone during the whole sampling period relative to other treatments. This result corroborates with the reports by Singh et al. (2007) who found that fertilization with NPK fertilizer improved the growth rate of rosemary plants.

3.2. Rosemary fat content (%)

Fat content as quantified using the conventional Soxhlet extraction method from fresh rosemary herbage harvested 360 days after planting is presented in Figure 1. The fat content ranged between 11.22% and 13.36%. The highest fat content was recorded in Cm treatment (13.36%) followed by Cm + F (11.98%) when compared to the other two treatments. The fat content in the present work was slightly higher compared to the yield (8.76%) obtained by Genena et al. (2008) when extracting essential oil from rosemary using the conventional Soxhlet extraction method. Hirondart et al. (2020) obtained higher fat content up to 26 ± 1% using the same method. The fat content of the control plots (11.48%) was slightly higher than that of F treatments (11.22%). Azizi et al. (2009) made similar observations while studying the oregano plants. Our rosemary fat content under different soil amendment plots was not statistically different at $P < 0.05$.

3.3. Rosemary essential oil yield (%, v/w)

The yield of rosemary essential oil from herbage harvested from different plots and extracted using the steam distillation method is illustrated in Figure 2. The oil yield varied from a low of 0.45% (v/w) in plots amended with F alone to a high of 0.59% (v/w) in plots treated with Cm + F. In comparison, our oil yield range (0.45–0.59%, v/w) aligned with the values (0.3–0.7%, v/w) of rosemary from different zones of Portugal (Serrano et al., 2002) and lower than that of the western and southern coastal belt of Turkey (0.71–0.94%, v/w) (Gurbuz et al., 2016) and southern Spain (1.03–1.81%) (Salido et al., 2003). The difference between these findings could perhap be due to different management practices such as agronomic practices, harvesting time and environmental conditions (Pirbalouti et al., 2013). The lower oil yield in fertilizer treatments obtained using the steam distillation method (Figure 2) is in line with the fat content results as determined using the Soxhlet method (Figure 1) in this study. In addition, the oil yield was not statistically different ($P < 0.05$) across the four levels of soil amendments. This observation concurs with the data of previously published articles (Singh et al., 2007; Singh and Guleria, 2013).

3.4. Compounds of rosemary essential oil

The identified relative proportions of the constituents of rosemary essential oils using the GC-MS method are arranged in order of magnitude. As an example, Figure 3 shows the trace of oil acquired from a cow manure treatment plot, replicate 3, consisting of 21 constituents. Similarly, the data for other treatments as well as their corresponding replicates resembled the same.

During data analysis, the data was pooled together and summarized in Table 2. Overall, twenty-six constituents were identified from the extracted rosemary oil under different treatments using the GC-MS analysis (Table 2). Treatments Cm, C, F and Cm + F contained 23, 22, 19 and 16 constituents, respectively. The major compounds of rosemary oil in this study were alpha-pinene (29.80%–34.34%), eucalyptol (27.15%–30.26%), verbenone (7.63%–8.14%), geraniol (4.47%–5.22%), endo-borneol (4.58%–4.93%), d-limonene (3.04%–3.13%), linalool (3.08%–3.62%), 1,6-dimethylhepta-1,3,5-triene (3.02%–3.44%), camphene (2.69%–2.99%), l-alpha-terpineol (2.19%–2.48) and camphor (2.09%–2.45%). While other compounds were revealed in much lower quantities (<2%) (Table 2). The percentage composition of alpha-pinene, eucalyptol and verbenone as major constituents aligned with the results of other similar studies (Ngân et al., 2019; Omer et al., 2020). In addition, multiple recent studies have enumerated the benefits of essential oils of rosemary with regard to chemical composition, applications and biological characteristics (Ban et al., 2016; Bajalan et al., 2017; González-Minero et al., 2020). As previously reported, the most commonly identified major compounds of rosemary essential oils are camphor, β-pinene, α-pinene and bornyl acetate, among others (Okoh et al., 2010; Ojeda-Sana et al., 2013; Bajalan et al., 2017). The main

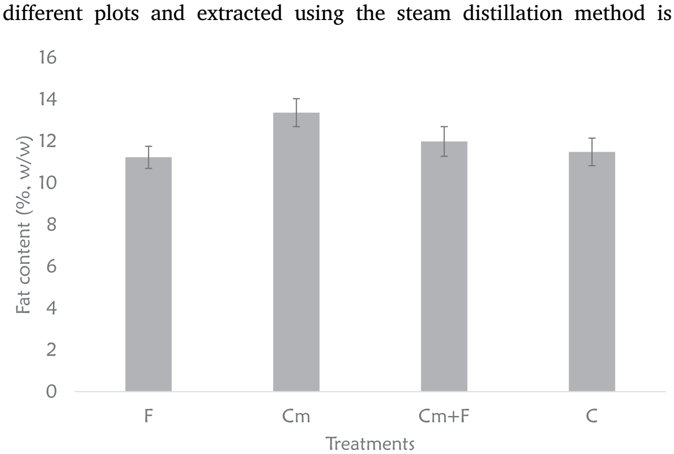


Figure 1. The fat content of rosemary herbage grown under four soil amendments (control (C), cow manure (Cm), cow manure + fertilizer (Cm + F), and fertilizer (F)) and extracted using the conventional Soxhlet method. The error bars represent standard deviation.

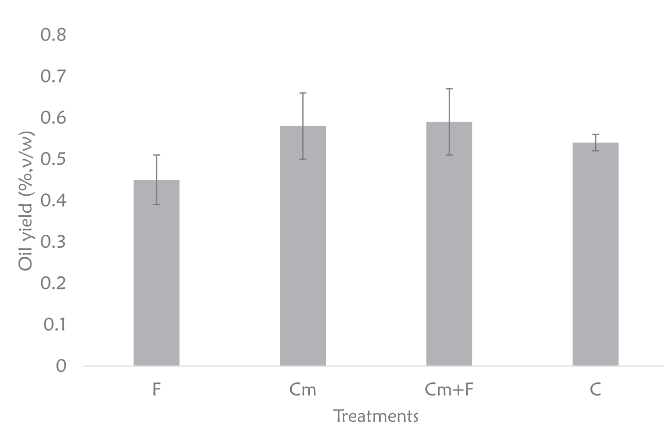
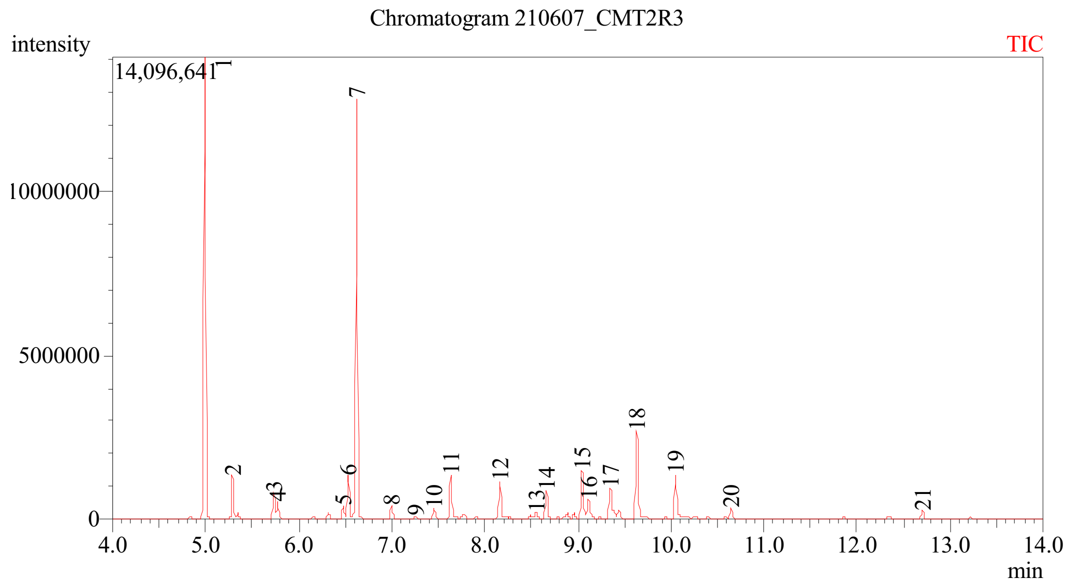


Figure 2. The essential oil yield of rosemary herbage grown under four soil amendments (control (C), cow manure (Cm), cow manure + fertilizer (Cm + F), and fertilizer (F)) and extracted using the steam distillation method. The error bars represent standard deviation.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.992	20978813	31.56	.alpha.-Pinene
2	5.290	1941168	2.92	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methy
3	5.735	1221836	1.84	.beta.-Pinene
4	5.774	694237	1.04	.beta.-Myrcene
5	6.478	515746	0.78	Benzene, 1-methyl-3-(1-methylethyl)-
6	6.532	2011176	3.03	D-Limonene
7	6.625	20108782	30.26	Eucalyptol
8	6.996	472972	0.71	.gamma.-Terpinene
9	7.257	40841	0.06	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-
10	7.453	378303	0.57	Cyclohexene, 3-methyl-6-(1-methylethyliden
11	7.636	2083922	3.14	Linalool
12	8.165	1781041	2.68	1,6-Dimethylhepta-1,3,5-triene
13	8.556	271909	0.41	trans-Verbenol
14	8.663	1501414	2.26	Camphor
15	9.048	2561426	3.85	endo-Borneol
16	9.117	1077949	1.62	3-Cyclohexen-1-ol, 4-methyl-1-(1-methyleth
17	9.350	1282252	1.93	L-.alpha.-Terpineol
18	9.635	4783392	7.20	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimeth
19	10.051	2181507	3.28	Geraniol
20	10.646	333095	0.50	Bornyl acetate
21	12.706	242465	0.36	Caryophyllene
		66464246	100.00	

Figure 3. The GC-MS trace obtained from essential oils of rosemary extracted using the steam distillation method from herbage cultivated on plots whose soil was amended with cow manure and harvested 360 days after planting. The IUPAC names: Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methy (camphene); Benzene, 1-methyl-3-(1-methylethyl)- (m-cymene); Cyclohexanol, 1-methyl-4-(1-methylethenyl)- (iso-β-terpineol); Cyclohexene, 3-methyl-6-(1-methylethyliden (isoterpinolene); 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethy; (terpinen-4-ol) and Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethy (verbenone) were substituted with respective common name in bracket and also in Table 2 using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and the NIST Chemistry WebBook database (https://webbook.nist.gov/chemistry/#).

compound of Italian rosemary oil is camphor with values ranging from 2% to 5% (Lo Presti et al., 2005). While Argentinian rosemary oil has α-pinene containing ratios between 4.9% and 31.2% (Ojeda-Sana et al., 2013).

Conversely, other compounds including linalool (3.08%–3.62%) and geraniol (4.47%–5.28%) (Table 2) exhibited variability compared to recently published articles (Melito et al., 2019; Omer et al., 2020) which found linalool ranged from 0.9% to 2.3%, while geraniol ranged between 0.0% and 0.1%. The difference in chemical composition between Kenyan rosemary oil and that of Egyptian and Sardinian may be attributed to the different geographical locations along with the environmental conditions. Previous work has also shown that rosemary essential oils are reliant on various factors for instance climatic factors, geographical location and soil structure and texture (Lo Presti et al., 2005; Gachkar et al., 2007).

The compounds alpha-pinene, linalool, m-cymene, and terpinen-4-ol differed considerably ($P < 0.05$) under the four treatment groups (Table 2). Rosemary plants treated with Cm produced higher percentages of terpinen-4-ol and m-cymene, whereas linalool and alpha-pinene were higher in Cm + F and F, respectively, compared to other treatments. Studies have elucidated that organic fertilizers contain complex materials such as minerals, plant growth hormones, pigments, polysaccharides and proteins that are absent in synthetic fertilizers (Gollan and Wright, 2006; Chojnacka et al., 2012). These compounds may induce positive effects in the biosynthesis of the plant activity hence influencing the quality of oil (Miguel et al., 2007).

Table 2. Compounds of essential oil of rosemary plants grown under four levels of soil amendment (cow manure (Cm), cow manure plus fertilizer (Cm + F), control (C) and fertilizer (F) alone) expressed as a percentage of total oil obtained using the GC-MS analysis.

Compound	Cm	Cm + F	C	F	*P*-value
4-carene	0.00a ± 0.000	0.00a ± 0.000	0.00a ± 0.000	0.09a ± 0.087	0.441
α-terpinene	0.13a ± 0.070	0.00a ± 0.000	0.10a ± 0.097	0.00a ± 0.000	0.283
1,6-dimethylhepta-1,3,5-triene	3.02a ± 0.182	3.39a ± 0.032	3.44a ± 0.190	3.06a ± 0.349	0.441
terpinen-4-ol	2.10a ± 0.135	0.00c ± 0.000	1.96a ± 0.125	0.92b ± 0.130	<0.001***
alpha-pinene	29.98ab ± 1.252	30.52ab ± 0.218	29.80b ± 1.194	34.34a ± 0.741	0.032*
m-cymene	0.91a ± 0.125	0.00b ± 0.000	0.81a ± 0.280	0.00b ± 0.000	0.002**
beta-pinene	1.73a ± 0.091	1.43a ± 0.568	1.47a ± 0.219	1.31a ± 0.105	0.769
beta-myrcene	0.68a ± 0.339	0.00a ± 0.000	0.17a ± 0.173	0.00a ± 0.000	0.148
camphene	2.72a ± 0.107	2.70a ± 0.113	2.69a ± 0.058	2.99a ± 0.063	0.135
verbenone	7.64a ± 0.239	8.14a ± 0.563	7.99a ± 0.566	7.63a ± 0.373	0.821
bornyl acetate	0.62a ± 0.115	0.54a ± 0.040	0.93a ± 0.125	0.61a ± 0.225	0.392
camphor	2.44a ± 0.169	2.37a ± 0.099	2.45a ± 0.110	2.09a ± 0.107	0.197
caryophyllene	0.88a ± 0.303	0.67a ± 0.079	0.97a ± 0.108	1.03a ± 0.197	0.593
6-terpineol	0.00a ± 0.000	0.00a ± 0.000	0.10a ± 0.000	0.00a ± 0.000	0.441
iso-β-terpineol	0.02a ± 0.020	0.00a ± 000	0.00a ± 0.000	0.00a ± 0.000	0.441
isoterpinolene	0.58a ± 0.010	0.48a ± 0.035	0.49a ± 0.010	0.63a ± 0.055	0.081
d-limonene	3.13a ± 0.058	3.08a ± 0.113	3.04a ± 0.132	3.07a ± 0.063	0.924
endo-borneol	4.78a ± 0.491	4.93a ± 0.019	4.87a ± 0.202	4.58a ± 0.258	0.85
eucalyptol	28.29a ± 1.430	30.26a ± 0.834	27.15a ± 0.619	27.43a ± 1.182	0.242
gamma-terpinene	0.73a ± 0.020	0.73a ± 0.035	0.75a ± 0.030	0.71a ± 0.090	0.948
geraniol	4.47a ± 0.638	5.07a ± 0.338	5.22a ± 0.445	4.64a ± 0.853	0.781
humulene	0.00a ± 0.000	0.00a ± 000	0.05a ± 0.050	0.00a ± 0.000	0.441
l-alpha-terpineol	2.28a ± 0.177	2.19a ± 0.392	2.48a ± 0.438	2.20a ± 0.235	0.925
linalool	3.27ab ± 0.085	3.62a ± 0.070	3.38ab ± 0.163	3.08b ± 0.100	0.049*
pinocarvone	0.08a ± 0.083	0.00a ± 0.000	0.00a ± 0.000	0.00a ± 0.000	0.441
trans-verbenol	0.14a ± 0.137	0.00a ± 0.000	0.00a ± 0.000	0.31a ± 0.310	0.582

Means followed by the same letters within the same row are not statistically different. *P < 0.05, **P < 0.01 and ***P < 0.001.

4. Conclusion

This study demonstrated that rosemary grown under cow manure alone (Cm) exhibited an overall greater value of plant height and number of primary branches per plant, while plants grown under fertilizer alone (F) had a higher survival rate relative to other treatments. The oil yield and fat content of rosemary were insignificantly influenced by the use of Cm and other treatments compared to the unamended check although the F treatment maintained the lowest oil yield and fat content. With regard to the chemical composition of the oil, our findings provide useful insights into the constituents of Kenyan rosemary oil for the first time. The Cm treatment showed a higher number of rosemary essential oil compounds as well as greatly affected the oil quality. Nonetheless, based on these results, long terms studies incorporating the influence of soil physical-chemical properties on rosemary oil are required. At present, we are evaluating the influence of different agroecological zones on rosemary essential oils quality and quantity.

Declarations

Author contribution statement

Gikuru Mwithiga; Samuel Maina: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Josiah Gitari; Phyllis Muturi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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