

Agronomic Performance, Oil Yield and Hydrolate Quality of Patchouli Cultivated in Different Soil Covers

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Abstract: This study describes the evaluation of the effectiveness of different soil covers on the development, productivity, yield and metabolic content of patchouli oil (*Pogostemon cablin*) and its hydrolate. The agronomic experiments were carried out in the field, including four types of soil cover and six replications (4 x 6), using green cover (peanut), straw (crotalaria and millet straw), white plastic cover, and no mulch (weeding). After transplanting, the initial growth of seedlings was analyzed through weekly monitoring of plant height, stem diameter, and the number of leaves. At harvest time, the harvest yield (green mass) was performed. After drying the leaves, the oil and hydrolate were extracted by steam distillation and then the yield of patchouli oil was determined. Regarding the agronomic analyses, white plastic and straw coverage was superior to the other treatments, with higher plant heights, number of leaves, whereas the plastic and straw coverage was superior to the other treatments, with higher plant heights, number of leaves and green mass yield. The metabolic content was evaluated using High Resolution Mass Spectrometry (HRMS), and the chemical markers were identified through the analysis of the MS/MS fragmentation spectra and chemotaxonomic data. No significant differences were observed in the essential oils yields and their hydrolates and the intensities of the major ions found in the samples identified as chemical markers for the quality control of *P. cablin*.

Key words: *Pogostemon cablin*, patchouli oil, pogostone, oil quality, hydrolate quality, Mass Spectrometry

1 Introduction

Essential oils have been used all over the world for many purposes including flavoring, and aroma additives for household products and cosmetics such as perfumes¹. A range of 15–21% of all essential oil produced is destined for a perfume and cosmetics industry. The projection for the world market of essential oils is that by the year 2026 it will reach US\$ 15.1 billion due to its growth in different sectors².

Pogostemon cablin (Lamiaceae), popularly known as patchouli, is a plant of great economic importance due to the presence of essential oil with woody fragrance and intense skin correction capacity, which is widely used by the cosmetics and perfumery industries^{3,4}, in addition to having medicinal properties⁵.

There is a wide quantitative and qualitative variation in the chemical composition of patchouli oil, from which more than 130 compounds have been identified. However, pa-

tchouli oil is rich in sesquiterpenes, mainly patchoulol, patchoulene, caryophyllene, pogostol, and pogostone. Other minor components such as guayene, cyclosylene, α -, β -bulnesene, and norpatchoulene were identified in its composition. Patchoulol and patchoulene are the main constituents of the oil and they are responsible for the quality and aroma of the essential oil^{2,4}.

Several factors influence the variation in the chemical composition of the essential oil, including environmental factors such as humidity, temperature, and light⁶. Other factors are material origin, agricultural practices, maturity, and post-harvest treatment. Concerning the patchouli oil, the influencing factors are the number of leaves to be distilled, the distillation method, the aging of the dry leaves, the cultivation practice, and the plants' age. There is evidence that the distillation process produces loss or enrichment of the chemical constituents of patchouli oil⁷.

In this context, considering the cultivation practices,

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management practices, and soil conservation, the soil cover becomes essential for crop productivity⁸). Soil covering can be done with organic materials such as straw and green manure, and inorganic materials such as plastic coverings. The choice of roofing material depends on the local climate, the cost-effectiveness, and the culture to be implemented⁹). The green manure has the potential to be applied as a soil cover, as it promotes the protection of the soil against climatic variations, maintains the soil organic matter content, and reduces the appearance of invasive plants¹⁰). Another efficient method for soil conservation is the use of plastic coverings, which are mainly indicated to protect the plant in its vegetative stage through thermal insulation and evaporation, promoting the maintenance of soil temperature and humidity¹¹). Another technique used in soil protection is the application of straw, as it provides soil resistance to erosion and promotes water infiltration¹²).

Therefore, it is evident that information on the use of different ground cover materials in the cultivation of patchouli is still incipient. Thus, the objective of this study was to determine the effect of different materials used as soil cover on productivity and chemical composition of hydrolate and essential oil from *P. cablin* (Blanco) Benth.

2 Materials and Methods

2.1 Soil cover application methodologies

The experiment was conducted under field conditions in the Horticulture Sector of the School of Agronomy from Federal University of Goiás (latitude 16° 35' 12" S, longitude 49° 21' 14" O Gr, at 730 m altitude). The region's climate is Aw, i.e., tropical savanna with a potential water deficit between June and August and global solar irradiance increasing globally between September and October (Fig. 1). The potential evapotranspiration and the maximum, average, and minimum air temperatures (Fig. 2) observed

were collected at the meteorological station of the School of Agronomy.

Soil analysis was done at a depth of 0-20 cm, whose physicochemical characteristics were pH-CaCl₂ (5.8), P-Mehlich (90.0 mg/dm³), K (64.0 mg/dm³), Ca (3.9 cmol/dm³), Mg (1.2 cmol/dm³), Al (0.0 cmol/dm³), H + Al (2.2 cmol/dm³), organic matter (18.0 g/dm³). The experiments were performed in soil with the following characteristics (46% clay, 44% sand and 10% silt).

The soil preparation was done conventional, with two plows, distribution of dolomitic limestone (PRNT 100%) with subsequent aggregation and leveling of the soil. Fertilization recommendations were performed according to the requirements of patchouli culture and interpretation of soil analyses. The fertilization was done with the commercial formulas 04-30-10 (NPP) and granulated potassium chloride (PC). Irrigation was carried out by drip.

The experimental design used was conducted in randomized blocks, with four treatments and six replications. The treatments consisted of different soil covers, being live cover, straw, white plastic cover, and without mulches/hoeing. Patchouli seedlings were purchased from the Instituto Agronômico de Campinas – IAC and were transplanted after acclimatization period. For the formation of straw, cultures *Penissetum americanum* L. and *Crotalaria juncea* were used, both of which were manually mowed for fifteen days before transplanting the patchouli seedlings. In live coverage, the forage peanut was adopted, which was sown 60 days before the transplanting of patchouli seedlings.

In the treatment without covering, the removal of invasive plants was carried out weekly through weeding, and in the plastic covering treatment, the plastic covering in white color was used, being this installed 60 days before the transplanting of the seedlings.

There was no need to apply pesticides from the group during the crop development cycle against damage to pest

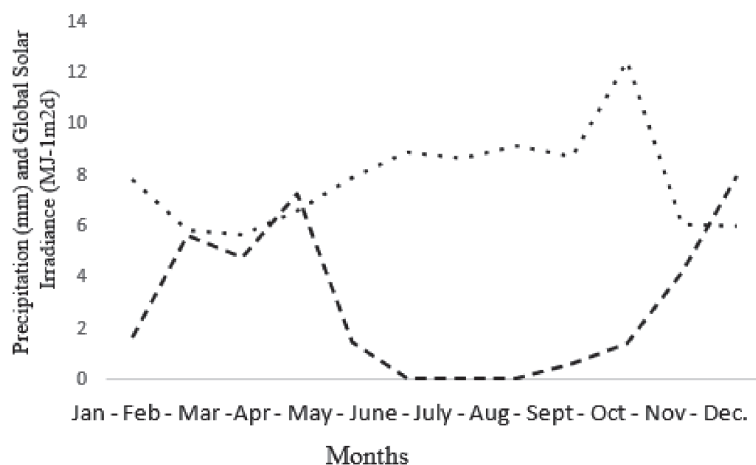


Fig. 1 Rainfall and sunshine distribution over an experimental area in 2019.

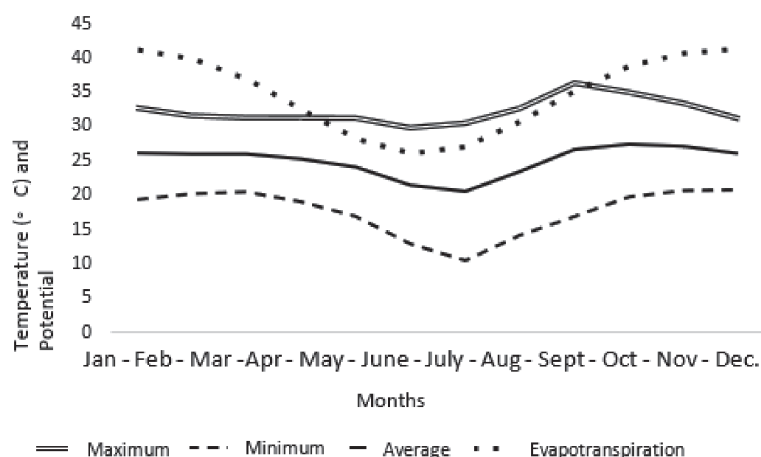


Fig. 2 Air temperature distribution and evapotranspiration throughout 2019.

attacks. Plants were measured to determine the following biometric variables: plant height (PH, m), stem diameter (SD, mm), number of leaves (NL). The measurement of plant height was performed using a graduated ruler. The stem diameter was obtained using a digital caliper. These three analyzes were performed after transplanting the seedlings for four consecutive weeks, to estimate the initial parameters of crop development. At harvest time, the yield parameters were determined, mass was analyzed, green mass yield (GMV) later, oil extraction was performed by steam drag to calculate the yield of patchouli oil, followed by chemical analysis of the hydrolate and patchouli oil. The data obtained were subjected to analysis of variance and Tukey's test ($p > 0.05$) using the SISVAR statistical software.

2.2 Extraction of patchouli essential oil

The leaves of *P. cablin* were dried for seven days at room temperature. The essential oils from dried leaves (525 g) were separately isolated by steam distillation for 2 h. The oil layers were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers, and kept at low temperature (4°C). The hydrolates produced by steam distillation were stored in hermetically sealed glass containers, kept under refrigeration (4°C), and submitted to ESI-MS analysis.

2.3 ESI-Orbitrap MS analysis and data processing for hydrolates

The analysis was performed by a Q-Exactive Orbitrap (Thermo Scientific, Bremen, Germany). The hydrolates obtained from *P. cablin* leaves were diluted with methanol (1:1, v/v) and infused via ESI-MS at 5 $\mu\text{L min}^{-1}$ by a syringe pump. The conditions used in the negative ion mode were: spray voltage 3.1 kV; sheath gas 10; S-lens 60; capillary temperature 320°C, 3 micro scans, and 50 scans. MS/MS was used to confirm the structure of compounds, and the

analysis was performed using higher-energy collisional dissociation (HCD) with a collision energy of 10 mu (manufacturer's units). The spectra were processed using the Xcalibur Analysis software package (version 2.2, Thermo Fisher Scientific, San Jose, CA). The results of the structural characterization were discussed in terms of measured mass-to-charge ratios (m/z), deprotonated molecule formulae, accurate molecular masses, and MS/MS data.

3 Results and Discussion

3.1 Plant biometrics

Regarding plant height, the number of leaves, and stem diameter, four measurements were taken after transplanting the seedlings, at 7 days after transplanting (DAT), 14 DAT, 21 DAT, and 28 DAT. The different ground covers influenced the height of the plants and the number of leaves in the evaluations carried out on the 14, 21, and 28 days after transplanting (Figs. 3 and 4). The treatments with live cover and weeding resulted in plants with smaller heights and fewer leaves compared to the other coverings. This result may have occurred due to nutrient competition caused by the live covering (forage peanut)¹³, and the direct incidence of the sun on the soil in the treatment weeding, raising the soil temperature¹⁴. These factors provide greater evaporation of water from the soil, unlike other covers, where it was observed that temperatures did not increase the soil temperature during the hottest periods of the day. Regarding the evaluation time (Figs. 3 and 4), differences were observed at all evaluation times. This is due to the normal growth of the plants.

Concerning the diameter of the stem (Fig. 5), there was no significant difference between the treatments and the periods of evaluation. After 120 days of seedlings transplanting, all leaves were collected and the green mass per hectare was calculated. The treatment of white plastic cov-

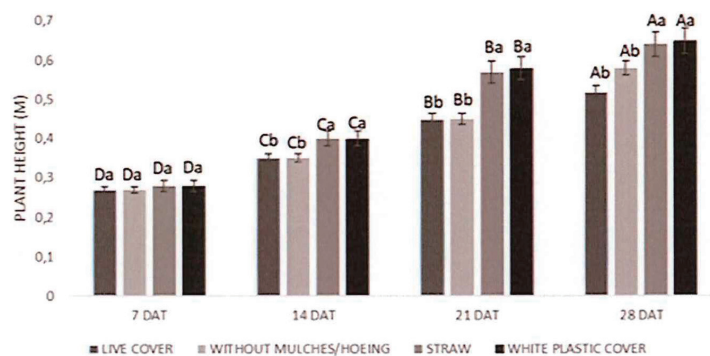


Fig. 3 Plant height (cm) at 7, 14, 21, and 28 DAT (days after transplanting) cultivated in different soil covers: live cover, without mulches/hoeing, straw, and white plastic cover. Means followed by the same uppercase letter between and lowercase within the treatments do not differ by the Tukey test ($p < 0.05$).

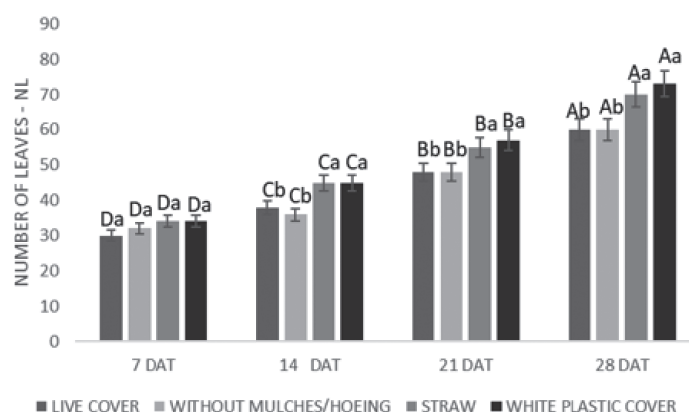


Fig. 4 Number of leaves at 7, 14, 21, and 28 DAT (days after transplanting) cultivated in different soil covers: live cover, without mulches/hoeing, straw, and white plastic cover. Means followed by the same uppercase letter between and lowercase within the treatments do not differ by the Tukey test ($p < 0.05$).

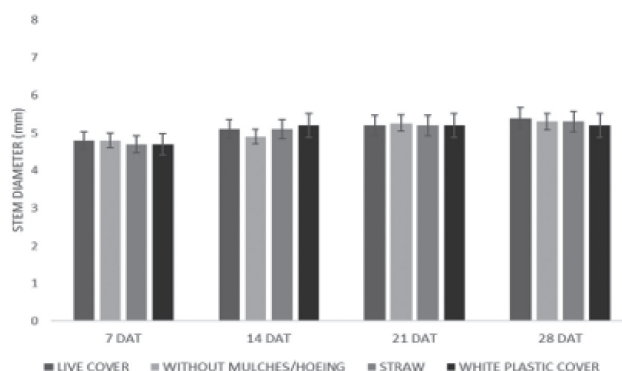


Fig. 5 Stem diameter (mm) at 7, 14, 21, and 28 DAT (days after transplanting) cultivated in different soil covers: live cover, without mulches/hoeing, straw, and white plastic cover. Means do not differ by the Tukey test ($p < 0.05$).

ering and straw allowed more outstanding production of green mass followed by weeding and live covering (Fig. 6). With fewer leaves and lower plant height, the production of photoassimilates ends up being restricted, which directly affects productivity.

3.2 Total essential oil yield

The essential oil yields obtained from hydrodistillation of dried leaves from *P. cablin* cultivated under four different soil coverings ranged from 0.46 ± 0.20 to 0.67 ± 0.31 % (Table 1). These findings are consistent with the literature, where yield values of approximately 0.3% from fresh leaves^{4, 15)} and between 0.54 to 5.2% from dry leaves are

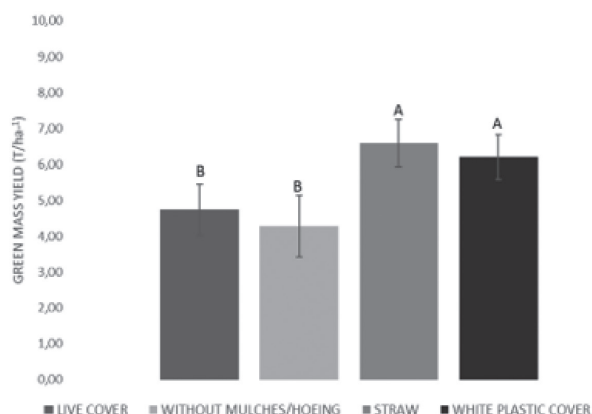


Fig. 6 Green mass yield per hectare (t/ha^{-1}) cultivated in different soil covers live cover, without mulches/hoeing, straw and white plastic cover. Means followed by the same capital letter the not differ between treatments by the Tukey test ($p > 0.05$).

Table 1 The total yield of *P. cablin* essential oil grown under different soil coating.

Repetition	Soil coating			
	L. COVER	HOEING	P. COVER	STRAW
1	0.86	0.60	0.73	1.04
2	0.97	0.53	0.70	0.55
3	0.80	0.38	0.87	0.49
4	0.84	0.32	0.20	0.26
5	0.21	0.20	0.43	0.41
6	0.33	0.74	0.70	0.33
Mean	0.67	0.46	0.60	0.51
SD	0.31	0.20	0.24	0.28

Table 2 Analysis of variance for essential oil yield *P. cablin* grown under different soil coating.

	DF	P-value
		EO Yield
Repetitions	5	0.05
Coating types	2	0.42

reported (2, 16, 4). The coating treatment variation did not interfere on essential oil yields. Analyses of variance were performed using two-way ANOVA. There was no significant difference ($p < 0.05$) for essential oil yield evaluating types of soil coverings (Table 2).

3.3 Hydrolates characterization

The characterization of *P. cablin* essential oil is widely reported in the literature, where the major constituent is patchoulol with more than 60% yield for some extractions. Other abundant constituents in *P. cablin* essential oil are guanine (3.7–23.3%), bulnesene (10.4–14.0%), seychellene (1.9–9.0%), patchoulene (2.0–6.0%), pogostol (0.3–5.2%), and caryophyllene (1.3–4.8%)^{2, 4, 17}. For the extraction of *P. cablin* essential oil, a large amount of hydrolates is generated, and because of this, understanding the constituents present in the hydrolates is essential for the fragrance industry.

Herein, the hydrolates obtained from *P. cablin* essential oil were analyzed by ESI(-)-Orbitrap MS/MS in order to obtain metabolic profiling and associate it with the quality of the products generated by the different soil covering methodologies (Fig. 7). The major components from hydrolates were identified by the fragmentation profile of the MS/MS spectrum and compared with chemosystematic data from *P. cablin*^{4, 18}. In the F m/z full Scan ESI(-)-MS, ions at m/z 223.09749, 209.08191, and 139.07640 were identified, which were present in all hydrolates samples analyzed (Fig. 7a), and have been used as a chemical marker for the quality control of patchouli oil¹⁹. The analysis of the fragmentation behavior by ESI-MS/MS of these compounds showed that the ion at m/z 139.07640 is a fragment generated from the fragmentation of the ion at m/z 223.09749, signaling that the patchouli oil hydrolate consists mainly of two compounds, the pogostone lactone at m/z 223.09749 (C₁₂H₁₅O₄) and its less hydrophobic analogue at m/z 209.08191 (C₁₁H₁₃O₄). The ion at m/z 209 is 14 Da lower than the ion at 223, indicating an analogue with one less methylene group in the methylpentane moiety. The ESI-MS/MS spectrum of the major compounds associated with deprotonated ions at m/z 223.09749 (Fig. 7b) and m/z 209.08191 (Fig. 7c) generated analogous fragments at m/z 139.07641 and m/z 125.06078, respectively.

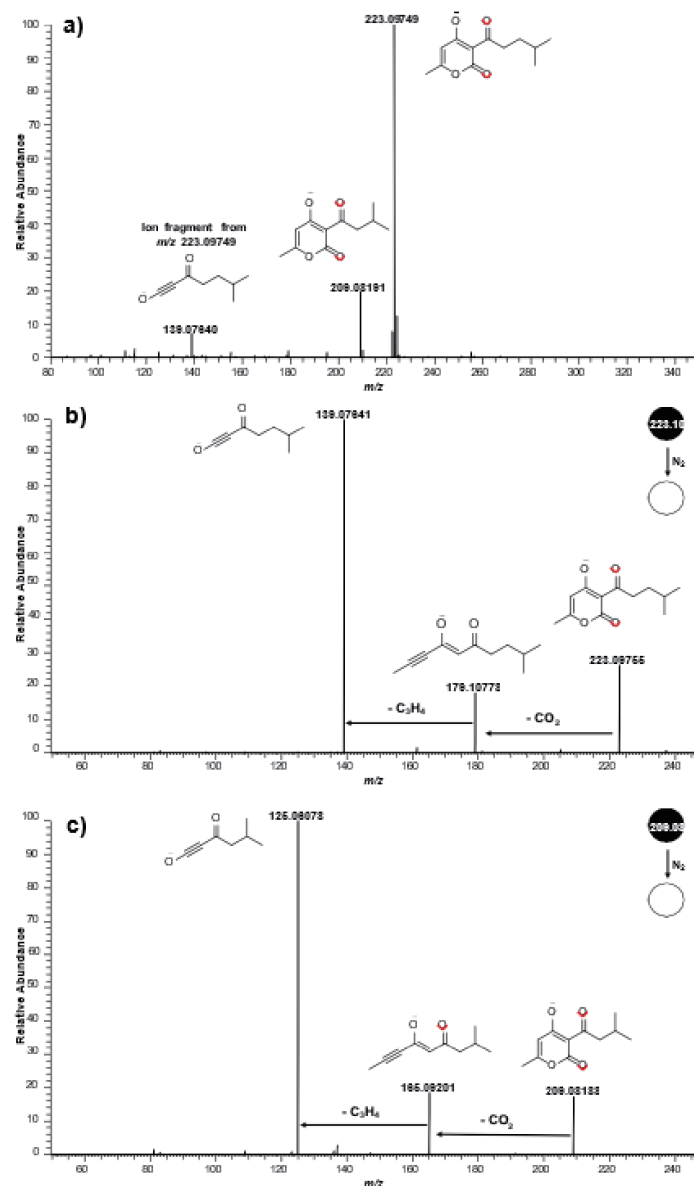


Fig. 7 ESI(-)-Orbitrap MS of hydrolates produced by hydrodistillation from *P. cablin*. a) Full scan MS in negative mode, b) ESI-MS/MS of m/z 223.09749, and c) ESI-MS/MS of m/z 209.08191.

These ions constitute the base peak of the MS spectrum and were generated by the loss of the lactone ring and they were used as a key element in the structural identification of both compounds, in addition to the ions related by the loss of a carbon dioxide moiety ($223 \rightarrow 179$) and C_3H_4 ($223 \rightarrow 139$). The fragmentation of the major compounds under positive ESI source (Fig. 8) showed ions in the MS/MS spectrum at m/z 207.10178 and 193.08615 formed by the loss of an H_2O molecule (18 Da) from the protonated parent molecule at m/z 225.11200 and 211.09639, respectively, which were used to confirm the identity of the chemical marker.

Table 3 shows the relative intensity followed by the re-

spective mean and standard deviation (SD) of pogostone and 4-hydroxy-6-methyl-3-(2-methyl-1-oxobutyl)-2H-pyran-2-one analyzed by ESI(-)-Orbitrap MS. Regarding the production of major compounds identified in hydrolates from *P. cablin* cultivated in different soil coverings, there was no significant difference, $p < 0.05$ (Table 4).

4 Conclusions

Our findings showed that the different ground covers influenced the height of the plants and the number of leaves in the assessment carried out on the 14, 21, and 28 days

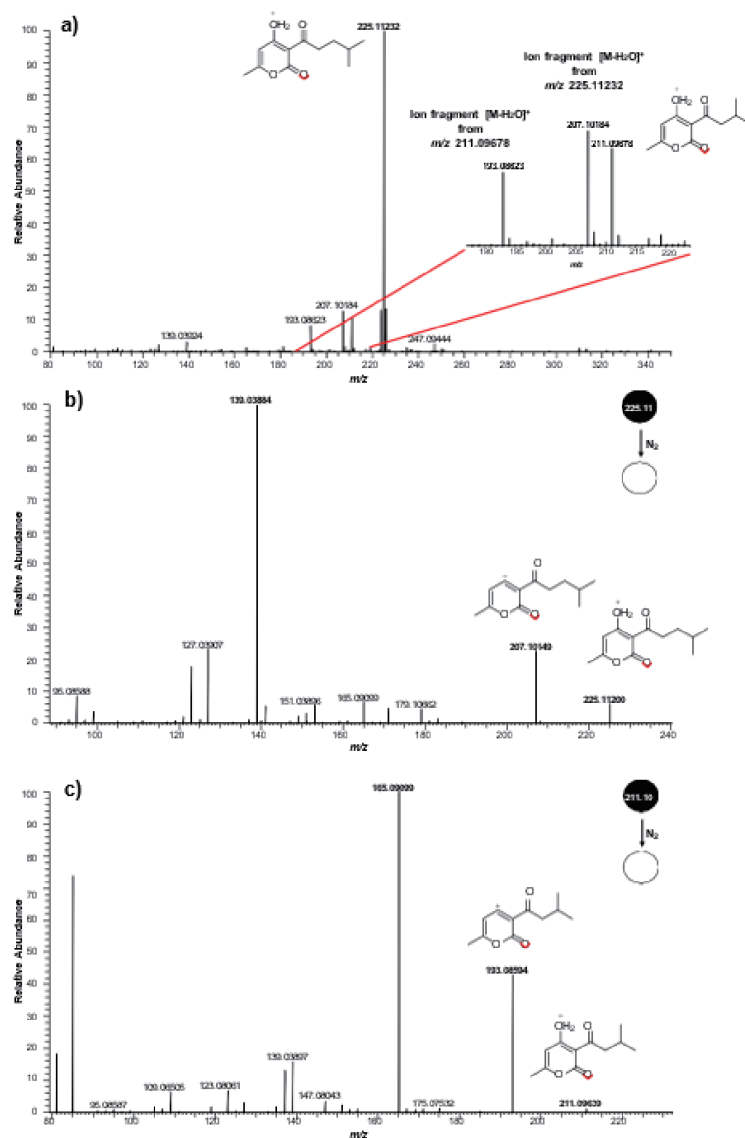


Fig. 8 ESI(+)-Orbitrap MS of hydrolates produced by hydrodistillation from *P. cablin*. a) Full scan MS in positive mode, b) ESI-MS/MS of m/z 225.11232, and c) ESI-MS/MS of m/z 211.09678.

Table 3 Relative intensity obtained by ESI(-)-Orbitrap MS for pogostone and 4-hydroxy-6-methyl-3-(2-methyl-1-oxobutyl)-2H-pyran-2-one identified in hydrolates from leaves of *P. cablin*.

Repetition	Compound 1*				Compound 2**			
	L. COVER	HOEING	P. COVER	STRAW	L. COVER	HOEING	P. COVER	STRAW
1	3.60E+06	6.66E+07	1.80E+06	1.80E+07	1.51E+06	2.85E+07	1.60E+06	4.11E+06
2	2.87E+06	1.41E+07	9.40E+08	2.90E+08	1.70E+06	9.55E+06	2.39E+08	1.58E+08
3	6.50E+08	1.22E+08	9.66E+08	1.82E+09	1.29E+08	4.36E+07	2.27E+08	2.50E+08
4	1.30E+09	8.20E+08	8.50E+08	2.73E+07	2.46E+08	1.96E+08	1.12E+08	4.10E+06
5	2.24E+06	5.78E+08	7.95E+08	4.27E+08	7.29E+05	1.19E+08	1.87E+08	9.63E+07
6	1.08E+09	2.25E+06	6.26E+08	2.18E+09	2.09E+08	5.36E+05	1.18E+08	3.48E+08
Mean	5.06E+08	2.67E+08	6.96E+08	7.94E+08	9.80E+07	6.62E+07	1.47E+08	1.43E+08
SD	5.89E+08	3.46E+08	3.61E+08	9.54E+08	1.12E+08	7.62E+08	8.90E+08	1.37E+08

*pogostone (m/z 223.09749)

**4-hydroxy-6-methyl-3-(2-methyl-1-oxobutyl)-2H-pyran-2-one (m/z 209.08191)

Table 4 Analysis of variance for compound 1 and compound 2 identified as majority compounds in hydrolates of *P. cablin* grown under different soil coating.

	DF	P-value	
		Compound 1	Compound 2
Repetitions	5	0.50	0.53
Coating types	2	0.34	0.37

after transplanting, and the treatments with live cover and weeding resulted in plants with smaller heights and fewer leaves compared to the other coverings. However, no significant difference in the essential oils yields and their hydrolates, as well as in the intensities of the major ions observed in MS spectrum identified as chemical markers for the quality control of the patchouli oil from *P. cablin* leaves cultivated under four different soil coverings.

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