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Influence of diurnal harvesting times on essential oil yield and chemical composition of lemongrass, palmarosa and tulsi

A Niranjana Kumar[1], Sahithi Audi[1], J Kotesh Kumar[1*], KVNS Srinivas[1*], GD Kiran Babu[1], AC Jnanesha[1], B Venkatesh[1] & Channayya Hiremath[2]

[1]*Phytochemistry Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Boduppal, Hyderabad-500092.*

[2]*CSIR - Central Institute of Medicinal and Aromatic Plants, Research Centre, Bangalore-560065.*

E-Mail: koteshkumarj@cimap.res.in

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Abstract

Essential oil yield and chemical composition of most of the aromatic crops are affected by environmental, agrochemical and genetic factors. One of such factors are Harvesting Time (HT's), optimizing which may result in higher essential oil production with better quality. The present study was carried out to determine the effect of HT's on three different aromatic crops [lemongrass, palmarosa and *Ocimum sanctum* (CIM-Ayu)] at different diurnal times (9.00 A.M, 12.00 P.M and 3.00 P.M) in a day and the same was repeated thrice on alternate days in the same fields. The results indicated that HT at 3.00 PM had higher essential oil content in all the three aromatic crops. But, the influence of HT on the quality of essential oil was specific to the different crops studied in this report. The quality of essential oil obtained from lemongrass harvested at 12.00 PM was found to be better as it showed more percentage of citral whereas the essential oils obtained from palmarosa and *O. sanctum* harvested at 3.00 PM were better as they showed more percentage of geraniol and eugenol respectively. This research not only refines essential oil production strategies but also underscores the crop-specific impact of harvesting times. The meticulous temporal analysis, coupled with statistical validation, contributes novel insights, providing practical benefits for industries reliant on these aromatic crops.

Keywords: CIM-Ayu, plamarosa, lemongrass, hydro-distillation, gas chromatography.

Introduction

Essential oils (EOs) are aromatic plant products widely used in perfumery, cosmetics, toiletry, preservatives, aromatherapy and pharmaceutical industry. The EO industry is growing globally at 9.3% compound annual growth rate and projected to reach USD 16.0 billion by 2026 (Essential Oil Market Report, 2021). The yield and chemical composition of EO is known to fluctuate with season, climate, land, soil and developmental stages. Although, the influence of seasonal variability and Time of Harvest (ToH) during the crop's life cycle were broadly researched but, influence of diurnal variation i.e., harvesting at different day times (9.00 A.M, 12.00 P.M and 3.00 P.M) on the same day on the yield and composition of EO was not studied earlier. lemongrass, palmarosa and *Ocimum sanctum* are grown in large acreage across the country due to the demand for their essential oil in perfumery and cosmetic industry. Hence these three crops were selected for determining the influence of diurnal HT's on the yield and quality of their essential oils.

Crop selection and rationale

Lemongrass (*Cymbopogon flexuosus*)

Cymbopogon flexuosus (Famil: Poaceae) commonly called as East Indian lemongrass is a perennial multi-harvest tropical and temperate aromatic and medicinal grass largely cultivated in India. The global lemongrass oil market size was USD 38.02 million in 2021 and is projected to grow to USD 81.43 million in 2028 at a CAGR of 9.93% annually. Fresh leaves of lemongrass are popularly used in making herbal teas and tender stems are used as culinary in foods made from poultry, fish, beef, soups and seafoods *etc*. The characteristic lemon note of lemongrass oil is due to the presence of citral (cis & trans) as a major component (Kulkarni *et al*. 2020; Benoudjit *et al*, 2022) and also consists of mineral elements (Nimenibo-Uadia and Nwosu. 2020; Okpo and Edeh, 2022). The lemongrass oil finds wide usage in fragrance, flavour, pharmaceutical, aromatherapy and pesticide industry. Besides this, lemongrass oil has shown numerous biological activities such as, anticancer (Kumar *et al*. 2008; Sharma *et al*. 2009), anti-inflammatory, analgesic (Chandrashekar *et al*. 2010), antihyperlipidemic (Kumar *et al*. 2011), antibacterial, antifungal, antiviral (Adinarayana *et al*. 2012; Chao *et al*. 2000), nematicidal (Tiyagi *et al*. 1990), antioxidant (Anand *et al*. 2011), and insecticidal (Moustafa *et al*. 2021) *etc.* All parts of the plant produce aromatic oil and the chemical profiles of oils obtained from leaf (Bhattacharya *et al*. 1997; Padalia *et al*. 2011), inflorescence (Sarma *et al*. 2004; Sarma *et al*. 2011), stems and rhizomes (Rajeswara Rao *et al*. 2015) have been previously reported.

Palmarosa (*Cymbopogon martini*)

Cymbopogon martini (Family: Poaceae), commonly known as palmarosa, is also a perennial multi harvest tropical and temperate aromatic and medicinal crop cultivated in major part of the world. The essential oil obtained from palmarosa finds wider applications in toiletry and cosmetics (Duarte *et al*. 2007; Muller *et al*. 2009). The characteristic note of palmarosa essential oil is due to the presence of geraniol as a major compound (apprx.80-85%) and many minor molecules such as myrcene, *cis*-β-ocimene, *trans*-β-ocimene, linalool, neral, geranial, geranylacetate, caryophyllene, geranyl isobutyrate and farnesol (Sastry *et al*. 2015) *etc.* Geraniol and geranyl acetate are widely used in pharmaceutical, cosmetics and flavoring industries. 76% of deodorants available in the European market are made of geraniol (Rastogi *et al*. 2001). Geraniol is a very effective plant-based insect repellent (Barnard & Xue 2004) and antimicrobial agent (Bard *et al*. 1988).

Holy basil (*Ocimum sanctum*)

Ocimum sanctum (Family: Lamiaceae) commonly known as Holy basil is also a perennial multi harvest aromatic and medicinal crop widely grown and also cultivated in the major part of the world. The variety named CIM-Ayu developed by CSIR-CIMAP was used in the present study. The characteristic note of essential oil obtained from areal parts of CIM-Ayu is due to the presence of eugenol as a major compound (45-65%) (Madhuri *et al*. 2023) and it is found to be anti-microbial (Nakamura *et al*. 1999), insecticidal (Paula *et al*. 2004), anti-oxidant (Ganiyu 2008) and analgesic (Franca *et al*. 2008) in nature.

Materials and methods

Collection and distillation of aerial parts

The present study was undertaken to evaluate the role of HT's during the day on quality and quantity of EO at three different time slots 9.00 A.M, 12.00 P.M and 3.00 P.M for three different aromatic crops (lemongrass, palmarosa and *O. sanctum* (variety CIM-Ayu)) in triplicates collected on alternate days in the month of June. The experiment was laid out at the research farm of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Boduppal, Hyderabad, India. The experimental site was located at an altitude of 542 m above mean sea level with a geographical bearing of 78°8′ longitude and 17°32′ latitude. Semi-arid tropical climate zone of Hyderabad has an average rainfall of 800 mm per year. The soil of the experimental field is a red sandy loam with pH 8.1 (1.25 soils to solution ratio), EC – 1.25 ds/m, organic C – 0.3%, total N – 0.03%, available P – 10 ug/g soil and exchangeable K – 128 ug/g soil.

The aerial parts of lemongrass, palmarosa and CIM-Ayu, 1 kg each, were collected from the CSIR-CIMAP, Research Centre's experimental farm field at 9.00 A.M, 12.00 P.M and 3.00 P.M for three alternative days. The freshly collected herbage was subjected to hydro-distillation using a Clevenger-type apparatus for 3.5 h. The essential oils obtained were collected and dried over anhydrous sodium sulphate and stored at 4 °C until the GC analysis was carried out. Essential oil content and quality of different harvests were analysed after two replications. The samples were also subjected to Gas Chromatography analysis.

Gas chromatography analysis

The relative peak area percentages of the constituents of oil samples were determined on a Varian CP-3800 gas chromatograph–flame ionization detector (GC–FID; Varian Chromatography Systems, Middelburg, Netherlands) equipped with a Varian

Galaxie chromatography data system (version 1.9 SP 1a), an electronic integrator and 100% dimethylpolysiloxane bonded phase Varian CP-Sil 5CB column (length = 50 m, internal diameter = 0.25 mm, film thickness = 0.25 μm). Injector temperature 250°C; FID temperature 300°C and the oil samples (0.2 μL) were injected neatly. The relative retention indexes (RRI) were generated with a standard solution of n-alkanes (C_6–C_{19}) (Sigma- Aldrich, St. Louis, USA) under identical conditions. The relative percentages of individual compounds were computed from GC peak areas by a normalization method without applying correction factors.

Specific analysis parameters

Lemongrass essential oil: The carrier gas nitrogen was introduced at a flow rate of 0.5 mL/minute and split injection with a 20:80:20 split ratio. The column temperature was programmed from 120°C (2 minutes) at to 240°C (10 minutes) at a rate of 8°C/minute.

Palmarosa essential oil: Nitrogen was used as the carrier gas at a constant flow rate of 0.4 mL/min. The column temperature was programmed from 120°C (held for 2 min.) to 240°C (held for 5 min.) at a rate of 8°C/min. Samples were injected with a 20:100:20 split ratio.

CIM-Ayu essential oil: Nitrogen was used as the carrier gas at a constant flow rate 0.5 ml/min. The column temperature was programmed from 120°C (2 min) to 240°C (6 min) at rate of 8°C/min. Samples were injected with a 20:80:20 split ratio.

Statistical analysis

Duncan's Multiple Range Test was executed utilizing IBM SPSS Statistics 23 to analyze and interpret the statistical outcomes. The measurements were carried out with a high confidence level set at P=0.001, and each test was replicated three times (n=3) to ensure robust statistical precision.

Results and discussion

Essential oil content variations

A gradual increase in the essential oil content was observed in samples collected from morning to evening with a significant enhancement noted in samples collected at 3.00 pm. Similar pattern was also observed in case of lemongrass and palmarosa oils except first day (Day I) harvested sample (Table 1).

Table 1. Diurnal variation in essential oil content

Time	Day	Oil yield* (%)		
		CIM-Ayu	Lemongrass	Palmarosa
9.00 AM	Day I	0.125 ±0.035[a]	0.35 ±0.070[a]	0.45 ±0.070[a]
	Day III	0.1 ±0.035[a]	0.305 ±0.007[a]	0.212 ±0.017[a]
	Day V	0.25 ±0.015b	0.575 ±0.035b	0.275 ±0.035b
	P (0.005)	0.026	0.019	0.031
12.00 PM	Day I	0.125 ±0.035[a]	0.55 ±0.001[a]	0.375 ±0.035[a]
	Day III	0.15 ±0.001[a]	0.61 ±0.014[a]	0.35 ±0.070[ab]
	Day V	0.137 ±0.017[a]	0.475 ±0.106[a]	0.35 ±0.005b
	P (0.005)	0.609	0.239	0.829
3.00 PM	Day I	0.2 ±0.001[a]	0.625 ±0.035[a]	0.375 ±0.035a
	Day III	0.25 ±0.001[a]	0.55 ±0.001[a]	0.35 ±0.070[a]
	Day V	0.625 ±0.035a	1.1 ±0.141b	0.775 ±0.035b
	P (0.005)	0.075	0.013	0.006

Numbers of replicates 3 n= 6. DMRT post hoc test was performed

Composition of lemongrass essential oil and influence of harvest time

Generally, the quality of lemongrass essential oil is assessed by quantifying four chemical constituents, and they are citral I, citral II (Total Citral), geraniol and geranyl acetate. From Table-2 it is observed that the major aldehyde total citral was expressed more (88.765%) at mid-day (12 PM) on day-I and least on day-III evening (81.235%). Another minor molecule geraniol was high in day-I morning (1.587%) and least was noticed in day-I evening (0.785%).

Table 2. Diurnal variation in lemongrass essential oil chemical composition

Time	Day	Chemical composition (%)*					Total (%)
		Citral-I	Citral-II	Total citral	Geraniol	Geranyl acetate	
	RI[np]	1215	1248		1234	1360	
9:00 AM	Day I	34.717 ±1.254[a]	46.941 ±1.620[a]	81.658	1.587 ±0.016a	0.611 ±0.007[b]	83.856
	Day III	34.63(±1.094[a]	48.949 ±2.066[a]	83.579	1.217 ±0.170a	0.906 ±0.222[b]	85.702
	Day V	35.644±2.202[a]	50.791 ±1.90)[a]	86.435	1.226 ±0.181a	0.660 ±0.044[b]	88.321
	P (0.005)	0.457	0.114		0.132	0.198	
12.00 PM	Day I	35.964 ±2.565[a]	52.801 ±2.870[a]	88.765	0.931(±0.139[a]	0.357 ±0.245[a]	90.053
	Day III	33.623 ±2.280[a]	50.197 ±2.155[a]	83.821	1.135 ±0.639[a]	0.329 ±0.050[a]	85.284
	Day V	35.969 ±1.674[a]	49.889 ±2.080[a]	85.858	0.877 ±0.121[a]	0.606 ±0.028[a]	87.341
	P (0.005)	0.528	0.423		0.793	0.098	
3.00 PM	Day I	36.103 (±1.468)[a]	50.417 ±2.311[a]	86.521	0.785 ±0.322[a]	0.158 ±0.069[a]	87.463
	Day III	33.550 (±1.231)[a]	47.685 ±1.125[a]	81.235	1.125 ±0.065[a]	0.494 ±0.232[a]	82.854
	Day V	34.203 (±1.876)[a]	48.680 ±1.37)[a]	82.883	0.953 ±0.024[a]	0.358 ±0.077[a]	84.194
	P (0.005)	0.195	0.61		0.337	0.119	

Numbers of replicates -3; n= 6. DMRT post hoc test was performed

The mean value of the three days harvests, as shown in Fig. 1a, the total citral was high in mid-day harvested samples (86.148%) and least in evening harvested samples (83.546%) but a marked difference was noted in their essential oil yields (0.213%; Table 1). Thus it is inferred that 3.00 PM is the ideal harvest time for lemongrass for getting good yields of essential oil. The remaining constituents decreased from morning to evening (Fig. 1b).

The lemongrass essential oil components were correlated with each other (Table 3). The lemon note caused by the constituents citral I and citral had significant positive correlation with each other and significant negative correlation with geraniol and geranyl acetate. This once again confirms the hypothesis that essential oil content and composition varies between varieties, and also varies with time, and changes in weather parameters (Sastry *et al*., 2014).

Table 3. Corrélation among lemongrass essential oil constituents

	Citral I	Citral II	Geraniol
Citral II	0.99595**		
Geraniol	-0.9445**	-0.95232**	
Geranyl acetate	-0.97623**	-0.98119**	0.944204**

* at 5% level of significance and ** at 1% level of significance

Palmarosa essential oil composition and harvest time Influence

Generally, the quality of palmarosa essential oil is assessed by quantifying three chemical constituents, linalool, geraniol and geranyl acetate as total geraniol. Table 1 indicates that the major alcohol geraniol and minor compound linalool were higher in day 1 morning sample (82.465% & 2.007%) whereas, geraniol was least (76.814%) and geranyl acetate was maximum (14.262%) in mid-day of first day sample. Geraniol was more in 9 AM sample (80.093%), geranyl acetate was higher in mid-day sample (13.499%) and linalool was higher in 3 PM sample (1.838%) (Fig. 1d). The variation of geraniol content was very less (0.698%) between 9 AM & 3 PM samples, but oil yields considerably differed (0.18%) (Table 1). Thus it is inferred that that 3.00 PM is the ideal harvest time for obtaining good amount of palmarosa essential oil.

Table 4. Diurnal variation in palmarosa essential oil chemical composition

Time	Day	Chemical composition (%)*			Total (%)
		Linalool	Geraniol	Geranyl acetate	
	RI[np]	1105	1238	1364	
9.00 AM	Day I	2.007 ±0.277[a]	82.465 ±1.195 [a]	9.900 ±0.474 [a]	94.372
	Day III	1.771±0.352 [a]	78.824 ±1.731) [a]	12.477 ±0.554 [a]	93.072
	Day V	1.465 ±0.365 [a]	78.991 ±1.279[a]	12.921 ±0.436 [a]	93.377
	P (0.005)	0.387	0.068	0.044	
12.00 PM	Day I	1.262 ±0.195 [a]	77.616 ±1.829 [a]	14.262 ±0.127 [a]	93.140
	Day III	1.760 ±0.033 [a]	76.814 ±1.796 [a]	11.731 ±0.698 [a]	90.305
	Day V	1.669 ±0.029 [a]	79.833 ±2.416 [a]	11.731 ±0.698 [a]	93.233
	P (0.005)	0.240	0.003	0.7470	
3.00 PM	Day I	2.136 ±0.408 [a]	79.701 ±1.222[a]	11.752 ±0.406 [a]	93.589
	Day III	1.800 ±0.565 [a]	79.039 ±2.905 [a]	12.643 ±0.844 [a]	93.482
	Day V	1.576 ±0.024	78.448 ±1.412 [a]	9.265 ±1.252 [a]	89.289
	P (0.005)	0.970	0.254	0.342	

Numbers of replicates -3; n= 6. DMRT post hoc test was performed

In correlation, the individual chemical constituents of the essential oil noticed at different times of harvest were also correlated among themselves (Table 5). The major constituent geraniol showed significant positive correlation with linalool and geranyl acetate. In earlier studies in palmarosa (Dubey *et al*. 2003), the essential oil analysis indicated a constant decrease in the geranyl acetate percentage with corresponding increase in the geraniol percentage.

Table 5. Correlation among palmarosa essential oil constituents

	Linalool	Geraniol
Geraniol	0.699**	
Geranyl acetate	0.419*	0.778**

* at 5% level of significance and ** at 1% level of significance

Essential oil composition of Cim-Ayu and the influence of harvest time

In the assessment of CIM-Ayu essential oil, eight chemical constituents were detected, *viz.* limonene (0.082-0.652%), linalool (0.104-0.462%), camphor (0.013-0.082%), methyl chavicol (0.073-0.127%), eugenol (42.898-65.823%), methyl eugenol (0.135-15.065%), β-elemene (8.216-15.065%), and caryophyllene (17.523-25.235%), collectively accounting for a range of 88.367% to 93.429% (Table 6). The major chemical constituent, eugenol, exhibited a higher concentration in the day-III harvested sample (65.823%) at 3:00 PM, while another significant compound, caryophyllene, was more abundant in the day-I harvested sample (25.235%) at 9:00 AM. Fig. 1e illustrates that eugenol, the primary chemical constituent, consistently showed higher levels in samples harvested at 3:00 PM [average value: 62.258% (±3.157)]. Conversely, the other two major compounds, β-elemene and caryophyllene, displayed a declining trend from 9:00 AM to 3:00 PM (Fig. 1f). A similar pattern was observed for the minor constituent methyl eugenol. Limonene, linalool, camphor, and methyl chavicol exhibited irregular patterns, influenced by microclimatic variations. Based on these findings, it can be deduced that the optimal quality of CIM-Ayu essential oil is obtained from plant material harvested at 3:00 PM.

Table 6. Diurnal variation in CIM-Ayu essential oil chemical composition.

Time	Day	Chemical composition (%)*								Total (%)
		Limonene	Linalool	Camphor	Methyl chavicol	Eugenol	Methyl eugenol	β-Elemene	Caryophyllene	
RI[np]		1030	1105	1130	1186	1340	1376	1396	1422	
9.00 AM	Day I	0.082±0.009[a]	0.104±0.004 [a]	0.013±0.003[a]	0.087±0.014 [a]	42.898±1.938 [a]	11.673±1.197 [a]	11.725 ±1.460 [b]	25.235 ± 0.90 [a]	91.817
	Day I	0.355± 0.034[a]	0.197 ± 0.082 [a]	0.082± 0.082[ab]	0.084± 0.008 [a]	52.701±2.375 [a]	0.994±1.079 [a]	15.065±0.214[ab]	17.523±1.90 [a]	87.001
	Day I	0.431±0.081 [a]	0.306±0.004 [a]	0.047±0.003[b]	0.09 ±0.005[a]	51.701±2.985 [a]	0.190±0.023 [a]	13.574±2.235[a]	22.246±2.01 [a]	88.585
	P(0.005)	0.330	0.0564	0.453	0.845	0.107	0.460	0.543	0.038	
12.00 PM	Day III	0.164±0.108 [a]	0.119±0.111 [a]	0.043±0.041 [a]	0.083±0.029 [a]	53.825±2.627 [a]	6.521±1.842 [a]	8.825 ±0.902 [a]	22.022 ± 1.911 [a]	91.602
	Day III	0.565±0.048 [b]	0.462±0.070 [b]	0.048±0.009 [a]	0.127±0.007 [a]	56.494±2.481 [a]	0.186±0.024 [a]	9.744±0.644 [a]	22.974±2.207 [a]	90.600
	Day III	0.42±0.021 [b]	0.170±0.028[a]	0.054±0.003[a]	0.073±0.009[a]	50.685±1.805 [a]	0.135±0.041 [a]	11.349±0.553 [a]	25.481±2.015 [a]	88.367
	P(0.005)	0.023	0.039	0.908	0.115	0.284	0.432	0.575	0.812	
3.00 PM	Day V	0.36 ± 0.008 [a]	0.33± .008 [ab]	0.072 ± 0.004 [b]	0.092 ±0.001 [a]	59.813±1.376 [a]	0.686±0.819 [a]	9.713 ±0 .480 [a]	18.760 ± 0.265 [a]	89.826
	Day V	0.347±0.055[a]	0.261±0.012[a]	0.035±0.007 [a]	0.077±0.001 [b]	65.823±1.673 [a]	0.225±0.065 [a]	8.216±0.528 [a]	18.445±0.1.346 [a]	93.429
	Day V	0.652±0.171 [a]	0.394±0.058[b]	0.025±0.002 [a]	0.097±0.004 [b]	61.140±2.761 [a]	0.177±0.068 [a]	11.369±1.595 [a]	18.483±1.839[a]	92.337
	P(0.005)	0.100	0.071	0.001	0.012	0.213	0.564	0.114	0.967	

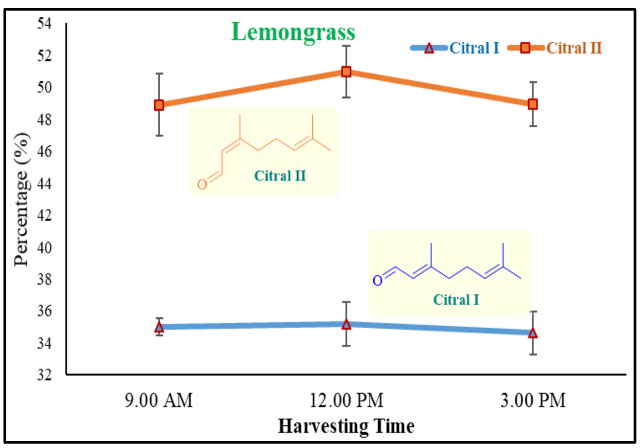
Numbers of replicates -3, n= 6. RI[np]: DMRT post hoc test was performed.

The essential oil components were analyzed for correlation with various harvesting times, as outlined in Table 7. The predominant constituent, eugenol, exhibited a significant positive correlation with the other two major components, β-elemene and caryophyllene. Conversely, there was a negative correlation observed with the remaining constituents. Additionally, the minor constituent linalool showed a significant positive correlation with methyl chavicol and camphor. Furthermore, caryophyllene displayed a positive correlation with methyl eugenol, β-elemene, and eugenol.

Table 7. Corrélation among Cim-Ayu essential oil constituents

	Limonene	Linalool	Camphor	Methyl chavicol	Eugenol	Methyl eugenol	β-Elemene
Linalool	0.862405**						
Camphor	0.766442**	0.978518**					
Methyl chavicol	0.768336**	0.980151**	0.999682**				
Eugenol	-0.5816*	-0.86257**	-0.92664**	-0.9274**			
Methyl eugenol	-0.28506	0.151509	0.280119	0.289216	-0.50676*		
β-Elemene	-0.34947	-0.53299*	-0.51644*	-0.52337*	0.3218	-0.19893	
Caryophyllene	-0.74379**	-0.84738**	-0.82977**	-0.82493**	0.613007**	0.012607	0.412667

* at 5% level of significance and ** at 5% level of significance



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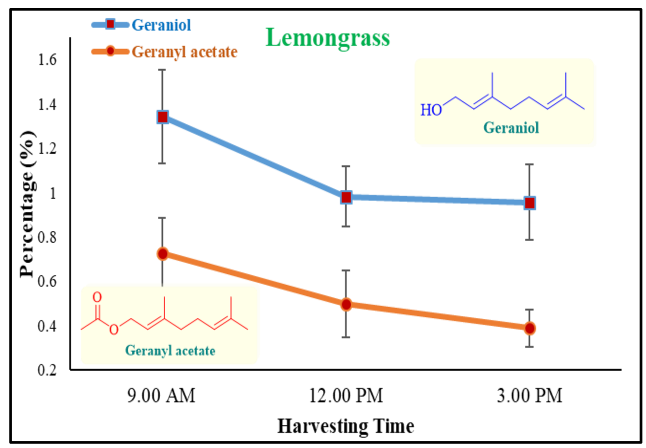

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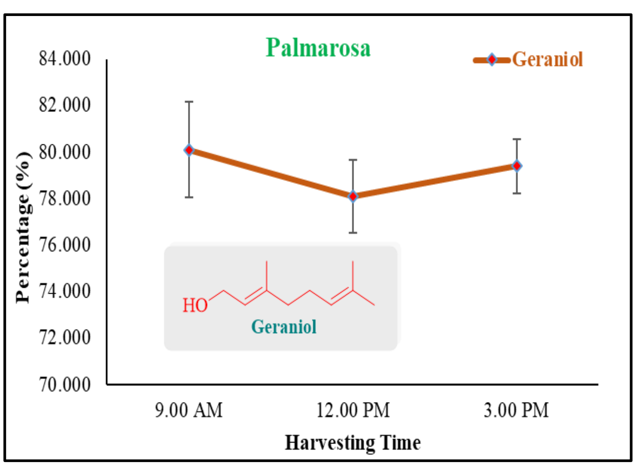

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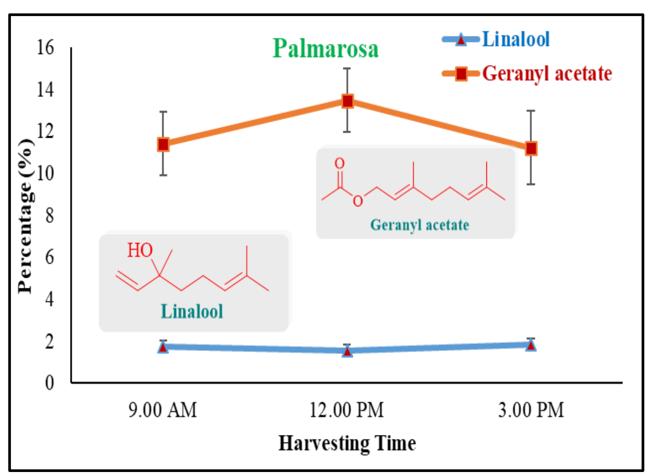

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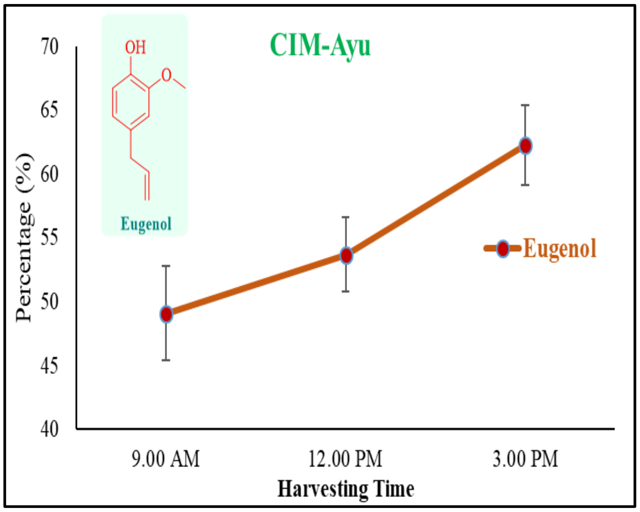

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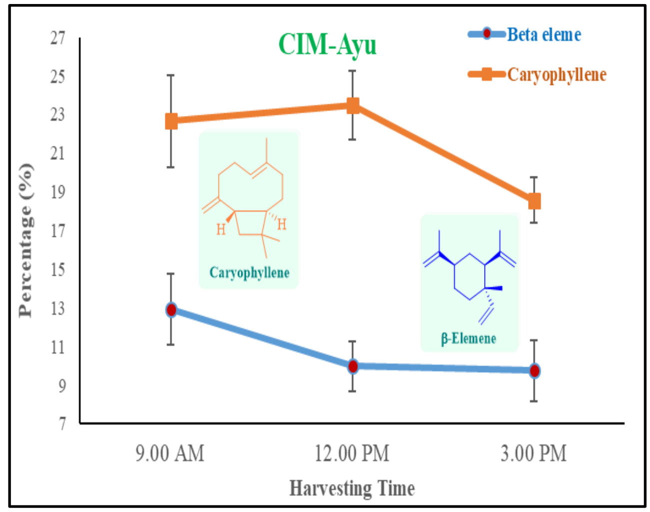

1f

Fig. 1. Variations of major chemical constituents (average values). [1a: *Lemongrass*-Citral I&II; 1b: *Lemongrass*-Geraniol & Geranyl acetate; 1c: *Palmarosa*-Geraniol; 1d: *Palmarosa*-Linalool & Geranyl acetate; 1e: *CIM-Ayu*-Eugenol; 1f: *CIM-Ayu*-β Elemene & Caryophyllene]

Conclusion

In conclusion, the quality and quantity parameters of CIM-Ayu essential oil harvested at 3.00 PM was optimum as the percentage of eugenol was found to be high. In palmarosa, although the geraniol was marginally high in the morning harvested crop compared to the evening harvested one, from the oil yield point of view harvesting at 3.00 PM is found to be better. In the case of lemongrass, mid-day harvest was found to be ideal for obtaining high citral in essential oil but from yield point of view harvesting at 3.00 PM is ideal.

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