

Impact of water pH on essential oil yield and quality in aromatic crops

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This study aimed to investigate the effects of using water with varying pH levels (pH 4, 7, and 9) instead of normal water during hydrodistillation. Four aromatic plants, Palmarosa, *Eucalyptus citriodora*, Lemongrass, and Citronella, were freshly distilled using a Clevenger apparatus with water at different pH levels. The study revealed that essential oil yields were significantly influenced by the distillation process. Soaking the plant material in water with varying pH levels overnight prior to hydrodistillation resulted in higher yields compared to distillation without soaking. In Palmarosa, geraniol was most abundant in the pH 7 sample (83.426%), while the yield increased by 0.2% when using pH 9 (soaked) water. It was concluded that pH 9 (soaked) water maximised essential oil yield, whereas pH 7 water was ideal for obtaining higher geraniol content. For *E. citriodora*, a notable yield increase (0.88%) was observed between pH 7 and pH 7 (soaked) samples, with the primary aldehyde Citronellal being most abundant in the pH 9 sample (84.432%). Similarly, Citronella showed higher essential oil yields (2.5%) when transitioning from pH 7 (soaked) to pH 9 (soaked), though Citronellal content was highest in the pH 7 sample (39.054%). In Lemongrass, the total citral content (86.702%) and essential oil yield (0.4%) were highest in the pH 7 (soaked) sample. These findings suggest that hydrodistillation with pH-adjusted water can optimise essential oil yields and quality, depending on the desired chemical constituents.

Keywords: Citronella, Essential oil, *Eucalyptus citrodora*, Lemongrass, Palmarosa, pH water

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Introduction

Aromatic plants contain odorous volatile substances, which occur as essential oils, green exudates, balsam and oleoresin in one or more plant parts, namely, roots, wood, stems, foliage, flowers and fruits. Essential oils are highly concentrated secondary metabolites with diverse functions in plant systems. They constitute hundreds of organic compounds, including terpenoids, benzenoids, organic sulfur and nitrogenous compounds, which work at different levels. Aromatic herbs, oils and aroma chemicals are extensively used in the perfumery, flavouring, cosmetic and drug industries. Moreover, they have now become indispensable ingredients for every human activity. Almost 300 plant species are predominantly traded for their essential oils, with over 3000 recognised as essential oil sources¹. The demand for natural essential oils is on the rise despite their relatively low yields, usually less than 8% of the plant's dry biomass². The most commonly employed

methods for extracting essential oils from aromatic plants include hydrodistillation, steam distillation, and organic solvent extraction^{3,4}.

Hydrodistillation with cohobation stands as a widely employed technique for extracting volatile oils from aromatic crops^{5,6}. However, the hydrodistillation processes significantly impact the ultimate yield and quality of volatile oil derived from aromatic crops. The quality of the water, including factors like purity, salinity, turbidity, heavy metals, and electrical conductivity, directly influences both the productivity and quality of volatile oil by affecting the boiling temperature and steam pressure in the distillation retorts.

The primary objective of this study was to investigate the effects of using water with varying pH levels (pH: 4, 7, and 9), along with overnight soaking (So), in place of distilled tap water or tap water, during the hydrodistillation process of four commercially significant aromatic crops: Palmarosa, *Eucalyptus citrodora*, Lemongrass, and Citronella. The changes in the essential yield and major marker compound in each crop were observed. Citral is the

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major marker compound found in the Lemongrass (*Cymbopogon flexuosus* (Steud.) Wats.) essential oil. There are different cultivar chemotypes popularly cultivated in India that are rich in citral, including Krishna, Nima, CIM-Shikhar, etc. The chemotypes popularly cultivated in India that are rich in geraniol include GRL-1 and CIM Atal⁷. Geraniol is the major marker compound found in Palmarosa (*Cymbopogon martini* (Roxb.) W. Watson) essential oil. There are different cultivar chemotypes popularly cultivated in India, including PRC-1 and Udaipur chemotypes. The PRC-1 showed geraniol as the major compound where whereas the Udaipur variety showed limonene epoxide (22.1%), trans carvyl formate and limone-10-ol as the major marker compound⁸.

Materials and Methods

Experimental site

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 is located at an altitude of 542 m above mean sea level with a geographical bearing of 78°8' longitude and 17°32' latitude. The 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.

Essential oil extraction

The aerial parts of Lemongrass, Palmarosa, and Citronella, as well as leaf parts of *E. citrodora*, were collected from the field in August 2021. The freshly collected herbage was subjected to hydrodistillation with a Clevenger-type apparatus for 3.0 h using different pH water (pH: 4, 7 and 9), and another experiment was carried out overnight, soaking the plant material in the different pH waters. The obtained

essential oils were collected and dried over anhydrous sodium sulphate and stored at 4°C until the GC analysis was carried out.

Gas chromatography analysis

Gas Chromatographic analysis of the essential oil sample was done on a Varian CP-3800 gas chromatograph with flame ionisation detector and split/splitless capillary injectors with Varian Galaxie chromatography data system (version 1.9 SP 1a) and 100% dimethylpolysiloxane bonded phase Varian CP-Sil 5CB column (50 m length, 0.25 mm internal diameter, 0.25 µm film thickness) was used to determine the relative peak area percentages of the constituents in the oil samples. The GC operating conditions included an injector temperature of 250°C and an FID temperature of 300°C. Neat injections of the oil samples (0.2 µL) were made. Relative retention indexes (RRIs) were generated using a standard solution of n-alkanes (C₆–C₁₉) under identical conditions. The relative percentages of individual compounds were calculated from GC peak areas through normalization. The Gas Chromatography analysis methodology (flow rate and temperature programme) for Lemongrass, Palmarosa, *E. citrodora*, and Citronella essential oils was followed by our previously reported methods⁹⁻¹².

Results and Discussion

Influence of water pH on the essential oil yields

The plant material was hydrodistilled using a Clevenger apparatus for 3 h in two experiments: one using water with varying pH levels (pH 4, 7, and 9) and another in which the plant material was soaked overnight in water with different pH values. Essential oil yields (v/w) obtained from these experiments are shown in Table 1.

In our present investigation, higher yields of essential oil were observed in the pH 9 (0.55±0.056%) overnight soaking (So) sample, with the lowest yield recorded for the pH 4 (0.3±0.020%)

Table 1 — Essential oil yields of aromatic crops

	Essential oil yields (%)*			
	Palmarosa	Lemongrass	<i>E. citrodora</i>	Citronella
pH-9	0.45±0.020	0.50±0.020	1.95±0.128	1.35±0.094
pH-9 (So)	0.55±0.056	0.65±0.020	1.20±0.032	2.15±0.112
pH-7	0.37±0.020	0.40±0.020	1.62±0.040	1.35±0.026
pH-7 (So)	0.35±0.036	0.65±0.088	2.50±0.204	2.15±0.096
pH-4	0.30±0.020	0.50±0.075	1.05±0.060	1.28±0.036
pH-4 (So)	0.40±0.020	0.70±0.080	1.25±0.020	2.10±0.055

*The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates.

sample of Palmarosa. The essential oil yield notably increased by 0.2% from pH 7 to pH 9 (So), indicating that hydrodistillation using pH9 (So) water enhanced essential oil yield. However, in *E. citriodora*, the highest aroma oil yield was observed for the pH 7 overnight soaking material ($2.5\pm 0.204\%$), while the lowest percentage was noted for the pH 4 sample ($1.05\pm 0.060\%$). The essential oil yield significantly increased by 0.88% from pH 7 (fresh) to pH 7 (So), suggesting that pH7 overnight soaking was optimal for maximising essential oil percentage. Similarly, in Citronella and Lemongrass, higher essential oil yields were obtained in the soaking samples compared to direct distillation samples for all pH values. Citronella yields were highest in the pH9 (So) and pH7 ($2.15\pm 0.112\%$) samples, while Lemongrass pH4 (So) samples resulted in a greater essential oil percentage. Table 1 clearly demonstrates that, with the exception of *E. citriodora*, all soaking samples contained higher amounts of essential oil compared to the other samples from the four aromatic crops. Thus, hydrodistillation of Palmarosa using pH-9 (So) water is optimal for maximising essential oil yield. Utilising *E. citriodora* and Citronella plants rich in the

aldehyde Citronellal at pH 7 (So) is also ideal for enhancing essential oil yield. However, Lemongrass at pH 4 (So) exhibits a notably high essential oil content.

Effect on essential oil composition

The oil samples, after drying over anhydrous Na_2SO_4 , were stored at $4-5^\circ\text{C}$ before being subjected to GC analysis. The oils obtained were analysed by GC using a CP-Sil 5CB column.

While analysis of essential oils by GC, in Palmarosa essential oil, the major alcohol geraniol exhibited higher abundance at pH 7 ($83.426\pm 1.482\%$) and lower abundance at pH 4 ($77.385\pm 1.622\%$) (Fig. 1a,b and Table 2). Conversely, geranyl acetate was more abundant in the pH-7 (So) sample ($12.636\pm 0.329\%$), while the minor compound linalool was more prevalent in the pH 4 sample ($6.651\pm 0.367\%$). Considering the quality of Palmarosa oil, a pH of 7 ($83.426\pm 1.482\%$) was deemed ideal due to the superior quality of the essential oil.

In Lemongrass, the quality is determined by the percentage of total citral content. As illustrated in Fig. 2a,b, the predominant chemical constituent in the

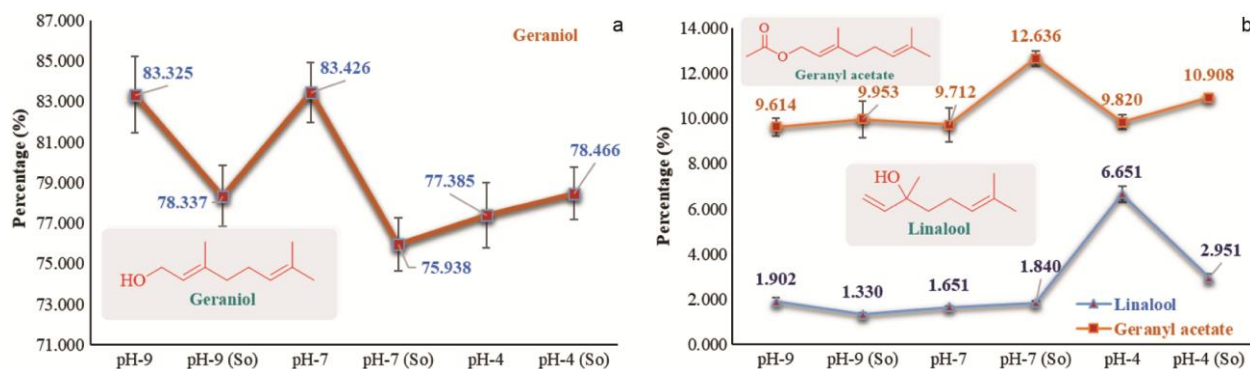


Fig. 1 — Variations in the chemical compositions of Palmarosa essential oils a) Geraniol, and b) Linalool and Geranyl acetate. *The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates.

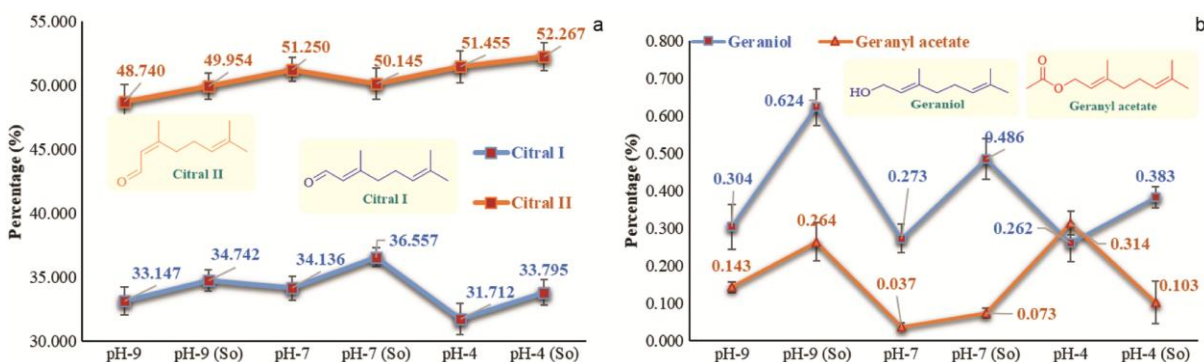


Fig. 2 — Variations in the chemical compositions of Lemongrass essential oils a) Citral I & II, and b) Geraniol & Geranyl acetate. *The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates.

total citral of Lemongrass was higher at pH 7 (86.702%), with the lowest percentage observed in the pH 9 sample (81.887%) (Table 3). However, the minor compounds geraniol and geranyl acetate exhibited greater abundance in the pH-9 (So) sample (0.624±0.049%) and pH-4 (0.314±0.032%) sample, respectively (Fig. 2b). A notable increase in essential oil yield from pH 7 to pH 4 (0.05%) was observed. The total citral content did not significantly vary from pH 7 to pH 4 (So). It was concluded that for high-quality essential oil, hydrodistillation of Lemongrass at pH 7 (So) was optimal for obtaining a favourable percentage of citral in the Lemongrass essential oil.

Whereas, in *E. citriodora*, the primary aldehyde Citronellal, exhibited higher abundance in the pH 9 sample (84.432±1.865%) (Fig. 3a,b). Additionally, another major chemical, citronellol, was more

prevalent in the pH-9 overnight soaking material (10.718±0.665%) (Table 4); considering the quality parameters, pH 9 offers high-quality oil. However, for farmers concerned with essential oil yields for income generation, the pH-7 overnight soaking technique is recommended.

Similarly, in Citronella, the predominant component, Citronellal, was most abundant in the pH 7 sample (39.054±1.766%) (Fig. 4a), while its lowest abundance was observed in the pH 4 sample (29.767±1.335%). Conversely, the alcohol, geraniol, was most abundant in the pH 4 sample (26.563±0.710%). However, citronellol exhibited higher abundance at pH 4 (So) (11.474±0.894%) (Table 5 and Fig. 4b). The increase in essential oil yield from pH 7 to pH 7 (So) exhibited a notable rise of 0.8%. Additionally, the Citronellal content

Table 2 — Chemical composition of Palmarosa essential oil

	Linalool	Geraniol	Geranyl acetate
RI ^{np}	1105	1238	1364
pH-9	1.902(±0.172)	83.325(±1.873)	9.614(±0.400)
pH-9 (So)	1.330(±0.063)	78.337(±1.501)	9.953(±0.816)
pH-7	1.651(±0.053)	83.426(±1.482)	9.712(±0.754)
pH-7 (So)	1.840(±0.075)	75.938(±1.306)	12.636(±0.329)
pH-4	6.651(±0.367)	77.385(±1.622)	9.820(±0.317)
pH-4 (So)	2.951(±0.181)	78.466(±1.277)	10.908(±0.094)

*The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates. RI^{np}: retention index; nonpolar column

Table 3 — Chemical composition of Lemongrass essential oil

	Citral I	Citral II	Total Citral	Geraniol	Geranyl acetate
RI ^{np}	1215	1248	---	1234	1360
pH-9	33.147(±1.072)	48.740(±1.343)	81.886	0.304(±0.060)	0.143(±0.013)
pH-9 (So)	34.742(±0.846)	49.954(±1.035)	84.696	0.624(±0.049)	0.264(±0.051)
pH-7	34.136(±0.945)	51.250(±0.902)	85.386	0.273(±0.037)	0.037(±0.011)
pH-7 (So)	36.557(±0.731)	50.145(±1.227)	86.702	0.486(±0.055)	0.073(±0.013)
pH-4	31.712(±1.209)	51.455(±1.249)	83.167	0.262(±0.049)	0.314(±0.032)
pH-4 (So)	33.795(±0.996)	52.267(±1.093)	86.062	0.383(±0.028)	0.103(±0.056)

*The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates. RI^{np}: retention index; nonpolar column

Table 4 — Chemical composition of *E. citriodora* essential oil

	Citronellal	Isopulegol	Citronellol
RI ^{np}	1136	1147	1211
pH-9	84.432(±1.865)	2.895(±0.089)	5.990(±0.766)
pH-9 (So)	75.037(±1.156)	3.508(±0.022)	10.718(±0.665)
pH-7	81.460(±2.168)	3.607(±0.075)	6.295(±0.197)
pH-7 (So)	74.754(±1.326)	4.243(±0.298)	9.885(±0.231)
pH-4	58.386(±1.923)	12.426(±0.324)	10.304(±0.176)
pH-4 (So)	64.340(±1.438)	9.467(±0.367)	9.784(±0.228)

*The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates. RI^{np}: retention index; nonpolar column

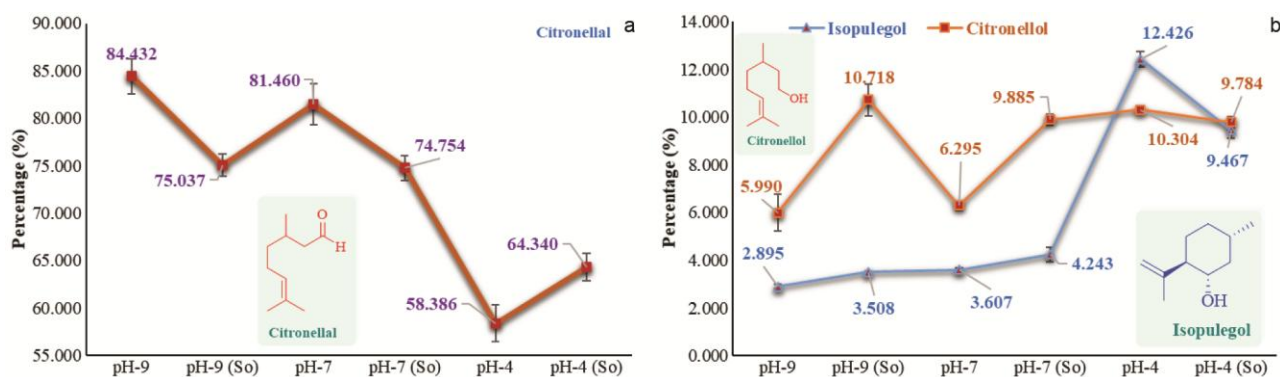


Fig. 3 — Variations in the chemical compositions of *E. citrodora* essential oils a) Citronellal, and b) Isopulegol & Citronellol.

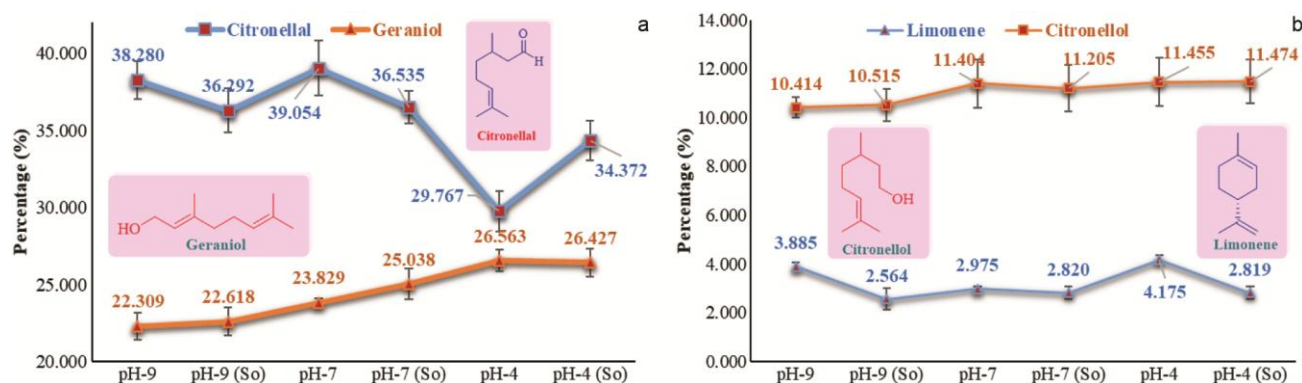


Fig. 4 — Variations in the chemical compositions of Citronella essential oils a) Citronellal & Geraniol, and b) Citronellol & Limonene.

Table 5 — Chemical composition of Citronella essential oil

Chemical composition (%)*

	Limonene	Linalool	Citronellal	Isopulegol	Citronellol	Citral I	Geraniol	Citral II	Citronellyl Acetate	Geranyl Acetate
RI ^{np}	1030	1105	1136	1147	1211	1222	1239	1239	1335	1364
pH-9	3.885	0.481	38.280	0.639	10.414	0.448	22.309	0.625	0.738	0.752
	(±0.196)	(±0.026)	(±1.210)	(±0.085)	(±0.432)	(±0.043)	(±0.892)	(±0.135)	(±0.027)	(±0.042)
pH-9 (So)	2.564	0.487	36.292	0.595	10.515	0.449	22.618	0.621	0.894	0.838
	(±0.428)	(±0.025)	(±1.429)	(±0.019)	(±0.649)	(±0.021)	(±0.909)	(±0.029)	(±0.020)	(±0.032)
pH-7	2.975	0.574	39.054	0.746	11.404	0.509	23.829	0.705	0.845	0.759
	(±0.115)	(±0.043)	(±1.766)	(±0.0159)	(±0.986)	(±0.019)	(±0.263)	(±0.038)	(±0.055)	(±0.067)
pH-7 (So)	2.820	0.558	36.535	0.705	11.205	0.497	25.038	0.699	0.929	0.874
	(±0.257)	(±0.031)	(±1.059)	(±0.075)	(±0.967)	(±0.012)	(±0.994)	(±0.011)	(±0.064)	(±0.025)
pH-4	4.175	1.548	29.767	1.254	11.455	0.569	26.563	0.828	0.977	1.031
	(±0.191)	(±0.064)	(±1.335)	(±0.043)	(±0.994)	(±0.061)	(±0.710)	(±0.033)	(±0.092)	(±0.147)
pH-4 (So)	2.819	0.818	34.372	0.868	11.474	0.522	26.427	0.752	0.821	0.836
	(±0.261)	(±0.018)	(±1.276)	(±0.052)	(±0.894)	(±0.032)	(±0.904)	(±0.070)	(±0.112)	(±0.082)

*The data shown in parentheses are the standard deviations from the corresponding mean values of three replicates. RI^{np}: retention index; nonpolar column

showed significant variation from pH 7 to pH 7 (So). Consequently, it was determined that for high-quality essential oil, hydrodistillation of Citronella at pH 7 was optimal for obtaining a favourable percentage of Citronellal in the Citronella essential oil.

Inter-relationships between essential oil constituents

Citronella

The inter-relationships among essential oil constituents in Citronella reveal significant biosynthetic and environmental influences (Table 6). Citronellal exhibits strong positive correlations with Citronellol

Table 6 — Correlation between essential oil constituents of Citronella distilled using water at different pH levels

	Limonene	Linalool	Citronellal	Isopulegol	Citronellol	Citral I	Geraniol	Citral II	C. acetate
Linalool	-0.40871								
Citronellal	0.72464	-0.89934							
Isopulegol	-0.73432	0.880865	-0.99329						
Citronellol	0.796636	-0.70187	0.933076	-0.94336					
Citral I	-0.80701	0.794213	-0.97427	0.986987	-0.97824				
Geraniol	0.808159	-0.64294	0.895797	-0.91758	0.992192	-0.96417			
Citral II	-0.80513	0.797714	-0.97552	0.987912	-0.9773	0.99998	-0.9626		
C. acetate	-0.81116	0.792483	-0.97451	0.986051	-0.97938	0.999779	-0.96528	0.99972	
G. acetate	-0.80489	0.797781	-0.97656	0.987482	-0.97931	0.99983	-0.96392	0.999833	0.999894

Table 7 — Correlation between essential oil constituents of Palmarosa (7a), Lemongrass (7b) and *E. citriodora* (7c) distilled using waters of different pH Levels

	Linalool	Geraniol	Citral I	Citral II	Geraniol
Geraniol	0.301298		Citral II	0.989471	
Geranyl acetate	0.252605	0.906683	Geraniol	-0.97427	-0.99158
			Geranyl acetate	-0.99513	-0.99589
					0.990501
	7a			7b	
		Isopulegol	Citronellal	Isopulegol	
		Citronellol	0.090095		
			0.559339	0.599917	
			7c		

($r = 0.933076$) and Geraniol ($r = 0.895797$), indicating selective biosynthetic links, while it shows no significant association with other components. Citronellol is positively correlated with Geraniol ($r = 0.992192$) but negatively correlated with Citral I ($r = -0.97824$), Citral II, and C. acetate ($r = -0.97938$). Similarly, Geraniol demonstrates a positive relationship with Citronellal and Citronellol but is negatively associated with citral, its isomers, and acetates (r values ranging from -0.96392 to -0.96528). Interestingly, citral and acetates show strong positive inter-correlations ($r > 0.99$), implying a shared biosynthetic pathway or environmental regulation, but they generally exhibit negative correlations with Citronellal, Citronellol, and Geraniol.

These patterns underscore the variability in essential oil composition between Citronella varieties and under differing environmental conditions. For instance, while Geraniol correlates positively with Citronellol¹³ in some studies, other varieties report a negative correlation¹⁴. Such variability highlights the influence of genetic, seasonal, and climatic factors on enzymatic pathways, which in turn dictate oil composition.

Palmarosa

The essential oil constituents of Palmarosa, obtained through hydrodistillation using waters of varying pH levels, were found to exhibit inter-correlations (Table 7a). Geraniol showed a significant positive correlation with geranyl acetate and linalool,

indicating a close biosynthetic relationship. Previous studies¹⁵ have revealed that as the percentage of geranyl acetate decreases, the proportion of geraniol increases, highlighting a dynamic shift between these constituents during oil production.

Lemongrass

The essential oil components of Lemongrass distilled with waters of different pH levels also displayed notable correlations (Table 7b). The primary constituent, Citral II, was significantly positively correlated with Citral I, but showed a significant negative correlation with geraniol and geranyl acetate. Additionally, Geraniol exhibited a strong positive correlation with its esterified form, geranyl acetate, underscoring their interdependent biosynthesis.

E. citriodora

In *Eucalyptus citriodora*, the essential oil constituents demonstrated specific correlations among themselves (Table 7c). The major aldehyde, Citronellal, was positively correlated with its alcohol derivative, Citronellol. Similarly, the minor component isopulegol exhibited a positive correlation with Citronellol, reflecting a consistent relationship between these compounds.

Conclusion

In Conclusion, considering essential oil yields in four aromatic crops, overnight soaking with pH-7

water was ideal for getting a good percentage of essential oil yields in *E. citrodora* and Citronella crops. Whereas, in Palmarosa, overnight soaking with pH-9 water and for Lemongrass, overnight soaking with pH-4 water is ideal. However, in terms of quality parameters, overnight soaking with pH-7 water was ideal for getting good quality essential oil in Palmarosa and Citronella crops. Similarly, in *E. citrodora*, soaked with pH-9 water was ideal, and in Lemongrass overnight, soaked with pH-4 water, was ideal for getting good quality oil. Chemical compositions in all four aromatic crops significant variations are not recorded. The essential yields played a major role in farmer economics. Considering the farmer's economic point, overnight soaking with pH-7 water was recommended for *E. citrodora* and Citronella crops. For palmarosa, overnight soaking with pH-9 water was recommended, and for lemongrass, overnight soaking with pH-4 water was recommended.

Conflict of interest

The authors of the present work declare that there are no conflicts of interest associated with this article.

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