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Effect of season on essential oil content and composition of Palmarosa [*Cymbopogon martini* (Roxb.) Will. Watson] cultivated in the mid-hill conditions of the Western Himalayas

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Palmarosa [*Cymbopogon martini* (Roxb.) Will. Watson] is an aromatic grass that is grown for the extraction of its valuable essential oil because it has extensive applications in various industries. A study was conducted in the Western Himalayan region from 2022 to 2023 to investigate the effects of different harvest seasons and dates on the quantity and quality of essential oil. The research spanned three phenological seasons: Summer, Autumn, and Winter. This study aims to address the existing gap in the literature regarding essential oil profiles under these environmental conditions. The highest essential oil yield was observed during summer (June 4). GC/GC-MS analysis identified seven compounds in the essential oil during chemical profiling. Amongst them, the major chemical compound was geraniol, which attained the highest percentage (93.48%) on January 21 during the winter season; this can be attributed to the maturity, quality stages of the plant, and polar nature of oxygenated compounds, which resulted in higher solubility in condensate water. In contrast, the rainy season led to a dilution of essential oil concentrations, adversely affecting key compounds like geraniol while also increasing the risk of fungal diseases. This study revealed that palmarosa can be harvested in the mature stage to increase the quality and quantity of essential oil in the Western Himalayas.

Keywords: Chemical profiling, *Cymbopogon martini* (Palmarosa), Geraniol, Harvest timing, Plant growth stages, Seasons

IPC code; Int. cl. (2021.01)− A01, A01D

Introduction

Palmarosa [*Cymbopogon martini* (Roxb.) Will. Watson] of the Poaceae family is an aromatic plant with a chromosomal count of (2n=2x=20) and holds industrial significance due to its multi-harvest nature. Its essential oil, obtained via steam distillation of flowering shoot biomass, exhibits a fragrance reminiscent of roses in both its blossoms and leaves. It demonstrates wide adaptability to various soil and climate conditions, with extensive occurrence in tropical and subtropical zones[1-3]. Research has indicated that palmarosa thrives best in well-drained sandy loam soils, which facilitate optimal root development and nutrient uptake, thereby enhancing essential oil yield[4].

According to Chauhan *et al.*, Indian palmarosa oil is most prominently imported by France, followed by the USA, UK, Germany, Spain, Switzerland, Sri Lanka, and the Philippines[5]. This could promote palmarosa cultivation on underutilised lands, like wastelands, without disrupting cereal and food crop production. The potential for palmarosa to improve the economic status of farmers in arid regions has been emphasised, indicating its role as a cash crop that requires minimal inputs[6]. Palmarosa is cultivated at an altitude of 300-1500 m amsl. It matures slowly, requiring three months to flower before harvest. Suitable for both rain-fed and irrigated conditions, it responds well to farmyard manure and fertilizers[7]. Studies have shown that the application of organic amendments significantly improves both the yield and quality of crops, indicating the importance of sustainable agricultural practices in its cultivation[8,9].

The essential oil from palmarosa has antifungal, antibacterial, anthelmintic, mosquito-repellent, antiviral, and cytotoxic properties. In aromatherapy, it promotes muscle and nerve relaxation, alleviating depression and anxiety[10-12]. Moreover, the essential oil's diverse biological activities make it a therapeutic

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agent for various applications, as evidenced by Gupta *et al.* who reviewed its efficacy against several pathogens. Essential oil has applications in the food, beverage, soap, perfume, and cosmetics industries[13].

The 'Motia' variety contains higher geraniol (85-92%) compared to the 'Sofia' variety (ginger grass), which holds about 60-70% geraniol[14]. Recent studies have shown that essential oil composition varies significantly between cultivars, which can be attributed to genetic factors and environmental conditions[15]. Palmarosa essential oil concentration is highest in the flowering tops with primary compounds geraniol, geranyl acetate, and geranial. Inflorescence yields the highest essential oil content (2.00%), followed by leaf lamina (1.40%), whole herb (0.75%), and leaf sheath (0.33%)[16].

Essential oil content in plants is stage-dependent, and essential oil composition alters with growth stages; immature Palmarosa inflorescences contain more geranyl acetate, whereas the seed oil is rich in geraniol, suggesting geraniol might derive from the breakdown of geranyl acetate during its development[17]. Additionally, environmental stressors, such as drought or nutrient deficiency, can significantly affect the composition of essential oil by affecting secondary metabolites, leading to variations in its bioactive properties[18].

Furthermore, recent studies highlight that environmental stressors, such as drought or nutrient deficiency, can significantly affect the oil composition, leading to variations in its bioactive properties[19]. In addition, the timing of harvesting plays a pivotal role in maximising oil yield, with certain phenological stages yielding higher concentrations of specific compounds[20].

Despite established factors that influence essential oil yields—such as genetics, phenological stages, altitude, soil type, and climate[21], there remains a significant lack of literature on the harvesting of Himalayan Palmarosa essential oils across different seasons and times. This gap in knowledge could hinder efforts to optimise production and establish best practices for sustainable cultivation.

This study aims to fill this gap by identifying the optimal seasons and timings for harvesting high-quality essential oils from Palmarosa. Furthermore, it will examine the variability in essential oil content and composition, providing valuable insights that can help regional growers enhance their cultivation practices.

Materials and Methods

Experimental site

For this study, specimens were procured from the CSIR-IHBT Chandpur farm accession IHBT/CM-1 in the Western Himalayan region, with taxonomic identification confirmed through morphological analysis and herbarium comparisons. A field trial was carried out in 2022-23 at Chandpur farm, CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India. This site is positioned at an elevation of 1325 m amsl, with coordinates at 32°06'05"N latitude and 76°34'10" E longitude. This area falls within the sub-temperate zone (1200-1700 m) climatically. The monsoon season begins with the June end and extends until mid-September. Meteorological data collected from agro-meteorological advisories during the growing season of the crops and "Crop weather outlook" and details of soil physicochemical analysis were given in Table 1. In 2022, an experiment was initiated using a statistical approach called a completely randomised block design (RBD) with three sets of replicates. To enhance the validity of treatment comparisons, the experimental area was divided into blocks based on initial soil fertility and moisture levels, which are known to influence palmarosa growth. Treatments were randomly assigned within each block to minimise bias and account for variability.

Experimental detail

The soil samples collected at 0–20 cm depth from the experimental plots were pooled, dried, sieved (2 mm sieve), and stored at room temperature until soil physicochemical analysis. The soil of the experimental area was clay loam in texture, with

Table 1 — Average meteorological data and physicochemical properties of soil

Average meteorological data during the crop growth period	Values	Physico-chemical properties of soil	Values
		pH	5.4
Maximum temperature (◦C)	15.0-29.5	Organic carbon (%)	0.7
Minimum temperature (◦C)	5.0-18.7	Electrical conductivity (m mhos/cm)	0.3
Total rainfall (mm)	2559.6	Available nitrogen (kg/ha)	292.6
Relative humidity (%)	73.4	Available phosphorous (kg/ha)	8.3
Sunshine hours	6.1	Available potassium (kg/ha)	873.9

acidic pH (5.4), medium in available N (292.6 kg/ha), and available P_2O_5 (8.3 kg/ha), while high in available K_2O (473.9 kg/ha). Palmarosa cultivation followed the standard agro-practices developed by CSIR-IHBT. Moreover, for quality production of biomass and essential oil produce, 10 t/ha farmyard manure (FYM) and N: P_2O_5: K_2O (150:50:50 kg/ha) as urea, single super phosphate (SSP) and muriate of potash (MOP) were applied. Nitrogen (N) was divided into two equal doses per harvest, initially applied with P and K; for succeeding harvests, the primary dose was applied proximately after the first harvest, while the subsequent dose was applied one month later. The experiment consists of three seasons, each having four dates of harvesting, i.e. Summer (May 4, May 20, June 4, and June 18), Autumn (September 3, September 17, October 3, and October 17), and Winter (December 7, December 22, January 6, and January 21), consisting in total 12 treatments.

Essential oil isolation

To extract the essential oil, 1 kg of fresh palmarosa aerial biomass was hydro-distilled by the European Pharmacopeia standards using an all-glass Clevenger-type apparatus. Palmarosa plant samples were hydro-distilled after 48 hours of post-harvest shade drying. In a 1:2 ratio, the content of EO was calculated as a weight percentage (v/w)[22]. The oil volume was directly measured using the extraction burette. The essential oil extracted from each treatment was dehydrated using anhydrous sodium sulphate (Merck) and subsequently stored at 4°C. This stored oil was later subjected to chemical analysis through both gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS), and component identification

The analysis involved the use of a Shimadzu GC 2010 gas chromatograph and QP 2010 mass spectrometer, both obtained from Shimadzu Corp. in Tokyo, Japan. These instruments were equipped with an AOC 5000 auto-injector. A 30-meter ZB-5 MS capillary column with dimensions of 0.25 mm i.d. and a 0.25 μm thick film sourced from SGE International in Ringwood, Australia, was utilised. For sample preparation, 5 μL of essential oil was mixed in 2 mL of dichloromethane (DCM) and auto-inoculated in split mode with 2 μL volume. Nitrogen served as the carrier gas at a flow rate of 1.05 mL/min. The initial oven temperature was maintained at 70°C for 3 minutes, followed by an increase to 220°C at a rate of 4°C/min. The injector temperature was set at 220°C, and the indicator temperature was held at 250°C. Retention indices (RIs) were evaluated for essential oil constituents using a sequence of n-alkanes (C8-C24) from SUPELCO Sigma-Aldrich. Quantification was accomplished through illustrative standard calibration curves generated by conducting GC analysis of illustrative components. Identification of essential oil components relied on comparing retention indices (RIs) and mass spectral disintegration designs with established values found in the literature[23], as well as NIST and Wiley libraries[24]. Detection limits and sensitivity for the gas chromatograph (GC) and gas chromatography-mass spectrometry (GC-MS) systems were evaluated. The limits of detection (LOD) for key components typically ranged from 0.1 to 1.0 μg/mL, demonstrating the instruments' ability to reliably identify trace amounts of compounds. This sensitivity is essential for the accurate quantification and characterisation of essential oil constituents, ensuring that even minor components are effectively detected and quantified. Calibration curves were established using standard solutions, enhancing the precision of the quantitative analyses.

Statistical analysis

Statistical analysis was carried out using SYSTAT-12 software and standard analysis of variance (ANOVA) in Chicago, IL, USA. To assess treatment variation, the least significant difference (LSD) at a 5% probability level was employed for comparisons. Additionally, multivariate principal component analysis was performed to analyse the impact of treatments on essential oil compounds using PAST4 software. These methods were chosen to evaluate treatment effects on essential oil yield and composition effectively, with LSD providing clear comparisons between groups. PCA helps simplify complex data, elucidating relationships among essential oil compounds and treatment impacts.

Results and Discussion

Moisture content and yield attributes

Harvesting season and date significantly influenced the essential oil content of palmarosa (Fig. 1). A notable increasing trend in essential oil content was observed from Autumn to Summer. Specifically, the essential oil content measured was 0.65% on September 3 (Autumn) and 1.71% on June

4 (Summer) (Fig. 1). These results indicate that the timing of harvesting plays a critical role in maximising essential oil yield.

Essential oil composition

The study of palmarosa essential oil composition leads to the identification of seven constituents accounting for 78.94-99.22% of the entire percentage. The GC-FID reported geraniol (52.93-93.48%), neryl acetate (1.15-21.53%), linalool (0.79-1.67%), and E-ocimene (0.57-1.53%), as the major compounds (Table 2). Seasonal variation significantly affected most of the essential oil components of palmarosa except myrcene. Among the different seasons, geraniol was recorded significantly higher in the winter harvest (January 21) compared to harvesting dates of other seasons; however, it remained statistically at par with January 6. Harvesting palmarosa on January 21 recorded 76.61% higher geraniol production as compared to the earliest harvesting date during the summer season (May 4). Neryl acetate and linaool were recorded significantly higher in May 4 harvesting date of summer season which were 94.65 and 52.69% higher as compared to

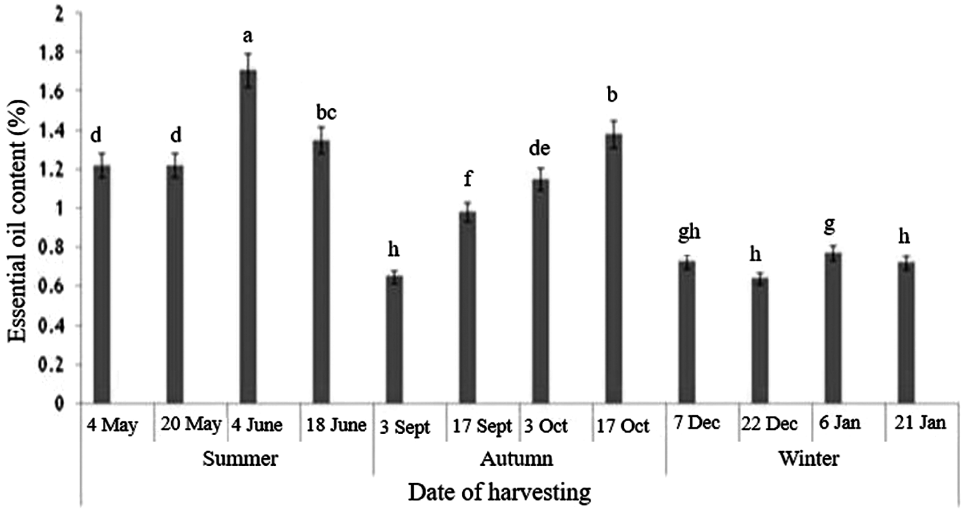

Fig. 1 — Seasonal variability on the essential oil content of palmarosa influenced by harvesting dates.

Table 2 — Seasonal variability on essential oil composition of Palmarosa influenced by harvesting dates.

Season	Date of harvesting	Area percentage (%)											
									Grouped components				
		Myrcene	*E*-ocimene	Linalool	Geraniol	Neryl acetate	Trans-caryophyllene	Farnesol	Acyclic monoterpene	Acyclic monoterpenoid	Sesquiterpene	Acyclic sesquiterpene alcohol	Total
	RI Lit.	988	1044	1095	1249	1359	1408	1714					
	RI Exp.	987	1044	1098	1251	1363	1420	1712					
Summer	May 4	0.45	1.21^{c}	1.67^{a}	52.93^{l}	21.53^{a}	0.47^{a}	0.65^{cd}	1.67	76.13^{k}	0.47^{c}	0.65^{cd}	78.94
	May 20	0.44	1.18^{cd}	1.12^{de}	77.00^{k}	18.68^{b}	0.38^{g}	0.35^{ef}	1.63	96.80^{bcd}	0.38^{cdef}	0.35^{efhij}	99.18
	June 4	0.38	0.57^{f}	1.19^{c}	79.95^{j}	16.19^{c}	0.41^{a}	0.31^{g}	0.95	97.33^{a}	0.41^{cde}	0.31^{hijk}	99.01
	June 18	0.36	0.79^{gfe}	1.00^{f}	81.32^{i}	14.93^{d}	0.46^{ab}	0.32^{e}	1.16	97.27^{ab}	0.46^{cd}	0.32^{fhijk}	99.22
Autumn	September 3	0.35	0.92^{gef}	1.09^{d}	89.99^{cd}	5.85^{fgh}	0.33^{b}	0.45^{d}	1.27	96.94^{abc}	0.33^{defgh}	0.45^{defghi}	99.02
	September 17	0.38	0.76^{gfe}	0.93^{fg}	85.24^{gh}	9.69^{e}	0.35^{a}	0.78^{c}	1.15	95.87^{abcdef}	0.35^{cdefg}	0.78^{c}	98.16
	October 3	0.33	0.83^{gfe}	0.83^{gh}	89.56^{cde}	6.31^{fg}	0.31^{cd}	0.49^{g}	1.16	96.71^{abcde}	0.31^{efghi}	0.49^{efg}	98.69
	October 17	0.36	1.53^{a}	1.28^{bc}	87.97^{f}	6.74^{f}	0.40^{a}	0.47^{de}	1.89	95.99^{abcdef}	0.41^{cde}	0.47^{efgh}	98.78
Winter	December 7	0.37	1.52^{ab}	1.29^{b}	86.94^{fg}	4.52^{i}	0.72^{e}	2.04^{a}	1.89	92.76^{ijk}	0.72^{a}	2.04^{a}	97.43
	December 22	0.29	0.71^{gfe}	1.19^{cd}	90.73^{c}	2.93^{j}	0.66^{f}	1.27^{ab}	1.01	$94.86^{abcdefghij}$	0.66^{ab}	1.27^{b}	97.81
	January 6	0.36	0.94^{e}	0.92^{f}	92.90^{ab}	1.15^{k}	0.33^{bc}	0.62^{c}	1.31	$94.98^{abcdefghi}$	0.33^{defgh}	0.62^{cde}	97.26
	January 21	0.26	0.82^{gfe}	0.79^{h}	93.48^{a}	1.25^{k}	0.25^{c}	0.59^{c}	1.08	$95.54^{abcdefgh}$	0.25^{fghi}	0.59^{cdef}	97.48

Note: RI; Retention Index. Means within each column with similar letters are not significantly different at the 5% probability level

June 6 harvesting date of winter season, respectively. There was a notable increase in the percentage of E-ocimene during the autumn season (October 17), which was 168.42% higher than on June 4 of the summer season. The autumn season, October 17, remained statistically at par with the December 7 harvesting date of the winter season. Trans-caryophyllene and farnesol were recorded significantly higher on the December 7 harvesting date of the winter season compared to other dates but remained at par with December 22. A representative chromatogram of the essential oil from Palmarosa, harvested in different seasons, is shown in (Fig. 2). These findings indicate that the essential oil composition varies markedly with seasonal changes, which is crucial for optimising harvest timing.

Grouped components of palmarosa mainly consist of acyclic monoterpene, acyclic monoterpenoid, sesquiterpene, and acyclic sesquiterpene alcohol (Table 2). Seasonal variation significantly affected most of the grouped components of palmarosa essential oil except acyclic monoterpene. Amongst different seasons, acyclic monoterpenoid was significantly higher (p=0.05) in the summer season on the June 4 harvesting date. However, it remained statistically at par with most of the other harvesting dates except for May 4 and December 7. Sesquiterpene and acyclic sesquiterpene alcohol were significantly higher (p=0.05) in the winter season when harvested on December 7 in comparison to other harvesting dates but remained statistically at par with December 22. The total area percentage was higher (p=0.05) in the summer season on June 18 harvesting. However, it remained statistically at par with most of the other harvesting dates except for May 4. These results provide valuable insights into how seasonal factors can influence the chemical profile of essential oils, which is important for industry applications.

Principal component analysis

Seasonal variations of palmarosa essential oil components were analysed using principal component analysis (PCA) to assess variability in treatment compositions. PC-1 and PC-2 were observed to be 94.37 and 5.45% of the variance, respectively, which together accounted for 99.82% of the total variance (Fig. 3). The PC-1 and PC-2 separated geraniol from other constituents and were positioned in the positive end of both PC-1 and PC-2. In contrast, myrcene, Z-ocimene, linalool, neryl acetate, trans caryophyllene, and farnesol were positioned in the negative end of PC-1. Neryl acetate was placed in the positive end of PC-2, while myrcene, Z-ocimene, linalool, trans caryophyllene, and farnesol were placed in the negative end of PC-2. The eigenvalue and variance (%) were also illustrated (Fig. 3), with PC-1 and PC-2 being the most informative with eigenvalues above ten. Principal Component Analysis (PCA) separated the treatments into four distinguishable clusters. (Fig. 3), where Cluster I comprised T2, T3, and T4, exhibiting the summer season with a lower content of geraniol and a higher content of neryl acetate. Cluster II consists of T5, T6, T7, and T8, exhibiting the autumn season with a lower content of geraniol. Cluster III consists of T9, T10, T11, and T12, exhibiting winter season with a higher content of geraniol and a lower content of neryl acetate. Cluster IV consists of T1, representing the earlier harvesting date with the lowest geraniol and highest neryl

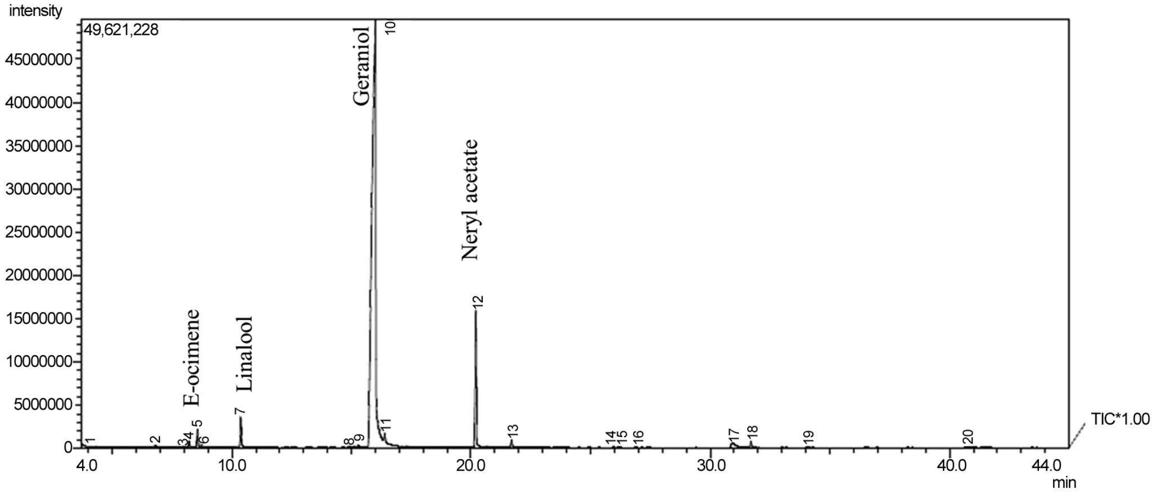

Fig. 2 — Representative Gas chromatogram of the essential oil of palmarosa.

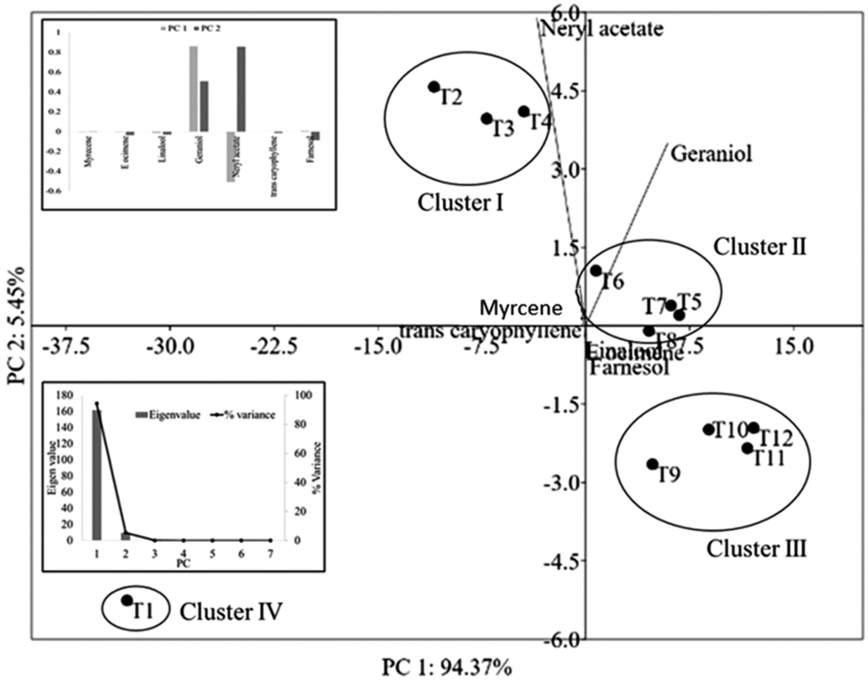

Fig. 3 — Bi-plot of principal components based on the mean value of composition of palmarosa. PCA explains 99.82% of the data variation. T1: Summer May 4, T2: Summer May 20, T3: Summer June 4, T4: Summer June 18, T5: Autumn Sep 3, T6: Autumn Sep17, T7: Autumn Oct 3, T8: Autumn Oct 17, T9: Winter Dec 7, T10: Winter Dec 22, T11: Winter Jan 6, T12: Winter Jan 21.

acetate. The PCA results demonstrated distinct treatment clusters that align with harvesting seasons, which can aid in strategic planning for oil extraction based on desired chemical profiles.

Hierarchical cluster analysis

To classify the examined species based on their unique compound profiles, we conducted hierarchical cluster analysis (HCA). This analysis was performed using the essential oil compositions of the twelve treatments, and a dendrogram was constructed using the unweighted pair-group method with square Euclidean distance (Fig. 4). Applying an arbitrary cut-off criterion allowed for the differentiation of treatments from one another. The dendrogram generated by hierarchical cluster analysis (HCA) revealed the presence of four primary groups, closely resembling the groups identified by PCA, thus highlighting the chemical similarities among treatments. Group A consisted of treatments T2, T3, and T4; treatments T5, T6, T7, T8, and T9 formed Group B; Group C consisted of treatments T10, T11, and T12; while treatment T1 established Group D. Group D was the farthest group and exhibiting distinct separation as of the remaining groups within the examination. This clustering provides a framework for understanding the relationships between treatment conditions and essential oil profiles, which is necessary for refining cultivation strategies.

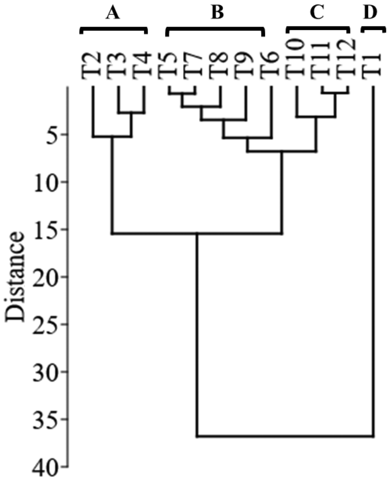

Fig. 4 — Hierarchical cluster analysis between essential oil compounds of palmarosa based on mean Euclidean distance. T1: Summer May 4, T2: Summer May 20, T3: Summer June 4, T4: Summer June 18, T5: Autumn Sep 3, T6: Autumn Sep 17, T7: Autumn Oct 3, T8: Autumn Oct 17, T9: Winter Dec 7, T10: Winter Dec 22, T11: Winter Jan 6, T12: Winter Jan 21.

Moisture reduction and yield attributes

The essential oil content exhibited a considerable rise during the harvest conducted on June 4th during the summer season compared to other harvest dates. This peak in oil yield can be attributed to the favourable climatic conditions and optimal plant maturity achieved during this period, as highlighted by Vuerich *et al*., who noted that temperature and humidity significantly influence essential oil production in aromatic plants[25]. The observed oil accumulation pattern indicates metabolic regulation of essential oil content during palmarosa vegetative and flowering stages. Furthermore, research by Ashraf *et al*. supports the notion that environmental stressors can alter metabolic pathways, thereby affecting essential oil synthesis during critical growth phases[26]. During Winter, when seed setting occurs, essential oil content decreases, possibly due to oil gland deterioration. Similar variations related to plant phenological stages have been reported in *Thymus serpyllum* by Verma *et al*.[27]. Additionally, Dubey *et al*. described variations in essential oil content and composition during palmarosa inflorescence development stages[28].

Essential oil composition

The identified seven essential oil constituents were significantly influenced by Seasonal variation. The study revealed that geraniol, linalool, and E-ocimene can serve as chemical markers for monitoring plant maturity and quality stages. The essential oil acquired through hydro-distillation exhibited a higher concentration of crucial oxygenated compounds, such as linalool, geraniol, E-ocimene, and geranial, while containing fewer terpene components[29]. This highlights the importance of extraction methods in determining essential oil composition and market value. This enrichment is attributed to the enhanced solubility of oxygenised constituents in condensate waters because of their polar characteristics[30].

Palmarosa essential oil is valued in the global market for its elevated geraniol content, which is isolated through fractional distillation and highly valued as a significant aroma compound extensively utilised in the fragrance industry[31]. Moreover, the findings underscore the importance of agricultural practices that align with the natural growth cycles of palmarosa. Implementing strategies that optimise harvesting schedules based on seasonal influences can lead to more sustainable production methods and increased economic returns for farmers[32]. This comprehensive understanding of the factors influencing essential oil composition benefits not only individual growers but also supports broader agricultural practices focused on improving the quality and marketability of essential oils[17]. Implementing these practices can foster sustainable agricultural systems that enhance economic viability while promoting environmental conservation.

A similar study was described by Smitha *et al*., which found that the trans-geraniol content in palmarosa exhibits an increase with maturity[33]. Over time, there was a notable rise in geraniol content and a consistent reduction in geranyl acetate[34]. Smitha *et al*. reported that the primary components found in palmarosa essential oil are geraniol (78.29%) and geranyl acetate (6.16%)[35,36]. Zheljazkov *et al*. noted similar findings in *Mentha spicata* L. and *Thymus daenensis* celak, highlighting that variations in configuration can be attributed to factors such as plant types, temperature, drying duration, and the timing of harvest[37]. These insights can help growers refine their cultivation practices, ensuring higher yields and improved essential oil quality, thereby contributing to the overall sustainability and profitability of palmarosa production in the market. Such improvements can lead to greater resilience within the industry, particularly in adapting to changing market demands and climatic conditions.

Conclusion

Harvesting biomass at the right stage ensures a consistent supply and contributes to the easy and high-quality marketing of the material. The study assessed changes in the essential oil content and composition with the different harvesting dates of palmarosa. Upon scrutiny of data, it was reported that the maximum essential oil yield was observed during the summer season on June 4, while the highest geraniol content was recorded on the January 21 harvesting date. These results not only inform growers about optimal harvesting times but also support broader agricultural practices aimed at enhancing the quality and marketability of essential oils. To increase the geraniol content in the essential oil, successively, palmarosa should be distilled at the proper maturity stage during the winter season under mid-hill conditions of the Western Himalayas. Future research should focus on exploring the impact of diverse extraction methods and environmental variables on the composition of palmarosa essential oils. Additionally, investigations aimed at optimising cultivation practices to increase geraniol yield are essential. Such a holistic approach could significantly enhance the resilience of the palmarosa industry, enabling it to adapt to changing market demands and climatic conditions. This integrated strategy has the potential to promote sustainable growth and provide benefits for both producers and consumers.

Conflicts of interest

The authors of this work declare that there is no conflict of interest.

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