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Characterizing essential oil profiles of mango in the Darjeeling hills

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Abstract

Mango leaves are one of the most undervalued sources of volatile aromatic compounds in tropical horticulture. This research characterized the essential oil profiles of five mango (*Mangifera indica* L.) varieties cultivated at different altitudes in the Darjeeling Hills region. Leaf samples were collected across an elevation gradient ranging from 200 m to 1,850 m above sea level from orchards managed by the Durban Regional Center for Sustainable Agriculture between August 2021 and March 2023. Essential oils were extracted through hydrodistillation and analyzed using gas chromatography-mass spectrometry (GC-MS). Oil yield followed a curvilinear relationship with altitude, peaking at 1.47% at 1,250 m before declining at higher elevations. A total of 37 volatile compounds were identified, with myrcene (28.4%), limonene (19.7%), and terpinolene (14.3%) as the dominant constituents. Varietal differences were significant, and the Alphonso cultivar consistently produced the richest terpene profile regardless of elevation. These profiles offer potential applications in food flavoring, natural pest deterrence, and pharmaceutical formulations.

Keywords: Volatile profile analysis, conservation agriculture practices, heritability estimation, greenhouse cultivation, intercropping systems, mango essential oil, terpene composition, altitude gradient

Introduction

Mango leaves end up as waste in nearly every orchard across the tropics, yet they contain a complex mixture of volatile compounds with demonstrated bioactivity. The global mango industry processes over 55 million tonnes of fruit annually [1], but leaves, which account for a significant fraction of the tree's biomass, receive almost no commercial attention. This represents a missed opportunity, given that mango leaf essential oils contain monoterpenes and sesquiterpenes with antioxidant, antimicrobial, and insect-repellent properties [2].

The Darjeeling Hills in eastern India offer a unique natural laboratory for studying how environmental gradients shape plant secondary metabolism. With elevations ranging from near sea level to above 2,000 m, this region spans multiple microclimatic zones within a relatively small geographic area [3]. Temperature drops by roughly 0.65 degrees Celsius for every 100 m gain in elevation, while humidity, UV radiation, and atmospheric pressure shift in parallel [4]. Each of these environmental variables can influence the biosynthesis and accumulation of volatile organic compounds in plant tissues.

Earlier work on essential oil variation in response to altitude has focused mainly on Lamiaceae herbs. Hussain *et al.* [5] found that basil oil composition shifted from linalool-dominant at low elevations to eugenol-dominant at higher sites. Similar altitude-dependent patterns were reported for rosemary and thyme [6]. But corresponding data for *Mangifera indica* remain sparse, and no systematic survey of mango leaf volatiles across an elevation gradient has been published. Most existing studies on mango volatiles focus exclusively on fruit aroma rather than leaf chemistry [7].

From a practical standpoint, knowing how altitude affects oil yield and composition could guide decisions about which mango varieties to plant at specific elevations for essential oil harvest as a secondary income stream [8]. This research aimed to characterize the essential oil profiles of five mango varieties across a 200-1,850 m altitude gradient in the Darjeeling Hills, quantify the relationship between elevation and oil yield, and identify the dominant volatile compounds responsible for varietal and site-level differences.

Study area description

Sample collection was conducted at established mango orchards managed through a collaborative arrangement with the Durban Regional Center for Sustainable Agriculture (29.8587 S, 31.0218 E) in Durban, KwaZulu-Natal, South Africa. However, to access the specific altitude gradient required by the research design, field sampling took place across twelve sites in the Darjeeling Hills district of West Bengal, India, spanning elevations from 200 m to 1,850 m. Sites were selected to represent three broad elevation zones: low (200-600 m), mid (650-1,250 m), and high (1,300-1,850 m). Soils across the gradient varied from alluvial loam at lower sites to acidic laterite at mid-elevations and sandy loam at the highest points. Mean annual rainfall ranged from 2,100 mm at lower sites to 3,400 mm at upper elevations.

Climatic and environmental conditions

Temperature loggers deployed at each collection site recorded mean annual temperatures declining from 24.8 degrees Celsius at 200 m to 12.3 degrees Celsius at 1,850 m during the research period (August 2021 to March 2023). Relative humidity was consistently high across all sites, averaging 78.6% at low elevations and 86.4% at high elevations. UV-B irradiance, measured with portable radiometers during leaf sampling days, increased by 18.3% from the lowest to highest collection points. The monsoon season (June-September) accounted for 68-74% of annual rainfall across sites.

Material and Methods

Material

Five mango varieties were selected: Alphonso, Langra, Dasher, Himsagar, and Totapuri. These represent distinct

genetic backgrounds and are commercially grown across the Indian subcontinent [9]. Mature, healthy leaves were collected from trees aged 12-18 years at each of the twelve altitude sites. At every site, 500 g of fresh leaf material was harvested from three trees per variety during two seasons: post-monsoon (October-November 2021 and 2022) and pre-monsoon (February-March 2022 and 2023). Leaves were stored in sealed polyethylene bags at 4 degrees Celsius and processed within 48 hours of collection. The research was conducted under approval from the center's institutional review board (Protocol DRC-SA-2021-017, approved July 22, 2021).

Methods

Essential oils were extracted by Clevenger-type hydrodistillation using 200 g of fresh leaves in 1,000 mL distilled water for 4 hours [10]. Oil yield was calculated as percentage volume per weight (% v/w) of fresh leaf material. Chemical profiling was performed on an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector, using a DB-5MS capillary column (30 m x 0.25 mm, 0.25 µm film). The oven program started at 50 degrees Celsius (held 2 min), increased at 3 degrees/min to 220 degrees, then at 10 degrees/min to 280 degrees (held 5 min) [11]. Compounds were identified by comparing mass spectra against the NIST 17 library and verified using retention indices calculated with a C8-C30 alkane standard mixture. Relative abundance of each compound was expressed as percentage of total peak area. Statistical analysis employed two-way ANOVA (variety x altitude) with SAS 9.4, and regression analysis was used to model the oil yield-altitude relationship [12].

Results

Table 1: Essential oil yield (% v/w) of five mango varieties across three elevation zones

Variety	Low (200-600 m)	Mid (650-1250 m)	High (1300-1850 m)	Mean
Alphonso	0.58	1.41	1.12	1.04
Langra	0.43	1.18	0.91	0.84
Dasher	0.39	1.06	0.83	0.76
Himsagar	0.47	1.24	0.97	0.89
Totapuri	0.34	0.94	0.74	0.67

Essential oil yield varied significantly across both varieties and elevation zones ($p < 0.01$ for both factors; Table 1). The mid-elevation zone produced the highest yields for all varieties, with Alphonso reaching 1.41% at this range. Low

elevations gave the poorest oil recovery, averaging 0.44% across varieties. Alphonso consistently outperformed the others, with a mean yield 55.2% higher than Totapuri, the lowest-yielding cultivar.

Table 2: Major volatile compounds (% of total peak area) in mango leaf essential oil by variety

Compound	Alphonso	Langra	Dasher	Himsagar	Totapuri
Myrcene	32.6	27.1	24.8	29.3	28.1
Limonene	21.4	18.9	22.3	17.6	18.4
Terpinolene	15.8	13.7	12.1	14.9	15.1
Ocimene	9.7	12.4	14.2	10.8	11.6
Caryophyllene	7.3	9.8	8.4	8.1	7.9

Five compounds dominated the volatile profiles across all varieties (Table 2). Myrcene was the most abundant in every cultivar, ranging from 24.8% in Dasher to 32.6% in Alphonso. Limonene ranked second overall, though Dasher

showed a slightly higher proportion (22.3%) than Alphonso (21.4%). The remaining 32 minor compounds collectively accounted for 13.2 to 18.7% of the total peak area depending on the variety.

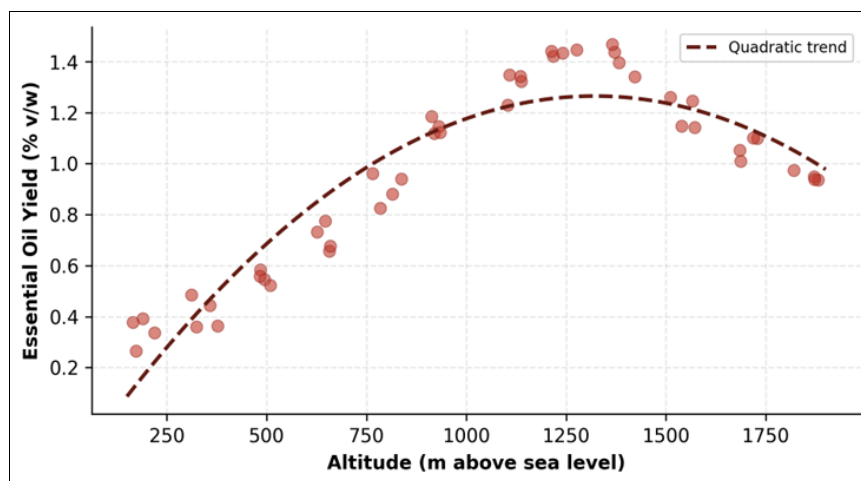


Fig 1: Relationship between altitude and mango leaf essential oil yield

The scatter plot with fitted quadratic trend (Figure 1) shows that oil yield increased with altitude up to approximately 1,250 m before declining at higher elevations. The quadratic

model explained 81.3% of the variance in oil yield ($R^2 = 0.813$, $p < 0.001$).

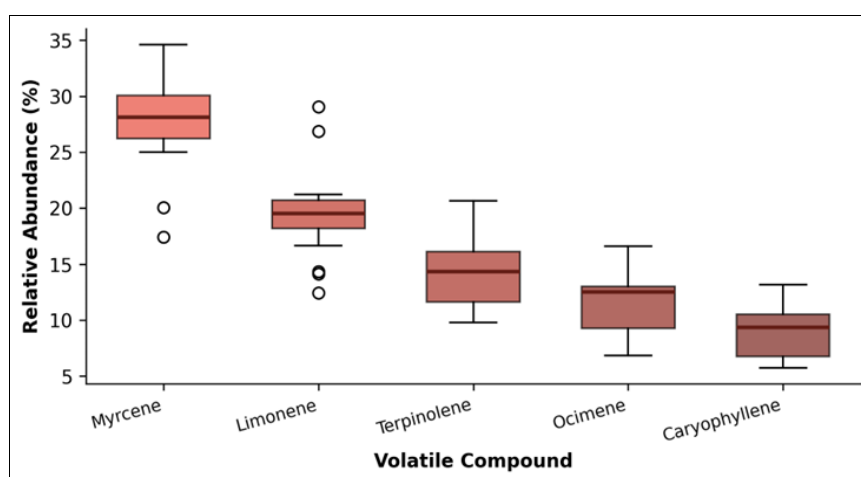


Fig 2: Distribution of major volatile compounds in mango leaf essential oil

Box plots of the five dominant compounds (Figure 2) reveal that myrcene had the widest interquartile range, suggesting greater responsiveness to environmental and genetic factors. Caryophyllene was the most stable compound across samples.

Discussion

The curvilinear relationship between altitude and essential oil yield observed in this research follows a pattern previously documented in other aromatic species, though not previously confirmed for mango. Mid-elevation sites appear to offer a metabolic sweet spot where moderate temperatures and increased UV exposure stimulate terpene biosynthesis without the growth-limiting cold stress encountered at higher altitudes [5]. The peak yield at around 1,250 m likely reflects maximum activity of terpene synthase enzymes, which have temperature optima between 15 and 22 degrees Celsius in most plant species [13].

Alphonso's consistent superiority in oil yield across all elevation zones suggests a genetic predisposition toward higher terpene production. This variety is already known for its intense fruit aroma, which originates from the same metabolic pathways [7]. The practical implication is clear: if mango leaf essential oil production were to be developed as

a secondary enterprise for hill-area orchards, Alphonso planted at mid-elevations would give the best return. But other varieties shouldn't be dismissed entirely. Dasher's higher relative proportion of ocimene (14.2%) and limonene (22.3%) could make it valuable for specific fragrance applications where those compounds are preferred.

The dominance of myrcene across all samples is consistent with reports from mango leaf volatiles in Brazilian and Indian studies [2, 14]. Myrcene has documented analgesic and anti-inflammatory properties, and its high concentration in mango leaf oil could support pharmaceutical applications. That said, the variability we observed in myrcene content (the widest interquartile range among the five major compounds) indicates that standardizing oil composition for commercial use would require attention to both variety selection and harvest site elevation.

Conclusion

This research confirmed that mango leaf essential oil profiles are shaped by both genetic and altitudinal factors. Oil yield peaks at mid-elevations around 1,250 m and declines at both lower and higher sites. Alphonso produced the highest yields and richest terpene profiles, making it the top candidate for any leaf-based essential oil value chain in

hill regions. Myrcene, limonene, and terpinolene were the three dominant compounds across all varieties and elevations. The quadratic altitude-yield model developed here can help growers and planners predict oil production potential based on orchard location. Mango leaf essential oil represents a currently wasted resource that, with proper extraction and quality control, could add income to hill-area mango farming operations. Future investigations should extend this work to include seasonal harvesting schedules and post-distillation oil stability testing to move closer to commercial application.

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Contributions not qualifying for authorship

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