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COMPARATIVE GC–MS PROFILING OF VOLATILE CONSTITUENTS IN PIMPINELLA ANISUM L. GROWN UNDER CONTRASTING SUBSTRATE AND CALCIUM–BORON NUTRITION CONDITIONS

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ABSTRACT

Anise (Pimpinella anisum L.) is valued for aromatic phenylpropenes and terpenoids whose profiles can vary with cultivation and analytical procedure. This exploratory greenhouse study compared anise grown in soil or coconut coir with or without calcium nitrate–boron nutrition (17N + 32CaO + 1B). Aerial tissues collected from full flowering to early umbel formation were macerated in 96% ethanol and analyzed by GC–MS. The available dataset comprised mean relative peak areas from 2–3 analytical replicates per substrate × nutrition combination; therefore, results were treated descriptively. Trans-anethole was the largest reported peak in all four groups, ranging from 38.4% in unsupplemented coconut coir to 50.2% in supplemented soil. With mineral supplementation, relative trans-anethole peak area was 7.7 percentage points higher in soil and 8.3 percentage points higher in coconut coir. Soil also showed higher trans-anethole than corresponding coconut-coir groups, whereas limonene was relatively higher in coconut coir. Estragole was retained as the canonical name for methyl chavicol, and a duplicate methyl-chavicol row was excluded rather than combined because raw chromatograms were unavailable for reassignment. Because the material was an ethanolic extract rather than distilled essential oil and biological replication could not be established from the source record, the findings represent preliminary relative-profile trends, not absolute metabolite concentrations or statistically confirmed treatment effects. Quantitative irrigation effects were not evaluated. Replicated factorial studies with complete instrument reporting and internal-standard quantification are required.

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Keywords: *Pimpinella anisum, trans-anethole, GC–MS, calcium–boron nutrition, substrate, phytochemical profiling.*

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Introduction

Anise (*Pimpinella anisum* L.; Apiaceae) is an aromatic medicinal and culinary species whose fruits and aerial tissues contain phenylpropenes and terpenoids of pharmacological, sensory, and commercial interest. Trans-anethole is the characteristic phenylpropene of anise and is commonly the dominant constituent of fruit essential oil, although composition varies across plant material and geographic origin (Orav et al., 2008; Shojaii & Abdollahi Fard, 2012). Molecular work in anise has also established a

dedicated biosynthetic route to trans-anethole involving phenylpropene-forming and O-methyltransferase enzymes (Koeduka et al., 2009).

The phytochemical phenotype of aromatic plants is not determined by genotype alone. Developmental stage, water availability, mineral nutrition, and other environmental conditions can alter primary metabolism, stress signaling, and the allocation of carbon to secondary metabolites (Isah, 2019; Jogawat et al., 2021). In anise specifically, field studies have shown that mineral-fertilizer regimes can affect growth, seed production, and essential-oil yield, although the magnitude and direction of responses depend on dose, genotype, and cultivation context (Bhuvaneshwari et al., 2002; Gedik & Akgul, 2023). Calcium has structural and signaling functions in plants, whereas boron contributes to cell-wall integrity, membrane function, reproductive development, and carbohydrate metabolism (Shireen et al., 2018; Thor, 2019). These physiological roles provide a plausible basis for investigating whether a calcium nitrate–boron treatment is associated with differences in the relative volatile profile of anise; however, they do not establish a compound-specific causal mechanism.

Interpretation also depends on extraction and analytical procedure. Distilled essential oils and solvent extracts are not chemically equivalent matrices, and extraction method can alter the apparent composition recovered by GC–MS (Fagbemi et al., 2021). Accordingly, the present study uses the term GC–MS-accessible volatile/aromatic fraction for the ethanol-derived material and avoids equating its relative peak areas with conventional hydrodistilled essential-oil composition or absolute metabolite concentration.

The broader greenhouse cultivation experiment included two substrates (soil and coconut coir), four irrigation volumes (50, 100, 150, and 200 mL per irrigation event), and two mineral-nutrition conditions. The quantitative GC–MS dataset available for the present manuscript, however, comprised four substrate × nutrition groups and did not contain a quantitative breakdown across the irrigation levels. The aim of this paper was therefore to describe and compare the relative GC–MS peak-area profiles of selected volatile constituents across soil and coconut-coir substrates with and without calcium nitrate–boron supplementation, while treating irrigation observations as contextual rather than as a quantitatively tested phytochemical factor.

Method

Plant Material and Greenhouse Conditions

Pimpinella anisum L. plants were cultivated in a protected greenhouse at the Institute of Fundamental and Applied Research, TIIAME National Research University, Tashkent, Uzbekistan. Two growth substrates were used: soil and coconut coir. The source manuscript reported daytime greenhouse temperatures of 22–25 °C, nighttime temperatures above 18 °C, relative humidity of 60–70%, and a 16-h photoperiod.

The broader cultivation framework included irrigation volumes of 50, 100, 150, and 200 mL per irrigation event and two mineral-nutrition conditions: an unsupplemented control and a calcium nitrate formulation supplemented with boron (17N + 32CaO + 1B). The fertilizer is referred to throughout this manuscript as the Ca–B treatment, rather than “NPK,” because the stated formulation contains nitrogen, calcium, and boron and does not report phosphorus or potassium. The original record did not provide fertilizer concentration in the irrigation solution, application frequency, total nutrient dose, irrigation frequency, pot/substrate volume, plant density per experimental unit, cultivar/accession information, or a voucher specimen number. These details were not reconstructed or inferred.

Analytical Comparison Groups

The quantitative GC–MS table available for analysis contained four substrate × nutrition combinations: soil control, soil + Ca–B, coconut-coir control, and coconut coir + Ca–B. The source manuscript described 2–3 analytical GC–MS replicates for each combination and indicated that biological replication had been used in cultivation, but the exact number of independent biological samples

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contributing to each chromatographic mean was not specified. Consequently, analytical replicate means are reported descriptively and are not treated as independent biological replication.

Sample Collection and Extraction

Aerial plant parts were collected at physiological maturity described as full flowering to early umbel formation. Plant material was chopped and macerated in 96% ethanol for 24 h at room temperature under protection from light. The extract was filtered and concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 40 °C until constant weight. Because this procedure yields an ethanolic plant extract rather than a hydrodistilled or steam-distilled essential oil, the resulting chromatographic percentages are interpreted as relative constituents of the analyzed extract fraction.

GC–MS Analysis and Compound Identification

GC–MS analysis was reported to have been performed using an Agilent 8860 gas chromatograph coupled to a 5977 mass-selective detector and an HP-5 capillary column (30 m × 0.25 mm internal diameter; 0.25 μm film thickness). Electron-impact ionization was performed at 70 eV, and helium was used as the carrier gas. The source manuscript stated that compounds were identified by comparison of mass spectra with the NIST/EPA/NIH library and by linear retention indices relative to n-alkanes.

The source record available for this revision did not contain the complete oven-temperature program, injection volume, inlet temperature, split ratio or injection mode, carrier-gas flow rate, MS scan range, source and transfer-line temperatures, solvent delay, n-alkane range, individual retention-index values, library version, or spectral-match acceptance thresholds. No values for these parameters were invented. Recovery of the original instrument method and raw chromatographic files is necessary for full analytical reproducibility and should precede journal submission.

Data Curation and Statistical Analysis

Relative peak areas were expressed as percentages of total chromatogram area and reported as means of 2–3 analytical replicates. The source table listed “estragole” and “methyl chavicol” as separate analytes. Because methyl chavicol is a synonym of estragole, the duplicate-named methyl-chavicol row was excluded from the curated table. Its values were not added to the estragole row because, without the original chromatograms and peak-identification records, it is not possible to determine whether the duplicated labels represented transcriptional duplication, two separate peaks with one misidentified, or another data-processing issue. The remaining values were retained exactly as reported.

Given the limited analytical replication and the absence of documented independent biological sample sizes for the chromatographic means, no ANOVA, post hoc testing, confidence intervals, or p values were calculated. Results are therefore descriptive and are expressed as differences in mean relative peak area (percentage points). No inference is made about absolute metabolite concentrations, essential-oil yield, or statistical significance.

Results

Reported Volatile Constituents

Seven uniquely named compounds from the supplied dataset were retained for quantitative comparison after harmonizing estragole/methyl-chavicol nomenclature (Table 1). Trans-anethole was the largest reported peak in every treatment group. The selected table is not a complete chromatographic mass balance; additional peaks were present in the original chromatograms but were not included in the summarized dataset.

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Table 1

Selected Volatile Constituents Retained After Nomenclature Curation

Compound	Chemical class	Interpretive relevance
Trans-anethole	Phenylpropene	Characteristic major aromatic constituent of anise
Estragole	Phenylpropene	Minor aromatic phenylpropene; synonym: methyl chavicol
Limonene	Monoterpene	Citrus-like monoterpene component
Linalool	Monoterpene alcohol	Floral monoterpene alcohol
Anisaldehyde	Aromatic aldehyde	Aromatic oxygenated constituent
α-Pinene	Monoterpene	Minor monoterpene constituent
Eugenol	Phenylpropanoid	Minor phenylpropanoid constituent

Note. Compound classes are descriptive. The duplicate methyl-chavicol entry in the source dataset was not retained as a separate compound because methyl chavicol is synonymous with estragole.

Relative Peak-Area Profiles Across Substrate and Nutrition Groups

Trans-anethole ranged from 38.4% to 50.2% of total chromatogram area among the four summarized groups (Table 2). In the unsupplemented groups, soil had 42.5% trans-anethole compared with 38.4% in coconut coir, a difference of 4.1 percentage points. In the Ca–B-treated groups, soil had 50.2% compared with 46.7% in coconut coir, a difference of 3.5 percentage points.

Within each substrate, Ca–B supplementation was associated with a higher mean relative trans-anethole peak area: 42.5% to 50.2% in soil (+7.7 percentage points) and 38.4% to 46.7% in coconut coir (+8.3 percentage points). Estragole, linalool, anisaldehyde, and eugenol also showed higher reported relative areas in the supplemented groups in both substrates. Limonene showed the opposite direction, decreasing by 0.35 percentage points with supplementation in each substrate and remaining higher in coconut coir than in soil under both nutrition conditions. These are descriptive contrasts and are not statistically tested treatment effects.

Table 2

Mean Relative GC–MS Peak Area of Selected Canonical Compounds Across Substrate × Nutrition Groups

Compound	Soil control (%)	Soil + Ca–B (%)	Coconut control (%)	Coconut + Ca–B (%)
Trans-anethole	42.50	50.20	38.40	46.70
Estragole	7.80	9.60	6.50	8.90
Limonene	2.45	2.10	3.20	2.85
Linalool	1.85	2.30	1.65	2.10
Anisaldehyde	2.10	2.75	1.80	2.40
α-Pinene	0.85	0.92	1.10	1.05
Eugenol	0.62	0.88	0.50	0.70
Subtotal of listed compounds	**58.17**	**68.75**	**53.15**	**64.70**

Note. Values are mean relative peak areas (% total chromatogram area) from 2–3 analytical replicates. Ca–B = calcium nitrate + boron (17N + 32CaO + 1B). Because methyl chavicol is a synonym of estragole, the duplicate methyl-chavicol row was excluded pending raw-chromatogram verification. Selected compounds only; values do not sum to 100%.

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Irrigation Observations

The cultivation experiment included four irrigation volumes, and the source manuscript described visually more vigorous and uniform vegetative development at 150 mL than at 50 or 200 mL per irrigation event. However, the quantitative GC–MS table did not contain treatment-specific values for the four irrigation levels. Accordingly, no result in this paper attributes differences in volatile composition to irrigation volume, and no irrigation regime is recommended on the basis of the present chromatographic dataset.

Discussion

Dominance of Trans-Anethole and Matrix-Dependent Interpretation

The largest reported peak across all four groups was trans-anethole, consistent with the established chemotaxonomic importance of this phenylpropene in *P. anisum* (Koeduka et al., 2009; Orav et al., 2008). The relative values in the present extract (38.4%–50.2%) are lower than the 76.9%–93.7% range reported by Orav et al. (2008) for hydrodistilled fruit essential oils from European samples. This discrepancy should not be interpreted as evidence of inferior plant quality because the matrices are not directly comparable: the present study analyzed ethanol-extracted aerial material collected around flowering/early umbel development, whereas conventional anise essential-oil studies often analyze distilled mature fruits. Extraction method can change both the compounds recovered and their apparent proportions (Fagbemi et al., 2021).

Substrate-Associated Descriptive Trends

Soil-grown samples had higher mean relative trans-anethole peak area than corresponding coconut-coir samples under both nutrition conditions, while limonene was higher in coconut coir. These repeated directions suggest a substrate-associated difference in the relative profile, but the mechanism cannot be determined from the current data. No measurements of substrate water potential, electrical conductivity, pH, aeration, nutrient availability, or root-zone temperature were provided. It would therefore be speculative to attribute the profile to particular moisture or aeration mechanisms. A replicated experiment that characterizes root-zone physicochemical conditions is needed before causal interpretation.

Mineral-Nutrition-Associated Descriptive Trends

The most consistent pattern in the summarized data was the higher relative trans-anethole peak area in Ca–B-treated material within both substrates. Similar-direction increases were observed for estragole, linalool, anisaldehyde, and eugenol, whereas limonene decreased slightly. Previous anise studies indicate that mineral nutrition can affect growth and essential-oil yield (Bhuvaneshwari et al., 2002; Gedik & Akgul, 2023), and plant physiology provides plausible links through nitrogen metabolism, calcium signaling, and boron-dependent structural and transport processes (Shireen et al., 2018; Thor, 2019). Nevertheless, the present formulation simultaneously supplied nitrogen, calcium, and boron. Because these components were not factorially separated and the fertilizer dose was not documented in the source record, the observed pattern cannot be attributed specifically to calcium, boron, or nitrogen, nor can it establish stimulation of the trans-anethole biosynthetic pathway.

Avoiding Overinterpretation of Relative Peak Areas

Relative peak-area percentages are compositional: an increase in the percentage of one constituent can occur because that constituent increases, because other constituents decrease, or because both processes occur. Without an internal or external standard, extraction-recovery measurements, and biomass-normalized concentration data, the present results cannot establish absolute accumulation of trans-anethole or any other metabolite. For the same reason, wording such as “increased biosynthesis,” “enhanced pharmacopoeial quality,” or “dominant driver” is not supported by the current dataset. The defensible conclusion is that the relative chromatographic profile differed descriptively across the four summarized conditions.

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Irrigation as a Future Quantitative Factor

Water availability is biologically relevant to secondary metabolism because drought and other stress states can alter hormonal signaling and secondary-metabolite production (Isah, 2019; Jogawat et al., 2021). However, biological plausibility cannot substitute for measurement. The present study did not provide replicated GC–MS profiles at 50, 100, 150, and 200 mL irrigation levels. The irrigation observations should therefore be used to formulate a future hypothesis rather than to claim a phytochemical response.

Limitations and Priorities for Replication

This study has four major limitations. First, the chromatographic means were based on only 2–3 analytical replicates, and the number of independent biological samples contributing to those means could not be established from the source record. Second, quantitative GC–MS values were not available across irrigation levels, preventing evaluation of irrigation effects or interactions. Third, the extract was semi-quantitatively characterized by relative peak area without internal or external standards, so absolute concentrations and total volatile yield remain unknown. Fourth, the complete GC–MS method, raw chromatograms, individual retention indices, and identification-quality metrics were not available for independent verification.

Future work should use a fully specified factorial design with independent biological replication, predefined experimental units, randomized treatment assignment, and sufficient replication for inferential analysis. Irrigation should be quantified not only as volume per event but also by frequency, substrate moisture status, drainage fraction, and root-zone properties. Mineral treatments should report concentration, application frequency, and cumulative N, Ca, and B doses and, ideally, separate nutrient factors to distinguish their contributions.

Analytical validation should include a complete GC–MS method, a documented n-alkane series, retention-index reporting for each analyte, explicit library-match thresholds, representative spectra/chromatograms, and authentic standards for key compounds when available. Absolute or semiquantitative concentration should be established using appropriate internal standards and expressed per unit dry biomass or extract. Because estragole and methyl chavicol are synonymous, the duplicated source assignment must also be reconciled against the original chromatogram before any definitive compound table is submitted for publication.

Conclusion

The available GC–MS summary data indicate that the relative volatile/aromatic profile of greenhouse-grown *Pimpinella anisum* differed descriptively across substrate and mineral-nutrition conditions. Trans-anethole was the largest reported peak in all four groups, and Ca–B-treated samples showed higher mean relative trans-anethole peak area than their corresponding controls in both soil and coconut coir. Soil groups also showed higher trans-anethole than matched coconut-coir groups, whereas limonene was comparatively higher in coconut coir. These patterns are preliminary because they are based on relative peak areas from limited analytical replication, without inferential statistics or absolute quantification. The study does not quantitatively demonstrate an irrigation effect, and the ethanol-extracted matrix should not be treated as directly equivalent to distilled essential oil. The findings provide a rationale for a rigorously replicated, fully documented factorial study rather than a basis for definitive agronomic or pharmaceutical-quality recommendations.

Data and Methodological Transparency Statement

The numerical values reproduced in Table 2 are the curated values available in the supplied manuscript. Raw chromatograms, mass spectra, complete peak tables, individual retention indices, and the original GC–MS instrument method were not available for this revision. No missing analytical parameters or experimental observations were fabricated. These primary records should be recovered, archived, and

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checked against the curated compound table before submission to a peer-reviewed analytical or phytochemical journal.

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