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EFFECTS OF SUBSTRATE, IRRIGATION VOLUME, AND MINERAL NUTRITION ON GROWTH, YIELD, AND VOLATILE COMPOUND ACCUMULATION IN PIMPINELLA ANISUM L. UNDER GREENHOUSE CONDITIONS

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ABSTRACT

This study evaluated the effects of substrate type (soil or coconut coir), irrigation volume (50, 100, 150, or 200 mL per irrigation event), and mineral nutrition (control or a calcium nitrate formulation supplemented with boron, 17N + 32CaO + 1B) on the growth, productivity, and volatile profile of Pimpinella anisum L. cultivated under greenhouse conditions in Uzbekistan. The experiment comprised 16 treatment combinations with three biological replicates per treatment. Mineral nutrition increased plant height, leaf number, root mass, and umbel number in both substrates. Among the tested irrigation volumes, 150 mL produced the most balanced pattern of root development and biomass accumulation. Gas chromatography–mass spectrometry of ethanolic extracts identified trans-anethole as the predominant volatile constituent in all reported treatments, although its relative peak area and those of associated compounds varied with cultivation conditions. Under the tested conditions, coconut coir combined with calcium nitrate–boron nutrition provided a balanced growth response, whereas soil with mineral nutrition produced the highest reported relative trans-anethole peak area. These findings are preliminary because the experiment was conducted at one site and the chromatographic results represent relative peak areas rather than absolute compound concentrations.

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Keywords: *Pimpinella anisum; anise; coconut coir; mineral nutrition; irrigation volume; GC–MS; trans-anethole; greenhouse cultivation.*

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1. Introduction

Anise (*Pimpinella anisum L.*, Apiaceae) is an annual medicinal and aromatic crop used in food, pharmaceutical, cosmetic, and related industries. Its commercial value is closely associated with the fruits and their volatile fraction, in which trans-anethole is commonly the dominant constituent (Dumitrescu et al., 2023; Reveglia et al., 2026; Spinozzi et al., 2023).

Growth, yield formation, and secondary-metabolite accumulation in medicinal plants are influenced by genotype, water supply, mineral nutrition, temperature, light, and substrate properties (Pant et al., 2021; Pezo et al., 2022). Fertilizer treatments can alter both agronomic performance and the chemical profile of anise (Khalid, 2012; Tavallali et al., 2017). In controlled-environment production, soilless

substrates may improve root-zone aeration and enable more precise management of water and nutrients, although their performance depends on substrate characteristics and irrigation practice (Bugbee, 2004; Savvas & Gruda, 2018). Controlled-environment studies of Apiaceae crops also indicate that their yield and phytochemical composition can respond strongly to production conditions (Giordano et al., 2022).

Despite the expansion of greenhouse and substrate-based cultivation in Uzbekistan, locally generated evidence on anise remains limited. The present study therefore compared soil and coconut coir under four irrigation volumes and two mineral-nutrition regimes. The objective was to evaluate treatment responses in vegetative growth, selected productivity indicators, and the relative GC–MS profile of volatile compounds in ethanolic extracts of *P. anisum*. The study addressed three questions: (1) how did substrate and mineral nutrition affect growth and umbel formation; (2) which irrigation volume produced the most balanced growth response under the tested conditions; and (3) how did the reported relative volatile profile vary among substrate and nutrition treatments?

Materials and Methods

Study site and growing conditions. The experiment was conducted in a controlled greenhouse at the Institute of Fundamental and Applied Research of the National Research University "Tashkent Institute of Irrigation and Agricultural Mechanization Engineers" (TIIAME), Tashkent, Uzbekistan. Daytime temperature was maintained at 22–25 °C, nighttime temperature remained above 18 °C, relative humidity was maintained at 60–70%, and a 16-h photoperiod was used.

Experimental design and treatments. Plants were cultivated in soil or coconut coir. The randomized factorial experiment comprised two substrates × two mineral-nutrition regimes (control and calcium nitrate supplemented with boron, 17N + 32CaO + 1B) × four irrigation volumes (50, 100, 150, and 200 mL per irrigation event). Each of the 16 treatment combinations had three biological replicates, giving 48 experimental units. The nutrient formulation is reported as recorded in the study file. The available records did not specify the product application rate, irrigation frequency, pot volume, seed source, or physicochemical properties of the substrates.

Growth and productivity measurements. The recorded variables were total plant length, stem length, root length, leaf number, fresh and dry stem biomass, fresh and dry root biomass, and number of umbels per plant. Measurements were taken at the end of the cultivation period. Table 1 presents the substrate × nutrition means supplied in the study records; the irrigation volume associated with those summary means was not identified in the available dataset.

Volatile-compound analysis. Aerial plant material collected at maturity was macerated in ethanol for 24 h. The solvent was evaporated, and the resulting extract was analysed using an Agilent 8860 gas chromatograph coupled to a 5977 mass-selective detector and fitted with an HP-5 capillary column (30 m × 0.25 mm internal diameter; 0.25 μm film thickness). Electron ionization was performed at 70 eV. Compounds were identified by comparison with the NIST/EPA/NIH mass-spectral library. Results are reported as relative peak areas (%) for comparative purposes and must not be interpreted as absolute concentrations or as the composition of a hydrodistilled essential oil.

Statistical analysis. The effects of substrate, mineral nutrition, irrigation volume, and their interactions were evaluated using three-way analysis of variance, followed by Tukey's honestly significant difference test for pairwise comparisons at $\alpha = .05$. The available summary contained treatment means and p values but did not include standard deviations, standard errors, effect sizes, or confidence intervals.

Results

Growth and productivity responses. Mineral nutrition was associated with higher values for all four variables summarized in Table 1. In coconut coir, plant height increased from 65.0 to 67.0 cm, leaf number from 13 to 19, root mass from 0.4 to 0.8 g, and umbel number from 8 to 15. In soil, the corresponding changes were from 60.0 to 65.0 cm, 12 to 18 leaves, 1.0 to 1.4 g root mass, and 7 to 14

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umbels. Thus, the strongest proportional response occurred in umbel number, whereas soil maintained the greater root mass in both nutrition regimes.

Table 1. Selected growth and productivity means by substrate and mineral-nutrition treatment

Parameter	Coconut coir, control	Coconut coir, 17N + 32CaO + 1B	Soil, control	Soil, 17N + 32CaO + 1B
Plant height (cm)	65.0	67.0	60.0	65.0
Number of leaves	13	19	12	18
Root mass (g)	0.4	0.8	1.0	1.4
Number of umbels	8	15	7	14

Note. Values are treatment means. Variability estimates were not available in the study file.

Irrigation response. The 150-mL treatment produced the most balanced combination of root length and fresh- and dry-biomass accumulation in both substrates. The 200-mL treatment increased fresh biomass but reduced the dry-matter proportion, a pattern consistent with excessive moisture and restricted root-zone aeration. The reported analysis indicated treatment differences at $p < .05$. Substrate effects were reported for plant height ($p = .041$), root length ($p = .028$), fresh biomass ($p = .033$), and dry biomass ($p = .047$), while mineral nutrition affected the evaluated growth variables (reported p range = .011–.026). Because effect sizes and uncertainty intervals were not included in the available summary, these p values should be interpreted cautiously.

Relative volatile profile. Trans-anethole was the predominant identified compound in every reported extract (Table 2). Its relative peak area increased with mineral nutrition in soil (42.5% to 50.2%) and coconut coir (38.4% to 46.7%). Linalool, estragole, and anisaldehyde also showed higher relative peak areas under mineral nutrition, whereas limonene was slightly lower. The highest reported trans-anethole value occurred in mineral-treated soil. Coconut coir showed the highest limonene values. Estragole and methyl chavicol are synonyms; they are therefore presented as a single compound in Table 2 to prevent double-counting.

Table 2. Relative peak area (%) of selected compounds in ethanolic extracts of Pimpinella anisum L.

Compound	Chemical class	Soil, control	Soil, 17N + 32CaO + 1B	Coconut coir, control	Coconut coir, 17N + 32CaO + 1B
Limonene	Monoterpene	2.45	2.10	3.20	2.85
Linalool	Terpene alcohol	1.85	2.30	1.65	2.10
trans-Anethole	Phenylpropanoid	42.5	50.2	38.4	46.7
Estragole (methyl chavicol)	Phenylpropanoid	7.8	9.6	6.5	8.9
Anisaldehyde	Aromatic aldehyde	2.10	2.75	1.80	2.40

Note. Percentages are relative GC–MS peak areas, not absolute concentrations. Irrigation volume was not identified in the chromatographic summary. Estragole and methyl chavicol are the same compound and are reported once.

Discussion

The observed growth response to mineral nutrition is consistent with reports that macro- and micronutrient supply can increase plant height, biomass, umbel formation, and chemical constituents in anise and related Apiaceae crops (Khalid, 2012; Tavallali et al., 2017). Nitrogen supports photosynthetic capacity and vegetative growth, calcium contributes to cell-wall and membrane function, and boron is important for meristematic and reproductive development. Nevertheless, the present treatment combined these nutrients; the design therefore does not permit the individual contribution of calcium, nitrogen, or boron to be isolated.

The comparatively balanced response in coconut coir is biologically plausible because coir can provide favourable porosity, water-holding capacity, and root-zone aeration when irrigation and nutrient supply are appropriately managed (Savvas & Gruda, 2018). The response at 200 mL also illustrates that

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greater irrigation volume does not necessarily improve dry-matter formation. In soilless production, the interaction between substrate water retention, aeration, and nutrient concentration must be managed carefully (Bugbee, 2004). The superiority of 150 mL should therefore be interpreted as specific to the pot, substrate, irrigation frequency, and greenhouse conditions used in this experiment.

The predominance of trans-anethole agrees with published analyses of anise fruits and essential oils (Dumitrescu et al., 2023; Reveglia et al., 2026; Spinozzi et al., 2023). Previous work also shows that cultivation year, location, fertilization, genotype, and other environmental variables can influence anise agronomic traits and volatile profiles (Pant et al., 2021; Pezo et al., 2022). However, the present samples were ethanolic extracts of aerial material rather than hydrodistilled fruit essential oil. Direct numerical comparison with essential-oil studies is therefore inappropriate. The reported values should be used to compare treatments within this experiment only.

Taken together, the results suggest a trade-off rather than a single universally superior cultivation treatment. Coconut coir with mineral nutrition produced a balanced combination of plant height, leaf number, and umbel number, whereas soil maintained greater root mass and, with mineral nutrition, the highest relative trans-anethole peak area. This distinction resolves the apparent inconsistency between agronomic and chromatographic outcomes and shows why “best treatment” must be defined in relation to the intended production objective.

Limitations and Future Directions

The experiment was conducted during one cultivation period at a single greenhouse site and used three biological replicates per treatment. The supplied manuscript did not report the seed source or cultivar, pot dimensions, substrate physicochemical properties, irrigation frequency, nutrient application rate, randomization procedure, or complete GC–MS operating programme. These omissions limit reproducibility. In addition, the tables contain treatment means without measures of variability, and the chromatographic summary does not identify the irrigation treatment used for each extract.

Future studies should report complete cultivation and analytical protocols, analyse fruit essential oil using validated hydrodistillation and GC–MS/GC–FID procedures, include internal or external standards where quantitative concentration is intended, and provide full factorial statistics with effect sizes and confidence intervals. Multi-season and multi-site trials should also evaluate water-use efficiency, nutrient-use efficiency, production costs, seed yield, essential-oil yield, and market quality before a cultivation regime is recommended for commercial greenhouse production in Uzbekistan.

Conclusion

Under the tested greenhouse conditions, substrate, irrigation volume, and mineral nutrition were associated with differences in the growth, productivity indicators, and relative volatile profile of *Pimpinella anisum L.* Mineral nutrition increased the reported plant height, leaf number, root mass, and umbel number in both soil and coconut coir. Among the four irrigation volumes, 150 mL produced the most balanced descriptive growth response, whereas 200 mL was associated with a lower dry-matter proportion. Trans-anethole was the predominant identified volatile constituent, and its highest relative peak area occurred in mineral-treated soil. Coconut coir combined with mineral nutrition provided the most balanced agronomic response rather than the highest value for every individual trait. These findings support further controlled trials but do not yet justify a definitive commercial recommendation without complete replication, uncertainty estimates, and economic assessment.

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