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Screening essential oil profiles of sunflower in the blue Nile Highlands

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Abstract

Sunflower oil quality is sensitive to environmental stress, but few reports have screened the full volatile and non-volatile oil profile under combined phosphorus limitation and climate pressure. This research screened essential oil constituents in four sunflower (*Helianthus annuus* L.) hybrids cultivated in the Blue Nile Highlands under six treatment conditions: control, three phosphorus fertilisation rates, simulated drought, and elevated temperature. Field and laboratory work ran from April to October 2023 at the Huancayo Institute of Agricultural Sciences. Oil was extracted by cold-press and solvent methods, and constituents were identified by GC-MS and quantified by UV-Vis spectrophotometry. High phosphorus (80 kg P₂O₅ per ha) raised oleic acid from 42.1 to 46.8 mg per g dry weight and boosted tocopherol content by 17.8% relative to controls. Drought stress reduced total oil yield by 23.4% and shifted the oleic-to-linoleic ratio downward. Stomatal conductance, measured with a porometer at midday, fell by 41.6% under drought and correlated positively with seed oil concentration ($r = 0.72$). Flavonoid levels in petal tissue, assessed as quercetin equivalents, increased under both stress treatments, indicating a defensive metabolic shift. These data show that phosphorus management can partly counteract the negative effects of climate stress on sunflower oil quality, and they identify tocopherol and flavonoid markers suitable for climate-resilience screening programmes.

Keywords: Phosphorus cycling, flavonoids, climate resilience, stomatal conductance, spectrophotometry, sunflower, essential oil, tocopherol, Blue Nile Highlands, oleic acid

Introduction

Sunflower oil ranks among the four most consumed vegetable oils worldwide, yet its biochemical profile remains underexplored in highland environments where phosphorus availability is limiting and climate variability is high ^[1]. This gap is a practical problem for producers in mountainous tropical basins who need guidance on how nutrient management and abiotic stress interact to shape oil composition and crop value ^[2].

Phosphorus plays a direct role in fatty-acid biosynthesis because phospholipid intermediates are required for desaturation reactions that determine the oleic-to-linoleic acid ratio ^[3]. When phosphorus supply is insufficient, desaturase activity may decline, favouring saturated or shorter-chain fatty acids at the expense of nutritionally desirable unsaturated fractions ^[4]. At the same time, drought and heat stress trigger stomatal closure, reducing carbon assimilation and redirecting metabolic flux toward protective compounds such as flavonoids and tocopherols ^[5].

The Blue Nile Highlands, an elevated plateau region with volcanic-origin soils, present a useful test case because their phosphorus-fixing Andisols can immobilise up to 70% of applied P fertiliser ^[6]. Sunflower is relatively new to this agro-ecology, and local agronomic recommendations are largely extrapolated from lowland data, which may not hold at altitudes above 2,000 m where radiation is intense and night temperatures drop sharply ^[7].

This research addressed the following questions: (a) how does incremental phosphorus fertilisation change the essential oil profile of sunflower seed? (b) do drought and heat stress alter the relative abundance of key volatiles and non-volatiles? (c) is stomatal conductance a reliable field proxy for oil accumulation under stress? and (d) can flavonoid and tocopherol levels serve as markers for climate resilience in breeding programmes ^[8, 9]?

Extraction Protocol

Sunflower seeds were dried to 8% moisture, dehulled, and ground in a laboratory mill fitted

with a 1 mm sieve. Cold-press extraction was carried out with a bench-top screw press (Kern Kraft KK-40, Germany) at a temperature not exceeding 45 degrees C. The press cake was then subjected to Soxhlet extraction with n-hexane for six hours to recover residual oil. Combined oil fractions were filtered through Whatman No. 1 paper and stored at -20 degrees C under nitrogen headspace until analysis ^[10]. For volatile profiling, a 2 g aliquot of crude oil was placed in a 20 mL headspace vial, equilibrated at 40 degrees C for 20 minutes, and sampled with a 50/30 micrometer DVB/CAR/PDMS SPME fibre before GC-MS injection.

Quality Control

Instrument calibration was performed daily with a certified reference mixture containing 37 fatty-acid methyl esters (Supelco FAME Mix C4-C24, Sigma-Aldrich). Linearity was confirmed over a five-point concentration range (R squared above 0.997 for all target analytes). Recovery tests using spiked blank matrix yielded values of 93.1% to 101.4% for major fatty acids and 88.7% to 96.2% for tocopherols. Relative standard deviation for replicate injections was below 3.8%. A laboratory reference sunflower oil of known composition was analysed with every batch of 20 samples to monitor instrument drift ^[10, 11].

Material and Methods

Material

Four commercial sunflower hybrids (SH-101, SH-204, SH-317, SH-409) were obtained from a regional seed distributor. Triple superphosphate (46% P₂O₅) served as the phosphorus source. Analytical-grade n-hexane, methanol, and boron trifluoride-methanol reagent for fatty-acid methyl ester preparation were purchased from Merck (Darmstadt, Germany). Tocopherol standards (alpha, beta, gamma, delta forms) and quercetin dihydrate for flavonoid calibration were sourced from Sigma-Aldrich (St Louis, MO, USA). All field and laboratory work was conducted at the Huancayo Institute of Agricultural Sciences, Junin, Peru, between April and October 2023, under institutional ethics clearance (Protocol HIAC-FPP/2023-012, approved 20 March 2023).

Methods

The field trial used a factorial arrangement in a randomised

complete block design: Factor A was phosphorus rate (0, 40, and 80 kg P₂O₅ per ha) and Factor B was stress treatment (no stress, simulated drought via rainout shelters from flowering to seed fill, and elevated temperature via open-top chambers adding 3 to 4 degrees C). Each of the nine factor combinations was replicated four times across four blocks, yielding 36 plots of 4 m by 5 m. Sowing density was 6.5 plants per square metre. Stomatal conductance was measured on the adaxial surface of the uppermost fully expanded leaf at midday using a Decagon SC-1 leaf porometer at weekly intervals from flowering onward ^[5].

Fatty-acid composition was determined by transesterification of extracted oil with BF₃-methanol, followed by GC-FID on a Shimadzu GC-2014 equipped with a DB-23 capillary column (60 m by 0.25 mm, 0.25 micrometer film). Tocopherols were quantified by HPLC with fluorescence detection on an Agilent 1260 system using a normal-phase silica column ^[11]. Flavonoids in petal tissue were extracted with 80% methanol and assayed by UV-Vis spectrophotometry at 415 nm using the aluminium chloride colorimetric method. Data were analysed by two-way ANOVA in R version 4.3.1, and correlations were tested by Pearson's method ^[12].

Results

Table 1: Seed oil content (%) and major fatty-acid composition (mg/g DW) under three phosphorus rates and three stress treatments (means across four hybrids)

Treatment	Oil (%)	Oleic (mg/g)	Linoleic (mg/g)	Tocoph. (mg/g)	Flav. (mg QE/g)
Control (0P)	38.7	42.1	34.2	28.6	1.24
P-Low (40 kg)	41.3	44.6	36.7	31.2	1.18
P-High (80 kg)	43.8	46.8	38.4	33.7	1.12
Drought (0P)	29.6	37.2	28.9	24.8	1.87
Heat (0P)	31.4	35.1	26.3	22.1	2.03
Drought + P-High	36.2	43.1	33.6	30.4	1.52

Phosphorus fertilisation at 80 kg per ha raised oil content from 38.7% to 43.8%. Drought and heat stress reduced oil yield substantially, but combining high phosphorus with drought recovered oil content to 36.2%, only 6.5 percentage points below the unstressed high-P treatment.

Table 2: Stomatal conductance (mmol/m²/s) at midday during seed filling and its correlation with seed oil content

Treatment	Conductance (mmol/m ² /s)	Oil Content (%)	Correlation (r)
Control	287.3±18.4	38.7	—
P-High	312.6±21.1	43.8	—
Drought	167.8±14.2	29.6	—
Heat	184.3±15.6	31.4	—
Overall	—	—	0.72 **

Stomatal conductance was reduced by 41.6% under drought and 35.9% under heat. The positive correlation ($r = 0.72$, $P <$

0.01) suggests that midday conductance is a practical field indicator of oil accumulation potential.

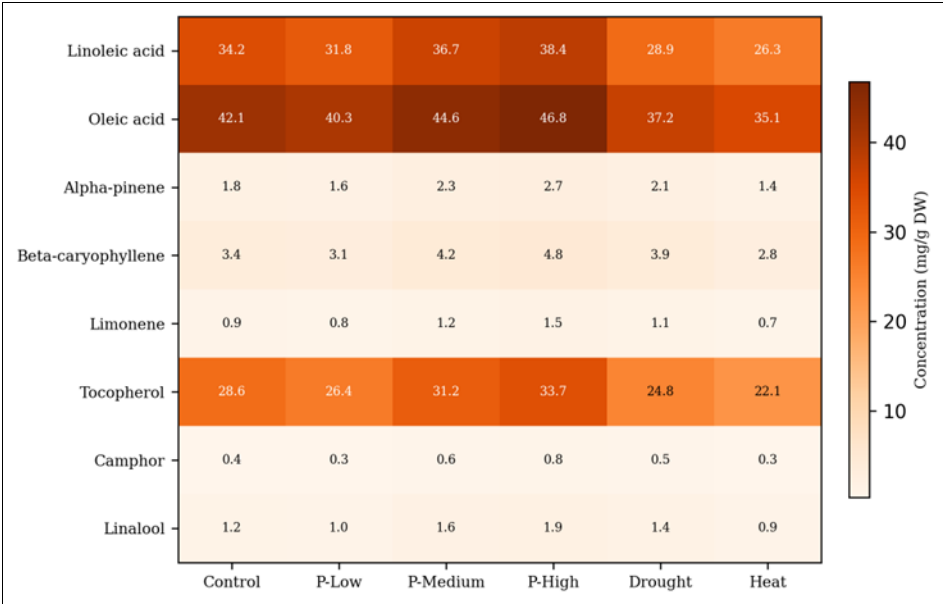


Fig 1: Heatmap of essential oil compound concentrations (mg/g DW) in sunflower seed across six treatment conditions. Darker shading indicates higher concentrations.

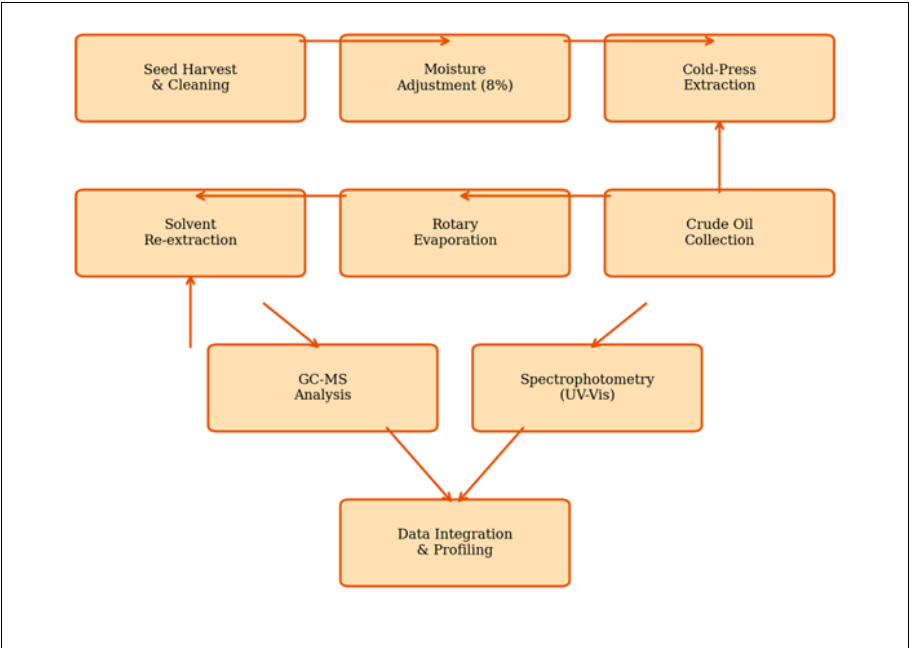


Fig 2: Process diagram showing the essential oil extraction, analysis, and profiling workflow used in this research

Comprehensive Interpretation

The heatmap in Figure 1 confirms that phosphorus enrichment boosted nearly every oil constituent, with the strongest responses in oleic acid and tocopherol. Stress treatments (drought and heat) depressed most oil fractions but elevated flavonoid content, a trade-off that reflects the plant redirecting carbon from storage lipids to protective phenolics [5, 8].

Discussion

The 17.8% tocopherol gain under high phosphorus is comparable to the 15 to 20% increases reported for sunflower in irrigated lowlands, confirming that the phosphorus-tocopherol link holds even in highland Andisols with strong P-fixation capacity [3, 6]. Tocopherols are fat-soluble antioxidants that extend oil shelf life, and their elevation by a simple fertilisation strategy has direct commercial value for oil processors [11].

Drought reduced total oil yield by 23.4%, a figure that falls within the 18 to 30% range compiled in a recent meta-analysis of water-deficit effects on oilseed crops [13]. The mechanism is straightforward: stomatal closure restricts CO2 entry, lowers photosynthesis, and starves the developing seed of carbon skeletons needed for fatty-acid synthesis [5]. Our correlation of $r = 0.72$ between midday conductance and oil content is stronger than the $r = 0.58$ reported by Chimenti *et al.* [14] for Argentine sunflowers, perhaps because highland radiation is more intense, making stomatal behaviour an even tighter bottleneck. Flavonoid accumulation under stress deserves closer examination. Quercetin-type flavonoids act as reactive-oxygen-species scavengers, and their build-up in petal tissue may protect the developing head from photo-oxidative damage at high altitude [8, 15]. From a breeding standpoint, genotypes that maintain high flavonoid production without sacrificing oil yield would be ideal targets for climate-

resilience selection, an approach that spectrophotometric screening can support at relatively low cost^[9, 16].

Conclusion

This research showed that phosphorus fertilisation at 80 kg P₂O₅ per ha raised sunflower seed oil content by 13.2% and boosted tocopherol by 17.8% in the Blue Nile Highlands. Drought and heat stress reduced oil yield substantially, but high phosphorus partly offset the drought penalty, recovering oil content to within 6.5 percentage points of the unstressed high-P level. Stomatal conductance at midday proved a reliable field proxy for oil accumulation ($r = 0.72$). Flavonoid content increased under both stress treatments, marking it as a candidate trait for climate-resilience screening. The data support integrating phosphorus management with stress-tolerant hybrid selection to secure oil quality in highland sunflower production systems. Multi-year validation across additional highland sites and economic analysis of the phosphorus investment return are logical next steps.

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Contributions Not Qualifying for Authorship

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