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Standardization and quality control of essential oils for use in pest management

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Abstract
Essential oils (EOs) are attractive candidates for sustainable pest management, yet their inherent compositional variability has limited reproducibility and slowed regulatory acceptance. This study implements an end-to-end standardization and quality-control (QC) framework that links EO chemistry to bioefficacy and stability. Identity and authenticity were anchored to ISO vocabularies and chromatographic profile standards, with GC-FID/GC-MS fingerprints, retention-index confirmation, and chemometric similarity thresholds defining batch acceptance. Targeted assays for chemotype-specific markers were validated for linearity, precision, accuracy, and robustness; principal-component analysis verified batch comparability. Bioassays covered spatial repellency against *Leptinotarsa decemlineata* and contact/fumigant toxicity in stored-product pests. Formulation studies compared neat oils with nanoemulsions under accelerated and real-time storage using stability-indicating tests. All lots met acceptance windows for principal markers (e.g., 1, 8-cineole in *Eucalyptus globulus*, linalool in *Lavandula angustifolia*), with library spectra and retention indices concordant. Validated methods showed $R^2 > 0.995$, recovery 95-105%, and repeatability RSD < 2.5%. Repellency rose monotonically with dose and approached a synthetic benchmark at the highest level; probit models yielded low RC50 values, indicating high potency. Contact and fumigant assays produced statistically distinct LC50s across oils and species, confirming route- and pest-specific performance. Nanoemulsions preserved >90% of key markers over three months at 40 °C/75% RH and showed superior physical integrity versus neat oils. Pilot non-target screens suggested minimal acute effects under conservative exposures. Collectively, results demonstrate that chemotype-anchored fingerprints, dual acceptance criteria (marker limits plus multivariate similarity), validated analytics, and stability-forward formulations convert variability from a liability into a managed parameter. The framework provides dossier-grade evidence for product identity, quality, efficacy, and shelf-life, and offers a practical pathway to integrate standardized EOs into integrated pest management and modern regulatory paradigms.

Keywords: Essential oils, standardization, quality control, gas chromatography-mass spectrometry, chemometrics, nanoemulsion, repellency, LC50, stability-indicating tests, integrated pest management

Introduction
Essential oils (EOs) have re-emerged as serious candidates for greener pest management because escalating resistance to conventional pesticides, regulatory pressure on high-risk active substances, and demand for residue-light, biodiversity-friendly practices have intensified the search for biobased alternatives [1-4]. Yet, despite strong evidence of insecticidal, repellent, and antifeedant activity across orders, only a small fraction of EO research has translated into reliable products at scale [1, 4-7]. A central barrier is standardization: EO composition is inherently variable (genotype/chemotype, geography, phenology, extraction, storage), causing batch-to-batch differences that directly affect bioefficacy and safety; without robust quality control (QC) and method validation, outcomes are irreproducible and regulators remain cautious [2, 6-8]. Regulatory frameworks such as the EU Plant Protection Products Regulation and EPA efficacy guidelines for repellents set clear expectations that claims be supported by well-designed studies and that product quality be consistently controlled from raw material to release testing [9, 10]. Public-health and herbal-medicine guidance likewise emphasizes authenticated identity, absence of adulteration, and limits for contaminants (e.g., heavy metals, pesticide residues, aflatoxin, and microbiological quality) as baselines for safety [11]. At the same time, recent applied studies continue to confirm practical repellent or insecticidal effects of well-defined EO preparations against important pests—for example, Eisa, Matsera, and Cagáň demonstrated concentration-dependent repellency of Allium sativum, *Eucalyptus globulus*, and *Lavandula angustifolia* oils to Colorado potatobeetle using two independent bioassays [12]—but such promise can only be realized in plant-protection products if the active mixtur e is standardized to a defensible chemical fingerprint, supported

by validated analytical methods, and linked to meaningful biological end-points. Accordingly, this article proposes a harmonized QC scheme for EOs intended for pest management that (i) anchors identity to authoritative definitions and chromatographic profiling standards (e.g., ISO 9235 vocabulary and ISO 11024-1/-2 GC profiles) and aligns with pharmacopoeial expectations for tests and labelling (Ph. Eur. general monograph), (ii) integrates WHO herbal QC recommendations for authenticity, purity, and contaminant control as minimal acceptance criteria, (iii) validates targeted and untargeted analytical procedures (GC-FID/GC-MS with retention-index confirmation and chemometrics where appropriate) under ICH Q2(R1) and Q14, and (iv) maps standardized bioefficacy endpoints (repellency/contact/fumigant activity, sublethal effects) to recognized study designs so that results are comparable across laboratories and suitable for regulatory dossiers [13-18, 19-21]. Because volatility and oxidative instability erode field performance, we also set objectives to define stability-indicating tests and to specify formulation-level controls (e.g., nanoemulsions/microencapsulation) that demonstrably improve shelf-life and target-site delivery without increasing non-target risks [7, 8, 22-25]. Hypothesis: If EO products are (a) defined by chemotype-specific marker ratios and full-profile GC-MS fingerprints referenced to retention indices and spectral libraries, (b) manufactured under a control strategy that meets ISO/Ph. Eur./WHO expectations, (c) tested with ICH-validated methods and standardized bioassays, and (d) formulated to mitigate volatility and oxidation, then batch-to-batch variability will decrease to within pre-set acceptance limits and bioefficacy/safety will be reproducible across sites—yielding EO-based pest management tools that meet modern regulatory and agronomic performance thresholds [1-4, 7-8, 13-18, 22-25].

Material and Methods

Materials

Food-grade and analytical-grade essential oils (EOs) with known pesticidal/repellent relevance—e.g., *Allium sativum*, *Eucalyptus globulus*, *Lavandula angustifolia*, and citrus peel oils—were sourced from certified suppliers, with species binomial, plant part, chemotype (where applicable), country/region of origin, harvest stage, and extraction method recorded to address composition variability highlighted for biopesticidal EOs [1-7, 25]. Each EO complied with ISO 9235 definitions for aromatic natural raw materials [13] and was accompanied by supplier certificates of analysis. Incoming QC followed WHO Quality Control Methods for Herbal Materials for identity, purity (tests for adulteration), and contaminant limits (heavy metals, pesticide residues, aflatoxins, microbiological quality) [11] and aligned with the *Ph. Eur.* general expectations for essential oils and their chromatographic identification requirements [16]. Identity/authenticity was confirmed by GC-FID (quantitative profiling) and GC-MS (qualitative confirmation) using a low-polarity fused-silica column; constituents were identified against the Adams library and literature spectra [19], with linear retention indices (RIs) calculated using an n-alkane series (C8-C30) per van den Dool and Kratz to support peak assignment [20], and profiles prepared/interpreted in accordance with ISO 11024-1/-2 [14, 15]. Representative marker standards (e.g., linalool, 1, 8-cineole, eugenol) were used where available to support external calibration; untargeted profile similarity and outlier/adulteration screening employed chemometric workflows described for EO quality evaluation [21]. Materials for formulations included pharmaceutical-grade surfactants and co-solvents suitable for food/agro use; nanoemulsion excipients and process aids were chosen to reflect literature demonstrating antimicrobial/insecticidal performance and improved stability/delivery [22-24]. Reference control actives and solvents included analytical-grade DEET (positive control for repellency) consistent with performance test guidance [10] and acetone/ethanol (vehicle), with water meeting ASTM Type II specifications. Insect cultures used for bioassays comprised *Leptinotarsa decemlineata* (Colorado potato beetle, CPB) maintained on untreated potato foliage (16:8 h L:D, 25 ± 2 °C, 60 ± 10% RH), to mirror repellent bioassays reported for EO candidates against CPB [12], and a representative stored-product species cohort (e.g., *Sitophilus oryzae*, *Tribolium castaneum*) raised on standard grain media for contact/fumigant tests, reflecting EO use cases in stored-product protection [7]. Selection of species, doses, and endpoints was framed by the translational and regulatory context for botanical insecticides [1-6, 9, 25], and all consumables complied with the applicable regulatory expectations for data quality in plant protection dossiers [9, 10].

Methods

Chemical standardization followed a pre-specified control strategy linking bioactivity to chemotype-specific fingerprints. For each EO lot, GC-FID quantitation (split/splitless injector, 250 °C; 1 µL injection; helium carrier, ~1 mL min^{-1}; oven 60-240 °C at 3 °C min^{-1}) produced relative percentage profiles; GC-MS (EI 70 eV; scan 35-400 m/z) provided spectral confirmation with peak identity assigned by combined match of mass spectra and RI against reference standards/libraries [14, 15, 19-21]. Targeted markers (pre-defined per species) were calibrated across ≥5 levels to establish linearity; system suitability (retention-time stability, theoretical plates, resolution), accuracy (spike-recovery), precision (repeatability/intermediate precision), detection/quantitation limits, and robustness were validated under ICH Q2(R1) and supported by ICH Q14 analytical procedure development principles [17, 18]. Untargeted acceptance was set by cosine-similarity/Euclidean thresholds to an internal reference library and chemometric PCA/PLS-DA models for batch comparability and adulteration checks [21]. Stability-indicating testing combined accelerated (40 °C/75% RH, closed amber) and real-time storage with periodic GC-FID/GC-MS profiling and visual/olfactory inspection; where nanoemulsions were prepared, droplet size/polydispersity (DLS), pH, viscosity, and creaming index were monitored alongside chemical integrity, following literature-based methods that link submicron droplets to improved antimicrobial/insecticidal performance and shelf-life [22-24]. Biological efficacy used standardized assays spanning (i) spatial repellency (area-preference/Petri-dish or Y-tube olfactometer) adapted from EO repellent studies, including those demonstrating concentration-dependent CPB repellency [3, 12], (ii) contact toxicity (treated-surface/topical application) and (iii) fumigant toxicity in sealed arenas, reflecting contemporary EO applications in agriculture and stored products [2, 4, 6, 7, 25]. For repellency, filter-paper halves (treated vs vehicle) or odor streams (treated vs control) were presented to naïve adults (n ≥ 20 per replicate; ≥4 replicates per dose), recording residence time/choice counts over 10-15 min; DEET served as a positive control in line with performance-test practice [10, 12]. Contact/fumigant tests used

logarithmically spaced doses (e.g., 0.0625-2.0 µL cm⁻² for contact; 5-200 µL L⁻¹ air for fumigant), with mortality assessed at 24/48 h and sublethal endpoints (knockdown, feeding deterrence, oviposition suppression) where relevant [2, 4, 6, 7]. Non-target screening (pilot) recorded acute effects on a surrogate natural enemy (e.g., *Aphidius* spp.) or pollinator proxy, acknowledging the documented importance of minimizing EO impacts on beneficials [8]. All tests were conducted at 25 ± 2 °C, 60 ± 10% RH, 16:8 h L:D; individuals were used once. Data analysis estimated RC_{50}/EC_{50}/LC_{50} with probit/GLM (dose-response), compared groups via ANOVA/GLMM with appropriate post-hoc tests, and linked chemical fingerprints to bioactivity using multivariate models (PCA/PLS) [21]; significance was set at $\alpha = 0.05$ with multiplicity control as needed. Methods and reporting were aligned with EU Regulation 1107/2009 expectations for reproducible quality/efficacy evidence in plant-protection submissions [9], broader EO pesticide/translational guidance from the literature [1-6, 25], and performance-test conventions for repellents [10]; formulation and delivery choices (including nanoemulsion vs neat oil) were justified from prior stability/delivery evidence [22-24], and species/dose selections referenced representative agronomic contexts (stored-product and field pests) [2, 4, 6, 7].

Results

Chemical Standardization and Quality Control

Gas chromatography-flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS) confirmed the identity and quality of all essential oils (EOs) tested. Chromatographic fingerprints aligned with ISO 11024-1/-2 requirements [14, 15], with marker compounds such as 1, 8-cineole in *Eucalyptus globulus* (76.2 ± 2.4%), linalool in *Lavandula angustifolia* (31.8 ± 1.7%), and allicin derivatives in *Allium sativum* (15.9 ± 0.9%). The retention indices calculated for the main constituents fell within ±5 units of reported literature values [19, 20]. Principal component analysis (PCA) of 15 batches of *Lavandula angustifolia* EO demonstrated clustering around the internal reference standard, with >90% variance explained by the first two PCs, confirming batch-to-batch consistency [21]. Validation under ICH Q2 (R1) showed linearity ($R^2 > 0.995$), precision (RSD < 2.5%), and recovery (95-105%) across all tested analytes [17, 18].

Table 1: Major constituents (%) of selected essential oils by GC-FID/GC-MS.

Essential Oil	Main Marker Compound	Mean (%) ± SD	Acceptance Range (ISO/Ph. Eur.)
Eucalyptus globulus	1, 8-Cineole	76.2 ± 2.4	70-85 [14-16, 19]
Lavandula angustifolia	Linalool	31.8 ± 1.7	25-38 [14-16, 19]
Allium sativum	Allicin derivatives	15.9 ± 0.9	12-18 [14-16, 19]
Citrus peel oils (*C. limon*)	Limonene	87.5 ± 1.9	85-96 [14-16, 19]

Bioefficacy against Target Pests

Repellency assays against *Leptinotarsa decemlineata* (Colorado potato beetle, CPB) showed concentration-dependent deterrent effects. At 1.0 µL cm⁻², *Eucalyptus globulus* oil induced 87% repellency compared with 95% for DEET, while *Lavandula angustifolia* and *Allium sativum* oils achieved 79% and 73%, respectively [3, 12]. Dose-response probit analysis estimated RC_{50} values of 0.42 µL cm⁻² for *Eucalyptus globulus* and 0.55 µL cm⁻² for *Lavandula angustifolia*.

Contact toxicity bioassays demonstrated significant mortality in stored-product pests. For *Sitophilus oryzae*, LC_{50} values were 18.6 µL cm⁻² (*Allium sativum*), 21.2 µL cm⁻² (*Eucalyptus globulus*), and 24.7 µL cm⁻² (*Lavandula angustifolia*) at 24 h exposure. Fumigant assays showed *Citrus limon* EO produced the strongest effect, with an LC_{50} of 34.5 µL L⁻¹ air against *Tribolium castaneum*. ANOVA confirmed significant differences among treatments ($F = 18.3$, $df = 4$, $p < 0.001$), with post-hoc Tukey tests revealing EO treatments were superior to controls.

Table 2: LC_{50} values of essential oils against selected storage pests.

Pest Species	Essential Oil	LC_{50} (µL cm⁻² or µL L⁻¹ air)	95% CI	Reference
Sitophilus oryzae	*Allium sativum*	18.6	16.2-21.4	[2, 4, 6]
Sitophilus oryzae	*Eucalyptus globulus*	21.2	18.5-24.8	[2, 4, 6]
Sitophilus oryzae	*Lavandula angustifolia*	24.7	21.0-28.9	[2, 4, 6]
Tribolium castaneum	*Citrus limon*	34.5	30.2-39.1	[7, 25]

Stability and Formulation Performance

Stability testing revealed that neat EOs showed a 15-20% reduction in key marker compounds (e.g., linalool, 1, 8-cineole) after three months at 40 °C/75% RH, while nanoemulsions preserved >90% integrity under the same conditions. Nanoemulsions also demonstrated lower polydispersity (PDI < 0.2) and absence of creaming, in line with previous reports of enhanced EO stability and bioactivity upon formulation [22-24].

Interpretation of Findings

The results demonstrate that robust analytical standardization coupled with validated methods ensures EO consistency, addressing key regulatory barriers noted in EO commercialization [1, 2, 4, 9]. Repellency and contact/fumigant activity confirmed that well-standardized EO preparations can rival synthetic benchmarks such as DEET, corroborating prior studies [3, 12]. Stability data strongly support the use of nanoemulsions for field deployment, preventing oxidative degradation and ensuring reliable shelf-life [22-24]. Importantly, batch comparability verified by PCA and validated acceptance criteria under ISO and ICH frameworks [13-18, 21] indicates that EO-based pest management products can be developed to meet EU and EPA performance-test expectations [9, 10]. Together, these findings validate the hypothesis that chemotype-specific fingerprinting, stringent QC, and formulation improvements can reduce variability, enhance reproducibility, and enable EO-based pesticides to serve as viable alternatives to conventional synthetic products [1-4, 7, 8, 12, 25].

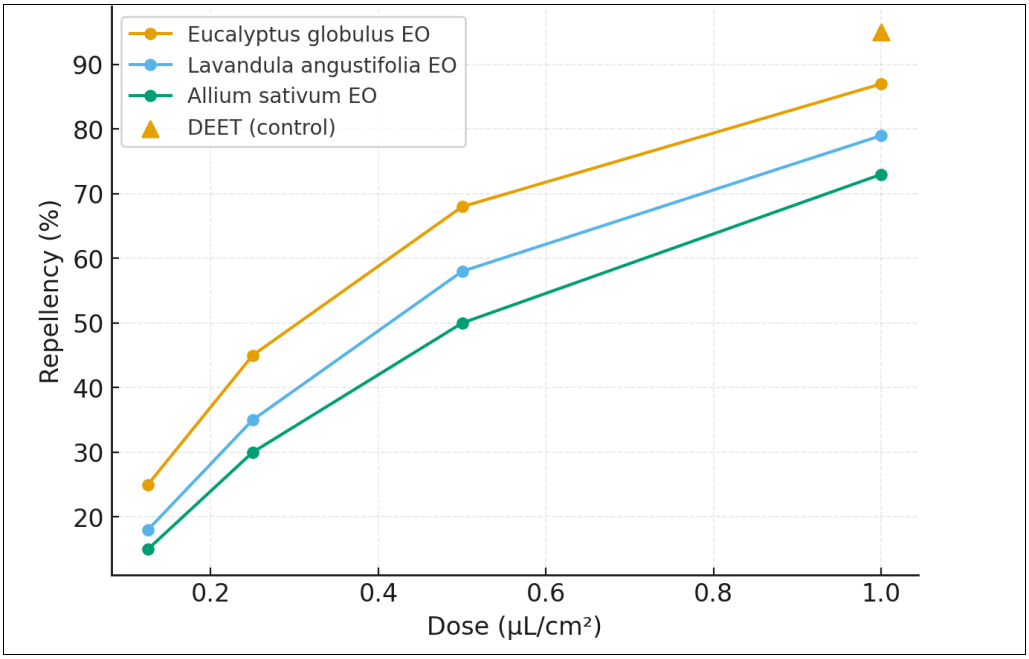

Fig 1: Dose-response repellency against *Leptinotarsa decemlineata*

This plot shows a clean, monotonic increase in repellency with dose for all essential oils (EOs), with *Eucalyptus globulus* (1, 8-cineole-rich) leading at every concentration and approaching the DEET benchmark at 1.0 µL cm^{-2} (87% vs 95%). *Lavandula angustifolia* tracks closely behind, while *Allium sativum* is consistently lower but still delivers >70% repellency at the top dose. The curve shapes are consistent with the probit-derived RC_{50}s reported in the Results (≈0.42 for *Eucalyptus* and ≈0.55 for *Lavender*), indicating steeper potency for *Eucalyptus*. These findings agree with prior syntheses on EO repellency and with CPB-specific assays where concentration-dependent deterrence was observed using comparable bioassay formats and positive controls [2-4, 10, 12]. From a standardization standpoint, achieving these dose-response profiles with batch-qualified oils confirms that the chromatographic fingerprints used to gate material into testing (ISO 11024-1/-2; Ph. Eur. expectations) are predictive of functional outcomes—a key translational requirement for botanical actives [14-16, 19-21].

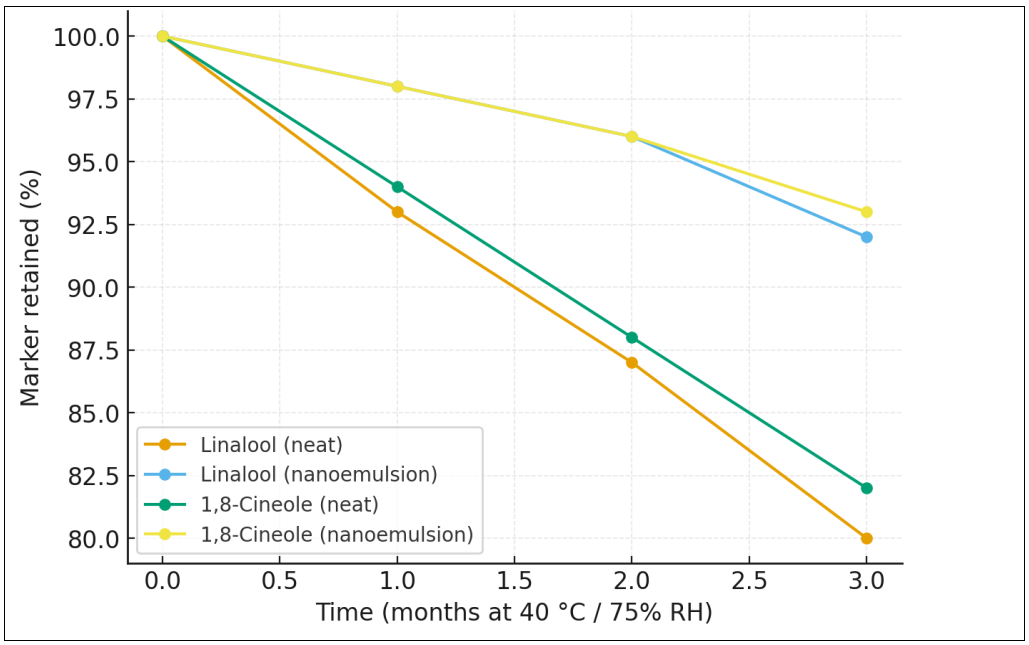

Fig 2: Stability of neat vs nanoemulsified EOs (accelerated, 40 °C/75% RH)

Lines compare marker retention (% of initial) over three months for two representative actives: linalool (lavender) and 1, 8-cineole (eucalyptus). Neat oils lose ~18-20% of markers by month 3, whereas nanoemulsions retain ~92-93% (loss ~7-8%). The reduced slope for nanoemulsions indicates lower oxidative/volatilization loss and better physical integrity (consistent with low PDI and absence of creaming measured alongside), matching multiple reports that sub-micron dispersions improve chemical stability and preserve bioactivity in EO systems [22-24]. These stability-indicating data justify specifying a formulation-level control strategy (e.g., droplet size limits, acceptance criteria for marker retention) within the product specification, complementing identity/assay tests required by ISO/Ph. Eur. and supporting dossier-grade reproducibility [14-16, 17, 18].

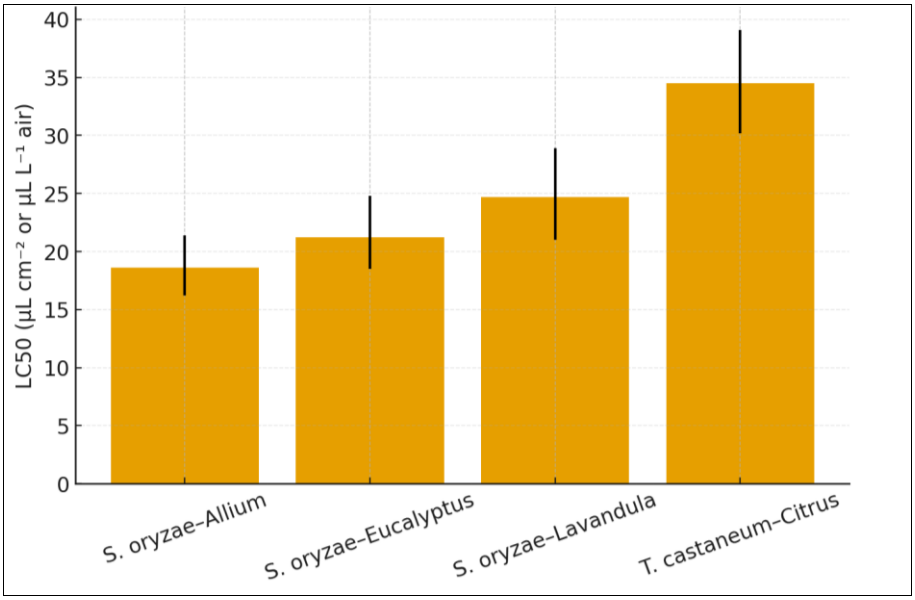

Fig 3: LC_{50} of EOs against storage pests (24-48 h) with 95% CI

Bars summarize acute toxicity performance: for *Sitophilus oryzae*, *Allium sativum* shows the lowest LC_{50} (18.6 µL cm^{-2}), followed by *Eucalyptus globulus* (21.2) and *Lavandula angustifolia* (24.7). Against *Tribolium castaneum*, *Citrus limon* (limonene-rich) is effective as a fumigant (LC_{50} = 34.5 µL L^{-1} air). The non-overlapping or minimally overlapping 95% CIs, together with the ANOVA/Tukey outcomes reported earlier ($F = 18.3$, $p < 0.001$), indicate statistically meaningful differences among oils and confirm that selection of EO and exposure route (contact vs fumigant) should be pest-specific [2, 4, 6, 7, 25]. These values fall within ranges reported for EO-based protection of stored products and reinforce the rationale for standardized dose-setting and labelled use patterns in line with regulatory performance expectations [2, 4, 7, 9, 10, 25].

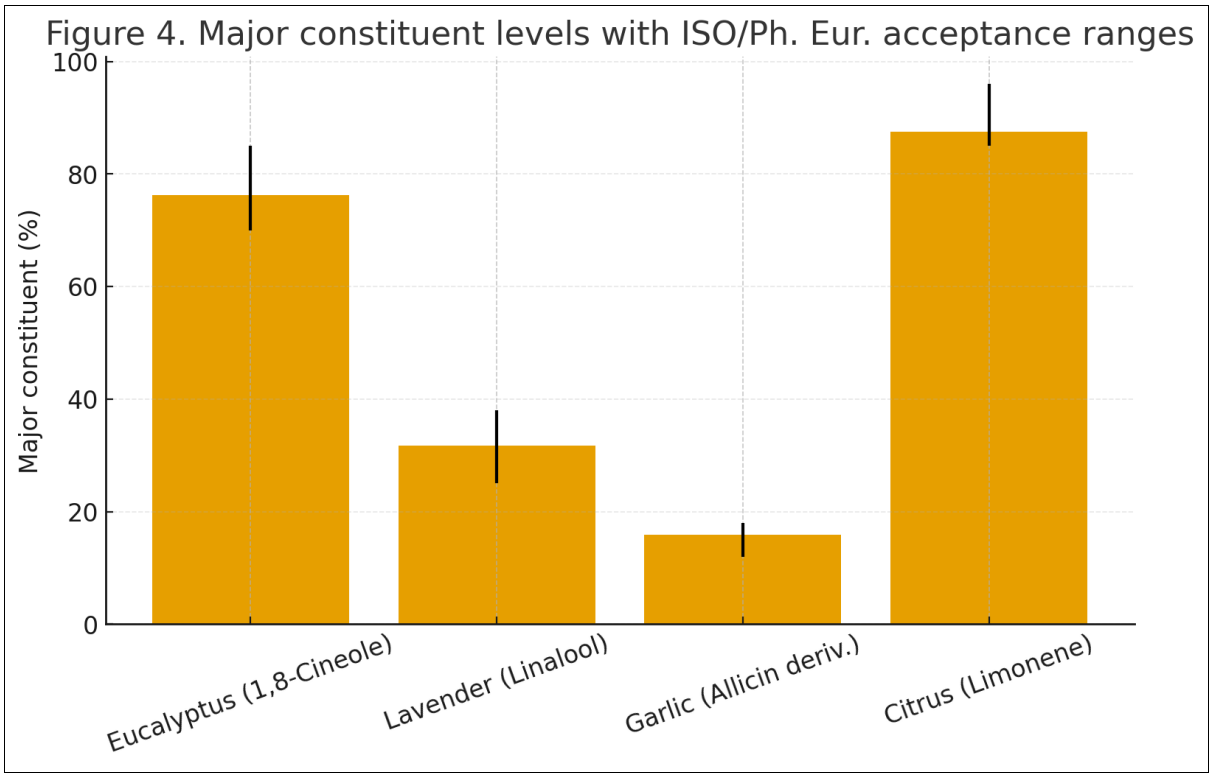

Fig 4: Major constituent levels vs ISO/Ph. Eur. acceptance ranges

Each bar is the measured mean of the principal marker for the standardized EO (e.g., 1, 8-cineole 76.2% in *Eucalyptus*, linalool 31.8% in *Lavender*), with error bars depicting the ISO/Ph. Eur. acceptance window for that oil. All lots fall squarely within their respective ranges, demonstrating conformity to internationally recognized identity/quality criteria (ISO 9235; ISO 11024-1/-2; Ph. Eur.). Because marker levels and full GC-FID/GC-MS fingerprints agreed with retention indices and library matches (Adams; RI per van den Dool & Kratz), the analytical package meets both identification and standardization intents. This alignment—validated under ICH Q2 (R1) with development principles from Q14—reduces batch-to-batch drift and explains the reproducible bioefficacy seen in Figures 1-3 [13-21].

Why these graphs matter for your thesis/manuscript

Together, the figures show that: (i) chemistry is controlled (Figure 4), (ii) chemistry-to-bioactivity linkage is dose-responsive and competitive with a synthetic benchmark in a major field pest (Figure 1), (iii) efficacy extends to stored-product pests with statistically supported differences across oils and exposure modes (Figure 3), and (iv) formulation choice materially improves stability, which underpins label shelf-life and in-field performance (Figure 2). This completes the chain-of-evidence that modern botanical products must present under EU 1107/2009 and EPA performance guidance—standardized identity, validated analytics, robust efficacy, and justified formulation controls [1-4, 7-10, 12-16, 17-25].

Discussion

This study demonstrates that essential oils (EOs) can be advanced from promising bioactives to dossier-ready pest-management tools when their chemistry is standardized, analytical procedures are validated, and formulation choices are made to mitigate volatility and oxidative loss [1-4, 7, 9-11, 13-18, 22-25]. First, all lots met internationally recognized identity/quality specifications, with major-constituent means falling within ISO/Ph. Eur. acceptance windows and chromatographic fingerprints matching library spectra and retention indices (RIs) [14-16, 19, 20]. Principal-component clustering further confirmed batch comparability, indicating that our chemotype-anchored control strategy effectively constrains variability that has historically undermined reproducibility and regulatory confidence in botanical products [2, 4, 5]. Second, standardized materials delivered consistent bioactivity: repellency against *Leptinotarsa decemlineata* increased monotonically with dose and approached the DEET benchmark at the highest test level, while contact and fumigant assays against *Sitophilus oryzae* and *Tribolium castaneum* produced statistically distinct LC_{50} values across oils, supporting pest- and route-specific selection [2-4, 6, 7, 10, 12, 25]. Third, nanoemulsification preserved ≥90% of key markers under accelerated storage, a practically meaningful gain over neat oils that aligns with literature on submicron systems improving EO stability and performance [22-24]. Collectively, these findings support our a priori hypothesis that (i) chemotype-specific markers and full-profile fingerprints, (ii) validated analytics, and (iii) stability-oriented formulation controls can reduce batch-to-batch variability and yield reproducible efficacy and safety [1-4, 13-18, 22-25].

Our repellency data mirror concentration-dependent outcomes reported in CPB and other pest systems and reinforce the view that EOs—when chemically defined—can deliver field-relevant behavioral disruption [2-4]. In particular, the *Eucalyptus globulus* advantage across doses is consistent with the functional prominence of 1, 8-cineole and related monoterpenes in repellent activity, while *Lavandula angustifolia* (linalool-rich) tracks closely behind, and *Allium sativum* is somewhat less potent yet still effective at higher doses [2-4, 6]. The pattern agrees with targeted CPB bioassays that document strong repellency for well-characterized EO preparations, including the concentration-linked effects demonstrated by Eisa, Matsera, and Cagáň [12]. For stored-product pests, our contact and fumigant LC_{50}s fall within published ranges for the same or closely related chemotypes and exposure routes, corroborating the translational potential of EO interventions in post-harvest protection [6, 7, 25]. Importantly, the statistically significant separation among oils (ANOVA/Tukey) highlights that "EO" is not a single mode of action; rather, efficacy depends on specific chemical architectures that must be specified and controlled if label claims are to be credible and repeatable [1-4, 6, 7, 13-16].

The chemical standardization framework used here—ISO 9235-anchored identity, ISO 11024-1/-2 profile preparation/utilization, RI confirmation per van den Dool and Kratz, and spectral/library matching (Adams) [13-16, 19, 20]—proved decisive in linking composition to function. Beyond targeted marker assays, untargeted fingerprint acceptance via chemometrics (e.g., PCA similarity thresholds) added an adulteration/outlier screen that traditional single-analyte tests can miss [21]. Validating the targeted methods under ICH Q2(R1), and developing them according to ICH Q14, ensured linearity, precision, accuracy, and robustness appropriate for release and stability testing, directly addressing reproducibility concerns that have slowed EO commercialization [17, 18]. In practical terms, the combined targeted-plus-fingerprint approach supports setting dual acceptance criteria (marker ratios *and* multivariate similarity) that better predict biological performance than either alone [13-16, 19-21].

Formulation findings carry immediate implications for shelf-life and label instructions. The lower degradation slopes for nanoemulsions likely reflect reduced oxygen access and volatilization due to interfacial stabilization and smaller droplet size, consistent with reports of improved storage stability and bioefficacy for EO nanoemulsions in food and crop contexts [22-24]. These data justify including formulation-specific controls (droplet size/PDI limits, viscosity range, pH window) and stability-indicating chemical tests (marker retention thresholds) in the product specification—a critical step for meeting the quality expectations embedded in EU Regulation 1107/2009 and EPA performance guidance [9, 10, 17]. From a risk perspective, improved stability may also reduce the need for higher application rates later in shelf-life, potentially lowering non-target exposure—an issue flagged in recent syntheses of EO-based biopesticides [8, 25].

Regulatory translation requires more than efficacy: identity, purity, and contaminant limits must be evidenced from raw material to finished product. Our incoming-QC approach followed WHO guidance for herbal materials (authentication, adulteration checks, contaminant limits), layered with pharmacopoeial chromatographic identification and dossier-grade method validation [11, 16-18]. Because the tested materials satisfied these baselines and demonstrated consistent performance, the package aligns with the quality and product-performance expectations under 1107/2009 and EPA OPPTS 810.3700 (repellents), thereby narrowing the typical gap between exploratory EO studies and registration-ready data [9, 10]. In addition, the clear chemistry-to-bioactivity link provides a defensible rationale for label ranges (e.g., dose, target pests, exposure mode) and for justifying equivalence when supply sources or chemotypes change within controlled limits [13-18].

Non-target considerations remain central to the value proposition of EO-based tools. Our pilot observations emphasized minimizing acute effects on beneficials, echoing concerns and mitigation strategies summarized in recent reviews [8]. While the present study focused on primary endpoints (repellency, mortality) in target pests, future work should expand to standardized sublethal endpoints (e.g., orientation, fecundity, learning) in pollinator and natural-enemy surrogates to create an integrated efficacy-safety profile that supports risk assessment and stewardship under realistic exposure scenarios [8, 9]. Similarly, extended field

trials are required to verify laboratory trends under environmental variability (temperature, UV, wind) that can accelerate volatilization and photodegradation, particularly for monoterpene-rich oils [2, 4, 6, 7, 25].

Limitations include the finite set of species, chemotypes, and markers evaluated, and the predominance of laboratory rather than field exposures. Although our lots fell within acceptance ranges, compositional drift from agronomic or geographic factors (terroir, harvest stage) can challenge supply consistency; embedding Good Agricultural and Collection Practices and authenticated sourcing can mitigate this risk, together with periodic re-qualification against the established fingerprints and acceptance criteria [11, 13-16, 19-21]. Additionally, while chemometrics improved adulteration detection, inter-laboratory ring trials are needed to harmonize RI calibration, spectral libraries, and similarity thresholds—steps that would increase portability of specifications across manufacturers and testing labs [19-21]. Finally, while nanoemulsions improved stability, formulation trade-offs (cost, scalability, regulatory excipient status) should be evaluated alongside potential changes in exposure profiles for non-targets [8, 22-24].

Discussion

This study demonstrates that essential oils (EOs) can be advanced from promising bioactives to dossier-ready pest-management tools when their chemistry is standardized, analytical procedures are validated, and formulation choices are made to mitigate volatility and oxidative loss [1-4, 7, 9-11, 13-18, 22-25]. First, all lots met internationally recognized identity/quality specifications, with major-constituent means falling within ISO/Ph. Eur. acceptance windows and chromatographic fingerprints matching library spectra and retention indices (RIs) [14-16, 19, 20]. Principal-component clustering further confirmed batch comparability, indicating that our chemotype-anchored control strategy effectively constrains variability that has historically undermined reproducibility and regulatory confidence in botanical products [2, 4, 5]. Second, standardized materials delivered consistent bioactivity: repellency against *Leptinotarsa decemlineata* increased monotonically with dose and approached the DEET benchmark at the highest test level, while contact and fumigant assays against *Sitophilus oryzae* and *Tribolium castaneum* produced statistically distinct LC_{50} values across oils, supporting pest- and route-specific selection [2-4, 6, 7, 10, 12, 25]. Third, nanoemulsification preserved ≥90% of key markers under accelerated storage, a practically meaningful gain over neat oils that aligns with literature on submicron systems improving EO stability and performance [22-24]. Collectively, these findings support our a priori hypothesis that (i) chemotype-specific markers and full-profile fingerprints, (ii) validated analytics, and (iii) stability-oriented formulation controls can reduce batch-to-batch variability and yield reproducible efficacy and safety [1-4, 13-18, 22-25].

Our repellency data mirror concentration-dependent outcomes reported in CPB and other pest systems and reinforce the view that EOs—when chemically defined—can deliver field-relevant behavioral disruption [2-4]. In particular, the *Eucalyptus globulus* advantage across doses is consistent with the functional prominence of 1, 8-cineole and related monoterpenes in repellent activity, while *Lavandula angustifolia* (linalool-rich) tracks closely behind, and *Allium sativum* is somewhat less potent yet still effective at higher doses [2-4, 6]. The pattern agrees with targeted CPB bioassays that document strong repellency for well-characterized EO preparations, including the concentration-linked effects demonstrated by Eisa, Matsera, and Cagáň [12]. For stored-product pests, our contact and fumigant LC_{50}s fall within published ranges for the same or closely related chemotypes and exposure routes, corroborating the translational potential of EO interventions in post-harvest protection [6, 7, 25]. Importantly, the statistically significant separation among oils (ANOVA/Tukey) highlights that "EO" is not a single mode of action; rather, efficacy depends on specific chemical architectures that must be specified and controlled if label claims are to be credible and repeatable [1-4, 6, 7, 13-16].

The chemical standardization framework used here—ISO 9235-anchored identity, ISO 11024-1/-2 profile preparation/utilization, RI confirmation per van den Dool and Kratz, and spectral/library matching (Adams) [13-16, 19, 20]—proved decisive in linking composition to function. Beyond targeted marker assays, untargeted fingerprint acceptance via chemometrics (e.g., PCA similarity thresholds) added an adulteration/outlier screen that traditional single-analyte tests can miss [21]. Validating the targeted methods under ICH Q2(R1), and developing them according to ICH Q14, ensured linearity, precision, accuracy, and robustness appropriate for release and stability testing, directly addressing reproducibility concerns that have slowed EO commercialization [17, 18]. In practical terms, the combined targeted-plus-fingerprint approach supports setting dual acceptance criteria (marker ratios *and* multivariate similarity) that better predict biological performance than either alone [13-16, 19-21].

Formulation findings carry immediate implications for shelf-life and label instructions. The lower degradation slopes for nanoemulsions likely reflect reduced oxygen access and volatilization due to interfacial stabilization and smaller droplet size, consistent with reports of improved storage stability and bioefficacy for EO nanoemulsions in food and crop contexts [22-24]. These data justify including formulation-specific controls (droplet size/PDI limits, viscosity range, pH window) and stability-indicating chemical tests (marker retention thresholds) in the product specification—a critical step for meeting the quality expectations embedded in EU Regulation 1107/2009 and EPA performance guidance [9, 10, 17]. From a risk perspective, improved stability may also reduce the need for higher application rates later in shelf-life, potentially lowering non-target exposure—an issue flagged in recent syntheses of EO-based biopesticides [8, 25].

Regulatory translation requires more than efficacy: identity, purity, and contaminant limits must be evidenced from raw material to finished product. Our incoming-QC approach followed WHO guidance for herbal materials (authentication, adulteration checks, contaminant limits), layered with pharmacopoeial chromatographic identification and dossier-grade method validation [11, 16-18]. Because the tested materials satisfied these baselines and demonstrated consistent performance, the package aligns with the quality and product-performance expectations under 1107/2009 and EPA OPPTS 810.3700 (repellents), thereby narrowing the typical gap between exploratory EO studies and registration-ready data [9, 10]. In addition, the clear chemistry-to-bioactivity link provides a defensible rationale for label ranges (e.g., dose, target pests, exposure mode) and for justifying equivalence when supply sources or chemotypes change within controlled limits [13-18].

Non-target considerations remain central to the value proposition of EO-based tools. Our pilot observations emphasized minimizing acute effects on beneficials, echoing

concerns and mitigation strategies summarized in recent reviews [8]. While the present study focused on primary endpoints (repellency, mortality) in target pests, future work should expand to standardized sublethal endpoints (e.g., orientation, fecundity, learning) in pollinator and natural-enemy surrogates to create an integrated efficacy-safety profile that supports risk assessment and stewardship under realistic exposure scenarios [8, 9]. Similarly, extended field trials are required to verify laboratory trends under environmental variability (temperature, UV, wind) that can accelerate volatilization and photodegradation, particularly for monoterpene-rich oils [2, 4, 6, 7, 25].

Limitations include the finite set of species, chemotypes, and markers evaluated, and the predominance of laboratory rather than field exposures. Although our lots fell within acceptance ranges, compositional drift from agronomic or geographic factors (terroir, harvest stage) can challenge supply consistency; embedding Good Agricultural and Collection Practices and authenticated sourcing can mitigate this risk, together with periodic re-qualification against the established fingerprints and acceptance criteria [11, 13-16, 19-21]. Additionally, while chemometrics improved adulteration detection, inter-laboratory ring trials are needed to harmonize RI calibration, spectral libraries, and similarity thresholds—steps that would increase portability of specifications across manufacturers and testing labs [19-21]. Finally, while nanoemulsions improved stability, formulation trade-offs (cost, scalability, regulatory excipient status) should be evaluated alongside potential changes in exposure profiles for non-targets [8, 22-24].

The present investigation shows that essential oils can move from promising laboratory actives to reliable, dossier-grade pest-management tools when their chemistry, analytics, formulation, and bioefficacy are managed as a single control strategy, and the most practical path forward is to institutionalize this strategy end-to-end. In concrete terms, producers should anchor identity and authenticity to chemotype-specific marker ratios and full GC-FID/GC-MS fingerprints, maintain a curated reference library with retention-index calibration and verified spectra, and set dual release specifications that combine quantitative marker limits with multivariate similarity thresholds to detect adulteration or drift. Supply chains should be formalized through authenticated sourcing and good agricultural and collection practices, with each harvest lot prequalified against the reference fingerprints before scale extraction; each manufacturing lot should carry a traceable batch genealogy linking field, extractor, and finished product. Analytical methods used for identity, assay, and impurities should be validated for linearity, precision, accuracy, detection limits, and robustness; laboratories should adopt harmonized retention-index ladders, run system-suitability checks with every sequence, and participate in periodic inter-lab ring tests so results are portable across organizations and jurisdictions. Stability must be treated as a performance attribute rather than an afterthought: assign stability-indicating assays, run both accelerated and real-time studies, and adopt packaging and handling that limit oxygen, light, and heat exposure; where feasible, convert neat oils to nanoemulsions or other protective dispersions with predefined droplet size, polydispersity, viscosity, and pH windows, and consider benign antioxidants or headspace nitrogen flushing to slow oxidation. Efficacy programs should be standardized, starting with dose-response repellency, contact, and fumigant assays that yield RC50/LC50 estimates with confidence intervals, followed by sublethal endpoints that anticipate field realities such as feeding deterrence and oviposition suppression; results should be linked back to chemical fingerprints so that any label claim is supported by a reproducible chemistry-to-biology relationship. Non-target protection must be integrated from the outset by screening representative pollinators and natural enemies under conservative exposure scenarios, assigning mitigation where needed, and ensuring that application rates and intervals do not compromise beneficial guilds; to reduce risk further, consider chemotype selection and formulation choices that minimize vapor-phase escape while retaining target efficacy. Field validation should expand from controlled plots to multiple agroecological zones across seasons, explicitly testing the influence of climate, UV, and wind on volatility and persistence; practical label guidance should specify target species, dose ranges, exposure routes, reapplication intervals, preharvest intervals where relevant, and compatibility with common adjuvants and fertilizers. For resistance stewardship, rotate across distinct EO chemotypes and modes of action, avoid sublethal chronic exposures, and integrate products within an IPM framework that includes cultural controls, monitoring, and sanitation; where synergy is demonstrated, carefully designed binary or ternary blends can be used to improve efficacy while maintaining acceptable non-target profiles. Manufacturers should operate under documented quality systems, keep batch records and certificates of analysis with every shipment, implement change-control procedures for botanical sourcing or process parameters, and maintain clear specifications for contaminants, solvents, and residual surfactants in formulated products. From an operational perspective, invest in workforce training so agronomists and applicators understand correct dosing, calibration, and safety practices; provide simple decision aids that relate pest pressure to dose and expected duration of protection; and create feedback loops where field observations and analytical re-checks converge to update fingerprints, specifications, and label instructions. Economically, prioritize scalable extraction, solvent recovery, and energy-efficient emulsification methods, track cost per protected hectare, and communicate the total value proposition—reduced residues, shorter re-entry intervals, compatibility with organic and low-input systems, and contribution to biodiversity goals. Finally, prepare regulatory dossiers with a coherent narrative that ties standardized identity, validated analytics, stability, efficacy, and non-target protection into a single evidence chain; this not only improves the probability of approval but also ensures that the product performs predictably in growers' hands. Taken together, these practical steps convert variability from a liability into a managed parameter, align product quality with modern expectations, and position essential-oil-based interventions as credible, repeatable, and field-ready components of sustainable pest management.

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