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Chemical Variability Is the Hidden Determinant of Botanical Insecticide Reproducibility, Environmental Safety, and Field-Level Performance

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ABSTRACT

Botanical insecticides are increasingly investigated as alternatives or complements to conventional pest-management products, yet the biological material described by a plant name rarely represents a chemically stable or operationally uniform intervention. Genetic provenance, chemotype, cultivation environment, harvest timing, post-harvest handling, extraction, analytical methods, formulation, storage, application, and environmental exposure can each alter the composition and performance of the tested product. This article develops an original, evidence-grounded, and explicitly non-validated standardization approach for managing this variability across botanical-insecticide development. It integrates plant-source qualification, chemotype and harvest control, processing and extraction specifications, assay-linked chemical characterization, formulation stability, environmental exposure, biological performance, and safety assessment. The central synthesis is that variability should be treated as a propagated product property rather than as experimental noise addressed only after efficacy testing. Botanical species identity cannot establish chemical equivalence; a reported analytical profile cannot establish batch standardization; repeatable laboratory activity cannot establish field performance; and natural origin cannot establish predictable exposure or environmental safety. The available evidence nevertheless remains heterogeneous across botanical materials, target organisms, extraction systems, formulations, endpoints, and environmental contexts. Associations between chemical markers and bioactivity are frequently context-dependent, while replicated batch series, interlaboratory comparisons, realistic exposure studies, and chemically qualified field evaluations remain limited. Product development should therefore move from identity-based candidate descriptions toward traceable evidence chains connecting source, process, chemical composition, formulation state, delivered exposure, efficacy, crop compatibility, and non-target effects. Such a structure can guide prospective validation and decision making, but its components and acceptance criteria must be established separately for each defined product class, intended use, target, formulation, and exposure scenario.

Keywords: Botanical insecticides, Chemical identity, Chemotype, Plant-source selection, Active constituents, Analytical standardization.

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INTRODUCTION

Botanical insecticides occupy an important but unsettled position between traditional plant use, natural-product discovery, formulation science, and operational pest management. Their attraction arises from extensive phytochemical diversity, multiple potential modes of action, renewable biological sources, and the possibility

of developing products that complement existing control strategies. These advantages, however, are often discussed at the level of a plant species, extract category, or dominant compound, even though the entity delivered to an insect, crop, worker, or non-target organism is a chemically and physically specific product. Botanical insecticides can provide useful pest-management functions, but their promise depends on replacing identity-based assumptions with chemically and operationally defined products [1]. The central scientific problem is therefore not simply whether a plant preparation can produce biological activity. It is whether the material can be defined, reproduced, formulated, stored, delivered, and evaluated with sufficient consistency for its efficacy and safety claims to remain interpretable across batches and use conditions.

Discovery-oriented studies commonly begin with laboratory mortality, repellency, feeding inhibition, developmental disruption, or another target-organism endpoint. These experiments are valuable for identifying biological potential, but they rarely resolve whether the same chemical entity can be produced repeatedly or whether its activity will persist after formulation, storage, dilution, application, and environmental exposure. Laboratory activity is therefore only the beginning of product development, because formulation, persistence, application context, and farm-level performance remain separate evidentiary questions [2]. A botanical preparation that performs strongly in a controlled assay may lose volatile constituents, separate during storage, deposit unevenly, degrade rapidly, injure the treated crop, or expose beneficial organisms through routes absent from the discovery experiment. Conversely, a preparation with moderate acute toxicity may provide useful crop protection through repellency, feeding deterrence, developmental effects, or repeated low-intensity exposure. Laboratory potency, product performance, and operational effectiveness must consequently remain analytically distinct.

A further difficulty is that evidence is accumulated across non-equivalent materials and methods. Studies may use different plant organs, accessions, harvest seasons, drying conditions, extraction solvents, concentration units, exposure routes, target stages, observation periods, and endpoint definitions while applying the same species or extract label. The evidence base also contains recurrent taxonomic, methodological, and reporting biases that can conceal product-level heterogeneity and overstate generalizability [3]. A pooled category such as “essential oils,” for example, may include chemically distinct mixtures delivered by fumigation, contact exposure, treated surfaces, emulsions, or nanocarriers. Apparent disagreement among studies may therefore reflect genuine biological heterogeneity, uncontrolled chemical variation, exposure differences, or incompatible outcome measures rather than simple experimental error. Meaningful synthesis requires the tested entity and its context to be reconstructed before results are compared.

This article addresses that gap by treating chemical variability as a product-development determinant generated across the complete botanical-insecticide chain. Its scope extends from botanical provenance and chemotype through harvest, processing, extraction, analytical characterization, formulation, degradation, environmental exposure, reproducibility, safety, and field-level performance. The central argument is that standardization cannot be reduced to taxonomic authentication, a dominant-marker assay, a chromatographic fingerprint, or a single potency test. It must connect controlled inputs and processes to measured chemical attributes, formulation behavior, delivered exposure, target performance, and relevant safety outcomes. The proposed contribution is an original scholarly organization of these relations rather than a validated universal standard. Its purpose is to identify the distinctions, decision points, failure modes, and validation requirements needed to transform a biologically active botanical material into a chemically interpretable and prospectively testable product class.

Sources of chemical variability in botanical materials

Chemical variability begins before extraction or bioassay and may be embedded in the source material through taxonomy, accession, genotype, cultivation, collection, phenology, geography, and supply-chain history. A reproducible botanical product begins with authenticated

provenance and a controlled supply chain, because local use records do not by themselves define a chemically equivalent material [4]. Ethnobotanical knowledge can identify useful species and preparation traditions, but vernacular identity and historical efficacy cannot establish that material collected from different regions, populations, or production systems has the same metabolite distribution. Source qualification therefore requires taxonomic authentication, plant-part definition, voucher or reference material, accession traceability where relevant, and documentation of cultivation or collection conditions. These controls establish provenance, not chemical equivalence; the chemical output of each qualified source must still be measured.

Within a single named species, essential-oil profiles and activity can vary materially among sources, demonstrating that species identity is not a chemical-equivalence criterion [5]. Variation may involve the presence or absence of constituents, shifts in the ratios among major compounds, changes in low-abundance metabolites, or differences in total recoverable oil or extract. Some compositional differences may alter penetration, volatility, target binding, mixture interactions, formulation compatibility, or degradation behavior. However, a correlation between a profile and an insecticidal response does not establish that the most abundant constituent caused the observed effect. Covarying compounds, extraction yield, exposure conditions, or assay sensitivity may provide competing explanations. Chemical markers should therefore be treated as provisional indicators until they demonstrate predictive relevance across independent batches and target-specific assays.

Preparation and environmental history further determine which chemical material reaches extraction. Preparation method is itself a source of chemical variability, and incomplete descriptions of plant state, solvent, ratio, duration, filtration, and dose prevent faithful replication [6]. Drying temperature, light, oxygen, moisture, storage duration, milling, transport, and contamination can alter volatile and non-volatile constituents before the formal extraction step begins. Geographic location, accession, and season can shift the composition of harvested botanical material even before extraction begins [7]. These influences may interact rather than operate independently: an accession may respond differently across seasons, while post-harvest processing may amplify or obscure source-related variation. The appropriate development response is not to presume that one favorable collection defines the species, but to specify a source-and-process envelope and verify each production lot against measured chemical attributes.

The evidence dimensions and interpretive boundaries for sources of chemical variability in botanical materials are summarized in **Table 1**.

Table 1. Sources of Chemical Variability in Botanical Materials: Chemical Identity, Mechanism, Standardization, Formulation, Safety, Resistance, and Translation Requirements

Candidate or product factor	Chemical or biological basis	Required characterization	Potential contribution	Formulation implication	Safety implication	Resistance implication	Development boundary
Botanical identity and provenance	Taxon, plant part, accession, geography, and production history constrain the starting chemical population	Voucher or reference material, plant-part confirmation, accession and chain-of-custody records	Prevents substitution and supports investigation of source-related deviations	Qualified source material reduces uncontrolled payload shifts but does not ensure formulation compatibility	Misidentification or source substitution may change hazardous constituents and exposure	Source consistency is required before resistance effects can be interpreted	Authentication establishes origin, not chemical or biological equivalence
Within-species chemical variation	Biosynthetic and environmental differences alter constituent identities and ratios	Multi-lot chromatographic fingerprinting, marker content, yield, and linked potency assays	Makes lot heterogeneity visible and supports product-specific acceptance regions	Variable constituent ratios may alter solubility, emulsification, encapsulation, release, and degradation	Similar species labels may conceal different target and non-target profiles	Changing mode-of-action mixtures may change selection pressure, but this must be tested	One species name or one representative profile cannot define a stable product
Preparation method	Plant state, solvent, ratio, extraction duration, filtration, and applied	Complete preparation and dose metadata with retained samples where feasible	Enables replication and distinguishes process variation from biological variation	Preparation inconsistency complicates later formulation and scale-up	Uncontrolled concentration or contaminants may alter user, crop, and ecological hazards	Variable exposure prevents defensible resistance comparisons	Traditional repeatability is not equivalent to controlled manufacturing reproducibility

	concentration determine the tested material						
Geography, accession, and season	Genotype-by-environment and seasonal regulation modify secondary metabolism	Repeated sampling across locations and seasons with compositional profiling	Supports risk-based source qualification and harvest planning	Seasonal payload shifts may alter carrier requirements and stability	Seasonal profiles may change hazardous or irritating constituents	Resistance relevance cannot be inferred without stable exposure and mode-of-action evidence	Compositional variation alone does not establish corresponding efficacy variation
Chemotype	Recurring profile classes reflect biosynthetic differences but retain within-class variation	Replicated chemotype classification, marker panel, reference material, and batch history	Narrows source heterogeneity and supports stratified specifications	Distinct chemotypes may require separate formulation or comparability studies	Chemotype identity does not define selectivity or environmental safety	Chemotype-specific mode balance may matter, but empirical selection studies are required	Chemotype is an input stratum, not a final release criterion
Whole mixture versus dominant marker	Mixture activity may differ from the activity of a major constituent through additive, antagonistic, or synergistic relations	Whole-product fingerprint, marker assay, constituent comparison, and endpoint-specific bioassays	Prevents unjustified single-marker standardization	Formulation may affect constituents differently and change mixture relations	A marker's safety cannot substitute for assessment of the complete product	Resistance claims based on presumed multi-target activity require direct evidence	Dominant abundance does not establish biological equivalence to the whole oil
Extraction process	Solvent and operating conditions selectively recover or transform chemical fractions	Locked process parameters, mass balance, fingerprint, marker recovery, impurities, and linked assays	Defines the extract as a process-specific chemical product	Materially different extracts may require separate product and formulation classes	Extraction can concentrate hazardous constituents or introduce residual-process concerns	Non-equivalent extracts cannot support pooled resistance conclusions	Equal crude yield does not mean equal chemical composition or activity

Chemotypes, harvest conditions, and plant processing

Chemotype classification is often proposed as a practical bridge between botanical identity and chemical specification. It can reduce heterogeneity by separating populations or accessions with recurrent compositional patterns, but it does not freeze constituent concentrations within a class. Chemotype classification narrows, but does not eliminate, within-source variability because constituent abundance can continue to shift across seasonal harvests [8]. A useful chemotype designation therefore requires replicated classification across locations, seasons, and production lots rather than assignment from one sample or one dominant peak. It should also be connected to the intended biological function. A chemically stable class may still lack predictive value if the distinguishing markers do not explain or forecast target activity, formulation behavior, degradation, or safety.

The convenience of a dominant chemical marker creates a particular standardization risk. A dominant constituent may be analytically convenient without being biologically equivalent to the whole oil, so a single-marker specification requires target-specific validation [9]. Whole mixtures can produce target-dependent effects that are not reproduced by the isolated major component, because minor constituents may alter penetration, volatility, metabolism, receptor interactions, or the effective ratio among active components. The opposite is also possible: a dominant constituent may account for much of the observed activity in one assay but not in another. Standardization should consequently combine marker content with a broader fingerprint and a relevant biological assay unless prospective evidence demonstrates that the marker reliably predicts whole-product performance across representative and edge-of-specification batches.

Harvest and post-harvest controls must also be interpreted in relation to the use environment and endpoint. The same applied material can produce different outcomes under different temperatures, showing that a batch specification cannot substitute for an exposure-context specification [10]. Temperature can influence volatilization, persistence, penetration, insect physiology, and effective exposure duration without changing the nominal dose. Processing introduces additional variability through drying, storage, milling, oxidation, moisture, light, and hold time. Chemical characterization must be

paired with endpoint-specific testing because toxicity, repellency, and other functions are not interchangeable measures of performance [11]. A product that meets a mortality specification may not meet a repellency, ovicidal, crop-protection, or residual-performance specification. Source qualification, harvest envelopes, controlled processing, chemical release tests, and endpoint definitions must therefore be designed as connected controls rather than independent labels.

Extraction and analytical characterization

Extraction is the transition at which variable botanical material becomes a defined oil, fraction, crude extract, or purified product. It should not be treated as a neutral laboratory preparation step. Extraction should be treated as a controlled manufacturing operation because it determines which chemical fraction enters all subsequent characterization and efficacy tests [12]. Solvent polarity, plant-to-solvent ratio, particle size, temperature, pressure, duration, mixing, distillation conditions, and separation procedures can change recovery, transform unstable constituents, or create materially different products. Reproducibility therefore requires specified critical process parameters, mass balance, yield, complete chemical profiling, impurity assessment, and retained samples where feasible. Scale-up must be assessed separately because equipment geometry, heat transfer, solvent recovery, residence time, and separation efficiency may change the product even when the nominal procedure remains similar.

Analytical characterization must be matched to the entity being evaluated. For formulated botanicals, analytical characterization must include both the chemical payload and the delivery system because droplet or particle properties can change release and exposure [13]. Chemical identity, marker content, fingerprint similarity, impurities, degradation products, carrier composition, particle or droplet distribution, encapsulation, interfacial properties, and release behavior answer different questions. No single measurement can establish all of them. Solvent and extraction design can change both the recovered chemical profile and the apparent spectrum of activity, so extraction equivalence must be demonstrated rather than presumed [14]. Extracts prepared by materially different processes should be classified as separate product entities unless bridging studies demonstrate comparable chemistry, biological performance, formulation behavior, and safety.

The principal interpretive boundary is the difference between describing a batch and standardizing a product. A credible specification should connect an analytical fingerprint to a defined biological assay, while avoiding the inference that either one alone proves batch standardization [15]. A chromatogram can reveal identity and variation, but standardization also requires validated methods, representative batch history, prespecified acceptance criteria, sampling rules, measurement uncertainty, deviation investigation, and evidence that accepted variation remains compatible with relevant potency and safety outcomes. Likewise, a repeatable bioassay can indicate biological consistency without identifying chemical drift or explaining a future failure. Analytical and biological methods should therefore operate as complementary components of an assay-linked specification. The required acceptance region must remain product-, target-, formulation-, and endpoint-specific, and its predictive value must be established prospectively rather than assumed from retrospective agreement.

Formulation, degradation, and environmental exposure

Formulation is the point at which a chemically characterized botanical payload becomes a finished delivery system, and the transformation can change both biological performance and environmental behavior. Formulation is not merely a convenience layer: it can alter degradation, persistence, deposition, release, and the environmental compartments that receive the active material [16]. Emulsifiers, encapsulating matrices, carriers, interfacial properties, droplet or particle distributions, viscosity, adhesion, and release kinetics influence how much material reaches the target, how long it remains available, and which non-target receptors are exposed. A finished product must therefore be identified by the botanical payload and its qualified chemical range together with its formulation composition, physical state, storage condition, container, dilution procedure,

application method, and intended exposure envelope. Greater laboratory potency after formulation may represent improved dispersion or exposure rather than increased intrinsic toxicity.

Once an oil is converted into a nanoemulsion, the product is no longer defined by the oil alone because the interfacial system and droplet properties become determinants of exposure and activity [17]. Formulation development must consequently evaluate chemical and physical stability as related but non-equivalent properties. Oxidation, hydrolysis, volatilization, constituent redistribution, aggregation, phase separation, or carrier degradation can change the delivered dose even when the product retains an acceptable appearance. Formulation stability and target toxicity are complementary outcomes: neither can be used as a proxy for the other, and both require prespecified acceptance criteria [18]. Shelf-life and in-use specifications should therefore be supported by stability-indicating chemical methods, appropriate physical measurements, release or deposition assessment, and a relevant potency assay. A formulation tested with one chemically favorable payload lot cannot be presumed robust to the full accepted range of botanical variability.

Environmental exposure extends the standardization problem beyond the container. Application rate, spray quality, temperature, humidity, rainfall, ultraviolet radiation, crop surface, soil, water, air movement, pest location, and receptor behavior determine the dose that reaches target and non-target organisms. Hazard and exposure must remain separate: a laboratory hazard does not by itself quantify field risk, but missing exposure data cannot be interpreted as evidence of safety. A formulation that increases target activity may still fail product development if it creates unacceptable crop injury or other exposure-dependent harms [19]. Intended-use scenarios should therefore specify crop or storage system, application route, environmental conditions, relevant receptors, and plausible exposure pathways. Natural origin is not equivalent to predictable environmental exposure, and labels such as natural, green, or eco-friendly cannot replace product-specific evidence on residues, fate, crop compatibility, worker contact, beneficial organisms, and other relevant environmental compartments.

Reproducibility, safety, and performance consequences

Chemical variability is propagated rather than erased as botanical material moves from source qualification through extraction, characterization, formulation, storage, application, and environmental exposure. Variability at an upstream stage can be attenuated by effective controls, amplified by formulation or environment, or concealed when only one biological endpoint is measured. Field evidence can demonstrate crop protection, yield, and beneficial-arthropod outcomes that are invisible to laboratory mortality assays, although uncontrolled batch chemistry still limits reproducibility [20]. Reproducibility should therefore be evaluated as agreement among defined chemical products under specified exposure conditions, not merely as repeated mortality within one assay. It includes within-laboratory repeatability, between-batch comparability, interlaboratory reproducibility, stability over time, and performance across relevant environments.

Figure 1 traces sources of chemical variability from plant genetics and cultivation to extraction, formulation, and field exposure within the analytical logic developed in this section.

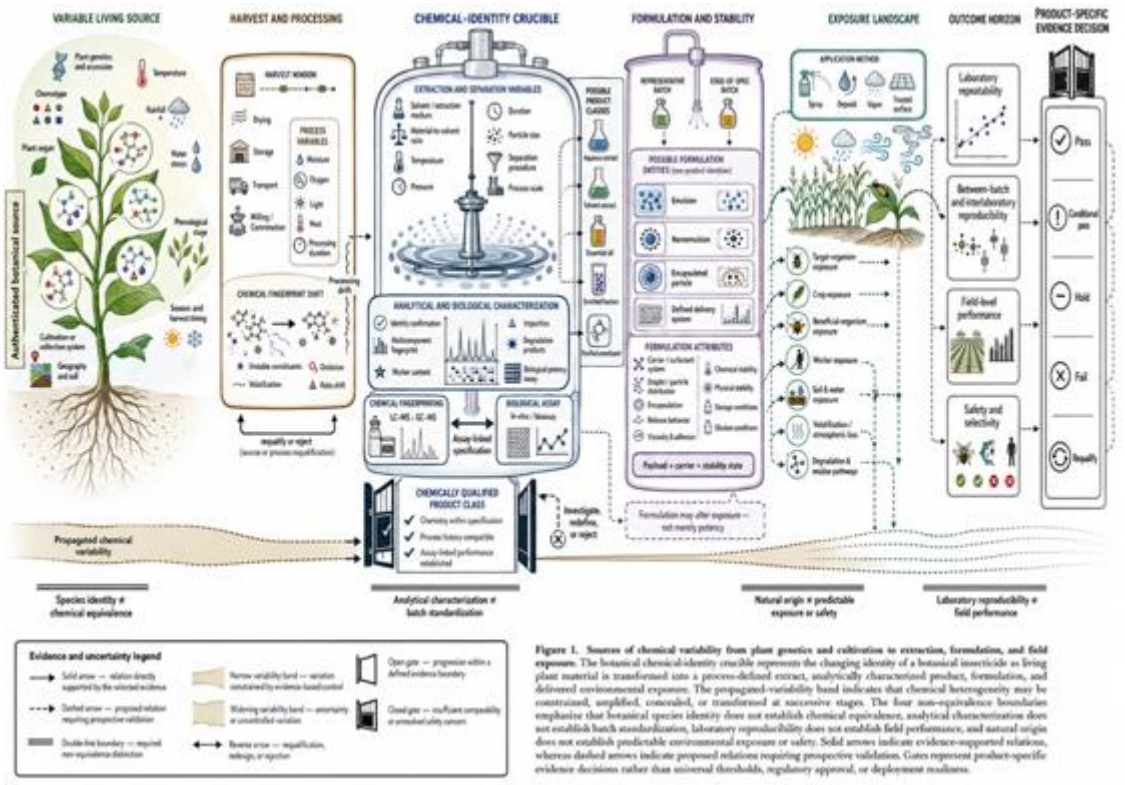

Figure 1. Sources of chemical variability from plant genetics and cultivation to extraction, formulation, and field exposure

Alt text

A structured conceptual diagram that traces sources of chemical variability from plant genetics and cultivation to extraction, formulation, and field exposure, with labelled components, directional relations, contextual modifiers, uncertainty points, and a clear boundary between observed evidence and proposed synthesis.

The practical meaning of reproducibility depends on matching the chemical profile, exposure route, target stage, and endpoint. Reproducibility requires matching chemical profile, exposure route, target stage, and endpoint because essential-oil performance is not a product-invariant property [21]. A batch may be repeatable in a contact-mortality assay yet differ in repellency, fumigant action, residual persistence, ovicidal activity, or crop-system performance. Product comparisons should therefore retain batch identifiers, analytical results, formulation state, application conditions, target stage, exposure route, observation period, and endpoint definition. Common reference materials and predefined comparability criteria are needed to separate product drift from assay imprecision. One successful replication cannot establish broader reproducibility, and a statistically similar mean cannot demonstrate equivalence when chemically important variation or endpoint-specific failures remain unresolved.

Safety and field performance further expand the relevant outcome space. A botanical treatment can influence crop growth and yield through pathways not captured by insect mortality, so product performance must be evaluated as a crop-system outcome [22]. Such effects may represent pest suppression, altered feeding, physiological plant responses, formulation effects, or interactions that cannot be inferred from acute mortality alone. Natural origin and target efficacy do not establish environmental safety, as an effective botanical nanoformulation can still produce consequential pollinator toxicity [23]. Hazard findings must be connected to realistic exposure before field risk is inferred, but beneficial-organism survival in one field context likewise cannot be generalized to all formulations, rates, crops, seasons, or receptors. Reproducibility, efficacy, crop compatibility, selectivity, and environmental safety are therefore related product attributes that require

separate evidence and an explicit intended-use context.

The evidence dimensions and interpretive boundaries for reproducibility safety and performance consequences are summarized in **Table 2**.

Table 2. Reproducibility, Safety, and Performance Consequences: Chemical Identity, Mechanism, Standardization, Formulation, Safety, Resistance, and Translation Requirements

Candidate or product factor	Chemical or biological basis	Required characterization	Potential contribution	Formulation implication	Safety implication	Resistance implication	Development boundary
Field performance	Crop protection depends on pest pressure, crop condition, application, environment, and product chemistry	Chemically qualified batch, field protocol, pest endpoints, yield or damage outcomes, and beneficial-organism observations	Demonstrates outcomes unavailable from laboratory mortality alone	Delivery and persistence must remain suitable under field application	Beneficial-organism outcomes require product- and context-specific interpretation	Field exposure quality must be known before selection pressure can be assessed	One field result cannot validate chemically uncontrolled batches or other environments
Reproducibility across batches and assays	Chemistry, target stage, exposure route, endpoint, and method contribute separate variance components	Batch fingerprints, potency assays, common protocols, reference material, and variance reporting	Distinguishes product drift from technical imprecision	Formulation lots must be compared alongside payload chemistry	Reproducible target activity does not prove reproducible safety	Consistent exposure is required for interpretable resistance studies	Technical replicate agreement is not product reproducibility
Crop-system performance	Botanical preparations may affect pest pressure, plant physiology, growth, or yield	Pest, crop-growth, injury, and yield endpoints with product and exposure metadata	Expands performance assessment beyond insect mortality	Excipients and formulation may contribute to crop responses	Beneficial effects do not rule out phytotoxicity or ecological harm	Indirect crop effects should not be presented as resistance management	Mechanisms and transferability require separate validation
Pollinator and non-target hazard	Formulation may alter target and non-target exposure	Relevant species, route, concentration, duration, formulation identity, and hazard endpoints	Identifies safety-critical failures hidden by target-only testing	Nanocarriers or emulsifiers may change uptake and persistence	Botanical origin cannot substitute for pollinator and ecological assessment	Non-target harm cannot be justified by presumed resistance benefits	Hazard must be integrated with realistic exposure to characterize risk
Endpoint specificity	Mortality, repellency, ovicidal effects, development, and field protection represent different functions	Prespecified endpoint definitions, observation periods, target stages, and assay validity	Prevents one assay from representing an entire performance profile	Formulation may improve one endpoint while weakening another	Safety endpoints require independent testing	Different endpoints may impose different selection pressures	No universal efficacy ranking is defensible across incompatible endpoints
Temperature and exposure context	Volatility, persistence, penetration, and organism physiology change with environment	Product chemistry, application conditions, temperature, exposure duration, and target response	Explains performance variation without assuming chemical batch failure	Environmental sensitivity may require use-condition restrictions	Changed volatility can alter worker and non-target exposure	Environmental variation complicates interpretation of resistance-related failures	A chemical specification cannot replace an exposure-context specification
Extraction-defined product	Extraction determines the fraction entering formulation and bioassay	Process parameters, fingerprint, yield, impurities, and biological profile	Supports comparison of genuinely equivalent extracts	Different extracts may require distinct carrier systems	Extraction may concentrate hazardous components	Resistance evidence cannot be pooled across non-equivalent fractions	Equal yield or plant identity does not establish extract equivalence

Proposed standardization requirements

The proposed standardization structure treats botanical-insecticide development as a connected evidence chain rather than a collection of independent tests. A defensible standard must connect raw-material control, chemical specification, formulation performance, biological efficacy, and safety rather than treating them as independent checklists [24]. The first requirement is an explicit product-class

definition that distinguishes raw powders, aqueous or solvent extracts, essential oils, purified constituents, analogues, emulsions, nanoformulations, and other materially different entities. Each class must be connected to an intended use, target, crop or storage setting, route, dose form, and exposure scenario. Botanical authentication and provenance then define the source, while chemotype, harvest, handling, and extraction controls constrain the chemical population entering manufacture. These upstream controls remain inputs; batch release must depend on measured product attributes.

The scarcity of dependable products relative to the number of biologically active candidates reflects failures distributed across the development chain. The small proportion of candidates that become dependable products reflects cumulative failures across standardization, formulation, evidence quality, safety, production, and governance rather than a simple shortage of active plants [25]. The proposed organization therefore links source qualification, processing and extraction parameters, orthogonal chemical methods, assay-linked specifications, formulation robustness, stability, exposure, crop safety, non-target assessment, and laboratory-to-field comparability. Decision outcomes should include pass, conditional pass, hold, fail, and requalification. A conditional pass would restrict interpretation to a stated source, formulation, target, or exposure boundary; a hold would require evidence capable of resolving uncertainty; and requalification would be triggered when changes to source, process, formulation, scale, packaging, or use conditions could invalidate earlier comparability.

A separate development pathway should be recognized for purified or structurally simplified natural-product leads. Purified or simplified natural-product leads offer a route to chemical definition, but they should not be treated as evidence that variable whole-botanical preparations have achieved batch equivalence [26]. Purification can improve identity, dosing, structure–activity analysis, and manufacturing control, yet it creates a different product from the source extract or oil and may alter efficacy, safety, environmental behavior, and resistance implications. The proposed standardization requirements are therefore product-class-specific and explicitly non-validated. They do not establish universal markers, numerical acceptance limits, regulatory sufficiency, or deployment readiness. Prospective multi-batch, multi-environment, and independently replicated studies are required to determine whether the proposed components measure distinct decision-relevant constructs and whether accepted chemical ranges predict formulation, efficacy, crop, and non-target outcomes.

The proposed components, evidence bases, boundary conditions, failure modes, and validation requirements are organized in **Table 3**.

Table 3. Proposed Standardization Requirements: Components, Evidence Basis, Relations, Boundary Conditions, Failure Modes, and Validation Requirements

Proposed component	Purpose	Evidence basis	Relation or mechanism	Input or precondition	Expected output	Boundary condition or failure mode	Validation requirement
Product-class definition	Prevent evidence from being pooled across materially different botanical entities	Product-development syntheses distinguish extracts, oils, purified compounds, and formulations	All later claims apply only to the defined entity	Intended use, target, route, dose form, crop or storage system	Unambiguous product identity and claim boundary	Oil, extract, constituent, and formulation evidence are treated as interchangeable	Prospective confirmation that classification predicts relevant process and performance differences
Botanical identity and provenance	Prevent substitution and establish traceable source control	Provenance and supply-chain evidence	Genetic and environmental source constrains biosynthetic potential	Voucher material, plant part, accession, geography, cultivation or collection history	Qualified source with investigation and deviation records	Species identity is treated as chemical equivalence	Authentication-method validation and periodic requalification
Chemotype and chemical envelope	Define accepted multivariate source variation	Within-species and seasonal chemical variation	Composition and constituent ratios affect potency	Replicated batch history and fit-for-purpose	Product-specific chemical acceptance region	One dominant marker or one typical chromatogram	Cross-season, cross-site, and target-linked

			and formulation behavior	analytical methods		defines equivalence	predictive validation
Controlled processing and extraction	Ensure that a reproducible chemical fraction enters later stages	Preparation and extraction studies	Process parameters selectively recover or transform constituents	Locked drying, storage, solvent, ratio, temperature, time, equipment, and separation parameters	Traceable extract with defined fingerprint, yield, and impurity profile	Equal mass yield is treated as equal chemical product	Process-capability, scale-up-comparability, and linked-bioassay studies
Assay-linked analytical specification	Connect measured chemistry with relevant biological function	Whole-mixture, marker, analytical, and bioassay evidence	Orthogonal identity, content, fingerprint, impurity, and potency information supports release decisions	Validated methods, reference materials, sampling plan, and batch history	Prespecified acceptance and deviation decisions	Characterization is reported without limits or biological linkage	Prospective batch prediction and interlaboratory analytical transfer
Formulation and stability comparability	Control payload delivery and drift during storage and use	Nanoformulation and stability evidence	Carrier and payload properties affect release, deposition, persistence, and exposure	Defined excipients, payload range, physical attributes, container, storage, and use conditions	Stable finished product within an intended-use envelope	One payload lot is assumed representative of all qualified lots	Real-time and accelerated studies using representative and edge chemical lots
Exposure and safety envelope	Connect hazard to realistic target, crop, worker, beneficial-organism, and environmental exposure	Crop-safety, environmental, and non-target evidence	Application and environment determine delivered doses	Intended-use scenarios, application conditions, relevant receptors, and hazard data	Explicit efficacy and risk interpretation boundary	Natural origin or target efficacy is used as safety evidence	Scenario-based exposure measurement and product-specific risk characterization
Laboratory-to-field comparability	Separate laboratory repeatability from operational performance	Translation and field evidence	Environment, application, crop, target pressure, and operator influence performance	Qualified lots, common comparators, multiple sites, seasons, batches, and endpoints	Separate estimates of laboratory reproducibility and field performance	Technical replicate precision is described as field effectiveness	Multi-environment field evaluation with chemically qualified batches
Change control and requalification	Prevent cumulative changes from creating an unrecognized new product	Product-development and governance evidence	Source, process, formulation, scale, packaging, and use changes may disrupt comparability	Predefined change-impact and bridging protocol	Accept, reject, bridge, or fully requalify with traceable rationale	Changed materials rely on evidence generated for an earlier configuration	Planned perturbation studies and prospective requalification testing

Implications for research and product development

Research priorities should shift from accumulating isolated activity reports toward producing comparable evidence that supports product definition and decision making. Future research should prioritize comparable chemical metadata, underrepresented targets and endpoints, negative results, and product-relevant tests rather than accumulating additional poorly connected mortality screens [27]. Minimum reporting should include authenticated source, plant part, accession or provenance where relevant, harvest and processing history, extraction parameters, batch identifier, chemical fingerprint, marker content, formulation composition, stability state, dose expression, exposure route, target stage, observation period, and endpoint definition. Retained samples, accessible analytical data, cross-platform reference materials, and explicit reporting of failed or inconsistent batches would help distinguish biological heterogeneity from analytical or publication bias. Progress would be demonstrated by studies that can be compared across laboratories without collapsing materially different products into one category.

Biological evaluation should also extend beyond acute mortality while maintaining a firm boundary between controlled outcomes and field effectiveness. Product-development studies should extend beyond acute mortality to life-history and population outcomes while preserving a clear boundary between controlled population effects and field effectiveness [28]. Development, fecundity, survival, behavior, feeding, repellency, ovicidal action, population growth, crop injury, pest damage, yield,

beneficial organisms, and environmental receptors may each reveal consequences missed by short-duration lethality tests. However, a life-table response remains a controlled population outcome rather than proof of crop protection or operational value. Progress would require chemically qualified batches tested through a staged evidence sequence that connects acute and sublethal effects to semi-field and field outcomes under realistic application and environmental conditions.

Formulation research should use direct comparators and characterize improvement as endpoint-specific rather than universal. Head-to-head comparisons of parent oil and formulation are essential because nanoformulation may change the pattern of mortality, ovicidal action, morphology, and volatile behavior rather than uniformly improve every endpoint [29]. Such comparisons should use the same qualified payload lot, matched dose expressions, explicit carrier controls, stability data, chemical and physical characterization, target and non-target endpoints, and realistic exposure conditions. Product development should also challenge formulations with representative and edge-of-specification botanical lots to determine whether the delivery system tolerates accepted chemical variability. Progress would be shown when formulation attributes prospectively predict stability, exposure, efficacy, crop compatibility, and safety across batches rather than merely producing a stronger response in one laboratory assay.

CONCLUSION

Chemical variability is a central determinant of whether botanical insecticides can move from biologically active preparations to reproducible and interpretable products. It originates in genetics, chemotype, cultivation, harvest, processing, and extraction and continues through formulation, storage, application, degradation, and environmental exposure. The strongest defensible synthesis is that standardization must connect traceable source and process controls with measured chemical attributes, assay-linked acceptance criteria, formulation robustness, intended-use exposure, efficacy, crop compatibility, and non-target safety. Botanical species identity is not chemical equivalence; analytical characterization is not batch standardization; laboratory reproducibility is not field performance; and natural origin is not predictable environmental exposure. The proposed requirements provide a non-validated structure for organizing these relations and identifying failure points, but no universal marker, threshold, or release rule is established. The highest-priority implication is the prospective evaluation of chemically qualified batches across laboratories, formulations, exposure scenarios, and field environments so that product variation can be measured, controlled, and connected to performance and safety rather than hidden within broad botanical labels.

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REFERENCES

1. Isman MB. Botanical insecticides in the twenty-first century—fulfilling their promise? Annu Rev Entomol. 2020;65:233-49. doi:10.1146/annurev-ento-011019-025010
2. Isman MB. Bridging the gap: Moving botanical insecticides from the laboratory to the farm. Ind Crops Prod. 2017;110:10-4. doi:10.1016/j.indcrop.2017.07.012
3. Turchen LM, Cosme-Júnior L, Guedes RNC. Plant-derived insecticides under meta-analyses: Status, biases, and knowledge gaps. Insects. 2020;11(8):532. doi:10.3390/insects11080532
4. Stevenson PC, Isman MB, Belmain SR. Pesticidal plants in Africa: A global vision of new biological control products from local uses. Ind Crops Prod. 2017;110:2-9. doi:10.1016/j.indcrop.2017.08.034
5. Kamanula JF, Belmain SR, Hall DR, Farman DI, Goyder DJ, Mvumi BM, et al. Chemical variation and insecticidal activity of Lippia javanica (Burm. f.) Spreng essential oil against Sitophilus zeamais Motschulsky. Ind Crops Prod. 2017;110:75-82. doi:10.1016/j.indcrop.2017.06.036

6. Dougoud J, Toepfer S, Bateman M, Jenner WH. Efficacy of homemade botanical insecticides based on traditional knowledge. A review. Agron Sustain Dev. 2019;39(4):37. doi:10.1007/s13593-019-0583-1
7. Sá Filho JCF, Nizio DAC, Oliveira AMS, Alves MF, Oliveira RC, Luz JMQ, et al. Geographic location and seasonality affect the chemical composition of essential oils of Lippia alba accessions. Ind Crops Prod. 2022;188:115602. doi:10.1016/j.indcrop.2022.115602
8. Gomes AF, Almeida MP, Leite MF, Schwaiger S, Stuppner H, Halabalaki M, et al. Seasonal variation in the chemical composition of two chemotypes of Lippia alba. Food Chem. 2019;273:186-93. doi:10.1016/j.foodchem.2017.11.089
9. Lima TA, Baptista NMQ, Oliveira APS, Silva PA, Gusmão NB, Correia MTDS, et al. Insecticidal activity of a chemotype VI essential oil from Lippia alba leaves collected at Caatinga and the major compound (1,8-cineole) against Nasutitermes corniger and Sitophilus zeamais. Pestic Biochem Physiol. 2021;177:104901. doi:10.1016/j.pestbp.2021.104901
10. Pavela R, Sedlák P. Post-application temperature as a factor influencing the insecticidal activity of essential oil from Thymus vulgaris. Ind Crops Prod. 2018;113:46-9. doi:10.1016/j.indcrop.2018.01.021
11. Yakhlef G, Hambaba L, Pinto DCGA, Silva AMS. Chemical composition and insecticidal, repellent and antifungal activities of essential oil of Mentha rotundifolia (L.) from Algeria. Ind Crops Prod. 2020;158:112988. doi:10.1016/j.indcrop.2020.112988
12. Velasques J, Cardoso MH, Abrantes G, Frihling BE, Franco OL, Migliolo L. The rescue of botanical insecticides: A bioinspiration for new niches and needs. Pestic Biochem Physiol. 2017;143:14-25. doi:10.1016/j.pestbp.2017.10.003
13. de Oliveira JL, Campos EVR, Fraceto LF. Recent developments and challenges for nanoscale formulation of botanical pesticides for use in sustainable agriculture. J Agric Food Chem. 2018;66(34):8898-913. doi:10.1021/acs.jafc.8b03183
14. Kavallieratos NG, Spinozzi E, Filintas CS, Nika EP, Skourti A, Panariti AME, et al. Acmella oleracea extracts as green pesticides against eight arthropods attacking stored products. Environ Sci Pollut Res Int. 2023;30(41):94904-27. doi:10.1007/s11356-023-28577-8
15. Szczepanik M, Walczak M, Zawitowska B, Michalska-Sionkowska M, Szumny A, Wawrzeńczyk C, et al. Chemical composition, antimicromicrobial activity and insecticidal activity against the lesser mealworm Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae) of Origanum vulgare L. ssp. hirtum (Link) and Artemisia dracunculus L. essential oils. J Sci Food Agric. 2018;98(2):767-74. doi:10.1002/jsfa.8524
16. Campos EVR, Proença PLF, Oliveira JL, Bakshi M, Abhilash PC, Fraceto LF. Use of botanical insecticides for sustainable agriculture: Future perspectives. Ecol Indic. 2019;105:483-95. doi:10.1016/j.ecolind.2018.04.038
17. Hashem AS, Awadalla SS, Zayed GM, Maggi F, Benelli G. Pimpinella anisum essential oil nanoemulsions against Tribolium castaneum—insecticidal activity and mode of action. Environ Sci Pollut Res Int. 2018;25(19):18802-12. doi:10.1007/s11356-018-2068-1
18. Mossa ATH, Afia SI, Mohafrash SMM, Abou-Awad BA. Formulation and characterization of garlic (Allium sativum L.) essential oil nanoemulsion and its acaricidal activity on eriophyid olive mites (Acari: Eriophyidae). Environ Sci Pollut Res Int. 2018;25(11):10526-37. doi:10.1007/s11356-017-0752-1
19. Giuliano G, Campolo O, Forte G, Urbaneja A, Pérez-Hedo M, Latella I, et al. Insecticidal activity of Allium sativum essential oil-based nanoemulsion against Spodoptera littoralis. Insects. 2024;15(7):476. doi:10.3390/insects15070476
20. Tembo Y, Mkindi AG, Mkenda PA, Mpumi N, Mwanauta R, Stevenson PC, et al. Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. Front Plant Sci. 2018;9:1425. doi:10.3389/fpls.2018.01425

21. Chaudhari AK, Singh VK, Kedia A, Das S, Dubey NK. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: Prospects and retrospects. Environ Sci Pollut Res Int. 2021;28(15):18918-40. doi:10.1007/s11356-021-12841-w
22. Mkindi AG, Tembo YLB, Mbega ER, Smith AK, Farrell IW, Ndakidemi PA, et al. Extracts of common pesticidal plants increase plant growth and yield in common bean plants. Plants (Basel). 2020;9(2):149. doi:10.3390/plants9020149
23. Modafferi A, Latella I, Longordo M, Pineda M, Cavaliere R, Forlano P, et al. Is natural always safe? Effective botanical nano-aphicide can be harmful to pollinators. Environ Sci Nano. 2025;12(12):5257-69. doi:10.1039/D5EN00498E
24. Souto AL, Sylvestre M, Tölke ED, Tavares JF, Barbosa-Filho JM, Cebrián-Torrejón G. Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. Molecules. 2021;26(16):4835. doi:10.3390/molecules26164835
25. Acheuk F, Basiouni S, Shehata AA, Dick K, Hajri H, Lasram S, et al. Status and prospects of botanical biopesticides in Europe and Mediterranean countries. Biomolecules. 2022;12(2):311. doi:10.3390/biom12020311
26. Qian H, Hu Y, Wang Z, Zhou Y, Tan X, Feng X, et al. In-depth structural simplification of celangulin V: Design, synthesis, and biological activity. J Agric Food Chem. 2024;72(27):15142-50. doi:10.1021/acs.jafc.4c01079
27. Collares LJ, Turchen LM, Guedes RNC. Research trends, biases, and gaps in phytochemicals as insecticides: Literature survey and meta-analysis. Plants (Basel). 2023;12(2):318. doi:10.3390/plants12020318
28. Ibrahim SS, El-Kholy MY, Shalaby SEM. Insecticidal effects of nano-encapsulated lemongrass essential oil on the population parameters of Spodoptera frugiperda using two-sex life table. Sci Rep. 2025;15(1):11138. doi:10.1038/s41598-025-93779-8
29. Apolinário RVC, Cruz JD, Neto WSMF, Soares JMC, Mpalantinos MA, Gomes SAO, et al. Insecticidal and ovicidal activity of Cymbopogon citratus essential oil and its nanoemulsion against hemipteran crop pests with mortality, antennal malformations, and volatile alterations. Insects. 2025;16(12):1254. doi:10.3390/insects16121254