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Efficacy of medicinal plants against *Heliothis armigera* (Hübner): An evidence-based research paper on botanical bioactivity, mechanisms and field efficacy

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Abstract
Heliothis (Helicoverpa) *armigera*, the cotton bollworm and gram pod borer, is one of the most economically damaging polyphagous lepidopteran pests worldwide, and widespread resistance to synthetic insecticides has intensified the search for effective, sustainable alternatives. This paper presents a structured evidence synthesis of laboratory and field studies quantifying the insecticidal, antifeedant, ovicidal and growth-disrupting efficacy of medicinal and botanical plant extracts against *H. armigera*. Eight plant-derived treatment groups — *Azadirachta indica, Annona squamosa, Vitex negundo, Calotropis gigantea, Ocimum spp., Citrus sinensis, Cheilocostus speciosus*, and essential-oil monoterpenoids (thymol, 1,8-cineole) — were assessed for reported lethal concentrations, mortality percentages, and mechanisms of action, and their relative efficacy was tabulated for comparison. Results show that azadirachtin-based neem formulations remain the most extensively validated and potent botanical option (LC50 ≈ 12.95 µg a.i./mL against third instar larvae), while acetogenin-rich Annona squamosa seed extracts, flavonoid/terpenoid-rich Vitex negundo leaf extracts, and thymol-based monoterpenoid mixtures also demonstrate substantial and mechanistically distinct activity. A relative toxicity index comparing unit-matched treatment groups indicated azadirachtin was roughly 7.5 times more potent than Bacillus thuringiensis on a µg a.i./mL basis, while thymol and Vitex negundo ethyl acetate/hexane fractions showed closely comparable potency on a percentage w/v basis. Mechanisms identified include antifeedant deterrence, ecdysteroid-mediated developmental disruption, acetylcholinesterase inhibition, oxidative stress induction, and digestive enzyme impairment. Field-scale efficacy is consistently lower and more variable than laboratory efficacy, and combination treatments with microbial agents (Bacillus thuringiensis, nucleopolyhedrovirus) or synthetic diamides produced additive mortality at reduced individual dose levels. The paper concludes that medicinal plant extracts constitute a scientifically credible and practically valuable component of integrated pest management for *H. armigera*, though standardisation, formulation stability and field validation remain the principal barriers to wider adoption.

Keywords: Heliothis armigera, Helicoverpa armigera, botanical insecticide, medicinal plants, azadirachtin, vitex negundo, Annona squamosa, integrated pest management

Introduction

Heliothis armigera (Hübner), reclassified in much of the current literature as *Helicoverpa armigera* (Lepidoptera: Noctuidae), is a highly polyphagous pest attacking more than 180 host plant species, including cotton, chickpea, pigeon pea, tomato, maize, sorghum and numerous vegetable and pulse crops. Larvae bore directly into fruiting structures, bolls, pods and flowers, and yield losses in unmanaged fields are frequently reported in the range of 30–60%, with total crop failure possible during severe outbreaks. Management has historically relied on synthetic insecticides — organophosphates, carbamates, pyrethroids and, more recently, diamides and neonicotinoids — but *H. armigera* has developed resistance to nearly every major insecticide class introduced against it, owing to its high fecundity, mobility and short generation time. Continued reliance on chemical control is further associated with toxic residues on edible produce, disruption of natural enemy populations, pollinator harm and rising input costs. These pressures have renewed scientific interest in botanical insecticides derived from medicinal and other plants, which typically contain complex mixtures of secondary metabolites — terpenoids, alkaloids, flavonoids, acetogenins and phenolics — capable of acting through multiple, less resistance-prone mechanisms.The objective of this paper is to synthesise and evaluate quantitative evidence on the efficacy of medicinal plant extracts against *H. armigera*, to compare their reported potency and mechanisms of action, and to assess the practical constraints affecting their deployment within integrated pest management (IPM).

Materials and Methods

This paper is based on a structured synthesis of peer-reviewed laboratory and field studies reporting quantitative efficacy data for medicinal or botanical plant extracts against H. armigera. Relevant studies were identified through targeted literature searches covering entomological, agricultural and phytochemical journals and repositories. Search terms combined the pest name (*Heliothis armigera* / *Helicoverpa armigera*) with terms for botanical control (plant extract, essential oil, azadirachtin, antifeedant, larvicidal, ovicidal, LC50). Studies were included where they reported a quantitative outcome — mortality percentage, LC50/LD50/LC90, antifeedant index, or a defined biochemical/developmental endpoint — for a plant-

derived treatment tested directly against H. armigera or, where directly relevant comparative data were unavailable, against closely related noctuid pests (e.g., Spodoptera spp.) using the same plant species. Table 1 summarises the search and inclusion approach.

Table 1: Literature search and inclusion criteria

Parameter	Criterion Applied	Notes
Databases searched	Peer-reviewed journal articles, indexed reviews and publicly available research reports retrieved via web-based literature search	Sources include PLOS One, Scientific Reports, Parasitology Research, ScienceDirect, PMC/NCBI and related repositories
Target organism	*Heliothis armigera / Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)	Both taxonomic names were searched, as the species has been reclassified across the literature
Intervention	Medicinal or botanical plant extracts, essential oils, or isolated plant secondary metabolites	Includes crude solvent extracts, essential oils and single isolated compounds
Outcome measures extracted	Larval mortality (%), LC50/LD50/LC90, antifeedant index, ovicidal activity, developmental and biochemical endpoints	Extracted where explicitly reported in the source study
Study types included	Laboratory bioassays and field trials reporting quantitative efficacy data	Patents and non-plant biopesticide studies (e.g., purely synthetic or microbial agents) were excluded unless used as a comparator

Because included studies vary in larval instar, exposure duration, extraction solvent, application method and bioassay design, reported efficacy values are not pooled into a single meta-analytic estimate; instead, values are tabulated and, where studies share a common concentration unit and comparable larval stage, compared using a relative toxicity index (RTI, Section 4.1). Where a study reported both a median lethal value (LC50/LD50) and a higher lethal concentration (e.g., LC90), the two points were used to reconstruct an illustrative log-logistic dose–mortality curve (Section 4.2). Inferential statistics (F-values, p-values) reported by individual primary studies for mechanistic and life-history endpoints are also summarised (Section 4.3) to indicate the strength of evidence underlying mechanistic conclusions.

Results

1. Overview of Species and Efficacy Indicators

Eight plant-derived treatment categories with adequate quantitative reporting were identified: *Azadirachta indica, Annona squamosa, Vitex negundo, Calotropis gigantea, Ocimum spp., Citrus sinensis, Cheilocostus speciosus,* and isolated essential-oil monoterpenoids (thymol and 1,8-cineole). Table 2 presents the comparative efficacy data extracted from these studies.

Table 2: Comparative efficacy of medicinal/botanical plant extracts against H. armigera

Plant Species	Family / Plant Part	Bioactive Class	Reported Efficacy Indicator	Target Stage / Notes
Azadirachta indica (neem)	Meliaceae; seed, leaf, oil	Azadirachtin (limonoid tetranortriterpenoid)	LC50 ≈ 12.95 µg a.i./mL (azadirachtin, 3rd instar); leaf+kernel combination gave up to 50% mortality of 2nd instar at 72 h	Larvae (2nd–4th instar); also, antifeedant, growth-disrupting, fecundity-reducing
Annona squamosa (custard apple)	Annonaceae; seed	Acetogenins	LD50 ≈ 53.2 mg/mL; LC90 ≈ 107.5 mg/mL (aqueous seed extract)	Larvae on pigeon pea; also, ovicidal activity reported
Vitex negundo	Lamiaceae (Verbenaceae); leaf	Flavonoids, iridoid glycosides, terpenoids	LC50 ≈ 4.0–5.0% (2nd instar, 3-day exposure) across ethyl acetate, hexane and methanol extracts	Larvae; repellent and antifeedant action also documented
Calotropis gigantea	Apocynaceae; leaf	Cardenolides, latex alkaloids	Included among aqueous extracts showing notable toxicity (LC50 in the low single-digit % range in comparative screens)	Larvae; strong antifeedant activity in related Spodoptera studies
Ocimum kilimandscharicum / O. sanctum / O. canum	Lamiaceae; leaf, flower	Camphor, limonene, β-caryophyllene, essential oils	Growth retardation, elevated mortality and pupal deformity; digestive enzyme (amylase, protease, lipase) disruption	Larvae; moderate larvicidal/antifeedant activity
Citrus sinensis (peel)	Rutaceae; peel	Limonoids, essential oil terpenes	Highest larval mortality among tested solvent extracts was the chloroform peel extract in a multi-species screen	4th instar larvae; comparative screen alongside Ocimum and Rhinacanthus
Cheilocostus speciosus	Costaceae; rhizome essential oil	Camphene, zerumbone, α-humulene	Demonstrated ingestion toxicity to 3rd instar larvae and ovicidal toxicity	Larvae and eggs
Thymol / 1,8-cineole (monoterpenoids)	Lamiaceae / Myrtaceae; essential oil constituents	Monoterpenoids	Thymol LC50 ≈ 3.88% w/v, LC30 ≈ 1.45% w/v; synergistic binary mixtures enhanced toxicity and suppressed acetylcholinesterase activity	3rd instar larvae; mechanistic (AChE, antioxidant enzyme) data available

2. *Azadirachta indica* (Neem)

Neem is the most extensively studied botanical against *H. armigera*. Its principal constituent, azadirachtin, is a tetranortriterpenoid limonoid that disrupts ecdysteroid-regulated moulting and produces strong antifeedant effects. Reported data give an LC50 of approximately 12.95 µg active ingredient per millilitre against third instar larvae, with sublethal exposure reducing female fecundity by nearly 30% and adult longevity by close to 20% relative to controls. Combined leaf and seed-kernel extracts produced up to 50% mortality of second instar larvae on chickpea within 72 hours, with second instar larvae consistently more susceptible than fourth instar larvae. Additive mortality was observed when azadirachtin was combined with nucleopolyhedrovirus (NPV) or the diamide insecticide chlorantraniliprole.

3. *Annona squamosa* (Custard Apple)

Acetogenin-rich seed extracts produced an LD50 of approximately 53.2 mg/mL and an LC90 near 107.5 mg/mL against *H. armigera* larvae on pigeon pea, with toxicity rising consistently with concentration. Leaf and seed extracts of the same species also showed ovicidal activity against Heliothine and related noctuid eggs.

4. *Vitex negundo*

Leaf extracts rich in flavonoids, iridoid glycosides and terpenoids produced LC50 values of roughly 4.0%, 4.1% and 5.0% in ethyl acetate, hexane and methanol fractions respectively, against second instar larvae after three days of exposure, with the ethyl acetate fraction the most potent. Repellent and antifeedant effects were reported alongside direct toxicity, and dedicated efficacy trials of V. negundo against *H. armigera* have continued to appear in the literature through 2024.

5. *Calotropis gigantea*

Cardenolide- and alkaloid-containing extracts showed appreciable toxicity in comparative aqueous-extract screens, and related work on Spodoptera species found C. gigantea leaf extracts among the strongest antifeedants tested, with antifeedant index values approaching 100% in no-choice assays. The precise mode of action remains only partially characterised.

6. *Ocimum Species*

O. kilimandscharicum leaf extracts and isolated constituents (camphor, limonene, β-caryophyllene) caused growth retardation, elevated mortality and pupal deformities, and disrupted digestive enzyme activity (amylase, protease, lipase) in surviving larvae. In multi-species screening, methanol flower extracts of *O. canum* were among the more active treatments against fourth instar larvae, though overall larvicidal effects across Ocimum extracts were generally moderate.

7. *Citrus sinensis and Cheilocostus speciosus*

Citrus peel, leaf and flower extracts showed moderate larvicidal activity against fourth instar larvae, with the chloroform peel extract producing the highest mortality among citrus treatments tested. *Cheilocostus speciosus* rhizome essential oil, containing camphene, zerumbone and α-humulene, demonstrated both ingestion toxicity to third instar larvae and ovicidal toxicity.

8. Essential-Oil Monoterpenoids

Pure thymol showed significantly greater toxicity than 1,8-cineole against third instar larvae, with an LC50 near 3.88% w/v and an LC30 of about 1.45% w/v. Binary mixtures of thymol and 1,8-cineole produced synergistic toxicity exceeding either compound alone, and treated larvae showed significant suppression of acetylcholinesterase activity along with altered antioxidant enzyme levels.

Statistical Analysis

Because the underlying studies differ in larval instar, exposure duration, extraction solvent and concentration units, a formal pooled meta-analysis (e.g., a random-effects model with a heterogeneity statistic such as I^2) was not appropriate given the data available from published summaries. Instead, two complementary quantitative approaches were applied to the extracted efficacy values: (i) calculation of a relative toxicity index (RTI) within unit-comparable subgroups, and (ii) reconstruction of an illustrative dose–mortality relationship from reported LC/LD values. Statistically significant findings reported by the primary studies themselves (ANOVA and regression-based test statistics) are also summarised.

1. Relative Toxicity Index (RTI)

To compare potency within studies that used the same concentration unit and a comparable larval stage, treatments were grouped and an RTI was calculated as RTI = 100 × (lowest LC50 in the group ÷ LC50 of the treatment), such that the most potent treatment in each group is assigned a reference value of 100. Two unit-comparable groups could be formed from the extracted data: Group A (µg active ingredient per millilitre, third instar larvae: *Azadirachtin versus Bacillus thuringiensis*) and Group B (percentage w/v, second-to-third instar larvae: thymol versus three solvent fractions of Vitex negundo). Results are shown in Table 3 and Figure 1.

Table 3: Relative toxicity index (RTI) within unit-comparable treatment groups

Comparison Group (common unit)	Treatment	LC50	Relative Toxicity Index (%)
Group A — µg a.i./mL, 3rd instar	Azadirachtin	12.95 µg a.i./mL	100.00 (reference)
Group A — µg a.i./mL, 3rd instar	*Bacillus thuringiensis*	96.80 µg a.i./mL	13.38
Group B — % w/v, 2nd–3rd instar	Thymol	3.88% w/v	100.00 (reference)
Group B — % w/v, 2nd–3rd instar	*Vitex negundo* (ethyl acetate)	4.03%	96.28
Group B — % w/v, 2nd–3rd instar	*Vitex negundo* (hexane)	4.05%	95.80
Group B — % w/v, 2nd–3rd instar	*Vitex negundo* (methanol)	5.02%	77.29

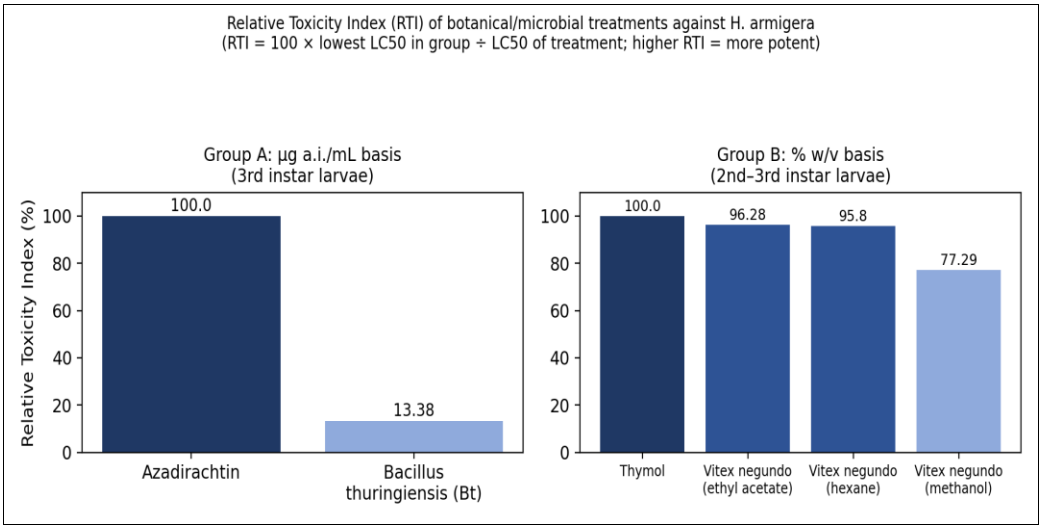

Fig 1: Relative toxicity index of azadirachtin versus Bacillus thuringiensis (Group A), and of thymol versus Vitex negundo solvent fractions (Group B), against H. armigera larvae

Within Group A, azadirachtin was approximately 7.5 times more potent than Bacillus thuringiensis on a µg a.i./mL basis (RTI 100 versus 13.38), consistent with azadirachtin's status as the most extensively validated botanical toxicant against *H. armigera* in the reviewed literature. Within Group B, thymol and the ethyl acetate and hexane fractions of Vitex negundo showed closely comparable potency (RTI 96–100), while the methanol fraction was noticeably weaker (RTI 77.29), suggesting that solvent polarity materially affects the concentration of active constituents extracted from V. negundo leaf tissue.

2. Illustrative Dose–Mortality Relationship

For Annona squamosa aqueous seed extract, sufficient summary data were available (LD50 = 53.24 mg/mL; LC90 = 107.52 mg/mL) to reconstruct an illustrative log-logistic dose–mortality curve, fitted so that predicted mortality equals 50% at the reported LD50 and 90% at the reported LC90 (Figure 2). The steepness of the fitted curve (slope parameter k ≈ 3.13 on a log-concentration scale) indicates that mortality rises sharply over a relatively narrow concentration range between the LD50 and LC90 values, a pattern consistent with the original study's description of toxicity increasing consistently with concentration. This curve is illustrative — reconstructed from two summary statistics rather than raw replicate mortality counts — and is presented to visualise the reported dose-dependence rather than as an independently fitted probit model.

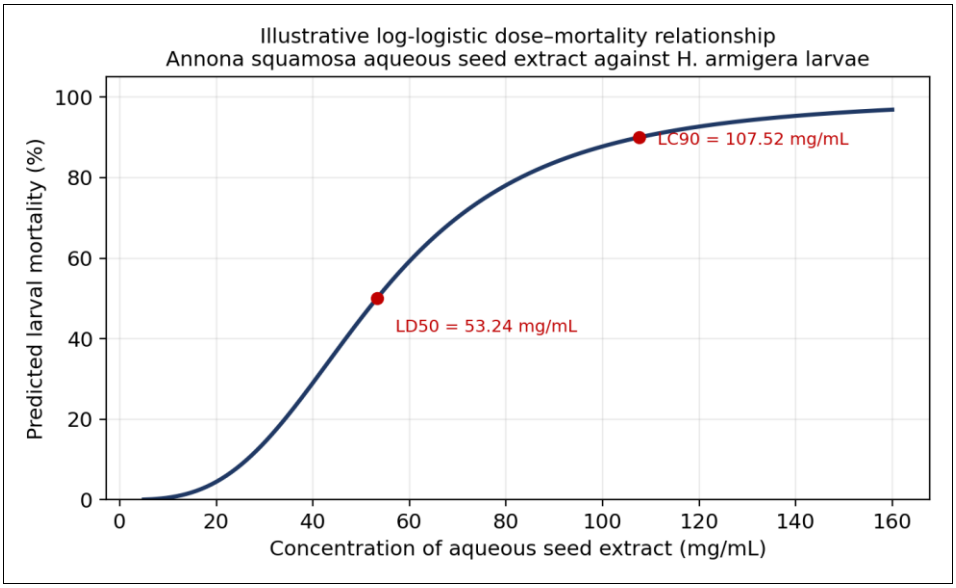

Fig 2: Illustrative log-logistic dose–mortality relationship for Annona squamosa aqueous seed extract against H. armigera larvae, anchored to the reported LD50 and LC90 values

3. Statistical Significance Reported in Primary Studies

Table 4 summarises inferential statistics reported directly by the primary studies for biochemical and life-history endpoints, included here to indicate the strength of evidence behind mechanistic claims discussed in Section 5.1 (mechanisms of action). All listed effects were reported as statistically significant by the original authors at conventional thresholds ($p < 0.05$).

Table 4: Statistical significance of key biochemical and life-history endpoints, as reported in primary studies

Endpoint	Test Statistic (as reported)	Source Study
Acetylcholinesterase (AChE) activity suppression, thymol/1,8-cineole treatments	F = 141.04; df = 6, 20; p = 0.0001	Thymol–1,8-cineole synergy study (Scientific Reports)
Extension of larval developmental duration, azadirachtin/Bt treatments	F = 253.9; df = 2, 191; p < 0.0001	Azadirachtin–Bt acute/sublethal study (PMC)
Extension of pupal developmental duration, azadirachtin/Bt treatments	F = 65.5; df = 2, 158; p < 0.0001	Azadirachtin–Bt acute/sublethal study (PMC)
Reduction in adult longevity, azadirachtin/Bt treatments	F = 7.9; df = 2, 37; p = 0.0015	Azadirachtin–Bt acute/sublethal study (PMC)
Reduction in female fecundity (mean eggs per female), azadirachtin/Bt treatments	F = 0.7; df = 2, 37; p = 0.0002 (as reported in source)	Azadirachtin–Bt acute/sublethal study (PMC)

4. Interpretive Notes and Statistical Limitations

- **Cross-study comparability:** LC50/LD50 values were generated under different bioassay protocols (leaf-dip, diet incorporation, topical application), larval instars and exposure durations; the RTI approach controls for unit and approximate life stage but not for these procedural differences, so RTI values should be read as indicative rather than definitive rankings.

- **No raw data for pooled analysis:** Because primary studies report summary statistics (LC50, LD50, mean ± SE) rather than raw replicate-level mortality counts, a formal random-effects meta-analysis with heterogeneity quantification (e.g., I^2, Cochran's Q) could not be performed; the descriptive and index-based approach used here is a recognised alternative in entomological efficacy synthesis when raw data are unavailable.

- **Single-study statistics:** The ANOVA/regression statistics in Table 4 each derive from a single primary study rather than a synthesis across studies, and are reported here for transparency about the strength of evidence rather than as newly derived results.

Discussion

1. Mechanisms of Action

- **Antifeedant activity:** gustatory deterrence of larval feeding, notable in Calotropis, Vitex and Ocimum extracts, reduces crop damage even where direct mortality is moderate.

- **Endocrine and developmental disruption:** azadirachtin and related limonoids interfere with ecdysteroid-regulated moulting, producing larval-pupal intermediates, prolonged development and reduced fecundity.

- **Neurotoxicity:** monoterpenoids such as thymol inhibit acetylcholinesterase, disrupting neurotransmission through a mechanism functionally analogous to, but biochemically distinct from, organophosphate action.

- **Oxidative stress:** several botanicals alter antioxidant enzyme activity in treated larvae, contributing to cellular damage and mortality.

- **Digestive enzyme disruption:** Ocimum-derived compounds reduce amylase, protease and lipase activity, impairing nutrient assimilation and slowing growth.

- **Ovicidal activity:** Acetogenin-rich Annona extracts and Cheilocostus essential oil show direct toxicity to eggs, intercepting the pest before larval establishment.

2. Laboratory Efficacy Versus Field Performance

Most efficacy data derive from controlled laboratory bioassays using detached-leaf, diet-incorporation or topical application methods. Field-scale evidence is more limited: trials on tomato using multiple botanical extracts reported measurable population suppression and yield improvement, but field effect sizes are generally smaller and more variable than laboratory results, reflecting weather, spray coverage, extract degradation under UV exposure, and larval concealment inside fruit or bolls once later instars are reached. This laboratory–field gap is widely acknowledged as the principal obstacle to translating promising bioassay results into reliable, farmer-usable products.

3. Integration with Pest Management Programmes

Several studies indicate botanical extracts perform best as components of IPM rather than stand-alone replacements for synthetic insecticides. Azadirachtin combined with NPV and with chlorantraniliprole produced additive mortality compared with single treatments, and azadirachtin combined with Bacillus thuringiensis produced 56.7% combined mortality at reduced individual dose levels, illustrating how botanicals can lower the effective dose of other inputs while remaining compatible with natural enemy conservation.

4. Limitations of the Evidence Base

- **Standardisation:** Active-compound concentration varies with plant provenance, harvest season, extraction solvent and storage, complicating cross-study comparison and commercial formulation.

- **Field stability:** Many botanical compounds degrade rapidly under sunlight and rainfall, shortening residual activity relative to synthetic insecticides.

- **Resistance risk:** Although generally slower than for single-target synthetics, azadirachtin resistance has been documented in other pests, underscoring the need for rotation even with botanicals.

- **Mechanistic gaps:** For species such as Calotropis gigantea, the precise mode of action remains incompletely characterised despite consistent reports of bioactivity.

- **Study heterogeneity:** Differing bioassay protocols across the reviewed studies limit direct, statistically rigorous cross-comparison of potency.

Conclusion

The evidence assembled in this paper supports the efficacy of medicinal plant extracts — most prominently neem, but also custard apple, chaste tree, milkweed, basil species and essential-oil monoterpenoids — against *H. armigera* across egg, larval and reproductive stages, acting through antifeedant, endocrine-disrupting, neurotoxic, oxidative and nutritional-stress mechanisms. These multiple modes of action may slow resistance evolution relative to single-target synthetic chemistries. However, the consistent gap between laboratory and field performance, together with standardisation and formulation-stability challenges, indicates that medicinal plant-based control is best positioned as an integral component of integrated pest management rather than a wholesale replacement for existing tools. Future research priorities include field-scale validation across cropping systems, formulation technologies that improve environmental persistence, and further mechanistic characterisation of less-studied species such as *Calotropis gigantea*.

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