Use of Oil, Aqueous Extracts, and Formulations of *Azadirachta indica* A. Jussieu against *Spodoptera frugiperda*: A Systematic Review (2015–2026)

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Systematic Review

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Abstract

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is one of the most economically damaging cereal pests globally, with yield losses exceeding 30%. The neem tree, *Azadirachta indica* A. Jussieu (Meliaceae), contains over 200 bioactive secondary metabolites, with azadirachtin A ($C_{35}H_{44}O_{16}$) as the most potent insecticidal compound. This review systematizes scientific evidence published between 2015 and 2026 on the use of oil, aqueous extracts, and formulations of *A. indica* against *S. frugiperda* under laboratory and field conditions, analyzing formulation efficacy, multi-target mechanisms of action, and the influence of high-altitude climatic conditions on biopesticide performance. Formulated neem oil at 500–1,500 µL/L achieves larval mortality exceeding 70% in early instars (L1–L3), with LC_{50} = 580–703 µL/L and LT_{50} = 21–67 h. Metabolite modes of action include ecdysteroid antagonism, gustatory inhibition, intestinal cytotoxicity, immunosuppression, and enzymatic inhibition of detoxification systems (Tables 1 and 3), constituting a multi-target strategy that minimizes resistance development risk in *S. frugiperda*. Emerging nanoformulations (microencapsulation, chitosan-azadirachtin nanoparticles) show LC_{50} values up to 2.5-fold lower and extended environmental half-lives of 7–21 days compared with conventional emulsifiable concentrates, constituting a priority area for future research and commercial development.

1. Introduction

1.1. Spodoptera frugiperda (J. E. Smith): Biology, Global Expansion, and Economic Impact

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) is recognized as one of the most economically important agricultural pests on a global scale (Montezano et al., 2018; Tay et al., 2023). This polyphagous lepidopteran, native to tropical and subtropical regions of the Americas, has the capacity to attack more than 400 plant species, with a marked preference for agronomically important grasses such as maize [*Zea mays* L.], sorghum [*Sorghum bicolor* (L.) Moench], rice [*Oryza sativa* L.], and cotton (*Gossypium hirsutum* L.) (Casmuz et al., 2010; Early et al., 2018). Yield losses in maize attributable to *S. frugiperda* are estimated at 18–30% of global production, representing billions of dollars annually (Montezano et al., 2018; Machekano et al., 2024).

Since its first detection outside the Americas in West Africa in 2016, *S. frugiperda* dispersed to 44 African countries within three years, reaching the Asian continent between 2018 and 2019 — India, China, Thailand, Myanmar, Bangladesh, and Sri Lanka — and Australia in 2020 (Goergen et al., 2016; Ganiger et al., 2018; Sun et al., 2021). In China, associated economic losses are estimated at USD 2.46–6.18 billion annually (Liu et al., 2025). Its biological characteristics — high fecundity (1,000–1,500 eggs per female), short life cycle (30–40 days), and flight capacity of up to 100 km per night — favor its persistence as a transboundary pest (Tay et al., 2023).

Control of *S. frugiperda* has historically depended on intensive use of synthetic organophosphate, pyrethroid, and carbamate insecticides. However, high-level resistance (> 100×) has been documented in populations from Brazil, Puerto Rico, Mexico, and several Asian countries (Carvalho et al., 2013; Gutiérrez-Moreno et al., 2019; Silva-Brandão et al., 2024). The use of Cry proteins from *Bacillus thuringiensis* Berliner in transgenic cultivars has also generated field-level resistance (Boaventura et al., 2020; Nascimento et al., 2021). Against this backdrop, the development of sustainable biorational alternatives within Integrated Pest Management (IPM) has become a priority in applied entomological research (Isman, 2020; Pavela & Benelli, 2023).

1.2. Azadirachta indica A. Jussieu: Secondary Metabolites and Insecticidal Activity

The neem tree, *Azadirachta indica* A. Jussieu (Meliaceae), contains more than 200 bioactive compounds distributed in four major groups (Table 1): (i) tetranortriterpene limonoids, of which the most active is azadirachtin A ($C_{35}H_{44}O_{16}$, MW = 720.7 g/mol, 16 stereocenters); (ii) secondary limonoids such as salannin ($C_{34}H_{44}O_9$), nimbin ($C_{30}H_{36}O_9$), gedunin ($C_{28}H_{34}O_7$), and azadiradione ($C_{28}H_{34}O_4$); (iii) flavonoids and polyphenols (catechin, epicatechin, quercetin, kaempferol) and diterpenoids (margolones); and (iv) the phytosterol β-sitosterol ($C_{29}H_{50}O$) and the fatty acids oleic and linoleic acids, which are the major components of seed oil (Schmutterer, 1990; Mordue & Nisbet, 2000; Koul, 2004).

Azadirachtin A exerts a multimodal insecticidal action including: (i) ecdysteroid and juvenile hormone antagonism through interference with the nuclear receptors EcR/USP and Met/FISC; (ii) feeding inhibition by blockade of gustatory chemoreceptors; (iii) intestinal cytotoxicity through apoptosis of epithelial cells; and (iv) immunotoxicity with suppression of phenoloxidase (PO) and cellular encapsulation (Mordue & Blackwell, 1993; Mordue et al., 2010; Kumar et al., 2024). This multi-target mechanism significantly reduces the risk of resistance development in *S. frugiperda* compared with single-site synthetic insecticides (Isman, 2015; Sparks & Nauen, 2015).

1.3. Review Objectives

The primary objective of this review is to critically and systematically analyze the scientific evidence published between 2015 and 2026 on the use of oil, aqueous extracts, and formulations of *A. indica* against *S. frugiperda* (J. E. Smith) under laboratory and field conditions (Table 2), integrating knowledge on the multi-target modes of action of its metabolites (Tables 1 and 3) to inform recommendations for IPM programs. As a secondary objective, the influence of high-altitude climatic conditions on formulation efficacy and the pest's ecophysiology is examined.

2. Bibliographic Search Methodology

The bibliographic search was conducted in the Scopus, Web of Science, PubMed, Google Scholar, SciELO, and AGRIS databases, covering publications from January 2015 to March 2026. Search terms employed, combined using Boolean operators (AND, OR, NOT), included: "*Spodoptera frugiperda*", "neem", "*Azadirachta indica*", "azadirachtin", "neem oil", "NSKE", "botanical insecticide", "biopesticide", "fall armyworm control", and their Spanish equivalents. Original research articles, systematic reviews, and indexed postgraduate theses published between 2015 and 2026 were included. Studies without access to full text, without quantitative efficacy results, or involving species other than *S. frugiperda* or formulations other than *A. indica* were excluded. Of 187 references initially identified, 68 met inclusion criteria. The 28 most representative studies and reviews are synthesized in Table 2.

3. Results and Discussion

3.1. Studies on the Use of Azadirachta indica against Spodoptera frugiperda

Table 2 synthesizes the studies and reviews identified that evaluated the use of *A. indica* formulations against *S. frugiperda* between 1990 and 2026. Of the total, ten correspond to laboratory trials, six to field or mixed (laboratory/field) trials, and seven to bibliographic reviews. Geographically, studies are concentrated in Latin America (Ecuador, Mexico, Nicaragua, Colombia), Asia (India, China), Africa (Zimbabwe, Botswana, South Africa), and Europe (Italy, Czech Republic). Formulations comprise emulsifiable concentrate (EC) oil, neem seed kernel extract (NSKE), technically purified azadirachtin, and emerging nanotechnology-based formulations.

Table 2
Studies on the use of *Azadirachta indica* A. Jussieu formulations against *Spodoptera frugiperda* (J. E. Smith) under laboratory and field conditions (1990–2026).

Author(s)	Year	Formulation/Product	Type	Setting	Country/Region	Main finding
Schmutterer	1990	Oil, botanical extracts	Review	Review	Germany	Foundational characterization of neem chemistry and insecticidal activity
Mordue & Blackwell	1993	Purified azadirachtin	Azadirachtin	Laboratory	United Kingdom	Elucidated multi-target mode of action of azadirachtin
García et al.	1999	Neem botanical extract	Aqueous extract	Field	Colombia	Field efficacy against S. frugiperda in maize
Mordue & Nisbet	2000	Purified azadirachtin	Azadirachtin	Laboratory	United Kingdom	Comprehensive review of azadirachtin action in insects
Nathan et al.	2005	Neem limonoids	Aqueous extract	Laboratory	India	Limonoid activity on vector mosquito Anopheles stephensi
Charleston et al.	2006	*A. indica* + *M. azedarach* extract	Aqueous extract	Field	South Africa	Impact on Plutella xylostella populations and natural enemies
Isman	2006	Botanical insecticides	Review	Review	Canada	Global overview of botanical insecticides in modern agriculture
Gutiérrez et al.	2010	Formulated neem oil (EC)	Formulated oil	Lab/Field	Mexico	LC_{50} = 720–950 µL/L for L3–L5 at 22°C
Senthil-Nathan	2013	Meliaceae extracts	Aqueous extract	Lab/Review	India	Biochemical effects of neem limonoids on Lepidoptera
Isman	2015	Botanical insecticides	Review	Review	Canada	Renaissance of botanical insecticides and regulatory outlook
Campos et al.	2016	Neem oil, NSKE	Oil/NSKE	Review	Brazil	Neem oil in crop protection: current status and future
Isman	2020	Botanical insecticides	Review	Review	Canada	21st-century botanical insecticides: innovation and going green
Almeida	2021	Neem oil + vegetable oil	Formulated oil	Laboratory	Ecuador	Larvicidal efficacy in maize against S. frugiperda

Note. EC = emulsifiable concentrate; NSKE = Neem Seed Kernel Extract; Lab. = laboratory; Rev. = bibliographic review; a.s.l. = above sea level. Source: compiled by the authors.

Author(s)	Year	Formulation/Product	Type	Setting	Country/Region	Main finding
Gámez	2021	Botanical extract (neem, *Eucalyptus* spp., *Capsicum annuum* L.)	Aqueous extract	Laboratory	Nicaragua	Mortality 58–78% (NSKE 5%) and 80–88% (NSKE 10%) in L2–L4
López et al.	2022	EC oil + NSKE 3–10%	Oil/NSKE	Lab+Field	Ecuador	LC_{50} = 650–840 µL/L for L2–L4; antifeedant effect 65–75%
Pavela & Benelli	2023	Essential oils and neem	Review	Review	Czech Rep./Italy	Challenges of botanical insecticides as ecofriendly biopesticides
Cantrell et al.	2024	Natural products as pesticides (incl. neem)	Review	Review	USA	Opportunities and constraints for natural product pesticides
Kumar et al.	2024	Oil, extracts, limonoids	Oil/extract	Review	India	Neem potential for animal and human health safeguarding
Machekano et al.	2024	Commercial neem formulations	Formulated oil	Field/Review	Africa (Zimbabwe, Botswana)	FAW biology, ecology, genetic diversity and management in Africa
Bezerra et al.	2025	Microencapsulated/photoresistant *A. indica* extract	Emerging form.	Lab/Semi-field	Brazil	Extended half-life and improved efficacy against S. frugiperda under semi-field conditions
Harrison et al.	2019	Agroecological options incl. neem	Review	Field/Review	Sub-Saharan Africa	FAW management options; neem as low-cost smallholder-friendly solution
Pavela et al.	2025	Botanical insecticides	Review	Review	Czech Rep./Italy	Criteria for biopesticide commercialization in the 21st century
Wu et al.	2025	O-carboxymethyl chitosan-azadirachtin nanoformulation	Emerging form.	Laboratory	China	Nanoformulation improves azadirachtin stability; enhanced antifeedant against S. frugiperda
Cañas-Meaury et al.	2025	NeemAZAL® 1.2% EC	Formulated oil	Laboratory	Colombia (Pamplona)	Instar-specific larvicidal efficacy at 2,586 m a.s.l.
Reatiga-Pulido et al.	2025	NeemAZAL® 1.2% EC	Formulated oil	Laboratory	Colombia (Pamplona)	Instar toxicity analysis under high-altitude laboratory conditions
Giraldo-Vanegas et al.	2026a	NeemAZAL® 1.2% EC — dose-response and survival analysis	Formulated oil	Laboratory	Colombia (Pamplona)	LC_{50} = 579.67–921.36 µL/L

Note. EC = emulsifiable concentrate; NSKE = Neem Seed Kernel Extract; Lab. = laboratory; Rev. = bibliographic review; a.s.l. = above sea level. Source: compiled by the authors.

Author(s)	Year	Formulation/Product	Type	Setting	Country/Region	Main finding
						across L1–L6; R^2 = 0.94–0.95
Giraldo-Vanegas et al.	2026b	NeemAZAL® 1.2% EC — ontogenetic thresholds and LT dynamics	Formulated oil	Laboratory	Colombia (Pamplona)	LT_{50} = 52.39 h (L1) to 196.69 h (L5); 271.3% ontogenetic gradient

Note. EC = emulsifiable concentrate; NSKE = Neem Seed Kernel Extract; Lab. = laboratory; Rev. = bibliographic review; a.s.l. = above sea level. Source: compiled by the authors.

The analysis of Table 2 reveals a sustained increase in publications from 2020 onward, coinciding with the global expansion of *S. frugiperda* to Asia and Oceania. The works of Giraldo-Vanegas et al. (2026a, b), Cañas-Meaury et al. (2025), and Reatiga-Pulido et al. (2025), conducted under high-altitude conditions in Pamplona, Norte de Santander, Colombia (2,586 m a.s.l., 17 ± 1°C), are the only studies that simultaneously evaluate all six larval instars of *S. frugiperda* with formulated azadirachtin, constituting the most ontogenetically comprehensive works available for the Colombian Andean region.

3.2. Phytochemical Characterization of the Defensive Complex of Azadirachta indica

The efficacy of neem formulations against *S. frugiperda* does not reside in a single active ingredient, but in the synergy of diverse chemical families (Koul, 2004; Schmutterer, 1990). More than 20 target metabolites form the core of the biological activity, classified into four main groups: limonoids and triterpenoids, phytosterols and amide alkaloids, oxygenated diterpenoids, and the flavonoid-polyphenol complex (Table 1).

Table 1

Principal secondary metabolites of *Azadirachta indica* A. Jussieu with documented biological activity against insect pests.

Metabolite	Mol. formula	MW (g/mol)	CAS No.	Plant part	Primary biological activity
Azadirachtin A	$C_{35}H_{44}O_{16}$	720.7	11141-17-6	Seed	Ecdysteroid antagonism, antifeedant (60–90%), intestinal cytotoxicity, immunosuppression, CYP/GST inhibition
Azadirachtin B	$C_{35}H_{44}O_{16}$	720.7	11141-17-6	Seed	Isomer B; lower biological activity than A
Salannin	$C_{34}H_{44}O_{9}$	596.7	992-20-1	Seed	Potent antifeedant; immediate action on gustatory chemoreceptors
Nimbin	$C_{30}H_{36}O_{9}$	556.6	13030-24-5	Seed, bark	Immunosuppressant; antifungal; synergist with azadirachtin
Gedunin	$C_{28}H_{34}O_{7}$	482.6	2753-30-2	Seed	Hsp90 inhibitor; amplifies EcR disruption; antimalarial
Azadiradione	$C_{28}H_{34}O_{4}$	450.6	18472-36-1	Seed, leaf	Contact insecticide; cuticle disruption; antibacterial
β-Sitosterol	$C_{29}H_{50}O$	414.7	83-46-5	Seed oil	Competitive antagonist EcR/USP receptor; analog of 20-HE
Quercetin	$C_{15}H_{10}O_{7}$	302.2	117-39-5	Leaf, bark	CYP P450 and GST inhibitor; pro-oxidant; antibacterial
Kaempferol	$C_{15}H_{10}O_{6}$	286.2	520-18-3	Leaf	Co-inhibitor CYP P450; antifungal; synergist with azadirachtin
Oleic acid (18:1 Δ^{9}-cis)	$C_{18}H_{34}O_{2}$	282.5	112-80-1	Seed oil	Primary lipid vehicle (50–60%); enhances transdermal penetration of azadirachtin
Linoleic acid (18:2 $\Delta^{9,12}$)	$C_{18}H_{32}O_{2}$	280.4	60-33-3	Seed oil	Co-vehicle (15–20%); generates LOOHs under UV → secondary oxidative stress
Salannol	$C_{32}H_{44}O_{8}$	564.7	78012-51-8	Seed	Complementary limonoid; synergistic antifeedant
Nimbinene	$C_{28}H_{34}O_{7}$	482.6	78916-54-8	Seed	High lipophilicity; synergistic activity with azadirachtin
Nimbolin A	$C_{39}H_{46}O_{8}$	650.8	24480-41-9	Seed	Antagonistic interactions with digestive enzymes
Azadiramide A	Amide alkaloid	—	No universal CAS	Leaf, seed	Neurotoxic properties; characterized by NMR
Margolone	$C_{19}H_{24}O_{3}$	300.4	120092-48-0	Seed	Tricyclic diterpenoid; intestinal epithelium cytotoxicity
Margolonone	$C_{19}H_{22}O_{4}$	314.4	120092-49-1	Seed	Oxidized derivative; lipid homeostasis disruption
(+)-Catechin	$C_{15}H_{14}O_{6}$	290.3	154-23-4	Leaf	CYP P450 and GST inhibition (trans configuration); synergistic oxidative stress
(−)-Epicatechin	$C_{15}H_{14}O_{6}$	290.3	490-46-0	Leaf	Isomer (cis); co-inhibitor of detoxification systems

Note. MW = molecular weight; CAS = Chemical Abstracts Service; 20-HE = 20-hydroxyecdysone; EcR/USP = ecdysone receptor/ultraspiracle protein; LOOHs = lipid hydroperoxides. Source: compiled by the authors after Schmutterer (1990), Mordue & Nisbet (2000), Koul (2004), and Nicoletti et al. (2023).

3.2.1. Limonoids and Triterpenoids

Tetranortriterpenoids constitute the most potent biological markers of neem, characterized by a highly oxygenated fused ring system (Kraus, 1995; Schmutterer, 1990). Azadirachtin A ($C_{35}H_{44}O_{16}$, CAS 11141-17-6) presents a pentacyclic skeleton (rings A–B–C–D–E) with 16 stereocenters. Salannin ($C_{34}H_{44}O_{9}$), a seco-limonoid with an open B ring, exhibits antifeedant potency superior to azadirachtin A at equivalent concentrations. Salannol ($C_{32}H_{44}O_{8}$), nimbinene ($C_{28}H_{34}O_{7}$), and nimbolin A ($C_{39}H_{46}O_{8}$) complement the limonoid profile with antagonistic interactions on insect digestive enzymes and chemoreceptors.

3.2.2. Phytosterols, Amide Alkaloids, and Diterpenoids

β-Sitosterol ($C_{29}H_{50}O$, CAS 83-46-5), present at 0.8–1.2% of seed oil, acts as a competitive antagonist of 20-hydroxyecdysone (20-HE) for the nuclear receptor EcR/USP (Behmer & Nes, 2003). Azadiramide A, an amide alkaloid characterized by NMR in leaves and seeds,

exhibits neurotoxic properties (Siddiqui et al., 2004). Margolones ($C_{19}H_{24}O_3$ and $C_{19}H_{22}O_4$, CAS 120092-48-0 and 120092-49-1) are tricyclic diterpenoids associated with cytotoxicity in the intestinal epithelium and disruption of larval lipid homeostasis (Koul, 2004).

3.2.3. Flavonoid and Polyphenolic Complex

Flavonoids are fundamental for the inhibition of detoxification systems and the generation of oxidative stress in polyphagous insects (Salminen & Karonen, 2011; Simmonds, 2001). Quercetin ($C_{15}H_{10}O_7$, CAS 117-39-5) and kaempferol ($C_{15}H_{10}O_6$, CAS 520-18-3), present in leaves of *A. indica*, competitively inhibit CYP6 and CYP9 monooxygenases and glutathione-S-transferases (GSTs). (+)-Catechin ($C_{15}H_{14}O_6$, CAS 154-23-4) and (−)-epicatechin ($C_{15}H_{14}O_6$, CAS 490-46-0) differ in the relative configuration of C2–C3 carbons (trans and cis, respectively), which determines variations in their affinity for detoxification enzymes. Both flavanols synergize with the limonoids of crude oil, incapacitating the insect from neutralizing the toxic complex (Riaz et al., 2014).

3.3. Formulation Efficacy under Laboratory Conditions

3.3.1. Acute Toxicity Parameters: LC_{50} and LT_{50}

Neem oil formulated as an emulsifiable concentrate (EC) with azadirachtin A content between 0.5% and 3% has demonstrated significant insecticidal activity against the different larval instars of *S. frugiperda* (Table 2). López et al. (2022) determined, through Probit analysis, LC_{50} values of 650–840 µL/L for L2–L4 larvae in Ecuador (25°C; R^2 = 0.93–0.95). Gutiérrez et al. (2010) reported LC_{50} values of 720–950 µL/L for L3–L5 in Mexico (22°C), and Gámez (2021) obtained 590–780 µL/L for L1–L3 in Nicaragua (27°C).

Giraldo-Vanegas et al. (2026a, b) determined the toxicological parameters of NeemAZAL® 1.2% EC for all six larval instars of *S. frugiperda* under Colombian high-altitude conditions, obtaining Probit models with R^2 = 0.94–0.95. LC_{50} values ranged from 579.67 µL/L (L1) to 921.36 µL/L (L6), with a 58.9% increase in the required concentration from first to sixth instar, adjustable to the model: LC_{50} = 472.8 + 74.3 × instar (R^2 = 0.986; $p < 0.001$). The LT_{50} was 52.39 h for L1, increasing to 196.69 h in L5 (271.3% increment). This ontogenetic susceptibility gradient is grounded in: (i) progressive cuticle thickening (~ 2 µm in L1 to ~ 15 µm in L6); (ii) a 4.2–6.8-fold increase in CYP6/CYP9 expression between L1 and L5 (Huang et al., 2023; Wang et al., 2023); (iii) toxicokinetic dilution due to body mass increase (~ 89-fold between L1 and L6); and (iv) a robust cellular immune response in advanced instars (Lavine & Strand, 2002).

3.3.2. Sublethal Effects on Biological Parameters

Beyond direct mortality, *A. indica* formulations exert significant sublethal effects. At sublethal concentrations (LC_{25}–LC_{10}), reductions in foliar consumption of 55–72% and weight gain of 40–65% are documented in L2–L4 larvae (Schmutterer, 1990; López et al., 2022). Exposure of L5–L6 larvae to 500–800 µL/L of formulated azadirachtin results in frequencies of malformed pupae of 20–45% and adults with deformed wings of 15–30%. Almeida (2021) reported a reduction in fecundity of 20–35% and egg viability of 15–40% in adults emerging from treated larvae, evidencing transgenerational effects (Isman, 2020; Pavela & Benelli, 2023).

3.4. Aqueous Extracts and Emerging Formulations

Neem seed kernel extract (NSKE) at 5% is one of the most studied formulations due to its low cost and availability (Campos et al., 2016; Isman, 2020). Gámez (2021) reported mortalities of 58–78% at 72 h with NSKE at 5% in L2–L4 larvae. López et al. (2022) demonstrated that NSKE at 10% achieved mortalities of 80–88% at 96 h, statistically equivalent to those of formulated oil at 1.0 mL/L, with antifeedant effects of 65–75% even at 3%. Extracts of *A. indica* leaves, with contributions of nimbin ($C_{30}H_{36}O_9$) and gedunin ($C_{28}H_{34}O_7$), show mortalities of 45–65% in L1–L2 at concentrations of 10% in water (Machekano et al., 2024).

Emerging formulations — nanoemulsions, microencapsulated azadirachtin, and chitosan-azadirachtin nanocomposites — present LC_{50} values up to 2.5-fold lower and extended environmental half-lives of 7–21 days compared with conventional EC formulations (Bezerra et al., 2025; Wu et al., 2025). Wu et al. (2025) demonstrated that O-carboxymethyl chitosan-based azadirachtin nanoformulations substantially improve the anti-degradation properties of azadirachtin and significantly enhance antifeedant activity against *S. frugiperda*, representing a major advance in the field of neem-based biopesticide technology. Cantrell et al. (2024) place these developments within the broader framework of natural product-based pesticide innovation, underscoring the importance of precision delivery systems in overcoming the photolability limitations of azadirachtin.

3.5. Field Efficacy

Field studies report efficacies of formulated neem oil (1.0–2.0 mL/L) of 40–85% reduction in foliage damage in *Z. mays* crops (Gutiérrez et al., 2010; López et al., 2022; Machekano et al., 2024). Harrison et al. (2019), in a review of sub-Saharan African trials, found that applications directed at L1–L2 achieved damage reductions of 65–85%, while late applications on L4–L6 showed efficacies of 30–50%.

Photodegradation of azadirachtin A under direct solar radiation reduces its half-life to 1–4 days, requiring applications every 5–7 days to maintain acceptable control levels (Stark & Walter, 1995; Barrek et al., 2004). In high-altitude Andean agroecosystems, Giraldo-Vanegas et al. (2026a, b) employed applications at 5–7-day intervals, evidencing the technical necessity of UV-protective microemulsions or co-adjuvants.

3.6. Multi-Target Modes of Action

The metabolites of *A. indica* act on *S. frugiperda* through complementary biochemical mechanisms that affect multiple insect systems simultaneously or sequentially (Table 3). This multi-target attack strategy saturates the phenotypic plasticity of the pest, minimizing the selection of resistant biotypes (Isman, 2020; Sparks & Nauen, 2015).

Table 3
Mode of action of principal metabolites of *Azadirachta indica* A. Jussieu on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae).

Metabolite	Mol. formula	Target / Site of action	Mechanism and main effect	Instar	References
Azadirachtin A	$C_{35}H_{44}O_{16}$	EcR/USP and Met/FISC receptors; gustatory chemoreceptors; intestinal epithelium; hemocytes	Ecdysteroid antagonism (molting blockage); antifeedant 60–90% ingesta; intestinal apoptosis; ↓PO; downregulates CYP6/CYP9/GSTs	L1–L6	Mordue & Blackwell, 1993; Kumar et al., 2024; Giraldo-Vanegas et al., 2026a, 2026b
Salannin	$C_{34}H_{44}O_{9}$	Chemoreceptor deterrent neurons (maxillary palps)	Immediate antifeedant (minutes); nanomolar EC_{50}; ↓foliar consumption 65–90% in L2–L4; no systemic absorption	L2–L4	Mordue & Blackwell, 1993; Senthil-Nathan, 2013; López et al., 2022
Nimbin	$C_{30}H_{36}O_{9}$	Hemocytes (PO, antimicrobial peptides); peritrophic membrane	Immunosuppression: ↓PO; ↑peritrophic permeability; facilitates secondary penetration of azadirachtin into hemocoel (L3–L5); synergist	L3–L5	Schmutterer, 1990; Senthil-Nathan, 2013; Machekano et al., 2024
Gedunin	$C_{28}H_{34}O_{7}$	Hsp90 (N-terminal/ATP domain); EcR receptor; fat body	Inhibits Hsp90 → ubiquitin-proteasome degradation of EcR complex; amplifies molting disruption in L3–L5	L3–L5	Senthil-Nathan, 2013; Kumar et al., 2024
Azadiradione	$C_{28}H_{34}O_{4}$	Epicuticular membrane (contact); intestinal microbiome	Contact insecticide: cuticle disruption (log P ≈ 4.2); broad-spectrum antibacterial on midgut microbiome	L1–L6	Schmutterer, 1990; Campos et al., 2016
β-Sitosterol	$C_{29}H_{50}O$	EcR/USP receptor; midgut lipid membrane domains	Competitive antagonism of 20-HE; saturates ecdysteroid signaling; interferes with membrane biosynthesis	L1–L6	Schmutterer, 1990; Behmer & Nes, 2003
Quercetin	$C_{15}H_{10}O_{7}$	CYP6/CYP9 (phase I); GSTs (phase II); hemocyte mitochondria; microbiome	↑azadirachtin half-life 15–30%; caspase-3/9 apoptosis at > 100 µM; bacteriostatic on intestinal microbiome	L2–L5	Huang et al., 2023; Simmonds, 2001
Kaempferol	$C_{15}H_{10}O_{6}$	CYP P450; intestinal microbiome	Co-inhibits CYP P450; antifungal on microbiome; synergist with azadirachtin in L2–L4	L2–L4	Senthil-Nathan, 2013
Oleic acid (Δ^9-cis)	$C_{18}H_{34}O_{2}$	Epicuticular surface lipids	Primary lipid vehicle (50–60%); Δ^9-cis geometry improves transcuticular diffusion of azadirachtin	L1–L6	Campos et al., 2016
Linoleic acid ($\Delta^{9,12}$)	$C_{18}H_{32}O_{2}$	Epicuticular surface; contact membranes	Co-vehicle (15–20%); generates LOOHs under UV → secondary oxidative stress on cuticle	L1–L6	Campos et al., 2016
Catechin/Epicatechin	$C_{15}H_{14}O_{6}$	CYP P450; GSTs; peritrophic membrane	Inhibition of detoxification phase I and II enzymes; synergistic oxidative stress	L2–L5	Riaz et al., 2014; Salminen & Karonen, 2011
Margolones	$C_{19}H_{24}/_{22}O_{3}/_{4}$	Intestinal epithelium; lipid membrane domains	Cytotoxicity in midgut mesenterone; peritrophic membrane destruction; lipid homeostasis disruption	L1–L4	Schmutterer, 1990; Koul, 2004

Note. EcR/USP = ecdysone receptor/ultraspiracle protein; Met/FISC = juvenile hormone receptor; PO = phenoloxidase; CYP = cytochrome P450; GSTs = glutathione-S-transferases; LOOHs = lipid hydroperoxides; 20-HE = 20-hydroxyecdysone; Hsp90 = 90 kDa chaperone protein. Source: compiled by the authors.

3.6.1. Azadirachtin A: Neuroendocrine Antagonism and Cytotoxicity

Azadirachtin A ($C_{35}H_{44}O_{16}$, MW = 720.7 g/mol) acts through four principal mechanisms on *S. frugiperda*: (i) *neuroendocrine antagonism*, competing with 20-HE and juvenile hormone (JH-III) for the nuclear receptors EcR/USP and Met/FISC, blocking early ecdysis genes (E74, Broad-Complex, HR3) and preventing synthesis of new cuticle, generating exuvia retention and mortality during ecdysis (Mordue &

Blackwell, 1993; Martinez & van Emden, 2001); (ii) *feeding inhibition*, blocking gustatory chemoreceptors of the maxillary palps and suppressing ingestion by 60–90% (Mordue et al., 2010); (iii) *intestinal cytotoxicity*, inducing apoptosis in columnar cells of the intestinal epithelium with cytoplasmic vacuolization, loss of microvilli, and permeabilization of the peritrophic membrane (Zhang et al., 2023); (iv) *immunosuppression and gene regulation*, suppressing phenoloxidase (PO) and negatively regulating CYP6, CYP9, and GSTs (Huang et al., 2023; Wang et al., 2023).

3.6.2. Salannin, Nimbin, Gedunin, and Azadiradione

Salannin ($C_{34}H_{44}O_9$) specifically activates deterrent chemoreceptor neurons with nanomolar binding constants, generating immediate rejection without systemic absorption (Senthil-Nathan, 2013). Nimbin ($C_{30}H_{36}O_9$) exerts immunosuppressive activity on granular hemocytes and plasmatocytes, inhibiting PO and antimicrobial peptide synthesis, and alters peritrophic membrane permeability, facilitating secondary penetration of azadirachtin A into the hemocoel in L3–L5 larvae (Schmutterer, 1990; Machekano et al., 2024). Gedunin ($C_{28}H_{34}O_7$) competitively inhibits Hsp90 of *S. frugiperda*, causing ubiquitin-proteasome degradation of the EcR complex and amplifying molting disruption in L3–L5 (Kumar et al., 2024). Azadiradione ($C_{28}H_{34}O_4$, log P ≈ 4.2) acts as a contact insecticide on the epicuticular membrane and exhibits antibacterial activity against the intestinal microbiome (Schmutterer, 1990; Campos et al., 2016).

3.6.3. Flavonoids and Fatty Acids: Enzymatic Synergy and Vehicular Function

Quercetin ($C_{15}H_{10}O_7$) competitively inhibits CYP6 and CYP9 monooxygenases (phase I) and GSTs (phase II), prolonging the systemic half-life of azadirachtin by 15–30% (Huang et al., 2023); at concentrations > 100 µM it acts as a pro-oxidant in hemocytes, activating caspase-3/9 apoptotic pathways (Simmonds, 2001). Kaempferol ($C_{15}H_{10}O_6$) co-inhibits CYP P450 and maintains antifungal activity and synergy with azadirachtin in L2–L4 (Senthil-Nathan, 2013). Catechin and epicatechin ($C_{15}H_{14}O_6$, trans and cis configurations, respectively) inhibit phase I and II detoxification enzymes, incapacitating the insect from neutralizing the toxic complex (Riaz et al., 2014). Oleic acid ($C_{18}H_{34}O_2$, 50–60% of the oil), through its Δ^9-cis geometry, improves the cuticular penetration of azadirachtin by interacting with epicuticular lipids. Linoleic acid ($C_{18}H_{32}O_2$, 15–20%) acts as a co-vehicle and can generate lipid hydroperoxides (LOOHs) under UV radiation, contributing to cuticular oxidative stress (Campos et al., 2016).

3.7. Chronic Impact and Suppression of Reproductive Dynamics

The impact of neem formulations on *S. frugiperda* transcends the lethal control of immature stages. Endocrine disruption significantly reduces juvenile hormone (JH) titers in females, blocking vitellogenin synthesis in the fat body, causing severe ovarian atrophy and subclinical sterility (Medina et al., 2004). Transfer of limonoid residues to egg chorion prevents embryogenesis (trans-ovarian ovicidal effect), while in exposed males a drastic reduction in sperm motility is documented (Sáenz-de-Cabezón et al., 2005). The high sensitivity of gravid females to polyphenols present on treated leaf surfaces triggers an oviposition deterrence response mediated by tarsal and ovipositor receptors (Viana & Prates, 2003). This set of chronic effects strengthens the role of neem as an integral tool in IPM of *S. frugiperda*.

3.8. Influence of Altitude and High-Altitude Climatic Conditions

In Andean agroecosystems such as those of Pamplona, Norte de Santander (2,586 m a.s.l., 17 ± 1°C), climatic variables substantially modify the stability of extracts and the ecophysiology of *S. frugiperda*. At higher altitude, the reduced atmospheric filtration increases the incidence of ultraviolet radiation, accelerating the photooxidation of photolabile limonoids and reducing their half-life on the foliar canopy (Barrek et al., 2004; Isman, 2020). The works of Giraldo-Vanegas et al. (2026a, b) under these conditions employed 5–7-day application intervals, evidencing the technical necessity of microemulsions or UV-protective adjuvants. The emerging nanoformulations reported by Wu et al. (2025) and Bezerra et al. (2025), which extend azadirachtin half-life to 7–21 days, represent particularly promising solutions for high-altitude Andean conditions.

From a thermodynamic standpoint, the high-altitude mountain climate reduces the basal metabolic rate of poikilotherm insects. In *S. frugiperda*, this prolongs the duration of each larval stadium (García-Roa et al., 1999; Núñez-García et al., 2024), extending the exposure window to the biopesticide, while also decreasing the foliar consumption rate and ingestion of active principles (Smirle et al., 1996). Nevertheless, low temperatures also depress the enzymatic efficiency of GST and oxidases in the insect, shifting the locus of control from acute mortality toward sustained endocrine disruption that collapses molting and sterilizes adult populations. The LT_{50} values documented by Giraldo-Vanegas et al. (2026b) — up to 196.69 h in L5 at 17°C — reflect this particular temporal dynamics of neem biological activity under Andean conditions.

4. Conclusions

The bibliographic review synthesized in Table 2 (28 studies, 1990–2026) and the analysis of the phytochemical profile of *A. indica* A. Jussieu (Tables 1 and 3) allow the following conclusions on the use of its formulations against *S. frugiperda* (J. E. Smith).

Formulations of *A. indica* — especially EC oil and NSKE — exert significant insecticidal and antifeedant activity on *S. frugiperda*, with differential susceptibility favoring control of L1–L3 instars (LC_{50} = 580–703 µL/L; LT_{50} = 21–67 h) compared with L4–L6 (LC_{50} = 795–921 µL/L; LT_{50} = 90–410 h). The critical physiological threshold between L3 and L4 is associated with cuticle thickening, increased CYP6/CYP9 expression, and toxicokinetic dilution documented by Wang et al. (2023) and Giraldo-Vanegas et al. (2026a, b). This threshold determines the optimal application window for maximum efficacy.

The multi-target mechanisms of action of *A. indica* metabolites — ecdysteroid antagonism, gustatory inhibition, intestinal cytotoxicity, immunosuppression, and enzymatic inhibition of detoxification systems — constitute a strategy that saturates the phenotypic plasticity of *S. frugiperda*, delaying the selection of resistant biotypes and consolidating neem as a cornerstone for IPM sustainability across different altitudinal zones. This multicomponent synergistic action explains the greater efficacy of crude *A. indica* oil over purified azadirachtin.

In field conditions, efficacy ranges between 40% and 85% damage reduction in *Z. mays* crops, conditioned primarily by the larval instar at the time of application, the environmental stability of the formulation, and the use of adjuvants. Emerging nanoformulations — microencapsulation, chitosan nanocomposites — represent a technological priority for overcoming the photolability limitation of conventional EC formulations, with demonstrated improvements in half-life and efficacy in both laboratory and semi-field conditions.

Key knowledge gaps identified include: (i) scarcity of field studies in high-altitude Andean ecosystems with *S. frugiperda*; (ii) insufficient field evidence for emerging formulations (nanotechnology, microencapsulation); (iii) absence of complete molecular characterization of the ontogenetic resistance of *S. frugiperda* to neem oil through comparative transcriptomics across all six larval instars; and (iv) the need for studies integrating the effect of the complete phytochemical complex of neem — including margolones, catechins, and amide alkaloids — on pest population dynamics in the field.

Declarations

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