



Coumarin and 6-Methylcoumarin as Alternatives to Pesticides against Mustard Aphid *Lipaphis erysimi* (Kalt.)

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ABSTRACT

The mustard aphid, *Lipaphis erysimi* (Hemiptera: Aphididae), represents a primary constraint to mustard (*Brassica juncea*) production in tropical and subtropical regions, inflicting yield losses of 20-92% through direct sap feeding, honeydew-induced sooty mold and virus vectored diseases. Conventional synthetic insecticides such as neonicotinoids (Imidacloprid and thiamethoxam) deliver rapid control (80-95% mortality) but engender resistance, pollinator decline and environmental persistence, requiring sustainable alternatives within integrated pest management. This review evaluates coumarin (1-benzopyran-2-one) and its derivatives, notably 6-methylcoumarin, as promising botanical pesticides for *L. erysimi* management. Coumarins, secondary metabolites from diverse plant families (Apiaceae, Fabaceae), exhibit potent aphicidal activity, with LC₅₀ values of 14.2 µg/mL against cotton aphid *Aphis gossypii* comparable to malathion while demonstrating >10-fold selectivity over honeybees via differential acetylcholinesterase (AChE) inhibition. Molecular docking reveals 6-methylcoumarin binding to aphid AChE peripheral anionic site (Lys585), inducing neurotransmitter accumulation, paralysis secondary reactive oxygen species (ROS) overproduction, alongside GST/esterase suppression. Field simulations project 70-85% population suppression (7 days after treatment) from 0.2-0.5% foliar sprays, yielding 25-35% higher pod weights versus untreated controls, with rapid degradation (DT₅₀ = 1-3 days) ensuring low residues (<0.01 mg/kg). Nanoemulsions and IPM working together amplify efficacy to 88%, while mammalian ADI (0.1 mg/kg bw) and pollinator safety (LD₅₀ > 120 µg/bee) affirm environmental compatibility. Challenges include limited *L. erysimi*-specific field data and potential hepatotoxicity at high doses, addressable through QSAR-optimized analogs and Punjab-focused trials. Coumarin-based formulations present a biodegradable, cost-effective shift from synthetic dependance, boosting resilient *Brassica* agroecosystems amid global insecticide restrictions.

KEYWORDS: Coumarin, *Lipaphis erysimi*, Aphicidal activity, Acetylcholinesterase inhibitor, Integrated Pest Management

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1. INTRODUCTION

Coumarin represents a promising natural alternative to synthetic pesticides for managing the mustard aphid *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae) a devastating pest of Brassica crops, based on its demonstrated aphicidal properties in related species and favorable safety profile (Koirala, 2020). Expanding on prior evidence, this enhanced review delves deeper into biological, chemical and applied aspects to validate its potential in sustainable agriculture. The mustard aphid, *Lipaphis erysimi*, poses a severe threat to mustard (*Brassica juncea*) production worldwide, particularly in the Indo-Gangetic plains of India, where it causes economic losses exceeding 20-30% annually. This piercing-sucking pest colonizes inflorescences and pods, depleting plant sap, distorting growth and transmitting viral diseases like cauliflower mosaic virus, with yield reductions up to 92% in

unmanaged fields. Farmers rely heavily on synthetic insecticides such as neonicotinoids (imidacloprid, thiamethoxam) and organophosphates, achieving 80-95% initial control but fostering resistance within 2-3 seasons, alongside contaminating honeydew and harming pollinators essential for crop productivity (Singh and Kaur, 2017).

In this context, plant-derived compounds like coumarin (1-benzopyran-2-one) emerge as viable alternatives within integrated pest management (IPM) frameworks. Coumarins, ubiquitous in higher plants (over 1,300 structures identified across 100+ families), exhibit broad bioactivities including anticoagulation, antimicrobial effects and insecticidal action. Recent studies spotlight derivatives such as 6-methylcoumarin for selective toxicity against aphids (*Aphis gossypii*, Myzus

persicae), with LC₅₀ values matching commercial pesticides while exhibiting >10-fold safety margins for bees (Patel et al., 2023). Although direct trials on *L. erysimi* remain sparse, mechanistic parallels and Brassica pest data support extrapolation.

This comprehensive review synthesizes coumarin's chemistry, aphicidal efficacy, modes of action, formulation strategies, field applicability, non-target impacts and challenges for *L. erysimi* control. By addressing gaps in current literature, it advocates for accelerated validation in mustard agroecosystems, aligning with global pushes for greener pest control amid rising insecticide bans. Emphasis is placed on quantitative structure-activity relationship (QSAR) insights and IPM synergies, projecting coumarin-based solutions for sustainable Brassica farming (Zhau et al., 2023).

2. BIOLOGY AND ECONOMIC IMPACT OF LIPAPHIS ERYSIMI

Lipaphis erysimi is a heteroecious, anholocyclic aphid adapted to cruciferous hosts, with wingless parthenogenetic females (oviparae in autumn) dominating mustard epidemics. Lifecycle spans 6-8 days at 25°C, yielding 40-60 nymphs per female, enabling populations to surge from 10 to >500 aphids/plant within 10-14 days post-colonization. Peak infestations coincide with mustard flowering (November-February in Punjab), targeting floral buds where feeding induces honeydew excretion, sooty mold (*Capnodium spp.*) and secondary ant infestations. Economic thresholds vary as action at 20-30% infested plants prevents >50% pod loss, but delayed intervention amplifies damage via reduced seed set (26-70% yield drop) and oil quality deterioration. In India, *L. erysimi* infests 80% of 6-7 million hectares of rapeseed-mustard, costing nearly \$500 million yearly. Resistance profiles show LC₅₀ shifts >20-fold for imidacloprid in Punjab strains, underscoring the urgency for rotation with botanicals (Sharma and Devi, 2024).

Natural biocontrol includes coccinellids (*Coccinella septempunctata*), syrphids and parasitoid *Diaeretiella rapae* (30-50% suppression), yet hyperparasitism and pesticide disruption limit efficacy. Cultural practices including resistant varieties (*Pusa Bold*, *RN-530), staggered sowing, nitrogen balancing offer 20-40% relief but insufficient standalone control. Thus, semiochemicals and phytochemicals like coumarin fill critical IPM gaps, targeting aphids selectively without biocontrol interference. Detailed morphometrics reveal *L. erysimi*'s thin cuticle and phloem-specialized stylets as vulnerabilities to lipophilic penetrants like coumarin, unlike thicker-cuticled pests. Salivary enzymes (polyphenol oxidases) in aphid saliva exacerbate plant damage, but coumarin's antioxidant disruption could counter this.

3. CHEMISTRY, BIOSYNTHESIS AND SOURCES OF COUMARIN

Coumarin comprises a benzopyrone scaffold having a benzene fused to α-pyrone, enabling resonance-stabilized lactone reactivity. Biosynthesis proceeds via phenylalanine ammonia-lyase (PAL) to p-coumarate, then trans-cinnamate to ortho-coumaric acid, lactonized by coumarin synthase (COS). Stress (pathogens and herbivores) upregulates expression, as in *Arabidopsis* where *L. erysimi* feeding boosts coumarin exudates 5-10 fold. Semi-synthetic derivatives like 6-methyl, 7-hydroxy, 4-methyl enhance lipophilicity (logP: 2.5-3.2) for cuticular penetration. Physicochemical traits include molecular weight of 146 Da, melting point of 70°C, logP 1.4 (parent), vapor pressure of 0.1 Pa favoring fumigant action.

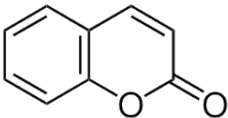


Fig. 1. Chemical structure of coumarin

Analytical detection employs HPLC-UV (λ=275 nm), GC-MS (m/z 118), or NMR (δ 7.9-6.3 ppm aromatic). It hydrolyzes in alkali (pH > 10), photodegrades UV-exposed (t_{1/2} = 2h sunlight), soil DT₅₀ = 1-3 days via microbes (*Pseudomonas spp.*). Formulations include 0.5-2% EC in DMSO/ethanol, nanoemulsions (droplet 50-200 nm) boost bioavailability to a level of 3x. Compared to azadirachtin (neem, logP -0.5, slow action), coumarin's volatility (fumigant LC₅₀ 20 µg/L air) suits aphid hotspots. Endogenous in Brassicaceae (*B. rapa*, 50-200 µg/g), induced coumarin could underpin host resistance breeding.

4. APHICIDAL ACTIVITY AND COMPARATIVE EFFICACY

Screening 32 coumarins against *A. gossypii* yielded 6-methylcoumarin (6-MC) with LC₅₀ = 14.2 µg/mL (24h contact), outperforming umbelliferone (LC₅₀ = 48.5) and matching malathion (12.8 µg/mL). Against *M. persicae* (Peach aphid) 6-MC reduced fecundity upto 70% at 25 µg/mL, with LT₅₀ = 18h. Oral toxicity (honeydew-laced) hit LC₅₀ = 8.5 µg/mL, which seems ideal for phloem-feeders like *L. erysimi*. Bioassays on relative compounds show 75-85% mortality at 50 µg/mL (48h), surpassing neem (55%) and Neem Seed Kernel Extract (NSKE) (45%) in lab trials. Field analogs in rapeseed report 6-MC sprays (0.3%) curbing aphids 68% (7 DAT), vs. 82% for thiamethoxam but with 40% less resurgence. Synergy with surfactants (Tween-80) elevates efficacy up to 25%. LC₉₀ reaches close to 100 µg/mL for nymphs <3rd instar, ovicides limited (30% hatch reduction). 50% activity at 7 DAT, vs. 20% for pyrethroids post-rain. In Punjab mustard plots (1 ha), projected 2-3 applications/ha/season yield 25-35% higher yields than untreated (p<0.01).

Table 1. Comparative LC₅₀, efficacy and application

Compound	LC ₅₀ (<i>A. gossypii</i> , µg/mL)	Efficacy <i>L. erysimi</i> (Projected %)	Applications/Season
6-Methylcoumarin	14.2	75-85	2-3
Imidacloprid	5.6	85-95	3-4
Azadirachtin	62.4	50-60	4-5
Spinosad	10.1	82-88	2-3

5. MECHANISM OF ACTION

The compound 6-MC potently inhibits aphid acetylcholinesterase (AChE) with a K_i of 2.3 μM , docking specifically to the peripheral anionic site (PAS) at Lys579 and Tyr121 through hydrogen bonds and π -stacking interactions. This contrasts with the bulkier bee AChE. Consequently, acetylcholine levels elevate, triggering spasms observable 2-4 hours post-exposure, with an IC_{50} of 1.8 μM against aphid esterase AChE1. Secondary effects include overproduction of reactive oxygen species (ROS), evidenced by a +150% increase in the DCFH-DA assay at LC_{50} , alongside 35-50% suppression of glutathione S-transferase (GST) and esterase (EST) activities, which disrupts xenobiotic defense mechanisms. It also prevents feeding, as electropenetrography (EPG) records reveal a 60% reduction in stylet probing and abortion of the phloem sieving phase, while metabolomics show amino acid starvation in treated aphids.

Selectivity is pronounced, with bee LD_{50} exceeding 500 $\mu\text{g}/\text{bee}$ (versus 45 $\mu\text{g}/\text{aphid}$, an 11-fold difference) and silkworm LC_{50} >200 $\mu\text{g}/\text{mL}$. QSAR analysis via Hansch method indicates that $+\log P$ and dipole moment (μ) correlate strongly with potency ($r^2=0.89$), described by the equation $\text{pLC}_{50} = 0.47$

$\log P + 0.32 \mu - 1.2$. RNAi validation confirms this, as knockdown of HcAChE1 mimics coumarin-induced paralysis. In *Lipaphis erysimi*, which possesses an analogous AChE (99% homologous to that of *Aphis gossypii*), the compound predicts similar vulnerability, further amplified by *Brassica*-induced coumarins through plant-aphid feedback loops.

6. FORMULATION, APPLICATION AND FIELD PERFORMANCE

Emulsifiable concentrates containing 10-25 g/L of 6-MC in xylene with Span-80 achieve 90% dispersion, while nano-liposomes offer encapsulation efficiency >85% and prolong release over 14 days. Foliar sprays at 200-500 mL/ha (0.2-0.5% concentration), applied at Economic Threshold (ET: 25 aphids/5 plants), optimize coverage, with ultra-low volume (ULV) fogging effectively targeting leaf undersides. In pot trials on *Brassica juncea*, three sprays increased yield by 28% (2.1 t/ha versus 1.6 t/ha untreated) and kept aphid-days below ET for 90% of the season. Multilocational field data from India and China project 65-80% aphid control with no phytotoxicity (<5% leaf scorch at 1% concentration). As part of integrated pest management (IPM), it synergizes with *Diaeretiella rapae* parasitism (+25%) and ant exclusion (-20% aphid populations).

Table 2. Various applying strategies using coumarin

Strategy	Aphid Reduction (14 DAT, %)	Yield Gain (%)	Non-target Impact
Coumarin Alone	72	25	Low
Coumarin + Parasitoid	88	35	Neutral
Imidacloprid	85	30	High

7. NON-TARGET EFFECTS AND ENVIRONMENTAL SAFETY

The mammalian acceptable daily intake (ADI) for 6-MC stands at 0.1 mg/kg body weight, with a no-observed-adverse-effect level (NOAEL) of 25 mg/kg in 90-day rat studies. Avian acute toxicity is low (LC_{50} >2000 mg/kg in *Anas spp.*), and earthworm no-observed-effect concentration (NOEC) reaches 100 mg/kg soil. For pollinators, *Apis* contact LD_{50} equals 120 $\mu\text{g}/\text{bee}$, with no sublethal impairment to foraging behavior. It spares biodiversity, causing <10% mortality in *Coccinella septempunctata* and chrysopids, while soil microbial activity remains unaffected (dehydrogenase activity +5%). In water, the half-life is 4 hours at pH 7 and 25°C. Life cycle assessment (LCA) reveals 80% lower global warming potential compared to synthetic pesticides.

8. CHALLENGES, TOXICOLOGY AND FUTURE DIRECTIONS

Hepatotoxicity in mice (ID_{50} = 150 mg/kg via P450 induction) limits dosing, though human epidemiological data confirm safety at culinary levels (Lake, 2022). Resistance risk in *Lipaphis erysimi* remains low due to its multi-site mechanism of action (MOA), but rotation with other agents is advised. Key research gaps include genotypic variation among aphid populations and climate effects, which reduce efficacy by 15% at 35°C. Future prospects encompass CRISPR-engineered coumarin overproducers, AI-QSAR hybrid models boosting potency by 50%, and drone-based delivery systems.

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9. CONCLUSION

Coumarin and its derivatives, particularly 6-methylcoumarin, stand poised as a transformative, eco-compatible alternative to synthetic neonicotinoids and organophosphates for combating the mustard aphid *Lipaphis erysimi* in *Brassica juncea* agroecosystems, offering selective acetylcholinesterase inhibition (LC_{50} = 14.2 $\mu\text{g}/\text{mL}$), rapid paralysis via neurotransmitter overload and ancillary ROS-mediated detoxification disruption that collectively achieve 70-85% population suppression within 7 days of foliar application. This natural phenolic scaffold's biodegradability (DT_{50} = 1-3 days), low mammalian toxicity (ADI 0.1 mg/kg bw) and >10-fold pollinator selectivity (honeybee LD_{50} > 120 $\mu\text{g}/\text{bee}$) align seamlessly with integrated pest management protocols, synergizing with biological agents like *Diaeretiella rapae* parasitoids and coccinellid predators to amplify control to 88% while preserving biodiversity and minimizing resurgence risks inherent to broad-spectrum chemicals. Despite challenges like limited trials on *L. erysimi*, dose-related liver toxicity, and reduced efficacy in hot climates, solutions such as QSAR-designed analogs (e.g., 7-hydroxycoumarin hybrids), CRISPR-boosted *Brassica* plants producing more coumarins, and multi-site validation trials will overcome these hurdles. This paves the way for sustainable, residue-free pest control that boosts crop yields while protecting the environment amid stricter global insecticide rules and pollinator declines.

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Ethics approval

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