



RNAi-Based Biopesticides: Silencing Insect Genes for Sustainable Pest Management

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Insect pest management has rested on synthetic chemistry for decades and that foundation is weakening. Resistance has eroded one molecule after another, while the damage to pollinators and natural enemies has become difficult to justify. RNA interference (RNAi) offers a different approach. An RNAi biopesticide delivers a short double-stranded RNA (dsRNA) whose sequence matches one essential gene in one target pest. When that pest feeds, the gene is silenced and the insect dies, while unrelated species remain unaffected. Two products are already registered: a transgenic maize trait against corn rootworm and a spray against Colorado potato beetle. This article examines how RNAi works, why some pests respond better than others, how dsRNA is delivered and produced, what is known about its safety, and what the technology may mean for Indian agriculture.

Introduction

Insects destroy up to 40 per cent of global crop production annually posing a major challenge to agricultural productivity and food security. To minimize these losses, pesticide use has increased worldwide. Global pesticide use rose from 1.8 million tonnes in 1990 to 3.53 million tonnes in 2021, yet insect pests continue to pose substantial challenges to crop protection (Ali *et al.*, 2026). The development of insecticide resistance across major chemical classes has further reduced the effectiveness of conventional pest-control strategies. Moreover, broad-spectrum insecticides can harm beneficial organisms, including parasitoids, predators, and pollinators. Sap-sucking pests, such as aphids, whiteflies, and planthoppers, present an additional challenge because Bt crops generally provide limited protection against these insects, which have become important pests in several Bt-based cropping systems (Jain *et al.*, 2021). These limitations highlight the need for selective and environmentally compatible pest-control strategies that can target harmful insects while minimizing effects on beneficial species.

RNAi has emerged as a promising approach to address these challenges because it enables sequence-specific suppression of target genes. It is a natural gene-regulatory mechanism conserved across plants, animals, and insects. It enables gene silencing without altering the underlying DNA sequence. The process is commonly triggered by double-stranded RNA (dsRNA), which is processed by cellular enzymes into small RNA molecules that guide the silencing machinery towards complementary messenger RNA (mRNA). The target mRNA is then degraded or its translation is inhibited, preventing or reducing the production of the corresponding protein. Consequently, although the target gene remains intact in the genome, its expression is suppressed. This mechanism plays important roles in

gene regulation, development and defence against certain viruses, making it a promising foundation for developing targeted pest-control technologies.

Building on this natural mechanism, RNAi-based biopesticides are being investigated as a more targeted approach to insect pest management. The strategy involves identifying an essential gene in the target pest, designing a complementary dsRNA molecule, and delivering it through methods such as topical application or expression in a crop plant. When a susceptible insect ingests the dsRNA, the RNAi machinery can silence the target gene, potentially disrupting essential biological functions and causing reduced growth, impaired development, or death, depending on the gene targeted and the effectiveness of delivery. The sequence-specific nature of RNAi allows carefully designed dsRNA molecules to target particular insect pests while potentially minimizing effects on beneficial and other non-target organisms. In addition, RNA is generally biodegradable, which may help reduce the persistence of residues in the environment compared with some conventional chemical pesticides. These characteristics make RNAi-based biopesticides a potential avenue to developing more targeted and environmentally compatible pest-management strategies.

How a gene is silenced

To appreciate why RNAi is so selective, it helps to follow the molecule step by step. Ingested dsRNA enters the insect cell through membrane channels or by endocytosis. In the cytoplasm it meets the enzyme Dicer, which cuts it into fragments of 21 to 25 nucleotides known as small interfering RNAs (siRNAs). Each siRNA is taken up by the RNA-induced silencing complex (RISC), and one of its strands serves as a guide that allows RISC to scan the cell for any mRNA with a complementary sequence. On finding a match, the complex cleaves the messenger in two. No intact messenger means no protein, and if that protein is indispensable, the insect cannot complete its development.

Selectivity rests on that act of recognition. RISC cuts only a messenger that matches its guide closely over a continuous stretch. A dsRNA designed against Colorado potato beetle will therefore do nothing to a honey bee unless, by coincidence, the bee carries a nearly identical sequence in the same gene. Such coincidences are screened for in advance by bioinformatic platforms that compare every candidate against the genomes of beneficial species (Cedden *et al.*, 2025).

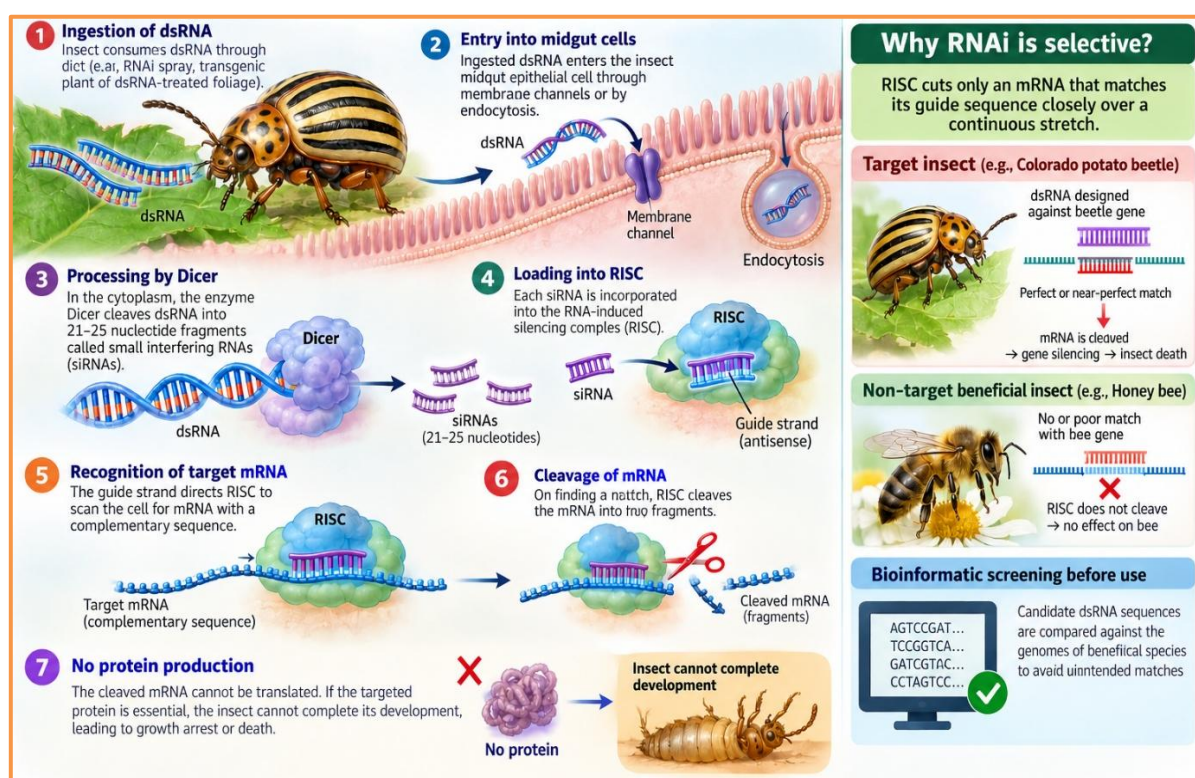


Figure1: How RNAi works

Why some pests respond and others resist

Not all insects respond equally. Beetles have proved remarkably amenable, even the modest doses of dsRNA bring about high mortality in corn rootworm, Colorado potato beetle, flea beetles and the 28-spotted ladybird beetle that troubles brinjal and potato growers in India. Caterpillars tell a different story. They ingest dsRNA readily, but much of it becomes trapped in endosomes and their guts are rich in RNA-degrading enzymes, so far larger doses or protective formulations are needed. Sap-sucking bugs fall in between. Their saliva and midgut harbour nucleases, and several species lack the transport proteins that spread the silencing signal between tissues. Even so, RNAi has been tested in some 38 hemipteran species from 14 families, among them aphids, whiteflies, brown planthopper and Asian citrus psyllid, with encouraging though variable results (Jain *et al.*, 2021). Table 1 draws together representative examples.

Table 1. Representative insect pests controlled by RNAi in laboratory and field studies

Order	Pest species	Target gene	Delivery method	Outcome
Coleoptera	<i>Diabrotica virgifera virgifera</i> (Western corn rootworm)	DvSnf7; V-ATPase	Transgenic maize	Larval mortality; commercial trait
Coleoptera	<i>Leptinotarsa decemlineata</i> (Colorado potato beetle)	Proteasome subunit beta-5	Foliar spray	>90% mortality; registered product
Coleoptera	<i>Henosepilachna vigintioctopunctata</i> (28-spotted ladybird)	HvUSP; Akirin	Leaf feeding; injection	High mortality; no effect on predatory ladybird
Lepidoptera	<i>Spodoptera exigua</i> (Beet armyworm)	Chitinase; non-midgut genes	Bacteria expressing dsRNA	Developmental delay; mortality
Lepidoptera	<i>Helicoverpa armigera</i> (Cotton bollworm)	Multiple stacked genes	Artificial diet	Reduced survival
Hemiptera	<i>Bemisia tabaci</i> (whitefly)	Cyclophilin B; HSP70	Transgenic tomato	82–86% mortality
Hemiptera	<i>Nilaparvata lugens</i> (Brown planthopper)	Trehalase-1 and -2	Microinjection	Moulting deformities

Source: Ali *et al.* (2026), Jain *et al.* (2021) and Rodrigues *et al.* (2021).

Getting dsRNA into the pest

Naked dsRNA is fragile. Sunlight degrades it on the leaf within hours, and nucleases in soil, saliva and midgut break it down further. Much of the effort of the past decade has gone into protecting the molecule long enough to reach its target. Three strategies have emerged.

1. Transgenic crops

The earliest and best validated route is to engineer the crop to produce the dsRNA itself, as a plant-incorporated protectant. Exposure is continuous and chewing pests cannot avoid the dose. SmartStax Pro maize, carrying a DvSnf7 dsRNA trait against corn rootworm, has been grown commercially in North America for several seasons. But has some drawbacks such as long development time, GM approval and public resistance to GM food.

2. Sprays

A more flexible alternative is to treat dsRNA like any other foliar product, i.e., spray-induced gene silencing. The same formulation can be used on any crop, applied only when needed. Most regulators regard a sprayed nucleic acid as a pesticide rather than a GMO, which simplifies approval. The landmark came on 28 December 2023, when the United States

Environmental Protection Agency registered Ledprona, marketed as Calantha, against Colorado potato beetle. It remains the only sprayable RNAi product on the market (Rodrigues *et al.*, 2021; Li *et al.*, 2026).

3. Carriers and nanoparticles

A carrier protects dsRNA from UV and enzymes and helps it cross the gut wall. Chitosan, liposomes, protamine-lipid complexes and layered double hydroxide clay nanosheets shield dsRNA from UV radiation and enzymes while assisting its passage across the gut wall. The clay system, developed in Australia as BioClay, kept dsRNA stable on leaves for weeks and protected even unsprayed leaves, suggesting the molecule had moved through the plant (Mitter *et al.*, 2017). For sap-feeders the carrier must also move dsRNA into the plant vascular system, since aphids and planthoppers never touch the leaf surface. Trunk injection and root drench have delivered dsRNA into citrus trees, where it persisted for nearly two months (Jain *et al.*, 2021).

Can farmers afford it?

Price was the biggest obstacle for a long time. In 2008, chemically synthesised dsRNA cost about US\$12,500 per gram. Fermentation in an engineered strain of the bacterium *Escherichia coli* that lacks the RNase III enzyme changed that. By 2016 the price had fallen to around US\$100 per gram. Encapsulated bacterial production brought it to roughly US\$2 per gram in 2017, and some manufacturers now report costs below US\$1 per gram (Li *et al.*, 2026). A field application needs 2 to 10 g of dsRNA per hectare. At current prices an RNAi spray already competes with conventional insecticides on many crops.

Safety for pollinators, natural enemies and the environment

RNAi is one of the few insecticidal technologies that can be designed to spare specific beneficial insects. The design starts with a sequence search. The candidate dsRNA is compared against the genomes of honey bees, ladybird beetles, parasitoids and other non-targets, and any stretch of close identity is removed. The dsRNA is then fed to those species in the laboratory before it goes to the field. Environmental persistence is short. dsRNA breaks down in agricultural soil within two to three days and in water even faster (Bachman *et al.*, 2020). It leaves no toxic residue on produce. Risk assessment is done in tiers. Laboratory feeding tests come first, then semi-field cage trials, then open-field monitoring (Ali *et al.*, 2026).

Resistance: a question of when, not whether

RNAi resistance can evolve rapidly as western corn rootworm showed reduced dsRNA uptake, while Colorado potato beetle developed over 11,000-fold resistance through reduced uptake and increased gut nuclease activity. However, beetles remained sensitive to chemical insecticides, supporting rotation strategies (Khajuria *et al.*, 2018; Mishra *et al.*, 2021). Practices that slow resistance are; stack two or more essential genes in one product so the pest must overcome both at once; Rotate dsRNA with Bt, chemical and biological controls that have different modes of action and; Monitor field populations with molecular tools so a shift is caught early (Christiaens *et al.*, 2020). Sprays have an advantage over transgenic traits here, because exposure is intermittent rather than constant

Where India stands

Regulation of dsRNA products differs by country. The United States classifies a sprayable dsRNA as a biochemical pesticide. The European Union, Canada, China and India treat it as a chemical pesticide under existing law. In India the authority is the Central Insecticides Board and Registration Committee under the Insecticides Act, 1968. No RNAi product has yet been registered in India, and the current rules were written for chemicals, not nucleic acids. Scientists have called for harmonised guidelines that address dsRNA stability, uptake and off-target testing (Gunasekara *et al.*, 2025). Indian research on RNAi against cotton bollworm, brown planthopper and whitefly is active, and the country has a large biologicals manufacturing base. Clear registration rules are now the main gap.

Conclusion

RNAi-based biopesticides have crossed from promise to product. They offer what chemical insecticides never could viz., a mode of action aimed at a single species, an active ingredient that disappears within days and a safety profile for pollinators and natural enemies that can be verified before the first spray. The economic objection has fallen away with the collapse in dsRNA prices. What remains is formulation and stewardship. Sprays must survive sunlight and gut enzymes, delivery to sap-feeders must improve, and resistance must be anticipated through gene stacking and rotation. For the Indian farming community, the first products will likely arrive for beetle pests, with whitefly, planthopper and bollworm to follow. The wisest course will be to treat them as one tool within integrated pest management rather than a replacement for it. Used with that restraint, RNAi may allow us to reduce our dependence on broad-spectrum chemistry without surrendering the harvest.

References

- Ali, A.A., Zhou, X., Zhang, Y. and Pan, H. (2026). RNAi-based biopesticides: A promising approach for sustainable pest management. *Entomologia Generalis*, early access, <https://doi.org/10.1127/entomologia/3780>
- Bachman, P., Fischer, J., Song, Z., Urbanczyk-Wochniak, E. and Watson, G. (2020). Environmental fate and dissipation of applied dsRNA in soil, aquatic systems, and plants. *Frontiers in Plant Science*, 11: 21.
- Cedden, D., Güney, G., Rostás, M. and Bucher, G. (2025). Optimizing dsRNA sequences for RNAi in pest control and research with the dsRIP web platform. *BMC Biology*, 23(1): 114.
- Christiaens, O., Whyard, S., Vélez, A.M. and Smagghe, G. (2020). Double-stranded RNA technology to control insect pests: Current status and challenges. *Frontiers in Plant Science*, 11: 451.
- Gunasekara, S., Fidelman, P., Fletcher, S., Gardiner, D., Manzie, N., Ashworth, P. and Mitter, N. (2025). The future of dsRNA-based biopesticides will require global regulatory cohesion. *Nature Plants*, 11(4): 664-667.
- Jain, R.G., Robinson, K.E., Asgari, S. and Mitter, N. (2021). Current scenario of RNAi-based hemipteran control. *Pest Management Science*, 77(5): 2188-2196.
- Khajuria, C., Ivashuta, S., Wiggins, E., Flagel, L., Moar, W., Pleau, M. and Clark, T. (2018). Development and characterization of the first dsRNA-resistant insect population from western corn rootworm, *Diabrotica virgifera virgifera* LeConte. *PLoS One*, 13(5): e0197059.
- Li, X., Lu, H., Zhao, C. and Tang, Q. (2026). Spray-applied RNA interference biopesticides: Mechanisms, technological advances, and challenges toward sustainable pest management. *Horticulturae*, 12(2): 137.
- Mishra, S., Dee, J., Moar, W., Dufner-Beattie, J., Baum, J., Dias, N.P. and Jurat-Fuentes, J.L. (2021). Selection for high levels of resistance to double-stranded RNA (dsRNA) in Colorado potato beetle (*Leptinotarsa decemlineata* Say) using non-transgenic foliar delivery. *Scientific Reports*, 11(1): 6523.
- Mitter, N., Worrall, E.A., Robinson, K.E., Li, P., Jain, R.G., Taochy, C. et al. (2017). Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature Plants*, 3: 16207.
- Rodrigues, T.B., Mishra, S.K., Sridharan, K., Barnes, E.R., Alyokhin, A., Tuttle, R. et al. (2021). First sprayable double-stranded RNA-based biopesticide product targets proteasome subunit beta type-5 in Colorado potato beetle (*Leptinotarsa decemlineata*). *Frontiers in Plant Science*, 12: 728652.