



REVIEW ARTICLE

# RNAi-based bioinsecticides for sustainable agriculture: from molecular discovery to field deployment and global policy perspectives

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pReceived: 23 February 2026; Accepted: 08 July 2026; Available online: Version 1.0: 22 September 2026

**Cite this article:** Ashutosh N, Chirasmitta B, Nihal R, Satyabrata N. RNAi-based bioinsecticides for sustainable agriculture: from molecular discovery to field deployment and global policy perspectives. *Plant Science Today* (Early Access). <https://doi.org/10.14719/pst.14204>

## Abstract

RNA interference (RNAi) is an effective technique for sustainable insect management by providing effective, targeted and species-specific solutions that can be deployed as an alternative to traditional chemical-based synthetic pesticides. RNAi is highly specific in nature, with a remarkable safety profile, rapid biodegradation and minimum risk to non-target organisms and reduces the environmental risk. In addition, the changing environmental and regulatory dynamics have made intergovernmental agencies and bodies initiate the approval of RNAi products, reflecting growing acceptability. Moreover, with the current advances in synthetic biology and artificial intelligence, the design and deployment of RNA-mediated insect control will be further facilitated. Thus, RNAi, which is regarded today as mostly a lab-scale experimental technique, can cement its place as a next-generation and sustainable integrated pest management solution. This review encompasses the theory and scientific principles behind RNAi-based biopesticides, from sequence identification to field deployment. It further addresses other critical issues in RNAi-mediated insect control, such as the discovery of target insect genes, double-stranded RNA degradation affecting efficacy and emerging delivery strategies, including foliar sprays, nanoparticle encapsulation and systemic approaches to enhance stability and cellular uptake. Moreover, the review provides an end-to-end comprehension and comparison of the molecular discovery, deployment and policy perspectives related to RNAi-based insect management. Together, these advances position RNAi as a promising but still evolving platform for sustainable pest management, with its impact ultimately depending on delivery efficiency, biosafety and regulatory readiness. Future progress will require coordinated advances in formulation science, target validation and policy support to enable reliable field deployment.

**Keywords:** bioinsecticides; plant protection; public policy; RNAi; sustainability

## Introduction

The continuous global population growth necessitates an increase in the amount of food production. To sustain such an endeavour, traditional chemical pesticides are extensively employed to safeguard crops from pathogens and pests. The exhaustive use of these synthetic chemicals has culminated in significant environmental and human health concerns because of their broad-spectrum activity and long-term ecosystem persistence in the food chain (1). This scenario underscores the urgent necessity for ecologically sustainable and novel pest management solutions that will ensure global food security, all while also minimising environmental damage. Crop protection thus must become more specifically tailored and ecologically sustainable to support environmentally conscious agriculture.

To address this issue, RNA interference (RNAi) technology has become a hopeful tool for creating biopesticides. Found in eukaryotic plants, mammals and fungi, RNAi is a naturally occurring gene silencing process deployed by organisms for their defence against foreign pathogens (2). This mechanism provides a very

precise, targeted method for pest control, as it may be used to specifically silence housekeeping genes in agricultural pests. By providing a more focused and ecologically safe method of pest management, thereby lowering the need for broad-spectrum chemical pesticides, the development and use of RNAi-based biopesticides have great promise for promoting sustainable agricultural practices (3).

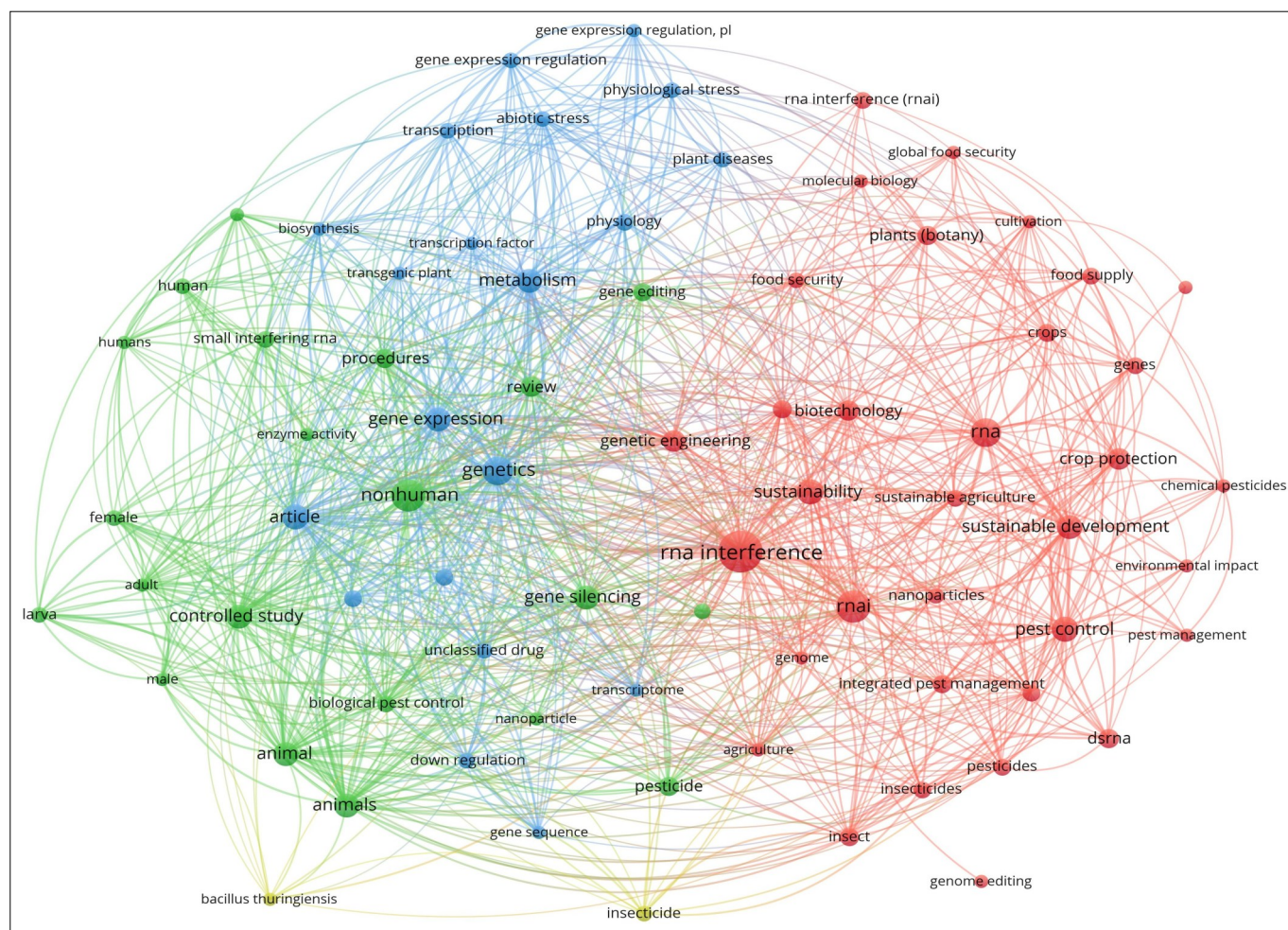
Double-stranded RNA (dsRNA) is used in RNAi, a gene silencing process, to specifically destroy target mRNA, which has become a highly effective tool for controlling pests. Its accuracy in focusing on genes vital for regular insect development and operation provides a possible substitute for traditional, dangerous insecticides (4). Initially found in *Caenorhabditis elegans*, RNAi has since been discovered in many eukaryotes and transformed functional genomics and pest control techniques, thus making it one of the ideal techniques to be harnessed for the development of pest-resilient crops. In agricultural uses, RNAi targets key genes in pests to provide species-specific management, therefore reducing impacts on beneficial or non-target species (5). Because of its scalability, environmental safety and flexibility throughout many pest taxa,

including insects, mites and nematodes, the technique seems very promising (6). Further greatly increasing its possibilities as a next-generation biopesticide platform are developments in bioinformatics, genomics and delivery systems.

The advancements in molecular biology, genomics and instrumentation have significantly transformed the application of RNAi in pest management in an economically feasible way (7). The idea of using RNA molecules to silence genes arose from preliminary work in *C. elegans* by Fire and Mello (1998), which demonstrated that exogenous dsRNA could induce strong, sequence-specific gene silencing. This discovery prepared the way for investigating RNAi in many species, including agricultural pests. In the early 2000s, researchers orally administered dsRNA to show effective RNAi-induced gene knockdown in coleopteran insects, marking the first significant step toward pest control. Western corn rootworm (*Diabrotica virgifera*) became a model system, especially since it demonstrated great sensitivity to dietary dsRNA (8), highlighting RNAi's possibility as a precision-targeted insecticide. Introduced in 2007, the idea of host-induced gene silencing (HIGS) lets transgenic plants generate dsRNA aiming at pest genes. This approach showed promise in offering constant, systematic defence against pests, including insects consuming the plant's vascular system and nematodes. Big Agri-biotech companies like Monsanto and Syngenta started commercial development of RNAi-based crops and foliar sprays by 2012 (9).

Research at the same time revealed notable variation in RNAi efficacy among insect species. Although certain lepidopterans

and coleopterans exhibited favourable responses to oral RNA interference, hemipterans and dipterans frequently demonstrated restricted gene silencing due to obstacles in dsRNA absorption, nuclease degradation or insufficient systemic response. These realisations spurred focused investigation on gene selection techniques, stability enhancement and RNAi delivery systems. Non-transformative delivery systems, including topical sprays, nanoparticle formulations and microbial-based RNAi systems, have become popular recently, providing substitutes for transgenic methods and a better fit with public and regulatory issues (10). Furthermore, international initiatives, especially in low- and middle-income nations (LMICs), have investigated RNAi's possible use in integrated pest management (IPM), thereby advancing it as a sustainable and ecologically benign choice (11). Driven by the need for focused, resistance-breaking biopesticides in an era of climate change, pesticide resistance and ecological awareness, the historical trajectory of RNAi in pest management reflects a shift from basic discovery to practical deployment. The thematic landscape of this field is highlighted in Fig. 1, which illustrates the co-occurrence of high-frequency keywords, emphasising the intersection of biotechnology, sustainability and integrated pest management. Although many reviews discuss RNAi mechanisms or delivery strategies separately, fewer integrate molecular target discovery, field deployment, environmental risk and policy considerations in a single framework. This review addresses that gap by combining these themes and highlighting the translational challenges that determine whether RNAi-based bioinsecticides can move from laboratory studies to practical agricultural use.



**Fig. 1.** Co-occurrence network of high-frequency keywords related to RNAi-based bioinsecticides. Node size corresponds to keyword frequency, link thickness indicates co-occurrence strength and colors denote thematic clusters generated by VOSviewer.

This review aims to provide a comprehensive overview of RNAi-based bioinsecticides, spanning from the fundamental molecular mechanisms to their field deployment and the global policy perspectives surrounding their use. Covering their development from early gene target discovery and functional validation to real-world agricultural uses, this review seeks to give a thorough and integrated picture of RNAi-based biopesticides. The review addresses a critical need for an integrated analysis of this technology, as existing literature often addresses molecular biology, delivery systems or regulatory issues in isolation. Specifically, this review: (i) synthesises advances in gene target discovery and functional validation across insect orders; (ii) critically evaluates delivery strategies and their comparative feasibility in real agricultural systems; (iii) examines environmental fate and risk assessment frameworks; (iv) analyses the evolving global regulatory landscape; and (v) discusses socio-economic and policy dimensions relevant to adoption in the Global South. By aggregating scientific, technological and policy viewpoints, this paper maps the status and future trajectory of RNAi in worldwide agriculture.

### Review methodology

This narrative review synthesises peer-reviewed literature and regulatory documents on RNAi-based bioinsecticides published primarily between 2000 and 2025. Relevant articles were identified in Web of Science, Scopus, PubMed and Google Scholar using combinations of the keywords “RNA interference”, “dsRNA”, “bioinsecticide”, “pest management”, “foliar spray”, “nanoparticle delivery”, “host-induced gene silencing”, “risk assessment”, “regulatory policy” and “smallholder agriculture”. Additional information on commercial products and regulatory evaluations was obtained from documents released by agencies such as the United States environmental protection agency (EPA), the European food safety authority (EFSA) and national pesticide regulatory authorities. Studies were prioritised if they (i) reported *in vivo* RNAi efficacy against agricultural pests; (ii) developed or evaluated delivery systems suitable for field use; (iii) quantified environmental fate and non-target effects of dsRNA or (iv) analysed regulatory and socio-economic dimensions of RNAi deployment. Review articles were used to frame historical context and identify seminal primary studies, but data interpretation relied on original research wherever possible. As this is not a systematic review, quantitative meta-analysis was not attempted; instead, emphasis was placed on identifying convergent findings, major limitations and critical knowledge gaps across studies.

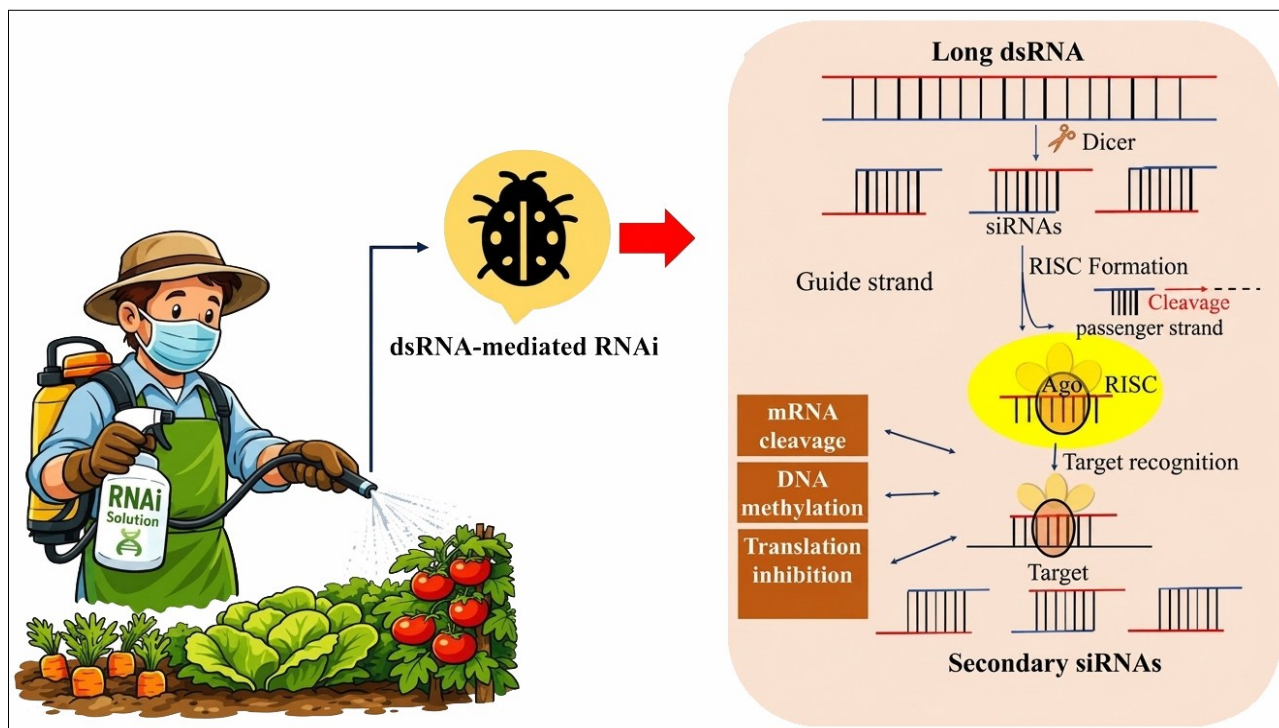
### Gene target discovery and functional genomics in insects

RNA interference works by exploiting endogenous gene silencing pathways and inhibiting the expression of essential genes. The basic mechanism of RNAi is that the dsRNA sequence is complementary to the translated mRNA of the targeted gene. Initially, the double-stranded RNA enters the insect's cells. The Dicer enzyme then cleaves it into small interfering RNAs (siRNA) (20–25 nt) (12). These siRNAs are then unwound and incorporated into the RNA-induced silencing complex (RISC). The guide strand then directs RISC to the targeted mRNA sequence, initiating its cleavage and finally degradation. For RNAi to be successful, it requires sufficient sequence matching between the siRNA and the host target transcript. Ideally, a sequence size of 21 nucleotides is more efficient for splicing; even in some cases, shorter matches around 15 to 20 nucleotides have shown silencing effects (13). Therefore, gene targets must be carefully chosen to maximise match specificity. As

RNAi acts after transcription, it efficiently silences the genes without interfering with the genome. Many insects possess an inherent RNAi capacity. After gut uptake, the silencing complex can reach other parts of the body, thereby amplifying the effect (14). However, RNAi uptake efficiency varies among insect species, so gene silencing results depend upon physiology and biochemical pathways. Unlike chemical toxins that target conserved pathways such as ion channels, dsRNA hypothetically can target any gene (15). This flexibility allows genome-based selection of novel targets, potentially yielding a unique mechanism that insects have not evolved resistance to. The process is initiated when dsRNA enters the insect's cells. Subsequently, the Dicer enzyme cleaves the dsRNA into siRNAs. These siRNAs are incorporated into the RISC, which then guides the degradation of target mRNA, effectively silencing the gene without altering the insect's genome (Fig. 2).

Identifying ideal targets for RNAi silencing will involve bioinformatic prediction and empirical screening. The exponential growth of genomic and transcriptomic resources for pest insects has enabled the *in silico* selection of candidate genes (16). Researchers put more effort into targeting genes involved in housekeeping roles because knockdown tends to be lethal. For example, genome-wide RNAi screens in *Tribolium castaneum* (red flour beetle) showed that the most effective targets belong to basic cellular processes like gene expression and protein homeostasis, compared to traditional insecticide targets (17). These gene targets are expected to be highly conserved across species, making them ideal for broad-spectrum RNAi application. In contrast, genes associated with classical pesticide action may not fully exploit the full potential of RNAi's unique mode of action (18). A recent review even argues that a knowledge-driven approach to gene target selection may miss superior candidates, instead advocating for large-scale unbiased screening. Such evidence-based screening strategies have revealed novel targets (proteasome subunits) that were missed from prior knowledge (19). Bioinformatics tools are also deployed to predict RNAi target sequences while decreasing the chances of off-target hits. Various databases of pests and off-target genomes support sequence searches (BLAST, etc.) to ensure no unintended matches (20). Tools like dsCheck (21) and OfftargetFinder (22) can scan transcriptomes of essential native species to avoid problematic regions. Moreover, such prediction requires robust genomic data, which may be lacking for most insects and this remains a limitation in practice.

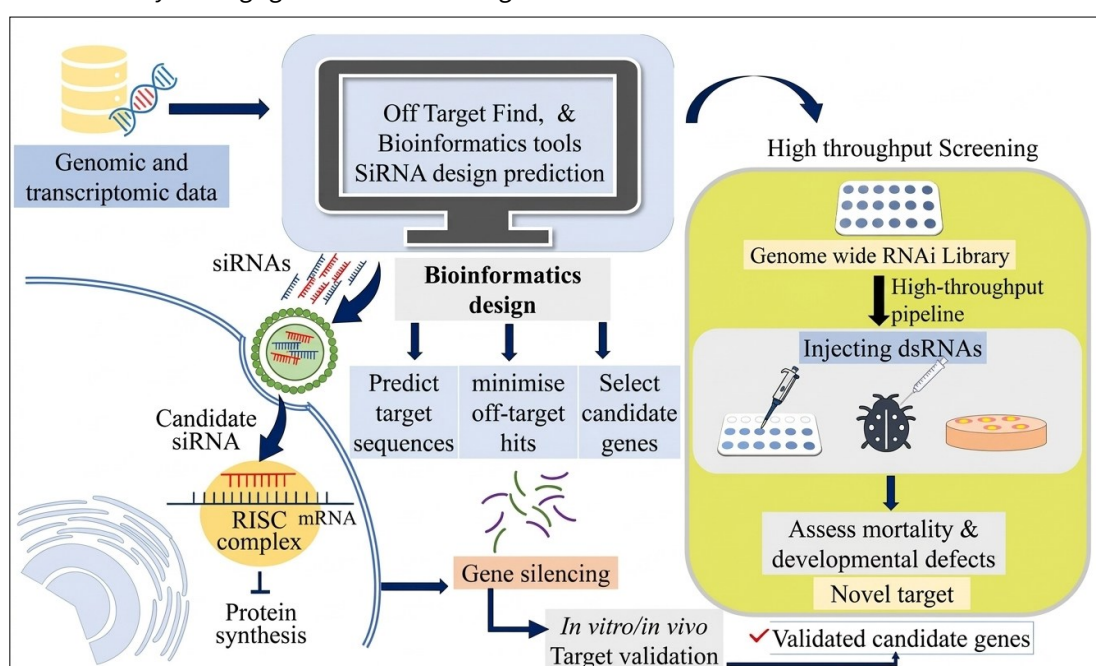
Advances in functional genomics provide platforms to discover RNAi targets. Insect research communities have developed genome-wide RNAi libraries and high-throughput screening pipelines. For instance, a genome-wide RNAi screen in *T. castaneum* identified ~900 candidate lethal genes; subsequent validation narrowed these to ~192 highly effective targets. Transferring this list to the related beetle pest (e.g., *Leptinotarsa decemlineata*) yielded a core set of 34 “superior” targets effective across species, demonstrating interspecies applicability (23). Such systematic screens rely on injecting or feeding individual dsRNAs (often in insect cell culture or whole insects) and assessing mortality or developmental defects. They can unravel novel targets that single-gene studies miss, including those in unexplored pathways. A simultaneous approach deploys CRISPR-based or transgenic reporter assays to complement RNAi findings (17). Gene knockout techniques by transposon mutagenesis or CRISPR in model pests can confirm that a candidate gene is essential, thereby providing



**Fig. 2.** Exogenously applied dsRNA on host plants is ingested by herbivorous insects, processed by Drosha/Dicer into siRNAs and incorporated into the RNA-Inducing Silencing Complex, guiding sequence-specific degradation of target mRNAs.

orthogonal validation (24). The integration of genomic editing with RNAi deepens our understanding of gene function and can effectively guide the design of more effective dsRNA constructs (Fig. 3). This involves leveraging genomic data to design siRNAs, followed by high-throughput screening using genome-wide RNAi libraries to assess mortality and developmental defects. Therefore, functional genomics is the key to RNAi target discovery. While the fundamental mechanism of RNAi is conserved across eukaryotes, the phenotypic outcomes vary significantly depending on the target species and the specific biological pathway being disrupted. This finding underscores the versatility of RNAi, demonstrating its success in achieving high mortality rates and significant growth reduction in major agricultural pests like the Western corn rootworm and the Colorado potato beetle. By leveraging omics data and high-

throughput assays, researchers can systematically identify and rank promising target genes for pest control (25). Table 1 provides a comprehensive summary of these applications, highlighting how different insect orders, ranging from Coleoptera to Hemiptera, respond to various gene-silencing strategies. This knowledge-based approach yields better outcomes than simply mimicking classic pesticide targets. Genome-wide RNAi screens have expanded the pool of candidate targets beyond classical pesticide pathways, revealing essential genes involved in proteostasis, transcription and cell survival. However, target selection must balance knockdown potency with species specificity, because highly conserved housekeeping genes may increase the risk of off-target effects in beneficial organisms.



**Fig. 3.** Strategies for field deployment of RNAi-based bio-insecticides in sustainable agriculture. These complementary technologies enable targeted, efficient and environmentally safe dissemination of RNAi molecules for pest management in crop systems.

**Table 1.** Representative instances of RNAi-mediated pest management aimed at several insect orders and plant-parasitic nematodes. A summary of the target species, RNAi gene targets, delivery techniques and documented phenotypic effects that show how effective Host-Induced Gene Silencing (HIGS) and exogenous dsRNA treatments are for protecting crops

| Target Pest                                                 | Order       | Target Gene/Pathway                   | Delivery Method                  | Observed Phenotype                                       | Reference |
|-------------------------------------------------------------|-------------|---------------------------------------|----------------------------------|----------------------------------------------------------|-----------|
| Western Corn Rootworm ( <i>Diabrotica virgifera</i> )       | Coleoptera  | V-ATPase A, Snf7                      | HIGS (Transgenic Maize)          | Stunted growth, mortality, root protection               | (26)      |
| Colorado Potato Beetle ( <i>Leptinotarsa decemlineata</i> ) | Coleoptera  | Actin, V-ATPase A                     | Topical Spray, HIGS (Potato)     | Mortality, reduced feeding                               | (27)      |
| Cotton Bollworm ( <i>Helicoverpa armigera</i> )             | Lepidoptera | Ecdysone receptor, P450 monooxygenase | HIGS (Transgenic Tobacco)        | Growth reduction, mortality, impaired gossypol tolerance | (28)      |
| Brownp lanthopper ( <i>Nilaparvata lugens</i> )             | Hemiptera   | Trehalose Phosphate Synthase          | HIGS (Transgenic Rice)           | Lethality                                                | (29)      |
| Cotton Aphid ( <i>Aphis gossypii</i> )                      | Hemiptera   | Odorant binding protein 2 (AgOBP2)    | HIGS (Transgenic Cotton)         | Failure to recognize host plant                          | (30)      |
| Root-Knot Nematode ( <i>Meloidogyne incognita</i> )         | Tylenchida  | Splicing factor, integrase            | HIGS (Transgenic Tobacco)        | >90 % reduction in established nematodes                 | (31)      |
| Striped Flea Beetle ( <i>Phyllotreta striolata</i> )        | Coleoptera  | Arginine kinase                       | Feeding (on dsRNA-coated leaves) | Retarded development, increased mortality                | (32)      |

### Dietary RNAi: mode of action and bioassay optimisation

Dietary RNAi relies on the intake of exogenous dsRNA into the gut cells of the insect. After ingestion, dsRNA must traverse the gut epithelium and enter the cytoplasm to trigger silencing. In organisms like *C. elegans*, dsRNA uptake is mediated by specific transmembrane proteins (SID-1, SID-2) that ferry dsRNA across the membrane. In insects, orthologous “SID-like” proteins have been identified in some species but appear to play variable roles (33). Experiments suggest that endocytosis is often the main route for dsRNA entry in insects, potentially involving clathrin- or caveolae-dependent pathways. For example, fluorescent dsRNA accumulates in the endosomes of *Drosophila* cells, thereby blocking endocytosis (34). After cellular uptake, dsRNA is released through endosomal escape into the cytosol, where the Dicer complex processes it into siRNAs. It is noteworthy that the physical characteristics of dsRNA (e.g., length) influence RNAi efficacy; longer dsRNAs generally produce more siRNAs and elicit stronger silencing (35). Indeed, *T. castaneum* studies indicate that dsRNAs below ~30 bp are less effective, likely due to inefficient uptake and fewer siRNA targets (36). Thus, typical biopesticide designs use dsRNAs of several hundred base pairs, encompassing multiple potential siRNA sites. As siRNAs are generated, they guide RISC to cleave target mRNAs (37). The silencing effect can spread systemically in some insects; cells that did not ingest dsRNA may exhibit knockdown, possibly via cell-to-cell transport of siRNAs or a secondary amplification mechanism. For example, coleopteran insects often show systemic RNAi, where initial gut uptake leads to gene silencing body-wide (38). This systemic trait is crucial for whole-body pest control using oral dsRNA.

The efficiency of dietary RNAi is highly species-specific. Coleopterans (beetles) generally exhibit a robust response to ingested dsRNA, whereas Lepidoptera (moths or butterflies), Diptera (flies) and Hemiptera (true bugs) often show weak or inconsistent silencing (39). A critical challenge for RNAi in insects is the degradation of dsRNA by nucleases (dsRNases) (40). These enzymes are secreted into the gut and haemolymph, where they can rapidly cleave dsRNA molecules into inert fragments, preventing them from reaching their target cells. Studies have shown that the fall webworm (*Hyphantria cunea*, Lepidoptera) has haemolymph and gut contents containing high dsRNase activity that can degrade dsRNA within minutes. Silencing of dsRNase genes in *H. cunea* significantly improved RNAi efficiency, confirming that dsRNases are a major barrier (41). Likewise, gut pH extremes may make dsRNA less stable. Alkaline midguts (common in Lepidoptera, pH>8) break

down dsRNA, whereas more neutral or acidic pH (as in many beetles) maintains it better (42). The peritrophic matrix, a layer of chitin and protein that lines the gut, may also prevent dsRNA from spreading. In certain species, it is also hard for dsRNA to get through cell membranes. Nematodes also have secondary amplification of the silencing signal (via RNA-dependent RNA polymerases), while insects do not (43). This means that a substantial initial dsRNA dosage is typically needed. Factors in the environment, such as temperature and gut flora, could potentially affect absorption and stability, although their impact is less well understood.

Before field deployment, candidate target genes must be rigorously validated. This can typically be demonstrated by testing dsRNA against the gene to determine whether it reduces its mRNA or protein levels, which induces the desired pest mortality or phenotype (44). Standard approaches include quantitative RT-PCR to confirm transcript knockdown after dsRNA treatment. Studies showed that feeding *Diabrotica* larvae dsRNA against the Snf7 gene results in an ~80 % reduction in Snf7 mRNA and high larval mortality. Such molecular assays verify the RNAi mechanism and help optimise dsRNA design (e.g., testing multiple target regions within the same gene). *In vivo* bioassays are essential: they involve treating live insects (by feeding, injection or injection of transgenic tissues) with dsRNA and observing survival, growth, reproduction or developmental defects. Positive targets typically yield significant mortality or abnormal development at nanogram to microgram per insect doses (45). Ideally, assays include dose-response curves to estimate lethal concentration (LC50) or lethal time (LT50). Similarly, a study on *Helicoverpa armigera* larvae fed on baculoviruses expressing dsRNA against juvenile hormone genes showed a much lower LC50 than controls (46). Critical controls include non-specific dsRNA (e.g., dsGFP) to check for immune or off-target effects and untreated or vehicle-only controls (47). Confirming the absence of effects in non-target species is also important in risk assessment. *In vitro* assays using insect cell lines or primary cells can complement whole-insect tests, allowing higher-throughput screening of many gene targets before costly large-scale bioassays.

Commercialisation of reliable bioassays requires attention to toxicological principles. Recent analyses applying machine learning to RNAi bioassay data have identified key factors influencing efficacy (48). For example, response time (duration until mortality) and choice of target gene were the strongest predictors of success, followed by dsRNA dose, construct length and insect species. This implies that assays must allow sufficient time for RNAi effects, use

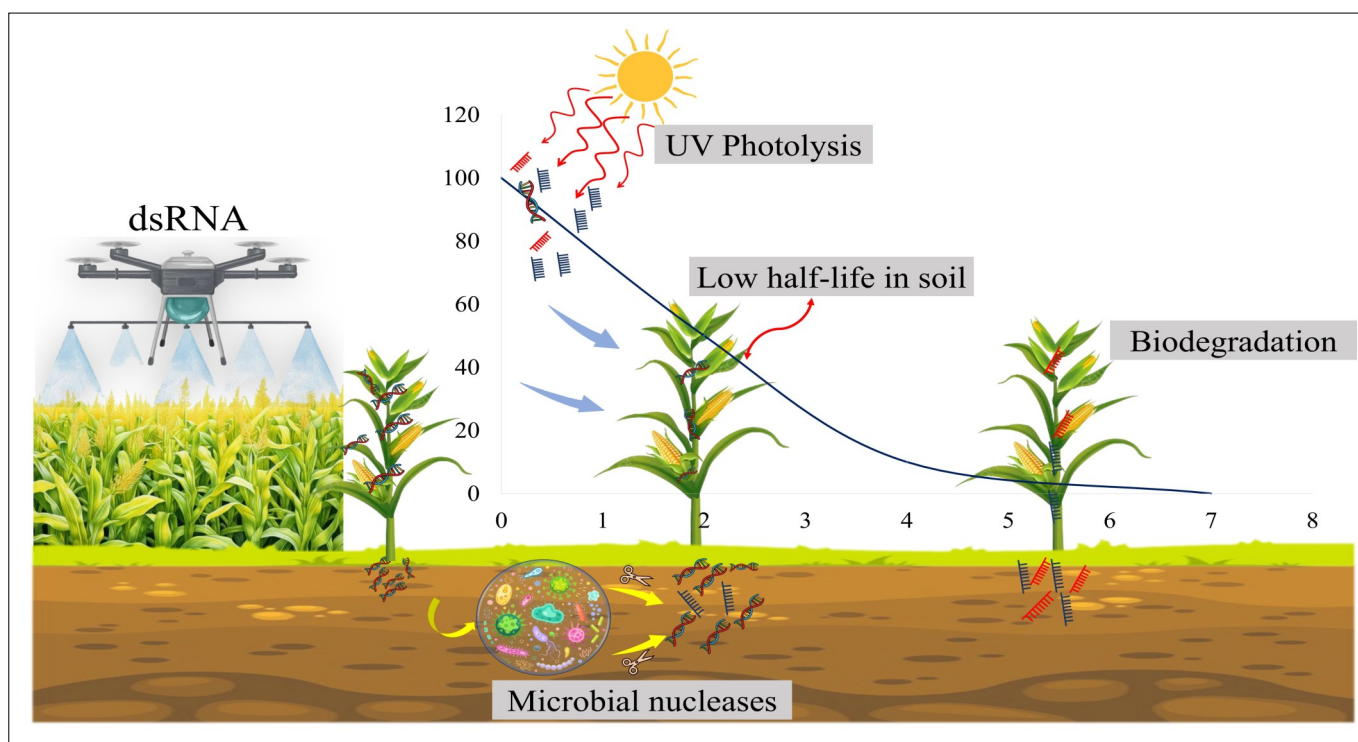
optimised dsRNA lengths and explore a range of doses (49). Standardisation is required; RNAi experiments have historically varied widely in methods and endpoints, making comparisons difficult. Future bioassays should mimic regulatory toxicology and include replicate trials, blank and sequence controls, and statistically meaningful replicates (50). Measuring target-gene knockdown by qPCR in parallel with mortality quantifies efficacy. New results suggest using traditional measures (e.g., LC50, LT50) as well as growth/developmental endpoints and considering sublethal impacts (e.g., reproduction) in target validation (51). Succinctly, successful gene validation for RNAi entails molecular confirmation of knockdown, dose/time-response mortality assays and careful controls. Combining principles from toxicology with generic screening yields robust candidate targets for bioinsecticide development. The efficiency of dietary RNAi varies widely across insect orders because of differences in dsRNA uptake, dsRNase activity, gut pH and systemic spread. These species-specific barriers make standardised bioassays essential for comparing efficacy across studies and for identifying delivery conditions that maximise silencing success.

### Delivery strategies in agricultural pest management

One of the most practical delivery methods for RNAi biopesticides is foliar spraying of dsRNA formulations. Sprayable dsRNA products provide flexibility akin to conventional pesticides: they can be applied with standard equipment and dosed as required during a growing season (52). Recent commercial success underscores the economic viability of this approach. GreenLight Calantha™ Bioscience product Calantha, containing the dsRNA “ledprona”, was approved by the US EPA on December 22, 2023, for Colorado potato beetle control (53). The foliar spray is applied at very low rates (~4 g/acre), yet it controls pests effectively. GreenLight Calantha™ Bioscience has reported that 4g/acre is very less to be spread over an entire American football field. The dsRNA rapidly degrades in the environment (within ~3 days), leaving minimal residue and further tests have shown it to have no off-target effects on species such as

honeybees, butterflies or mammals (54). Various companies and institutions are developing similar spray products. For eg, synthetic dsRNA against the cotton bollworm or fall armyworm has been tested in greenhouse and field trials, achieving significant mortality with controlled-release formulations (55). The U.S. Environmental Protection Agency (EPA) has also proposed registering a sprayable dsRNA (Vadescana) for Varroa mite control in beehives (56). These commercialisation and regulatory successes reflect the potential of sprays: they allow targeted control without genetic modification of the crop. Challenges for spray delivery include ensuring dsRNA stability on plant surfaces, uptake by the pest and rainfastness. Spray formulation often uses surfactants or protective agents to help dsRNA adhere to leaves and resist wash-off (57). However, UV radiation, high temperature and nucleases on plant surfaces can degrade naked dsRNA. Field trials have shown that frequent application (timed according to the pest life cycle) may be needed to maintain an effective dose; this depends on how long the dsRNA remains active on or in the plant (58). The benefits of foliar sprays are their broad applicability to any treatable pest, compatibility with existing spray programs and flexibility. The Calantha™ case also demonstrates that existing equipment can apply dsRNA with high precision and low environmental impact. As production methods improve and costs decline, direct sprays could become part of mainstream IPM. The intended lifecycle is depicted in fig. 4, where exogenously applied dsRNA is subject to UV photolysis and microbial nuclease degradation, resulting in a low half-life in soil and water, often disappearing entirely within a few days.

To overcome the limitations of naked dsRNA (degradation, poor uptake), researchers have developed nanoparticle carriers and encapsulation technologies. Nanoparticles can effectively protect dsRNA from nucleases and enhance delivery across biological barriers (59). Clay nanosheets (e.g., “BioClay”) adsorb dsRNA and slowly release it on the plant surface, increasing the protective potential. Chitosan nanoparticles have been used to condense dsRNA into stable complexes that can pass through the insect cuticle



**Fig. 4.** Environmental fate and degradation of dsRNA-based pesticides. This schematic illustrates the processes affecting double-stranded RNA (dsRNA) after it is applied to a cornfield by a drone.

or gut lining more efficiently (60). Surface functionalisation has further enhanced targeting. Researchers have attached specific ligands like peptides, proteins or antibodies to nanocarriers that bind to insect gut receptors, thereby enhancing uptake (61). Nanoparticle coating can also be modulated to release dsRNA only in the insect midgut, thereby providing controlled release protection and potentially targeted delivery of RNAi molecules (62). Limitations still exist, as some carriers may be economically unviable or complex to manufacture at scale. The environmental safety of some novel nanoparticles warrants further assessments. Early trials have shown promising results; field studies with dsRNA-loaded liposomes or chitosan particles have achieved mortality in target pests at lower dsRNA doses than naked dsDNA sprays (63). New findings have shown promising results, validating that nanoparticle-based RNAi delivery can be an effective complement to standard foliar sprays (64).

For large trees and woody plants, systemic delivery methods like trunk injection are being explored. Trunk injection involves infusing a dsRNA solution directly into the tree vascular system, allowing transport throughout the canopy (65). It was demonstrated that this approach in 20-year-old apple trees injected with dsRNA resulted in detectable levels of mRNA in their foliage for several months (65). Quantitative PCR confirmed that dsRNA concentration in leaves remained significantly above background for up to 141 days post-injection. The peak levels of around 242 pg per 30 mg leaf tissue were within the range needed to affect gene expression. This suggests that a single injection early in the season could protect a tree for an entire growing cycle. Trunk injection has some practical advantages over traditional methods, as it bypasses environmental degradation factors (UV, rain) entirely by delivering RNA inside the plant (66). It can target pests that feed on leaves or bark. Moreover, it avoids spray drift and reduces exposure of non-target flora and fauna. Drawbacks include labour intensity (each tree must be injected individually) and the potential for injury at injection sites (66). The technique is more feasible for high-value fruit trees or ornamental trees rather than broad-acre crops. Related systemic approaches include root drenching or trunk injection in forest trees. These exploit plant vasculature to circulate RNAi throughout the plant. In theory, grafting or aerosolised trunk injection could distribute dsRNA. Research is nascent but growing: pilot studies are examining whether translocation can protect entire orchards. Trunk injection thus represents a niche but promising delivery method for perennial systems where foliar spraying is unfeasible.

Microbial and viral carriers offer innovative delivery routes by harnessing biological agents to produce or deliver dsRNA *in situ*. An efficient way is paratransgenics, which involves engineered symbiotic bacteria or yeasts that naturally colonise an insect's gut to produce dsRNA continuously. Prior research has indicated modifying symbiotic bacteria from *Rhodnius prolixus* and *Frankliniella occidentalis*. These bioengineered bacteria were fed to insects, where they colonised their gut and expressed dsRNA targeting essential insect genes (67). The result was efficient gene silencing and pest mortality. Similarly, *Saccharomyces cerevisiae* can be genetically modified to express dsRNA (68). Studies have shown that *Drosophila suzukii* had reduced survival and reproduction after feeding on a formulated bait containing dried yeast engineered to produce dsRNA against a critical gene. Viral vectors offer another avenue; several insect-specific viruses (e.g., baculoviruses) have been repurposed to express dsRNA. For

example, *H. armigera*, a polyhedrovirus, was engineered to carry inverted repeat sequences of hormone regulation genes (69). Larvae that ingested the recombinant baculovirus had significantly faster mortality (LT50 reduced by ~46 %) and lower lethal doses (LC50 decreased more than six-fold) compared to the wild-type virus, due to RNAi of the target genes (70). Baculoviruses naturally cause infection in insects and are already deployed as biopesticides: adding a dsRNA payload significantly enhances their lethality. Similarly, other insect viruses (densoviruses or entomopoxviruses) could likewise be engineered, although host range and stability must be considered. These biological delivery strategies have intriguing potential; they can continuously produce dsRNA within the pest population, potentially overcoming the limitation of single-dose sprays. They can also exploit host-seeking behaviours (e.g., insects naturally picking up pathogens) for spread. However, deploying living agents raises regulatory and ecological questions (e.g., horizontal gene transfer, off-target spread) that need careful study. Symbiont and viral delivery remain largely experimental but represent a frontier for RNAi biopesticides.

The delivery strategies may differ in scope, cost and practicality. Foliar sprays are the most versatile for annual or recurring crops, fitting existing pest control infrastructure, but require frequent application or face environmental degradation. Nanoparticle carriers sometimes help mitigate these limitations by protecting dsRNA and enhancing uptake, but they add complexity and cost. Trunk injection offers systemic coverage for tree crops, but is intensive and scale-limited, thereby making it economically unviable. Symbiont and viral methods promise sustained, self-perpetuating control but involve biological complexity and regulatory hurdles. While choosing a delivery method, it is necessary to balance efficacy, cost, target pest biology and cropping system. For example, a fast-replicating pest like *D. suzukii* might be best tackled with a microbial bait (yeast) in high-value berry crops, whereas a lepidopteran defoliator might require potent larvicidal sprays with nanoparticle carriers (71). Comprehensive comparative field trials will need to be done to determine which approaches provide the greatest control per investment. Early indications from trials with *Calantha* (spray) and systemic injection (trees) show that each method can deliver a lethal concentration to the pest while maintaining crop safety. Importantly, delivery methods can be combined. For instance, dsRNA could be applied as a foliar spray for broad coverage and also delivered via trunk injection or seed treatment for residual protection. Conversely, RNAi could be paired with traditional insecticides or biological controls in an IPM framework, reducing reliance on any single tactic and potentially delaying resistance.

Implementation of RNAi in the field faces significant challenges, primarily related to environmental stability and scaling. Naked dsRNA is highly susceptible to rapid degradation by light, moisture and ambient nucleases, which can break it down within hours to days. Indeed, GreenLight *Calantha*<sup>TM</sup> reports that ledprona dsRNA degrades fully in about three days under field conditions (72). While this rapid breakdown reduces environmental bioaccumulation, it means that spray application must account for quick dissipation over hours. Formulating dsRNA with protective agents (e.g., encapsulating nanoparticles, sunscreen additives) can extend its longevity. However, any new additive must itself be safe and not inhibit dsRNA function. Furthermore, additives also must go through the entire regulatory process, thereby increasing cost and

time. Another practical challenge is cost and production. Early RNAi applications in plants required transgenic expression of dsRNA, which had high regulatory and time barriers. For sprayed applications, large amounts of dsRNA are needed (grams per hectare). Recent advances have lowered production costs: *in vitro* transcription and microbial fermentation methods can now yield dsRNA for under USD ~0.50 per gram. However, producing tonnes of dsRNA for large-scale agriculture is still non-trivial. Manufacturing platforms (like that of GreenLight Calantha™) use cell-free bioreactors or bacteria to scale up production economically. Capitalising on these platforms is crucial to making RNAi competitive with conventional chemicals in terms of cost. Environmental variability is also a concern. Soil and canopy conditions (temperature, humidity, microbial activity) can affect dsRNA persistence and uptake. Rain, for example, could wash away spray applications, requiring re-application. Field trials must therefore carefully consider formulation and timing to match pest life cycles and weather patterns (73). Furthermore, regulatory and consumer acceptance of sprayable RNAi may depend on clear evidence of safety under real-world conditions. In sum, while the toolbox of delivery methods for RNAi bioinsecticides is expanding, each approach has trade-offs. Overcoming stability and cost hurdles will require advances in formulation science and industrial production. Ongoing field tests and refinements will be needed to ensure that dsRNA-based products can be reliably delivered where pests feed, providing consistent control without unintended impacts. Moving from molecular discovery to field application requires delivery systems that can withstand environmental degradation while ensuring sufficient cellular uptake by the pest. A comparative analysis of these methodologies is presented in Table 2, which evaluates various delivery routes from traditional foliar sprays to advanced nanoparticle encapsulation and systemic trunk injections. The table illustrates the critical trade-offs between stability, cost and labour intensity, providing a roadmap for selecting the most economically viable delivery method for specific cropping systems. Among the available delivery platforms, foliar sprays offer the greatest operational flexibility for annual crops, whereas nanoparticle formulations improve dsRNA stability and uptake. Trunk injection is better suited to perennial tree crops, while microbial and viral carriers offer sustained delivery but require further validation for safety, scalability and regulatory acceptance.

### Risk assessment and off-target effects

A key safety question for RNAi biopesticides is the risk of off-target gene silencing in non-target organisms (NTOs). Off-target effects could occur if the dsRNA, or its siRNA fragments, have partial sequence identity to genes in beneficial or neutral species (86). Since the RNAi mechanism operates on complementarity, even imperfect matches can sometimes trigger silencing. Therefore, it becomes imperative that this aspect of RNAi is well studied before any field trials or commercialisation are attempted. Studies indicate that continuous 21-nt matching segments are the usual requirement, but as few as 15-nt of identity may elicit an effect, depending on context (87). Mismatches in siRNAs reduce efficacy, but the threshold of allowed mismatches without effect is not fully studied. Because siRNAs derived from dsRNA are heterogeneous in nature, one designer dsRNA may yield dozens of distinct siRNA sequences. Each of these has the potential to target a transcript in a non-target species if there is a stretch of near complementarity (88). Beyond sequence-specific off-targeting, high doses of dsRNA might theoretically saturate the RNAi machinery or stimulate innate immune responses in non-target organisms, leading to nonspecific effects. However, studies to date suggest this is of low concern in arthropods: for instance, injections of dsGFP (a control sequence) did not cause lethal effects in honey bees or fruit flies. Nonetheless, intergovernmental regulators consider sequence-specific off-targets as the primary concern because they can result in subtle but ecologically relevant effects (89). To mitigate off-target risk, dsRNA design employs *in silico* filters. In practice, any candidate dsRNA sequence BLAST (Basic local alignment search tool) analysis of any candidate dsRNA sequence is performed against genomes or transcriptomes of known beneficials (bees, lady beetles, earthworms, etc.) (90). Only sequences with no significant matches (beyond short, rare motifs) to important non-target genes are chosen. Tools like dsCheck (sensitive short-match finder) or Offtarget Finder can automate this by screening thousands of sequences for minimal cross-reactivity. However, the efficacy of such prediction hinges on the completeness of genomic databases; many non-target invertebrates have poorly annotated or missing genomes, making complete off-target assessment challenging. Thus, conservative design and post-application monitoring remain essential components of risk management.

**Table 2.** Comparative analysis of RNAi delivery techniques for agricultural pest control. Overview of principal RNAi delivery methodologies, their modes of application, stability characteristics, benefits, drawbacks and notable commercial or experimental instances illustrating contemporary advancements and translational prospects in RNAi-mediated crop protection

| Delivery method          | Application        | Stability  | Advantages                    | Limitations          | Commercial examples | References |
|--------------------------|--------------------|------------|-------------------------------|----------------------|---------------------|------------|
| Foliar Spray             | Topical            | Low        | Existing equipment compatible | Degrades fast        | Calantha            | (74)       |
| Nanoparticle Carriers    | Topical/Systemic   | High       | Enhanced uptake               | Costly manufacturing | BioClay             | (75)       |
| Trunk Injection          | Systemic           | High       | Long protection               | Labor intensive      | Apple trials        | (76)       |
| Microbial Carriers       | Biological         | Continuous | Self-sustaining               | Regulatory hurdles   | Engineered yeast    | (77, 78)   |
| Viral Vectors            | Biological         | Continuous | High lethality                | Containment issues   | Baculovirus         | (79)       |
| Root Drench              | Systemic           | Moderate   | Systemic protection           | Variable uptake      | Limited trials      | (80)       |
| Seed Treatment           | Systemic           | High       | Season-long control           | Germination only     | Developmental       | (81)       |
| Soil Drench              | Systemic           | Moderate   | Root targeting                | Leaching risk        | Research trials     | (81)       |
| Hydroponic Delivery      | Systemic           | Controlled | Precise dosing                | Infrastructure need  | Hydroponics         | (81)       |
| Aerosol Application      | Topical            | Low        | Uniform coverage              | Weather dependent    | Greenhouse use      | (81)       |
| Bark Application         | Topical/Systemic   | Moderate   | Weather resistant             | Limited penetration  | Tree care           | (81)       |
| Granular Formulation     | Soil-based         | Moderate   | Slow release                  | Non-target exposure  | Soil trials         | (82)       |
| Transgenic Expression    | Plant-incorporated | Lifetime   | No reapplication              | Public acceptance    | SmartStax PRO       | (83)       |
| Electroporation Delivery | Direct cellular    | High       | High efficiency               | Equipment needed     | Research use        | (84)       |
| Liposome Encapsulation   | Targeted delivery  | High       | Protected delivery            | Stability issues     | Pharma models       | (85)       |

Understanding what happens to dsRNA after it is applied is essential when weighing the possible risks of using it in agriculture. How long does it remain on plants, in water and in soil? Determining whether non-target organisms may be exposed requires this information. It is interesting to note that dsRNA tends to degrade in the environment rather quickly, according to multiple studies. For example, studies found that DvSnf7 dsRNA degraded by 90 % in four days in small aquatic setups, and had a half-life of less than three days (91). In just seven days, it vanished entirely in a variety of water-sediment systems, demonstrating how quickly it biodegrades. Likewise, the half-life of DvSnf7 dsRNA in soil is only a few days (92). Analysis of these results indicates that dsRNA does not normally accumulate to long-lasting levels in soil or water. This rapid degradation is driven by nuclease-rich microbial communities, UV rays from the sun and the way dsRNA adheres to soil or plant matter also plays a part. The concentration of dsRNA in a field would still decrease far more quickly than that of chemical pesticides, even though binding to materials like clay can somewhat slow down degradation. It is also diluted and washed away by irrigation and rain. This rapid disappearance is comforting from a risk perspective because it indicates that non-target organisms are likely to be exposed only for a brief period of time immediately following application. However, for extremely sensitive species, even a short exposure could be problematic. Because of this, ecological risk assessments frequently adopt a very cautious approach when assessing the safety of dsRNA, assuming that non-target organisms consume fresh dsRNA. As a result, dsRNA appears to have an environmental half-life of a few days rather than weeks or months. For animals that are not the target, this transient nature reduces the likelihood of long-term exposure. This implies for lawmakers that any post-application environmental monitoring should concentrate on the initial weeks rather than the entire year. Additionally, it implies that follow-up treatments can be scheduled to avoid times when non-target organisms are most active, such as by spraying after bees have completed their daily foraging. In the end, a crucial component of developing a thorough risk assessment is researching and quantifying the fate of dsRNA.

The global shift toward sustainable agricultural biotechnologies necessitates a rigorous evaluation of their potential externalities, particularly regarding the preservation of essential ecosystem services provided by pollinators and native species. The EPA and EFSA are two examples of regulators that are keeping a close eye on this. The fundamental advantage of RNA interference (RNAi) technology lies in its unparalleled nucleotide-level precision. The active component, double-stranded RNA (dsRNA), functions essentially as a molecular “key” that mandates a near-perfect sequence match within the target organism to trigger gene silencing. Most of these products only work on genes that are unique to the pest they are meant to kill. Recently commercialized dsRNA targets a gene that is only found in the Colorado potato beetle. Studies have shown that it did not affect bees, butterflies, or even earthworms because those organisms lack the complementary sequence required for silencing. That’s why risk assessments use both bioinformatics analysis and tests in the field to make sure that these products are safe. It is paramount to protect these beneficial species from a larger ecological point of view. A bee could drink nectar from a treated plant, or a tiny parasitic wasp could eat a pest that has eaten dsRNA. The good news is that dsRNA breaks down quickly and doesn’t seem to stay in nectar for very long. Studies have shown

that almost none of it gets into honey. Many predator species don’t seem to be affected at all when they eat a pest containing dsRNA. This is probably because the dsRNA does not get into their bodies very well by endocytosis. In the end, regulators see these sprays in the same way as regular chemicals. But dsRNA breaks down so quickly that there isn’t much time for that to happen. The risk to other animals is very low as long as the genetic sequence is unique to the pest.

Rapid sequencing of genomes and AI-based algorithms have given rise to bioinformatics tools tailor-made for predicting RNAi off-targets. These tools allow developers to pre-screen dsRNA sequences against sequence databases of non-target organisms. For example, dsCheck is an online platform that checks long dsRNA sequences for potential off-target 19–21-mer matches across a variety of genomes. OfftargetFinder similarly automates the search for unique (species-specific) dsRNA regions, leveraging the expanding transcriptome datasets for arthropods (93). These predictive tools work by ensuring that no continuous stretch of nucleotides in the dsRNA aligns with any gene of a non-target organism. They can also rank off-target risk by counting the number of siRNAs with partial matches. The accuracy of such predictions often depends on the availability of detailed, comprehensive genomic data. Many beneficial insects and soil organisms are poorly sequenced or annotated, so risk prediction is often limited. Therefore, bioinformatics tools are to be used in conjunction with *in vitro* testing. However, they do allow rational design of dsRNA to minimise sequence overlap with non-target species. Regulatory guidelines have begun to incorporate these predictive methods into their approval processes. The OECD (Organisation for Economic Co-operation and Development) countries and the various agencies under its umbrella have recommend for conducting a kind of exposure sequence homology assessment, which identifies any non-target genera that might encounter the dsRNA and checks for shared gene sequences (94). The output informs which species should be included in ecotoxicological testing and helps justify any exemptions (95). As genomic and transcriptomic databases grow, these bioinformatic approaches will become increasingly powerful, enabling more confident assurance that RNAi biopesticides have minimal unintended gene effects. Because RNAi depends on sequence complementarity, rigorous *in silico* filtering and cross-species validation are necessary to reduce off-target risk. Environmental persistence of dsRNA is usually short, but actual exposure depends on formulation, weather conditions and local soil or canopy environments, making post-application monitoring an important part of risk assessment.

Despite the progress, there are notable gaps in our understanding of dsRNA toxicology and exposure. Unlike chemical toxicants, dsRNA does not have a clear toxicodynamic profile; its toxicity is gene-specific. So, traditional concepts like LD50 must be interpreted in the context of gene knockdown efficiency. Long-term and generational studies in non-target organisms are missing. If a sublethal gene knockdown occurred in a pollinator, could this affect its reproductive potential? Cumulative effects with other stress factors (pathogens, chemicals) are unexplored. The toxicokinetics of dsRNA in non-target organisms is also poorly characterised. How much dsRNA crosses insect gut barriers, which are often more restrictive than those of target pests? Is there any bioaccumulation in soil organisms? Answering these questions requires new exploration techniques. Furthermore, public and regulatory bodies are still

refining their opinions. Most studies use worst-case assumptions, like assuming that 100 % of the spray applied ends up on the insect, which may overestimate exposure limits. Field residue monitoring and modelling of spray drift are needed to refine exposure estimates. Risk assessments often focus on arthropods, but dsRNA entry into vertebrates (through food or environmental routes) is an area of inquiry, though dsRNA is generally considered non-toxic to mammals (because it is digested as RNA); formal hazard evaluations (like allergenicity or unintended immune effects) are still a future task. Current knowledge supports the view that well-designed dsRNA products pose low risk to non-target organisms, but comprehensive assessment requires filling data gaps on chronic exposure, ecological interactions and more extensive taxonomic coverage. Ongoing work by regulatory bodies and academic consortia (e.g., OECD expert groups) aims to establish standardised protocols and guidelines for RNAi risk evaluation.

### Regulatory landscape and public acceptance

Regulatory pathways for RNAi-based products remain uneven across regions, reflecting differences in data requirements, biosafety frameworks and public communication strategies. Beyond regulatory approval, adoption will also depend on consumer trust, transparent safety evaluation and practical access for growers. One new area of pesticide supervision is the regulation of products based on RNA interference. The United States Environmental Protection Agency (EPA) handles dsRNA bioinsecticides under the current pesticide laws (FIFRA). For instance, the initial EPA registrations of dsRNA products (which target Varroa mites and Colorado potato beetles) were handled similarly to traditional pesticides, involving an evaluation of safety and efficacy data. In order to approve the beetle dsRNA (ledprona), the EPA determined that it is extremely specific and does not endanger non-target organisms. Due to its precise mode of action, the EPA once again found no environmental or human health concerns when it proposed to register a dsRNA product (Vadescana) for Varroa mites in May 2025 (96). These regulatory actions show that when data support low off-target hazard, RNAi products can meet current safety standards. Similar recognition of dsRNA as a new class of “pest control products” under the Pest Control Products Act has been given by Health Canada’s Pest Management Regulatory Agency (PMRA). Since 2016, the organisation has been interacting with developers and issuing experimental-use permits for RNAi trials. Canada makes it clear that dsRNA products will be assessed individually, with data requirements specific to this cutting-edge technology (97). Since no dsRNA products have been fully registered in Canada as of January 2024, developers still need to submit thorough risk and efficacy data before approval. In order to standardise risk assessment methodologies for dsRNA pesticides, Canada is also taking part in international discussions through the OECD. Plant-incorporated protectants (PIP) that generate dsRNA (such as RNAi transgenic crops) are subject to GMO regulations and must undergo an EFSA risk assessment, making the regulation of dsRNA products in the EU complicated. There is currently no specific EU regulation for dsRNA (sprays) applied externally; in reality, they are regarded as chemical pesticides. With a focus on sequence specificity and off-target analysis, European Food Safety Authority (EFSA) has issued guidelines for RNAi risk assessment in GM plants (98). Nonetheless, some EU legislators are debating whether or not novel organism frameworks should govern RNAi sprays. The EU is generally cautious

and demands thorough environmental risk assessments (99). Other areas are starting to react. In the context of their biopesticide frameworks, Brazilian agricultural regulators are investigating RNA interference products (99). The Australian Pesticides and Veterinary Medicines Authority (APVMA), has released preliminary guidelines indicating that, subject to exposure and hazard data, exogenously applied dsRNA may be evaluated as a pesticide (100). However, many developing and underdeveloped nations do not have written regulations for RNAi products. This highlights the necessity of international communication and capacity building and may lead to regulatory uncertainty that slows adoption.

In low- and middle-income countries, regulatory systems often face resource constraints. Many such countries lack the technical expertise or infrastructure to evaluate complex biotechnologies. As a result, even if RNAi products become available, local registration may be slow. On the other hand, developing countries stand to benefit greatly from RNAi’s sustainability. The challenge is to build capacity for risk assessment and product stewardship. International collaboration is important. For example, the OECD’s work on RNAi safety is a global resource, and regional cooperatives (such as the International Centre for Pesticide Regulation) could adapt guidelines for local conditions. Public sector research institutes in Africa and Asia are beginning to test RNAi against local pests, which will generate data relevant for national regulators. However, without a clear path for submission and approval, private companies may be hesitant to market RNAi products in these regions. Clear regulatory pathways, possibly starting with experimental use and moving to accelerated evaluations, will be needed to harness RNAi’s benefits in the Global South.

The development of RNAi bioinsecticides involves substantial investment in research and production technologies, leading to patent filings. Companies like GreenLight Biosciences Inc. have patented their dsRNA manufacturing platforms and specific dsRNA sequences. This intellectual property (IP) can spur innovation but also raises access concerns about its exclusivity. Broad patents on production methods or common target genes could limit smaller companies or public programs from using RNAi. A balance is needed: IP rights encourage the development of reliable products, but licensing or open-access initiatives could ensure that critical targets (especially for staple crop pests) are widely available. Another commercialisation issue is the integration with existing market channels. Farmers and distributors need training on these new products, including how to store dsRNA (often as dry powder), how to mix and spray it, and how to rotate it with other chemistries. Extension services and agro-dealers must adapt. The cost per treatment, although low by weight (grams/acre), may still be higher per hectare than that of cheaper older pesticides. Economic incentives such as public subsidies and tax concessions might be needed initially to encourage adoption. The transition from laboratory success to commercial availability is dictated by the evolving regulatory landscape, which varies significantly by region. Table 3 provides a snapshot of the current regulatory status, key governing authorities and public acceptance levels across major agricultural nations. This overview highlights a clear divide between proactive frameworks in USA and Brazil and the more cautious, research-oriented approaches currently seen in the EU and parts of Asia.

**Table 3.** Overview of the regulatory progress, key authorities, approval timelines, public acceptance and market readiness across major agricultural regions for RNAi-based pest control products

| Country/Region | Regulatory status | Key authority                                                    | First approval | Public acceptance | Market status  |
|----------------|-------------------|------------------------------------------------------------------|----------------|-------------------|----------------|
| USA            | Approved          | EPA (Environmental Protection Agency)                            | 2017           | Moderate          | Commercial     |
| EU             | Under Development | EFSA (European Food Safety Authority)                            | Not approved   | Low-Moderate      | Pre-commercial |
| Australia      | Approved          | APVMA (Australian Pesticides and Veterinary Medicines Authority) | 2019           | High              | Pre-commercial |
| Canada         | Under Development | PMRA (Pest Management Regulatory Agency)                         | Research only  | Moderate          | Research       |
| Brazil         | Approved          | CTNBio (National Technical Biosafety Commission)                 | 2018           | Moderate          | Pre-commercial |
| China          | Under Development | ICAMA (Institute for Control of Agrochemicals)                   | Under review   | Moderate          | Research       |
| Japan          | Under Development | Ministry of Agriculture, Forestry and Fisheries                  | Under review   | Moderate          | Research       |
| India          | Under Development | Department of Biotechnology                                      | Under review   | Low               | Research       |
| Malaysia       | Under Development | Department of Biosafety                                          | Under review   | Low               | Research       |
| New Zealand    | Under Development | EPA New Zealand                                                  | Under review   | Moderate          | Research       |
| Argentina      | Under Development | SENASA (National Service of Agri-Food Health)                    | 2024           | Moderate          | Pre-commercial |
| South Africa   | Under Development | Department of Agriculture                                        | Under review   | Low               | Research       |

Government regulators, lawmakers and international bodies must study the pros and cons of RNAi biopesticides (targeted pest control, reduced chemical loads, potential to break resistance) against the risks (non-target effects, regulatory uncertainty, socio-economic factors). This involves integrated risk-benefit analysis and consultations with all stakeholders. For instance, the EPA noted that Calantha™'s benefits include addressing a critical pest and mitigating chemical resistance, while acknowledging rigorous safety reviews. The PMRA (Canada) indicates that it will require evidence of value in addition to safety data before approval. In decision-making, considerations include: can RNAi improve yields or reduce losses in ways that conventional methods cannot? Is there a particular pest crisis (e.g., invasive fall armyworm) that RNAi uniquely addresses? Policymakers may also consider international harmony: divergent regulations can cause trade issues if only some countries accept RNAi products. Harmonising guidelines (through bodies such as the OECD or the FAO/IPPC of the United Nations) could streamline approvals globally.

### RNAi for sustainable agriculture in the Global South

RNAi technologies have a lot of potential for agriculture in developing areas, where pests can destroy crops and put food security at risk. RNAi's specificity can be very helpful against invasive pests, like the fall armyworm in Africa and Asia, that don't have good native controls. RNAi gives smallholder farmers a chance to use fewer harmful pesticides and keep beneficial insects that help with pollination and natural pest control. If dsRNA can be made or formulated locally, it could be a new, long-lasting tool for integrated pest management. Also, the small amounts needed (grams per hectare) work well for small plots. RNAi sprays could be added to current crop protection methods. For example, one spray near planting could protect crops early in the season with little effect on the environment. RNAi could also be used on crops that are important to the South's economy or culture, like cassava, sorghum and legumes, by targeting pests that are common in the area.

There are technical and financial problems that make it hard to use RNAi in the Global South. Implementing RNAi in the Global South faces technical and financial hurdles. Biotech infrastructure (labs, production facilities) is often limited and scarce. Even if an RNAi

product is registered, local capability to produce it at scale may be lacking. Proper validated QA/QC protocols, which are also lacking, hamper implementation. Shipping dsRNA internationally could be an interim solution, but cold-chain requirements and costs could be barriers. Partnerships with international companies or establishing regional manufacturing hubs may be necessary. Scientific capacity is another challenge. Crop pest genomics and RNAi expertise are less developed in many low-income countries. Training programs and collaborations with universities can build this capacity. Extension services must be educated about RNAi so they can advise farmers appropriately. Furthermore, regulatory agencies in these countries often have resource constraints; they might need technical support (training, funding) to evaluate RNAi products responsibly. The global interest in climate-smart agriculture aligns well with RNAi, given its potential to reduce chemical inputs and greenhouse gas impacts of pesticide manufacture. RNAi should not be a stand-alone "silver bullet" for sustainable agriculture. Instead, it should be part of a larger plan for managing pests. In smallholder settings, traditional practices such as crop rotation, intercropping, the use of botanical pesticides and the timing of planting are commonly employed. RNAi can help these by filling in the gaps where other methods don't work. For instance, farmers might plant crops that keep bugs away, but they could still get a certain bug; an RNAi spray could be used as a targeted knockdown when needed. On the other hand, using only RNAi might miss out on cultural and ecological knowledge. Extension programs should stress how RNAi fits into IPM. For example, it could help keep natural enemies alive by avoiding broad-spectrum sprays, thereby supporting biological control. Adaptation to the local area is very important. Formulations or delivery methods ought to take into account local circumstances. For example, if sprayers are hard to obtain, it might be better to develop cheap manual applicators or seed-coating methods. Also, if drinking water is hard to find, it is important to make sure that dsRNA can dissolve in small amounts of water. Getting farmers involved early on to learn about their methods will help make RNAi solutions that meet their needs. The success of any new agricultural technology depends on social acceptance and economic viability. In many regions, there is scepticism of new "magic bullets", often based on past experiences with failed GM or chemical initiatives. Transparent stakeholder

engagement is needed to address cultural beliefs (e.g., worries that RNAi is unnatural or harmful). Farmers may require demonstrations in their villages to build trust. Extension materials should be in local languages and culturally relevant. Socioeconomically, access is a key issue. If RNAi products are patented and pricey, only wealthier farmers might use them, exacerbating inequality. Subsidies or credit programs could help smallholders try RNAi methods. Alternatively, community-based production (e.g., cooperatively maintained fermentation units that produce dsRNA) could democratize access. Data on cost-effectiveness will influence adoption; if RNAi sprays can be shown to increase yields or reduce loss enough to pay for themselves, farmers will be more willing to invest. Finally, the regulatory acceptance of RNAi may vary across cultures. Some countries may apply more precaution due to public opinion, while others may welcome it as a needed innovation. Policymakers must balance these forces to ensure that beneficial technologies reach farmers without undue delay.

From a business point of view, RNAi products need to be economically viable. Even though production costs are going down, they are still high for low- and middle-income countries. Private investors may not be interested in smaller markets at first, like Africa. International development funds or public-private partnerships may need to support the first deployment in these areas. Demonstrating economic viability is very important. Pilot projects could gather data on how much RNAi affects yield per dollar spent compared to traditional inputs. RNAi's low active ingredient usage (grams instead of kilograms) is interesting because it could mean lower costs for logistics and more frequent applications. For example, using 4 g/acre of insecticide instead of 1 kg/acre could mean lighter loads and fewer problems with transportation. For small-scale farmers who live at subsistence levels, lowering crop losses also has a direct economic benefit. Life-cycle analyses of RNAi versus chemical pest control, considering health and environmental externalities, will probably demonstrate long-term societal benefits, thereby justifying public support. In conclusion, the technology has potential for the Global South, but it needs to be worked on in terms of technical, infrastructural, cultural and economic aspects. If used correctly, RNAi bioinsecticides could help small farmers keep their crops safe, which would help with sustainable intensification and poverty reduction. For smallholder systems, successful RNAi deployment will depend not only on efficacy but also on affordability, formulation stability, local manufacturing capacity and extension support. Without these enabling conditions, even technically effective RNAi products may remain inaccessible to resource-limited farming communities.

### Future directions and emerging trends

RNAi is changing quickly, with new ideas coming from the intersection of molecular biology and gene editing. A new idea is to use RNAi and CRISPR-based technologies together. For instance, CRISPR interference (CRISPRi) uses Cas proteins that don't cut DNA to stop gene expression. CRISPRi constructs are mostly being studied in prokaryotes or cell cultures right now. However, in theory, they could be used in insects to silence genes in a way similar to RNAi, but with different levels of effectiveness or specificity. On the other hand, CRISPR-Cas systems that target RNA directly, like Cas13, could be developed as programmable RNA-targeting tools for insects. While still hypothetical and speculative in nature, these CRISPR RNAi hybrids could facilitate improved targeting precision and promote self-replicating RNA systems. There are new ways to

more efficiently produce and deliver dsRNA thanks to synthetic biology. It is possible to engineer plants or designer microbes to generate large quantities of Dicer substrates or dsRNA. One could envision, for example, endophytic bacteria colonising crop plants and continuously releasing dsRNA into the vascular system of the plant to provide protection. In cell factories that generate and encapsulate dsRNA upon demand, synthetic gene circuits may be constructed. The possibility of producing dsRNA in the field by merely adding water to a prepackaged factory kit is even being investigated for cell-free systems. These future advancements in synthetic biology have the potential to significantly lower input cost and boost the feasibility of dsRNA production.

The amalgamation of big data and artificial intelligence (AI) is beginning to transform dsRNA design and development. Databases of insect genomes and RNAi outcomes allow machine learning models to predict highly active dsRNA sequences and optimal target sites. Researchers are developing deep learning predictors to rank dsRNA fragments by expected silencing efficiency, based on sequence features, such as GC content, secondary structure and absence of polyA tracts. These tools speed up the design cycle. Rather than empirically manually testing dozens of sequences per gene, one can computationally focus on a handful of top candidates. AI can also optimise formulations and deployment strategies. By analysing datasets from multiple trials, machine learning models might identify hidden factors (climate conditions, pest pressure) that influence RNAi success. In risk assessment, AI could predict off-target matches more efficiently across large genomic datasets. Furthermore, data-driven agriculture platforms could integrate RNAi into decision-support systems. For instance, a software tool could be developed that could suggest which dsRNA product to use and when, based on crop, pest surveillance data and environmental parameters. Such integration with precision agriculture technology (drones, sensors) may enhance the field-level implementation of RNAi. Machine learning approaches for dsRNA design are increasingly being trained on sequence composition, GC content, thermodynamic stability, predicted accessibility and experimentally validated silencing outcomes. However, their performance is still constrained by limited high-quality training data, heterogeneous assay conditions and the risk of overfitting, which can reduce transferability across insect species. Future integration will therefore depend on larger curated datasets, rigorous external validation and iterative experimental feedback to make AI a reliable support tool for dsRNA design.

A concise strategy is required for widespread use. In the short term, this means increasing production, conducting field trials and getting regulatory approvals in new and emerging markets. Public-private partnerships can speed this up by funding demonstration projects in different crop systems and geographical areas. To make sure that farmers receive training on sustainable product use and best practices as soon as they are available, extension networks should be involved early on. In the medium term, RNAi biopesticides will need to be included in IPM programs. It will be important to monitor the development of insect resistance to dsRNA over time. To overcome this challenge, strategies like rotating targets or combining RNAi with other modes of action have to be employed. Ongoing research should work to improve delivery methods and create multi-target dsRNA cocktails to lower the risk of resistance development. To advance RNAi biopesticides globally, concerted actions are needed. Research should prioritize,

(A) expanding genomic resources for pests and beneficials to inform target design and off-target screening; (B) optimizing dsRNA production methods to further lower costs; (C) developing robust field-scale formulation and delivery systems (e.g. stable nanoparticles or spray enhancers) that can withstand diverse climates; and (D) establishing standardized bioassay protocols and data sharing for cross-study comparability. Collaborative trials in different agroecosystems will generate the data necessary for broad adoption. On the policy side, regulators should provide clear guidance on the risk assessment of dsRNA products.

## Conclusion

RNAi-based bioinsecticides represent a transformative approach to pest management. At the molecular level, they exploit a natural gene silencing mechanism to knock down essential genes in target insects with high specificity. Advances in genomics and screening have identified optimal gene targets, often in core cellular pathways, yielding greater efficacy than traditional pesticide targets. The EPA's recent approvals set precedents, but globally harmonised frameworks (perhaps under the OECD or Codex Alimentarius) would reduce uncertainty for developers. Governments in the Global South may need support to evaluate RNAi pesticides; international assistance and capacity-building programs can help. Public communication strategies must be proactive: sharing the science, addressing misconceptions (especially confusion with GMOs) and highlighting the human and environmental safety of RNAi. Funding agencies should consider the dual goals of innovation and accessibility. To ensure that RNAi benefits reach smallholder farmers, grant programs could subsidise early-stage development of region-specific RNAi solutions and ensure that licensing models allow low-cost distribution. Intellectual property policies might encourage open licensing for key targets (especially those affecting staple crops) or support patent pools for biological control innovations. The goal is to make RNAi a common tool in agriculture worldwide. This could mean building large factories in developing countries, having clear global systems for keeping track of environmental impact and developing regulations that evolve with advances in science and technology. Overall, RNAi-based bioinsecticides offer strong promise for sustainable pest management, but their wider adoption will require better standardisation, improved delivery platforms and more robust ecological validation. Progress in these areas will be essential to translate laboratory success into durable field performance.

## Acknowledgements

The authors acknowledge the full-time PhD fellowship from Centurion University of Technology and Management (CUTM), Odisha. All authors thank CUTM for the support and encouragements.

## Authors' contributions

AN and SN conceptualised the work. AN and CB collected data, analysed and wrote the manuscript. NR and SN reviewed the manuscript and supervised the work. All authors have read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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