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REVIEW

Role of dsRNA-Based Insecticides in Agriculture: Current Scenario and Future Prospects

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ABSTRACT

Insect pests cause severe crop damage, resulting in substantial economic losses and threats to global food security. Conventional insecticides are low-cost chemical agents that kill the target insects and some non-specific beneficial organisms. Due to their toxic and non-biodegradable nature, these conventional insecticides persist in the environment, thus causing pollution and accumulating in the food chain. The development of novel insecticidal products based on double-stranded (dsRNA)-based RNA interference (RNAi) technology is a sustainable tool to effectively control insect pests. The dsRNA-based insecticides are known for their specificity, non-toxicity, and biodegradability. The current review introduces the dsRNA-based RNAi technique as a novel tool to control crop insect pests. The review highlights the mechanism behind dsRNA uptake into insect cells. Furthermore, it discusses the commercial aspects of different dsRNA-based products available in the market, their penetration rates, and public acceptance. The review details the latest developments in the field and the regulatory landscape regarding the technology. The advantages and limitations of dsRNA-based insecticides are discussed, and future research directions to overcome the potential challenges have been briefly suggested. The dsRNA-based insecticidal products may be a better alternative to conventional insecticides, thus delineating the resistance among insects and increasing agricultural productivity.

KEYWORDS

dsRNA; insecticide; integrated pest management; RNA interference; sustainable agriculture

1 Introduction

The introduction of the Green Revolution in the 1940s was a stepping stone in agriculture that led to high agricultural productivity and made several nations self-sufficient in food production. The Rockefeller Foundation spearheaded the revolution, thus making Mexico a self-reliant nation in wheat production by 1956 [1]. The increase in agricultural productivity is quite evident as the population grows. The farm sector has resorted to injudicious farming practices to meet global food demands and achieve higher productivity rates [2]. These include higher dependence on nitrogen fertilizers and intensive cropping practices, resulting in higher pest infestations and crop damage [2]. Insecticides are harmful substances that control insect infestations in crops and crop products. The sole aim of the insecticides has been to

maximize crop yields and minimize post-harvest losses [3]. Insecticides play a significant role in safeguarding crops, ensuring sufficient food supply to the growing global population of more than 9 billion people [4]. Most of the insecticides used in agriculture are of chemical origin and contain heavy metals and sulfur. Besides chemical insecticides, organic substances such as plant extracts and metabolites have also been used as an alternative. However, most farmers prefer inorganic chemical pesticides to organic ones because of their low cost and stability, the most important of which are neonicotinoids, organophosphates, and carbamates, which account for almost all insecticidal products [5].

The use of conventional insecticides has resulted in a significant number of deaths among agricultural workers, with estimates ranging from 5000 to 20,000 per year. These pesticides are primarily toxic in nature, which has contributed to the alarming number of fatalities. The ingestion of these pesticides has been linked to several adverse health effects, including renal failure, cardiovascular disease, and respiratory problems [6]. Furthermore, the excessive utilization of these insecticides has resulted in the emergence of insect and pest resistance. The development of resistance can occur via two distinct mechanisms. Exposure to insecticides may sometimes result in the mutation of specific genes, thus leading to the development of insensitivity to the chemicals among the insects. Alternatively, insects can alter the quantity and quality of their detoxifying genes and enzymes, thus creating metabolic resistance [7]. Some insecticides may persist in the environment and accumulate in different parts of the food chain, causing widespread toxicity [8]. There is an urgent need to develop new alternatives to conventional insecticides to prevent the spread of environmental toxicity and counteract the development of insect resistance.

RNA interference (RNAi) technology has emerged as a reasonably sustainable alternative to conventional insecticides, thus balancing agricultural productivity and environmental health. This process induces instability in the messenger RNA (mRNA) and disrupts transcription and translation in specific targeted cells or organisms mediated by microRNAs (miRNAs), Small interfering RNAs (siRNAs), and Argonuate (Ago) family protein complexes. RNAi mechanism leads to disruption of gene functions and a reduction in the levels of gene products, mainly proteins [9]. RNAi has contributed significantly to advancing our insights into insect biology. The importance of RNAi in modern agriculture is clearly demonstrated by the growing number of publications in this field. The top three countries with the highest number of publications in this field are the China, the USA, and India [10]. Several attractive features of RNAi make it an efficient and responsible solution for breeders to induce varietal plant improvement rather than going for more complicated and costly procedures like CRISPR/Cas9 or TALENS. RNAi can achieve a knockdown effect (by blocking or degrading mRNA transcripts) in a gene rather than complete gene knockout. Besides their unique characteristic features of high-spread mobility inside the plant system, they can also be used as a topical formulation, thus making them a highly competitive product in the market [11]. Furthermore, the demand for these products in the agricultural field is increasing due to their sustainable approach, wherein they can induce mortality in specific target insect pests or lead to the loss of a particular function in the organism without killing them [12]. The increase in agricultural productivity has also led to large-scale insect infestations, with these insects accounting for 18%–20% of global crop loss, estimated to be worth 470 billion USD [13]. RNAi represent a potential tool to the control of crop loss and sustainably improve agricultural productivity in such a situation.

2 Mechanism of Action

Double-stranded RNA plays a significant role in RNAi. Large-scale delivery of dsRNA can specifically target genes in insects responsible for development and overall survival [14]. Particular genes accountable for energy metabolism, digestion, internal cellular activities, chitin production, hormone balance, immunity, and insecticidal resistance can be targeted. Besides, some other target genes, primarily responsible for different

molecular processes like replication, transcription, translation, and fertility, can also be considered [15]. Table 1 accounts for different knockdown targets of dsRNA delivery and their impact on insect pests.

Table 1: Functional and knockdown targets in different insects of crops and their corresponding knockdown effects

Functional targets	Knockdown targets	Insects	Effect	Reference
Energy metabolism	V-ATPase subunits (A, B, D, E, G)	Coleopteran (*Diabrotica virgifera virgifera*) (Coccinellidae), Hemipteran (*Myzus persicae*), Orthopteran (*Locusta migratoria*), Lepidopteran (Tuta absoluta), and Dipteran (*Drosophila melanogaster*)	Control efficacy of 61% in 3 days against *Myzus persicae*. Transcriptional suppression of two host genes in *L. migratoria* nymphs (5th instar). 60% reduced target gene transcript in *T. absoluta*. Selective killing of *D. melanogaster.*	[15–17]
	Arginine kinase	*Nylanderia fulva*	15% higher larval mortality	[18]
	ADP/ATP translocase	*Diabrotica virgifera*	Stunted growth and death	[19]
	NADH dehydrogenase	*Chilo suppressalis*	30% increased larval mortality	[20]
Intracellular components	α-tubulin	*Bemisia tabaci*	Upto 97% mortality after 6 days of feeding containing 6 ng of dsRNA per day	[21]
	Shibire	*Dendroctonus frontalis*	Mortality rate exceeding 80%	[22]
	Ribosomal proteins	*Diabrotica virgifera*	Larval death	[19]
	SNAP gene	*Tetranychus urticae*	More than 80% mortality	[23]
Hormone balance	Ecdysone receptor	*Helicoverpa armigera*	Mortality rates exceeding 90% in adults after 24 h	[24]
	Juvenile Hormone Binding Protein	*Tuta absolute*	55% increase in mortality rate	[25]
	Bursicon	*Phenacoccus solenopsis*	28%–37% mortality	[26]
Chitin metabolism	Trehalase gene	*Aphis glycines*	51% increased mortality	[27]
	Hexokinase gene	*Diaphorina citri*	10% increase in larval mortality	[28]
	Chitin deacetylases (CDA1)	*Spodoptera frugiperda*	Retardation of growth and mortality (40%) in the larva	[29]
	Cuticle protein gene	*Aphis citricidus*, *Acyrthosiphon pisum*, and *Myzus persicae*	Up to 64.2% gene silencing, and 55.8% mortality	[30]

(Continued)

Table 1 (continued)

Functional targets	Knockdown targets	Insects	Effect	Reference
Digestion	Snakeskin gene (SSK)	*Anoplophora glabripennis*	80% mortality	[31]
	Kunitz-type Trypsin Inhibitors	*Ostrinia furnacalis*	19.5% increased mortality	[32]
	α-amylase	*Helicoverpa armigera*	17.7% increased mortality	[32]
Immunity and insecticidal resistance	Hemocytin	*Myzus persicae*	Higher mortality rates	[33]
	Apoptosis inhibitor proteins	*Anoplophora glabripennis*	90% mortality	[34]
	P450 genes	*Nilaparvata lugens*	Approximately 50% mortality in 5 days	[35]
	Acetylcholinesterase	*Plutella xylostella*	Lower larvae weight gain and high mortality rates	[36]
	Synapsin	*Aphis gossypii*	93.3% mortality in 4 days	[37]
Other genes	Calmodulin gene	*Varroa destructor*	Significant inhibition of reproduction	[38]
	Tektin 1 gene	*Bactrocera dorsalis*	Caused 64.5% sterility in males	[39]
	Hexamerin II gene	*Reticulitermes flavipes*	86.7% mortality	[40]
	Integrin β1	*Plutella xylostella*	Extended developmental time, low pupa weight, and rate of pupation and mortality	[41]

The mechanism of RNAi in insect pests can be categorized into two main phases (Fig. 1). The first phase involves the uptake of the dsRNA by the insect cells, which is then processed by the core RNAi system. It has been demonstrated that insect cells are capable of efficiently uptaking dsRNA through clathrin-mediated endocytosis [42]. Additionally, specific proteins, such as SID-1 like proteins, RNA binding proteins (RBP), or extracellular vesicles may also facilitate the uptake process by fusing the dsRNA molecules through the plasma membrane. These molecules are also thought to play a significant role during the transfer of the silencing signal from one cell to another cell of an insect pest, thereby conferring systemic silencing [2]. Three SID1-related genes were studied in *Spodoptera litura* by Gong et al. [43]. Analysis using qPCR found homologoues of the SID1-like genes to be involved in the transfer of dsRNA into the cell of *S. litura*. In another study, qPCR analysis revealed the expression of SID1 genes in various tissues of *Aphis glycines*, thus implicating its important role in RNAi-based gene knockdown in the species [44]. In insects like *Apis mellifera*, the expression levels of the SIL gene increased upon exposure to dsRNA, thus confirming its role in RNAi. Knockdown of the SIL mRNA in *Leptinotarsa decemlineata* resulted in reduced RNAi efficiencies [42]. However, in certain other insects, the SID1 proteins are found to have no particular role in the process of RNAi. In a study, the silencing of three orthologs of SID1 in *Tribolium castaneum* had no effect on RNAi efficiency [45]. Similarly, SID1 proteins or their homologs have not been found to play any role in RNAi [46]. This suggests variable roles of the protein or its homologs in

different insects. In certain insects, it may facilitate the uptake of dsRNA, while in some other insects, it may not. Once inside the cell, the dsRNA is cleaved into sRNA with the aid of the enzyme DICER. Subsequently, the sRNA is then further loaded onto specific proteins of the AGO (Argonaute) family, forming the RNA-induced silencing complex (RISC). The sRNA's guide strand facilitates the attachment of the RISC complex to the target RNA, which results in the degradation or inhibition of translation of the target mRNA and, consequently, causes post-transcriptional gene silencing. The process primarily occurs in the cytoplasm of the insect cell.; however, it can also occur by modifying chromatin within the nucleus [47,48].

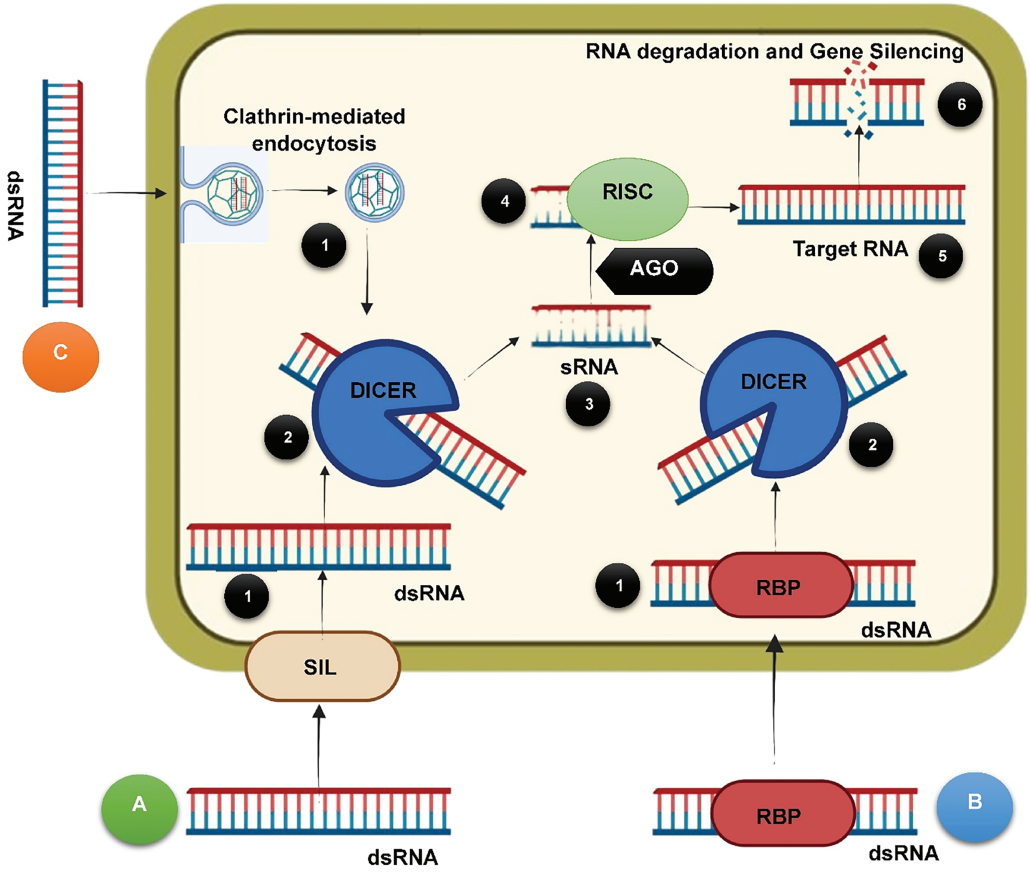

Figure 1: Mechanism of RNAi in insects. The dsRNA can be taken up by insect cells by either of the three mechanisms or pathways—(A) Delivery mediated by SIL, (B) RBP, or (C) receptor-mediated endocytosis. Step 1: Uptake of dsRNA by any of the pathways. Steps 2 and 3: Cleaving of long dsRNA fragments into sRNA. Step 4: sRNA binds to proteins of the AGO family. Step 5: sRNA and AGO form an RISC complex that guides the sRNA to the target sequence. Step 6: The sRNA binds with the target gene sequence, thus degrading it to induce a silencing effect. (SIL: SID1-like proteins, RBP: RNA binding protein, AGO: Argonaute, RISC: RNA-induced silencing complex)

The RNAi process can be broadly classified into two types—Cell Autonomous RNAi (CAR) and Non-Cell Autonomous RNAi (NCAR) (Fig. 2). In CAR, the silencing effect of the dsRNA is limited to a single cell where it is applied [49]. In NCAR, the silencing can be further divided into environmental RNAi and systemic RNAi. Environmental RNAi involves the uptake of exogenously supplied dsRNA by cells from the environment. This can affect those particular cells that uptake it. Sometimes, the silencing effect can also pass from the primary cells that have taken up the dsRNA to secondary cells and tissues [50].

Environmental RNAi is primarily achieved in insects by feeding them the dsRNA or soaking them in a dsRNA solution. Systemic RNAi involves the delivery of dsRNA to a single healthy cell from where the silencing signal can further be spread to other cells and tissues [49]. In certain insects like red flour beetle, extracellular vesicles have been found to transport dsRNA from one cell to another, thus causing a silencing effect [51]. REXD-1, TBC-3, and SID-5 genes in *C. elegans* help promote systemic RNAi by transporting dsRNA intracellularly [52].

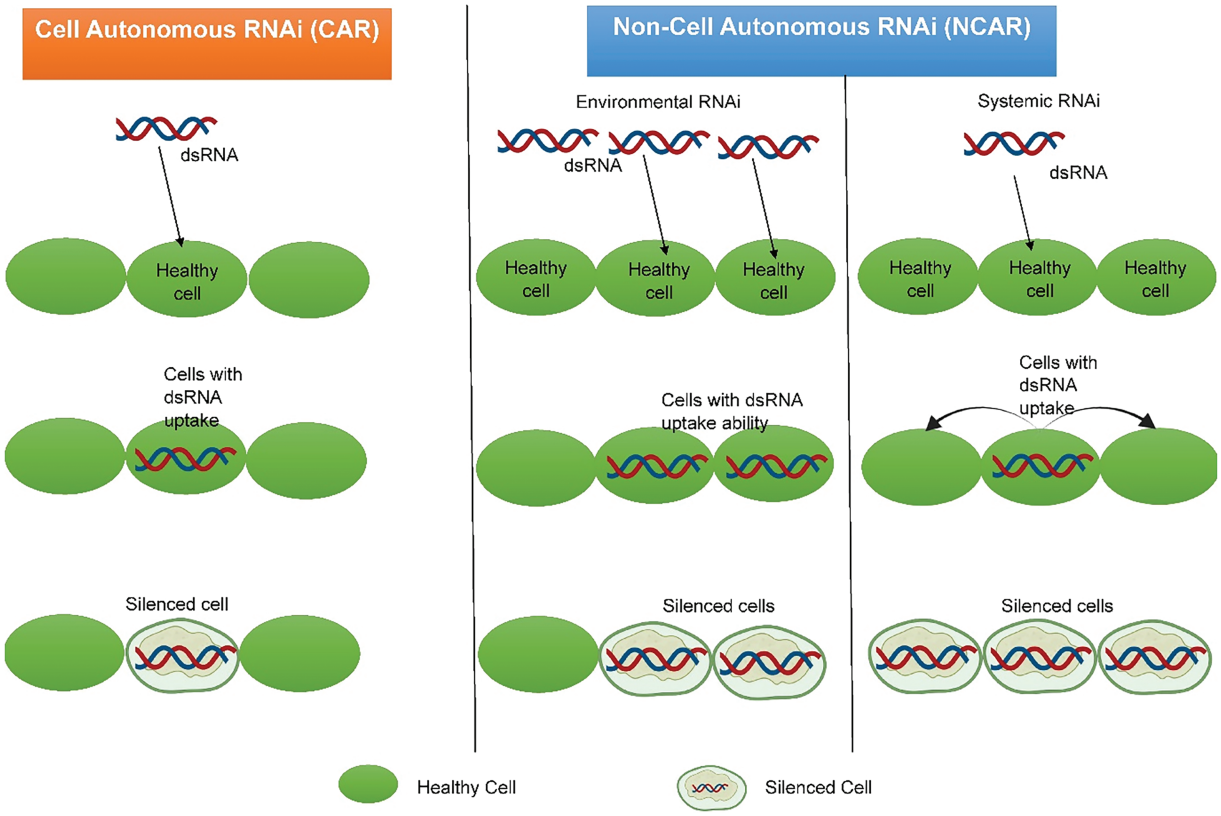

Figure 2: The cell-autonomous and non-cell-autonomous RNAi processes

Several methods achieve delivery of dsRNA, including microinjection, ingestion, topical application, and nanoparticle delivery. Microinjection can directly apply the dsRNA to the target insect tissues or hemolymph [53]. This ensures the safe passage of the dsRNA and prevents its degradation from the harsh gut environment of the insect. However, the microinjection technique is too laborious and requires experienced personnel. It is also impossible to use the technique in the field [54].

Ingestion is one of the simplest methods for delivering dsRNA into insects and is a feasible method for field application [55]. The midgut cells can take up the dsRNA and be transported to other tissues [56]. The delivery agent is basically a liquid formulation that can be applied to the foliar parts of the plant by soaking or mixing with insect diets to facilitate their ingestion by the target insects. The dsRNA for the purpose is mostly synthesized *in vitro* or by certain microbes [57]. The development of transgenic plants with the ability to continuously produce dsRNA has been instrumental in inducing RNAi in insects feeding upon the plant parts. Plant chloroplasts lack RNAi machinery, thus allowing the production and accumulation of stable dsRNA [58]. Non-transgenic delivery options are less time-consuming, and cheaper as compared to the

transgenic approaches. Transgenic plant development reduces the need for pesticides but faces the problem of practical application in different plants and low public acceptance [59].

Topical applications, also referred to as spray-based formulations, penetrate the cuticle of insects to cause mortality [60]. Topical applications are quite easy to use but face the drawbacks of low silencing efficiency in insects with thicker cuticles, limiting the dsRNA's penetration [29].

Nanoparticles have been found to act as an efficient delivery vehicle for promoting the efficient uptake and stability of the dsRNA. Chitosan nanoparticles, liposomes, and cationic dendrimers have been found to prevent the degradation of dsRNA in the environment as well as inside the insect's gut epithelium and further help in the translocation into different cells. Nanoparticles are no doubt one of the best options for the safe and efficient delivery of dsRNA, but they are too costly and may pose certain risks [61].

3 Current Scenario in dsRNA-Based Insecticides

There have been multiple research studies to formulate different dsRNA-based insecticides specifically targeting insect pests in crops. The subsequent sub-sections deal with these insecticides' commercial, R&D, and regulatory aspects.

3.1 Commercially Available dsRNA Insecticides

The use of dsRNA-based products in agriculture is still in its nascent stage, with very few of them commercialized to date. The first commercially available RNAi product was SmartStax Pro, a pioneering transgenic corn crop developed by Bayer Crop Science (earlier known as Monsanto). The product in question comprised a combination of two Bt proteins and a dsRNA, which targeted the snf7 gene and conferred resistance against western corn rootworms [62]. Once the Bt proteins enter the gut epithelium, they cause death by inducing gut paralysis. In contrast, the dsRNA leads to the downregulation of the snf7 gene, which plays a significant role in the protein trafficking process, ultimately resulting in death [63]. Some of the recent product developments in the field are provided in Table 2.

Table 2: Details of commercially available dsRNA products

Company	Product	Composition/Process	Current stage of development	Reference
Bayer crop science	SmartStax Pro	Combination of 2 Bt proteins and dsRNA targeting snf7 gene in western corn rootworms	Commercialized	[63]
RNAissance Ag	Sprayable biopesticide	Safe and cost-effective production of dsRNA using industrial fermentative bacterial species against diamondback moth	Early field trials	[64]
RNAgri	APSE RNA Containers	Use of *E. coli* for mass production of encapsulated dsRNA	Developed	[65]
Dow AgroSciences, in collaboration with Fraunhofer Institute for Molecular Biology and Applied Ecology, Europe	Transgenic plants against coleopteran and hemipteran pests	–	Unknown	[66]

It is too early to compare the different dsRNA-based products that have been commercialized or are in the process of development. This is because of the slow penetration of such products into the market and the lack of sufficient customer feedback data. Silencing through the RNAi mechanism is broadly classified into two types. When the dsRNA-based formulation is delivered on plant surfaces by spraying, it is known as spray-induced gene silencing (SIGS). When the exogenous dsRNA is inserted into the plants to confer resistance against specific pests, it is called host-induced gene silencing (HIGS) [67]. Plant-induced gene silencing is known to offer long-lasting protection from insects. However, it faces several difficulties associated with the genetic transformation of plants and regulatory issues, which are not a constraint in the case of spray-based dsRNA products [68]. Here, the authors anticipate spray-based dsRNA products to have an advantage owing to their non-transgenic nature and ease of use.

There has been a slow penetration of the dsRNA-based biopesticides used to treat insect pests. Although several patents and intellectual property (IP) applications have been filed in the field of dsRNA-based insecticides, but certain other limiting factors are responsible for the slow commercialization of these products. These factors include the cost of production, the requirement for high-end infrastructure, skilled labor, and stringent regulatory norms [69].

3.2 Research and Development

There has been a notable increase in research activity within the field of dsRNA-based insecticides. The field of sustainable management of agricultural productivity and pest infestations has been a point of attraction for numerous researchers. In recent years, several dsRNA-based insecticidal formulations and strategies have been developed with the specific aim of targeting particular insects affecting crops. Recent research has produced a dsRNA-based insecticide (Calantha), which contains ledprona as an active ingredient against *Leptinotarsa decemlineata* (Colorado potato beetle) through RNAi interference. Low doses of the insecticide were found to interfere with the pupation of the larvae (fourth instar) and reduce mobility and fertility among the adults [70]. A recent study by Li et al. [71] aimed to knockdown the Rdl2 gene in *Plutella xylostella*, thus increasing the insect's sensitivity towards γ-aminobutyric acid receptor targeting compounds like fipronil, pyrazoloquinazolines, and isoxazolines via RNAi mediated gene silencing.

The stability of the dsRNA within the insect gut poses a significant bottleneck in the process of RNAi. The gut environment of most insect pests is highly alkaline, thus leading to degradation of the dsRNA. Besides, several microbiota present in the gut can also lead to the degradation of the dsRNA. Lepidopterans express specific nucleases not found in other insects and can quickly degrade the dsRNA. A gene termed 'up56' was identified in *O. furnacalis* and encodes for a protein previously uncharacterized and found to be homologous in seven other lepidopteran species (while absent in other insects) with an ability to degrade dsDNA, dsRNA, and ssRNA, both *in-vivo* and *in-vitro*. Guan et al. (2018) named the gene as RNAse efficiency-related nuclease (Rease) [72]. Similarly, Hemipterans can degrade the dsRNA in their saliva and within the digestive tract [73]. The degradation of naked dsRNA molecules under several biotic and abiotic stresses invites the development of various carrier molecules for safe delivery, efficient uptake, and systematic distribution of the same in insect target cells. Several recent research studies have focused more on the efficient delivery of the dsRNA molecule to confer effective silencing of the target genes.

Protection of dsRNA is quite essential, so as to prevent its degradation and improve the RNAi efficiency. This can be achieved by several techniques, which include encapsulation of the dsRNA within liposomes (lipid bilayer-based nanoparticles) [74], nanoparticles [75], or embedded onto nano clay sheets [76]. Delivery of dsRNA could also be facilitated through root absorption in plants. The dsRNA is integrated into the crop irrigation system [77]. Insecticidal dsRNA can also be supplied via microorganisms like yeasts and *E. coli* [78] and other gut microbiota of the insect [79]. Table 3 provides different methods

employed for efficient dsRNA delivery. The advanced delivery methods protect the dsRNA from degradation under high alkaline gut pH and help the molecule escape from the endosomes, thus protecting them. Moreover, these delivery agents also protect the dsRNA from harsh environmental conditions like fluctuations in temperature, pH, soil microbes, thereby ensuring the efficacy of the RNAi approach.

Table 3: Novel methods for safe and efficient delivery and uptake of dsRNA in insects

Method	Target gene	Insect	Impact	Reference
Egg soaking method	V-ATPase	*Amphitetranychus viennensis*	Dose dependent RNAi effect. Acts as both contact and stomach toxicity. 8 ng/μL dsRNA induced 100% dark body color and mortality	[80]
Nanocarrier star polycation-based transdermal delivery	CYP6CY3	*Aphis gossypii*	84.3% reduction in expression levels at 48 h and 67.21% at 96 h due to increased susceptibility of 4th instar aphids to imidacloprid and prolonged doubling time and development of the insect population	[81]
Nanoparticle-mediated delivery system	CaM	*Grapholita molesta* and *Cacopsylla chinensis*	Increased susceptibility to cyantraniliprole	[82]
Chitosan nanoparticles mediated delivery	GRK2	*Apolygus lucorum*	Stability of dsRNA up to 48 h. 50% increased mortality, 26.54% reduction in weight, 8.04% increase in developmental period	[83]
Plastid-mediated RNAi and foliar application of gut bacteria—*Pseudomonas putida*	β-Actin and Srp54k	*Plagiodera versicolora*	Enhanced RNAi effectiveness	[84]
Galanthus nivalis agglutinin protein-mediated delivery	V-ATPase A	*Spodoptera exigua*	Mortality rate increased to 48% as compared to naked dsRNA treatments (8.3%)	[85]
Encapsulation with guanylated polymers	Chitin synthase B	*Spodoptera exigua*	37% increase in RNAi efficiency	[86]

3.3 Regulatory Landscape

The regulatory landscape of dsRNA-based insecticides is quite complicated. The stringent regulatory frameworks are the main reason for the availability of very few dsRNA-based insecticidal products in the market. dsRNA-based crops are considered to be genetically modified and hence are strictly evaluated. Being tagged as genetically modified, these crops lose acceptance among the common masses. The European Union mentions that genetic engineering approaches are linked to specific safety concerns and

must be regulated. On the other hand, the regulatory system in Canada mentions that plants that only have novel traits or foods can be sold in a marketplace [87]. The United States also follows a similar approach wherein GM crops are riskier than crops cultivated conventionally and will be subjected to different safety evaluations [87]. To date, there have been no clear guidelines on using dsRNA-based insecticides. Suppose a biotech product in the form of a plant protectant is developed and is to be registered as an insecticide. In that case, the developer must obtain an experimental usage permit from the Environmental Protection Agency (EPA) before field testing on a minimum of 10 acres of land [88]. In the US, dsRNA-based products are considered biopesticides, while in the EU, they fall under the category of chemical pesticides. Similarly, in Australia, these products are labeled under agricultural chemicals. In December 2023, the USEPA approved the registration of the dsRNA pesticide Ledprona developed by GreenLight BioSciences [69].

4 Advantages of dsRNA-Based Insecticides

Despite many uncertainties, dsRNA-based insecticides offer a number of advantages over their conventional ones (Fig. 3). Conventional insecticides are mostly inorganic in composition and can easily get transferred into the food chain as a consequence of their non-biodegradable nature and prolonger persistence in the environment. However, in this case, dsRNA-based insecticides are mostly biodegradable and non-toxic. This is due to the fact that these molecules are developed with the specific intention of targeting particular genes, thereby being specific to a particular insect pest. In comparison to their toxic inorganic counterparts, biodegradable dsRNA-based insecticides are less harmful to the environment. Inorganic insecticides poison a wide range of insects and disrupt soil ecology and fertility [89]. In contrast, dsRNA-based pesticides are natural products that can control insect infestations in crops by acting as a sustainable tool. They can be easily included under integrated pest management (IPM) strategies to confer resistance in plants or eradicate specific insect pests without harming the beneficial ones.

Figure 3: Advantages of dsRNA-based insecticides over conventional chemical insecticides

5 Challenges and Limitations

The RNAi technology via delivery of dsRNA faces several challenges and limitations. The stability of the dsRNA is questionable in the environment. Several environmental factors like temperature, photodegradation due to exposure to UV, microbial degradation, and wash-off due to rain may affect the stability of the molecules [90–93]. Off-target effects are another limitation of dsRNA-based insecticides.

Although these insecticides are specifically designed to target a specific gene in a particular insect, sometimes, any other organism with the same sequence identity as that of the dsRNA will be affected [94–97]. In a study conducted by Pan et al. [98], dsRNA designed to target the V-ATPase gene in *D. virgifera* was found to induce RNAi in four other species that shared several 21 nucleotides continuous matches with the dsRNA sequence. One of the major bottlenecks in dsRNA-based insecticides is the development of resistance among the insects. Insects can bring about mutations to the target genes or to the genes involved in core RNAi machinery, thus leading to degradation of the dsRNA. Insects have been thought to bring about alterations in critical genes involved in RNAi mechanisms, thereby developing resistance that is quite impossible to mitigate. Recent studies found that *D. v. virgifera* and *L. decemlineata* developed resistance against Snf7 dsRNA and IAP dsRNA without any mutations to the dsRNA target site [99,100]. This confirms that the mutation of genes involved in the uptake and transport of dsRNA in both organisms is a potential reason behind the development of resistance. The production of dsRNA, which involves acquiring trained researchers, chemicals, and equipment, increases the cost. Increased production costs lead to increased market prices, a significant reason behind the non-acceptance of the product among marginal farmers. There is a fear amongst the common masses regarding the acceptability of dsRNA-based products similar to that of genetically modified ones. Moreover, certain industrial insecticide producers feel the onset of these next-generation bioinsecticides is a looming threat to their existing business.

6 Future Prospects

6.1 Technological Innovations

Several research studies are now focussed on delivering the dsRNA molecules and their stability inside the insect pests and the environment [101–103,53]. Moreover, preparing the bio-insecticidal formulation is a vital part of the journey. The addition of certain surfactants generally improves the applicability of these products. Similarly, LDH-nano clays enhance the stability of up to 20 days in the foliar parts of a crop. Natural polymers like chitosan are frequently utilized as encapsulating agents, thus confirming efficient uptake of the dsRNA and protecting it from harsh alkaline pH conditions in the pest gut. The dsRNA molecules are known to be quite unstable in the soil due to several factors like temperature, other chemicals, and microbial degradation. Star cationic polymers have been developed as an efficient delivery agent, which can improve the molecule's stability for up to 3 weeks [95]. Plant plastids have emerged as one of the most fascinating options for expressing and delivering dsRNAs in insects. Whitfield et al. successfully induced the production of long dsRNAs in the chloroplasts of potato plants [104]. The dsRNA produced from the transplastomic plants efficiently targeted the β-actin gene of the Colorado potato beetle, thus providing crop protection [105]. Plastid based RNAi approach has proven successful in tobacco plants against non-chewing herbivores like *Frankliniella occidentalis* thus causing high mortality [106]. The gut microbiota in insects also plays a major role in sustaining them under varied environmental conditions. They supplement insects with important nutrients, provide protection from pathogens, and help circumvent plant defense systems [107]. These microbial symbionts also help insects degrade pesticides, thus conferring high levels of resistance [108]. Targeting bacterial symbiosis in target insects has been found to efficiently cause RNAi effects. Sap-feeding insects like pea aphids demonstrated reduced growth and reproduction when two genes, namely amiD1 and LdcA1, acquired from bacterial symbiont Buchnera, were targeted [109]. The symbiotic relationship between the insect and its gut microbes has been efficiently explored for RNAi by genetically modifying the bacterial symbiont. Genetically modified yeast expressing γ-tubulin dsRNA in *Drosophila suzuki* have been found to reduce survivability in larvae and locomotor and reproductive activity in adults [110].

6.2 Broader Applications

The development of dsRNA-based insecticides was initially concentrated on a few selective crops and their insect pests. However, recent studies have broadened the research horizon by experimenting with insect pests of several plants and animals, such as honey bees. The amount of dsRNA required for a successful application needs to be analyzed. As a rough estimation, 10 g of dsRNA is required per hectare of cultivable land to manage insects [111]. However, the dose may vary from insect to insect and crop to crop, thus requiring the confirmation of the doses differently in each case [112,113]. The dsRNA can also be used with certain pesticides to improve their efficiency. The synergistic effects of combining various pest control strategies can be analyzed to enhance RNAi efficiency.

6.3 Research Directions

The Organization for Economic Co-operation and Development states that dsRNA-based products have no ill effects on humans or the environment [114]. However, the non-target effects of RNAi make it highly important to design the dsRNA properly to avoid sequence identity. Further research needs to be carried out to improve the stability of these bioinsecticides in a way similar to conventional ones. In some cases, resistance to dsRNA can develop due to reduced uptake of dsRNA. To counter such issues, paperclip RNAs (pcRNAs) have been developed. These are the short-sized dsRNAs, and due to their closed-end structure, they can be quickly taken up through a clathrin-independent manner. These can also be applied to insects that do not respond to dsRNA-based RNAi machinery [115–118]. Developing such alternatives to dsRNA that can effectively induce silencing amongst the insects becomes essential.

The production of dsRNA-based insecticides is costly and needs collaboration between industrial, academic, and governing bodies to develop and market new sustainable pest control products. The players in the agribusiness sector will have to play a crucial part in commercializing RNAi-based insecticides.

7 Conclusion

The increasing global population is well substantiated by a corresponding increase in agricultural production. Large-scale use of fertilizers and deviation from proper agricultural practices have led to a rise in the population of insect pests in crops. These insects destroy crops and their products, thus accounting for substantial annual losses. Conventional insecticides have been successful to some extent in controlling these infestations. However, there has been an upsurge in resistance development in pests. Conventional chemical pesticides are also responsible for causing large-scale environmental pollution and negatively impacting certain beneficial organisms. The development of novel bioinsecticides has led to using dsRNA molecules as bioinsecticides to induce lethality among pests through the RNAi mechanism. The dsRNA-based bioinsecticides are characterized by their non-toxic, biodegradable nature and specificity. These modern-day bioinsecticides are being widely explored for sustainable pest management in agriculture. These molecules can induce mortality and target specific functions like mobility, reproduction, fertility, replication, and many other insect factors. The dsRNA molecule is sensitive to several biotic and abiotic stresses and prone to easy degradation. Several research and advancements in the field have led to the development of different methods for safely delivering these molecules and their efficient uptake within insect cells. Using dsRNA as an RNAi agent is also associated with several drawbacks related to its stability, efficiency, dosage, resistance, and off-target effects. Several research projects are being run to overcome these limitations. Few dsRNA products have been commercialized in the global market. The market penetration of such products and their acceptance is relatively slow due to the high costs involved, the need for a skilled workforce, and the fear of using genetically modified products. The stringent regulatory compliances of different countries and regions towards dsRNA-based insecticidal products hinder their commercialization. The paper advocates in favor of dsRNA-based

insecticides as a sustainable tool for use in integrated pest management and highlights future research directions. Moreover, the authors emphasize the need for collaboration among industries, academics, and government bodies for efficient commercialization and acceptance of these products.

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