

RNA interference in agricultural insect pest management: Status and perspectives

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In recent years, RNA interference (RNAi) has emerged as a potential tool to manage the pest population in agricultural fields and storage systems, however so far it has not come up in a big way. RNAi is a basic molecular biology mechanism occurring in all eukaryotic cells wherein a dsRNA (specific to a particular target messenger RNA) is used to inhibit/silence/knockdown gene expression. This dsRNA undergoes nucleolytic cleavage and several modifications to bind the complementary mRNA and induce endo-nucleolytic cleavage or translation repression. This basic molecular biological phenomenon can be exploited to silence the key genes (virulence and disease progression, toxin production, pest-specific key genes) in pests thus challenging their fecundity and survival. This mechanism is suitable for managing most insects (lepidopteran, dipteran, coleopteran, hemipteran, *etc.*) and nematodes. Application of RNAi differs based on the mode of delivery system of dsRNA to the pest. The efficiency and efficacy of RNAi depend upon targeted genes, type of dsRNA produced, length of dsRNA, feeding methods, stability of dsRNA in insect guts, and easy uptake by gut epithelium, *etc.* However, this technology is not utilised up to its full potential and therefore the present review is aimed to assess the possibility of RNAi in agricultural pest control and the way forward.

Keywords: RNA interference (RNAi), Gene silencing, Integrated pest management (IPM), Insect-pest, siRNA, Biological control

Introduction

Insect infestation in crops has been estimated to affect 10.8% of crop yield globally. According to the Food and Agricultural Organization (FAO), 1.3 billion tons of food grains are wasted every year due to insect pests. The rapid growth cycle of insect pests, changing climatic patterns, rising temperature, the narrow genetic base of crop varieties, mono-cropping, increased resistance in insects to chemicals, *etc.* are the major setbacks to present-day agricultural pest management¹. With the increase in the temperature, there will be a considerable increase in the metabolic rate and reproductive ability of insect pests, leading to more crop losses in the near future². For example, in recent years, insects like fall armyworms (FAW) in maize have spread across the whole world. They are a recent example of climate change affecting the behaviour of insect pests².

Chemical management of agricultural pests is hazardous, pollution-causing, costly, and un-economical to the resource-poor farmers³. Other mechanical, cultural, and biological methods include solarisation, larva picking, trapping, crop rotation, multi-tier cropping, use of resistant varieties, predators, parasitoids, sterile insects, *etc.* Although these methods are very effective, economical, and easy to incorporate but can only be applied to a small area over time⁴. Morpho-physiological features of insect pests such as small body size, ability to fly, tough skeleton, high reproductive potential, adaptability, polyphagous nature, inherent capacity to evolve, *etc.* have given an advantage to their dominance in nature⁴.

Advancements in genomics have assisted in the development of novel concepts of breeding resistant cultivars by offering different opportunities to address biotic and abiotic stresses⁵. If the genes for resistant sources are present in the crop germplasm, the breeding efforts with marker-assisted backcrossing, genomic selection, introgressive hybridisation, and marker-

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assisted recurrent selection are easy. However, variations for resistance to insect pests are uncommon, less durable, inefficient, and problematic to transfer⁵. Therefore, methods such as RNA interference, gene editing technologies, transgenic approaches, *etc.* are offering great promises⁶. Transgenic approaches depend on introducing foreign genes into the target plant's genome to make it resistant to specific insect pests (affecting physiology and growth of pests)⁷. Gene editing is generally done for plant genes that are targets of pathogenic effectors making them inaccessible to be targeted by pathogen⁶. RNA interference, if artificially induced mimics the natural processes occurring in the organism to silence the genes in pests that are vital for their growth and survival or to silence their effector genes^{6,7}. However, a thorough analysis of the present-day usage of RNAi in agricultural pest management and understanding of RNAi needs to be updated. In the present review, we have critically analysed the potentiality of RNA interference in crop pest management in today's era and its drawbacks and limitations. A brief description of the present understanding of the molecular mechanism of RNAi, various methods of delivery, potential target genes, *etc.* are also mentioned. This review aims to bring forward the concepts and futuristic approach to the usage of RNAi in agricultural insect pest management.

Mechanism of RNA interference

RNA interference was first reported as the Host Induced Gene Silencing (HIGS) method to control the attack of viruses, activation of transposons, and changing the spatiotemporal expression of host genes^{6,8}. RNAi controls the expression of target genes by destroying their messenger RNA (mRNA) or by blocking their translation. Artificially, this can be used to target specific key genes involved in virulence and disease progression in pests, toxin production in pests, metabolising pest/host material for defence, strengthening basic plant resistance mechanism, *etc.*⁶. It is an easy-to-use, and highly target specific technology for agricultural pest control⁴. This natural phenomenon has also been used as a powerful tool to generate variations in plant traits, gene identification, metabolic pathway determination, changes in gene expression, imparting abiotic stress tolerance *etc.*⁷.

RNA interference (RNAi) works via a long double stranded RNA (dsRNA) which may be of exogenous or endogenous origin⁹. When a long dsRNA is present in a eukaryotic cell, an RNase called Dicer cleaves it into a short ~20-25 nucleotide long dsRNA molecule with a 3'

overhang. This short RNA called small RNA may be converted into small interfering RNA (siRNA) or micro RNA (miRNA) based on their origin⁸. An RNA-induced silencing complex (RISC - made up of Argonaut class of protein) is then attached to this RNA and one of the strands called the passenger strand is removed⁹. The single-stranded small RNA with RISC is then attached to the targeted mRNA through complementary sequence pairing (complete pairing – siRNA; incomplete pairing – miRNA) and may degrade the mRNA by endonucleolytic cleavage (in case of siRNA) or may block translation (in case of miRNA)^{3,4,6}. A figure containing an outline of the mechanism of RNA interference in eukaryotic organisms is given (Fig. 1).

RNAi mechanism in pest control

Naturally, RNA interference is found in all eukaryotes including insects, and may be a systemic occurrence or by cellular uptake of dsRNA molecule⁴. Systemic RNAi phenomenon has been found to have trans-generational and trans-developmental effects¹⁰. Based on reports on several insect species, it has been reported that insects do have a mechanism analogous to RNA-dependent RNA polymerase (RdRP) mediated amplification of siRNA as found in plants, fungi, and nematodes⁸. Most eukaryotic organisms have genes called miR genes, which are responsible for the production of micro-RNA (miRNA) and work in stress response, developmental pathways, growth, and reproduction. They are also vital for the survival of organisms¹¹. Cellular uptake of dsRNA and their movement has been reported in various organisms such as plants to fungi, bacteria to nematodes, plants to nematodes, fungal pathogens, insect pests, human to insect parasites *etc.*¹¹. Small interfering RNA (siRNA) can be directly taken up from the environment or tissue and transfer signal from cell to cell in insect pest⁸. Therefore, both the process of autonomous production of dsRNA from internal genes and the external application of dsRNA for the generation of RNA interference in target organisms have been exploited to control insect pests¹². A figure explaining available strategies employed to utilise RNAi in pest control is explained (Fig. 2).

Systemic RNAi

Systemic RNAi for pest control is a production of dsRNA and its utilisation for gene silencing at the same cell⁸, which may have trans-generational and trans-developmental effects¹⁰. Artificially, systemic RNAi phenomenon can be induced *via* a transgenic approach

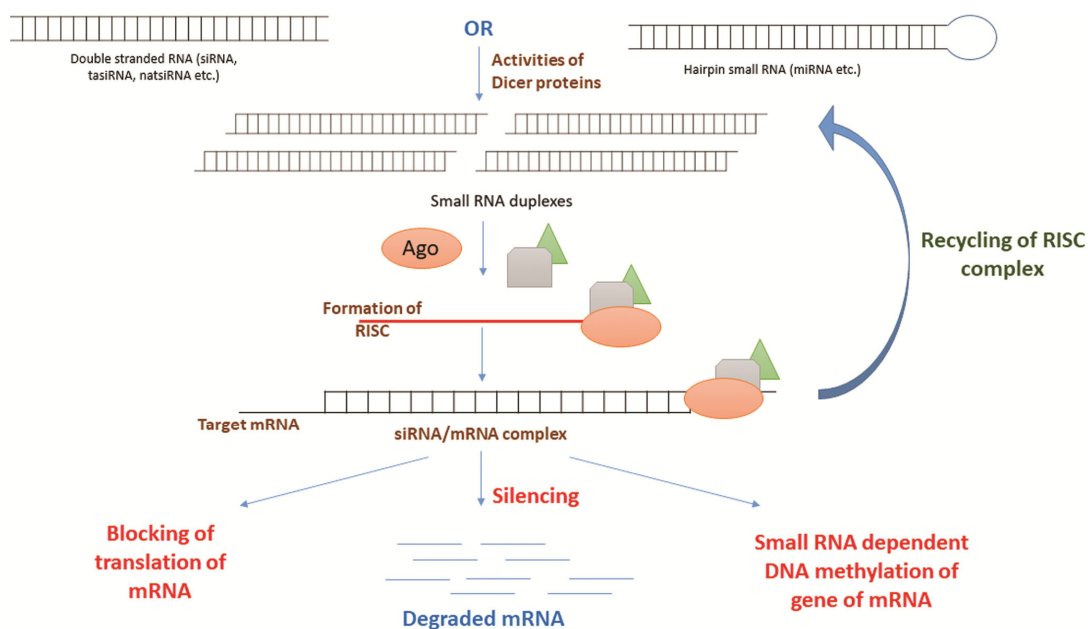


Fig. 1 — Schematic diagram showing RNAi-mediated gene silencing in eukaryotes viz., ds RNA, hpRNA, siRNA, RISC – RNA induced silencing complex (modified from Majumdar *et al.*⁴.)

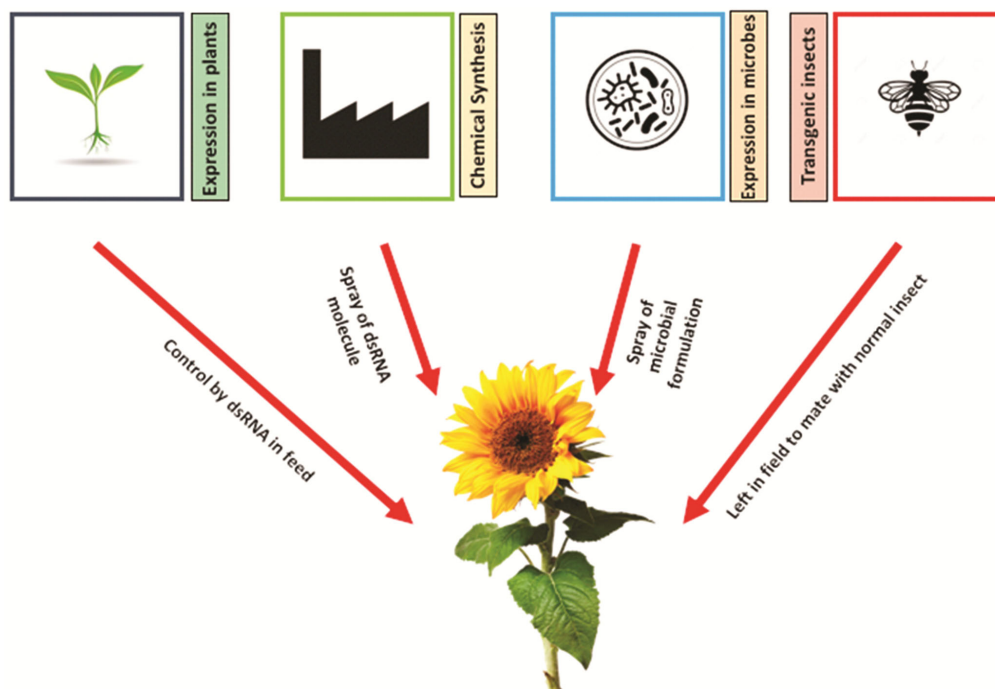


Fig. 2 — Use of RNAi as future insecticide. Target gene expressed in plant will produce dsRNA which will be made available in gut of insects and through transmembrane uptake of dsRNA insect will get killed; chemically synthesised dsRNA formulation can be sprayed on leaf and after ingestion it will kill insect pest; microbial formulation will work in same way as chemical; Transgenic insect of same species will compete for mating and will produce sterile progeny or deformed progeny, thus controlling pest population.

by inserting a gene that can produce a dsRNA targeting gene of interest in a particular insect pest⁸. To have such a gene construct, one must consider the gene target, the tissue where the gene must be targeted, the desired

developmental stages, and the level of expression¹². Generally, genes related to fertility, growth, development, and physiological traits are targeted via this method^{8,12}. A transgenic insect pest with RNAi

construct which can transfer dsRNA via gamete can also be used to produce sterile progeny or deformed progeny^{8,10}. Double-stranded RNA interference for insect pest-specific genes can be produced in the plant by designing a gene construct with plant-specific promoter fused with dsRNA called a host-induced RNAi approach¹³. In this method, a crop plant is engineered to produce a hairpin RNAi vector which can produce dsRNA¹². When insects feed on such plant parts, dsRNA enters into its body and induces RNA-induced silencing of the target gene¹⁴.

Externally applied small RNAs

Signals of RNA interference in the form of dsRNA molecule can move from cell to cell, tissue to tissue within the organism, and even among organisms of different kingdoms¹⁰. Different mechanisms have been proposed to explain the uptake of and transfer of dsRNA and one of the mechanisms explains the presence of receptor-mediated endocytosis of dsRNA signal uptake and vesicle-mediated intra-cellular trafficking for RNAi effects^{12,15}. The external dsRNA can be transferred to the insect by external application of dsRNA formulation on plant parts or *in-situ* production of dsRNA in plant tissue using a transgenic approach¹². External delivery methods used for dsRNA delivery to the target site are soaking or incubation, injection, spraying, feeding, and dusting⁸. The different delivery methods employed in the external application of dsRNA for RNAi mechanism are explained in Table 1.

RNA interference in agricultural pest control

RNAi as a tool has been demonstrated to control various agricultural pests such as insects, nematodes,

weeds, fungi, viral and bacterial pathogens, *etc.* in laboratories and field condition^{6,8,12}. Chemical, biological, cultural, mechanical, and novel tools like RNAi can be combined for pest control^{8,12}. Double-stranded RNA can be produced using transgenic approaches to control lepidopteran, coleopteran, dipteran, and hemipteran insects as well as nematodes⁴.

Target insects and nematodes

Coleopteran insects were the first target of the RNAi mechanism for which a product had been developed¹⁶. Western corn rootworm is the most important coleopteran insect in which RNAi has been developed with little effort, fewer side effects, and an easily identified target gene. This was achieved by producing a hairpin dsRNA for the *v-ATPase* gene^{5,16}. Similarly, the Colorado potato beetle (*Leptinotarsa decemlineata*), an important pest of potato has been controlled by making a transgenic potato plant producing a hairpin version of β -actin and *Shrub* genes in chloroplast or in cytoplasm¹⁷. These plants were producing hairpin versions of β -actin and *Shrub* genes in chloroplast. Interestingly, the dsRNA gene expressed in the nucleus could not produce much RNAi effect^{16,17}. This may be explained by the fact that chloroplasts do not have cellular RNAi machinery therefore dsRNA produced will be transferred to the insect gut after feeding whereas; cytoplasmic dsRNA will be digested by the dicer in the cells of potato¹⁸. A list of some of the target organisms along with their target gene used for RNA interference is given in Table 2.

Genes targeted

The use of RNAi for successful insect control depends on the identification of the target gene

Table 1 — Different modes of delivery of external dsRNA for RNAi in pest control

Mode of Delivery	Remarks
Soaking or incubation	The cell culture or tissue is soaked in a dsRNA solution containing a transfecting agent for a particular time. The transfecting agent assists the easy uptake of dsRNA by cells. This system can be rarely used for field control of insect pests.
Injection	It is a microinjection-based delivery method where dsRNA is directly inserted into the body fluids of a pest by micro-needle. It is not economical for pest control in the field.
Spraying	Spraying of dsRNA molecules produced by bacterial culture or chemical synthesis of nucleotide can be done on the plant surface or the pest. After feeding onto the sprayed plant part, the insect will die due to the action of RNAi. It may be requiring a large-scale production of dsRNA which makes this method costlier. Internal feeders and root pests cannot be controlled by this method.
Feeding	The most important and economical method to apply dsRNA to control pests is by feeding them with dsRNA formulations. Double-stranded RNA or a transgenic bacterium capable of producing dsRNA is mixed with an artificial diet and used as feed for pests. It can be applied to the leaf or other plant parts affected by pests to control them.
Nanoparticle-based delivery	Organic molecules coating inorganic nanoparticles can act as delivery agents for dsRNA-based formulation. They offer elongated exposure, longevity of efficient delivery and easy and fast spread onto the plant tissue and insect body, and easy uptake by the host cell.

Table 2 — List of several genes targeted to manage different insect pests using RNAi approach

Name of insects/ pest and their family	Gene and their function	Methods	Effects of RNAi
Silkworm (<i>Bombyx mori</i>) (family- Bombycidae)	Circadian clock gene <i>per</i>	Transgenic	Disruption of egg-hatching rhythm ¹⁹
	Ecdysis-triggering hormone gene <i>ETH</i>	Transgenic	Lethal at pharate second-instar larval stage ²⁰
Egyptian cotton leaf worm, <i>Spodoptera littoralis</i> (family-Noctuidae)	β -actin gene	Injection	Disruption of sperm release ²¹
	Circadian clock gene <i>per</i>	Injection	Delayed sperm release ²²
Light brown apple moth, <i>Epiphyas postvittana</i> (Family-Tortricidae)	Carboxylesterase gene <i>EposCXE1</i> and pheromone binding protein gene <i>EposPBPI</i>	Feeding	Inhibition of gene expression ^{23,24}
	Cytochrome P450 gene <i>CYP6AE14</i>	Feeding	Inhibition of larval growth ²⁵
Cotton bollworm, <i>Helicoverpa armigera</i> (Family-Gelechiidae)	Glutathione-S-transferase gene <i>GSTI</i>	Feeding	Successful inhibition of gene expression ²⁶
	Protease genes including Trypsin (Ha-TRY39 and Ha-TRY96), Chymotrypsin (Ha-CHY), and Cathepsin L (Ha-CAT)	Ingestion by feeding and Injection in Larva	Disruption of digestion ²⁷
Beet armyworm, <i>Spodoptera exigua</i> (Family-Noctuidae)	<i>Chitin synthase</i> gene	Injection	Disorder in the insect cuticle, no expansion of the larval trachea epithelial wall, and other larval abnormalities ^{28,29}
	Chymotrypsin gene (<i>SeCHY2</i>)	Bacterial feeding	Digestive system disorder with high mortality ³⁰
Japanese pine sawyer, <i>Monochamus alternatus</i> (Family-Cerambycidae)	Laccase gene <i>MaLac2</i>	Injection	Pupal and adult cuticle sclerotisation, death at a high dose ³¹
	Chitin synthase genes <i>TcCHS1</i> and <i>TcCHS2</i>	Injection	Disruption in all types of moulting (larva-larva, larva- pupa, and pupa-adult), cessation of ingestion, decrease in larval size, and reduction of chitin content in the midgut ^{32,33}
Red flour beetle, <i>Tribolium castaneum</i> (Family-Tenebrionidae)	Chitinase-like proteins <i>TcCHT5</i> , <i>TcCHT10</i> , <i>TcCHT7</i> , and <i>TcIDGF4</i>	Injection	Effects on pupal-adult moulting Effects on egg hatching, larval moulting, pupation, and adult metamorphosis. Effects on abdominal contraction and wing/elytra extension. Effects on adult eclosion ³⁴
	Vacuolar ATPase (<i>v-ATP</i>)	Feeding	Delayed larval development and increased mortality ³⁵
Western corn rootworm, <i>Diabrotica virgifera virgifera</i> (Family-Chrysomelidae)	<i>Snf7</i> homolog is part of Endosomal Sorting Complex (ESCRT) pathway; Crucial in internalisation, transport, sorting and lysosomal degradation of transmembrane proteins.	Feeding on Transgenic plants	Ubiquitinated proteins are accumulated in <i>DvSnf7</i> dsRNA-fed larval tissues and process of autophagy is impaired ³⁶
Striped flea beetle, <i>Phyllotreta striolata</i> (Family-Chrysomelidae)	Arginine kinase gene <i>AK</i>	Feeding	Delayed development, increased mortality, and reduced fertility ³⁷
Mediterranean field cricket, <i>Gryllus bimaculatus</i> (Family-Gryllidae)	Circadian clock gene <i>per</i>	Injection	Complete loss of circadian control of locomotor activity and electrical activity in the optic lobe ^{38,39}
	Nitric oxide synthase gene <i>NOS</i>	Injection	Destruction of long-term memory ^{40,41}

(Contd.)

Table 2 — List of several genes targeted to manage different insect pests using RNAi approach

Name of insects/ pest and their family	Gene and their function	Methods	Effects of RNAi
German cockroach, <i>Blattella germanica</i> (Family-Ectobiidae)	<i>BgRXR</i> gene Pigment-dispersing factor gene <i>pdf</i>	Injection Injection	Inhibition of pupal eclosion ^{42,43} Effects on insect night activity ⁴⁴
American grasshopper, <i>Schistocerca americana</i> (Family-Acrididae)	Eye colour gene <i>vermilion</i>	Injection	Suppression of ommochrome formation and systematic expression ⁴⁵
Brown plant hopper, <i>Nilaparvata lugens</i> (Family-Delphacidae)	Trehalose phosphate synthase (<i>TPS</i>) (<i>NITPS</i> mRNA)	Feeding	Disturbed development through disruption in the <i>TPS</i> enzymatic activity, reduction of insect survival rate ^{46,47}
Turnip sawfly, <i>Athalia rosae</i> (Family-Tenthredinidae)	<i>white</i> gene	Injection	White phenocopy in embryonic eye pigmentation ⁴⁸
European honey bee, <i>Apis mellifera</i> (Family-Apidae)	Transcription factor gene <i>Relish</i>	Injection	Inhibition of <i>Relish</i> gene expression and reduction in the expression of two other immune genes, <i>abaecin</i> and <i>hymenoptaecin</i> ⁴⁹
Triatomid bug, <i>Rhodnius prolixus</i> (Family-Reduviidae)	Salivary nitrophorin 2 gene <i>NP2</i>	Injection and feeding	Shortened plasma coagulation time ⁵⁰
Savannah tsetse fly, <i>Glossina morsitans</i> (Family-Glossinidae)	<i>TsetseEP</i> gene and transferrin gene <i>2A192</i>	Feeding	Inhibition of <i>TsetseEP</i> gene expression, but no inhibition of <i>2A192</i> gene expression ^{41,51,52}
Eastern subterranean termite, <i>Reticulitermes flavipes</i> (Family-Rhinotermitidae)	Cellulase enzyme gene <i>Cell-1</i> and caste- regulatory hexamerin storage protein gene <i>Hex-2</i>	Feeding	Reduction in group fitness and increased mortality ^{53,54}
Asian corn borer, <i>Ostrinia furnacalis</i> (Family-Crambidae)	Chitinase like gene <i>CHT10</i>	Nanoparticle	Knock down midgut specific <i>Chitinase</i> gene and induced mortality ⁵⁵
Whitefly, <i>Bemisia tabaci</i> (Family-Aleyrodidae)	Acetylcholinesterase gene (<i>AChE</i>) Vacuolar-ATPase A subunit	Feeding Feeding	90 % mortality in adult after 4 days ^{56,57,58} 70% Mortality is caused by disruption in ionic transport in mid-gut ⁵⁹
Colorado potato beetle, <i>Leptinotarsa decemlineata</i> , (Family – Chrysomelidae)	β -Actin gene N-glycosylation-related genes	Feeding Larval injection	Disruption of sperm release ⁶⁰ High mortality was observed in the larval stage with the silencing of early NGRGs, as <i>STT3a</i> , <i>DAD1</i> and <i>GCS1</i> ⁶¹
Root knot nematode, <i>Meloidogyne Incognita</i> ,	Parasitism gene <i>16D10</i> ; which encodes a secretory peptide acting as a ligand for a plant transcription factor. Chitin biosynthetic pathway genes (chitin synthase, glucose-6-phosphate isomerase, and trehalose)	Feeding on transgenic plants Feeding on transgenic plants	Reduced nematode infectivity ⁶² Number of eggs and root knots were significantly decreased by 60–90% in RNAi transgenic lines ⁶³

responsible for deleterious effects on insect growth and development, which is a tedious process¹². The dsRNA must be sequence-specific, have great impact, organism-specific, and functionally known⁶⁴. Baum *et al.*¹⁶ developed the transgenic corn expressing dsRNA targeting the vacuolar *ATPase* gene (v-ATP). This can easily reduce the damage caused by *Diabrotica virgifera* (LeConte). RNAi response in western corn rootworm (WCR) has been reported by several authors for many genes and a product based on oral

administration of a dsRNA-based compound has been reported to cause RNAi. Based on this, a product has been developed and released in the United States and was commercially successful⁶⁵. Similarly, various dsRNA, each targeting a particular mRNA has been reported in different crops for various insects¹². RNAi or gene silencing offers an excellent alternative way to traditional methods to manage nematode-pests of important crops⁸. Lists of genes targeted in a particular organism to control them are given in Table 2.

Genetic and physiological mechanism for successful RNAi

A successful RNAi construct or formulation must consider the fact that when and where it is effective and economical. Transgenic plants carrying dsRNA genes must consider the availability and effectiveness of target genes on pests for spatiotemporal expression⁶⁵. To become applicable as an insecticide, transcript reduction of the intended target gene must additionally lead to mortality in the insect. Therefore, major things affecting the success of RNAi as a pest management strategy are the selection of the target gene, the effect of the target gene on the pest, length of dsRNA produced, stability of hairpin dsRNA, digestion of dsRNA by insect gut nucleases, chemical hydrolysis by gut pH, amount of dsRNA produced, life stage of insects, mode of dsRNA delivery methods, off-target effects of target genes *etc*⁶⁶. For example, RNAi as a pest management strategy, combined with other methods or alone, will only be used if it is cheap, easy to use, non-hazardous, non-polluting, and effective^{65,66}.

Efficiency of dsRNA uptake

Cooper *et al.*⁶⁶ reported the highly variable efficiency of environmental RNAi (RNAi triggered through exogenous application of dsRNA) in different insect orders, occurring due to various factors like dsRNA instability, variation in uptake of dsRNA by the insects, differences in ability to up-regulate or express RNAi genes, different copy number of RNAi pathway genes in insects of different species and orders, differential rate of processing of dsRNA to sRNA & degradation by dsRNases among different insect orders⁶⁷. Deletion & duplication of the RNAi pathway genes may lead to reduction or abundance respectively of the proteins due to reduced/enhanced or novel function, subsequently affecting the gene silencing. Enhanced levels of dsRNases may lead to the degradation of exogenously applied dsRNA before its conversion to sRNA^{67,68}.

Systemic spread of RNAi in insects

Systemic spread of RNAi in insects (non-cell autonomous RNAi) and transmission of RNAi in its progeny (parental RNAi) has been reported in plants and nematodes but in very few insects, affecting RNAi's efficiency in pest control⁶⁹. Genes encoding RNA-dependent RNA polymerases (*RdRPs*) generate secondary RNAs (sRNAs) which facilitate amplification & systemic spread of dsRNA but the absence of these genes (encoding *RdRPs*) in insects

affects the success of insect control through this mechanism^{70,71}. Another gene *SID-1* (*Systemic RNA interference deficient 1*) encodes for transmembrane channel protein which facilitates dsRNA uptake and cell-to-cell movement of dsRNA in nematodes. The absence of genes encoding *SID-1-like* (*SIL*) proteins in many insect genomes is another factor that impacts the commercial success in attaining pest control through RNAi^{66,72,73}. However, *SIL* (*SID-1-like*) independent mechanism for dsRNA uptake *e.g.* clathrin-dependent receptor mediates endocytosis may also operate in the insects lacking *SIL* proteins⁷⁴.

Selection of target genes

Targeting the essential gene reduces insect activity and fitness, but the targeting of successful gene silencing is highly variable^{71,75}. The optimum length & dose of the dsRNA also determines RNAi's success in pest control, which is a critical task to achieve. Generally, molecules of size >60bp have given the best result and small RNAs of about ~20bp have also been effective^{73,75}. The stability of dsRNA in the insects is another concern that influences this mechanism in insect control. Exogenously applied dsRNA (environmental RNAi) may get degraded by the microorganisms before being taken in by insects. After intake, dsRNA can be degraded by the nucleases present in the saliva or gut lumen of the insects^{76,77}. High or low pH of the gut lumen regulates the activity of the nucleases which in turn reduces the stability of dsRNA⁶⁶. The life stage of the insects also is an important factor that decides the dsRNA activity and stability^{61,77}. These are the several factors that hinder and make it harder to develop and optimise an effective RNAi-mediated control strategy.

Biosafety aspects

RNAi may lead to silencing of the non-intended off-target genes as well as off-target organisms⁷⁸. Qiu *et al.*⁷⁹ reported 5% to 80% of the off-target effects through this technology in three diverse organisms. Another concern is the risk of toxicity in human beings or other non-target organisms and disturbance in their immune system due to the use of RNAi-mediated pest control mechanisms^{80,81}. The biosafety issue can be addressed either by enhancing the targeted delivery of dsRNA and specificity to target RNA⁸¹. The biosafety assessment can utilise bioinformatics approaches to deriving sequence homology to minimise off-target effects precisely. The assessment of external dsRNA compatibility with

host RNAi machinery must also be performed beforehand to minimise any negative effects⁸². Efforts should also address the impacts of dsRNA delivery to non-target non-pest species which have lesser capability of digesting dsRNA in their gut. These can be addressed by the very high specificity of target RNA⁸³. Resistance development in insects against RNAi in insects is another aspect of biosafety. Evolution and mutation a very common phenomena in insects that may lead to genetic variation in insects. Further studies should assess the off-target effects/toxicity, development of insect resistance, and persistence of dsRNA in the environment, which will help address these biosafety issues and make this efficient mechanism commercially successful⁸⁴.

RNAi vs. Gene editing in pest control

Gene editing has come up in a big way in stress-tolerant plant types and is very much expected to give genetic solutions to major challenges in crop cultivation. However, it has several limitations as it can be highly target-specific and crop-specific. The general external application of RNAi offers an opportunity for pest control without knowing the mechanism of plants and without any need to transform and culture them⁸⁵. Many crop species are very difficult to culture and transform and for them, RNAi can be the best approach. Besides this, the development of a robust RNAi tool can target several pests of a single family and hence can address many problems at a time⁸⁶. The complete alteration of genes may be sometimes detrimental to host species via gene editing and for this, RNAi may offer an opportunity to selectively modulate the expression⁸⁷.

Conclusion and Future Prospects

RNAi technologies have the enormous potential to control a wide range of insect pests on crops. Many successes have been achieved in this area, but a few drawbacks have impaired their commercial success, which need to be studied to overcome these drawbacks. Future studies to identify the specific target gene in different genomes and reduce the risk of its effect on off-target genes/genomes will improve its efficacy. The development of improved delivery methods and optimisation of the length and dose of dsRNA for different insect species will give better control over pests. Identifying novel methods that can enhance the stability and uptake of the dsRNA can improve the efficacy of RNAi in insect pest control. Double-stranded RNAs (dsRNA) coated with

biocompatible and biodegradable nanoparticles have been reported to enhance the stability of dsRNA. Double-stranded RNAs (dsRNA) loaded on layered double hydroxide (LDH) clay nano-sheets showed greater stability and less degradation after treatment with RNase⁸⁸. These strategies will help attain commercial success and minimise the risk of the evolution of resistance in targeted insects.

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Authors contribution

NRP conceived the idea of writing this review. NRP, AA1, SK, KK, VS, and DJ contributed to writing the body of the manuscript. NRP and AA2 finalised the manuscript.

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