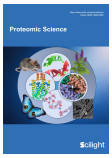
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Review

Engineering Nanocarrier-Mediated dsRNA Delivery Systems for Aphid Control: From AI-Based Target Discovery to Proteomic to Systems-Level Responses

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Abstract: Wheat aphids, particularly *Sitobion avenae*, are among the most destructive insect pests affecting wheat production worldwide, causing substantial yield losses through direct feeding damage and virus transmission. Conventional chemical pesticides remain the primary control strategy; however, their extensive use has led to insecticide resistance, environmental contamination, and concerns about non-target effects. RNA interference (RNAi) has emerged as a promising and highly specific alternative for aphid management, enabling the targeted silencing of essential genes involved in survival, development, and reproduction. Nevertheless, the practical application of RNAi in field conditions is constrained by the instability of double-stranded RNA (dsRNA), susceptibility to nuclease degradation, and inefficient cellular uptake. Nanocarrier-based delivery systems have been developed to overcome these limitations by enhancing dsRNA stability, protecting it from environmental degradation, facilitating cellular internalization, and enabling controlled release. Various nanoplatforms, including polymeric nanoparticles, lipid-based carriers, mesoporous silica nanoparticles, metal–organic frameworks and bio-based nanomaterials, have demonstrated significant potential for improving RNAi efficiency in insect pest management. Beyond delivery enhancement, increasing evidence suggests that nanocarrier-mediated RNAi can induce substantial molecular and physiological changes that extend from transcriptomic alterations to downstream proteomic responses. This review provides a comprehensive overview of nanocarrier-assisted dsRNA delivery strategies for wheat aphid control, with particular emphasis on the integration of proteomic and multi-omics approaches for evaluating RNAi outcomes. We discuss advances in bioinformatics-guided target gene selection, engineering principles of nanocarrier systems, mechanisms of dsRNA uptake and release, and the protein-level consequences of gene silencing. Furthermore, the review highlights the importance of combining transcriptomic, proteomic, and systems biology analyses to achieve a more comprehensive understanding of RNAi-mediated pest suppression. By bridging nanotechnology, RNAi, and proteomics, this review provides a systems-level perspective for the development of sustainable and precision-based strategies for aphid management.

Keywords: RNA interference; dsRNA; proteomics; AI; grain aphid; BAPC



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1. Introduction

The global population is growing at an unprecedented rate, with estimates projecting it to increase by 26% by 2050 [1]. This rapid population growth has intensified the demand for food production, placing immense pressure on agricultural systems to ensure global food security. Among staple cereal crops, wheat (*Triticum aestivum* L.) plays a pivotal role, providing approximately 40% of energy calories and 20% of protein supply to more than 36% of the world's population [2]. However, wheat productivity is influenced by various factors, including varietal characteristics, inadequate agricultural inputs, weed growth, unpredictable rainfall, and biotic stresses. Among these challenges, pests such as aphids stand out as a major threat, leading to considerable yield losses ranging from 7.9% to 34.2%, with direct aphid infestations contributing to 10–50% of the losses and indirect impacts accounting for 20–80% [3,4]. Among all aphids, English grain aphid (*Sitobion avenae*) is the most infesting, dominant, and destructive, representing a major pest of wheat [3].

Conventional aphids' management relies predominantly on broad-spectrum chemical pesticides [5]. Despite their effectiveness: extensive and repeated application has accelerated the insecticide resistance resulting in compromised long-term control. Along with it has raised the serious environmental concerns through off-target toxicity to surrounding ecosystem including beneficial insects, pollinators, soil microbiota, and the end users [6].

Bacillus thuringiensis (Bt) proteins are the insecticidal proteins introduced to control insects and pests. Insects had developed the resistance to Bt-derived Cry protein series [7]. So alternate strategies named as pyramids of proteins are used against insects. Head et al. [8] reported the Bt derivatives Cry3Bb1 and the Cry34Ab1/Cry35Ab1 combination to control Western and Northern corn rootworm in maize and named it SmartStax and SmartStax PRO. Corn rootworm larvae after ingestion of these transgenic root tissues, disrupt digestion, stop larval feeding, and cause mortality. Stacked Bt proteins target major leaf- and stalk-feeding pests like European corn borer, fall armyworm, and corn earworm. But due to the development of resistance to the Cry series, alternatives are used.

RNA interference (RNAi) is a naturally conserved gene silencing mechanism in which double-stranded RNA triggers sequence-specific degradation of complementary RNA, thereby suppressing the gene expression. RNAi has emerged as one of the most promising next-generation technologies for species-specific insect control. Double-stranded RNA (dsRNA), small interfering RNA (siRNA), or hairpin RNA (hpRNA) mediated interference is reported for RNA interference [4,9–11]. Intron-containing hairpin RNA (ihpRNA) gene silencing with various restriction-ligation steps was used, followed by Golden Gate cloning with a single restriction-ligation step with a PCR product flanked with type IIs restriction enzyme recognition sites, for ihpRNA formation [3,12,13]. It is substantially utilized to silence the essential genes involved in feeding, reproduction, development, detoxification, neural signaling, metabolism [3,4,9,12,13]. This sequence specificity substantially minimizes adverse effects on non-target organisms while offering new opportunities to manage pesticide-resistant insect populations [3,10,11].

Despite of RNAi remarkable specificity, the practical use in agriculture remains fundamentally constrained by inefficient dsRNA delivery. Following field application, naked dsRNA molecules are rapidly degraded by ultraviolet radiation, and extracellular nuclease present on plant surfaces. Low penetration across biological membranes can be because of inefficient endosomal escape after internalization in the cell, low absorption of RNAi targets to insect gut and hemolymph. These biological and physicochemical barriers collectively reduce the efficiency of the gene-silencing process [3,10,11].

Naked dsRNA delivery to the insect by HIGS or SIGS is used. SIGS had constrained of dsRNA degradation occur before it can target the gene. While coleopterans have a mechanism of stable dsRNA transfer across the midgut membrane, spreading systemically through the insect's hemolymph led to the discovery of the 1st approved commercial biopesticide (Calantha™) targeting VATPase [14]. Due to the development of cry protein susceptibility and unstable integration of dsRNA in insect classes other than coleopterans, there is a need for stable delivery of dsRNA [14].

Another breakthrough comes from the introduction of peptide nanomaterials, Branched Amphiphilic Peptide Capsules (BAPCs), for facilitation of dsRNA cellular uptake by insects. BAPCs are reported to protect dsRNA from degradation in the insect gut and successfully shuttle it across the gut epithelium. The insect diets were adjuvanted with dsRNA (BiP and Armet: crucial transcripts) and BAPCs. Pea aphid (BiP-dsRNA + BAPCs) and red flour beetle (Armet-dsRNA + BAPCs) led to the premature death of the aphids, resulting in 75% mortality in comparison to 30% without BAPCs [15]. Peritrophin gene-dsRNA-BAPC) suppress gut peritrophin synthesis and transport across the larval midgut of *P. japonica* [16]. Delivery challenges had marked the nanotechnology-driven delivery systems: are reported as an an alternate to deliver RNAi in aphids [17]. Engineered nanocarriers protect dsRNA from premature degradation, enhance its stability on plant surfaces, and improve delivery into target insects through controlled and

sustained release mechanisms [17,18]. In this context, a range of nanocarriers will be critically discussed in this review with respect to their structural feature, loading capacity, and delivery efficiency.

Concurrently, high-throughput sequencing technologies, computational biology, and bioinformatics approaches play an increasingly important role in optimizing RNAi-based strategies, including identification of essential gene targets, refinement of dsRNA constructs, off-target prediction, RNA secondary structure modelling, and system-level pathway analysis to improve silencing efficiency and specificity [19]. Aphid Base: Gene identification (Aphid gene e.g., *A. pisum*, *A. gossypii*, *A. glycines*), BIPAA (Comparative genomics + transcriptomics), NCBI (RNA Seq), InsectBase 3.0 (Comparative Omics), AphidNet (Gene sources). Proteomics data PRIDE (MS; differential protein analysis), UniProt (Protein sequences, and functional annotation for protein identification and annotation). RNAi targets with no off-targets had been identified by the ERNAi (Boutros lab, E-RNAi-Version 3.2) [20]. Sequences are compared on the AI and ML tool [19,21]. With the growing number of genome and transcriptome resources available for aphids, systematic searches for such candidates have been conducted using comparative transcriptomic analyses [22]. This integration of in silico and nanoscale engineering approaches is progressively redefining the paradigm from empirical formulation toward predictive, design-driven pest control.

RNAi is dependent on the important genes, transcript, and protein expressed post feeding. As proteomics is a final expression of the genes so the differential protein expressed in response to feeding is an important RNAi targets by Mass spectrometry [23,24]. The major proteins identified in saliva were glucose dehydrogenase, lipophorin, chitinase, CiV16.8g1-like, lava lamp, trehalase, β-N-acetyl glucosaminidase, calreticulin, aminopeptidase, acetylglucosaminyltransferase, hydroxymethylglutaryl-CoA lyase, acyltransferase, ficolin-3, retinaldehyde-binding protein. Few studies present the differential transcriptomics of aphids post feeding Zhang et al. [22]. Phenotypic effects after RNAi treatment are often affected by post-transcriptional control, differential protein turnover, translational control, and compensatory cellular mechanisms. Thereby, the use of proteomics has become an essential tool to validate responses to the RNAi treatment by characterizing the subsequent molecular changes, which will reflect the insect physiology and survival. Gel-free and gel-based approaches gave a detailed picture of the remodeling of metabolic pathways, stress responses, detoxification systems, reproductive functions, and signaling networks after RNAi treatment, allowing for a more complete picture of RNAi-mediated phenotypes [25].

In this context, this review presents an integrated overview of nanocarrier-mediated dsRNA delivery for RNAi-based management of wheat aphids with particular emphasis on computational target discovery, proteomic validation, and intelligent delivery strategies. By integrating these complementary disciplines, it provides a comprehensive framework for advancing precision RNAi technologies and accelerating their translation into sustainable crop protection.

2. RNA Interference Technology in Aphid Management

2.1. Gene Silencing Mechanisms: From Transcriptional Regulation to RNAi

Nuclear processing RNAs present in the nucleus include snRNA (Small Nuclear RNA) for mRNA maturation and snoRNA (small nucleolar RNA) for rRNA, tRNA, and snRNA methylation. Gene silencing is a regulatory mechanism controlling gene expression for protection of eukaryotic organisms against invasive genetic elements, along with cellular homeostasis and developmental processes regulation [11]. Gene silencing can be generally divided into two categories: transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS), depending on the stage at which expression is silenced. Gene silencing is governed by regulatory RNA including miRNA target mRNAs for degradation or translational repression by PTGS. TGS induce silencing by epigenetic modifications, including RNA-directed DNA methylation and histone modifications; heterochromatin blocks access for RNA polymerases, represses gene transcription, and regulates genomic expression by lncRNA (long Non-Coding RNA) (200 nucleotides) [26,27]. PTGS inactivate gene expression after transcription in the cytoplasm and block protein synthesis. Small interfering RNAs (siRNAs) or microRNAs (miRNAs) guide an RNA-induced silencing complex (RISC) to cleave target transcripts. In insects, miRNAs, siRNAs (21 nt), and P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNAs; 23–29 nt) are established. siRNA regulates endogenous development and stress response genes, along with its role against foreign nucleic acids, including RNA viruses. PTGS by siRNA has become the basis of sequence-specific, Dicer-dependent aphid gene knockdown [11,26–28]. PIWI is Dicer-independent and can result in TGS and PTGS. In TGS, it can silence transposons: important in germline stability and, recruit DNA-and histone- modifying methyltransferases to promote heterochromatin formation [28]. In PTGS, they can bind target mRNAs to inhibit translation. piRNA pathway is mainly expressed in germline tissues, so the siRNA pathway is one of these pathways that is uniquely capable of recognizing long dsRNAs and launching the highly specific degradation of homologous mRNA transcripts [10].

With the increasing number of insect genome assemblies, insect transcriptomic resources, and databases of functional annotation, the potential of RNAi has been greatly enlarged, with a systematic approach to identifying candidate genes that play roles in development, reproduction, feeding, detoxification, immunity and stress adaptive mechanisms. The integration of comparative genomics, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, orthology prediction, and protein–protein interaction network analysis now makes it possible to prioritize highly conserved molecular targets that vary between species for RNAi-mediated suppression [19,22,29]. These bioinformatics-driven strategies not only optimize target selection but also lower the risk of off-target effects, which boosts the accuracy and biosafety of RNAi-based pest management methods.

2.2. Molecular Mechanism of RNAi

The success of RNAi relies largely on a suitable molecular target followed by its efficient activation of the relevant endogenous gene silencing machinery. RNAi targets can be identified by genomics, transcriptomics, and proteomics [3,4,22,25]. The activated RNA-induced silencing complex catalyzes several rounds of transcript cleavage, leading to more efficient and persistent gene silencing by a single guide strand [10,11]. The effect of RNAi is determined to a considerable extent by the biological importance of the gene being targeted [3,4,11]. Suppression of genes that are important for transport, embryonic growth, cuticle production, molting, endocrine control, neural communication, detoxification, immunity, or reproduction affects key physiological pathways, which ultimately result in growth deficits, lower fecundity, changes in feeding patterns, or death [3,4,9,13]. siRNA and dsRNA with no sequence homology with other species are being identified by the ERNAi (Boutros lab, E-RNAi-Version 3.2), along with machine learning (ML) tools [19–21]. Off-target effects for environmental safety are important in RNAi-based biopesticides. Proteomics has been used to assess RNAi targets but not to assess RNAi efficacy [25,26]. With the growing number of genome and transcriptome resources available for aphids, systematic searches for such candidates have been conducted using comparative genomic and transcriptomic analyses (Figure 1). Metabolic pathways, stress responses, immune signaling, and compensatory regulatory networks need to be explored by proteomics. Combining transcriptomic and proteomic data can offer systems-level understanding of physiological responses to RNAi, while allowing for the testing of the computationally predicted targets before their adoption in actual pest management. RNAi efficiency can vary between different insect species. Generally, Coleoptera, Lepidoptera, and Diptera are susceptible to RNAi, with much lower efficiencies [3,4,9,13]. Among hemipterans, lower to higher efficiency is reported. dsRNA efficiency is determined by its stability, cell uptake, intracellular transport, and systemic distribution [10].

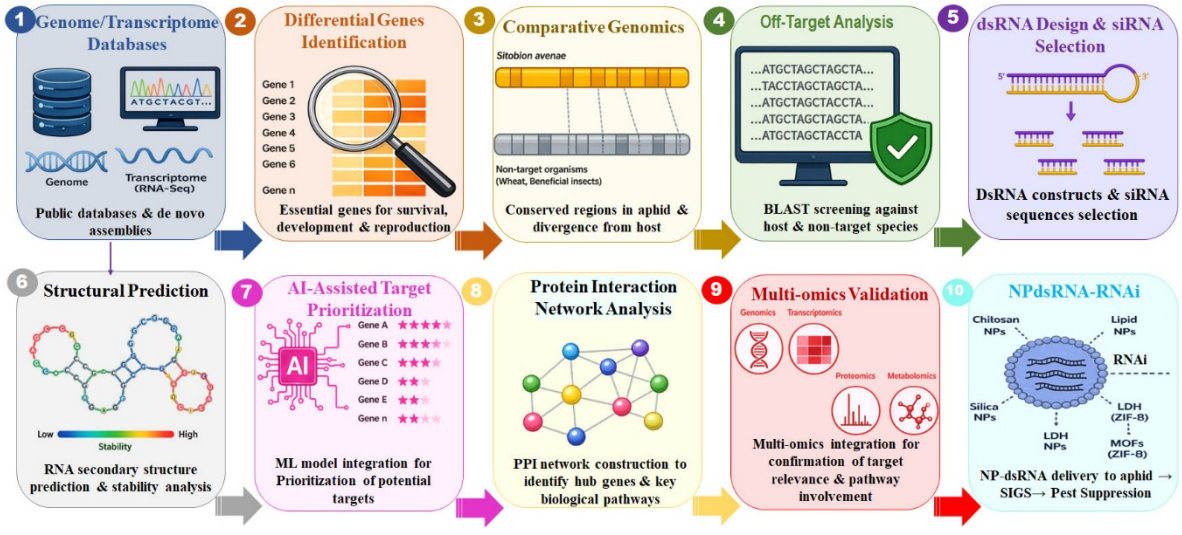


Figure 1. Integrated workflow for bioinformatic target discovery, multi-omics validation and nanocarrier-mediated dsRNA delivery for RNAi-based management of wheat aphids.

RNA sequencing and proteomics identify genes that are expressed constitutively, in specific tissues or under stress conditions, with more efficient silencing [25,26]. Several cereal aphid species, such as *S. avenae*, have incomplete genome annotations and a limited number of functional datasets. All of which are still challenges for computational target discovery and the need for further generation of species-specific multi-omics resources [27].

2.3. Artificial Intelligence (AI) and Machine Learning (ML) for RNAi Optimization

Genomic, transcriptomic, and proteomic data for RNAi target prediction are revolutionized by AI algorithms. Random Forest (RF) and Support Vector Machines (SVM) are ML algorithms used for analyzing biological data and predicting RNAi targets. They can prioritize potential RNAi target genes, optimize dsRNA sequences, and identify off-targets; they can predict RNAi for novel dsRNA sequences [30]. RF classifies data and predicts RNAi outcomes, and SVMs differentiate between classes by identifying optimal decision boundaries. In addition, gradient-boosting algorithms such as XGBoost sequentially construct decision trees to enhance prediction accuracy [31–33]. Convolutional neural networks (CNNs) can predict RNAi efficacy by off-target prediction and can also predict the functional outcomes of siRNA by RNAi. Accuracy of these methods depends upon the relevant data available. Therefore, their application to aphid RNAi research requires validation unless tested using an appropriate aphid-specific dataset [22,29,33]. Workflow for the aphid-wheat interaction starts with (Aphid Base + NCBI SRA/GEO) + publicly available transcriptomic data. The next step is to find collective differential expression by DESeq2/edgeR, followed by functional analysis by (GO + KEGG + protein interaction networks + WGCNA). All of it is followed by AI help using RF + XGBoost + LASSO + SHAP to identify the potential RNAi targets for dsRNA design in experiments. The next important step is to integrate proteomics data with transcriptomics to validate the targets efficiently [4,23–25]. It will involve wheat proteomics by gel-based assay (2D-PAGE followed by MALDI-TOF/TOF MS), identified by MASCOT using peptide mass fingerprinting and MS/MS spectra, and Label-free quantitative proteomics (MaxQuant). Perseus, limma, DEP (R package), and MS stats are bioinformatics and statistical software tools for quantitative proteomics data analysis. This is followed by AI and ML tools to predict RNAi targets, along with important feature predictions including RF and XGBoost. This could help identify aphid genes involved in feeding, digestion, detoxification, metabolism, and plant–insect interactions.

2.4. Prioritization of Aphid RNAi Targets and Transcriptome-Guided Identification

Few transcriptomic data are available to identify potential RNAi targets in aphids [22,29]. Differential gene expression analysis, functional annotation, Gene Ontology and KEGG enhancement to investigate digestion, defense, and host-plant responses. These approaches help identify genes linked to important biological functions or adaptation to the host plant. However, these approaches do not directly involve AI-based prediction [29]. AI parameters for RNAi can be patterns, functional significance, sequence conservation, predicted knockdown effects, and potential non-target effects. RF or gradient-boosting models could combine these features when adequate, sufficiently large, and consistently annotated experimental datasets become available [30–33].

2.5. ML for Sequence Optimization and dsRNA Efficacy Prediction

Insect RNAi bioassay data for developing an RF model was published [21]. The Model had 73% accuracy for predicting mortality by identifying the most important predictors such as Response time and target gene, followed by dsRNA dose, construct size, and target species [21]. Cedden et al. [19] introduced dsRIP, a web-based platform that used experimentally investigated sequence features of the red flour beetle (*Tribolium castaneum*) for optimizing dsRNA. The web utilizes the RNA sequence, siRNA thermodynamics, and its secondary structure for functional validation. Species-specific experimental data is required for validation [19]. dsRNAEngineer is used for dsRNA design using target screening, on-target and off-target analysis, with multi-target sequence design using pest and non-pest transcriptomes. dsRNAEngineer is a computational platform for designing dsRNA sequences rather than a validated AI algorithm for aphid target prediction [34]. Comparative transcriptomics of *M. persicae* was performed to find RNAi targets, three-gene fusion dsRNA, with weighted gene co-expression network analysis (WGCNA) was used [35,36].

2.6. AI and Nanocarrier-Mediated RNAi Integration

AI is also used to select delivery systems like nanocarriers for RNAi. AI potential model can integrate gene-expression profiles, sequence features, predicted off-target matches, dsRNA stability, carrier properties, cellular uptake, and experimentally measured silencing [37]. This integrated framework is still not a validated system for aphid control. ML helps predict RNAi efficacy where sufficient training data exist. Combining these approaches with nanocarrier-mediated delivery may improve the consistency, selectivity, and practical performance of RNAi-based aphid management [19,34].

2.7. RNAi Delivery Strategies for Aphid Management

RNAi in insect pest management relies on its efficiency to activate intracellular RNAi machinery and its ability to reach the target tissues. The RNAi sensitivity of *S. avenae,* as a hemipteran (phloem-feeder), is relatively low compared to coleopterans because their salivary and gut nucleases rapidly degrade the dsRNA, and cells do not take up much dsRNA, resulting in a less responsive RNAi pathway [10,11]. These biological barriers emphasize the need for efficient nanocarrier delivery systems that can shield dsRNA from degradation and increase cellular internalization [38,39]. Microinjection was the most direct method for testing RNAi in aphids (Figure 2). This approach had the advantage of avoiding extracellular barriers like gut nucleases and epithelial transport, introducing dsRNA directly into the hemocoel. Silencing of the sodium voltage gated channels, and laccase gene, for example, made *S. avenae* more susceptible to pirimicarb and simultaneously affected aphid fecundity, thereby highlighting the potential of RNAi for functional gene validation and resistance studies [3,4]. Artificial feeding has been a key tool for the identification of candidate RNAi targets in *S. avenae* and post-transcriptomics of salivary glands [22]. Host-Induced Gene Silencing causes the production of the dsRNA targeting essential insect genes [3,10,11]. However, the need for genetically modified crops, laws and regulations, and public concerns about transgenic agriculture are still significant challenges to widespread use of host-induced gene silencing. To overcome this, a non-transgenic strategy uses synthetic dsRNA applied directly to plant surfaces, called Spray-Induced Gene Silencing (SIGS) [11,40]. The aphids feeding on the host plants are sprayed with the dsRNA [40]. An artificial diet causes significant aphid mortality, in comparison to foliar application of naked dsRNA, which caused only low levels of biological activity [10]. The results highlight the importance of environmental exposure, inadequate plant uptake, and the instability of unprotected dsRNA for the successful management of aphids through SIGS. The naked dsRNA is very susceptible to degradation from UV radiation, temperature changes, rainfall, microorganisms, and endogenous nucleases, which significantly limits its stability in the field [38,40]. Furthermore, several physiological barriers to entry, such as movement restriction within plant tissues, inefficient uptake across the insect gut epithelium, inefficient internalization, sequestration in the insect endosomes, and systemic spread limitations, impede the biologically active amount of dsRNA from reaching the cytoplasm where RNAi is initiated [10,11]. Hence, more stable, bioavailable, and intracellular delivery of dsRNA has been an important research goal, and more sophisticated delivery methods need to be developed to overcome these limitations and provide a more reliable way to manage wheat aphids via RNAi. Systemic spread of dsRNA within the plant is influenced by environmental conditions and can be highly variable [3]. Microbial-mediated RNAi and symbiont-mediated RNAi have emerged as promising alternatives to produce long-term dsRNA but have not been extensively tested in the wheat–*S. avenae* interaction [11].

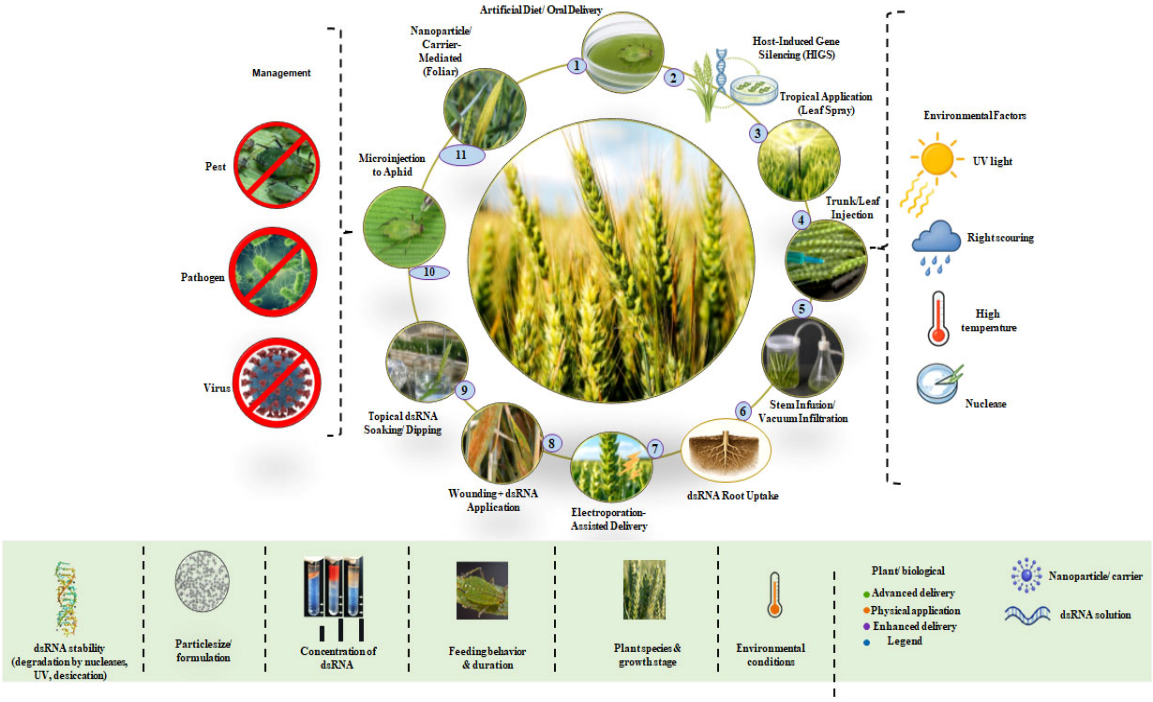


Figure 2. Conventional dsRNA delivery strategies for RNAi- based insect control and major challenges limiting delivery efficiency.

3. Nanocarrier-Mediated dsRNA Delivery Systems for RNAi-Based Wheat Aphid Management

The multifunctional properties make nanocarriers an attractive option to conventional formulation strategies and have attracted them to become one of the promising approaches for next-generation RNAi technologies for sustainable crop protection. Nanotechnology is a useful approach to overcome the dsRNA delivery challenges, such as its protection from enzymatic degradation, enhancing the stability, transport, and cellular uptake. Nanocarriers improve dsRNA delivery by electrostatic complexation, encapsulation, adsorption, or chemical conjugation. Moreover, well-designed nanomaterials can enhance the adhesion of the nanoconjugates to the plant surfaces, facilitate the penetration of plant tissues, promote internalization of the nanoconjugates into cells, and facilitate controlled release into the cells, which will lead to more efficient RNAi-mediated gene silencing [17,18].

The performance of a nanocarrier depends mainly on the following physicochemical properties: particle size, surface charge, morphology, porosity, biodegradability, release behavior. An ideal delivery system should efficiently bind or encapsulate dsRNA, be able to protect dsRNA from degradation during storage and application, encourage uptake by target insects, facilitate endosomal escape, and release intact dsRNA in the cytoplasm without inactivation [41,42]. Furthermore, the field implementation of BMPs requires successful adaptation to existing agricultural practices regarding biosafety and scalability.

Over the last few years, a variety of nanomaterials such as polymeric nanocarriers based on lipid, silica, carbon, metal, clay, and metal–organic framework nanocarriers have been studied for the delivery of RNAi (Figure 3). The materials, varying in composition and delivery method, have the shared goal of overcoming biological barriers to naked dsRNA. Nanocarrier classes are discussed in subsequent sections, in terms of their mechanisms of dsRNA delivery, benefits and drawbacks, and possible use in RNAi-based management of wheat aphids.



Figure 3. Classification of nanocarriers used for dsRNA delivery, highlighting major classes and representative examples employed to enhance RNA stability, cellular uptake, and RNA interference efficiency in insect pests.

3.1. Polymeric Nanocarriers

Polymeric nanocarriers have been the most widely studied organic delivery system for RNAi because of their structural versatility, biodegradability, and ability to form complexes with nucleic acids efficiently. They rely on functional groups along the macromolecular chains to form stable dsRNA nanoscale complexes by electrostatic interactions. They have fine NPs assembly, dsRNA loading, and membrane interactions, followed by their release. Polymeric nanocarriers are divided into natural and synthetic polymers, exhibiting structural and functional variability for their potential applications in the field.

3.1.1. Natural Polymeric Nanocarriers

Natural polymeric nanocarriers are favored for agricultural RNAi applications due to their biodegradability, low toxicity, and resulting biosafety. They are natural polysaccharides having variable reactive functional groups that are utilized for dsRNA delivery. Chitosan is most widely studied as a natural carrier due to its intrinsic positive nature, which enables strong binding to nucleic acids [6,42]. Alginate and cellulose, other natural polymers, are structurally modified/ combined with other polymers for use.

3.1.2. Chitosan

Chitosan is the most broadly studied natural polymer and has been used as a model system to build polymer-assisted RNAi for insects [11,42]. Chitosan is a linear polysaccharide containing both D-glucosamine and N-acetyl-D-glucosamine units linked together by the β (1 → 4) linkages; produced by chitin deacetylation; the resulting amino group can be protonated under mildly acidic conditions [43]. The nitrogen-to-phosphorus (N/P) ratio controls dsRNA condensation for complex formation, its stability, particle size, encapsulation efficiency, and colloidal stability of the NPs form critical parameters [42–44].

For the NPs' stability: chitosan is cross-linked to ionic gelation (sodium tripolyphosphate), releasing dsRNA for a longer period. After NP-dsRNA ingestion: +ve charged NPs interact with −ve charged insect epithelium membrane and enter inside via endocytosis. The polymer–RNA dissociates in the intracellular environment, releasing dsRNA available to endogenous RNAi [43]. Although mechanisms leading to chitosan promotion for intracellular release are unclear but chitosan/naked dsRNA role in successful RNAi is cited [43]. Chitosan-based nanocarriers have been reported in hemipteran sap-sucking insects suggesting that the improvement of dsRNA stability and cellular delivery is key to achieving the high degree of RNAi responsiveness that is missing in sap-feeding insects, which have relatively low RNAi responsiveness [44]. Together, these results indicate chitosan as the most experimentally proven natural polymeric carrier for agricultural RNAi and suggest the need to work on the formulation of chitosan for cereal aphids. Other naturally derived polysaccharides, however, are not inherently cationic enough to be able to directly condense dsRNA and must be utilized in any other way for efficient nucleic acid loading and delivery.

3.1.3. Alginate- and Cellulose-Based Nanocarriers

For agricultural biotechnology, environmentally friendly alternatives to EDTA to reduce nuclease action on dsRNA: alginate is used. It is an anionic polysaccharide from brown algae and is a linear polymer with (1 → 4)-β-D-mannuronate and α-L-guluronate. Alginate can bind metal ions, causing the formation of alginate hydrogels [45]. Alginate can form an interpolyelectrolyte complex with chitosan [46]. Alginate, in RNAi, works as a controlled-release matrix and not as a nucleic acid condensing agent. Electrostatic repulsion between alginate and dsRNA has been addressed by incorporating cationic polymers, particularly chitosan, in the preparation of multilayer or polyelectrolyte-complex nanoparticles (NPs), which achieve good structural stability and sustained release properties thanks to alginate while exploiting the excellent dsRNA-binding property of chitosan. There is great potential as sprays under field conditions: owing to superior biocompatibility, biodegradability, low price, and availability from renewable sources, high integrity of the NPs, extended cargo retention, and increased environmental stability [45,46].

Another new class of natural carriers is cellulose-based nanomaterials. Cellulose has many hydroxyl groups, giving it mechanical strength with minor affinity for binding dsRNA. Longer dsRNA molecules have supportive hydrogen-bonding with the cellulose matrix compared to short siRNA [47]. 15% ethanol further improves dsRNA binding by reducing hydration around the polysaccharide. Cellulose is also useful as a supporting matrix that can enhance the dispersion of NPs, adherence to leaves, and the release of the encapsulated dsRNA. A commercial biopesticide (PYR@UiO-66@HPC) encapsulated with pyraclostrobin and hydroxypropyl cellulose in a metal–organic framework was found beneficial and biosafe against *Rhizoctonia solani* infestation [48].

3.2. Synthetic Polymeric Nanocarriers

Natural polymers have the advantage of high biocompatibility and biosafety with certain limitations. They have limited structural tunability, variable physicochemical properties, and little control over intracellular trafficking, paving the way for RNAi delivery alternatives. Synthetic polymeric nanocarriers have been designed according to dsRNA compatibility, their stability, to enhance cellular uptake, and their release. Optimized polymers provide more flexibility to control charge density, branching, molecular weight, and surface chemistry to create delivery systems with optimized RNAi [11,49]. Efficient condensation and intracellular delivery of nucleic acids have been observed for a variety of synthetic polymers, including polyethyleneimine (PEI), polyamidoamine (PAMAM) dendrimers, and star polycations (SPc), all of which have been the subject of particular interest [38,50]. Biodegradable polymers: polylactic-co-glycolic acid (PLGA) and polycaprolactone (PCL) are also shown as promising platforms for controlled release of dsRNA and prolonged drug delivery [50,51].

3.2.1. PEI

PEI is a synthetic cationic polymer used to design polymer-based systems for RNAi delivery [38,50]. PEI consists of repeating EI units with primary, secondary, and tertiary amine groups: linear or branched. Branched PEIs have higher charge density and stronger nucleic acid condensation. The charged amine groups allow efficient electrostatic complexation with dsRNA, leading to compact NPs [52,53]. PEIs have a proton sponge effect: osmotic swelling and destabilization of endosomal membranes, resulting in dsRNA escape before its degradation by the lysosomes. This mechanism greatly facilitates the availability of dsRNA in the cytoplasm for processing by the RNAi machinery. RNAi efficiency is related to molecular weight, branching, and cationic formulations can be toxic and may not be directly applicable in agriculture [38]. PEI has proven to be effective in RNA delivery in several insect systems, but its application to aphid management is not as extensively used.

3.2.2. PAMAM Dendrimers

The development of PAMAM dendrimers, as a result of polymer engineering, has improved the structural precision of polymers over the traditional linear or branched types of polymers. PAMAM dendrimers are highly ordered three-dimensional structures with a central core and repeated branched layers, leading to a high number of terminal amine groups that can bind dsRNA [54,55]. Due to their monodisperse nature, proton sponge effect, size precision, and surface chemistry: produce effective drug delivery systems. Despite these benefits, there are no studies for PAMAM to deliver dsRNA as a pesticide to control aphid pests. Structural accuracy attained with dendritic polymers: simultaneous improvement of loading into dsRNA, intracellular transport, and environmental adaptation needed to be explored.

3.2.3. SPc

SPc is specially engineered and extensively optimized to be able to cross the biological and physicochemical barriers for delivering dsRNA in crop protection [56–58]. SPc is a biodegradable, highly branched polymeric nanocarrier that was originally designed to be a low-cost, versatile gene delivery system to insects, but that can improve the stability of dsRNA in water and enhance plant uptake. The structure of SPc is hydrophobic, with several polymeric arms that have positive charges, creating a three-dimensional structure with a high number of accessible amine groups. The multivalent configuration offers numerous binding sites for dsRNA and high water dispersibility and formulation flexibility, which makes SPc well suited for foliar RNAi application [57]. When dsRNA is added to the polymer, the positively charged polymer arms bind to the negatively charged phosphate backbone of the dsRNA in a spontaneous process by electrostatic interactions, along with hydrogen bonding and van der Waals attractions, to form stable nanoscale polyplexes. This condensation also provides resistance to enzyme hydrolysis of the dsRNA and enhances its colloidal stability and uniformity when applied to plant surfaces.

SPc has been reported in mostly artificial feeding assays on variable developmental stages of aphids and mites [56–59]. Plant-mediated dsRNA is reported for maize and *Arabidopsis* [58]. SPc enhanced the dsRNA efficiency of miR-184 agomir against *S. avenae*. It had a negative effect on the rate of increase, the finite rate of increase, the net rate of increase, and the mean generation time. 95.8% reduction in the population size after 60D-postr treatment [57]. dsRNA translocation via the vascular tissues increased the likelihood of dsRNA distribution to phloem sap, which will be taken by piercing–sucking pests/aphids. Glutamate decarboxylase is silenced in cotton aphids by SPc [59]. SPc is the most well-rounded solution to various challenges in RNAi delivery, as it simultaneously enhances protection of the dsRNA, plant transport, cellular uptake, and biological efficacy.

3.2.4. Biodegradable Polyesters: PLGA and PCL

PLGA and PCL encapsulate nucleic acids in polymeric matrices (nano capsules), and protect them from environmental degradation, while providing for prolonged release over time [50,51]. PLGA has been used in biomedical drugs due to its high biocompatibility, adjustability of degradation rates, and regulatory acceptance. The PLGA degradation rate can be regulated by changing the lactic acid and glycolic acid ratio, molecular weight, and particle size, which creates specific release profiles for different applications. There is limited direct research on the dsRNA delivery by PLGA in insect pests. Benefits of protecting nucleic acids and prolonged release have led to increased interest in using PLGA for agricultural RNAi. PLGA and PEG-arginine nanocarriers were used against Orthopteran pests (*Schistocerca gregaria*) and (*Melanoplus sanguinipes*). PLGA, after oral feeding of dsRNA, caused infusion and stabilization in hemolymph [60].

PCL degrades at a slower rate than PLGA and is more hydrophobic: resulting in a more stable structure and longer retention. Its properties have been exploited in the development of nano capsules for sustained delivery of

drugs but agrochemicals such as pesticides are not focusses [51]. Biodegradable polyesters contribute relatively less to cellular uptake and endosomal escape than cationic polymeric carriers but have certain advantages in terms of formulation stability, environmental persistence, and controlled release.

3.3. Lipid-Based Nanocarriers

Lipid-based nanocarriers are an important class of delivery vehicles of RNA due to their excellent biocompatibility, membrane-mimicking architecture, and efficient encapsulation of nucleic acids [38,50]. Lipid-based systems are based on the ability to self-assemble lipid structures, which protect the RNA from enzymatic degradation and promote its internalization by cells. Structural similarity to biological membranes also facilitates interactions with cell membranes, which boosts delivery of RNA cargo into the cytoplasm and intracellular delivery. The benefits have supported lipid nanocarriers as the most widely used platform for therapeutic RNA delivery and have increasingly sparked interest to use them for agricultural RNAi applications.

3.3.1. Liposomes

Liposomes are the most thoroughly studied of the lipid-based delivery systems used to carry nucleic acids. They are spherical vesicles containing one or more phospholipid bilayers that enclose an aqueous core, allowing hydrophilic molecules to be trapped inside and hydrophobic molecules to be incorporated into the membrane [50]. Lipid NPs have been used to deliver siRNA, mRNA, and plasmid DNA. These encapsulated nucleic acids can be protected from degradation and released into the cytoplasm after endosome escape to achieve the desired purposes. Currently, LNPs are used in the development of RNA medicine and have contributed a lot in disease prevention like Pfizer-BioNTech used LNP to deliver mRNA vaccine against the COVID [61].

In RNAi applications, cationic or ionizable lipids are frequently added to the phospholipid bilayer to allow the electrostatic association with the negatively charged dsRNA and to result in stable lipoplexes with greater capacity to protect the RNA and to improve the delivery efficiency [50]. Nanoliposomes (LNPs) modified with PEI deliver the dsRNA of the *Methoprene-tolerant gene* in fall armyworm, significantly improving RNAi efficiency from 25% to 91.7% [50]. Co-delivery of chlorantraniliprole, an insecticide, and poly-siRNA targeting cytochrome P450 genes for controlling resistant *Plutella xylostella* (diamond black moth) [62]. The efficient interaction with biological membranes by endocytosis or membrane fusion allows the membrane-like structure of liposomes to be used to transport the encapsulated dsRNA into the cells. One the inside, the destabilization of the lipid membrane allows for the release of dsRNA into the cytoplasm, where it will be acted upon by endogenous RNAi machinery. These properties have led to liposomes being one of the most successful non-viral RNA delivery systems and the basis for many nucleic acid therapeutics.

Liposome-assisted RNAi has been proven to work in *Drosophila melanogaster*, *Blattella germanica*, and the neotropical brown stink bug *Euschistus heros*, in which the use of liposomes to package the dsRNA caused an increase in gene knockdown and intracellular uptake compared with naked dsRNA [50,61]. These studies show that lipid-mediated encapsulation is an effective way to overcome the cellular barriers that delay the effectiveness of RNAi in insects and could be used as an effective framework in which to gain insight into how to adapt the lipid-based delivery system for use in agriculture for pest management.

LNPs use ionizable lipids in combination with helper phospholipids, cholesterol, and PEG-lipids to create compact NPs that achieve high RNA encapsulation efficiency and enhanced endosomal escape compared to conventional liposomes, mainly for therapeutic RNA delivery applications [61,62]. Due to the ongoing developments of research on sprayable RNA pesticides, the use of lipid based as delivery systems is likely to increase, as it is a promising solution that can improve the stability and intracellular delivery of dsRNA.

3.3.2. Silica-Based Nanocarriers

Mesoporous silica NPs with highly ordered pore networks from 2–50 nm in diameter have been the major drive for the development of silica nanocarriers for RNAi delivery. Mesoporous silica is negatively charged, and low affinity for dsRNA is achieved by 3-aminopropyltriethoxysilane: attaches amino groups (+ve charged) to silica, leading to strong electrostatic adsorption of dsRNA. Amines enrich the dispersion of the particles and allow dsRNA release in a prolonged manner via reversible electrostatic interactions [63]. Surface amination gives the silica NPS versatile anchoring sites for subsequent modification with polymers, peptides, or responsive ligands. SiO_2 NPs have immense potential to mitigate both biotic and abiotic stresses through hormonal, defense, and metabolic regulation. As NPs, they improve pesticide accuracy without environmental hazard, promoting plant growth. SiO_2-NPs synthesis includes physical–chemical techniques to natural biomaterials and peptides. SiO_2-NPs

green synthesis has a biosafety advantage but long-term environmental and ecological impacts, and regulatory context need to be addressed [63,64].

They are efficient carriers of drugs and pesticide formulations. Against *Aphis gossypii*, ampicillin and imidacloprid co-delivery mesoporous NP, greatly reduced the cotton aphids. *A. gossypii* cytochrome P450 genes were significantly suppressed, associated with imidacloprid resistance [63]. 100 mg L^{-1} Chitosan-Mesoporous NPs reduced aphid reproduction to half in comparison to the acetamiprid pesticide. Along with this, it resulted in reduced oxidative damage caused by aphids by upregulation of antioxidants [64]. Size-based dsRNA-loaded mesoporous NPs infiltrated in tobacco leaves and roots caused a reduction in Tomato leaf curl New Delhi virus. Among 10, 32, and 66 nm NPS, 32 nm NPs show an eleven-fold reduction of viral load [65]. The porous structure of silica NPs allows large amounts of nucleic acids to be transported and also slows the molecules' mobility, preventing them from escaping the target. Along with this, dsRNA SiNPs are protected from nuclease degradation. Loading capacity of the nanopores, molecular diffusion, and release rates are dependent on the pore diameter [65].

3.3.3. Carbon-Based Nanocarriers

Carbon nanotubes are known for their exceptional morphological, physical, electrical, thermal, and magnetic properties. CNPs family mainly includes carbon dots (CDs), carbon nanotubes (CNTs), carbon fullerenes, graphene, graphene oxide, nanohorns, and carbon nanofibers. The use of CNPs to improve plant stress tolerance and thus agricultural production. CNTs are cylindrical carbon fibers composed of hexagonal carbon atoms linked together [66].

CNTs, besides single-walled carbon nanotubes, can penetrate plant cells well. Multiwalled carbon nanotubes enter cells by endosome escape in plant protoplasts [66]. CDs (<10 nm) have good water dispersibility, intrinsic fluorescence, and low cytotoxicity and are a good option for dsRNA delivery [10]. Imidazole-modified graphene quantum dots were reported for cuticular protein dsRNA delivery. Cuticular protein, vital for epidermal development and larval survival, is silenced, resulting in epidermal contraction, decrease in feeding, and *Spodoptera litura* (Lepidoptera) larval mortality. Quantum dots caused a 4.65-fold greater reduction in cuticular protein compared to naked double-stranded RNA and elevated larval mortality from 38−56% [67]. Carbon dots have numerous oxygen- and nitrogen-containing functional groups functionalized with cationic polymers like PEI to create positively charged NPs effective at complexing with dsRNA [53]. Further studies then showed the potential of carbon dots in spray-induced RNAi. DsRNA with and without CDs was delivered to cucumber leaves. 50-fold more dsRNA was detected compared to naked dsRNA. Systemic dsRNA was observed when delivered as a nanocomposite [68].

In addition to CDs, CNTs have also emerged as a highly promising platform for dsRNA delivery due to their unusually high aspect ratio, large specific surface area, and unique needle-like morphology [39,53,66,68]. dsRNA and shRNA-mediated silencing technology with single-walled carbon nanotubes against tomato mosaic, spotted wilt, and yellow leaf curl virus using SIGS. The CNT-RNA composite shows promising silencing with reduced viral growth [69]. Single-walled CNTs successfully deliver siRNA and silence endogenous genes by *Agrobacterium*-mediated transformation [68]. Collectively, carbon-based nanocarriers provide an excellent example of the potential, along with CDs are the most advanced carbon-based platform to deliver foliar RNA. CNTs have an outstanding ability to transport RNA into cells and graphene oxide is a multifunctional scaffold for formulations. They have not yet been widely used for aphid RNAi, but recent results show great potential for their use.

3.3.4. Metal, Metal Oxide NPs

Metal and metal oxide NPs have stimulating optical, magnetic, catalytic, and surface physicochemical properties. Most metal NPs have been used in biomedical gene delivery and diagnostics [17,18]. Metallic NPs: Au, Ag, and Fe_3O_4 were emphasized as siRNA delivery vehicles with the integration of AI for their clinical applications [70]. The dsRNA can also be further coated with PEI, chitosan, or thiolated oligonucleotides, which further enhance dsRNA adsorption and protect the RNA from nuclease degradation [42,52]. The efficiency of PEI-modified zirconium-based metal-organic frameworks (MOFs) was found to be better in plasmid delivery [52]. siRNA-Polyarginine/siRNA-Polyarginine-20 nm Au NPs were used for RNAi of the luciferase gene in the plant pest *Spodoptera frugiperda* cell line. Electrostatic binding (layer-by-layer method) of AuNPs (<100 nm) resulted in maximum gene knockdown [71]. Hemipteran (*P. citri*, *Diaphorina citri*, *A. gossypii*) chitinases were silenced by foliar spray of Fe^{+3} linked layered double hydroxide (LDH)-dsRNA, promoting RNAi efficiency [72]. LDHs are comprised of positively charged metal hydroxide layers with interlayer galleries filled with holes and exchangeable anions and water molecules. Their general chemical formula, $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}(A^{n-})_{x/n}\cdot mH_2O$, generally consists of divalent cations (Mg^{2+}, Zn^{2+}, Ca^{2+}) and trivalent ones (Al^{3+}, Fe^{3+}) [66]. Trivalent ions can

substitute for divalent ions in the hydroxide layers, leaving a permanent positive charge that will promote efficient electrostatic interactions with the negatively charged dsRNA molecules. Foliar, as well as root absorption of laccase (lac) loaded dsRNA-LDH efficiently silenced whitefly lac expression for white fly effective control with 92% mortality, and 66% fecundity [73].

LDH (Bio Clay) NPs sheets-RNAi for resistance against plant viruses and *Botrytis cinerea* in tomato and chickpea is reported [74]. Later research focuses on the use of LDHs as a protection against insect pests controlling viruses indirectly [75]. SIGSs of cowpea with bean common mosaic virus-coat protein gene-Bio Clay after 5D protect plants from viral and aphid infestation [75]. LDH: high loading capacity, colloidal stability, and release behavior enhanced dsRNA stability and minimized UV degradation [71,72].

3.4. MOFs

MOFs are crystalline networks that are built from the coordination of metal ions or metal clusters, which create highly ordered porous structures that can contain large amounts of biomolecules. The incredible structural versatility of MOFs enables the fine-tuning of particle size, pore diameter, surface chemistry, and degradation behavior, which makes them highly versatile for the design of intelligent dsRNA delivery systems for agricultural RNAi [17,18,38–40,76]. Molecular dynamics (MD) simulations predict the behavior of MOFs under variable environments; without many experiments results are predicted [77]. MOFs are porous crystalline metal ions/clusters with a central atom and one or more organic ligands. MOFs' unique feature is large surface areas, adjustable apertures, and diverse structures applied in many fields. Carboxylic acid ligands are part of MOF materials, along with zinc, iron, copper, transition elements, and lanthanide. MOFs have been used to control the release of pesticides [76,77]. Carboxymethyl-chitosan-modified MOFs had enhanced loading capacity, helpful for rapid release of MOFs as an effective pesticide [78]. MOF modification is particularly suitable for interaction, loading process, and release mechanism of MOF–drugs/biomolecules (e.g., proteins, DNA, lipids, etc.) complexes [77]. Yu et al. [79] reported that MOF-encapsulated imidacloprid (insecticide)-dsRNA within zeolite NPs (195 nm) improved the susceptibility of brown planthopper to imidacloprid. Survival rates of hoppers treated with MOF-dsRNA-insecticide resulted in 8% hopper survival. Chitosan and MOFs improve the efficacy of dsRNA and cycloxaprid against *Diaphorina citri*, causing 80% mortality [80]. The loading mechanism of dsRNA in MOFs is highly dependent on the structure of the MOF and the physical and chemical characteristics of the nucleic acids. dsRNA can be encapsulated into internal cavities, adsorbed onto the external surface by electrostatic interaction, or immobilized after functionalization of the surface with positively charged polymers, depending on the pore dimensions [79,80].

MOFs also offer a more significant physical barrier to nucleases, UV radiation, and environmental degradation, compared to adsorption-based carriers. Programmed dsRNA release from MOFs can be in response to physiological or environmental factors [76,81]. At neutral pH, ZIF-8 will not change; but the endosomes at mildly acidic pH degrade, liberating intact dsRNA in the cell cytoplasm. The biomimetic mineralization process can be used during the synthesis process to encapsulate dsRNA; by using the nucleic acid molecules as nucleation templates, the MOF crystals grow without exposing the RNA to harsh solvents or high temperatures, resulting in high encapsulation efficiencies [41]. Other MOFs besides ZIF-8 have been studied for RNA delivery, including UiO-66, MIL-101, and HKUST-1, which possess unique structural and chemical properties [41,52,81]. The adsorption of dsRNA can be further optimized by surface modification with polymers like polyethyleneimine, chitosan, or polyethylene glycol, in addition to conferring stimulus-controlled release depending on the physiological environment [42]. Studies of RNAi in *S. avenae* using MOFs have not been reported but have shown great promise in other insect pests and plant systems.

4. Uptake of dsRNA and Internalization Inside the Cell

The RNAi machinery in the cell is highly conserved, but activation requires delivery of intact dsRNA into the cytoplasm (Figure 4). To induce gene silencing by Dicer-2, the exogenous dsRNA must pass through multiple biological barriers, each of which contributes to gene silencing efficiency. Upon ingestion, dsRNA initially encounters the digestive tract, where unprotected RNA molecules are rapidly degraded by salivary and gut-associated ribonucleases (dsRNases). Insects do not have the classic SID-1 uptake system like *Caenorhabditis elegans*, but have receptor-dependent endocytosis and tissue-dependent pathways [66,82]. Clathrin-mediated endocytosis is the dsRNA entry mechanism in most insects [82]. However, endosome maturation continues to lysosomal degradation; NPs stabilize dsRNA until released into the cytoplasm [68,83]. If endosomal escape does not occur, the dsRNA will be sequestered in endosomes and degraded by an enzyme with no RNAi [10,72]. This is a reason for less RNAi in hemipteran insects. Combining proteomic information with functional RNAi

experiments is thus helping to understand the dynamics of dsRNA transport and the rational design of more efficient delivery systems.

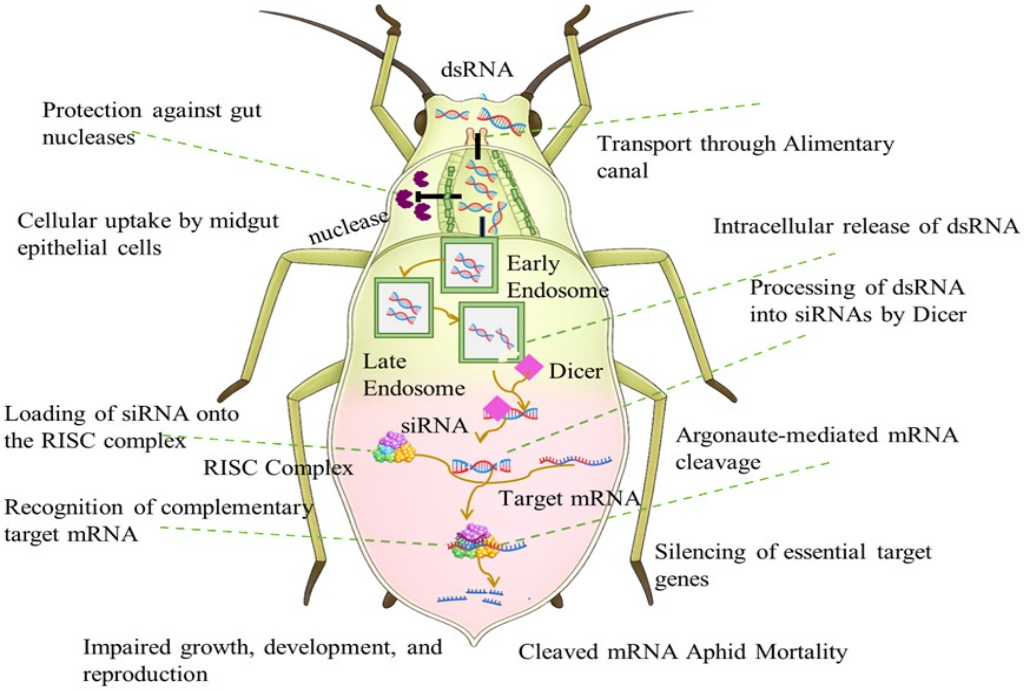


Figure 4. Schematic illustration of nanocarrier-mediated dsRNA uptake and intracellular RNAi mechanism in aphids.

5. Proteomics as a Functional Validation Tool for RNAi

Proteomic research is gaining importance in insect molecular biology because proteins are the functional executors of any developmental, metabolic, stress adaptation, and host interaction processes. While transcriptomic data can be used to find genes that might regulate a process, proteomic data can be used to identify which molecular components are currently synthesized and/or maintained in a given biological context. New platforms of mass spectrometry, especially liquid chromatography and tandem mass spectrometry (LC–MS/MS), label-free quantification, tandem mass tag (TMT), and data-independent acquisition (DIA) enable robust coverage of complex insect proteomes with high sensitivity and reproducibility [84]. RNAi technologies are rapidly emerging as a new platform for sequence-specific suppression of essential genes, fundamentally changing the approach for sustainable insect management. Salivary transcriptomic and proteomic data have identified candidate targets for RNAi in the model pea aphid, *M. persicae*, *Lipaphis erysimi*, and *Buchnera aphidicola* [85–87]. Grain aphid transcriptomics and proteomics databases are available and have provided a great margin for further RNAi experiments [4,22–25].

RNAi experiments measure mRNA expression at the transcript level. mRNA levels related to reduced protein function are not reported. Western blot with a specific antibody may be provided but the whole protein of the cell/tissue/organism is not reported. While whole protein expression can give a better idea about the RNAi fate [87]. Change in expression of a single gene does not fully describe the molecular response to RNAi. By directly capturing changes in protein function, Proteomics offers the missing link between gene silencing and physiological responses, allowing more confident target validation and understanding of RNAi responses [86,87]. Gel-free techniques can identify many proteins present in small quantities, to characterize post-translational modifications, and to reconstruct molecular pathways involved in insect survival and adaptation [84]. Aphids' secretome enzymes and effector proteins help aphids to penetrate the stylet, obtain food from the phloem, suppress plant immunity, and manipulate host metabolism. They are direct factors of aphid survival for RNAi-based intervention. *C*ereal aphid that has whose transcriptome and proteome analysis have given candidates for RNAi [4,22–25]. Zhang et al. [22] identified *S. avenae* proteins related to metabolism, oxidative stress regulation, calcium binding, and molecular chaperoning. LC-MS/MS suggested that several identified proteins are involved in feeding adaptation and host interaction. Comparative proteomic analyses of *S. avenae* and *Metopolophium dirhodum* salivary proteins with *A. pisum* by tandem mass spectrometry [87]. Glucose dehydrogenase and carbonic anhydrase were conserved candidate proteins in all aphids and were presented as RNAi targets. Voltage-gated sodium channels found as RNAi targets were used for grain aphid [4]. Proteomics offers significant scope to identify metabolic pathways beyond aphid interference.

6. BAPC as dsRNA Carrier

DsRNA complexed with protein peptide (BAPC's) make an attractive RNAi target. Pea aphid (BiP-dsRNA + BAPCs), red flour beetle (Armet-dsRNA + BAPCs), and *P. japonica* (Peritrophin gene-dsRNA-BAPC) led to the 75% mortality in comparison to 30% without BAPCs [15,16]. Delivery challenges had marked the nanotechnology-driven delivery systems: are reported as an alternate to deliver RNAi in aphids [17]. BAPC's mode of action is dependent on the positive surface charges of usually lysine, to bind negatively charged dsRNA. dsRNA binding to the BAPC's surface resulted in positive lysine residues blockage. This can reduce its efficiency for dsRNA delivery to the insect midgut by reducing positive charge (zeta potential). High production & purification costs can make it slightly difficult task in comparison to the traditional insecticides. On the other hand, dsRNA stability on basis of four free lysine Ɛ-amino groups pKa values (9–10.5) remain stable in neutral as well as alkaline insect guts. According with DLS, the BAPCs-dsRNA complexes form compact clusters with size ranging from 250–350 nm in a pH environment consistent with the gut of *P. japonica* adults [16].

7. Conclusions and Future Perspective

Aphids not only directly affect cereal plants but also indirectly, as virus carriers and outsmart traditional chemical control. Chemical insecticides are not only the major causes of environmental contamination, but also affect non-target organisms. They have spurred efforts to sustainable and precision-based alternatives. RNAi has allowed sequence-specific control of targeted insects. RNAi utility is still limited by dsRNA instability, inefficient uptake, degradation without reaching the target, and inconsistent efficacy under field conditions. Nanocarrier-mediated dsRNA delivery is a step forward to overcome these limitations. It enhances dsRNA stability, cellular internalization, and the controlled release of RNA molecules. Nanomaterials such as polymeric NPs, lipid-based systems, silica-based carriers, layered materials, and MOFs have great potential for improving insect RNAi. Molecular interactions between nanocarriers, dsRNA molecules, and the aphid cellular machinery need to be better understood. BAPC make an attractive alternate to the NP for dsRNA delivery with improved efficiency and stability. Future delivery systems should thus go beyond protecting the dsRNA and attempt to design intelligent carriers that can improve the stability, target specific tissues, and respond to biological conditions, reducing environmental impacts. Next-generation RNAi development involves the incorporation of computational biology, AI, transcriptomics, and proteomics to target discovery and validation process. AI identification and system dynamics simulations and prioritization of essential genes identified by genomic, transcriptomic, or proteomics data allow for functional confirmation and identification of proteins and pathways directly involved in aphid survival, feeding, and fecundity. More aphid transcriptomic and proteomic data at different developmental stages will be needed to find crucial RNAi target selection. Along with this is there is a lot of margins to find differential proteomics analysis of the aphids fed with dsRNA. That can help to find the regulatory pathways post-dsRNA feeding of resistant and susceptible plants in aphids. There are still several challenges that need to be overcome to make nanocarrier-based RNAi a viable and effective tool in sustainable aphid management. Further work is required to enhance the scalability and reproducibility of nanocarrier production, assess their long-term environmental safety, investigate the RNAi responses of different species, and create standardized methods for delivery efficiency and ecological compatibility. In the future, the combination of molecular biology and nanotechnology with proteomics and computational intelligence will play a pivotal role in the management of wheat aphids using RNAi.

Author Contributions

A.A.: conceptualization, supervision, Editing; S.A.: Data curation, writing—original draft preparation; I.A. and H.A.: Visualization, investigation; G.A.: Validation; N.Z.: reviewing and editing. All authors have read and agreed to the published version of the manuscript.

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No AI tools were utilized for this paper.

List of Abbreviations

RNA interference: RNAi; Double-stranded RNA: dsRNA; Transcriptional gene silencing: TGS; Small interfering RNA: siRNA; Post-transcriptional gene silencing: PTGS; Branched Amphiphilic Peptide Capsules: BAPCs; P-element-induced wimpy testis (PIWI)-interacting RNAs: piRNAs; Spray-Induced Gene Silencing: SIGS; Nanoparticles: NPs; Artificial Intelligence: AI; Machine Learning: ML; Random Forest: RF; Support vector machines: SVMs; Convolutional neural networks: CNNs; Kyoto Encyclopedia of Genes and Genomes: KEGG; Polyethyleneimine: PEI; Polyamidoamine: PAMAM; Star polycations: SPc; Polylactic-co-glycolic acid: PLGA; Polycaprolactone: PCL.

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