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RNA INTERFERENCE-BASED MYCOHERBICIDES FOR AQUATIC WEED MANAGEMENT: SCIENTIFIC BASIS, EMERGING TECHNOLOGIES, AND FUTURE PROSPECTS

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ABSTRACT

Invasive aquatic weeds such as Eichhornia crassipes (water hyacinth), Salvinia molesta (giant salvinia), Pistia stratiotes (water lettuce), and Hydrilla verticillata pose serious threats to freshwater ecosystems, irrigation systems, fisheries, and agricultural productivity worldwide. In India, their rapid proliferation has resulted in significant ecological degradation and economic losses through obstruction of water flow, depletion of dissolved oxygen, suppression of native biodiversity, and impairment of aquatic resources. Conventional management approaches, including mechanical removal, chemical herbicides, and classical biological control, often provide limited and short-term control while raising concerns regarding cost, environmental safety, and herbicide resistance.

Recent advances in RNA interference (RNAi) technology offer a novel and highly specific approach for sustainable weed management. RNAi functions through sequence-specific silencing of essential genes using double-stranded RNA (dsRNA), enabling targeted suppression of weed growth, development, and reproduction with minimal impacts on non-target organisms. Emerging strategies such as Spray-Induced Gene Silencing (SIGS), Host-Induced Gene Silencing (HIGS), and trans-kingdom RNAi have expanded the potential applications of RNAi in plant protection and invasive weed control.

Mycoherbicides, based on phytopathogenic fungi, provide an attractive platform for RNAi delivery and enhanced weed suppression. Fungal genera including Acremonium, Colletotrichum, Phytophthora, Alternaria, and Fusarium have demonstrated potential for the biological control of aquatic weeds and may serve as vehicles for RNAi-mediated gene silencing. The integration of RNAi technology with mycoherbicidal fungi could enable the development of next-generation bioherbicides that combine fungal pathogenicity with precise molecular targeting, resulting in improved efficacy, host specificity, and environmental compatibility.

This review examines current advances in RNAi technology and its potential integration with mycoherbicides for aquatic weed management. Major topics include the biology and impacts of invasive aquatic weeds, molecular mechanisms of RNAi, target gene selection, fungal candidates for mycoherbicide development, trans-kingdom RNAi, nanotechnology-assisted delivery systems, biosafety considerations, and regulatory challenges. Future opportunities and research priorities for the development of RNAi-based mycoherbicides are also discussed. The convergence of RNAi biotechnology, fungal biocontrol, genomics, and advanced formulation technologies offers promising prospects for sustainable and species-specific management of invasive aquatic weeds in India and worldwide.

KEYWORDS: *RNA interference, RNAi, mycoherbicides, aquatic weeds, Eichhornia crassipes, trans-kingdom RNAi, spray-induced gene silencing, biological weed control, dsRNA delivery.*

1. INTRODUCTION

Aquatic weeds are among the most destructive invasive plants affecting freshwater ecosystems worldwide. Their rapid growth, prolific vegetative reproduction, and broad ecological adaptability enable them to form dense floating or submerged mats that disrupt aquatic habitats, reduce biodiversity, and impair ecosystem services (Gopal 1987; Villamagna and Murphy 2010). In tropical and subtropical regions, invasive aquatic weeds interfere with irrigation, hydropower generation, fisheries, navigation, and water supply systems, causing substantial ecological and economic losses (Hussner et al. 2017). Increasing eutrophication, climate change, and altered hydrological conditions have further accelerated their spread and persistence, making sustainable management a global priority.

1.1 Global Importance of Aquatic Weeds

Invasive aquatic weeds such as *Eichhornia crassipes* (water hyacinth), *Salvinia molesta* (giant salvinia), *Pistia stratiotes* (water lettuce), and *Hydrilla verticillata* have become major environmental and agricultural challenges across Asia, Africa, Australia, and the Americas (Villamagna and Murphy 2010). Among these, water hyacinth is regarded as one of the world's most problematic aquatic weeds due to its exceptional growth rate and ability to rapidly form dense mats that obstruct waterways, reduce dissolved oxygen, impair fisheries, and degrade aquatic ecosystems (Gopal 1987). Management of these infestations requires considerable financial resources for mechanical removal, herbicide application, and maintenance of affected water infrastructure (Hussner et al. 2017).

1.2 Major Invasive Aquatic Weeds in India

India's extensive network of rivers, reservoirs, lakes, wetlands, and irrigation systems provides favorable conditions for invasive aquatic weeds. *Eichhornia crassipes* is the most widespread and economically damaging species, infesting numerous freshwater bodies and reducing water-use efficiency (Gopal 1987). Other problematic species include *Salvinia molesta*, *Pistia stratiotes*, and the submerged weed *Hydrilla verticillata*. These weeds form dense infestations that restrict light penetration, alter ecosystem processes, interfere with fisheries and irrigation systems, and cause significant ecological and economic losses across the country (Mitchell and Tur 1975; Hussner et al. 2017).

1.3 Limitations of Conventional Management

Current management strategies for aquatic weeds primarily include mechanical removal, chemical herbicides, and classical biological control. Although these approaches have achieved varying degrees of success, each suffers from important limitations. Mechanical harvesting provides immediate reduction of weed biomass but is labor-intensive, expensive, and often ineffective for long-term control because of rapid regrowth from surviving plant fragments (Saxena and Singh 2001). Repeated harvesting operations are frequently required, increasing management costs substantially.

Chemical herbicides such as glyphosate and 2,4-D can provide effective short-term suppression of aquatic weeds; however, concerns regarding environmental contamination, non-target effects, herbicide residues, and regulatory restrictions limit their widespread application in aquatic ecosystems (Folmar et al. 1979; Hussner et al. 2017). Furthermore, repeated herbicide use may contribute to the evolution of herbicide-resistant weed populations and negatively affect aquatic biodiversity.

Classical biological control using insects and fungal pathogens has provided long-term suppression in certain situations but often exhibits inconsistent efficacy due to environmental variability, limited host-pathogen interactions, and challenges associated with establishment and persistence of biological control agents (Schroeder and Müller-Schärer 2007). Consequently, there remains an urgent need for innovative, environmentally sustainable, and species-specific approaches for aquatic weed management. Biological weed management using fungal pathogens and their metabolites has received increasing attention as a sustainable alternative to synthetic herbicides (Singh and Pandey 2019a, 2019b).

1.4 Emergence of RNAi-Based Weed Management

Recent advances in molecular biology have introduced RNA interference (RNAi) as a promising technology for next-generation crop protection and weed management. RNAi is a highly conserved gene-silencing mechanism in which double-stranded RNA (dsRNA) triggers sequence-specific degradation of complementary messenger RNA, resulting in suppression of target gene expression (Fire et al. 1998; Agrawal et al. 2003). Because RNAi acts through precise nucleotide sequence recognition, it offers unparalleled specificity compared with conventional herbicides.

The development of RNAi-based technologies such as Spray-Induced Gene Silencing (SIGS), Host-Induced Gene Silencing (HIGS), and trans-kingdom RNAi has expanded opportunities for managing plant pathogens, insect pests, and invasive weeds (Weiberg et al. 2013; Christiaens et al. 2020). By targeting genes involved in photosynthesis, respiration, nutrient uptake, growth regulation, and reproduction, RNAi-based bioherbicides have the potential to suppress invasive weeds with minimal impacts on non-target organisms and aquatic ecosystems. Advances in dsRNA synthesis, nanocarrier-mediated delivery systems, and bioinformatics-assisted target identification are further accelerating the development of practical RNAi-based weed management solutions. RNAi technology has emerged as a highly specific and environmentally compatible approach for weed management, with increasing interest in its application against invasive weed species (Singh and Pandey 2023).

1.5 Scope and Objectives of the Review

The integration of RNAi technology with fungal biocontrol agents presents a novel opportunity for developing highly specific and environmentally compatible mycoherbicides for aquatic weed management. Fungal pathogens naturally infect and suppress aquatic weeds, while RNAi technology offers precise molecular targeting of essential weed genes. Combining these approaches could generate next-generation RNAi-assisted mycoherbicides with enhanced efficacy, host specificity, and sustainability.

This review critically examines current knowledge on RNAi technology and its potential application in the development of mycoherbicides for invasive aquatic weed management. Specifically, the review discusses the biology and impacts of major aquatic weeds, molecular mechanisms of RNA interference, potential gene targets, fungal candidates for mycoherbicide development, trans-kingdom RNAi interactions, delivery technologies, biosafety considerations, regulatory challenges, and future research directions. The review aims to provide a comprehensive framework for researchers, policymakers, and biotechnology developers working toward sustainable management of invasive aquatic weeds through advanced molecular and biological control technologies. At present, no commercial RNAi-assisted mycoherbicide has been registered for aquatic weed management, and current developments remain largely at the proof-of-concept and experimental stages.

2. INVASIVE AQUATIC WEEDS AND THEIR IMPACTS

Invasive aquatic weeds are among the most damaging biological invaders of freshwater ecosystems. Their rapid growth, efficient reproduction, and broad environmental adaptability enable them to colonize lakes, rivers, reservoirs, wetlands, and irrigation systems, where they reduce biodiversity, impair water utilization, disrupt ecosystem processes, and generate substantial economic losses (Gopal 1987; Villamagna and Murphy 2010; Hussner et al. 2017). In India, nutrient enrichment of water bodies and favorable climatic conditions have facilitated the spread of several invasive species, particularly *Eichhornia crassipes*, *Salvinia molesta*, *Pistia stratiotes*, and *Hydrilla verticillata*.

2.1 *Eichhornia crassipes* (Water Hyacinth)

Eichhornia crassipes is widely regarded as the world's most problematic aquatic weed. Native to South America, it has invaded freshwater ecosystems throughout tropical and subtropical regions, including India (Gopal 1987). The species reproduces rapidly through vegetative stolons and can double its biomass within 10–15 days under favorable conditions. Dense floating mats obstruct waterways, reduce light penetration, deplete dissolved oxygen, impair fisheries, and interfere with irrigation and navigation (Villamagna and Murphy 2010). Its high nutrient uptake efficiency, rapid growth, and environmental adaptability contribute to its persistence despite extensive control efforts.

2.2 *Salvinia molesta* (Giant Salvinia)

Salvinia molesta is a highly invasive floating fern that reproduces through vegetative fragmentation and rapidly colonizes freshwater habitats (Mitchell and Tur 1975). It forms dense mats that block sunlight, reduce oxygen levels, alter nutrient cycling, and suppress aquatic biodiversity. In India, giant salvinia has become a major problem in wetlands, reservoirs, and irrigation systems. Although biological control using the salvinia weevil (*Cyrtobagous salviniae*) has shown success, management remains challenging due to rapid regrowth and environmental variability affecting control efficacy.

2.3 *Pistia stratiotes* (Water Lettuce)

Pistia stratiotes is a free-floating aquatic weed that forms dense rosettes on the water surface. Rapid vegetative reproduction allows it to spread quickly, obstruct waterways, reduce light penetration, and compete with native aquatic vegetation. Large infestations impair fisheries, irrigation, and water transport while creating favorable habitats for mosquito breeding. Its persistence in nutrient-rich water bodies makes long-term management difficult.

2.4 *Hydrilla verticillata* and Other Submerged Weeds

Unlike floating weeds, *Hydrilla verticillata* grows entirely beneath the water surface, making detection and management more difficult. The species reproduces through fragments, tubers, and turions, enabling rapid spread and persistence. Dense infestations obstruct water flow, interfere with recreation and hydropower operations, and alter aquatic habitats. Other submerged weeds such as *Najas* spp. and *Ceratophyllum* spp. present similar challenges due to their hidden growth habit and strong regenerative capacity.

2.5 Economic and Ecological Impacts

Invasive aquatic weeds cause significant ecological and economic damage by reducing biodiversity, disrupting food webs, lowering dissolved oxygen levels, and degrading water quality (Villamagna and Murphy 2010). Economically, they increase costs associated with irrigation maintenance, fisheries losses, water transport disruption, and weed management operations (Hussner et al. 2017). In addition, dense weed mats promote mosquito breeding and increase public health risks. These impacts highlight the urgent need for innovative and sustainable management strategies for invasive aquatic weeds (Table 1).

Table 1. Major Invasive Aquatic Weeds of India and Their Ecological and Economic Impacts

Weed Species	Common Name	Growth Habit	Major Impacts	Management Challenges
Eichhornia crassipes	Water Hyacinth	Free-Floating	Blocks waterways, reduces dissolved oxygen, biodiversity loss, fish mortality	Rapid vegetative growth, high regeneration capacity
Salvinia molesta	Giant Salvinia	Free-Floating Fern	Dense mats, light exclusion, oxygen depletion, impaired fisheries	Rapid fragmentation and regrowth
Pistia stratiotes	Water Lettuce	Free-Floating	Water obstruction, mosquito breeding, competition with native species	High growth rate and rapid spread
Hydrilla verticillata	Hydrilla	Submerged	Water flow obstruction, habitat alteration, recreational losses	Hidden growth habit, tuber formation
Najas spp.	Naiad Weeds	Submerged	Competition with native vegetation, fisheries interference	Difficult detection and repeated regrowth
Ceratophyllum spp.	Hornwort	Submerged	Dense underwater biomass, altered ecosystem processes	Fragment-based dispersal and persistence
Azolla spp. (in nuisance situations)	Mosquito fern	Floating fern	Surface coverage, oxygen reduction under excessive growth	Rapid multiplication under nutrient-rich conditions
Lemna spp.	Duckweed	Floating	Water surface coverage and reduced light penetration	Rapid population expansion under eutrophic conditions

3. RNA INTERFERENCE TECHNOLOGY

RNA interference (RNAi) is a conserved gene-silencing mechanism that regulates gene expression through small RNA molecules. Since its discovery, RNAi has become a powerful tool in functional genomics, crop protection, disease management, and emerging weed control technologies. Its high specificity enables selective suppression of target genes with minimal effects on non-target organisms, making it an attractive platform for environmentally sustainable bioherbicide development (Baulcombe 2004; Koch et al. 2016; Christiaens et al. 2020).

3.1 Historical Development of RNAi

RNA-mediated gene silencing was first observed in transgenic petunia plants, where introduction of additional pigment genes unexpectedly suppressed flower coloration, a phenomenon known as co-suppression (Napoli et al. 1990). Similar observations in fungi further indicated the existence of a conserved gene-silencing mechanism (Romano and Macino 1992).

The molecular basis of RNAi was established by Fire et al. (1998), who demonstrated that double-stranded RNA (dsRNA) could induce sequence-specific gene silencing in *Caenorhabditis elegans*. Subsequent studies revealed that RNAi is widely conserved across plants, fungi, and animals, where it functions in gene regulation, antiviral defense, and genome protection (Baulcombe 2004; Waterhouse et al. 2001). More recently, technologies such as Spray-Induced Gene Silencing (SIGS), Host-Induced Gene Silencing (HIGS), and environmental RNAi have expanded RNAi applications in sustainable crop protection and weed management (Koch et al. 2016; Mitter et al. 2017).

3.2 Molecular Mechanism of RNAi

RNAi is initiated when dsRNA molecules enter the cell and are processed by Dicer-like (DCL) enzymes into 21–24 nucleotide small interfering RNAs (siRNAs) (Baulcombe 2004). These siRNAs are incorporated into the RNA-Induced Silencing Complex (RISC), where one strand serves as a guide to recognize complementary messenger RNA (mRNA) molecules.

Upon target recognition, RISC mediates mRNA cleavage or translational repression, resulting in suppression of gene expression (Agrawal et al. 2003; Vaucheret 2008). In plants, RNAi operates through both post-transcriptional gene silencing (PTGS), which degrades target mRNA, and transcriptional gene silencing (TGS), which involves DNA methylation and chromatin modification (Matzke and Mosher 2014). Together, these pathways provide an efficient regulatory system that can be exploited for precise suppression of essential genes in invasive aquatic weeds.

3.3 Dicer, siRNA, Argonaute and RISC

The RNAi pathway is governed by four key components: Dicer-like (DCL) enzymes, small interfering RNAs (siRNAs), Argonaute (AGO) proteins, and the RNA-Induced Silencing Complex (RISC). DCL enzymes process double-stranded RNA (dsRNA) into 21–24 nucleotide siRNAs, which provide sequence specificity for gene silencing. In plants, DCL4 primarily produces 21-nt siRNAs involved in post-transcriptional gene silencing, DCL2 generates 22-nt siRNAs associated with secondary silencing, and DCL3 produces 24-nt siRNAs involved in RNA-directed DNA methylation (Xie et al. 2004; Matzke and Mosher 2014).

The resulting siRNAs are loaded onto AGO proteins, which form the catalytic core of RISC. Guided by siRNA sequence complementarity, AGO-containing RISC complexes recognize target mRNAs and induce their degradation or translational repression. AGO1 primarily functions in post-transcriptional gene silencing, whereas AGO4 is involved in transcriptional silencing and DNA methylation (Vaucheret 2008). Together, DCLs, siRNAs, AGO proteins, and RISC constitute the central machinery responsible for sequence-specific gene regulation in plants.

3.4 Gene Silencing Pathways in Plants

Plants possess multiple RNA silencing pathways that regulate gene expression, maintain genome stability, and mediate responses to biotic and abiotic stresses. The most common pathway is Post-Transcriptional Gene Silencing (PTGS), in which siRNA-guided RISC complexes degrade complementary mRNA molecules in the cytoplasm, thereby preventing protein synthesis (Baulcombe 2004).

A second pathway, Transcriptional Gene Silencing (TGS), operates in the nucleus through DNA methylation and chromatin modification, resulting in suppression of gene transcription (Matzke and Mosher 2014). A specialized form of TGS, known as RNA-Directed DNA Methylation (RdDM), utilizes 24-nt siRNAs and AGO4 to direct de novo DNA methylation and silence transposable elements and repetitive sequences.

Plants also employ the Natural Antisense Transcript (NAT) pathway, in which complementary endogenous transcripts form dsRNA molecules that are subsequently processed into siRNAs involved in developmental regulation and stress adaptation (Baulcombe 2004). Collectively, these interconnected pathways form a sophisticated RNA-based regulatory network that provides the molecular foundation for RNAi-based crop protection and weed management technologies.

3.5 Trans-Kingdom RNAi

Trans-kingdom RNAi is the transfer of small RNA molecules between interacting organisms, resulting in gene silencing across species boundaries. This phenomenon was first demonstrated by Weiberg et al. (2013), who showed that the fungal pathogen *Botrytis cinerea* delivers small RNAs into plant cells to suppress host immunity. Subsequent studies revealed that plants can also transfer small RNAs to fungal pathogens, insects, and nematodes, thereby inhibiting genes essential for virulence and survival (Koch et al. 2016; Christiaens et al. 2020).

This bidirectional RNA exchange provides a strong foundation for RNAi-assisted mycoherbicides. Fungal pathogens may serve as natural delivery systems for dsRNA or siRNA molecules targeting essential genes involved in photosynthesis, respiration, nutrient uptake, growth, and reproduction in invasive aquatic weeds. Although still largely at the proof-of-concept stage, advances in fungal biotechnology, RNA production, and nanocarrier-based delivery systems are rapidly expanding the potential of trans-kingdom RNAi for developing highly specific and environmentally sustainable aquatic weed management strategies.

4. RNAi-BASED WEED MANAGEMENT TECHNOLOGIES

RNA interference (RNAi) has emerged as a promising tool for sustainable weed management due to its ability to silence specific genes with high precision. Unlike conventional herbicides, RNAi-based technologies target essential genes involved in growth, metabolism, and reproduction, thereby minimizing impacts on non-target organisms and the environment. Recent advances in RNA synthesis, genomics, and nanotechnology have facilitated the development of approaches such as Host-Induced Gene Silencing (HIGS), Spray-Induced Gene Silencing (SIGS), and environmental RNAi for crop protection and weed control (Koch et al. 2016; Mitter et al. 2017; Christiaens et al. 2020). The application of RNAi for weed suppression relies on sequence-specific degradation of target mRNAs, enabling precise regulation of essential physiological processes (Singh and Pandey 2026b).

4.1 Host-Induced Gene Silencing (HIGS)

Host-Induced Gene Silencing (HIGS) is a transgenic approach in which plants are engineered to produce dsRNA molecules targeting essential genes in pests, pathogens, or weeds (Nowara et al. 2010; Koch et al. 2016). The expressed dsRNA is processed into siRNAs and transferred to the target organism, resulting in gene silencing. HIGS provides long-term protection but requires genetically modified plants and faces regulatory and public acceptance challenges. Although theoretically applicable to aquatic weed management, its practical use remains limited because invasive aquatic weeds are not suitable targets for transgenic crop-based systems.

4.2 Spray-Induced Gene Silencing (SIGS)

Spray-Induced Gene Silencing (SIGS) is a non-transgenic RNAi strategy involving external application of dsRNA onto plant surfaces. After uptake, dsRNA is processed into siRNAs that silence target genes, providing a highly specific and environmentally friendly alternative to conventional herbicides (Koch et al. 2016; Mitter et al. 2017). SIGS has shown considerable success against plant pathogens and insect pests and is regarded as the most promising RNAi technology for weed management.

For aquatic weeds, species-specific dsRNA can be designed to target genes associated with photosynthesis, respiration, nutrient uptake, stress tolerance, and vegetative reproduction. However, successful field application depends on overcoming challenges related to dsRNA stability, environmental degradation, dilution in aquatic habitats, and efficient uptake by target weeds (Christiaens et al. 2020).

4.3 Environmental RNAi

Environmental RNAi refers to gene silencing triggered by the uptake of externally supplied dsRNA from sources such as water, soil, microorganisms, or artificial delivery systems (Christiaens et al. 2020). In plants, exogenous dsRNA can enter cells through natural openings, wounds, or endocytosis and is subsequently processed into siRNAs that activate RNAi pathways. This approach is particularly relevant for aquatic weed management because dsRNA can potentially be delivered through water-based formulations. However, factors such as dsRNA stability, environmental persistence, uptake efficiency, and non-target exposure require further investigation before field application.

4.4 dsRNA Production Technologies

Cost-effective production of dsRNA is essential for commercial RNAi-based weed management. Early applications relied on in vitro transcription, which produces high-purity dsRNA but remains relatively expensive for large-scale use. Advances in bacterial expression systems, particularly *Escherichia coli* HT115, have significantly reduced production costs by enabling large-scale fermentation-based synthesis (Bennett et al. 2020). Engineered yeasts such as *Saccharomyces cerevisiae* and *Yarrowia lipolytica* provide additional scalable and biosafe production platforms. Emerging cell-free and synthetic biology-based systems further enhance production efficiency and may support future commercialization of RNAi bioherbicides.

4.5 RNAi Delivery Platforms

Efficient delivery of dsRNA remains a major challenge because naked RNA molecules are rapidly degraded by ultraviolet radiation, nucleases, and environmental factors (Mitter et al. 2017). Direct application of naked dsRNA is simple but often provides limited field persistence. Consequently, advanced delivery systems have been developed to improve RNA stability and uptake.

Nanotechnology-based carriers, including chitosan nanoparticles, layered double hydroxides (BioClay), silica nanoparticles, lipid nanoparticles, and polymeric nanocarriers, protect dsRNA from degradation and enhance cellular uptake (Mitter et al. 2017). Liposomal systems further improve stability and penetration into plant tissues. In addition, engineered bacteria, yeasts, and fungal pathogens can function as biological delivery vehicles, offering opportunities for sustained RNA production and targeted delivery.

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Such platforms are expected to play a crucial role in the development of effective RNAi-based bioherbicides and RNAi-assisted mycoherbicides for aquatic weed management.

Controlled-Release Formulations

Encapsulation technologies using alginate, chitosan, starch, and biodegradable polymers enable slow release of dsRNA under field conditions. Controlled-release formulations may improve persistence and reduce application frequency in aquatic environments.

The continued advancement of delivery technologies is expected to play a critical role in the successful development of RNAi-based bioherbicides and mycoherbicides for sustainable management of invasive aquatic weeds (Table 2).

Table 2. RNAi Delivery Systems and Their Characteristics

Delivery System	Principle	Advantages	Limitations	Potential Application in Aquatic Weed Management
Naked dsRNA spray	Direct application of dsRNA	Simple, low-cost, non-transgenic	Rapid degradation	Short-term weed suppression
SIGS	Foliar application of dsRNA	Highly specific, environmentally friendly	Uptake efficiency varies	Floating aquatic weeds
HIGS	Transgenic expression of dsRNA	Continuous RNA production	Regulatory challenges	Limited practical use
Chitosan nanoparticles	Encapsulation within chitosan matrix	Improved stability and uptake	Formulation complexity	Aquatic bioherbicides
BioClay (LDH nanoparticles)	Layered double hydroxide protection	Slow release and UV protection	Higher production cost	Long-term RNA delivery
Liposomes	Lipid-based encapsulation	Enhanced cellular penetration	Storage stability issues	Foliar RNA delivery
Polymeric nanoparticles	Controlled-release systems	Extended persistence	Manufacturing complexity	Sustained weed control
Bacterial delivery	dsRNA-producing bacteria	Low production cost	Regulatory concerns	Environmental RNAi
Yeast-based delivery	Recombinant yeast systems	Scalable production	Limited field validation	Bioherbicide formulations
Fungal delivery systems	Mycoherbicide-mediated RNA delivery	Combined pathogenicity and RNAi	Experimental stage	RNAi-assisted mycoherbicides

5. RNAi TARGETS FOR AQUATIC WEED MANAGEMENT

The effectiveness of RNAi-based weed management depends on the identification of essential genes whose silencing leads to growth inhibition, metabolic disruption, reproductive failure, or plant death. Ideal targets should be critical for weed survival, highly expressed, and sufficiently distinct from non-target species to ensure specificity (Bennett et al. 2020; Christiaens et al. 2020). Advances in genomics and transcriptomics have facilitated the discovery of candidate genes in major aquatic weeds such as *Eichhornia crassipes*, *Salvinia molesta*, *Pistia stratiotes*, and *Hydrilla verticillata*.

5.1 Photosynthesis-Related Genes

Photosynthesis-related genes are attractive RNAi targets because they are essential for energy production and biomass accumulation. Key targets include *psbA*, *psaA*, and *rbcL*, which are involved in photosynthetic electron transport and carbon fixation. Genes associated with chlorophyll biosynthesis, such as *CHLH* and *POR*, are also promising targets, as their silencing induces chlorosis, reduced photosynthetic efficiency, and growth inhibition (Baulcombe 2004; Koch et al. 2016).

5.2 Energy Metabolism Genes

Genes involved in respiration and ATP production are critical for plant survival and represent important RNAi targets. Potential candidates include ATP synthase genes (*atpA*, *atpB*, *atpE*), NADH dehydrogenase genes (*ndh* family), cytochrome oxidase genes (*cox1*, *cox2*), and enzymes of the tricarboxylic acid (TCA) cycle. Silencing these genes disrupts cellular energy production, resulting in metabolic stress and reduced weed vigor (Agrawal et al. 2003).

5.3 Growth and Development Genes

Genes regulating cell division, hormone signaling, and plant development are essential for invasive growth and biomass accumulation. Promising targets include auxin transporters (*PIN*), gibberellin biosynthesis genes (*GA20ox*, *GA3ox*), cytokinin response regulators (*ARR*), cyclins (*CYC*), and cyclin-dependent kinases (*CDKs*). RNAi-mediated suppression of these genes can cause stunted growth, abnormal development, reduced vegetative propagation, and decreased competitive ability of aquatic weeds (Vaucheret 2008).

5.4 Reproduction and Flowering Genes

Genes involved in flowering, seed production, and vegetative propagation are attractive RNAi targets because they directly influence weed persistence and spread. Key flowering regulators include *FLOWERING LOCUS T (FT)*, *LEAFY (LFY)*, *APETALA1 (AP1)*, and *CONSTANS (CO)*, whose silencing can delay flowering and reduce reproductive success (Bennett et al. 2020). In aquatic weeds, genes controlling stolon formation, rhizome development, tuber production, and vegetative propagation are particularly important. For example, *Eichhornia crassipes* spreads through stolons, whereas *Hydrilla verticillata* reproduces via tubers and turions. Suppression of these pathways can reduce dispersal, population growth, and reinfestation potential.

5.5 Nutrient Uptake Genes

The invasive success of aquatic weeds is closely linked to their efficient uptake of nutrients from eutrophic water bodies (Villamagna and Murphy 2010). Consequently, genes encoding nutrient transporters represent promising RNAi targets. Important candidates include nitrate transporters (*NRT1*, *NRT2*), ammonium transporters (*AMT1*), phosphate transporters (*PHT1* family), potassium transporters (*HAK*, *KT/KUP*), and iron transporters (*IRT1*). Silencing these genes impairs nutrient acquisition, resulting in nutrient deficiency, reduced growth, and diminished competitive ability. Combining nutrient uptake targets with genes involved in photosynthesis and respiration may further enhance weed suppression through synergistic effects (Table 3).

Table 3. Potential Target Genes for RNAi-Based Management of Invasive Aquatic Weeds

Target Category	Representative Genes	Biological Function	Expected Effect of Gene Silencing
Photosynthesis	*psbA*, *psaA*, *rbcL*	Photosynthetic electron transport and carbon fixation	Chlorosis, reduced photosynthesis, growth inhibition
Chlorophyll biosynthesis	*CHLH*, *POR*	Chlorophyll synthesis	Leaf bleaching, impaired photosynthesis
Energy metabolism	*atpA*, *atpB*, *ndh*, *cox*	ATP generation and respiration	Energy deficiency and metabolic collapse
Cell cycle regulation	*CYC*, *CDK*	Cell division and growth	Growth arrest and reduced biomass
Auxin signalling	*PIN*, *ARF*	Plant growth and development	Abnormal morphology and reduced vigor
Gibberellin biosynthesis	*GA20ox*, *GA3ox*	Stem elongation and growth	Dwarfism and suppressed growth
Flowering regulation	*FT*, *LFY*, *CO*, *AP1*	Floral induction and reproduction	Delayed flowering and reduced seed production
Vegetative propagation	Stolon/rhizome-associated genes	Clonal reproduction	Reduced spread and persistence
Nitrogen uptake	*NRT1*, *NRT2*, *AMT1*	Nitrogen transport	Nutrient deficiency and reduced growth
Phosphorus uptake	*PHT1* family	Phosphate transport	Reduced nutrient acquisition
Potassium uptake	*HAK*, *KT/KUP*	Potassium transport	Growth reduction and stress sensitivity
Stress response	*HSP70*, *SOD*, *CAT*	Abiotic stress tolerance	Increased susceptibility to environmental stress

6. Mycoherbicides for Aquatic Weed Control

Mycoherbicides, which utilize plant-pathogenic fungi for weed suppression, represent an environmentally sustainable alternative to chemical herbicides. These biological agents infect target weeds, causing disease, growth inhibition, and mortality while generally exhibiting high host specificity and environmental compatibility (Charudattan 2001; Boyette et al. 2014). Their potential is particularly significant for managing invasive aquatic weeds such as *Eichhornia crassipes*, *Salvinia molesta*, *Pistia stratiotes*, and *Hydrilla verticillata*. Moreover, fungal pathogens provide a promising platform for the future development of RNAi-assisted weed management technologies. Fungal pathogens have demonstrated significant potential as bioherbicides against a wide range of invasive and noxious weeds (Singh and Pandey 2019a; Singh and Pandey 2019b).

6.1 Concept of Mycoherbicides

Mycoherbicides are formulations containing fungal propagules (spores, mycelia, or chlamydospores) or fungal-derived metabolites developed specifically for weed control. Applied in an inundative manner, fungal pathogens infect susceptible weeds and induce symptoms such as chlorosis, necrosis, wilting, defoliation, and plant death (Templeton et al. 1986; Charudattan 2001). Compared with conventional herbicides, mycoherbicides offer advantages including biodegradability, host specificity, compatibility with integrated weed management, and suitability for organic agriculture. However, their performance can be influenced by environmental conditions and formulation stability.

6.2 Commercial Mycoherbicides Worldwide

Several fungal bioherbicides have demonstrated commercial feasibility. Notable examples include DeVine®, based on *Phytophthora palmivora* for control of stranglervine, and Collego®, containing *Colletotrichum gloeosporioides* f. sp. *aeschynomene* for management of northern jointvetch (Charudattan 2001). Although commercial adoption remains limited compared with chemical herbicides, these products established the proof-of-concept for fungal weed control. To date, no mycoherbicide has achieved widespread commercial use specifically against aquatic weeds, despite encouraging research results.

6.3 Fungal Pathogens of Aquatic Weeds

Numerous fungal pathogens naturally infect aquatic weeds and have been investigated as biological control agents. Important genera include *Acremonium*, *Alternaria*, *Cercospora*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Exserohilum*, *Fusarium*, *Myrothecium*, and *Phytophthora* (Charudattan 2001; Boyette et al. 2014). These fungi attack leaves, petioles, stolons, roots, and reproductive structures, reducing plant vigor, biomass, and reproductive capacity.

Many fungal pathogens produce phytotoxic metabolites and cell wall-degrading enzymes that enhance disease severity and weed suppression. In addition to direct pathogenic effects, fungal infection may increase susceptibility of aquatic weeds to environmental stress and secondary pathogens, thereby improving overall control efficacy (Table 4).

Table 4. Major Fungal Pathogens Evaluated Against Aquatic Weeds

Fungal Pathogen	Target Aquatic Weed	Major Disease Symptoms	Development Status
Acremonium zonatum	*Eichhornia crassipes*	Leaf spots, chlorosis, necrosis	Experimental
Alternaria eichhorniae	*Eichhornia crassipes*	Leaf blight, tissue necrosis	Advanced research
Cercospora piaropi	*Eichhornia crassipes*	Leaf lesions and blight	Experimental
Myrothecium roridum	*Eichhornia crassipes*	Severe foliar necrosis	Experimental
Curvularia lunata	*Eichhornia crassipes*	Chlorosis and leaf blight	Experimental
Fusarium pallidoroseum	*Eichhornia crassipes*	Wilt and tissue collapse	Experimental
Phytophthora palmivora	*Pistia stratiotes*, *Salvinia molesta*	Root rot, wilting, plant death	Field evaluation
Colletotrichum gloeosporioides	Aquatic broadleaf weeds	Anthracnose and necrosis	Research stage
Drechslera gigantea	Various aquatic weeds	Foliar blight	Experimental
Exserohilum longirostratum	Aquatic grasses and sedges	Leaf blight	Experimental
Cercospora rodmanii	Water hyacinth	Severe leaf blight	Advanced research
Alternaria alternata	Multiple aquatic weeds	Foliar necrosis	Experimental

The diversity of fungal pathogens available for aquatic weed control highlights the considerable potential of mycoherbicides as sustainable management tools and as biological platforms for future RNAi-assisted weed suppression strategies.

6.4 *Acremonium* spp.

Acremonium zonatum and related species are among the most studied pathogens of water hyacinth. They cause leaf spots, chlorosis, necrosis, and destruction of photosynthetic tissues, leading to significant biomass reduction under humid conditions (Shabana 1997). Their ease of culture and demonstrated efficacy against *Eichhornia crassipes* make them promising candidates for future RNAi-assisted mycoherbicide development.

6.5 *Colletotrichum* spp.

Colletotrichum species are important bioherbicidal fungi that infect aquatic weeds and induce anthracnose symptoms, including necrotic lesions, blight, defoliation, and reduced photosynthesis (Templeton et al. 1986). Their prolific conidial production and well-developed genetic tools make them attractive platforms for next-generation RNAi-assisted bioherbicides.

6.6 *Phytophthora* spp.

Phytophthora palmivora and related species are effective pathogens of aquatic weeds such as water lettuce and salvinia. They cause water-soaked lesions, tissue maceration, wilting, and plant death. Their adaptation to wet environments and availability of genomic resources support their potential use in RNAi-based weed management (Charudattan 2001).

6.7 Emerging Fungal Candidates

Emerging fungal pathogens, including *Alternaria eichhorniae, Fusarium, Curvularia*, *Myrothecium*, *Exserohilum*, and *Cercospora*, have shown promise for aquatic weed control through the production of phytotoxic metabolites and rapid tissue necrosis (Evidente et al. 2006; Boyette et al. 2014). Advances in genomics, transcriptomics, and metabolomics are accelerating the discovery of highly virulent isolates and supporting the development of more effective RNAi-assisted mycoherbicides. Several phytopathogenic fungi and their metabolites have been investigated for weed suppression and may provide valuable resources for future RNAi-assisted mycoherbicide development (Singh and Pandey 2019c).

7. RNAi-ASSISTED MYCOHERBICIDES

The integration of RNA interference (RNAi) with fungal bioherbicides represents an emerging strategy for sustainable weed management. While conventional mycoherbicides suppress weeds through infection and disease development, RNAi enables targeted silencing of essential weed genes. Combining these approaches may enhance efficacy, host specificity, and environmental safety while reducing application rates (Weiberg et al. 2013; Koch et al. 2016; Christiaens et al. 2020). The integration of RNAi technology with fungal bioherbicides builds upon extensive research demonstrating the effectiveness of fungal pathogens and phytotoxic metabolites in weed management (Singh and Pandey 2019a, 2019c). To date, direct experimental evidence demonstrating fungal-mediated delivery of dsRNA for suppression of aquatic weed genes is not available. The proposed RNAi-assisted mycoherbicide concept is derived from advances in trans-kingdom RNAi, fungal biotechnology, and RNA delivery systems.

7.1 Conceptual Framework

RNAi-assisted mycoherbicides use fungal pathogens as both disease-causing agents and carriers of double-stranded RNA (dsRNA) targeting critical weed genes. After infection, the fungus colonizes plant tissues and delivers dsRNA molecules that activate the weed's RNAi machinery, resulting in sequence-specific gene silencing. The combined effects of pathogen infection and suppression of vital physiological processes can accelerate weed decline and mortality. Integration of RNAi with biological control agents may provide synergistic effects by combining pathogen-mediated damage with targeted gene silencing (Singh and Pandey 2023; Singh and Pandey 2026b).

The framework involves:

1. Identification of essential weed target genes.
2. Design of species-specific dsRNA molecules.
3. Development of fungal RNA delivery systems.
4. Transfer of dsRNA into weed tissues.
5. RNAi-mediated suppression of key biological functions.

This dual mode of action offers significant advantages over conventional mycoherbicides.

7.2 Trans-Kingdom RNAi

Trans-kingdom RNAi refers to the transfer of RNA molecules between different organisms, leading to gene silencing across species boundaries. This mechanism provides the scientific basis for RNAi-assisted mycoherbicides (Weiberg et al. 2013).

Studies have shown that fungal pathogens such as *Botrytis cinerea* can deliver small RNAs into plant cells to suppress host genes, while plants can also transfer RNAs to pathogens and pests to inhibit their growth and survival (Weiberg et al. 2013; Cai et al. 2018). In aquatic weed management, fungal pathogens could similarly deliver dsRNA molecules targeting genes involved in photosynthesis, growth, nutrient uptake, and reproduction, thereby enhancing weed suppression. The discovery of extracellular vesicles as RNA carriers further supports the feasibility of this approach (Cai et al. 2018).

For a review paper, the following concise version is more suitable:

7.3 Fungal-Mediated dsRNA Delivery

Efficient delivery of dsRNA is a major challenge in RNAi-based weed management. Fungal pathogens offer a natural delivery platform because their infection structures, such as appressoria and haustoria, establish close contact with host tissues and may facilitate RNA transfer (Koch et al. 2016). Fungal-mediated delivery can protect dsRNA from environmental degradation, enable continuous in situ production, improve tissue penetration, enhance host specificity, and reduce application frequency. Genera such as *Acremonium*, *Colletotrichum*, and *Phytophthora* are considered promising candidates for future RNAi delivery systems.

7.4 Genetic Engineering Strategies

Genetic engineering enables fungal pathogens to produce dsRNA molecules targeting essential weed genes. Common approaches include:

- Hairpin RNA (hpRNA) constructs: Generate self-complementary dsRNA processed into siRNAs by fungal RNAi machinery.
- Agrobacterium-mediated transformation (ATMT): Provides stable integration and efficient expression of RNAi constructs.
- CRISPR/Cas technologies: Allow precise genome editing for optimized dsRNA expression and enhanced delivery efficiency.

- Synthetic biology approaches: Enable development of regulatory gene circuits for controlled dsRNA production during infection.

These technologies provide the foundation for engineering fungal strains that combine pathogenicity with targeted gene silencing.

7.5 Current Research Progress

Although no commercial RNAi-assisted mycoherbicides are currently available, significant progress has been made in related fields. Trans-kingdom RNAi has demonstrated RNA transfer between plants and fungal pathogens, while Host-Induced Gene Silencing (HIGS) and Spray-Induced Gene Silencing (SIGS) have successfully suppressed target organisms through RNAi-mediated gene silencing (Weiberg et al. 2013; Koch et al. 2016; Cai et al. 2018; Mitter et al. 2017). Advances in fungal genomics, transformation systems, and molecular biology have identified several promising fungal genera, including *Colletotrichum*, *Fusarium*, *Trichoderma*, and *Phytophthora*, as potential RNA delivery platforms. These developments indicate that RNAi-assisted mycoherbicides are scientifically feasible and represent a promising next-generation strategy for aquatic weed management.

7.6 Technical Limitations

Despite their potential, RNAi-assisted mycoherbicides face several challenges. dsRNA molecules are vulnerable to degradation by nucleases, UV radiation, and environmental conditions, affecting stability and field performance (Christiaens et al. 2020). Efficient transfer of dsRNA from fungal cells to weed tissues remains poorly understood, while long-term stability of engineered RNAi constructs in fungal genomes is essential for consistent efficacy. Although RNAi is highly specific, potential off-target effects on non-target plants require careful assessment. Regulatory frameworks for genetically modified fungi and RNA-based products are still evolving, creating commercialization uncertainties. In addition, production costs must be reduced to compete with conventional herbicides. Nevertheless, advances in RNA biology, fungal biotechnology, synthetic biology, nanotechnology, and precision agriculture are expected to accelerate the development of RNAi-assisted mycoherbicides in the coming years.

8. NANOTECHNOLOGY AND ADVANCED DELIVERY SYSTEMS

Efficient delivery of dsRNA remains a major challenge in RNAi-based weed management because naked dsRNA is rapidly degraded by UV radiation, nucleases, temperature fluctuations, and microbial activity. In aquatic environments, dilution and continuous water movement further reduce RNA persistence. Nanotechnology offers promising solutions by protecting dsRNA, enhancing cellular uptake, improving translocation, and enabling controlled release, thereby increasing the efficacy of RNAi-based weed control (Mitter et al. 2017; Christiaens et al. 2020; Fletcher et al. 2020). Advanced formulation technologies, including nanoparticles, BioClay, nanoemulsions, and controlled-release carriers, have been proposed to overcome major barriers associated with dsRNA uptake and environmental stability (Singh and Pandey 2026a).

8.1 Chitosan Nanoparticles

Chitosan is a biodegradable and biocompatible polymer that forms stable complexes with negatively charged dsRNA molecules (Malik et al. 2016). Chitosan nanoparticles protect dsRNA from degradation, enhance cellular uptake, promote adhesion to plant surfaces, and provide controlled release. Their potential to improve RNA persistence on floating aquatic weeds makes them promising carriers for RNAi-based weed management.

8.2 BioClay Technology

BioClay, developed by Mitter et al. (2017), consists of dsRNA adsorbed onto nanoscale clay particles that protect RNA from environmental degradation and enable sustained release. This technology prolongs dsRNA stability, reduces application frequency, improves field efficacy, and offers an environmentally safe delivery platform. Its extended persistence is particularly advantageous for managing aquatic weeds over large water bodies.

8.3 Layered Double Hydroxides (LDHs)

Layered double hydroxides (LDHs) are clay-based nanomaterials widely used in BioClay formulations. They bind dsRNA through electrostatic interactions, protect it from nuclease degradation and UV damage, and provide sustained release over time (Mitter et al. 2017). Their high loading capacity, biodegradability, and environmental compatibility make LDHs highly suitable for aquatic weed management applications.

8.4 Lipid Nanoparticles (LNPs)

Lipid nanoparticles are highly efficient RNA carriers that encapsulate dsRNA and protect it from degradation. By promoting cellular uptake through endocytosis, LNPs enhance RNA delivery across biological barriers and improve translocation within plant tissues (Fletcher et al. 2020). Their high encapsulation efficiency and strong delivery performance make them promising, although current production costs remain relatively high.

8.5 Nanoemulsions

Nanoemulsions are nanoscale oil–water dispersions that improve the stability, spreading, and retention of dsRNA formulations. They enhance foliar adhesion, penetration through cuticular barriers, and uptake efficiency while remaining

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compatible with conventional spraying equipment (Kah et al. 2018). These properties make nanoemulsions particularly suitable for floating aquatic weeds such as *Eichhornia crassipes* and *Pistia stratiotes*.

8.6 Controlled-Release Formulations

Controlled-release systems use biodegradable carriers such as alginate, chitosan, starch, PLGA, cellulose derivatives, and hydrogels to gradually release dsRNA over time (Christiaens et al. 2020). These formulations enhance RNA persistence, reduce degradation and application frequency, improve cost-effectiveness, and provide sustained weed control. In aquatic environments, controlled-release technologies are especially valuable for overcoming dilution and environmental instability.

Advances in nanotechnology, synthetic biology, smart polymers, and precision agriculture are expected to further improve RNA delivery efficiency and accelerate the commercialization of RNAi-based bioherbicides and RNAi-assisted mycoherbicides (Table 5).

Table 5. Nanocarriers and Advanced Delivery Systems for RNAi-Based Weed Management

Delivery System	Carrier Material	Mechanism of RNA Protection	Major Advantages	Limitations	Potential Application
Chitosan nanoparticles	Chitosan polymer	Electrostatic complex formation	Biodegradable, biocompatible, enhanced uptake	Moderate loading capacity	Foliar RNA delivery
BioClay	Clay nanoparticles + dsRNA	Adsorption and slow release	Extended stability and persistence	Production complexity	Aquatic weed management
Layered Double Hydroxides (LDHs)	Clay nanomaterials	Electrostatic binding and controlled release	UV protection and nuclease resistance	Cost considerations	Long-term RNA delivery
Lipid nanoparticles	Lipid vesicles	Encapsulation within lipid bilayers	High cellular uptake efficiency	Relatively expensive	Advanced RNA formulations
Nanoemulsions	Oil-water nanosystems	Physical encapsulation	Improved spreading and adhesion	Limited long-term stability	Foliar application
Polymeric nanoparticles	Synthetic biodegradable polymers	Encapsulation and controlled release	Sustained delivery	Manufacturing complexity	RNA bioherbicides
Alginate microcapsules	Alginate biopolymers	Controlled release matrix	Low cost and biodegradable	Slower release rates	Aquatic deployment
Hydrogel systems	Cross-linked polymers	Water-responsive release	Extended persistence	Formulation optimization required	Wetland applications
Silica nanoparticles	Mesoporous silica	Surface adsorption and protection	High loading capacity	Regulatory evaluation needed	Experimental RNA delivery
Fungal nanocarriers	Biological nanoparticles	Natural RNA transport systems	High biological compatibility	Early-stage research	RNAi-assisted mycoherbicides

Nanotechnology is likely to be the enabling technology that determines the commercial success of RNAi-based aquatic weed management. Among currently available platforms, BioClay (LDH-based systems), chitosan nanoparticles, and controlled-release formulations appear most promising for field-scale deployment because they combine environmental safety, prolonged RNA stability, and economic feasibility.

9. DEVELOPMENT PIPELINE FOR RNAI-BASED AQUATIC WEED BIOHERBICIDES

The development of RNAi-based bioherbicides involves an integrated pipeline combining genomics, molecular biology, bioinformatics, formulation science, and field validation. Unlike conventional herbicides, these products suppress weeds through sequence-specific gene silencing, requiring precise target selection, efficient dsRNA design, effective delivery systems, and comprehensive biosafety assessment. Advances in sequencing technologies, transcriptomics, artificial intelligence, dsRNA production, and nanotechnology have significantly accelerated this process (Koch et al. 2016; Mitter et al. 2017; Bennett et al. 2020).

9.1 Target Identification

Target identification is the most critical step in RNAi bioherbicide development. Ideal target genes should be essential for weed survival, growth, reproduction, or stress tolerance and sufficiently divergent from non-target species (Bennett et al. 2020). Common targets include genes involved in photosynthesis (*psbA, psaA, rbcL*), respiration, nutrient uptake, hormone biosynthesis, cell division, and flowering. Transcriptomic and functional analyses help identify highly expressed genes in aquatic weeds such as

Eichhornia crassipes, *Salvinia molesta*, and *Pistia stratiotes*. Effective targets are essential, highly expressed, conserved within the weed population, and capable of producing strong phenotypic effects when silenced. Identification of essential and weed-specific target genes represents the foundation of successful RNAi bioherbicide development (Singh and Pandey 2026b).

9.2 Bioinformatics and dsRNA Design

Following target selection, bioinformatics tools are used to design highly specific dsRNA molecules while minimizing off-target effects (Christiaens et al. 2020). The process includes sequence retrieval, identification of conserved target regions, off-target screening against crops and non-target organisms, prediction of effective siRNA-generating sequences, and RNA secondary structure analysis. Increasingly, artificial intelligence and machine learning are being applied to optimize target selection and predict silencing efficiency, improving the precision and effectiveness of RNAi-based bioherbicides. Bioinformatic tools play a critical role in optimizing target specificity and minimizing off-target effects (Singh and Pandey 2026b).

9.3 dsRNA Synthesis

Production of high-quality dsRNA is essential for RNAi bioherbicide development. Common production platforms include in vitro transcription, which provides high-purity dsRNA for research applications; bacterial expression systems such as *Escherichia coli* HT115, which offer cost-effective large-scale production; yeast-based systems, which provide scalable and biosafe alternatives; and cell-free synthesis platforms, which enable rapid production with high product quality. Reducing dsRNA production costs remains a key requirement for commercial viability. Advances in fermentation-based production systems have significantly improved the economic feasibility of large-scale dsRNA manufacturing (Singh and Pandey 2026b).

9.4 Formulation Development

Formulation is critical for ensuring dsRNA stability, delivery efficiency, shelf life, and field performance. Because naked dsRNA is highly susceptible to degradation, protective carriers are required to enhance nuclease resistance, UV protection, adhesion, cellular uptake, and controlled release (Mitter et al. 2017). Common delivery systems include chitosan nanoparticles, BioClay, layered double hydroxides (LDHs), lipid nanoparticles, nanoemulsions, polymeric encapsulation systems, and hydrogels. For aquatic weeds, formulations must also withstand dilution and prolonged water exposure.

9.5 Laboratory Screening

Laboratory screening provides initial proof-of-concept by evaluating target gene silencing and biological activity under controlled conditions. Assessments typically include gene silencing efficiency (qRT-PCR or RNA sequencing), physiological responses such as chlorophyll content and growth reduction, visible symptoms including chlorosis and necrosis, dose–response relationships, and validation of RNAi pathway activation. These studies identify the most promising dsRNA targets and formulations for further testing.

9.6 Greenhouse Testing

Greenhouse experiments evaluate RNAi bioherbicides under semi-controlled conditions and serve as a bridge between laboratory and field studies. Key parameters include weed mortality, biomass reduction, growth inhibition, reproductive suppression, persistence of RNAi effects, formulation stability, and application frequency. Greenhouse trials also provide preliminary biosafety data on non-target aquatic plants and associated organisms. For aquatic weeds, artificial ponds, tanks, and mesocosms are commonly used to simulate natural aquatic environments while maintaining experimental control.

9.7 Field Evaluation

Field evaluation is the final stage in the development of RNAi-based bioherbicides and provides the most realistic assessment of efficacy, environmental safety, and economic feasibility. Large-scale trials evaluate weed suppression (biomass reduction, population decline, and long-term control), environmental persistence of dsRNA and formulations, effects on non-target organisms and water quality, and overall cost-effectiveness. These studies also generate essential data for environmental risk assessment and regulatory approval. Successful field validation supports product registration, commercial manufacturing, and integration into aquatic weed management programs.

Key Research Bottlenecks

Despite significant advances, several challenges remain:

1. Identification of highly effective weed-specific target genes.
2. Reduction of dsRNA production costs.
3. Development of stable formulations for aquatic environments.
4. Efficient delivery of dsRNA into weed tissues.
5. Long-term environmental and ecological risk assessment.
6. Establishment of clear regulatory approval pathways.

Overcoming these bottlenecks will be crucial for the successful commercialization and widespread adoption of RNAi-based aquatic weed bioherbicides.

10. BIOSAFETY AND RISK ASSESSMENT

Biosafety and environmental risk assessment are essential for the development of RNAi-based aquatic weed bioherbicides. Although RNAi is highly species-specific and dsRNA molecules are biodegradable and generally non-toxic to vertebrates, potential concerns regarding off-target effects, environmental persistence, ecological interactions, and resistance evolution must be carefully evaluated (Roberts et al. 2015; Petrick et al. 2016; Christiaens et al. 2020). Comprehensive risk assessment is therefore required to ensure effective weed control while maintaining environmental sustainability. The species-specific mode of action of RNAi offers significant biosafety advantages over conventional herbicides, although comprehensive ecological assessments remain essential (Singh and Pandey 2023).

10.1 Off-Target Effects

Off-target effects occur when siRNAs derived from dsRNA inadvertently silence genes in non-target organisms due to sequence similarity (Roberts et al. 2015). Risk depends on sequence homology, dsRNA stability, environmental persistence, uptake efficiency, and sensitivity of non-target species. Modern bioinformatic screening against genomes of crops, native aquatic plants, microorganisms, fish, insects, and other organisms greatly minimizes these risks (Bachman et al. 2016). In aquatic weed management, careful target selection is particularly important to protect native aquatic vegetation. Current evidence indicates that RNAi-based approaches pose substantially lower non-target risks than conventional herbicides.

10.2 Effects on Aquatic Biodiversity

Assessment of impacts on aquatic biodiversity is a key component of environmental risk evaluation. Available evidence suggests that dsRNA molecules have very low toxicity to vertebrates and unrelated organisms because they are rapidly degraded and require high sequence specificity for activity (Roberts et al. 2015). However, potential effects on native aquatic plants, beneficial microorganisms, microbial communities, and food webs should be examined. Importantly, successful control of invasive aquatic weeds can provide significant ecological benefits, including restoration of native biodiversity, improved water quality, enhanced oxygen availability, and better ecosystem functioning (Villamagna and Murphy 2010). Mesocosm studies, ecological monitoring, and long-term field evaluations are recommended to assess both risks and benefits under realistic conditions.

10.3 Environmental Fate of dsRNA

The environmental fate of dsRNA influences its persistence, mobility, and ecological exposure. Unlike conventional herbicides, dsRNA molecules are naturally biodegradable and are rapidly degraded by microbial ribonucleases, UV radiation, hydrolysis, and other environmental factors (Petrick et al. 2016). Adsorption to sediments and organic particles can temporarily influence stability. Advanced delivery systems such as BioClay and chitosan nanoparticles can extend dsRNA persistence while maintaining biodegradability (Mitter et al. 2017). Overall, the rapid environmental degradation of dsRNA is considered a major ecological advantage of RNAi-based weed management.

10.4 Resistance Management

Although resistance to RNAi-based weed control has not yet been reported in aquatic weeds, long-term use may select for adaptations such as target-gene mutations, reduced dsRNA uptake, altered RNAi machinery, increased nuclease activity, or epigenetic changes (Christiaens et al. 2020). To minimize resistance risk, strategies should include targeting multiple genes simultaneously, rotating RNAi targets, combining RNAi with mycoherbicides and mechanical control, integrating RNAi into broader Integrated Weed Management (IWM) programs, and monitoring treated populations. Multi-target RNAi approaches are particularly promising because they substantially reduce the likelihood of resistance development.

10.5 Regulatory Biosafety Requirements

Regulatory approval of RNAi-based bioherbicides requires comprehensive biosafety and environmental risk assessments. Major regulatory agencies involved include the United States Environmental Protection Agency, European Food Safety Authority, Australian Pesticides and Veterinary Medicines Authority, Central Insecticides Board and Registration Committee, and Department of Biotechnology (Petrick et al. 2016) (Table 6).

Table 6. Major Biosafety Considerations for RNAi-Based Aquatic Weed Bioherbicides

Biosafety Aspect	Potential Concern	Risk Mitigation Strategy
Off-target gene silencing	Unintended effects on non-target species	Bioinformatic sequence screening and target-specific design
Native aquatic plants	Accidental suppression of related species	Species-specific target selection and ecological testing
Aquatic biodiversity	Community-level ecological effects	Mesocosm and field biodiversity assessments
Environmental persistence	Prolonged exposure of non-target organisms	Biodegradable formulations and degradation studies
Water quality	Effects of large-scale weed suppression	Monitoring of oxygen levels and nutrient dynamics
Resistance evolution	Reduced long-term efficacy	Multi-target RNAi and integrated weed management
Human and animal safety	Potential exposure concerns	Toxicological and allergenicity evaluation
Genetically engineered fungi	Environmental release risks	Containment studies and regulatory compliance

Key data requirements typically include:

- Product characterization: target gene selection, dsRNA sequence information, manufacturing process, and quality specifications.
- Environmental risk assessment: persistence, degradation, mobility, exposure, and fate in aquatic ecosystems.
- Non-target organism testing: aquatic plants, fish, aquatic invertebrates, beneficial insects, and microorganisms.
- Human and animal safety: toxicity, allergenicity, and exposure assessments.
- Resistance management: evaluation of resistance risks, mitigation strategies, and post-registration monitoring.

For RNAi-assisted mycoherbicides based on genetically modified fungal strains, additional requirements related to microbial biosafety, environmental release, and gene flow may apply. Therefore, integrating biosafety and regulatory considerations early in product development is essential for successful commercialization.

Current scientific evidence indicates that RNAi-based bioherbicides possess a favorable biosafety profile due to their high target specificity, biodegradability, and minimal inherent toxicity. Nevertheless, comprehensive risk assessment addressing off-target effects, aquatic biodiversity, environmental fate, resistance management, and regulatory requirements remains essential for responsible development and commercialization of RNAi-based aquatic weed management technologies.

11. REGULATORY FRAMEWORK

The commercialization of RNAi-based aquatic weed bioherbicides requires compliance with evolving regulatory frameworks that evaluate product safety, efficacy, environmental fate, and non-target impacts. Because RNAi products function through sequence-specific gene silencing rather than chemical toxicity, regulatory assessments focus on molecular characterization, environmental exposure, off-target effects, ecological safety, and resistance management (Roberts et al. 2015; Petrick et al. 2016). Additional biosafety requirements may apply to RNAi-assisted mycoherbicides based on genetically modified fungal strains.

11.1 United States Environmental Protection Agency (EPA)

The United States Environmental Protection Agency regulates RNAi-based crop protection products as biopesticides under FIFRA. Regulatory evaluations include product characterization, dsRNA sequence information, mode of action, environmental fate, non-target organism safety, human health risks, and ecological exposure pathways (EPA 2013). The EPA emphasizes a weight-of-evidence approach integrating molecular, toxicological, ecological, and environmental data, with particular attention to bioinformatics-based off-target assessments (Petrick et al. 2016).

11.2 European Union – EFSA

The European Food Safety Authority provides guidance for the assessment of RNAi-based products and genetically modified organisms within the European Union. Key evaluation criteria include dsRNA sequence specificity, potential off-target effects, environmental persistence, biodiversity impacts, food and feed safety, and horizontal gene transfer potential (EFSA 2014). The EU follows a precautionary regulatory approach, requiring extensive environmental risk assessment before commercialization. For RNAi-assisted mycoherbicides involving genetically modified fungi, both RNAi-related and microbial biosafety requirements must be addressed.

11.3 Australia – APVMA

The Australian Pesticides and Veterinary Medicines Authority regulate RNAi-based agricultural products through a science-based, product-specific risk assessment framework (APVMA 2021). Evaluations focus on product composition, environmental fate, ecotoxicology, non-target organism effects, human health risks, and efficacy. Given Australia's extensive experience in invasive aquatic weed management, the APVMA framework provides valuable guidance for assessing RNAi-based weed control technologies.

11.4 India – GEAC and DBT

In India, biotechnology-derived products are regulated primarily by the Genetic Engineering Appraisal Committee and the Department of Biotechnology under the Environment (Protection) Act, 1986. Additional oversight may involve the Central Insecticides Board and Registration Committee and the Ministry of Environment, Forest and Climate Change. Although India lacks a dedicated framework for RNAi bioherbicides, future products are likely to be evaluated based on molecular characterization, environmental risk assessment, non-target testing, ecotoxicology, efficacy, and manufacturing quality standards. Establishing clear regulatory pathways will be important for commercialization in India.

11.5 Regulatory Challenges for RNAi Products

Despite rapid scientific progress, several regulatory challenges remain:

- Lack of harmonized international guidelines for RNAi products.
- Need for risk assessment frameworks tailored to gene-silencing mechanisms.
- Standardization of bioinformatics-based off-target analyses.
- Limited data and models for environmental exposure and persistence.
- Complex regulation of RNAi-assisted mycoherbicides combining RNAi, biological control, and genetic engineering.

- Public perception and acceptance of biotechnology-derived products.

Future regulatory systems are expected to adopt risk-proportionate, science-based approaches that recognize the unique properties of RNA molecules while ensuring protection of human health and the environment (Table 7).

Table 7. Regulatory Requirements for RNAi-Based Weed Management Products

Regulatory Component	EPA (USA)	EFSA (EU)	APVMA (Australia)	GEAC/DBT (India)
Product characterization	Required	Required	Required	Required
dsRNA sequence analysis	Required	Required	Required	Expected
Mode-of-action studies	Required	Required	Required	Expected
Environmental fate studies	Required	Required	Required	Required
Off-target assessment	Required	Required	Required	Required
Non-target organism testing	Required	Required	Required	Required
Ecotoxicological evaluation	Required	Required	Required	Required
Human health risk assessment	Required	Required	Required	Required
Environmental risk assessment	Required	Required	Required	Required
Resistance management plan	Recommended	Recommended	Recommended	Emerging requirement
Field efficacy data	Required	Required	Required	Required
Manufacturing quality standards	Required	Required	Required	Required
GMO-specific assessment*	Case-specific	Case-specific	Case-specific	Required when applicable

*Applicable primarily to RNAi-assisted mycoherbicides involving genetically engineered fungal strains.

Key Regulatory Considerations for RNAi-Based Aquatic Weed Bioherbicides

1. Demonstration of high target specificity.
2. Comprehensive off-target risk assessment.
3. Environmental fate and degradation studies.
4. Evaluation of impacts on aquatic biodiversity.
5. Long-term resistance management strategies.
6. Compliance with biotechnology and pesticide regulations.
7. Post-registration environmental monitoring.

Regulatory approval represents one of the major determinants of commercial success for RNAi-based aquatic weed bioherbicides. Although regulatory frameworks continue to evolve, most authorities recognize the favorable safety profile of dsRNA molecules and are moving toward science-based assessment approaches emphasizing target specificity, environmental fate, and ecological safety. Development of harmonized international guidelines and dedicated regulatory pathways for RNAi technologies will be essential for accelerating commercialization of next-generation RNAi-assisted weed management products.

12. CHALLENGES AND FUTURE PERSPECTIVES

RNAi-based aquatic weed bioherbicides offer a highly promising, species-specific, and environmentally friendly approach to weed management. Integration with fungal biocontrol agents, nanotechnology, and precision agriculture could further enhance efficacy and sustainability. However, challenges related to RNA stability, delivery efficiency, production costs, commercialization, and regulatory approval must be overcome before large-scale adoption. Advances in molecular biology, genomics, synthetic biology, artificial intelligence, and formulation science are expected to accelerate the development of practical RNAi-based weed management technologies.

12.1 RNA Stability

The limited environmental stability of dsRNA remains a major constraint for field applications. In aquatic ecosystems, degradation by ribonucleases, UV radiation, microbial activity, temperature fluctuations, dilution, and water movement can significantly reduce efficacy (Christiaens et al. 2020). Nanotechnology-based carriers such as chitosan nanoparticles, BioClay, layered double hydroxides (LDHs), lipid nanoparticles, and biodegradable polymers have shown considerable potential for protecting dsRNA and enabling controlled release (Mitter et al. 2017). Future research should focus on developing cost-effective formulations with prolonged stability under aquatic conditions.

12.2 Delivery Efficiency

Efficient delivery of dsRNA into weed tissues is essential for successful gene silencing but remains a significant challenge due to barriers such as plant cuticles, cell walls, and membranes (Fletcher et al. 2020). Uptake efficiency varies among aquatic weed species depending on their morphology and growth habits. Current strategies to improve delivery include nanocarriers, liposomal systems, controlled-release formulations, extracellular vesicles, smart polymers, and fungal-mediated RNA delivery. Among these, RNAi-assisted mycoherbicides are particularly promising because fungal pathogens naturally penetrate plant tissues and may facilitate direct transfer of RNA molecules into target cells. Improving delivery efficiency will be critical for enhancing field performance and reducing application rates.

12.3 Cost of dsRNA Production

The commercial viability of RNAi-based bioherbicides largely depends on reducing dsRNA production costs. Although advances in bacterial fermentation, recombinant yeast systems, cell-free synthesis, synthetic biology, and bioprocess optimization have substantially lowered costs, further reductions are needed for broad agricultural adoption (Bennett et al. 2020). Continued innovations in industrial biotechnology and large-scale manufacturing are expected to improve scalability, consistency, and economic competitiveness. Recent advances in industrial-scale dsRNA production technologies are expected to further improve the economic viability of RNAi-based bioherbicides (Singh and Pandey 2026b).

12.4 Large-Scale Commercialization

Successful commercialization requires more than technical efficacy; it also depends on scalable production, stable formulations, regulatory approval, distribution systems, and user acceptance. Major challenges include limited field validation, variable environmental performance, production costs, shelf-life requirements, and regulatory uncertainty. For aquatic weed management, large-scale application in lakes, reservoirs, canals, and wetlands adds further operational complexity. Collaboration among research institutions, industry, regulators, and end users will be critical for accelerating commercialization.

12.5 Integration with Artificial Intelligence and Precision Agriculture

The integration of RNAi technology with artificial intelligence (AI), machine learning, remote sensing, and precision agriculture offers significant opportunities to enhance weed management. AI can assist in target gene discovery, dsRNA design, efficacy prediction, off-target assessment, and genomic analysis. Precision agriculture tools such as drones, satellite imaging, automated weed mapping, and site-specific application systems can improve monitoring and deployment of RNAi-based bioherbicides. In aquatic ecosystems, UAVs, autonomous platforms, and remote sensing technologies may enable precise detection and targeted treatment of invasive weeds. The convergence of RNAi, AI, and precision agriculture is expected to play a major role in the future of sustainable weed management. Integration of RNAi technologies with advanced formulation systems and precision agriculture platforms may enhance field efficacy and reduce operational costs (Singh and Pandey 2026a).

12.6 Future Research Priorities

To advance RNAi-based aquatic weed bioherbicides toward commercialization, several key research areas require attention:

- Novel target gene discovery through genomic and transcriptomic analyses.
- Multi-gene RNAi strategies to enhance efficacy and minimize resistance development.
- Advanced delivery systems, including nanocarriers, controlled-release formulations, extracellular vesicles, and fungal-mediated delivery platforms.
- RNAi-assisted mycoherbicides, with emphasis on trans-kingdom RNAi and fungal RNA delivery mechanisms.
- Long-term environmental risk assessment to evaluate ecological impacts and environmental fate.
- Regulatory science for development of harmonized risk assessment and approval frameworks.
- Integration with digital agriculture, including AI, remote sensing, and automated application technologies.

RNAi-based aquatic weed bioherbicides have the potential to become highly specific, environmentally friendly, and sustainable alternatives to conventional herbicides. Continued advances in genomics, synthetic biology, nanotechnology, fungal biotechnology, artificial intelligence, and precision agriculture are expected to overcome current limitations related to RNA stability, delivery efficiency, production costs, and regulatory approval. Although significant challenges remain, ongoing interdisciplinary research is likely to accelerate the transition of RNAi-based technologies from experimental systems to commercially viable weed management tools, contributing to the sustainable control of invasive aquatic weeds and the restoration of freshwater ecosystems. Advances in fungal biotechnology, phytotoxin discovery, and molecular weed management are expected to accelerate the development of next-generation bioherbicides (Singh and Pandey 2020). Continued innovation in target discovery, formulation science, and delivery technologies will be critical for successful commercialization of RNAi-based weed management products (Singh and Pandey 2023; Singh and Pandey 2026a; Singh and Pandey 2026b).

13. CONCLUSION

RNA interference (RNAi) represents a highly promising approach for the sustainable management of invasive aquatic weeds through sequence-specific silencing of essential genes. Advances in dsRNA production, nanotechnology-based delivery systems, trans-kingdom RNAi, and fungal biotechnology have greatly improved the feasibility of RNAi-based bioherbicides. In particular, RNAi-assisted mycoherbicides combine the natural pathogenicity of fungal biocontrol agents with the precision of gene silencing, offering enhanced efficacy, improved host specificity, and reduced environmental impact.

Despite these advances, challenges related to dsRNA stability, delivery efficiency, production costs, biosafety assessment, regulatory approval, and field validation must be addressed before large-scale commercialization. Continued progress in genomics, synthetic biology, nanotechnology, artificial intelligence, and precision agriculture is expected to overcome these limitations. Recent studies have highlighted the growing potential of RNAi-based bioherbicides and advanced sprayable delivery systems for sustainable weed management, providing a strong foundation for future development of RNAi-assisted mycoherbicides (Singh and Pandey 2023; Singh and Pandey 2026a; Singh and Pandey 2026b).

Overall, RNAi-based bioherbicides and RNAi-assisted mycoherbicides have the potential to become transformative tools for environmentally sustainable aquatic weed management, contributing to the restoration of freshwater ecosystems, conservation of biodiversity, and long-term control of invasive aquatic weeds.

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