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dsRNA-Biopesticides for Crop Protection

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Preface

Pests and pathogens of economic significance are serious threat to agriculture production. Use of chemical pesticides has played a key role in reducing crop loss and improving crop production. However, long-term and non-judicial use of chemical pesticides have caused adverse effects on the environment and human health. One of the most promising and up-coming alternatives of chemical pesticides is double stranded RNA (dsRNA)-biopesticides. The approach works by triggering RNA interference (RNAi) in plant through exogenous application of dsRNA. The RNAi-based defence mechanism is being utilized as a powerful non-GMO strategy to develop disease resistance in crop plants against pests and pathogens. However, there are many challenges and issues for its wider application as an alternative crop protection technology.

In order to discuss the issues of dsRNA-biopesticides and to bring out the prospects and policy guidelines, the National Academy of Agricultural Sciences (NAAS) organized a Brainstorming Session (BSS) on “dsRNA-based Biopesticides for Crop Protection” in a hybrid mode and more than 140 participants including scientists and research scholars from various parts of the country attended the session. The presentations, discussion and debate on the prospects and current scientific and regulatory status resulted in valuable insights and recommendations.

On behalf of the Academy, I appreciate the efforts of Dr. Bikash Mandal (Convener) for bringing out this policy paper, which I hope will help in developing implementable strategies for safe and effective plant protection measure ensuring sustainable crop production goal. My thanks are due to the distinguished Reviewers (Prof. Neena Mitter & Dr. B.L. Patil) for their valuable comments and Editors (Dr. V.K. Baranwal & Dr. R. K. Jain) for critical editorial support.

September 2025 **(Himanshu Pathak)**
New Delhi *President, NAAS*

dsRNA-Biopesticides for Crop Protection

1. INTRODUCTION

Several management approaches are practiced to minimize the losses caused by pests and pathogens, of which the chemical management approach by using pesticides plays a key role in food production. However, long-term and non-judicial use of chemical pesticides created adverse effects on the environment and human health. One of the most promising and up-coming alternatives of chemical pesticides against insects, fungi, mites, nematodes and viruses is double-stranded RNA (dsRNA)-biopesticides. The approach works by triggering RNA interference (RNAi) through exogenous application of dsRNA (Voloudakis *et al*., 2022).

The RNAi has been extensively used against virus and insect pests through transgenic approach, which is also referred as host-induced gene silencing (HIGS), where the host plant is genetically modified with the foreign nucleotide sequence to induce gene silencing. The technology was demonstrated to be highly effective against pests and pathogens in a variety of food and fibre crops, but environmental, ethical, political, and regulatory issues constrained its wide adaptability. As an alternative to transgenic or HIGS technology, dsRNA-biopesticides are gaining importance, where RNAi can also be induced against pests and pathogens through exogenous application of dsRNA without the need of genetic modification. The exogenous dsRNA approach, being a non-GMO in nature, provides distinct advantages over HIGS. The dsRNA can be exogenously applied by various methods based on the type of target organisms and purposes. Spray application of dsRNA is one of the most convenient methods of application for inducing RNAi, which is referred as spray-induced gene silencing (SIGS) (He *et al*., 2024). During last two decades, the exogenous dsRNA mediated protection has been documented in controlling different pests and pathogens including insects, fungi, nematodes, weeds and viruses (Kumari *et al*., 2017; Christiaens *et al*., 2020; Hough *et al*., 2022; Voloudakis *et al*., 2022; Zhang *et al*., 2022; Mann *et al*., 2023; Singewar and Fladung, 2023; Bairwa *et al*., 2024; Venu *et al*., 2025). The dsRNA-biopesticide is referred here as a class of non-transgenic or non-GMO RNAi-biopesticides and therefore are distinct from other class of RNAi-biopesticide that works through HIGS (GMO) approach.

Despite the successful experimental demonstrations of dsRNA-biopesticides against various pests and pathogens, further refinements are needed for its utilization in the practical field situations. It is to be noted that numerous laboratories worldwide are actively involved in dsRNA research for developing chemical alternative and non-GMO plant protection measures. In India too, many laboratories have developed significant capacity on handling this emerging approach of crop protection. The National Academy of Agricultural Sciences (NAAS) made a maiden effort in India for brainstorming this emerging topic to assess the current status at the national and international level. The present policy paper, based on the deliberations during the brainstorming session, includes the current approaches of crop protection, dsRNA working principle, target genes, dsRNA production, dsRNA delivery, dsRNA-biopesticides developmental process, risk, biosafety, regulatory framework and major recommendations. Deployment of dsRNA-biopesticides is expected to have a greater impact for the innovative management of pests and pathogens especially for the difficult pathogens like viruses. The policy support is required to encourage the development and adoption of dsRNA-biopesticides for managing various biotic stresses in practical field conditions for strengthening sustainable production practices.

2. CURRENT METHODS AND ISSUES OF CROP PROTECTION

Of all the crop protection measures, resistant genotype is the most suitable approach. The genetic and biotechnological approaches of crop protection are highly effective, but the successful field application involves a long drawn effort to overcome scientific, financial and regulatory issues.

The chemical approach with synthetic agro-chemicals is highly effective for direct control of pests and pathogens. However, indiscriminate and long-term use of pesticides results in to emergence and outbreak of pesticide resistant variants of pests and pathogens. Increasingly high bench-mark of regulatory demands, ecological and health issues, pest resistance, and high developmental costs are the major challenges in chemical pesticides industries. For the safe and sustainable production of food crops, the chemical substitutes of crop protection are advocated.

The biologicals in crop protection are referred as biopesticides that include agents or compounds or genes sourced from plants, animals and microorganisms. Microbial pesticides include biocontrol agents such as fungus, bacteria, viruses, nematodes, protozoa, and other microorganisms. Biochemical pesticides are naturally occurring compounds and have many relative advantages such as easy to source, specific in action, cost effective and environmentally sustainable having no effects of residue or greenhouse gas emission. Despite promise of biopesticides as sustainable crop protection measure, consistency in quality and efficacy are yet the major issues of attaining as effective substitute of chemical pesticides.

3. BASIC WORKING PRINCIPLE OF dsRNA TECHNOLOGY

The loss of gene function through RNA degradation was observed in plant, fungus and virus in 1990s, however the RNAi phenomenon was first described in nematode (*Caenorhabditis elegans*), where injection of dsRNA in *C. elegans* led to sequence specific silencing. The dsRNA-biopesticide in plant system works by limiting the proliferation of the invading pests and pathogens by interfering the specific gene functions through RNAi pathway. In case of weeds, insects, and mites, etc, dsRNA-biopesticide has the ability to eliminate these unwanted organisms through directly silencing of critical genes required for their growth and development. The dsRNA-biopesticides are applied exogenously or topically on the surface of the host plant or the target organism, which enters in the cells and disable the target gene function through induction of RNAi.

The RNAi takes place through four major steps, where (i) the dsRNA is recognised and cleaved by cellular nuclease known as Dicer-like enzymes (DCL) in to duplex small interfering RNA (siRNA) of 21-25 nucleotides; (ii) the siRNA undergoes modification at the 3' overhang, where 2'-O is methylated to protect the siRNAs from degradation by the cellular nuclease. Subsequently, the modified duplex siRNA interacts with the Argonaute (AGO), and other associated proteins and forms an RNA-induced silencing complex (RISC), where following unwinding of the duplex siRNA the sense strand is degraded, while the antisense strand remains to form active RISC; (iii) the RISC having antisense single stranded siRNA capture the target mRNA transcripts in the cell through complementary base-pairing and forms ds mRNA, which is subsequently cleaved into siRNA. As a result, the mRNA is silenced and no longer is able to produce the desired protein; and (iv) at this stage, siRNA is used as primers for RNA dependent RNA polymerase-I (RDR) to generate further dsRNA from the target mRNA, which becomes the target of DCL cleavage. The RDR activity, therefore, amplify the siRNA pool through generating secondary siRNA expanding the cellular defence window against the invading pathogens.

4. THE dsRNA TARGET GENES

Identification of single or multiple target gene(s) are crucial for the success of dsRNA-biopesticides. Several target genes have been demonstrated to be effective against pests and pathogens. The length of target gene, RNAi cleavage sites and non-off target effects are important considerations while designing for dsRNA. It has been known that the length of dsRNA ranging from 300 to 1000 nucleotides works well against various target pests and pathogens. As the dsRNA-biopesticides works through sequence homology, the siRNA generated from the target organism may find similarities with the host gene(s) resulting in undesirable silencing. Care needs to be taken to avoid high sequence similarity with the host gene while designing the dsRNA target through sequence analysis of both the target and the host organisms by using NCBI BLAST database (http://www.ncbi.nlm.nih.gov/BLAST), siRNA finder (https://github.com /snowformatics/siFi21), ERNAi (https://www.dkfz.de/signaling/e-rnai3/), dsCheck (http://dscheck.rnai.jp/) etc. The potential target genes used in dsRNA mediated protection against virus, fungus, insect, nematode and weed are briefly described below:

4.1. Viruses

Plant viruses are cellular parasites and have limited genes for performing essential functions such as replication, encapsidation, movement and suppression of host defence. All these genes alone or in combination were shown to be the good targets to disrupt their replication, movement and pathogenesis. The viral suppressor of gene silencing (VSR) that helps virus to overcome host defence through suppressing RNAi have been identified in a large number of diverse plant viruses. VSR genes has been shown to be the effective target to prevent viral disease development in different plant species. The VSR gene, HC-Pro of potyvirus is shown to provide significant protection in cucurbits (Kaldis *et al*., 2018). The other viral genes such as CP (coat protein), Rep (replication) and MP (movement protein) are the other potential targets against viruses (Vadlamudi *et al*., 2020; Patil *et al*., 2021).

4.2 Fungi

So far, limited studies have been undertaken to assess the potential target genes against fungal pathogens. However, the genes associated with the fungal growth, survival and pathogenicity would be the potential targets to prevent development of fungal disease in crop plants. Targeting several fungal genes are shown to significantly reduce the disease symptoms. Some of the promising fungal genes are *28S ribosomal* RNA,*β-tubulin*, translation elongation factor 1-α, Cytochrome P450 monooxygenase, MAPK, acetyl CoA-transferase and glycine cleavage system-H (Donaldson and Saville, 2013).

4.3 Insects

The dsRNA target genes for topical and oral applications against insects are those involved in energy metabolism, synthesis of essential cellular components, hormone homeostasis, chitin metabolism, the digestive system, immunity, detoxification, insecticide resistance, and other processes. More than 100 genes targeting various functions have been identified for pests control (Lu *et al*., 2023). For the growth and survival of insect, energy metabolism is critical. Genes responsible for energy metabolism in insect such asvacuolar ATPase and arginine kinase are known to cause mortality. dsRNA-biopesticides are effective against Coleopteran, Hemipteran, Lepidopteran, Dipteran, and Orthopteran insects and mites (Lu *et al*., 2023).

4.4 Nematodes

In case of nematode the genes critical for survival and parasitism are targeted for the reduction of nematode fitness and infestation. Nine FMRFamide-like peptides (FLPs) genes (flp-1, flp-3, flp-6, flp-7, flp-11, flp-12, flp-14, flp-16 and flp-18) and a partial neuropeptide receptor gene (flp-18) of *Meloidogyne incognita* were identified as broad-spectrum RNAi targets to manage the root-knot nematode problem in rice and wheat crops (Kumari *et al*., 2017). The neuropeptide genes such as flp-32c, flp-32p and flp-2, which affect the locomotory behaviour of juveniles of potato cyst nematode, *Globodera pallida* were promising targets (Bairwa *et al*., 2024).

4.5 Weeds

There are only limited studies available for application of dsRNA-biopesticides for weed management. The genes encoding chlorophyll a/b-binding proteins, which are essential for the growth of *Mikania micrantha*, an invasive tropical weed were demonstrated to be the potential target for controlling *M. micrantha* (Mai *et al*., 2021). In 2025, Green Light Biosciences developed dsRNA-bioherbicide for managing horseweed (*Conyza canadensis*) (https://www.greenlightbiosciences.com/articles/breakthrough-in-rna-herbicide-development).

5. dsRNA PRODUCTION

The economic and large-scale production of dsRNA is important for the successful utilisation of the Biopesticides. The production process of dsRNA mainly involves two major steps: synthesis and its purification. The dsRNA corresponding to the target gene can be synthesised either by *in vitro* transcription method or by *in vivo* method of expressing transcripts in bacterial system.

In vitro method of production of dsRNA, generally, bacteriophage T7 DNA dependant RNA polymerase (DdRp) is used for enzymatic synthesis of RNA over DNA template. *In vitro* method generates high quality dsRNA for precision down-stream experimentations, however, it is expensive to produce large quantity of dsRNA through *in vitro* method for agricultural usages (Voloudakis *et al*., 2022).

In vivo method, where dsRNA is produced in bacteria is cost-effective and scalable. In this method, the target sequence is amplified by PCR and then ligated into L4440 vector containing T7 polymerase promoter sites at both ends of the cloning site. The recombinant plasmid is then transferred into *E. coli* strain HT115 (DE3) lacking the RNase III enzyme activity.

Other than *E. coli*, dsRNA can also be produced in other bacterial and yeast species. *Pseudomonas syringae*, *Corynebacterium glutamicum* and *Bacillus thuringiensis* were utilised as alternative bacterial species for the production of dsRNA (Hough *et al*., 2022). The other microbial system that was used for production of dsRNA through *in vivo* method is yeast. Recent studies showed that the yeast species, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* were potential alternative systems for the production and application of dsRNA-biopesticide. The modified yeast has advantages as live bait agent as it lacks the RNAi machinery supporting the processing of dsRNA and also it is biologically safe for human consumption.

The cost of dsRNA is an important aspect of viability of this approach for crop protection. In the early days, the high cost of synthesis of dsRNA was a major limitation in running laboratory experiments. The advancement of dsRNA synthesis protocols contributed to significant cost cutting (US$ 1-2/g of dsRNA) and made the technology feasible for field-level application. Large scale and economic production system of dsRNA is now available using engineered bacteria and yeast in fed-batch fermenter (Zotti *et al*., 2018).

6. DELIVERY OF dsRNA-BIOPESTICIDES

A wide variety of delivery methods of dsRNA in the plant system are known, e.g. syringe infiltrations, vacuum infiltration, injections, root dipping, seed soaking, petiole absorption, spraying, and mechanical rubbing. Application of the delivery method depends on the type of host, type of pest and pathogens, site of application, amount or volume of dsRNA to be delivered, etc.

For delivering dsRNA in the plant leaf, high-pressure spraying is convenient and more effective for penetrating dsRNA. For woody plants, trunk injections of dsRNA can be achieved using trunk drilling equipment and air gun bombardment devices. dsRNA is prone to nuclease degradation in the cytoplasm. Carriers such as carbon dots, single-walled carbon nanotubes, and clay nanosheets are effective in improving the stability and effectiveness of dsRNA in plant. Conjugation of dsRNA with nano-carriers like chitosan, carbon quantum dot, and amine-functionalized silica nanoparticle were effective in silencing target genes in different organisms including plant, shrimp, pod-borer and mosquito. Clay nanosheets (Bioclay) conjugated with dsRNAs are shown to reduce infection of pepper mild mottle virus and cucumber mosaic virus in tobacco and cowpea plants (Mitter *et al*., 2017).

Oral ingestion of dsRNA can be administered directly to insect through artificial diet and indirectly spraying on leaf surface or soaking plant roots. The method is more suitable for RNAi in the midgut tissues. dsRNAs also can be orally delivered in insect through genetically modified microbe. Successful gene silencing in *Drosophila suzukii* was demonstrated through oral delivery of genetically modified yeast (*Saccharomyces cerevisae*).

A breakthrough study demonstrated delivery of dsRNA derived from arginine kinase to citrus plant system through root drench and injections. A single treatment of about 2.0 g dsRNA in 15 liters of water showed seven weeks long persistence of dsRNA in a grown up six-year-old Mexican lime plants. The study demonstrated useful field application of dsRNA-biopesticide to reduce infestation of psyllid in citrus plant (Hunter *et al*., 2012).

Lipid-based artificial nanovesicle has been demonstrated to be effective delivery method of dsRNA through spray application against the fungal pathogen, *Botrytis cinerea* in tomato and grape (Qiao *et al*., 2023). In this method, the encapsulated dsRNA, provided stronger and prolonged protection. The nanovesicle has the advantage of overcoming the instability issues of dsRNA by protecting the dsRNA from nuclease degradation and removal by washing from the application surface.

7. EFFECTIVENESS OF dsRNA-BIOPESTICIDES

RNAi is well known for cellular gene silencing in plants and animals, and has largely been used in agriculture through transgenic approach for the management of pests and pathogens in crop plants. During more than last two decades, several studies have shown successful induction of RNAi through the exogenous application of dsRNA for the inhibition of infection caused by virus, fungus and insects in plants. The effectiveness of exogenous application of dsRNA was also documented in controlling insect pests and weeds.

7.1 Against Plant Virus

The first evidence of exogenous application of dsRNA through mechanical rubbing on the leaf surface against virus was demonstrated in 2001 (Tenllado and Diaz-Ruiz, 2001). Subsequently, foliar application of dsRNA was found effective against diverse plant viruses in many different plant species. The level of efficacy widely differed (14-99%) based on the type of plant species, virus and doses of dsRNA used. Some studies reported excellent protection of virus through exogenous application of dsRNA. The dsRNA prepared from coat protein and HC-Pro genes of papaya ring spot virus (PRSV, Potyvirus) provided 81-100% protection in papaya against different isolates of PRSV (Vadlamudi *et al*., 2020).

Begomovirus being DNA containing plant virus appeared to be less responsive to dsRNA treatment. However, spray application of cocktail of dsRNAs containing three suppressor genes, C2, V2, and C4 of Chilli leaf curl virus (ChiLCV, family *Geminiviridae*) reduced disease incidence up to 66.7% in tobacco plants till two weeks (Singh *et al*., 2021).

In order to enhance cellular delivery, dsRNA is conjugated with the nanocarriers. Clay-nanosheets (Bioclay) conjugated dsRNA provided protection for at least 20 days in tobacco and cowpea plants against pepper mild mottle virus and cucumber mosaic virus (Mitter *et al*., 2017). Although, a large body of literature is available on lab scale efficacy of dsRNA against different plant viruses, there is hardly any field data to assess the usefulness under field conditions.

7.2 Against Fungus

The potential of dsRNA-biopesticide against fungus (subsequently referred as dsRNA-biofungicide) has been demonstrated against more than a dozens of fungal pathogens, including *Botritis cinerea, Fusarium graminearum, Sclerotinia sclerotiorum, F. asiaticum, F. oxysporum, Phakopsora pachyrhizi, Aspergillus niger* and *Rhizoctonia solani* (Mann *et al*., 2023). The major limitation in this approach against fungus is short half-life and active uptake of dsRNA by the invading fungus and also the host plant. The dsRNA-based fungicide appears to be more effective against fungal pathogens that colonise on the surface of plant parts.

7.3 Against Insect and Mite

In general, dsRNA-biopesticide is more effective against insects belonging to Coleoptera than Hemiptera and Lepidoptera. Plant surface applied dsRNA has shown to be effective against chewing insects. For the sucking pests, dsRNA needs to be systemically distributed in the plant system. Several studies showed silencing of target genes, reduction in fecundity and or high mortality in a dozen of aphid species infesting barley and wheat. Several studies showed that dsRNA-biopesticide delivered through injection in forest tree resulted up to 100% mortality of moth, borer and beetle. The advancement of research on dsRNA-biopesticide firming the ground to develop commercial product for field application. The first marketable dsRNA-biopesticide product is developed by GreenLight Biosciences against the Colorado potato beetle (Welsh, 2024).

Honey bee (*Apis mellifera*) is seriously affected by the varroa mite (*Varroa destructor*). The field study first time demonstrated successful reduction of varroa mite without affecting the survival of honey bee by feeding mixture of dsRNA containing acetyl-CoA carboxylase, Na+/K+ ATPase and end-ochitinase genes from *V. destructor* under natural rearing conditions (Bortolin *et al*., 2025). GreenLight Biosciences developed another dsRNA biopesticide product known as Vadescana against varroa mite. In May 2025, the U.S. Environmental Protection Agency has proposed the registration of Vadescana as a sustainable alternative to the traditional chemical pesticide against mite infestation in honeybee (https://www.epa.gov/pesticides/epa-proposes-register-new-pesticide-varroa-mite-control).

7.4 Against Nematode

The effective delivery of dsRNA to the target nematode through plant system or directly to the root zone is a major challenge in the application of dsRNA-biopesticide for nematode management in agriculture. Application of dsRNA through foliar spray can be targeted to foliar and stem nematodes such as *Bursaphelenchus xylophilus*, *Ditylenchus dipsaci*, *D. Angustus* and *Aphelenchoides besseyi*. There are few promising studies that showed potential of dsRNA-biopesticide against important crop nematodes. Root dipping treatment of rice seedlings in dsRNA-flp12 solution showed aberrant behaviour of *M. graminicola* juveniles (Kumari *et al*., 2017). In another study, drenching of potato cultivar Kufri Jyoti with dsRNA-kaolinite nanoclay formulation targeting neuropeptide genes, flp-32c, flp-32p and flp-2 successfully induced deformation and premature death of juvenile-2 of *Globodera pallida*, an obligate sedentary endoparasitic cyst nematode of potato (Bairwa *et al*., 2024). This study signifies the potential of developing nano-carrier formulation of dsRNA-bionematicide for soil drenching application.

7.5 Against Weed

dsRNA-bioherbicide is an attractive approach for weed management. However, at present, limited studies are available targeting weeds. Identification of candidate dsRNA with high selectivity and minimal off-target effect is critical in developing dsRNA-bioherbicide for a specific weed. A pioneering study to manage an invasive weed, *Mikania micrantha* through foliar spray of dsRNA showed the potential of developing dsRNA-Bioherbicide (Mai *et al*., 2021). In this study, dsRNA was derived from genes encoding the chlorophyll a/b-binding proteins of *M. micrantha.* Direct spray application of dsRNA on *M. micrantha* leaves resulted in yellowing and eventually death of plan. In 2025, GreenLight Biosciences made an announces of developing dsRNA-bioherbicide for managing horseweed (*Conyza canadensis*) (https://www.greenlightbiosciences.com/articles/breakthrough-in-rna-herbicide-development).

8. RISK, BIOSAFETY AND REGULATORY FRAMEWORK

The advancement of research on dsRNA-biopesticides is firming the ground to develop commercial products for field applications. The first marketable dsRNA-biopesticide product is Calantha, a sprayable formulation developed by GreenLight Biosciences against the Colorado potato beetle (Welsh, 2024). Another field study first time demonstrated successful reduction of varroa mite *(Varrora destructor*) without affecting the survival of honey bee by feeding mixture of dsRNA containingacetyl-CoAcarboxylase, Na+/K+ ATPase, and endo-chitinase genes from *V. destructor* under natural rearing conditions (Bortolin *et al*., 2025). GreenLight Biosciences has developed another dsRNA biopesticide product known as Vadescana against varroa mite. In May 2025, the U.S. Environmental Protection Agency has proposed the registration of Vadescana as a sustainable alternative to the traditional chemical pesticide against mite infestation in honeybee (https://www.epa.gov/pesticides/epa-proposes-register-new-pesticide-varroa-mite-control).

Despite rapid advancements in dsRNA-biopesticides against pests and pathogens, there is a significant gap in understanding its environmental fate and biosafety issues. The growing body of research and the potential future market release of exogenous RNAi technologies pose challenges for regulatory bodies, prompting the need for stringent regulations. Like any cutting-edge technology, this one comes with both advantages and drawbacks. Among its benefits are efficient pest protection, precise targeting to minimize off-target effects, and environmentally friendly pest control, reducing harm to non-target organisms and pollution, and less expensive and less time consuming for developing a product compared to chemical pesticides. Overall, the exogenously induced RNAi provides effective pest protection while mitigating the adverse effects typically linked with conventional chemical pesticide.

The state-of-the-art dsRNA technology, characterized by its ability to precisely target gene expression in various organisms, presents several associated risks due to its rapid growth and increasing commercial interest. Despite the specificity of dsRNA molecules, there is a risk of unintended off-target effects. The widespread use of dsRNA technology, particularly in agriculture for pest control, raises concerns about its environmental impact. The application of dsRNA technology raises broader ethical and social considerations, including issues related to genetic modification, intellectual property rights etc. Exogenous RNAi applications in plants are currently evaluated and regulated under the existing frameworks for genetically modified organisms (GMOs). However, there is a pressing requirement to establish science-based risk assessment protocols specifically tailored for topical RNAi applications within the current legislation governing plant protection products.

In 2018, the New Zealand Environmental Protection Authority (EPA) made a decision that places the use of externally applied dsRNA molecules on eukaryotic cells or organisms outside the scope of legislation on new organisms. Consequently, risk assessments for such treatments in the open environment were deemed unnecessary. The decision was grounded in the EPA's determination that this treatment does not result in the creation of new or genetically modified organisms. Furthermore, the EPA concluded that dsRNA was not heritable and did not act as a mutagen.

In contrast, countries like the USA, Europe, and Australia have established both moderate and stringent regulations for exogenous dsRNA. The European Union classifies it within the category of PIPs under biopesticides regulations. For instance, in the EU, any pesticide intended to protect crops or other useful plants, known as a plant protection product (PPP), requires authorization for market placement under Regulation (EC) No 1107/2009 (EC, 2009). According to EU regulations, PPPs utilizing dsRNA are currently classified as chemical PPPs. In the United States, specific data requirements for sprayed or externally applied dsRNA-biopesticides have not yet been enacted. However, the EPA's regulations for these pesticides are based on the biochemical pesticide requirements outlined in the legal framework of Pesticide Regulation40 CFR 158. Similarly, Australia regulates dsRNA-biopesticides as chemical pesticides under the legal framework of the Agricultural and Veterinary Chemicals Code Act 1994.

The OECD Conference, (April 2019) on Regulation of externally applied dsRNA-based products for management of pest held in Paris (2019) addressed regulatory needs, risk assessment, and policy implications (https://www.oecd.org/content/dam/oecd/en/topics/policy-sub-issues/pestices-and-biocides/oecd-conference-on-rnai-based-pesticides/conference-on-rnai-based-pesticides-programme.pdf).It facilitated collaboration among policymakers, academia, and industry, aiming to guide research funding and harmonize regulatory approaches. Outputs include Working Documents on environmental and human health risk assessments, offering recommendations for regulating externally applied dsRNA-biopesticides in agriculture while considering potential impacts on health and the environment.

9. RECOMMENDATIONS

1. Enhancing technical capacity and expanding area of focus of dsRNA-biopesticide research

The dsRNA-biopesticide research in India appears to be more focused on plant viruses and insects. Greater focus of study is necessary against fungi, nematodes and weeds. dsRNA-biopesticides having a wider potential, there is a need for continued research and collaboration to unlock its full potential for sustainable pest and pathogen management in diverse agricultural landscapes.

2. Improvement of the design of dsRNA with a combination of multiple targets for higher protection

As the dsRNA-biopesticides works on the principle of degradation of mRNA responsible for translation of protein that supports the basic functions for growth and development of pest and pathogens, it is imperative to identify the most effective target sequence. Studies showed greater efficacy when combined with multiple target genes or cocktail dsRNA (Singh *et al*., 2021). During the cellular processing of dsRNA, DCL generates a large pool of siRNA of which only a few siRNAs are effective siRNAs (esiRNA) that perform AGO-catalyzed cleavage, resulting effective gene silencing. It is necessary to functionally characterise esiRNA to develop edsRNA for higher protection against pests and pathogens.

3. Developing methods of sustained delivery of dsRNA for better efficacy in field situation

More research is necessary to develop sustained delivery system that is feasible in large field cultivation situation. One promising and economic method of continuous delivery of dsRNA in plant tissues is through symbiotic microbial delivery system. Such symbiotic microbes are modified for producing specific dsRNA and allowed to colonize the treated plant, where the dsRNA is continuously delivered to the plant cells.

4. Developing formulation for enhancing stability and window of efficacy

Concerted efforts are required to utilise various synthetic and natural polymer- and peptide-based carriers to overcome the specific hurdles such as aquatic environment, strong alkaline condition of insect gut and plant surface environments to cross the cell wall barrier. The other approaches such as use of biodegradable nano-carrier and encapsulated dsRNA with mini cells and inactivated whole organism need greater attention for the development of formulation for better stability and uptake of dsRNA in the target organisms.

5. Greater focus on field experiments

Over all, there is a serious lacuna of field data to know the doses/rate, cost, stability, persistence, uptake, systemic activation of RNAi state, durability etc. The field experiments addressing the challenges in a real-world setting need to be undertaken.

6. Establishing a biosafety and regulatory framework for exogenous dsRNA-biopesticides

The regulatory status of exogenous dsRNA-biopesticides so far is not yet well established in the different countries. There is a wide difference in perception on the application of exogenous dsRNA-biopesticides. For example, New Zealand adopted a liberal stance, the USA and Australia have a moderate approach and the EU is stringent on regulatory approach. The dsRNA in EU and Australia is considered as chemical and is subjected to pesticide regulation act, whereas, in USA, it is considered as a biochemical. The clear recognition of the definition of exogenous dsRNA is required to establish an internationally acceptable distinction between biochemical and chemical pesticide categories.

Biopesticides are primarily distinguished from chemical pesticides by their origin from biological materials such as animals, plants and microbes, or their metabolites and genetic materials. Broadly, biopesticides are sub-grouped as microbial, botanicals, semiochemicals, natural enemies, PIPs and biochemical pesticide. In India, biopesticides are regulated by the Central Insecticides Board and Registration Committee under the Insecticides Act of 1968 and the Insecticides Rules of 1971. There are 970 biopesticide products registered in India, which mostly fall under the sub-categories of microbial and biochemical pesticides. The guidelines for registration of biopesticides like botanical and microbial pesticides are available (https://ppqs.gov.in/en/divisions/cib-rc/guidelines). However, so far, there is no specific regulatory framework for guidelines for registration of exogenous dsRNA-biopesticide in India.

As dsRNA is sourced from the genetic material of the target pests and pathogens, it is recommended to consider it as a biochemical pesticide. The experts during the debate and discussion suggested on the nomenclature of the product category as dsRNA-Bioprotectant instead of biopesticide. Considering the significance of the chemical alternative crop protection approach, it is recommended to establish the regulatory guidelines on dsRNA-biopesticide/dsRNA-bioprotectant, which will encourage the R&D process to develop commercial products of dsRNA-biopesticide/dsRNA-bioprotectant for the sustainable agriculture production practice in the country.

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