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DNA INSECTICIDE: AN EMERGING CROP PROTECTION TECHNOLOGY

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ABSTRACT

The agriculture industry faces a challenge in balancing the need for pest management and environmental protection. This review describes DNA insecticides, composed of small, single-stranded oligonucleotides that are environment-friendly and target pests efficiently and specifically DNA insecticide stems from the discovery of coevolution between baculo viruses and insects, where the virus exploit inhibitors of apoptosis (IAPs) genes to stop insect-induced apoptosis. Historically, the journey started by targeting IAP genes. Butthis context has now changed as DNA insecticides work best by targeting ribosomal RNAs (rRNA) of insect pests, where the oligonucleotide from rRNAs or any gene can be artificially designed using Contact Unmodified Antisense DNA (CUAD) Biotechnology to against the host target genes. DNA insecticides being operative in nature got later uncovered by humans showcases a novel, advantageous, and secure approach to manage insect pests.

Key words: Apoptosis, baculoviruses, coevolution, IAP genes, RING, DNA insecticide, oligonucleotide, rRNA, *Ld*MNPV, RNAse-H, CUAD, 28 S RNA, olinside

Agriculture has become more industrialized in modern times, with improved practices and developments in all spheres of crop cultivation adding crop production and crop protection aspects. It has been seen that in crop protection sciences, improved cultivation along with commercial agriculture has in turn led to more prevalence of insect pests (Tudi et al., 2021). As a result, a variety of insect pest management methods are used to manage these pests, including cultural, mechanical, physical, biological, bio rational and chemical methods in agroecosystems (Han et al., 2024). Undoubtedly the most efficient of all is the chemical control showing instant impact (Adeniyi et al., 2024). Unfortunately, less than 0.1% of pesticides used for pest control reach the insect pest, thus causing negative impacts on the environment and human health. Hence, more than 99.9% of the pesticides in use end up accumulating in the environment, in some cases human health hazard, in other cases hazard for useful biota contaminating soil, water and air of the ecosystem, and certain insects acquire insecticide resistance (Pimentel and Burgess, 2012). In a world where agriculture is the primary source of food, it remains very difficult to maintain this balance of managing pests and maintaining the natural environment within the agricultural ecosystem.

Chemical pesticide applications have resulted in unsustainable generation and disruption of the ecosystem, which led to the inevitable crucial demand for inventive formulations that have broad applications without damaging our environment. One more important reason behind the high demand for pesticide usage is to increase food production productivity for supporting the fast-growing population satisfactorily. For efficient pest control solutions, an effective collaboration between scientists and manufacturers was needed to tackle significant challenges due to plant-eating insects that should be safe, affordable and effective against pests. Using nucleic acid-based insecticide preparations (DNA insecticides), which are single stranded and short DNA fragments appeared to be novel and pioneering approach for the inception of DNA insecticide was enacted by Oberemok et al. (2024). Interestingly, the researchers and collaborators in this field call it an "intelligent insecticide" as it acts selectively and effectively with good results. The inspiration behind this insecticide is the natural interactions between insects and pathogens, which also aligns with ecological balance concomitantly and offers a promising solution to pest management problems (Oberemok et al., 2022; Oberemok and Gal'chinsky, 2024). Pioneer works in this field have already been done regarding, lab trials and field trials of

DNA insecticide on target insects, safety of non-target organism, biosafety and environmental safety. In the upcoming days, this new idea of nucleic acid-based insecticides (low carbon insecticides, Oligonucleotide insecticides and olinscides) that even think before acting on their target which was never thought before will revolutionize the field of sustainable agriculture and integrated pest management if implemented properly (Oberemok et al., 2017a; Oberemok et al., 2022; Oberemok and Gal'chinsky, 2024).

Baculoviruses: an inspiration

The journey of DNA insecticides begins with the diversity of baculoviruses in various insects, their natural evolution, and the genetic modification of insect baculoviruses for improved pest control (Ikeda et al., 2015). Despite numerous successful field applications of baculoviral formulations, their full effectiveness is constrained by host factors such as gut pH, peritrophic membrane structure, endocytosis, nuclear incorporation, enzymes, and apoptosis. Among these, apoptosis acted as a particularly predominant factor, as it significantly hindered viruses' ability to propagate inside the insect cell (Rohrmann,2019). Over years of continuous research, it was discovered that baculoviruses can thwart insect-induced apoptosis using inhibitors of apoptosis (IAPs) (Srinivasula and Ashwell, 2008) to replicate within insect cells. This finding inspired researchers to develop an antisense oligonucleotide, derived from the DNA sequence of the IAP gene that was later named as DNA insecticide (Wang and Hu, 2019) (Fig. 1). History suggests that the viral IAP genes were first described from the *Orgyia pseudotsugata* Multicapsid nucleopolyhedrovirus and *Cydia pomonella* granulovirus, the *Cp*-IAP. Structurally, IAPs are characterized by the presence of two signature

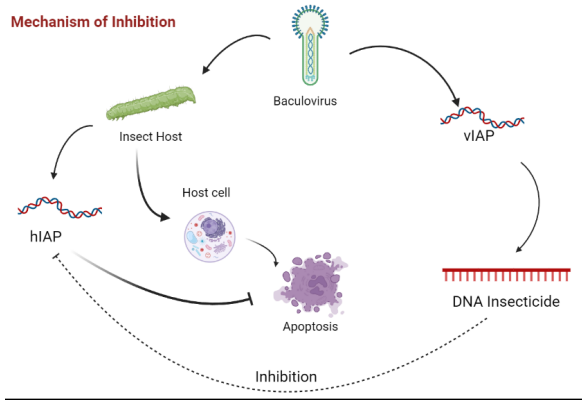

Fig. 1. Mechanism of inhibition of DNA insecticides. vIAP-Virus Inhibitors of apoptosis, hIAP-host Inhibitors of apoptosis (Designed by using https://www.biorender.com/) (Oberemok et al., 2014).

conserved motifs: the so-called baculovirus IAP repeats (BIR) and the Really Interesting New Gene (RING) domain, which spares the caspase enzymes from inhibiting the apoptotic process (Cossu et al., 2019).

Initially, the concept of creating a DNA insecticide was inspired by Inhibitors of Apoptosis (IAP) genes found in both insect hosts and baculoviruses because of coevolution (Byers et al., 2016). These genes, known as host IAP genes (hIAPs) and viral IAP genes (vIAPs), help their respective organisms survive by counteracting the detrimental effects of apoptosis (Li et al., 2008; Byers et al., 2016) (Fig. 2). For the first time, based on research knowledge DNA insecticide formulations were created using long double-stranded RNA fragments and short single-stranded fragments derived from IAP genes of the *Lymantria dispar* multicapsid nuclear polyhedrosis virus (*Ld*MNPV). When these formulations were applied *to Lymantria dispar*, they demonstrated promising control results. Later on, researchers started using rRNA as a target gene in insect pests which revolutionised ongoing research in the field of DNA insecticide. Thus, DNA insecticide was effectively derived from nature (Oberemok et al., 2014; Gu and Knipple, 2013; Wang et al., 2019).

Timeline

The history of DNA insecticides goes back to the 1978 by using unmodified antisense oligonucleotides against the Rous sarcoma virus, Paul Zamecnik and Mary Stephenson (1978) for the first time described the DNA insecticide. Next year, in 1979 possibility of a surprizing mechanism was noticed by researchers about the mechanism of action of the antisense oligonucleotides and reported that RNase H cleaved the RNA strand in RNA–DNA hetero duplexes in a site-specific manner (Donis-Keller, 1979). It took three decades for unmodified antisense oligonucleotides to be

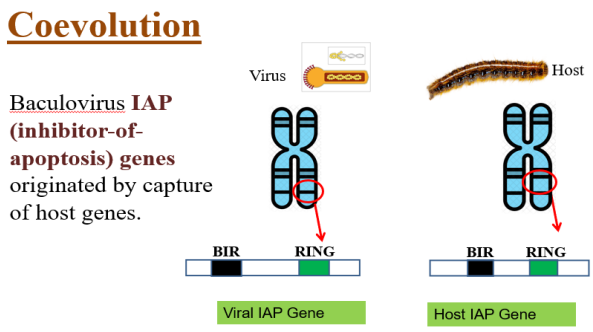

Fig. 2. Coevolution of baculovirus by capturing the insect host genes with specific conserved domain, Baculovirus Inhibitors Repeats (BIR) and Really Interesting New Gene (RING) to counter attack the effect of anti-apoptotic genes (*Source*: Oberemok et al., 2014).

conceptually applied as contact unmodified antisense DNA (CUAD) biotechnology (Oberemok et al., 2024), leading to the development of oligonucleotide insecticides (olinscides or DNA insecticides) for plant protection. This innovation harnesses the potential of DNA-based pest control, offering a novel approach to safeguard crops from insect damage (Gal'chinsky et al., 2024; Oberemok et al., 2024). In 2008, the unmodified antisense DNA oligonucleotides and contact insecticides were assigned as an equal entity (Oberemok et al., 2024). The development of phosphoramidite DNA synthesis marked a pivotal moment, enabling the large-scale synthesis and testing of antisense DNA fragments against numerous pests at a cost-effective price (Hoose et al., 2023). Phosphoramidite, a nucleoside derivative of natural nucleosides, is a crucial building block in solid-phase nucleic acid synthesis, allowing for the incorporation of specific structural features at targeted locations (Maydanovych et al., 2007). For the first time, oligonucleotide insecticides were tested on spongy moth, *Lymantria dispar*. Applying antisense DNA oligonucleotides that target IAP genes effectively controlled both baculovirus-free and *Ld*MNPV-infected spongy moth caterpillars (Oberemok et al., 2014; Oberemok et al., 2016).

The most important year for the development and advancement of CUAD biotechnology was 2019, when, three path-breaking changes happened that significantly changed the area of DNA insecticide science. Earlier research was only targeting IAP genes, that showed less prominent results. The important change that occurred was the use of rRNAs of insect pests began as targets for oligonucleotide insecticides. It happened because the efficacy of DNA insecticide was increased due to rRNA that makes up 80% of all RNA in the cell (Oberemok et al., 2019a, Oberemok et al., 2024). Followed by first change, second change was made to provide sufficient selectivity and cost-effective phosphoramidite DNA synthesis further the length of oligonucleotide insecticides was successfully reduced to 11 nucleotides (Oberemok et al., 2022). Earlier the target of DNA insecticide was concise to only lepidopteran but the newest discovery made targeted new orders like representatives of the suborder Sternorrhyncha, which is also a significant pest in agriculture and forestry worldwide (Gal'chinsky et al., 2020; Useinov et al., 2020; Oberemok et al., 2022; Gal'chinsky et al., 2023; Oberemok et al., 2023; Puzanova et al., 2023).

Design and application

For designing DNA insecticide or oligonucleotide insecticides the sequences of respective genes can be obtained using the DNA Insector program (dnainsector.com). Alternatively, these sequences can be manually retrieved from pest rRNA data available in the GenBank database. The development of DNA insecticides, or olinscides, employs both phosphoramidite solid-phase and liquid-phase oligonucleotide synthesis techniques as suggested by researchers because the contemporary production of oligonucleotide insecticides via solid-phase synthesis on DNA synthesizers employing phosphoramidites results in negligible emissions of greenhouse gases, including nitrogen oxide, ozone, methane, and carbon dioxide (Gal'chinsky et al., 2023; Oberemok et al., 2024).

In the case of oligonucleotide insecticides, these reagents are dissolved in nuclease-free water at a concentration of 1 mg/ 10 ml of solution that is applied to the foliage of plants at a rate per square meter targeting insect pests. Oberemok et al. (2019) reported that contact-based application, CUAD, of unmodified antisense DNA was more effective than oral administration, ODUAD. This decreased efficiency can be attributed to active DNases in the insect digestive system. These degrade the antisense DNA during ingestion and therefore reduce its activity. On the other hand, contact delivery prevents enzymatic degradation of the active agent, hence a larger concentration of it causes pesticidal effects. This result highlights the fact that the application method is a critical component for the efficacy of antisense DNA-based insecticides and suggests the potential benefits of CUAD in pest management strategies (Schernthaner et al., 2002; Keyel, 2017; Oberemok et al., 2024). The DNA insecticide or single-stranded DNA molecules may pass through both the polar and non-polar layers of insect tissues because of it hydrophilic (polar sugar-phosphate backbone) and hydrophobic (nitrogenous bases) regions. Due to its hydrophobic nature, nitrogenous bases usually face the double helix's central axis and point away from the surrounding aquatic environment. The nitrogenous bases in single-stranded DNA have a propensity to interact with hydrophobic compounds in the environment, such as the triacylglycerides in the cytoplasmic membrane and wax in the epicuticle (Oberemok et al., 2013).

In terms of practical application, oligonucleotide-based insecticides are remarkably straightforward to deploy and suitable for use with hand sprayers or cold fog generators. These DNA insecticides exhibit several notable properties, including high specificity in targeting pests while ensuring safety for non-target organisms. Furthermore, they are environment

friendly, characterized by a low carbon footprint and rapid biodegradability. This innovative approach offers the potential for developing long-lasting insecticides by utilizing conserved sequences of pest ribosomal RNA genes, promising a multi-decade efficacy (Oberemok et al., 2019, 2022; Gal'chinsky et al., 2023; Puzanova et al., 2023; Gal'chinsky et al., 2024).To solve the issues associated with moulting, scientists are also experimenting with *polyphosphagenes*, a multifunctional, biodegradable, and gene-delivery molecule. In the event of target-site resistance to oligonucleotide-based insecticides (olinscides) observed in a given insect pest, it is noteworthy that the development of a new olinscide can be effectively achieved by shifting the target site within the target rRNA either to the left or to the right of the resistance site (Gal'chinsky et al., 2024). This approach allows for the circumvention of existing resistance mechanisms, thereby enabling the continued efficacy of olinscides in pest management strategies.

Mode of action

Applying DNA insecticides externally, having the characteristic of contact insecticides, is one feasible and practical way to apply it. The permeability of an insect's exoskeleton to most pesticides is known to be somewhat constrained by the presence of developed epicuticle (Oberemok et al., 2013). DNA insecticide study, the oligonucleotides were designed using the sequences of the highly conserved RING domain inIAP genes. An antisense strand of 18-20 nucleotides was designed and named OligoRING (DNA insecticides) from the baculoviral IAP gene of the *Lymantria dispar* multicapsid nuclear polyhedrosis virus (*Ld*MNPV). TheoligoRING (5′-CGACGTGGTGGCACGGCG-3′), acting as an antisense RNase H-dependent oligonucleotide, induces the degradation of target mRNA for the host IAP-Z gene (very homologous to *Ld*MNPV IAP-3 gene). As a result, the expression of the target protein was down regulated. This marked the existence of DNA insecticide (Oberemok et al., 2017b).

In 2019, Oberemokand coworkers observed that both unmodified and modified antisense oligonucleotides used in medicine share comparable mechanisms of action as DNA insecticides. DNA insecticides produce their antisense effects by a process that is RNase-Hdependent. The enzyme RNase-H hydrolyses the RNA strand of an RNA/DNA duplex. For that, they took the 5.8S ribosomal RNA representing the most stable reference genes, as revealed in several studies that offer a precise and repeatable real-time PCR experiment. 28Sand 5.8S rRNAs constitute about 85–90per cent of total cellular RNA and are very useful as internal controls. After 6th day of treatment, the concentration of the 5.8S ribosomal RNA in oligoRIBO-11-treated insects were significantly lower (16.5-fold) compared to that of the controls. Thus, proving that the DNA insecticides work on the principle of RNase-H.

As the research on DNA insecticides progresses, the oligonucleotides' true mode of action emerged differently. Oligonucleotide insecticides function via the DNA containment (DNAc) mechanism, which involves two distinct steps. This evolving mechanism underscores the complexity and potential of DNA-based pest control strategies, offering new insights into its effectiveness and applications in pest management (Oberemok and Gal'chinsky, 2024). The initial phase of DNAc entails the complementary binding of antisense DNA oligonucleotides to their target rRNA. This binding disrupts the normal functioning of ribosomes, leading to their inactivation and resulting in significant insect pest mortality. Thereafter, rRNA hyper compensation by DNA-dependent RNA polymerase becomes the sole remaining path of survival for the insect cell when target rRNAs and/or polycistronic rRNA transcripts are "arrested" deleteriously via antisense DNA oligonucleotides. Next, the target rRNA is cleaved by RNase H which diminishes its amount automatically resulting in the death of its insect host (Gal'chinsky et al., 2024) (Fig. 3).

Efficacy on insects: List of trials conducted

Oberemok et al. (2017) designed an antisense RING domain fragment of the LdMNPV*iap-3*gene,

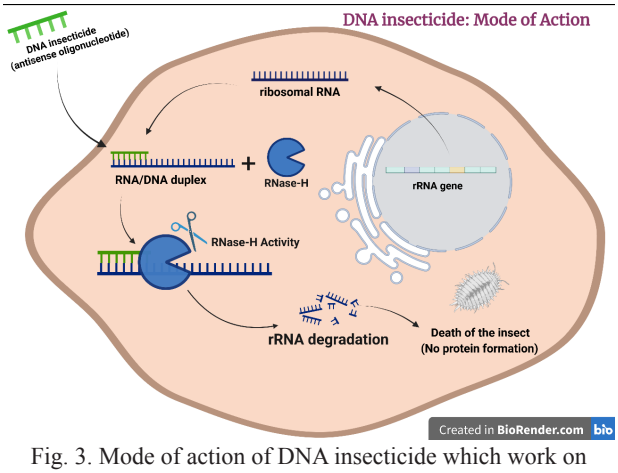

Fig. 3. Mode of action of DNA insecticide which work on the principle ofDNA containment (DNAc) mechanism (using https://www.biorender.com/) (Oberemok et al., 2024).

5′-CGACGTGGTGGCACGGCG-3′ (antisense strand; experimental group; oligoRING) and control, 5′-CGCGCGCGCGCGCGCGCG-3′ (oligoCpG) and it was evaluated that gene expression of both the target host *IAP-Z* gene and the non-target host *IAP-1* gene was significantly down-regulated (2.04± 0.29-fold stronger) compared with that of control ($p < 0.05$) on the 14th day (Table 1). Several apoptotic patterns in larvae were also observed including structural deformation, involution of cells, and condensation (pyknosis) and fragmentation (karyorrhexis) of nuclear material. The field trial conducted in 2018 by Oberemok and co-

Table 1. The Successful employment of DNA insecticide or oligonucleotide

Sl. No.	Targeted insect Pest	Sequence of the target genes	Target gene	Concentration and Mortality	References
1.	Gypsy moth, *Lymantria dispar*	oligoRIBO-11 (5′-TGC-GTT-CGA-AA-3′)	5.8S rRNA	Concentration:72 ng/ µℓ 46.9% ± 9.3% mortality rate	Oberemok et al., 2019
2.	The euonymous scale, *Unaspis euonymi*	oligoUE-11 (5′-AGA-CCG-ACG-AC-3′)	28S rRNA	Concentration: 100 ng/ µℓ 99.24%± 1.32% mortality rate	Gal'chinsky et al., 2020; Oberemok et al., 2020
3.	The holly scale, *Dynaspidiotus britannicus*	oligoDB-11 (5′-ATA-CCG-ACG-AT-3′)	28S rRNA	Concentration: 100 ng/ µℓ 82.44%± 15.62% mortality rate	Gal'chinsky et al., 2020
4.	The Japanese wax scale, *C. japonicus*	oligoCJ-11 (5′-CGA-CCG-ACG-AA-3′)	28S rRNA	Concentration: 100 ng/ µℓ 78.82%± 18.60% mortality rate	Useinov et al., 2020
5.	The invasive scale insect, *Diaspis echinocacti*	Cactus-NBG (5′-ATC-GCT-GCG-GA-3′)	28S rRNA	Concentration: 100 ng/ µℓ 84.2% ± 2.2% mortality rate	Plugatar et al., 2021
6.	The soft scale insect, *Coccus hesperidum*	Coccus-11 (5′-CCA-TCT-TTC-GG-3′)	28S rRNA	Concentration: 100 ng/ µℓ 95.59%± 1.63% mortality rate	Oberemok et al., 2022
7.	The bay sucker, *Trioza alacris*	Alacris-11 (5′-CCA-CCG-GGT-AG-3′)	ITS2 of poly-cistronic rRNA	71.02%± 5.21% mortality rate	Novikov et al., 2022
		Laura-11 (5′-GAC-ACG-CGC-GC-3′)	ITS2 of polycistronic rRNA	72.39%± 6.48% mortality rate	
		Concentration: 100 ng/ µℓ			
8.	The cottony cushion scale, *Icerya purchasi*	oligoICER-11 (5′-ACA-CCG-ACG-AC-3′)	28S rRNA	Concentration: 100 ng/ µℓ 70.55%± 0.77% mortality rate	Gal'chinsky et al., 2023
9.	The chrysanthemum aphid, *Macrosiphoniella sanborni*	Macsan-11 (5′-TGT-GTT-CGT-TA-3′)	ITS2 of polycistronic rRNA	Concentration: 100 g/ µℓ 67.15%± 3.32% mortality rate for single treatment and 97.38%± 2.49% mortality rate for double treatment	Puzanova et al., 2023
10.	The mealybug, *Pseudococcus viburni*	Alpha-11 (5′-GGT-CGC-GAC-GT-3′)	28S rRNA	63.42%± 3.1% mortality rate	Novikov et al., 2023a
		Beta-11 (5′-GGA-ATC-GAA-CC-3′)	18S rRNA	78.31%± 4.5% mortality rate	
		Gamma-11 (5′-CCT-CAG-ACA-GG-3′)	5.8S rRNA	66.96%± 2.9% mortality rate	
		Concentration: 100 ng/ µℓ			
11.	The laurel scale, *Aonidia lauri*	oligoAL-11 (5′-ATG-CCA-ACG-AT-3′)	28S rRNA	98.19%± 3.12% mortality rate	Gal'chinsky et al., 2024
12.	The two-spotted spider mite *Tetranychus urticae*	Tur-3 (5′-AAA-ACA-TCA-AG-3′)	ITS2 of polycistronic rRNA	72.85%± 4.55% mortality rate	Novikov et al., 2023b
		Turka (5′-AGC-GAC-GTC-GC-3′)	28S rRNA	77%± 0.4% mortality rate	
		Concentration:100 ng/ µℓ			

workers also supports the idea of using DNA pesticides in forests. The data demonstrated for the first time that using contact oligoRING pesticide is efficient for field experiments and reduced the *Lymantria dispar* larval survival by 73.7% in 14 days after treatment when compared with the control group which was treated with water. The results showed that DNA insecticides were selective and that the sequence of oligonucleotides in the fragment is the determining factor of its efficiency (Oberemok et al., 2018).

Oberemok et al. (2019) studied the inhibition of protein synthesis by specific anti 5.8S rRNA oligonucleotides, an 11 nucleotide long (5′-TGCGTTCGAAA-3′) antisense oligonucleotide (oligoRIBO-11) from *Lymantria dispar*. They designed an oligonucleotide that included the universally conserved antisense 5′-GTTC-3′ sequence. When it was applied as a contact insecticide, the insect mortality increased significantly with 35.3% in oligoRIBO-11 compared to 8.3, and 4.2%, inoligoRING, and water (control) respectively. It was demonstrated that *Coccus hesperidum* insects can be controlled in a targeted and efficient manner by topically applying DNA insecticides. The mortality of *C. hesperidum* larvae exposed to the contact oligonucleotide insecticide *Coccus*-11 at a concentration of 100 ng/ ℓ was 95.59± 1.63% after 12 days (Oberemok et al., 2022).

Gal'chinsky et al. (2023) successfully used a brief single-stranded fragment of the 28S ribosomal RNA gene known as 'oligoICER-11' for the first time to control cottony cushion scale. The mortality of *Icerya purchasi* larvae exposed to the contact oligonucleotide insecticide oligoICER-11 at a concentration of 100 ng/ ℓ was 70.55±0.77% after 10 days of treatment. Recently Gal'chinsky and co-workers in 2024 using sequencing data found that *Aonidia lauri* Bouche predominates in the mixed populations of insect pests *Dynaspidiotus britannicus* Newstead and *Aonidia lauri*. 80% of the population consisted of individuals of *A. lauri* carrying the sequence 3′-ATC-GTT-GGC-AT-5′ at the 28S rRNA site, whereas twenty per cent of the population contained individuals of *D. britannicus* carrying the same sequence. With perfect complementarity to each of the sequences, they produced the olinscides Diasp80-11 (5′-ATG-CCA-ACG-AT-3′) and Diasp20-11 (5′-ATA-CCG-ACG-AT-3′). On the 14th day, it was observed that the insect mortality was 3.77± 0.94% in the control group, 64.66± 0.67% in the Diasp20-11 group ($p < 0.05$), and 98.19± 3.12% in the Diasp80-11 group (Gal'chinsky et al., 2024).

Successful attempts

Based on several studies, it has been demonstrated that CUAD biotechnology's straight forward design, adaptability, and efficiency in managing sap-feeding pests like aphids, psyllids, and various types of scales are remarkable (Oberemok., 2024). By leveraging the specific conserved sequences of target rRNAs in insect pests, the likelihood of developing target-site resistance can be significantly reduced. After that, several research on deoxyribonucleases found in the cell homogenates of the spongy moth, Colorado potato beetle, cottony cushion scale, and their host plants (*Quercus pubescens*, *Solanum tuberosum*, *P. tobira*) exhibit a high biodegradability potential for oligonucleotide insecticides, ensuring their rapid degradation (usually within 24 hr) upon interaction (Oberemok et al., 2018; Oberemok et al., 2019; Gal'chinsky et al., 2023). Those nitrogenous base combinations will ensure that an oligonucleotide insecticide is very good at killing one single pest. The success stories of DNA insecticides in various insects are enhancing the field of agroecosystems (Table 1).

Safety towards non-target organism

In English oak (Quercus robur) and apple seedlings (*Malus domestica*), applying a RING domain fragment at 5 pmol/ cm² significantly decreased alkaline phosphatase activity in apple seedlings ($p<0.05$) and glucose concentration in oak leaves ($p<0.01$) after 24 hr. However, these effects were insignificant by the 7th day, indicating no long-term negative impact (Zaitsev et al., 2015).The study on the impact of DNA insecticides on wheat, *Triticum aestivum* sprouts' glucose levels and alkaline phosphatase activity investigated that a DNA insecticide based on a single-stranded DNA fragment from the gypsy moth virus significantly decreased glucose and enzyme activity by the second day, but these effects diminished after one week. No significant differences in dried biomass were observed between the treated and control groups after one and three weeks (Oberemok et al., 2013). A study examined the impact of ssDNA oligonucleotides, employed as DNA insecticides, on wheat (*T. aestivum*) plant biomass, organs, and certain biochemical markers to assess non-target safety. Results indicated that ssDNA oligonucleotides at concentrations of 0.01, 0.1, and 1 pmolμl^{-1} affected plant biomass compared to the control but was not detrimental to growth or development. At 0.001 pmolμl^{-1}, no impact was observed. Glucose, protein, and phosphorus levels, evaluated after 21

days, confirmed their safety (Nuadar et al., 2019). Oberemok et al. (2015) discovered that single-stranded fragments (Oligonucleotide) of the LdMNPVIAP3 gene had no significant impact on the survival of tobacco hornworm, *Manduca sexta* and black cutworm, *Agrotis ipsilon* second-instar caterpillars after 7 days, when compared to controls. Even after 14 days, mortality rate remained unchanged, highlighting the specificity of DNA insecticides (Oberemok et al., 2015).

Oberemok et al. (2019a) investigated the effects of oligoRIBO-11 and oligoRING on the lab-grown greater wax moth, *Galleria mellonella* larvae, a non-target lepidopteran species. After a period of 6-day observation, there was no statistically significant difference in larval viability between the treated groups and water-treated controls ($p > 0.05$), with all groups showing a 0% mortality rate (Oberemok et al., 2019).This study highlights the selectivity of DNA insecticides, emphasizing that their specificity is influenced by the particular combination of nitrogenous bases in a fragment, consistent with findings in other non-target insect species. The high specificity of DNA insecticides presents a very potential tool for providing precise pest control, reducing collateral damage to beneficial insects. Targeted approaches such as this one will be important for sustainable practices in pest management and environmental protection.

Way forward

DNA insecticides are advanced nucleic acid-based insecticides composed of natural polymers that operate on the principle of complementarity, allowing them to act highly selectively. They integrate the advantages of modern insecticides while mitigating their drawbacks. These substances can be produced in large quantities using automated systems. However, it's essential to establish their environmental safety, as current data on their ecological impacts are limited. With the global population projected to exceed 9 billion in the next 50 years, food demand is expected to rise, leading to increased pesticide use, potentially 2.7 times more by 2050. This escalation poses greater risks to both humans and ecosystems. Effective pest control is likely to require a collaborative effort for the development of insecticides that are efficient, cost-effective, and environmentally safe. DNA insecticides offer a promising alternative, as they are selective, biodegradable, and economically viable, with minimal impact on plants and a lower carbon footprint.

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AUTHOR CONTRIBUTION STATEMENT

HK, RKB, MS and SV conceived and designed the review. HK, RKB, AC, MS and SV wrote the manuscript. MS, DS and SV corrected the article. All authors read and approved the manuscript.

CONFLICT OF INTEREST

No conflict of interest.

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