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DNA INSECTICIDES: AN EMERGING TOOL IN PEST MANAGEMENT

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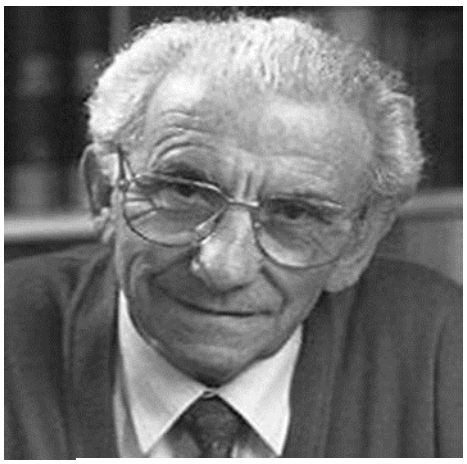


V.V OBEREMOK

Insecticides are chemicals which kill insects. Considering the rapid growth of global population, annual reduction in cultivated lands, and considerable loss due to pests, the need for insecticides cannot be questioned but many of them cause great harm to the environment. This demands further research aimed at production of safer insecticides. The most marketable insecticides are those combine the best properties of existing insecticides. This condition is met by DNA insecticides, preparations based on nucleic acids. This concept was formulated and expanded by V. V Oberemok. They have unique features like use of ssDNA molecules, cost effective, target specific, fast action, topical application and the use of viral anti-apoptotic genes, can even replace organophosphates as reported by Useinov*et al.*, 2020. The action is similar to methods of blocking expression of genes using the mechanisms of RNA interference (Wang *et al*., 2011), DNA interference (Kawai-Toyooka *et al*., 2004) and application of antisense technologies. These features make DNA insecticides distinct from the other post-genomic means of crop protection. But this technique is still in its infant stage. Researches provide on how to rearrange current attempts and supplement for the production of such insecticides and make them available for common man.

DNA insecticides are known as intellectual insecticides because they act target specific. It is a technology based on application of single stranded viral DNA fragments possessing insecticidal activity. These are introduced exogenously which can act in a manner similar to antisense molecules. It combines the techniques of RNA interference (RNAi), DNA interference (DNAi) and antisense oligo nucleotides (ASOs).

RNA Interference

RNAi is a biological process involving double stranded RNA which can suppress gene expression through translational or transcriptional repression. It was first documented in animals in 1996 by Gou and Kemphues in *Caenorhabditis elegans.* This includes two components, namely, micro RNA (miRNA) and small interfering RNA (siRNA).

The RNAi can occur in a cell due to the presence of antisense RNA. The complementarity of antisense RNA and cellular mRNA cause them to bind with each other. The antisense RNA can be introduced into a cell through different ways such as, by a researcher for any research purpose or a virus when they multiply within a cell.

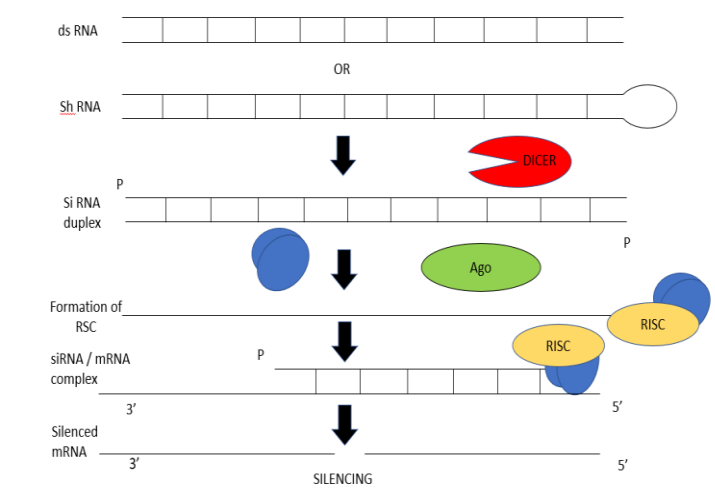


Fig 1: RNA interference

Source: Clipart edited by authors

RNAi involves two steps:

- Creation of RISC (RNA Induced Silencing Complex)
- Degradation of cellular RNA by RISC

An endonuclease known as "dicer" identifies dsRNA in a cell. It cuts the dsRNA into pieces of 21-23 nucleotide long. The antisense RNA associated with proteins like Argonaute proteins forms RISC.

RISC associate with cellular mRNA which has a sequence homologous with RNA fragment in RISC. The RISC attaches and thus degrades the cellular RNA. Thus, the gene expression is blocked.

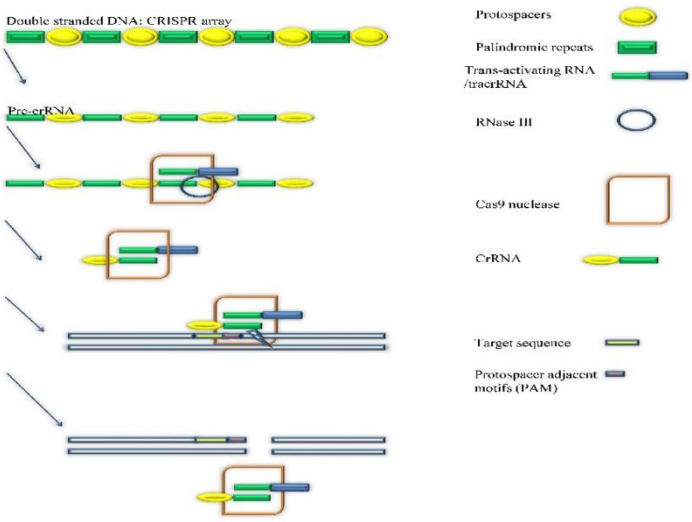


Fig 2: DNA interference

Source: Shengfu Shen *et al.*, 2016

DNA interference

The DNAi can be best explained by the help of CRISPR/Cas9 technique. It

works on dsDNA. The components of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are mainly an endonuclease called Cas9 and a guide RNA. Guide RNA has two components, they are tracrRNA and crRNA. The tracrRNA binds to one of the palindromic sequences and the ribonuclease 3 will cut the tracrRNA. This is called the crRNA. The crRNA, tracrRNA and the Cas9 enzyme forms a complex. The crRNA is complementary to a DNA sequence of the target. So, crRNA guide Cas9 nuclease to the target. PAM (Protospacer Adjacent Motifs) acts as an initiator of attaching crRNA to its complementary strand. The complex will thus cut the target DNA and blocks its genetic expression.

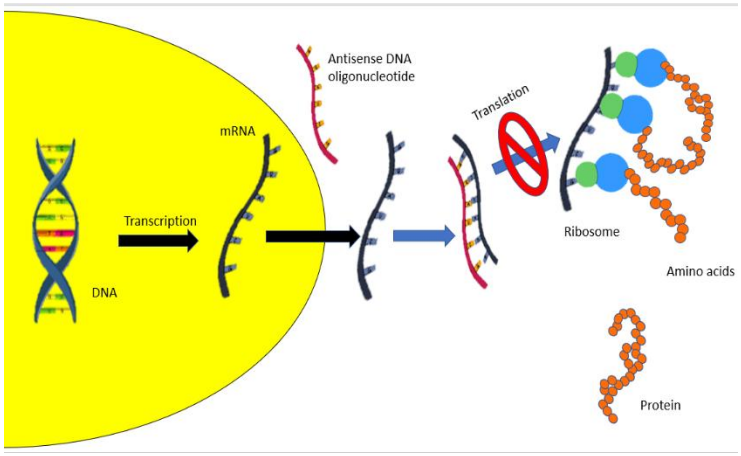


Fig: Antisense Oligo nucleotide Technique

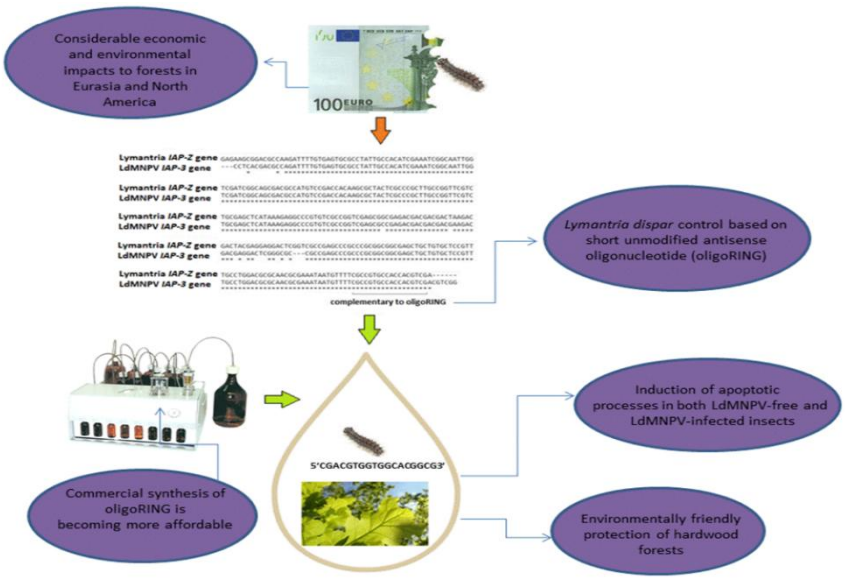
Source: Clipart edited by authors

Antisense oligo nucleotides

Antisense oligo nucleotides are short strands of nucleotides which are complimentary to the mRNA and can bind with each other.

Mode of Action

The DNA insecticides have been studied only with the anti apoptosis gene. The anti apoptosis gene is found in Baculo viruses. The Baculo viruses have IAP gene 1-5, among which the IAP3 is the most effective against insects. The IAP3 gene is extracted from the baculo viruses. They contain two domains namely, BIR (baculo viruses IAP repeats) and RING (Really Interesting New Genes). RING is the most effective and act homologous to the target site in insects. The insects also possess anti apoptosis gene. When IAP3 is introduced into insects as ssDNA fragments, they act complementary to the anti apoptosis site of insects. Thus, bind together and blocks the expression of anti apoptosis gene in insects which lead apoptosis in them. Thus, the insect dies. Most of the studies are done in Gypsy moth, *Lymantria dispar,* this is because it is a major pest in Russian forest which have a capacity to feed upon $1m^2$/larvae area in its caterpillar stage. This also have more than 80 host plants including agricultural crops.



(Source: V. V. Oberemok*et al.*, 2018*)*

The DNA insecticides are basically single stranded DNA fragments in water solution which can be applied topically. Cold fog generators are used for topical application or spraying of these insecticides. The cold fog generators are mainly used in the application because the atomization of the insecticide particles is more which help in more penetration into insects. These act like hand sprayers. The carriers mostly used are poly-L-lysine, polyethyleneimine, poly[(organo) phosphazenes], etc**.** The better the carriers are, the more the penetration and absorption of the insecticides applied.

The symptoms are shown by caterpillars within three to seven days of application. The caterpillars infected stop eating, move slowly, appear dehydrated and appear to be smaller than the healthy ones.

The Advantages of Using DNA Insecticides

They are very selective in action and cost effective, no long term negative effects are shown on host plants. They are very suitable for lepidopteran pests in caterpillar stage specially second instar. They resolve the problem of developing insecticide resistance in insect pests. They work at lower concentration (in picomolar concentration). It is more natural containing water dissolved with antisense oligonucleotides.

The Disadvantages of using DNA Insecticides

They are hard to penetrate cryptic feeding insects and adult beetles due to hard elytra. They may not be successful in controlling insect pests in an emergency situation like pest outbreak. The carriers used are very expensive.

Challenges of DNA Insecticides

This approach must be made profitable and marketable. This can be made marketable only by creating a product composed of multiple treatments such as a solution containing several ASOs that target different genes. Optimal delivery is critical for achieving maximum potency. We must ensure more penetrability of DNA insecticides with the use of suitable carriers. DNA insecticides can only be produced if we know about the gene sequences of agricultural pests. So the sequencing of all insect genomes, especially that of pest is necessary. As of 2019, the genomes of only 28 species have been sequenced. One of the most pressing challenges while creating DNA insecticides is the need to increase insect mortality as compared to the present insecticides.

Future Prospects

Investigation in the field of post genomic approach deserve attention and detailed study. Researches provide on how to rearrange current attempts and supplement for the production of such insecticides. Further improvement for establishing more precise mechanism of their action. Researches for extending use to controlling other serious insect pests

Conclusion

` The DNA insecticides can combine the best properties of modern insecticides; the low cost and the fast action of chemical preparations on one hand and the selectivity of biological preparations on the other. Considering the rapid growth of the global population, annual reduction of cultivated lands, and considerable agricultural losses due to pests, more feasible alternatives to insecticides are to be discovered. The new insecticides must be selective, feasible and eco-friendly. Such requirements are fulfilled by DNA insecticides, arising as a new promising insecticide group. So, the studies in this area have to be more encouraged and supported for a better tomorrow.

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