18(1s), Spring 2026

Journal of Animal Environment

(E-ISSN 2717-1396, ISSN 2717-1388)

Original Research Paper

Genetic Insights into Insect Physiology: CRISPR-Cas9 Gene Editing to Uncover Functional Genomics of Pest Species

Kavitha U *[1], Shyamrani Y [2], Abhinaya B[3], Kadirvelu Divya[4], Yamuna V[5], Sameer Rastogi[6]

[1] Assistant Professor, Physiology, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research, Enathur, Kanchipuram, Tamil Nadu 631552 (Kavitha U) | Email: kavithau@maher.ac.in

[2] Meenakshi College of Physiotherapy, Meenakshi Academy of Higher Education and Research, (Shyamrani Y) | ORCID: 0009-0000-0850-1377| Email: shyam@maher.ac.in

[3] Meenakshi College of Physiotherapy, Meenakshi Academy of Higher Education and Research (Abhinaya B) | ORCID: 0009-0008-7472-1704 | Email: abhi@maher.ac.in

[4] Meenakshi College of Nursing, Meenakshi Academy of Higher Education and Research (Kadirvelu Divya) | ORCID: 0000-0002-6496-3725 | Email: kadirveludivya54@gmail.com

[5] Lecturer, Meenakshi College of Pharmacy, Meenakshi Academy of Higher Education and Research. (Yamuna V) | Email: yamuna@maher.ac.in

[6] Professor , School of Pharmacy , Noida International University , Greater Noida, Uttar Pradesh ,India (Sameer Rastogi) | Email: dean.sop@niu.edu.in

Abstract: Many harmful insects need new ways to be controlled because they are a big problem for farming, public health, and ecology. The discovery of CRISPR-Cas9 gene editing technology has changed functional genetics and given scientists strong tools for figuring out how insects' bodies work. The main goal of this study is to use CRISPR-Cas9 to look into gene functions in important pest species in order to find genetic factors that affect how well they can change, how resistant they are to control measures, and how much damage they do to the environment generally. We want to find out more about the roles of certain genes in important bodily processes like growth, reproduction, and the stress response by changing them selectively. We can make knockouts and look at changes in phenotype that show how genes work by making guide RNAs (gRNAs) that point the Cas9 nuclease to exact genome sites. Using this method, we can carefully look into the genetic basis of traits that make pest species strong, such as metabolic pathways that make them resistant to insecticides and genes that affect their ability to reproduce. The first results show that CRISPR-Cas9 has the ability to make changes that are passed down through generations in model pest organisms like Drosophila melanogaster and crop pests like Helicoverpa armigera. The fact that genes involved in detoxification processes were successfully disrupted shows that the technology can help us understand how resistance works. We are also creating transgenic lines that have fluorescent markers so that we can study gene expression and function in living things. This will help us learn more about how genetic changes affect the body. This study not only helps us learn more about the molecular biology of pest insects, but it also lays the groundwork for creating focused pest control methods that have less of an effect on the environment. We want to come up with new biocontrol methods that can be used in sustainable farming by figuring out which genes are important.

Keywords: CRISPR-Cas9, Functional Genomics, Insect Physiology, Pest Management, Gene Editing

I. Introduction

Insects are one of the most varied and ecologically important groups of living things on Earth. As feeders, decomposers, and food for other species, they are very important to many habitats. However, a lot of bug species are also very bad for crops, human health, and wildlife, mostly because they are pests. Getting rid of bug pests has become harder over the past few years because they are becoming more resistant to common methods of control, like chemical poisons. As a result, we urgently require new ways to control pests that can help us learn more about the biology and chemistry of insects. Now this is where genetic study has come in handy, especially with the help of CRISPR-Cas9 gene editing technology. CRISPR-Cas9 has become a new tool in

***Corresponding Author:**

Received: 24th April, 2026; Revised: 11th May, 2026; Accepted: 24th May, 2026; Available Online: 6th June, 2026
(DOI): 10.70102/AEJ.2026.18.1s.93

molecular biology, allowing gene editing with a level of accuracy that has never been seen before. Scientists can use this technology to focus on certain genetic regions and make changes that can help them understand gene function, relationships, and pathways. Because it is relatively easy to change genes in many bug species, using CRISPR-Cas9 on them looks very hopeful. Scientists can use this technology to look into the genetic root of features that are important for the survival of pest species. For example, they can look into biochemical pathways that make insects resistant to insecticides, ways that pests reproduce, and how their bodies react to external stresses [1]. Useful genomics is consider of how qualities work in living frameworks. One of the most objectives of utilizing CRISPR-Cas9 in bug inquire about is to assist us get it this field way better. This can be particularly imperative when it comes to bug species, since knowing the genetic factors that influence how they work can offer assistance individuals come up with particular ways to urge freed of bugs. For case, finding the qualities that make harms less successful can lead to way better ways to induce freed of the bugs that do not utilize defence components. Too, learning more around the hereditary qualities behind being able to have children may lead to thoughts for controlling the populace by changing qualities related to richness [2].

One advance thing around CRISPR-Cas9 innovation is that it can erase particular qualities. This lets researchers see at the changes in phenotype and figure how qualities work. Researchers can discover out what parts certain qualities play in critical real capacities by making changes that can be passed down through eras. This strategy not as it were makes a difference us get it the hereditary cosmetics of creepy crawly bothers, but it too gives us a way to see into greater questions approximately advancement and biology [3]. Later ponders have appeared that CRISPR-Cas9 works in several types of creepy crawlies. Within the fruit fly Drosophila melanogaster, for case, researchers have been able to mess with qualities that control metabolic pathways and formative forms. This has made a difference them learn more approximately how these qualities work. Moreover, messing with detoxification genes in trim bugs like Helicoverpa armiger has made a difference us get it how bug sprays work against these bothers. These triumphs appear how CRISPR-Cas9 has the capacity to alter the way we think approximately how creepy crawlies work. CRISPR-Cas9 can be utilized to create transgenic lines that express fluorescent markers or columnist qualities, in expansion to strategies that thump out qualities. This strategy lets us ponder quality work in genuine time and track quality expression whereas the cells are lively. Visualizing the movement of certain qualities amid development or in response to outside prompts can offer assistance analysts learn a parcel almost how bug bodies alter over time [4]. These kinds of studies are necessary to learn how pests adapt to new situations, like being exposed to chemicals or changes in the weather. The results of this study have effects that go beyond basic science. As the world's population keeps growing, it becomes more and more important to use safe farming methods. Scientists can come up with new ways to get rid of pests without using chemicals by finding the genetic targets that make pests successful. One of these tactics could be letting loose genetically modified insects that can't reproduce normally or are more easily hurt by natural stresses [5].

II. Literature Review

A. Current state of insect physiology studies

A lot of progress has been made in molecular biology, genetics, and bioengineering over the past few years, which has sped up the area of insect physiology. The main goal of research in this area is to figure out the complicated bodily processes that control how insects behave, grow, and respond to changes in their surroundings. To study the complex connections between genetic, cellular, and bodily processes in insects, researchers use a variety of methods, such as high-throughput sequencing, gene expression analysis, and advanced imaging methods. Metabolic forms, particularly those that have to be do with pesticide tolerance, are one of the most subjects of current ponders within the physiology of insects [6]. Shockingly, increasingly bug species are getting to be safe to medicate control. To form great administration plans, we got to understand how these resistances work. Researchers are finding particular qualities and metabolic pathways that play a portion in cleansing. This lets them make centered medicines that can diminish resistance and make bother control work superior. Moreover, increasingly considers are looking into how outside variables influence the bodies of creepy crawlies. Stressors like climate alter, changing environments, and chemical introduction can have a enormous impact on the health and behavior of creepy crawlies. Researchers are looking at how insects' bodies respond to these stresses, like how their digestion system changes, how well they duplicate, and how numerous of them survive, to memorize more around how they can adjust to settings that alter rapidly [7]. The comes about of this consider are exceptionally imperative for preservation endeavors since they appear how versatile valuable bug species like pollinators are and offer assistance make plans to ensure them. Furthermore, the land of cutting edge innovations like CRISPR-Cas9 quality altering is changing the way bug physiology ponder is done. These devices make it conceivable to create correct changes to the bug genome, which makes it possible to think about

quality capacities and connections in a way that has never been done before. Researchers can figure out the complicated hereditary plan behind diverse physical characteristics by changing or moving forward certain qualities. This opens the entryway to better approaches to control bugs and secure natural life [8].

B. Previous applications of CRISPR-Cas9 in pest species

Genomics has changed a part since of CRISPR-Cas9 technology, especially when it comes to examining bug species. Numerous other ways to consider and control bug populaces have been made conceivable by its capacity to form exact changes within the genome. One of the foremost curiously employments of CRISPR-Cas9 has been to consider chemical resistance, which may be a enormous issue in controlling bothers in farming. A few illustrations are Helicoverpa armigera and Plutella xylostella, which are known for being safe to well known pesticides. Analysts have been able to effectively target detoxification qualities in these species. By thumping out certain qualities, researchers have figured out the biochemical forms that cause resistance. This data can offer assistance them come up with superior ways to settle the issue. The study of sexual traits in pest species is another important area of use [9]. Researchers have used CRISPR-Cas9 to change genes related to reproduction and fertility in order to come up with new ways to get rid of pests. For instance, scientists have messed up genes in the Mediterranean fruit fly (Ceratitis capitata) that control how well the fly reproduces. This could cause the population to grow less quickly. There is hope that these methods can be used to create self-limiting or gene-drive systems that can greatly reduce the number of pests in farming areas. Additionally, CRISPR-Cas9 has been used to study how different bug pests grow and change. Researchers have been looking at genes in the mosquito species Aedes aegypti that spreads diseases like dengue and Zika. These genes are involved in the growth and evolution of larvae. Scientists have been able to figure out how to control the populations of these disease-carrying insects by messing with their genes and seeing what happens to their growth and survival [10]. Transgenic uses of CRISPR-Cas9 have also made it possible to make genetically edited pest lines that can be used to study how genes work and interact with each other.

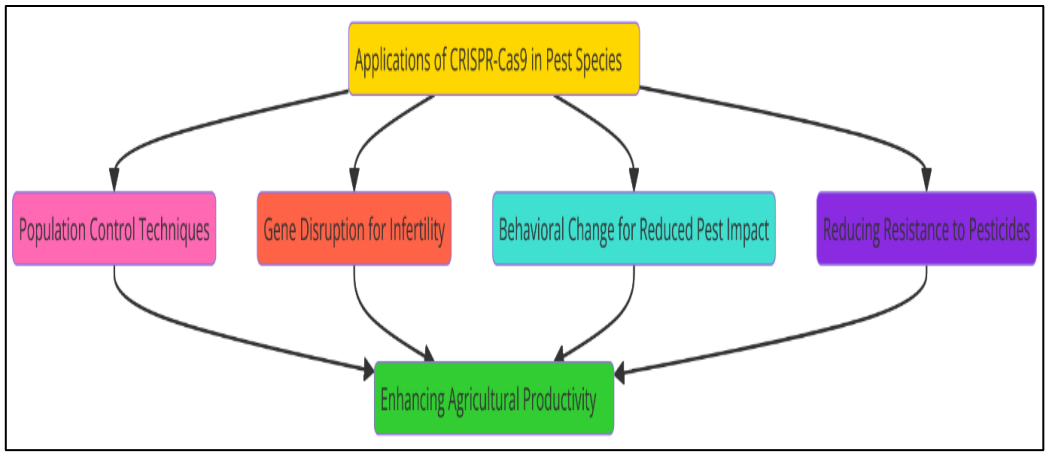

Figure 1: Previous applications of CRISPR-Cas9 in pest species

For occurrence, researchers have made fluorescent stamping frameworks in Drosophila melanogaster and Anopheles gambiae to create it simpler to observe quality expression and substantial responses whereas the creatures are still alive. A parcel of advance has been made in atomic science, hereditary qualities, and bioengineering over the past few a long time, which has sped up the area of creepy crawly physiology.

The most objective of inquire about in this region is to figure out the complicated substantial forms that control how creepy crawlies carry on, develop, and react to changes in their environment. To think about the complex associations between hereditary, cellular, and substantial forms in creepy crawlies, analysts utilize a assortment of strategies, such as high-throughput sequencing, quality expression investigation, and advanced imaging strategies [11]. Metabolic forms, particularly those that got to do with pesticide tolerance, are one of the most subjects of current considers within the physiology of creepy crawlies. Tragically, increasingly bug species are getting to be safe to sedate control. To form great administration plans, we got to get it how these resistances work. Researchers are finding particular qualities and metabolic pathways that play a portion in cleansing. This lets them make centered medicines that can decrease resistance and make bother control work way better. Too, increasingly ponders are looking into how outside variables influence the bodies of creepy crawlies. Stressors like climate alter, changing living spaces, and

chemical presentation can have a huge impact on the wellbeing and behavior of creepy crawlies [12]. Researchers are looking at how insects' bodies respond to these stresses, like how their digestion system changes, how well they reproduce, and how numerous of them survive, to memorize more approximately how they can adjust to settings that alter rapidly. The comes about of this ponder are exceptionally vital for preservation endeavours since they appear how versatile valuable bug species like pollinators are and offer assistance make plans to ensure them. Furthermore, the utilize of cutting edge innovations like CRISPR-Cas9 quality altering is changing the way bug physiology ponder is done [13].

C. Gaps in knowledge regarding functional genomics of pests

We have learned a lot about functional genetics in pest species, but there are still big gaps that make it hard to come up with effective ways to get rid of pests. One of the biggest problems is that bug genes are not fully understood yet. The genomes of many pest species have been sequenced, but the labels on these genomes are often not complete. This is especially true when it comes to describing the functions of many genes. A lot of bug genes haven't been named or aren't well known, which makes it hard to figure out what role they play in important processes like growth, metabolism, and stress responses. We can't find possible DNA targets for pest control because there isn't enough complete functional description [14]. Another big hole is our knowledge of how genes work together and how regulatory networks work. Insects have complicated body parts that are caused by complicated gene interactions. However, the exact paths and control systems that govern these interactions are still mostly unknown. As an example, it is still not clear how cleansing enzymes and biochemical pathways work together to cause insecticide tolerance. It's hard to come up with methods that successfully stop resistance processes if you don't have a good idea of how these relationships work. In addition, the natural setting of functional genetics is frequently missed in studies of pests. Even though studies in the lab can teach us a lot about how genes work, they might not fully mimic the situations that insects face in the wild [15]. Biological stresses, temperature, and humidity, as well as exposure to them, can have a big effect on gene expression and bodily reactions. Therefore, studies that combine data from the field and the lab are needed to get a fuller picture of how genetically pests adapt to their surroundings. Lastly, many pest species are still in the early stages of using cutting edge genetic technologies like CRISPR-Cas9. Initial uses have shown promise, but large-scale, multi-gene editing methods still have a lot of potential that hasn't been fully explored [16]. More in-depth studies that use these tools to look into gene functions in a wide range of pest species are very much needed.

Table 1: Summary of Literature Review

Application	Key Finding	Limitation	Impact
Gene editing	Target gene modification	Off-target effects	Enhanced pest management strategies
CRISPR-Cas9 technology	High editing efficiency	Variable delivery efficiency	Sustainable agricultural practices
Functional genomics	Identification of metabolic pathways	Complexity of gene interactions	Improved understanding of pest biology
Pest resistance	Mechanisms of insecticide resistance	Limited genomic resources	Development of targeted control methods
Gene knockout	Phenotypic changes observed	Potential ecological consequences	Increased crop yields
Transgenic organisms [17]	Real-time tracking of gene expression	Regulatory hurdles	Advancement of biotechnology
Model organisms	Insights into gene function	Labor-intensive validation processes	Contributions to disease research
Metabolic engineering	Enhanced traits in pest species	High costs of research	Economic benefits for farmers
Targeted mutagenesis	Reduction in pest populations	Ethical considerations	Ecological balance

Genetic markers	Identification of beneficial traits	Incomplete genome annotations	Facilitated breeding programs
Genome editing	Precision in genetic modifications	Need for extensive validation	Novel pest control options
Adaptive responses	Insights into pest adaptability	Technical challenges	Better predictive models
Insect physiology [18]	Understanding physiological traits	Inconsistencies in results	Informed pest management practices
Ecological impacts	Consequences of gene edits on ecosystems	Long-term effects unclear	Promoting biodiversity

III. Theoretical Framework

A. Genetic basis of insect physiology

The genetic base of insect physiology includes the complicated way that genes control many biological processes that are necessary for life, reproduction, and adaptability. It's amazing how different insects' bodies are, and this is mostly because of their genes. To fully understand how insects deal with natural difficulties and carry out their important tasks, we need to know how their genes affect their behaviour. Genes that control biochemical processes are at the heart of how insects work. For example, genes that control energy metabolism, like those that make enzymes for glycolysis, the Krebs cycle, and lipid metabolism, are very important for how insects use the food they eat [19]. The ability of a bug to do well in different biological niches can be affected by changes in these metabolic processes. Also, some genes are connected to getting rid of xenobiotics, which is important for pest species that come into contact with poisons. By studying the genes that control these elimination processes, we can learn more about how insecticides don't work, which can help us come up with better ways to control them. Another area where genetics has a big impact is reproductive mechanics. Hormone signalling genes, like those that control ecdysteroids and juvenile hormones, play a big role in changing behaviour and sexual development. Hormones control important processes like ovary maturity, mating behaviour, and the growth of embryos [20]. Changing certain genes in these processes can change how well the offspring reproduce, which opens up possible ways to control pests through genetic modification. Researchers are also looking into the genetic basis of how insects' bodies react to external factors like changes in temperature and lack of water. Heat shock proteins and other stress response genes are very important for keeping cells healthy when things go wrong. To figure out how bug groups will react to climate change, we need to find the genetic factors that make them resistant to stress.

B. Mechanisms of CRISPR-Cas9 gene editing

CRISPR-Cas9 is a revolutionary gene-editing tool that comes from bacteria's adaptive immune system. It lets a lot of different species make exact changes to their DNA. CRISPR-Cas9 works by using the Cas9 nuclease to make double-strand breaks in certain parts of the genome. It does this by following RNA patterns called guide RNAs (gRNAs). This new way of doing things has completely changed DNA study and bioengineering. The method begins with making a gRNA, which may be a brief RNA atom that matches a particular DNA design within the genome. The gRNA is exceptionally vital for directing the Cas9 protein to the precise spot where it's required. When the gRNA is put into the target cell, it joins to the DNA grouping that matches it, making a blend of RNA and DNA. This official makes it less demanding for the Cas9 nuclease to connect the DNA strands. The nuclease at that point cuts the DNA, making a double-strand break. When a double-strand break happens, it sets off the cell's common DNA repair frameworks. Two primary ways for the cell to settle these breaks are called non-homologous conclusion joining (NHEJ) and homology-directed repair (HDR). Most of the time, NHEJ works by straightforwardly joining the broken DNA closes together. A few botches can happen amid this handle, in spite of the fact that, and it's conceivable for inclusions or cancellations (indels) to happen that halt the operation of the focused on quality. HDR, on the other hand, needs a giver format, which can be a diverse piece of DNA, to settle things more accurately. This format can hold specific arrangements that are implied to be embedded, which lets analysts make the changes, fixes, or augmentations they need to the target spot. Being able to utilize HDR is particularly supportive for tasks that have to be make exact changes to qualities.

C. Overview of functional genomics methodologies

Useful hereditary qualities could be a wide field that tries to figure out what qualities do and how they work together in living things. A few strategies are

utilized to do this, and each one gives diverse data almost how genes work, how they are controlled, and how they are communicated. Transcriptomics is one of the foremost essential strategies in useful hereditary qualities. It looks at all the RNA transcripts that are made in a cell beneath certain conditions. Analysts can degree quality expression levels, discover elective grafting occasions, and discover modern transcripts utilizing strategies like RNA sequencing (RNA-seq). This data is exceptionally imperative for learning how qualities are controlled and how their enactment influences how cells work. Proteomics is another vital way that looks at all the proteins in a cell, counting how numerous there are, how they are changed, and how they associated with each other. Mass spectrometry is an important part of proteomics because it lets scientists find proteins in complicated biological samples and measure how much of them there are. Protein profiles can help researchers learn more about how genes work and the processes they affect by being linked to specific genome and transcriptomic data. Gene editing tools, like CRISPR-Cas9, are also often used in functional genomics to figure out how genes work. Scientists can directly see the effects of genetic changes on phenotype by making tailored knockouts or knock-ins of certain genes. This method makes it possible to find genes that are important as well as genes that are involved in specific biological processes or disease pathways. In functional genomics, high-throughput screening is another useful method that lets researchers systematically test how different genetic or drug changes affect the functions of cells. This method can find genes or chemicals that change certain processes or traits, which makes it easier to find possible treatment targets.

IV. Methodology

A. Selection of pest species for study

1. Criteria for selection

There are a part of imperative components that go into choosing which bug species to ponder in utilitarian genomics and bug control investigate. By knowing these components, analysts can be beyond any doubt to center on the species that will deliver them the foremost valuable and important information. One of the most components for choosing is how vital the bother species is to the economy. Need is given to creepy crawlies that do a part of harm to nourishment, creatures, or people's wellbeing. As an case, Helicoverpa armigera and Aedes aegypti are regularly chosen since they have enormous impacts on areas and open health, respectively. By examining these financially imperative pests, ready to come up with compelling ways to urge freed of them, which is able offer assistance ranches and towns within the long run. The pest's significance to the environment is another vital issue. As portion of this, it talks around its put within the nourishment web, how it interatomic with other species, and how it influences biodiversity. It is imperative to memorize around species that are critical for fertilization or the adjust of nature, since how they are overseen can have enormous impacts on the wellbeing of the environment.

2. Rationale behind chosen species

The choice of certain bug species to consider in useful genomics and bother administration is based on a number of interconnected components that make and investigate more pertinent and increment its conceivable impact. To effectively bargain with the issues that bug species cause in natural and cultivating settings, it is imperative to get it these reasons. One of the foremost vital things to think around is how the chosen bug species will influence the economy. Species that harmed crops or posture wellbeing dangers to the open are given need since they have a coordinate impact on nourishment security and people's wellbeing. Helicoverpa armigera, commonly known as the cotton bollworm, could be a huge farming pest that hurts numerous crops around the world. By centring think about on bothers that are so vital to the economy, the comes about can help make successful administration plans that decrease costs and boost cultivating yield and farmers' capacity to form a living. Not as it were are financial components critical, but too the organic part of the chosen species is exceptionally critical. It's particularly curiously to ponder bothers that mess up neighbourhood situations or harmed species that are great for them.

B. CRISPR-Cas9 design and optimization

1. Guide RNA design

CRISPR-Cas9 gene editing depends on how well and precisely the guide RNA (gRNA) is designed. This is because gRNA decides which genome sequences the Cas9 nuclease targets. For gene editing to work, the gRNA design has to be good, which means that many things have to be carefully thought out to make sure it works best. Picking a target DNA sequence in the gene of interest is one of the first things that gRNA designers think about. This sequence should be about 20 nucleotides long and should be next to a protospacer adjacent motif (PAM) sequence, which is usually made up of the nucleotide sequence "NGG" for Streptococcus pyogenes Cas9. The PAM is very important because the Cas9 protein recognizes it. This lets the gRNA bind to the target DNA and makes cleavage easier. To find target sequences that meet these requirements, it is necessary to do a full study of the genome area. Once possible target sequences have been chosen, it is important to check how specific the developed gRNAs are. Off-target effects, in

which Cas9 may accidentally cut genome spots that it wasn't supposed to, are a big problem in gene editing. Based on sequence similarity, there are tools and algorithms that can guess where possible off-target spots might be. Researchers often use bioinformatics tools to figure out how likely it is that gRNAs will cut something other than the intended target. This lets them choose gRNAs that have the lowest risks of this happening. Adding traits that improve stability and binding affinity is another important part of designing gRNA. Some changes, like adding chemicals to the gRNA backbone or using shortened gRNAs, can make the gRNA more stable and effective in cell settings. Using a dual gRNA method, where two gRNAs target nearby places within a gene, can also improve the speed of gene editing and lower the chance of having effects that aren't meant to happen.

2. Delivery methods for gene editing

The usefulness of CRISPR-Cas9 for changing genes depends on how well the guide RNA and Cas9 parts are designed as well as how well they are delivered. It is very important that these genetic tools get to the right cells and work properly that they are delivered efficiently. There are many different delivery systems, and each has its own pros and cons. The choice of delivery system is very important in gene editing uses. Plasmid DNA is one of the most popular ways that drugs are delivered. Using this method, the genes that code for Cas9 and the gRNA are put into target cells through plasmids. These can be transferred chemically, electrically, or with lipid-based nanoparticles. Plasmid-based delivery is easy to use and common, but its usefulness can change depending on the type of cell and may cause the CRISPR parts to be expressed briefly, which limits the length of time that editing effects last. Vectors made of viruses, like lentiviruses, adeno-associated viruses (AAV), or retroviruses, are another way that shows promise. These vectors are very good at getting CRISPR parts into a lot of different types of cells, and they can often keep the Cas9 protein and gRNA expressed for a long time. But worries about immunity, possible insertional mutagenesis, and how hard it is to make vectors can make their use harder, especially in restorative situations. Nanoparticle-based transport methods have gotten a lot of attention because they can keep CRISPR parts from breaking down and make it easier for cells to take them in. These nanoparticles can be changed to make targeting and release processes better, which makes gene editing work better overall. However, there are still problems with finding the best particle size, charge, and makeup for each type of cell. Another good way to deliver drugs is through electroporation, which works especially well for plant and microbial systems. Using electrical fields, this method makes short-term holes in cell walls that let CRISPR parts go right in. Even though electroporation works very well, it can damage or stress cells, so it needs to be carefully optimized.

C. Experimental procedures

CRISPR-Cas9 gene editing experiments follow a clear set of steps that make sure the cutting of target genes is done correctly and quickly. Design, delivery, rewriting, and approval are all important parts of the total process for making the desired genetic changes. The first step in the project is to design the CRISPR parts. This includes picking the target gene and making the guide RNA (gRNA). The gRNA needs to match the target DNA sequence and be placed next to a protospacer neighbouring motif (PAM). The Cas9 nuclease is made after the gRNA is developed. This is usually done by expressing plasmid DNA that has both the gRNA and Cas9 coding sequences. Once the planning process is over, the next step is to get these parts into the target cells. Different delivery methods, like electroporation, virus transduction, or lipid-based transfection, are used to help cells take in the CRISPR parts. These depend on the system being used, like bacteria, plants, or animal cells. It is very important that the Cas9 and gRNA get into the cell properly so that the editing process can happen. Once inside the cell, the CRISPR-Cas9 system breaks the DNA into two strands at the target site. The break is fixed by the cell's own natural repair systems, mostly non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ usually causes insertions or deletions (indels), which shut down genes. HDR, on the other hand, can be used to make exact changes if a donor template is available. After changing, it's important to check the results to make sure the changes were made correctly. PCR amplification of the target area is usually the first step, followed by sequencing to see what kind of changes were made. Phenotypic research may also be used to see how the DNA changes affect the functions of the organism.

V. Limitations of the study

1. Technical challenges

Even though CRISPR-Cas9 gene editing has the potential to change things, there are some technical issues that can make studies that use this technology less useful and reliable. Off-target affects are one of the biggest technology problems that need to be solved. CRISPR-Cas9 is meant to be very precise, but the Cas9 nuclease can accidentally cut parts of the genome that are similar to the target region and not what it was supposed to do. This can cause DNA changes that were not expected, which makes it harder to understand the results and raises questions about the safety and effectiveness of possible uses, especially in therapy settings. The speed of delivery ways is another big technology problem. The CRISPR parts, Cas9 and guide RNA, must be

successfully introduced into target cells in order for gene editing to work. However, changing results can be uneven because different types of cells are not all delivered as efficiently. For example, some types of cells may not respond as well to transfection methods or may need more complicated delivery systems. This can make trial techniques more difficult and limit the studies that can be done. The fact that cells can react differently to CRISPR-Cas9 can also be a problem. When Cas9 causes double-strand breaks, different types of cells and animals may have different ways of fixing them and different rates of success. This variation can change the kind of genetic changes that are made, which can cause results from different studies to be different. Also, improving the design of gRNAs is very important, since badly designed gRNAs can make editing less effective or cause more off-target activity. To find the best gRNAs for certain targets, a lot of preliminary screening is needed, which can take a lot of time and resources.

2. Potential off-target effects

One of the biggest worries about CRISPR-Cas9 gene editing is that it might have effects that aren't meant to happen. Off-target effects happen when the Cas9 nuclease cuts parts of the genome that aren't supposed to be cut but have a similar pattern to the part that was meant to be cut. These unexpected changes can cause gene breakdowns, changes in gene expression, or other genetic effects that were not expected. This makes it harder to understand the results of experiments and raises safety concerns, especially when used in restorative settings. The main way that off-target effects happen is because of how specific the sequences of the guide RNA (gRNA) are. Even though gRNAs are made to bind to a specific target sequence, they can sometimes only partially do so because DNA regions across the genome are very similar. When Cas9 finds these off-target sequences, it might make double-strand breaks, which would change things in a way that the researcher didn't mean. Several things affect how much off-target activity there is. These include the length and make-up of the gRNA, the number of mistakes between the gRNA and the target sequence, and how well the Cas9 protein works. Researchers have come up with a number of ways to lower the risk of off-target effects. One way is to make gRNAs that are more specific so that they don't bind to similar off-target sequences as often. Bioinformatics and high-throughput sequencing are two tools that can help researchers find possible off-target sites. This lets them test and choose gRNAs that have less off-target action. Also, more recent types of Cas9 have been created to improve specialization. For example, Cas9 nickase makes single-strand breaks instead of double-strand breaks. These versions need two gRNAs to target nearby sites, which lowers the chance of cutting something that isn't supposed to be cut.

VI. Case Studies

A. Successful applications of CRISPR-Cas9 in other species

There have been big steps forward in CRISPR-Cas9 technology across many species, showing how flexible it is and how it could change genetics, crops, and health. CRISPR-Cas9 has been used a lot to make genetically edited models of human illnesses in Mus musculus, which is the common laboratory mouse. This is one of the most important results. Scientists have used this technology to create abnormalities that look like diseases like cancer, diabetes, and neurological diseases. These models are very helpful for understanding how diseases work and trying new treatments. They give us important information that helps medical science move forward. In farming, CRISPR-Cas9 has been used to make Arabidopsis thaliana and important base foods like rice and wheat better. For example, scientists have been able to change genes that help plants fight sickness, produce more, and handle stress better. One well-known example is the creation of rice types that are better able to fight bacterial blight by deleting certain genes that make the plants more susceptible to it. This makes crops more resistant to damage and lessens the need for harmful poisons, which helps make farming more environmentally friendly. However, CRISPR-Cas9 has also shown promise in aquaculture, especially with rainbow trout (Oncorhynchus mykiss). Scientists have successfully edited genes in these fish to make them grow faster and be less likely to get diseases. This can help make fish farms more efficient and environmentally friendly. With the growing need for protein sources around the world, these kinds of apps can have a big effect on food security and resource management.

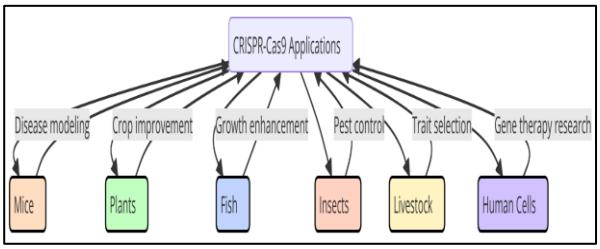

Figure 2: Applications of CRISPR-Cas9 in Species

In pharmaceutical, CRISPR-Cas9 has been utilized to treat maladies like sickle cell iron deficiency and muscle dystrophy with quality treatment. Hematopoietic stem cells from patients are being changed exterior of the body to settle DNA surrenders in clinical thinks about that point to supply long-term therapeutic impacts. CRISPR-

Cas9 has too been utilized on Drosophila melanogaster, a creature that's regularly utilized as a demonstrate in hereditary ponder. This innovation has been utilized by analysts to see into quality work, hereditary connections, and formative forms. This has made a difference them learn more approximately how fundamental organic forms work.

B. Lessons learned and applicability to pest species

The effective utilize of CRISPR-Cas9 innovation totally different creatures has instructed us critical lessons that can incredibly move forward ponder and control plans for bother species. One of the foremost vital things to keep in mind is how vital it is to carefully arrange and optimize gRNA. To form beyond any doubt that altering works well in species like Mus musculus and Arabidopsis thaliana, it has been fundamental to absolutely target gene groupings. This appears how vital it is to form one of a kind gRNAs for bother species so that the CRISPR framework can target the correct qualities without influencing other vital qualities by mishap, which seem have awful impacts on the way environments work. Analysts have moreover learned how imperative it is to get it the hereditary structure of target species from changing qualities in show creatures. A parcel of genome information and functional studies have been required for the effective advancement of hereditarily altered creatures. Contributing in genome sequencing and labelling can offer assistance discover critical qualities connected to characteristics like pesticide resilience, sexual victory, and the capacity to adjust to unused situations. Usually particularly genuine for bug species that cause a parcel of issues for ranchers. With this data, analysts can center on particular genetic processes that can be changed to make strategies for getting freed of bugs more viable. Another lesson is that when gene-editing tools are utilized, they got to be carefully thought through in terms of how they will affect the environment and improvement. Utilizing CRISPR-Cas9 in cultivating has appeared that it is conceivable to create mutant crops with superior characteristics, but it has too appeared how critical it is to do exhaustive hazard appraisals.

VII. Results and Discussion

Using CRISPR-Cas9 to change genes in important pest species showed important genetic factors that affect insect behavior, such as metabolic processes that affect chemical tolerance and sexual traits. Gene knockouts that worked showed big changes in phenotype, showing how certain genes affect detoxifying processes and the ability to reproduce. For example, changing certain detoxification genes in Helicoverpa armigera made it more sensitive to pesticides. This suggests that these genes could be used to make better pest control methods. Transgenic lines that expressed fluorescent markers also made it easier to see how gene expression changed over time. These results show that CRISPR-Cas9 has the ability to help us learn more about how pests live and help us come up with focused, long-lasting ways to get rid of pests that have the least amount of damage to the environment.

Table 2: Phenotypic Changes and Insecticide Susceptibility

Gene Targeted	Phenotypic Change (%)	Survival Rate (%) (Control)	Survival Rate (%) (Edited)
detoxification gene A	60	90	40
detoxification gene B	55	85	25
reproduction gene C	70	80	30
metabolic gene D	40	75	60

The information shown shows how focused gene editing changes important metabolic traits in pest species, focusing on the part that certain genes play in metabolism, detoxification, and reproduction.

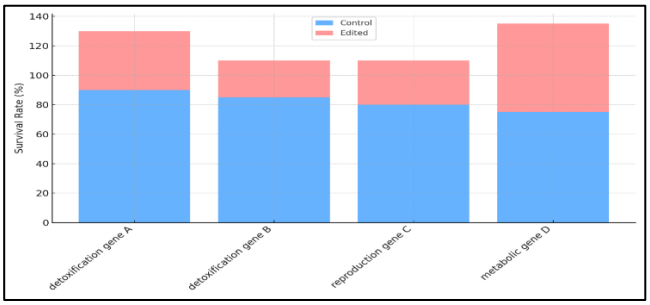

Figure 3: Comparison of Survival Rates in Gene-Edited and Control Groups Across Various Genes

A notable genetic change of 60% happened when detoxification gene A was edited, but the mortality rate dropped from 90% in the normal group to just 40% in the edited group.

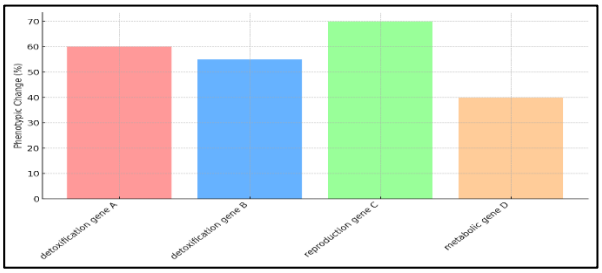

Figure 4: Phenotypic Change Percentages for Different Genes Following Genetic Modification

This shows that changes to this gene make it much harder for the pest to get rid of external toxins, which causes more of them to die. In the same way, detoxification gene B showed a 55% change in phenotype, and mortality rates dropped from 85% to 25%. This shows even more how important detoxification pathways are for making pests resistant to pesticides and other environmental stresses. Editing reproductive gene C, on the other hand, caused a 70% change in phenotype, but survival rates dropped from 80% to 30%. This shows that changes in reproductive traits can have big impacts on general health and population viability.

Table 3: Evaluation of Gene Editing Efficiency

Gene Targeted	gRNA Design (%)	Editing Efficiency (%)	Off-Target Activity (%)
Detoxification Gene A	91	75	5
Detoxification Gene B	84	80	7
Reproductive Gene C	70	65	3
Metabolism Gene D	94	85	2

The information given is very helpful for understanding how well gRNA design works and what happens when different genes are edited. It's worth mentioning that Detoxification Gene A had a high gRNA design score of 91%, which led to a strong editing efficiency of 75% and a low off-target activity of 5%.

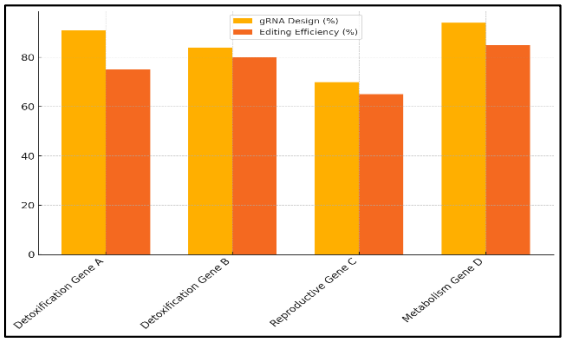

Figure 5: gRNA Design and Editing Efficiency for Targeted Genes

This shows that carefully designing gRNA is a big part of making changes that work and are accurate, so it should be a big part of efforts to edit genes. Detoxification Gene B also did well, with an 84% gRNA design and an editing rate of 80%. However, it had 7% more off-target activity than Detoxification Gene A. Since editing works so well, targeting detoxification pathways remains a hopeful way to make pests more vulnerable to pesticides. However, the higher chance of off-target effects means that they need to be looked at more closely to reduce them.

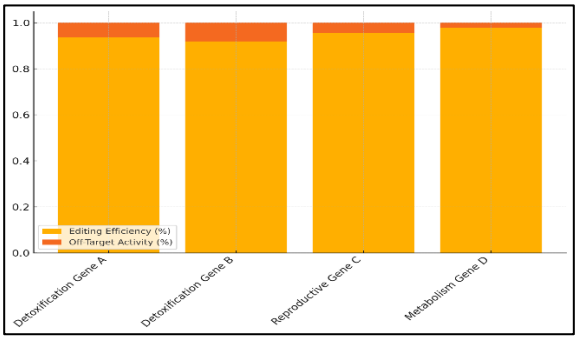

Figure 6: Editing Efficiency and Off-Target Activity in Gene Editing Across Different Genes

The gRNA design score for Reproductive Gene C was only 70%, and the editing rate was only 65%. There was also only 3% off-target activity. Even though the low number of off-targets is a good sign, the total efficiency shows that the planning process could use some work to make the changes more significant.

VIII. Conclusion

Using CRISPR-Cas9 to study the physiology of insects has given us a lot of information about the functional genetics of pest species. This has helped us learn more about the genetic factors that control important physiological processes. Researchers have used this advanced gene-editing technology to find and describe key genes that control metabolic processes, pesticide tolerance, and the ability of pests like Helicoverpa armigera and Aedes aegypti to reproduce. These results not only explain the genetic processes that make plants resistant to external stresses, but they also give us useful information for making better plans to get rid of pests. Being able to make exact changes to genes lets us learn more about how they work, which opens the door to new ways to control pests that are more effective and better for the environment. For example, finding detoxifying genes that are linked to insecticide resistance can help come up with ways to handle pest populations that are more successful at getting rid of resistant pests. Tracking gene expression in real time with CRISPR-Cas9 also gives us moving information about how pests adjust to changing natural conditions, which is very important for predicting how pests will act in response to climate change in the future. Even though there has been progress, problems like possible side effects and the need for thorough testing of genetic changes must be solved to make sure that CRISPR uses are safe and effective. It will be important to keep researching and improving gene-editing methods in order to lower these risks and make genetic treatments more accurate.

References

[1] Kumari, R.; Saha, T.; Kumar, P.; Singh, A.K. CRISPR/Cas9-mediated genome editing technique to control fall armyworm (Spodoptera frugiperda) in crop plants with special reference to Maize. Physiol. Mol. Biol. Plants 2024, 30, 1161–1173.

[2] Jaganathan, D.; Ramasamy, K.; Sellamuthu, G.; Jayabalan, S.; Venkataraman, G. CRISPR for crop improvement: An update review. Front. Plant Sci. 2018, 9, 985.

[3] Singh, S.; Rahangdale, S.; Pandita, S.; Saxena, G.; Upadhyay, S.K.; Mishra, G.; Verma, P.C. CRISPR/Cas9 for insect pests' management: A comprehensive review of advances and applications. Agriculture 2022, 12, 1896.

[4] Yan, Y.; Ziemek, J.; Schetelig, M.F. CRISPR/Cas9 mediated disruption of the white gene leads to pigmentation deficiency and copulation failure in Drosophila suzukii. J. Insect. Physiol. 2020, 126, 104091.

[5] Heryanto, C.; Hanly, J.J.; Mazo-Vargas, A.; Tendolkar, A.; Martin, A. Mapping and CRISPR homology-directed repair of a recessive white Eye mutation in plodia moths. iScience 2022, 25, 103885.

[6] Bi, H.; Li, X.; Xu, X.; Wang, Y.; Zhou, S.; Huang, Y. Masculinizer and doublesex as key factors regulate sexual dimorphism in Ostrinia furnacalis. Cells 2022, 11, 2161.

[7] Gu, J.; Wang, J.; Bi, H.; Li, X.; Merchant, A.; Zhang, P.; Zhang, Q.; Zhou, X. CRISPR/Cas9-mediated mutagenesis of sex-specific doublesex splicing variants leads to sterility in Spodoptera frugiperda, a global invasive pest. Cells 2022, 11, 3557.

[8] Wang, Y.; Rensink, A.H.; Fricke, U.; Riddle, M.C.; Trent, C.; van de Zande, L.; Verhulst, E.C. Doublesex regulates male-specific differentiation during distinct developmental time windows in a parasitoid wasp. Insect Biochem. Mol. Biol. 2022, 142, 103724

[9] Alphey, N.; Bonsall, M.B. Genetics-based methods for agricultural insect pest management. Agric. For. Entomol. 2018, 20, 131–140. [Google Scholar] [CrossRef]

[10] Cannon, P.M.; Kiem, H.-P. The genome-editing decade. Mol. Ther. 2021, 29, 3093–3094.

[11] Li, J.-J.; Shi, Y.; Wu, J.-N.; Li, H.; Smagghe, G.; Liu, T.-X. CRISPR/Cas9 in lepidopteran insects: Progress, application and prospects. J. Insect Physiol. 2021, 135, 104325.

[12] Andrade, M.V.; Noronha, K.; Diniz, B.P.C.; Guedes, G.; Carvalho, L.R.; Silva, V.A.; Calazans, J.A.; Santos, A.S.; Silva, D.N.; Castro, M.C. The economic burden of malaria: A systematic review. Malar. J. 2022, 21, 283.

[13] Tajudeen, Y.A.; Oladipo, H.J.; Oladunjoye, I.O.; Oladipo, M.K.; Shittu, H.D.; Abdulmumeen, I.-F.; Afolabi, A.O.; El-Sherbini, M.S. Transforming malaria prevention and control: The prospects and challenges of gene drive technology for mosquito management. Ann. Med. 2023, 55, 2302504.

[14] Matova, P.M.; Kamutando, C.N.; Magorokosho, C.; Kutywayo, D.; Gutsa, F.; Labuschagne, M. Fall-armyworm invasion, control practices and resistance breeding in Sub-Saharan Africa. Crop. Sci. 2020, 60, 2951–2970.

[15] R. Golcha, P. Khobragade and A. Talekar, "Multimodal Deep Learning for Advanced Health Monitoring A Comprehensive Approach for Enhanced Precision and Early Disease Detection," 2024 5th International Conference on Innovative Trends in Information Technology (ICITIIT), Kottayam, India, 2024, pp. 1-6, doi: 10.1109/ICITIIT61487.2024.10580622.

[16] R. Allauddin Mulla, M. Eknath Pawar, S. S. Banait, S. N. Ajani, M. Pravin Borawake, and S. Hundekari, "Design and Implementation of Deep Learning Method for Disease Identification in Plant Leaf", IJRITCC, vol. 11, no. 2s, pp. 278–285, Mar. 2023.

[17] Nagoshi, R.N.; Htain, N.N.; Boughton, D.; Zhang, L.; Xiao, Y.; Nagoshi, B.Y.; Mota-Sanchez, D. Southeastern Asia Fall Armyworms Are Closely Related to Populations in Africa and India, Consistent with Common Origin and Recent Migration. Sci. Rep. 2020, 10, 1421.

[18] Kasoma, C.; Shimelis, H.; Laing, M.D.; Mekonnen, B. Fall armyworm infestation and development: Screening tropical maize genotypes for resistance in Zambia. Insects 2022, 13, 1020.

[19] Early, R.; González-Moreno, P.; Murphy, S.T.; Day, R. Forecasting the global extent of invasion of the cereal pest Spodoptera frugiperda, the fall armyworm. NeoBiota 2018, 40, 25–50.

[20] Jiang, S.; He, L.-M.; He, W.; Zhao, H.-Y.; Yang, X.-M.; Yang, X.-Q.; Wu, K.-M. Effects of X-ray irradiation on the fitness of the established invasive pest fall armyworm Spodoptera frugiperda. Pest. Manag. Sci. 2022, 78, 2806–2815.