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New Crucial Areas for Frontier Research in Entomology

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ABSTRACT: Entomology is the study of all aspects of insects and allied species, including their developmental morphology, biology, ecology, biochemistry, physiology, behaviour, systematics, and molecular genetics. The application of artificial intelligence is being used in insect pest detection and monitoring in crops/ fruit plants, pest management in warehouses/ grain reserves. This review brings to light the alarming status of DNA barcoding, where India had correctly documented DNA barcodes just 3.97% of the known insect species. Unmanned vehicle/ Drone technology is being utilised for detecting, identifying and managing pests more quickly and effectively to assist farmers and agricultural experts. The CRISPR-Cas system has become a potent tool in genetic engineering because of its ease of use and extraordinary accuracy with low cost. Its use enables the development of genetically modified creatures across a wide range of plants, animals and microbes.

Keywords: CRISPR-Cas system, DNA barcoding, entomology, genetic engineering, RNA interference.

INTRODUCTION

Agricultural entomology involves the study of insects and other arthropods that have an impact on agricultural production. It also deals with insects that are beneficial to crops/ pollinators and harmful pests and their pathogens. It is necessary to understand the biology and behaviour of these insect pests for developing integrated management methods for protecting crops and improve yields. It is divided into A) Harmful insects and B) Beneficial insects.

Harmful pests: Despite man's intelligence and efforts in controlling insect pests; insect pests play a direct role in yield losses with development of resistance against various insecticides. Hence environment friendly techniques should be prioritized for timely detection and their management.

Crucial areas to be focused: Molecular detection techniques through DNA Barcoding, Drone Technology, Artificial Intelligence and Management through Genetic Engineering (CRISPR, RNAi) have to be emphasized.

Detection techniques:

DNA Barcoding: Interception of potential invasive species at entry points such as ports is essential for efficient biosecurity and bio surveillance programmes. Identifying the immature stages of most arthropods assessment is a major challenges for taxonomists. To overcome this, as a method of identification, Novel technologies i.e. DNA barcoding is being employed for identification of both known and unknown species through identifying arrangement pattern of nucleotides in a specific DNA fragment. Unfortunately, a little progress made by India for documentation of entire biota before it becomes extinct is not sufficient. Consequently, there is a growing momentum in adopting DNA barcoding which is rapid and cost-effective for biodiversity documentation. DNA barcoding in India, where only 3.97% of the described species of insects have been successfully identified and captured. So, DNA barcoding-based novel technique for detection studies must be conducted.

Artificial Intelligence: Artificial Intelligence (AI) stands as one of the most significant advancements and key driving force behind the fourth industrial revolution. Emulation of human intelligence in machines for enabling them to think and learn in a manner akin to human being. Artificial Intelligence systems can be trained to accomplish various tasks, including language translation, visual perception, speech recognition, language comprehension and decision-making. Artificial Intelligence seeks to replicate human intelligence, while machine learning (ML) aims to enhance task performance through experience, and deep learning (DL) empowers software to self-train by leveraging vast amounts of already existing data. Artificial intelligence in pest detection can be utilized in various areas, such as monitoring insects in crop plants, managing pests in grain stocks/ reserves, controlling pests in warehouses, and implementing pest control measures in food manufacturing units.

Drone Technology: Unmanned vehicle/ Drone technology is increasingly being used in pest identification more efficiently and in effectively managing pests to help farmers and agricultural experts. Drones can be used for collecting high-resolution images and data on pests and crop health through scientific equipped cameras. Analysed data can be helpful in to identify the presence and severity of pest infestations using machine learning algorithms. Once pests are identified, necessary action can be taken for the control of infestation through pesticides or using other pest management methods. Drone technology is an efficient tool for monitoring pests and crop health and used to survey large areas of land quickly and effectively at low cost. The applications of drones in pest management can be classified in two categories: Sensing Drones and Actuation Drones are used to identify pest hotspots and employed for targeted pesticide distribution respectively. Sensing drones can expedite the process of monitoring and scouting for pests, while actuation drones can minimize the targeted pesticide application area by reducing costs and effectively releasing natural enemies. Park *et al*., (2021) revealed that drones can effectively and accurately identify cocoons from elevated positions using aerial surveys. Specifically, their research focused on developing an aerial survey method by employing a rotary-wing drone to locate cocoons of the oriental moth species called *Monema flavescens* Walker. An aerial survey was conducted on 15 trees, flying at a height of 3-5 meters above the tree canopy. Additionally, they performed a conventional ground survey of *M. flavescens* cocoons on the same trees, considering two conditions: open cocoons and closed cocoons.

Genetic Engineering: Genetic engineering involves the manipulation of an organism's genetic material through addition, removal, or modification of specific genes to alter the characteristics of the target organism or to introduce desired traits in plants, animals, and microorganisms. Genetic engineering can be used in developing crops for resistance against pests and diseases. Furthermore, genetic engineering plays a significant role in research, facilitating the study of specific gene functions for rapid advancement with a wide range of applications and significant transformations in various areas of science and industry. Techniques employed in genetic engineering are ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-like Effector Nucleases), and CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats).

Clustered Regularly Interspaced Palindromic Repeats (CRISPR/Cas): In nature, CRISPR/Cas immune systems are used by bacterial and archaeal species to repel phages and foreign genetic material (Sorek *et al*., 2008). The development of techniques based on CRISPR and Cas proteins revolutionised the area of genome editing in 2012 (Mali *et al*., 2013). Using the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas (CRISPR-associated) enzymes, a genetic engineering technique known as CRISPR-Cas modifies the DNA of living things in a precise, targeted manner. The genomes of many bacteria and certain archaea contain short, repetitive DNA sequences known as CRISPRs. Proteins called Cas enzymes, like Cas9, may bind to particular DNA sequences and create specific cuts. When using the CRISPR-Cas system for genetic engineering, scientists create a guide RNA (gRNA) that resembles the particular DNA sequence they want to target. The Cas enzyme then follows the gRNA to the desired site in the genome and cleaves the DNA there. The DNA at that precise position can then be added to, deleted from, or modified by researchers using other methods. The CRISPR-Cas system is an effective tool for genetic engineering due to its relative simplicity, low cost, and extreme precision. In a wide range of taxa, including plants, animals, and microbes, it has been employed to produce genetically engineered creatures. The CRISPR/Cas9 technology may offer promising approaches for the management of insect pests.

Why CRISPR/Cas is mostly used than other genome editing options? A module that binds to certain DNA sequences and a FokI nuclease domain that needs dimerization are the components of ZFNs and TALENs, respectively. Two modules must be created for targeting closely spaced DNA sequences and allow FokI dimerization. However, it is costly and difficult to create functional nucleases. It requires the time-consuming creation and expression of two distinct DNA binding domains for each target location. On the other hand, the CRISPR/Cas9 system is an incredible effective genome editing technique of low-cost and easy-to-use. It depends on DNA-RNA interaction and calls for the production of an oligonucleotide with 18 to 20 bases which helped CRISPR/Cas9 to become the most popular genome editing method. The CRISPR/Cas9 system's capacity to concurrently edit numerous target genes is a key benefit.

In a two-step experiment, Zsögön *et al*., (2017) targeted six genes and caused mutations in four of them, and the second round of genome editing successfully modified all six genes simultaneously. This shows that multiplex genome editing with CRISPR/Cas9 may be used to easily domesticate novel plants by quickly introducing desired features. Simultaneous targeting of multiple sites might result in deletions between the target sites of a certain size and using this capacity, knockout mutants can be produced with altered gene functions and disrupt regulatory sequence. S-genes are the plant genes that facilitate infection and support compatibility and it codes for the operative target that plays a negative role in plant defence. It can be beneficial to target S-genes using multiplex CRISPR/Cas9 genome editing in order to strengthen plant defence system.

There are several genes that CRISPR/Cas9 can target to control insect pests:

1. Male related genes: double sex (*DSX*), Transformer, Chromatin-linked adapter for MSL proteins (clamp).
2. Female related genes: Egg specific proteins (*Esp*) and Double sex (*DSX*)

3. Pheromone related genes; Pheromone binding proteins, Olfactory receptor and Odorant receptor
4. Segmentation related genes: Paired, *Wnt*-1, Abdominal-A *and Ago*1
5. Metabolism related genes: Succinyl- CoA, P450and NPC1b
6. Phenotype related genes: Tyrosine hydroxylase, Troponin C and *BLOS2*

Case study: The Vitelline membrane proteins (VMPs), which are the major proteins that make up the inner shell (vitelline membrane layer) of insect eggs and are a crucial component of egg production and embryo development, were the subject of this technology's application. Zhai *et al*., (2022) described the molecular characteristics and patterns of the VMP26 gene's expression in the diamondback moth, *Plutella xylostella* (L.), a migratory pest of cruciferous plants. PxVMP26 was primarily expressed in female adults and was very highly expressed in the ovary, according to both qPCR and western blot studies. Using CRISPR/Cas9 mutations in 8 and 46 bp that cause frame shifts were successfully used to construct a PxVMP26 knockout. CRISPR/Cas9 technology used for egg collapse resulted from the deletion of the PxVMP26 gene, which also altered the average egg size, harmed the structure of the vitelline membrane, and raised the fraction of defective eggs. VMP gene in the oocyte formation and embryonic development of *P. xylostella* serves as a foundation for searching for novel genetic control of *P. xylostella by using* CRISPR/Cas9 technology.

RNA interference: Regulatory RNAs was the discovery of RNA interference is a significant turning point (RNAi) (Fire *et al*., 1998). In RNA interference cleaving or blocking larger RNA molecules with a matching sequence, tiny RNA molecules of 18–31 nucleotides in length activate a sequence-specific gene silencing response throughout this process. Therefore, the expression of this gene can be inhibited at the post-transcriptional level by delivering dsRNA targeting any endogenous gene transcript to the desired pest organism. Therefore, this technique has the potential to cause insect death by the precise selection of a crucial target gene. Baum *et al*., (2007) were able to manage coleopteran pest using RNA interference technology.

Beneficial insects: Insects that have a good effect on the environment or on human activities are said to be beneficial insects may be pollinators, predators, and parasites, all of which are crucial for preserving the equilibrium of ecosystems and promoting agriculture. These insects are useful because they pollinate plants, reduce pest populations and preserve biodiversity. Sericulture, Apiculture, and Lac Culture are among them.

Sericulture: Sericulture is the practise of raising silkworms for the purpose of producing silk. To create high-quality silk, silkworms, which are the larvae of silk moths, need to be given a lot of care and attention. Sericulture is a significant cultural and economic endeavour in addition to being a substantial agricultural industry.

Sericulture + Biotechnology = Seri biotechnology: Seri-biotechnology is a branch of biotechnology that improves silkworm breeding and silk production by using the concepts of biochemistry and genetic engineering. It involves the application of genetic engineering, molecular biology, and other biotechnological approaches to improve the silkworm's capacity to produce silk and enhancing the usefulness and quality of silk production. This can involve creating genetically altered silkworms with higher disease resistance, better silk output and improved climatic adaptation. Seri-biotechnology can be utilised to manufacture novel silk-based goods and to develop new techniques for processing silk. It has the potential to completely transform the silk business and make it more efficient and sustainable.

India may be the second largest producer in sericulture after China, but it is still in early stage in seri biotechnology. The advantages of seri biotechnology will result in improved types of silkworms that generate more silk and are more resilient to illness and environmental changes. Techniques involving recombinant DNA, genetic engineering that manipulates desirable features, and methods used in stem cell research are all areas where focus might be placed.

Green, orange luminous and red silks were produced utilising a vector derived from the fibroin H chain gene using traditional breeding process. The findings of Iizuka *et al*., (2013) show that transgenic silkworms can produce significant volumes of genetically modified silk, and that this silk may be used as functional silk fibre for textiles and medicinal purposes.

Spider (*Araneus ventricosus*) Dragline Silk Protein-Expressed Silkworm Produces High-Toughness Silk. A natural fibre with outstanding tensile characteristics, spider dragline silk is challenging to artificially create as a long, strong thread. The spider dragline protein gene (*Araneus ventricosus*) was cloned and transgenic silkworms were produced that expressed the fibroin heavy chain and spider dragline protein in cocoon silk. The natural silkworm fibroin content of spider silk ranged from 0.37 to 0.61% w/w (1.4–2.4 mol %). The transgenic silkworm can initially produce raw silk from its cocoons using an excellent silk-producing strain, C515. After the addition of spider dragline silk protein, the raw silk's tensile properties improved by 53% (Kuwana *et al*., 2014).

Apiculture: Apiculture, also known as beekeeping, is the practice of maintaining honey bee colonies in hives for the purpose of collecting honey and other products such as propolis, beeswax, pollen, royal jelly and bee venom. It also involves the raising of queens and package bees for the purpose of increasing or repopulating colonies. Honeybees are important pollinators for many crops, including vegetables, fruits and nuts. Beekeeping is a challenging task that requires knowledge of bee biology, behaviour and diseases, as well as proper equipment and maintenance. Beekeepers have to carefully monitor their hives, keep records of their colonies, and take steps to protect their bees from disease and other threats. In terms of honey production, India ranked eighth in the world in 2019, and Punjab is the primary state for beekeeping. The value of the Indian honey market was Rs. 17.3 billion.

What is the urgent need for new research in Apiculture: Rising domestic market and increasing the quality standards of honey is need of the present time. Consumer preferences for wellness foods and natural alternatives to artificial sweeteners, together with changing eating patterns, are projected to drive up demand for honey in upcoming years. People have increased its use due to its anti-microbial and anti-inflammatory effects in response to the risk of COVID-19 infection. Only three out of 13 brands passed a recent study by the Centre for Science and Environment (CSE), India, which discovered that 77% of the samples were contaminated with sugar syrup.

Flow Hive technology: Flow Hive technology, developed in Australia, allows honey to flow down freely (Grekov *et al*., 2021). Flow Hive beehive technology relies on tough plastic movable honeycombs as its secret weapon. The vertical gaps are adjusted by one-half of a cell by twisting a particular knob, allowing the honey to flow through the cells, into a channel at the bottom of each frame and out into a collection jar. This invention reduced the price of basic production while simultaneously increasing revenues.

Varroa Control by Hyperthermic Device: The Bee Ethic system is a technological hive that make up of an electronic control panel and a series of heated frames. Trials were conducted in the years 2018, 2020, and 2021 to compare colony health and mite infestation in heat-treated and untreated control beehives. The findings demonstrated that, even in the face of significant re-infestation events, hyperthermia was a successful method for controlling *V. destructor* within an IPM strategy (Porporato *et al*., 2022).

Biological waste decomposition: A potential biological waste decomposer of organic wastes is the use of black soldier fly (BSF) larvae (*Hermetia illucens*). In addition, the larva itself is a rich source of protein and minerals, making it suitable for use as animal feed in poultry (Anand *et al*., 2008; Khusro *et al*., 2012) and pig farms as well as fish cultures (Tran *et al*., 2015). The larvae of the species are voracious consumers, and several reports exist that have examined the efficacy of the BSF larvae in the conversion of organic wastes from fishery industries, bio-leaching, domestic kitchen and municipal wastes, as well as manure from cattle farms.

Forensic Entomology: Forensic entomology is the discipline of forensic science which studies insects and other arthropods in order to provide legal evidence. Forensic entomologists use their knowledge of the life cycles and behaviours of insects to help determine the time of death of a corpse, or to provide other details about a crime scene. In order to estimate the post-mortem interval (PMI), or the amount of time that has transpired after death, forensic entomologists examine the presence and stage of insects discovered on a body. They can determine the time of death by looking at the types of insects present on a body and their developmental stages. It is widely used in many developed countries and it's considered as an important tool for criminal investigations. Among the 29 insect orders Diptera and Coleoptera plays important role in investigation.

Recent advances in forensic entomology: Insect toxicology is emerging as new sub branch of forensic entomology which is used in detection of poisons, toxins or drugs from various insect's developmental stages. J C Beyer is known as father of entomo-toxicology for his pioneer significant contribution into the field. The entomological evidences collected from crime sites can be used to determine the cause of death due to poisoning.

Wildlife Forensic Entomology: Forensic entomology can be effectively utilized in wildlife crime investigations, such as cases involving illegal hunting, poaching, and trade, which fall under The Wildlife Protection Act 1972. Two highly publicized incidents in 2010, concerning the deaths of tiger cub's in Ranthambhore and a tiger in Sariska National Park (a tiger reserve in Rajasthan), were successfully resolved through the collection and taxonomic identification of insects found on the bodies of the deceased animals, specifically, the entomofauna collected from the tiger cubs and tigers included species of *Chrysomya*, commonly known as 'hairy maggot blowflies.'

Medical Entomology: Medical entomology is the study of insects and arthropods that have an impact on human health. This includes insects and arthropods that transmit diseases, as well as those that cause physical damage or irritation to the skin, eyes, or other parts of the body. Some examples of insects and arthropods studied in medical entomology include mosquitoes, ticks, lice, and fleas with their management strategies to prevent the spread of diseases they transmit.

Why study Medical Entomology: The impacts of insect pests on human health in two types: direct effects and indirect effects. Direct effects encompass mechanical reactions like dermatitis, dermatosis, and itching as well as exsanguination resulting in blood loss and annoyance. It also includes myiasis, which refers to the invasion of living tissues by fly larvae, and the effects of toxins and paralysis caused by insects. Insects are collectively responsible for over one million deaths per year worldwide. It is estimated that in 2020 more than half of a million malaria deaths occurred worldwide (WHO World Malaria Report, 2021). Allergic reactions are another form of direct effect. Indirect effects primarily involve the transmission of diseases through vectors, such as insects.

Veterinary Entomology: Veterinary entomology is a field of study that focuses on insects and other arthropods that affect the health and welfare of both domestic and wild animals. Its scope encompasses the examination of insects that transmit diseases to animals, as well as those that cause physical discomfort or irritation. Veterinary entomology is closely related to disciplines such as medical entomology, parasitology, animal sciences, veterinary medicine, and epidemiology. They are categorized into (A) Permanent Ectoparasites, (B) Semipermanent Ectoparasites and (C) Occasional Parasites. They cause damage to animals through: Loss of blood and tissue fluids, pain and interference with activities, allergic responses to saliva and diseases.

Entomophagy: Entomophagy is the practice of eating insects as food by human being. It is a traditional dietary practice in many parts of the world, and is increasingly being considered as a sustainable source of protein in areas where traditional protein sources, such as beef and pork, are not readily available or are too expensive. Insects are a highly nutritious food source, as they are high in protein, fat, minerals, and vitamins. Additionally, they are more environment friendly to produce than traditional livestock, as they require less land, water and feed. Tribes in Africa and Australia eat anything from ants to beetle larvae as part of their subsistence meals, while in Thailand, crispy-fried locusts and beetles are a favourite snack. It is believed that at least 2 billion people worldwide frequently consume insects. Beetles, locusts,caterpillars, grasshoppers,bees, wasps, ants, cicadas, crickets, leaf and planthoppers, scale insects and true bugs, termites, dragonflies, and flies are the insect families that are most frequently consumed.

Why eat insects: Overall, there are three reasons to encourage entomophagy: **Health**: Many insects are excellent in calcium, iron, zinc, and healthy fats as well as protein. **Environmentally** speaking, insects that are marketed as food because they emit considerably fewer greenhouse gases (GHGs) than most livestock. **Livelihoods (economic and social factors)**: It provides low-cost means of subsistence for both urban and rural residents.

Entomophagy in India: In India, termites were consumed by native tribes in Mysore and the Karnataka region (Forbes, 1813). Approximately 255 different types of insects are used as food by Indian tribes. Coleopteran species made up the majority of these edible insect species - roughly 34% - followed by Orthoptera (24%), Hemiptera (17%), Hymanoptera (10%), Odonatae (8%), Lepidoptera (4%), Isoptera (2%), and Ephimeroptera (1%).

New frontiers in vector control:

1. Paratransgenesis - Recently *Serratia* (bacteria) was been isolated in China that not only spread through mosquitoes but also by natural vectors (without genetic engineering) strongly prevents *Plasmodium* growth in mosquitoes (Gao *et al*., 2021).

2. Improved nets - Utilization of Interceptor G2 nets, which are treated with a combination of a pyrethroid and chlorfenapyr (a distinct class of chemical not previously employed for vector control), demonstrated a significant improvement in malaria prevention compared to nets treated with pyrethroid alone in United Republic of Tanzania.

3. Attractive targeted sugar baits (ATSBs) - Recently undergoing testing in Zambia, Mali, and Kenya, there is a novel intervention being evaluated that resembles the size of an A4 sheet. This intervention comprises small compartments containing a sugary substance infused with insecticide. Preliminary results have already been observed as encouraging and complete results are expected to be available by 2025.

4. Endectocides (Humans as vector control) - Involves the use of endectocides or medications by humans is the developing field in malaria control having an impact on the mosquitoes that bite them. Initially employed to treat onchocerciasis, ivermectin is the most extensively studied endectocide so far.

According to Dr. Sunil Parikh, an associate professor of Epidemiology and Infectious Diseases at the Yale School of Public Health, that ivermectin appears to have an adverse effect on mosquitoes as well. Mosquitoes that had ingested blood from individuals treated with ivermectin exhibited noticeably reduced survival rates when captured.

5. Gene drive: changing mosquito DNA - Dr. Mamadou Coulibaly, the principal investigator at Target Malaria, provides an explanation stating that the implementation of gene drive technology in male mosquitoes can lead to a reduction in population size. When a genetically modified male mosquito carrying this technology is released, it hampers the fertility of the female mosquito during mating, resulting in no offspring being produced. As a result, the overall mosquito population reduced (WHO, 2022).

Recent Novel Control Tactics and Resistance Management:

Vesicular stomatitis (VSV) is an illness that causes flu-like symptoms in people and painful lesions in horses. To lessen the danger or consequences on the equestrian sector, Peck *et al*. (2020) identified the three most typical insect vectors of VSV and created workable vector abatement and husbandry measures. A qPCR-based Melt-MAMA analysis to detect three resistance mutations and a High-Resolution Melt qPCR method that enables the detection of previously described single-nucleotide polymorphisms in a single PCR were developed by Thomas *et al*., (2020) in an effort to find a faster method for determining acaricide resistance.

CONCLUSION

This study offers viewpoints on the potential for increasing insect-based detection and control. Encouraging new crucial areas for research could help us to manage harmful pests and also make use of beneficial insects. Not only in the area of agriculture, but entomology can also be used in various other areas like forensic, veterinary, medical etc. Insects are not always pests but can also be used for food, providing livelihood. Encouraging insect-based companies would aid in efforts to conserve biodiversity and promote human well-being.

FUTURE SCOPE

The future of entomology research lies in the integration of cutting-edge technologies and innovative methodologies. Molecular detection techniques, artificial intelligence and drone technology, and genetic engineering offer exciting opportunities to enhance our understanding of insects and develop effective management strategies. By exploring these crucial areas, entomologists can make significant strides towards sustainable agriculture, ecological conservation, and human well-being. It is imperative for researchers to embrace these advancements and collaborate across disciplines to unlock the full potential of entomology in the years to come.

Conflict of Interest: The authors declare that they have no conflict of interest regarding the publication of this article. They have no financial or personal relationships with individuals or organizations that could influence their objectivity or the content of the article. The research presented in this article is based on independent and unbiased findings.

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