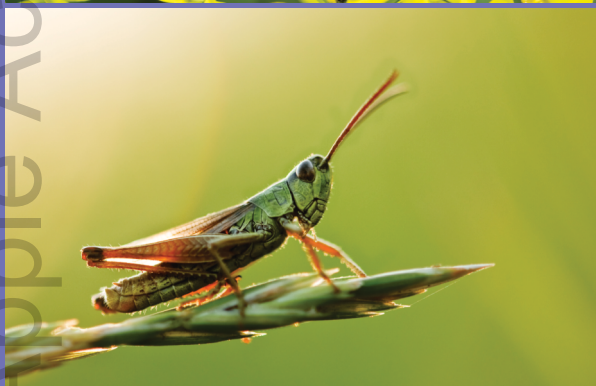


Insect Diversity and Ecosystem Services

Volume 2: Environmental Indicators,
Molecular Approaches, and Management Strategies



Younis Ahmad Hajam | Sajad Hussain Parey | Rouf Ahmad Bhat
Editors



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CHAPTER 8

Medical Entomology: Advanced Applications and Future Prospects

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ABSTRACT

Entomology is the branch of science that studies insects. It specifically refers to those insects which affects human being and causes diseases in medical entomology. Medical entomology is associated with biomedical study of insects and their morphology, biology, and anatomy. The epidemiology, precautive measures, and methods of infection or infestations are controlling, which are caused by insect vectors. It is also interacted with insect behavior and history of lifespan of vector and hosts. Nowadays, medical entomology is directly linked with biomedical sciences, for example, medical virology, medical parasitology, epidemiology, and public health, etc. Phylum Arthropoda causes direct effect on human being

or other animals such as biting, tickling, and ticking which are commonly known as ectoparasites. It also causes some other vector-borne or infectious diseases such as dengue fever, malaria, and some others. Innovative ideas related to medical entomology has been currently projected. These ideas should be systematically considered due the effects of arthropods on human health and control measures. PCR and MALDI-TOF techniques are mostly used in identification of hematophagous arthropods such as mosquitoes, ticks, and lice. This chapter summarizes the medical entomology, its applications, and future perspectives.

8.1 INTRODUCTION

Entomology is the branch of science that studies insects. It specifically refers to those insects which affects human being and causes diseases in medical entomology. Medical entomology is associated with biomedical study of insects and their morphology, biology, and anatomy (Tyagi, 2003). Additionally, the epidemiology, precautive measures, and methods of infection or infestations are controlling, which are caused by insect vectors. It also interacts with insect behavior and history of lifespan of vector and hosts. Nowadays, medical entomology is directly linked with biomedical sciences, for example, medical virology, medical parasitology, epidemiology, public health, etc. Phylum Arthropoda causes direct effect on human being or other animals such as biting, tickling, and ticking, which are commonly known as ectoparasites. It also causes some other vector-borne or infectious diseases such as dengue fever, malaria, and some others. Innovative ideas related to medical entomology has been currently projected. These ideas should be systematically considered due to the effects of arthropods on human health and control measures. After that, the responsibilities of “medical entomology” must be distinctly widened to accept the cognitively and reasonably critical issues, yet neglected.

In entomological studies, the phylum Arthropoda comprises important classes including “Pentastomida,” “Arachnida,” “Crustacea,” “Chilopoda,” “Diplopoda,” and “Insecta.” Majority of taxa belong to this phylum, approximately 90%, as comparison to other species, and prominent by the exoskeleton presence. The class insecta is the most important class in medicinal practice (Schmid-Hempel, 1998).

TABLE 8.1 Showing Most Important Orders and Some Examples Used in Medical Entomology.

S. No.	Order	Examples
1.	Diptera	Mosquitoes (anopheles, aedes, and culex)
2.	Hemiptera	Bugs
3.	Suctoria	Fleas
4.	Anoplura	Louse
5.	Coleoptera	Beetles
6.	Blattaria	Cockroaches
7.	Lepidoptera	Butterflies
8.	Hymenoptera	Ants, hornets

These species are liable for vector-borne/infectious diseases, that is, malaria, yellow fever, trypanosomiasis, encephalitis, and dengue fever. These are showing the transmission of diseases by arthropods which are known as arthropod-borne diseases. It has a high burden on mortality and morbidity worldwide, especially in developing countries. Those diseases signify the different epidemiological factors and increases year after year. Currently, the addition of these factors has been prominent in a new medical science and supports medical entomology or ecoepidemiology study. In medical entomology, the discipline offered some tools and approaches, as well as public health. It is also correlated with supplementary objectives, for example, anticipation, forecast, and estimation of infectious/vector-borne diseases. These diseases are transmitted by insects under class insecta.

In classes Arachnida and Acarina, which are ticks and mites responsible for such diseases as scabies, allergic, rickettsiosis, and dermatoses. Presently, the main observational studies about arthropods stay with conventional taxonomical classification. It is the first primary device for the categorization of collected samples, excluding the revolution of biotechnologies in molecular biology and has impacted new molecular entomology. The authority exploring the new tools and techniques to manage the vector-borne diseases by manipulation of genes is known as genetic manipulation.

In genetic technology, the growth of virus or parasites is enabled by using gene transfer technology, and eventually leads to suppress the diseases. Another individual area is the modification of genes in transgenic mosquitoes, and avoiding the spread of various diseases, for example, dengue fever and malaria. Additionally, these are existing accessibility of

sequence of genes such as *Anopheles gambiae*. Medical entomology has been also closely applicable in forensic science; insect necrophagy are significant in corpse decay. The association between cadaver and arthropods (insects) for the use of insects in medico-criminal consideration is known as forensic entomology. In addition to this, it help in determining the insects necrophagy feeding on cadaver, and investigation of insects. Another utilization of medical entomology is the toxicological examination of larval necrophagy from cadaver to classify and estimate various toxicants (PCBs) and allopathic drugs assimilated with the evidence of feasible post-mortem investigations (Amendt et al., 2004).

Arthropods are invertebrate animals of around 1 million species, and approximately 80% of living species (Giribet and Edgecombe, 2012). A number of these are used as vectors and affects the transmission of pathogenic bacteria, virus, and parasite from one animal to another, by taking meal and mix up with blood (Mathison and Pritt, 2014). Every year, the main vector such as mosquitoes causes malaria and dengue fever mostly in tropical countries. In addition, the Chikungunya virus and Zika virus have twisted community in the last decade and increased the development of vector-borne diseases throughout the globe (Simon et al., 2008; Musso and Gubler, 2016). Furthermore, the penalties of insects (ticks) plague are eminent to veterinary field under medical entomology. On the contrary, in 1980s, its medical significance has reappeared with the category of “Lyme disease.” The overall depiction of some tick-borne diseases is the most common ricektsia prevailing since last 15 years (Parola et al., 2013).

Among arthropods, ticks are now known to be infectious diseases (second main vector) in humans spreading all over the world. Considerate biology of vectors helps expect the appearance of various vector or viral diseases. In fact, anthropophilic vectors (mosquito) are spreading virus and are also responsible for interrelated outbreaks (Parola et al., 2006). A public health emergency of international concern are vector-borne diseases and Chikungunya or Zika virus, which have become dangerous throughout the world (Parola et al., 2016). Medical entomology is basically persistent toward insects under arthropoda, and affects human health. In one health concept, some different categories are mentioned such as environment science and veterinary entomology. In medical entomology, to understand the vector biology, regular monitoring of arthropods (insects) are a basic necessity to prevent vector-borne/infectious diseases. Therefore, fighting and proper monitoring against infectious diseases is necessary.

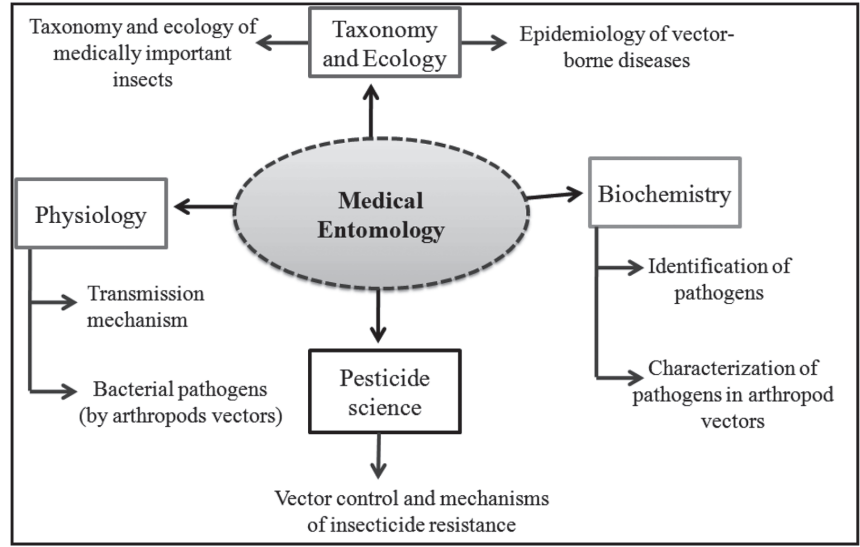


FIGURE 8.1 Medical entomology focused on basic and applied researches for taxonomy and ecology, physiology, and pesticide science in insect pests and vector-borne diseases.

8.2 APPLICATIONS OF MEDICAL ENTOMOLOGY

8.2.1 EMERGENCE OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION—TIME OF FLIGHT (MALDI-TOF) IN ENTOMOLOGY

In transmitted diseases, the essential factors are the detection of vectors, important vector control, facts regarding distribution, and contribution to estimate diseases risk. However, this study area mentions about planning and protection against infectious diseases and vector control. It provides protection to humans against exposed insects. Vector is known to influence human health and provide entomological investigating area such as pathogen transmission risk to humans (Aubry et al., 2016). Morphological classification is the most common to detect insects depends upon dichotomic keys (Mathison and Pritt, 2014). Although, it requires documentation of comprehension and entomological proficiency. Comprehensive recognition keys are required in some insects such as ticks, mites, and mosquitoes which are spread thought-out the geographic area (Estrada-Pena et al., 2004; Brunhes et al., 2000). Furthermore, numerous trials can change or cause concession of critical morphological criterion, that is, the worsening

of the samples throughout gathering or carrying the specimen, or the enlargement of the samples. In criteria morphology, it can also be deficient for the undeveloped stages, except kind complex species morphologically identical (Yssouf et al., 2016). It can lead to incorrect identification effect on the understanding possibility of infectious risk. In addition, the number of species in biological systematic has declined. In fact, entity entomologists can scarcely become species on extensive variety of class insecta under arthropoda. In medical entomology, polymerase chain reaction (PCR) has been used in identification of insects in molecular biology. For the identification of different insect genes, various molecular level techniques are used (Norris, 2004; Fang et al., 2002). Certainly, arthropods identifying primers are not available, and these approaches are dependent on entirety and consistency of the sequences available in the genebank database (Lv et al., 2014). The MALDI-TOF mass spectrometry (MS) is used for identification of insects. These tools and techniques is primarily based on different protein profile and genome sequencing according to the database spectra. It has revolutionized the field of clinical microbiology (Seng et al., 2010; Laroche et al., 2015). The use of MALDI-TOF MS for the identification of insects for the identification involves a number of steps.

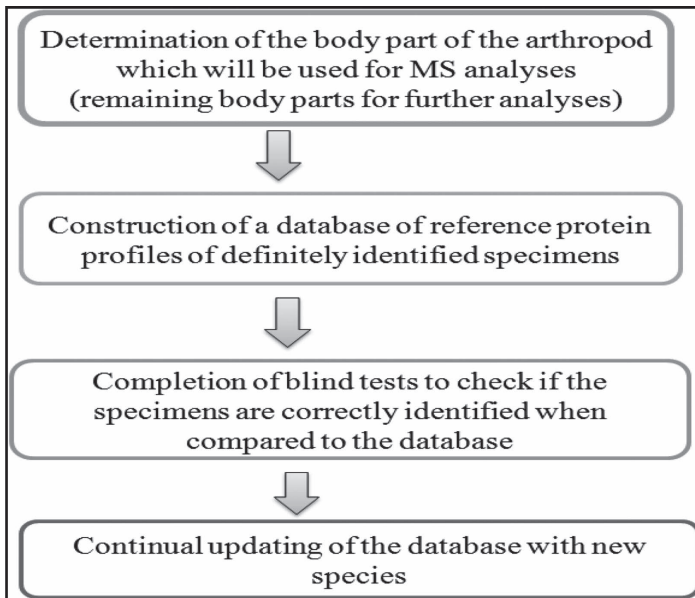


FIGURE 8.2 Different steps involved in identification of insects.

PCR and MALDI-TOF techniques are mostly used in the identification of hematophagous arthropods such as mosquitoes, ticks, and lice (Yssouf et al., 2014; Yssouf et al., 2013; Yssouf et al., 2014). The most accurate technique, that is, the MALDI-TOF technique is used to identify the insects body parts such as sand flies (thorax, legs, and wings) (Lafri et al., 2016). Medical entomology/veterinary entomology are identifying and challenging more than 60 arthropod species (Sambou et al., 2016). To identify or use ticks composed by MS and using different body parts to recognize pathogens by molecular technique. Currently, *Rickettsia conorii* and other non-infected ticks are identified by MALDI-TOF mass spectroscopy technique (Yssouf et al., 2015; Fotso et al., 2014). It was challenging to detect protozoan or plasmodium parasites by MADI-TOF techniques. In addition to this, protozoan parasites such as *Plasmodium berghei* were identified on the basis of database spectra of cephalothorax from infected anopheles' mosquitoes (Laroche et al., 2017). Beside this, the key information was identified by sources of blood meal, and better understanding about malaria endemic or host-vector relations. *Anopheles gambiae* were distended to precise the feed-pattern determination, and observe post-blood meal in the abdomen proteins (Niare et al., 2016).

Since the past 15 years, molecular tools (MT) having ability to utilize and recognize the distribution, disease control, various health-related factors, and geographical monitoring of micro-organisms that are carried by the arthropods are in use. The particular information about the distribution, disease, etc., of micro-organisms has been obtained with the help of this technique or method to increase the list of communicable diseases in geographical area of interest that also helps to inform all the microbiologists about that particular area having pathogen presence (Niare et al., 2016; Parola et al., 2013). The African tick bite fever is one of the best examples in the world. The people who come from Guadeloupe (a French Caribbean Island) has rickettsiosis, and in 18th century, an African tick (*Amblyom-mavariegatum*) was diagnosed in West Indies and was reported in literature and also in cattle from Senegal. In the present days, the causative agent of bovine cow deiosis is known to be the vector of Ehrlichia ruminantium that reported tick-bite fever in sub-Saharan Africa human. R. Africae also analyzed that it was an etiological agent in their index case (Parola et al., 1998). Parola et al. (1999) reported that an entomological survey helps to isolate as well as the detect *A. variegatum* ticks (extracted from cattle) present in *R. africae*. Also, *R. africae* presence was confirmed by many

people in different studies on surrounding islands (Kelly, 2006), that lead to the other example that contributes broadly in North Africa toward range of rickettsioses. However, a Mediterranean spotted fever (caused by *R. conorii*) reported in the area since 1990. Further, the MT development and their expansion (such as PCR and arthropods sequencing) helps to identify the different new pathogens found in tick that was collected from North Africa and a complemented knowledge was stored on arthropod-born pathogen (in Maghreb) (Parola et al., 2013; Kernif et al., 2012).

Tick-borne lymphadenopathy (TIBOLA), also known as Scalp Eschar and Neck Lymphadenopathy (SENLAT), is caused by *Rickettsia slovaca*. There are different agents such as *Rickettsia aeschlimanni*, *Rickettsia massiliae*, and *Rickettsia monacensis* of rickettsioses group (Kernif et al., 2012). In fleas, studies were conducted from Africa which detected *Rickettsia typhi* and *Rickettsia felis*, a murine typhus agent and pathogenic species of *Rickettsia* (Leulmi et al., 2014). A causative agent Borreliagarrinii of disease Lyme was detected (in Ixodes ricinus ticks) in Algeria but still the epidemiology of this disease is not identified (Benredjem et al., 2014; Kernif et al., 2012). In some mosquito-borne viruses (Zika virus) and sand-fly viruses, this technique has utilized more knowledge in the arboviruses field. Similarly, the Toscana virus was detected in Southern Europe; different studies have been conducted in particular cases also. Also, it was demonstrated that the virus was broadly spread more than we have imagined in about all North Africa and some parts of Europe (Charrel et al., 2012; Charrel et al., 2007). Such techniques help to demonstrate and isolate the various unknown or new viruses present in sand-fly worldwide (Alkan et al., 2015). In different fields, such as entomologists, virologists, parasitologists, ecologists, and veterinary doctors, the latter is one of the good examples of good and interested research studies. Bichaud et al. (2016) reported that cell culture, electronic microscopy (EM, seroneutralization (some classic techniques) mutualized with molecular techniques. Phlebotomies collection can be oriented from Leishmanial parasites, human leishmaniosis, and serorevalence results for phleboviruses and phleboviruses detection data. Since a decade, about >50% new viruses were reported that transmitted through phlebotomies in worldwide: Pinique and Medjerda alley viruses in Tunisia (Bichaud et al., 2015; Zhioua et al., 2010), Massilia virus in France (Charrel et al., 2009), and Zerdali, Toros, and Adana viruses in Turkey (Alkan et al., 2016; Alkan et al., 2015).

8.3 ADVANCED APPLICATIONS IN VECTOR GENETICS

Various considerable improvements have been carried since the last year in vector genetics, and also novel and enhanced genome of significant vectors of human disease has been published. Firstly, the genome of Tsetse fly was analyzed comparatively, in which the sequencing of six species of glossina were done across each of the three sub-genera, such as, Morsitans, Palpalis, and Fusca. All these genera of glossina serve as vectors for the disease African sleeping sickness (Attardo et al., 2019). There are various resemblances and disparities found on the comparative examination to other Diptera, with the protection of the chromosome X and the fortification of particular genes exceptional to the species of Glossina, additionally to those that are concerned with lactation (Attardo et al., 2019). Of interest, considerable difference in the photoreceptors that differentiate color was observed that distinguishes Glossina attraction to diverse types of traps and techniques used for Glossina vector control (Attardo et al., 2019). Various researchers have introduced substantial advancements to the DNA of *Aedes aegypti* (L.) (Matthews et al., 2018), by attaching contigs (known genomic sequence) to the three chromosomes of *Aedes aegypti* and in capacitating numerous obstacles of its greatly monotonous DNA that had afflicted the former association (Nene et al., 2007). From side-to-side improvements in DNA-sequencing engineering, these researchers reported reduction in the amount of map-making DNA sequences (contigs) by 93%, engendering an association sustained by cytological and physical gene maps along with a whole re-explanation (Matthews et al., 2018), significantly improving the effectiveness of this reference DNA for the technical communal reviewing mosquitoes. Some of the researchers have demonstrated the effectiveness of the CRISPR-Cas9 in *Culex fasciatus* by generating sensations in pigmentation genes of eye to create an inherited “white-eye” constellation (Anderson et al., 2019; Li et al., 2020). These kind of evidences of standard experimentations validated the solicitation of technologies of gene-excision in a significant until now regularly mistreated mosquito vector (Anderson et al., 2019; Li et al., 2020). Prominently, such investigations flabbergasted problems in *Culex* mosquito micro-injection that have formerly restricted genomic modification in *Culex* to particular research (Allen et al., 2001), consequently demonstrating a noteworthy improvement for *Culex* heredities. Supplementary solicitations of CRISPR-Cas9 applied sciences in

mosquitoes have introduced pretty importantly completed the previous time, with progresses in genomic alteration (Chaverra-Rodriguez et al., 2018) and genetically engineered conflict to Zika virus (Buchman et al., 2019). By pointing an Ir8a (olfactory receptor), by CRISPR-Cas9 convoluted in appreciation of human lactic acid, mosquito fascination to humans was suggestively damaged (Raji et al., 2019). Even though mutation in Ir8a didn't entirely avoid recognition of human, these testings deliver sustenance for strategies to hereditarily change olfactory constituents to decrease transmission of mosquito-borne diseases.

8.3.1 GENETIC EPIDEMIOLOGY

There is a fast increase of the genomic epidemiology field due to the new advancements in genetic sequencing. The outbreaks of virus can be examined in the past or in actual time by the use of sequences and phylogenetics of virus. It helps in taking decisions about people for decreasing the multiplication and expanding of the disease or the detection of the basis of its introduction (Grubaugh et al., 2019a; Pollett et al., 2020). Due to this novel approach, that is, genetic epidemiology, the outbreak of Zika virus has been identified in Cuba in 2017 (Grubaugh et al., 2019b). The identification of transmission on the Cuba island has been done by the authors after outbreaks in the remaining Caribbean with the help of isolating data from the travel-related cases of the Zika virus. The authors also traced this transmission which was undetected, as a minimum of three independent introductions of Zika virus (Grubaugh et al., 2019b). Same kind of information regarding the spread of West Nile virus in the America was also shared by the use of active sequence of virus (Hadfield et al., 2019).

8.3.2 MEDICAL ENTOMOLOGY VS. MEDICAL SCIENCE

In an increasingly connected world aided by the accessibility of information from the internet and social media, the application of citizen science is beginning to play a larger role in the collection of arthropod vector samples and public health surveillance. This was exemplified by the engagement of a Maryland community to deploy a network of Gravid Aedes traps to reduce populations of *Aedes albopictus* (Skuse) (Johnson et al., 2018). Through individual "buy-in" and ownership by the community, the program was able to achieve high trap coverage for sustained periods throughout the

mosquito season that significantly reduced *Aedes albopictus* abundance, demonstrating that citizen science can achieve long-term and sustainable methods of mosquito control (Johnson et al., 2018). Similarly, the engagement of citizen science also led to the acquisition of human behavioral data associated with tick exposure (Fernandez et al., 2019). Through the use of a smartphone application, The Tick App (<https://thetickapp.org/>) enrolled almost 1500 users to collect information from May to September of 2018 regarding location, pet ownership, and the engagement in outdoor activities (Fernandez et al., 2019). This initial survey provided tick identification services and a diary function for users to record their daily functions that led to tick encounters (Fernandez et al., 2019). Through this interface, the analysis of these human activities could better inform public health interventions to reduce the risk of tickborne disease.

8.3.3 MEDICALLY IMPORTANT ARTHROPODS (INSECTS)

There are various ways that arthropods choose to affect animal health. The major impact that arthropods involve is their function as main vectors and alternate hosts of numerous infectious disease agents. Parasitic agents transmitted by hematophagous arthropods include filariae, protozoa, bacteria, rickettsiae, and viruses. There is direct effect of arthropods on the health of vertebrates by activating rehabilitated mental conditions, allergies, feeding vexatious, anaemia, envenoming, and infestation. Due to these arthropods, millions of human deaths occur and bring illness in various peoples (Kenis and Branco, 2010). The highest impact of these arthropods can be seen in the tropical counties which is the one of main cause of reducing production of animals, agriculture, enhancement of economic, and goodness. Many vector-dependent disease including plague, leishmaniasis, yellow fever, malaria, dengue, etc., was conveyed from old world to North America through ship crew. Partz et al., 2008 reported that co-species of various insects were competent to sustains and communicate these inserted parasites. Over the last few years, various indigenous but earlier unknown arthropods-born contagions such as Lyme borreliosis, canine rickettsiosis, etc., have been exposed in the United States and somewhere else. In zones of the world, there are various recognized disease including dengue and malaria which has been recurring. Pages et al. (2010) studied that some other disease like West Nile fever have lately been familiarized into new

area with destructive effects. The factors which are responsible for the growing threat of vector-borne diseases are increasing population growth, hurried people movement, and environmental interruption. Coker et al. (2011) reported that these diseases are difficult to control due to lack of investment in the public health and progress of drug-resistant vermin and insecticide-resistant vectors.

Many classes of phylum Arthropoda has wide medical significance. Beside insect, these classes contain arachnids, millipedes, and centipedes. Some Arthropods such as sowbugs, copepods (Crustaceans), snails (molluscs), oribatid mites (arachnids), and beetle larvae (insects) are transitional hosts for parasitic worm (Capinera, 2011). Most of the arthropods are involved in the transmission of these microparasites. However, these arthropod ectoparasites feeds upon human cause blood loss, anaemia, discomfort, stress, allergic reactions, and reduced output. Some insects are specialized human ectoparasites such as bed bug and kissing bug whereas some parasites have always occupied human abodes and feeds in nights. Pubic louse, head louse, and body louse are the three species of sucking lice which specialized in different regions of human body. Mosquito, black flies, deer flies, and biting midge are some biting flies can be able to accomplish aggravation levels, and wide range of insects. Venomous insects are mostly belonging to order hymenoptera includes ants, hornet, honey bees, and bumble bees. Those insects and arachnids can cause venoms and harm to human being. Most of coleopterans also cause toxic secretions for their defence mechanism. These toxic secretions are secreted by special glands known as hemolymph. These secretions are especially toxic when exposed to mucus or lachrymal glands. Therefore, all spiders and scorpion cause toxic effect and venomous stings that have fatal to humans (Habermehl, 2012).

Some arthropods under class insecta causes allergens which leads to acute asthma in humans. It is associated with *Dermatophagoids* sp. commonly known as house dust (mites) and *periplaneta* sp. (cockroaches). Therefore, some other insects such as mayflies and caddis fly which causes acute asthma or allergic reactions. Obligate parasites that are dipterans family includes calliphoridae, oestridae, and sarcophagidae. The blood-sucking or other insects exist inside the fine hair, nasal passages, and lungs (Edman, 2009).

8.4 DISEASE AND VECTOR MANAGEMENT

Various efforts are adopted for the development of vaccines for a broad range of vector-borne diseases such as malaria, but the success rate was very slow. Major disincentive is parasitic variation, and treatment of new drugs. Numerous drugs are used for the treatment of helminthes, protozoan, and parasitic diseases. However, the first line defence mechanism is vector control in opposition to disease transmission. During epidemic condition, vertebrates have infrequently controlled the diseases, for example, wild bovine reservoirs in Africa. These bovine reservoirs were controlling some diseases in human and cattle such as trypanosomiasis and nagana disease. A diversity of tools and techniques are accessible to control vector-borne diseases such as alterations in habitat, pesticides, repellents, biological control, etc. In addition to this, genetic control and most favorable programs are incorporated management strategy of vectors, and preserve the entry or transmission of vectors. It is more efficient and cost-effective to target the immature vector stages and transmission of diseases. These diseases have greatest impact on public health throughout the worldwide (Edman, 2009).

8.5 FUTURE PERSPECTIVES AND CHALLENGES

The study about the medical entomology and diseases spread by various vectors is a frequently varying field. A number of ongoing movements of vectors of arthropods and their relevant pathogens is gradually increasing globally which is strongly influencing the field of medical entomology. Now-a-days, rapid increase in shipping and air traveling has caused persistent threats of introduction and increase in vector species and the capability for the transmission of disease. Regrettably, at the nation or state level, a number of surveillance actions are done in separation; thus, vectors of arthropods do not admire these conventional restrictions. Due to this, a foremost challenge comes for the control of the diseases that are vector-borne and this elevates the significance of sharing of open data and association inside the vector community. In United States, it is clear from the mosquito data, which was collected abundantly that more than one thousand agencies of mosquito control execute surveillance; thus far mosquito abundance data is mostly not available. Presently, some agencies share data in public, making the requirement for a countrywide open-access information repository of surveillance data of mosquitoes.

This data-sharing type helps various agencies such as mosquito control and public health to increase the efforts to control mosquitoes and also supply the required infrastructure to measure the transmission risks of local disease. Furthermore, chances of this data sharing can expand with no trouble and likewise help other arthropod vectors of ailments of human beings. One more challenge that comes in front of the community of vectors is the precise and appropriate communication of findings of the scientists to the common people. In today's world, the effect of social media is critical to any program on the control of vector, where the need for clear-cut messaging for the common reader is most important. The evidence regarding the genetics of *Aedes aegypti* before and after the discharge of a transgenic suppressing strain in Brazil has been provided. It explained that the mating suppression was not complete. There is an increase in the importance of genetic technologies and applications related to genetic technology, which turn out to be more practicable scientifically, and the success of their utilization is gradually more dependent on the responses of the common public.

KEYWORDS

- **insects**
- **arthropods**
- **vector borne diseases**
- **polymerase chain reaction**
- **entomology**

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