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REVIEW

A patent review on strategies for biological control of mosquito vector

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Abstract
Mosquitoes are the vectors responsible for transmitting serious and life-threatening diseases such as malaria, dengue, yellow fever, chikungunya and lymphatic filariasis. Very few effective vaccines or drugs have been developed so far to prevent or treat these diseases, highlighting a need for vector control. This paper presents a comprehensive technology overview of patent documents disclosing biological agents for mosquito control. The patent analysis revealed that comparable number of patent documents were filed in two technology categories: non-recombinant agents and genetically modified (GM) agents. In the category of non-recombinant agents, toxic peptides from microbes and biological consortia seemed to be the earliest technology noted right from the year 1965 whereas the patent filings for suppression of mosquito population using genetic modification techniques have emerged from the year 2000 onwards. The United States of America is the leading patent filing jurisdiction followed by China and the Great Britain. Academic institutes have filed higher number of patent applications as compared to private companies. University of Florida was found to be the leading patent filing entity and its patents were focused on suppression of vector population using techniques such as release of insects with dominant lethal (RIDL) and RNA interference (RNAi).

Keywords Biocontrol · Genetic modification · Mosquito vector · Patent landscape

Abbreviation

RIDL	Release of insect with dominant lethal
RNAi	Ribo Nucleic Acid interference
TMOF	Trypsin Modulating Oostatic Factor
IPC	International Patent Classification
CPC	Cooperative Patent Classification
dsRNA	Double stranded RNA
ATCC	American Type Culture Collection
NCIMB	National Collection of Industrial, Food and Marine Bacteria
NRRL	Northern Regional Research Laboratory
IIT	Incompatible Insect Technique
CRISPR	Clustered regularly interspersed short palindromic repeats

Introduction

Vector borne diseases account for more than 7,00,000 deaths globally every year and may be caused by parasitic, bacterial and viral infections (WHO- vector-borne-diseases, 2020). Mosquitoes are one of the most important insect vectors responsible for transmitting various pathogens to humans. Female mosquitoes feed on the blood of humans as a source of nutrition to complete their lifecycle. They are instrumental in transmitting pathogens when they suck blood of an infected human and then bite another healthy individual. Numerous mosquito genera spread human diseases. *Aedes* mosquitos are responsible for the transmission of dengue, yellow fever, lymphatic filariasis, Rift valley fever, chikungunya, and Zika viruses, mainly in tropical and subtropical regions of the world. *Anopheles*, another mosquito genus, is mainly responsible for the transmission of malaria and other diseases like encephalitis and lymphatic filariasis, whereas, *Culex* genus is known for spreading lymphatic filariasis, Japanese encephalitis and West Nile virus in the humans (WHO- vector-borne-diseases. 2020; USAID 2020).

The incidence of mosquito-transmitted diseases continues to create a worldwide public health crisis by causing millions of deaths. According to the latest World malaria

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report released in December 2019, over 220 million cases of malaria have been reported in the years 2017 and 2018 and a large size of the population is still at the risk of contracting the disease (WHO- malaria, 2020). Dengue outbreaks are estimated to affect more than 3.9 billion people in over 128 countries with 96 million cases per year. Recently, the emergence of viruses such as chikungunya, Zika, and re-emergence of Yellow fever are becoming major global health concerns (WHO- vector-borne-diseases. 2020; USAID 2020). Moreover, factors such as high reproduction rate and genomic flexibility of mosquitoes, rapid urban growth (André et al. 2019) and widespread global air travel (Luis et al. 2017) augment the global spread of these vectors and the related diseases.

Research groups world over are working on the development of safe and effective vaccines against human diseases transmitted by mosquitoes. Although the vaccine for yellow fever is available, the leading vaccine candidate for dengue has shown disappointing results in a recent large trial. In the absence of a suitable vaccine or a specific drug (prophylactic or therapeutic), dengue control is still focused on the control of the major mosquito vector. The development of a malaria vaccine is a very difficult task owing to the complexity of the malarial parasite. Although the Phase 3 trial of the RTS,S/AS01 candidate vaccine is promising, a commercial malaria vaccine is not yet available. Irrespective of the availability of a vaccine, vector control is expected to remain essential to curb the threat of mosquito- borne diseases (Luke et al. 2013; WHO- immunization- malaria, 2020). Therefore, management of these diseases demands preventive treatment for high risk human population as well as eradication of vectors and disruption of the disease transmission cycle (Rosemary et al. 2015). The prevalent methods to eradicate mosquitoes include physical and chemical control. While physical control such as use of bed nets, sound traps, and removal of mosquito breeding or swarming sites has met with some success; the use of chemical control in the form of synthetic insecticides and repellent sprays have raised concerns of growing insecticide resistance in mosquitoes, potential human toxicity and mortality in non-target organisms with other environmental risks (Giovanni et al. 2016). Continued high mortality and morbidity rates due to mosquito borne diseases indicate that the conventional mosquito control methods are largely inadequate to cope with disease outbreaks. Thus, there is a sustained demand for novel, effective, sustainable and eco-friendly approaches to control mosquito populations.

It is evident from the large number of research publications and patent documents that several strategies for mosquito control using various biological agents have been tried over the past decades. These include release of mosquitoes that are either sterile or unable to transmit disease (Sterile insect technique, RIDL), genetic modification strategies (CRISPR, TMOF), use of protein-based insecticides and certain non-recombinant techniques (mosquito predators, botanical repellents) which specifically target the vectors and seem to be more efficient and environment-friendly. Although both journal publications and novel inventions captured in patents are crucial, the latter carry an unmatched wealth of comprehensive technical information which might be commercially important. Patent analysis highlights the prominent research areas and identifies gaps for a new product or process development, which can in turn influence vital business decisions. A patent review can also shed light on the technology trends, patenting trends and major assignees. To our knowledge, the patents related with this topic have not been reviewed so far.

Thus, the present work aims to analyse technical aspects of inventions related with biological control of mosquito population that are disclosed in patent documents as well as to discuss their drawbacks. This review excludes the patents focusing on chemical control agents, repellents, attractants and plant essential oils/ bio-actives used as insect repellents. However, patents claiming plant molecules in combination with other biocontrol agents such as bacterial toxic peptides are included in the study.

Patent search methodology

The patent search strategy was based on keywords and important concepts relevant to biological control of mosquitoes in combination with relevant patent classification codes viz. International Patent Classification (IPC) codes and Cooperative Patent Classification (CPC) codes. A thorough patent search was then conducted using different proprietary databases such as PatBase, Thomson Innovation, Patseer and open-access databases like INPASS, Espacenet, Google patents. The search was not restricted by country or priority year to ensure maximum coverage. Datasets retrieved from various sources were combined, duplicate records were removed, and patents were reduced to one representative member per family. Further data processing excluded patents disclosing pure chemical-based mosquito control agents (insecticide, pesticide, mosquitocide, and larvicide) and the use of plant-derived products such as tissue extract/essential oils. The resultant set of 179 unique patent records was carefully read and analysed in detail.

Patent analysis and results

Patent filing trend

The progress of research and development activity in a technology area can be illustrated by the number of patent

applications filed annually Fig. 1. The analysis showed that patent filing in this technology arena spanned the last six decades with the earliest filing in the year 1965. A significant increase in patent filings was observed after 1998. Year 2011 recorded the maximum number of patent filings, however, in the recent years the numbers have tapered off. The overall trend reflected fluctuations in patent filing. The number of patent documents filed in the year 2018 and 2019 would be incomplete as patent applications are published only after 18 months from the date of filing.

Geographical distribution of patents

Priority country is the country of the earliest patent filing or from where the invention originates. The number of priority filings is indicative of the extent of research and development in a technology area within that jurisdiction. Figure 2 represents priority patent filings related with biological control of mosquitoes. The United States of America topped the list with ninety-two patent applications. China was the second major jurisdiction where nineteen patents were filed. China was followed by Great Britain with 14 patent applications. EP represented the number of patent families (5) for which European Patent application was the earliest filed document.

Nature of invention

The first claim or the independent claim(s) of a patent document is indicative of the nature of invention. Patents were classified based on the first or independent claim into different categories such as product, process or both product and process. Figure 3 illustrates the distribution of patents with respect to the nature of invention. It can be observed that the maximum number of patent documents (80) claimed products related with mosquito control using biological agents. A

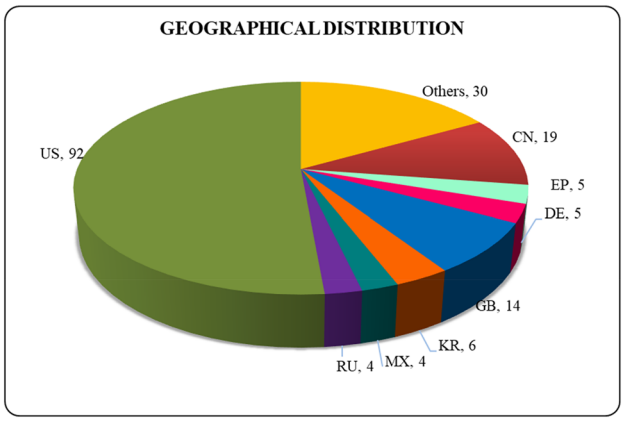

Fig. 2 Geographical distribution of patents with respect to priority country

comparable number of patent applications (67) claimed both product and related processes. Thirty-two patent documents claimed a process.

Citation analysis

One of the important value indicators of any patent application is the forward citations. Highly cited patents are thought to be of greater value because further innovations in the area are more likely based on the technical content of the referred patent. Figure 4 represents the top 10 patent applications based on the number of forward citations. Two patent documents with the highest number of forward citations have been described in detail. Patent application AU6414294A filed by Ciba-Geigy AG received the highest number (267) of forward citations. The patent disclosed pesticidal proteins from *Bacillus* strains and methods for using the strains, proteins, and genes for controlling plant and non-plant pests. These pesticidal proteins were produced during the vegetative growth of the said *Bacillus* strains. The mosquito

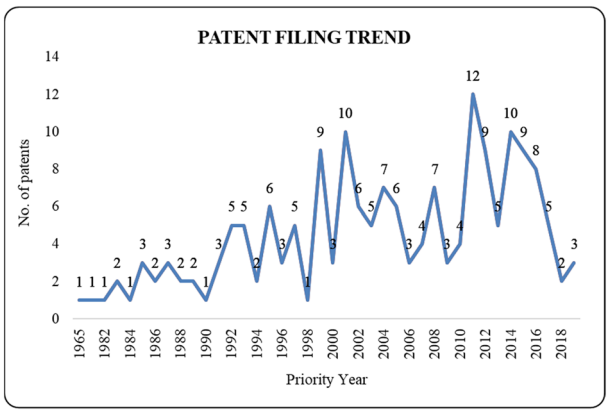

Fig. 1 Year wise patent filing for use of biological agents in mosquito control

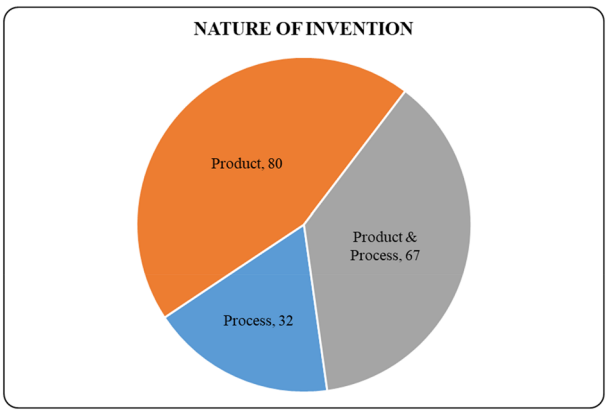

Fig. 3 Nature of invention based on the first claim of the patents

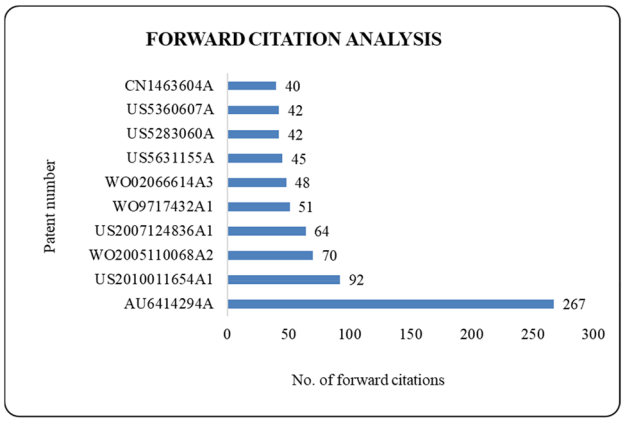

Fig. 4 Forward citation analysis (Top 10 patent applications)

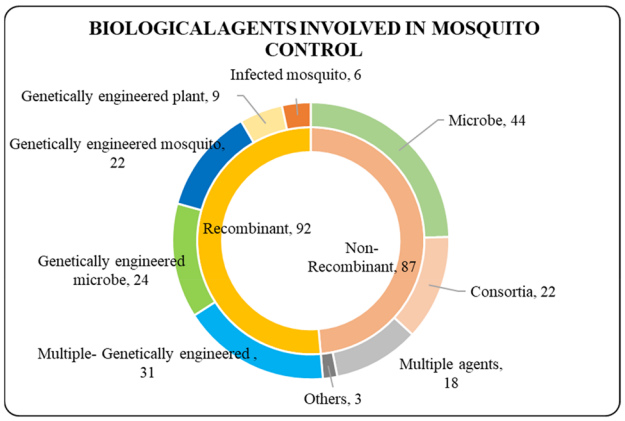

Fig. 5 Biological agents involved in mosquito control

species disclosed in the patent specification included *Culex pipiens* (northern house mosquito) that feeds on avian or mammalian blood and is known to transmit several avian pathogens to humans (Ary et al. 2011).

Another highly cited patent US2010011654A1 with ninety-two forward citations was assigned to Devgen N V. It disclosed an RNA interference (RNAi) based approach wherein gene silencing in insect species resulted into insect control. It further described an insecticidal formulation containing a double stranded RNA (dsRNA) as an active ingredient. The sequence alignment between the said dsRNA and a vital insect gene prevented the expression of the corresponding insect protein and hence resulted into death, growth arrest or sterility of the insect. However, the claimed nucleic acid sequences were described to be active against numerous insects or arachnid species including mosquito species of *Anopheles* and *Aedes*.

Moreover, both these patents claimed to control wide range of insect pests that affect different crops, as well as mosquito vectors that transmit human diseases such as malaria, dengue, yellow fever, chikungunya and lymphatic filariasis.

Technology trend

The patent documents have been classified into two broad categories depending on the nature of biological agents used viz. genetically modified or non-recombinant. It was observed that ninety-two patents were related to genetically modified (GM) biological agents and eighty-seven patents disclosed non-recombinant biological agents for mosquito control. Figure 5 illustrates the subcategories of biological agents involved in mosquito control. Patent analysis revealed that majority of the documents were focused on the control of mosquito species such as *Culex, Anopheles* and *Aedes*. Representative examples of biocontrol agents along with their control mechanisms as disclosed in the patents in each technical category are listed in Table 1.

Non-recombinant biological agents for mosquito control

In the category of non-recombinant agents for mosquito control, patent applications were classified based on the use of single species of microbes, microbial consortia and multiple agents not limiting to microbes. For the ease of categorization, bacteria and fungi were put under the category of 'microbes' which was further classified based on the mechanisms of control of the non-recombinant agents (Fig. 6; Table 2). Out of a total of 87 patent applications in this category, nearly 50% of the patents revealed the use of single species (cells, spores or endotoxins) in formulations related to mosquito control. Examples of microbes used as single species included bacteria such as *Bacillus thuringiensis israelensis*, *Bacillus thuringiensis kurstaki*, *Bacillus cereus, Bacillus sphaericus*, *Serratia marcescens*, *Chromobacterium* sp. Panama, *Photorhabdus temperate*, *Brevibacillus laterosporus*, *Streptomyces culicidicuslan*, *Xenorhabdus* (ATCC PTA-6826) and fungi such as *Beauveria bassiana* and *Metarrhizium anisopliae*. Almost one-third of the patents (25%) in non- recombinant biocontrol agent category disclosed the use of consortia of microorganisms. Majority of patent documents belonging to this category specified the combination of *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* in their compositions. Patent application US2005266036A1 disclosed a fermentation broth isolated from one of the microbial agents such as *Pseudomonas* species, including *P. aeruginosa*, *P. putida*, *P. florescens*, *P. fragi*, and *P. syringae*, *Flavobacterium* species, *Bacillus* species, including *B. subtilis*, *B. pumillus*, *B. cereus*, *B. licheniformis*, *Candida* species, including *C. albicans*, *C. rugosa*, *C. tropicalis*, *C. lipolytica*, and *C. torulopsis*, *Rhodococcus* species, *Arthrobacter* species, *Campylobacter* species and

Table 1 Representative examples of biocontrol agents with respective mosquito control mechanisms

Biocontrol agent	Example	Agent- name	Mechanism of control
Non- recombinant biocontrol agents with mechanism			
Microbe	Bacteria	*Bacillus thuringiensis var* WHO/CCBC 1897 (US4166112A)	Toxic peptide
		Bacillus sphaericus strain 2362 (CU22461A1)	Toxic peptide
		Brevibacillus laterosporus NCIMB 41419 (WO2008031887A2)	Toxic peptide
		Chromobacterium sp Panamam (WO2016033396A1)	Mosquito gut colonization
		Photorhabdus temperata strain ECOWIN_104 (KR20140044435A)	Toxic peptide
		Serratia marcescens KH-001 (CN110192557A)	Prodigiosin (tripyrroles)
		Xenorhabdus ATCC PTA-6826 (WO2012040062A1)	Toxic peptide
	Fungi	*Metarhizium anisopliae* (CN1435483A)	Toxic peptide
Multiple		*Bacillus thuringiensis israelensis*| Chemical insecticide: pyrethroid, organophosphate or carbamate (WO2013159023A1)	Endotoxin+chemical insecticide
Consortia		*Streptomyces culicidicus* ACCC-41132| *Bacillus sphaericus* ACCC-11096| *Bacillus thuringiensis israelensis* ACCC-10038 (CN105076216A)	Toxic peptide
Others	Nematode	Mosquito parasitic nematodes (KR20120095607A)	Mosquito gut infiltration
	Ciliated protozoa	*Chilodonella uncinate* (US20040219692A1)	Consumption
	Fish	*Leucaspius delineates* (SU1720613A1)	Consumption
Genetically engineered (GM) agents with mechanism			
GM microbe	Genes from *Bacillus thuringiensis*	Transgenic *Cyanobacterium Anabaena* PCC 7120 (Bacteria); Gene: *Cty/VA, Cty/VD, p20. of Bacillus thuringiensis israelensis* (US6503500B1)	Recombinant toxic peptide
		Transgenic *Saccharomyces cerevisiae* (yeast); Gene: cry11A of *Bacillus thuringiensis israelensis* (MX2002008706A1)	Recombinant toxic peptide
		Transgenic *Chlamydomonas reinhardti* (Fungi); Gene: cytlAa gene expressing cytlAa protoxin (Cry4Aa, Cry4Ba, Cry4Aa7oo, Cry4Ba675 and Cryl lAa) from *Bacillus thuringiensis israelensis* (US2016345590A1)	Recombinant toxic peptide
	Gene encoding TMOF protein	Genetically modified *Metarhizium anisopliae* or *Beauveria bassiana* (Fungi) produce Trypsm Modulating Oostatic Factor (TMOF) (WO2013052536A2)	Recombinant toxic peptide
Infected mosquito		Mosquito artificial infection: *Wolbachia* (US7868222B1)	Cytoplasmic incompatibility
GM plant		Transformed plant cell produces endotoxin: Cry ll gene from *Bacillus thuringiensis subsp. aizawia*. (US5986177A)	Recombinant toxic peptide
		Transformed plant cell carries gene encoding *Anopheles gambiae* chitinase. (US2005054821A1)	Mosquito gut infiltration
GM mosquito	Mosquito	Chitin synthase gene suppression: Genetically modified mosquitoes have increased susceptibility to pesticides (US8841272B2)	RNAi based insecticide
GM Multiple	Recombinant toxic peptide produced by several host systems	Toxin producing gene from Photorhabdus is transformed in a plant, *Baculovirus*, or heterologous microbial host. (AU1050997A)	Recombinant toxic peptide

Cornybacterium species to prepare a biosurfactant to control mosquitoes. Eighteen patents claimed the use of 'multiple agents'. An example of this category disclosed a composition with bioactive agents and chemical pesticides as its active ingredients. Bioactive agents included microbial consortia of *Bacillus thuringiensis israelensis*, *Bacillus sphaericus*, a watermold namely *Lagenidiuin giganteum* and chemical based pesticides such as Methoprene, diflubenzuron,

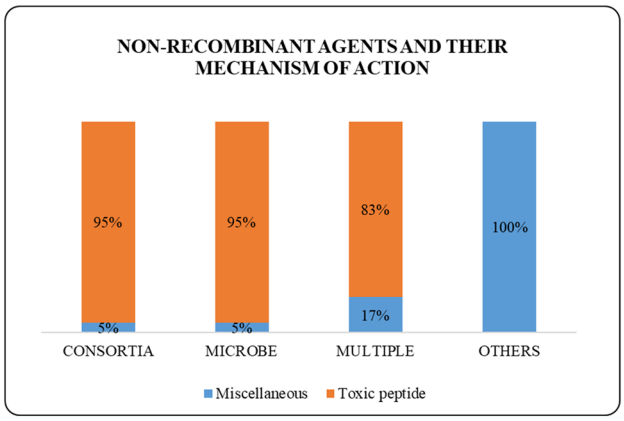

Fig. 6 Distribution of non-recombinant mosquito bio-control agents with respect to their mechanism of action

pyriproxyfen, temephos, chlorpyrifos, primiphos-methyl, lambda cyhalothrin, pyrethrins, ethoxylate of isostearyl alcohol, lecithins, or petroleum oils, and combinations thereof. Three patents were categorized as 'others' wherein they disclosed the use of nematodes, ciliated protozoa and fish in controlling mosquito populations.

Genetically modified (GM) agents for mosquito control

Patents disclosing GM agents for mosquito control were further classified according to the use of a single GM microbial species or multiple agents or GM mosquitoes or GM plants. Patents disclosing mosquito strains obtained by artificial infection with *Wolbachia* or genetically engineered *Wolbachia* were also included under the GM agent category.

Further, these patent documents were also classified based on the mechanisms of control by the various GM agents as disclosed. Out of the total 92 patent applications in this category, a number of patents (31) disclosed the use of multiple GM agents. Examples of multiple GM microbes used included transformed entomopathogenic fungi: *Hypho-mycetes, Metarhizium anisopliae* or *Beauveria bassiana*. Another example included *Bt* toxin and TMOF peptides produced by transformed microbes such as bacteria (*E. coli* or *Bacillus* species) or algal cells (*Chlorella*) or yeast cells or a viral particles. Twenty-four patents, categorized as GM microbes claimed the use of a single transformed microbial species that included bacteria, fungi and viruses. An example of this category included a host microorganism (*Bacillus megaterium* VT-1660 or a mutant thereof) transformed with a vector (pBC16) with expressible heterologous genetic information coding for a toxic protein (*Bacillus thuringiensis israelensis* δ-endotoxin gene).Twenty-two patents disclosed the use of GM mosquitoes and techniques such as RIDL, RNAi for vector control. Nine patent applications claimed the use of GM plants for producing mosquitocidal peptides. Six patents, categorized as 'infected mosquito', made use of mosquito species infected by an endosymbiotic bacteria *Wolbachia*.

51% of total 179 patent applications were filed under the GM category indicating continued research and development of novel agents for effective control of mosquitoes.

Mechanisms of mosquito control

The patent documents were further classified based on mechanisms of mosquito control by non-recombinant and GM agents as described in the patents. Maximum number of patents (78) were related to toxic peptides produced by microbes in the category of non-recombinant mosquito control agents followed by recombinant toxic peptides (54) under the category of GM bio-control agents (Figs. 6 and 7). It was further noted that the use of toxic peptides from microbes and consortia represented the earliest technology right from 1965 and has till date sustained a lead over other technologies in terms of the number of patent filings. It appears that toxic peptides (both non-recombinant and GM) have been the major focus of research worldwide. Patent filing in the case of mosquito control mechanisms such as gene knock down with CRISPR/ Cas, RNAi based vector control, RIDL, and IIT has seen marked increase in the recent years.

Toxic peptide

Seventy-eight patent records disclosed toxic peptides with mosquitocidal or larvicidal activity produced by non-recombinant microbes and consortia. Majority of the patent documents disclosed compositions comprising endotoxins extracted from microbes/ consortia or toxin producing microbial cells (30). Other patents claimed either formulations with microbial toxin in combination with other biocontrol agents (25) or formulations with microbial toxin along with carriers (23). Bacterial genus *Bacillus* was extensively claimed for use in mosquito control wherein *Bacillus thuringiensis israelensis* or its endotoxins was the popular choice followed by *Bacillus sphaericus.* During its vegetative and sporulation phase, *Bacillus thuringiensis* produces insecticidal Cry (Crystal toxins) and Cyt (Cytolytic) proteins that form pores in the mosquito gut. Crystal proteins are toxic to the larval form of the insect only after ingestion of the protein. These proteins work on a complex mechanism involving interaction with many proteins in the insect gut such as aminopeptidase N (APN), alkaline phosphatase (ALP) and cadherin (CAD) (Qi et al., 2016). Patent application CN102154171A, for example, claimed *Bacillus thuringiensis* WFS-97 strain (strain collection number of CGMCC No.3946) with insecticidal crystal protein-coding genes cry30 and cryt2 which were associated with high virulence for the larvae of *Culex*

Table 2 List of non-recombinant mosquito control agents specifying microbial genera and strains disclosed in the patents

Non-Recombinant microbial mosquito control agents		
	Genera	Species
Bacteria	*Bacillus thuringiensis*	*Bacillus thuringiensis israelensis* (US6898898B1, GB2376887B, US5283060A); *Bacillus thuringiensis israelensis*, strains SAN 402; ABG-6164 (WO2013110594A1); *Bacillus thuringiensis* var. WHO/CCBC 1897 (US4166112A); *Bacillus thuringiensis kurstaki* (US5484600A); *Bacillus thuringiensis* Tm13–14, CCTCC NO: M202004 (CN1368549A); *Bacillus thuringiensis israelensis* (H14) or var. *morrisoni* (WO1997016972A1); *Bacillus thuringiensis* JQD117 (CN105838634A); *Bacillus thuringiensis kurstaki* strain CAB565 (KR20160052265A); *Bacillus thuringiensis* strain NRRL B-50434 (MX2011002100A); *Bacillus thuringiensis* WFS-97 (CN102154171A); *Bacillus thuringiensis israelensis* BEC/19131461 (RS20070243A); *Bacillus thuringiensis* M-H-14 (EP1306008A1); *Bacillus thuringiensis israelensis* VKPM B-6405 (RU2122791C); *Bacillus thuringiensis* 92-KU-137-4 (FERM P-15230) (JPH09266787A); *Bacillus thuringiensis* VKPM B-6562 (EP0409438A1); *Bacillus thuringiensis* var. *morrisoni* PS71M3–69, *Bacillus thuringiensis israelensis* PS123D1–45 (CA2019442A1); *Bacillus thuringiensis israelensis* DSM 3439, *Bacillus thuringiensis israelensis* DSM 3440 (US5277906A)
	Bacillus sphaericus	*Bacillus sphaericus* MBI5, MBI6 and MBI7 (US2014273160A1); *Bacillus sphaericus* 1593 M, 2362, 2297 (US2003064060A1); *Bacillus sphaericus* 16 - S 25 (VKPM B - B-6408) (RU94018420A); *Bacillus sphaericus* 2362 (CU22461A1)
	Other *Bacillus*	*Bacillus brevis* (CN104542727A); *Bacillus* strain VB17 (US2011243906A1); *Bacillus cereus* (CN104542727A, US5560909A, US2005266036A1); *Lactobacillus coagulans* (IN03595MU2014A); *Bacillus pumillus* (US2005266036A1); *Bacillus popilliae* (US5560909A); *Bacillus lentimorbus* (US5560909A); *Bacillus fribourgensis* (US5560909A); *Bacillus licheniformis* (US2005266036A1);
Bacteria other than *Bacillus*	*Brevibacillius*	*Brevibacillus laterosporus* (UNISS18 deposited with number) NCIMB 41419 (WO2008031887A2)
	Clostridium	*Clostridium bifermentans* (WO1997016972A1)
	Chromobacterium	*Chromobacterium* sp. *Panamam* (WO2016033396A1); *Chromobacterium vaccinii* strains MWU205, MWU300 or MWU328 (US9339039B1)
	Photorhabdus	*Photorhabdus temperata* subsp. ECOWIN_104 (KR20140044435A)
	Pseudomonas	*P. aeruginosa*, *P. putida*, *P. florescens*, *P. fragi*, and *P. syringae* (US2005266036A1)
	Saccharopolyspora	*Saccharopolyspora spinosa* NRRL 18823 (US5631155A)
	Streptomyces	*Streptomyces culicidicus* ACCC-41132 (CN105076216A)
	Xenorhabdus	*Xenorhabdus* (ATCC PTA-6826) (WO2012040062A1)
	Others	*Rhodococcus* species; *Arthrobacter* species; *Campylobacter* species; *Cornybacterium* species (US2005266036A1)
Fungi	*Beauveria*	*Beauveria bassiana* K4B3 (WO2013030792A1)
	Candida	*C. albicans, C. rugosa, C. tropicalis, C. lipolytica*, and *C. torulopsis* (US2005266036A1)
	Lecanicillium	*Lecanicillium muscarium* strain K4V1/2/4 (WO2013030792A1)
	Metarhizium	*Metarhizium anisopliae* (CN1463604A)
	Paecilomyces	*Paecilomyces fumosoroeus* ATCC No. 20874 (US5360607A)

pipiens pallens and *Aedes albopictus*. Also, various strains of *Bacillus sphaericus*, *Xenorhabdus, Morganella* and *Chromobacterium* were disclosed frequently as a source of mosquitocidal toxin. An insecticidal composition comprising mosquitocidal toxin(s) produced by *Xenorhabdus* MT (American Type Culture Collection, PTA-6826) was claimed in patent US2012088719A1. Another patent US2017280730A1 disclosed a biologically pure culture of *Chromobacterium sp* Panamam (Csp P) that could effectively colonize the midgut of *A. gambiae* and *A. aegypti* mosquitoes when introduced

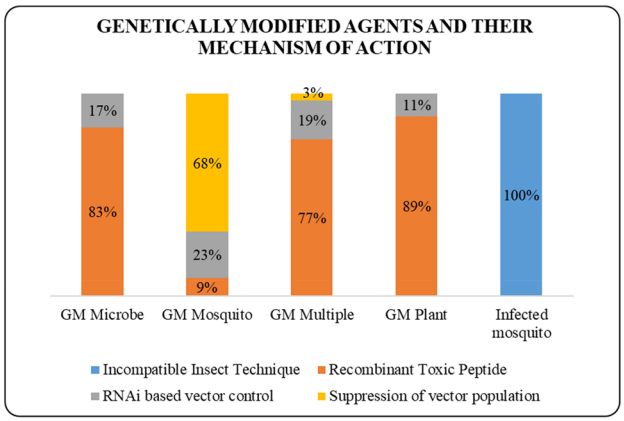

Fig. 7 Distribution of genetically modified biocontrol agents with respect to their mechanism of action

through an artificial nectar meal. Csp P exposure reduced the survival of both the larval and adult mosquito stages thereby representing a potent entomopathogenic agent. A few patent applications revealed mosquito larvicides with enhanced mortality rate derived from a combination of bacterial toxins and essential oils obtained from plants. Patent application KR101618190B1, for instance, disclosed a mixture of cassia oil and *Bacillus thuringiensis israelensis* (Bti) wherein the most effective insecticidal activity was obtained when the two actives were used in the ratio of 7: 3. Another Korean patent application KR101618197B1 claimed mosquito larvicide derived from star anise or anethole oil, and *Bacillus thuringiensis israelensis* in a weight ratio ranging from 5:5 to 7:3. Patent US2008287662A1 disclosed encapsulation of *Bacillus thuringiensis israelensis* toxin in *Balanites* saponin nanovesicles that protected the toxin against inactivation and enabled significant extension of its larvicidal activity for about 14 days. Patent application CN101347129A claimed a mosquitocidal product 'Bactivec' comprising viable bacterium *Bacillus thuringiensis* (7–9 x l06 cfu per ml) with suspension formulation consisting of corn Starch 4%, soybean meal 3.5 to 4.5%, 0.7–0.8% yeast extract, 0.3–0.5% peptone, fish meal 1.0–1.2% and water. Although, majority of the patents under this category disclosed the use of different bacteria, a few patents disclosed the use of fungi. For example, patent application CN102669185A claimed a release method of entomopathogenic fungal consortia including *Metarhizium anisopliae, Beauveria bassiana, Paecilomyces ilacinus* to control Culex larvae. None of the Patent applications claiming entomopathogenic fungi as the mosquitocidal agent specified the mechanism of control. However, the research in this area has showed that the spores of *Beauveria bassiana* and *Metarhizium anisopliae* germinate on the surface of mosquito host, penetrate through the cuticle owing to subtilisin-like serine protease. This initiates cuticle protein degradation which is followed by the action of exopeptidases, spread systemically in the hemolymph, resulting in the death of mosquito (Benjamin et al. 2016). Hence, toxic peptides from microbes have been a rich source of potential mosquitocide or larvicide that were found to combine the desirable attributes of high potency, specificity and were easily employed as sprays, foams, gels, suspensions, emulsifiable concentrates, or the like.

Miscellaneous

A total of nine patent applications were clubbed under the miscellaneous category of non-recombinant biocontrol agents. Patent application KR20120095607A claimed a biological extermination method using mosquito parasitic nematodes. Another patent application US2015164069A1 disclosed the use of lipid agents isolated from Entomopathogenic fungi, *Beauveria bassiana* K4B3 and *Lecanicillium muscarium strain* K4V1/2/4 as an active ingredient for mosquito control. US2004219692A1 disclosed a method of killing mosquito larvae by allowing the ciliated protozoa *Chilodonella uncinate* to form a hole in the body wall of the larvae and to enter the haemocoelomic cavity of the larvae. SU1720613A1 claimed the use of freshwater fish (*Leucaspius delineatus*) that consume mosquito larvae and pupae and could withstand a wide range of aqueous environments. A few patent applications claimed macrocyclic lactone from *Saccharopolyspora spinosa* NRRL 18823 (US5631155A) and rhamnolipids along with consortia of microbes (US2005266036A1) as mosquitocidal agents. Patent application US6663860B1 claimed a method for exterminating pests using digestive proteases from multiple cells (animal, plant, bacterium, or fungus) wherein the proteases directly attack the protein zipper which holds the halves of the insect exoskeleton together until moulting.

From the technology trend, it appears that mosquito control with toxic peptides has taken a lead over other technologies in terms of the number of patents filed. The year 2015 recorded the maximum number of patents (7) filings in a single year in this category. On the other hand, seven patents in all have been filed under miscellaneous category over the years.

GM toxic peptide

Majority of the patents (54) based on recombinant DNA technology belonged to this category. Patents placed under this category claimed recombinant cells producing mosquito-toxic polypeptide. The use of recombinant toxic peptide emerged in the early 1980s and peaked till 2001 after which a steady decline was seen.

Cry toxins form *Bacillus thuringiensis israelensis* have been used for effective and safe biocontrol against larvae of many mosquito species. However, a few shortcomings in their use such as the development of resistance to Bti

category. Patent application WO2014089581A1 explored the use of the RNAi technique to repress the activity of the transactivator (tTA) gene (lethal gene) in a Transformer-2 RNAi regulatory system using aTA RNAi construct wherein the tTA RNAi construct turned off the male biasing effect and restored progeny to a 1:1 sex ratio. This lead to population replacement effect in *Culicidae* mosquitoes. IN201917007705A claimed a method of reducing a wild insect population comprising the steps of contacting said wild mosquito population with a plurality of the male GM mosquitoes. The patent application disclosed a gene expression system with doublesex (dsx) splice control module comprising at least one substitution, insertion, and/or deletion to form an open reading frame for the entire exon and operably linked to a polynucleotide that encodes a heterologous protein that had a lethal, deleterious or sterilizing effect to mosquito. The protein was selected from the group consisting of a synthetic tetracycline repressive transcriptional activator protein (tTAV), an apoptosis-inducing factor, Hid, Reaper (Rpr), and NipplDm. Patent application WO2015040449A1 disclosed the interference effect of the Transformer 2 RNAi system that was expressed during male spermatogenesis and resulted in a severe male bias in the progeny. The interference effect was also thought to be lethal in early female zygote stages of the organism.

Patent applications under this category disclosed RNAi technology to target sequences unique to the mosquito vector and it could be easily implemented with existing mosquito control tools such as sprays, suspensions and toxic bait. Additionally, this technology was proved to have far greater specificity when compared to chemical insecticides.

Table 3 List of GM mosquito control agents specifying microbial genera and species, gene source and host cells disclosed in the patents

Genetically modified microbial mosquito control agents		
	Genera	Species
Genes source	*Bacillus thuringiensis*	*Bacillus thuringiensis israelensis* (US5304484A, WO1998039974A2, US4652628A); *Bacillus thuringiensis* NRRL B-21060, NRRL B-21224/ NRRL B-21225/ NRRL B-21226/ NRRL B-21227 (WO1994021795A1); *Bacillus thuringiensis* strain LRC3 (ATCC PTA-6248) (US2006083726A1); *Bacillus thuringiensis* PS92J (NRRL B-18747)/ PS196S1 (NRRL B-18748)/ PS201L1 (NRRL B-18749)/ PS201T6 (NRRL B-18750) (WO1995002693A1); *Bacillus thuringiensis israelensis* HD567–61-9 (EP0195285A2); *Bacillus bacteria* FERM P-20949 (JP2008048682A); *Bacillus thuringiensis kurstaki* (US6335008B1)
	Bacillus sphaericus	*Bacillus sphaericus* strain 1693 (WO1995015383A2)
	Bacillus cereus	*Bacillus cereus* Accession No. B-21058 (WO1994021795A1);
	Clostridium	*Clostridium bifermentens* (WO2001016305A2)
	Photorhabadus	*Photorhabdus luminescens* strain designated ATCC 55397 (WO1997017432A1)
	Xenorhabdus	*Xenorhabdus* species ATCC 19061, NCIMB 40886/ NCIMB 40887 (WO1998008388A1)
Host cells	*Bacteria*	*Bacillus subtilis* (US4652628A); *Bacillus thuringiensis israelensis* 4Q2–72 (US5304484A); *Bacillus thuringiensis israelensis* EG12341 (US6482636B1); *Bacillus licheniformis* (US2006083726A1); *Bacillus megaterium* VT-1660, *Bacillus megaterium* VB131 (EP0195285A2); *Caulobacter* (EP0454485A2); *Cyanobacterium synechocystis* 6803 (US6335008B1); *Clavibacterxyli* (US2006083726A1); *Cyanobacterium Anabaena* PCC 7120 (WO1998039974A2); *Cyanobacterium, Agmenellum quadruplicatum* PR-6 (*Synechococcus* sp. strain PCC7002) (US5518897A); *Escherichia coli* MGl 655 (WO2011022435A2); *Pseudomonas* (EP0308199A1); *Pseudomonas fluorescens* (US2006083726A1);
	Fungi	*Aspergillus niger, Aspergillus ficuum, Aspergillus awamori, Aspergillus oryzae, Kluyveromyces lactis, Mucor miehei, Trichoderma reesei* (US2006083726A1); *Metarhizium anisopliae; Beauveria bassiana* (WO2011022435A2; WO2013052536A2);
	Algae	*Blue green algae* (WO1988010305A1); *Chlamydomonas reinhardtii* (WO2016179086A2);
	Plant cell	Family *Solanacae*, *Solanum, Glycine*, Family *Fabacae*, *Zea mays*, Zea or *Nicotina* (US2003140371A)
	Yeast cell	*Pichia pastoris, Saccharomyces cerevisiae* (US2006083726A1)

Table 4 List of GM mosquito control agents specifying microbial gene source and host disclosed in the patents

Gene Source	Gene details	Host
Gene from *Bacillus thuringiensis*	δ-endotoxin: *Bacillus thuringiensis israelensis* (US4652628A)	*E. coli*; *Bacillus subtilis*
	δ-endotoxin: *Bacillus thuringiensis israelensis* strain HD567–61-9 (EP0195285A2)	*Bacillus megaterium* VT-1660 or a mutant, *Bacillus megaterium* VB131 or a mutant
	Cty/VA, Cty/VD, p20. of *Bacillus thuringiensis israelensis* (WO1998039974A2)	*Cyanobacterium Anabaena* PCC 7120
	cytlAa gene expressing cytlAa protoxin (Cry4Aa, Cry4Ba, Cry4Aa7oo, Cry4Ba675 and Cryl lAa) from *Bacillus thuringiensis israelensis* (WO2016179086A2)	*Chlamydomonas* chloroplast
	Cytl A gene expressing Cytl A variant polypeptide: *Bacillus thuringiensis* (WO2017199078A2)	Plant cell; Bacterial cells: *Bacillus thuringiensis* 407 *acrystalliferous* strain
	CryII from *Bacillus thuringiensis* (BtC-18) (WO1998030700A1)	Plant cell: Tomato (Microinjection)
	δ-endotoxin: *Bacillus thuringiensis* HD73 (US5281532A)	*Pseudomonas aeruginosa* NRRL B-15977
	CrylAc from *Bacillus thuringiensis kurstaki* (WO2017015559A2)	Monocot plant cell; Dicot plant cell; Bacterial cell (*E. coli*) Dicot plant cell is selected from the group consisting of a tomato, cotton, potato, soybean, tobacco, alfalfa, broccoli, cauliflower, citrus, sugar beet, rapeseed, fava bean, pea, bean, apple, cherry, pear, strawberry, raspberry, legume, tuber, and fruit plant cell.The recombinant cell of monocot plant cell is further selected from the group consisting of a corn, rice, wheat, barley, oats, rye, millet, sorghum, sugarcane, asparagus, turfgrass, any grain, and cereal plant cell.
	Cryl from *Bacillus thuringiensis* (WO2018005411A1)	Plant (plants, plant cells, tissues and seeds); Bacterial cell (*Agrobacterium, Bacillus, Escherichia, Salmonella, Pseudomonas* and *Rhizobium* bacterial host cells)Plant species: *Athyrium*, *Platycerium*, *Pteris*, *Colysis*, *Nephrolepis*, *Polystichium*, *Thelypteris, Tectaria, Davallia*
	Cry4A, Cry4B, Cry4D and CytA from *Bacillus thuringiensis israelensis* (US6482636B1)	*Bacillus thuringiensis israelensis* EG12341; *Bacillus thuringiensis kurstaki* EG12368; *Bacillus thuringiensis subsp. jegathesan* EG12410
	cryIVD protein encoding gene from *Bacillus thuringiensis israelensis* (US5518897A)	*Cyanobacteria* (*Agmenellum quadruplicatum* BG-1 (*Synechococcus* 73,109) (ATCC 29404), *Coccochloris elabens* 17-A (*Synechococcus* 7003) (ATCC 27265), *Aphanocapsa (Synechocystis* 6714) (ATCC 27178), *Nostoc muscorum* UTEX 1545 (*Nostoc* 6314) (ATCC 27904), *Nostoc* sp. MAC (*Nostoc* 73,102) (ATCC 29133), *Chloroglea fritschii (Chlorogloeopsis fritschii*) (*Chlorogloeopsis* 6912) (ATCC 27193), *Anabaena flos* (*aquae*) (ATCC 22664), and *Anabaena variabilis* (*Anabaena* 7118) (ATCC 27892))
	Chimeric gene from *Bacillus thuringiensis israelensis* obtained from the 72 mD plasmid (WO1988010305A1)	Blue green algae; *Escherichia coli*; Plant cell

Table 4 (continued)

Gene Source	Gene details	Host
	B282 plasmid carrying cry4A, cry4B, cry10A, cry11A, cyt1A, cyt2B genes: *Bacillus thuringiensis* (JP2008048682A)	Bacteria: *E. coli, Bacillus*, *actinomycetes; E*ukaryotic cells: yeast
	Plasmid pJC2297–2 *Bacillus sphaericus* toxin genes; Genes from *Bacillus thuringiensis israelensis* (EP0454485A2)	Caulobacter: Aquatic organism
	Endotoxin genes for encoding Cry1A, Cry1B, Cry1F, Cry1H, Cry1I, Cry1K, Cry2 from *Bacillus thuringiensis* strain LRC3 deposited as ATCC PTA-6248 (US2006083726A1)	*Aspergillus niger, Aspergillus ficuum, Aspergillus awamori, Aspergillus oryzae, Bacillus subtilis or licheniformis, Clavibacterxyli, Escherichia coli, Kluyveromyces lactis, Mucor miehei, Pichia pastoris, Pseudomonas fluorescens, Saccharomyces cerevisiae, Trichoderma reesei*, and a plant cell.
	cry11A gene of *Bacillus thuringiensis israelensis* (MX2002008706A1)	Yeast: *Saccharomyces cerevisiae*
	Gene from a *Bacillus thuringiensis* isolate PS92J; *Bacillus thuringiensis* PS196S1; *Bacillus thuringiensis* PS201L1; *Bacillus thuringiensis* PS201T6 (WO1995002693A1)	Not specified
	cytA gene present on the 72 MDa resident plasmid from *Bacillus thuringiensis israelensis* (US5304484A)	*Bacillus thuringiensis israelensis* 4Q2–72 (Δcyt A)
	Gene from *Bacillus thuringiensis israelensis* (EP0349769A1)	*Bacillus sphaericus* CNCM I-746
	Gene from *Bacillus thuringiensis* (EP0308199A1)	*Escherichia coli* strain BB3(pBT13,82–5), NRRL B-18252; pigmented bacterium, yeast, or fungus;*Pseudomonas* (preferred), *Azotobacter, Erwinia, Serratia, Klebsiella, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter or Alcaligenes;Prokaryote cells* selected from *Enterobacteriaceae, Bacillaceae, Rhizobiaceae, Spirillaceae, Lactobacillaceae, Pseudomonadaceae* (preferred), *Azotobacteraceae* and *Nitrobacteraceae,* or *eukaryote cells* selected from *Phycomycetes, Ascomycetes* and *Basidiomycetes.*
	Gene encoding endotoxin from *Bacillus thuringiensis, Bacillus sphaericus* or *Bacillus kurstaki* strain (US6335008B1)	*Cyanobacterium synechocystis* 6803
	crya, cry2, cry3, cry4, cry 5, cry6, cry8, cry9, cryl J, cry 14, cry21, cytl or cyt 2 genes; cpcR regulator gene (EP3565826A1)	Not specified
Gene expressed in *Bacillus thuringiensis*	Bacterium is mutated by inactivation or disruption of its mutS gene (WO2019030529A1)	*Bacillus thuringiensis* strain: *Bacillus thunngiensis* entomocidus, *Bacillus thuringiensis* aizawai or *Bacillus thuringiensis kurstaki.*
Gene in addition to endotoxin gene from *Bacillus thuringiensis*	Gene encoding PRAP protein| Endotoxin gene from *Bacillus thuringiensis* (WO2009108180A2)	Plant cell
	Gene encoding *B. bassiana* proteins| gene encoding insecticidal *Bacillus thuringiensis* (Bt) toxin (US2007044179A)	Yeast cell.
Gene from *Photorhabdus*	Toxin producing gene from *Photorhabdus luminescens* strain W-14 (WO1997017432A1)	Plant, *Baculovirus*, or heterologous microbial host; purified culture of *Photorhabdus*

Table 4 (continued)

Gene Source	Gene details	Host
	Toxin producing gene from *Photorhabdus luminescens* (WO1998008388A1)	Cultures of cells of *Xenorhabdus* species: *Xenorhabdus nematophilus* species is ATCC 19061, NCIMB 40886 or NCIMB 40887; virus pathogenic to insects (*baculovirus*); plant.
Gene from *Brevibacillus*	Toxin producing gene from *Brevibacillus laterosporus* (WO2014102697A2)	Plant: Tobacco, Sorghum, cotton, wheat, maize,
Gene from *Bacillus sphaericus*	Cyt1Aa1 gene from *Bacillus sphaericus*: binary toxin (WO2002013606A2)	*Bacillus thuringiensis*
	Bacillus sphaericus mtx gene (WO1995015383A2)	*Bacillus sphaericus* 1693; *Bacillus sphaericus* 4525
	Gene encoding Toxic protein such as the P43 protein, P56 protein produced by *Bacillus sphaericus* (FR2639959A1)	*Bacillus thuringiensis israelensis* morrisoni strain; *E. coli*
Gene from *Baculovirus*	AcNPV L1LC-galcat gene from *Baculovirus* (US5004687A)	Mosquito: Adult female mosquitoes were inoculated with approximately 2×107 virus per midgut.
Genes from multiple microbes	Toxin producing gene from *Bacillus cereus* having Accession No. B-21058; *Bacillus thuringiensis* selected from Accession Numbers NRRL B-21060, NRRL B-21224, NRRL B-21225, NRRL B-21226 and NRRL B-21227 (WO1994021795A1)	Plant: maize, soybean, cotton, wheat, sunflower, tomato, potato, and oilseed rape;Microorganism: *Bacillus, Pseudomonas, Saccharomyces, Clavibacter, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Agrobacterium,* insect pathogenic viruses, fungi, protozoans and nematodes
	Gene encoding patatin, a *Bacillus thuringiensis* insecticidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporus* insecticidal protein, and a *Bacillus sphaericus* insecticidal protein. (US20077124836A1)	Not specified
	Gene from *Serratia entomophila* or *Serratia proteamaculans*; Genes encoding *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins. (WO2001016305A2)	A transgenic plant, bacterium, virus or fungus
Trypsin Modulating Oostatic Factor	Gene encoding TMOF protein (WO2000063233A2)	Plants, animals, fungi, and viruses; green algae, *Chlorella*, yeast
	Gene encoding TMOF protein (WO2013052536A2)	*Metarhizium anisopliae* or *Beauveria bassiana*
	Gene encoding TMOF protein (US6593299B1)	Bacteria, algae, fungi, plants, or other cells
	Gene encoding TMOF protein; Gene encoding NPF polypeptide is a fusion polypeptide (US20030108520A1)	Algae, *Clorella* species; yeast cell
	Gene encoding TMOF protein (WO2000062620A2)	Algae *chlorella desicata*; Yeast
	Gene encoding TMOF protein (WO2000063235A2)	Bacteria, algae, yeasts, insect viruses, and plants

Table 4 (continued)

Gene Source	Gene details	Host
	Gene encoding TMOF protein (US2002132302A1)	Plant claimed; can be applied to prokaryotes, i.e., bacteria; and eukaryotes, such as fungi, including yeasts, algae, protozoa, molds, and the like, as well as plant cells, both in culture or in planta, and animal cells. Specific bacteria which are susceptible to transformation include members of the *Enterobacteriaceae*, such as strains of *Escherichia coli; Salmonella; Bacillaceae, such as Bacillus subtilis; Pseudomonas; Pneumococcus; Streptococcus; Haemophilus influenzae*, and yeasts such as *Saccharomyces*, among others.
	Gene encoding TMOF protein (US5629196A)	Not specified
TMOF+Endotoxin gene from *Bacillus thuringiensis*	Gene encoding Cry4Aa, Cry4Ba, Cryl lAa, or CytlAa and TMOF peptide (WO2010077672A2)	Bacterial cells (*E. coli* or a *Bacillus* species); algal cells (*Chlorella*.); or yeast cells.
Gene source other than microbe	U-ACTX polynucleotides: Gene expressing polypeptide that is a component of the venom of a spider of the genera *Atrax* or *Hadronyche* (WO2006052806A2)	Plant cell; Insect cell; Bacterial (*Escherichia coli* BL21), yeast, insect, amphibian, or mammalian cell
	Gene encoding sex peptide (SPR orthologue) (WO2009071672A1)	Plant
	Gene encoding trait that is not naturally associated with said microorganism (WO2011022435A2)	Fungus: *Metarhizium anisopliae, Metarhizium flavoviridae, or Beauveria bassiana*; Bacteria: *E. coli* strain MGl 655
	Gene encoding mosquito arrestin (US20030082637A1)	*E. coli*
	CAATCH genes from *Manduca sexta* (tobacco hornworm) encoding CAATCH protein (US2003140371A)	Mosquito; plants, algae, bacteria, viruses, virus particles, and yeastPlant: Family Solanacae; *Solanum spp.; Glycine spp.; Family Fabaceae; Zea mays; Zea spp. and Nicotina spp.*
	Autogenous mosquito gene, AatHex-1.2; cDNA encoding AatHex-1.2 (WO2003018827A2)	Bacteria, insect, plant cell, fungal cell, mammalian cell.
	Gene encoding *Anopheles gambiae* chitinase (WO2003014150A2)	Plant cell or plant
	Gene encoding a chimeric RXR ligand binding domain (LBD) (WO2002066614A2)	Bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell
	Insect-antagonistic gene is selected from the group consisting of: toxins, insect hormones, insect hormone mimics, and sterilization peptides (US6521454B1)	
	SmTPS11 gene for sesquiterpenoid synthesis isolated from Radix *Salviae Miltiorrhizae* (herbal medicine from China) (CN108893483A)	The synthesis of terpenoid is participated in bacterium, fungi and higher plant by genetic engineering means. Bacteria (*E. coli*); Plant: Tobacco

in mosquitoes and short shelf life have been reported (Dov et al. 2011; Giovanni et al. 2016). Expressing Bti toxins in organisms that are readily eaten by mosquito larvae was tried as one of the possible solutions. Majority of the patent applications (25) in this category disclosed the expression of a heterologous gene derived from strains of *Bacillus thuringiensis* in various host cells. For example, patent application US5281532A claimed a *Pseudomonas fluorescens* strain comprising a heterologous gene derived from a strain of *Bacillus thuringiensis* that produces Cry protein. KR890701744A disclosed expression of Cry toxin-encoding DNA fragments from *Bacillus thuringiensis* in *Escherichia coli*, blue green algae and even a plant cell. A list of several recombinant strains producing toxic peptides has been presented in Tables 3 and 4.

Patents disclosing GM agents in the form of Trypsin Modulating Oostatic Factor (TMOF) formed another interesting group. A total of nine patent applications disclosed the production of a mosquito decapeptide hormone- TMOF that inhibits the biosynthesis of trypsin and chymotrypsin-like enzymes (collectively referred to as 'TTLE') in the midgut epithelial cells of female and larval mosquitoes. In the larvae, TMOF prevents food digestion in the gut, starvation, anorexia, and death. In the adults, TMOF causes inhibition of food digestion leading to sterility. As TMOF has a tendency to transverse the gut epithelial cells to enter the hemolymph, it binds TMOF specific receptor(s) on the epithelial cells and stops trypsin biosynthesis and egg formation. When fed orally, TMOF can be used as an effective larvicide against many mosquito species (Dov et al., 2011). Patent applications US2002132302A1and US5629196A claimed the production of TMOF using genetically engineered plant cells. Use of transformed cells such as non-human animals, bacteria, algae, fungi, yeast, and viruses has also been disclosed for the production of TMOF.

Therefore, various genetically engineered microbes, recombinant mosquitocidal or larvicidal toxic peptides have remained a significant focus of the researchers around the globe and provided potential products and methods to manage mosquito vectors.

RNA interference-based vector control

RNA interference (RNAi) refers to the process of post-transcriptional silencing of crucial genes by the application of double-stranded RNA (dsRNA) (Agieshkumar et al. 2016). Strategies for mosquito control based on RNAi have emerged from the year 2000 onwards and research in this direction is in progress. A total of sixteen patent records under this category disclosed use of interfering RNA for suppression of mosquito population wherein mechanisms such as RNAi based insecticides and RNAi based gene knock down were applied to control the vector population and the details have been depicted in Table 5.

Microbe based insecticides using RNAi

Thirteen patent applications claimed compositions containing genetically engineered microbial cells (bacteria, algae, yeast) comprising interfering ribonucleic acid (iRNA) or double-stranded RNA (dsRNA) that down-regulate expression of vital genes endogenous to a mosquito. The mode of delivery of these compositions was either oral feeding or contacting mosquitoes by spraying, dusting or suspending the composition in water. Two Chinese patent applications CN110042102A and CN110241114A disclosed feeding of yellow fever mosquitoes with transgenic microalgae (*Clamydomonas* or *Chlorella*) comprising dsRNA that lead to silencing of *Aedes aegypti* HR3 gene and *Aedes aegypti* 3HKT gene, respectively. Hormone receptor 3 (HR3) gene plays an important role in insect metamorphosis. Because of inhibition of HR3 expression, an insect cannot carry out normal husking which eventually leads to death of the larvae. On the other hand, 3- Hydroxykynurenine transaminase (3HKT) gene helps in catalysis of 3- Hydroxykynurenine transaminase (3HKT) to hydroxyl cynruin (3-HK) and generation of non-toxic intermediates urealic acid and the silencing of this gene leads to accumulation of toxic intermediates and death of the mosquitoes. Patent application WO2018026992A1 disclosed a composition containing a transformed bacterium expressing dsRNA that could suppress several mosquito target genes such as genes encoding a proteasome protein, vesicle protein, ribosomal proteins, gene encoding a glycolysis and energy metabolism protein.

The cuticle in the exoskeleton of mosquito is composed of Chitin and has an enormous contribution towards their survival in the environment as it provides strength, protection and prevents water loss. Patent application US8841272B2 claimed dsRNA-based nanoparticles for Chitin synthase (CHS1 and/or CHS2) gene silencing through larval feeding. Chitin synthases are encoded by two different genes: *CHS1* and *CHS2*. *CHS1* codes for an enzyme that catalyzes the production of chitin used in the formation of cuticle and tracheae and is mainly expressed in the exoskeleton structure. *CHS2* is expressed in the midgut and is responsible for the production of chitin required in the midgut membrane (Raman et al. 2012). Hence, feeding these compositions to mosquito lead to the silencing of these crucial genes, ultimately killing several mosquito species.

Gene knock down in mosquitoes with RNAi

Three patent applications disclosing GM mosquitoes comprising interfering ribonucleic acid (RNAi) were included in this

Table 5 Details of RNAi based vector control

Patent application No.	Mechanism	Gene source and Vector	Mode of dsRNA delivery	Effect of silencing
CN110042102A	RNAi BASED INSECTICIDE	Gene: *Aedes aegypti* HR3 gene;Vector: Genetically modified Microalgae with dsRNA are fed to larvae	Oral feeding	Exoskeleton malformation
CN110241114A	RNAi BASED INSECTICIDE	Gene: *Aedes aegypti* 3HKT gene;Vector: Genetically modified Microalgae with dsRNA are fed to larvae	Oral feeding	Accumulation of acid
PH12019500583A1	RNAi BASED INSECTICIDE	Gene: Gene not specified;Vector: Genetically modified bacteria (*E. coli* strain JC8031)	Oral feeding	Down regulates pathogenic genes in mosquito
US8841272B2	RNAi BASED INSECTICIDE	Gene: Chitin synthase gene;Vector: Mixing said biopolymer and said dsRNA, wherein said biopolymer and dsRNA self-assemble into said nanoparticle	Oral feeding	Exoskeleton malformation
WO2018026992A1	RNAi BASED INSECTICIDE	Gene: Genes encoding various such as: proteasome proteins, ATPase proteins; ribosomal proteins; vesicle proteins; actin; protein related to behaviour; glycolysis and energy metabolism proteins; protein turnover and mitosis proteins; gene expression protein; protein-folding, stress, and heat shock response protein; miscellaneous protein	Oral feeding	Inactivate many vital functions in mosquito

Table 5 (continued)

Patent application No.	Mechanism	Gene source and Vector	Mode of dsRNA delivery	Effect of silencing
WO2018013801A1	RNAi BASED INSECTICIDE	Gene: AGAP000891, AGAP008903, AGAP003014, AGAP01 1489, AGAP007987, AGAP007596, AGAP007254, AGAP009777, AGAP01 1133, AGAP010510, AGAP004548, AGAP010265, AGAP009978, AGAP003349, AGAP011320, AGAP007942, AGAP008656, AGAP007247, AGAP008822, AGAP010163, AGAR) 101 47. AGAPO 10242, AGAP010307, AGAP009777, AGAPO 12189, AGAPO 10138, AGAPQ02731, AGAP007242, AGAP000591, AGAP004438, AGAP01 1655, AGAP003893, AGAP008200, AGAP008201, AGAP005010, AGAP002374, AGAP005095, AGAP011516, AGAP00431 L AGAP000254, AGAP000254, AGAP001651, AGAP006089, AGAP002974, AGAP008656, AGAP006089, AGAPO 11985, AGAP002974, AGAP008656, AGAP001650, AGAP001651, AGAP004613, AGAP000254, AGAPO 11038, AGAP004405;Vector: bacterium, algal, plant, or yeast cell (*Saccharomyces cerevisiae*) engineered to produce the iRNA from the DNA construct;	Oral feeding	Inactivate maturation of mosquito species from larvae to adult; Crucial for survival of mosquito (*A. aegypti, A. albopictus, A. gambiae, A. coluzzii, A. merus, A. arabiensis, A. me las, A. fimestes, A. sinensis, A. dims, A. quadriannulatus, and A. chrisiyi*.)
WO2015040574A1	RNAi BASED INSECTICIDE	Gene: dsRNA that inhibits expression of a testis-specific coding region; RNA (dsRNA) that inhibits expression of a coding region encoding a doublesex female splice variantVector: Bacterial cells codes hairpin dsRNA.Target: *Culicidae* is *Aedes* spp., *Anopheles* spp., or *Culex* spp	Oral feeding	Reduced fertility, reduced fecundity, or a combination of male insect; inhibits expression of a testis-specific coding region; and allowing the juvenile insect to mature into an adult, wherein the adult insect has reduced fertility, reduced fecundity, or a combination thereof, compared to a control insect.
WO2006129204A2	RNAi BASED INSECTICIDE	Gene: Gene not specified; Vector: Bacterial cell	Oral feeding	Inactivate many vital functions in mosquito
US2005095199	RNAi BASED INSECTICIDE	Gene: Gene not specified; Vector: Bacterial cell	Oral feeding	Inactivate many vital functions in mosquito

Table 5 (continued)

Patent application No.	Mechanism	Gene source and Vector	Mode of dsRNA delivery	Effect of silencing
AU2018261075A1	RNAi BASED INSECTICIDE	Gene: Microbe-induced gut specific genes-insect RNAi target genes (MIGGS-IRTG) Vector: L4440 plasmids; bacterial cells (HT115 bacteria), fungal cells, yeast cells, plant cells, plant organelles (e.g., including plastids), and mammalian cells	Oral feeding	Mosquito gut infiltration
WO2005110068A2	RNAi BASED INSECTICIDE: Produced by plant	Gene: Encoding ribosomal protein L19 (rp1.19); V-ATPase A ortholog gene; tp119 orthologs gene Endotoxin: *Bacillus thuringiensis* insecticidal protein is selected from the group consisting of a Cryl, a Cry3, a TIC851, a CryET70, a Cry22, a binary insecticidal protein CryET33 and CryET34, a binary insecticidal protein CryET80 and CryET76, a binary insecticidal protein TIClOO and TIC101, and a binary insecticidal protein PS 149B1.Vector: Diet is selected from the group consisting of an artificial diet, a plant cell, a plurality of plant cells, a plant tissue, a plant root, a plant seed, and a plant grown from a plant seed, wherein said diet comprises a pest inhibitory amount of said RNA molecule.	Oral feeding	Reduction in feeding capacity;Coding region of the V-ATPase do show significant stunting and mortality at a range of concentrations.Target sequence encode proteins that are critical for regulation of mosquito cell membrane, anatomy, digestion, life-cycle, synthesis of pheromone, hormones, enzymes.
CA2945736	RNAi BASED INSECTICIDE	Gene: Aub (AAEL007698) and Argonaute-3 (AAEL007823); Vector: algal cell, bacterial cell+ Chemical insecticides+ *Bacillus sphaericus*, and *Bacillus thuringiensis israelensis* toxinsHost: *Aedes aegypti* and *Anopheles gambiae.* Composition contain siRNA, shRNA and miRNA	Suspension, Granules contacted to mosquitoes	(i) affecting larval survival; (ii) interfering with metamorphosis of larval stage to adulthood; (iii) affecting susceptibility of mosquito larvae to a larvicide; (iv) affecting susceptibility of an adult mosquito to an adulticide/insecticide; and (v) affecting fertility or fecundity of a male or female mosquito.

Table 5 (continued)

Patent application No.	Mechanism	Gene source and Vector	Mode of dsRNA delivery	Effect of silencing
US20130011372A1	RNAi BASED INSECTICIDE	Gene: Receptor genes (allatostatin, diuretic hormone, octopamine/tyramine, PBAN peptide, adipokinetic hormone, neuropeptide, dopamine, serotonin, gonadotropin receptors, as well as olfactory or rhodopsin-like receptors) coupled with G proteins (GPCR); Vector: bacteria, yeasts, fungi, protozoa, or into a eukaryotic cell in culture selected from insect cells, mammalian cells or plant cells for dsRNA expression	Suspension, Granules contacted to mosquitoes	Inactivate many vital functions in mosquito
WO2014089581A1	GENE KNOCK DOWN: RNAi	Gene: Transformer-2 gene	Biological population replacement	Male only progeny
WO2018029534A1	GENE KNOCK DOWN: RNAi	Gene: Tetracycline repressible Trans-Activator and variant (tTA or a Ttav)	Biological population replacement	Lethal effect resulting in sterilization
WO2015040449A1	GENE KNOCK DOWN: RNAi	Gene: Transformer-2 gene	Biological population replacement	Mortality of X (m) chromosome bearing sperm; producing an all-male mosquito population

Incompatible Insect Technique

A total of six patent applications claimed the Incompatible Insect Technique (IIT) using *Wolbachia* bacterium which is an endosymbiotic bacterium that infects mosquitoes. Approximately 40% of insect species are naturally infected by *Wolbachia*. It induces cytoplasmic incompatibility in mosquitoes (Giovanni et al. 2016). When artificially transferred into the mosquito, *Wolbachia* effectively modifies one or more biological properties of a mosquito host. Patent filing trend showed that IIT emerged from the year 2000 onwards and research in this direction is in progress.

Patent application US20110145939A1 claimed mosquito (*Aedes aegypti*, and *Anopheles gambiae*) adapted- recombinant *Wolbachia pipientis* that had improved protection against virus, protozoan, bacterium, fungus. Thus, this technology claimed to make the mosquitoes far less capable of transmitting alphavirus (Chikungunya virus), a flavivirus (West Nile virus, Yellow Fever virus) and protozoans (plasmodium) to humans. The patent application further claimed recombinant *Wolbachia pipientis* to impart host mosquito with reduced ability to feed, reduced desiccation tolerance of eggs and reduction of an average lifespan. Patent application US7868222B1 disclosed a novel approach for artificially infecting *Culicidae* (mosquito) species with one or more *Wolbachia* strains using microinjection technique. Another patent application WO2013026994A1 disclosed *Wolbachia bacterium* (strain iiMel) infected mosquito (*Aedes albopictus*) wherein the mosquito had enhanced resistance to viral pathogens such as dengue virus, Chikungunya virus, West Nile virus, St. Louis encephalitis virus, Rift Valley fever virus, and yellow fever virus. Thus, the patents under this category disclosed a promising, affordable and self-sustaining tool for controlling mosquito vector.

Suppression of vector populations

A total of sixteen patent records under this category disclosed suppression of mosquito population wherein mechanisms such as incorporating self-limiting gene into mosquito and CRISPR were applied to control the mosquito population. Methodologies for suppression of vector population such as Release of Insect with Dominant Lethal (RIDL) and gene knock down using CRISPR/Cas started to develop in the early 1990s and a steady rise in patent filings in this direction was observed. Moreover, patent study showed that these site-specific gene editing tools may offer beneficial insights towards understanding and further exploring mosquito genetics and suppression of mosquito vector populations.

Gene knock down with CRISPR

This category included four inventions claiming genetically modified mosquitoes wherein expression of clustered regularly interspaced short palindromic repeats (CRISPR) associated protein 9 (Cas9) was either used to disrupt the expression of an ionotropic receptor polypeptide in wild-type mosquitoes (US2019153451A1) or to target multiple loci within a target genome (US2019194632A1) or to reduce competitive fitness of an organism hemizygous for a transgenic locus compared to the organism homozygous for the same (US2015373937A1). In patent application US2018291382A1, the autocatalytic CRISPR/Cas genome editing system was used to target a pathogen *Plasmodium falciparum*. Application of mutagenic chain reaction (MCR) to attenuate mosquito-borne malaria wherein an effector cassette encoding the SMI peptide (limits passage of *P. falciparum* through the gut), was conditionally activated by a blood meal (AgCP promoter). Alternatively, single-chain antibody (scFvs) directed against the malarial agent *P. falciparum*, was inserted along with core MCR components (Cas9 and gRNA) into a non- coding region of the mosquito genome.

Release of Insect Dominant Lethal

A total of twelve patents disclosed methods for the control of mosquitoes using a dominant sex-specific lethal genetic system named as Release of Insects carrying a Dominant Lethal (RIDL). RIDL is conditional i.e. expressed when the mosquito is in its natural environment. Patent application GB2355459A disclosed a conditional dominant sex-specific lethal genetic system, which was expressed in the restrictive conditions of the natural environment of an organism. The patent further disclosed how this system may be conditional on temperature or a dietary additive that suppressed expression when supplied to insects in the laboratory. This suppression was removed once the insect was in its natural environment where the additive is not found in its food. The lethal effect might be expressed in the laboratory or natural environment so that only one sex e.g. males were released or survived to interbreed with the wild population thus passing on the genetic system. Alternatively, the lethal system might be sex-specific in an adult organism but be lethal to both males and females in the larval stage. Another such conditional expression system was disclosed in patent application US20150237838A1. The invention disclosed production of genetically modified *Anopheles* mosquitoes containing the *pac* gene with the dsx sex-specific intron. The adult of both sexes was transferred to puromycin-supplemented diet. Following reproduction to the desired population level, the puromycin-supplemented diet selectively killed all female progeny at the first instar larval stage of development. Their inability to splice the male-specific dsx intron and express a functional *pac* resistance gene resulted in only males surviving to adulthood. Another aspect of the invention involved the generation of genetically engineered *Anopheles* mosquitoes that contained the neo gene with the dsx sex-specific intron wherein the expression of *neo* gene product conferred resistance to neomycin and geneticin (G418). When these transformed adults (of both sexes) were transferred to the G418 supplemented diet, it allowed survival of male-only population. G418 antibiotic is an aminoglycoside that interferes with the function of 80S ribosomes and protein synthesis and thus provide a positive selection. After antibiotic-based (puromycin or G418) sex sorting, adult males could be sterilized by irradiation and continually released in the wild in high numbers. US20060242717A1 claimed for conditional gene expression system wherein mediation of alternative splicing was in a sex-specific, stage-specific, germline-specific and tissue-specific manner. US20110283945A1 claimed a novel nucleic acid molecule encoding a sex peptide resistant to proteolytic degradation. Due to its presence following mating with sterile transgenic males introduced into the insect population, the females became non-receptive to males for extended periods of time, thus being incapable of producing offspring. Patent application US2006123489A1 related to populations of male and female mosquitoes, which could be induced to produce a single-sex population. The transformed population comprised a heterologous pro-apoptotic gene of interest which was expressed in a tissue-specific manner. The pro-apoptotic gene was selected from the group consisting of head involution defective, reaper, grim, hid-ala, ICE, and ced-3.

Assignee trend

Assignee analysis is performed to understand the active players in the field under study and their areas of technical expertise. Figure 8 helps in understanding the assignee distribution filing for patents wherein the assignees were grouped into the categories of Academia/ research institutes or company or individual inventors or their collaborations. The Fig. 8 outlines that 90 documents of the total patent applications had academia/ research institutes as assignees followed by private companies with 60 patents to their credit. Twenty patents had individual inventors as assignee. Collaborations of research institutes accounted for 4 patents and 5 of the patents were jointly filed by academia and companies.

Assignees with a minimum of two patents to their credit have been included in the graph (Fig. 9). Analysis showed that the University of Florida was the most prolific assignee. It had 12 patent applications to its credit which were filed independently and a total of four patent documents filed in collaboration with the University of California; the

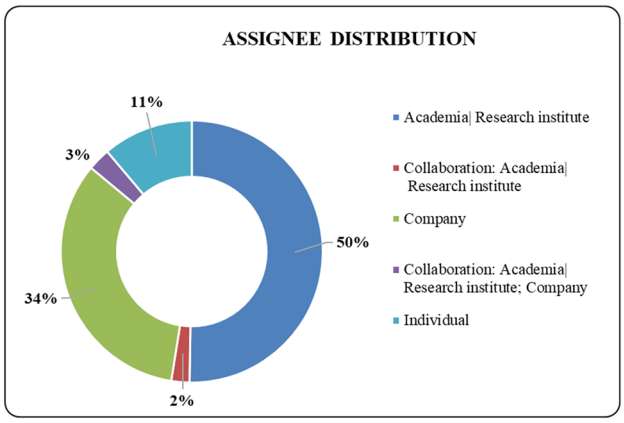

Fig. 8 Assignee distribution based on patent filing entities

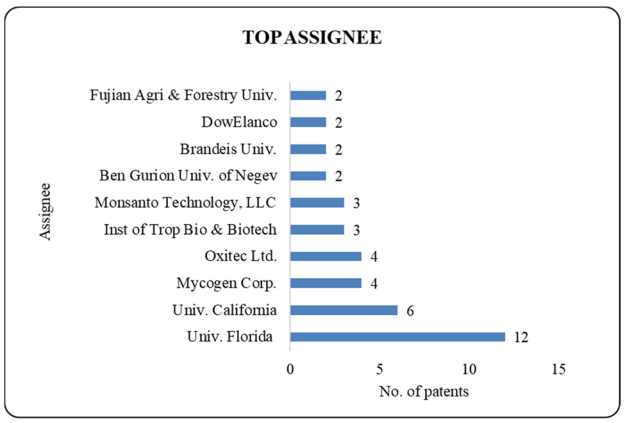

Fig. 9 Assignee wise patenting activity

University of Ben-Gurion and Insect Biotechnology Inc. There were 118 assignees with one patent each to their credit.

A comparative study between the top assignee and their technology focus (Fig. 9) showed that patents by the University of Florida were mainly focused on a range of genome modification techniques like RIDL, RNAi, microinjection to produce transformed cells and toxic fusion proteins. Their technology revolved around neuropeptide F and trypsin modulating Oostatic factor (TMOF) production. The next top assignee was university of California with a total of six patents out of which one patent application was filed in collaboration with University of Florida. Two of their inventions disclosed vector suppression using CRISPR/Cas9 system. Three of their patents primarily disclosed transforming arthropods with exogenous DNA wherein the conditional expression proved detrimental to the viability or reproduction of the arthropod's progeny. The inventions by Mycogen Corp. claimed toxins produced by novel and mutant *Bacillus thuringiensis*. Oxitec technologies has filed patents disclosing release of mosquito carrying self-limiting genes, with conditional expression modules, in the environment. One of their patents claimed the release of genetically engineered mosquito male population that was homozygous sensitive to *Bt* toxins, thereby diluting the resistance in the gene pool, consequently suppressing the wild populations. Oxitec patents claimed a cost effective and non-labour-intensive solution to a long-standing problem of manually separating male/female sexes in mosquito. This further consisted of gene expression systems comprising of self-limiting genes linked to splice control sequences leading to alternative splicing of RNA transcripts of the coding sequence. The ingenious technology by Oxitec. Ltd. under field conditions has been employed in suppressing the vector population in sex-specific, stage-specific and germline-specific and tissue-specific manner.

Year-wise patenting activity of the assignees depicted in Fig. 10 represents the inventive activities of the assignees over the period. The analysis of patenting activities of top assignees over the years showed that the University of Florida has been consistent in patent filing in this technology domain from the early '90s till the year 2012. The University of California started filing patents in the early '90s and Oxitec Ltd. in 2003, respectively, and are seen to be active till recent years. Institute of Trop Bio & Biotech and Fujian Agriculture & Forestry University have recently entered the domain with patent filing beginning in the year 2013. The analysis indicated that although academia and research institutes were the top assignees in filing patent applications, companies were more actively researching this field in the recent years. Also, the focus of research was more on suppression of vector population using GM mosquito (eg. use of RIDL).

Discussion

Use of chemical insecticides is still the most widely used approach towards mosquito control. However, research on effective and eco-friendly bio-control agents has gained importance due to the adverse health and environment-related effects of the chemical insecticides. Patent analysis revealed that both non-recombinant and GM biological agents have been extensively studied for mosquito control. However, greater number of patents have been filed in the last decade as compared to the recent years. Academic institutions have been the active players in this field contributing more than half of the filed patent applications. A number of the patent documents have claimed larvicidal formulations comprising bacterial cultures (novel/mutant/GM) and isolated toxic peptides and other related products. Of these, a significant number of patents have claimed the use

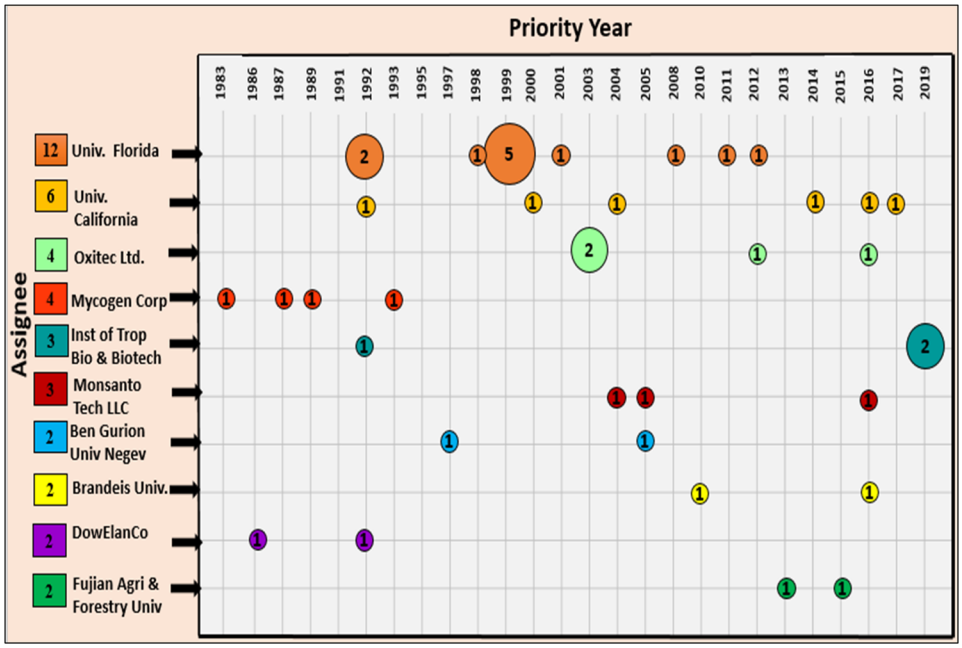

Fig. 10 Year wise patenting activity of top assignee

of Bacillus species such as *Bacillus thuringiensis israelensis,* compared to any other bacteria owing to its efficacy. A number of patents have also disclosed the use of microbial consortia or more than one biocontrol agents putting forth a synergistic approach as a mosquito biocontrol strategy. Various laboratory studies have claimed these bacterial agents as ideal mosquitocidal agents as it is difficult for the mosquitoes to develop resistance against them as compared to chemical insecticides (Federici et al. 2003).

With the advent of genetic engineering, researchers around the globe have tried new ways to control mosquito vectors. Patents based on genetic engineering technology mainly disclosed the use of recombinant toxic peptides for mosquito control. Although existing recombinant bacterial strains have limited activity against Aedine and Anopheline mosquitoes when compared to *Bacillus thuringiensis* toxin, GM bacteria transformed with newly discovered bacterial Mtx proteins and trypsin-modulating oostatic factor have shown to mitigate these limitations (Armelle et al. 2000).

Genetic engineering approaches such as releasing GM male mosquitoes (*Aedes aegypti)* with dominant lethal gene and RNAi based gene suppression have been tried in the field (Benjamin et al. 2019; Elise et al. 2019). Also, IIT is already in use to control mosquito population (Pei-Shi and Anna-Bella 2020). Studies so far have shown that the field-testing using such mosquito vectors have not had any negative impact on human population. Moreover, the GM species produced using these approaches are responsible for vertical gene transmission, easing further dispersion of the transferred genes, and consequently decreasing the target mosquito population. In addition, these approaches assure environmental safety owing to their target specificity without affecting the non-target pest and insect populations, further eliminating the need of harmful chemical pesticides (Luke and Nina 2014).

Despite the desired outcome, specificity and efficacy, the implementation of these gene driven approaches is also facing certain challenges such a s relative lack of control post-release (Luke and Nina 2014), logistical issues, lack of resources (funding) and unavailability of large scale field trials (treatment and control sites) (André et al. 2019). Moreover, one cannot accurately predict the future changes that a genetically engineered species would bring to the environment and likelihood of giving rise to unknown side effects on other living organisms and humans (André et al. 2019). These factors might contribute to comparatively lesser patent filing in this category.

While technology for suppression of mosquito population has been around for nearly two decades, it has remained largely in the domain of releasing GM mosquitoes expressing self-limiting dominant lethal genes. This technology was first applied by Oxitec Ltd. and till date it remains one of the active assignees pursuing research and field operations in this area. The release of genetically engineered mosquito has led to significant transfer of its genome into the natural population of mosquito vector carrying devastating viruses such as Zika and dengue. However, with time, ethical and economic issues related to this patented technology might emerge. For example, recent study has shown that the release of OX513A into natural Jacobina population of *Aedes aegypti* has led up to 10–60% of introgression of OX513A genome in all individuals. Although there has been no evidences showing ill-effect of these hybrids on human health, un-anticipated consequences may follow in the form

of more robust mosquito populations, further giving rise to many ethical issues (Benjamin et al. 2019).

The cost of these patented GM mosquitoes would vary with different situations and would be subject to the country implementing these interventions. Current estimates suggest that, for an urban population of 50,000, the cost of using the Oxitec GM mosquito in the first year would be approximately US$1.9 million and US$384,000 for subsequent years. Although factors such as recurrent relicensing and subsidizing the cost may be considered in near future, at present substantial economic burden of this technology seems to be on the public health budgets of developing countries (Zahra and Christophe 2018).

While other microbial agents such as entomopathogenic fungi show promising applicability in vector control, a paucity of studies describing their effects on mosquito populations indicates the need of further research to determine efficacy and viability of this technology in mosquito field populations. Finally, very few patents claimed biological agents such as nematode, protozoa and fish. One reason might be their threat to native aquatic fauna, highlighting a need to carefully assess the environmental cost of introducing predatory species intended to contribute to mosquito control.

Taken together, these findings highlight the promise of biological agents as potential control tools against various mosquito vectors. These agents could be very specific and effective in their action posing them as interesting alternative or supportive option to mechanical and chemical means to control mosquitoes. A synergistic approach towards these biological control agents and their mechanism of action may be explored further to create new avenues in mosquito suppression. Although such biocontrol approaches might be efficient and specific, their long-term effects on the ecosystem need to be examined and monitored carefully.

Considerations

Patent documents are a rich source of technical, legal and business information. However, the techno-legal language of patent information makes it difficult for researchers and inventors to interpret. One needs to read and analyse a large number of patent documents to understand the state of the art. Patent landscape is a comprehensive study performed by knowledgeable patent experts, with both, legal and technical understanding of the patents. Therefore, it is a specialized field of study. The findings of this review might help the readers in understanding various technical approaches followed by inventors around the world. For example, Target Malaria is an international consortium exploring the use of genetically modified mosquitoes to suppress malaria transmission. As the first initiative to explore the use of gene-drive modified mosquitoes for malaria control, Target Malaria has attracted a significant degree of attention (Nourou et al. 2020). This review might aid the readers in appreciating the nuances of the genetic modification techniques instrumental in the creation of GM mosquitoes. Randomized field trials of mosquitoes carrying *Wolbachia* is being conducted in many countries by the 'World Mosquito Program' (Katherine et al. 2018).

Certain commercially available products or those under development can be mapped to patents based on the product description, press releases and identity of the patent assignees. Such information might take a long time and effort to collect as the information sources tend to be diverse. For example, BACTIVEC® is a commercially available water-based suspension containing spores and toxic crystals of *Bacillus thuringiensis israelensis* type H-14 for the control of mosquito larvae. A patent (CN101347129A) for this technology has been assigned to Foshan Standard Bio-Tech Co. Mosquito and fly control products based on the patented technology of University of Florida have been developed by Florida Insect Control Group after licensing the technologies. The mosquito products are in the last phases of approval by the Environmental Protection Agency and the European Union. This is a noteworthy example of public-private partnership which has been equally beneficial to academics and industry and has resulted in to the products becoming available to the military as well as the general public (University of Florida News 2020).

Overall, it can be said that the present patent landscape might serve as a window to observe greater expanse in the field of biological mosquito control.

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Data availability Not applicable (as the work did not involve or use any novel microbial strain, plasmid, virus, other material such as prions or cell lines and nucleotide or amino acid sequence data).

Code availability Not applicable (as the work did not involve software application or custom code).

Compliance with ethical standards

Conflict of interests The authors declare no conflict of interests regarding the publication of this paper.

Ethics approval This is an observational study and hence no ethical approval is required (as the research did not involve human or animal participation).

Research involving human or animal rights Not applicable (as the research did not involve human or animal participation).

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