Current status and future prospects of bacilli−based vector control

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ABSTRACT

Mosquito-borne diseases such as malaria, filariasis, dengue, chikungunya, Japanese encephalitis, yellow fever and Zika contribute significantly to health problems of developing as well as developed nations. Vector control is central to control of vector borne diseases. In the last four-five decades, biological control methods have been inducted in the integrated vector management strategy, advocated nationally as well as globally by the World Health Organization. Currently, biological control of vectors is globally acknowledged as the best available strategy in the wake of growing concerns about vector resistance as well as adverse effects of insecticides on the environment and non-target fauna co-inhabiting the same ecological niches as vectors. In India and elsewhere, efforts are ongoing to screen newer isolates to bring forth new biolarvicidal products of public health importance. In this review, by carrying out extensive literature survey, we discuss advances thus far and the prospects of bacilli-based control of vectors and vector borne diseases.

KEYWORDS: Mosquito-borne diseases; Vector control; Biological control; Biolarvicide

1. Introduction

Mosquitoes are associated with transmission of pathogens to humans and other vertebrates resulting in significant morbidity and mortality due to the difficulty of controlling mosquitoes[1]. The most important disease vectors belong to the subfamily Anophelinae (*Anopheles* mosquitoes) which transmits malaria; Culicinae *i.e. Culex* species transmit filariasis; West Nile virus, Japanese encephalitis and *Aedes* mosquitoes which primarily transmit dengue, chikungunya, yellow fever and Zika. These diseases account for more than 17% of all infectious diseases, causing 7 00 000 deaths annually with 80% of the world's population at risk of one or more vector-borne diseases[2]. In recent years, changes in public health policy and social factors as well as reports of resistance in both vector mosquitoes and the pathogens transmitted by them have caused a resurgence in the incidence of mosquito borne diseases[3].

Vector control is a key strategy to control these diseases. In India, vector control is primarily based on the use of long-lasting insecticide treated nets (LLINs) in addition to indoor residual spraying of insecticides in rural areas and anti-larval operations in urban areas[4]. Larval control may be particularly valuable in regions where the eradication or elimination of vector borne diseases is being targeted, as a means of reducing the mosquito larval populations before they emerge to the adult stage[5].

However, with regards to mosquito control strategies, chemical control agents still play a major role. Insecticides applied with the aim of eliminating mosquitoes have given rise to other serious problems[6]. Not only have mosquitoes developed resistance, but these insecticides also pose threat to human, animal health and the ecosystem as a whole. Chemical insecticide exposure among

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humans has been linked to immune dysfunction, neurological disorders, various forms of cancer, birth defects, liver damage and infertility[7]. These adverse effects have led to the discovery of alternatives to these insecticides. Microbial control agents are effective and proven to be a method effective against mosquito immatures of both *Anophelines* and *Culicines*. Commercial biolarvicide formulations of gram positive and spore forming bacteria, *Bacillus (B.) thuringiensis israelensis* and *B. sphaericus* are now being widely used across the globe in the vector control programmes. These strains have been well characterized both at the microbiological and molecular level. Based on these two bacilli, there are several effective and well tested formulations commercially available including the wettable powder, slow release granules, briquettes, tablets and emulsifiable concentrates. These formulations are often deployed as an integral components of the integrated vector management strategy advocated by the World Health Organization and adopted by vector borne disease endemic countries.

In this review article, extensive literature search was done to collect and collate published information on bacilli-based biolarvicides and their control in different parts of the world, especially the articles published on the recent advancements in the field of bacilli-based vector control.

2. Bacilli as bio–control agents

In nature, a wide variety of organisms including viruses, protozoans, fungi and bacteria, effectively control mosquitoes[8]. Among many bacteria that have been tested, strains of *B. thuringiensis* (*Bt*) and *B. sphaericus* (*Bs*) are the most promising for vector control so far. *B. thuringiensis* var. *israelensis* (*Bti*) has an advantage of a broader host range. While *B. sphaericus* has a narrow spectrum, it has an advantage of increased duration of larvicidal activity against specific mosquito species like *Culex (Cx.) quinquefasciatus* and possess recycling ability within mosquito cadavers[9]. There are options available for 'stand-alone' and combined formulations of these two *Bacilli* species and their strains for vector control programmes.

2.1. B. thuringiensis

B. thuringiensis is a ubiquitous, gram positive, sporulating aerobic bacterium which can be easily grown and cultured on routinely used media like nutrient agar. It can be isolated from a variety of sources[10]. On sporulation, it produces two types of insecticidal crystal proteins or δ-endotoxins, Cry (for crystal) and Cyt (for cytolytic) proteins and further variations of each of these types. Cry proteins target lepidopteran insects, while few are toxic to dipteran or coleopteran insects. Cyt proteins show moderate toxicity to mosquitoes and black fly larvae occurring mostly in mosquitocidal

subspecies *e.g. B. thuringiensis* subsp. *israelensis*[11].

Cyt proteins have been studied less in comparison to Cry proteins. Based mainly on studies of Cyt1Aa, their importance is in the biology of mosquitocidal strains as they synergize with other mosquitocidal Cry proteins (Cry4Aa, Cry4Ba, and Cry11A) resulting in delay in the phenotypic expression of resistance which would require multiple mutations at different loci[12].

The high degree of host specificity and the complexity of *B. thuringiensis* mode of action results from the interaction of the mosquitocidal toxins within the complex environment of the insect's midgut lumen. Although researchers discovered relatively early that the midgut was the primary site of δ-endotoxin activity as seen in (Figure 1A and 1B), the molecular mechanisms of *Bt* intoxication have continued to be the subject of intensive research[13].

Although *Bti* is proven to be effective against many mosquito species, operational application showed that it is more suitable against *Aedes* species. *Aedes (Ae.) aegypti* and *Ae. albopictus* were most susceptible to *B. thuringiensis* H-14 in comparison to other vector mosquitoes[14]. More advantages for *Aedes* control may be due to their feeding behaviour, as most of the *Bti* toxins sediment to the base of the container during treatment where *Aedes* larvae frequently feed[15]. On the other hand, *Anopheles (An.) balabacensis* and *Mansonia* (Mansonioides) *indiana* were found comparatively less susceptible to the *Bti* (H-14)[16].

Cx. quinquefasciatus was also found to be highly susceptible to *B. thuringiensis* H-14[17]. This mosquito species, however, is more susceptible to *B. sphaericus*, the latter being more effective in polluted water with high organic contents where *Cx. quiquefasciatus* prefers to breed as *B. sphaericus* is known to recycle in polluted water and persists longer than *B. thuringiensis* H-14[18].

2.2. B. sphaericus

B. sphaericus is a common aerobic, rod-shaped, endospore forming gram positive soil bacterium with a few entomopathogenic strains. The first discovery of a strain toxic to mosquito larvae was reported by Kellen *et al.* in 1965[19]. The biolarvicide based on *B. sphaericus* is unique in that it consists of two binary proteins BinA (42 kDa) and BinB (51 kDa), both of which are required for toxicity to mosquito larval midgut. These binary proteins are cleaved by mosquito gut proteases, forming the active toxin by yielding peptides of 39 kDa and 43 kDa respectively. These associate and bind to the α-glucosidase receptor located on the midgut microvilli, resulting in lysis of midgut cells upon internalization[20,21]. It is suspected that reported loss of toxicity *i.e.* resistance in target mosquito species to *B. sphaericus* may be due to the reduction or loss of interactions between BinA and BinB or BinB and its receptor[22]. In addition, another 100 kDa mosquitocidal protein appears to be synthesized in lesser toxic and some highly toxic strains. This polypeptide is
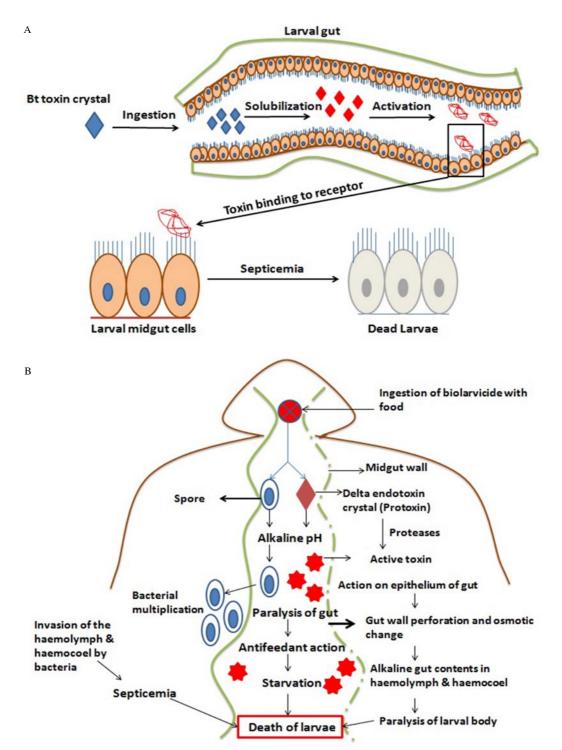
A

B

Figure 1. (A) Diagrammatic representation of mode of action of *Bacillus thuringiensis* israelensis toxin; (B) Cartoon showing death of mosquito larvae due to toxin action of biolarvicide.

expressed during the vegetative phase and is not homologous with the 51 and 42 kDa proteins[23].

2.3. B. subtilis

A *B. subtilis* strain producing mosquitocidal (larvicidal and pupicidal) toxin was isolated from mangrove forests of Andaman and Nicobar Islands of India and found to kill larval and pupal stages of three species of mosquitoes *viz.*, *An. stephensi*, *Cx quinquefasciatus* and *Ae. aegypti*. It is the first gram-positive bacterium highly toxic

to mosquito pupae[24]. Its mosquitocidal activity is associated with an exotoxin identified as surfactin, a cyclic lipopeptide highly active at both acidic and basic pH, temperature range of 25 ℃-42 ℃, and UV stability, suitable features for the development of a biolarvicide. Preliminary toxicity studies with crude surfactin showed that it is non-toxic to mammals[25]. The arsenal of biocontrol agents is further augmented with this potential mosquitocidal bacterium. The overview of different bio-control agents, their strains, activity profile against target species, toxin genes and strain modifications with recombinant technology is shown in Table 1.

Table 1. Overview of different bio-control agents, their strain, activity profile against target species, toxin genes and strain modifications with recombinant technology.

Sr. No.	Bacteria reported	Strain	Activity against mosquito species	Toxin gene identified	Recombinant technology	Reference
1	*Bacillus thuringiensis*	*israelensis* (*Bti*) H-14 and VCRC B17	*Ae. aegypti*, *Cx. quinquefasciatus*, *An. stephensi*, *Ae. nigromaculis*, *An. quadrimaculatus*, *Cx. tarsalis*	Cry (crystal) and Cyt (cytolytic) proteins	130 kDa toxin from *Bti* introduced into plasmid pRK248 expressed in *Caulobacter crescentus* CB15.	[26–28]
		kurstaki (*Btk*)	*Ae. aegypti*	Cry protein	NK	[28]
		morrisoni	NK	Cry protein	NK	[29]
		jegathesan (*Btj*)	*An. stephensi*, *Cx. pipiens*, *Ae. aegypti*	Cry protein (Cry19Aa1, Cry11Ba1)	NK	[30]
		medellin	*Ae. aegypti*, *An. albimanus*, *Cx. quinquefasciatus*	Cry protein (Cry11Bb1, Cyt1Ab1)	NK	[31]
		higo	*Cx. pipiens*	Cry protein (Cry 21Aa1)	NK	[32]
		fukuokaensis	*Ae. aegypti*	Cry protein (Cry 20Aa1)	NK	[32]
		kyushuensis	*Ae. aegypti*, *An. stephensi*, *Cx. pipiens*	Cry protein (Cyt2Aa1)	NK	[32]
		tochigiensis	*Ae. aegypti*, *Cx. quinquefasciatus*	Cry protein	NK	[32]
2	*Brevibacillus laterosporus*	strains 921 and 615	*Ae. aegypti*, *An. stephensi* and *Cx. pipiens*.	Toxic factors likely to be proteins of about 14 kDa	Enhanced larvicidal activity by bio-encapsulation in Protozoa.	[33–36]
3	*Bacillus sphaericus*	*Neide* H5a5b and VCRC B42	*Culex* sp., *Anopheles* sp., *Aedes* sp.	Binary (Bin) toxins of 42 (BinA) and 51 (BinB) kDa + MtX (mosquitocidal) toxins	(1) Use of cyt1A promoters + mRNA stabilizing sequence to synthesize high levels of *Bs* binary toxin in *Bti* strains. (2) Cry4Ba and cyt1Aa genes expressed in *Bs*2362 produced stable transformants 10 times more toxic to *Aedes* larvae than the host strain.	[37–41]
4	*Clostridium bifermentans*	*malaysia*	*An.* sp., *Ae. detritus*, *Ae. caspius*, *Ae. aegypti*, *Cx. pipiens*, blackfly larvae	Toxic extract contains three major proteins of 66, 18 and 16 kDa	Toxin encoding gene cloned and induced to be expressed by transformed *Bt*, exhibited toxicity against mosquitoes.	[42]
5	*Bacillus alvei*	NK	*Culiseta longiareolata*	NK	NK	[43]
6	*Bacillus brevis*	NK	*Culiseta longiareolata*	NK	NK	[43]
7	*Bacillus circulans*	NK	*Cx. quinquefasciatus*, *An. gambiae*	Toxicity due to spores	NK	[44]
8	*Pseudomonas fluorescens*	*Migula* strain VCRC B426	*An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*	Mosquitocidal exotoxin (44 kDa)	NK	[45–47]
9	*Bacillus subtilis*	*subtilis*	*An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*	Exotoxin- surfactin	NK	[23]
10	*Bacillus amyloliquefaciens*	strain VCRC B483	*An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*	Production of lipopeptide (s)	NK	[48]
11	*Peanibacills macerans*	NK	*Ae. aegypti*	80 kDa parasporal protein	NK	[49]
12	*Chromobacterium* spp	*Chromobacterium* strain Csp-P	*Ae. aegypti*	Colonize the insect midgut and displays entomopathogenic and anti-pathogen properties	NK	[50]
13	*Bacillus cereus*	VCRC B540	*An. stephensi*, *Ae. aegypti*	NK	NK	[51,52]
14	*Pseudomonas aeruginosa*	strain KUN2	*Ae. aegypti*	Extracellular toxins	NK	[53]

*NK-not known, *Bs*-*Bacillus sphaericus*, *Bt*-*Bacillus thuringiensis*, *Bti*-*Bacillus thuringiensis israelensis*, *An*-*Anopheles*, *Ae*-*Aedes*, *Cx*-*Culex*, sp-species.

3. Bioassays, isolation, characterization and identification

Microbial isolates are constantly screened and isolated from terrestrial and aquatic environments for mosquito control programmes[54]. The earlier method of isolating mosquito pathogenic *Bacillus* strains was cumbersome and time consuming, hence Dhindsa *et al.* in 2002 devised a new soil screening method that could reveal the presence of mosquito pathogenic bacilli in the soil samples. This method involves the use of LB broth (buffered with Sodium acetate) and a heat shock step at 65 ℃[55]. Using this method, eight different *Bacillus* strains, *B. pumilus* (KSD-1), *B.*

sphaericus (KSD-2), *B. brevis* (KSD-3), *B. sphaericus* (KSD-4), *B. subtilis* (KSD-5), *B. stereothermophilus* (KSD-6), *Bacillus* sp. (KSD-7) and *B. sphaericus* (KSD-8) were successfully isolated, identified and evaluated for their larvicidal activity in Goa, India[55].

In a screening assay carried out by Radhika *et al.* in 2011, ten bacilli were isolated from Tamil Nadu, India and tested for larvicidal activity against *Ae. aegypti* mosquito. Two microbial isolates (*B. megaterium* and *Acinetobacter* sp.) effectively caused 97% larval mortality at 48-hour incubation at bacterial concentrations of (4.1±0.39) and (3.6±0.71) mg/L[56]. Another study by Allwin *et al.* in 2007 showed native strains of *B. thuringiensis* were isolated from soil samples collected from different locations and characterizations in India[57]. Samples collected from mangroves of Vellar estuary in India yielded mosquitocidal bacteria *B. subtilis* with increased activity against *An. stephensi* and *Ae. aegypti*[58]. Many other reports on the frequent occurrence of mosquito pathogenic bacterial isolates in the natural environment showed high possibility of isolating novel strains[59].

4. Resistance phenomenon and overcoming resistance

Since insecticide resistance can undermine efforts to control vector borne diseases, effective mosquito control can be successfully achieved only by overcoming insecticide resistance. Resistance is a complex genetic, evolutionary and ecological phenomenon. Resistance to microbial insecticides formulations is a serious threat to their success in public health settings[60].

4.1. Strategies for management of resistance to biolarvicides

Some measures to counter resistance include: (1) rotation or alternation of bacterial biolarvicidal toxins with other toxins, insecticides or biological control strategies; (2) less frequent biocide treatments; (3) use of slow-release, ultra-low volume (ULV) and thermal formulations which are active for longer durations; (4) use of source reduction methods and (5) constant resistance surveillance and monitoring. These principles when combined are essentially a blueprint for integrated pest management which will successfully delay or prevent the development of resistance in vector populations[61].

4.2. Insecticide mixtures

Studies have shown that by combining different classes of insecticides or their application by mosaic design can effectively overcome resistance in the target insects. However, unless insecticides of different classes are combined and judiciously used, there is possibility of cross resistance if insecticides induce similar mechanism of resistance and have mode of action in target insect. If mixtures are used, there is inherent risk of resistance build up to multiple classes of insecticides rendering them eventually useless. But there is a drawback in this approach for their practical application mainly due to higher cost and practical difficulty as both compounds need to be present in equally high and persistent concentrations[62]. In such a scenario, the use of two or more interventions has been advocated so that mosquitoes that survive contact with one (*e.g.* LLINs) are killed due to exposure to the second (*e.g.* indoor residual spraying). In such a scenario, the use of biolarvicides where feasible, can also delay the onset of resistance and ease selection pressure of insecticide on target vectors.

4.3. ULV and thermal application

The dengue vector *Ae. aegypti* is a container breeder, hence use of *Bti* for its control is limited due to difficulty in its effective application. In this respect, ULV cold fogging can be used effectively for larviciding purposes when the agent is applied correctly and under required conditions[63]. Seleena *et al.* 1996 found that ULV fogging of *B. thuringiensis* H-14 was highly effective in *Aedes* larval control and when used together with malathion it induced complete adult mortality[64].

In addition, the effectiveness of the thermal application of an aqueous suspension of *Bti* with and without pyrethroids using a thermal fogger has been reported without loss of its larvicidal activity[65–67].

4.4. Application of ice granules containing endotoxins of microbial agents

A novel method for the aerial delivery of microbial mosquito control agents into vast aquatic sites in the form of ice granules was developed by Becker *et al.* in 2003. The solutions containing powder formulations of *Bti* or *Bs* were transformed into ice pellets (named IcyPearls) using a special ice-making machine and applied aerially. Successful field tests using IcyPearls applied at the rate of 5 and 10 kg/ha containing various dosages of 100, 200, and 400 g of VectoBac® WDG (3 000 ITU/mg) were conducted against larvae of *Ae. vexans* with mortality rates of 91%-98%[68].

5. Commercial bio–larvicide formulations and their field efficacy

Two biolarvicide formulations-Bacticide® and VectoBac® containing viable endospore and delta endotoxin of *Bti* H-14 were evaluated in 2001 in Surat city, India against *An. subpictus* and *Cx. quinquefasciatus*. Both formulations were equally effective on larvae after second application[69].

Field testing and evaluation of the efficacy of bio-larvicide,

Bactivec® SC (*Bti* H-14) was carried out in Bengaluru, India. It was found to be operationally feasible and easy to handle[70].

Kumar *et al.* 1995 and 1996 tested a formulation of Bactoculicide (*Bti* strain 164) in construction sites, abandoned overhead tanks and curing waters and a formulation of Spherix (*B. sphaericus* H5a5b) in Goa, India respectively and found them highly effective[71,72].

The weekly application of biolarvicide *B. sphaericus* (Strain 101, Serotype H5a5b) in Panaji, Goa, India helped in malaria control and was identified as a useful biocontrol agent of *An. stephensi*[73]. Similarly, application of biolarvicide *Bti* strain 164 at 1 g/m^2 and introduction of larvivorous fish *Aplocheilus blocki* in major breeding

habitats of *An. stephensi* was carried out in order to control malaria in Goa, India. This was found to successfully replace DDT and pyrethrum fogging[74].

In addition, the efficacy of various formulations of *Bti* (Bactimos®, Teknar®, VectoBac®, Bactisand®, VectoPrime®, VectoMax®) and *Bs* (HIL-9® & HIL-10®, VectoLex®) in the form of tablets, granules, wettable powder, pellet, aqueous suspension, *etc.* were tested against mosquito vectors and found to be highly effective.

Table 2 provides a list of the available commercial bio-larvicide formulations, their type, potency and field evaluation of these formulations.

Table 2. A list of the available commercial bio-larvicide formulations, their type, potency and field evaluation of these formulations.

Sr. No.	Formulation	Active ingredient	Type	Potency	Field evaluation	Reference
1	HIL-9 & HIL-10	*Bacillus sphaericus* strain 1593	Dust	NK	V/s *An. culicifacies* (doses-0.05, 0.1 and 0.5 g/0.1 m^2) 100% mortality in third and fourth instar larvae.	[75]
2	Bactimos®	*Bacillus thuringiensis israelensis* strain AM 65-52	PT	3 000 ITU/mg against *Ae. aegypti* larvae	High mortality (96%-100%) in late larval instars of *Ae. albopictus* and *Cx. quinquefasciatus* from lab and field 24 hours after application.	[76]
3	Bactisand®	*Bacillus thuringiensis israelensis* H-14	FG	112 ITU/mg against *Ae. aegypti* larvae	NK	[77]
4	Bactoculicide	*Bti* (strain 164)	Suspension	993 ITU/mg against *Ae. aegypti* larvae	*Culex, Aedes* and *Anopheles* larvae breeding controlled (96%-100%, for up to 5 weeks, dose-0.5 g/m^2) in industrial scrap in UP, India.	[78]
5	Spherix	*Bacillus sphaericus,* serotype H5a5b, strain B101	WDG	NK	In lab evaluation in Assam, India 90% mortality observed in *Cx. quinquefasciatus* third instar larvae at 0.6 ppm.	[79]
6	VectoBac®	*Bacillus thuringiensis israelensis* H-14 strain AM 65-52	WDG 12 AS, SCG	3 000 ITU/mg 1 200 ITU/mg 200 ITU/mg against *Ae. aegypti* larvae	(1) In a study in Malaysia, VectoBac® G and VectoBac® 12AS effective for 24 hrs v/s *Ae. albopictus* in discarded tires with > 80% mortality. (2) VectoBac® WDG evaluated in the lab and field in Bangalore, India v/s *An. culicifacies* and *An. stephensi* revealed increased efficacy against *An. stephensi*.	[80,81]
7	Teknar®	*Bacillus thuringiensis israelensis,* strain SA3A	SC	1 200 ITU/mg against *Ae. aegypti* larvae	Larvicidal efficacy v/s *Cx. quinquefasciatus* was determined in lab and field in Pondicherry, India. In cesspits >80% reduction of pupae up to day 6 post-treatment and in unused wells >80% reduction of pupae for 17 days post treatment was observed.	[82]
8	VectoLex®	*Bacillus sphaericus* 2362, Serotype H5a5b strain ABTS 1743	FG WDG	50 BSITU/mg against *Cx. quinquefasciatus* larvae 650 BSITU/mg against *Cx. quinquefasciatus* larvae	Efficacy against third instar larvae of *Culex* sp. and *Ae. aegypti* was studied in Queensland, Australia. Both formulations were most effective against *Culex* spp, with the WDG 10-100 times more effective than the FG on an ITU/mosquito basis.	[83]
9	VectoMax®	*Bacillus sphaericus* 2362, Serotype H5a5b, Strain ABTS 1743 + *Bacillus thuringiensis israelensis* Serotype H-14 Strain AM 65-52	FG WSP	50 BSITU/mg against *Cx. quinquefasciatus* larvae 50 BSITU/mg against *Cx. quinquefasciatus* larvae	A trial v/s *Ae. albopictus* larvae in Spain took place over 2 seasons in the same water at dosages of 10, 50, and 577 kg/ha. At all 3 concentrations the efficiency was close to 100% for up to 345 days post-treatment. Residual effectiveness of VectoMax® WSP when applied to septic tanks against 3rd and 4th stage larvae of *Cx. pipiens* was evaluated in a study in Turkey, at operational application rates of 1 pouch (10 g) and 2 pouches (20 g) per septic tank. Both application rates resulted in >96% control of larvae for 24 days.	[84] [85]
10	VectoPrime®	*Bacillus thuringiensis israelensis* strain AM 65-52 + (S)-methoprene	FG	400 ITU/mg	NK	[86]

*FG=Fine granule, G=Granule, SC=suspension concentrate, WDG=water-dispersible granule, WSP=water soluble pouch, SC=Suspension concentrate, PT=pellet, AS=aqueous suspension, NK= not known, *An-Anopheles*, *Ae-Aedes*, *Cx-Culex*, sp-species.

6. Future prospects

Future prospects for the use of biolarvicide formulations against mosquito vectors will depend on low cost production and development of cost-effective formulations. Cheaper formulations designed from the seeds of legumes, dried cow blood and mineral salts as well as the use of potato-based culture medium, bird feather waste and de-oiled rice bran waste as culture medium when assessed for growth and production of insecticidal toxins of *Bti* were shown to be more economical and effective against *Ae. aegypti*, *Cx. quinquefasciatus* and *An. gambiae*[87–89]. This is very important from the point of media optimization for the economical production of *Bacillus* based insecticides in mosquito control programs[90].

In addition, enhanced activity of protein toxins by use of recombinant bacteria containing a mixture of endotoxins having different modes of action shows great promise. A few examples include newly discovered mosquitocidal proteins and peptides such as Mtx proteins and trypsin modulating oostatic factor which can be genetically engineered for development and use in vector control programs[91].

Research is also underway with respect to transgenic algae and cyanobacteria by expressing larvicidal endotoxins of *Bti* and *B. sphaericus* to allow the toxins to persist in the feeding zone for a longer duration as well as providing increased protection from sunlight (UV light). The most promising results were obtained when Cry4Aa and Cry11Aa alone or with Cyt1Aa were expressed in the filamentous, nitrogen-fixing cyanobacterium *Anabaena* PCC 7120[92]. A transgenic strain of *Anabaena* PCC 7120 was reported to protect the expressed δ-endotoxins of *Bti* from damage inflicted by UV-B. This organism has an added advantage as it has the ability to multiply in the breeding sites as well as serving as a food source to mosquito larvae[93].

Recently there has been focus on the development of novel biolarvicides and their applications. The use of entomopathogenic bacteria and fungi mainly ascomycetes fungi such as *Metarhizium anisopliae* and *Beauveria bassiana*, for control of both larval and adult stages of mosquito vectors such as *Aedes*[94]. The use of spatial repellents has been advocated to release volatile chemicals into the air, to induce modifications in insect behaviour and to reduce human-vector contact thereby reducing pathogen transmission[95].

Although mosquito traps have been used effectively as surveillance tools in order to capture vector mosquitoes for population and disease transmission studies, they have recently been considered a control strategy by the introduction of the lethal ovitrap. These traps such as attractive baited lethal ovitrap are being developed to attract and kill the egg-bearing females. They have shown promise in both lab and field settings for significant reduction in *Aedes* populations[96]. The use of attractive toxic sugar baits which work by attracting mosquitoes and having them feed on toxic sugar meals could also be a potential vector control tool.

The future vector control includes the use of sterile insect technique (SIT) which has been successfully demonstrated against *Ae. albopictus* mosquitoes[97]. SIT appears very promising to control mosquito populations and has been recently combined with auto-dissemination *i.e.*, adult females contaminated with dissemination stations of juvenile hormone to treat breeding habitats, especially for the control of *Aedes* species, but this technique has not been used in large scale at present. Recently, a new control concept has been devised, named "boosted SIT" that might enable the area-wide eradication of mosquitoes[98]. In addition, the exploitation of cytoplasmic incompatibility can be an advantageous mosquito control method[99]. Cytoplasmic incompatibility is induced by the bacterial endosymbiont *Wolbachia* which is widespread and its use is a promising tool for mosquito control either alone or associated with SIT[100]. Lately, mosquitoes modified with gene drive systems are being proposed as new tools that will complement the existing ones[101]. The synergistic utilization and application of these control measures to protect against mosquito borne diseases could have a major impact on the socio-economic health of populations particularly in developing countries. These methods are currently in the pipeline and could complement the integrated vector management programmes when available.

7. Conclusions

Vector borne diseases transmitted by mosquitoes are a major public health concern. Effective vector control requires the deployment of a range of integrated interventions. This review focuses on the current status and future prospects of bacilli-based vector control to explore additional options and potentially augment existing strategies. It is of immense importance to focus on the development, evaluation and deployment of alternative vector control products and strategies. However, for effective control and elimination of the mosquito vector and vector borne diseases, these strategies will have to be locally adapted to account for vector biology and the intensity of disease transmission keeping in mind both human and financial resources. In addition, we are waiting for the discovery of a novel bacterium from nature which could be developed into an ideal biolarvicide having a broad spectrum of activity at very low concentrations without developing resistance in the target mosquito species.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Authors' contributions

A.K. designed the study, J.S.A and A.K.M. carried out the data collection, data analysis and interpretation. J.S.A and A.K.M. drafted the article. A.K, S.K and S.L.H. edited the article. All authors read and approved the final article.

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