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BIOCONTROL AND LARVICIDAL ACTIVITY OF *BACILLUS THURINGINESIS* AGAINST THE LARVAE OF MOSQUITO TO COMBAT DENGUE AND MALARIA

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ABSTRACT

With the increasing awareness of the harmful effects of chemicals on man and his environment, the immediate need for sustainable, eco-friendly pest management has been felt very strongly for providing an impetus to research and development of microbial pesticides. A major challenge for achieving successful mosquito control is overcoming insecticide resistance. This study was carried out to assess the larvicidal activity of Bacillus thuringiensis isolated from different soil samples in the regions of Bangalore and Hyderabad using standard methods. Among the different microbial agents developed and tested, bacteria, viruses and fungi are considered promising for the management of insect-pests. The isolate organisms were confirmed as Bacillus thuringiensis based on simple staining, gram staining, coomassie blue staining and their microscopic observation as well by their biochemical characterization. The larvacidal activity of Bacillus thuringiensis isolates was tested against the larval of mosquito by using the standard cup bioassay method using three dilutions of the Bacillus culture. The isolates of Bacillus thuringiensis showed a significant level of variation in their larvacidal activity. From this study, it is concluded that Bacillus thuringiensis is a very potent biolarvicide that can be used to reduce and possibly eradicate the nuisance of disease-causing mosquitoes and aid in the rollback of malaria and other vector borne viral diseases.

KEYWORDS: Dengue, Bacillus thuringiensis, malaria, insecticide, larvicidal, bioassay.

1. INTRODUCTION

Dengue is an acute arboviral infection with potential fatal complications. Dengue is an endemic disease worldwide. According to estimates of the World Health Organization (WHO), around two fifths of the world's population in tropical and subtropical countries is at constant risk of contracting this infection. Dengue is endemic in many parts of India and recently in the last few years many places have even experienced epidemics. Dengue is a worldwide serious public health problem spread throughout the tropical and subtropical zones. It is endemic in South-East Asia, the Pacific, East and West Africa, the Caribbean and the America. In India, dengue virus was first isolated in 1945 and the first outbreak of Dengue hemorrhagic fever (DHF) in Calcutta occurred during 1963 and subsequent DHF/ dengue shock syndrome (DSS) outbreak was documented in Delhi in 1988. The dengue epidemiology in India has been very complex and significantly changed over almost the past six decades in terms of prevailing strains, the severity of disease and geographical locations that were affected. DF, DHF and DSS has been identified as a re-emerging disease and already reported from 35 states including union territory by the National Vector Borne Disease Control Programme (NVBDCP) during the last decade. A total of 75858 dengue cases with 193 deaths in 2013 and 36486 dengue cases with 92 deaths in 2014 were reported by the NVBDCP from all states of the country. Among the arbovirus in India, distribution of all the dengue virus types (DEN 1, 2, 3 & 4) is continuously expanding. Dengue, the most common arboviral disease, is caused by four strains of dengue virus (DEN1, DEN2, DEN3 and DEN4) a member of flavivirus group in the family-flaviviridae and transmitted by female *Aedes aegypti* mosquitoes. Dengue virus has also been recently detected in *Ae. albopictus*, a secondary vector of dengue. Now this vector has spread to rural areas also and spreading in areas which were so far free from this disease due to increasing urban population, unplanned urbanization, rapid transportation (movement of human carriers and infected mosquitoes), unreliable water supply and storage practices. Dengue haemorrhagic fever (DHF) and Dengue fever are the clinical manifestations causing a wide spectrum of illness. However, the infection, transmission, diverse in manifestation and pathogenicity is the exaggeration for the stiff challenge to public health. The chikungunya virus is a rare form of alpha virus, which is spread by *Aedes* mosquitoes and characterized by fever, rash, and arthralgia. In India, immunity to malaria is unstable, allowing people of all ages to become ill during an epidemic. In Karnataka state in 2005, 83,181 cases of malaria was reported, out of

them, 21,984 were cases of *Plasmodium falciparum*. There were 26 malaria-related deaths. *Nopheles* mosquitos prefer to breed in clean water in and around houses. *Aedes* mosquitos prefer to breed in artificial collections of water, *Culex* mosquitoes prefer to breed in dirty water, and *Mansonia* prefers to breed in aquatic vegetations. A low literacy rate, especially in women, a lack of knowledge of the proper disposal of solid wastes, sewage and excreta, intermittent or inadequate water supply, the lack of drainage facilities, a high rate of unskilled workers and unemployment, an unhygienic lifestyle, slum and cluster dwellings, high population density, low per-capita income, and a poor knowledge regarding vector-borne diseases and mosquito breeding sites and their preventive measures are playing a pivotal role in the transmission and propagation of the vector-borne diseases in the area, *viz.* dengue, malaria, and chikungunya. In this respect controlling the vectors is a crucial step and thus insecticide-based inhibition methods with organophosphate based insecticides and insect growth regulators are commonly applied in controlling these pests of medical importance. Chemical insecticides provide many benefits to food production and human health and have proven very effective at increasing agriculture and forestry productivities. However, they also pose some hazards as contamination of water and food sources, poisoning of non-target fauna and flora, concentration in the food chain and selection of insect pest populations resistent to the chemical insecticides (Wojciech & Korsten 2002). It is well documented that chemical pesticides reduced natural-enemy populations and chemical applications can disrupt biological control and may cause outbreaks of secundary pests previously suppressed by natural enemies (Bartlett, 1964) and pest species develop pesticide resistance but natural enemies not (Johnson & Tabashnick, 1999). The use of synthetic organic pesticides has had serious economic, social and environmental ramifications. Economically, the rapidy increasing cost for development and production of petrochemically derived insecticides, together with the declining effectiveness due to widespread insect resistance. . New strategies were created to replace the use of chemical insecticides. They include Integrated Pest Management (IPM) that has guidelines. Guidelines of which are based on environmental planning, public awareness and biological control that control the mosquitoes more efficiently while preserving the environment from contamination. One such alternative is the use of microbial insecticides that contain microorganisms or their by-products. Microbial insecticides are especially valuable because their toxicity to non-target animals and humans is extremely low. Compared to other commonly used insecticides, they are safe for both, the pesticide user and consumers of treated crops. Microbial insecticides also are known as biological pathogens, and biological control agents. A number of biological control agents formulated with bacteria, fungi, virus, pheromones, and plant extracts have been in use mainly for the control of insects responsible for the destruction of forests and agriculture crops (McDonald & Linde, 2002). Bacteria received limited attention as microbial control agents of mosquitoes before the discovery of Bacillus thuringiensis (Berliner) serovariety israelensis de Barjac (Bti) and efficacious strains of Bacillus sphaericus Neide. Bacillus thuringrensis (Bt) is a well known and widely studied bacterium which is known for its use in pest management. Today it is the most successful commercial xenobiotic with its worldwide application when compared with the chemical pesticides; Bacillus thuringiensis has the advantages of being biologically degradable, selectively active on pests and less likely to cause resistance. Safety of Bacillus thuringiensis formulations for humans, beneficial animals and plants explains the replacement of chemical pesticides in many countries with these environmentally friendly pest control agents. Bacillus thuringiensis (Bt) was first identified in the beginning of the twentieth century in 1902 by Ishiwata, who reported the microorganism infecting Bombyx mori and causing damage in the silk industry of Japan (Beegle & Yamamoto, 1992). At that time, the author named it Bacillus sotto, which means soft and flabby, in reference to the appearance of the infected larvae. Subsequently, in the city of Thuringia (Germany), Berliner isolated a Gram-positive bacterium in the moth Ephestia kuehniella larvae, and, ignoring the nomenclature of Ishiwata, named it Bacillus thuringiensis. This name has persisted until now. Although Berliner proved the toxic effects of repeated ingestion, leading to the death of insects, the author did not consider the applicability of Bt to the control of moth larvae, since at that time little was known about the characteristics or potential of the Bt bacteria (Coˆte´, 2007). The microorganism was again isolated by Mattes in 1927 and was used in the following years for the biological control of Ostrinia nubilalis (Lepidoptera: Crambidae) (Beegle & Yamamoto, 1992). The first formulation of Bt biopesticide emerged in the following decade, and the product Sporeine was applied to control the various Lepidoptera species that adversely affected crops in France (Milner, 1994). Since then, the spectrum of susceptible organisms has expanded and now also includes the orders of Culicidae, Coleoptera, Simuliidae, Hymenoptera, Homoptera, Mallophaga and others (Sanchis, 2012). Subsequently, with the advent of molecular biology, crystallized genes of Bt toxins were inserted into cultivars, making these genetically modified plants resistant to various pests (Roh et al., 2007). Like all organisms, insect are susceptible to infection by pathogenic microorganisms, many of these infections agents have a narrow host range and therefore, do not cause uncontrolled destruction of beneficial insects and are not toxic to vertebrates. Bt is a Gram-positive, rod-shaped bacterium with the capacity to form resistant spores, classified in the family Bacillaceae. Bt cells, while sporulating, characteristically form a parasporal crystal composed of proteins which are active against a number of insect species from different orders such as Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Orthoptera, as well as other organisms such

as mites and nematodes. Bacillus thuringiensis is a major microorganism, which shows entamopathogenic activity (Glazer & Nikaido, 1995, Schnepf, et al. 1998) which forms parasporal crystals during the stationary phase of its growth cycle. Most Bacillus thuringiensis preparations available on the market contain spores with parasporal inclusion bodies composed of δ – endotoxins. Bacillus thuringiensis insecticides are divided into three groups, group one has been used for the control of lepidopterans. These groups of insecticides are formulated with Bacillus thuringiensis Subspecies. Kurstaki, group two contains the sandiego and tenebrionis strains of Bacillus thuringiensis and has been applied for the control of certain celopterans and their larvae. Group three contains the Israelensis strains of Bacillus thuringiensis which has been used to control black flies and mosquitoes. The larvicidal activity of Bt is due to the toxins found in the parasporal inclusions (also known as parasporal bodies and crystals) that are produced at the time of sporulation. Collectively referred to as d-endotoxins (delta-endotoxins) they comprise a diverse group of proteinaceous toxins, inclusion shapes and host ranges. The parasporal inclusions of Bti must be ingested in order to be active in susceptible species. The inclusions are solubilized in the alkaline midgut and cleaved to the larvicidal moieties by proteases. The lethal effect of Bti is due to the proteinaceous toxins binding to the surface membranes of the epithelial cells in the larval midgut and disrupting osmotic balance of the cells. The 3-domain structure of the Cry 4Ba toxin has recently been described by Boonserm et al. (2005). Although it is similar to that reported for the Cry 3A and Cry1Aa toxins, Boonserm et al. (2003, 2005) report some distinctions. The toxin components have 2 major functions related to larvicidal activity: receptor binding and membrane insertion. Domain 1 is believed to be the portion of the toxin that is inserted into the brush border membrane of midgut cells. This insertion results in the formation of channels and loss of osmotic balance and ultimately in the lysis of the cell (Schwartz and Laprade 2000, Boonserm et al. 2005). Domain 2 contains a receptor-binding region and is believed to determine insect specificity. Domain 3 also has a receptor-binding site and contributes to insect specificity. Within minutes of ingestion of a lethal dose of Bti toxins, there is noticeable swelling in the brush border of epithelial cells (blebbing). In most accounts, lytic vacuoles become apparent soon after and cells begin to lyse in as little as 30– 60 min depending on dose of toxin. Eventually the affected cells are sloughed with only the basal membrane of the midgut remaining. Death of the insect follows soon thereafter. Lahkim-Tsror et al. (1983) reported that mortality in some larvae started as early as 6 min after application of high concentrations of Bti. The mode of action and histopathology of Bti toxins in mosquito larvae and that of other Bt strains in other insect targets are similar (Lu¨thy and Wolfersberger 2000). Despite the potential of Bt to be exploited for a long time as a pest control, for the production of genetically modified plants, and to control the vector of human diseases, research on this microorganism is still incomplete. Every year, new approaches are found for the study of bacteria, and these pave the way for new applications (Bravo et al., 2007; Dash et al., 2013; Ernandes et al., 2012; Kroeger et al., 2013; Tan et al., 2010). This review focuses on recent discoveries involving the mechanism of action of Bt, the study of toxin resistance, the resistance management and an overview of the current studies on this bacteria and its applications, such as the production of chitinases and its use as a toxin for cancer cells.

The present study was donw to isolate the *B. thuringinesis* from the various soil samples and to further investigate the entomological and larvicidal activity of the B. thuringinesis against the larvae of the mosquito species responsible for causing the dengue and maleria.

2. MATERIAL AND METHOD

2.1. Collection of samples

The 6 soil samples were procured from the local regions of the Bangalore and Hyderabad. All the soil samples were collected from a depth of 7-10cm deep down the soil surface with the help of a sterile spatula. The samples were then immediately transferred into the aseptic plastic bags, which were labelled according to the location from where they are being collected. The soil samples were then brought to the microbiology lab and stored in the room temperature for further use.

2.2. Formulation and preparation of the culture media

There were two culture media used for the isolation of the desired bacterium in the present study. Those were 1) T3 media and 2) TCHA medium. The components of both the media were purchased from the HIMEDIA Laboratories Pvt.Ltd, Mumbai, India and Thermo Fisher Scientific. The media components were weighed accurately in a weighing balance according to the data given in the table 1 and table 2, further they were dissolved in distilled water taken in a conical flask and a cotton plug was plunged on the mouth of the conical flask. The flasks containing the media were then sent for the autoclave at 121ºC at 15 Lbs pressure maintained for 15-20 minutes. After autoclave, the flasks were removed carefully and cooled under running tap water for sometime to bring it to room temperature. The media was then poured into the sterile petriplates under the laminar air flow hood and allowed to solidify for the further usage.

Table 1: Composition of T3 medium (For 1L).

1.	Tryptone	3.0g
2.	Tryptose	2.0g
3.	Yeast extract	1.0g
4.	Magnesium chloride	0.005g
5.	Sodium dihydrogenphosphate	6.9g
6.	Di-sodium phosphate	8.9g
7.	Agar - Agar	20.0g
8.	Distilled water	1000ml

Table 2: Composition of TCHA medium (For 1L)

1.	Tryptone	5g
2.	Caesin hydrolysate	2g
3.	K_2HPO_4	12.5mM
4.	$MgSO_4$	12.5mM
5.	$MnSO_4$	0.05mM
6.	$ZnSO_4$	1.2mM
7.	$Fe_2(SO_4)_3$	1.2mM
8.	H_2SO_4	0.5%
9.	$CaCl_2$	25mM
10.	Agar - Agar	20.0g
11.	Distilled water	1000ml

2.3. Isolation of *Bacillus thuringinesis* from the samples

For the isolation of the B. Thuringinesis, acetate selection method described by Travers et al. (1987) was used. The complete isolation method was done in 2 phases. In the first phase, the pre treatment of the soil was done in order to remove the unwanted bacteria from the soil samples. During this, approximately 2 g of the soil sample was weighed and added to 20ml of sterile Luria – bertani broth taken in a conical flask. It was then incubated in a shaking incubator which was operated at 160rpm and the temperature was maintained at 37ºC for 24 hours. After the incubation, in the 2nd phase, a small amount of sample i.e 0.5 ml from the flask was pipetted out and transferred into a test tube under the aseptic conditions. The test tube containing the sample was then placed in a water bath maintained at 80ºC for the removal and killing of the other unwanted organisms and the non- sporing bacteria.

2.4. Culturing of the *Bacillus thuringinesis*

After the completion of the pre – treatment process, a loopful of the sample was streaked in the T3 media and TCHA media in separate petriplates. The plated were then incubated at 37ºC for 24 hours for the colony development.On the next day after the incubation, the plates were observed for the formation of the colonies resembling the appearance of B. Thuringinesis that were rough, white and spread out over the plate.

2.5. Purification of the obtained isolates

The colonies obtained on the plates after the incubation were observed, analyzed, selected and sub - cultured individually on the nutrient agar pates and blood agar to check the type of haemolysis and incubated again at 37ºC for 24 hours. Further the characterization of the isolates was performed with the staining procedues and series of biochemical reactions.

2.6. Characterization and identification of the isolates

The isolates obtained were then intified by conventional biochemical procedures according to Claus and Berkeley (1986) and Barrow and Feltham (1993) also, Gram staining and spore staining procedures were performed for each isolates.

2.6.1. Simple staining

The colonies were picked using a sterile inoculation loop and a smear was perpared on a clean greese free slide. The smear was then heat fixed and flooded with methylene blue and kept for 1 minute. The slide was then washed gently under the running tap water to remove the excess stain and air dried. Following this, the slide was 1st observed under 10X in a compound microscope then focused under the 100X using oil immersion.

2.6.2 Gram staining

A smear of colonies isolated after the identification was made on clean glass slides using a sterile wire loop. They were air dried and fixed. The smears were flooded with crystal violet for about 1 minute and were washed with tap water. They gram's iodine was added for 20 seconds and then washed with tap water. They were decolourized with ethanol (95%) and washed off with tap water. The fixed smears were counterstained with safranin and allowed for 1 minute and then washed off with tap water and allow to air dry. Immersion oil was added to the stained slides and viewed under a microscope using 100X objective for the morphological characteristics of the isolates.

2.6.3. Coomassie blue staining

The coomassie blue staining was done for the observation of the parasporal inclusion bodies which is a characteristic feature of the *Bacillus thuringinesis*. This staining method increases the resolution where by the crystal bodies can be easily viewed. All the isolates were inoculated individually into a sterilized 100ml conical flask containing the 50ml of nutrient broth. The flasks were then incubated in an orbital shaker at 250rpm for 50-60 hours for the sporulation to occur. After incubation, the samples were smeared onto a glass slide followed by heat fixation. The slides were then flooded with 0.133% coomassie blue stain solution and left for 1-2 minutes. The slides were then washed under running tap water gently to remove the excess stain and air dried. The slides were then observed under 100X oil immersion for the presence of the parasporal inculsion bodies.

2.6.4. Biochemical tests

1. Indole test: This test is used to determine the ability of microorganisms to degrade the amino acid tryptophan. Using sterile techniques the organism was innoclated into tryptophan booth. This was incubated at 37 ºC for 24-48 hours. After incubation 0.2 ml of kovac's reagent was added and shaken to observe the result.

2. Methyl red test: This test is to determine the capacity of some organisms to ferment carbohydrates with the production of acidic end products depending upon the specific enzymatic pathway as a product of glucose fermentation. MR/VP broth were prepared and sterilized. Sample was inoculated into the MR/VP broth and incubated at 37 ºC for 24 hours. After incubation, 5to 6 drops of methyl red solution was added into the same culture tubes.

3. Voges Proskauer test (V.P.test): This is to determine the capacity of some microorganism to ferment carbohydrates with the production of non-acidic or neutral end products such as methyl carbinol as a product of glucose fermentation. MR/VP broth were prepared and sterilized, sample was inoculated into the MR/VP broth and incubated at 37 ºC for 24 hours.

4. Citrate Utilisation test: This test is used to differentiate among enteric organism on the basis of their ability to ferment citrate as sole carbon source. Using sterile technique, the organism was inoculated in Simmons citrate agar slant. The tubes were incubated at 37 ºC for 24 hours.

5. Sugar utilization test: An inoculum from a pure culture of all the isolates was transferred aspectically to a sterile tube of glucose broth, lactose broth, mannitol broth, sucrose broth with phenol red to see the frementation of glucose, lactose, mannitol, sucrose respectively. The inoculated tubes were incubated at 35-37 ºC for 24 hours. A positive test consists of a colour change from red to yellow indicating a ph change to acidic.

6. Catalase test: This test is used to determine the ability of an organism to produce the enzyme catalase. 2-3 ml of H_2O_2 solution was poured into the test tube containing growth of microorganisms. Immediate liberation of air bubbles indicate that organism was catalase positive and no liberation of air bubbles indicates that the organisms was catalyse negative.

7. Starch hydrolysis test: Starch agar plates were prepared. A single line inoculation with test culture was made. The plate was incubated at 37 degree Celsius 24-48 hours. The formation of clear zone around the colonies upon additon of the gram's iodine indicates starch hydrolysis.

2.7. Bioassay

The *Bacillus thuringiensis* isolates selected were tested against larvae of mosquito. 10 larvae were transferred into each disposable cup with 30ml sterile distilled water. The stock suspension of cultures of Bacillus thuringiensis from broth in the conical flask was diluted to 10^{-1}, 10^{-2} and 10^{-3} in sterile water and 2ml of each dilution was added to each of the cups. The cups were kept at 30^{O}C. Larval mortality was observed after 6 hours and 12 hours respectively.

3. RESULT

3.1. Sample collection

The soil samples collected showed the presence of B. thuringiensis.The isolates which showed the morphology similar to that of the desired bacteria was given a unique primary code to identify them furthter. Among all the isolates there were four colonies which showed the maximum similar characteristics when compared to the morphology of B. thuringiensis. Those were named as Btr1, BtL2, BtC3, and BtK4. The result of the 1st phase of pre – treatment process showed the formation of the turbid layer on the top of the conical flask after completion of incubation period (Fig 1).

Fig 1: Formation of the turbid layer after 1st phase.

3.2. Culturing and purification of the isolates

The results after the 2nd phase of the pre – treatment process resulted in the formation of the colonies which were creamy to whitish in colour, rough, opaque and spreaded over the plate (Fig 2). When these isolated when subjected to purification, it resulted in the formation of the colonies similar to the *B. thuringiensis* (Fig 3). The streaking of these isolates on the blood agar showed the formation of beta hemolysis around the colonies developed after the incubation (Fig 4).

Fig 2: Visualization of colonies similar to the B. Thuringiensis.

Fig 3: Purification of the isolated Colonies.

Fig 4: Beta haemolysis was observed by the isolates after incubation period.

3.3. Characterization and identification

The simple staining showed the presence of the rod shaped bacteria stained with methylene blue and found to be appeared in blue colour. The gram staining resulted in the visualization of the gram positive rods with spore formers under the 100X microscopic view (Fig 5). The result of the coomassie blue staining done for all the 4 suspected isolates showed the stained parasporal bodies in the Bacillus cells (Fig 6). These isolates were then subjected to biochemical characterization to confirm the isolated being Bt.

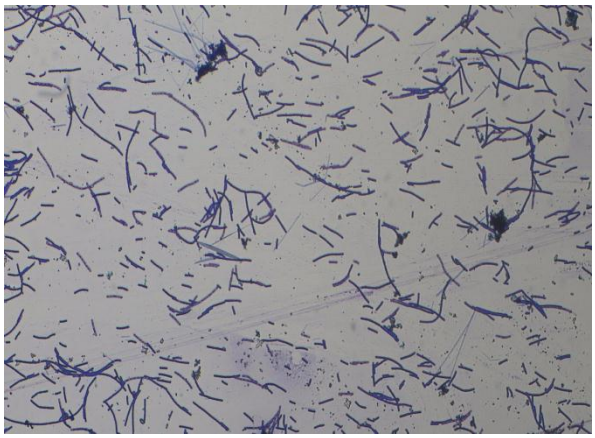

Fig 5: Gram positive rods under 100X.

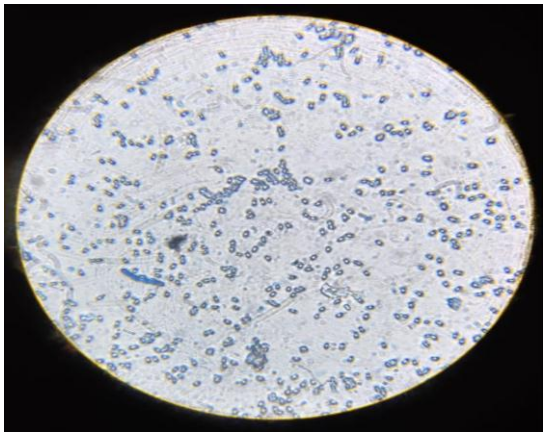

Fig 6: Coomassie blue staining under 100X.

All the isolates were tested for biochemical tests and results of these tests are listed below in table 3 and 4.

Table 3: Biochemcial test results for the 4 isolates.

Biochemical tests	BtR1	BtL2	BtC3	BtK4
Indole test	+	+	+	-
MR test	-	+	+	+
VP test	+	+	+	+
Citrate utilization test	+	+	+	+
Catalase test	+	+	+	+
Starch hydrolysis test	+	+	+	+

Table 4: Sugar utilization results for the 4 isolates.

Sugars	BtR1	BtL2	BtC3	BtK4
Glucose	+	+	+	+
Lactose	-	-	-	-
Mannitol	-	-	+	+
Sucrose	+	+	+	+

3.4. Bioassay results

Table 5-8 shows the results for the bioassay performed on the larvae of the mosquito using the bacterial suspensions of the 4 isolates.

Table 5: Bioassay results for BtR1.

Dilution	Time of incubation					
	0hr		6hr		12hr	
	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae
10^{-1}	10	0	1	9	0	10
10^{-2}	10	0	1	9	0	10
10^{-3}	10	0	2	8	0	10

Table 6: Bioassay results for BtL2.

Dilution	Time of incubation					
	0hr		6hr		12hr	
	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae
10^{-1}	10	0	1	9	0	10
10^{-2}	10	0	2	8	0	10
10^{-3}	10	0	2	8	0	10

Table 7: Bioassay result for BtC3.

Dilution	Time of incubation					
	0hr		6hr		12hr	
	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae
10^{-1}	10	0	1	9	0	10
10^{-2}	10	0	1	9	0	10
10^{-3}	10	0	1	9	0	10

Table 8: Bioassay result for BtK4.

Dilution	Time of incubation					
	0hr		6hr		12hr	
	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae	No of live larvae	No of dead larvae
10^{-1}	10	0	1	9	0	10
10^{-2}	10	0	2	8	0	10
10^{-3}	10	0	2	8	0	10

4. DISCUSSION

Bacillus thuringiensisis a ubiquitous gram positive and spore - forming bacterium that produces insecticidal proteins during sporulation. Mosquitoes are a great nuisance and they pose a serious threat to human health in society. Many chemical insecticides have been produced for the control of mosquitoes in the past years, some of which have been very effective while others have done little or no good at all. In this regard, biological pesticides based on Bacillus thuringiensis are becoming increasingly important in pest management programs, accounting for 80-90% of all biological pest control agents used worldwide. Their compatibility with other biological control agents will enable a more sustainable approach to mosquito control than would be possible with conventional chemical larvicides. The use of these bacteria in control programs has often seen great success. Specific applications have included control of malaria vectors, blackflies as a part of the highly successful onchocerciasis control program and against nuisance mosquitoes in developed countries.

5. CONCLUSION

Bacillus thuringiensis has been used for a long time as a bioplesticide. However it is still necessary to search for more toxins to control insect orders which have the ability to develop resistance against such pesticide and to also provide alternative for chemical insecticide. Mosquito – toxin stains of Bacillus thuringiensis have been reported from different continent and their source include soil, plant, insect, animal feces and water. The organism and it product can be further studied to search for novel compounds that can be used in the control of mosquito-borne diseases such as malaria. The results obtained in this study clearly showed the efficiency of the Bacillus thuringiensis in controlling mosquito larvae. A better understanding of the mode of action for individual proteins against their targets, as well as the molecular interactions occurring between them will help to develop a greater range of tools in the fight against multiple larvae and insects, and help us overcome the appearance of resistance in the future.

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7. DATA AVAILABILITY

The article contains the appropriate and proper data obtained during the experiment which supports the result, discussion and conclusion of the research article.

8. CONSENT FOR PUBLICATION

Not applicable.

9. CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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