Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

ISSN 2320-7078
JEZS 2014; 2 (3): 121-125

Received: 23-05-2014
Accepted: 10-06-2014

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Evaluation of *Gambusia affinis* and *Bacillus thuringiensis var. israelensis* as *Culex quinquefasciatus* Control Agents

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ABSTRACT
Objectives: Biological control strategies for the filarial vector *Culex quinquefasciatus* using *Bacillus thuringiensis israelensis (Bti)* and *Gambusia affinis*. **Methods:** The potential of *Bti* as a bio-control agent was investigated under laboratory conditions. *Bti* strain H-14 when assayed for its residual toxicity against *Culex quinquefasciatus* revealed that the susceptibility status varies according to the developmental stage of the vector. Various Sizes of *Gambusia affinis* were taken to evaluate the consumption of *Culex quinquefasciatus.* **Results:** *Culex quinquefasciatus* larvae appeared highly sensitive for the higher concentrations (ppm) of *Bti*; various sizes of *Gambusia affinis* consumed *Culex quinquefasciatus* as potential larvicidal agent. **Conclusion:** The present investigation clearly exhibits that both *Bacillus thuringiensis israelensis* and *Gambusia affinis* serve as a potential larvicidal agent.

Keywords: *Bti, Gambusia affinis*, Mosquito Vector, *Cx. quinquefasciatus*, Larvicidal Control.

1. Introduction
Biological pest control involves the use of another living organism to kill a pest. No chemicals are needed, there is no environmental contamination with pesticides and the pests don't become resistant to the control method. Many different chemicals are used to kill pests. These pesticides often work well, but since they're designed to kill living things they may cause serious problems in humans or pets. Pesticides contaminate the environment. They sometimes harm other organisms in addition to their target pest.

Biological pest control has some distinct advantages compared to chemical pest control. There are no toxic chemicals to store. There are no pesticides to give off dangerous vapors, accumulate in the soil or collect in water. Clearly there is a need for alternative methods that are more effective, less expensive and eco-friendly [1].

Gravid *Culex quinquefasciatus* females fly during the night to nutrient-rich standing water where they will lay their eggs [2].

The larvae feed on biotic material in the water and require between five to eight days completing their development at 30 °C. The larvae progress through four larval instars, and towards the end of the fourth instar they stop eating and molt to the pupal stage. Following 36 hours at 27 °C the adults emerge from the pupal stage. The time of development under natural conditions for all stages is variable and dependant on temperature [3].

The larval head is short and stout becoming darker toward the base. The mouth brushes have long yellow filaments that are used for filtering organic materials. The abdomen consists of eight segments, the siphon, and the saddle. Each segment has a unique setae pattern. The saddle is barrel shaped and located on the ventral side of the abdomen with four long anal papillae protruding from the posterior end [4]. The siphon is on the dorsal side of the abdomen, and in *Culex quinquefasciatus* the siphon is four times longer than it is wide with multiple setae tufts [5].

Culex quinquefasciatus is a vector of Lymphatic filariasis, affecting 120 million people worldwide, and approximately 400 million people are at risk of contracting filariasis world wide, resulting into the annual economic loss of 1.5 billion dollars. To combat this disease, World Health Assembly has passed a resolution to eliminate Lymphatic filariasis by the year 2020, for which Global Programme for Elimination of Lymphatic Filariasis began during 1999 in all the Lymphatic filariasis endemic countries [6].

The National Filariasis Control Programme which has been in operation, since 1955, has estimated in 1982 that over 42% of the Indian population was exposed to infection with filarial parasites [7]. The diseases are endemic all over India except in few States. Biological control of mosquitoes is also a necessary part of a complete mosquito control. Biological control mechanism is using *Bti* & *Gambusia affinis* are that specifically target mosquito larvae.

Bacillus thuringiensis israelensis (Bti) The active ingredient in two of the most popular and environmentally-sensitive products is a Bacillus bacterium. The bacterial cells of Bacillus thuringiensis israelensis (Bti) produce a spore and a crystalline protein toxin (endotoxin) as they develop. When the mosquito larvae ingest the spores, the endotoxin is activated by alkaline conditions and enzyme activity in the larval gut. The activated endotoxin attaches to specific receptor sites resulting in larvae paralysis and destruction of the gut wall. Larvae usually die quickly from the activity of the toxin or stop feeding and die within 2 or 3 days from the effects of septicaemia (blood poisoning). Because the majority of aquatic invertebrates do not have alkaline guts, Bti has no effect on the majority of potential non-target organisms, although it might affect some other dipteran larvae [8].

Gambusia affinis (Baird and Girard) have been used for more than 100 years. *Gambusia* was introduced to more than 60 countries in last century and remains as preferred larvivorous fishes on mosquitoes [9, 10]. However, many ichthyologists and ecologist concerned the potential negative impact of Gambusia on non-targets and natural ecosystems [11, 12]. Mosquito fish easy to culture and capable of rapidly producing large populations in laboratory colony or field aquatic habitats. The mosquito fish were used to evaluate non-target impact for mosquitocides [13].

Alternative control technologies for *Culex quinquefasciatus* include the use of *Bacillus* and *Gambusia affinis.* Gambusia is surface feeding fish for bio control of mosquito was recognized as early as and the top minnow gambusia and guppy, poecilia have been extensively used for all over the world.

The goal of this study was to quantify the potential role of *Gambusia affinis* as predators and *Bti* destroy of larval mosquitoes through a series of laboratory feeding trails. The objective of this study was to 1. Quantify the maximum number of mosquito larvae a five different size of *Gambusia affinis* could consume over a 24 hour period. 2. To estimate the larvicidal efficacy of microbial agents viz. *Bti* against the larvae of culex in the laboratory conditions. 3. Compare mosquito larvae eradication by *Bti* and *Gambusia affinis*.

2. Materials and Methods

2.1 Collection of *Culex quinquefasciatus*

Culex quinquefasciatus immature stages collected from various places of Vellore, Tamilnadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified and species confirmed before rearing. Cyclic generations of *Culex quinquefasciatus* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of 27±2 ºC and a relative humidity of 70- 80 percent were maintained in the insectary. The adult mosquitoes were fed on ten percent glucose solution. The adult female mosquitoes were blood fed with the laboratory rearing albino mice. Ovitraps were placed inside the cages for eggs lying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with dog biscuits and yeast in 3:1 ratio. The 1st and 2nd instar larvae becoming 3rd and 4th instar larvae were collected and transferred to experimental trays. Ten 3rd and 4th instar larvae were exposed to separate experimental trays.

2.2 Effect of *Bti* on the immature stages:

In general, 3rd and 4th instar larvae of *Culex quinquefasciatus* served as test insects. Distilled water was used for preparing the bacterial dilution (ppm). Laboratory temperature ranged between 27–30 °C.

2.3 Concentrations of *Bti*:

Six concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ppm/100 ml distilled water), were tested against 3rd and 4th instar larvae.

2.4 Test insects:

Larvae of 3rd and 4th instar larvae of *Culex quinquefasciatus* were used in this series of tests. Hundred larvae used for each concentration were divided into four replicates.

2.5 Mortality reading:

Mortality data were recorded after 24 and 48 hours of application by counting both dead and alive larvae.

2.6 Collection of *Gambusia affinis*

Gambusia affinis were collected from chetpet fish farm, with the help of fisherman using net and kept in a plastic drum. The lake water was used for transportation. Fishes were transported quickly to the laboratory. The fishes were acclimatized slowly, in tap water and the experiments were conducted for 48 hours in two replicates. Individual experimental and control tanks were used in the laboratory *Gambusia affinis* were measured and weighed before and after of the experiment. A control was also maintained separately throughout the experiment conducted in individual fish of five different size groups (3.0, 3.3, 3.6, 3.9, 4.2, and 4.5) against the mosquito larvae of 3rd and 4th instar stage of *Culex quinquefasciatus*. The larval feeding rate was recorded.

3. Results and Discussion:

Table 1 indicates that, the effect of *Bti*, on the survival of the 3rd and 4th instar larvae of mosquito. It is clear that the highly percentage of mortality (100%) was recorded with 30 ppm, but the concentration of 5 ppm caused low mortality even after 48 hours. This result may attribute the 4th instar larvae metamorphosis to the pupae and it could not become as able to resist the toxicity of *Bti*.

The larvicide had caused significant mortality of larvae during the experiment. Reduction of larval population was pronounced at each experiment, except the control experiment.

The mortality rate of 100 percent was observed at 24 hours. In Chart 1 the 3rd and 4th instar larvae of *Culex quinquefasciatus* L3 larvae population shows lower mortality then L4 larvae. *Culex quinquefasciatus* 3rd and 4th instar larvae had virtually the same sensitivity to *Bti*.

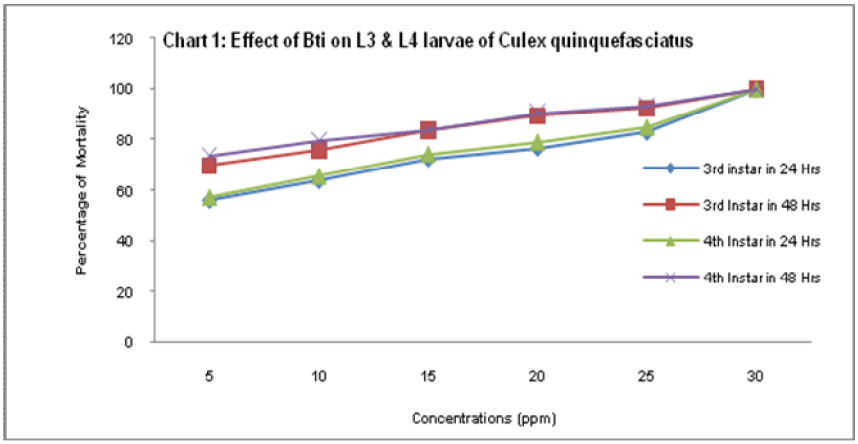
3.1 Effect of *Bti* on *Cx. quinquefasciatus*

Bti was found to be relatively specific to Diptera and was quickly shown to be toxic to a range of mosquito and black fly species. Therefore, it was considered to have commercial potential as a control agent of nuisance Diptera around the world. Rapid development of *Bti* strains occurred in the early 1980 and several products were developed. The need for a more environmentally benign mosquito control agent and rising incidence of resistance to chemical pesticides provided a platform for rapid *Bti* development.

Classification of subspecies or varieties based on serotyping using H-serovars resulted in identification of almost 60 varieties [14]. Serotype does not necessarily relate to the presence of δ-endotoxins, which determine host specificity, as flagellar genes are carried on the chromosome, while toxin genes are usually encoded on plasmids. A number of DNA based methods have been developed for characterization: specific primed polymerase chain reaction (PCR); Random amplified polymorphic DNA (RAPD), DNA: DNA colony hybridization and rRNA-based probe [15]. These methods can distinguish individual strains and isolates, allowing the tracking of the environmental fate of strains used for pest control. Such methods can also be used to identify the presence/absence of specific endotoxin genes, which mean it is possible to establish whether a particular strain has lost or acquired specific δ- endotoxin genes in the environment. The products contain the spores and parasporal crystals of *Bti* H-14 serotype which must be ingested by the larval stage of the mosquito to cause mortality. Following ingestion, the parasporal crystals are solubilized in the alkaline larval midgut, followed by proteolytic activation of the soluble insecticidal crystal proteins. The toxin binds to a receptor on the midgut cell wall resulting in pore formation in the cell, which leads to death of the larva. *Bacillus thuringiensis* var. *israelensis* treated mosquito larvae generally cease feeding within 1 hour, show reduced activity by two hours, extreme sluggishness by four hours and general paralysis by six hours after ingestion [16, 17, 18].

Table 1: Mean±SD of effect of *Bti* on susceptibility of the 3rd & 4th instar larvae of *Cx. Quinquefasciatus*

Concentration (ppm)	Mortality % of 3rd Instar larvae		Mortality % of 3rd Instar larvae	
	After 24 Hrs	After 48 Hrs	After 24 Hrs	After 48 Hrs
5	56.25±2.99	70.00±2.83	57.50±2.08	73.75±1.71
10	64.00±2.16	76.00±3.37	65.75±2.22	79.50±2.08
15	72.50±1.29	83.75±3.50	74.50±2.08	84.00±2.94
20	76.75±1.50	89.50±3.42	79.25±2.22	90.75±2.22
25	83.00±2.58	92.50±1.91	85.00±2.16	93.50±2.38
30	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00



The Value of some surface feeding fish for bio-control of mosquito was recognized as early as 1907 [19] and the top minnow *Gambusia* and *Guppy, Poecilia* have been extensively used all over the world [20]. Have showed that in some places in Bangkok, *Poecilia reticulate* developed satisfactorily in polluted breeding places [21]. Similar studies in other species of fish have correlated the predatory rate and the values as *Gambusia affinis* – 17.4 larva/individual, *Poecilia reticulate* 19.8 larva/individual, and *Culex lutzia* – 3.8 larva/individual [22]. *Mesogomphus lineatus* 23.2 larva/individual, *Orthetrum Sabina* 22.2 larva/individual, *Anisops sp*. 6.8 larva/individual and *Notonecta undulate* 55.5 *Aedes* larva/individual [23, 24, 25].

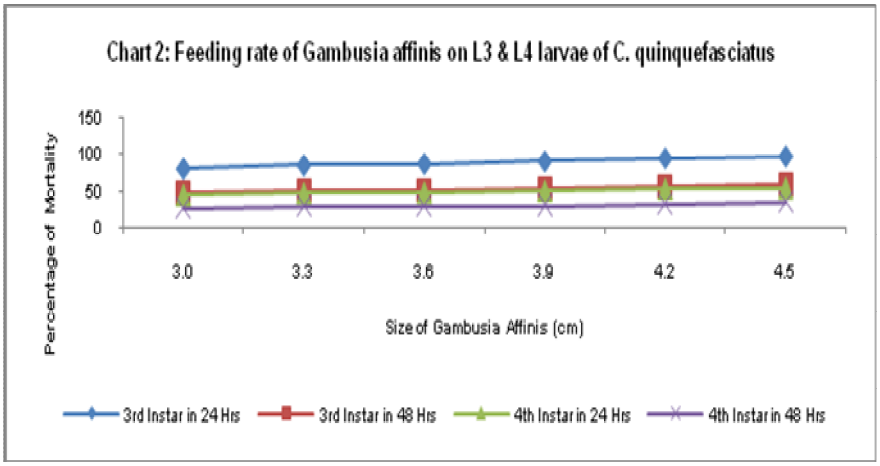
Behavior in fish is associated with varied and numerous 'visual' biologically significant stimuli [26]. In fishes which are active visual feeders, the feeding activities and prey predator relations are known to be markedly influenced by the

illuminations [27, 28]. The fish *Gambusia affinis* has been extensively used as an effective predator of mosquito larvae and it is observed to be an active visual feeder [29, 30, 31]. Illumination might be the reason of high consumption of mosquito larvae in the present study. The fish consumed more number of larvae during the day time feeding when compared to night, where the feeding rate was less.

The occurrence and success of aquatic predators is pronounced to be largely dependent on physicochemical factors operative in natural waters [32]. The physicochemical complex of fresh water bodies where mosquitoes breed are known to fluctuate from time to time [33, 34], observed that the feeding behavior of *Gambusia affinis* was a direct response to the water temperature. They found that the feeding rate was decreased at 20 °C and at 30 °C, the fish consumed more larvae. The conservative parameters (pH and water temperature) analyzed along with the predatory rate of *Gambusia affinis* did not fluctuate very much in the present study and hence did not show any appreciable effect on larval intake by *Gambusia affinis*.

Table 3: Mean±SD of effect of *G. affinis* on susceptibility of the 3rd & 4th instar larvae of *Cx. quinquefasciatus*

Size (Cm)	Feeding rate of 3rd Instar Larvae		Feeding rate of 4th Instar Larvae	
	After 24 Hrs	After 48 Hrs	After 24 Hrs	After 48 Hrs
3.0	79.00±1.41	47.50±0.71	43.50±0.71	26.00±0.00
3.3	83.50±2.12	50.00±1.41	46.00±1.41	27.50±0.71
3.6	85.50±2.12	51.00±1.41	47.00±1.41	28.50±0.71
3.9	89.00±1.41	53.50±0.71	49.00±1.41	29.50±0.71
4.2	93.00±1.41	55.50±0.71	51.50±0.71	31.00±0.00
4.5	95.50±0.71	57.50±0.71	52.50±0.71	33.00±1.41



4. Conclusion

The Mosquito control process requires alternative simple and sustainable methods of control. Biological control has many advantages as compared to chemicals. Because it can be effective and safe to human and non-target populations. It has low cost of production and lower risk of resistance development. The *Bti* and *Gambusia affinis* are excellent agents for use as biological control of mosquito larvae.

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