Tropical Biomedicine 29(1): 81–91 (2012)

A novel cost-effective medium for the production of *Bacillus thuringiensis* subsp. *israelensis* for mosquito control

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Received 27 April 2011; received in revised form 18 August 2011; accepted 28 September 2011

Abstract. *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) has been used for mosquito-control programmes the world-wide. Indeed, the large-scale production of *Bti* for mosquito control is very expensive due to the high cost of its culture. In the present study, we attempted to widen the scope in developing cost-effective culture medium for *Bti* production, based on the raw materials available on the biosphere, including coconut cake powder, CCP (*Cocos nucifera*), neem cake powder, NCP (*Azadirachta indica*) and groundnut cake powder, GCP (*Arachis hypogea*). Among these raw materials, the biomass production of *Bti*, sporulation and toxin synthesizing from 'CCP' in combination with mineral salt ($MnCl_2$) was comfortably satisfactory. Bioassays with mosquito species (*Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*) and field trials were also satisfactory. The present investigation suggests that coconut cake-based culture medium can be used as an alternative for industrial production of *Bti* in mosquito-control programme. Therefore, the study is very important from the point of effective production of *Bti* from cost-effective culture medium for the control of mosquito vectors.

INTRODUCTION

Mosquitoes are a source of great nuisance to human beings and pose a threat to public health as vectors of diseases like malaria, filariasis, dengue, Japanese encephalitis, West Nile fever. Annually, 500 million people are estimated to be affected by malaria, transmitted by the *Anopheles* spp., with over 2 million deaths (Suh *et al.*, 2004). The total global population at risk of lymphatic filariasis transmitted primarily by *Culex* spp., and to some extent by *Anopheles* spp. is estimated to be 1,307 million people (WHO, 2006). About 50 million people are infected every year by dengue viruses transmitted by the *Aedes* spp., with about 24,000 deaths (Kroger *et al.*, 2006). Hence, vector control has a direct impact on the reduction of mosquito-borne diseases. Several strategies have been adopted to control these dipteran pests and to reduce vector-borne diseases. Synthetic insecticides have been effectively used during the past several decades for mosquito-control operations. But the chemical approach has several demerits, such as the development of insecticide resistance, environmental pollution, bio-amplification of contamination of food chain, and harmful effects to beneficial insects. Hence, there has been an increased interest, in recent years, in the use of biological agents for mosquito control.

The discovery of bacillus like *Bacillus thuringiensis* subsp. *israelensis* (*Bti*), highly toxic to dipteran larvae, opened up the possibility of the use of this biolarvicide in mosquito eradication programmes (deBarjac & Larget-Thiery, 1984; Delecluse *et al.*, 1993; Charles *et al.*, 1997; Poopathi *et al.*, 2003; Becker *et al.*, 2010). Mosquito pathogenic bacilli have some advantages over

conventional insecticides in mosquito-control operations, because they have a narrower host spectrum and thus are safer to non-target organisms (including humans) and are more environment friendly. *Bti* synthesizes intracellular crystal inclusions by sporulation that contains multiple protein components of 134, 125, 67, and 27 kDa (Hofte & Whitely, 1989; Federici *et al.*,1990; Wirth *et al.*, 1998). These proteins have been cloned individually and are toxic to mosquito larvae and other blackflies (Delecluse *et al.*, 1993).

Though the high efficacy and specificity of *Bti* are useful in controlling mosquitoes, the cost to grow and produce *Bti*, through highly refined laboratory bacterial culture medium, is high. The cost of *Bti* production depends on many factors; however, the raw material cost is one of the most important criteria, which may comprise >70% of the overall production cost (Ejiofor, 1991). Therefore, the selection of growth medium or raw material is critical for commercial production of these biopesticides. In order to encourage the commercial production of biopesticides, utilization of less expensive raw material is advisable (Mummigatti & Raghunathan, 1990). Several raw materials (industrial and agricultural by-products) have been tested successfully in mosquito-control programme, as an alternative culture media, for the production of *Bacillus sphaericus* and *Bti* (Saalma *et al.*, 1983; Obeta & Okafor, 1984; Kumar *et al.*, 2000; Poopathi *et al.*, 2002a).

In the present study, we attempted to develop a cost-effective medium, based on inexpensive, locally available raw materials including CCP (*Cocos nucifera*), NCP (*Azadirachta indica*) and GCP (*Arachis hypogea*). However, we found that, except CCP, all the other raw materials were not suitable for satisfactory *Bti* production. A combination of this substrate with mineral salt (COC+$MnCl_2$) also gave appreciable results in the production of bacterial toxins to control mosquito larvae. So, in the present study, we suggest that this medium can be used as a prospective alternative medium in mosquito-control programmes involving bacterial biopesticides. Therefore, the object of the present study is to (i) facilitate the combination of COC+$MnCl_2$ as an alternative bacterial culture medium instead of the existing conventional medium (Luria Bertani, LB) for the production of biopesticide (*Bti*); (2) the toxic effect of the bacterial endotoxins produced in cultures from the media (CCP, COC+$MnCl_2$ and LB) for the control of mosquito vectors (*Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*) in the laboratory as well as in the field and (3) assess the cost-effectiveness of raw materials involved in preparing the culture medium.

MATERIALS AND METHODS

Bacterial strains

Bti, H-14 (IPS-82) available in the laboratory (stored at –80°C, Ultra Low Freezer, Thermo Electron Corporation, USA) was used in the present study. The bacterial strain was earlier received by gratis from Bacteries Entomopathogenes, Institute Pasteur, Paris, France (courtesy: Dr. Jean Francois Charles).

Conventional culture medium

Luria Bertani, LB (standard conventional culture medium) was used as reference: peptone 20, yeast extract 10 and NaCl 20 (g l^{-1}), pH 7.8.

Agro-industrial by-products

The agro-industrial by-products, such as *C. nucifera*, *A. indica* and *A. hypogea*, commonly known as CCP, NCP and GCP, respectively, were used in the present study. A known quantity of the above cake powders (50g l^{-1}) were boiled separately in tap water for 30 min. After cooling, the cake powders were filtered and the pH of the filtrate was adjusted (pH 7.8). The extract from each sample, i.e. CCP, NCP and GCP (5%), and its combinations, i.e. CCP+NCP, CCP+GCP and NCP+GCP (1:1), were dispensed separately into Erlenmeyer flasks (vol. 2 l) for culturing *Bti*. A similar combination from the extracts (COC/NOC/GOC) was made with mineral salts ($MnCl_2$, $MgCl_2$, $CaCl_2$, NaCl, KCl, and $Na_2HPO_4.H_2O$) also (5:1). The control (medium without *Bti* inoculation) and

reference (LB) media were also kept. From the above test culture media, the medium which showed maximum *Bti* biomass production was selected for further studies. The culture media were autoclaved (120°C/ 20 lb/in^2/20 min).

Bacterial inoculation

Bti pre-culture (50µl) was inoculated into all culture media and was allowed to grow under constant agitation on a rotary shaker (200 rpm at 30°C for 72 h). Sample from each culture medium was drawn (vol. 2.5 ml) every 6 h, up to 72 h of bacterial growth. The pH and culture density (turbidity at 650 nm) were measured using digital pH meter (Genei, India) and SP-75 UV-VIS spectrophotometer (Sanyo, UK). The bacterial stages (vegetative to sporulative stage) were also examined by LEICA DM 1000 LED microscope (made in Germany).

Bacterial toxin separation

Bti spores/crystals were purified according to Charles *et al.* (1997). The spore/crystals were harvested by centrifugation (10,000 x g/30 min/4°C) using SORVALL Evolution RC super speed centrifuge (Kendro, USA), and the culture supernatants were discarded. The biomass of pellets (cell mass) containing spore/crystal toxins was quantified before washing (0.1 M NaCl and sterile water). The purified toxins were treated finally with protease inhibitor (phenyl methyl sulphonyl fluoride, 1 mM, Sigma) and resuspended in sterile distilled water. The spores/crystals were sonicated (30% scale output, 25% duty cycle for 4 min), the crystals were separated by the discontinuous sucrose gradient (72 and 79% mass/vol.) method (Payne & Davidson 1984) and finally the crystals were solubilized (50mM NaOH for 90 min at 30°C). The solubilized crystals were dialyzed after neutralization against 20 mM Na_2HPO_4/ NaH_2PO_4, pH 8, and the protein content was determined (Lowry *et al.*, 1951). The crystal protein containing mosquitocidal toxins (*Bti*: 134, 125, 67 and 25 kDa) was studied qualitatively using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) (Lammeli, 1970).

Laboratory bioassay

Culex quinquefasciatus, *An. stephensi* and *Ae. aegypti*, indented from the division of "Mosquito Rearing and Colonization," Vector Control Research Centre, Pondicherry, India, were used for the present study. Bioassays for *Bti* were carried out as per WHO (1982). A stock solution was prepared from *Bti* (100 mg l^{-1}), and serial dilutions were made (0.001 to 0.05 mg l^{-1}). Bioassays were conducted in disposable wax-coated paper cups, containing 300 ml of water with suitable dosage. Twenty five early third instar larvae from respective mosquito species were exposed into the paper cups separately. Food supplement (dog biscuit and yeast mixture, 2:1) were not provided for larvae under *Bti* treatment as per WHO (1982). Bioassays were conducted at room temperature (28–30°C) and the larval mortality was monitored (24 h). Moribund larvae (if any) in the replicates were counted as dead. Control mortality (if any) was corrected (Abbott, 1925).

Mosquito control in the field

Bti produced from new and conventional culture media were field tested against the filarial vector (*Cx. quinquefasciatus*) in an urban area at Pondicherry. Fifteen cesspits with high larval density were selected. Bacterial spores and crystals produced from new and conventional culture media were sprayed in the respective cesspits with hand-compression sprayer (vol 2.5 l) at the rate of 0.25 g/m^2 as per the recommendations (Arunachalam *et al.*, 1991; Poopathi *et al.*, 2003). Pre- and post-treatment counts of immatures of *Cx. quinquefasciatus* in all habitats were done by taking three dips per cesspit using a standard 350 ml dipper, and the data were recorded. Three replicates per treatment (experimental and control) were placed for the homogeneous observation of mosquito larvae. The larval samples were brought to the laboratory for identification (Barraud, 1934). Post-treatment counts of larvae were made on alternate days until immature population reached approximately pre-treatment levels.

The percentage reduction (%R) of immatures was calculated by the Mulla's formula (Mulla *et al.*, 1971):

$$(\%R) = 100 - \frac{(C_1 \times T_2)}{(T_1 \times C_2)} \times 100$$

where C_1 is the number of larvae in control cesspits before treatment, C_2 is the number of larvae in control cesspits after treatment, T_1 is the number of larvae in treated cesspits before treatment and T_2 is the number of larvae in treated cesspits after treatment.

Statistical analysis

Data on the production of *Bti* from different culture media were subjected to Student's *t*-test to analyze the significance of difference ($P \leq 0.05$). The LC_{50} and LC_{90} values were calculated by regression analysis using SPSS software package ASSAY (Courtesy: Dr. C.F. Curtis, London School of Tropical Medicine and Hygiene, UK). Data on field trials were subjected to analysis of variance (ANOVA) and variations in the treatment types, periods and their interactions were analyzed critically using EpiInfo v6.041 PLUS (CDC, Atlanta, USA).

RESULTS

Biomass production

The biomass of *Bti* produced by different media varied. As summarized in Table 1, the biomass dry weight (g l^{-1}) was 1.10 in medium 'A', 6.18 in 'B' and 0.85 in 'C'. The biomass was 3.43 between the combination medium 'D', 2.10 in 'E', 1.78 in 'F' and 1.75 in LB. Thus, medium 'B' had the highest biomass production compared to all media, including the conventional one. The proportion of media solids in the freeze dried powder also varied among the media combinations with mineral salts (Table 2). The biomass in medium 'A', 'B' and 'C' in combination with $MnCl_2$ was 4.88, 8.56 and 2.58 respectively. Thus, in this test also, the medium 'B' had the highest biomass production in combination with $MnCl_2$. Therefore, further studies on bacterial growth pattern, mosquitocidal toxin synthesis, bioassays and field trials were conducted only with medium 'B' (CCP) and its combination with salt (CCP+$MnCl_2$) in comparison with conventional medium (LB).

Bacterial growth

It was observed from all culture media (CCP, CCP+$MnCl_2$ and LB) that the *Bti* exponential phase was initiated from the sixth hour onwards. This extended up to 48 h after which the *Bti* entered into the stationary phase of growth (48–72 h). There was a rapid multiplication of bacterial cells starting from this stationary phase, followed by an increase in culture density. Sporulation started in 48 h of growth and complete sporulation was achieved at 54 h and in 72 h, the spores were found to have been released from the cells (Fig. 1). It indicated that the bacteria was able to digest the nutrients from all the culture media completely in 72 h. It was further observed that, the growth pattern of *Bti* from experimental media (CCP, CCP+$MnCl_2$) was higher than the conventional medium (LB), corroborating the results of biomass production in the present study. Microscopic observation of bacterial growth revealed that, the sporulation rate from experimental media was as good as that of the conventional medium (figure not shown).

Table 1. Biomass production of *Bti* from different culture media

Sl. No.	Culture Media	Freeze dried powder weight (dry biomass, gmL^{-1})
	Agro-industrial byproducts:	
A	Neem oil cake (5%)	1.10 ± 0.14*
B	Coconut oil cake (5%)	6.18 ± 0.21
C	Groundnut oil cake (5%)	0.85 ± 0.10
	Combinations:	
D	Neem oil cake + Coconut oil cake (2.5 % + 2.5 %)	3.43 ± 0.16
E	Coconut oil cake + Groundnut oil cake (2.5 % + 2.5 %)	2.10 ± 0.18
F	Neem oil cake + Groundnut oil cake (2.5 % + 2.5 %)	1.78 ± 0.11
G	Luria Bertani (LB)	1.75 ± 0.13

*Average performance of six individual observations

Table 2. Biomass production of *Bti* from Agro-industrial byproducts in combination with mineral salts

Sl. No	Culture Media	$MnCl_2$ (1%)	$MgCl_2$ (1%)	$CaCl_2$ (1%)	NaCl (1%)	KCl (1%)	$Na_2HPO_4.H_2O$ (1%)
A.	Neem oil cake (5%)	4.88 ± 0.12*	3.18 ± 0.12	4.05 ± 0.10	2.06 ± 0.10	1.56 ± 0.12	1.98 ± 0.08
B.	Coconut oil cake (5%)	8.56 ± 0.12	5.10 ± 0.12	4.76 ± 0.10	2.60 ± 0.09	4.96 ± 0.12	3.50 ± 0.09
C.	Groundnut oil cake (5%)	2.58 ± 0.15	0.51 ± 0.12	1.80 ± 0.07	0.40 ± 0.12	1.26 ± 0.08	0.93 ± 0.10

*Freeze dried powder weight (dry biomass, gm/L): Average performance of six individual observations. *Bti* Biomass from Luria Bertani (conventional) = 1.86 gm/L

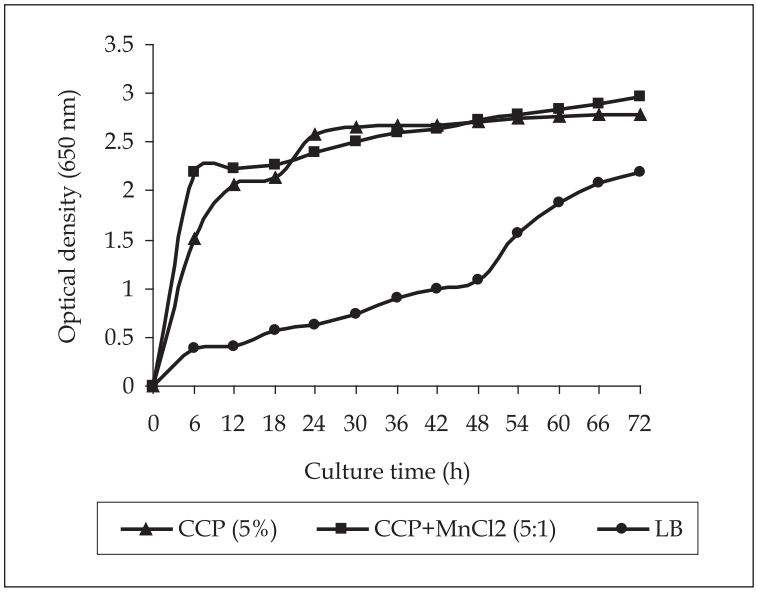

Figure 1. Growth pattern of *Bti* produced from different culture media

Mosquitocidal toxin synthesis

Figure 2 shows the dynamics of mosquitocidal toxin synthesized by *Bti* grown in various culture media (CCP, CCP+$MnCl_2$ and LB). Toxin production showed the following trends, viz., CCP+$MnCl_2$>CCP>LB, having marginally a higher value for the combination medium (0.05mg protein ml^{-1}). Thus, the combination medium shows good correlation between the growth, sporulation and the kinetics of mosquitocidal toxin synthesis in *Bti*.

Protein profiles of *Bti* from various culture media were examined by SDS-PAGE (10%) and it was observed that all mosquitocidal polypeptides (134, 125, 67 and 27 kDa) were present (Fig. 3).

Bioassays

Comparative bioassays of *Bti* produced from different culture media (CCP, CCP+$MnCl_2$ and LB), as evident from laboratory assays, are summarized in Table 3. There was no significant difference between the LC_{50}/LC_{90} values of bacterium grown in the media of experimental and conventional media (due to overlap of 95% fiducial limits). The LC_{50} value of *Bti* against *Cx. quinquefasciatus* was 0.006 mg l^{-1} for both 'LB' and 'CCP' and 0.005 mg l^{-1} for CCP+$MnCl_2$. This LC_{50} value

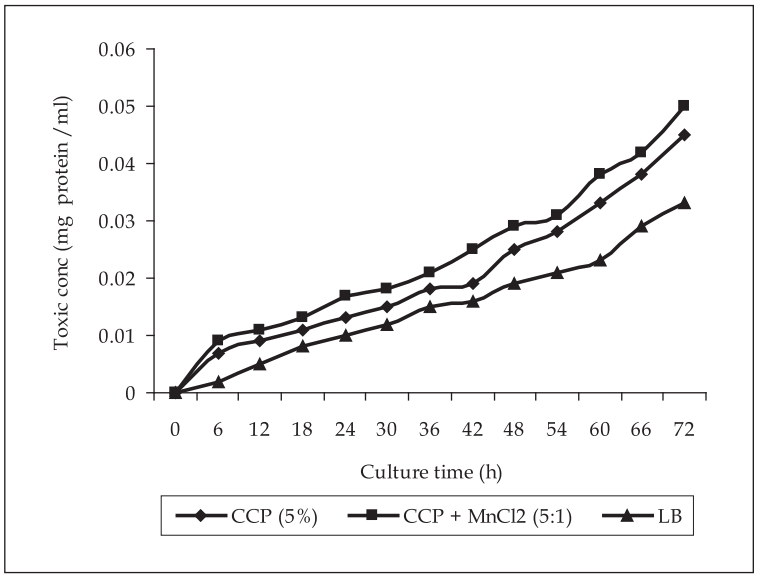

Figure 2. Dynamics of mosquitocidal toxin produced from *Bti*

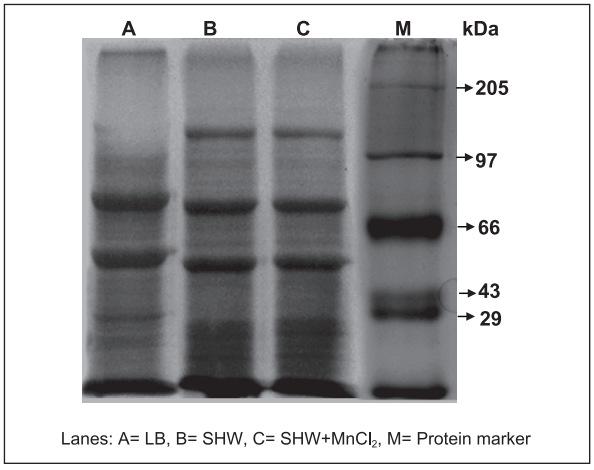

Figure 3. SDS-PAGE showing the expression of *Bti* toxins

Table 3. Laboratory bioassays with *Bti* produced from various culture media against Mosquito vectors

Culture media	Mosquito species	Intercept	Slope	LC 50 (mg/L)* (90% UCL-LCL)**	LC 90 (mg/L)* (90% UCL-LCL)	X^2 (df)
Luria Bertani (LB)	*Culex quinquefasciatus*	10.09	1.01	0.006 (0.007-0.005)	0.02 (0.032-0.014)	4.18 (7)
	Anopheles stephensi	17.36	3.06	0.017 (0.018-0.016)	0.026 (0.030-0.022)	1.07 (7)
	Aedes aegypti	23.57	5.01	0.024 (0.025-0.023)	0.031 (0.033-0.028)	2.55 (7)
Coconut cake (5%)	*Culex quinquefasciatus*	18.07	1.11	0.006 (0.007-0.005)	0.019 (0.029-0.012)	4.64 (7)
	Anopheles stephensi	17.73	3.14	0.017 (0.018-0.016)	0.026 (0.032-0.028)	2.16 (7)
	Aedes aegypti	23.10	4.84	0.023 (0.025-0.022)	0.031 (0.032-0.028)	1.35 (7)
Coconut cake + $MnCl_2$ (5:1)	*Culex quinquefasciatus*	10.3	1.03	0.005 (0.006-0.004)	0.014 (0.029-0.012)	6.84 (7)
	Anopheles stephensi	18.8	3.4	0.017 (0.018-0.016)	0.025 (0.032-0.028)	1.18 (7)
	Aedes aegypti	25.8	5.5	0.024 (0.025-0.023)	0.030 (0.032-0.028)	0.43 (7)

* Average performance of six individual observations. ** 90% confidential limits at upper and lower levels

against *An. stephensi* was 0.006 mg l^{-1} in all the three media. Similarly, the LC_{50} against *Ae. aegypti* was 0.024 mg l^{-1} for both 'LB' and 'CCP+$MnCl_2$' and 0.023 mg l^{-1} for CCP. Thus, bacterial toxins produced from all culture media were effective against mosquito species, and their lethal effects were also within the limit of standard toxicity range.

Field trials

The potency of *Bti* produced from various culture media (CCP, CCP+$MnCl_2$ and LB) were compared against the filarial vector of *Cx. quinquefasciatus* in the cesspits of urban area, on the outskirts of Pondicherry (India). There was no significant difference in the efficacy of *Bti* produced from these media against the mosquito vector. At the application rate of 0.25 g/m^2, the larval density was brought down by 92.4–100% with *Bti* produced from experimental media (CCP, CCP+$MnCl_2$) 24 h post-treatment. This significant reduction in the abundance of larval density (>90%) was noticed for 19 days. But from the 21st day onwards, the larval density started increasing (Fig. 4). Similarly, 24 h post-treatment at the application rate of 0.25g m^2, the larval density was brought down by 91.2–100 % with *Bti* produced from LB. Here also, there was a significant reduction in the abundance of larval density (>90%) for 19 days, but from the 21st day onwards the larval density started increasing. Our result are corroborate earlier findings. The mosquito larvae killed as a result of *Bti* were easily identifiable due a dark colouration of the dead larvae.

For convenience of interpretation of the effectiveness of the bacterial toxin produced from the different culture media (CCP, CCP+$MnCl_2$, LB), ANOVA was conducted on the observations, which indicated that there were significant differences (P<0.0001) due to treatments (two factors), period of exposure (24 factors) and their interactions (treatment × periods) where *Bti* toxins were used (Table 4). Further pairwise comparison was made to examine critically on which pairs values were statistically significant. It was observed from Table 5 that the *Bti* produced from all the culture media gave a significant effect (P<0.05) compared to control. However, the efficacy of bacterial toxins produced from 'CCP' with 'LB' and 'CCP+$MnCl_2$' with 'LB' did not show significant variations (P=0.008, 0.193), implying that all culture media (CCP, CCP+$MnCl_2$ and LB) had a similar effect on mosquito larvae of *Cx. quinquefasciatus*.

Cost analysis

As summarized in Table 6, the amount of CPP required to prepare 1 l culture of medium A was 50.0 g, which costs US$ 0.05. In comparison, preparation of 1 l of the LB medium costs US$ 2.50. Hence medium 'A' (CPP) was found to be 50 times less expensive than the conventional medium. The other medium (C) was 49.01 times less expensive than LB. Thus the use of an agro-industrial by-product-based medium (CCP+$MnCl_2$) is highly economical for large-scale industrial production of this mosquito pathogenic bacillus.

DISCUSSION

In the present study, we have studied the possibility of using three agro-industrial by-products (CCP, NCP and GCP) either individually or in combination with mineral

Table 4. Analysis of Variance (ANOVA) from *Bti* field trials for the control of mosquito vector (*Culex quinquefasciatus*)

Source	Degree of Freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	F	Level of Significance
Periods	24	8394779.919	349782.497	1998.251	0.000
Treatment	2	2890.470	1445.235	8.256	0.000
Interactions	48	21492.586	447.762	2.558	0.000
Error	150	26256.647	175.044	–	–

Table 5. Comparison of Mean density between the bacterial culture media

Culture Media	Media Comparison	Mean Difference	Std Error	Significant Error	95% Confidence Interval Lower Bound	Upper Bound
A	B	5.784*	2.1605	0.008	1.515	10.053
	C	-2.828	2.1605	0.193	-7.097	1.440
B	A	-5.784*	2.1605	0.008	-10.053	-1.515
	C	-8.612*	2.1605	0.000	-12.881	-4.343
C	A	2.828	2.1605	0.193	-1.441	7.097
	B	8.612	2.1605	0.000	4.343	12.881

*Mean difference is significant at 0.05 level. A – Luria Bertani; B – Coconut oil cake; C – Coconut oil cake+$MnCl_2$

Table 6. Comparative costing for producing *Bti* from conventional and experimental culture media

Culture medium	Main constituent of the medium	Quantity for culture preparation (gm/L)	Cost of quantum used (US $)	Total cost for culture medium used (US $)	Net difference in cost between Coconut oil cake and LB (in ratio)
Conventional:					
A. Luria Bertani (LB)	Peptone + Yeast extract + Sodium chloride	10 + 5 + 10	1.0 + 1.0 + 0.50	2.50	
Experimental:					
B. Coconut cake (5%)	Coconut cake	50	0.05	0.05	1: 50
C. Coconut cake + $MnCl_2$ (5:1)	Coconut cake + $MnCl_2$	50 + 10	0.05 + 0.001	0.051	1: 49.01

salts. It was observed that 'CCP' gave a promising result in combination with $MnCl_2$ in the production of mosquitocidal toxin. This product normally accumulates lipids and carbohydrates useful for manure and cattle feeding (Krishnan & Chandra, 1982; Rao *et al.*, 1985; Nalini *et al.*, 2004; Ramachandran *et al.*, 2004). However, this product has not been studied for the production of a biopesticide, like *Bti* in mosquito vector control programme, and this has been the objective of the present study. The result revealed that, the biomass production of bacterial toxin from the experimental culture medium (CCP+$MnCl_2$) was much higher than the conventional medium (LB). Bioassays with mosquito vectors (*Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*) were found to be comparable with LB. It is specific to mention here that, the bacterium produced from CCP+$MnCl_2$ controlled the mosquito larvae (*Cx. quinquefasciatus*) for about 3 weeks in the field trials. Thus, our results indicate that CCP+$MnCl_2$ can effectively replace synthetic chemicals, like, peptone and yeast extract from the conventional medium (LB) currently in use for large-scale preparation of biopesticide (*Bti*). Further, it is pertinent to emphasize that the use of CCP+$MnCl_2$ as the medium for the production of *Bti* has many advantages because, it is available throughout the year, permits faster sporulation, provides high biomass production and is also convenient to handle and apply. The bioremediation method thus envisaged proved to be cost-effective in the preparation of the culture media with high efficacy in bacterial toxin production and mosquito vector control, especially in

tropical countries. Thus, this study possesses the dual benefits of efficient utilization of an agro-industrial by-product (CCP) and effective production of mosquitocidal biopesticide.

Literature surveys revealed that media formulated from seeds of legumes (groundnut cake, cow pea of white and black varieties, soya bean and bambara beans), dried cow blood and mineral salts were ideal for the production of *Bti* (Obeta & Okafor, 1984). Potatoes, coconuts, fishmeal and corn steep liquor have also been reported to be efficient for the production of biopesticides (Saalma *et al.*, 1983; Kuppusamy, 1990; Ejiofor, 1991). Tyagi and his co-workers had recently evaluated the improvement in biopesticides and alkaline proteases using *Bti* from waste water and sewage sludge (Tyagi *et al.*, 2002; Ghribi *et al.*, 2004; Brar *et al.*, 2006). Gruel and fish meal media have also been used for *Bacillus thuringiensis* delta-endotoxin production in agriculture (Zouari *et al.*, 2002). The present study also corroborates the earlier reports that *Bti*, as potential bacterium for mosquito control, also uses an agro product (CCP) for toxin synthesis.

Bioassays with *Bti* against mosquito larvae in the present study have shown considerable toxicity. It is evident from the mode of action of toxins that the toxicity is due to binding of active toxins to specific receptor sites on the larval midgut brush border membrane (MBBM) (Charles *et al.*, 1997). Initially the crystal toxins from the spore/crystal complex are ingested along with food materials by the mosquito larvae, and after solubilization and proteolytic cleavage, the activated toxin interacts with midgut epithelium, leading to the death of the larvae (Poopathi *et al.*, 2002b). We also suggest, therefore, that the *Bti* produced from coconut cake-based medium as in the present study would also have a similar effects on the larvae, agreeing with the earlier findings.

Acknowledgements. Our thanks are due to the Department of Science, Technology and Environment (DSTE), Government of Pondicherry, India, for funding the project (Ref: 10/DSTE/GIA/RP/JSA-1/2008/887 dated 18th April, 2008), to the Director, Vector Control Research Centre (ICMR), Pondicherry, for permission and to Dr M. Kalyanasundaram, Scientist-F for critically reviewing the MS.

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