Production of *Bacillus thuringiensis* subsp. *israelensis* from agro-based product (corncob)

Bacillus thuringiensis subsp. *israelensis* (*Bti*) is advantageous to conventional insecticides, due to its narrow host spectrum and safety to the environment[1–3]. Though the high efficacy and specificity of *Bti* is useful in controlling mosquitoes, the cost of production is high. The raw material cost alone may comprise > 70% of the overall production cost[4]. Therefore, selection of inexpensive growth medium or raw material is essential[5–8]. Nitrogen and carbon (N : C) are the essential source for bacterial proliferation[9,10]. *Triticum aestivum* (wheat bran, WB), *Cicer arietinum* (chickpea husk, CH) and *Zea mays* (corncob, CC) are tested in the present study on judicious combination with mineral salts to derive more yield of *Bti* than the reference medium (Luria Bertani, LB).

Powdered agro-industrial by-products (WB, CH and CC) were boiled separately (50 g/l) with tap water (15 min) and extracted (pH 7.8). They were dispensed separately and in combinations (WB + CH, WB + CC and CH + CC, 1 : 1). Similar combinations were also made with mineral salts ($MnCl_2$, $MgCl_2$, $CaCl_2$, NaCl, KCl and $Na_2HPO_4{\cdot}H_2O$; 5 : 1). The medium which showed maximum biomass production was selected for further analysis. LB (LB: peptone + yeast extract + NaCl, 1 : 0.5 : 1) was used as reference. Culture media were autoclaved (120°C/20 lb/in^2/20 min).

Bti H-14 (15,000 ITU/mg) was inoculated into the culture medium and grown under orbital shaker (120 rev/min, 30°C, 72 h). Samples were drawn (2.5 ml) at every 6 h up to 72 h for turbidity (650 nm) measurement. Bacterial spores/crystals were harvested (10,000 g/30 min/4°C) for biomass and toxin analysis (SDS–PAGE)[11,12].

Bioassays were conducted with *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*[13]. Twenty-five early, third-instar larvae were subjected to different concentrations at dosage ranging from 0.001 to 0.05 (mg/l) and mortality was observed after 24 h. Control mortality was corrected[14]. The LC_{50} and LC_{90} values were calculated from regression analysis. *Bti* produced from the test and reference media were sprayed in the field (15 cesspits) with dosage 0.25 g/m^2. Replicates were maintained with unsprayed control[15]. Post-treatment counts from immature population were done every alternate day and percentage reduction in the population was calculated[16].

The growth pattern of *Bti* starts primarily with the vegetative phase and culminates with the sporulative phase. In all experimental culture medium, after an hour-long lag phase, there was rapid multiplication of bacterial cells and maturation of spores (Figure 1). The culture density increased with time and reached a plateau. Growth and release of spore/crystal toxin was maximal at 72 h. Microscopic observation also supported this finding. The data were subjected to regression analysis and results showed that the range of production of *Bti* was 0.37–2.20 for LB, whereas it was 1.09–2.93 for the combination medium (CC + $MnCl_2$), indicating that the latter had the maximum bacterial growth rate ($P < 0.05$). Biomass, an indicator of bacterial growth, was also examined and the result showed that CC had a greater level of bacterial production (data not shown). Therefore, the growth of bacteria corroborated with its biomass production, and suggested that the combination medium (CC + $MnCl_2$) has good potential of *Bti* production. Results from SDS–PAGE indicated that the polypeptides of *Bti* (134, 125, 67 and 27 kDa) produced from test and reference media were identical (Figure 2).

The comparative toxicities of *Bti* produced from various culture media (CC, CC + $MnCl_2$ and LB) are shown in Table 1. The LC_{50} against *Cx. quinquefasciatus* was 0.006 mg/l for both LB and CC and 0.005 mg/l for CC + $MnCl_2$. Thus the toxicity levels were statistically similar in both test and reference media (fiducial limit overlapping).

There was significant reduction in the population of *Cx. quinquefasciatus* in the cesspits treated with *Bti* produced from CC and LB compared with control ($F = 16.14$, d$f = 2$, $P < 0.0001$). This significant reduction in abundance of larval density (> 90%) was observed for 19 days. From the 21st day onwards, the density started increasing. However, 50% reduction in the population of larval density was noticed up to 33 days (data not shown).

ANOVA indicated that there was significant difference ($P < 0.0001$) in the treatments (two factors), periods (24 factors)

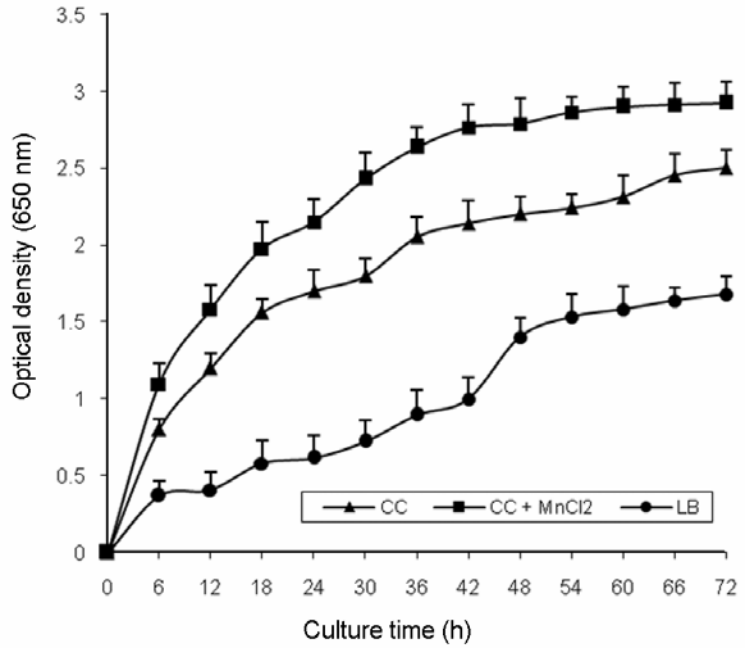

Figure 1. Growth pattern of *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) produced from different culture media.

Table 1. Mosquito toxic effect of *Bacillus thuringiensis* subsp. *israelensis* produced from test culture media in comparison with the reference medium

Culture medium	Mosquito species	Intercept	Slope	LC_{50} (mg/l)* (90% UCL–LCL)**	LC_{90} (mg/l)* (90% UCL–LCL)	χ^2 (d*f*)
Luria Bertani	*Culex quinquefasciatus*	9.59	0.90	0.006 (0.007–0.004)	0.025 (0.043–0.014)	3.80 (7)
	Anopheles stephensi	19.17	3.51	0.017 (0.018–0.016)	0.025 (0.028–0.022)	1.01 (7)
	Aedes aegypti	23.9	5.10	0.024 (0.015–0.023)	0.031 (0.034–0.028)	0.65 (7)
Corncob (5%)	*Cx. quinquefasciatus*	9.53	0.89	0.006 (0.007–0.004)	0.026 (0.046–0.014)	4.35 (7)
	A. stephensi	19.72	3.63	0.017 (0.018–0.016)	0.024 (0.027–0.022)	1.55 (7)
	A. aegypti	23.75	5.06	0.023 (0.025–0.023)	0.031 (0.034–0.029)	0.47 (7)
Corncob + $MnCl_2$ (5 + 1%)	*Cx. quinquefasciatus*	9.96	0.95	0.005 (0.007–0.004)	0.021 (0.033–0.014)	7.74 (7)
	A. stephensi	20.01	3.69	0.017 (0.018–0.016)	0.024 (0.027–0.021)	1.63 (7)
	A. aegypti	23.37	4.93	0.024 (0.025–0.023)	0.030 (0.034–0.028)	1.11 (7)

*Average performance of six individual observations. **90% confidence limits at upper (UCL) and lower (LCL) levels.

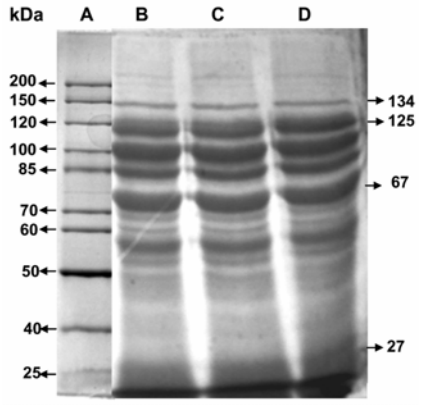

Figure 2. *Bti* toxins produced from different bacterial culture media. Lane A, Protein marker; lane B, CC; lane C, CC + $MnCl_2$ and lane D, LB. CC, Corncob; LB, Luria Bertani medium.

and their interactions (treatment × periods). Pairwise assessment indicated that the *Bti* produced from the test medium gave significant effect ($P < 0.05$) compared to the control. Nevertheless, the efficacy of *Bti* produced from CC with LB and CC + $MnCl_2$ with LB did not show significant variations ($P = 0.0001, 0.85$), implicating that all culture media had similar effect over *Cx. quinquefasciatus* (data not shown).

It is plausible from the foregoing observation that the *Bti* produced from corncob-based culture medium (CC + $MnCl_2$) is highly economical and efficacious. Many effective strains of spore-forming bacteria, *B. sphaericus*, *Bti* and *B.t. kurstaki* have been successfully used for producing bacterial toxins in different culture media[5–8]. The present result also agrees with the earlier findings. The amount of CC + $MnCl_2$ required to prepare 10 l of culture medium was 600 + 6 g, with a total cost of US$ 0.50. In comparison, preparation of 10 l of LB costs US$ 25.0. Hence, the test medium was found to be 50 times less expensive than the reference medium. In view of this cost factor, the application of corncob-based culture medium appears to be quite promising and feasible for mosquito-control operations, especially in the developing countries.

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SUBBIAH POOPATHI*
BALARAMAN ARCHANA

Unit of Microbiology and Immunology, Vector Control Research Centre (ICMR), Indira Nagar, Puducherry 605 006, India.
**For correspondence.*
e-mail: Subbiahpoopathi@rediffmail.com