# Evaluation of sugarcane-based byproducts media for improvement in production of *Bacillus thuringiensis israelensis*

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***Abstract***

*This study optimized Bacillus thuringiensis israelensis (Bti)-based biopesticide production using cost-effective substrates molasses, jaggery and sugarcane juice, in batch and fed-batch fermentations. Molasses was pre-treated to hydrolyse sucrose into glucose and fructose using enzyme invertase, produced via solid-state fermentation using Aspergillus niger, enhancing substrate suitability. Bti growth was evaluated under varying substrate concentrations, aeration conditions and nutrient supplements. Fed-batch fermentation proved superior to batch fermentation, mitigating catabolite repression at high substrate concentrations and supporting enhanced growth and toxin production. Sugarcane juice at 10 g/L sugar concentration yielded the highest optical density (OD) in fed-batch mode. Calcium chloride supplementation (0.1%) further improved Bti biomass yield and sporulation.*

*Larvicidal assays against Aedes aegypti larvae revealed 90% mortality rate for media supplemented with sugarcane juice and molasses under fed-batch conditions with calcium chloride while 70% mortality was observed in batch fermentation. These findings highlight fed-batch fermentation as an efficient strategy to maximize Bti productivity and bioactivity. The study emphasises on the economic viability of using sugarcane-derived substrates, leveraging India's position as the second-largest sugarcane producer globally. By integrating low-cost substrates and optimized fermentation, this scalable approach offers a sustainable solution for mosquito control programs.*

**Keywords:** *Bacillus thuringiensis israelensis*, pre-treated molasses, mosquito control.

## Introduction

Mosquitoes (Diptera: Culicidae) are significant vectors of numerous diseases including malaria, dengue, chikungunya, filariasis, yellow fever and West Nile fever, posing a major global health threat. Effective vector control measures are crucial for disease prevention and often involve using insect growth regulators, microbial agents and organophosphates to target mosquito larvae. Additionally, tropical regions frequently employ strategies like insecticide-treated bed nets and indoor residual spraying to reduce mosquito-borne disease transmission[13]. However, the widespread use of these chemical interventions has led to resistance in several mosquito species, along with adverse effects on human health and the environment. To counter these challenges, environmentally sustainable control methods have been introduced[4,5]. One such solution is *Bti*, a subspecies of *Bacillus thuringiensis*, which has proven to be an effective biological control agent against dipteran larvae, offering a safer alternative to chemical insecticides[21].

*Bti* is an environmentally friendly, Gram-positive, spore-forming bacterium recognized as one of the most effective biological agents for controlling disease-carrying vectors[7]. *Bti* produces crystalline parasporal bodies composed of protein protoxins known as δ-endotoxins. These crystals contain three primary antidipteran toxins: Cry4A, Cry4B and Cry11A, which act synergistically to enhance the overall toxicity of the crystals[12,19,24]. In addition to Cry toxins, *Bti* produces Cyt toxins, smaller polypeptides (25–28 kDa) that exhibit hemolytic and cytolytic activity *in vitro* and are highly specific to dipteran larvae *in vivo*. Cyt toxins have been categorized into several types including Cyt1 (formerly CytA), Cyt2 (formerly CytB), CytC and the newly proposed CytD[23].

Cyt1A is predominantly found in *Bacillus thuringiensis israelensis* and *morrisoni PG14*[11], while Cyt2 is present in subspecies *darmstadiensis* and *kyushuensis*[22]. CytC is associated with subspecies f*ukuokaensis* and CytD has been identified in subspecies *jegathesan*[27]. Although these Cyt toxins do not share sequence homology with Cry toxins, they both rely on a cytolytic mechanism involving colloid-osmotic lysis, although their mechanisms of pore formation differ. Cyt1A and Cyt2A have been shown to form cation-selective channels and exhibit broad cytolytic activity *in vitro*.

However, their insect-specific activity *in vivo* likely depends on the presence of specific receptors in target larvae. This receptor-mediated interaction could explain the high specificity of Cyt toxins for dipteran species, making Bti a highly selective and potent biological control agent[20].

Sugarcane juice is primarily composed of sucrose, accounting for approximately 66% of the total metabolites in sugarcane juice. It also contains phenolic compounds, organic acids, sterols, amino acids etc.[25] Molasses, a by-product of sugarcane processing, is more concentrated in certain nutrients like carbohydrate mainly sucrose with glucose and fructose. It has minerals like potassium, calcium, magnesium and proteins[2]. Jaggery, a non-

centrifugal sugar made from sugarcane or palm sap, is rich in nutrients and bioactive compounds.

Jaggery contains about 70-85% sucrose, making it a natural sweetener with a lower glycaemic index than refined sugar. It also has small amounts of glucose and fructose. It is a significant source of iron, calcium, magnesium and potassium. Jaggery retains polyphenols like hydroxycinnamic and gentisic acids, which contribute to its antioxidant properties. These compounds have anti-inflammatory and protective health effects. Good-quality jaggery has low moisture content (1-2%), which helps in prolonged storage[6].

India, as the second-largest producer of sugarcane, generates abundant and cost-effective by-products like molasses and jaggery, making them ideal carbon sources for industrial-scale *Bti* production. Utilizing these sugarcane derivatives reduces reliance on expensive synthetic substrates, lowering production costs and enabling the development of affordable biopesticides. Their local availability minimizes transportation costs and enhances economic feasibility, while repurposing agro-industrial by-products supports rural economies, fosters a circular economy and reduces environmental waste. This study underscores the dual benefits of sugarcane derivatives: improved biopesticide efficacy and significant economic advantages. Integrating these substrates into industrial fermentation processes could transform sustainable pest control practices in India and similar agricultural economies.

## Material and Methods

**Sample collection:** Molasses, jaggery and sugarcane juice were purchased for the current study from The Corporative Sugars Ltd. in Chittur, Palakkad. *Bti* was obtained from the Institute of Microbial Technology in Chandigarh, India (MTCC 869). Other chemicals were purchased from Merck, Himedia and Sigma.

**Preparation of inoculum:** The standard protocol was followed when preparing the inoculum. To prepare the inoculum, the frozen culture of *Bti* was thawed in a water bath set at 30ºC. 1 ml of the preculture was inoculated to 50 ml of nutrient broth and cultivated for 24 hours at $30^0$C till the optical density reaches 2.5-3 at 600 nm. The culture was then centrifuged at 10ºC at 5000 rpm for 30 minutes. Following that nutrient broth was used to dilute the pellet until its concentration was approximately $10^8$ CFU/ml. This culture was further used as inoculum for basal medium[8].

**Pre-treatment of molasses:** *Aspergillus niger* was utilized to produce invertase enzyme, which hydrolyses sucrose in molasses into glucose and fructose, enhancing its uptake by bacteria. Solid-state fermentation was employed for invertase production, using a mixture of rice bran and rice husk in 1:4 ratio and then soaked in water for 12 hours. After draining the excess water, the mixture was combined with 5 mL of molasses and transferred to Petri plates, which were then sterilized at 121°C for 20 minutes. Once cooled, the plates were inoculated with a pure culture of *Aspergillus niger* at $10^3$ spores/mL and incubated at 32°C for 5 days.

The grown material was subsequently pressed to extract the crude enzyme. 5 mL portion of the enzyme extract was mixed with 95 mL of molasses, thoroughly combined and left at room temperature for 2 hours. The resulting pre-treated molasses was then utilized in subsequent experiments[9].

**Fermentation of *Bti* using various substrates:** *Bti* was cultivated using specialized medium formulations containing various carbon sources through both batch and fixed volume fed-batch fermentation. The carbon sources tested were glucose, sucrose, fructose, lactose, sugarcane (*Saccharum officinarum*) juice, pre-treated molasses *(Saccharum officinarum molasses (*SOM*))* and jaggery. In this study, the basal medium was supplemented with various carbon sources to achieve a carbon concentration of 10 g/L. The pH of the media was initially adjusted to 7.2 and sterilized by autoclaving at 121°C (15 psi) for 15 minutes. After cooling, 0.1 mL of *Bti* preculture was used to inoculate the media. In fed-batch fermentation, 25 mL of sterile media with 2g/L sugar concentration was added every 12 hours post-inoculation. This process was repeated four more times using the carbon additive, gradually increasing the sugar concentration to 10g/L. OD at 600 nm was measured after 48 hours of aeration[16].

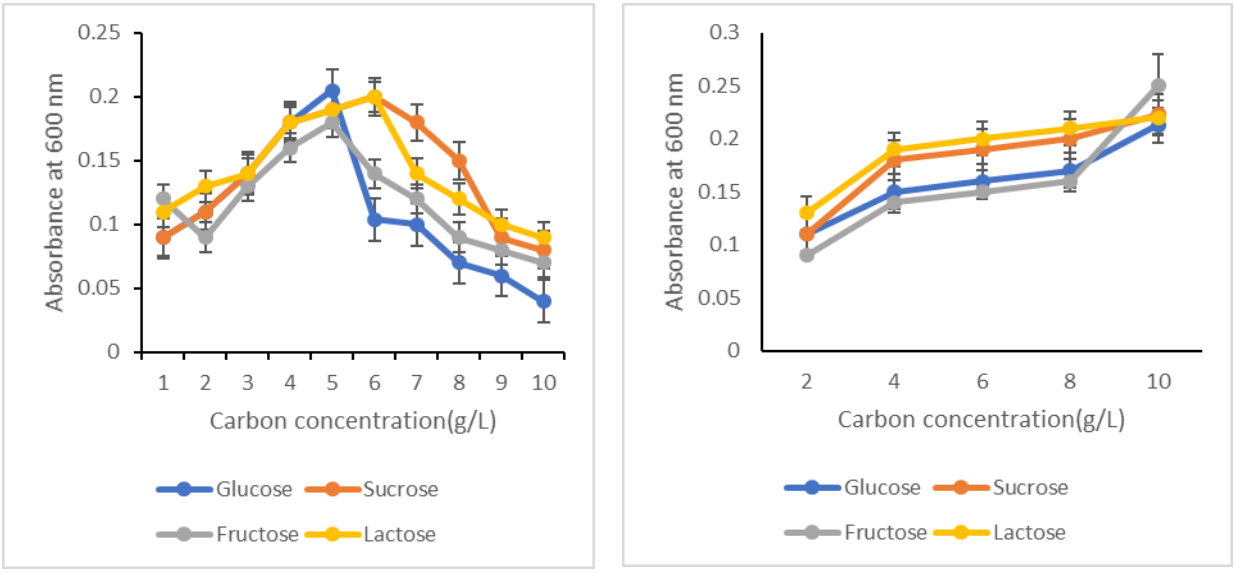
For batch fermentation, sugar concentration was initially kept to 10 g/L. Experiments were conducted to evaluate the effects of variable timing for substrate addition, aeration and calcium chloride enriched media. For variable timing of substrate addition, the substrate was introduced at intervals of 3, 6 and 9 hours after the initial addition, resulting in a final concentration of 10 g/L. To assess the influence of calcium chloride on *Bti* growth, 0.1% calcium chloride was added to the medium, with a control setup maintained without calcium chloride. The effect of aeration was studied by introducing air into the medium using an aerator, while a separate control experiment was conducted under agitation in a shaker.

**Toxicity assay:** Larvae were collected from the Departmental Garden of the University of Calicut's Department of Biotechnology and identified by Dr. Raghu, Assistant Director at the Center for Disease Control, Kallayi, as *Aedes aegypti*. The larvae were kept in plastic containers with tap water. Test setups were conducted in Petri plates following a slightly modified version of the standard WHO methodology for time-dependent testing. Each experiment included three replicates and a control, with dechlorinated tap water used as the control. The bioassay involved collecting a 100 ml sample post-fermentation, which was centrifuged at 8000 rpm for 15 minutes. The resulting pellets were lyophilized and the lyophilized *Bti* powder was dissolved in distilled water to achieve a final concentration

of 15 ppm[10]. Twenty third-instar larvae were then introduced into a Petri plate containing 90 ml of water and 10 ml of the prepared test solution. Larvae were considered dead if they remained motionless after probing the siphon or cervical region with a needle. Experiments were carried out in a laboratory setting at 25–30°C and 80–90% relative humidity. Mortality rates were recorded as an average of three replicate[17].

## Results and Discussion

The success of industrial fermentation largely depends on choosing an appropriate medium and fermentation mode. Since both the fermentation medium and microbial cells predominantly consist of carbon, the choice of carbon sources plays a pivotal role in determining the overall production cost. A significant portion of the total expenses in industrial fermentation can be attributed to the cost of the production medium[14,26]. As the concentration of the carbon source rises, *Bti* experiences catabolite repression, as depicted in figure 1. Fed-batch processes gradually supply glucose, preventing its accumulation to inhibitory levels and supporting balanced growth and toxin production. Among all the tested carbon sources, the highest growth was observed at a 5% carbon concentration in batch fermentation[3].




**(a)** **(b)**

**Figure 1: Investigation on the catabolite repression using different carbon sources as substrates using (a) batch fermentation (b) fixed volume fed batch fermentation. This graph shows the effect of different pure carbon sources (Glucose, Sucrose, Fructose and Lactose) on bacterial growth, measured at 600 nm. In batch fermentation, highest absorption was reported at 5g/L carbon source. While in fed batch fermentation highest absorbance was at 10g/L.**

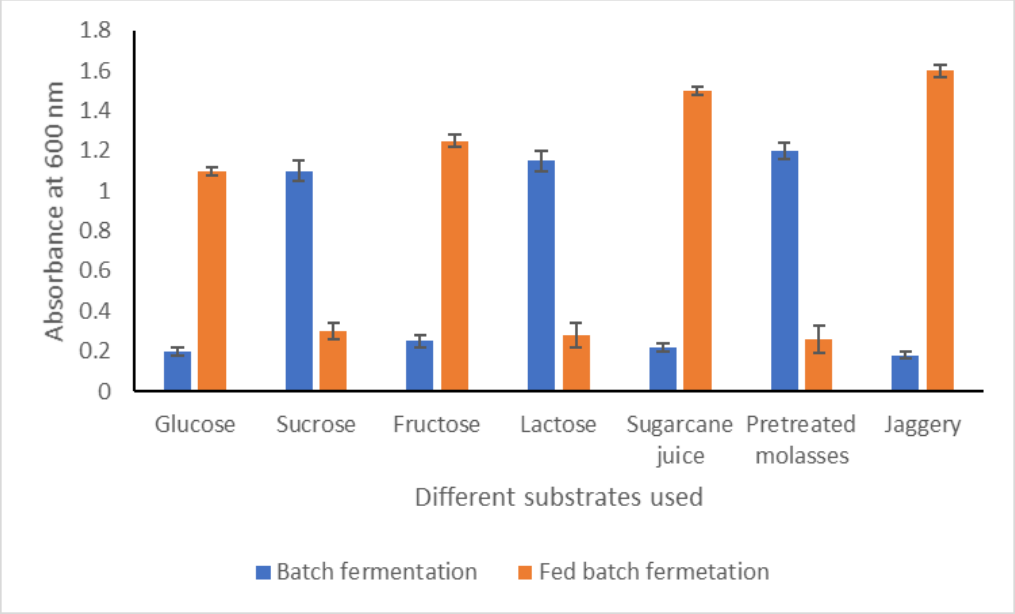



**Figure 2: Comparison of growth using different carbon substrates (Fructose, Glucose, Jaggery, Lactose, Pre-treated Molasses, Sucrose, Sugarcane Juice) under two fermentation methods (Batch and Fed-Batch). Here, carbon concentration used was 10g/L.**

In this study, fixed volume fed-batch fermentation was employed due to its economic advantages compared to batch fermentation. Additionally, we explored the potential of producing *Bti* using sugarcane and its related products. Figure 1 illustrates the growth of *Bti* under fed-batch and batch fermentation conditions. Fixed-volume fed-batch fermentation yielded higher biomass compared to batch fermentation. This increase in biomass is attributed to the distinctive operational method of fed-batch fermentation which alleviates the stress on microbial cells caused by excessive substrate concentrations and the accumulation of toxic metabolites. In this study, 10g/L sugar concentration was used as the substrate. While batch fermentation exhibited catabolite repression at this high sugar level, fed-batch fermentation facilitated improved growth[15].

Figure 2 clearly shows that fed-batch fermentation exceeds batch fermentation for all tested substrates. This is especially evident with jaggery and pre-treated molasses which yielded the highest absorbance values under fed-batch conditions. Consistently, fed-batch produced higher biomass or product compared to batch fermentation across all substrates. By incrementally feeding substrates at fixed intervals in a fixed-volume fed-batch setup, we observed rapid *Bti* growth. Considering India's significant sugarcane production, this approach holds substantial economic potential. By optimizing the fermentation process, higher yields of valuable products can be achieved, leading to increased economic benefits. This approach can contribute to the sustainable and efficient utilization of agricultural resources.

A comparative analysis of fixed-volume fed-batch and batch fermentation under various conditions revealed that the highest growth of *Bacillus thuringiensis israelensis* (*Bti*) occurred when calcium chloride was incorporated into the medium (figure 3). 0.1% calcium chloride plays a vital role in stabilizing cell membranes, enhancing sporulation and supporting enzyme activity, which are essential for optimal microbial growth and productivity. The fed-batch system maximized these benefits by ensuring a controlled, continuous supply of calcium ions and other nutrients, preventing nutrient depletion and minimizing the accumulation of toxic by-products. In contrast, batch fermentation showed limited growth under the same conditions, likely due to the rapid exhaustion of nutrients and metabolic stress, emphasizing the superiority of fed-batch fermentation for enhancing *Bti* production.

After a 48-hour exposure period, the third instar *Aedes aegypti* larvae showed a moderate level of harmful effects across all the media formulations tested. Figure 4 compares the mortality rates of a target organism under two fermentation types: batch fermentation and fed-batch fermentation across seven different substrates (glucose, sucrose, fructose, lactose, sugarcane juice, molasses and jaggery). Fed-batch fermentation consistently shows higher mortality rates compared to batch fermentation for all substrates. Substrates such as sugarcane juice, molasses and jaggery demonstrate the highest mortality rates in both fermentation types.

Figure 5 suggests that the presence of calcium chloride in the medium enhanced the effectiveness of *Bti* in killing the larvae. Upon closer examination, it was observed that all the larvae exposed to the *Bti*-treated medium were dead after the 48-hour period, indicating the strong toxicity of the fermented medium containing calcium chloride. This highlights the potential of calcium chloride supplementation in improving the efficacy of *Bti* as a biopesticide for controlling mosquito larvae[1].

Figure 5 illustrates the mortality rate of a target organism under three experimental conditions: variable time interval, aeration and calcium chloride. Among these, the highest mortality rate (80%) is observed under the calcium chloride condition, indicating its greater effectiveness.

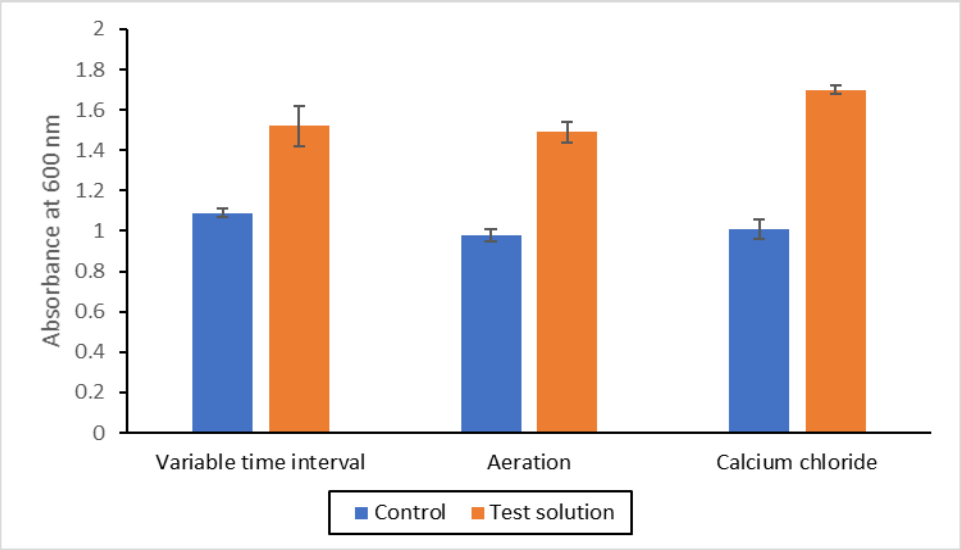



**Figure 3: Fixed volume fed batch and batch fermentation under various conditions are compared. Here, maximum growth was found in *Bti* grown along calcium chloride.**

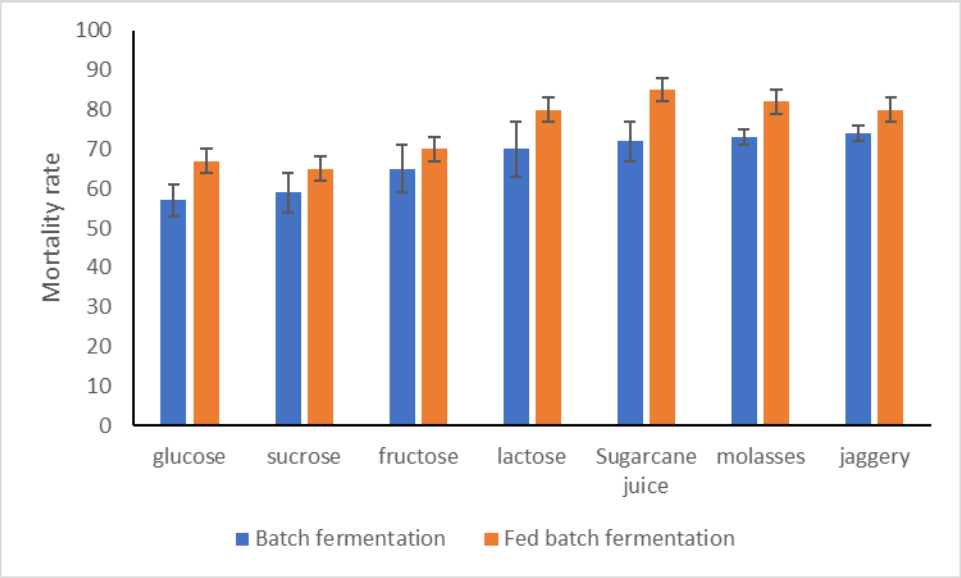



**Figure 4: Larvicidal assay employing medium supplemented with different substrates after 48 hours under two different fermentation types. Highest mortality was obtained was found in *Bti* grown in media supplemented with sugarcane juice. This suggests that fed-batch fermentation may be more effective in achieving higher mortality rates.**

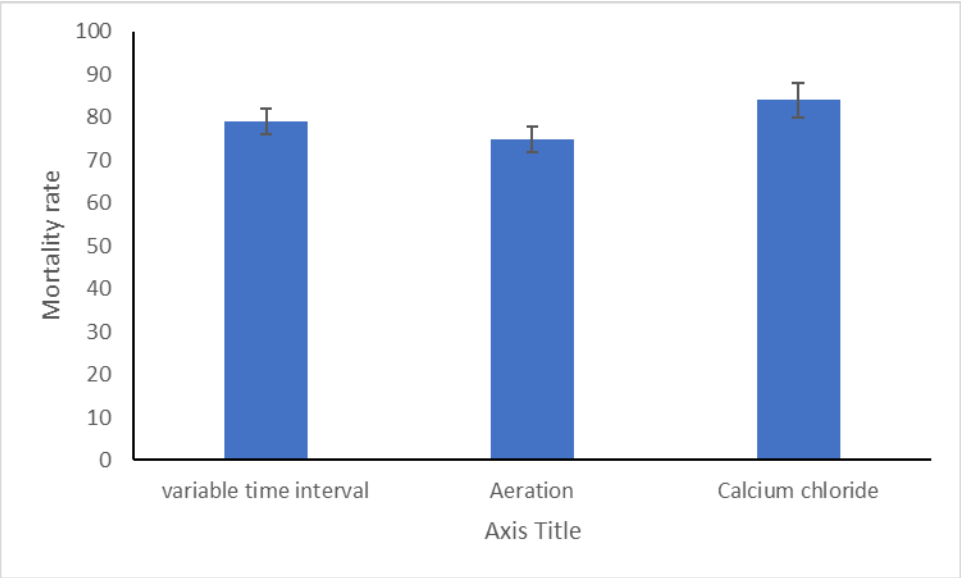



**Figure 5: Fixed volume fed batch fermentation utilizing media supplemented with various experimental conditions like aeration, 0.1% calcium chloride and variable time interval using jaggery as carbon source. The *Bti* that was cultivated with calcium chloride displayed the highest percentage mortality.**

The aeration condition shows the lowest mortality rate (70%), while the variable time interval condition results in a slightly higher rate (75%). This figure highlights the significant impact of experimental conditions on the mortality rate with calcium chloride emerging as the most effective variable[18].

## Conclusion

The present study aimed to optimize the production of *Bacillus thuringiensis israelensis* (*Bti*) through fermentation using different substrates with the goal of enhancing its larvicidal activity against *Aedes aegypti* larvae. The results demonstrated that fixed-volume fed-batch fermentation significantly outperformed batch fermentation in terms of biomass production. This was particularly evident when calcium chloride was included in the medium, as it helped to stabilize cell membranes, enhance sporulation and support enzyme activity which are critical for optimal microbial growth and toxin production.

When *Bti* was cultivated using various substrates, pre-treated molasses emerged as the most effective, showing the highest mortality rate in the larvicidal assay. In the experiment, *Bti* cultivated using pre-treated molasses resulted in a substantially higher percentage of larval mortality compared to other substrates including jaggery, sugarcane juice and untreated molasses. The pre-treatment of molasses, which involved enzymatic hydrolysis of sucrose into glucose and

fructose enhanced the substrate's nutritional profile, making it more suitable for *Bti* growth and toxin production.

Furthermore, the results from larvicidal assays confirmed that the presence of calcium chloride in the fermentation medium further improved the efficacy of *Bti* as a biopesticide. After 48 hours of exposure, larvae treated with *Bti* grown in calcium chloride-supplemented media showed the highest mortality rate, indicating the synergistic effect of calcium chloride in promoting larvicidal activity. In addition to pre-treated molasses, other sugarcane-based by-products, such as sugarcane juice and jaggery, also showed potential as substrates for *Bti* cultivation, making them viable alternatives for large-scale production.

The use of these industrial by-products as low-cost substrates not only supports the sustainable production of Bti but also offers an economically viable solution for biopesticide development. Overall, the study underscores the importance of optimizing both the fermentation process and substrate selection to maximize the productivity and efficacy of *Bti*. The combination of fixed-volume fed-batch fermentation, calcium chloride and pretreated molasses provides a cost-effective and environmentally friendly approach for producing highly effective *Bti*-based biopesticides for mosquito control.

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## References

1. Ahmed A.M., Hussein H.I., El-Kersh T.A., Al-Sheikh Y.A., Ayaad T.H., El-Sadawy H.A., Al-Mekhlafi F.A., Ibrahim M.S., Al-Tamimi J. and Nasr F.A., Larvicidal Activities of Indigenous Bacillus thuringiensis Isolates and Nematode Symbiotic Bacterial Toxins against the Mosquito Vector, Culex pipiens (Diptera: Culicidae), *J. Arthropod-Borne Dis*, **11**, 260–277 **(2017)**

2. Ali S.E., El Gedaily R.A., Mocan A., Farag M.A. and El-Seedi H.R., Profiling Metabolites and Biological Activities of Sugarcane (Saccharum officinarum Linn.) Juice and its Product Molasses via a Multiplex Metabolomics Approach, *Molecules*, **24**, 934 **(2019)**

3. Ben Khedher S. et al, Overcome of Carbon Catabolite Repression of Bioinsecticides Production by Sporeless *Bacillus thuringiensis* through Adequate Fermentation Technology, *Biotechnol. Res. Int*, doi: 10.1155/2014/698587 **(2014)**

4. Benelli G., Research in mosquito control: current challenges for a brighter future, *Parasitol. Res*, **114**, 2801–2805 **(2015)**

5. Benelli G. and Beier J.C., Current vector control challenges in the fight against malaria, *Acta Trop*, **174**, 91–96 **(2017)**

6. Bhattacharya S., Sugars, sweeteners, chocolates and sweet snacks, In Snack Foods, Elsevier, 211–249 **(2023)**

7. Brühl C.A., Després L., Frör O., Patil C.D., Poulin B., Tetreau G. and Allgeier S., Environmental and socioeconomic effects of mosquito control in Europe using the biocide Bacillus thuringiensis subsp. israelensis (Bti), *Sci. Total Environ*, **724**, 137800 **(2020)**

8. Çalik G., Berk M., Boyaci F.G., Çalik P., Takaç S. and Özdamar T.H., Pretreatment Processes of Molasses for the Utilization in Fermentation Processes, In Hofman M. and Thonart P., Eds., Engineering and Manufacturing for Biotechnology, Focus on Biotechnology, Kluwer Academic Publishers, Dordrecht, 21–28 **(2002)**

9. Chittimalla Srinivasulu, Pochampally Jalapathi, Thumma Vishnu, Nalla Umapathi and Matta Raghavender, Synthesis, antimicrobial activity and molecular docking study of (e)-4-(4-((3-benzylidene-2-oxoindolin-1-yl) methyl)-1h-1,2,3-triazol-1-yl)benzoic acid derivatives, *Res. J. Chem. Environ.*, **27(9)**, 39-45 **(2023)**

10. Devidas P.C., Pandit B.H. and Vitthalrao P.S., Evaluation of different culture media for improvement in bioinsecticides production by indigenous Bacillus thuringiensis and their application against larvae of Aedes aegypti, *Scientific World Journal*, **2014**, 273030 **(2014)**

11. Ibarra J., Parasporal bodies of Bacillus thuringiensis subsp. morrisoni (PG-14) and Bacillus thuringiensis subsp. israelensis are similar in protein composition and toxicity, *FEMS Microbiol. Lett*, **34**, 79–84 **(1986)**

12. Lacey L.A., Bacillus thuringiensis serovariety israelensis and bacillus sphaericus for mosquito control, *J. Am. Mosq. Control Assoc*, **23**, 133–163 **(2007)**

13. Lees R.S., Knols B., Bellini R., Benedict M.Q., Bheecarry A., Bossin H.C., Chadee D.D., Charlwood J., Dabiré R.K., Djogbenou L., Egyir-Yawson A., Gato R., Gouagna L.C., Hassan M.M., Khan S.A., Koekemoer L.L., Lemperiere G., Manoukis N.C., Mozuraitis R., Pitts R.J., Simard F. and Gilles J.R.L., Review: Improving our knowledge of male mosquito biology in relation to genetic control programmes, *Acta Trop*, **132**, S2–S11 **(2014)**

14. Link H. and Weuster-Botz D., Medium Formulation and Development, In Comprehensive Biotechnology, Elsevier, 119–134 **(2011)**

15. Longobardi G.P., Fed-batch versus batch fermentation, *Bioprocess Eng*., **10**, 185–194 **(1994)**

16. Loutfi H., Fayad N., Pellen F., Le Jeune B., Chakroun M., Benfarhat D., Lteif R., Kallassy M., Le Brun G. and Abboud M., Morphological Study of Bacillus thuringiensis Crystals and Spores, *Appl. Sci*., **11**, 155 **(2020)**

17. Mol K.E.H., Rosalin T. and Elyas K.K., Iron oxide nanoparticles derived from Polyalthia korintii (Dunal) Benth. & Hook. F leaves extract exhibits biological and dye degradation potentials, *Bio Metals*, **37**, 1289–1303 **(2024)**

18. Monette R., Potvin L., Baines D., Laprade R. and Schwartz J.L., Interaction between Calcium Ions and Bacillus thuringiensis Toxin Activity against Sf9 Cells (Spodoptera frugiperda, Lepidoptera), *Applied Enviornmental Microbiology*, **63(2)**, 440-447 **(1997)**

19. Noran E.M. et al, Isolation and characterization of halophilic Bacillus thuringiensis from local Egyptian sites and their potential

against Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae), *Egypt. J. Biol. Pest Control*, **34**, 62 **(2024)**

20. Palma L., Muñoz D., Berry C., Murillo J. and Caballero P., Bacillus thuringiensis Toxins: An Overview of Their Biocidal Activity, *Toxins*, **6**, 3296–3325 **(2014)**

21. Poulin B., Lefebvre G., Hilaire S. and Després L., Long-term persistence and recycling of Bacillus thuringiensis israelensis spores in wetlands sprayed for mosquito control, *Ecotoxicol. Environ. Saf*., **243**, 114004 **(2022)**

22. Ragni A., Thiéry I. and Delécluse A., Characterization of Six Highly Mosquitocidal Bacillus thuringiensis Strains That Do Not Belong to H-14 Serotype, *Curr. Microbiol*, **32**, 48–54 **(1996)**

23. Rechcigl J.E. and Rechcigl N.A., Eds., Insect Pest Management: Techniques for Environmental Protection, CRC Press **(2016)**

24. Rudd S.R., Miranda L.S., Curtis H.R., Bigot Y., Diaz-Mendoza M., Hice R., Nizet V., Park H.W., Blaha G., Federici B.A. and Bideshi D.K., The Parasporal Body of Bacillus thuringiensis subsp. israelensis: A Unique Phage Capsid-Associated Prokaryotic Insecticidal Organelle, *Biology*, **12**, 1421 **(2023)**

25. Singh A., Lal U., Mukhtar H., Singh P., Shah G. and Dhawan R., Phytochemical profile of sugarcane and its potential health aspects, *Pharmacogn. Rev*, **9**, 45 **(2015)**

26. Stanbury P.F., Whitaker A. and Hall S.J., Media for industrial fermentations, In Principles of Fermentation Technology, Elsevier, 213–272 **(2017)**

27. Wirth M.C., Delécluse A. and Walton W.E., Laboratory Selection for Resistance to *Bacillus thuringiensis* subsp. *jegathesan* or a Component Toxin, Cry11B, In Culex quinquefasciatus (Diptera: Culicidae), *J. Med. Entomol*, **41**, 435–441 **(2004)**.

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