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Alternative culture medium based on *Manihot esculenta* (Cassava) for the growth of *Bacillus thuringiensis* aimed at the control of *Aedes aegypti*

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ABSTRACT

The objective was to evaluate alternative culture media for the growth of the *Bacillus thuringiensis* strains BtMA-690, BtMA-750, and BtMA-1114, assessing growth parameters such as pH, spore concentration, optical density, and biomass, as well as selective and quantitative bioassays with *Aedes aegypti* larvae to estimate the lethal concentration (LC_{50} and LC_{90}). The isolates BtMA-690 and BtMA-750 remained around neutrality (pH 7.0) throughout the fermentation period in the conventional media, while in the alternative medium they maintained a pH of 6.0, in contrast, BtMA-1114 showed a pH variation (6.0–8.0) across the media tested. In terms of spore concentration, for BtMA-690 and BtMA-750, the cassava-based alternative medium showed higher spores/mL values compared to the conventional media. Absorbance readings were higher for the conventional Nutrient Broth medium for isolates BtMA-690 and BtMA-750, whereas for BtMA-1114 the highest values were obtained with the Luria Bertani (LB) medium and the cassava-based alternative medium. Regarding biomass after 72 h, the LB medium showed higher values for all three isolates tested. The selective bioassays showed 100 % larval mortality within 24 h for the isolates BtMA-690 and BtMA-750 in both the conventional Nutrient Broth and the cassava-based alternative medium. The lowest LC_{50} and LC_{90} values obtained for the isolates were 0.0102 mg/mL and 0.0253 mg/mL, respectively, for BtMA-690. The use of alternative media improved bacterial yield, making them competitive in the market and environmentally beneficial, with potential application in the production of bioproducts.

1. Introduction

The control of mosquito vectors of human pathogens, such as *Aedes aegypti* (Linnaeus, 1762), the vector of dengue, chikungunya, and Zika, is a challenge for national public health. It is considered one of the main arbovirus vectors in Brazil, as it transmits different viruses that affect thousands of Brazilians (Ferreira and Castro, 2016; Lima-Camara, 2016; BRASIL/MS. Ministério da Saúde, 2025). New alternatives for controlling these species have been discussed, as several studies have demonstrated resistance in mosquito populations to different classes of chemical insecticides (Flores-Suarez et al., 2016; Moyes et al., 2017; Vargas et al., 2022). Therefore, there is a need to replace chemical insecticides, especially with biological insecticides, which are considered environmentally safe (Scopel and Roza-Gomes, 2011; Guo et al., 2015; Brühl et al., 2020).

Bacillus thuringiensis (Bt) (Berliner, 1911) is one of the main active agents used in the production of biolarvicides for biological control due to its insecticidal properties. It produces protein crystals composed of δ-endotoxins, Cry and/or Cyt proteins, which are toxic to a wide range of insects, although specifically targeted to each group. Bt offers multiple

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advantages, especially when compared with chemical control, which causes serious problems for human health and the environment (Alves, 1998; Ben-Dov, 2014; Heckel, 2020; Peña et al., 2022; Crickmore et al., 2023). Due to the high effectiveness of *B. thuringiensis* in vector control, several studies have sought alternative methods for producing this bacterium under laboratory conditions (Pereira and Martins, 2016; Mourão, 2017; Lobo et al., 2018; Vieira-Neta et al., 2020). Selecting an appropriate culture medium is extremely important for successful bacterial production; optimal growth of the strains should enable maximum production of toxic crystals at minimal cost (Couch, 2000; Mourão, 2017).

Commercial culture media for *B. thuringiensis* are expensive, making the production of bioproducts difficult (Mourão, 2017; Viana et al., 2021a). Therefore, research has sought more economical alternatives, especially for regional and small-scale production, taking advantage of Brazil's agro-industrial diversity (Angelo et al., 2010; Pereira and Martins, 2016; Viana et al., 2021a). These media are used for the cultivation and isolation of microorganisms, and recent studies have identified more efficient and sustainable alternatives to increase Bt production on a large scale (Santos et al., 2022). Alternative culture media are innovative options for the cultivation of microorganisms, offering advantages over traditional media such as reduced cost, being cheaper than conventional media, sustainability, by reducing environmental impact, and flexibility in adapting to the specific needs of each bacterium (Silva et al., 2013). In Brazil, cassava (macaxeira) is widely used as a food product and in agro-industrial applications; therefore, its chemical components have been extensively studied. According to data from the Brazilian Food Composition Table (TABNUT), raw cassava contains significant amounts of minerals. The root contains approximately 65 mg of calcium, 86 mg of magnesium, and 110 mg of phosphorus. The presence of potassium (1106 mg) is noteworthy, in addition to 57 mg of sodium and 1.39 mg of zinc (Tabela Brasileira de Composição de Alimentos – TABNUT, 2025). Thus, the mineral composition of raw cassava root indicates a promising potential as an alternative growth medium for *B. thuringiensis*, since this microorganism depends on adequate sources of macro and micronutrients for vegetative growth, sporulation, and the production of insecticidal toxins (Ernandes et al., 2013; Alamu et al., 2020).

For the efficient cultivation of *B. thuringiensis* in the laboratory or on an industrial scale, it is essential to choose an appropriate culture medium that promotes both vegetative growth and sporulation, as well as the production of protein crystals. The cultivation of Bt for bioinsecticide production has traditionally been carried out in synthetic and complex media; however, the high cost of these media can hinder large-scale production. Thus, there is growing interest in developing alternative culture media using agro-industrial residues and low-cost materials that allow effective growth, sporulation, and Cry toxin production. Therefore, the use of alternative culture media, such as agro-industrial residues and food by-products, has proven to be viable and effective for cultivating Bt, as these media offer a sustainable and economical alternative for bioinsecticide production (Polanczyk et al., 2004; Lopes et al., 2005; Mummigatti and Rajashekhar, 2012; Silva et al., 2013). The Entomopathogenic Bacilli Bank of Maranhão (BBENMA) is one of the largest in the Northeast region of Brazil, maintaining strains with high toxicity to disease-vector larvae, which were isolated from different biomes (Soares-da-Silva et al., 2017; Lobo et al., 2018; Viana et al., 2021b; Vieira-Neta et al., 2020). Due to the high toxicity of these strains to arbovirus vector larvae, it is necessary to optimize their growth in the laboratory using accessible alternative methods, in order to increase the production of insecticidal toxins and support the development of a future low-cost bioproduct for the control of these vectors. Therefore, the objective was to evaluate alternative culture media for the growth of *B. thuringiensis* to be used in mosquito vector control.

2. Materials and methods

2.1. Selection of Bacillus thuringiensis isolates

For fermentation using conventional and alternative culture media, three strains, BtMA-690, BtMA-750, and BtMA-1114, from the BBENMA were selected. These strains were isolated from soil and dead insects and originated from the Cerrado and Amazon biomes. They exhibited higher toxicity against *A. aegypti* larvae, with lower LC_{50} values of 0.004 mg/L (0.003–0.006), 0.041 mg/L (0.030–0.063), and 0.025 mg/L (0.019–0.030), respectively, and also showed the presence of insecticidal toxins with different gene profiles, including combinations of mosquitocidal genes (Soares-da-Silva et al., 2017; Viana et al., 2021b; Vieira-Neta et al., 2020).

The evaluated isolates exhibited different toxin gene profiles, including combinations of diptera-specific genes similar to those described for *B. thuringiensis* subsp. *israelensis* (Bti), as previously reported in the literature (Soares-da-Silva et al., 2017; Vieira-Neta et al., 2020). The isolates BtMA-690 and BtMA-750 displayed dipteran-specific genes consistent with classical mosquitocidal profiles. In turn, the isolate BtMA-1114 showed positive amplification for the genes *cry4Aa*, *cry4Ba*, *cry11Aa*, and *cyt1Aa*, with no amplification for *cry10Aa*, *cry11Ba*, *cyt1Ab*, and *cry2Aa*, indicating a distinct toxic profile (Table 1). The isolates were also sequenced for a 300-base-pair region of the *cyt1Aa* gene, revealing that BtMA-690, BtMA-750, and BtMA-1114 exhibited 100 % similarity among themselves (Soares-da-Silva et al., 2022). This result demonstrates the high conservation of this gene among the evaluated isolates.

2.2. Selection of alternative and conventional culture media for Bacillus thuringiensis fermentation

Seven culture media were used: four commonly cited in the literature for the growth of *B. thuringiensis* and three alternative media based on cassava (*Manihot esculenta*) at 0.25 % (Table 2). All culture media were adjusted to the optimal pH (7.0) using 0.5 M sodium hydroxide and sterilized in an autoclave at 120 °C for 15 min.

The crude starches were crushed and dissolved in water (1 % m/v) at room temperature for 24 h. The pH was adjusted to 7.0 with NaOH solution, and NaCl was then added. The subsequent steps included filtration and precipitation in ethanol. The precipitated material was washed with ethanol and acetone and dried at 40 °C. To remove sodium ions, the starch was redissolved in distilled water and precipitated as previously described.

2.3. Bacillus thuringiensis inoculum in alternative and conventional media

The inoculum was obtained from cultures of the *B. thuringiensis* strains BtMA-690, BtMA-750, and BtMA-1114 from BBENMA, grown on Nutrient Agar for 24 h at 30 °C. After this period, the cell mass was scraped off and added to 25 mL of Nutrient Broth (NB), under constant agitation at 200 rpm in a shaker incubator (Novatecnica) for 24 h at 30 °C.

After the 24-h period, a volume of 20 mL of the total culture grown in NB was used and added to the alternative and conventional culture media under testing, for analysis of growth parameters at 8-h intervals. For bacterial growth, three replicates were prepared for each medium, each in an Erlenmeyer flask with 200 mL, totaling 600 mL per culture medium. The media were previously sterilized and, after the addition of *B. thuringiensis*, they were kept under constant agitation at 200 rpm in a shaker (Novatecnica) for 72 h at 30 °C.

Table 1
Bacillus thuringiensis strains from BBENMA, with their respective origin data and gene content, selected for growth parameter studies in culture media.

isolate	Isolation/substrate	City (MA, Brazil)	Diptera-specific genes								
			cry4Aa	*cry4Ba*	*cry10Aa*	*cry11Aa*	*cry11Ba*	*cyt1Aa*	*cyt1Ab*	*cyt2Aa*	*chi*
BtMA-690	Cerrado/Soil	Duque Bacelar	+	+	+	+	+	+	+	+	+
BtMA-750	Amazon/Soil	São José de Ribamar	+	+	+	+	+	+	+	+	+
BtMA-1114	Cerrado/Insects	Mirador	+	+		+		+			
Bti[a]	–	–	+	+	+	+	+	+	+	+	+

[a] *Bacillus thuringiensis* subsp. *israelensis* – Bti T14 001 (Institut Pasteur, France), provided by the Laboratory of Bacterial Genetics and Applied Biotechnology (LGBBA), FCAV/UNESP, Jaboticabal, São Paulo, Brazil. (+) presence of the gene studied. Source: Soares-da-Silva et al., 2017; Vieira-Neta et al. (2020).

Table 2
Conventional and alternative (0.25 %) culture media evaluated for the growth of *Bacillus thuringiensis*.

Culture Medium		Composition
Standard		
01	Luria Bertani (LB)	(1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl)
02	Luria Bertani + Salts	(1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl) + salt solution ($FeSO_4$, $ZnSO_4$, $MnSO_4$, $MgSO_4$)
03	Nutrient Broth (NB)	(Meat extract – 1.0 g/L, Yeast extract – 2.0 g/L, Peptone – 5.0 g/L, Sodium chloride – 5.0 g/L)
04	Nutrient Broth + Salts	(Meat extract – 1.0 g/L, Yeast extract – 2.0 g/L, Peptone – 5.0 g/L, Sodium chloride – 5.0 g/L) + salt solution ($FeSO_4$, $ZnSO_4$, $MnSO_4$, $MgSO_4$)
Alternative		
01	Cassava (MX) (*Manihot esculenta*)	Purified cassava (*M. esculenta*) starch
02	Cassava (*M. esculenta*) + Salts	Purified cassava starch + salt solution ($FeSO_4$, $ZnSO_4$, $MnSO_4$, $MgSO_4$)
03	Cassava (*M. esculenta*) + Salts and NaCl	Purified cassava starch + salt solution ($FeSO_4$, $ZnSO_4$, $MnSO_4$, $MgSO_4$) + 0.5 % NaCl

2.4. Microbial growth parameters of Bacillus thuringiensis in alternative and conventional media

Growth parameter evaluations were performed every 8 h at the following time intervals: 8, 16, 24, 32, 40, 48, 56, 64, and 72 h. At each interval, a 1 mL aliquot was taken from each sample to analyze the following parameters: (I) pH determination; (II) Spore concentration; (III) Optical density (OD). Finally, after 72 h, the cell mass from all culture media was obtained.

Aliquots from all fermented media of the three isolates were collected at all time intervals. For each observation time, pH was determined using sterile pH indicator paper strips (Kasvi) ranging from 0 to 14. Spore concentration (spores/mL) was determined by counting viable spores on Petri dishes (Alves and Moraes, 1998). Optical density readings were performed at 600 nm using a spectrophotometer (Agilent Technologies/Cary 60 UV–Vis). At the time of measurement, the instrument was blanked using the corresponding culture media without the addition of the isolates.

For cell mass quantification, the entire 600 mL volume of each fermented culture (culture medium + bacterial cells) from each isolate grown in the different tested media was centrifuged at 5000 rpm for 20 min, followed by three washes with sterilized distilled water. The supernatant was discarded, and the pellet was frozen and subsequently lyophilized in an Enterprise (Terroni) freeze dryer. The resulting material was used to calculate cell mass, which was expressed in g/L.

2.5. Bioassays with Bacillus thuringiensis in alternative and conventional media

The bioassays were conducted at the Medical Entomology Laboratory of UEMA, Caxias campus, under a temperature of 28 ± 2 °C and relative humidity of 85 %. Third-instar *A. aegypti* larvae were used, obtained from the colony maintained in the insectary of the institution's laboratory.

2.5.1. Selective bioassays

The cultures obtained from the three isolates in the different culture media were tested against *A. aegypti* larvae. The bioassays consisted of one trial, with triplicate 50-mL plastic cups containing 10 mL of water, 10 third-instar *A. aegypti* larvae, and 1 mL of the solution prepared after the lyophilization process of the isolates from each fermented medium (0.05 mg/mL or 50 mg/L). In each bioassay, a negative control was prepared, consisting of a single replicate without bacterial inoculation.

Mortality readings of the larvae were taken at 24 and 48 h after the application of the bacterium. In the readings, larvae that did not respond to touch with sterile sticks were considered dead. All living and dead individuals were counted, thus obtaining the larval mortality percentage (Dulmage et al., 1990).

2.5.2. Quantitative bioassays for determining the lethal concentration (LC_{50} and LC_{90})

Quantitative bioassays were conducted with the isolates grown in the culture media that yielded the highest spore concentration (spores/mL) and the greatest mortality in the selective bioassays, under the same laboratory conditions. For these bioassays, the lyophilized bacterial suspension of the three isolates fermented in the conventional and alternative media tested was used for the subsequent preparation of the solutions to be applied to the larvae.

These bioassays were carried out following the WHO - World Health Organization (2005) protocol and consisted of preparing two serial solutions, 5.0 mg/mL (solution I) and 0.05 mg/mL (solution II), made from the lyophilized isolate. Eight to eleven concentrations prepared from solution II (0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.008, 0.005, 0.004, 0.003, and 0.002 mg/mL) were used, each tested in five replicates and in three repetitions (triplicate) for the three Bt isolates in the media with the highest yield. Each repetition consisted of five plastic cups with a capacity of 200 mL, containing 150 mL of water, 20 third-instar *A. aegypti* larvae, and the bacterial dose to be tested. The standard strain Bti VectoBac® WG was used as a positive control and was evaluated under the same conditions as the isolates. As a negative control, one cup containing only water and larvae, without bacterial application, was included in each repetition. After the application of the bacillus, larval survival readings were taken at 24, 48, and 72 h intervals.

2.6. Data analysis

All statistical analyses of the growth parameters were carried out in the R environment using the tidyverse and rstatix packages. The tests applied included the non-parametric Kruskal–Wallis test for group comparison, followed by Dunn's post-hoc test with Benjamini–Hochberg adjustment for multiple comparisons when appropriate.

The determination of the Lethal Concentration (LC_{50} and LC_{90}) was performed through Probit analysis ($p < 0.05$), using the POLO PLUS software (LeOra, 2005), calculated from larval mortality data obtained in the quantitative bioassays (Finney, 1981; Haddad, 1998).

3. Results

3.1. pH determination

For BtMA-690, it was observed that, when comparing the culture media studied, during the fermentation process in NB and NB + salts media, the isolate remained neutral up to 64 h of the experiment with pH 7.0. At 72 h, the pH increased, becoming slightly basic (pH 8.0). In the growth using LB and LB + salts media, there was a decline in pH up to 24 h of fermentation; after this period, the values increased, remaining neutral up to 56 h, and after 64 h of the experiment, the pH rose to 8.0. In the alternative media MX, MX + salts, and MX + salts + NaCl, the isolate maintained the same value throughout the entire experiment, with pH 6.0 (Table 3). The Kruskal–Wallis test indicated a significant difference among the media ($\chi^2 = 38.644$; df = 6; $p < 0.000001$). Dunn's post-hoc test showed lower pH variation in the MX, MX + salts, and MX + salts + NaCl media compared to NB and NB + salts ($p < 0.001$).

In the fermentation with the BtMA-750 isolate, it was observed that in the NB and NB + salts culture media, the isolate maintained a neutral pH up to 64 h of the experiment, increasing to 8.0 at 72 h. In the LB medium, the pH dropped to 6.0 during the first 24 h of isolate growth; from 32 to 56 h the values remained at pH 7.0, and after 64 h the pH increased to 8.0, remaining so until the end of the experiment. In the alternative MX medium, the isolate remained at pH 6.0 up to 64 h of fermentation, increasing to 7.0 at 72 h, while in the MX + salts and MX + salts + NaCl media it maintained a pH of 6.0 throughout the fermentation process (Table 3). The Kruskal–Wallis test indicated a significant difference among the mean pH values of the media ($\chi^2 = 38.644$; df = 6; $p < 0.000001$). Dunn's post-hoc test revealed that the significant differences occurred mainly between the NB, NB + salts, LB and LB + salts groups in comparison with MX, MX + salts and MX + salts + NaCl, with no relevant differences among similar media.

In the experiment with BtMA-1114, a greater variation in pH values was observed across all tested media. The NB and NB + salts media started the fermentation process with pH 7.0, then showed pH 6.0 from 16 to 32 h, after which the pH returned to neutrality until 64 h, rising to pH 8.0 at 72 h. In the LB culture medium, the isolate showed pH 6.0 up to 24 h of fermentation, returning to pH 7.0 between 32 and 40 h and reaching pH 8.0 after 48 h, maintaining this value until 72 h. In the MX medium, the pH dropped to 6.0 at the first fermentation time point and subsequently remained neutral until the end of the experiment; in the MX + salts and MX + salts + NaCl media, the pH ranged from 6.0 at the beginning to 7.0 at the end of the experiment with the isolate (Table 3). The Kruskal–Wallis test did not detect any statistically significant difference among the pH values of the evaluated media ($\chi^2 = 4.08$; df = 6; $p = 0.665$).

Table 3
Descriptive analysis of pH variations during the fermentation process of *Bacillus thuringiensis* isolates evaluated in different culture media.

Isolate	Medium	n	Mean pH	Standard Deviation	Minimum	Maximum
BtMA-690	NB	9	7.11a	0.33	7	8
	NB + salts	9	7.11a	0.33	7	8
	LB	9	6.89a	0.78	6	8
	LB + salts	9	6.89a	0.78	6	8
	MX	9	6b	0	6	6
	MX + salts	9	6b	0	6	6
	MX + salts + NaCl	9	6b	0	6	6
BtMA-750	NB	9	7.111a	0.333	7	8
	NB + salts	9	7.111a	0.333	7	8
	LB	9	6.889a	0.782	6	8
	LB + salts	9	6.889a	0.782	6	8
	MX	9	6.111b	0.333	6	7
	MX + salts	9	6.000b	0.000	6	6
	MX + salts + NaCl	9	6.000b	0.000	6	6
BtMA-1114	NB	9	6.78a	0.67	6	8
	NB + salts	9	6.78a	0.67	6	8
	LB	9	7.11a	0.93	6	8
	LB + salts	9	7.11a	0.93	6	8
	MX	9	6.89a	0.33	6	7
	MX + salts	9	6.78a	0.44	6	7
	MX + salts + NaCl	9	6.56a	0.53	6	7

BtMA = *Bacillus thuringiensis* from Maranhão; NB = Nutrient Broth; LB = Luria Bertani; MX = Cassava.

3.2. Spore concentration

For the BtMA-690 isolate, it was observed that the alternative medium MX and its variants showed higher spore/mL values when compared to the conventional media. In MX + salts + NaCl, the isolate reached its highest spore production peak between 24 and 48 h of the experiment, with 8.30 x 10^7 at 32 h of fermentation. In MX + salts, the highest concentration (5.80 x 10^7 spores/mL) occurred at 48 h, and in MX similarly high values were recorded, with 5.80 x 10^7 at 24 h. In the NB medium, BtMA-690 showed its highest spore concentration at the end of the experiment, 6.60 x 10^7 at 56 h and 5.10 x 10^7 at 64 h in NB and NB + salts, respectively. The LB medium showed lower values compared to the other media throughout the fermentation process, with its highest concentration reaching only 1.0 x 10^5 spores/mL (Fig. 1). The Kruskal–Wallis test indicated a significant difference in spore quantity among the culture media ($\chi^2 = 38.42$; df = 6; $p = 9.3x10^{-7}$). Dunn's post-hoc test showed that LB and LB + salts produced fewer spores than NB and NB + salts ($p < 0.005$); however, no significant difference was observed between NB and MX or their variants.

The fermentation of the BtMA-750 isolate also showed higher spore concentrations (spores/mL) for the alternative medium compared to the conventional media. In MX and its variants, the isolate exhibited elevated spore concentrations from 24 to 48 h of the experiment. In MX and MX + salts + NaCl, the 24-h interval showed the highest concentrations, 6.20 x 10^7 and 7.0 x 10^7, respectively. In MX + salts, the highest value was observed at 48 h, with 7.30 x 10^7. In the NB medium, the isolate showed the highest spore concentrations at the final fermentation time, with 7.30 x 10^7 and 4.20 x 10^7 in NB and NB + salts, respectively. The LB medium also showed lower values compared to the other media analyzed for BtMA-750 throughout the experiment, with its highest concentration reaching only 8.80 x 10^4 at 8 and 24 h in LB + salts and LB, respectively (Fig. 2). The Kruskal–Wallis test indicated statistically significant differences in spore production among the media ($p = 9.3$ x 10^{-7}). Dunn's post-hoc test revealed significant differences between NB and LB ($p = 4.29$ x 10^{-4}), NB and LB + salts ($p = 2.17$ x 10^{-3}), NB + salts and LB ($p = 4.29$ x 10^{-4}), and NB + salts and LB + salts ($p = 2.16$ x 10^{-3}). No significant differences were observed between NB and MX or its variations.

For BtMA-1114, the spore concentrations (spores/mL) were lower in all culture media evaluated when compared to the other isolates. The highest value was observed only at the end of fermentation in the NB medium, reaching 1.40 x 10^7 at 72 h; in NB + salts, the isolate showed concentrations that did not exceed 7.0 x 10^2. In LB medium, the highest concentration was recorded at 16 h, with 3.0 x 10^5, while in LB + salts the maximum concentration was 6.20 x 10^4 at 24 h of the experiment. In the alternative medium, the isolate showed its highest concentrations in MX + salts and MX + salts + NaCl at the first fermentation time, with 4.20 x 10^4 and 5.50 x 10^4, respectively; in MX, only 1.40 x 10^4 spores/mL were observed at 48 and 72 h (Table 4). The Kruskal–Wallis test revealed statistically significant differences in the mean number of spores among the media tested, with a chi-square value of 31.3, 6 degrees of freedom, and an extremely low p-value ($p = 0.000022$). Dunn's post-hoc test, adjusted using the Benjamini–Hochberg method, indicated that production in NB was significantly higher than in NB + salts ($p = 2.22x10^{-4}$), and NB + salts differed significantly from the LB

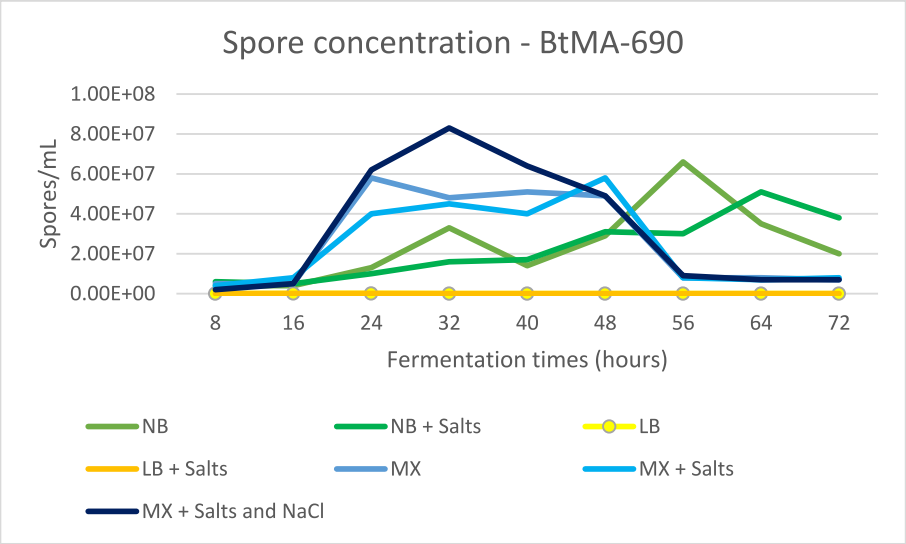

Fig. 1. Spore/crystal concentration of the BtMA-690 isolate grown in conventional and alternative culture media at different fermentation times. NB – Nutrient Broth; LB – Luria Bertani; MX – Cassava.

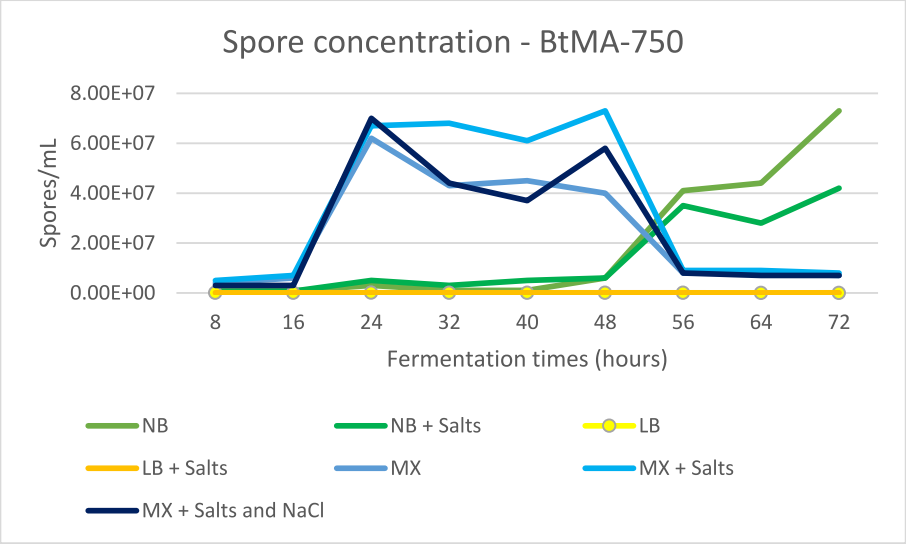

Fig. 2. Spore/crystal concentration of the BtMA-750 isolate grown in conventional and alternative culture media at different fermentation times. NB – Nutrient Broth; LB – Luria Bertani; MX – Cassava.

Table 4
Descriptive analysis of the spore concentration (spores/mL) of the BtMA-1114 isolate by culture medium.

Medium	n	Mean	Standard Deviation	Minimum	Maximum
NB	9	2.468.156a	4.888.941	1.300	14.000.000
NB + salts	9	167c	224	0	700
LB	9	45.267b	96.572	1.100	300.000
LB + salts	9	11.956b	19.124	1.800	62.000
MX	9	9.389b	3.741	5.500	14.000
MX + salts	9	6.178b	13.457	900	42.000
MX + salts + NaCl	9	10.844b	17.359	200	55.000

NB–Nutrient Broth; LB–Luria Bertani; MX–Cassava.

medium ($p = 2.18x10^{-4}$) as well as from MX and its variants ($p = 1.33x10^{-4}$).

3.3. Optical density

For the BtMA-690 isolate, absorbance readings remained practically constant between 24 and 40 h for all tested media. However, the culture medium that showed the highest optical density values was the conventional NB medium, exhibiting higher readings at the end of the experiment, with values of 1.7596 at 64 h and 1.4693 after 72 h, and at the beginning of the tests with the NB + salts medium, with an absorbance of 1.2291 after 8 h of the experiment (Table 5). The Kruskal–Wallis test indicated a significant difference among the media ($\chi^2 = 24.066$; df = 6; $p = 0.0005$). Dunn's post-hoc test showed differences between NB + salts and LB, LB + salts, MX, and MX + salts ($p < 0.05$).

For the BtMA-750 isolate, the readings also remained stable between 24 and 40 h of fermentation for all tested culture media. With this isolate, it was observed that the readings at the beginning of the fermentation process in the NB medium were quite high, showing an absorbance of 2.0716 8 h after the start of the experiment. In the NB + salts medium, however, the highest reading occurred at the end of the

Table 5
Descriptive statistics of Optical Density (OD) by culture medium for the *Bacillus thuringiensis* isolates.

Isolate	Medium	n	Mean OD	Standard Deviation	Minimum	Maximum
BtMA-690	NB	9	0.9a	0.443	0.423	1.76
	NB + salts	9	0.96a	0.136	0.796	1.229
	LB	9	0.559b	0.345	0.083	0.935
	LB + salts	9	0.501b	0.273	0.068	0.962
	MX	9	0.575c	0.189	0.201	0.691
	MX + salts	9	0.48c	0.163	0.134	0.62
	MX + salts + NaCl	9	0.501b	0.281	0.015	0.806
BtMA-750	NB	9	0.989a	0.430	0.675	2.072
	NB + salts	9	0.988a	0.246	0.768	1.596
	LB	9	0.709a	0.349	0.065	1.060
	LB + salts	9	0.622a	0.434	0.030	1.023
	MX	9	0.513b	0.253	0.034	0.714
	MX + salts	9	0.480b	0.190	0.127	0.598
	MX + salts + NaCl	9	0.652b	0.156	0.294	0.809
BtMA-1114	NB	9	0.73 ab	0.235	0.372	1.1579
	NB + salts	9	0.819a	0.228	0.5024	1.2337
	LB	9	0.45b	0.529	0.0662	1.7674
	LB + salts	9	0.496b	0.239	0.0949	0.8289
	MX	9	0.791 ab	0.24	0.3638	0.9673
	MX + salts	9	0.989 ab	0.365	0.4365	1.3629
	MX + salts + NaCl	9	0.602 ab	0.369	0.2571	1.1307

BtMA = *Bacillus thuringiensis* from Maranhão; NB = Nutrient Broth; LB = Luria Bertani; MX = Cassava.

test, with an absorbance of 1.596 at 72 h (Table 5). The Kruskal–Wallis test confirmed differences among the OD means of the media ($\chi^2 = 27.232$; $p = 0.000131$), and Dunn's post-hoc test indicated that the NB and NB + salts media have significantly higher optical density than the MX medium and its variations.

In the fermentation with BtMA-1114, the values differed from those of the other isolates, showing the highest absorbance reading for the conventional LB medium, at 1.7674 after 8 h of the experiment. However, after 72 h, the media that displayed the highest readings were the MX medium and its variants, with 1.3456 in the MX + salts medium and 0.9596 in the MX medium. The alternative MX + salts + NaCl medium also showed elevated values at the 24- and 40-h intervals of the fermentation process (Table 5). The OD analysis reveals that cell density varied significantly among the different media (Kruskal–Wallis test: $\chi^2 = 18.2$; $p = 0.00577$). Dunn's post-hoc test, with Benjamini–Hochberg correction, indicated statistically significant differences ($p < 0.0524$) between NB + salts and the LB media.

3.4. Cell mass (biomass)

Regarding cell mass after 72 h of the experiment, the LB culture medium showed the highest values for the three isolates tested. In the LB + salts medium, BtMA-690 reached 0.63 g/L, BtMA-750 reached 0.83 g/L, and BtMA-1114 reached 0.98 g/L, while in the LB medium the three isolates exhibited cell masses of 0.79 g/L, 0.80 g/L, and 0.75 g/L, respectively. This was followed by the NB and NB + salts media, in which isolates BtMA-690 and BtMA-750 showed values below 0.5 g/L; however, BtMA-1114 showed higher values, reaching 0.62 g/L and 0.58 g/L in the NB + salts and NB media, respectively. In the alternative MX medium and its variants, the three isolates showed lower biomass values, with the MX + salts + NaCl medium standing out, with cell masses of 0.51 g/L, 0.32 g/L, and 0.41 g/L for isolates BtMA-690, BtMA-750, and BtMA-1114, respectively (Fig. 3).

3.5. Selective bioassays

The selective bioassays showed high mortality in *A. aegypti* larvae for two isolates in five of the culture media tested (both conventional and alternative). In the NB and NB + salts media, isolates BtMA-690 and BtMA-750 reached 100 % mortality in just 24 h of the experiment, while BtMA-1114 showed 73.3 % mortality at 48 h in the NB medium and only 6.6 % in the NB + salts medium within the same time interval. In the LB culture medium, isolate BtMA-690 showed 13.3 % and 50 % mortality after 48 h in the LB and LB + salts media, respectively. Isolate BtMA-750 showed 63.3 % and 33.3 % mortality under the same growth conditions, and BtMA-1114 reached only 33.3 % in LB + salts. Larval mortality values were also high in the MX, MX + salts, and MX + salts + NaCl media, with 100 % mortality in just 24 h of the experiment for isolates BtMA-690 and BtMA-750, whereas BtMA-1114 showed larval mortality below 10 % (Table 6).

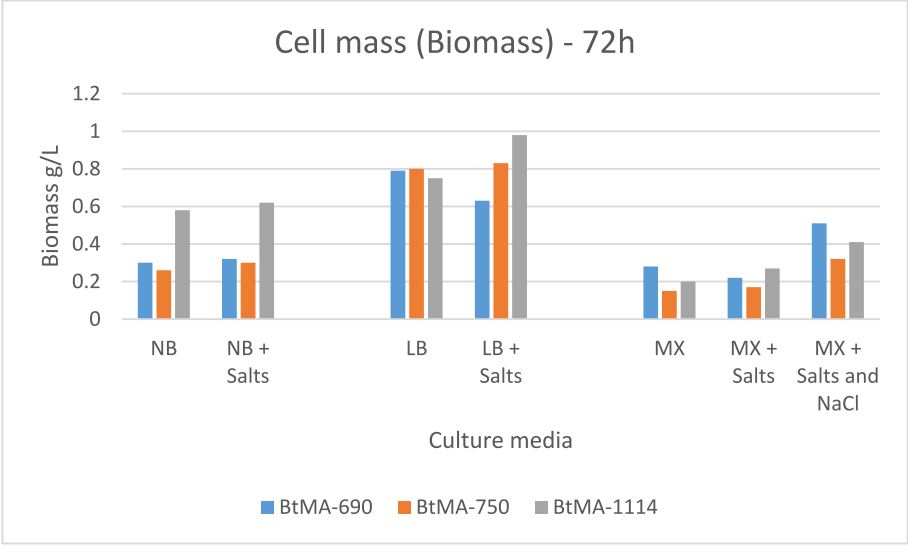

Fig. 3. Cell mass (biomass) of *Bacillus thuringiensis* isolates grown in conventional and alternative culture media after 72 h of fermentation.
NB – Nutrient Broth; LB – Luria Bertani; MX – Cassava.

Table 6
Mortality of *Aedes aegypti* larvae in selective bioassays with *Bacillus thuringiensis* isolates grown in conventional and alternative culture media.

ISOLATE	CULTURE MEDIUM	MORTALITY (%)	
		24 h	48 h
BtMA-690	NB	100	–
	NB + Salts	100	–
	LB	6.6	13.3
	LB + Salts	40	50
	MX	100	–
	MX + Salts	100	–
	MX + Salts/NaCl	100	–
BtMA-750	NB	100	–
	NB + Salts	100	–
	LB	60	63.3
	LB + Salts	33.3	33.3
	MX	100	–
	MX + Salts	100	–
	MX + Salts/NaCl	100	–
BtMA-1114	NB	70	73.3
	NB + Salts	3.3	6.6
	LB	0	0
	LB + Salts	33.3	33.3
	MX	10	10
	MX + Salts	3.3	3.3
	MX + Salts/NaCl	0	0

BtMA = *Bacillus thuringiensis* from Maranhão; NB = Nutrient Broth; LB = Luria Bertani; MX = Cassava.

3.6. Quantitative bioassays (lethal concentration – LC_{50} and LC_{90})

The quantitative bioassays with isolates BtMA-690 and BtMA-750 were carried out using the strains grown in the alternative macaxeira-based culture medium, in which they showed higher spore concentrations (spores/mL) and 100 % mortality in the selective bioassays. For BtMA-1114, the same bioassay was conducted using the isolate obtained from fermentation in the conventional Nutrient Broth medium, which showed a higher spore count and larval mortality above 70 % in the previous bioassay.

A similarity was observed among the concentrations required for the isolates to cause 50 % mortality (LC_{50}) in the *A. aegypti* population, which ranged from the lowest value of 0.0102 mg/mL, obtained with the BtMA-690 isolate at the 72-h interval, to the highest value of 0.0200 mg/mL of *B. thuringiensis* spores/crystals, recorded for BtMA-750 at the 24-h reading. The lowest and highest concentrations required to cause 90 % mortality (LC_{90}) in the *A. aegypti* population were obtained with the BtMA-690 isolate, with 0.0253 mg/mL and 0.0554 mg/mL of Bt spores/crystals after 72 and 24 h of the experiment, respectively (Table 7).

The lowest LC_{50} and LC_{90} values were obtained with the standard Bti strain used as the control, with 0.0036 mg/mL and 0.0073 mg/mL, respectively, both at 72 h of the bioassay. This strain proved to be more toxic when compared to the two isolates tested (Table 7).

The BtMA-1114 isolate showed mortality well below 50 % for all concentrations tested in the three readings performed in the bioassay, making it impossible to estimate the LC_{50} and LC_{90} for this strain.

4. Discussion

With the alternative media tested in this research, it was observed that the cassava-starch-based medium showed excellent Bt growth, displaying a high number of spores/mL produced by the bacterium and high larval mortality for the two isolates studied. The growth and multiplication of *B. thuringiensis*, which are essential for the production of bioinsecticides, depend directly on the balanced presence of macro and micronutrients in the culture medium. These nutrients provide carbon and nitrogen for biomass and spore synthesis, as well as mineral ions that act as enzymatic cofactors. Macronutrients such as carbon and nitrogen are crucial for energy generation and the construction of cellular structures, whereas micronutrients such as $FeSO_4$, $ZnSO_4$, $MnSO_4$, and $MgSO_4$ play catalytic roles in essential metabolic pathways, influencing both bacterial growth and sporulation. Carbon and nitrogen serve as energy and structural sources, while mineral salts function as cofactors (Mourão, 2017; Tortora et al., 2016). Classical studies demonstrate that a balanced combination of these nutritional sources in alternative culture media can be as effective as traditional media for Bt, including approaches that seek to use agro-industrial by-products such as cassava starch or cassava wastewater (manipueira) to reduce costs without compromising microbial development or the production of spores or entomopathogenic toxins (Ernandes et al., 2013).

Table 7
Lethal Concentration (LC_{50} and LC_{90}) in mg/mL at 24, 48, and 72 h for *Bacillus thuringiensis* isolates with toxicity to *Aedes aegypti* larvae.

Isolate	LC_{50} (CI 95 %)	LC_{90} (CI 95 %)	Slope ± SE	χ2 (DF = 3)
	24 h			
BtMA-690	0.0181 (0.0157–0.0208)a	0.0554 (0.0452–0.0727)a	2.632 (0.113)	4.8905
BtMA-750	0.0200 (0.0185–0.0218)a	0.0524 (0.0461–0.0610)a	3.069 (0.140)	2.178
Bti	0.0040 (0.0038–0.0042)b	0.0086 (0.0080–0.0093)b	3.891 (0.170)	0.717
	48 h			
BtMA-690	0.0132 (0.0125–0.0140)a	0.0323 (0.0297–0.0356)a	3.307 (0.152)	1.460
BtMA-750	0.0163 (0.0144–0.0189)a	0.0419 (0.0341–0.0549)a	3.136 (0.146)	3.3544
Bti	0.0037 (0.0035–0.0039)b	0.0078 (0.0073–0.0085)b	3.889 (0.174)	1.616
	72 h			
BtMA-690	0.0102 (0.0087–0.0119)a	0.0253 (0.0208–0.0328)a	3.247 (0.136)	6.3384
BtMA-750	0.0145 (0.0125–0.0167)b	0.0397 (0.0330–0.0500)b	2.927 (0.122)	6.4494
Bti	0.0036 (0.0032–0.0039)c	0.0073 (0.0067–0.0081)c	4.115 (0.356)	1.941

LC_{50} e LC_{90} = Lethal Concentration; CI=Confidence interval; Slope±SE=Slope ± Standard Error; χ2 = Chi-square; DF = Degree of freedom; BtMA = *Bacillus thuringiensis* from Maranhão; Bti = *Bacillus thuringiensis* var. *israelensis*.

The results obtained by Alamu et al. (2020) showed that potassium was the most abundant macronutrient in yellow-fleshed cassava roots, followed by calcium and magnesium, while sodium presented the lowest levels. Among the micronutrients, manganese stood out as the most abundant element, followed by iron and zinc, whereas molybdenum and cobalt occurred at low concentrations. Overall, a greater accumulation of macro and micronutrients was observed in the experimental set involving cassava, indicating a genotypic influence on the mineral composition of the roots. These findings reinforce the potential of cassava as a relevant source of essential minerals, highlighting its importance in the production of biotechnological products. Different studies have analyzed alternative culture media with the aim of increasing the production of *B. thuringiensis* cells, seeking to improve the cultivation conditions of this bacterium, since the production of insecticidal toxins depends on the nutrients present in the fermentation medium. Sarrafzadeh (2012) conducted studies on the effects of culture medium compositions on the growth of *B. thuringiensis*, and their results demonstrate that for the successful commercialization of Bt production, the development of an optimal fermentation process is necessary.

Poopathi and Abidha (2012) analyzed the feasibility of clarified butter sediment waste as a culture medium for the production of mosquitocidal bacteria, indicating excellent cell growth and larvicidal

activity against vector mosquitoes. This result was similar to that obtained in this study with the alternative cassava-based medium, which showed high mortality of third-instar *A. aegypti* larvae. Likewise, Ernandes et al. (2013), who used a submerged fermentation process aiming to obtain Bti cell growth for the control of *A. aegypti*, found that corn steep liquor has better efficiency when compared to tryptose for spore production. Devidas et al. (2014) evaluated conventional and non-conventional carbon sources and nitrogen sources for biomass and spore/crystal production and determined the cost-effectiveness of potential substrates in the production of *B. thuringiensis*. In assessing cost-benefit, they found that the production of biopesticides using alternative media is highly economical, showing effective results, and in the toxicity tests it was observed that the toxic effect was doubled compared to conventional culture media.

Research has demonstrated other low-cost formulations, such as mixtures of wheat bran with carbon sources (molasses or glycerol), which promote good yields of spores and Cry crystals and can replace commercial media in semi-industrial production. Polanczyk et al. (2004) supported the discussion on replacing synthetic media with agro-industrial residues for Bt production; Lopes et al. (2005) discussed the challenges of industrial Bt production and the importance of reducing input costs; Mummigatti and Rajashekhar (2012) presented low-cost alternative formulations with good yields of spores and Cry toxins; Silva et al. (2013) addressed the techno-economic feasibility of using residues in the formulation of culture media; and Santos et al. (2022) explored alternative media to improve efficiency and optimize Bt production, such as the use of agro-industrial by-products or other more accessible and sustainable materials.

Poopathi and Abidha (2011) developed a culture medium using coffee husk waste for biopesticide production and reported that the delta-endotoxins produced by Bti were similar to those produced in the conventional medium studied. In addition, the toxins showed excellent larvicidal activity against vector mosquitoes and strong production of insecticidal toxins. Poopathi and Kumar (2003) analyzed three alternative culture media, potato extract, sweetened potato extract, and potato extract combined with chickpea, for the growth and toxin production of Bti, and in the bioassays they observed toxicity toward *A. aegypti* larvae; the toxicity was similarly comparable to that obtained with the conventional Luria Bertani (LB) culture medium. These studies differ from the present research, as the cassava-based alternative culture medium showed superior results when compared to the conventional media tested with respect to isolate growth.

The conventional culture media under study showed lower spore concentrations compared to the alternative media (cassava, cassava + salts, and cassava + salts + NaCl); however, they resulted in high larval mortality rates for the three isolates grown in Nutrient Broth, which provides nutrient sources such as nitrogen, vitamins, minerals, and amino acids essential for bacterial growth. Nutrient Broth is a versatile culture medium, suitable for initial growth and widely used for cultivating Bt. It is a simple but effective medium for the vegetative growth of Bt, commonly employed in growth assays, culture maintenance, and cell viability tests. This medium provides basic sources of nitrogen, vitamins, minerals, and osmotic balance, creating a favorable environment for bacterial growth. However, when the goal is the production of spores and insecticidal toxins, its formulation can be supplemented with additives such as salts, showing good performance during the vegetative phase (Atlas, 2010; Rangaswamy and Daniel, 2011; López-Pérez et al., 2014). The Luria Bertani culture medium is widely used for the initial growth of Bt on plates or in tubes, especially for culture maintenance and colony multiplication. LB medium favors rapid growth; however, it is not ideal for inducing sporulation or crystal production (Atlas, 2010; Green and Sambrook, 2012).

Regarding the variation in pH values of the three isolates during the fermentation process, in the present study it was observed that in the conventional culture media the pH remained close to neutrality for most of the experiment, whereas in the alternative media the isolates showed a reduction in pH, with values below 7.0 in most readings, although displaying less variation throughout the entire fermentation. Saksinchai et al. (2001) report that during the first 10 h of fermentation there was a decrease in pH in different media analyzed to 5.5, starting from pH 7.2, and after this period a slight increase occurs followed by stabilization.

Results obtained by Morris et al. (1996), Vidyarthi et al. (2002), and Valicente and Mourão (2008) corroborate the findings of this study regarding the fermentation of the isolates in conventional media, in which a slight decrease in pH occurs during the initial hours of fermentation, followed by a return to neutrality and subsequently a slight increase, tending toward basicity in the final hours of fermentation. Other studies describe that the drop in pH values during the first hours of fermentation is due to the release of acetic acid and organic acids produced during the growth of *B. thuringiensis*. However, subsequently, because these acids are consumed by the bacterium itself, an increase in pH occurs (Hanson et al., 1963; Içgen et al., 2002).

The spore concentration (spores/mL) of two of the three isolates tested in this study was considerably higher in the alternative cassava-based culture media compared to the values observed in the conventional media, also indicating a reduced sporulation time for these isolates. Both showed high larval mortality rates in the bioassays, demonstrating their importance from a biological activity standpoint. Studies indicate that, in addition to the toxins, the spores of *B. thuringiensis* also contribute to its toxicity, as they can germinate and grow vegetatively inside the target insect, causing septicemia or enhancing the effect of the toxins through a synergistic action (Glare and O'Callaghan, 2000; Raymond et al., 2008; Galzer e Azevedo-Filho, 2016). Several lines of evidence suggest that the vegetative growth of *B. thuringiensis* in the insect gut may play an important role in lethal infections. The importance of viable spores in the synergy of mortality caused by endotoxins indicates that the vegetative growth of *B. thuringiensis* contributes to toxin lethality (Raymond et al., 2008).

Prabakaran et al. (2008) developed a coconut water–based culture medium and observed a sporulation level of $3.4x10^{11}$ and larvicidal activity against *A. aegypti* larvae through the cultivation of Bti. Studies evaluating different culture media report that the addition of micronutrients to the media formulation increased the final average concentration of spores/mL after growth. These findings corroborate the results obtained in the present study, in which the culture media supplemented with salts showed an increase in the concentration of spores/mL during the fermentation process (Salama et al., 1983; Angelo et al., 2012). Bressuire-Isoard et al. (2018), in a study on the influence of the environment on sporulation and spore properties in *Bacillus*, showed how the structure, composition, and properties of spores are shaped by the environmental conditions under which they are formed, profoundly affecting spore production and characteristics. Therefore, controlling the sporulation environment of *Bacillus* is essential for ensuring the efficiency of fermentation processes.

The optical density value is an important measure for monitoring bacterial growth and understanding its optimal cultivation conditions, and the higher the optical density, the greater the cell density in the bacterial culture. In the present study, the three isolates tested showed a more pronounced increase in optical density during the initial hours of the fermentation process with the conventional culture media, followed by a tendency toward stabilization in the subsequent hours for all tested media, including the alternative ones. Similar results were reported by Içgen et al. (2002) in tests with different culture media, which suggest a common trend among all media tested, where an increase in absorbance occurs during the initial hours of growth, followed by stabilization afterward. This phenomenon was also described by Poopathi and Kumar (2003), who observed linear growth during the first hours of cultivation, with subsequent stabilization in later fermentation readings. In contrast, Poopathi and Abidha (2011), using an alternative culture medium, observed that the optical density of the medium, between 6 h and 72 h, reached absorbance values from 0.5 to 2.62, showing a tendency toward continuous increase in readings throughout the entire fermentation

process.

However, the values for cell mass in the three isolates studied were higher when grown in the conventional LB medium, which showed lower larval mortality compared to the other media. Overall, the media supplemented with salts showed higher cell mass values. Angelo et al. (2012) reported that the addition of salts led to an increase in cell mass production, raising biomass yield from 1.7 g/L to 2.7 g/L, regardless of variations in the amounts of carbon and nitrogen sources. Poopathi and Abidha (2012), also analyzing biomass at the end of the experiment (72 h) in alternative media for the fermentation of bacteria with larvicidal activity, showed that the yield after this growth period was 9.7 g/L, higher than the values found in the present study after 72 h of fermentation for both the conventional and alternative culture media. Rojas et al. (2018), investigating a low-cost alternative process for large-scale Bt production in a simple and innovative culture system, concluded that both the use of non-conventional sources and the development of processes for biomass production are important for the economic production of Bt-based insecticides in mosquito control programs.

The bacterium *B. thuringiensis* has been extensively studied due to its insecticidal properties, as it produces protein crystals composed of δ-endotoxins, or Cry and Cyt proteins, which are toxic to a wide range of insects, albeit in a group-specific manner. The Cry proteins produced by Bt become toxic to target insects when ingested by larvae. After ingestion of the crystalline inclusions, the protein crystals are solubilized under alkaline pH conditions in the larval midgut, releasing protoxins. Once activated, these toxins form pores in the epithelial cell membrane of the intestine, causing ionic imbalance, increased water absorption, rupture, and disintegration of midgut cells, leading to insect paralysis and, ultimately, death. Cyt toxins, unlike Cry proteins, do not bind to receptors but instead insert directly into the cell membrane, thereby enhancing the insecticidal action of Cry proteins (Viana et al., 2021b; Ragavendran et al., 2024; Aravinth et al., 2024).

In the selective bioassays, two of the three isolates studied, grown in both conventional and alternative media, showed high mortality of *A. aegypti* larvae. Regarding the estimated LC_{50} and LC_{90} values for these isolates in alternative media, it was observed that BtMA-690 presented the lowest values for both lethal concentrations. Other studies have already tested the toxicity of these same isolates against larvae of disease-vector insects; however, the fermentation process in those cases was carried out only in conventional culture media (Soares-da-Silva et al., 2017; Vieira-Neta et al., 2020).

Vieira-Neta et al. (2020) performed the isolation and testing of BtMA-750 on *A. aegypti* larvae and obtained LC_{50} and LC_{90} values of 0.0044 mg/mL and 0.01 mg/mL, respectively, values lower than those found in the present study with the isolate grown in the cassava-based alternative medium. Soares-da-Silva et al. (2017), studying the isolates BtMA-690 and BtMA-1114 against *A. aegypti* and *A. darlingi* larvae, also observed lower LC_{50} and LC_{90} values for BtMA-690, corroborating the results found in this research, in which this strain showed greater toxicity. However, in the aforementioned study, low LC_{50} and LC_{90} values were also obtained for BtMA-1114. Therefore, the low larval mortality observed for this isolate fermented in an alternative medium in the present study may be attributed to poor sporulation. Although this isolate showed larval mortality when grown in conventional medium, it is suggested that this strain requires a longer fermentation period for better sporulation, since the aforementioned research conducted experiments with the same isolate and reported 100 % mortality of *A. aegypti* larvae in bioassays, but using a longer growth period, with five days of fermentation prior to the lyophilization process.

Devidas et al. (2014), evaluating conventional and non-conventional carbon and nitrogen sources for Bt spore/crystal production, also reported effective results. In their toxicity tests, it was observed that the toxic effect using alternative media was greater or similar compared to the conventional medium, with LC_{50} values of 1.57 μg/L when using beans and 3.17 μg/L when using soybeans against *A. aegypti* larvae. Angelo et al. (2012), analyzing the toxicity of cultures against *A. aegypti* larvae using a pupal-flour–based medium, reported an LC_{50} of 3.64 mg/L, achieving maximum toxicity with the alternative medium compared to the value obtained for the conventional medium.

Therefore, the analysis of all these parameters shows that the culture medium can alter the degree of toxicity of *B. thuringiensis* strains. These data demonstrate that an appropriate medium can enhance the larvicidal activity of each strain evaluated in this study, identifying the isolate as a promising candidate for use as a biolarvicide agent against *A. aegypti*. Thus, the productivity of insecticidal toxins can be improved by optimizing nutritional parameters, environmental conditions, and the selection of carbon and nitrogen sources.

5. Conclusion

The alternative cassava-based medium showed superior performance compared with traditional media, especially for the isolates BtMA-690 and BtMA-750, with higher concentrations of spores/mL. Regarding pH, the traditional media maintained values close to neutrality throughout fermentation for BtMA-690 and BtMA-750. In the cassava medium, pH remained stable at around 6.0 during the entire evaluated period. The isolate BtMA-1114 showed greater pH variation in all media, indicating a distinct metabolic behavior. Optical density readings were higher in Nutrient Broth for the isolates BtMA-690 and BtMA-750, whereas for BtMA-1114 the best results were observed in Luria–Bertani and cassava-based media. In terms of biomass, the Luria Bertani medium presented the highest values for all three isolates. Selective bioassays showed 100 % larval mortality within 24 h for the isolates BtMA-690 and BtMA-750 grown in both Nutrient Broth and the alternative cassava medium. The lowest LC_{50} and LC_{90} values were recorded for the isolate BtMA-690, at 0.0102 mg/mL and 0.0253 mg/mL, respectively, indicating high larvicidal efficiency.

CRediT authorship contribution statement

Katiane dos Santos Lobo: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Juliete Lima Viana:** Writing – review & editing, Writing – original draft, Methodology. **Fátima Maria de Souza Pereira:** Methodology. **Pedro Alberto Pavão Pessoa:** Writing – review & editing. **Jefferson Almeida Rocha:** Writing – review & editing, Methodology. **Joelma Soares da Silva:** Writing – review & editing, Project administration, Funding acquisition, Data curation. **Valéria Cristina Soares Pinheiro:** Writing – review & editing, Supervision. **Rosemary Aparecida Roque:** Writing – review & editing, Supervision, Data curation.

Declaration of competing interest

All authors declare that there is no conflict of interest in this study.

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Data availability

The authors do not have permission to share data.

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