

# Low-cost media for Bt-based biopesticides mass production and their efficacy against *Spodoptera frugiperda*

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**Abstract.** Djunaedy A, Julivia AR, Apriliawati, Pawana G, Megasari D, Khoiri S. 2026. Low-cost media for Bt-based biopesticides mass production and their efficacy against *Spodoptera frugiperda*. *Asian J Agric* 10 (1): g100164. <https://doi.org/10.13057/asianjagric/g100164>. Fall armyworm, *Spodoptera frugiperda*, is a destructive pest that threatens maize production worldwide. Biopesticides derived from *Bacillus thuringiensis* (Bt) offer a sustainable alternative to chemical insecticides; however, their widespread adoption is often constrained by the high cost of production media. This study aimed to develop low-cost media using locally available flours (tapioca, arrowroot, mung bean, and soybean) for Bt-based biopesticide production and to evaluate their efficacy against *S. frugiperda* larvae. *Bacillus thuringiensis* strain Bt21 was inoculated into each low-cost medium, and their bacterial growth was recorded. Bacterial viability during eight weeks of storage was monitored weekly using the spread agar methods. Insecticidal activity was evaluated through larval bioassays using ten larvae per experimental unit with four replications. Larval mortality was recorded and determined using probit analysis to estimate Lethal Concentration (LC) and Lethal Time (LT) values. The results showed that among the four flour, tapioca-based medium supported the highest bacterial growth and maintained viability over time compared with others. At 15% concentration, Bt formulations using tapioca and arrowroot resulted in the highest larval mortality (95% and 90%, respectively), with low LC<sub>50</sub> values (1.67% and 1.80%) and rapid LT<sub>50</sub> value (3.20 h and 9.07 h). In contrast, mung bean and soybean-based media were found less effective, exhibiting higher LC and LT values. These findings demonstrate that substrate composition significantly influences bacterial viability and insecticidal activity. In conclusion, tapioca and arrowroot flours are effective and low-cost substrates for Bt-based biopesticide production and offer promising options for sustainable pest management. The use of locally available agricultural resources can support more economical and environmentally friendly biopesticide development for farming systems.

**Keywords:** Arrowroot, *Bacillus thuringiensis*, biopesticide, pest management, *Spodoptera frugiperda*

**Abbreviations:** Bt: *Bacillus thuringiensis*, CFU: Colony Forming Unit, LC: Lethal Concentration, LT: Lethal Time

## INTRODUCTION

Maize (*Zea mays*) production faces numerous challenges, one of the most pressing being pest infestations. The fall armyworm (*Spodoptera frugiperda*) is one such pest that has rapidly expanded across continents, including Asia, Africa, and the Americas, leading to yield losses ranging from 20% to 80% in severe outbreaks (Assefa 2018; Ganiger et al. 2018; Assefa and Ayalew 2019; Prabath 2019; Mukkun et al. 2021; Rizali et al. 2021). Originally endemic to the Americas, *S. frugiperda* has proven to be a formidable adversary due to its high fecundity, broad host range exceeding 80 plant species, and resistance to conventional insecticides (Mao et al. 2023). The pest was first reported in West Sumatra, Indonesia, in 2019 and has since been detected in nearly all maize-growing provinces, wreaking havoc on both subsistence and commercial farms (Maharani et al. 2019; Mamahit et al. 2020; Megasari and Khoiri 2021; Sataral et al. 2023).

The prevalent use of chemical insecticides in managing *S. frugiperda* raises significant concerns regarding environmental sustainability, non-target species toxicity, and pest resistance (Boaventura et al. 2020; Malaquias et al. 2020). These challenges underscore the need for alternative, eco-friendly pest control strategies. Among these, biopesticides derived from microbial agents, particularly *Bacillus thuringiensis* (Bt), have gained prominence due to their specificity and safety profile (Pinto et al. 2012).

*Bacillus thuringiensis* (Bt) is a gram-positive, spore-forming bacterium that synthesizes insecticidal crystalline proteins (Cry and Cyt toxins) during sporulation (Akhmad et al. 2017). These proteins are toxic to specific insect orders, including Lepidoptera, Diptera, and Coleoptera (Luiz et al. 2022). Upon ingestion by susceptible larvae, the Cry toxins bind to receptors in the insect midgut, leading to cell lysis and eventual death. Commercial formulations of Bt have been successfully employed in Integrated Pest Management (IPM) programs globally (Bharti and Ibrahim

2020; Šunjka and Mechora 2022; Djunaedy et al. 2024a, b). Despite its effectiveness, the commercial application of Bt faces limitations, particularly in resource-constrained settings. High production costs, driven largely by the expense of synthetic growth media and formulation processes, hinder widespread adoption (Tamez-Guerra et al. 2000; Šunjka and Mechora 2022). Thus, identifying and validating alternative, low-cost substrates for Bt cultivation and formulation is critical for the development of accessible biopesticide solutions.

Previous studies have explored various organic materials, including agricultural residues, plant-based starches, and legumes, as potential substrates for microbial growth (McGuire et al. 1996; Rojas et al. 2018; Djunaedy et al. 2024a, b). Tapioca and arrowroot are starch-rich materials with high carbohydrate content, providing readily metabolizable sugars for microbial growth (Edhirej et al. 2017; Ariyantoro et al. 2024). Mung bean and soybean flours offer substantial protein content, supporting nitrogen requirements for microbial metabolism. Each substrate's physicochemical properties, including pH, moisture content, and nutrient profile, influence microbial viability and biopesticide efficacy (Wolf 1970; Gunathilake et al. 2016; Yi-Shen et al. 2018). Past research also indicates that combinations of carbon and nitrogen sources can optimize Bt sporulation and toxin production (Gunathilake et al. 2016; Saberi et al. 2020). Tapioca (cassava starch), arrowroot, mung bean, and soybean flours are readily available in many developing countries and offer diverse nutritional profiles conducive to bacterial proliferation (Sakhale and Giri 2019; Li et al. 2023). Their utility in Bt-based biopesticide formulations, however, remains underexplored, particularly in terms of comparative efficacy.

The aim of this study was to evaluate and compare the efficacy of liquid biopesticide formulations of *B. thuringiensis* using four local flours (tapioca, arrowroot, mung bean, and soybean) as carrier substrates. Specific objectives include: (i) to determine the viability of Bt in each formulation over an 8-week storage period, (ii) to assess the insecticidal activity of each formulation against *S. frugiperda* larvae in vitro, (iii) to estimate the LC<sub>50</sub> and LC<sub>90</sub> values for each formulation, and (iv) to estimate the LT<sub>50</sub> and LT<sub>90</sub> values for each formulation. By utilizing locally available and low-cost substrates, this research contributes to the development of sustainable and accessible pest management tools. The findings may

inform policy and practice in agricultural technology, enabling smallholder farmers to adopt eco-friendly solutions for crop protection.

## MATERIALS AND METHODS

### Bacterial strain and preparation of bacterial culture

*Bacillus thuringiensis* strain BT21 isolate used in this study was identified previously and maintained in the Laboratory of Plant Protection and Environment Culture Collection (collection number 21). Prior to experimentation, the isolate was re-cultured by streaking onto Nutrient Agar (NA) and incubated at room temperature ( $\pm 30^\circ\text{C}$ ) for 24 hours. A single colony was then inoculated into 50 mL of NA broth and incubated in a rotary shaker at 150 rpm and room temperature for 24 hours.

For the experimental media, the basal formulation consisted of nutrient broth media. The peptone was replaced with 5 g L<sup>-1</sup> of one of the four test flours: tapioca, arrowroot, mung bean, or soybean. Each flour was sieved through a 60-mesh screen prior to use (each medium composition see Table 1). In same times, the price each ingredient was recorded based on Indonesian Product Catalogue (<https://katalog.inaproc.id/>) that was accessed on November 17<sup>th</sup>, 2025. All media were sterilized by autoclaving at 121°C for 20 minutes.

### Microbial growth analysis on low-cost media

Five millilitres of NA broth Bt culture was inoculated into each 250 mL Erlenmeyer flask containing the experimental medium. The flasks were incubated in a rotary shaker at 150 rpm and room temperature ( $\pm 30^\circ\text{C}$ ) for 48 hours to allow sufficient bacterial growth. Bacterial growth was measured by sampling 1 mL of each culture every 4 hours up to 24 hours, followed by serial dilution and spread plate methods. After 48 hours, Bt culture was mixed with additives to enhance formulation stability, including 0.1% citric acid, 1% NaNO<sub>3</sub>, 20% glycerol, 1% TiO<sub>2</sub>, and distilled water same as previous report (Djunaedy et al. 2024a, b). Each formulation was stored in sterile glass vials at room temperature ( $\pm 30^\circ\text{C}$ ). Each formula was homogenized and sampled weekly for viability testing up to eight weeks (same sample) followed by serial dilution and spread plate methods.

**Table 1.** Composition of low-cost medium and nutrient broth per liter

Ingredients	Nutrient Agar broth	Low-cost medium			
		Tapioca	Arrowroot	Mung bean	Soybean
Peptone	5.0 g	-	-	-	-
Tapioca	-	5.0 g	-	-	-
Arrowroot	-	-	5.0 g	-	-
Mung bean	-	-	-	5.0 g	-
Soybean	-	-	-	-	5.0 g
Yeast extract	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
Beef extract	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g
NaCl	5.0 g	5.0 g	5.0 g	5.0 g	5.0 g
pH	7.4 $\pm$ 0.2	7.0 $\pm$ 0.3	7.1 $\pm$ 0.1	7.2 $\pm$ 0.2	7.2 $\pm$ 0.2

### Bioassay of biopesticide against *S. frugiperda* larvae

The initial stock of *S. frugiperda* eggs was obtained from Balai Perakitan dan Pengujian Tanaman Aneka Kacang (BRMP Aneka Kacang) or Center for Development and Testing of Legume Crops, Indonesian Ministry of Agriculture. Eggs were reared until 2<sup>nd</sup> instar larvae. The 2<sup>nd</sup> instar larvae of *S. frugiperda* were reared on untreated green bean leaves as previous study (Afifah et al. 2023). Bioassays were conducted using the leaf-dip method. Larvae were placed individually in Petri dishes lined with moistened filter paper and fed leaves ( $\pm 2$  cm in diameter size) dipped in each formulation at concentrations of 5%, 10%, and 15%. A control group was fed leaves dipped in sterile distilled water (as control) and NA broth (as comparative medium). Each treatment included four replicates of 10 larvae each. Larval mortality was recorded at 12-hour intervals for up to 168 hours. Dead larvae were recorded, and cumulative mortality rates were calculated (Formula 1). Then calculated using Abbott's formula to check corrected mortality (%) (Formula 2) (Piepho et al. 2024).

$$\text{Larval mortality (\%)} = \frac{\text{Larval death number}}{\text{Total larva}} \times 100\% \quad [1]$$

$$\text{Corrected mortality (\%)} = \frac{\text{mortality of threated larvae} - \text{mortality of control larvae}}{100 - \text{mortality of control larvae}} \times 100\% \quad [2]$$

### Data collection and analysis

Viability of Bt was assessed weekly by dilution plating and colony count (CFU mL<sup>-1</sup>) (Formula 3). Mortality data were analyzed using probit analysis to estimate LC<sub>50</sub> and LT<sub>50</sub> values. Estimates were calculated with 95% confidence intervals using R software (Djunaedy et al. 2024a, b). The LC and LT estimation was based on a linear regression equation,  $Y = a + bx$ , where Y represents the probability of unit mortality (%) and X represents the test concentration for LC calculation or Y the probability of unit mortality (%) and X represents the probability of time to death (hours) for LC calculation.

$$\text{Bacterial population (CFU mL}^{-1}\text{)} = \frac{\text{Colonies counted}}{\text{Dilution factors} \times \text{volume transferred to plate (mL)}} \quad [3]$$

## RESULTS AND DISCUSSION

### Comparing medium with low-cost media

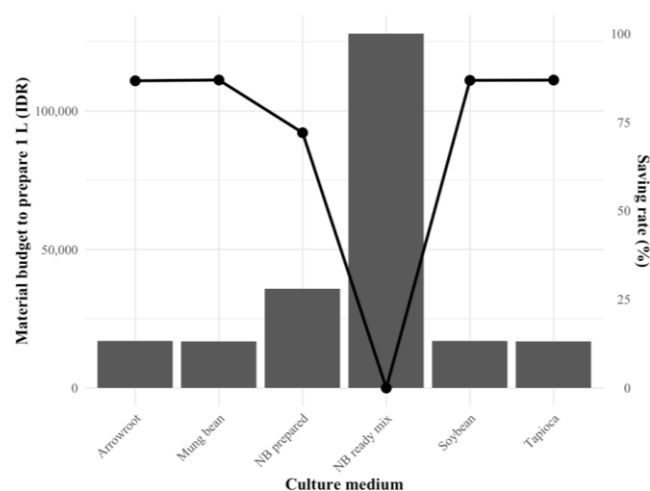
Based on Figure 1, a substantial cost difference was evident between alternative low-cost media and commercial NA broth both ready mix or manual prepared-media. Commercial NA broth exhibit the highest production costs per liter, primarily due to the use of high-purity ingredients, standardized formulations, strict quality control, sterilization, packaging, and distribution processes required to ensure reproducibility and regulatory compliance. These factors significantly increase the final price. In contrast, low-cost media formulated from locally available plant-based ingredients, such as soybean, mung bean, arrowroot, and tapioca show dramatically lower

production costs. These alternative media rely on inexpensive, local raw materials and simple preparation procedures, eliminating many industrial processing expenses. Previous studies have demonstrated that plant-based or locally sourced media can reduce production costs by 80% compared with commercial media, while still supporting satisfactory microbial growth (Shojaaddini et al. 2010; Vargas et al. 2024; Fenibo and Matambo 2025).

### Bacterial growth on low-cost media

Figure 2 shows the colony-forming units (CFU mL<sup>-1</sup>) in logarithmic scale overnight Bt growth. Tapioca exhibited the highest bacterial growth, peaking at 16 hours with over 10.25 log<sub>10</sub> CFU mL<sup>-1</sup>, and maintaining high viability up to 24 hours. Mung bean showed stable performance, with a noticeable increase at 12 hours and consistent values after, staying around 10.0 log<sub>10</sub> CFU mL<sup>-1</sup>. Soybean had a sharp increase by 12 hours, followed by a mild decline. Arrowroot and Nutrient Broth both showed moderate peaks at 12-16 hours but declined slightly by 24 hours (Figure 2). Tapioca was the most effective alternative medium for enhancing Bt growth. This result showed potential as low-cost sustainable medium substitutes to nutrient broth.

In this study, different agro-based substrates-tapioca, arrowroot, mung bean, and soybean-were evaluated for their capacity to support Bt growth. Among these, tapioca demonstrated superior performance, reaching a peak cell density of over 10.25 log<sub>10</sub> CFU mL<sup>-1</sup> at 16 hours and maintaining high viability throughout the observation period. This finding underscores the importance of readily metabolizable carbohydrate sources in promoting Bt biomass. Tapioca, being rich in starch, provides a high level of fermentable sugars that facilitate rapid bacterial metabolism and energy production, thereby enhancing cell division and sporulation (Wronkowska et al. 2008; Gurbanov et al. 2021). Arrowroot also showed substantial support for bacterial growth with high protein (Jaison et al. 2023).



**Figure 1.** Comparison of production costs between low-cost media and commercial standard media (per liter)

Mung bean and soybean, while nutritionally rich, contain more complex compounds such as proteins and anti-nutritional factors (e.g., trypsin inhibitors), which may have interfered with Bt proliferation. Additionally, the nitrogen-to-carbon (N:C) balance in legume-based media may have been suboptimal, as excessive protein can suppress sporulation (Wolf 1970; Gunathilake et al. 2016).

These results are consistent with previous reports indicating that starch-rich media, especially cassava-derived products like tapioca, favour microbial growth and toxin yield in entomopathogenic bacteria (Chang et al. 2008; Neves et al. 2017). Notably, the growth patterns observed in the present study suggest that not only nutrient composition but also the bioavailability of carbon sources and the absence of growth inhibitors. Previous studies have reported that nutrient composition, bioavailability, and inhibitor play a crucial role in supporting high-density cultures (Silva-Sánchez et al. 2019; Ramos et al. 2023).

### Viability of Bt over an 8-week storage period

Long-term viability is a critical parameter in assessing the shelf-life and practical applicability of *B. thuringiensis* (Bt)-based biopesticides. In this study, the formulations were monitored over an 8-week storage period to evaluate their ability to maintain viable Bt populations in different substrate media (Bharti and Ibrahim 2020; Šunjka and Mechora 2022; Djunaedy et al. 2024a, b). There was practically no difference because the bacterial population still over  $10^8$  CFU mL<sup>-1</sup>. In details, Figure 3 illustrated that tapioca-based medium showed the highest increase in Bt viability, reaching nearly  $3.0 \times 10^8$  CFU mL<sup>-1</sup> at 8 weeks. This indicates excellent support for long-term bacterial viability. Arrowroot medium also supported good bacterial survival, with a steady increase in CFU and a final value slightly below tapioca. Mung bean medium showed a more moderate increase in cell density but still performed better than the control by week 8. Soybean medium resulted in the lowest cell densities throughout the storage period among the alternative media, though it showed a gradual increase. Nutrient Broth, as control, started with a relatively high cell density ( $\sim 2.1 \times 10^8$  CFU mL<sup>-1</sup>) but showed little to no significant increase, and even a slight decrease at week 6, suggesting limited stability over long-term storage compared to tapioca and arrowroot. Tapioca and arrowroot-based media are the most effective formulations for maintaining *Bacillus* viability over 8 weeks, surpassing even the standard Nutrient Broth.

Tapioca has a high carbon content (>40%), which may affect the efficiency of spore formation. In *Bacillus megaterium*, sporulation efficiency reaches up to 80% and continues to increase until 120 h of incubation (Mahariawan et al. 2020). Additionally, 5% tapioca concentration has been reported as the estimated optimal composition for the production of *B. thuringiensis* spores (Liu and Tzeng 1998). Arrowroot, another starch-rich medium, also showed strong viability retention, though slightly lower than tapioca. Arrowroot powder enhanced the persistence of *B. thuringiensis* (Bt) (Singh et al. 2007). The superior performance of tapioca and arrowroot may be attributed to their balanced nutrient profiles and low

microbial antagonism. Starch-based media likely reduce osmotic stress and oxidative damage, both of which can negatively affect spore viability during storage. In contrast, mung bean and soybean formulations exhibited moderate to low viability, and nutrient broth, used as the control, showed a gradual decline in CFU after week 6, suggesting that standard synthetic media may not provide the same long-term stability as carbohydrate-rich alternatives.

Previous studies have reported that Bt spores can lose viability rapidly if not formulated properly, especially under fluctuating environmental conditions (Berninger et al. 2018). Growth medium has enhanced shelf stability by maintaining pH (Brar et al. 2005). A similar approach using tapioca-based media optimized via response surface methodology achieved high spore yields of *B. thuringiensis* (Liu and Tzeng 1998). The synergy between nutrient source and stabilizing agents appears essential in formulating Bt products with long shelf life (Bharti and Ibrahim 2020; Šunjka and Mechora 2022). From a practical standpoint, these findings suggest that low-cost, starch-rich media like tapioca not only support high initial growth but also prolong the viability of Bt cultures, making them highly suitable for decentralized production and distribution (Brar et al. 2006; Li et al. 2017).

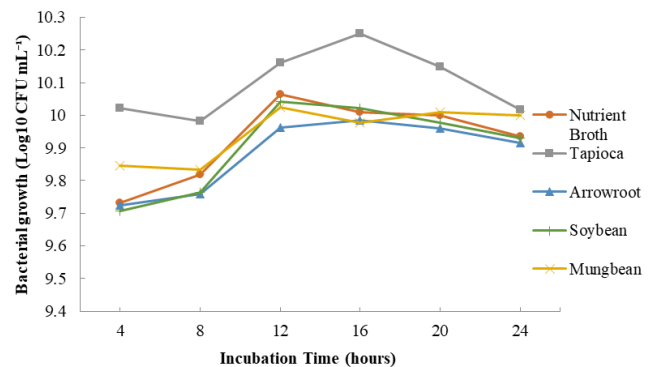


Figure 2. Logarithmic growth of Bt on different effective media

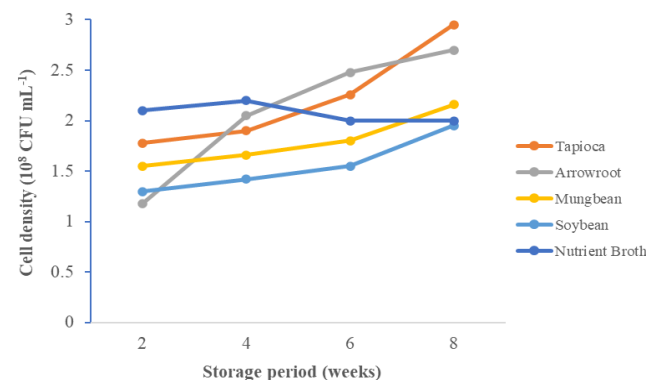
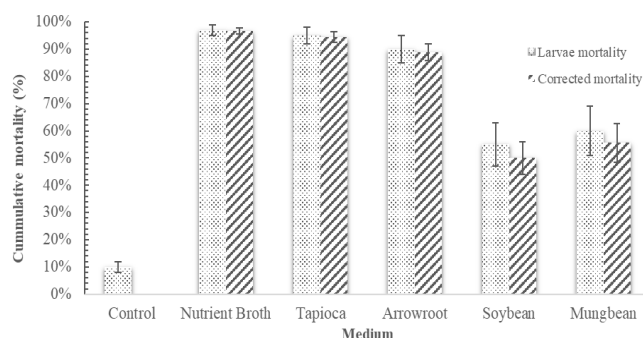


Figure 3. Effect of different liquid media compositions on the viability of *Bacillus* (measured as cell density in CFU mL<sup>-1</sup>) during 8-week storage period

**Table 2.** Estimation of LC and LT value of Bt using different low-cost media based on probit analysis

Media	Concentration (LC) (% v/v)		Mortality time (LT) (hours)*	
	LC <sub>50</sub>	LC <sub>90</sub>	LT <sub>50</sub>	LT <sub>90</sub>
Tapioca	1.67	3.20	3.20	30.20
Arrowroot	1.80	4.50	9.07	24.10
Soybean	13.60	82.70	71.40	214.10
Mung bean	10.60	65.70	70.20	156.50

Note: \*LT was calculated using mortality data of 15% concentration medium

**Figure 4.** Larval mortality of *Spodoptera frugiperda* exposed to a 15% Bt formulation at 168 hours post-treatment

### Larval mortality

The larval mortality assay provides direct evidence of the insecticidal efficacy of Bt formulations against *S. frugiperda*. In this study, the application of 15% Bt formulations resulted in significantly different mortality rates depending on the media used for cultivation. Tapioca-based Bt formulations achieved 95% mortality, closely following the NA broth comparative medium (97%), and surpassing all other test formulations. Arrowroot also produced high mortality (90%), whereas mung bean (60%) and soybean (55%) treatments were significantly less effective (Figure 4). The control treatment (sterile water) produced only 10% mortality, confirming the specificity and effectiveness of the formulations. Mortality was positively correlated with concentration, highlighting dose-dependent toxicity. Previous study demonstrated that cassava-based Bt formulations achieved over 50% mortality against *S. frugiperda* in “On Farm” biofactories (Freire et al. 2024). The mortality trend also confirms that the effectiveness of a formulation correlates not only with bacterial viability but also with the ability of the substrate to promote Cry toxin expression (Nyouki et al. 1996). Interestingly, while mung bean supported moderate bacterial growth, its larvicidal effect was limited. This suggests that bacterial viability alone does not guarantee insecticidal potency. Substrate composition may influence the metabolic pathways involved in Cry toxin expression, or potentially inhibit optimal sporulation due to imbalanced carbon-nitrogen ratios or the presence of anti-nutritional compounds (Nyouki et al. 1996).

### Lethal concentration and time

Lethal Concentration (LC) and Lethal Time (LT) are critical indicators for evaluating the potency and speed of action of biopesticide formulations (Djunaedy et al. 2024a, b). In this study, Bt formulations derived from tapioca and arrowroot exhibited superior virulence and rapid action against *S. frugiperda*, as reflected in their low LC<sub>50</sub> and LT<sub>50</sub> values. Tapioca achieved the lowest LC<sub>50</sub> (1.67%) and LT<sub>50</sub> (3.20 h), indicating that even at low concentrations, the formulation caused high mortality within a short time. Arrowroot followed closely with LC<sub>50</sub> of 1.80% and LT<sub>50</sub> of 9.07 h, suggesting high efficacy with slightly delayed action. Interestingly, in LT<sub>90</sub> arrowroot more rapid action than tapioca (Table 2). In contrast, soybean and mung bean-based formulations required substantially higher concentrations and longer exposure times to reach equivalent mortality thresholds. Soybean showed the highest LC<sub>50</sub> (13.6%) and LT<sub>50</sub> (71.4 h), while mung bean required 10.6% concentration with LT<sub>50</sub> of 70.2 h. These results indicate limited toxicity and delayed onset of mortality, likely due to suboptimal Bt virulence in legume-based media.

The observed differences were closely tied to the substrate composition. Tapioca and arrowroot, being starch-rich, may enhance sporulation and toxin expression, which were essential for larvicidal action. In previous study, upon ingestion by larvae, Cry toxins bind to midgut receptors and cause lysis of epithelial cells (Gómez et al. 2007; Liu et al. 2021). Meanwhile, legume-based media (soybean, mung bean) may interfere with toxin expression due to their higher protein and anti-nutritional content, or due to less favourable carbon-to-nitrogen ratios, which can suppress sporulation and toxin crystallization. Furthermore, the rapid action of tapioca-based Bt (LT<sub>90</sub> = 30.20 h) supports its use in time-sensitive pest management scenarios. Arrowroot, though slightly slower (LT<sub>90</sub> = 24.10 h), falls within an effective range. The prolonged LT<sub>90</sub> in soybean (214.1 h) and mung bean (156.5 h) reflects delayed mortality, which may be insufficient to prevent crop damage in field conditions. These findings emphasize that virulence may cause by Cry toxin bioavailability, midgut receptor affinity, and substrate-induced metabolic regulation all contribute to insecticidal performance. The data further confirm that growth rate alone is not a proxy for effectiveness; rather, the biochemical compatibility of the substrate with Bt’s growth and virulence is key.

In conclusion, this study concluded that locally available, low-cost substrates (particularly tapioca and arrowroot) are effective media for the production of *B. thuringiensis* (Bt)-based biopesticides. Tapioca supported the highest bacterial growth, maintained viability during eight weeks of storage, and exhibited superior insecticidal efficacy against *S. frugiperda*, as evidenced by its lowest LC<sub>50</sub> and LT<sub>50</sub> values. Arrowroot also performed well, showing comparable effectiveness and rapid action. In contrast, mung bean and soybean-based formulations exhibited significantly lower bioefficacy, requiring higher concentrations and longer exposure times to achieve similar levels of mortality. These results suggest that

starch-rich substrates enhance both microbial viability and virulence, making them suitable for low-cost biopesticide development. The use of tapioca and arrowroot provides a promising strategy for sustainable pest management, especially in resource-limited agricultural systems. Future studies should be done to quantify Cry protein and endospore production and explore formulation optimization and field-scale applications to further validate their practical use.

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