

RESEARCH PAPER

Development of *Bacillus thuringiensis*-based liquid and paste formulations for controlling invasive pest species *Spodoptera frugiperda* J. E. Smith

Achmad Djunaedy¹, Syaiful Khoiri¹, Dheananda Fyora Hermansyah Azari¹, Zahratus Syamsiyah¹, Gita Pawana¹, Dita Megasari², & Giyanto³

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ABSTRACT

Spodoptera frugiperda J.E. Smith (Spodoptera: Noctuidae) is an invasive pest of maize that has been reported around the world. Control efforts using biological agents continue to be developed, including the use of entomopathogen bacteria such as *Bacillus thuringiensis*. To boost the efficacy and efficiency of biological control, formulations are required. The objective of this study was to develop biopesticide formulations and evaluate their efficacy. The research was carried out by formulating *B. thuringiensis* strain BtJ2 (10^{10} cfu mL⁻¹) in liquid and paste formulations. The effectiveness of the formulations was evaluated using the feed dipping method. The results showed that paste formulations at a concentration of 10% caused 100% mortality, whereas the liquid formulation resulted in 85% mortality. The LC₉₀ for the paste formulation was 6.66%, while the LC₉₀ for the liquid formulation was 12.90%. Both the liquid and paste formulations had similar effects on mortality and viability. Based on the LC₉₀ and LT₉₀, the paste formulation was more efficient and faster in killing *S. frugiperda* than the liquid formulation. The results of this study provide recommendations that *B. thuringiensis* as a bioinsecticide is better formulated in a paste than in a liquid form.

Key words: entomopathogen, fall armyworm, formulation, mortality

INTRODUCTION

Spodoptera frugiperda J.E. Smith is a highly destructive invasive pest of maize that has been documented all over the world (Milano et al., 2008; Shylesha et al., 2018; Sisay et al., 2019; Bajracharya et al., 2020; Cokola et al., 2020; Ashok et al., 2021; Yan et al., 2022). The incidence and intensity of *S. frugiperda* attacks can reach 100% (Sisay et al., 2019; Megasari & Khoiri, 2021). *S. frugiperda* attacks maize crops and causes significant losses (Kebede & Shimalis, 2019). In African and European countries, the economic losses caused by this pest amount to 20.6 million tonnes per year, with a total loss of US\$ 2.5-6.2 billion (FAO & CABI, 2019).

This pest's imago can lay up to 2000 eggs. The

egg stage lasts 2–3 days and the larval period 14–30 days. The larval stage of this pest prefers leaves and sensitive shoots, particularly buds, and develops into a plant tissue chewer (He et al., 2020). Pupae formation occurs on the ground and lasts 8–30 days (da Silva et al., 2016; Sharanabasappa et al., 2018). The short life cycle and high reproductive capacity make this pest one of the most destructive.

In general, synthetic pesticides such as carbaryl, diazinon, cypermethrin, methyl parathion, and methomyl have been used to control *S. frugiperda* (Paredes-Sánchez et al., 2021). However, several studies have reported that pest resistance builds up quickly, making synthetic insecticides ineffective (Kumela et al., 2019). Pest resistance is induced by mutations in genes that confer resistance to pyrethroids, organic phosphates, and carbamates (Boaventura et al., 2020). Therefore, alternative control methods are needed. One example is utilizing the entomopathogenic bacterium, *B. thuringiensis*. The use of *B. thuringiensis* as a biological agent is environmentally friendly because its crystal protein is selective (Arsi et al., 2019).

Previously, *B. thuringiensis* strains were found to be efficient biological controls in prior investigations (Pinto et al., 2012; Gazali et al., 2017; de Oliveira et al., 2022). However, *B. thuringiensis* must be prepared

Corresponding author:

Syaiful Khoiri (syaiful.khoiri@trunojoyo.ac.id)

¹Program study of Agroecotechnology, Department of Agricultural Science and Technology, Faculty of Agriculture, Universitas Trunojoyo Madura, Bangkalan, Indonesia 69162

²Program study of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional “Veteran” Jawa Timur, Surabaya, Indonesia 60294

³Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia 16680

for large-scale manufacturing. Formulations can help sustain population density while also increasing the efficacy of active compounds (Fravel et al., 1998; Yulensri, 2020). Furthermore, the appropriate formulation can aid in availability, mass distribution, storage, transportation, packaging, application, and marketing (Fravel et al., 1998; Wardati & Erawati, 2015).

Based on this, two *B. thuringiensis* formulations, liquid and paste, were created. The liquid formulation is the simplest and has the benefit of being more stable in terms of color, scent, and appearance, as it does not alter from the start of manufacturing (Wardati & Erawati, 2015). The paste formulation was developed for comparison. The purpose of this study was to determine the toxicity of liquid and paste formulations with the active ingredient *B. thuringiensis* in killing *S. frugiperda*, estimate lethal concentration and lethal time, and compare viability. The findings of this study provide recommendations for the development of the *B. thuringiensis* formulation.

MATERIALS AND METHODS

Research Site. This research was carried out at the Laboratory of Plant Protection and Environment, Major of Agroecotechnology, Department of Agricultural Science and Technology, Faculty of Agriculture, Universitas Trunojoyo Madura, Indonesia.

Materials. The *B. thuringiensis* strain BtJ2 was an isolated collection from the Plant Protection and Environment Laboratory. *S. frugiperda* was provided by Balai Penelitian Tanaman Pemanis dan Serat (*The Indonesian Sweetener and Fiber Crops Research*

Institute), Malang, Indonesia. All chemicals used in this research were of analytical grade and purchased from Sigma (St. Louis, MO), Merck (Darmstadt, Germany), and HiMedia (Maharashtra, India).

Preparation of *S. frugiperda* Larvae. The larvae of *S. frugiperda* were maintained at the Laboratory of Plant Protection and Environment, Universitas Trunojoyo Madura. The rearing box used were cages made of insect netting, measuring 50 cm × 50 cm × 50 cm, and the larvae were fed baby beans.

Preparation of Formulation. Preparation of *B. thuringiensis* strain BtJ2 began with re-culture isolation from cold storage, followed by quadrant streaking on tryptic soy agar (TSA) and incubation at 37 °C for 24 hours. Novel liquid and paste formulations were created by combining the *B. thuringiensis* culture with additional components (Table 1). The active component was *B. thuringiensis*. Glycerol acts as a protector, preventing cell damage and preserving bacterial viability during storage (Stevenson et al., 2017). Sodium nitrate serve as a nitrogen source, citric acid is an organic acid compound (Zhang et al., 2013), molasse is a carbon source, and TiO₂ acts as an adjuvant, protecting cells and making them more resistant to UV light.

Bioassays. The toxicity test was carried out by bioassay using the feed dipping method (Balfas & Willis, 2009). The test feed used 5 cm pieces of baby beans, which were prepared by dipping them into the formulation according to the treatment. The formulations used were two weeks old. Next, the beans were dried for one hour (Turhadi et al., 2020). Second instar larvae

Table 1. Composition of liquid and paste formulation

Ingredient	Liquid formulation	Paste formulation
Cell culture	50 mL	50 mL
TiO ₂	40 mL (in acetic acid 0.6%)	39 g
Sodium nitrate (NaNO ₃) 1%	2.4 g	-
Citric acid (C ₆ H ₈ O ₇) 0.1%	0.24 g	-
Glycerol 20%	58.82 mL	15 mL
Sterile-Aquadest	98.54 mL	-
Talc powder	-	225 g
Molase	-	15 g
Tween 80	-	6.5 mL
CMC	-	5 g
Xanthan gum	-	7.5 g

were fasted for 24 hours, then placed into plastic vials with two larvae per vial and fed according to treatment (Asmaliyah et al., 2010). This bioassay consisted of five concentration treatments: 0% (control), 0.5%, 1%, 5%, and 10%. The treatment concentrations were determined to facilitate the calculation of LC and LD. In the control group, green beans were dipped in distilled water only. Each experimental unit consisted of ten larvae and was repeated four times. The feed was changed every day without any treatment. Observations were made every day for three days. Every two hours for 72 hours, larval mortality was recorded and documented. After the first three days, observations were made once a day. Mortality observation continued until one concentration resulted in 100% larval death. Mortality was calculated using the formula below (Hidayati et al., 2013).

$$M = \frac{n}{N} \times 100\%$$

M = Larvae mortality (%);

n = The number of dead larvae;

N = The number of treated larvae.

Formulation Effect on Viability. The viability test was performed by combining 1 mL of the compound with 9 mL of distilled water. Following that, dilution were performed up to 10^{-8} . Then, 1 mL of the diluted suspension solution was added to 9 mL of warm nutrient agar and vortexed. The medium was then poured into the petri dishes. Observations were made after 24 hours to calculate the bacterial population, which can be computed using the method below (Habazar et al., 2015).

$$PB = A \times C$$

PB = The population of bacteria (cfu mL⁻¹);

A = The colony number of bacteria;

C = The dilution factor.

Data Analysis. Mortality data were analyzed using probit analysis to estimate the LC₅₀, LC₉₀, LT₅₀, and LT₉₀ values, utilizing IBM SPSS Statistics software

(Umaru & Simarani, 2020).

RESULTS AND DISCUSSION

Insecticidal Effect of the Formulations on *S. frugiperda*. Based on the results of observations, it can be seen that in the liquid formulation, the highest mortality was 85% at a concentration of 10%, and the lowest was 18% at a concentration of 0.5%. In the paste formulation, the highest mortality was 100% at a concentration of 10%, while the lowest mortality was 0% at a concentration of 0% (Table 2). This indicates that the paste formulation was more effective in killing *S. frugiperda* larvae.

Salaki & Watung (2022) classify virulence levels based on the mortality value of the test insects: high virulence if the mortality value is above 50%, moderate virulence if the mortality value is 30%–<50%, low virulence if the mortality value is <30%, and no virulence if the mortality value is 0%. Both formulations showed high virulence starting from a 5% concentration. A previous study reported that the mortality value increases if the treatment is given to the larvae for a long period (Zulfiana et al., 2017). Factors that affect virulence include pH, temperature, humidity, and light (Motta, 2021).

The results of the probit analysis for concentration estimation showed that the liquid formulation required a concentration of 5.83% to kill 50% of the larvae, and 12.9% to kill 90% of the larvae. Meanwhile, the paste formulation required a concentration of 4.308% to cause 50% mortality and 6.61% to cause 90% (Table 3).

The results of linear regression analysis with the equation $Y = a + bX$ showed that for the liquid formulation, the value of a is -0.2857 and the value of b is 1.7857, with an R² value of 0.928 (in equation $Y = -0.2857 + 1.7857X$). Meanwhile, the linear regression for the paste formulation showed a value of a as -4.294 and a value of b as 6.770, with an R² value of 0.939 (in equation $Y = -4.294 + 6.770X$). The two regression equations indicated that the concentration variable (X)

Table 2. Comparison of mortality effects on *S. frugiperda* between liquid and paste formulation

Concentration (%)	Liquid formulation		Paste formulation	
	Mortality (%)	Virulence level	Mortality (%)	Virulence level
0 (control)	0	-	0	-
0.5	18.00	Low	27.50	Low
1	25.00	Low	30.00	Low
5	53.00	High	77.50	High
10	85.00	High	100	High

affected the larval mortality variable (Y).

The results of the probit analysis for estimating the time of death showed that the liquid formulation required 65.32 hours to kill 50% of the larvae and 165.63 hours to kill 90% of the larvae. In contrast, the paste formulation required 50.115 hours to kill 50% of the larvae and 63.701 hours to kill 90% of the larvae (Table 4).

The results of linear regression analysis with the equation $Y = a + bX$ showed that for the liquid formulation, the value of a is -4.5 and the value of b is 2.5, with an R^2 value of 0.98 (in equation $Y = -4.50 + 2.50X$). Meanwhile, for the paste formulation, the value of a is -20.913 and the value of b is 12.302, with an R^2 value of 0.542 (in equation $Y = -20.913 + 12.302X$). These two regression equations indicated the concentration variable (X) affects the larval death time variable (Y). The closer the R (coefficient of determination) value is to 1, the stronger the effect of the independent variable (X) on the dependent variable (Y) (Muckoya et al., 2020).

This study showed that paste formulation

faster than liquid formulation in killing larvae. One of the purpose formulations was enhance activity of microbial agents. The density of the suspension was not an important factor (Bharti & Ibrahim, 2020). This result may be caused by composition of formulation. Tamez-Guerra et al. (2000) tested 80 formulation and determined optimal combinations of ingredients such as corn flours, lignin, and pregelatinized corn flour (PCF). The addition of PCF increase the effectivity.

Mortality Symptoms. There were no different symptoms between the liquid and paste formulations. Symptoms of poisoning caused by the application of a bioinsecticide paste formulation with active *B. thuringiensis* began with paralysis of the *S. frugiperda* larvae, commonly known as the knockdown effect. Poisoned larvae initially became yellow-brown with a soft texture and exhibited passive movement. The symptoms of death from the observed after the application included physical changes in the larvae. Initially, the body of the *S. frugiperda* larva, which was light brown with active movements, turned green-

Table 3. The results of the probit analysis to estimate the lethal concentration formula (LC_{50-90}) on larval mortality

Mortality (%)	Probability of concentration (%)	
	Liquid formulation	Paste formulation
50	5.83	4.31
55	6.30	4.50
60	6.82	4.70
65	7.40	4.91
70	8.07	5.15
75	8.85	5.42
80	9.82	5.74
85	11.08	6.13
90	12.90	6.66

Table 4. The results of the probit analysis to estimate the lethal time formula ($LT_{50,90}$) based on larval

Mortality (%)	Estimation of lethal time (hours)	
	Liquid formulation	Paste formulation
50	65.32	50.11
55	71.56	51.30
60	78.51	52.54
65	86.41	53.86
70	95.59	55.28
75	106.59	56.85
80	120.34	58.66
85	138.62	60.84
90	165.63	63.70

brown to dark brown, and then black-brown, with its body size decreasing. The texture of the body became soft (Figure 1). These symptoms were caused by the presence of parasporal crystals (Cry protein) produced by *B. thuringiensis* and the attachment of protoxins to receptors in the digestive tract, assisted by protease enzymes, which then led to proteolysis (Schünemann et al., 2014; Zulfiana et al., 2017).

The Bacterial Viability. A bacterial viability test was carried out to determine the survival ability of bacteria during storage in the formulations. Based on the viability test, the number of bacterial colonies after storage for 8 weeks, in both the liquid and paste formulations, did not decrease and remained at a concentration of 10^{10} cfu mL⁻¹ (Table 4). This indicated that the bacteria could survive during storage. Both formulations showed stable characteristics in storage. The right formulation of biological agents can maintain the stability of the agent during storage and distribution, increase the persistence of the agent in the field, facilitate the application of these products in the field, protect the agent from unfavorable environmental

factors, and increase the activity of the agent on the target host (Leland & Behle, 2004).

CONCLUSION

The results showed that paste formulation at a concentration of 10% could cause 100% mortality, whereas the liquid formulation resulted in 85% mortality. The LC₉₀ in the paste formulation was 6.66%, while the LC₉₀ in the liquid formulation it was 12.90%. Both liquid and paste formulations had similar effects on mortality and cell viability. Based on the LC₉₀ and LT₉₀, the paste formulation is more efficient and faster in killing *S. frugiperda* compared to the liquid formulation.

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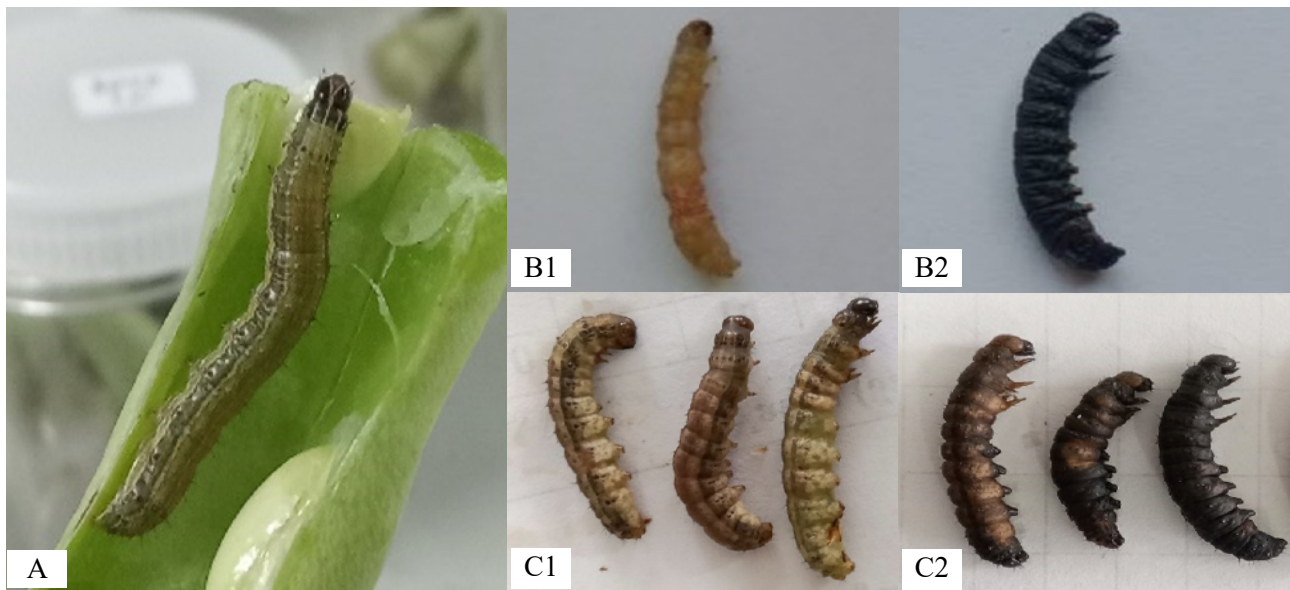


Figure 1. Effect of *B. thuringiensis* BtJ2-base formulation treatment on *S. frugiperda* larvae. A. Healthy larvae in the control treatment; B. Symptoms of larval death due to liquid formulation treatment; C. Symptoms of larval death due to paste formulation treatment. 1. After 24 hours; 2. After 48 hours.

Table 4. Viability of *B. thuringiensis* strain BtJ2 in the formulation over 8 weeks

Storage period (Weeks)	Population of <i>B. thuringiensis</i> strain BtJ2 (x 10 ¹⁰ cfu mL ⁻¹)	
	Liquid formulation	Paste formulation
0	3.64	1.74
4	3.65	1.74
8	3.65	1.79

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AUTHORS' CONTRIBUTIONS

AD and SK conceived and planned the experiment. G carried out the isolation and pathogenicity test of *B. thuringiensis*. DM managed rearing and identification of *S. frugiperda*. DFHA and ZS collected bioassay data and also performed analysis data. SK and GP interpreted the data. SF prepared the manuscript. The authors provided responses and comments on the research flow, data analysis, and interpretation as well as the manuscript's structure. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there is no competing interest regarding the publication of manuscripts.

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