INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 21, No. 5, p. 258-267, 2022
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Efficacy of a Bt-based biopesticide formulation of the Kurstaki HD1 variety against *Spodoptera frugiperda* larvae

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Article published on November 25, 2022

Key words: Biopesticide Btk HD-1, Test, *Spodoptera frugiperda*, Mortality

Abstract

Maize cultivation is subject to many constraints that cause yield reduction. *Spodoptera frugiperda* is one of the insect pests of this crop in Côte d'Ivoire. In recent years, microbial insecticides have become a viable alternative to control the armyworm and are considered a safe tool in integrated pest management. Various Bt-based biopesticide formulations were tested on the armyworm larvae to identify the most effective ones. A rearing of *S. frugiperda* was carried out in the entomology laboratory of University Nangui Abrogoua. Thus, larvae from L_1 to L_6 stages were tested. Successive decimal dilutions of bacterial suspension of Btk HD1 were made, and five different doses of each biopesticide and a control were used. All bioinsecticides tested had an effect on the mortality of the larvae. However, the biopesticide formulation M_{10} had a much greater effect on the mortality for the most concentrated dose (10^{11} spores/mL) with a mortality rate of 92.1% followed by the M_5 formulation with a mortality rate of 76.9% at a dose of $8.6x10^{10}$ spores/mL. For the M_{15} formulation, only the first dose D_1 at $4.6x10^{11}$ spores/mL killed 50% of the stage 2 larvae, whereas the M_0 formulae at the most concentrated dose of $1.3x10^{11}$ spores/mL, produced a mortality rate of 26.7%. The M_{10} formulae can be used in integrated pest management scheme against *S. frugiperda*.

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Introduction

Maize (*Zea mays* L.) is a cereal that belongs to the grass family (Poaceae) and to the subfamily Panicoideae (Barrière, 2000). In Côte d'Ivoire, maize plays a predominant role as subsistence, commercial and socio-cultural crop. Its production is used for human consumption in the form of semolina in the northern part of the country, where it is a staple food for the population. National production is estimated at 654,738 tons for a total sown area of 327,800 ha (Countrystat, 2013). However, maize cultivation is subject to many constraints causing yield decline. Various factors are responsible for low maize production, the main ones being insect pests. These insects damage all parts of the plant from the roots to the seeds. Fall armyworm (FAW) also called *Spodoptera frugiperda*, a native insect pest to America, is one of the most important against maize in Côte d'Ivoire. This insect causes serious economic losses to farmers of various crops besides maize, such as soybean and cotton (Bueno *et al.*, 2010; Nagoshi *et al.*, 2007). The use of synthetic insecticide is most often preferred control method. It should be noted that the extensive use of pesticides is expensive for the farmers. It oftentimes leads to pest resistance (Gene, 2018). In recent years, microbial insecticides have become a viable alternative to control armyworm, and are considered a safe tool in IPM (Integrated Pest Management) (Moscardi, 1999; Valicente & da Costa, 1995). One of the most important insect pathogens in the world today is the bacterium *Bacillus thuringiensis*, which accounts for 1-2% of the global insecticide market (Lambert & Peferoen, 1992), and more than 90% of the commercial biopesticide sales with over 100 products reported in the literature (Glare & O'Callaghan, 2000). *B. thuringiensis* is a Gram-positive rod-shaped bacterium that occurs naturally in soil, on dead insects, in water and in grain dust (Lambert & Peferoen, 1992). During sporulation, this bacterium produces a large amount of larvicidal proteins called δ-endotoxins. These proteins are toxic to many insect pests. When ingested by sensitive insects, these crystalline proteins are solubilized in the midgut to form the active proteins called δ-endotoxins. The toxicity of these crystals to insects is determined by the presence of specific receptors in the midgut epithelium (Lambert & Peferoen, 1992). δ-endotoxins produced as parasporal bodies or crystalline inclusions are highly host-specific. To curb caterpillar invasion, the FAO (2017) has developed an action plan to not only promote good agricultural practices, but especially the use of effective biopesticides instead of synthetic pesticides. This method of control participates in the preservation of human, animal and environmental health. The objective of this study was to select for an effective home-made biopesticide against *S. frugiperda*.

Materials and methods

Entomological and plant material

The entomological material used was the larva of *S. frugiperda*. Larvae of different stages were chosen because they all feed on large quantities of leaves and the shells from which they originate before dispersing in search of food after 4 to 10 hours of immobility. All larvae, from stages 1 to 6, were used in the experiments in order to know which ones are the most sensitive to biopesticides. The plant material consisted of one month old maize plants and the leaves of *Panicum maximum* which are privileged targets of *S. frugiperda* on which this caterpillar proliferates naturally.

Biological control materials

B. thuringiensis var. *kurstaki* HD_1-based biological insecticide used in this study was formulated, in the course of this work, from sugarcane molasses enriched with 100mg/L yeast extract for the M_5 formulation or 200mg/L yeast extract for the M_{10} formulation or 300mg/L yeast extract for the M_{15} formulation. The M_0 formulation was made from pure sugarcane molasses with no yeast extract supplementation. These formulations were all in a liquid form. The concentrations of the microbial products are shown in Table 1. A commercial biopesticide with the name brand of Bioprotect PLUS manufactured by AEF Global (Quebec, Canada) with a concentration of 5% was used as a positive control. Its active ingredient is *B. thuringiensis* subspecies

kurstaki registered under the number 68030-71-1. The registration number of the bio-insecticide is 32425. Distilled water was used as a negative control.

Table 1. Biopesticide Concentration.

Biopesticides	Cells (UFC/mL)	Spores (UFC/mL)
M_0	$8,9.10^{11}$	$1,3.\ 10^{12}$
M_5	$9,9.\ 10^{11}$	$8,6.10^{11}$
M_{10}	$7,1.\ 10^{11}$	1.10^{12}
M_{15}	$2,8.\ 10^{8}$	$4,6.\ 10^{12}$

Materials methods

Rearing of Spodoptera frugiperda (J.E. Smith, 1797)

To study the effect of biological control agents, *B. thuringiensis* variety *kurstaki* HD 1 (Btk HD 1), a rearing was set up. The strain of *S. frugiperda* attacking mainly maize, was reared in the Laboratory of Agricultural Entomology of the Nangui Abrogoua University of Abidjan.

Efficacy tests of biopesticides M_0; M_5; M_{10} and M_{15} on the Spodoptera frugiperda population

Larvae from the laboratory were used for the biopesticide efficacy tests. The IRAC (Insecticide Resistance Action Committee) 020 method, version 3.2 of May 2011 approved for *S. frugiperda* larvae, was used for the various susceptibility tests. Initially, diets were to be used for the different tests. Soft corn leaves of about 6 cm in length were used for food. To improve the adhesion of the biopesticides to the leaves, triton X was used as a wetting agent. Larvae stages from L_1 to L_6 were collected from growing eggs of a strain of S. *frugiperda*. Five (5) different doses of each biopesticide formulations and of the positive and negative controls were used. Each trial was repeated three times. The choice of these doses was based on the protocol established by Gadji *et al.* (Gadji *et al.*, 2016) for testing the efficacy of Btk-based biopesticides. Successive decimal dilutions of the Btk HD-1 bacterial suspension were performed, in sterilized test tubes, with sterile distilled water. To do so, a volume of 5mL of the stock solution or subsequent dilution of the Btk HD-1 bacterial suspension was pipetted into a test tube containing 45mL of sterile distilled water to generate a range of concentrations (doses) from 10^{-2}% to 100% (Table 2).

Table 3 shows concentration values of microbial products of different formulated biopesticides.

Table 2. Decimal dilution process of biopesticide.

Tubes	1	2	3	4	5
Sterile distilled water (mL)	-	45	45	45	45
Dilution process	100mL of Btk HD-1	Added 5mL of tube 1	Added 5mL of tube 2	Added 5mL of tube 3	Added 5mL of tube 4
Dose (%)	100	10	1	0,1	0,01

Dose (%): percentage concentration of Btk HD-1 biopesticide

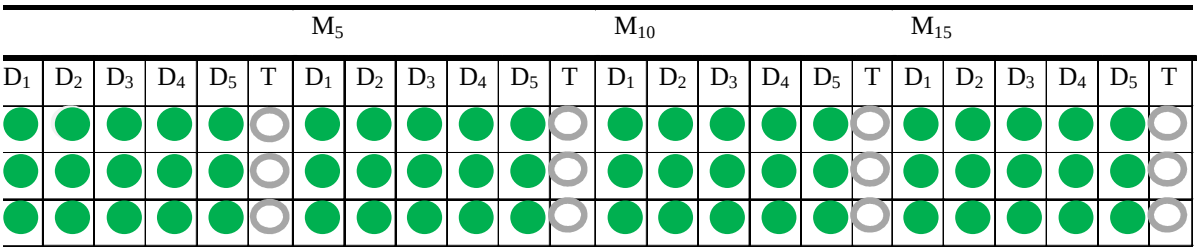
Table 3. Serial dilution of different biopesticides.

	Initial dose	D_1	D_2	D_3	D_4	D_5
Medium M_0						
Cells (CFU/mL)	$8,9\times 10^{11}$	$8,9\times 10^{10}$	$8,9\times 10^{9}$	$8,9\times 10^{8}$	$8,9\times 10^{7}$	$8,9\times 10^{6}$
Spores (CFU/mL)	$1,3\times 10^{12}$	$1,3\times 10^{11}$	$1,3\times 10^{10}$	$1,3\times 10^{9}$	$1,3\times 10^{8}$	$1,3\times 10^{7}$
Medium M_5						
Cells (CFU/mL)	$9,9\times 10^{11}$	$9,9\times 10^{10}$	$9,9\times 10^{9}$	$9,9\times 10^{8}$	$9,9\times 10^{7}$	$9,9\times 10^{6}$
Spores (CFU/mL)	$8,6\times 10^{11}$	$8,6\times 10^{10}$	$8,6\times 10^{9}$	$8,6\times 10^{8}$	$8,6\times 10^{7}$	$8,6\times 10^{6}$
Medium M_{10}						
Cells (CFU/mL)	$7,1\times 10^{11}$	$7,1\times 10^{10}$	$7,1\times 10^{9}$	$7,1\times 10^{8}$	$7,1\times 10^{7}$	$7,1\times 10^{6}$
Spores (CFU/mL)	1×10^{12}	1×10^{11}	1×10^{10}	1×10^{9}	1×10^{8}	1×10^{7}
Medium M_{15}						
Cells (CFU/mL)	$2,8\times 10^{8}$	$2,8\times 10^{7}$	$2,8\times 10^{6}$	$2,8\times 10^{5}$	$2,8\times 10^{4}$	$2,8\times 10^{3}$
Spores (CFU/mL)	$4,6\times 10^{12}$	$4,6\times 10^{11}$	$4,6\times 10^{10}$	$4,6\times 10^{9}$	$4,6\times 10^{8}$	$4,6\times 10^{7}$

Toxicological test setup and treatment structure

To evaluate the efficacy of the biopesticide in controlling S. *frugiperda*, toxicological tests were set up in a chamber where the temperature and relative humidity were maintained at 28 ± 1°C and 70 ± 5%. A completely randomized block design was adopted with one factor under study. This included the biopesticide and its five modalities or doses (D_1, D_2, D_3, D_4 and D_5). The trials were conducted in Petri dishes. A stage 2 larva was carefully placed with fine-tipped tweezers in the petri dish. Ten Petri dishes were used for each experiment and dose. The experiments were repeated three times. For all the tests (including the negative control), 180 larvae were used for each modality for a total of 720 larvae.

The experimental set-up consisted of four (04) batches, that is one batch per treatment of 18 Petri dishes each including 15 Petri dishes for B. *thuringiensis* applications and three for the negative control. A total of 72 Petri dishes were used. In each Petri dish, one larva was subjected to the biopesticide formulation treatment that is 10 larvae per replicate per dose for a total of 720 larvae. The Petri dishes were reused by rinsing them with 100% ethanol followed with distilled water. Treated dishes were transferred to a culture chamber at a temperature of 28 ±1°C and a relative humidity of 70 ± 10% (Fig. 1).



M : Biopesticide 0, 5, 10, 15 R (1;2;3) : Repetition T : negative control D : dose (%) ($D_1 = 10^{-2}$; $D_2 = 10^{-1}$; $D_3 = 1$; $D_4 = 10$; $D_5 = 100$)

● Petri dish + corn husk +1 larva ⬭ Petri dish + distilled water + 1larva

Fig. 1. Experimental set-up of Btk HD-1 efficacy tests on *Spodoptera frugiperda* larvae in the laboratory.

Efficacy tests of bioprotect PLUS on the different larval stages of Spodoptera frugiperda

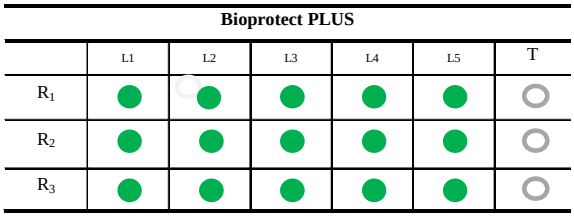
The commercially available Bioprotect PLUS was used to evaluate the impact of Bt on all larval stages of *S. frugiperda*. One larva was placed with fine-tipped tweezers in a Petri dish. Ten dishes were used for each experiment and for each stage of the larvae. The experiments were repeated three times. A total of 180 larvae were used including those of the negative control (Fig. 2). Pieces of soft young corn leaves of about 6 cm long were dipped into each concentration for 30 seconds and then air dried on a sterile paper for 1 hour. To improve the adhesion of the product to the leaves, Triton X was added to the different preparations at a rate of one drop per 40mL of biopesticide solution. The leaves were then placed in Petri dishes containing a slightly moistened filter paper covering the bottom of each dish to keep the leaf moisturized. A single larvea was added to each Petri dish using flexible forceps. Once the infestation was complete, Petri dishes were sealed with a tight lid to prevent the larvae from escaping. Petri dishes were stored in a bioassay room with a temperature of 28 ± 1 °C and a relative humidity of 70 ±10%. The room was illuminated for 12 hours per day. The mortality of the larvae was assessed every 24 hours up until day 7. A larva was considered dead if it was unable to right itself after being placed on a dorsal position. The gross and adjusted mortality rate were assessed using the Abbott's equation (Abbott, 1925).

$$\text{Gross mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Number of introduced larvae}} \times 100 \quad (1)$$

$$\text{Adjusted mortality (\%)} = \left(1 - \frac{\text{nT after treatment}}{\text{nCo after treatment}}\right) \times 100 \quad (2)$$

Où n T = Number of survivors in treatments;

n Co = Number of survivors in controls.



Stage L1: first larval stage; Stage L2: second larval stage; Stage L3: third larval stage; Stage L4: fourth larval stage; Stage L5: fifth larval stage; Stage L6: sixth larval stage

● Petri dish + corn husk +1 larva ⬭ Petri dish + distilled water + 1larva

Fig. 2. Experimental set-up for testing the effectiveness of bioprotect PLUS on the different larval stages of *Spodoptera frugiperda*.

The data on the mortality was used to perform a dose-response analysis in order to estimate the lethal concentration 50 and 90 (LC_{50} and LC_{90}), for each of the biopesticides. To evaluate the efficacy of each of the different biopesticides tested on *S. frugiperda* larvae, the mortality rate was log-transformed.

Statistical Analysis

Statistical analysis of the data was done using GraphPad Prism version 6.0 c software. Corrected larval mortality data were analyzed by ANOVA test using biopesticide concentrations and percent mortality as factors. Mean mortalities were compared using Dunnet's test for multiple comparisons at the 0.05 significance level. This included the comparison of mortalities of the different formulated biopesticides to that of the reference biopestcide Bioprotect PLUS and also the comparison of larval mortalities obtained with different formulated biopesticides. The plots of the percentage of larval mortality corrected according to the dose of the biopesticide were carried out by the GraphPad Prism software version 6.0 c. Median lethal concentrations (LC_{50}) and concentration for 90% mortality (LC_{90}) were extrapolated from the regression curves.

Results

The rearing of *S. frugiperda* yielded 150 to 200 eggs per cluster laid on *P. maximum* leaves. The number of newly hatched larvae after 3 to 4 days ranged from 90 to 120 L_1. About 110 L_1 reached the L_2 stage. And 95 L1 proceeded to L_3 and L_4 stages. The number of L_1 that reached the L_5 and L_6 stages was 80. Dose-response curves showed an increase in mortality of L_2. These results indicated that majority of the formulated biopesticides caused mortality of L_2 larvae after one week post-treatment. The M_{10} formulation had the greatest effect on the mortality rate with a maximum dose at 10^{11} spores/mL and a minimum at 10^7 spores/mL. The mortality rates were 92.1% and 30.1% respectively for these doses. The M_{10} formulation recorded an LD_{50} of 6.9 $x10^7$ spores/mL and an LD_{90} of 3.8 $x10^{10}$ spores/mL (Fig. 3). The M_5 formulation only allowed the determination of an LD_{50} of $1.05x10^9$ spores/mL (Fig. 4).

For the M_{15} formulation, only the first dose D_1 ($4.6x10^{11}$ spores/mL) killed 50% of the stage 2 larval population (Fig. 5). The M_0 formulation had the lowest mortality rates no matter the doses (Fig. 6). The curves representing the insecticidal effect of M_0 and M_{15} bioinsecticides on *S. frugiperda* larvae showed convex shapes. These two bioinsecticides M_0 and M_{15} failed to determine lethal doses that kill 50% and 90% of the L_2. These biopesticides resulted in mortality percentages below 50%. The in vitro activities of the Bioprotect PLUS on the different stages of larvae of *S. frugiperda* after one week of treatment are shown in fig. 7. All stages of larvae were sensitive to the commercial bioinsecticide Bioprotect PLUS. The mortality rates were 100% for the first four stages. A significant difference was observed in the mortality rates of L_5 (p 0.05) and L_6 (p 0.00001). Thus, L_5 and L_6 seemed less sensitive to the Bioprotect PLUS. The insecticidal effect of the Bioprotect PLUS and of the M_0; M_5; M_{10} and M_{15} are compared in fig. 8. There was no significant difference ($p < 0.05$) between the M_{10} formulation and the Bioprotect PLUS with regard to their insecticidal effects on the L_2 after one week of treatment. However, a superior effect (p 0.00001) was noted with the Bioprotect PLUS compared to the other formulations (M_0; M_5; M_{15}) reflecting the poor performance of these formulated bioinsecticides.

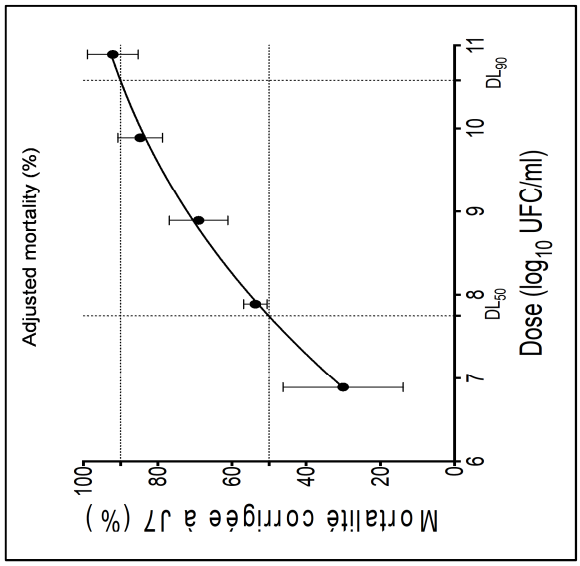


Fig. 3. Concentration-response curves for biopesticide M_{10}.

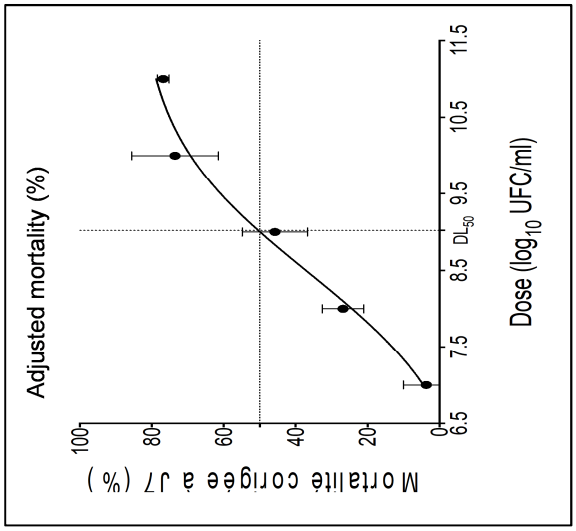


Fig. 4. Concentration-response curves for biopesticide M_5.

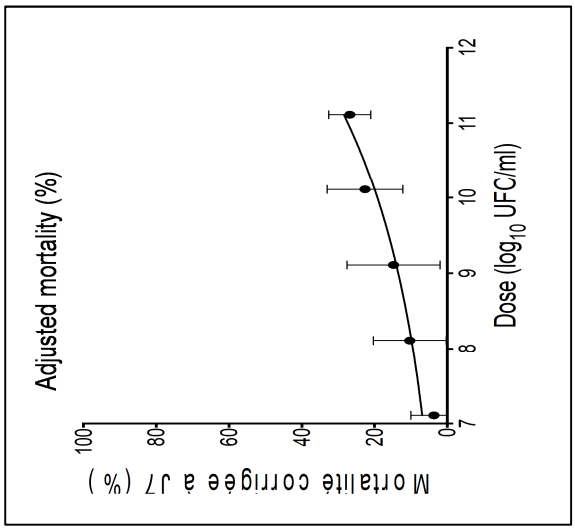


Fig. 5. Concentration-response curves for M_0 biopesticide.

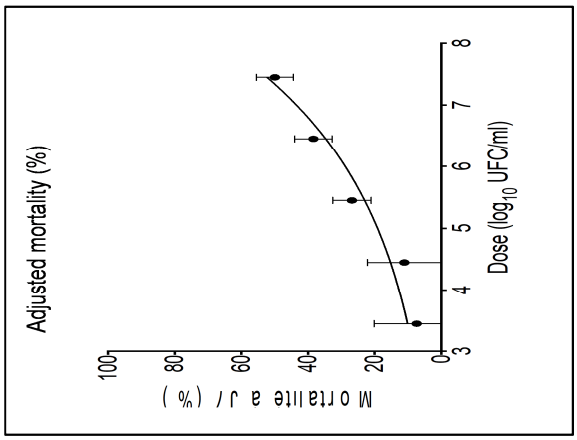


Fig. 6. Concentration-response curves for biopesticide M_{15}.

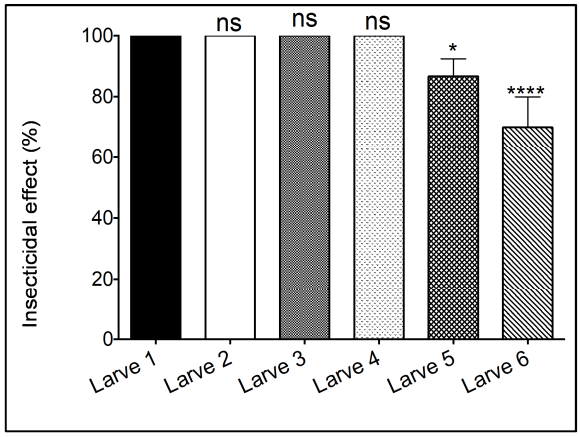


Fig. 7. Toxicity of Bioprotect PLUS biopestcide on the different larval stages of Spodoptera frugiperda.

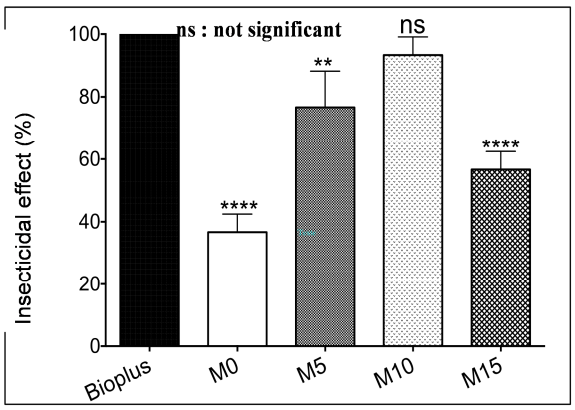


Fig. 8. Effect of different biopesticides (M_0; M_5; M_{10}; M_{15}) and the commercial product bioprotect PLUS (Bioprotect plus) on mortality of *Spodoptera frugiperda* larvae at stage 2 after one week of treatment.

M_0: sugarcane molasses medium; M_5: molasses +100mg/L yeast extract;

M_{10}: molasses +200mg/L yeast extract; M_{15}: molasses +300mg/L yeast extract

Discussion

The objective of the rearing was to have a large number of larvae for testing. However, some losses in larvae were recorded. These larvae deaths could be explained on one hand by the high density of individuals per jar which favors cannibalism. On the other hand, some larvae morphed into chrysalids before they could be processed for the intended experiments. The effects of high density population of larvae on their survival was mentioned by Da Silva et Parra (2013).

Their results suggest a density of 40 larvae per jar to prevent the cannibalistic behavior given that it is a common behavior during laboratory rearing of *S. frugiperda*. Even when the food source is abundant, a cannibalism rate of 40% to 60% has been reported (Chapman *et al.*, 1999). Studies conducted by Chapman *et al.* (1999) showed that individuals derived from cannibalism in *S. frugiperda* had low live weight, low developmental rate and low survival rate. This explains the high percentage of mortality observed during laboratory rearing. The results of the current study showed the effectiveness of some of the insecticides used in the control of the armyworm. The biopesticide M_{10} was the most effective compared to all the other formulations. This could be explained by the fact that the crystals produced by the bacteria in this culture medium may be more toxic. The nutrients provided through that culture medium were optimal to drive the bacterial sporulation. Indeed, the differences in composition of the culture media influence the entomotoxic activity of the *B. thuringiensis* products as shown by Obeta and Okafor (Obeta et Okafor, 1984). The same observations were reported by Black et Snyman (2021), who argued that the availability of carbon sources for *B. thuringiensis* can influence the time required to reach the stationary growth phase and therefore the time to produce the spore-crystal complex. Variations in the levels of spore production as well as in the entomotoxicity of *B. thuringiensis* cultures obtained using molasses enriched or not with yeast extract at different concentrations are common.

In addition, during the sporulation phase, metabolism is based on the use of polyhydroxybutyrate and amino acids as an energy source for spore and crystal maturation and for cell lysis (Rowe, 1990). For all the doses of biopesticide used in this work, the results showed a higher larval mortality rate. These results corroborate those of Chirag *et al.* (2020) who assessed the efficacy of different biopesticides against the same maize pest, and showed a reduction of the larvae population to 2.03 larvae / 10 plants post-treatment. A study by Buntin *et al.* (2003) demonstrated that Bt corn expressing the Cry 1A(b) gene was effective against that pest. A bioassay with a strain susceptible to *S. frugiperda* showed high mortality (97 and 82%), stunted growth and reduced larval weight (Machado *et al.*, 2020). The dose-dependent increase in mortality is in agreement with the results published by Kumar *et al.* (2021) that noted a mortality increases according to the microbial pesticide doses and the time of exposure.

The biopesticide M_{10} exhibited the highest LD_{50} and LD_{90} values. This indicates that more concentrated biopesticide solutions are needed in fall armyworm to achieve a mortality response of 50% and 90% of the fall armyworm population (stage 2 larvae). These results are consistent with previous published data documenting the toxicity of the Bt-based biopesticide on the Fall armyworm larvae that showed that the genus Spodoptera has low susceptibility to them (Rabelo *et al.* 2020; Bohorova *et al.*(1996)) requiring high concentrations of Cry proteins synthesized by Bt. Furthermore, the Cry1Aa, Cry1Ab, and Cry1Ac proteins secreted by *B. thuringiensis* during the sporulation phase do not show a strict correlation between binding and toxicity in *S. frugiperda*. This was also reported by Aranda *et al.* (1996) for whom Bt Cry1 proteins were found to have weak interactions with the brush border membrane of epithelial cells of the gut of *S. frugiperda* larvae. The armyworm, *S. frugiperda* is a species that exhibits genetic variability within populations (Clark *et al.*, 2007; Monnerat *et al.*, 2006). This characteristic may be one of the reasons for such different values in lethal concentration estimates between formulated biopesticides. The data from the analysis of variance and the multiple comparison test of corrected mortality rates demonstrated that the Bioprotect PLUS efficiently reduced the population of younger larvae compared to older ones. These results highlight the insecticidal properties of *B. thuringiensis* which stipulate that Bt are gram + bacteria that during the sporulation phase produce insecticidal proteins in the form of parasporal crystals consisting mainly of one or more crystalline (Cry) and cytolytic (Cyt) proteins that are toxins also called δ-endotoxins (Bravo *et al.*, 2007).

It was thus observed that there may some kind of resistance to the Biopesticide PLUS as the pest grows. This could be explained by the fact that the first, second, third and fourth stages of the larvae are fragile and had developed less defensive mechanisms; whereas those of the 4th and 5th stages showed decreased sensitivity to Bioprotect PLUS. Therefore, an effective biological control with the use of biopesticides is the one that is applied early on during infestation of the maize fields. Any delay in treatment could make the action of the biopesticides less effective as resistance increases with the age. The same finding was shown in biological control trials using parasitoids. Several studies have shown that the rate of parasitism is highest at the egg stage up to larval stage 2 and decreases as the larvae develops (Kakimoto *et al.*, 2009; Sigsgaard *et al.*, 2002). These results are in line with studies done by Steinkraus & Young (1999) on the armyworm. They revealed that Bt-based insecticides were effective against the early stages of larvae development, but less effective during the later stages. This requires constant surveillance of the maize field to ensure proper timing of spraying. A 100% efficacy was achieved with the Bioprotect PLUS. Similar results were reported by Polanczyk *et al.* (2000). A study by Polanczyk *et al*ves (2005) showed that Bt affects biological parameters (weight of female larvae and pupae, oviposition and fecundity) of S. frugiperda. The biopesticide M_{10} was the only formulation capable of closing in the efficacy of the Bioprotect PLUS due to the quality of its toxins and the killing of *S. frugiperda* through similar mechanisms. Therefore, it could be recommended for the biological control of the armyworm.

Conclusion

This study demonstrated the efficacy of laboratory-formulated biopesticides against *S. frugiperda*, an insect pest of nearly 80 plant species. It showed that the commercial biopesticide caused high mortality to all stages of this caterpillar larvae. The mortality was 100% for the first four stages. To all the insecticides formulated, the biopesticide M_{10} was the most effective with LD_{50} and LD_{90} of 6.9. 10^7 spores/mL and 3.8. 10^{10} spores/mL respectively. These features are similar to those of the commercially available Bioprotect PLUS.

Acknowledgements

We would like to sincerely thank the managers of the Agricultural Entomology Unit of the Plant Production Pole of the Nangui Abrogoua University of Abidajn. Special thanks go to Mr. Koffi Kwadjo Eric, a university lecturer who made all the rearing material available to us. Our thanks also go to Mr. Kimou and Mr. Doué, both members of the aforementioned unit for their contribution.

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