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Comparison of the efficacy of a local and commercial formulation of *Bacillus thuringiensis* on *Spodoptera frugiperda* of Maize in Côte D'Ivoire

Achi Stanislas Maurice Akpo[*1], Yapo Atsé Alain[1], Koffi Eric Kwadjo[2], Yapo Ossey Bernard[1,3], Tyagi Dayal Rajeshwar[4]

[1]*Laboratory of Environmental Sciences (LSE), Training and Research Unit in Environmental Sciences and Management (UFR-SGE), University Nangui Abrogoua, Abidjan, Côte d'Ivoire*

[2]*Research and Training Unit of the Nature Sciences, University Nangui Abrogoua, Abidjan, Côte d'Ivoire*

[3]*Central Laboratory for the Environment of the Ivorian Antipollution Center (LCE-CIAPOL), Abidjan, Côte d'Ivoire*

[4]*University of Quebec, National Institute for Scientific Research (INRS-Water Earth and Environment), Laboratory of Wastewater and Sewage Sludge Bioconversion into High Value Added Product, Rue de la Couronne, Québec, Canada*

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Abstract

Spodoptera frugiperda is one of the most important pests of maize, which can cause significant economic losses, up to 4.8 billion dollars. This study aims to evaluate the bioinsecticidal activity of *Bacillus thuringiensis* var. *kurstaki* HD-1 on this caterpillar. For this purpose, two formulations based on Btk HD -1 were tested in vitro against L2 and L3 stage larvae of S. *frugiperda* at different concentrations for seven days. After one to five days of treatments the local formulation induced reduced mobility, cessation of feeding, color change, desiccation and mortality of larvae. However, the commercial formulation was more effective with the 6.5% concentration. It induced 100% mortality in three days of testing on L2 larvae versus five days for the local formulation and four days on L3 larvae. The LC_{50} obtained with L2 and L3 were 2.14 and 2.29% for the commercial formulation against 5.3 and 5.5% for the local formulation. This local formulation could be an interesting alternative in an integrated strategy for the control of *Spodoptera frugiperda* in Côte d'Ivoire in maize crops.

***Corresponding Author:** Achi Stanislas Maurice Akpo ✉ stanisakpo/77@gmail.com

Introduction

Maize (*Zea mays* L.) is the most widely grown crop in the world and the leading grain produced ahead of wheat (*Triticum aestivum* L. subsp. *aestivum*). The world production of maize in 2013 was 839 million tons, compared to 653 million tons for wheat Deffan *et al.* (2013). According to, Agreste, (2021) world maize production in 2021 would have reached a record level of 1131 million tons. In Côte d'Ivoire and most West African countries, maize is the staple diet of rural populations and is consumed by over 300 million African smallholder households (Devi, 2018). It is used for food and feed and as a raw material in some industries. National production is estimated at 654,738 tons, with a total area of 327,800 ha Deffan *et al.* (2013). However, in recent years, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), has appeared in several West African countries, causing considerable damage to maize.

In Côte d'Ivoire, since 2016, sustainable maize production has been confronted with damage caused by *Spodoptera frugiperda* originating from the Americas Kouakou *et al.* (2019). The rapid spread of S. *frugiperda* is facilitated by its high dispersal ability and the wide range of host plants, including grasses and cereals (Montezano *et al.*, 2018). The hardest hit crop is largely maize (Abrahams *et al.*, 2018; Baudron *et al.*, 2019). Indeed, according to FAO (2018), this pest threatens the food security of more than 300 million people in Africa and can cause significant economic losses, up to $4.8 billion in maize production alone.

To this end, emergency actions against this pest in several African countries have focused primarily on chemical control (Feldmann *et al.*, 2019; Kumela *et al.*, 2018). However, insecticides are generally considered harmful to the environment (Todorova and Kozhuharova, 2010) and can also lead to resistance in the insect pest population to insecticides. In addition, some chemical pesticides (Pyrethroids, organophosphates and carbamates) commonly used against S. *frugiperda* remain in soil samples, which can have adverse effects on soil organisms, other soil-dwelling organisms, and other non-target species Togola *et al.* (2018). Therefore, an integrated management approach for S. f*rugiperda* is desirable. In Africa, the main integrated control strategy is based on the use of plant protection products.and lower risk methods, such as biopesticides, biological control and agronomic practices Prasanna *et al.* (2018). Furthermore, to find biological control solutions on S. *frugiperda* resistance to many plant protection products, the objective of this study is to compare and evaluate the entomotoxic efficacy of two formulations of Btk HD-1 biopesticides on S. *frugiperda* stage 2 and 3 (L2, L3) larvae in Côte d'Ivoire. The bioassays were carried out in the entomology laboratory of the University Nangui Abrogoua in Côte d'Ivoire.

Material and methods

Plant material

The plant material consisted of maize leaves susceptible to S. *frugiperda*. These leaves came from an experimental plot located next to the entomology laboratory of the University of Nangui Abrogoua in Côte d'Ivoire.

Entomological material

The entomological material used is S. *frugiperda*. It is a lepidopteran whose young larvae are greenish in color with a black head. The width of the head capsules range from about 0.3 mm (stage 1) to 2.6 mm (stage 6), and the larvae reach lengths of about 1 mm (stage 1) to 45 mm (stage 6) (Prasanna *et al.*, 2018). Only stage 2, 3 were used in the experiment. Caterpillar samples were collected from the farmers' field and reared in the laboratory.

Biological control material

It was essentially made up of different concentrations of the locally formulated biopesticide based on Btk HD-1 on the one hand, whose characteristics are defined as follows: a biopesticide based on Btk HD-1 in liquid form, with a pH (5.2). It is essentially composed of cells (1.42.109 CFU/mL), spores (1.30.109 CFU/mL) and other active components not quantified in this study but synthesized by the

bacteria during the growth and sporulation phases, and on the other hand, a commercial formulation of biopesticide based on Btk HD-1 "bioprotec plus", manufactured in Canada by AEF Global used as a positive control. It is a concentrated liquid formulation with a concentration of 17,500 international units (IU)/mg of product with a pH (5-5.6). It is a registered insecticide, approved for organic agriculture and effective for most caterpillars. A control containing only distilled water was also used as a negative control for the tests.

Methods

Collection and rearing of Spodoptera frugiperda

Rearing consisted of collecting caterpillars between 7:00 and 9:00 a.m. from farmers' untreated maize fields around the city of Yamoussoukro. Using fine-tipped brushes, the insects were detached from the maize leaves and placed in the collection boxes. The collection boxes were covered with fine mesh cloth to allow aeration of the insects Fig. 1. After collection, the boxes containing the caterpillars were taken to the entomology laboratory at Nangui Abrogoua University. These caterpillars were reared one by one, in Petri dishes and all arranged on a rearing shelf in the laboratory at room temperature. The caterpillars were fed with fresh maize leaves, while regularly maintaining the hygiene of the petri dishes, until they reached the chrysalis stage. After the emergence of the butterflies, pairs were formed, each of which was distributed in a well-ventilated plastic jar ; the butterflies fed on honey put in well adapted tubes at the same time the corn (host plant) was sown in polyethylene bags, installed in adapted and well ventilated cages. Then, the pairs of S. *frugiperda* were released in these cages to reproduce. Each cage contained two feet of corn and two pairs of butterflies, two males and two females, which were intended to mate and lay eggs. The laid eggs were reared until they hatched to obtain young larvae that were used to test the larval toxicity efficiency of the tested biopesticides. As for the monitoring, it was done every day to check if there was oviposition or if there was a hatching of the eggs or a moult for the passage from one larval stage to the next.

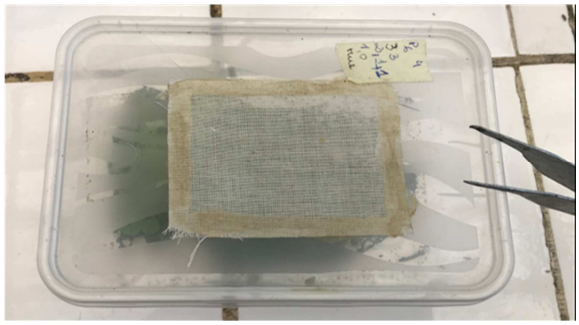


Fig. 1. Collection box for *Spodoptera frugiperda* from corn.

Preparation of different concentrations of biopesticides

It was opted, in our experimental trials, the use of eight (08) concentrations on two types of formulations (the local formulation of Biopesticide and a commercial formulation) and a control (C0) with three (03) repetitions. For the selection of concentrations, the method of Elouissi, (2016) was used. To do this, we based ourselves on the concentration recommended by the manufacturer of the commercial product "bioprotec plus" against lepidopterans, which is 5 /100 (v/v) of distilled water. Based on this reference concentration, preliminary tests were conducted in the laboratory to determine the different concentrations to be tested. Consequently, a series of four (04) concentrations for each product was selected: 2, 3.5, 5, 6.5% designated as C1, C2, C3 and C4 (local formulation) and B1, B2, B3 and B4 (commercial formulation).

Laboratory testing of biopesticides on S. frugiperda

Regarding the bioassays against S. f*rugiperda* in the laboratory, two types of experiments were conducted and concerned the L2, L3 stages. Each trial was bifactorial, with the primary factor being the type of Btk HD-1 biopesticide (the local formulation and the commercial product). The secondary factor was the concentrations used, with four variations: 2%, 3.5%, 5%, 6.5% for each of the biopesticides tested and the control. The bioassay was conducted using the modified leaf dip method (Pandey *et al.* (2009); Kandil *et al.* (2020)). This method involved washing the corn leaves harvested in the field with distilled water, air dry them, 30 pieces of leaves of 5 cm length were cut with a pair of scissors. Each cut leaf piece was dipped into the first concentration (C1) of 2%

suspension of Btk HD-1 prepared in the petri dishes. It was dipped by the C1 concentration bacterial suspension of biopesticide and then placed in another Petri dish containing a paper towel.

A total of 30 Petri dishes were prepared for this first concentration experiment (C1). One larva, left fasting for 24 hours, was deposited on the treated leaf pieces with fine-tip forceps in each prepared Petri dish. This combination constituted one replicate and each test had 30 larvae and the test was done in 3 replicates (n = 3). This same method was applied for the other concentrations and test products. In the case of the control, the leaf pieces were soaked in distilled water only. A total of 90 S. *frugiperda* stage 2 larvae were used per test (test concentration), or 810 individuals for the eight concentrations of bioproducts tested including the control.

A total of 1620 larvae for both stages were used. To avoid early death of the insects due to lack of food, the corn leaves were renewed in the Petri dishes every day. These new leaves were not impregnated with the Btk HD-1 suspension to avoid the double dose effect. Petri dishes were placed in a room at room temperature (25°C) and a 12-hour photoperiod, following a completely randomized design. The results were recorded every 24 hours for 7 days after treatment by noting the mobility and mortality of the larvae. The efficacy of the preparations is evaluated in relation to the mortality of the larvae after treatment.

Calculation of the mortality rate and determination of the LC_{50}

Calculation of mortality rate

Observations of insect mortality were made at a regular frequency, every 24 hours, over seven days after which dead individuals were counted. Mortality rates were expressed as percent mortality according to formula (1), correcting for "untreated" (control) mortality using Abbott's (1925) formula (2) recommended by FAO and WHO in insecticide tests (Mawussi, 2008).

$$\text{Mortality (\%)} = \frac{\text{Number of deaths}}{\text{Total number of individuals}} \times 100 \quad (1)$$

$$\text{M.C (\%)} = \frac{\text{M2} - \text{M1}}{100 - \text{M1}} \times 100 \quad (2)$$

M.C: Percentage of corrected mortality.

M1: Percentage of mortality in the control lot.

M2: Percentage of mortality in the treated batch.

LC_{50} Determination

Lethal concentrations of the local Btk HD-1 biopesticide killing 50% of the larval population (LC_{50}) were determined using the method of Miller and Tainter (Randhawa, 2009). Observed mortality percentages are transformed into probits by referring to the probit table. Regression of data against Log concentration of Btk HD-1 biopesticide was obtained using XLstat software version 2016. This method was used to determine the LC_{50} values.

Statistical analyses of the data

The collected data were subjected to analysis of variance (ANOVA), this type of analysis was used to test the effect of the factors concentrations of the Btk HD-1 biopesticide and test duration on the mortality rate of S. *frugiperda*. If there was a significant difference, at the 5% level, Tukey's test was used to average the insect mortality rate caused by the different concentrations. The data were processed using XLstat 2016 software.

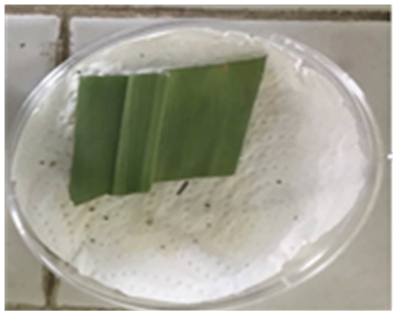
Results and discussion

Results

Effect of the biopesticide on the behavior of the larvae after treatment

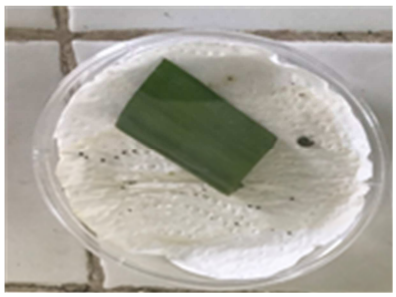
In this part some images were represented with the test of the biopesticide with the local formulation. Fig. 2 shows the appearance of the larvae after treatment with the biopesticide. Biopesticide treatments affect the mobility and morphology of the larvae.

After ingesting the leaves treated with B. *thuringiensis* after 1 to 2 days of treatment the larvae stopped feeding with a reduced mobility and followed by a reduction of its shape (Fig. 2a), after 4 days of treatment a total stop of mobility was observed (Fig. 2b). After 5 days of treatment a total paralysis followed and the larva died (Fig. 2c).



a

b



c

Figs. 2 (d and **e)** also show the appearance of the larva before and after application of the Btk HD-1 biopesticide. The larva after treatment dies, becomes discolored, desiccated and shows a reduction in shape.

(a): Reduced mobility of the larva after 1 to 2 days of treatment

(b): Stopping of the mobility of the larva after 3 to 4 days of treatment

(c): Death of the larva after 4 to 5 days of treatment

(d): Larva appearance before treatment **(e):** Larval appearance after treatment

Fig. 2. Larvae appearance after treatment of Btk HD-1 bioproduct.

Effect of biopesticide and test duration on L2 stage mortality of S frugiperda

The results of efficacy tests of the two formulations of Btk HD-1 biopesticide and a control on the L2 stage larval population of S. *frugiperda* were presented in Table 1. Analysis of the data revealed significant variances and differences in mortality rates ($p < 0.05$). Indeed, the results presented show that mortality of L2 stage individuals occurs 24 h (J1) after exposure of insects to commercial biopesticide at B4 (6.5%) and B3 (5%) concentrations of Btk HD-1 with a significant difference. The respective mortality rates obtained were 80% and 30%. The maximum entomotoxic effect of the commercial bioproduct at 6.5% concentration was reached on day 3. In addition, statistical analysis of the data by a Tukey test at the 5% threshold revealed no statistical difference between the two concentrations (6.5 and 5%) on the fourth day of testing ($p > 0.0001$). Regarding the concentrations of the local biopesticide Btk HD-1, these did not cause any effect on the insects during the 48 h of testing.

The minimal bioinsecticidal effects of the concentrations C4 (6.5%), C3 (5%) and C2 (3.5%) of the local formulation were observed after 72 h (J3) of submission of the subjects to the tests, causing the mortality of 33.33% for C4, 13.33% for C3 and 6.67% for C2. Statistical analysis of the data showed significant differences ($p < 0.0001$) in comparison with the bioproduct commercial product at different concentrations B4, B3 and B2 causing 100, 56.67 and 50% mortality.

On the other hand, the maximum entomotoxic effect of the C4 concentration was reached on the fifth day as well as those of the commercial product at concentrations B4, B3 and B2 causing 100% mortality of individuals. On the seventh day of testing, four effects were recorded after statistical analysis. Concentrations B4, B3, B2 and C4 causing 100% mortality of insects caused the very high (maximum) effect, then the medium effect was caused by concentrations C3 and B1 causing 43.33% and 40% mortality, the low effect was

observed with concentration C2 (30%) and very low with C1(16.67%) and Co (9.99%). Concerning the control lot (Co) and the concentration C1, similar mortalities were observed on the seventh day of the tests after statistical analysis by a Tukey test ($p > 0.0001$).

Table 1. Efficacy tests of different concentrations on the mortality of S. *frugiperda* stage 2 (L2) larvae after seven days.

days / Concentrations	% Daily mortality after treatment						
	J1	J2	J3	J4	J5	J6	J7
B4	80,00 a	83,33 a	100,00 a	100,00 a	100,00 a	100,00 a	100,00 a
B3	30,00 b	50,00 b	56,67 b	100,00 a	100,00 a	100,00 a	100,00 a
B2	0,00 c	16,67 c	50,00 b	66,67 b	100,00 a	100,00 a	100,00 a
C4	0,00 c	0,00 d	33,33 c	56,66 c	100,00 a	100,00 a	100,00 a
C3	0,00 c	0,00 d	13,33 d	26,67 d	30,00 b	40,00 b	43,33 b
B1	0,00 c	0,00 d	10,00 d	23,33 d	26,67 bc	36,67 b	40,00 b
C2	0,00 c	0,00 d	6,67 de	20,00 d	23,33 c	26,67 c	30,00 c
C1	0,00 c	0,00 d	0,00 e	0,00 e	6,67 d	10,00 d	16,67 d
Co	0,00 c	0,00 d	0,00 e	0,00 e	0,00 e	0,00 e	9,99 d
Pr > F	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Significatif	Oui	Oui	Oui	Oui	Oui	Oui	Oui

The values in each column assigned with the same letter are not significantly different according to Tukey's test at the 5% level.

The change in the corrected mortality rate of the L2 stage larval population of S. *frugiperda* after treatment with different concentrations of the local formulation was shown in Fig. 3. The L2 stage larvae tested were sensitive to the different test concentrations. Also, the bioinsecticidal effect of the local formulated product increased over time and this effect increased significantly on the test subjects by increasing the biopesticide concentration. Indeed, no mortality was observed on the first and second days of the tests. Statistical analysis of the data showed no significant difference ($p > 0.05$). L2 stage larvae were more sensitive to C4 (6.5%) concentrations of Btk HD-1 than C3 (5%), C2 (3.5%), and C1 (2%) concentrations of locally formulated Btk HD-1. Statistical analysis revealed no statistical difference between C3 and C2 concentrations at day 4. In fact, the death of all (100%) of the insects tested occurred on day 5 with concentration C4. After seven days of testing, the statistical analysis of variance of concentrations C4, C3, C2 and C1 revealed statistical differences ($p < 0.05$). To this effect, the recorded mortalities were 100% for C4, 37% for C3, 22.16% for C2 and 7.56% for C1.

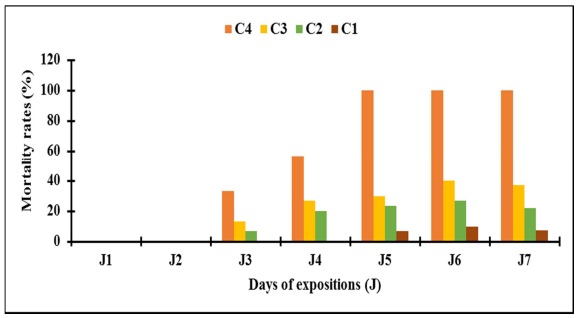


Fig. 3. Evolution of the corrected mortality rate of S. *frugiperda* L2 larvae subjected to different concentrations of Btk HD-1 as a function of test duration.

Effect of biopesticide and test duration on L3 stage mortality of S. frugiperda

The results of the efficacy tests of the two formulations of Btk HD-1 biopesticide and a control on the L3 stage larval population of S. *frugiperda* were presented in Table 2. Analysis of the results shows that mortality of L3 stage individuals occurs 24 h after exposure of the insects to the commercial biopesticide at concentrations B3 (5%) and B4 (6.5%).

The mortality rates recorded for this effect were 20% and 60%. However, the maximum entomotoxic effect of the two concentrations was reached after four days of submission of the larvae to the products, causing the death of 100% of the L3 larvae. As for concentrations C2, C3 and C4, they did not cause any effect on L3 larvae during the 48 hours (J2) of testing. The minimal entomotoxic effect of these concentrations started 72 h after treatment. Mortalities obtained were 3.33% for C2, 10% for C3 and 26.67% for C4. However, these mortality rates evolved to reach their maximum on the seventh day causing the death of 23.33%, 36.67% and 76.67% of the insects tested. Concentrations C1 and Co (control) had the lowest mortality. The statistical analysis revealed similarities between these two concentrations with ($P > 0.05$). On the last day of testing, different effects emerged after statistical analysis of the data by a Tukey test. The maximum effect causing the mortality of the totality was caused by the concentrations B4, B3 of the commercial product. As for the C4 concentration of the local formulation, it registered a medium effect and the other concentrations weak effects with highly statistical differences ($p < 0.05$). Concerning, the concentrations Co and C1, the statistical analysis revealed no statistical difference ($p > 0.05$) between the two concentrations.

Table 2. Efficacy tests of the different concentrations on the mortality of S. *frugiperda* stage 3 (L3) larvae after seven days.

Day / Concentrations	% Daily mortality after treatment						
	J1	J2	J3	J4	J5	J6	J7
B4	60,00 a	73,33 a	80,00 a	100,00 a	100,00 a	100,00 a	100,00 a
B3	20,00 b	46,67 b	50,00 b	100,00 a	100,00 a	100,00 a	100,00 a
B2	0,00 c	13,33 c	46,67 b	63,33 b	76,67 b	86,67 b	93,33 a
C4	0,00 c	0,00 d	26,67 c	50,00 c	66,67 c	70,00 c	76,67 b
C3	0,00 c	0,00 d	10,00 d	20,00 d	26,67 d	30,00 d	36,67 c
B1	0,00 c	0,00 d	6,67 de	16,67 de	23,33 de	26,67 de	33,33 c
C2	0,00 c	0,00 d	3,33 de	10,00 e	16,67 e	20,00 e	23,33 d
C1	0,00 c	0,00 d	0,00 e	0,00 f	0,00 f	6,67 f	10,00 e
Co	0,00 c	0,00 d	0,00 e	0,00 f	0,00 f	0,00 f	6,66 e
Pr > F	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Significatif	Oui	Oui	Oui	Oui	Oui	Oui	Oui

The values in each column assigned with the same letter are not significantly different according to Tukey's test at the 5% level.

The evolution of the corrected mortality rate of the L3 stage larval population of S. *frugiperda* after treatment with the local biopesticide Btk HD-1 was shown in Fig. 4. The L3 stage larvae were sensitive to the different concentrations of biopesticide being tested. By increasing the biopesticide concentration, the entomotoxic effect of Btk HD-1 on the tested individuals increased significantly. Also, the observed entomotoxic effect of Btk HD-1 increases over time. The L3 stage larvae were very sensitive to the highest concentration C4 causing 75.03% mortality at the end of the trials. As for the concentrations C3, C2 and C1, they were less sensitive causing less than 40% mortality of the subjects tested. Mortality of L3 larvae changed significantly until the last day (J7) of testing. However, the daily mortality rates induced by the C1, C2, C3, and C4 concentrations were statistically different ($p < 0.05$).

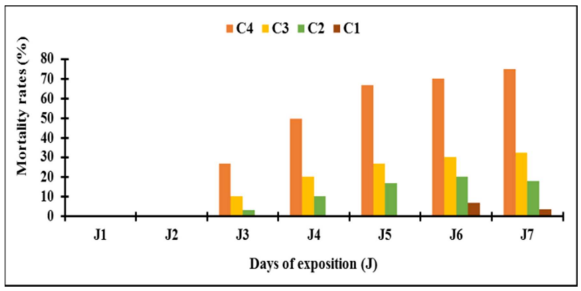


Fig. 4. Evolution of the corrected mortality rate of L3 S. *frugiperda* larvae subjected to different concentrations of Btk HD-1 as a function of the test duration.

Lethal concentration causing 50% (LC_{50}) mortality of S. frugiperda larval populations

The probit-transformed mortality percentages after seven days of exposure of S. *frugiperda* larvae to

biopesticides yielded the LC_{50} and equation lines summarized in Table 3. The comparison of LC_{50} of the tested biological insecticides shows that the local formulation (F.L) reveals to be the less toxic insecticide against L2 and L3 larvae of S. *frugipetrda* than the commercial formulation (F.C). The LC_{50} recorded for this purpose, were 5.3; 5.5% against 2.09; 2.14% for the commercial formulation (F.C).

Table 3. LC_{50} of local and commercial biopesticides on L2 and L3 larval stages of S. *frugiperda*.

Stage	CL 50 (%)		Regression equation	
	F.L	F.C	F.L	F.C
L2	5,3	2,14	Y= -2,637+4,316X	Y= -8, 045+25,885X
L3	5,5	2,29	Y=-2,284 +3,762X	Y=-2,448+8,189X

Discussion

Effect of biopesticide on larval behavior after treatment

The results of observations on larval behavior (mobility, feeding cessation, paralysis, color change, desiccation and mortality) obtained after application of the local bioproduct on S. *frugiperda* larvae were similar to those of Devi *et al.* (2005) and Elouissi, (2016). The first authors, by applying a formulation of Bt powder produced from three media at concentrations of 0.1 or 0.2% on *Semiloope castor* larvae, the results showed an immediate cessation of larval feeding, followed by their mortality after 5 days of treatment. As for the second author, after application of different doses (0.200, 0.350, 0.500, 0.650 and 0.800g/l.) of Bt on larvae of the L1 to L4 stages of *Tuta absoluta* observed discoloration, desiccation and mortality of treated larvae. These different manifestations in larvae are probably related to the presence of protein crystals contained in the Bt preparation. In this regard, some authors, such as Aronson *et al.* (1986) and Hofte and Whiteley (1989), indicate that the crystallized proteins of Bt exert their effect on the host by lysing the epithelial cells of the midget and causing paralysis of the digestive tube. The infected larva then stops feeding within hours of ingestion (2-4 hours) and eventually dies. Once ingested, the crystals dissolve in the alkaline environment of the host midget. Proteolysis of the solubilized crystallized protein or protoxin produces the toxic fragment (toxin). Once bound to specific receptors on the membranes of the midget epithelial cells, the toxin induces the formation of pores in the epithelial cell membrane and, in so doing, causes the death of the cells and the infected larva.

Effect of bioproducts and test duration on mortality of S. frugiperda larval populations

Regardless of the larval stages, in general the efficacy tests of B. *thuringiensis* on larval populations of S. *frugiperda* conducted in the laboratory showed a high and significant efficacy in reducing damage caused by the different larval stages (L2, L3) of S. *frugiperda* at different concentrations of the bioproducts tested compared to the untreated control.

Larvae of both stages recorded high mortalities to both bioproducts. The mortality of infected larvae could be due to the non-digestion of ingested food caused by the physiological disruption of the hemolymph (Lotfy, 1988). Also, the results showed that, the insecticidal activity of the commercial formulation based on Btk HD-1 was effective on both larval stages of S. *frugiperda* at 100% mortality, with low LC_{50}s (2.14 and 2.29). These results obtained were similar to those of these authors (Sneh *et al.* (1981); Polanczyk *et al.* (2000) and Vlicente *et al.* (2008)). The first authors obtained an efficiency of 100 to 70% mortality on all larval stages (L1 to L6) of S. *littoralis* using a strain of Bt 24. As for the second, using a suspension of Bt *aizawai* HD 68 and Bt *thuringiensis* 4412 containing 3.10^8 cells/mL on stage 2 (L2) larvae of S. *frugiperda* obtained 100% and 80.4% mortality with LC50s of $6.7.10^6$ cells/mL and $8.6.\ 10^6$ cells/mL, and finally the last authors obtained 100% mortality on S. *frugiperda* stage 2 (L2) larvae using a concentration of $1.14.10^9$ spores/mL of Bt produced in a commercial mineral-enriched Luria Bertani (LB) medium.

Also, like the commercial formulation all developmental stages of S. *frugiperda* were found to be sensitive to the local Btk HD-1 formulation. This sensitivity is thought to be due to the Btk strain being predominantly composed of crystalline endotoxins, mainly cry1Aa, Ab, Ac, and cry2, which are highly toxic to insects of *Spodoptera* species (Schnepf *et al.* (1998); Boucias and Pendland (1999)).

However, this sensitivity differs between larval stages. L2 stage larvae were more sensitive to the effects of the bioproducts tested than L3 larvae. The local formulation at high concentration C4 ($1.30.10^9$ CFU/mL) was also more effective on L2 than L3 larvae of S. *frugiperda*. The results obtained are similar to those of Valicente *et al.* (2008) and Elouissi, (2016). The first authors, obtained 100% mortality on stage 2 (L2) larvae of S. *frugiperda* using a concentration of $3.02.10^9$ spores/mL of Bt produced in a medium composed of 1% glucose and 3% soybean powder. Also, the second author also achieved 100% mortality on L2 stages of *Tuta absoluta* using high doses of 0.650g/L and 0.800g/L of B. *thuringiensis*. This author reported that this susceptibility of L2 larvae would be closely related to the physiological and histological characteristics of the site of action of the bacterium, the insect's digestive tract. Also, this corroborates the results of the work of several authors, especially on other Lepidoptera. These authors have shown that young larvae, more sensitive and vulnerable, have important food needs and a high metabolic activity (Beevers, (1990); Behle, (1997)).

Based on the observed results, the commercial biopesticide based on Btk HD-1 was more effective than the local formulation in both stages (L2 and L3) of S. *frugiperda* larvae development with lower LC_{50}s than the local formulation. This difference would be due to the composition of the formulated products, the culture methods and the formulation conditions. This same observation was reported by Srinivasan *et al.* (2001), according to these authors, the modification of the media, the culture methods and the growth conditions affected the toxicity and therefore the efficacy of a Bt strain on insects. In this regard, Boucias and Pendland (1999) reported that the difference in activity of the different formulations was attributed to the different levels of toxins present in them and as larvae were fed with preparations containing a spore-crystal complex, mortality was more rapid. In terms of spore productivity, the commercial product would contain a higher spore load than the local formulation. This high spore concentration would be one of the causes of its efficacy and toxicity on S. *frugiperda* larvae. On this basis, Pruett *et al.* (1980) reported that the higher the number of viable spores in a *Bacillus thuringiensis* preparation, the more effective it is and the higher the insect mortality. Therefore, the number of viable spores may be one of the indices to predict the effective dose of a *Bacillus thuringiensis* preparation. The effective dose depended more on the number of viable spores than on the crystals because the crystals degrade much faster than the spores.

Conclusion

The high entomotoxicity of 50 to 100% mortality presented by the local formulation against S. *frugiperda* indicates its application in integrated pest management systems. For the time being, while waiting for some improvements, it can be considered as an alternative biological control against S. *frugiperda* in Côte d'Ivoire as a replacement to chemical products toxic for humans and the environment.

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