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Full Length Article

Assessment of the Entomotoxic Potential of the *Bacillus thuringiensis* var. *kurstaki* HD-1-Based Biopesticide against *Sahlbergella singularis* (Hemiptera: Miridae): A Cocoa Mirid in Côte d'Ivoire

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Abstract

The mirid *Sahlbergella singularis* Hagl. causes major production losses in cocoa trees in Côte d'Ivoire. To deal with this problem, farmers make excessive use of synthetic chemical insecticides to improve crop yield. In order to reduce the chemical pollution risk, this study was initiated to assess the efficacy of a *Bacillus thuringiensis* var *kurstaki* HD-1 (Btk HD-1)-based biopesticide against *S. singularis*. The study was carried out in the laboratory in Petri dishes on two L4 larvae using the triplet method and, in the field, on a homogeneous population of L4 larvae, using a scattered block design. Observations were made using four doses [D1 (10%), D2 (1%), D3 (0.1%), D4 (0.01%) (v/v)] of biopesticide versus a negative control (D0). The mortality rates and the severity of damage to cocoa organs (immature cocoa pods and twigs) caused by mirid bites were measured. Laboratory results revealed the effectiveness of the tested doses. D1 induced 100% mortality of L4 larvae, while D4, D3 and D2 caused 41.61, 47.55 and 69.41% mortality respectively, compared with 4.17% mortality in control (D0), on the fifth day of laboratory tests. In the field tests, in a natural environment, D1 and D2 provided more than 77% phytosanitary protection of cocoa organs against mirids. The LD_{50} values obtained on the fifth and seventh day of treatment were 0.038%, respectively for the laboratory tests and varied between 0.015 and 0.066% for the field tests. The demonstrated bioinsecticidal effect of Btk HD-1 is dose sensitive and increases with time. This biopesticide could be an alternative to the conventional chemical insecticides used to control cocoa mirids. © 2024 Friends Science Publishers

Keywords: *Bacillus thuringiensis* var. *kurstaki* HD-1; Biopesticide; Cocoa tree; Côte d'Ivoire; Efficacy test; *Sahlbergella singularis*

Introduction

The cocoa tree (*Theobroma cacao* L.) is economically important for its seeds, commonly known as cocoa beans, which are used as a raw material in the manufacture of chocolate and its derivatives. For more than four decades, this cash crop has been the driving force behind Côte d'Ivoire's economic and social development (Tano 2012). However, the cocoa tree is subject to numerous constraints, including biotic constraints. These include attacks by insect pests, diseases, and damage caused by rodents and parasitic plants (Kébé *et al*. 2006). In this context, attacks by mirid pests are a major problem for growers (Coulibaly *et al*. 1998).

In Côte d'Ivoire, *Sahlbergella singularis* is the predominant mirid species in cocoa orchards (Kouamé *et al*. 2014, 2015). In the event of infestation, these insects cause partial or total degradation of the cocoa plantation, a reduction in the trees' production (Kouame *et al*. 2014) and poor cocoa bean quality (Akpesse *et al*. 2014). Annual production losses caused by mirids in Côte d'Ivoire vary between 30 and 40% (CNRA 2010).

To this end, several control approaches are used to manage pests on cocoa plantations. However, the main means of effectively controlling these pests is still the use of chemical insecticides (Gidoin 2013). Although this method has helped to improve crop yields, overuse poses a real

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threat to the environment and human health, and can lead to the emergence of resistant mirid populations (Asogwa *et al*. 2006; Biégo *et al*. 2009; ICCO 2015; Lenteren *et al*. 2018). This greatly reduces the sustainability of this control method. One potentially promising management approach is the application of beneficial microorganisms (Deravel *et al*. 2014).

In Côte d'Ivoire, projects have been launched to evaluate and promote the application of a *Bacillus thuringiensis* var. *kurstaki* HD-1 (Btk HD-1) biopesticide in cocoa orchards. This biopesticide is a mixture of a suspension of insecticidal spores and protein crystals obtained after growth of the bacterium (Btk HD-1) in starch wastewater. The active components (insecticidal spores and protein crystals) produced by the bacteria are target specific, biodegradable, environmentally friendly and harmless to human and mammals (Roh *et al*. 2010; Czaja *et al*. 2015; Duchet *et al*. 2015). Several studies have highlighted their effectiveness in controlling a wide range of insects (Bravo *et al*. 2007; Pardo-Lopez *et al*. 2009; Lagadic *et al*. 2014; Duchet *et al*. 2015). This makes it the most widely used biological insecticide in the world (Helassa 2008). The aim of this study was to assess the efficacy of the Btk HD-1-based biopesticide against *S. singularis*, a cocoa mirid in Côte d'Ivoire, as potential preventing tools against this pest.

Materials and Methods

Study site

The present study was carried out from July 2015 to February 2017 in two phases. The first phase involved studying the *In vitro* effect of the *Bacillus thuringiensis* var. *kurstaki* HD-1 (Btk HD-1)-based biopesticide against *Sahlbergella singularis* Hagl. (Hemiptera: Miridae) in the entomology laboratory of the Cocoa Programme of the "Centre National de Recherche Agronomique (CNRA)" based in Divo, in the centre-west of Côte d'Ivoire (5°46'22"N, 5°13'46"W). The second phase of the study involved evaluating the product in the field, in cocoa plantations at Guéyo (Fig. 1), in the South-West region of Côte d'Ivoire (5°41′16″N, 6°04′15″W). This locality is placed in a humid forest zone characterised by a humid tropical climate where mean annual rainfall during study period varied from 1254.13 to 1390.05 mm. Average temperature and relative humidity fluctuated between 28 and 35.7°C and 73 and 78.3% RH. This south-western region is Côte d'Ivoire's new cocoa loop, supplying 34% of national production alone that corresponds about 15% of world production (Tano 2012).

Characteristics of Btk HD-1-based biopesticide

Btk HD-1-based biopesticide was developed in the laboratory for the bioconversion of wastewater and sewage sludge into high value-added products at INRS-ETE, Université de Québec, Canada. It is produced by bioreaction of the bacteria (Btk HD-1) using wastewater from starch industries as a fermentation substrate. The liquid substrate obtained after 48 h of fermentation contains bacterial cells (3.13×10^9 CFU/mL), spores (2.46×10^9 CFU/mL) and other active components including delta-endotoxins, chitinases, zwittermicin A and vegetative insecticidal proteins (Vu 2009). This biopesticide is marketed in Canada under the name Bioval. It was applied against cocoa mirids in Côte d'Ivoire as part of a research project in collaboration with the aforementioned laboratory.

Collection of plants and animals' material

For the laboratory tests, green, tender and healthy twigs of the same diameter (0.5 cm) from a cocoa clone (T79/501) susceptible to *S. singularis* were used as food for the insects (N'Guessan *et al*. 2010). They were collected in an experimental plot not treated with chemical pesticides at the CNRA research station in Divo. Animal material consisting of *S. singularis* mirids was also captured in this plot, on cocoa pods and small branches, using fine-tipped brushes. Capture was made early in the morning, between 7.00 and 9.00 a.m., as mirids hide in the darkest cracks of the cocoa tree in sunlight. The captured insects were placed in Plexiglas collection boxes containing immature pods and twig fragments as a food source. This material was taken to the laboratory for *in vitro* bioassays.

Laboratory bioassays

Btk HD-1 biopesticide preparation: The direct plating technique, following the method described by Yezza *et al*. (2005), was used on trypticase soya agar (TSA) culture medium to determine the total number of viable bacteria and spores in the Btk HD-1 biopesticide. After checking the viability of the cells and spores, four successive decimal dilutions were made in sterilised test tubes using sterile distilled water. The different dilutions performed or test doses [10; 1; 0.1; 0.01 and 0% (v/v)] are shown in the table below (Table 1), and designated as D1, D2, D3, D4 and D0 (control), respectively. These were tested on L4 larvae of *S. singularis*.

Conducting bioassays in the laboratory: The twigs of cocoa clone T79/501 were cut into fragments 5 cm long and 0.5 cm wide, soaked in diluted suspensions of the biopesticide, depending on the doses to be tested. The treated twig fragments were then placed in Petri dishes of 14 cm in diameter and 2 cm high, lined with blotting paper, using the triplet method described by Badegana *et al*. (2005). Two L4 larvae, starved for 24 h, were carefully placed in the middle of the triplet branch fragments using a fine-tipped brush (N'Guessan *et al*. 2010). The test was carried out in two series of 120 L4 larvae each, with 24 L4 larvae per diluted suspension of the biopesticide. A total of 240 individuals from the natural population of *S. singularis*

Table 1: Different doses of the *Bacillus thuringiensis* var. *kurstaki* HD-1 (Btk HD-1) biopesticide evaluated on *S. singularis*

			Concentration (CFU/mL)	
Treatments	Dilutions	Doses % (v/v)	Cells	Spores
D0	0	0	-	-
D1	10^{-1}	10	4.1 x10^{8}	3.2x10^{8}
D2	10^{-2}	1	3.2x10^{7}	2.4x10^{7}
D3	10^{-3}	0.1	5.1x10^{6}	4.3x10^{6}
D4	10^{-4}	0.01	3.13x10^{5}	2.5x10^{5}

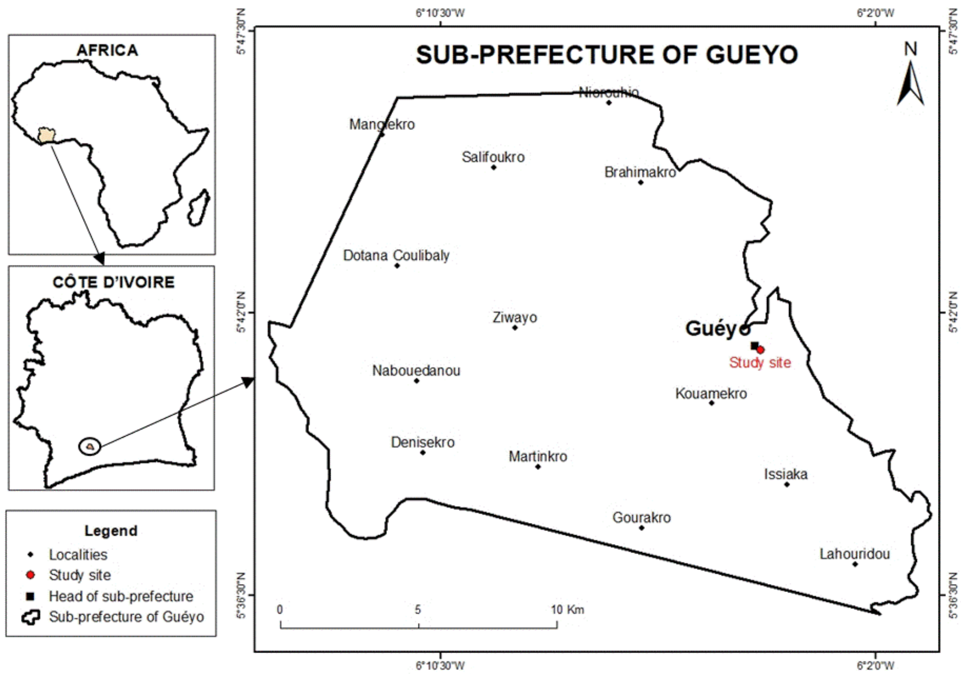

Fig. 1: Map showing the study site in the cocoa plantations

were used for bioassays in the laboratory. To prevent insect dying due to lack of food, the twig fragments in the Petri dishes were replaced after every 48 h. These new shoot fragments were not treated with biopesticide to avoid a double-dose effect. The Petri dishes were placed at room temperature (27°C) and photoperiod (12L: 12D) using completely randomised design. The duration of each test was five days.

Field trials

Insect breeding: *S. singularis* mirids were reared in the field at Guéyo, in western Côte d'Ivoire, using the mosquito netting technique described by Babin (2009). The experiment consisted of selecting tender, green twigs and green immature cocoa pods from cocoa trees for each generation of *S. singularis*. The selected cocoa trees were identified in six untreated plots far from areas where chemical pesticides are widely used. Four cocoa trees were identified per generation of *S. singularis*, with eight cocoa bushes and four branches in one plot. Two female larvae and one male larva per generation were placed on two immature pods or one twig (branch) per cocoa tree. There were L3, L4 and L5 larvae, and adults of *S. singularis*. However, only L4 larvae of the third generation were used to evaluate the efficacy of biopesticide's doses.

Bioassays in open fields: The study was carried out in the same plots and under the same conditions as those adopted for the rearing of *S. singularis* mirids, using a dispersed block design. It was carried out on 90 cocoa trees, with thirty trees per cocoa plot. Six immature cocoa pods and six tender twigs in mid-shade were treated per dose, an immature pod and one twig per cocoa tree. The dilutions or doses were applied to the cocoa part at a distance of approximately 10 cm, using a 100 mL spray bottle. Each cocoa part received approximately 2 mL of each dilution, corresponding to four sprays from the spray bottle. Fifteen minutes after treatment, three L4 larvae (one male and two females) were carefully placed on each treated cocoa part and covered with mosquito netting. In total, 180 L4 larvae spread over 30 cocoa trees per plot were exposed to different doses or dilutions of the biopesticide. A plot of 30 cocoa trees constituted one replication. The untreated control cocoa trees were separated by 10 m from the treated cocoa trees. Field bioassays required 540 L4 larvae, 90 immature cocoa pods and 90 cocoa branches. Observations of the number of deaths were made 24 h after the L4 larvae had been exposed to different doses of biopesticide for a fortnight.

Assessment of insect damage to immature cocoa pods and twigs: Mirid damage to immature cocoa pods and twigs was assessed visually at 30 days interval for 90 days. Affectations were estimated according to four damage rating classes in accordance with the modified Brun *et al*. (1997) method:

- Class 0: immature cocoa pods or twigs without damage;
- Class 1: 25% of the immature cocoa pods or twigs affected;
- Class 2: 50% of the immature cocoa pods or twigs affected;
- Class 3: more than 50% of the immature cocoa pods or twigs affected.

Calculation of mortality rate

Average mortality rates were calculated per week over two weeks according to the doses applied. When the insect mortality rate in the control batch was less than 10%, the Abbott (1925) formula was applied to correct the mortality rate according to the equation below:

$$\mathrm{Mc} = \left(\frac{\mathrm{Mo} - \mathrm{Me}}{100 - \mathrm{Me}}\right) \times 100$$

Mc: corrected mortality (%),
Mo: mortality observed in treated batches (%),
Me: average mortality in the untreated control.

Statistical analysis of data

The data collected were subjected to a one-factor analysis of variance (ANOVA 1) to assess the effect of each dose on the insects. In the event of a significant difference at the 5% threshold, the Newman-Keuls (Duncan) test was applied to segregate the means between insect mortality rates. In addition, the non-parametric Kruskal Wallis test at the 5% significance level was applied to data relating to the severity of cocoa organ diseases caused by mirid bites. The data were processed using SPSS 22.0 software. *In vitro* and field LD_{50} values were determined using the method of Finney (1971). Corrected mortality percentages were transformed into probits using the probit table. The regression of the data as a function of the biopesticide dose was obtained using MS Excel version 2019.

Results

In vitro effect of biopesticide against larvae of *S. singularis*

The results of the laboratory bioassays (Table 2) showed that there were significant differences ($F_{(4, 115)} \geq 31.703$; $P = 0.0001$) between the doses of biopesticide applied for the mortality rates of L4 larvae of *S. singularis*. The dose D1 [10% (v/v)] induced 100% corrected mortality on the fifth day, whereas this rate was 41.6, 47.6 and 69.4% respectively for D4 [0.01% (v/v)], D3 [0.1% (v/v)] and D2 (1%), compared with 4.2% for the control (D0). The variability of mortality shows that the biopesticide becomes more toxic for the insects when the dose applied is high and it increase in time (Table 2). At least 50% mortality was observed at the fourth day after exposure L4 *S. singularis* larvae to the biopesticide. However, the first mortalities were observed as soon as 24 h (Day 1 and 2) after exposure of the L4 larvae to the biopesticide. The LD_{50} at five days after exposure of L4 larvae to different doses of biopesticide was 0.038%.

Effect of biopesticide doses in open fields

Mirid mortality on immature cocoa pods: the *In vitro* results were confirmed by conducting the field assays with cocoa plantations to evaluate the efficacy of the biopesticide against *S. singularis*. Insect mortality rates varied according to the doses tested. Highly significant differences ($F_{(4, 85)} \geq 52.328$; $P = 0.0001$) between biopesticide doses were observed for the mean corrected mortality rate. Thus, the high doses (D1 and D2) of biopesticide induced respectively 96.3 and 83.3% mortality rate during the first week compared to the lowest doses (D3 and D4) where mortality was 44.4 and 33.3%, respectively (Table 3). During the second week, 100% mortality was recorded for the high doses, while this rate was 77.78 and 59.26% for doses D3 and D4 respectively. In contrast, no mirid mortality was observed in the untreated control (D0). The LD50 one week after exposure of *S. singularis* mirids was 0.066%.

Mirid mortality on cocoa twigs: a variation in mirid mortality rates according to doses of the Btk HD-1 based biopesticide was also observed on cocoa twigs. Analysis of variance indicated statistically significant differences ($F_{(4, 85)} \geq 16.703$; $P = 0.0001$) between the doses studied. Insect mortality evolved in the same way as that observed on cocoa pods. Except that on cocoa twigs the rate was higher than that observed on cocoa pods during the first week. Doses D1, D2, D3 and D4 induced mortalities of 100, 92.6, 63 and 51.9% respectively (Table 4). In the second week, however, the mortality rate was similar, except for the mortality rates (3.7 and 75%) observed with the untreated control (D0) and dose D3. The LD50 one week after exposure of the insects was 0.015%.

Classification of mirid damage to immature cocoa pods and twigs

Damage to immature cocoa pods: Mirid damage to cocoa pods was classified according to severity. The Kruskal Wallis test showed that the distribution of affected severity classes, in particular class 0 and class 3, was significantly different ($P \leq 0.023$) between the doses applied. Doses D1 and D2 each recorded more than 14 cocoa pods out of 18 in class 0 (healthy pods) compared to the untreated control pods (D0)

Table 2: Corrected mortality rate of *S. singularis* L4 larvae per day as a function of the doses of Btk HD-1-based biopesticide tested

		Mean corrected mortality (%)				
Treatment	Doses [% (v/v)]	Day 1	Day 2	Day 3	Day 4	Day 5
D0	0	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}	0.00 ± 0.0^{c}	0.0 ± 0.0^{d}	4.2 ± 14.1^{d}
D1	10	16.7 ± 24.1^{a}	31.3 ± 24.7^{a}	45.8 ± 14.1^{a}	89.6 ± 20.7^{a}	100 ± 0.0^{a}
D2	1	0.0 ± 0.0^{b}	16.7 ± 24.1^{b}	35.4 ± 23.2^{ab}	50.0 ± 0.0^{b}	69.4 ± 26.4^{b}
D3	0.1	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}	33.3 ± 24.1^{b}	43.8 ± 16.9^{b}	47.6 ± 0.0^{c}
D4	0.01	0.0 ± 0.0^{b}	0.0 ± 0.0^{c}	29.2 ± 25.2^{b}	33.3 ± 24.1^{c}	41.6 ± 16.1^{c}
$F_{(4, 115)}$		184.000	183.048	42.993	31.703	43.325
P		*0.0001*	*0.0001*	*0.0001*	*0.0001*	*0.0001*
LD_{50}		nd	nd	nd	1	0.038

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$)
nd: not defined

Table 3: Corrected mortality rate of *S. singularis* mirids on cocoa pods as a function of biopesticide doses

		Mean corrected mortality (%)	
Treatment	Doses [% (v/v)]	Week 1	Week 2
D0	0	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
D1	10	96.3 ± 10.78^{a}	100 ± 0.0^{a}
D2	1	83.3 ± 17.15^{b}	100 ± 0.0^{a}
D3	0.1	44.4 ± 16.17^{c}	77.8 ± 16.27^{b}
D4	0.01	33.3 ± 0.0^{d}	59.3 ± 14.26^{c}
$F_{(4, 85)}$		56.807	52.328
P		*0.0001*	*0.0001*
LD_{50}		0.066	nd

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$)

where more than 12 cocoa pods belonged to class 3 (more than 50% affected) (Fig. 2a). For doses D3 and D4, more than 8 cocoa pods were not affected (class 0) by mirids compared at 2 to 4 cocoa pods that were severely affected (class 3). At the same time, the median of independent samples test for classes 0 and 3 showed significant differences ($P \leq 0.047$) in the doses assessed. The median of class 3 for untreated cocoa pods (D0) was highest (15 affected pods) than the median obtained with treated pods, which varied from 0 to 4 affected pods. However, the distribution of classes 1 and 2 was not significantly different ($P \geq 0.104$) according to the doses applied (Fig. 2a).

Damage to cocoa twigs: For cocoa twigs, the distribution of classes 0 and 3 was significantly different ($P \leq 0.041$) between the doses of biopesticide applied. The most severe damage was observed on untreated cocoa twigs (D0), with more than 12 twigs recorded in class 3 (more than 50% affected), compared with less than 5 twigs for the lowest biopesticide dose (D4) (Fig. 2b). However, the highest doses (D1 and D2) limited the damage to less than 2 shoots. The median of independent samples test for classes 0 and 3 showed significant differences ($P \leq 0.047$) between the doses tested. The medians of these classes were the highest (16 and 15). These medians were obtained respectively with doses D1, D2 and D0 (untreated twigs). For doses D3 and D4, the medians were between 6 and 7 twigs for class 0, compared to 3 and 4 for class 3. At the same time, the distribution of classes 1 and 2 showed no significant difference ($P \geq 0.51$) between biopesticide doses. The damage to cocoa twigs was more severe than that observed on cocoa pods, regardless of the dose used.

Effectiveness of Btk HD-1-based biopesticide doses in protecting immature cocoa pods and twigs against mirids

The biopesticide doses provided protection levels of 51.85 to 87.04% for immature cocoa pods and 38.89 to 87.04% for cocoa twigs (Tables 4, 5) against *S. singularis* mirids. Protection level variability shows that the entomotoxic potential of Btk HD-1 depends on the tested doses. The highly significant differences ($F_{(4, 85)} \geq 34$ with $P = 0.0001$) were found between doses of the biopesticide. The highest doses or less diluted suspensions (D1 and D2) produced at least 80% protection of cocoa pods and twigs against damage caused by *S. singularis* mirids. However, a significant difference ($P > 0.05$) between D1 and D2 was observed only in cocoa pods. Furthermore, these results showed that cocoa pods were better protected against *S. singularis* mirid attacks, with an average level of protection of 58%, unlike cocoa twigs, which had an average protection level of 50.31%.

Discussion

This study shows the bioinsecticidal efficacy of *Bacillus thuringiensis* var. *kurstaki* HD-1 (Btk HD-1) against the mirid *Sahlbergella singularis,* one of the major biotic constraints on cocoa in Côte d'Ivoire. Bioefficacy tests carried out in the laboratory and in the field were used to assess the entomotoxic potential of this biopesticide against this cocoa pest. In the laboratory tests, the study showed that the bioinsecticidal effect of Btk HD-1 on *S. singularis* individuals (L4 larvae) increased progressively over time to reach maximum effect (100% mortality) 5 days after exposure of the larvae to the biopesticide. This could

Table 4: Corrected mortality rate of *S. singularis* mirids on cocoa twigs as a function of biopesticide doses

		Mean corrected mortality (%)	
Treatment	Doses [% (v/v)]	Week 1	Week 2
D0	0	0.0 ± 0.0^{e}	3.7 ± 10.78^{d}
D1	10	100 ± 0.0^{a}	100 ± 0.0^{a}
D2	1	92.6 ± 14.26^{b}	100 ± 0.0^{a}
D3	0.1	63.0 ± 10.78^{c}	75.0 ± 15.95^{b}
D4	0.01	51.9 ± 17.04^{d}	59.62 ± 13.27^{c}
$F_{(4, 85)}$		34.268	16.703
P		*0.0001*	*0.0001*
LD_{50}		0.015	nd

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$)

Table 5: Level of protection of immature cocoa pods and twigs according to the doses of Btk HD-1-based biopesticide

		Protection level (%)	
Treatment	Doses [% (v/v)]	Twigs	Cocoa pods
D0	0	0.00 ± 0.0^{c}	0.00 ± 0.0^{e}
D1	10	87.04 ± 2.69^{a}	87.04 ± 2.69^{a}
D2	1	84.88 ± 2.56^{a}	$79{,}63 \pm 2.69^{b}$
D3	0.1	40.74 ± 7.13^{b}	$70{,}37 \pm 5.39^{c}$
D4	0.01	38.89 ± 8.08^{b}	$51{,}85 \pm 7.13^{d}$
Protection level mean		50.31± 33.1	58.0 ± 31.65
$F_{(4, 85)}$		42.179	34.0
P		*0.0001*	*0.0001*

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P > 0.05$)

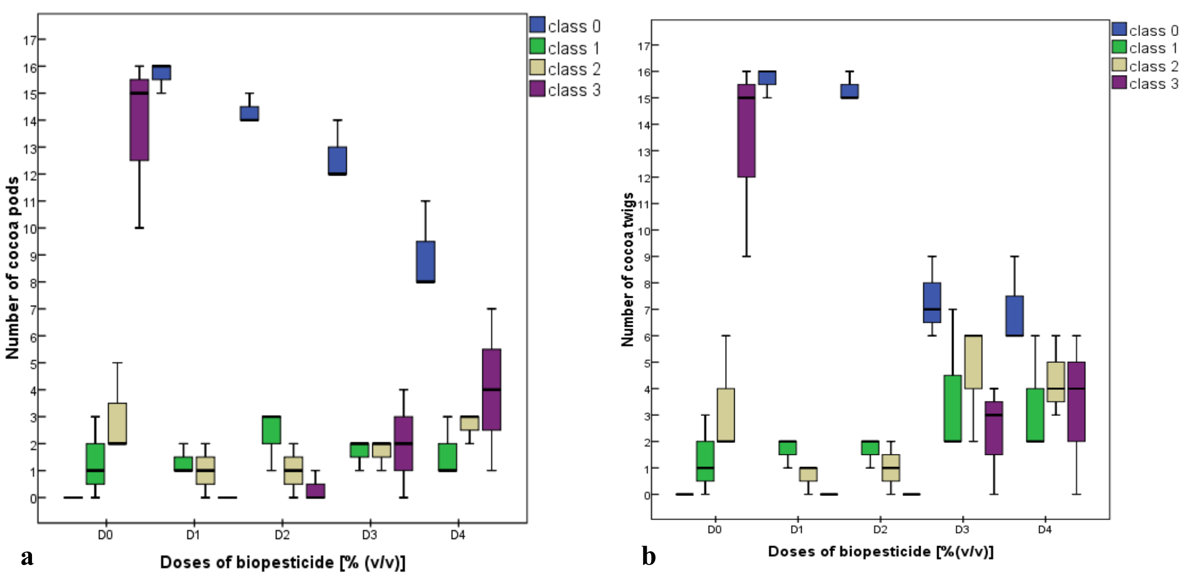

Fig. 2: Severity of damage to cocoa pods (**a**) and twigs (**b**) by class as a function of biopesticide doses

explain the low mortality of L4 larvae observed during the first two days of the tests. The same observation was made by Alsaedi *et al.* (2017) against the tomato leaf miner (*Tuta absoluta*), using the Btk HD-1-based biopesticide. In addition, these authors demonstrated a strong positive correlation between the daily mortality rates observed and the concentrations of the biopesticide.

In fact, the mechanism of action of Bt-based biopesticides is slow, compared to conventional chemical insecticides. This hypothesis is confirmed by the study carried out by Mboussi *et al.* (2018) on the management of cocoa mirids in Cameroon. Their results indicate a 100% mortality rate of cocoa mirids, within 48 h, for the chemical insecticide (Acta) used as a positive control. Regarding Bt's mechanism of action, Mendoza-Almanza *et al.* (2020) showed that the mechanism of action of Bt's main toxic proteins against insect larvae is the membrane pore formation model. This has also been confirmed by several authors (Zhuang *et al.* 2002; Bravo *et al.* 2004; Rodríguez-Almazán *et al.* 2009). Consequently, the death of L4 larvae is due to the ingestion of virulence compounds or active compounds (spores, protein crystals, vegetative insecticidal proteins, enzymes, *etc.*) contained in the biopesticide by them during their feeding. In fact, mirids, which are piercing-sucking insects, inject lytic saliva into the cells and then suck out the liquefied cells, possibly containing certain toxins such as those found in *Bt* (Harmel *et al.* 2010). Fragments of cocoa twigs treated by immersion with different suspensions of the biopesticide were used as a feeding medium for the larvae tested. After ingestion of these toxins, the symptoms typical of the insecticidal activity of Btk HD-1 very quickly manifested themselves as major lesions in the intestine and paralysis of the digestive tract, leading to an immediate cessation of feeding activity. The insect dies which may or may not be accompanied by septicaemia (Schnepf *et al.* 1998; Ambang *et al.* 2002; Maagd *et al.* 2003; Pardo-Lopez *et al.* 2013). However, the mode of action of the Btk HD-1-based biopesticide appears faster and more effective against cocoa mirids compared to the *Beauvoria bassiana*-and *Metarhizium anisopliae*-based formulations used by Mahot *et al.* (2019) against these same pests in Cameroon.

To our knowledge, there are no studies on the use of Btk HD-1-based biopesticides against cocoa mirids. However, the results of this study are similar to those obtained by Ambang *et al*. (2002) on *Andrector ruficornis* larvae and imagos on *Solanum tuberosum* plants in Cameroon. The difference is that these authors used *Bt* protein crystals as a wettable powder, which had a maximum effect (100% mortality) 72 h after treatment. This is not the case in this study, where the liquid formulation of Btk HD-1 was used against cocoa mirids. The results obtained in the laboratory were confirmed during trials carried out in the field. The application of doses of biopesticide to the twigs (young stems) and immature cocoa pods gave very satisfactory results for the use of this biopesticide in the management of *S. singularis* mirids. However, a variation in the mortality rate of mirids was observed on twigs (young stems) and immature cocoa pods. This variability in mirid mortality on these cocoa organs could be linked to mirid feeding preference. In fact, mirids cause as much damage to pods as to young stems. However, the effects of this damage are seen more quickly on the young stems than on the pods, with the upper part of the pods drying out. This is why Babin (2009) might say that most cocoa production losses in Africa are due to mirid attacks on twigs and branches. According to this author, the high and rapid severity of disease on young stems (gourmands or twigs) compared to young pods could mean that the latter are not naturally adapted to *S. singularis* feeding. Previous studies have shown that pods are better adapted to the feeding needs of *S. singularis* (Babin 2009; N'Guessan *et al*. 2010). In addition, these authors reveal that competition for pods, or their disappearance at harvest time, would have forced *S. singularis* to adapt to the young branches and gourmands of the cocoa tree for its feeding and oviposition.

This study shows good phytosanitary protection of cocoa twigs and pods against one of the main cocoa pests in Côte d'Ivoire. The treatment method used proved effective in killing mirids and limiting the damage they cause by feeding on immature cocoa twigs and pods. A similar study carried out by Frem *et al.* (2023) on tomatoes against *Tuta absoluta*, using the Btk HD-1-based biopesticide, showed a very satisfactory level of phytosanitary protection against this tomato leaf miner. The efficacy of this biopesticide shows that it is an interesting alternative to chemical control in the management of cocoa mirids.

In addition to being effective against a wide range of insects belonging to orders such as Hymenoptera, Coleoptera, Homoptera, Orthoptera and Malleoptera, as well as nematodes, mites and protozoa (Christou *et al*. 2006; Baig *et al*. 2010; Sharma *et al*. 2010; Pardo-Lopez *et al.* 2013), the Btk HD-1-based biopesticide also has antifungal activity on certain phytopathogenic parasites, in particular *Phytophthora palmivora*, the agent responsible for cocoa black pod rot in Côte d'Ivoire (Kamenek *et al.* 2012; Gadji *et al*. 2018). Is it not in view of the above that Baranek *et al*. (2017) argue that *B. thuringiensis*-based formulations may be one of the best ways to control crop pests. Controlling cocoa mirids using environmentally friendly methods, such as biological methods, could provide an ecological benefit by reducing the use of chemical insecticides in agriculture (Roh *et al.* 2007; Gadji *et al.* 2018).

Conclusion

This study shows that the use of the Btk HD-1-based biopesticide to control *S. singularis* in cocoa plantations is an interesting alternative approach to conventional insecticide treatments. The efficacy of Btk HD-1-based biopesticide was demonstrated in this study at different doses. The toxicity of the biopesticide increases with the concentration of Btk HD-1 spores and cells. The least diluted suspensions of Btk HD-1 caused the highest mortality of *S. singularis*. A suspension [1% (v/v)] of the biopesticide significantly reduces mirid damage to both young cocoa stems and pods. This could be the ideal dose to ensure good phytosanitary protection of cocoa trees against mirids.

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Author Contributions

This research work is part of Gadji André's doctoral thesis on the formulation of *Bacillus thuringiensis* var. *kurstaki* HD-1 based biopesticide and its efficacy on two cocoa pests in Côte d'Ivoire: *Phytophthora* *palmivora* and *Salhbergella singularis*. This study was carried out in perfect collaboration among all the collaborators involved in the research project. The doctoral thesis was co-directed by Prof. Yapo O. Bernard and Abo Kouabenan, Prof. Tyagi and Prof. Satinder. The Btk HD-1-based biopesticide was designed by Prof. Tyagi and his team (Prof. Satinder) at the INRS-ETE laboratory. Laboratory efficacy tests of the biopesticide on larvae (L4) of *S. singularis* were carried out by Gadji André and N'Guessan Walet. Prof. Kwadjo K. Eric and Dr. Gadji André defined the protocol for the field tests. Satinder K. Brar corrected the article translated into English and contributed to its drafting and finalisation. All authors analyzed the results and contributed to the writing of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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