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Efficacy of *Beauveria bassiana* against the cotton leaf roller, *Haritalodes* (*Syllepte*) *derogata* (Fabricius, 1775) (Lepidoptera: Crambidae) under laboratory conditions.

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ABSTRACT

Objective: The leaf-roller caterpillar *Haritalodes (=Syllepte) derogata* (Fabricius, 1775) (Lepidoptera: Crambidae) induces high yield losses by damaging cotton leaves and reducing the photosynthetic activity of the plant. Laboratory bioassays were carried to evaluate the effect of *Beauveria bassiana* on the survival of *H. derogata* larvae.

Methodology and results: In the first trial, screening of thirteen *B. bassiana* isolates was performed on third larval instars at 10^7 conidia.mL^{-1}. In the second trial, effects of five concentrations (10^5 to 10^9 conidia.mL^{-1}) of the three best isolates of the fungus were tested. Conidia suspension was applied on each larva topically. Germination rates of conidia used varied between 90.2% to 95.7%, 24 hours after incubation. Five isolates were found to be the most promising namely Bb116, Bb3, Bb11, Bb6 and Bb115. In the second bioassay, caterpillar mortality increased with fungal concentration. Lethal Concentration (LC50) was estimated to $1.18x10^{15}$ conidia.mL^{-1}, $1.75x10^{13}$ conidia.mL^{-1}, $1.75x10^{13}$ conidia.mL^{-1}, 9 days after inoculation for Bb3, Bb11 and Bb115, respectively.

Conclusion and application of results: The use of *B. bassiana* as a biopesticide against *H. derogata* could be a good alternative method to control the pest. It is an environmentally friendly method with less side effects compared to the application of synthetic pesticides on cotton. This method could be tested in future station and field experiments.

Keywords: Cotton, Integrated pest management, *Haritalodes (=Syllepte) derogata*, *Beauveria bassiana*, Lethal Concentration.

INTRODUCTION

Cotton crop is the first cash crop of Benin that contributes up to 80% to official export earnings and 13% to GDP (Great Development Product) (Afouda *et al.,* 2013). In 2018-2019 season, the recorded production was 700,000 TM ranking Benin as the largest producer in West Africa (Tonavoh, 2019). This increase in cotton production is related to the expansion of cultivated areas and the use of new cotton varieties but not to an increase in yield (CRA-CF 2018). Yield is heavily limited by pressure from insect pests. In West Africa, insect pests become a major constraint on increasing cotton production (Brevault *et al.,* 2017). Indeed, cotton is the one of the most damaged crops with more than 1300 species of insect pests, heavily limiting its productivity (UNCTAD, 2008 cited by Douro Kpindou *et al.,* 2013). Among these, phyllophagous and carpophagous caterpillars are the most destructive pests of cotton in Benin. There are two main groups of caterpillar pests according to their feeding preferences (Héma *et al.,* 2009). The first group consisted of defoliators such as the leaf-roller caterpillar *Haritalodes* (=*Syllepte*) *derogate* F. (Crambidae)), the leaf-eating noctuids *Anomis flava* F. and *Spodoptera littoralis* (Boisduval). On the other hand the second group includes bollworms or fruit-feeders namely the old world bollworm *Helicoverpa armigera* (Hubner), the red bollworm *Diparopsis watersi* Rothschild and the spiny bollworms *Earias insulana* (Boisduval) and *Earias biplaga* (Walker) which feed on fruiting organs (squares, flowers, bolls) (Silvie *et al.,* 2013). Expansion of cultivated area led to higher consumption of chemical pesticides in order to overcome insect pests damage (Westerberg, 2017). The side effects of chemical insecticide misuses included the contamination of cotton production area, human hazards (frequent pesticide poisoning, skin and stomach irritation), insect pest resurgence and resistance, environmental pollution (Lawani *et al.,* 2017; Djihinto *et al.,* 2016; Djihinto *et al.,* 2009). Cotton is an income generating for all stakeholders in the value chain and insect resistance mainly of Lepidopteran species became a major issue to be solved for boosting cotton production in Benin. Among these caterpillars of cotton, *H. derogata* (Fabricius, 1775) (Lepidoptera: Crambidae) inducing up to 20-60% of cotton yield loss (Silvie, 1993). Thus, this phyllophagous could alter the photosynthetic activity of the plant. In order to economically and ecologically manage cotton insect pests, biological control remains an attractive option. This control option includes the use of natural enemies of target insect pest species. Several studies reported various natural enemies (parasitoids and pathogens) of *H. derogata* (Gahramanova *et al.,* 2020). In this perspective, the entomopathogen *Beauveria* bassiana (Bals.-Criv.) Vuill., 1912, is a promising candidate. Different isolates were identified infecting a wide range of insects (707 species belong to 15 orders) and mites (13 species) (Zimmerman 2007; Lambert 2010). The use of *B. bassiana* is an environmentally friendly control mean and harmless to human health compared to chemical pesticides (Althouse *et al.,* 1997; Faria and Wraight, 2001). Fungus kills both susceptible adult and immature stages (eggs, larvae) causing the so-called *''white muscardine''* disease by a simple contact. Pest mortality may occur with the conidia development process and dead host becomes a new source of contamination. Four steps are known for the infection of the fungus namely adhesion, germination and differentiation, penetration, and dissemination within the host and to another host (Dannon *et al.,* 2020). It is not theoretically possible for insects to develop resistance to *B. bassiana* because the fungus simultaneously uses several modes of action such as infection by conidia and toxins (Mascarin and Jaronski, 2016) and as a living organism, it can adapt to various host changes (Sabbahi, 2008). The present study was designed to assess the susceptibility of *H. derogata* to various *B. bassiana* isolates under laboratory conditions.

MATERIALS AND METHODS

Insects: Larvae of *H. derogata* were collected during the period of 12 to 15 October 2017 on untreated cotton plants samples at the experimental site of the agricultural research center, cotton and fiber (CRA-CF), in two regions of central Benin namely Savalou (07°54'.032''N; 001°55'.024''E; 179 m of altitude) and Savè (08°00'.003'' N; 002°25'.054''E; 175 m of altitude). They were reared at (26 ± 1) °C, (70 ± 5) % HR and a photoperiod of 12:12 (L: D) h in the laboratory at International Institute of Tropical Agriculture (IITA), Benin station. They were fed using untreated cotton leaves. The first generation was used in the different bioassays. Only, the third larval instars were tested with *B. bassiana* isolates.

Fungal isolates: Thirteen isolates were used in the first trial and the best three ones were retained for the second experiment. All isolates were obtained from the collection unit of IITA-Benin (**Table 1**).

Table 1: Information about *B. bassiana* isolates used in different trials.

Range	Abbreviation of fungal isolates	Register N°	Host (Country of origin. district)	Auteur (Year of isolation)
1	Bb2	5644	*Eldana sacharina (Benin, Atlantique)*	-IITA-Benin (1997)
2	Bb3	5645	*Eldana sacharina (Benin, Atlantique))*	-IITA-Benin (1997)
3	Bb5	5647	*Acigona sp. (Nigeria, Ikom)*	- IITA-Benin (1997)-
4	Bb6	5648	*Acigona sp. (Benin, Alibori)*	- IITA-Benin (1997)-
5	Bb11	5653	*Sesamia calamistis (Benin, Atlantique))*	-IITA-Benin (1997)
6	Bb115	I93-841	*Locusta migratoria (Madagascar, Toliara)*	-Madagascar (1993)
7	Bb69	I91-623	*Zonocerus variegatus (Benin, Atlantique)*	-IITA-Benin (1991)
8	Bb71	I91-592	*Zonocerus variegatus (Benin, Atlantique)*	-IITA-Benin (1991)
9	Bb84	I91-679	*Hieroglyphus (Benin, Alibori)*	-IITA-Benin (1998)
10	Bb353	-	*Callosobruchus sp. (Benin, Ouémé)*	-IITA-Benin (2001)
11	Bb116	I93-842	*Locusta migratoria (Madagascar, vatolalaka)*	-Madagascar (1993)
12	Bb338	2191 ARSEF	*Pentatomidae Oebalus (Brazil, Fazenda)*	- Cornell University (USA, 1986)
13	Bb339	3086 ARSEF	*Leptoglossus fulvicorni (USA, Florida)*	-Cornell University (USA, 1990)

Isolates were mass cultured in petri dishes (9 cm diameter) containing Potato Dextrose Agar (PDA) and incubated at 26 ± 1°C. When isolates sporulated after fifteen days, conidia of each isolate were harvested with 0.05% of Tween 80 and filtered. After serial dilution of initial conidial suspension of each isolate, viability of conidia was checked on PDA inoculated with 100 µL of the lowest diluted suspension (~10^4 conidia.mL^{-1}). Twenty-four (24) hours later, conidia were counted using a sub-sample of 100, and viable conidia were estimated (formula 1) and conidia concentration to be used was calculated accordingly (formula 2) (Douro Kpindou *et al.,* 2012).

$$\%Viable = \left[\frac{a}{a+b}\right] \text{x } 100$$

a=number of germinated conidia within 24 hours; b= number of non-germinated conidia.

$$C' = \frac{\text{Co x Vo}}{\text{Vo} + \text{V}'}$$

C' = concentration to be used, Co = concentration of initial conidial suspension;
Vo= volume needed and V'= volume to be added.

Bioassays

Screening of B. bassiana isolates: Isolates of *B. bassiana* were formulated with Tween 80 (0.05%) at the concentration 10^7 conidia. mL^{-1}. Hundred-twenty larvae of *H. derogata* were used for the bioassay. Third instars larvae were individually placed in rearing boxes (3.8 cm x 2.9 cm x 4.0 cm) with punched tiny holes for ventilation. There were thirteen isolates used and each of these was tested on twenty larvae replicated three times in a completely randomized block. Larvae were inoculated by the method of Bateman *et al.* (1996) and Peveling *et al.* (1997). Each larva received topically 1µL of the fungal suspension on the pronotum at a concentration of 10^7 conidia.mL^{-1}. The rates of germination varied from 90.2% to 95.7%, 24 hours after incubation. Conditions of the laboratory were 26 ± 1°C and 70 ± 5% for averages of temperature (T) and relative humidity (RH) respectively. Mortality of larvae was checked daily over 15 days. Cadavers were dried for 48-72 hours, and transferred in petri dishes (9 cm diameter) containing humidified Whatman filter paper, sealed with parafilm and incubated at 26 °C to check sporulation. The number of pupae formed, dead and sporulated larvae and adults emerged were also recorded.

Assessing of effect of different concentrations* of *B. bassiana* on the survival of *H. derogata larvae: Three isolates (Bb3, Bb11 and Bb115) of *B. bassiana* were used in the current bioassay. Treatments consisted of a control (Tween 80 à 0.05% without fungus) and five different concentrations (10^5, 10^6, 10^7, 10^8, 10^9 conidia.mL^{-1} corresponding to 10^2, 10^3, 10^4, 10^5, 10^6 conidia per insect) tested using 3rd larval instars of *H. derogata*.

Data analysis: Mortality rate was estimated based on the OECD approach (OECD, 2010).

$$\text{MR} = \frac{\text{Nd}}{\text{Nu}}$$

MR= mortality rate, Nd= number of dead larvae, Nu= number of larvae used (normally 20). Sporulation rate was assessed basing on the number of larvae dead in each treatment. Likewise, the emergence rate was estimated based on the number of pupae formed by treatment. Percentages were converted in "Arcsin (square (p)) transformation" before statistical analysis. Transformed data related to mortality, sporulation and emergence were subjected to Fisher's analysis of variance (ANOVA) (Steel *et al.,* 1997), using the general linear model (GLM) procedure of SAS (Version 9.2). The post hoc test of Tukey (HSD) at 95% probability was used to separate the means after ANOVA indicated at 5%. The lethal concentrations and lethal times were estimated using the model of Cox regression (SPSS, 1989- 2007) as described by Douro Kpindou *et al.* (2012).

RESULTS

Screening test of isolates: Results from the screening of *B. bassiana* isolates revealed the pathogenicity of *B. bassiana* isolates on third larval instars of *H. derogata* (**Fig. 1**).

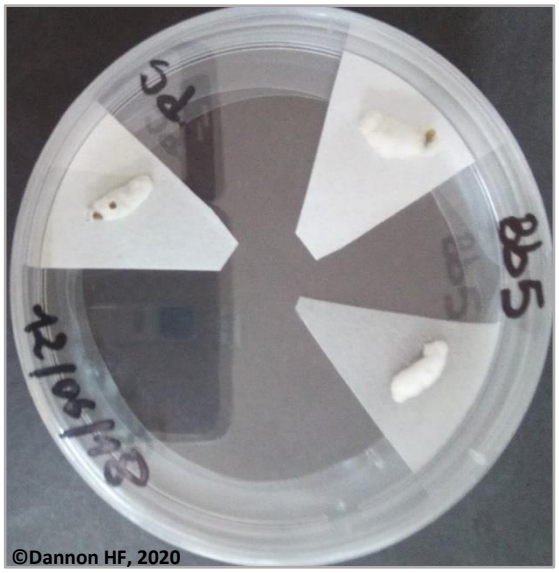

Fig. 1 Sporulation of *B. bassiana* on third larval instars of *Haritalodes (=Syllepte) derogata.*

Significant differences were observed between the tested isolates when considered larval mortality (*F*=8.1464, *P*<0.0001) and sporulation rate of dead larvae (*F*=3.9333, P<0.01) (**Fig. 2 and Fig. 3**). Indeed, after the fifteenth days of inoculating larvae, the cumulative mortality varied from 16.67 ± 4.40% (isolate Bb69) to 73.33 ± 1.66% (Bb2) compared to the control (3.33 ± 1.66%). The highest recorded mortality rates were 73.33 ± 1.66%, 46.66 ± 4.40%, 43.33 ± 7.26%, 41.66 ± 3.33%, 41.66 ± 8.81%, 33.33 ± 3.33%, 33.33 ± 6.66% and 31.66 ± 4.44% for Bb2, Bb5, Bb116, Bb3, Bb11, Bb6, Bb71 and Bb115, respectively. On the other hand, the sporulation rate of dead larvae was from 3.70 ± 3.70% (Bb5) to 43.61 ± 3.95% (Bb116) after incubation. The highest recorded sporulation rates were 43.60 ± 3.95%, 26.98 ± 6.34%, 25.00 ± 14.43%, 21.54 ± 17.20% and 20.55 ± 2.42% for Bb116, Bb3, Bb6, Bb11 and Bb115 respectively. However, the sporulation rate was lower on larvae dead following the activity of Bb2 (9.04 ± 2.14%), Bb5 (3.70 ± 3.70%) and Bb71 (8.33 ± 8.33%). Moreover, no sporulation was observed for the isolates Bb338, Bb353, Bb69, and Bb84.

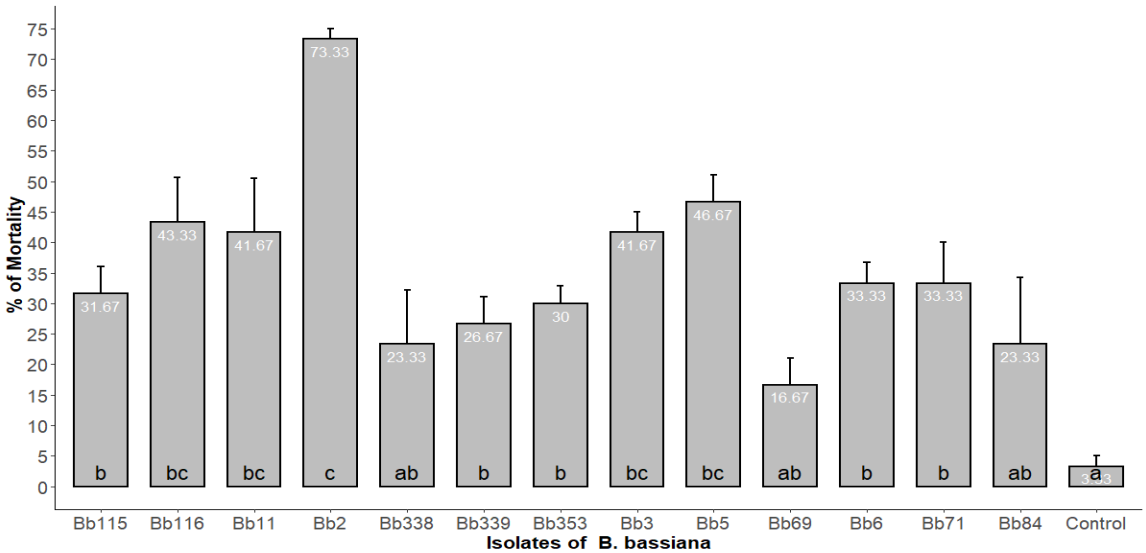

Fig. 2 Mean mortality rates induced by 13 *B. bassiana* isolates at 10^7 conidia.mL^{-1} on third larval instars (L3) of *H. derogate*

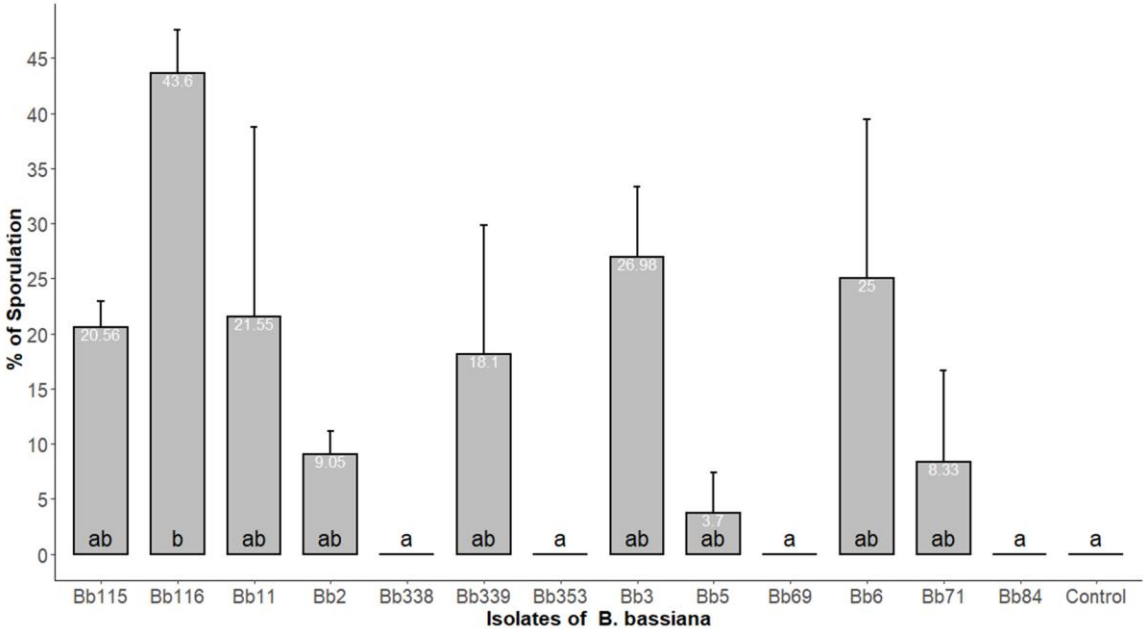

Fig. 3 Mean sporulation rates of 13 *B. bassiana* isolates at 10^7 conidia.mL^{-1} on third larval instars (L3) of *H. derogata.*

Assessing of effect of different B. bassiana concentrations* on the survival of *H. derogata larvae

Effect of B. bassiana on third larvae stage and adults emergence: The larval mortality rates increased with fungal concentrations (**Table 2**). The mortality induced varied from 31.66 ± 4.40 % to 63.33 ± 4.40 %, 33.33 ± 3.33 % to 76.66 ± 1.66 % and 31.66 ± 1.66 % to 58.33 ± 4.41% for the isolates Bb3, Bb11 and Bb115, respectively. For the isolate Bb3, there was no difference for larval mortality obtained at the first and the second concentrations, and at the third and fourth concentrations. The highest mortality rate was recorded (63.33 ± 4.40%) at the concentration of 10^9 conidia.mL^{-1}. In isolate Bb11, there was a significant difference between the mortality rates (60.00 ± 5.77% and 76.66 ± 1.66%) at 10^8 and 10^9 conidia.mL^{-1}, respectively. In the isolate Bb115 any significant differences were not observed between the first 4 concentrations and the highest mortality was obtained (58.33 ± 4.41%) at the concentration of 10^9 conidia.mL^{-1}. When comparing isolates within each concentration, there was a significant difference within isolates at 10^9 conidia.mL^{-1}. The isolate Bb11 induced the highest mortality rate (76.66 ± 1.66%) compared to Bb3 and Bb115 at 10^9 conidia. mL^{-1}.

Table 2: Effects of *B. bassiana* on third larval instars of *Haritalodes derogata*

Bb Concentration (Conidia/ml)	Mortality rate of larvae (%)			
	Bb3	**Bb11**	**Bb115**	**ANOVA**
10^5	31.66 ± 4.40 bA	33.33 ± 3.33 bA	31.66 ± 1.66 bA	F=0.0904 P=0.9154
10^6	33.33 ± 3.33 bA	40.00 ± 2.88 bA	35.00 ± 2.88 bA	F=1.1331 P=0.4075
10^7	43.33 ± 9.27 bcA	45.00 ± 5.00 bA	36.66 ± 1.66 bA	F=0.5900 P=0.5963
10^8	53.33 ± 3.33 bcA	60.00 ± 5.77 bcA	41.66 ± 4.40 bA	F=6.0523 P=0.06169
10^9	63.33 ± 4.40 cA	76.66 ± 1.66 cB	58.33 ± 4.41 cA	F=14.8527 P=0.01408
Control	8.33 ± 1.66 aA	8.33 ± 4.40 aA	8.33 ± 4.40 aA	F=0.3001 P= 0.7540
ANOVA	F=15.5130 P=0.0001961	F=18.6442 P=8.824e-05	F=32.2840 P=7.355e-06	

*There is no significant difference between means followed by the same lowercase letters shown in the same column (ANOVA followed by Tukey test at 5%).
*There is no significant difference between means followed by the same capital letters shown in the same line (ANOVA followed by Tukey test at 5%).

The emergence rate of *H. derogata* adults decreased with the increase in fungal concentrations (**Table 3**). Adult emergence was significantly affected by concentrations in each isolate ($P<0.05$). Nevertheless, when comparing isolates for each concentration, no significant differences occurred.

Table 3: Effects of *B. bassiana* on emergence of *Haritalodes derogata*

Bb Concentration (Conidia/ml)	Emergence rate of moths (%)			
	Bb3	**Bb11**	**Bb115**	**ANOVA**
10^5	79.84 ± 7.56 abA	75.39 ± 5.72 abA	80.58 ± 4.58 abA	F=0.3723 P=0.7107
10^6	70.63 ± 7.57 aA	88.38 ± 5.82 bA	79.80 ± 4.19 aA	F=4.8867 P=0.08434
10^7	85.55 ± 8.67abA	61.28 ± 4.70 abA	76.06±5.19 aA	F=2.3823 P=0.2083
10^8	68.33 ± 4.40 aA	55.55 ± 5.55 abA	73.97 ± 2.06 aA	F=4.7484 P=0.08783
10^9	71.95 ± 3.21 aA	33.33 ± 17.63 aA	63.21 ± 3.72 aA	F=3.0347 P=0.1578
Control	96.29 ± 3.70 bA	92.96 ± 3.53 bA	96.29 ± 3.70 bA	F=0.2310 P= 0.8043
ANOVA	F=4.9185 P=0.01568	F=4.899 P=0.01129	F=6.9204 P=0.004876	

*There is no significant difference between means followed by the same lowercase letters shown in the same column (ANOVA followed by Tukey test at 5%).
*There is no significant difference between means followed by the same capital letters shown in the same line (ANOVA followed by Tukey test at 5%).

Lethal concentration (LC50) of *B. bassiana* on third larvae stage: Analysis of Cox regression indicated that the different fungal concentrations used were significant and affected larval survival ($P < 0.05$) (**Table 4**). The model provided a good estimation of the different parameters. Values of B showed the existence of a dose-response relationship. This relationship was strong for the Bb11 isolate giving the highest B value (B = 0.087).

Table 4: Estimation of B values and Wald coefficients with Cox regression model for *B. bassiana* and third instars larvae of *H. derogata*

B. bassiana **isolate**	***H. derogata*** **stage**	**B**	**SE**	**Wald**	**df**	**Sig.**
Bb3	L3	0.069	0.019	13.755	1	0.000
Bb11	L3	0.087	0.019	20.589	1	0.000
Bb115	L3	0.055	0.018	8.932	1	0.003

B: B value of the Cox regression; **SE:** standard error; Wald: Wald coefficient; **df:** degree of freedom; **Sig:** probability.

The LC50 curves for isolates are depicted in **figures (5, 6 and 7**). For all isolates, the dose-response effect was significant and depends on the B value. Higher is B value, the narrower are the confidence intervals. It had to 3.52×10^{26}, 5.17×10^{20} and 1.18×10^{15} conidia.mL^{-1} of isolate Bb3, i.e. 3.52×10^{23}, 5.17×10^{17} and 1.18×10^{12} conidia by insects to kill 50% of the 3rd larval instars, in 5, 7 and 9 days respectively (**Fig. 5**) on the one hand. Likewise, 8.78×10^{35}, 4.08×10^{29} and 2.19×10^{23} conidia.mL^{-1} of Bb115 isolate i.e. 8.78×10^{32}, 4.08×10^{26} and 2.19×10^{20} conidia per insect were needed to kill 50% of 3rd larval instars, in 5, 7 and 9 days (**Fig. 6**) on the other hand. The isolate Bb11 gave the highest B value (0.087) and therefore the lowest LC50 values. The width of the confidence intervals was narrower for Bb11 compared to those of the other isolates. Thus, 1.73×10^{19}, 4.91×10^{15} and 1.75×10^{13} conidia.mL^{-1} of Bb11 i.e. 1.73×10^{16}, 4.91×10^{12} and 1.75×10^{10} conidia per insect were needed to kill 50% 3rd larval instars of *H. derogata* in 5, 7 and 9 days respectively (**Fig. 7**).

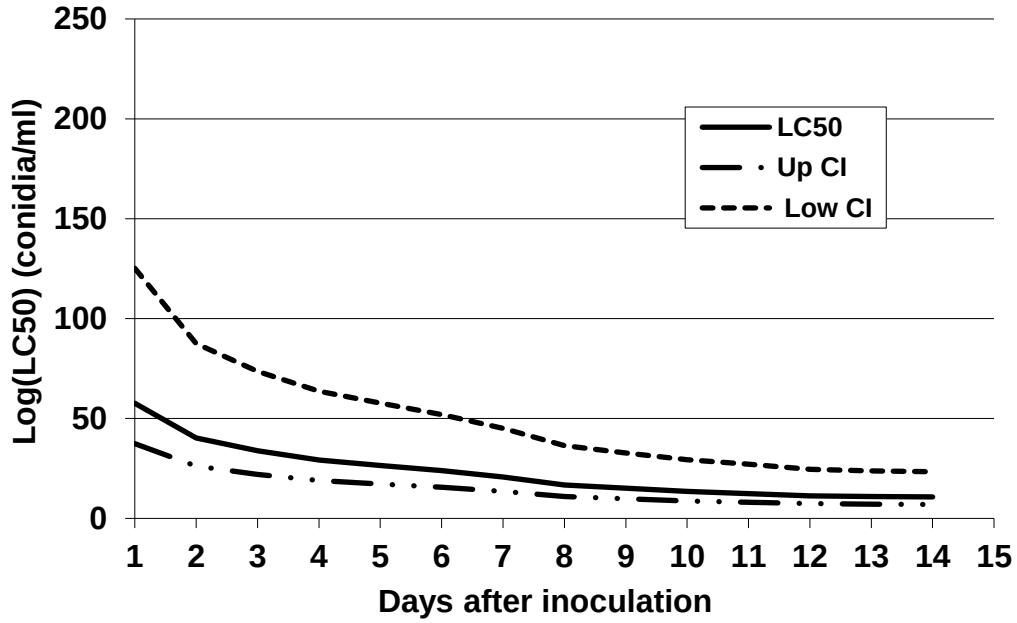

Fig. 5 LC50 values after treatment of the third stage of *Haritalodes (=Syllepte) derogata* to various doses of Bb3.

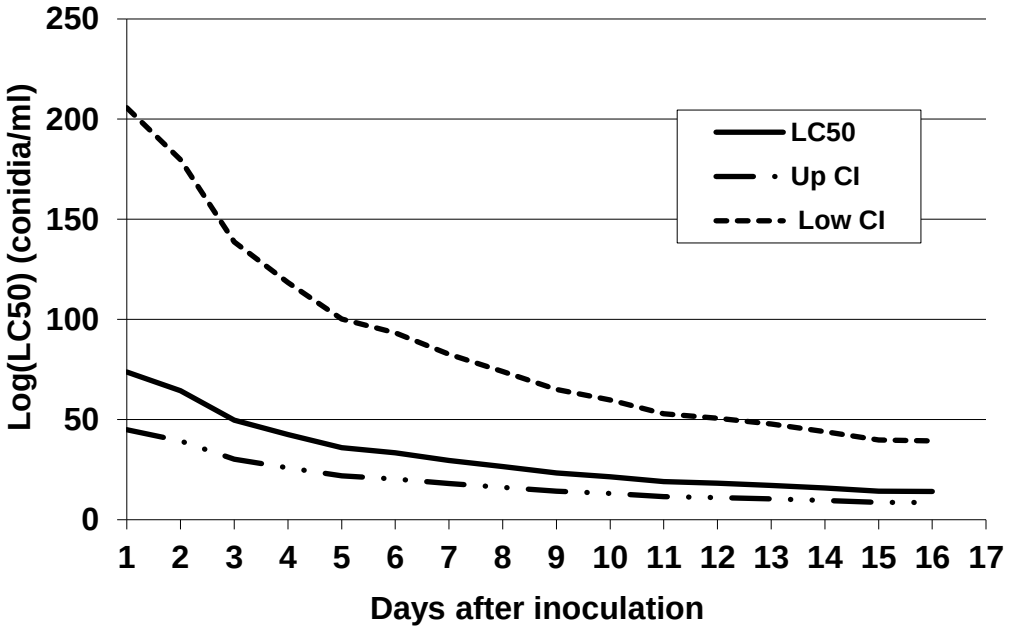

Fig. 6 LC50 values after treatment of the third stage of *Haritalodes (=Syllepte) derogata* to various doses of Bb115.

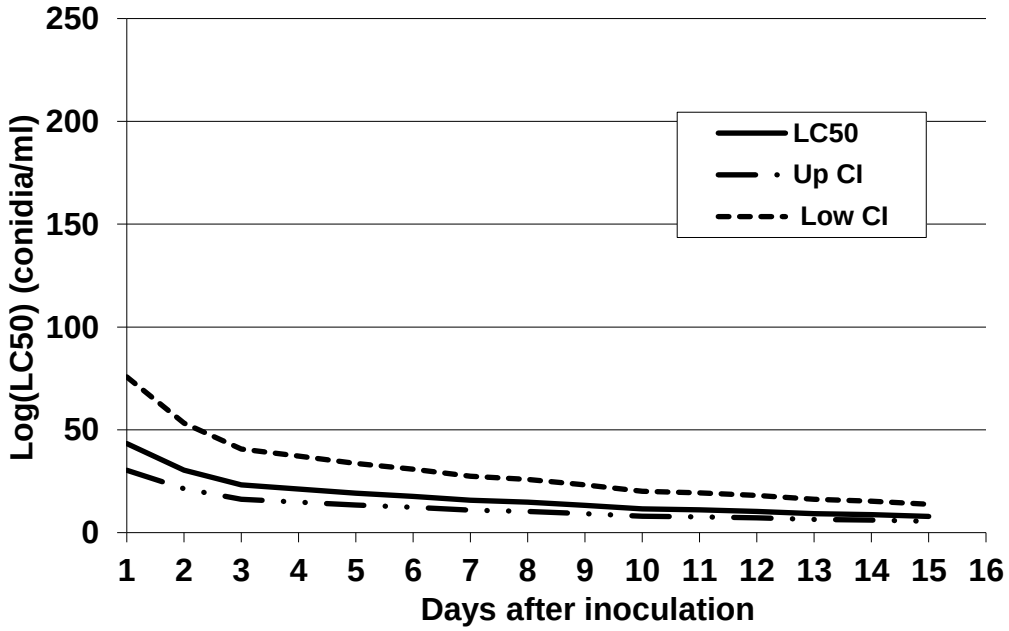

Fig. 7 LC50 values after treatment of the third stage of *Haritalodes (=Syllepte) derogata* to various doses of Bb11.

DISCUSSION

Mortality and sporulation of larvae: Results of this study indicated that the third larval stages were susceptible to infection of *B. bassiana* isolates tested. Mortality rate is depending on the type of isolate. Indeed, the highest mortality rate was obtained with the isolate Bb2. Such difference could be explained by its great capacity to produce enzymes and more toxic metabolites than others (Ferron, 1981). Valda et al. (2003) obtained similar results with mortality that ranging between 70 and 96 % in *Plutella xylostella* treated with different *B. bassiana* isolates. In addition to Bb2, other isolates namely Bb116, Bb11, Bb3 and Bb5 induced statistically similar mortality rates and higher virulence on the tested insect host. The difference in virulence between the various isolates may be related to their origin f and in some cases to the first species from which the isolate was cultured (same insect family or order). Our findings were in concordance with previous studies done by McCoy *et al.* (1988) and Goettel, (1992), who stated that an isolate was generally more virulent to the family and host area from which it has been isolated. Likewise, sporulation rate varied from isolate to isolate and the highest on dead larvae resulted from the use of Bb116. In this perspective, some isolates induced a germination of the fungus on the cuticle of incubated cadavers. This variability, which due to their difference of pathogenicity results from their virulence on the larvae of *H. derogata*. Similar results have been reported by Douro Kpindou *et al.* (2012) and Toffa-Mehinto (2014) on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and *Maruca vitrata* Fabricius (Lepidoptera; Crambidae), respectively. In addition, dead larvae from some isolates such as Bb338, Bb353, Bb69 and Bb84 did not sporulate. Moreover, other isolates namely Bb2, Bb5 and Bb71 had a low sporulation rate less than 10% but a higher mortality as mentioned previously. The susceptibility of the insect would therefore be influenced by their innate virulence. This observation was in agreement with the views of Soetopo (2004) who reported that the innate virulence of the isolate could induce high mortality with low sporulation rate. However, the choice of isolates depends primarily on their pathogenicity and the susceptibility of the host insect (Prior, 1990; Tanada and Kaya, 1993;). In the present case, when identifying the most virulent isolates after screening, sporulation and mortality could be determining parameters in the choice of the most virulent isolates as suggested by Jamal (2008) and Groden & Lockwood (1991). Thus, five isolates are found to be promising due to their ability to cause a high rate of mortality and to induce a relatively high sporulation rate. These included the isolates Bb116, Bb3, Bb11, Bb6 and Bb115.

Effect of concentrations on larvae and adults' emergence: The most promising isolates Bb3, Bb11 and Bb115 used for assessing the effect of different concentrations were chosen taking into account their origin from Benin (case of Bb3 and Bb11) and their effectiveness on other Lepidopteran pests such as *H. armigera* (Douro Kpindou *et al.,* 2012) and *M. vitrata* (Toffa Mehinto, 2014) for the cases of Bb11 and Bb115, respectively. The mortality rates increased with the concentration within each isolate. This suggests that the mortality rate depends on the quantity of conidia received. In some isolates, several concentrations induced similar mortality rate. For example, in isolate Bb11, no significant difference was obtained for concentrations 10^5, 10^6 and 10^7 conidia per mL, and the highest mortality was recorded at 10^8 and 10^9 conidia per mL using the third larval instars of *H. derogata*. The highest mortality rate was observed in Bb11 (76.66 ± 1.66%) with the lowest emergence rate (33.33 ± 17.63%) of adults suggests that Bb11 was better compared to the other isolates Bb 3 and Bb115.

Lethal Concentration (LC50) of B. bassiana on third H. derogata larval stage: Models that combine time and dose effects seem to be more appropriate for assessing the effectiveness of a pathogen or pesticide on target insect species (Robertson *et al.*, 1992). Of these Cox regression model was more flexible in the analysis of bioassays on biopesticides than other models such as Probit and Logit analyses (Douro Kpindou *et al.*, 2012). Probit analysis and Logit regression analysis have found wide use in modelling the probability of a dose response (Finney, 1971; Robertson *et al.*, 1992). Cox regression models based on the time-dose relationship of *B. bassiana* isolates for the survival of infected larvae of *H. derogata* fitted well in the current study. All B values given by Cox regression model were all significant ($P<0.05$). In consequent, our data showed a significant effect / dose response. Similarly, the LC50 curves had good trend and their confidence intervals were relatively narrower. Our results were comparable to those obtained by Douro Kpindou *et al.* (2012) when using virulent isolates of fungi *M. anisopliae* and *B. bassiana* on *H. armigera*. Based on LC50, isolate Bb11 was found to be more virulent followed by Bb3 and Bb115.

CONCLUSION AND APPLICATION OF RESULTS

Among *B. bassiana* isolates used for the screening, some showed their potential for the control of *H. derogata* through their virulence. These isolates delayed the growth of treated caterpillars and finally killed them. Three of virulent isolates were more promising (Bb11, Bb3 and Bb115) and could be included in a new strategy of cotton integrated pest management.

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REFERENCES

Afouda R, Ahlonsou R, Amoussouga TB, Djihoun M, 2013. La filière coton tisse sa toile au Bénin, AgriProfocus [The cotton sector weaves its web in Benin, AgriProfocus].

Althouse CM, Petersen BE, McEwen LC, 1997. Effects of young American kestrels (*Falco sparvericus*) exposed to *Beauveria bassiana* bioinsecticide. Bull. Environ. Contam. Toxicol, 59: 507–12.

Bateman RP, Carey M, Moore D, Prior C, 1994. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations at low humidities. Ann. Appl. Biol., 122:145–52.

Brevault T, Renou A, Goebel R, Clouvel P, 2017. Repenser la gestion des ravageurs du cotonnier. *Les Zones Cotonnières Africaines, Dynamiques et Durabilité* [Rethinking the management of cotton pests. African Cotton Zones, Dynamics and Sustainability], 399–412.

CRA-CF, 2018. Rapport de la campagne cotonnière de 2017-2018 [Cotton campaign report for 2017-2018]

Dannon HF, Dannon AE, Douro-Kpindou OK, Zinsou AV, Houndeté AT, Toffa-Mehinto J, Elégbédé IATM, Olou BD and TAMÒ M, 2020. Toward the efficient use of *Beauveria bassiana* in integrated cotton insect pest management. Journal of Cotton Research 3**(**1): 1-21. https://doi.org/10.1186/s42397-020-00061-5

Djihinto AC, Katary A, Prudent P, Vassal JM, Vaissayre M, 2009. Variation in Resistance to Pyrethroids in *Helicoverpa armigera from Benin Republic, West Africa. Journal of Economic Entomology*, 102(5): 1928-1934. https://doi.org/10.1603/029.102.0525

Djihinto CO, Affokpon A, Dannon E, Aboua C, 2016. Réduction de doses de cyperméthrine-triazophos et lutte raisonnée en culture cotonnière au Bénin [Reduction of doses of cypermethrin-triazophos and reasoned control in cotton cultivation in Benin]. Journal of Applied Biosciences, *98:* 9261-9269.https://doi.org/10.4314/jab.v98i1.3

Douro Kpindou O, Djegui DA, Glitho IA, Tamò M, 2012. Sensitivity of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidea) to the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana* in laboratory. ARPN Journal of Agricultural and Biological Science, **7**: 1007-1015.https://hdl.handle.net/10568/80862

Douro Kpindou O, Djegui DA, Glitho IA, Tamò M, 2012. Réponse des stades larvaires de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) à l'application de champignons entomopathogènes *Metarhizium anisopliae* et *Beauveria bassiana* [Response of the larval stages of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) by the using of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*]. Biotechnology, Agronomy and Society and Environment, 16(3): 283–293. https://hdl.handle.net/10568/80842

Douro Kpindou OK, Paraïso A, Djegui DA, Glitho IA, Maffon S and Tamo M, 2013. Comparative study of the efficacy of Entomopathogenic fungi, chemicals and botanical pesticides in the management of cotton pests and their natural enemies in Benin. *International Journal of Science and Advanced Technology*,*3*(1):21–33. https://doi.org/https://www.researchgate.net/publication/236159740

Fari M, Wraight SP, 2001. Biological control of *Bemisia* with fungi. Crop Protect, 20: 767-778.

Ferron P, 1981. Pest control by the fungi *Beauveria and Metarhizium*. Microbial control of pests and plant diseases*, 1970-1980*.

Finney DJ, 1971. Probit analysis. 3rd ed. Cambridge: Cambridge University Press.

Gahramanova G, Mamay M, Mammadov Z, 2020. Biological characteristics and efficacy of *Bacillus thuringiensis* var. thuringiensis against the cotton leaf roller, *Syllepta derogata* (Fabricius, 1775) (Lepidoptera : Crambidae). Egyptian Journal of Biological Pest Control, *30*(85): 1–7. https://doi.org/https://doi.org/10.1186/s41938-020-00289-y

Ganda H, Togbé EC, Houndété TA, Zannou Boukari ET, Gogan M, Dagbénonbakin GD and Kossou DK, 2018. Effectiveness of neem seed oil (Azadirachta *indica* A. Juss : Meliaceae) on *Syllepte derogata* Fabricius, Lepidoptera : Pyralidae. Journal of Applied Biosciences, *129*:13029–13038. https://doi.org/http://dx.doi.org/10.4314/jab.v129i1.6

Goettel MS, 1992**.** Biological control of locusts and grasshoppers. Wallingford, UK: *CAB. International*, 122-130.

Groden E and Lockwood JL, 1991. Effects of soil fungistasis on *Beauveria bassiana* and its relationship to disease incidence in the Colorado potato beetle, *Leptinotarsa decemlineata*, in Michigan and Rhode Island soils. Journal of Invertebrate Pathology, *57*(1): 7–16. https://doi.org/10.1016/0022-2011(91)90036-P

Héma O, Somé Hugues N, Traoré O, Greenplate J, Abdennadher M, 2009. Efficacy of transgenic cotton plant containing the Cry1Ac and Cry2Ab genes of Bacillus thuringiensis against *Helicoverpa armigera* and *Syllepte derogata* in cotton cultivation in Burkina Faso. *Crop Protection*, *28* :205–214. https://doi.org/10.1016/j.cropro.2008.09.014

Jamal Z, 2008. Application de *Beauveria bassiana* contre la punaise terne *Lygus lineolaris* (palisot de beauvois) (hémiptères: miridés) dans les vignobles [*Application of Beauveria bassiana against Lygus lineolaris (palisot de beauvois) (hemiptera: miridae) in vineyards*] [Thèse de doctorat]. Montréal: Université du Québec.

Lambert N, 2010. *Lutte biologique aux ravageurs : applicabilité au Québec* [mémoire de maîtrise en environnement (M.Env.)]. Québec: Université de Sherbrooke.

Lawani RAN, Kelome NC, Agassounon D, 2017. Effets des pratiques agricoles sur la pollution des eaux de surface en République du Bénin [Effects of agricultural practices on the pollution of surface water in Benin Republic]. Larhyss Journal; 30:173-190.

Mascarin MG and Jaronski TS, 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. World J. Microbiol Biotechnol, 32(177): 1–26.

McCoy CW, Samson RA, Boucias DG, 1988. Entomogenous fungi. In: CM Ignoffo and NB Mandava (Editors), *Handb. Nat. Pestic.*, Volume V: Microbial Insecticides, Part A, Entomogenous Protozoa and Fungi. CRC Press, Boca Raton, FL, pp. 151- 236.

OECD, 2010. Sediment-water chironomid life-cycle toxicity test using spiked water or spiked sediment. OECD guidelines for the testing of chemicals. 233, 1-30.

Peveling R and Demba SA, 1997. Virulence of the entomopathogenic fungus *Metarhizium flavoviride* gams and rozsypal and toxicity of diflubenzuron, fenitrothion- esfenvalerate and profenofos-cypermethrin to nontarget arthropods in Mauritania. Arch. Environ. Contam. Toxicol., 32: 69-79.

Prior C, 1990. The biological basis for regulating the release of micro-organism, with particular reference to the use of fungi for pest control. Asp. Appl. Biol.*,* 24: 231–238.

Robertson JL and Preisler HK, 1992. Pesticide bioassay with arthropods. London: CRC Press.

Sabbahi R, 2008. *Utilisation du champignon entomopathogène Beauveria bassiana dans une stratégie de gestion phytosanitaire des principaux insectes ravageurs en fraiseraies* (Thèse de Philosophiae Doctor en Biologie) [*Use of the entomopathogenic fungus Beauveria bassiana in a strategy of phytosanitary management of the main insect pests in strawberry plantations (Doctoral dissertation in Biology)].*Université du Québec : Institut national de la recherche scientifique, INRS-Institut Armand-Frappier.

Sahayaraj K, Namachivayam SKR, 2011. Field evaluation of three entomopathogenic fungi on

groundnut pest field evaluation of three entomopathogenic fungi on groundnut pests. *Tropicultura*, *29*(3) :143–147. https://doi.org/https://www.researchgate.net/publication/265729758

Silvie P, 1993. Les parasitoïdes de *Syllepte derogata* (Fabricius, 1775) (Lepidoptera, Crambidae) au Togo. *J. Afr. Zool.*, *107* : 363–372.

Silvie PJ, Renou A, Vodounnon S, Bonni G, Adegnika MO, Héma O, Prudent P, Sorèze J, … Brévault T, 2013. Threshold-based interventions for cotton pest control in West Africa : What's up 10 years later ? *Crop Protection*, *43*: 57–165. https://doi.org/10.1016/j.cropro.2012.09.006

Soetopo D, 2004. Efficacy of selected *Beauveria bassiana* (Bals.) Vuill. isolates in combination with a resistant cotton variety (PSB-Ct 9) against the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) PhD diss., University of The Philippines Los Banos.

Steel RGD, Torrie JH and Dickey DA, 1997. Principles and procedures of statistics: a biological approach. McGraw-Hill, 666 pp.

Streito JC, Nibouche S, 1997. First observations on the parasitoids associated with Lepidopterous pests of cotton in Burkina-Faso. *Entomophaga*, *42*(4) : 543–557.

Tanada Y, Kaya H 1993. Insect pathology. Academic Press, San Diego, CA. 666 pp.

Toffa Mehinto J, Atachi P, Douro-Kpindou OK, Tamò M, 2014. Pathogenecity of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* on larvae of the legume pod borer *Maruca vitrata* (Lepidoptera : Crambidae). ARPN Journal of Agricultural and Biological Science. 9(2): 55–64.

Tonavoh F, 2019. Le Bénin premier producteur du coton en Afrique de l'Ouest. Economie, news.orificegroup.net [Benin, the leading producer of cotton in West Africa. Economy, news.orificegroup.net].

Valda CA, Silva RB, Edmilson JM, Jorge BT, 2003. Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) to fungus *Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* (Metsch.) Sorok. *Neotrop. Entomol.* 32 (4): 653-658.

Westerberg V, 2017. The Economics of conventional and organic cotton production. A case study from the municipality of Banikoara, Benin. Report for the Economics of Land Degradation Initiative, 36p.

Zimmermann G, 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology, 17(6): 553–596.https://doi.org/10.1080/09583150701309006