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Improving the efficiency of *Beauveria bassiana* applications for sustainable management of *Plutella xylostella* (Lepidoptera: Plutellidae) in West Africa

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ABSTRACT

The effectiveness of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin isolates Bb11, Bb115, Bb116 and Bb362 against the diamondback moth (DBM), *Plutella xylostella* (L.) population from Cotonou, Benin, was investigated in the laboratory and on station. In the laboratory, six concentrations, 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 conidia/ml of each isolate were used to assess *B. bassiana* virulence. Third instar DBM larvae were inoculated with 2 µl of each isolate's concentrations by topical application method. Control larvae were inoculated with sterilized Tween 80 solution (0.05%). Compared with other isolates, Bb11 was more virulent against DBM with LD_{50} value estimated to 4.96×10^6 conidia/ml and the highest rates of cadaver sporulation. The effectiveness of Bb11 against DBM on cabbage was subsequently assessed on station and on farm in Benin and Togo, at a low doses of 53 g/ha once or twice a week and a high dose of 315 g/ha weekly. Compared with unsprayed control and deltamethrin treatments, the dose of 53 g/ha of Bb11 applied twice a week at intervals of 4 days was able to reduce on farm the density of DBM by 83% and 93%, respectively. Also, the marketable cabbage yield obtained on farm with this dose was significantly higher compared with the other treatments, with a 199% and 452% increase in Danyi and Cotonou over the unsprayed control, respectively. These results confirm the better performance of Bb11 over other isolates, and also indicate that the newly investigated application dosage and frequency of Bb11 could be an efficient management option to control DBM in Benin and Togo.

1. Introduction

Cabbage, *Brassica oleraceae* L. (Brassicaceae) is one of the most common vegetables in West Africa (James et al., 2010). In Togo and Benin, as in most part of the world, the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the major cabbage pest, causing considerable crop losses (Talekar and Shelton, 1993; Martin et al., 2006; Ayalew, 2006; Nyambo et al., 2011; Zhou et al., 2011; Zalucki et al., 2012; Legwaila et al., 2014; Mondédji et al., 2014; Ramya et al., 2016). The frequent application of synthetic insecticides remains the prevalent control practice to manage DBM in Togo and Benin, which has led to the development of resistant populations to several types of chemical insecticides and certainly adverse impacts on humans and the environment (Grzywacz et al., 2010; Ansari et al., 2013; Agboyi et al., 2016; Togola et al., 2018). Hence, the search for alternative options to sustainably manage insecticide-resistant DBM populations has become a priority. The development of biological control approaches – including bio-pesticides - could offer promising solutions (Sarfraz et al., 2005; Sow et al., 2013). Among them, the entomopathogenic fungi have gained a lot of interest in recent years so much that many fungus-based biopesticides have been developed for biological control of insect pests (De Groote et al., 2001; De Faria and Wraight, 2007). Generally, entomopathogenic fungi act by contact and as they are quite host specific, their use is considered as environmentally friendly control method (Shahid et al., 2012). Two fungus species, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Ophiocordycipitaceae) are the most common commercial fungus-based biopesticides worldwide (Feng et al., 1994; de Faria and Wraight, 2007). In this study, however, *B. bassiana* has been singled out

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as one of the best candidates because some of its isolates have already been successfully tested against DBM (Wraight et al., 2003). In Benin, Godonou et al. (2009) showed that *B. bassiana* isolate Bb11 (also referred to as Bba5653) collected and isolated from *Sesamia calamistis* (Hampson) (Lepidoptera: Noctuidae) was effective against DBM by causing up to 94% mortality in laboratory experiments, and an important decrease of the pest population density on farm. The dose of 1000 g conidia powder considered by their study as effective against DBM was however far too high for practical implementation by vegetable farmers, making its application inefficient. Indeed, Pu et al. (2005) showed evidence that the application methods of *B. bassiana*-based mycoinsecticides could impact their efficacy, thereby the dose. Another constraint in using *B. bassiana* as a bio-pesticide is the fact that most farmers use watering cans for daily irrigation and this practice might have a negative impact on the persistence of *B. bassiana* conidia on plants (Inglis et al., 1995). Moreover, Leland and Behle (2005) showed a rapid deactivation of *B. bassiana* conidia by UV radiation. Hence, there is a need to revise both application dosage and frequencies in order to minimize the impact of these unfavorable factors and to improve *B. bassiana* efficiency for crop protection.

In the present study, the virulence of Bb11 to a DBM population from Cotonou, Benin was compared in the laboratory to that of three other *B. bassiana* isolates. Subsequently, the most virulent isolate was tested on-station and on-farm at different doses and application frequencies for its efficacy in controlling DBM populations both in Benin and Togo.

2. Materials and methods

2.1. Laboratory bioassay

2.1.1. Insects

DBM Larvae were collected during January 2013 from one of the most important cabbage production sites (N06°22.067′; E002°23.800′) around Cotonou, the main city in Benin. Larvae were mass-reared in ventilated cages on 6–8 true leaf cabbage plants (stage 3 *B. oleracea* L., var. kk-cross plants) (Andaloro et al., 1983) grown in a greenhouse at 27 ± 1 °C, 81 ± 5% RH and 12:12 (L:D) h. Adults were fed with a 10% sugar solution and were used to produce subsequent generation larvae for different tests.

2.1.2. Susceptibility of DBM to B. bassiana

The tests were carried out in the laboratory of the International Institute of Tropical Agriculture, IITA, near Cotonou, Benin. Third instar larvae issued from the above rearing were used to study the virulence of *B. bassiana* isolates Bb362, Bb116, Bb115 and Bb11 obtained from IITA-Benin microbial collection (Table 1). The DBM susceptibility to the various isolates was assessed in two stages: (i) conditioning of the isolates to DBM and (ii) virulence tests.

2.1.2.1. Conditioning of B. bassiana isolates to DBM. The process of conditioning consisted in exposing DBM larvae to *B. bassiana* isolates obtained from other insect species (Table 1), in order to induce metabolic changes for better adaptation to the target pest (Xiao et al., 2012). The isolate Bb11 being the reference strain already adapted to DBM (Godonou et al., 2009), this process was only applied to isolates Bb115, Bb116 and Bb362. Conidia of *B. bassiana* isolates conserved on silica gel since 1998 in the IITA-Benin microbial collection were produced on Potato Dextrose Agar (PDA) plates in Petri dishes (Ø = 90 mm). The Petri dishes were sealed with Parafilm ® and incubated at 26 °C ± 1 °C in a dark incubator for 5 days. The new conidia obtained from these colonies were subsequently multiplied on new PDA plates, harvested and dried. Conidia of *B. bassiana* from each isolate were mixed with a sterilized Tween 80 solution (0.05%). For each isolate, 2 µl of a 10^8 conidia/ml concentration was applied topically to third instar larvae of DBM. Dead larvae were dried in Petri dishes at 26 ± 1 °C in laboratory for 7 days, and then incubated separately in sterilized Petri dishes containing a single moistened Whatman no. 1 filter paper at 26 °C ± 1 °C. Conidia from the sporulated dead larvae were transferred to fresh PDA plates, and subsequently sub-cultured again on new PDA plates in order to have sufficient amount of conidia powder for further experiments.

To obtain the required concentrations, the conidia powder of the different *B. bassiana* isolates was suspended in a solution of Tween 80 (0.05%) and homogenized with a magnetic stirrer. The fungal suspensions were filtered using a laboratory sieve (mesh Ø: 90 µm) in order to remove mycelium residues. Conidia in the filtered suspensions were counted using a Neubauer hemocytometer. The concentrations of 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 conidia/ml of each *B. bassiana* isolate were obtained by successive dilution. Prior to the virulence test, the rate of conidia germination in each fungal suspension was assessed and ranked overall between 90 and 95%.

2.1.2.2. Virulence test. The virulence of *B. bassiana* isolates was tested on third instar larvae of DBM reared in cages in a ventilated screen house. The tests were carried out in laboratory at 26 ± 1 °C, 65 ± 2% RH and 12:12 (L:D) h. Under a laminar air flow cabinet, 10 larvae were released in each Petri dish (Ø = 90 mm) containing a leaf disc (88 mm diameter) cut from a tender leaf (from a 6–8 true leaf stage cabbage plant) and laid on a moistened Whatman no.1 filter paper to serve as food. The cabbage leaf discs were previously disinfected by dipping them in 10% sodium hypochlorite for 3 min and then rinsing them 3 times with sterilized distilled water. The infection method used was a topical application of 2 µl of each *B. bassiana* isolates concentration on the body of the larvae. The control larvae were inoculated with 2 µl of sterilized distilled water containing 0.05% of Tween 80. Each concentration of *B. bassiana* isolate was repeated 5 times, with 10 larvae per repetition, for a total of 50 larvae per concentration.

Mortality was assessed every 24 h until adult emergence. Dead larvae were air-dried for 7 days and then incubated separately in Petri dishes containing moistened Whatman no 1 filter paper for 7 days at 26 ± 1 °C. At the end of incubation, the dead larvae which showed signs of fungal growth (sporulated larvae) were recorded in order to estimate the sporulation rate following the formula:

Table 1
Details of *Beauveria bassiana* isolates tested in our study.

Isolate of *Beauveria bassiana*		Host insect (Order, Family)	Location	Collector's institute
Sample owner reference	Accession code			
ODA 581	Bb 11	*Sesamia calamistis* (Lepidoptera, Noctuidae)	Benin	IITA-Benin
I93-841	Bb 115	*Locusta* sp. (Ortoptera, Acrididae)	Madagascar	MSU/LUBILOSA
I93-842	Bb 116	*Locusta* sp. (Ortoptera, Acrididae)	Madagascar	MSU/LUBILOSA
53 a	Bb 362	*Callosobruchus* sp. (Coleoptera, Bruchidae)	Benin	IITA-Benin

$S = [Ns/No] \times 100$

with:

S: sporulation rate; N_s: number of sporulated dead larvae; *No*: total number of dead larvae incubated.

2.2. Field experiments

2.2.1. Study Site

The trial was conducted from 14 November 2013 to 17 January 2014 at IITA-Benin Station (N 06°24.917′; E 002°19.920′) located 13 km north of Cotonou, Benin. At the same time, the trial was replicated on farms located in horticultural settings of Cotonou (N06°22,067′; E002°23,800′) and Danyi, Togo (N 07° 16.579′; E000° 42.643′), where, as opposed to the experimental fields at IITA-Benin Station, cabbage is grown continuously with excessive use of synthetic insecticides by farmers. The experiments took place during the dry season, when DBM attacks on cabbage were most important, with mean temperature and relative humidity (%RH) around 28 °C and 80% for Cotonou and 23 °C and 82% for Danyi.

2.2.2. Insecticide application

The isolate Bb11 was selected for the field experiments because of its rapid virulence to DBM (eliciting 100% mortality in 4 days) observed in the preceding laboratory tests. Suspensions of Bb11 conidia powder were sprayed on cabbage plots and compared with a commonly used synthetic insecticide, deltamethrin (Deltacal 25 g/l EC, Arysta LifeScience, France), and untreated plots (Table 2).

In this study, two doses of Bb11 conidia powder, 53 g/ha and 315 g/ha were tested. They are far lower than the dose of 1000 g/ha Bb11 conidia powder used by Godonou et al. (2009). In addition, the low dose of 53 g/ha Bb11 conidia powder suspended in 315 L of water corresponding to a concentration of 1.68×10^7 conidia/ml was applied at two different frequencies: (i) one application per week (LBb1) and (ii) two applications at interval of 4 days (LBb2). The high dose of 315 g/ha Bb11 conidia powder (HBb) suspended in 315 L of water corresponding to the concentration of 10^8 conidia/ml was used as reference and tested only at one application per week. The HBb corresponds to the concentration of 10^8 conidia/ml considered as very effective against DBM on farm in Benin by Godonou et al. (2009). All applications were performed using commonly available manually operated knapsack sprayers (OSATU; model: STAR 16 AGRO), calibrated to spray 315 L/ha in well dispersed fine droplets. It is important to mention that the efficiency of microbial insecticides applications is closely related to droplet size and spore dispersal, rather than to high volume application (Chapple et al., 2007).

The insecticide deltamethrin was applied, using a separate sprayer of the same type, at the dose of 12.5 g/ha in 315 L of water at 7 days intervals according to farmer practices in Benin (James et al., 2006).

2.2.3. Experimental design

A randomized complete blocks design as described by Hoepting (2018) was followed, using 5 treatments replicated 4 times in both on station and on farm. Plot size was three rows of 5 m length each with a spacing of 40 cm between cabbage plants (*B. oleracea* L., var. kk-cross) and 40 cm between rows. Spacing between plots and replications were 1 m and 1.5 m respectively. Cattle manure was used for soil amendment at a dose of 12 kg per plot or 20 t/ha. The cabbage seedlings were transplanted at 3 weeks after emergence. A granular fertilizer NPK 15-15-15 was applied twice on each plot at the dose of 100 kg/ha, two weeks after cabbage seedling transplantation and at the early heading stage. Insecticides were applied starting one week after seedling transplantation and stopped 2 weeks before harvesting, for a total of 6 or 12 applications, according to the common application practices (Table 2). Daily watering −33 L of water per plot, 2 times per day- was applied on cabbage plants, using watering cans.

2.2.4. Data collection

The effectiveness of insecticides was assessed by checking 8 randomly selected cabbage plants weekly from the central row of each plot. The number of DBM larvae and pupae were recorded. At maturity, marketable heads – with no or negligible damage – were harvested on the central row and their weight recorded in order to estimate the yield following the formula:

$$Y = \frac{W}{A} \times 10000$$

with

Y: marketable cabbage heads yield per hectare; *W*: weight of marketable cabbage heads on the central row; *A*: area of the central cabbage row per plot (1.92 m^2).

2.3. Statistical analysis

For the laboratory bioassays, daily percentage mortality data (P) were first arcsine√(P/100) transformed before being subjected to GLM procedure of SAS followed by Student Newman Keuls (SNK) test at 5% for the separation of means. The LC_{50s} values were estimated by probit analysis (Finney, 1971) using WINDL software Version 2.0 (Giner et al., 1999) at 6 days after conidia inoculation to larvae, where there was no or insignificant progression in the mortalities. Prior to probit analysis, the mortalities were corrected with Abbott's formula (Abbott, 1925). The LC_{50s} values were considered significantly different when their confidence intervals (95%) did not overlap. The relation between sporulation of DBM dead larvae and the dose of *B. bassiana* conidia inoculated was estimated by a linear regression model using R (version 3.5.3) statistical package.

For the field experiments, the average numbers of DBM recorded under different treatments were √(x + 1) transformed before being subjected to ANOVA, and means were separated by SNK test at 5%, using the SAS statistical software 9.2. The total monetary revenue of marketable cabbage heads of each treatment was estimated by using the obtained yield times the average price of a cabbage head at the local market (0.5 US$/kg). The net return per hectare was estimated by the total revenue from the yield minus the costs of cabbage protection by insecticides; all other costs including inputs and labor are assumed to be the same for all the treatments. The cost of deltamethrin and spinosad, necessary for the 6 treatments throughout a cabbage growing season,

Table 2
Description of insecticides used in the field trial.

Commercial name (Active ingredient)	Concentration of active ingredient	Dose applied per spray	Number of sprays[a]	Total amount of insecticide per ha/season	Code of Treatment
Beauveria bassiana,isolate Bb11	53. 10^{11} conidia/ha	53 g/ha	6	318 g	LBb1
		53 g/ha	12	636 g	LBb2
	315. 10^{11} conidia/ha	315 g/ha	6	1890 g	HBb
Deltacal 25 EC (Deltamethrin)	12.5 g/ha	0.5 l/ha	6	3 l	Deltamethrin
Control	–	–	–	–	T_0

[a] Insecticides were applied weekly from 21 November to 26 December 2013 for all the treatment, except LBb2 with two applications per week at interval of 4-days.

was 54 and 768 US$/ha, respectively. The amount of *B. bassiana* powder required for the treatments LBb1, LBb2 and HBb during one growing season of cabbage was estimated at 70, 140 and 416 US$/ha respectively, according to De Groote et al. (2001). The marginal revenue was estimated for cabbage protection with *B. bassiana*, in order to analyze the profitability of the doses LBb1, LBb2 and HBb, computed as the increase in revenue that results from the application of an additional unit (1 g) of *B. bassiana*, when all other factors are kept equal. It was calculated by dividing the change in total revenue by the change in *B. bassiana* dose, according to the following formulas (adapted from Adegeye and Dittoh, 1985):

$$MR = \Delta TR/\Delta q$$

With

MR: Marginal revenue; ΔTR: Change in total revenue obtained with *B. bassiana* doses; Δq: Change in dose of *B. bassiana*

3. Results

3.1. Laboratory assay

3.1.1. Virulence of B. bassiana isolates

The DBM larval mortality obtained with each *B. bassiana* isolate depended on the conidia concentration and on the time elapsed after larvae inoculation (Fig. 1). In fact, the mortalities induced were significantly different between the various concentrations of each *B. bassiana* isolate ($P < 0.05$). Except for Bb11, the mortalities due to the same concentration during the first four days were however similar for the isolates Bb115, Bb116 and Bb362 ($P > 0.05$) (Fig. 1). In addition, the overall mortalities recorded for *B. bassiana* isolate were lower than 50% during the first three days after larvae inoculation, except for Bb11 at 10^8 and 10^9 conidia/ml and Bb115 at 10^9 conidia/ml. With the dose of 10^9 conidia/ml, Bb11 elicited the most rapid mortality estimated to 64%, 86% and 98% on second, third and fourth day after inoculation, respectively (2nd day: $F = 6.80$; df = 3; $P = 0.006$; 3rd day: $F = 15.66$; df = 3; $P = 0.0002$ and 4th day: $F = 32.78$; df = 3; $P = 0.0001$). At the dose 10^8 conidia/ml, Bb11 elicited a higher mortality than Bb362 but statistically similar to those of Bb115 and Bb116. From the fourth to eighth day after larval inoculation, no significant ($P > 0.05$) differences were observed between the mortalities induced by any of the *B. bassiana* isolates within the dose ranging from 10^4 to 10^7 conidia/ml. At 10^8 and 10^9 conidia/ml, however, Bb11 displayed the highest mortality (Fig. 1).

Due to its efficiency in killing DBM larvae faster and in higher proportion, the isolate Bb11 displayed the lowest LD_{50} value, compared with others isolates (Table 3). The LD_{50} values of the isolates Bb115 and Bb116 were quite the same but lower as compared with Bb362 (Table 3).

3.1.2. Sporulation of dead DBM larvae

The sporulation rate of dead larvae was dose-dependent for all the *B. bassiana* isolates, with high coefficients of determination (R^2) estimated to 0.82, 0.85 0.87 and 0.78, for Bb11, Bb115, Bb116 and Bb362, respectively (Fig. 2). At the lower dose (10^4 conidia/ml), the sporulation rates were very low and quite the same for all isolates (Fig. 2). From 10^5 to 10^8 conidia/ml, isolates Bb11 and Bb362 displayed the highest sporulation rates. The slope value obtained with Bb362 was higher than those of Bb115 and Bb116 but similar to that of Bb11 (Fig. 2).

3.2. Field experiments

3.2.1. Diamondback moth population dynamic and density

On-station, DBM larval densities in both control and treated cabbage plots were lower than 0.5 larva/8 plants the first week after transplanting, corresponding to the beginning of insecticide sprays (Fig. 3A). In the on-farm plots in Cotonou and Danyi, DBM larval densities were already high the first week after transplanting reaching 0.8 and 8 larvae/8 plants, respectively. Starting from the third week after transplanting, control and deltamethrin-treated plots showed the highest increase of DBM populations both on-station (2.50 ± 0.58 and 3.25 ± 0.58 larvae/8 plants, respectively) and on-farm (Cotonou: 2.19 ± 0.48 and 3.21 ± 0.48 larvae/8 plants; Danyi: 10.43 ± 4.29 and 11.07 ± 2.72 larvae/8 plants, respectively). Overall, plots treated with Bb11 displayed low DBM populations, under 1.5 larvae/8plants on-station and 3.2 and 4.2 larvae/8 plants on-farm in Cotonou and Danyi, respectively. Among the treatments, LBb2 applied at the interval

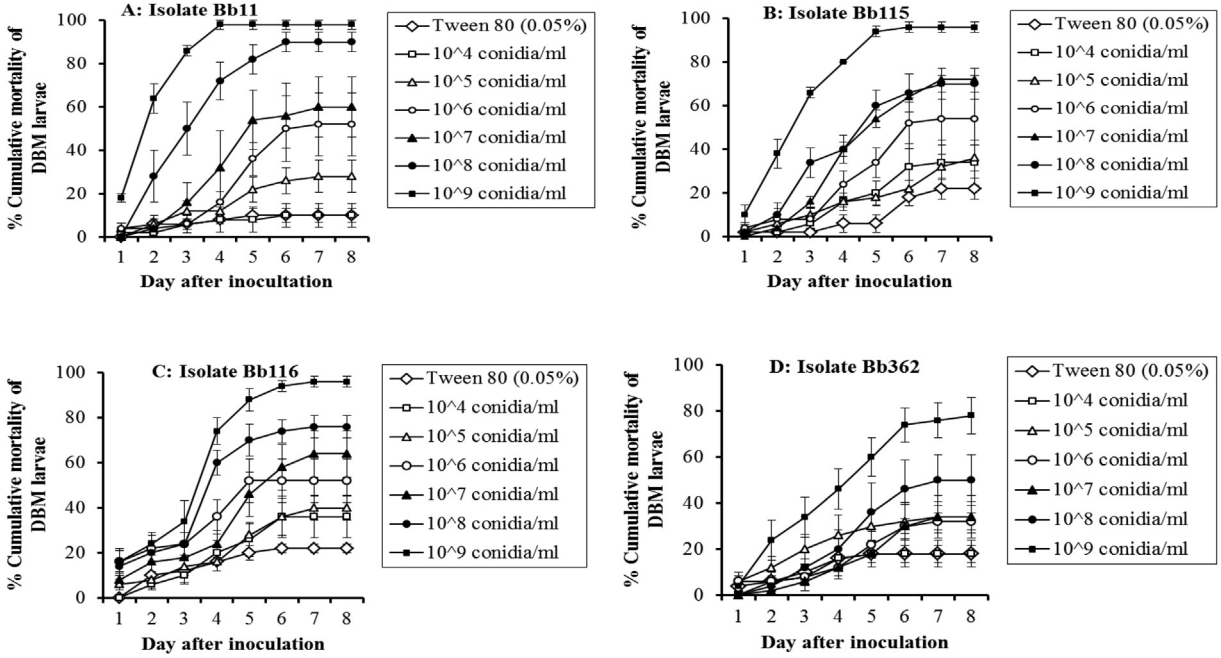

Fig. 1. Mortality of third instar diamondback moth (DBM) larvae obtained with various conidia concentrations of *Beauveria bassiana* isolates Bb11 (A), Bb115 (B), Bb116 (C) and Bb362 (D).

Table 3
LD_{50} values of *B. bassiana* isolates, 6 days after conidia inoculation to third instar DBM larvae.

B. bassiana isolates	n[a]	Slope ± SE	χ^2	LD_{50} (95% CI) (conidia [AI]/ml)
Bb11	300	0.752 ± 0.120	5	4.96×10^6 1.40×10^6–1.17×10^7)
Bb115	300	0.563 ± 0.216	12	8.97×10^6 (1.43×10^5–5.64×10^8)
Bb116	300	0.527 ± 0.132	4	9.24×10^6 (2.99×10^5–4.47×10^7)
Bb362	300	0.857 ± 0.266	4	3.68×10^8 (1.09×10^8–1.19×10^9)

CI: Confidence interval. χ^2: Chi Square. SE: Standard Error. [a]Number of larvae of *P. xylostella* tested. Isolates with confidence intervals (95%) overlap are not significantly different.

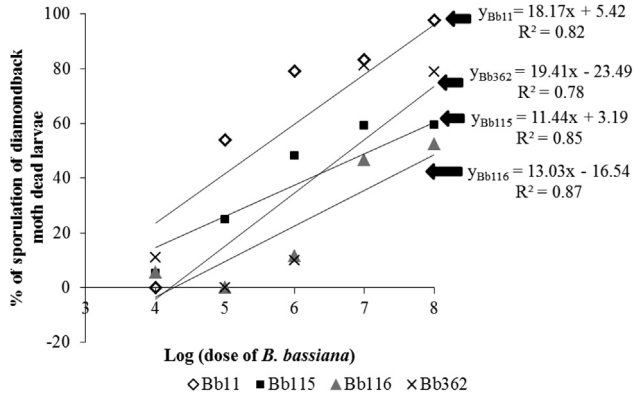

Fig. 2. Sporulation rates of dead diamondback moth (DBM) larvae as a function of the conidia concentrations of *Beauveria bassiana* isolates Bb11, Bb115, Bb116 and Bb362. N = 5 replications.

of 4 days considerably reduced the density of DBM populations to < 0.5 larva/8 plants on-station, throughout the period of observation (Fig. 3A), followed by HBb and LBb1, while the control and deltamethrin plots consistently recorded the highest densities. Similarly, LBb2 was able to maintain low DBM populations on-farm, with < 1.2 larva/8 plants and < 2.3 larvae/8 plants in Cotonou and Danyi, respectively (Fig. 3B and C). Compared with LBb2, the high dose of *B. bassiana* sprays once a week (HBb), both on-station and on-farm, led to a similar reduction of DBM populations density.

The pooled data over sampling dates (Fig. 4) clearly illustrate that LBb2 substantially reduced of the larval population both on-station and on-farm (Fig. 4). On-station, the reduction was estimated to 83% and 86% as compared with the unsprayed control and deltamethrin treatments, respectively (F = 19.38; df = 4; P < 0.00001). On-farm, LBb2 displayed similar results, reducing DBM larval population by 83% and 84% in Cotonou and by 83% and 93% in Danyi, compared with the control and deltamethrin treatments, respectively (Cotonou: F = 20.37 ; df = 4; P < 0.00001; Danyi: F = 41.12 ; df = 4; P < 0.0000).

3.2.2. Yield

Marketable cabbage yields obtained on-station with LBb2 were higher but not statistically significantly different than the other treatments (F = 0.92; df = 4; *P* < 0.48) (Fig. 5A). When exposed to higher DBM populations densities on-farm, however, treatments induced significant differences in marketable yields (Cotonou: F = 26.15; df = 4; P < 0.0000; Danyi: F = 10.46; df = 4; P < 0.0003). At both localities, LBb2 and HBb treatments obtained the highest cabbage yields, with LBb2 being highest in Cotonou (17.49 ± 3.89 t/ha, representing a 452% increase over the control plot) and HBb in Danyi (35.17 ± 5.95 t/ha, 199% increase over the control plot) (Fig. 5B and C). Cabbage yields on plots treated with deltamethrin were not statistically different to the control.

3.2.3. Profitability of insecticides use

Overall, the highest total revenue and net returns were obtained

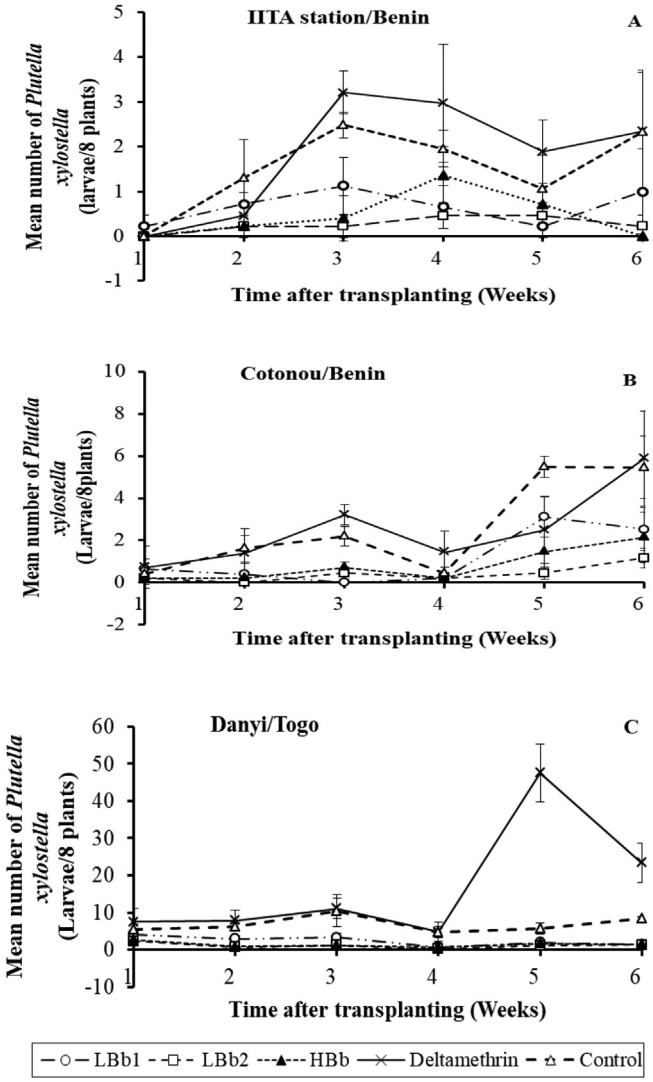

Fig. 3. Fluctuation of diamondback moth (DBM) population density under the influence of the different treatments on station (A) and on farm (B and C). LBb1: low dose of *B. bassiana* (53 g/ha) applied once a week; LBb2: low dose of *B. bassiana* (53 g/ha) applied twice a week; HBb: High dose of *B. bassiana* (315 g/ha) applied once a week; Control: cabbage plots not treated with insecticide. N = 4 replications.

with the application of *B. bassiana* (Table 4). Two applications of 53 g/ha of *B. bassiana* (LBb2) per week doubled the total revenue and net returns, compared with the control. The results obtained with LBb2 were similar to those of HBb (315 g/ha of *B. bassiana*), while the application of the synthetic insecticide deltamethrin (Delta) lead to low total revenues and net returns, similar to the control (Table 4).

The marginal revenue obtained by spraying 53 g/ha of *B. bassiana* twice a week (LBb2) ranged from US$ 69 to US$ 148 (Table 5). It was

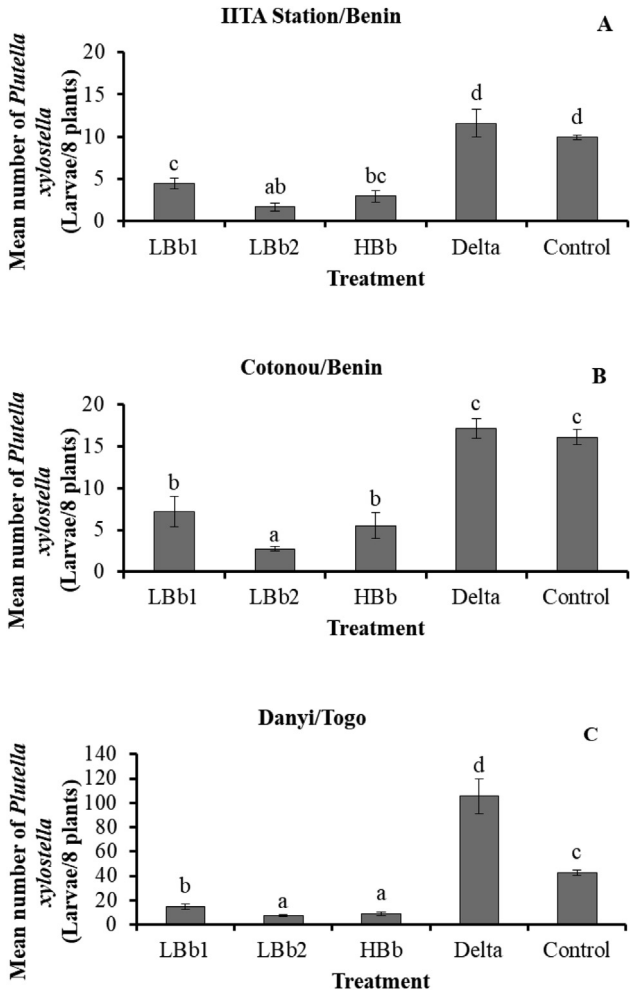

Fig. 4. Density of diamondback moth (DBM) populations under the influence of the different treatments on station (A) and on farm (B and C). Mean (± SE) with the same letter are not significantly different (ANOVA; SNK test; $P < 5\%$); LBb1: low dose of *B. bassiana* (53 g/ha) applied once a week; LBb2: low dose of *B. bassiana* (53 g/ha) applied twice a week; HBb: High dose of *B. bassiana* (315 g/ha) applied once a week; Control: cabbage plots not treated with insecticide. N = 4 replications.

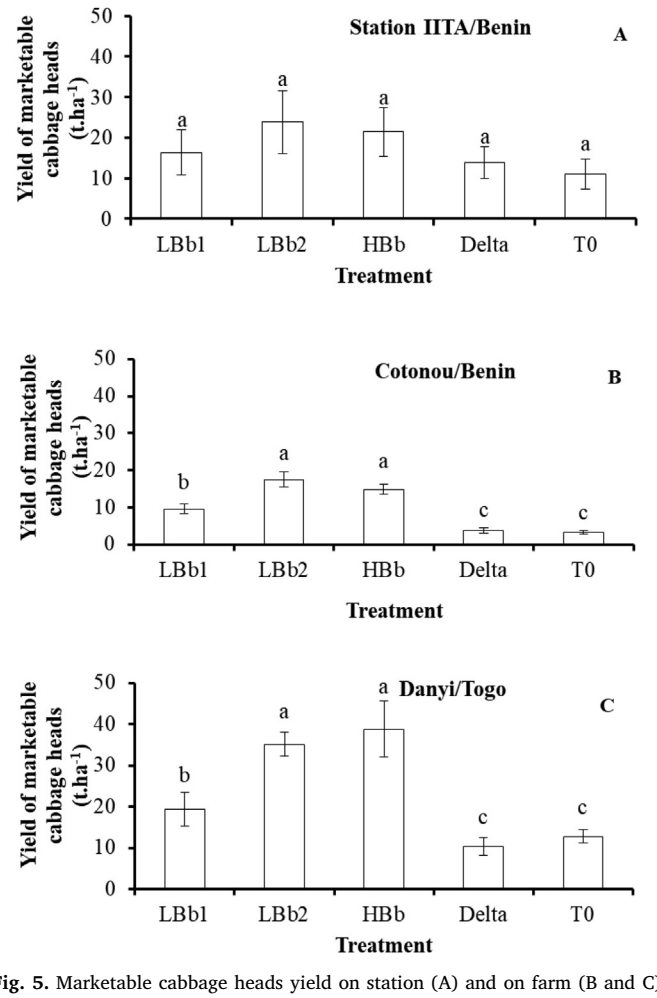

Fig. 5. Marketable cabbage heads yield on station (A) and on farm (B and C) per season. Mean (± SE) with the same letter are not significantly different (ANOVA; SNK test; $P < 5\%$). LBb1: low dose of *B. bassiana* (53 g/ha) applied once a week; LBb2: low dose of *B. bassiana* (53 g/ha) applied twice a week; HBb: High dose of *B. bassiana* (315 g/ha) applied once a week; Control: cabbage plots not treated with insecticide. N = 4 replications.

higher than those obtained with the weekly application of 53 g/ha (LBb1) and 315 g/ha (HBb). The weekly application of the high dose of *B. bassiana* HBb decreased the marginal revenue which reached negative values such as −8 US$ and −7 US$ in Cotonou and IITA Station respectively (Table 5).

4. Discussion

In this study, *B. bassiana* isolates displayed different levels of virulence to DBM depending on dose of conidia and time after inoculation. This confirms observations by Durbec and Stora (1990) whereby time after inoculation was a key factor in the computation of mortalities induced by toxic products. Accordingly, the time conidia of each *B. bassiana* isolate will take to achieve the different steps leading to the colonization of the host body can influence the virulence potential. As shown by our results, the isolate Bb11 induced the fastest mortality and its LD_{50} was lowest, indicating better adaptation to DBM. An earlier study by Godonou et al. (2009) had shown that Bb11 (called Bba5653 at that time) was effective against DBM in Benin causing 94% mortality, but did not assess its LD_{50} value. In contrast, Bb362 showed the highest LD_{50} 6 days after inoculation, while Bb115 and Bb116 still required more time to induce sufficient larval mortality, indicating their lower efficiency against DBM, compared with Bb11. This confirms previous observations by Shaw et al. (2002) and Shahid et al. (2012) that isolates of entomopathogenic fungi belonging to the same species could display varying reactions with regard to the number of hosts infected, infestation and germination rates, and optimal development temperature. The lower performance of the three isolates, Bb362, Bb115 and Bb116, might be due to various factors affecting the process of adhesion or passage of the fungus through the insect cuticle, as demonstrated by Holder and Keyhani (2005). According to their studies, aerial conidia characterized by single-cell propagule, as in our case with *B. bassiana*, adhered poorly to weakly polar surfaces, and more rapidly to hydrophobic surfaces similar to insect cuticle. The adhesion kinetics of *B. bassiana* conidia can also be influenced by electrostatic charges and low relative humidity, in addition to factors contributing to cuticle properties such as nutrient levels, endogenous microbial flora, and cross-linked proteins (St Leger, 1991; Pedrini et al., 2013).

The isolate Bb11 was initially obtained from *S. calamistis,* also a Lepidoptera like *P. xylostella*, whereas Bb115 and Bb116 were isolated from *Locusta* sp. (Orthoptera), and Bb362 from *Callosobruchus* sp. (Coleoptera). This could explain, at least partially, why conidia of Bb11 are more adapted to the cuticle of DBM larvae, and their hyphae can more easily penetrate into the host haemolymph after degrading the cuticle (Bidochka and Khachatourians, 1987). In fact, Bb11 could have evolved to produce more lepidopera-specific cuticle-degrading

Table 4
Total revenue and net return of cabbage growing under various treatments in Togo and Benin.

Site	Treatment	Yield kg/ha	Total revenue* US$	Cost of cabbage protection/ha** US$	Net return*** US$
IITA station	LBb1	16,380	8190	93.96	8096
	LBb2	23,840	11,920	187.92	11,732
	HBb	21,440	10,720	439.8	10,280
	Delta	13,840	6920	78	6842
	Control	10,990	5495	0	5495
Cotonou	LBb1	9580	4790	93.96	4696
	LBb2	17,490	8745	187.92	8557
	HBb	14,850	7425	439.8	6985
	Delta	3780	1890	78	1812
	Control	3160	1580	0	1580
Danyi	LBb1	19,440	9720	93.96	9626
	LBb2	35,170	17,585	187.92	17,397
	HBb	38,860	19,430	439.8	18,990
	Delta	10,370	5185	78	5107
	Control	12,800	6400	0	6400

Delta: deltamethrin (12.5 g [a.i]/ha); LBb1: One application of a low dose (53 g/ha) of *B. bassiana* isolate Bb11 conidia powder per week; LBb2: Two applications of a low dose (53 g/ha) of *B. bassiana* isolate Bb11 conidia powder per week; One application of the high dose (315 g/ha) of *B. bassiana* isolate Bb11 conidia powder per week.

* Total revenue = Yield × 0.5 US$, with the mean price of cabbages on local markets estimated to 0.5 US$/kg.

** Cabbage protection cost = insecticide cost + spraying cost.

*** Net return = Total revenue − Cost of cabbage protection; the net return here is that of the first harvest.

Table 5
Marginal revenue obtained by cabbage protection with *B. bassiana*, isolate Bb11.

Site	Treatment	*B. bassiana*		Revenue with *B. bassiana*		
		Dose (g/ha)	Change in dose Δq* (g/ha)	Total revenue *TR* (US $/ha)	Change in Total revenue ΔTR** (US$/ha)	Marginal revenue *MR**** (US$)
IITA station	Control	0	–	5495	–	–
	LBb1	53	53	8190	2601.04	49
	LBb2	106	53	11,920	3636.04	69
	HBb	315	209	10,720	−1451.88	−7
Cotonou	Control	0	–	1580	–	–
	LBb1	53	53	4790	3116.04	59
	LBb2	106	53	8745	3861.04	73
	HBb	315	209	7425	−1571.88	−8
Danyi	Control	0	–	6400	–	–
	LBb1	53	53	9720	3320	63
	LBb2	106	53	17,585	7865	148
	HBb	315	209	19,430	1845	9

Delta: deltamethrin (12.5 g [a.i]/ha); LBb1: One application of a low dose (53 g/ha) of *B. bassiana* isolate Bb11 conidia powder per week; LBb2: Two applications of a low dose (53 g/ha) of *B. bassiana* isolate Bb11 conidia powder per week; One application of the high dose (315 g/ha) of *B. bassiana* isolate Bb11 conidia powder per week.

* Δq = Change in dose of *B. bassiana*.

** ΔTR = Change in total revenue obtained with *B. bassiana* doses.

*** Marginal revenue $MR = \Delta TR/\Delta q$.

proteases (Dias et al., 2008) and mechanisms to evade phagocytic haemocyte cells circulating in the haemolymph (Bidochka et al., 2010). This was also confirmed by results of Douro Kpindou et al. (2012) demonstrating that Bb11 was pathogenic to another lepidopteran species, *Helicoverpa armigera* (Hübner) causing considerable larval death and sporulation rates.

In our study, we observed that despite the low virulence of Bb362 against DBM, larvae killed by this isolate displayed a high sporulation rate, similar to that of Bb11. This might be a further indication that the low effectiveness of Bb362 stems merely from its low performance in the germination and penetration process, rather than from the colonization of the host body (Khachatourians, 1992; Kouassi et al., 2002; Shahid et al., 2012). The isolate Bb11 was able to induce the highest mortality and produce the highest sporulation rate, showing great potential with regard to auto-dissemination and subsequent infection of surrounding larvae as observed by Furlong and Pell (2001) for the genus *Beauveria.* Its efficiency will still depend, however, on environmental factors, such as relative humidity, rain intensity and frequency, and UV radiation (Maniania, 1993; Meyling and Eilenberg, 2007; Rodrigues et al., 2016).

Our field trials indicated that the spray frequency of Bb11 was the key factor for improving its application efficacy against the target pest. The reduction of DBM larval populations obtained with a high dose of Bb11 (HBb) applied once a week was similar to that of a low dose applied twice a week (LBb2) at intervals of 4 days. As reported by Inglis et al. (1995) for wheat and alfalfa, the daily watering of cabbage plants practiced by farmers was likely to flush *B. bassiana* conidia from cabbage leaves, thereby reducing the duration of exposure of DBM larvae to Bb11 conidia. The application of *B. bassiana* twice a week appeared to improve the persistence of Bb11 conidia on cabbage plants, thereby strengthening its effectiveness against DBM larvae. Also, as demonstrated by Ugine et al. (2007), decreasing spraying intervals from 7 to 5 days increased *B. bassiana* conidia load on the thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Our approach of applying 53 g/ha of Bb11 every 4 days has considerably reduced the dosage of conidia powder, as compared with the dosage of 1000 g/ha reported by Godonou et al. (2009). In addition, limiting factors affecting the efficacy of *B. bassiana* such as conidia deactivation by UV radiation (Inglis et al., 1995; Leland and Behle, 2005) and relative humidity (RH) could be further mitigated by the 4-days interval spray. Additional reduction of UV radiation and increase in RH could be achieved, however, by covering the cabbage crop with proper insect netting (Muleke et al., 2014; Simon et al., 2014; Martin et al., 2015).

With regard to the economic performance, the use of 53 g/ha of Bb11 applied every 4 days (LBb2) led to the highest marginal revenue, due to most efficient protection of cabbage at lowest cost. Moreover, this bi-weekly application strategy could be easily adopted by farmers as it is similar to their own chemical sprays frequency on cabbage crops in Benin (James et al. 2006).

As opposed to *B. bassiana*, the synthetic insecticide deltamethrin was ineffective against DBM, with observed larval densities, marketable cabbage head yield and net revenue similar to those of the untreated control. These results confirm previous observations that DBM populations of Benin and Togo are increasingly resistant to deltamethrin, (Goudegnon et al. 2000; Agboyi et al. 2016), but also highlight the use of *B. bassiana* isolate Bb11 as a promising bio-pesticide against deltamethrin-resistant DBM populations.

CRediT authorship contribution statement

Lakpo K. Agboyi: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing - original draft. **Guillaume K. Ketoh:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Orou K. Douro Kpindou:** Methodology, Writing - original draft. **Thibaud Martin:** Conceptualization, Methodology, Writing - review & editing. **Isabelle A. Glitho:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Manuele Tamò:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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