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Effect of *Bacillus thuringiensis* var. *kurstaki* HD-1-based biopesticide on the pathogenicity of *Phytophthora palmivora*

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Abstract

Black pod disease caused by *Phytophthora* spp limits cocoa yield. This study aims at assessing the antifungal activity of *Bacillus thuringiensis* var. *kurstaki* HD-1 on pathogenicity of the parasite. The pea-based agar medium was treated either with Btk HD-1 suspension at different concentrations (C1 = 100%, C2 = 50% and C3 = 25%) or with the reference fungicide (3.33g/L). The medium distributed in petri dishes was inoculated with a 6 mm-diameter mycelial disc. After five days of incubation, safe and immature pods were inoculated with extracts taken from the previous culture medium. The control pods were infected by black pod, 48 hours after inoculation; whereas, those which have been inoculated with extracts taken from the medium treated with biopesticide showed necrotic lesions on the 3rd and 5th days of incubation. On the contrary, with reference fungicide showed no necrotic lesions on cocoa pods. The size of the necrotic lesions varied from 0 to 10.37 ± 0.46cm diameter.The rate of pods infected was 25%, 50% and 100%, respectively, for C1, C2 and C3. The infectivity of *P. palmivora* decreased significantly ($p < 0.001$) in the presence of biopesticide concentrations. The effect of the biopesticide evolves according to its concentration. This formulation could be an interesting alternative in an integrated strategy for the control of this cocoa disease.

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Introduction

Black pod caused by *Phytophthora* spp. is the most devastating fungal disease that occurs in cocoa orchards in West and Central Africa. It is caused by pests, including mirids, a great concern in cocoa cropping. This disease is directly expressed by yield losses. The infected pods produce cocoa beans unfit for consumption (Coulibaly *et al.*, 2013). Crop losses due to this disease are in the order of 30 to 40%, according to the International Cocoa Organization (ICCO, 2009), and can even reach 100% in the absence of control (Ndoumbe-Nkeng *et al.*, 2004).

In Côte d'Ivoire, one of the species blamed for black pod disease is *Phytophthora palmivora*. This species constitutes one of the major constraints to the sustainability of cocoa cultivation. In order to control this parasite, copper and metalaxyl-based chemical fungicides are applied by farmers. These chemicals have helped improve crop yields. Their application in agriculture has increased because of their relatively rapid and effective action. However, the excessive use of these products can lead the pathogen to develop resistance against these chemical fungicides (Fontem *et al.*, 2005). They can also contribute to soil and water pollution from application derivatives or rainfall runoff (Orisajo *et al.*, 2011).

In view of the aforestated adverse impacts, chemical Safety advocates an integrated pest control approach combining different techniques and methods in the management of crop pests and disease vectors (Mawussi, 2008). Among them, biological control constitutes a promising alternative for the integrated control strategy. One of the biological control methods using microbial pesticides, including *Bacillus thuringiensis* (Bt)-based, has been developed. Many studies have shown the effectiveness of this biopesticide against insects (Lagadic *et al.*, 2014. Duchet *et al.*, 2015) and other parasites including Oomycetes (Zhou *et al.*, 2008 ; Gadji *et al.*, 2015) and nematodes (Hu *et al.*, 2010). This efficiency makes it the most used biological pesticide in the world. The mass adoption of this biopesticide is also justified by its specificity and especially its almost non-existent negative impact on the environment and human health (Roh *et al.*, 2007; Lagadic *et al.*, 2014; Duchet *et al.*, 2015). This study aims at testing and comparing the inhibitory effect of Btk HD-1-based biopesticide to that of the reference chemical fungicide (Ridomil Gold Plus 66 WP) used as a positive control, on pathogenicity of the parasite responsible for cocoa black pod disease in Côte d'Ivoire.

Materials and methods

Plant material

The plant material comprised cocoa pods disease stemming from the variety Amelonado. This variety is highly sensitive to black pod caused by *Phytophthora* spp.

Fungal material

A strain of *Phytophthora palmivora* whose aggressiveness is proven on plant material was used for this study. This strain called BL7/11.2 was isolated from a pod naturally infected by black pod disease. The pod was taken from the experimental plot BL7 of the National Center for Agricultural Research (CNRA) station of Bingerville, in Southern Côte d'Ivoire. The strain was kept in a fungus culture collection at 26°C on a pea-based agar medium in test tubes.

Biological and chemical control equipment

The control equipment was essentially made up of a *Bacillus thuringiensis* var. *kurstaki* HD-1 (Btk HD-1)-based biopesticide and the reference chemical fungicide, Ridomil Gold Plus 66 WP. The biological control material is made in the bioconversion of wastewater and wastewater sludge into value added products laboratory of Water-Earth-Environment, National Scientific Research Institute of Quebec University, Canada. This bioproduct was synthesized in a bioreactor using the starch industry wastewater (SIW) as raw material. It is in liquid form and contains cells (3.13 x 10^9 CFU/mL), spores (2.46 x 10^9 CFU/mL) and other virulence factors such as delta-endotoxin, chitinase, zwittermicin A, produced by the bacterium during fermentation. However, the reference chemical fungicide was composed of 60g/kg metalaxyl and 600g/kg copper oxide as active ingredients.

Restoration of aggressiveness

The strain of *P. palmivora* (BL7/11.2) was removed from the fungus culture collection and its aggressiveness was renewed on safe and immature pods stemming from the variety Amelonado. The pods were inoculated by opening the cortex in the middle of the lateral side of pods, under aseptic conditions in a laminar flow hood. After 72 hours of incubation at 26°C in crystallizing dishes in saturated moisture (80%), the pods showing necrotic lesions were identified and fragments were taken from the growth front of black pod. These pod fragments (a cubic necrotic piece of 0.5 cm^3) were transferred to water agar medium at 1.5% in 90 mm-diameter Petri dishes. Incubation was performed at 26 °C for three to four days. After thallus formation, mycelial fragments were then taken from the growth front of the crop and transferred to pea-based agar medium in a 90 mm-diameter petri dish. Incubation was performed under the same conditions as mentioned earlier (Coulibaly *et al.*, 2013).

Preparation of inoculum and treatment concentrations

Phytophthora palmivora inoculum

The strain of *P. palmivora* was first purified by two or three successive subcultures on pea-based agar medium in 90 mm-diameter petri dishes. In order to avoid the obvious heterogeneity of zoospores, the cloning of the strain by mono zoospore isolation was performed according to the technique described by Babacauh (1980) and Ortiz-Garcia (1996). The strain was then cultured on pea-based agar medium in Roux flasks and incubated in darkness for 6 days at 26°C. Finally, the resulting culture was exposed to a photoperiod of 12 hours for at least two days to induce the formation of sporocysts. Sporocyst germination was caused by thermal shock by placing the wet culture with 40mL of distilled water in a refrigerator at 4°C for 15 min. Then, the culture was exposed to the light of an incandescent lamp of 60 W for 45 minutes. The zoospore suspension obtained in the Roux flask was counted with a Malassez cell and adjusted to a concentration of 10^3 zoospores/ mL. A quantity of 100uL of this suspension was spread, using a Pasteur pipette, on pea-based agar medium in 90 mm-diameter Petri dishes.

The Petri dishes were incubated in reverse position at 26°C for three days. After incubation, the mycelial discs calibrated at 6mm diameter e were used as *P. palmivora* inoculum for bioassays.

Concentration of Btk HD-1-based biopesticide and Ridomil Gold Plus 66 WP

Dilutions of Btk HD-1 suspension were made according to the technique described by Gadji *et al.* (2015). The 25, 50 and 100% (v/v) Btk HD-1 concentrations, respectively designated by C1, C2 and C3 were selected for bioassays. These concentrations were chosen after a preliminary laboratory tests. The different concentrations are listed in Table 1. A reference fungicide, Ridomil Gold Plus 66 WP, was used as a positive control. The approved dose is 50g of wettable powder of the chemical in 15L of water, that is, 3.33g/L. It is designated by D in this study.

Table 1. Btk HD-1-based biopesticide concentrations in cells and spores.

Btk HD-1-based components of the biopesticide	Concentration (CFU/mL)		
Cells	C1 (100%)	C2 (50%)	C3 (25%)
	3.13 $x10^9$	1.565 $x10^9$	7.825 $x\ 10^8$
Spores	2.46 $x10^9$	1.23$x10^9$	6.15 X 10^8

Assessment of Btk HD-1-based biopesticide effect on the pathogenicity of *P. palmivora* on detached pods

Cocoa pods harvesting and preparation

Safe and immature pods, aged four to five months, were harvested on four cocoa trees. The pods were washed with tap water and rinsed with distilled water. Later, they were placed one by one in crystallizing dishes on a foam soaked in sterile distilled water. Twenty (20) pods were used at a rate of four fruits per concentration including the control. Using a 6 mm-diameter punch, a 1 cm-deep cut was made in the middle of the lateral side, on the pod cortex. The test was made in two sets of 20 pods each.

Inoculation of detached pods

P. palmivora (inoculum) mycelial discs and agar discs taken from culture medium added to Btk HD-1-based biopesticide, after five days of incubation, were transferred to the cuts of detached pods. Mycelial disks that were used to inoculate the control pods were taken from untreated culture medium, after five days of incubation. Determination of the rate of pods infected by black pod

The observations were made, regularly, 24 hours after inoculation, for a week. The pods showing a necrotic lesion at the inoculation point, were counted and the rate of pods infected by black pod (RPI) was calculated according to the Equation 1:

$$RPI(\%) = \frac{\text{Number of inoculated pods infected by black pod}}{\text{Total number of inoculated pods}} X\ 100\% \quad (1)$$

RPI (%) refers to the rate of pods infected by black pod

Size of necrotic lesions and inhibition rate

Size of necrotic lesions

Lesion diameter (LD) was determined according to the formula of Shuman (2001), based on daily measurements of brown spot diameters following two perpendicular axes of the fruit for Equation 2:

$$LD\ (cm) = \frac{\text{Lesion length} + \text{Lesion width}}{2} X\ 100\% \quad ..(2)$$

Calculation of the inhibition rate of necrotic lesions

The rate was calculated by reference according to the formula of Sunpapao and Pornsuriya (2014) as given below in Equation 3:

$$PICD\ (\%) = \frac{A - B}{A} x\ 100\% \quad .....(3)$$

Where, A refers to the average lesion diameter of *P. palmivora* on the pods inoculated with mycelia disks taken from untreated culture medium. B refers to the average lesion diameter on the pods inoculed with mycelia taken from medium treated either with Btk HD-1-based biopesticide or with Ridomil Gold Plus 66 WP.

Statistical analysis of data

All data were analyzed using Statistica software version 7.1. Multivariate analyzes of variance (MANOVA) were applied to mean diameter and to inhibition rate of cocoa pods necrotic lesions to study the interaction concentrations - time of incubation on the pathogenicity of *P. palmivora*. Comparison of the means between the diameters of the necrotic lesions and their inhibition rates was carried out by the analysis of variance (ANOVA). Newman-Keuls test was used to make the mean segregation for significant differences ($p < 0.05$). This test enable to compare all the levels of the factors (Bar-Hen, 1998).

Results

Effect of Btk HD-1-based biopesticide on the expression of black pod disease

The inoculated pods showed symptoms of black pod at various rates on different dates. The analysis of variance of black pod data according to concentrations of Btk HD-1-based biopesticide revealed differences in black pod rates that were statistically significant between concentrations at 5% threshold, from the third to the fifth day of incubation (Table 2). The rates of pods infected by black pod fluctuated depending on the concentrations. The control pods inoculated with concentration Co showed symptoms of the disease, on the second day of incubation. However, with concentrations, C2 and C3, the rate of pods infected by black pod have gradually evolved from the third to the fourth day of incubation, respectively, from 25 to 50% and from 75 to 100%. These rates were highly significant ($p < 0.001$) than the one induced by concentration C1 over the same period up to the fifth day of incubation when 25% of pods showed necrotic lesions.

Effect of concentrations and incubation time on necrotic lesions of cocoa pods

Multivariate analyses of variance (MANOVA) performed with mean rates of germination and inhibition of *P. palmivora* zoospores revealed identical levels of differentiation ($p < 0.001$) between test concentrations and incubation time (Table 3). These results show that concentrations and incubation times had a synergistic effect on the pathogenicity of *P. palmivora* against cocoa pods of Amelonado variety.

Table 2. Rate of pods infected by black pod per day in the presence of concentration tested.

Treatment	Rate of pods infected by black pod (%)					
	Incubation period (day)					
	2	3	4	5	6	7
Co (Control: P. palmivora)	100	100 ± 0.0^{d}	100 ± 0.0^{c}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
C1 (100% of biopesticide)	0	0 ± 0.0^{a}	0 ± 0.0^{a}	25 ± 0.0^{c}	25 ± 0.0^{c}	25 ± 0.0^{c}
C2 (50% of biopesticide)	0	25 ± 0.0^{b}	50 ± 5.7^{b}	50 ± 5.7^{d}	50 ± 5.7^{d}	50 ± 5.7^{d}
C3 (25% of biopesticide)	0	75 ± 4.0^{c}	100 ± 0.0^{c}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
D (3,33 g/L Ridomil Gold Plus 66 WP)	0	0 ± 0.0^{a}	0 ± 0.0^{a}	0 ± 0.0^{b}	0 ± 0.0^{b}	0 ± 0.0^{b}
F	nd	2475.0	1500.0	1200.0	600.0	797.3
p	nd	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The averages in each column followed by the same letter are not significantly different, according to the Newman Keuls test, at 5% threshold.
nd: not defined.

Analysis of the variance, based on the two factors (concentrations and incubation times) studied, showed a significant interaction as indicated in Table 3. That result has been interpreted using ANOVA 2. The mean diameter of necrotic lesions and the mean inhibition rate varied from one level of factor to another (Table 4). Concerning the mean diameter, it varied from 0 to 10.37 ± 0.48 cm, from the second to the fourth day of incubation. On the other hand, the mean inhibition rate varied from 31.14 ± 7.12 to 100%, from the second to the seventh day of incubation and from concentration C3 of the biopesticide to concentration D of the reference chemical fungicide (Ridomil Gold Plus 66 WP). Significant differences between the mean diameters of the necrotic lesions on the cocoa pods ($F = 27.932$ with $p < 0.001$) for the tested concentrations and the incubation time were observed. The control pods showed necrotic lesions with mean diameter ranging from 1.10 ± 0.18 to 10.37 ± 0.48cm, from the second to the seventh day of incubation. This mean diameter of the necrotic lesions of the untreated pods (control) was higher than the mean diameter of the necrotic lesions observed on the pods treated with the lowest concentration biopesticide suspension (C3). Analysis of the variance revealed significant differences ($p < 0.05$) between the mean diameters of the necrotic lesions on the cocoa pods. On the other hand, no symptoms of black pod on pods treated with the concentration C1 of the biopesticide or the reference chemical fungicide were observed during the same period (Table 4).

Table 3. Probability values associated with MANOVA analysis according to the factors concentration and incubation time.

Factors	F	*p*
Concentration	384.84	< 0.001
Incubation time	61.48	< 0.001
Concentration*time of incubation	39.05	< 0.001

F: Statistic value of MANOVA test *p*: probability value of MANOVA test.

Table 4. Interaction concentration-time of incubation on mean diameters of necrotic lesions and inhibition rates of black pod.

Concentrations	Incubation time (Day)	Mean diameter of necrotic lesions (cm)	Inhibition rate (%)
Co (control : P. palmivora)	2	1.00 ± 0.05^{ab}	
	3	4.84 ± 0.10^{ef}	
	4	10.37 ± 0.47^{g}	
	5	10.37 ± 0.47^{g}	
	6	10.37 ± 0.47^{g}	
	7	10.37 ± 0.47^{g}	
C1 (100% of biopesticide)	2	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	3	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	4	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	5	0.67 ± 0.14^{ab}	93.47 ± 1.44^{ghi}
	6	0.75 ± 0.28^{ab}	92.74 ± 2.79^{ghi}
	7	0.75 ± 0.28^{ab}	92.74 ± 2.79^{ghi}
C2 (50% of biopesticide)	2	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	3	0.77 ± 0.32^{ab}	82.41 ± 6.49^{f}
	4	1.43 ± 1.14^{b}	86.87 ± 7.76^{fg}
	5	2.33 ± 1.98^{c}	83.09 ± 11.80^{f}
	6	2.80 ± 2.43^{c}	81.45 ± 13.53^{f}
	7	3.30 ± 2.89^{cd}	80.51 ± 14.54^{f}
C3 (25% of biopesticide)	2	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	3	1.03 ± 0.46^{ab}	43.21 ± 22.29^{d}
	4	2.62 ± 0.62^{c}	58.72 ± 6.74^{e}
	5	4.00 ± 1.01^{de}	41.57 ± 2.93^{d}
	6	4.65 ± 1.12^{ef}	30.24 ± 3.57^{c}
	7	5.60 ± 0.84^{f}	21.07 ± 3.90^{b}
D (3,33 g/L of Ridomil Gold Plus 66 WP)	2	0,00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	3	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	4	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	5	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	6	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
	7	0.00 ± 0.00^{ab}	100.00 ± 0.00^{hi}
F		27,93	27.40
p		< 0.001	< 0.001

F: value of the ANOVA test statistics ; *p*: probability value of the ANOVA test.

Concerning the inhibition rates of black pod symptoms, highly significant differences (F = 27.400 with $p < 0.001$) were also observed for the studied factors. The inhibition rate induced by the concentration C1 from to fifth day of the incubation was unlike to that induced by the reference chemical fungicide composed of 6% metalaxyl and 60% copper oxide. Pods treated with these concentrations did not show symptoms of black pod due to *P. palmivora*. On the other hand, with the concentration C2, necrotic lesions appeared on the pods only from the third day of incubation. The samples treated with C3 concentration showed necrotic lesions from the second day of incubation (Table 4). Statistical analysis revealed significant differences ($p < 0.05$) between inhibition rates induced by concentrations. Fig. 1 shows the size of necrotic lesions on artificial inoculated pods with explants taken from control culture medium and culture medium treated with Btk HD-1-based biopesticide.

Discussion

This study has highlighted the antifungal activity of Btk HD-1 on Phytophthora palmivora, causal agent of the cocoa black pod disease in Cote d'Ivoire. Btk HD-1-based biopesticide exerted an inhibitory effect on the growth and appearance of brown spots due to P. palmivora on pods. In fact, the incidence of the disease and the evolution of the necrotic lesions size on the treated pods with Btk HD-1 suspension were lower compare to the control. This inhibitory effect of Btk HD-1 on P. palmivora might be due to some active components synthesized by this bacterium (Zhou et al., 2008). Virulence factors include zwittermicin A and chitinase. The zwittermicin A, a linear aminopolyol antibiotic was very active against oomycetes (Zhou et al., 2008). As for chitinase, it had a proven fungistatic activity against several soil fungi (Arora et al., 2013), according to supprimer, study conducted by Arora *et al.* (2013).

In fact, the time for appearance of symptoms and the size of necrotic lesions on pods that have been inoculated with extracts taken from treated culture medium differs from that of witness pods. The bacterial biopesticide significantly reduces the infectivity of the parasite on cocoa tree fruits. The biological activity of Btk HD-1 induced the inhibition of zoospore germination or limited the biological activity of infectious particles of the parasite thereby reducing its pathogenicity on plant material.

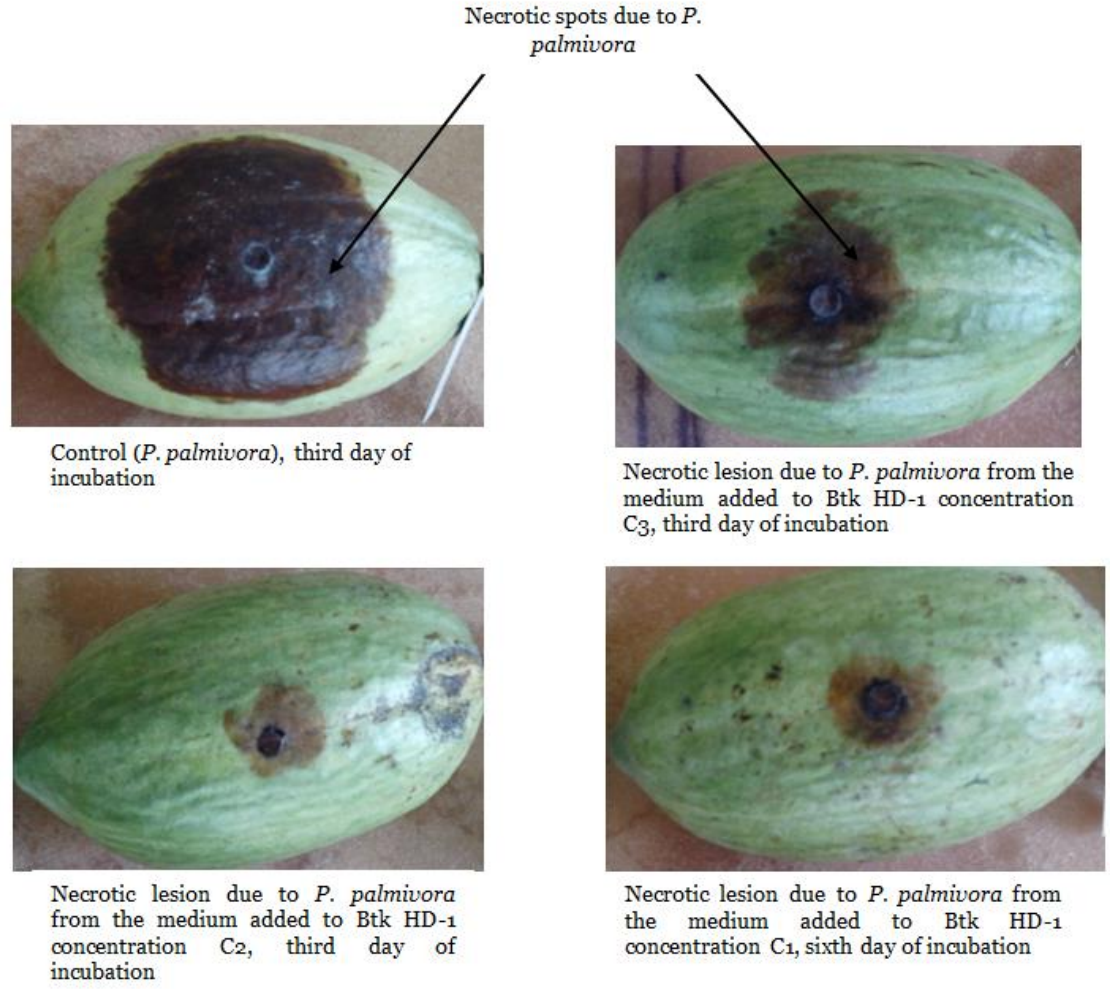

Fig. 1. Size of necrotic lesions on pods artificially inoculated with *P. palmivora* parasite taken from previously treated medium.

Furthermore, the treatment performed with Ridomil Gold Plus 66 WP stopped the appearance of black pod symptom on treated pods. The pathogenicity of the parasite was thus neutralized by the recommended dose (3.33g/L) of the reference chemical fungicide. A recent study conducted by Sutan *et al.* (2014) showed that Ridomil Gold Plus 42.5 WP might cause a decrease in the mitotic index and caused chromosomal aberrations in meristem cells of *Allium cepa* L. roots. Thus, it is known unequivocally that chemical fungicides constitute a threat to the sustainability of natural resources and a growing threat to the environment.

Their frequency of use in cocoa cultivation should be reduced in favor of other biological products such as microbial biopesticides. The inhibitory activity of the highest concentration in Btk HD-1-based biopesticide (C1) was identical to that of the reference fungicide on culture medium according to Gadji *et al.* (2015). However, a significant difference ($p < 0.05$) was observed with the reference chemical on plant material. Nevertheless, this biopesticide concentration could be used in an integrated strategy for cocoa black pod control.

Conclusion

This study highlighted the fungistatic activity of Btk HD-1-based biopesticide on the infectivity of *P. palmivora,* pathogen of cocoa black pod. The concentration C1 of the Btk HD-1-based biopesticide controls the symptoms of cocoa black pod the first day of incubation until the fifth day where it appeared 0.8 cm diameter necrotic lesion on the cocoa pods. Twenty-five percent (25%) of the pods treated with this concentration showed slight black pod less than 1,10 cm of diameter the seventh day of incubation.The effect of Btk HD-1-based biopesticide was less important for the pathogenicity of the fungus than that of the reference fungicide (Ridomil Gold Plus 66 WP). On-farm trials should be conducted to consolidate the results, for an efficient use of microbial biopesticide as one of the means for integrated control of cocoa black pod disease.

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Références

Arora N, Sachdev B, Gupta R, Vimala Y, Bhatnagar RK, 2013. Characterization of a Chitin-Binding Protein from *Bacillus thuringiensis* HD-1. PLoS ONE **8(6),** e66603. doi:10.1371/journal.pone.0066603.

Babacauh KD. 1980. Structure et dynamique des populations de *Phytophthora* sp parasite du cacaoyer (*Theobroma cacao* L.). Thèse d'Etat, Université de Paris-Sud., Paris, France. 153 p.

Bar-Hen A. 1998. Quelques méthodes statistiques pour l'analyse des dispositifs forestiers. Vol. 5, Série FORAFRI. 118 p.

Coulibaly K, Kébé BI, Koffi KN, Mpika J, Koné D. 2013. Caractérisation des isolats de *Phytophthora* spp du verger cacaoyers de Cote d'Ivoire. Journal of Applied Biosciences **70,** 5567-5579.

Duchet C, Franquet E, Lagadic L, Lagneau C. 2015. Effects of *Bacillus thuringiensis* israelensis and spinosad on adult emergence of the non-biting midges *Polypedilum nubifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae) in coastal wetlands. Ecotoxicology and Environmental Safety **115,** 272-278.

Fontem DA, Olanya OM, Tsopmbeng GR, Owona MAP. 2005. Pathogenicity and metalaxyl sensitivity of *Phytophthora infestans* isolates obtained from garden huckleberry, potato and tomato in Cameroon. Crop Protection **24,** 449-456.

Gadji AAG, Yapo OB, Abo K, Coulibaly K, Kébé BI, Gnépé JR, Tyagi RD, 2015. *In vitro* assessment of biopesticide *Bacillus thuringiensis* var. *kurstaki* HD-1 effectiveness on *phytophthora palmivora*, agent of cocoa black pod rot in Côte d'Ivoire. European Scientific Journal **11,** 276-292.

ICCO. 2009. ICCO (International Cocoa Organization) quarterly bulletin of cocoa statistics. Cocoa Year 2008/09, 27 May 2009, **35(2).**

Lagadic K, Roucaute M, Caquet T. 2014. *Bti* sprays do not adversely affect non-target aquatic invertebrates in French Atlantic coastal wetlands. Journal of Applied Ecology **51,** 102-113.

Mawussi G. 2008. Bilan environnemental de l'utilisation de pesticides organochlorés dans les cultures de coton, café et cacao au Togo et recherche d'alternatives par l'évaluation du pouvoir insecticide d'extraits de plantes locales contre le scolyte du café (*Hypothenemus hampei* Ferrari). Thèse de doctorat, Université de Toulouse, France 154 p.

Ndoumbe-Nkeng M, Cilas C, Nyemb E, Nyasse S, Bieysse D, Flori A, Sache I. 2004. Impact of removing diseased pods on cocoa black pod caused by *Phytophthora megakarya* and on cocoa production in Cameroon. Crop Protection **23,** 415-424.

Orisajo SB, Dongo LN, Agbeniyi SO, Adedeji AR, Fademi OA, Otuonye AH, Okeniyi MO, Adeniyi DO, Oduwaye OF Kolawole OO. 2011. Assessment of Ultimax Plus 72 W.P. for the Control of Black Pod Disease of Cocoa in Nigeria. Journal of Basic and Applied Scientific Research **1,** 880-884.

Ortiz-Garcia CF. 1996. Etude de la diversité génétique de populations de Phytophthora pathogènes du cacaoyer (Theobroma cacao L.) et du cocotier (Cocos nucefera L). Thèse de Doctorat, Université Paul Sabatier, France, 85 p.

Roh, Yul J, Choi YJ, Shun, Li M, Jin BR, Je YH. 2007. *Bacillus thuringiensis* as a Specific, Safe, and Effective Tool for Insect Pest Control. Journal of Microbiology and Biotechnology **17,** 547-559.

Shuman JL. 2001. Anthracnose Fruit Rot Resistance in Strawberry. PhD thesis, North Carolina State University, USA. 121 p.

Sunpapao A, Pornsuriya C. 2014. Effects of chitosan treatments on para rubber leaf fall disease caused by *Phytophthora palmivora* Butler - a laboratory study. Songklanakarin Journal Sciences and Technology **36,** 507-512.

Şuţan AN, Popescu A, Mihăescu C, Soare CL, Marinescu VM. 2014. Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi s. II a. Biologie Vegetală **60,** 5-12.

Zhou Y, Choi Y-L, Sun M, Yu Z, 2008. Novel roles of *Bacillus thuringiensis* to control plant diseases. Applied of Microbiology Biotechnology **80,** 563-572.