VOL. 7, NO. 12, DECEMBER 2012 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science
©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

www.arpnjournals.com

SENSITIVITY OF *Helicoverpa armigera* (HÜBNER) (LEPIDOPTERA: NOCTUIDEA) TO THE ENTOMOPATHOGENIC FUNGI, *Metarhizium anisopliae* AND *Beauveria bassiana* IN LABORATORY

O. K. Douro Kpindou[1], D. A. Djegui[1], I. A. Glitho[2] and M. Tamò[1]
[1]International Institute of Tropical Agriculture, Tri Postal, Cotonou, Benin
[2]Laboratoire d'Entomologie Appliquée, Faculté des Sciences, Université de Lomé, Lomé, Togo
E-Mail: d.kpindou@cgiar.org

ABSTRACT

The present work aims to assess the pathogenicity of the conidia of six isolates of *Metarhizium anisopliae* and two isolates of *Beauveria bassiana* on *Helicoverpa armigera* in order to select the most promising ones for developing a microbiological product to be used in cotton fields. The pathogenicity of six isolates of the entomopathogenic fungi, *M. anisopliae* (Met 28, Met 32, Met 92, Met 31, Met 34 and Met 341) and two isolates of *B. bassiana* (Bb11 and Bb 12) was evaluated in the laboratory by applying topically 1 µl of an oil-based formulation of conidia at a concentration of 10^8 conidia/ml on the third, fourth, fifth and the sixth instars of *H. armigera*. Taken separately, each isolate caused less mortality among the larvae of the sixth stage than in younger stages. Corrected mortality rates varied from 3.6 ± 1.8 % (Met 28, sixth instar) to 56.3 ± 0.8% (Met 31, third instar) for *M. anisopliae* and from 3.6 ± 1.8 % (Bb 12, sixth instar) to 34.4 ± 4.2% (Bb 11, third instar) for *B. bassiana*. Certain isolates of *Metarhizium* were infective to pupa. Host development stage at inoculation affected both survival times compared to control. The results of the presented study showed that the isolates of *Metarhizium* (Met 31) and *Beauveria* (Bb 11, known as Bba 5653), the last being tested successfully against *Plutella xylostella* (Lepidoptera: Noctuidae), are virulent isolates and can be promising isolates for the control of the cotton bollworm *H. armigera*.

Keywords: *Helicoverpa armigera*, microbial control, entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*, pathogenicity.

1. INTRODUCTION

Cotton is produced on more than 1.8 million hectares in West Africa by more than 2 million farmers (Alavo *et al*., 2011). Indeed, cotton is the main export crop and contributes to the socioeconomic development of Benin. It constituted the basis of agro-industry in Benin and represents about 60% of local industry. In 2009, it contributed in terms of value, for 13% to the Gross Domestic Product (GDP). Cotton has also contributed for 45% of the Internal Revenue (taxes and Treasury). It is the main source of currency in Benin despite its poor performance in recent years (MEF, 2010). From 2000 to 2005 the production increased from 376, 141 tons to 426, 251 tons, representing an increase of 13.3% (MEF, 2010); this increase of production also rhythm with the level of pesticides consumption. Thus, at the national level, the consumption of pesticides has increased from 1, 972, 764 litres in 1993 to 2, 314, 127 litres in 2000, representing an increase of 17.30% within eight years, then to 2, 453, 880 litres in 2010 representing an increase of 6.04% (MEF, 2010).

The use of synthetic pesticides to protect crops, leads to some unfortunate consequences such as environmental pollution, pest resistance and toxicity to other non-target organisms. The limited success of these control methods explains the need for developing alternatives that are more effective, healthy and respectful of the environment and human health and more economically profitable.

Such alternative methods include the use of natural enemies of insects such as bacteria, viruses and fungi (Quintela and McCoy, 1997; McCoy *et al*., 2000; Sabbour and Sahab, 2005). *Metarhizium anisopliae* (Metchinikoff) Sorokin (Hypocreales: Clavicipitaceae) was the first pathogen deliberately used to control insect pest by the Russian Metchnikoff (1880) (the father of microbial control) in the 1880s. *Beauveria bassiana* (Bals. - Criv.) Vuill. (Hypocreales: Ophiocordycipitaceae) microfungus is a pathogen of many insects. Pathogenicity has been demonstrated for the first time by Agostino Bassi de Lodi (1835), the precursor studies of infectious diseases, demonstrating for the first time a micro-organism could be responsible for infectious disease for animals. To date, approximately 750 fungi species are known to infect insects and mites (Ferron, 1978; Burges, 1981; Hall and Papierok, 1982; Cloutier, 1992; Nielson *et al*., 2007). Biopesticides based on *B. bassiana*, a wide range of entomopathogenic fungi, are marketed and used in pest management (Reddy *et al*., 2008). These entomopathogenic have great potential as biological control agents against insects and are an important component in integrated pest management. They are developed worldwide for the control of many pests of agricultural importance (Ferron, 1985; Thungrabeab and Tongma, 2007). It has emerged as one of the biological control agents of the most promising and extensive research of these pathogens that may allow the removal of a wide range of insect pests of economic importance (Coates *et al*., 2002; McGurire *et al*., 2005; Kaur and

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


Padmaja, 2008). Fortunately many moths are among the hosts of these fungi, including several other species of pests of agricultural crops of economic importance (Abdel-Razek *et al*., 2006, Lozano-Gutierrez and España-Luna, 2008).

In view of diversifying strategies to control *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), the present work aims at assessing control methods of *H. armigera* using biopesticides based on the entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, which are neither harmful to human being nor to animals and the environment (Tanada and Kaya, 1993), making them potential candidates for crop pest control. More specifically, this paper presents the results of the pathogenicity of the conidia of six isolates of *M. anisopliae* and two isolates of *B. bassiana* on *H. armigera* in order to select the most promising ones for developing a microbiological product to be used in cotton fields.

2. MATERIALS AND METHODS

2.1. Insect colonies

Helicoverpa armigera larvae were collected from tomato and cotton fields at different localities of the agro-ecological zones of northern Benin namely the far north, the cotton belt of the North and the food crop Zone of South Borgou (between N 8° 53'; E 2° 35' and N 11° 55'; E 3° 13'). They were maintained on an artificial diet composed of beans, agar, corn, brewer's yeast, ascorbic acid, sorbic acid, honey, formaldehyde and p-hydroxy benzoate Metyl (Teakle and Jensen, 1985) in the laboratory at IITA-Benin. The experimental conditions were kept at 70 ± 5% RH, 26 ± 2°C with a photoperiod of L: D 12: 12. Larvae of the third (L3; 7.4 ± 0.1 days), fourth (L4; 10.2 ± 0.2 days), fifth (L5; 13.6 ± 0.2 days) and sixth (L6; 17.0 ± 0.2 days) stages were used for the bioassays, because these are the stages which cause the greatest damage (Saour and Causse, 1996).

2.2. Fungal isolates

Height entomopathogenic fungi isolates, two of *B. bassiana* and six *of M. anisopliae*, obtained from the IITA-Benin collection unit were used in this study (Table-1).

Conidia of different isolates were produced in Petri dishes (Ø = 9 cm) containing Sabouraud Dextrose Agar (SDA) and incubated at 26 ± 2°C. After 10 days of incubation, conidia were harvested using peanut oil and filtered. To have a concentration needed, three plates of each isolate were used. Germination test was done by counting conidia on a sub-sample of 100, and viable conidia were calculated as followed:

$$\%Viable = [a/(a+b)]x100,$$

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

Before inoculating the larvae, the concentration of conidia to be used was calculated as follows:

$C' = \frac{CxV}{V \pm V'}$, where C' = concentration to be used; C = concentration obtained after harvesting; V = volume of the suspension recuperated after harvesting; V' = volume to be used.

Table-1. Different isolates of fungi used in different trials.

Fungal species	Register N°	Abbreviations	Host (Country of origin)	Author (Year of isolation)
M. anisopliae	28	Met 28	*Eldana saccharina*, (Benin)	IITA-Benin (1996)
M. anisopliae	32	Met 32	*Sesamia calamistis* (Benin)	IITA-Benin (1996)
M. anisopliae	92	Met 92	*Busseola fusca*, (Kenya)	ICIPE (1989)
B. bassiana	12	Bb 12	*Sesamia calamistis*, (Benin)	IITA-Benin (1997)
M. anisopliae	31	Met 31	*Sesamia calamistis*, (Benin)	IITA-Benin (1996)
M. anisopliae	34	Met 34	*Eldana saccharina*, (Benin)	IITA-Benin (1996)
M. anisopliae	341	Met 341	*Nezara viridula* (Bresil)	Cornell University (1996)
B. bassiana	11	Bb 11	*Sesamia calamistis*, (Benin)	IITA-Benin (1997)

2.3. Bioassays

Conidia from the eight isolates were formulated with peanut oil at a concentration of 10^8 conidia per ml.

Six hundred larvae of each stage (i.e., third,

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


fourth, fifth, sixth) of *H. armigera* were used for this bioassay. Different larval instars were tested separately.

Twenty larvae of *H. armigera* were used per repetition. The larvae were put individually in a 3.8 cm x 2.9 cm x 4.0 cm boxes with perforated cover for ventilation. The test consisted of eight isolates and one control, repeated each one three times in a completely random block. 1µl of the formulation was deposited (topical application) on the pronotum of the larva. The rates of germination of the isolates used varied from 91.1% to 99.2%, 24 hours after incubation Mean temperatures varied between 24.2 ± 2°C and 25.8 ± 1.5°C with a mean relative humidity between 80.40 ± 4.5% and 87.6 ± 9.0%. Cadavers were collected daily. They were put in Petri dishes (Ø = 9 cm) for 24 hours to dry out, and subsequently incubated in Petri dishes containing wet filter paper and the presence or absence of sporulation was noted. The number of pupa formed, died and sporulated and the emergence of the adults were also recorded.

2.4. Data analysis

Cumulative percentages of mortality data were corrected from the natural mortality using Abbott's formula (Abbott, 1925) and have been used for comparison between treatments.

The infection rates were calculated on the basis of the percentage of cadavers (nymphs or pupa) with sporulating fungus:

$$\%Infection = \frac{NCF}{NC} x100.$$

NCF stands for the number of cadavers (nymphs or pupa) with sporulating fungus and NC for the total number of cadavers (nymphs or pupa).

Percentages of emergence were calculated on the basis of the number of pupa formed:

$$\%Emergence = \frac{NAE}{NPF} x100.$$

NAE stands for the number of adults emerged and NPF for the total number of pupae formed. Median survival times (MST) were calculated using Kaplan Meier Survivorship analysis (SPSS, 1989-2003).

Differences in mortality and sporulation rates, mean survival time (MST), percentages of pupae formed, dead pupae, pupae with mycosis and adults emergence rates were subjected to analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS (SAS, 2002-2008). In case of significant F values, means were compared by using SNK (Student-Newman-Keuls) at the probability level of 5%. Percentages were arcsine transformed and the MSTs were log transformed, before being subjected to analysis of variance (ANOVA). The efficacy of the pathogen was compared using the final mortality (i.e., 21 days cumulative mortalities).

3. RESULTS

3.1. Susceptibility of larvae of *H. armigera* to pathogens

3.1.1. Mortality

Table-2 shows that entomopathogenic fungi used were pathogenic to all tested instars. Taken separately, each isolate caused less mortality among sixth instar larvae than in younger stages. Indeed, after inoculation of the larvae, the average corrected mortality varied from 3.6 ± 1.8% (Met 28, sixth stages) to 56.3 ± 0.8% (Met 31, third stage). Fungal isolates Met 28, Met 32 and Bb 12 caused the lowest mortality rate in all larval stages. The isolate Met 31 was by far the most virulent, causing significantly higher mortality than those caused by other isolates of each for all larval stages ($F_{7,14}$ = 16.90, P <0.0001; $F_{7,14}$ = 15.22; P <0.0001; $F_{7,14}$= 8.72; P = 0.0003; $F_{7,14}$ = 11.13; P <0.0001, for third, fourth, fifth and sixth, respectively). Isolates Met 34, Met 341 and Bb 11 caused globally similar mortality (Table-2).

An analysis of variance performed to compare the mortality rates caused by the isolates used, showed a significant decrease in mortality rates with increasing host age ($F_{21, 64}$ = 26.99, P <0, 0001) (Table-3). For all fungal isolates combined, the corrected rates of mortality ranged from 8.4 ± 1.9% (sixth stage) to 26.2 ± 3.7% (third stage). The interaction pathogens-larval also revealed that mortality rates caused by each of fungal isolates depends on the larval stage ($F_{21, 64}$ = 1.82, P = 0.0356).

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


Table-2. Corrected average mortality rate of larvae L3, L4, L5 and L6 of *H. armigera*, 21 days after application of different isolates of entomopathogenic fungi used.

Treatments	Larval stages			
	L3 (n = 20 ; r = 3)	**L4** (n = 20 ; r = 3)	**L5** (n = 20 ; r = 3)	**L6** (n = 20 ; r = 3)
Met 28	7, 3 ± 1, 9 c	5, 5 ± 0, 1 d	3, 6 ± 1, 8 b	3, 6 ± 1, 8 b
Met 32	7, 3 ±1 , 9 c	5, 5 ± 0, 1 d	5, 5 ± 0, 1 b	5, 4 ± 3, 0 b
Met 92	30, 7 ± 5, 9 b	14, 4 ± 3, 3 cd	9, 1 ± 1, 8 b	5, 5 ± 3, 2 b
Met 31	56, 3 ± 0, 8 a	45, 3 ± 4, 0 a	30, 7 ± 5, 9 a	23, 5 ± 4, 4 a
Met 34	30, 6 ± 8, 4 b	25, 1 ± 8, 5 bc	16, 1 ± 7, 9 b	9, 1 ± 1, 8 b
Met 341	36, 2 ± 7, 4 b	30, 7 ± 7, 5 b	18, 0 ± 4, 4 b	9, 1 ± 1, 8 b
Bb 11	34, 4 ± 4, 2 b	28, 9 ± 4, 3 b	9, 0 ± 3, 4 b	7, 2 ± 1, 7 b
Bb 12	8, 9 ± 6, 3 c	7, 2 ± 3, 6 d	5, 4 ± 3, 0 b	3, 6 ± 1, 8 b
Control	-	-	-	-
$F_{7,14}$ *P*	16, 90 < 0, 0001	15, 22 < 0, 0001	8, 72 0, 0003	11, 13 <0, 0001

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%)

3.1.2. Sporulation

Dead insects, following the application of Bb 11, Met 34 and Met 31 have better sporulated. These average rates of sporulation significantly decreased with the age of the treated larval stages ($F_{3, 72} = 3.14$, P = 0.0305) (Table-4). In contrast to mortality, the interaction, larval age-isolates, was not significant for all isolates ($F_{24, 72} = 0.38$, P = 0.9951). All fungal isolates combined, these sporulation rates varied from 14.0 ± 3.0% (sixth stage) to 27.5 ± 4.8% (third stage) (Table-3). No sporulation was observed on dead insects in the controls.

3.1.3. Larval mean survival time

Compared to all other isolates used, the survival of the third larval stage was significantly affected by Met 31 and Bb 11 ($F_{8, 16} = 16.79$, P = <0.0001). Larvae treated with isolates Met 28, Met 32, Met 92 and Bb 12 gave similar survival times. Also, from the fourth larval stage, no significant difference was observed between the isolates Met 31, Met 34, Met 341 and Bb 11. The values of mean survival times (MST) are summarized in Table-5. Irrespectively to the larval stage, MST decreased from control to the treated; the lowest MST being recorded within nymphs treated with isolates Met 31 and Bb11. The average of MST calculated for all stages combined treated with these two isolates, was about 15.9 ± 0.7 and 16.6 ± 0.9 days, respectively and this was 6 days and 5 days less compared to control respectively. The MST increased from the third to sixth instar, varying from 17.2 ± 0.6 days (third stage) to 19.7 ± 0.2 days (sixth stage) (Table-6).

Table-3. Effects of pathogens used (all species and isolates combined) on different larval stages of *H. armigera*, 21 days after application.

Larval stages	**Mortality** (%)	**Sporulation** (%)	**Pupae formed** (%)	**Dead pupae** (%)	**Adults emerged** (%)
Control (n=20; r=24)	-	0, 0 ± 0, 0 a	91, 2 ± 0, 9 a	14, 0 ± 0, 8 a	78, 3 ± 1, 2 a
L3 (n=20; r=24)	26,2 ± 3,7 a	27,5 ± 4,8 b	69,1 ± 3,3 b	39, 4 ± 3, 4 b	42, 9 ± 3, 3 b
L4 (n=20; r=24)	20, 3 ± 3, 2 b	20, 7 ± 3, 6 bc	72, 9 ± 2, 8 c	36, 2 ± 2, 8 b	46, 9 ± 2, 9 b
L5 (n=20; r=24)	12, 1 ± 2, 2 c	22, 5 ± 3, 3 bc	80, 4 ± 1, 9 d	31, 1 ± 2, 3 c	55, 6 ± 2, 3 c
L6 (n=20; r=24)	8, 4 ± 1, 9 c	14, 0 ± 3, 0 c	84, 0 ± 1, 3 e	20, 1 ± 2, 0 d	67, 1 ± 1, 9 d
F *P*	26,99 < 0, 0001	15,22 < 0, 0001	32,74 < 0, 0001	21,09 <0, 0001	47,48 < 0, 0001

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%)

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


Table-4. Average rates of sporulated dead insects in different batches of larval stages L3, L4, L5 and L6 of *H. armigera* after incubation.

Treatments	Larval stages			
	L3 (n = 20 ; r = 3)	**L4** (n = 20 ; r = 3)	**L5** (n = 20 ; r = 3)	**L6** (n = 20 ; r = 3)
Control	0, 0 ± 0, 0 b	0, 0 ± 0, 0 b	0, 0 ± 0, 0 a	0, 0 ± 0, 0 a
Met 28	25, 0±14, 4 ab	27, 8 ± 14, 7 ab	27, 8 ± 14, 7 a	16, 7 ± 16, 7 a
Met 32	11, 1 ± 11, 1 ab	11, 1 ± 11, 1 ab	11, 1 ± 11, 1 a	11, 1 ± 11, 1 a
Met 92	13, 9 ± 7, 3 ab	15, 0 ± 7, 6 ab	11, 1 ± 11, 1 a	11, 1 ± 11, 1 a
Met 31	42, 6 ± 10, 3 a	41, 8 ± 2, 7 a	35, 0 ± 5, 0 a	24, 6 ± 4, 5 a
Met 34	45, 8 ± 19, 7 a	32, 5 ± 6, 3 ab	24, 2 ± 8, 2 a	17, 5 ± 2, 5 a
Met 341	34, 7 ± 9, 7 ab	22, 4 ± 1, 4 ab	14, 5 ± 2, 8 a	10, 8 ± 5, 8 a
Bb 11	48, 6 ± 8, 4 a	37, 9 ± 2, 8 ab	33, 7 ± 5, 2 a	20, 0 ± 6, 7 a
Bb 12	0, 0 ± 0, 0 b	8, 3 ± 8, 3 ab	11, 1 ± 11, 1 a	0, 0 ± 0, 0 a
$F_{8,16}$ P	4, 68 0, 0042	3, 14 0, 0244	1, 65 0, 1863	1, 26 0, 3280

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%)

Table-5. Mean survival time (MST) (days ± SE) and 95% CI of larvae L3, L4, L5 and L6 of *H. armigera* inoculated with fungal isolates.

Fungi isolates	**Mean Survival Times** (Days ± SE) (95%CI)			
	L3 (n = 20 ; r = 3)	**L4** (n = 20 ; r = 3)	**L5** (n = 20 ; r = 3)	**L6** (n = 20 ; r = 3)
Control	21, 5 ±0, 3 a (20, 9 -22, 2)	21, 4 ±0, 4 a (20, 6 -22, 1)	21, 3 ±0, 4 a (20, 3 -22, 0)	21, 4 ±0, 2 a (21, 0 -21, 9)
Met 28	19, 9 ± 0, 7 a (18, 5 - 21, 2)	20, 0 ± 0, 7 a (18, 8 - 21, 3)	20, 3 ± 0, 6 a (19, 1 - 21, 5)	20, 3 ± 0, 6 ab (19, 1 - 21, 5)
Met 32	19, 9 ± 0, 7 a (18, 6 - 21, 3)	20, 1 ± 0, 7 a (18, 8 - 21, 4)	20, 2 ± 0, 6 a (18, 9 - 21, 4)	20, 2 ± 0, 6 ab (18, 9 - 21, 4)
Met 92	18, 5 ± 0, 9 ab (16, 7 - 20, 3)	19, 1 ± 0, 8 a (17, 6 - 20, 6)	19, 8 ± 0, 7 a (18, 5 - 21, 1)	20, 0 ± 0, 6 ab (18, 7 - 21, 4)
Met 31	12, 8 ± 1, 0 d (10, 8 - 14, 8)	15, 6 ± 1, 0 b (13, 7 - 17, 5)	17, 0 ± 0, 9 c (15, 1 - 18, 8)	18, 6 ± 0, 8 b (17, 1 - 20, 1)
Met 34	16, 6 ± 1, 0 bc (14, 7 - 18, 5)	17, 9 ± 0, 8 ab (16, 2 - 19, 5)	18, 6 ± 0, 8 ab (17, 0 - 20, 2)	19, 7 ± 0, 7 abc (18, 4 - 21, 1)
Met 341	15, 8 ± 1, 0 c (13, 9 - 17, 8)	17, 5 ± 0, 9 ab (15, 7 - 19, 2)	19, 0 ± 0, 7 ab (17, 6 - 20, 5)	19, 7 ± 0, 7 ab (18, 4 - 21, 0)
Bb 11	14, 7 ± 1, 1 cd (12, 4 - 16, 9)	15, 0 ± 1, 1 b (13, 5 - 17, 8)	18, 6 ± 0, 9 ab (17, 3 - 20, 7)	18, 9 ± 0, 8 ab (17, 6 - 20, 9)
Bb 12	19, 8 ± 0, 7 a (18, 5 - 21, 1)	19, 6 ± 0, 8 a (18, 2 - 21, 1)	19, 9 ± 0, 7 a (18, 7 - 21, 3)	20, 2 ± 0, 6 ab (18, 9 - 21, 5)
$F_{8,16}$ P	16, 79 <0, 0001	6, 37 0, 0009	4, 46 0, 0053	2, 38 0, 0493

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%)

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


Table-6. Mean survival time (MST) (days ± SE) and 95% CI by larval stage of *H. armigera* (all fungal isolates combined).

Fungi isolates	**MST** (Days ± SE) (95 % CI)
Control	21, 4 ±0, 0 a (20, 8 - 22, 0)
L3	17, 2 ± 0, 6 c (15, 5 - 19, 0)
L4	18, 1 ± 0, 5 bc (16, 6 - 19, 8)
L5	19, 2 ± 0, 3 b (17, 8 - 20, 7)
L6	19, 7 ± 0, 2 b (18, 4 - 21, 1)
$F_{4,103}$ *P*	11, 97 <0, 0001

In the same column means followed by the same letter
are not significantly different (ANOVA followed by SNK test at 5%)

3.2. Effect of entomopathogenic fungi on host development

3.2.1. Pupae formed

The low average rate of pupae formed was recorded for the third larval stage treated with Met 31 (40.7 ± 0.7%) and the highest with the sixth stage in control (95.0 ± 1, 4%) (Table-7). For the third larval stage, the difference between the average rates of pupae formed was significant between the isolate Met 31 and the others ($F_{8, 16}$ = 18.89, P <0.0001). Contrary to mortality rates, the rate of pupae formed increased with increasing host age, regardless of isolates used ($F_{3, 74}$ = 32.74, P <0.0001) (Table-3).

Table-7. Average rates of pupae formed for each of the larval stages of *H. armigera*, 21 days after application of different entomopathogenic fungi isolates.

Treatments	**Larval stages**			
	L3 (n = 20 ; r = 3)	**L4** (n = 20 ; r = 3)	**L5** (n = 20 ; r = 3)	**L6** (n = 20 ; r = 3)
Control	91, 5 ±0, 8 a	89, 2 ± 0, 8 a	89, 2 ± 1, 7 a	95, 0 ± 1, 4 a
Met 28	85, 0 ± 2, 9 ab	86, 7 ± 1, 7 a	88, 3 ± 1, 7 a	88, 3 ± 1, 7 ab
Met 32	85, 0±2, 9 ab	86, 7 ± 1, 7 a	86, 7 ± 1, 7 ab	86, 7± 1, 7 b
Met 92	74, 5 ± 5, 2 bc	78, 3± 1, 7 ab	83, 3 ± 1, 7 ab	86, 7± 3, 3 b
Met 31	40, 7 ± 0, 7 e	50, 0 ± 2, 9 d	63, 3 ± 4, 4 c	70, 0 ± 2, 9 c
Met 34	64, 2 ± 5, 8 dc	68, 3 ± 6, 7 bc	76, 7 ± 6, 0 ab	83, 3 ± 1, 7 b
Met 341	58, 3 ± 6, 0 d	63, 3 ± 6, 0 c	75, 0 ± 2, 9 b	83, 3 ± 1, 7 b
Bb 11	60, 0 ± 2, 9 d	65, 0± 2, 9 c	83, 3 ± 1, 7 ab	85, 0± 0, 0 b
Bb 12	83, 3 ± 4, 4 ab	85, 0 ± 2, 9 a	86, 7 ± 1, 7 ab	88, 3± 1, 7 ab
$F_{8,16}$ *P*	18, 89 < 0, 0001	16, 00 < 0, 0001	8, 91 0, 0001	12, 61 0, 0001

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%)

3.2.2. Mortality and sporulation of pupae formed

Overall, pupal mortality rates varied from 20.1 ± 2.0% (sixth stage) to 39.4 ± 3.4% (third stage) (Table-3). The highest pupal mortality was caused by Met 31 with 70.8 ± 4.2% (third stage) (Table-8). Some dead pupae resulting from larvae inoculated with Met 28, Met 32 and Met 92 had sporulated with 5.6 ± 5.6%, 9.5 ± 9.5% and 13.1 ± 7.2%, respectively.

3.2.3. Emergence of adults

As is the formation of pupae, emergence of the adults was low in the batches of pupae resulting from inoculation of larvae with isolates Met31 and Bb11. These

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


percentages of adults emerged varied from 13.7 ± 3.7% (third stage) to 53.3 ± 4.4% (sixth stage) for Met 31. For isolate Bb 11, the emergence rates were higher than for Met 31 and varied from 30.0 ± 2.9% (third stage) to 75.0 ± 0.0% (sixth stage). For all treated larvae, control had the highest emergence rate, but the rate of emergence in the controls were only significantly different from those in the treated for the younger stages (third and fourth) ($F_{8, 16}$ = 15.31, P <0.001, $F_{8, 16}$ = 14.39, P <0.001, respectively) (Table-8). The adult emerged rate for the third larval stage treated with Met 28, Met 32, Met 92, Met 34, Met 341 and Bb 12 were generally similar (Table-8).

Table-8. Average rates of dead pupae for each larval stages of *H. armigera*, 21 days after application of different isolates.

Treatments	Larval Stages			
	L3 (n = 20 ; r = 3)	**L4** (n = 20 ; r = 3)	**L5** (n = 20 ; r = 3)	**L6** (n = 20 ; r = 3)
Control	15, 3 ± 0, 9 c	15, 6 ± 1, 9 c	12, 8 ± 0, 7 b	12, 3 ± 2, 0 cd
Met 28	40, 9 ± 4, 6 bc	40, 2 ± 4, 9 abc	35, 7 ± 4, 5 ab	33, 9 ± 2, 7 a
Met 32	37, 1±3, 0 bc	34, 6 ± 3, 5 bc	28, 9 ± 0, 5 ab	28, 9 ± 0, 5 ab
Met 92	33, 0±9, 3 bc	31, 7 ± 6, 9 bc	30, 0 ± 6, 8 ab	21, 1 ± 3, 4 bcd
Met 31	70, 8 ± 4, 2 a	57, 1 ± 5, 0 a	45, 5 ± 5, 4 a	24, 1 ± 3, 4 abc
Met 34	20, 0 ± 8, 0 bc	18, 6 ± 7, 5 c	16, 9 ± 7, 3 b	8, 0 ± 5, 2 d
Met 341	35, 0 ± 3, 4 bc	32, 1 ± 2, 9 bc	26, 7 ± 1, 0 ab	14, 0 ± 1, 9 cd
Bb 11	50, 2 ± 2, 4 ab	46, 3 ± 2, 1 ab	36, 0 ± 0, 7 ab	11, 8 ± 0, 0 cd
Bb 12	30, 6 ± 10, 2 bc	29, 0 ± 8, 6 bc	28, 8 ± 8, 8 ab	18, 8 ± 4, 8 bcd
$F_{8,16}$ P	6, 03 0, 0012	5, 59 0, 0017	3, 45 0, 0168	7, 59 0, 0003

In the same column means followed by the same letter are not significantly different (ANOVA followed by SNK test at 5%)

4. DISCUSSIONS

In general, the results of various experiments in the laboratory showed that all fungal isolates used are pathogenic to *H. armigera* larvae. These results are similar to that of other authors who reported sensitiveness of *H. armigera* larvae to *Metarhizium* and *Beauveria* under laboratory conditions (Gundannavar *et al*., 2007; Lozano-Luna-España and Gutierrez, 2008; Kumar and Chowdhry, 2004). Mortality rates of larvae and pupae, pupal development and adult emergence were affected by the isolates used and the host age. Younger larvae were almost more sensitive compare to the olders. The two genus of fungi used were infectious for all stages of *H. armigera* in adequacy with the results obtained by Kulkarni *et al*., (2008).

Dead pupae resulting from larvae treated with certain isolates of *Metarhizium* have sporulated, indicating that conidia can remain in a latent state in the insect and become virulent when the latter does not feed more (pupa). Indeed, in Australia, Wilson (1983) noted 15 to 20% mortality of pupae due to *B. bassiana*.

Among the *Metarhizium* isolates used, Met 31 is the one that caused the highest mortality to *H. armigera* larvae and pupae. The sporulation rate was the highest on cadavers resulting from the application of Met 31; this rate is among the best rate of sporulation recorded for these experiments. Indeed, sporulation of insects is one of the factors determining the choice of an isolate as a biopesticide in the field. It allows the increase of the inoculum, thus promoting the transfer of conidia to insect unaffected during field applications (Langewald *et al*., 1997; Adu-Mensah, 2002; Douro Kpindou *et al*., 2005). The combination of these parameters (higher mortality rates of larvae and pupae, the relatively high rate of sporulation, the lowest rate of pupae formed and adult emerged) makes Met 31 a good candidate among isolates of *Metarhizium* tested for further study with a view to its use as a biopesticide.

The highest rates of sporulation were observed with Bb 11. Of the two isolates of *Beauveria* used, Bb 11 is the one that caused the highest mortality of larvae and pupae and consequently a low rate of formation of pupae and adult emergence. Dead insects from the inoculation of larvae by Bb 12 showed no sporulation for the third and sixth instars. For other stages treated, this rate is very low, making this isolate an inappropriate candidate for its use as a biopesticide.

Larval development as well as MST was affected by the pathogens compared to control. Confidence intervals (CI) of MST of Met 31 and Bb 11 showed the possibility to kill caterpillars in 11 days or 13 days respectively compared to the lowest level of TMS recorded in control (20 days).

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.


From the results obtained it appears that all isolates of entomopathogenic fungi used are pathogenic to *H. armigera* larvae. The isolates Met 31 and Bb 11 were the most virulent and have the potential to be used as biopesticides. Investigations should continue for obtaining effective and efficient doses to be used in the field. In addition, other studies such as fungal conidia production, growth of hyphae in artificial media, and the effects of various environmental conditions that influence persistence must also be evaluated to fully assess the potentiality of these isolates as biological control agents against the pest, *H. armigera*.

ACKNOWLEDGEMENTS

We thank the Dutch Embassy in Benin for their financial support. We also thank the other members of the project "Méthodes Alternatives de Lutte contre les Ravageurs du Cotonnier au Bénin" and the partners CRA-CF, OBEPAB, UAC and UL.

REFERENCES

Abbott W. S. 1925. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology. 18: 265-267.

Abdel-Razek A.S., M.H. Abbas, M. El-Khouly and A. Abdel-Rahman. 2006. Potential of microbial control of diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) on two cabbage cultivars under different fertilization treatments. Journal of Applied Science Research. 2: 942-948.

Adu-Mensah J. 2002. Primary and secondary uptake of conidia of entomopathogenic fungi and the effect of oxybenzone as a chemical sunlight protector. Ghana Journal Science. 42: 3-10.

Alavo T. B. C., A. Z. Abagli, K. J.C. Tégbéssou and G.B. Dunphy. 2011. Kaolin potential for the integrated management of Aphis gossypii Glov. (Homoptera: Aphididae) on cotton. Archives of Phytopathology and Plant Protection. 44: 764-770.

Burges D. H. 1981. Microbial control of pests and diseases 1970-1980. Academic Press. New York. p. 949.

Cloutier C. 1992. La solution biologique de lutte pour la répression des insectes et acariens ravageurs des cultures: in Vincent, C. et Coderre D, (eds), Gaétan Morin, Boucheville, Québec, canada. pp. 19-88.

Coates BS, Hellmich RL and Lewis LC. 2002. Allelic variation of a *Beauveria bassiana* (Ascomycotina: Hyphpocreales) minisatellite is independent of host range and geographic origin. Genome. 45: 125-132.

Douro Kpindou O. K., C. Gbongboui, R. Badou, E. Paa-Kwessi, J. B. Ackonor and J. Langewald. 2005. Optimisation de l'application du *Metarhizium anisopliae* var. *acridum* sur le criquet puant, *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). International Journal of Tropical Insect Science. 25: 251-258.

Ferron P. 1978. Biological control of insect pests by entomopathogenous fungi. Annual Review of Entomology. 23: 409-442.

Ferron P. 1985. Fungal control. In: Kerkut, G.A. and Gilbert, L.I. (Eds.). Comparative insect physiology, biochemistry and pharmacology. Oxford, Pergamon press. pp. 313-346.

Gundannavar K. P., S. Lingappa and R. S. Giraddi. 2007. Study of dose mortality response between instars of *Helicoverpa armigera* (Hübner) and *Metarhizium anisopliae* (Metschinkoff) Sorokin. Karnataka Journal of Agricultural Sciences. 20: 140-141.

Hall R. A. and B. Papierok. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology. 84: 205-240.

Kaur G. and V. Padmaja. 2008. Evaluation of *Beauveria bassiana* isolates for virulence against Spodoptera litura (Fab.) (Lepidoptera; Noctuidae) and their characterization by RAPD-PCR. African journal of Microbiology Research. 2: 299-307.

Kulkarni S. A., V. Ghormade, G. Kulkarni, M. Kapoor, S. B. Chavan, A. Rajendran, S. K. Patil, Y. Shouche and M. V. Deshpande. 2008. Comparison of *Metarhizium* isolates for biocontrol of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea. Biocontrol Science and Technology. 18: 809-828.

Kumar V. and P.N. Chowdhry. 2004. Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against tomato fruit borer, *Helicoverpa armigera*. Indian Phytopathological Society. 54: 208-212.

Langewald J., M. B. Thomas, O-K. Douro-Kpindou and C. J. Lomer. 1997. Use of *Metarhizium flavoviride* for control of *Zonocerus variegatus*: A model, linking dispersal and secondary infection from the spray residue with mortality in caged field samples. Entomologia Experimentalis et Applicata. 82: 1-8.

Lozano-Gutiérre J. and M. P. Espaňa-Luna. 2008. Pathogenicity of *Beauveria bassiana* (Deuteromytcotina: Hyphomycetes) against the White Grub *Lanlifera cyclades* (Lepidoptera: Pylaridae) under field an greenhouse conditions. Florida Entomologis. 91: 664-668.

McCoy C. W., W. D. I. Shapiro and L. W. Ducan. 2000. Application and Evaluation of Entomopathogens. Application and Evaluation of Entomopathogens for

©2006-2012 Asian Research Publishing Network (ARPN). All rights reserved.

Citrus Pest Control. In: Field Manual of Techniques in Invertebrate Pathology; application and Evaluation of Pathogens for insects and other Invertebrate Pests. Lacy, L.A. and H.K. Kaya (Eds.). Kluwer Academic publishers, Dordrecht, (In Press). p. 33.

McGurire R. M., M. Ulloa, P. Young-Hoon and N. Hudson. 2005. Biological and molecular characteristic of *Beauveria bassiana* isolates from California Lygus Hesperus (Hemiptera: Miridae) populations. Biological Control. 33: 307-314.

MEF (Minstère de l'Economie et des Finances). 2010. Evaluation ex-ante de la mise en œuvre des stratégies de relance du pole coton-textile au Bénin. Rapport Final. p. 86.

Nielsen C., A. B. Jensen and J. Eilenberg. 2007. Survival of entomophthoralean fungi infecting aphids and higher flies during unfavorable conditions and implications for conservation biological control. In: Use of entomopathogenic fungi in biological pest management. Ekesi, S. and Maniania, N. K. (Eds.). CABI International, Wallingford. pp. 13-38.

Quintela E. D. and C. W. McCoy. 1997. Effect of imidacloprid on development locomotory response and survival of first instars of *Diaprepes abbreniatus*. Journal of Economical Entomology. 90: 988-995.

Reddy N. P., A. P. A. Khan, K. Uma Devi, S. J. Victor and H. C. Sharma. 2008. Assessment of the suitability of Tinopal as an enhancing adjuvant in formulations of the insect pathogenic fungus *Behauveria bassiana* (Bals.) Vuillemin. Journal of Pest Management Science. 3: 15-19.

Sabbour M. M. and A. F. Sahab. 2005. Efficacy of some microbial control agents against cabbage pest in Egypt. Pakistan Journal of Biological Sciences. 8: 1351-1356.

Saour G. and R. Causse. 1996. Comportement alimentaire des chenilles d'Helicoverpa armigera Hbn. (Lep, Noctuidae) sur tomate cultivée sous serre. Journal of Applied Entomology. 120, 1-5: 87-92.

SAS. 2002-2008. SAS® 9.2, Copyright (c) 2002-2008 by SAS Institute Inc., Cary, NC, USA.

SPSS 16.0. 2007. Copyright (c) SPSS Inc., 1989-2007. All right reserved. This product uses WinWrap Basic, copyright 19932007, Polar engineering and consulting, http://www.winwrap.com. Licensed to Team EQX, 6th Birth days, 1337.

Tanada Y. and H. K. Kaya. 1993. Insect pathology. Academic Press, London. p. 666.

Teakle RE and Jensen JM. 1985. *Heliothis punctigera*. In: Handbook of Insect Rearing. P Singh and RF Moore (Eds.). Elsevier, Amsterdam, Netherlands. pp. 313-322.

Thungrabeab M. and S. Tongma. 2007. Effect of entomopathogenic fungi, *Beauveria bassiana* (Balsam) and *Metarhizuim anisopliae* (Metsch). KMITL Science technology. 7(S1).

Wilson A. G. L. 1983. Abundance and mortality of overwintering and spring emergence of Heliothis spp. J. Australian Entomol Society. 22: 191-199. www.oecd.org/dataoecd/19/10/34352710.pdf.