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PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI *Metarhizium anisopliae* AND *Beauveria bassiana* ON LARVAE OF THE LEGUME POD BORER *Maruca vitrata* (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Experiments were performed to evaluate the pathogenicity of conidia from seven isolates of *Beauveria bassiana* (Bb6, Bb115, Bb116, Bb17, Bb42, Bb362, Bb24) and four isolates of *Metarhizium anisopliae* (Ma361, Ma29, Ma356, Ma 359) on larvae of the legume pod borer *Maruca vitrata* in order to select the most promising isolates to be used in cowpea fields. Pathogenicity was evaluated under laboratory conditions by applying 2 µl of conidia suspension, formulated in peanut oil, on different larval stages (first, second, third, fourth and the fifth instars) of *M. vitrata*. The concentrations of 10^7, 10^8, 10^9 and 10^{10} conidia/ml were used and mortality and sporulation of dead larvae were recorded. Taken separately, each isolate caused less mortality among fifth instar larvae than in younger instars. Mortality rates and high sporulation were recorded for Bb 115 and Ma29. Mortality rates ranged from 65.8 ± 3.5% (fifth instar) to 79.0 ± 3.0% (first instar) for *B. bassiana* isolate Bb 115. The *M.* anisopliae isolate Ma 29 showed significantly higher mortality rates ranging from 31.7 ± 3.3% (fifth instar) to 50.4 ± 1.5% (second instar). Host development stage at inoculation affected both survival times compared to controls (control and absolute control with oil). The results of the presented study showed that the isolates of *Beauveria* (Bb 115) can be promising isolates for the control of the legume pod borer, *M vitrata*.

Keywords: *Maruca vitrata*, microbial control, entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana,* pathogenicity.

1. INTRODUCTION

The legume flower and pod borer *Maruca vitrata* Fabricius, (Lepidoptera, Crambidae) is reported to cause serious damage on cowpea in tropical and subtropical regions of Asia, Latin America and Africa (Liao and Lin., 2000). The caterpillars attack flowers and pods of cowpea causing yield losses up to 80% (Singh *et al.,* 1991). So far, chemical control has shown to be most efficient option to protect cowpea crops against this pest (Rao *et al*., 1992). Unfortunately, besides their high costs, the overreliance on synthetic pesticides and their inappropriate use in cowpea has led to considerable health problems in human and animals, the development of pest resistance to chemical pesticides, the destruction of natural enemies and the resurgence of other pests (Tamò *et al*., 2003). The limited applicability of chemical control in West African subsistence agriculture explains the need for developing alternatives that are effective, but more sustainable and 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; Sabour and Saab, 2005). 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). These entomopathogens have great potential as biological control agents against insects and are an important component for integrated pest management. Various bio-pesticides based on entomopathogenic fungi, *Metarhizium anisopliae* (Metchinikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Bals. Criv.) Vuill. (Hypocreales: Ophiocordycipitaceae) are available for the management of economically important insect pests such as Diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) (Deshpande *et al*., 2001; Abdel-Razek *et al*., 2006; James *et al*., 2009; Godonou *et al*., 2009) and locusts and grasshoppers (Johson *et al*., 1993; Bateman, 1997; Lomer *et al*., 1999; 2001), Several isolates of these fungi were tested on pod sucking bugs of cowpea *Clavigralla tomentosicollis* (Stål) (Heteroptera: Coreidae), inducing mortalities ranging from 52% to 94% (Toffa, 2004). Similarly, Douro Kpindou *et al*. (2012) performed lab tests with isolates of *M. anisopliae* and *B. bassiana* on larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and reported mortality rates ranging from 58% to 74%. The same study indicated that, for all isolates tested and irrespective of the larval stage of *H. armigera*, the dose/effect responses were always significant.

Although Soundararajan and Chitra (2011) showed that *B. bassiana* significantly reduced the damage caused by *M. vitrata* in the field in India, very little work has been carried out so far on the use of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* in controlling the larval populations of this pod borer on cowpea in Africa.

Therefore, the aim of the present study is to evaluate the pathogenicity of various isolates of *M. anisopliae* and *B. bassiana* against larval stages of *M. vitrata,* in order to select the most promising ones and to develop a bio-pesticide formulation for use in cowpea fields.

2. MATERIALS AND METHODS

2.1. Study site

The experiments were conducted at the laboratory of insect pathology of the International Institute of Tropical Agriculture (IITA), Benin Station, near Cotonou, Benin, in controlled climate cabinets at the temperature of 26 ± 0.50ºC and a relative humidity of 65.5 ± 5%.

2.2. Mass rearing of *M. vitrata* larvae

Larvae of *M. vitrata* were supplied from stock cultures at IITA-Benin. Mated female moths were transferred in groups of 4 or 5 individuals in transparent small plastic cups (3 cm diameter x 3.5 cm height) and kept for oviposition, which took place on the inner walls of the cup. Upon emergence of neonate larvae, they were transferred to meridic rearing diet prepared according to Jackai and Raulston (1988) for rearing until obtainment of the desired larval stages to be used for the experiments.

2.3. Origin of the entomopathogenic fungi

The isolates of *M. anisopliae* and *B. bassiana* used in the current study were obtained from the fungal collection of IITA-Benin. Preliminary screening carried out on 106 isolates (64 of *M. anisopliae* and 42 of *B. bassiana*) resulted in a first selection of seven isolates of *B. Bassiana* and four isolates of *M. anisopliae*. This preliminary selection was based on the observed mortality and cadaver sporulation rates. The selected isolates used in this study (Table-1) were subsequently produced in Petri dishes (Ø = 9 cm) containing standard Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) for *B. bassiana* and *M. anisopliae*, respectively and incubated at 26 ± 2°C. After 10 days of incubation, conidia were harvested using peanut oil and filtered. To obtain the required concentration, three plates of each isolate were used. Germination test was performed 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:

$$C' = (C\ x\ V) / V \pm V'$$

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 for the pathogenicity experiments.

Fungal species	Register N°	Abbreviations	Host (Country of origin)	Author (Year of isolation)
B. bassiana	6	Bb6	*Sesamia calamistis* (Benin)	IITA-Benin (1996)
B. bassiana	115	Bb115	*Sesamia calamistis*, (Benin)	IITA-Benin (1997)
B. bassiana	116	Bb116	*Eldana saccharina*, (Benin)	IITA-Benin (1996)
B. bassiana	17	Bb17	*Eldana saccharina*, (Benin)	IITA-Benin (1996)
B. bassiana	42	Bb42	*Cyclas Puncticolis* (Benin)	IITA-Benin (1996)
B. bassiana	362	Bb362	*Sesamia calamistis* (Benin)	IITA-Benin (1997)
B. bassiana	24	Bb24	*Eldana saccharina*, (Benin)	IITA-Benin (1996)
M. anisopliae	361	Ma361	*Eldana saccharina*, (Benin)	IITA-Benin (1996)
M. anisopliae	29	Ma29	*Sesamia calamistis*, (Benin)	IITA-Benin (1996)
M. anisopliae	356	Ma356	*Nezara viridula* (Bresil)	Cornell University (1996)
M. anisopliae	359	Ma 359	*Callosobruchus* sp Benin	IITA-Benin (1996)

2.4. Experimental design

Different concentrations of conidia (10^7, 10^8, 10^9 and 10^{10} conidia/ml) obtained from 7 isolates of *B. bassiana* and 4 of *M. anisopliae* were obtained by mixing the conidia with the appropriate quantity of peanut oil prior to application. Forty *M. vitrata* larvae of the same instar were used per replicate, with a total of 120 larvae per larval instar (i.e., first, second, third, fourth, fifth) and

per treatment. Different larval instars were tested separately. The larvae were put individually in transparent small plastic cups (3.8 x 2.9 x 4.0 cm) with perforated cover for ventilation. The test consisted of 11 isolates and two controls (one consisting of peanut oil only and the other one being the absolute control with no treatment), repeated each one three times in a completely random block.

The inoculation was performed by topic application as described in Bateman *et al*., 1996 by dropping 2µl of the formulation on the larva. Germination rates of the isolates were measured prior to inoculation, and varied from 87.4% to 96.8%. Mean temperatures varied between 24.6 ± 1.8°C and 26 ± 0.50 ºC with a mean relative humidity between 63.3 ± 4.3% and 65.5 ± 5%. 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.

2.5. Data analysis

Data on larval mortality and sporulation 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 square root arcsine transformed before being subjected to analysis of variance (ANOVA). The efficacy of the pathogen was compared using the final mortality (i.e., 15 days cumulative mortalities).

3. RESULTS

3.1. Larval mortality

First larval instar (L1): All isolates were pathogenic to L1 instars regardless of the concentration (Table-2). Mortality rates ranged from 25.8 ± 3.0 (Bb 6) to 100, 0 ± 0.0% (Bb 115). Fungal isolates Ma369, Ma356 and Bb6 caused the lowest mortality rate. Significant differences were observed between isolates at the concentrations of 10^8, 10^9 and 10^{10} conidia/ml but not at 10^7conidia/ml. Moreover, at 10^9 ($F_{12, 26}$ = 39.31, P <0.0001) and 10^{10} conidia/ml ($F_{12, 26}$ = 72.89, *P* <0.0001), the mortality rates induced by *B. bassiana* isolates were significantly different when compared to *M. anisopliae* isolates. For the highest virulent isolate Bb115, the mortality rate significantly increased with the concentration. However, no significant differences were observed between 10^9 and 10^{10} conidia/ml.

Second larval instar (L2): Mortality rates for all treatment ranged from 25.8 ± 3.6% (Bb6, 10^7conidia/ml) to 100.0 ± 0.0% (Bb115, 10^{10}conidia/ml) (Table-3). They were significantly different between isolates at the concentrations of 10^8, 10^9 and 10^{10} conidia/ml. Again, isolate Bb 115 was by far the most virulent; causing significantly higher mortalities, but no significant differences were observed between concentrations of 10^9 and 10^{10} conidia/ml.

Third larval instar (L3): Mortality rates ranged from 21.7 ± 9.4% (Bb362, 10^7 conidia/ml) and 99.2 ± 0.8% (BB115, 10^9conidia/ml) (Table-4). Larval mortality was generally low at concentrations of 10^7 and 10^8conidia/ml. At 10^9conidia/ml ($F_{12, 26}$ = 15.24, *P* <0.0001), the mortality rates induced by *B. bassiana* isolates were significantly different when compared to *M. anisop*liae isolates. These results obtained with the concentration 10^9 are globally similar to those obtained with the concentration 10^{10} conidia/ml ($F_{12, 26}$ = 7.82, *P* <0.0001)

Fourth larval instar (L4): All isolates were pathogenic to L4 instars regardless of the concentration (Table-5). Isolates Ma361; Ma29; Ma356 and Ma359 caused the lowest mortality, while Bb 115 was by far the most virulent.

Fifth larval instar (L5): Mortality rates for all treatment ranged from 13.3±3.5 % (Ma361) to 56.7±2.2 % (Bb115) (Table-6). Mortality rates were low and not statistically different at 10^7 and 10^8conidia/ml. However, at 10^9conidia/ml, the mortality due to isolate Bb115 was higher than those caused by the other isolates ($F_{12, 26}$ = 34.20, *P* <0.0001). These results obtained with the concentration 10^9 are globally similar to those obtained with the concentration 10^{10} conidia/ml ($F_{12, 26}$ = 30.48, *P* <0.0001). However, no significant differences were observed between 10^9 and 10^{10} conidia/ml.

Table-2. Average *Maruca vitrata* first larval stage (L1) mortality rates15 days after application of different concentrations of fungal isolates (Bb: *Beauveria bassiana* and Ma: *Metarizhium anisopliae*).

Treatments	**Concentrations** (conidia/ml) (n=40; r=3)			
	10^7	10^8	10^9	10^{10}
Control1	1.8±0.2bB	0.8±0.4 cA	0.4±0.4 cA	1.3±0.7 dA
Control2	2.1±1.1 bB	0.4±0.4 cA	1.3±0.7 cA	0.8±0.4 dA
Bb6	25.8±3.0 aC	50.8±3.6 abA	86.7±3.3 aAB	85.8±3.0 bAB
Bb115	45.8±8.7aA	70.0±3.8 aC	100.0±0.0 aB	100.0±0.0 aB
Bb116	32.5±3.8 aA	68.3±6.8 aB	90.8±4.4 aC	90.8±3.0 bBC
Bb17	43.3±3.6 aA	58.3±7.1 abB	87.5±1.4 aB	87.5±1.4 bB
Bb42	43.3±4.2 aA	58.3±5.8 abA	95.8±1.7 aB	95.0±1.4 abB
Bb362	35.0±10.0aAB	60.0±7.6 abB	86.7±9.6 aA	89.2±8.3 bBC
Bb24	40.0±9.0 aAB	59.2±6.0 abB	91.7±1.7 aBC	92.5±1.4 bA
Ma361	30.0±6.3 aAB	35.0±11.8 bB	41.7±6.8 bA	53.3±5.8 cA
Ma29	40.0±6.6 aA	45.8±7.9 abA	48.3±7.9 bA	56.7±2.2 cA
Ma356	31.7±8.5 aA	52.5±6.6 abA	56.7±8.5 bA	65.0±1.4 cB
Ma359	27.5±4.3 aA	30.8±3.0 bA	59.2±12.6 bB	55.0±7.6 cB
$F_{12,26}$	9.23	19.83	39.31	72.89
P	<0,1985	<0,2224	<.0001	<.0001

In the same column, means followed by the same lowercase letter are not significantly different (between isolates comparison) (ANOVA followed by SNK test at 5%)
In the row, means followed by the same uppercase letter are not significantly different (within concentrations comparison) (ANOVA followed by SNK test at 5%)

Table-3. Mortality rate of larvae of *M. vitrata* L2, 15 days after application of different concentrations of fungal isolates.

Treatments	**Concentrations** (conidia/ml) (n=40; r=3)			
	10^7	10^8	10^9	10^{10}
Control1	1.3±0.7 bA	1.9±0.2 bA	0.4±0.4 cA	0.4± 0.4 dA
Control2	0.4±0.4 bA	0.0±0.0 bA	1.3±1.3 cA	0.4±0.4 dA
Bb6	25.8±3.6 aAB	48.3±4.4 abA	86.7±5.1 bB	93.3±1.7 baB
Bb115	49.2±5.8 abC	64.2±1.7 abB	100.0±0.0 aA	100.0±0.0 aA
Bb116	32.5±4.3 aC	67.5±5.0 aB	82.5±7.5 aA	90.0±5.2 abA
Bb17	40.0±3.8 aB	45.8±5.8 bcB	87.5±11.3 aA	90.0±4.3 abA
Bb42	45.0±0.0 aC	46.7±5.8 bcAC	92.5±2.5 aA	86.7±8.5 bB
Bb362	25.0±2.9 aAB	65.0±7.3 abB	83.3±7.2 aA	91.7±5.0 abA
Bb24	41.7±10.6 aB	55.8±4.6 abcB	88.3±0.8 aA	84.2±4.6 bA
Ma361	30.0±10.8 aAC	49.2±5.5 abcC	54.2±3.0 bB	63.3±0.8 cA
Ma29	45.8±5.8 aA	52.5±3.8 abcA	49.2±2.2 bA	54.2±3.6 cA
Ma356	38.3±4.4 aB	40.8±4.6 cB	61.7±1.7 bA	52.5±6.3 cA
Ma359	41.7±8.3 aAB	50.0±5.2abcB	60.0±3.8 bBC	67.5±1.4 cA
$F_{12,26}$	14.20	40.02	33.04	57.83
P	<0,3287	<0,1758	<.0001	<.0001

In the same column, means followed by the same lowercase letter are not significantly different (between isolates comparison) (ANOVA followed by SNK test at 5%)
In the row, means followed by the same uppercase letter are not significantly different (within concentrations comparison) (ANOVA followed by SNK test at 5%)

Table-4. Average rates of total mortality of larvae L3 of *M. vitrata*, 15 days after application of different concentrations of fungal isolates.

Treatments	**Concentrations** (conidia/ml) (n=40; r=3)			
	10^7	10^8	10^9	10^{10}
Control1	1.3±1.3 cA	0.4±0.4 dC	0.4±0.4 fC	0.8±0.8d B
Control2	0.4±0.4 cA	0.4±0.4 dA	0.8±0.4 fA	1.3±0.0 dA
Bb6	25.8±1.7 bAB	40.0±2.9 abcB	92.5±1.4 bA	94.3±1.3 aA
Bb115	52.5±1.4 aC	62.5±1.4 aB	99.2±0.8 aA	96.7±3.3 aA
Bb116	25.0±0.0 bAC	55.0±5.8 abC	87.5±2.9 bB	92.5±3.8 aA
Bb17	25.8±4.4 bAB	45.0±6.3 abcB	73.3±2.2 cA	78.3±5.1 bA
Bb42	31.7±2.2 bB	25.0±0.0 cB	87.5±2.9 bA	89.2±3.3 abA
Bb362	21.7±9.4 bAB	45.8±6.8 abcB	90.0±1.4 bA	89.2±4.7 abA
Bb24	31.7±5.5 bAB	48.3±4.4 abB	90.8±3.0 bA	93.3±0.8 aA
Ma361	25.0±9.4 bB	40.8±10.2 abcAB	56.7±3.0 dA	59.2±6.5 cA
Ma29	40.0±2.9 abA	39.2±2.2 abcA	44.2±2.3 eA	57.5±8.7 cB
Ma356	25.0±1.4 bB	35.0±2.9 bcA	39.2±3.0 eA	43.3±2.2 cA
Ma359	40.0±6.6 abA	43.3±5.8 abcA	45.0±0.0 eA	49.2±2.2 cA
$F_{12,26}$	15.82	26.37	15.24	7.82
P	<0,2163	<0,3441	<.0001	<.0001

In the same column, means followed by the same lowercase letter are not significantly different (between isolates comparison) (ANOVA followed by SNK test at 5%)
In the row, means followed by the same uppercase letter are not significantly different (within concentrations comparison) (ANOVA followed by SNK test at 5%)

Table-5. Mortality rate of L4 larvae of *M. vitrata*, 15 days after application of different concentrations of fungal isolates.

Treatments	**Concentrations** (conidia/ml) (n=40; r=3)			
	10^7	10^8	10^9	10^{10}
Control1	0.8±0.4 cD	0.0±0.0 cD	0.4±0.4 gD	0.0±0.0 dD
Control2	0.4±0.4 cD	0.8±0.8 cD	0.0±0.0 gD	0.0±0.0 dD
Bb6	17.5±2.9 bC	38.3±0.8 abB	75.0±2.5 bcA	72.5±2.9 aA
Bb115	40.0±9.0 aC	52.5±1.4 aB	81.0±1.4 aA	78.8±1.7 aA
Bb116	22.5±2.9 abB	30.8±0.8 bB	66.28±1.7 cdA	70.3±4.6 aA
Bb17	23.3±0.8 abC	36.7±4.4 abB	65.5±2.9 cdA	61.8±1.7 abA
Bb42	25.8±0.8 abB	27.5±2.5 bB	57.3±2.2 deA	63.8±4.6 abA
Bb362	21.7±3.3 abB	25.8±3.6 bB	68.7±4.6 cdA	63.0±4.6 abA
Bb24	17.5±1.4 bC	37.5±9.5 abB	72.2±6.0 abA	73.0±5.2 aA
Ma361	28.3±11.0 abB	38.3±5.8 abB	52.7±0.8 eA	57.2±7.9 bcA
Ma29	24.2±6.8 abA	27.5±3.8 bA	40.8±8.8 fB	46.7±13.4 cB
Ma356	26.7±0.8 abA	29.2±0.8 bA	36.7±3.6 fA	37.5±6.6 cA
Ma359	20.0±1.4 abA	33.3±5.1 bA	55.8±3.0 eB	60.0±3.8 bcB
$F_{12,26}$	12.55	23.97	82.84	40.35
P	<.0001	<0, 0005	<0, 0005	<0, 0001

In the same column, means followed by the same lowercase letter are not significantly different (between isolates comparison) (ANOVA followed by SNK test at 5%)
In the row, means followed by the same uppercase letter are not significantly different (within concentrations comparison) (ANOVA followed by SNK test at 5%)

Comparison across concentrations: The mortality inflicted by each isolate, pooling all concentrations, is presented in Figure-1. Each of the isolates caused less mortality among fifth instars larvae than in younger stages. The average mortality varied from 8.8 ± 3.7% (Ma 359, L5 stages) to 79.0 ± 3.0% (Bb 115, L1 stages). Isolates Ma361, Ma29, Ma359, Ma35 caused the lowest mortality regardless of larval stage. Bb isolate 115 induced the highest mortality rate to all tested instars. These mortality rates were significantly higher than those caused by each of the other isolates for all stages. ($F_{12, 26}$ = 154.74, P <0.0001; $F_{12, 26}$ = 81.45, P <0.0003; $F_{12, 26}$ = 243.61, P <0.0001; $F_{12, 26}$ = 134.55, P <0.0001: $F_{12, 26}$ = 243.61; P <0.0001 for larval stages respectively L1, L2, L3, L4 and L5. The interaction between pathogens and larval stages have also showed that the mortality rate caused by each of the isolate depends on the larval stage ($F_{12, 26}$ = 1.04, P <0.0432).

Table-6. Mortality rate of larvae of *M. vitrata* L5, 15 days after application of different concentrations of fungal isolates.

Treatments	**Concentrations** (conidia/ml) (n=40; r=3)			
	10^7	10^8	10^9	10^{10}
Control1	0.0±0.0 dD	0.0±0.0 cD	0.0±0.0 fD	0.4±0.4 fD
Control2	0.0±0.0 dD	0.8±0.8 cD	0.0±0.0 fD	0.0±0.0 fD
Bb6	18.3±1.7 abcA	27.5±1.4 bAB	48.5±2.5 bB	52.5±4.3 aB
Bb115	30.8±5.8 aA	45.0±2.9 aA	56.7±2.2 aB	53.8±5.8 aB
Bb116	25.0±3.8 abcB	28.3±1.7 bB	42.3±7.1 bcdA	39.7±11.0 abcA
Bb17	15.8±0.8 abcC	35.0±5.2 abB	45.5±5.2 bcdA	33.7±0.8 cdA
Bb42	20.0±1.4 abcB	26.7±0.8 bB	41.0±3.8 bcA	40.0±4.3 abcA
Bb362	20.8±3.3 abcB	25.0±1.4 bB	39.7±7.6 bcA	43.5±9.1 abcA
Bb24	15.0±2.5 bcB	31.7±2.2 abB	39.3±4.4 bA	48.7±3.0 abA
Ma361	13.3±3.5 cB	20.8±5.5 bB	50.0±5.2 cdeA	52.08±5.2 cdA
Ma29	18.3±4.4 abcB	22.5±5.2 bB	35.0±15.1 deAB	29.8±7.9 cdA
Ma356	17.5±0.0 abcB	25.0±1.4 bAB	30.8±9.6 eA	31.7±8.7 deA
Ma359	27.5±2.9 abA	31.7±3.0 abA	35.0±1.4 deA	20.8±4.6 eA
$F_{12,26}$	17.15	27.57	34.20	30.48
P	<.0001	<.0001	<.0003	<.0001

In the same column, means followed by the same lowercase letter are not significantly different (between isolates comparison) (ANOVA followed by SNK test at 5%)
In the row, means followed by the same uppercase letter are not significantly different (within concentrations comparison) (ANOVA followed by SNK test at 5%)

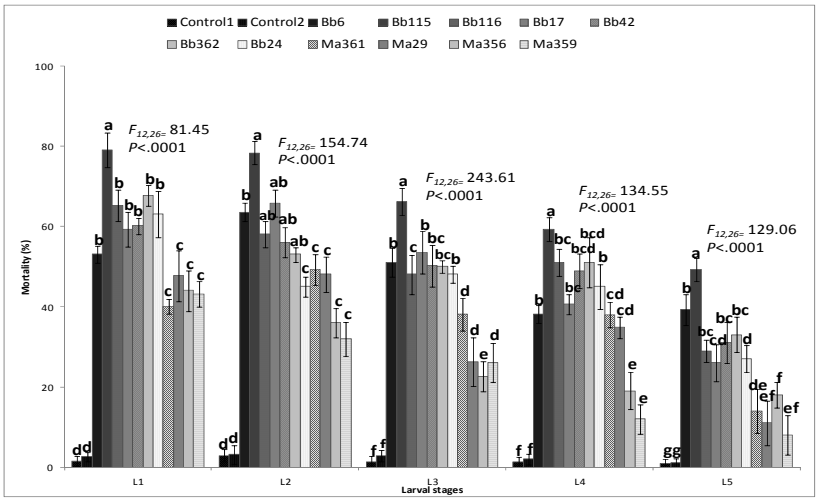

Figure-1. Mortality rate of larvae of *M. vitrata* (all concentrations combined), 15 days after application of entomopathogenic fungi.

3.2. Sporulation

The sporulation rates of infected cadavers (pooled from all concentrations) varied from 1.9 ± 0.8% (Ma 359, L4 stages) to 33.5 ± 1.5% (Bb 115, L1 stages) (Figure-2). The degree of sporulation varied significantly between larval stages and fungi, significantly decreasing with the larval age.

Contrary to mortality, isolate x larval age interactions was not significantly determinant for all isolates ($F_{12.\ 26}$ =79; P< 0.9737). No sporulation was observed on dead insects in the controls.

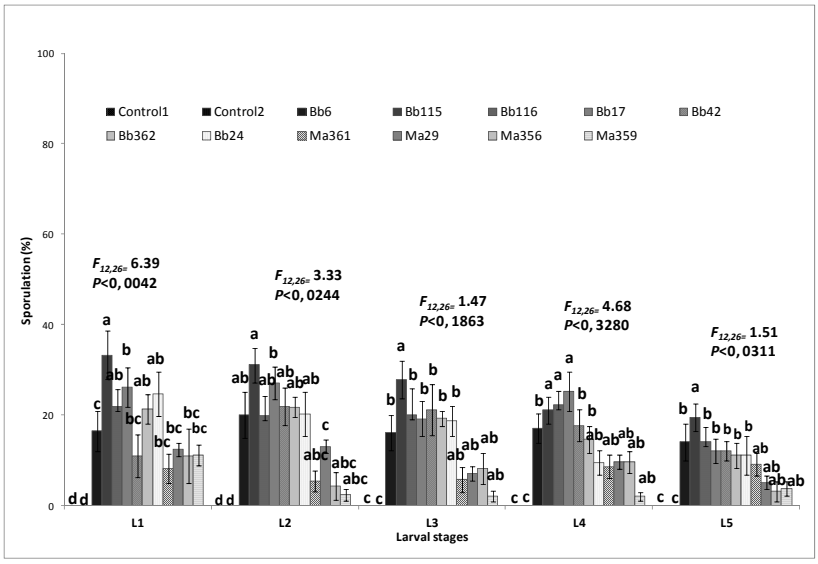

Figure-2. Average rates of total sporulation (all concentrations combined) *M. vitrata* after application of fungal isolates.

4. DISCUSSIONS

The susceptibility of *M. vitrata* larval stages to *M. anisopliae* and *B. bassiana* evaluated in the laboratory showed that all isolates were pathogenic, corroborating observations by Gundannavar *et al*., (2006) and Douro *et al*., (2012). The induced mortality rates depended on fungal species and concentration. Kulkarni *et al*. (2008) reported similar results when using different doses to treat larvae of *H. armigera*.

However, *B. bassiana* significantly induced higher mortality rate compared to *M. anisopliae*. Such differences could be attributed to a stronger ability of *B. bassiana* to produce enzymes and other toxic metabolites as compared to *M. anisopliae* (Ferron, 1981). Similar results were obtained by Valda *et al*. (2003) who observed 70-96% and 26% mortality of *P. xylostella* larvae when treated with *B. bassiana* and *M. anisopliae*, respectively. Among the *B. bassiana* isolates, Bb115 caused the highest mortality to *M. vitrata* larvae within short period after inoculation (2-8 days). The higher virulence of Bb115 isolate could be explained by the fact that this isolate was isolated from an insect of the family Pyralidae in Central Benin. These results corroborate several earlier studies, such as the ones by Mc Coy *et al*. (1988) and Goettel, (1992), who reported that an isolate is usually more virulent to the host family, from which it was isolated. However, the virulence of a isolate reveals the complexity of the factors involved in the specificity of entomopathogen (climate of the region of collection, the storage conditions of the inoculum, the species of insect host on which the fungus was originally isolated, number of re-inoculation of artificial media, etc.).

Larval mortality varied according to the amount of conidia received. Concentrations of 10^9 and 10^{10} conidia/ml of *B. bassiana* Bb 115 induced higher larval mortality compared to 10^7 and 10^8 conidia ml^{-1}, showing a characteristic dose-dependent response as reported e.g. by Lozano and Espaňa-Luna, (2008).

The highest mortality rates were obtained from younger larvae (L1, L2 and L3). Irrespective of isolate and dose, younger larvae were more vulnerable compare to older ones (L4 and L5). This is probably due to the larger size of L4 and L5 larva, compared to that of L1, L2, and L3, confirming findings by Bateman *et al*. (1996) who found that infection of insects by fungi depends on their weight. Similarly, Gundannavar *et al*. (2006) found that, after application of different concentrations of *B. bassiana* on larvae of *H. armigera*, young larvae were more susceptible than older.

The absence of significant differences between the concentrations 10^9 and 10^{10} conidia ml^{-1} suggests that 10^9 could be an optimal concentration for practical purposes. It appears that a concentration of 10^9conidia ml^{-1} will induce the similar mortality rate as 10^{10} conidies.ml^{-1} in less than two weeks. After infection, a latent period is observed for larvae before their death (Thomas *et al*., 1997). This period could be shortened at higher concentrations (Tanada and Kaya, 1993).

Moreover, the sporulation rate was highest on cadavers resulting from the application of Bb115; this rate

is among the best 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 natural increase of the inoculum, promoting secondary transfer of conidia to insects unaffected during field applications (Adu-Mensah, 2002; Douro Kpindou *et al*., 2005). The low sporulation of dead larvae (L4, L5) at concentrations of 10^7 and 10^8 conidia/m is probably due to the small amount of conidia received in relation to their weight or high humidity in the incubation boxes of dead insects, favouring more rapid onset of bacteria and therefore rotting of cadavers.

From the results obtained it appears that all isolates of entomopathogenic fungi tested are pathogenic to *M. vitrata* larvae. The combination of these parameters (high mortality rates of larvae and the relatively high rate of sporulation) makes Bb-115 a good candidate among isolates of *B. bassiana* warranting further studies in view of developing this strain into a viable biopesticide. Further studies should address how to obtain effective and efficient doses for field applications. In addition, studies investigating the growth of hyphae in artificial media, and the effects of various environmental conditions that influence persistence of fungal conidia must also be carried out to fully assess the potentiality of these isolates as biological control agents against *M. vitrata.*

5. CONCLUSIONS

From These experiments, we can conclude that:

- Both *B. bassiana* and *M. anisopliae* isolates tested were pathogenic on all *M. vitrata* larval stages
- Dose/response effect was significant for all larval stages of *M. vitrata* tested and all fungi used
- Isolates Bb 115 (*B. bassiana)* was the most effective on *M. vitrata*
- The young larval stages (L2, L3) of *M. vitrata* are the most vulnerable
- Concentrations 10^9 and 10^{10}conidia/ml gave similar results
- The optimum concentration of 10^9conidia/ml identified should be object of further testing under field conditions for the control of *M. vitrata*.

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