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Thermal response and horizontal transmission of cameroonian isolates of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* – Candidates for microbial controls of the banana root borer *Cosmopolites sordidus*

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ABSTRACT

Beauveria bassiana and *Metarhizium anisopliae* are two promising microbial agents for biopesticides development against the banana root borer *Cosmopolites sordidus*. In this study, germination, mycelial growth, and sporulation of six local Cameroonian isolates of those two species were assessed under seven different thermal conditions (13, 15, 20, 25, 29 33, and 37 °C) to select thermo-tolerant isolates. The Transmission potential of the thermo-tolerant isolates was determined at 25 ± 1 °C by dipping adult weevils in conidial suspensions (3.2×10^8) conidia/ml and mixing these with uninoculated weevils in different proportions (0, 10, 30 and 50%), in groups of 30, and assessing the spread of the mycosis within the group over 35 d of co-incubation. Incubation temperature and isolates significantly affected germination, mycelial growth and conidial production. All isolates had large thermal tolerance ranges (13 −33 °C) except MIITAC6.4.2 (20−29 °C). Horizontal transmission resulted in mortality of non-inoculated weevils from 4.63 ± 1.77 to 53.3 ± 11.9%. The isolate BIITAC6.2.2 exhibited high auto-dissemination potential and high conidia yield in cadavers. These results demonstrate the potential use of these isolates for biopesticides development against *C. sordidus* in Central Africa.

1. Introduction

Several species and strains of entomopathogenic fungi (EPF) originating from a wide diversity of environments have been used in microbial control of mites and insects (Vega et al., 2012). The entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae,* by their easy mass production on artificial media, are the most widely used myco-insecticides (Jaronski, 2013; Brunner-Mendoza et al., 2019). The virulence and multiple modes of action of these two species contribute to the rarity of resistance among host arthropods and enhances the value of EPF as tools in integrated pest management programs (Butt et al., 2016; Petrisor and Stoian, 2017; Abdelaziz et al., 2018). Specific ecological and biophysical requirements, however, continue to pose serious challenges to the development and utilization of myco-insecticides (Maina et al., 2018).

Numerous studies have shown that abiotic and biotic factors such as temperature, relative humidity, solar radiation, competition with other microbes, and arthropod density and diversity affect the infection process during exposure of the arthropods to the EPFs. The effects of these factors are usually determined through evaluation of the action of environmental variables on biological parameters, fungus virulence, persistence, and dissemination of inoculum in the field of which one or more of these factors can limit

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myco-insecticide use (Nankinga et al., 1999; Vänninen et al., 2000; Inglis et al., 2001; Vega et al., 2012). Among the abiotic factors, temperature is the most critical in both arthropod host biology (e.g., life cycle duration, host activity and consequently host population growth rate) and entomopathogen performance (i.e., fungus growth and pathogenicity) (Katsaruware-Chapoto et al., 2017; Taylor et al., 2018; Alali et al., 2019). Temperature has been linked to inconsistent or reduced field performance of some EPF strains. Thompson and Reddy (2016) even recommended that with a changing climate, greater attention should be placed on the effect of temperature on the efficacy of myco-insecticides. Extreme temperature changes can cause cellular stress or death, and initiate appropriate responses that enable them to survive (Shapiro and Cowen, 2012; Braga et al., 2015). Knowledge of thermal tolerance range is fundamental for conservation biological control and for selection of fungi that can offer good ecosystem service or be manipulated depending on insect habitat (Meylind and Eilenberg, 2007). EPF strains that can tolerate a wide thermal range should be therefore an essential component of the selection of EPF in anticipation of the environmental and ecological challenges of the development of sustainable biopesticide products (Alali et al., 2019). The complexity of climatic and agro-ecological zones in Cameroon known to harbor major agro-ecologies of Africa (Robiglio and Sinclair, 2011; Yengoh et al., 2011) could be an advantage for suitable selection of biocontrol agents that can tolerate a wide range of temperatures and maintain its efficient performance widely.

Banana root borer *Cosmopolites sordidus* (Coleoptera: Curculionidae) is the most destructive insect pest of banana and plantain worldwide. It feeds, develops, and oviposits in the corm and rhizomes of banana plants in the soil. Several *B. bassiana* isolates have been tested for the development of myco-insecticides for BRB control with variable levels of success (Schoeman and Schoeman 1999; Godonou et al., 2000; Lopes et al., 2011; Omukoko et al., 2014). In contrast, *M. anisopliae* though effective has been rarely tested against BRB (Kaaya et al., 1993; Aby et al., 2010).

Poor performance of biopesticides against BRB in the field combined with short environmental persistence and difficulty to reach the target, are major challenges of biopesticide use in banana agro-system worldwide (Grzywacz et al., 2009). The efficacy of EPF depends on its ability to spread in the target insect population, typically through horizontal transmission (or auto-dissemination) from infected to healthy individuals, which generally improves disease persistence and the intensity of epizootics that can limit the target host arthropod population (Vega et al., 2007; Lopes et al., 2011; Sharifiifard et al., 2012). Earlier reports even supported the idea that auto-dissemination potential of EPF can be exploited to increase biopesticide performance under field conditions (Nankinga et al., 1999; Godonou et al., 2000; Nankinga and Moore, 2000). The auto-dissemination potential of EPF targeting BRB has inspired several innovative delivery systems of biopesticide applications including attract-kill strategy where an attractant such as kairomone (phenolic odor of banana and plantain corm or pseudostem) and/or sex pheromone (cosmolure) are combined with EPF to favor inoculum dissemination in host populations (Nankinga et al., 1999; Lopes et al., 2014; Uzakah et al., 2015). To date, the attract-kill strategy has been one of the best biopesticide delivery systems in the field for the control *C. sordidus* (Godonou et al., 2000; Nankinga and Moore, 2000; Tinzaara et al., 2007, 2015; Fanceli et al., 2013; González et al., 2018; Moreira et al., 2017; Opisa et al., 2019). However, conidiogenesis is a highly sensitive process that requires special attention given that it also affects auto-dissemination of the fungus and consequently its persistence in the field, which, in turn, will affect the costs of industrialization for a virulent strain. Ahmad et al. (2016) even suggested that thermal tolerance of EPFs should be considered as the main factor for the industrialization of products that can adapt to various environments.

In Central Africa, very limited efforts have been made to identify and use local EPF strains in the fight against BRB. Recently, Membang (2013) isolated seven *B. bassiana* and 33 *M. anisopoliae* isolates from plantain rhizosphere in Cameroon. Some of these isolates were highly pathogenic to *Sahlbergella singularis* (Mahot et al., 2019), *Nisotra uniformis* (Niyibizi, 2018) and BRB (Membang et al., 2020). Six of the isolates showed high infectivity against multiple life stages of BRB (Membang et al., 2020).

The broad objective of this study is to contribute knowledge that can be used in the development of biopesticides based on locally-adapted strains of *B. bassiana* and *M. anisopliae* for the control of BRB in Central African countries and beyond. In this study, we determined the range of thermal response and horizontal transmission potential of six isolates of *B. bassiana* and *M. anisopliae* that had shown high levels of pathogenicity to BRB (Membang et al., 2020).

2. Materials and methods

The experiments were carried out in the laboratories of the International Institute of Tropical Agriculture (IITA) in Yaoundé, Cameroon (N 03° 51′ 84”; E 11° 27′ 76”).

2.1. Weevil and fungi cultures

A laboratory BRB colony was initiated with adult BRB collected with pseudo-stem traps (Tinzaara et al., 2011) from plantain fields at the experimental farm of IITA-Cameroon. One hundred BRB adults were added to containers housing plantain suckers. Three days after the addition of adults, BRB eggs were removed from the corms and inserted (with fine-tip hairbrush) into small holes in weevil-free corms of young suckers (never infested with BRB). The newly infested suckers were placed in opaque plastic containers (40 × 14 mm D x H) lined at their bottoms with wet filter paper and sterilized river sand and incubated in continuous dark conditions at 25 ± 1 °C. At 45 d post-incubation, BRB adults were hand-harvested for their use in fungus isolate re-isolation and horizontal transmission experiments. Prior to their use in the experiments, adult weevils were disinfected by dipping in 1% sodium hypochlorite solution for about 30 s and rinsed 3 or 4 times with sterile distilled water under laminar flow.

Six EPF isolates, three *B. bassiana* (BIITAC6.2.2, BIITAC8.1.5, and BIITAC10.3.3), and three *M. anisopliae* (MIITAC6.2.2, MIITAC6.4.2, and MIITAC11.3.4), were used (Table 1). The six isolates were identified using the identification key to Humber (2012), the molecular characterization could have been useful but logistically impossible due to COVID-19. These isolates were selected in previous studies (Membang, 2013; Mahot et al., 2019) and were preserved at −80 °C as conidia suspension and PDA plates in the IITA-Cameroon entomopathogen collection. Before the inception of the experiment, all isolates were re-isolated from healthy adult BRB (from our lab colonies), to ensure full potential in viability and virulence, and were cultured on PDA medium (LAB M Limited 1, United Kingdom). Conidia were recovered from pure cultures for subsequent studies.

2.2. Germination, mycelial growth, and conidiation under constant temperatures

Conidia of each isolate were harvested from the surface of 15-d-old pure culture of each isolate and suspended in 0.1% Tween 80. The suspension was then stirred with a vortex mixer and filtered with a sterile cheesecloth before conidia counting under a

Table 1
Origin and code of *Beauveria bassiana* and *Metarhizium anisopliae* isolates studied.

Fungus species	Isolate	Origin	Geographic coordinates	Year of 1st isolation
M. anisopliae	MIITAC11.3.4	Bouidon-Ombessa	N04° 35.680, E011° 15.813	2012
M. anisopliae	MIITAC6.4.2	Nyassakounou	N04° 53.633, E011° 25.443	2012
M. anisopliae	MIITAC6.2.2	Nyassakounou	N04° 53.352, E011° 25.142	2012
B. bassiana	BIITAC10.3.3	Kiki	N04° 41.867, E011° 10.876	2012
B. bassiana	BIITAC8.1.5	Yangafock	N04° 47.020, E011° 26.175	2012
B. bassiana	BIITAC6.2.2	Nyassakounou	N04° 53.352, E011° 25.142	2012

dissecting microscope using a Malassez cell (Marienfeld, Germany). Conidial viability of each isolate was determined by spreading 0.1 ml of conidial suspension with a concentration of 3.2×10^6 conidia/ml on PDA plates using a spatula. Four replicates were established for each isolate. Covered glass Petri dishes were tightly sealed with parafilm and transferred to climate control chambers (Percival Scientific, USA) set at seven constant temperatures (13, 15, 20, 25, 29, 33, and 37 °C), 70–80% relative humidity and total darkness. The seven constant temperatures were chosen to cover the range of temperatures in regions with banana and plantain cultivations worldwide (above 13 °C and under 38 °C) and where BRB occurs (above 12 °C and under 34 °C) (Traore et al., 1993; Duyck et al., 2012). The germination rate of 400 conidia was assessed at 40X magnification (Leica, Germany). Conidia were considered as germinated when the germinal tube became longer than normal conidia (Petlamul and Prasertsan, 2012).

To determine the mycelial growth capacity of each isolate under the seven constant temperatures, a 4-mm colony disc of each isolate was removed from the same growing plate of 3 to 5-d-old pure culture incubated at 25 °C and placed in the center of Petri dishes, similar to the description of this process in Petlamul and Prasertsan (2012). Inoculated plates were sealed with parafilm and transferred for incubation at the same conditions set for conidia germination. There were 4–5 replicates per isolate at each temperature. Colony radial extent (mm) in each plate was measured daily for 21 d across two perpendicular diameters (mm) drawn on the bottom of the Petri plates. Conidia from each plate were harvested by scraping the media surface with a sterile scalpel and suspending the whole colony in 10 ml of 0.1% Tween 80 solution. Conidia yield was estimated by diluting the conidia suspension and counting the number of spores with a hemocytometer under a light microscope at 40X magnification (Petlamul and Prasertsan, 2012).

2.3. Horizontal transmission

Horizontal transmission potential was determined for *B. bassiana* isolates BIITAC6.2.2 and BIITAC10.3.3 and for *M. anisopliae* isolates MIITAC6.2.2 and MIITAC11.3.4, which had the broadest temperature range for germination and sporulation (see results below). The horizontal transmission was evaluated using fungus-treated and control (uninfected) adult weevils in four vector ratios (i.e, percentages of fungus-treated weevils in groups of 30 insects) of 0, 10, 30, and 50%, in each of 4 replications per isolate (Lopes et al., 2011). Fungus-treated weevils were air-dried at room temperature after marking them with white paint (Nr. Igle-147) on their dorsum, then dipping them individually for 30 s in 0.1 ml conidial suspension of each isolate prepared at a concentration of 3.2×10^8 conidia/ml. Control insects were dipped in a solution of Tween 80 for 30 s. Both fungus-treated and control insects were placed at the diagonally opposite corners of a sterile plastic container (7 × 5 cm) containing 20-g pieces of plantain corm as food which was changed at 5-d intervals. Containers were incubated for 35 d in the laboratory (in darkness, at 25 °C, which is the optimal temperature for adult weevil activity (Cuillé, 1950) and 70–80% relative humidity). Dead, non-inoculated insects were removed and incubated on moist filter paper in Petri dishes at the condition mentioned above to favor mycosis development.

To evaluate sporulation, ten cadavers of non-inoculated insects for each isolate, presenting signs of infection by the pathogens after 7 d (i.e., insect from mycosis confirmation test) were randomly collected to determine conidial yield per cadaver. This process consisted of transferring cadavers individually in a 10-ml sterile solution of 0.1% Tween 80 and shake for 3 min using vortex to break down the chain and facilitate dispersion of conidia (Latifian and Rad, 2012). The suspension was then serially diluted, and spores were counted under Haemocytometer Malassez cell to estimate conidial yields.

2.4. Statistical analysis

Percentage germination, mycelial growth and conidial production of each of the six isolates across seven constant temperatures were analyzed separately with a 2-factor analysis of variance using a complete randomized design. The significance of pairwise differences was assessed using Tukey HSD ($P < 0.05$). The response variables were log-transformed [Log (x+1)] where needed to correct the heterogeneity of the error variances inherent in the types of data presented in this manuscript. A Pearson's correlation test was performed to assess the relationship between germination, mycelial growth, and conidial production.

Mortality and mycosis values from the horizontal transmission experiment were arcsine-transformed to correct for the heterogeneity of error variances but since transformed data were still not normally distributed, a non-parametric Kruskal Wallis test was used to assess the effect of isolate on percentage BRB infection and mortality for each vector ratio. Means were separated using the Wilcoxon test at a threshold of 5%. The conidial yield of the four fungal isolates infecting BRB in the horizontal transmission tests was analyzed with 1-factor ANOVA using a complete randomized design. Significance was tested with Tukey's HSD test.

ANOVA and regression analyses were performed with R while non-parametric tests were done using SAS (version 9.2).

3. Results

3.1. Thermal effect on fungal conidia germination on a solid substrate

Conidia germination rate was highly affected by temperature ($F = 787.7$; $df = 6, 209$; $P < 0.001$) and isolates ($F = 67.4$; $df = 5, 209$; $P < 0.001$), and the effect of temperature on conidia germination was significantly different between isolates (temperature x isolate: $F = 34.4$ $df = 30, 209$; $P < 0.001$) (Table 2). Conidia germinated at all temperatures except at 37 °C. Optimal germination rates of *B. bassiana* BIITAC8.1.5 were from 20 to 25 °C, while a wider optimal thermal range from 20 to 29 °C was recorded for BIITAC10.3.3 and BIITAC6.2.2 (Table 2). An even wider optimal range was obtained for

Table 2
Effect of seven constant temperatures on conidia viability (mean % viability ± SE) of six entomopathogenic fungus isolates.

Temperatures	BIITAC10.3.3	BIITAC6.2.2	BIITAC8.1.5	MIITAC11.3.4	MIITAC6.2.2	MIITAC6.4.2
13 °C	10.0 ± 1.15**bA**	6.05 ± 1.70 **dA**	7.55 ± 0.95**bcA**	0 **cB**	4.70 ± 2.03 **cA**	5.10 ± 2.60 **bcA**
15 °C	12.6 ± 0.48**bBC**	6.55 ± 0.77**dD**	24.4 ± 1.61**bA**	2.85 ± 1.16 **bcE**	7.65 ± 0.62b**cCD**	13.6 ± 1.34**bB**
20 °C	92.7 ± 0.54 **aA**	68.5 ± 1.70**bB**	92.5 ± 0.80 **aA**	90.9 ± 0.28 **abcA**	71.5 ± 3.71**abcB**	94.7 ± 0.36 **aA**
25 °C	76.9 ± 10.10**aAB**	88.0 ± 0.69**aAB**	70.2 ± 16.4 **aB**	99.6 ± 0.13 **aA**	99.4 ± 1.17 **abA**	98.1 ± 0.57**aAB**
29 °C	78.4 ± 5.72 **aB**	82.0 ± 3.67**abB**	11.8 ± 1.52**bC**	99.4 ± 0.15 **abA**	99.7 ± 0.24 **abA**	99.7 ± 0.12 **aA**
33 °C	24.9 ± 2.00**bB**	42.7 ± 9.56 **cB**	9.50 ± 1.05 **bcC**	99.6 ± 2.15 **aA**	100 ± 0.00 **aA**	99.4 ± 0.36 **aA**
37 °C	0**c**	0**e**	0c	0 ± 0**c**	0**c**	0**c**
F, P	F = 82.7; P < 0.0001	F = 115.5; P < 0.0001	F = 36.12; P < 0.0001	F = 1196; P < 0.0001	F = 727.8; P < 0.0001	F = 262.9; P < 0.0001

Means followed by same lower (upper) case letter within column (row) are not significantly different with Tukey HSD test, $\alpha = 5\%$ for temperatures (isolates).

the three *M. anisopliae* isolates from 20 to 33 °C (Table 2). Conidia germination rates of all fungal isolates were less than 15% at 13 and 15 °C.

3.2. Effect of temperature on mycelial growth

Similar to conidia germination (i.e., viability), mycelial growth was strongly affected by temperature (F = 3491.9; df = 6, 4058; P < 0.001) and isolate F = 97.81; df = 5, 4058; P < 0.001), and highly significant interactions between temperature and isolate were recorded on mycelial growth (F = 48.80; df = 30, 4058; P < 0.001).

For all isolates tested, mycelial growth was completely absent at 37 °C and below 1 mm/d at 13 °C. The *B. bassiana* isolates, BIITAC8.1.5 and BIITAC6.2.2 produced the largest colonies, particularly at 20 °C (2.97 ± 0.09 and 2.70 ± 0.06 mm/d respectively). All *B. bassiana* isolates displayed optimum mycelial growth at 15–29 °C except BIITAC6.2.2 for which optimum range occurred at 20–29 °C. All the *M. anisopliae* isolates showed optimum mycelial growth between 20 and 29 °C except for MIITAC6.2.2 which has wider optimum growth at 20–33 °C (Fig. 1).

3.3. Effect of temperature on conidial production

Total conidial production by the six tested fungi was highly significantly affected by temperature (F = 1821.6; df = 6, 167; P < 0.001) and isolates (F = 729.5; df = 5, 167; P < 0.001), and highly significant interactions between temperature and isolate were recorded on conidial production (F = 92.4; df = 30, 167; P < 0.001). All three *B. bassiana*'s isolates and one *M. anisopliae* isolate - MIITAC6.2.2 − produced conidia in the range of 13–33 °C compared with *M. anisopliae's* isolates MIITAC11.3.4 and MIITAC6.4.2 which, respectively, produced conidia in the range of 13–29 °C and 20–29 °C (Table 3). The widest optimum range of temperature for the highest conidial production was at 13–29 °C for MIITAC6.2.2, followed by BIITAC10.3.3 (13–25 °C) and MIITAC11.3.4 and BIITAC8.1.5 which had both highest conidial production at 15–25 °C. The isolates BIITAC6.2.2, MIITAC11.3.4 and BIITAC8.1.5, and MIITAC6.4.2 showed narrow optimum temperature range for high conidia yield, 13 and 25 °C respectively (Table 3).

Pearson's correlation analysis showed that there was a strong relationship between germination and mycelial growth (r = 0.75; n = 208; P < 0.001) while there was no correlation between germination and conidial production (r = −0.02; n = 208; P = 0.80), nor between mycelial growth and conidial production (r = 0.05; n = 208; P = 0.50).

3.4. Horizontal fungal transmission in adult C. sordidus at different vector ratios and conidial production

All isolates showed the ability to disseminate from inoculated to non-inoculated insects, but transmission potential varied with vector ratio. When 10, and 30% of adult weevils were treated with fungal isolate, mortalities were significantly different from control ($\chi 2 = 17.72$; df = 4; P = 0.0014 and $\chi 2 = 16.92$; df = 4; P = 0.002

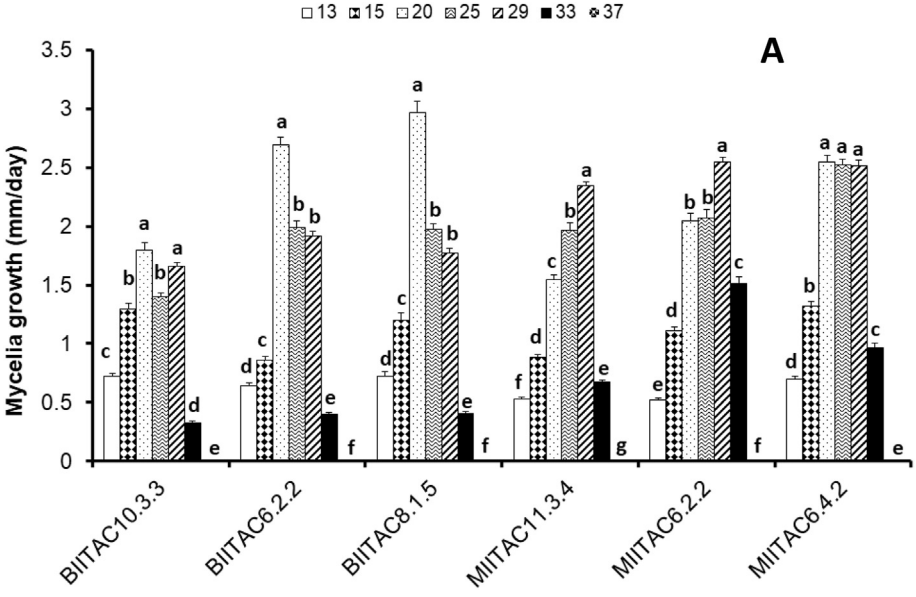

Fig. 1. Mycelial growth of six entomopathogenic fungal isolates incubated on PDA medium at seven constant temperatures (13, 15, 20, 25, 29, 33 and 37 °C).

Table 3
Effect of seven constant temperatures on conidia production (mean unit ± SE) of six entomopathogenic fungus isolates.

Temperature	BIITAC-10.3.3	BIITAC6.2.2	BIITAC-8.1.5	MIITAC-11.3.4	MIITAC-6.2.2	MIITAC-6.4.2
13 °C	(6.00 ± 3.10) x10^8**aB**	(7.50 ± 3.30) x10^9**aA**	(2.50 ± 0.64) x10^8**bB**	(3.52 ± 2.02) x10^7**cC**	(4.35 ± 0.30) x10^7**abBC**	0**dD**
15 °C	(8.61 ± 1.56) x10^8**aA**	(1.62 ± 0.23) x10^9**bA**	(5.43 ± 0.55) x10^8**abA**	(4.10 ± 0.51) x10^8**aA**	(4.93 ± 0.56) x10^7**abA**	0 **dB**
20 °C	(2.01 ± 0.56) x10^9**aA**	(1.88 ± 0.60) x10^9**bA**	(4.45 ± 2.36) x10^8**abB**	(2.83 ± 1.11) x10^8**aB**	(3.35 ± 0.63) x10^8**aB**	(8.18 ± 1.20) x10^5**bC**
25 °C	(2.02 ± 0.80) x10^9**aA**	(2.12 ± 0.65) x10^9**bA**	(2.16 ± 1.10) x10^9**aA**	(2.02 ± 0.70) x10^8**abB**	(1.64 ± 0.32) x10^8**abB**	(6.90 ± 0.22) x10^6**aC**
29 °C	(7.54 ± 5.53) x10^7**bA**	(1.73 ± 0.17) x10^8**cA**	(1.55 ± 0.42) x10^7**cA**	(3.57 ± 1.40) x10^7**bcA**	(1.33 ± 0.23) x10^8**abA**	(1.43 ± 0.45) x10^5**cB**
33 °C	(2.20 ± 0.34) x10^6**bB**	(8.87 ± 1.80) x10^5**dB**	(2.85 ± 0.53) x10^6**dB**	0 **dC**	(6.60 ± 2.27) x10^7**bA**	0 **dC**
37 °C	0**c**	0**e**	0**d**	0**d**	0**c**	0**d**

Means followed by same lower (upper) case letter within column (row) are not significantly different with Tukey HSD test, $\alpha = 5\%$ for temperatures (isolates).

respectively). However, no significant differences were found among the four isolates assessed. At vector ratio 50%, the mortality rates were significantly high with BIITAC6.2.2 followed by the other three isolates which were higher compare to the control ($\chi 2 = 21.40$; df = 4; P = 0.0003). The infection rates of non-inoculated insects was significant high for the four isolates at vector ratio of 10% ($\chi 2 = 14$; df = 4; P = 0.007). A similar trend was observed at vector ratio 30% ($\chi 2 = 12.23$; df = 4, 19; P = 0.016). When 50% of adult weevils were treated, the infection rate of the four isolates were significantly higher than that of the control ($\chi 2 = 20.70$; df = 4, 19; P = 0.0004) with high infection rates for BIITAC6.2.2, BIITAC10.3.3 and MIITAC11.3.4, while MIITAC6.2.2 was not significantly different from MIITAC11.3.4. The mean mortality of control insects was below 1% (0.83%) and none of the dead insects showed mycosis (Fig. 2A and B).

The mean conidia yield by non-inoculated insects was higher for isolate BIITAC6.2.2, ($3.43 \pm 1.90 \times 10^{10}$ conidia/mL compared with the other isolates (F = 59.7; df = 3, 39; P < 0.001)) while BIITAC10.3.3 and MIITAC11.3.4 produced similar numbers of conidia ($1.08 \pm 0.05 \times 10^{10}$ and $7.80 \pm 2.58 \times 10^9$conidia/ml respectively). MIITAC6.2.2 produced the lowest number of conidia ($4.70 \pm 1.61 \times 10^8$conidia/ml) in this study (Fig. 3).

4. Discussion

The success of bio-insecticides based on entomopathogenic fungi (EPF) depends, among other factors, on favorable properties of three successive stages in the growth of entomopathogenic fungi, conidia germination, mycelial growth and conidiogenesis, and their response to environmental variables such as the range of temperatures to which the fungus will be exposed. The three stages determine the ease of inoculum mass production and affect the capacity of the fungus to cause infection and to multiply in the target host. Challenges of environmental conditions have caused field failure and withdrawal of many biopesticides (Maina et al., 2018). Furthermore, the capacity of EPF to disperse within the host populations through horizontal transmission is critical for the ability of the fungus to cause disease epidemics and therefore suppression of the host populations. Studies of the three biological parameters and horizontal transmission are critical in the selection, eventual development, and success of a bio-insecticide.

In this study, we determined the thermal response of three isolates of *B. bassiana* and of *M. anisopliae,* which were isolated from plantain fields in Cameroon (Membang 2013; Membang et al., 2020). This is the first report of the thermal response and

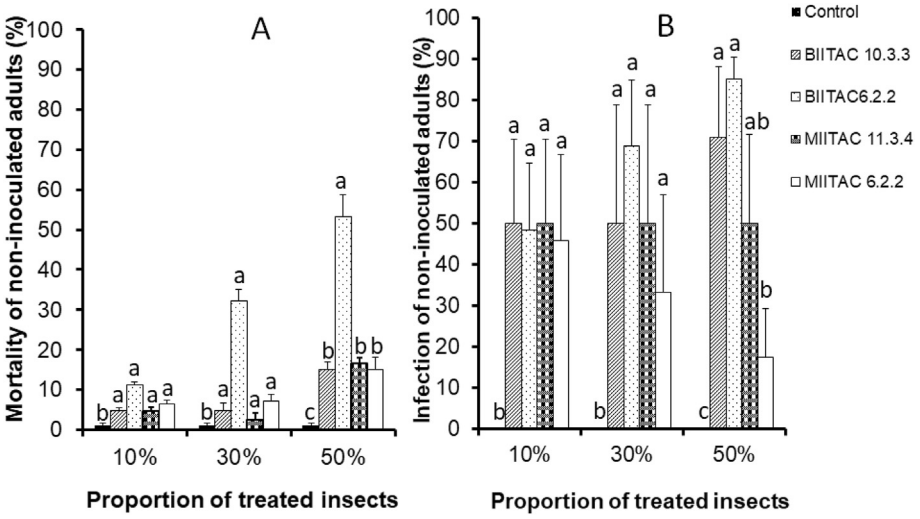

Fig. 2. (A) Percent mortality and (B) mycosis (mean + SE) in experiments on horizontal transmission potential of four entomopathogenic fungi isolates (*B. bassiana* isolates BIITAC10.3.3, BIITAC6.2.2 and *M. anisopliae* isolates MIITAC11.3.4, MIITAC6.2.2) with four increasing levels of vector (inoculated weevils) ratios.

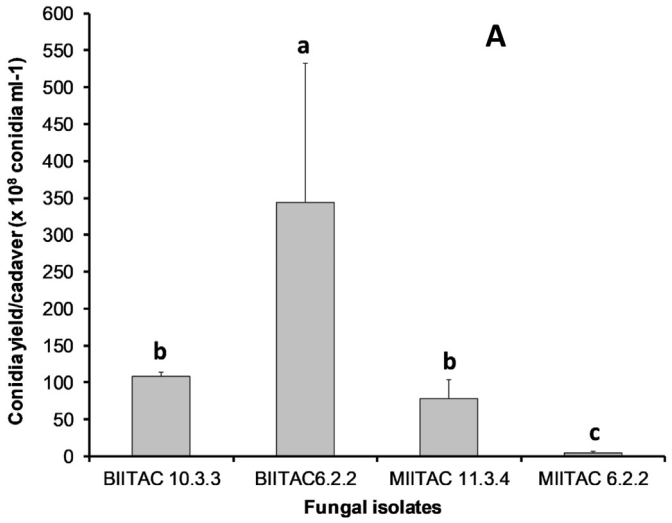

Fig. 3. Fungal conidia yield (mean ± SE) per insect cadaver for four fungal isolates. Means (bars) followed by the same letter are not significantly different (Tukey HSD, $\alpha = 0.05$).

horizontal transmission under laboratory conditions of *B. bassiana* and *M. anisopliae* isolates from Central Africa.

The EPFs used in our study exhibited remarkable adaptability reflected by their ability to germinate, grow and produce conidia at a wide thermal tolerance range with fungal activity inhibited only at 37 °C. The optimum temperature range for maximum conidia germination, high mycelial growth and high conidial production was respectively wide, 20–33 °C, 20–29 °C, and 13–29 °C for *M. anisopliae* and *B. bassiana* isolates studied. This largely agrees with previous studies on the effects of temperature on *B. bassiana* and *M. anisopliae,* which revealed that these fungus species are mostly mesophilic with optima temperature ranges between 20 and 30 °C (Fargues et al., 1992, 1997; Tefera and Pringle, 2003; Lawrence and Khan, 2009; Alali et al., 2019).

Additionally, the thermal response (tolerance and optimum ranges) was fungus isolate-dependent with most isolates tolerating 13–33 °C except MIITAC11.3.4, MIITAC6.4.2 which tolerated, 13–29 °C and 15–33 °C respectively. Earlier reports show that variation in fungi thermal profile could be interspecific (Borisade and Magan, 2014), intraspecific between isolates of same origin (Borisade and Magan, 2014; Heviefo et al., 2019) and also between isolates of different origin (Fargues et al., 1997; Vidal et al., 1997; Teja and Rahaman, 2016; Alali et al., 2019; Heviefo et al., 2019). However, isolates from different origin can also show the same thermal tolerance range, as was the case with *B. bassiana* isolates originating from hot environments in Syria and isolates from Benin which tolerated, 20–30 °C (Alali et al., 2019; Heviefo et al., 2019). EPF strains from the forest can also grow better at low temperatures compared with strains from agricultural fields which could tolerate high temperatures (Augustyniuk-Kram and Kram, 2012). In our study, all the strains were from a forest-savanna transition zone, the microclimate and microhabitat may influence the adaptation potential of a fungus as well as enzymatic, genetics, and molecular regulation that determine the expression or repression of morphological features (Papagianni, 2004; Abdelaziz et al., 2018). Similar to our findings from Cameroon, a report on the thermal sensitivity of five Ethiopian EPF isolates, one *B. bassiana* and four *M. anisopliae,* revealed that *B. bassiana* tolerated 15–30 °C, whereas *Metarhizium* spp. isolates germinated, grew, and sporulated between 15 and 35 °C (Tefera and Pringle, 2003).

The optimum temperature ranges for germination and sporulation obtained in the present study have been favorable for the development of fungus infection in several hosts including BRB (Lawrence and Khan, 2009; Taylor and Khan, 2010; Lopes et al., 2011; Sharififard et al., 2012; Mishra et al., 2015). While testing the thermal effect on the infectivity of two *B. bassiana* strains originating from a warmer region of Brazil, Lopes et al. (2011) demonstrated that some isolates can have a wide thermal range of significantly high infectivity of BRB (21–29 °C), while it is narrow for others (25–29 °C). Similarly, the thermal tolerance range and optimum thermal ranges for fungus *in vitro* growth obtained in the present study is close to both the thermal ranges for growing banana crop (thermal tolerance 19–29 °C and optimum banana growth 26–30 °C) and development of the target pest in our study (thermal tolerance 12 °C to less than 34 °C, optimum ranges 20–30 °C). This means that these fungi can remain active in the banana rhizosphere.

Additionally, all isolates exhibited high activity at 25 °C which is the optimal temperature for adult BRB activity and favorable for disease occurrence (Cuillé, 1950; Lopes et al., 2011*)*. This is important for the dissemination of inoculum in the host through direct contamination, indirect contamination, and secondary transmission of conidia (Vega et al., 2007). It has been exploited for effective control of insect pests using attractants (Nankinga and Moore, 2000; El-Sufty et al., 2010; Tinzaara et al., 2015; Opisa et al., 2019). The wide thermal tolerance of our isolates can also be favorable for the development of a biopesticide product that may withstand wide temperature changes. The tolerance of these isolates to other environmental factors such as humidity, solar radiation, and pH still needs to be assessed.

We observed that the conidial production of *B. bassiana* isolates was 12 times higher compared with *M. anisopliae* isolates**.** Similarly, Lopes et al. (2013), while studying the diversity of indigenous *B. bassiana* and *Metarhizium* spp. in a commercial banana field of Brazil and their virulence toward *C. sordidus*, found that *B. bassiana* produced between 5 and 95 times more conidia per colonized host than indigenous *Metarhizium* spp. isolates. Gandarilla-Pacheco et al. (2012) also reported high sporulation of *B. bassiana* strains at almost all tested temperatures while evaluating conidial production and mycelial growth in solid culture media for native strains of entomopathogenic fungi isolated from citrus-growing areas of México.

The present study results also revealed that conidial production was not directly influenced by mycelial growth and germination. Contrary to our findings, Teja and Rahaman (2016) while testing temperature tolerance of four *M. anisopliae* isolates, obtained a positive correlation between conidia yield and radial growth, and between conidia yield and germination rate. Given that the biological traits studied depend on the nutritive requirement, the type of culture media used, methodology of assessment of conidial production, and fungus specificity may explain the difference between their results and ours. Teja and Rahaman (2016) used PDAY medium, to evaluate conidia yield on 10 mm disc colony for only one fungus species (*M. anisopliae*) incubated at 25–45 °C, while in our case we used PDA, assessed conidial production on the old colony of 21 d using two fungus species (*B. bassiana* and *M. anisopliae*). Those differences imply that detailed studies of individual species and traits are necessary to select suitable microbial agents as a tool for pest management.

Our study showed that the mortality of non-inoculated BRB increased with vector ratio for the more virulent isolate BIITAC6.2.2, with up to 53% mortality at vector ratio 50%. The Mortality of non-inoculated BRB obtained in present study was high compared with those of Schoeman and Schoeman (1999) and Omukoko et al. (2014) who found respectively 27% and 24–26% mortality. Our results are close to those of Lopes et al. (2011) who found 45% mortality of non-inoculated BRB at a vector ratio 50%. A

positive correlation was observed between the proportion of inoculated insects and infection of non-inoculated individuals in studies with entomopathogenic fungi and ants (Pereira and Stimac, 1992), for *C. sordidus* (Lopes et al., 2011), and for *Microcerotermes diversus* (Isoptera: Termitidae) (Cheraghi et al., 2012). The living BRB were effective carriers of inoculum, in contrast to other coleopterans that detect and avoid entomopathogenic fungi (Villani et al., 1994). The number of inoculated insects in the host population may have increased mortality given that the highest mortality was achieved at a high vector ratio 50% (Kocaçevik et al., 2016).

Two studies compared the pathogenicity of the six isolates used in our study and found high virulence of BIITAC10.3.3, BIITAC6.2.2, MIITAC11.3.4, and MIITAC6.2.2 against the cocoa mirid *S. singulris* (Mahot et al., 2019) and high virulence of the six against adult BRB (Membang et al., 2020) respectively causing up to 100.0 ± 00% and 96.7 ± 3.33% mortality. These findings can explain the high potential of transmission, surely related to the level of virulence of the isolates. Ewald (1994) and Myers et al. (1995) reported that efficient transmission of disease by vectors is heavily associated with greater virulence. The high virulence of BIITAC6.2.2 compared with others could be related to pathogenicity factors involved in fungus-host interaction. Pathogenicity factors such as destructive enzymes (protease, chitinase, and lipases) and metabolites can differ in type and amount depending on fungus species and strain (Butt et al., 2016; Petrisor and Stoian, 2017; Abdelaziz et al., 2018).

Conidial production on BRB cadavers was fungus isolate-dependent with significantly higher conidiation for BIITAC6.2.2 compared with all other isolates. The difference in conidial yield between EPF isolates and species was also reported by Bayissa et al. (2016) and Mweke et al. (2018) on aphid cadavers. High conidia yield is important for explosive epizootics, which can lead to disease outbreaks in favorable conditions and consequently lead to reductions in the host population (Myers et al., 1995). The sporulation potential of BIITAC6.2.2 is of great importance for commercialization and the potential for field epizootics.

Author contribution

- Gertrude MEMBANG, Zachee AMBANG, Apollin Fotso KUATE, and Rachid HANNA conceived research
- Gertrude MEMBANG conducted experiment
- Zachee AMBANG, Hermine Claudine MAHOT, Apollin Fotso KUATE, Komi Kouma Mokpokpo FIABOE, and Rachid provided research materials, tools and intellectual support during research execution
- Gertrude MEMBANG, Komi Kouma Mokpokpo FIABOE, and Rachid HANNA conducted statistical analyses
- Gertrude MEMBANG, Zachee AMBANG, Hermine Claudine MAHOT, Apollin Fotso KUATE, Komi Kouma Mokpokpo FIABOE, and Rachid HANNA wrote the manuscript
- Komi Kouma Mokpokpo FIABOE, and Rachid HANNA secured funding
- All authors read and approved the manuscript

Data statement

The dataset can be view using any of the links below.

- DOI: https://doi.org/10.25502/v2pj-g452/d
- CKAN link: http://data.iita.org/dataset/thermal-tolerance-of-cameroonian-fungal-isolates

Declaration of competing interest

No conflict of interest declared.

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