## Title

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# *Cosmopolites sordidus* (Germar) susceptibility to indigenous Cameroonian *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) isolates

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## Abstract

Management of the banana root borer (BRB), *Cosmopolites sordidus* (Germar; Coleoptera: Curculionidae), remains a challenge in banana and plantain production worldwide. Synthetic pesticides remain the most widely used solution while mycoinsecticides are increasingly being recommended. In this study, we selected indigenous isolates of *Beauveria bassiana* and *Metarhizium anisopliae* collected from plantain fields in Cameroon, and tested them in the laboratory for their viability, pathogenicity and virulence against all *C. sordidus* life stages. Of 13 isolates initially screened for spore germination and pathogenicity to adult weevils in conidial suspension of $3.2 \times 10^8$ conidia/ml, eight isolates with high to moderate germination and highest weevil mortality were selected for dose–response bioassays with four concentrations per isolate: $3.2 \times 10^2$, $3.2 \times 10^4$, $3.2 \times 10^6$ and $3.2 \times 10^8$ conidia/ml. The virulent isolates from adult bioassays were tested with eggs, larva and pupae in conidial suspension of $3.2 \times 10^8$ conidia/ml. Isolates performance depended on insect life stage with significantly high pathogenicity and virulence against larval, pupa and adult stages. The *Beauveria* isolate BIITAC6.2.2 caused the highest mortality rates followed by MIITAC1.1.5. Lethal times and lethal concentrations were relatively low for the three *M. anisopliae* isolates and three *B. bassiana* isolates which were the best isolates in almost all insect life stages. Apart from being effective in multiple life stages, these isolates were transmitted horizontally from one stage to another when eggs and pupae were treated. The implication of these findings for integrated management of the BRB, and potential biopesticides development and commercialization are discussed.

**KEYWORDS**
biodiversity, inoculum transmission, microbial biopesticides, virulence

## 1 | INTRODUCTION

Banana and plantain are important commodities for food and nutrition security of more than 400 million persons (Paranthaman, Sudha, & Kumaravel, 2012), with about 187 million metric tons produced annually worldwide (FAOSTAT, 2020) and an international banana trade value between US$ 4.5 and 5 billion per year (Arias, Dankers, Liu, & Pilkauskas, 2003). In the central Africa region, banana and plantain together occupy the second rank in crop production, after cassava (FAOSTAT, 2020). Cameroon, with its annual production

of 5.6 million tons, is the 8th producer worldwide and the highest producer in Africa (FAOSTAT, 2020). Several biotic and abiotic constraints, however, continue to challenge banana and plantain production, regardless of region or country.

In central Africa, the major biotic constraints include banana bunchy top disease (BBTD), Banana *Xanthomonas* Wilt (BXW), fungal wilts (Panama disease and Sigatoka) (Blomme et al., 2017; Ngatat et al., 2017), nematodes (Jones, 2000a) and insect pests (Masanza, 2003). The banana root borer (BRB) *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is the most widespread of all insect pest constraints (Gold, Pena, & Karamura, 2001; Okolle, Gagriel, Franklin, Patrick, & Loubana, 2009;Stover, 2000), in addition to the banana aphid *Pentalonia nigronervosa* (Cocquerel; Hemipetera: Aphididae), the vector of banana bunchy top virus, the causal agent of BBTD (Kumar et al., 2011).

Larvae of BRB tunnel through banana and plantain corms leading to several types of damage to banana and plantain (Gold & Messiaen, 2000). These include (a) reduction in planting material availability through pre-mature death of suckers, reduced number of suckers, reduced sucker vigour and development of water suckers; (b) decline in fruit production through stunting of plant growth, delayed fruit maturation, reduced bunch size, and plant toppling resulting in reduction of plantation lifespan and massive toppling of bananas. In the absence of effective control measures, severe infestations of BRB can lead to 100% yield losses, affecting livelihood and food security of vulnerable communities (Koppenhfer, Seshu Reddy, & Sikora, 1994; Muñoz, Cañas, Urrea, & Guarín, 2013; Ocan, Musaka, Rubaihayo, Tinzaara, & Blomme, 2008; Sengooba, 1986; Ysenbrandt, Fogain, Messiaen, & Lang, 2000). In Cameroon, BRB is found in all area of banana and plantain production (Okolle et al., 2009).

Several strategies have been used in banana farming systems for the management of BRB, including the use of various cultural, genetic, chemical and biological methods (Akmal, Freed, Malik, & Gul, 2013; Arinaitwe et al., 2014; Kiggundu et al., 2003b; Mongyeh, Ndamukong, & Okolle, 2015; Osorio-Osorio et al., 2017; Sivirihauma et al., 2017). Due to its cryptic nature, repeated synthetic and systemic insecticides' applications remain, however, the most widely used option for BRB control, increasing the risk of pesticide residues in fruits and leaves (Carvalho, 2017; Kim, Kabir, & Jahan, 2017; Nicolopoulou-Stamati, Maipas, Kotampasi, Stamatis, & Hens, 2016 ;Paranthaman et al., 2012). Entomopathogenic fungi, with their capacity to cause high BRB mortality and to spread locally in BRB populations, have been considered as a safer alternative to synthetic and systemic insecticides (Hasyim, Azwana, & Syafril, 2009; Nankinga et al., 1999).

The entomopathogens *Beauveria bassiana* ((Bals.) Vuill.; Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* ((Metsch.) Sorokin; Hypocreales: Clavicipitaceae) have been shown to be the most promising microbial agents against adult BRB (Aby et al., 2010; Nankinga et al., 1999; Schoeman & Schoeman, 1999; Tinzaara et al., 2015). There is little experimental evidence of the potential of local isolates of entomopathogenic fungi in BRB microbial control in Central Africa. Furthermore, most studies in other regions of Africa targeted BRB adult stages, and very few of them considered susceptibility of immature stages (Godonou, Green, Oduro, Lomer, & Afreh-nuamah, 2000; Kaaya, Seshu Reddy, Kokwaro, & Munyinyi, 1993). In Cameroon, there have been limited researches in developing biopesticides against BRB Indigenous isolates offer the triple advantage of being adapted to local environmental conditions, suitable for use against local strains of pests and do not require permits for introduction in the country. Forty isolates of *B. bassiana* and *M. anisopoliae* were collected from soils under plantain production in Cameroon using baited method with BRB larvae (Membang, 2013). A recent report by Mahot et al. (2019) assessing the performance of these isolates against the cocoa mirid, *Sahlbergella singularis* Hagl. (Heteroptera: Miridae) demonstrated high pathogenicity of six of the forty isolates.

Several methods have been used for the delivery of entomopathogenic fungi, including application of liquid or powder formulation, through attract-and-kill techniques or plant inoculation as endophytes (Akello, Dubois, Coyne, & Kyamanywa, 2008; Akutse, Maniania, Fiaboe, Van den Berg, & Ekesi, 2013; Backman & Sikora, 2008; Gathage et al., 2016; Lacey & Goettel, 1995; Vega et al., 2007). Knowledge of an entomopathogen's effect on the target organism's mortality rate and lethal time and concentration to kill at least 50% of individuals are fundamental for isolate selection (Bayissa et al., 2016; Kaaya et al., 1993; Lopes et al., 2011; Mahot et al., 2019; Mweke et al., 2018). For a cryptic insect like *C. sordidus* living in corms, the fact that infected insects do not die immediately after infection gives the opportunity for further horizontal transmission of inoculum to different developmental stages and adults living inside the corm is of great importance in terms of durability and dissemination of the epizooties.

The main objective of this study is to evaluate the pathogenicity and virulence of 13 indigenous Cameroonian *B. bassiana* and *M. anisopliae* isolates against all life stages of *C. sordidus* to select isolates for further development and commercialization of biopesticide against the pest. It is expected that if found effective against the pest, integration of the most promising isolate into a fungus-based biopesticide formulation in the country will be faster and cheaper for companies desiring to invest in formulating *B. bassiana* or *M. anisopliae* biopesticide for Cameroon and the larger Central African market for the control of the BRB.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental site

The experiments were conducted under laboratory conditions of 25 ± 1°C, 70%–80% relative humidity (RH) and total darkness, in the laboratory of the International Institute of Tropical Agriculture (IITA) in Yaoundé, Cameroon (N03°51′84″, E11°27′76″).

## 2.2 | Banana root borer colony maintenance

Adults BRB were collected from naturally infested banana fields at IITA-Cameroon using pseudo-stem traps (Tinzaara, Gold, Dicke, Van Huis, & Ragama, 2011). These weevils were confined in an aerated plastic container (40 mm × 14 mm) with disinfected rhizomes of the local plantain variety Elat (AAB) as food. Rhizomes were washed with tap water, then disinfected by dipping in 1% sodium hypochlorite and rinsed thrice in sterile distilled water. The first 2 weeks in the laboratory were considered as quarantine period during which collected insects were inspected daily for possible occurrence of infection prior experimental setup. The dead insects were incubated on moist filter paper in Petri dishes for mycosis observation. None of the adult BRB at that stage showed any signs of fungal infection. The remaining living weevils were divided into two groups of equal number of adults which formed the cohorts of our study. One cohort was directly used in the experiment with adults. The second cohort served for mass rearing where eggs, larvae and pupae were harvested for subsequent experiments. For this purpose, adults were placed in containers with a trimmed plantain sucker (variety Elat) for weevil multiplication, following method of Musabyimana, Saxena, Kairu, Ogol, and Khan (2001) and Tinzaara et al. (2011). Eggs laid by second cohort adult BRB were harvested daily. The eggs were then transferred into a Petri dish lined with moist filter paper (Night, Gold, & Power, 2010) to conserve them in incubation chamber at conditions described above. Larvae obtained from hatched eggs were transferred daily into a plastic container with crushed plantain corm as larval food. To obtain crushed plantain corm, rhizome was trimmed, disinfected with sodium hypochlorite 1% and rinsed three times before mashing in a blender (Severin, KM 3881 made in Germany). Larval food was changed twice per week. One-day-old weevil 5th instar larvae and pupae were harvested from the containers as needed for the various bioassays.

## 2.3 | Entomopathogenic fungi cultures

The 13 isolates of entomopathogenic fungi used in this study were obtained from the IITA-Cameroon fungi collection germplasm: seven *B. bassiana* isolates and six *M. anisopliae* isolates (Table 1). All the isolates were obtained from bait insect method (Meyling, 2007) in soil samples collected in Cameroon during various banana field surveys. The fungal isolates were identified based on Humber (2012) identification key (Membang, 2013). Information on isolates collection is provided in Table 1 (Membang, 2013). They were first re-isolated from adult weevils and cultured on potato dextrose agar (PDA) for 21 days at 25 ± 1°C in dark and at 70%–80% RH. Fungal conidia of each isolate were scrapped and suspended in 10 ml Tween 80 solution. Conidia concentrations were then quantified using haemocytometer under a microscope at magnification 40×. The suspensions were then diluted to obtain the needed concentrations.

All bioassays were carried out through artificial infection of 5th instar larvae, pupae and adults using immersion method. Conidia germination was evaluated by spreading conidial suspension each isolate, label $3.2 \times 10^6$ on PDA plates using inoculation loop. The plates were sealed with parafilm, and incubated for 16 hr at 25 ± 1°C in the dark (Table 1). Percentage germination was determined by counting 400 conidia per plate with four repetition by isolate. Conidia showing longer germ tube than normal conidia were considered as an indicator of spore viability.

**TABLE 1** Source localities and conidia germination rate (mean ± *SE*) of 13 isolates of *Beauveria bassiana* and *Metarhizium anisopliae* 16 hr post-incubation on PDA at 25°C

| Species | Isolate code | Source locality | Geographic coordinates | | Germination (%) (Mean ± SE) |
|---|---|---|---|---|---|
| *M. anisopliae* | MIITAC11.3.4 | Bouidon-Ombessa | N04°35′68.0″ | E011°15′81.3″ | 99.7 ± 0.08a |
| *M. anisopliae* | MIITAC8.1.2 | Yangafock | N04°47′02.0″ | E011°26′17.5″ | 98.8 ± 0.46a |
| *M. anisopliae* | MIITAC6.4.2 | Nyassakounou | N04°53′63.3″ | E011°25′44.3″ | 99.8 ± 0.08a |
| *M. anisopliae* | MIITAC6.2.2 | Nyassakounou | N04°53′35.2″ | E011°25′14.2″ | 99.4 ± 0.16a |
| *M. anisopliae* | MIITAC5.3.5 | Talba | N04°36′71.4″ | E011°29′64.4″ | 99.2 ± 0.22a |
| *M. anisopliae* | MIITAC1.1.5 | Atinodzoe | N03°48′14.9″ | E011°22′70.8″ | 99.3 ± 0.43a |
| *B. bassiana* | BIITAC10.3.3 | Kiki | N04°41′86.7″ | E011°10′87.6″ | 58.7 ± 2.38e |
| *B. bassiana* | BIITAC10.2.2 | Bep Kiki | N04°40′56.0″ | E011°07′56.4″ | 93.8 ± 0.36b |
| *B. bassiana* | BIITAC8.2.5 | Yangafock | N04°46′77.3″ | E011°26′36.3″ | 78.4 ± 1.33d |
| *B. bassiana* | BIITAC8.1.5 | Yangafock | N04°47′02.0″ | E011°26′17.5″ | 85.8 ± 0.79c |
| *B. bassiana* | BIITAC6.4.4 | Nyassakounou | N04°53′63.3″ | E011°25′44.3″ | 26.3 ± 0.68f |
| *B. bassiana* | BIITAC6.2.2 | Nyassakounou | N04°53′35.2″ | E011°25′14.2″ | 75.8 ± 1.45d |
| *B. bassiana* | BIITAC4.2.5 | Nguete | N04°26′33.0″ | E011°34′90.4″ | 73.0 ± 0.43d |

*Note:* Means followed by the same letter in the same column are not significantly different with Tukey's HSD test, α = 5%. Isolates were collected from seven localities in Cameroon in the year 2012.

## 2.4 | Pathogenicity and virulence test on adults

Live adult *C. sordidus*, collected from the rearing units were first sterilized with 1% sodium hypochlorite and rinsed three times in sterile distilled water. The adults were then immersed individually for ~30 s in 1 ml conidial suspension of $3.2 \times 10^8$ conidia/ml of each isolate, as described by Hasyim et al. (2009). Control insects were also dipped for ~30 s but in a solution of Tween 80 (0.1% v/v), prepared with sterile distilled water. Treated insects were transferred to sterile Petri dishes covered with cheese cloth and starved for 24 hr before food (20 g crushed plantain corms) was added. Ten treated insects were grouped as a replicate and three replicates for each treatment (each fungus isolate). The dishes were incubated in an insectarium maintained at 25 ± 1°C in darkness and 70%–80% RH. Weevils were individually inspected for dead every 3 days for 36 days (Kaaya et al., 1993; Lopes et al., 2011). Dead weevils were removed and disinfected with 1% of hypochlorite as described previously and incubated on humid filter paper to assess fungal growth.

## 2.5 | Dose–response

Four *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2, MIITAC1.1.5 and MIITAC6.4.2) and four *B. bassiana* isolates (BIITAC6.2.2, BIITAC8.1.5, BIITAC10.3.3 and BIITAC10.2.2) with mortality rate above 65% and relative short $LT_{50}$ were selected for dose–response assays. Each isolate was prepared according to the direct enumeration procedures (Inglis, Enkerli, & Goettel, 2012) and adjusted at four concentrations: $3.2 \times 10^2$, $3.2 \times 10^4$, $3.2 \times 10^6$ and $3.2 \times 10^8$ conidia/ ml (Hasyim et al., 2009). Disinfected adult *C. sordidus* were treated by immersion method following the same process as described above. Control insects were treated with Tween 80 solution (0.1%v/v). Each concentration level consisted of 10 inoculated insects in each of the three replicates. The dishes containing the treated insects were transferred and maintained as described earlier. Weevil mortality was recorded every 3 days during the experimental period (36 days), and weevil was disinfected with 1% sodium hypochlorite and rinsed thrice in sterile distilled water before incubation on humid filter paper for mycosis confirmation.

## 2.6 | Pathogenicity and virulence on immature stages

For the study of fungal isolates pathogenicity and virulence on immature stages, eggs of less than 24-hr-old, 5th-instar larvae and 1-day-old pupae, the three most potent *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2) and *B. bassiana* isolates (BIITAC6.2.2, BIITAC8.1.5 and BIITAC10.3.3), which showed relatively low $LC_{50}$ or $LC_{90}$ values were used.

Newly laid eggs (<24 hr old) from the weevil culture were used in the egg bioassay. Collected eggs were disinfected with 1% sodium hypochlorite and rinsed three times in sterile distilled water before immersing them in a conidial suspension. A batch of ten eggs was dipped for ~30 s in the fungal suspension at $3.2 \times 10^8$ conidia/ml. Control eggs were similarly immersed but in 0.1% solution Tween 80. The egg bioassay consisted of 10 eggs in each treatment, replicated five times. The treated eggs were placed in Petri dishes with humid filter paper, sealed with parafilm, and incubated for 10 days at 25 ± 1°C in darkness. Mortality was abnormal in the control groups which did not allow for proper estimation of egg mortality caused by the fungal isolated. The experiment provided, however information on horizontal transmission of infection from eggs to larvae by relating signs of disease on eggs (i.e. colonization of egg surface by fungi observed under a dissecting microscope) and infection of emerging larvae (Gindin, Levski, Glazer, & Soroker, 2006).

Larvae and pupae were similarly treated as eggs and adults as described above. Fungus-treated and control insects were transferred to sterile cups containing mashed corm as food and incubated in a growth chamber at 25 ± 1°C with constant darkness; and 70%–80% RH. Weevil mortality was monitored every 2 days for a period of 14 days for larvae and 10 days for pupae. Each treatment consisted of five inoculated larvae or pupae per treatment, replicated five or six times respectively. Dead weevils were disinfected as described above and incubated on humid filter paper for the fungal growth confirmation. Mortality and mycosis were recorded in both larvae and pupae bioassays, in addition to mortality and mycosis of emerged adult mycosis in the pupae bioassay (Gindin et al., 2006).

## 2.7 | Data analysis

Data obtained from the experiment were statistically analysed with R software version 3.4.3. Cumulative mortality rates were corrected using Abbott's formula (Abbott, 1925). Arcsine or square root transformation was used to correct error distributions and normality of germination, corrected mortality and mycosis rates of adults, eggs, larvae and pupae. The percentage of emerged larva and emerged adult BRB was used as response variables in univariate 1-factor (isolate) ANOVA after arcsine or square root transformation of the response variables to correct for heteroscedasticity inherent in our type of data. Where significant factor *F*-tests ($p < .05$) were found, means were separated with Tukey Honestly Significant Difference (HSD) test at $\alpha = .05$ using R software version mentioned above. The values of $LT_{50}$, $LT_{90}$, $LC_{50}$ and $LC_{90}$ of the isolates were estimated at 95% confidence limits (CL) using probit analysis with Package "ecotox" using the same version of R software (Wheeler, Park, & Bailey, 2006). Insect mortality was analysed between doses for each isolate using Kaplan–Meier survival analysis (log-rank method) using JMP 8.0.2 software.

## 3 | RESULTS

### 3.1 | Conidia viability

Germination rates of *Metarhizium* isolates were significantly higher than those obtained for *Beauveria* isolates ($F = 308$; $df = 12$; $p < .001$). *Metarhizium* isolates had similar conidia viability while *Beauveria* isolates could be grouped into three categories of conidia viability: >85% (BIITAC10.2.2 and BIITAC8.1.5), 73%–78% (BIITAC8.2.5, BIITAC6.2.2 and BIITAC4.2.5), and <60% (BIITAC10.3.3 and BIITAC6.4.4) (Table 1).

### 3.2 | Susceptibility of *Cosmopolites sordidus* adults to entomopathogenic fungal isolates

Mortality rates of adult BRB that were treated with the eight isolates with the high to moderate germination rates are summarized in Table 2. Adult mortality rates varied significantly among the isolates ($F = 7.05$; $df = 12$; $p < .001$). At 36 days post-treatment results showed that *M. anisopliae* isolate MIITAC5.3.5 was the least virulent while *B. bassiana* BIITAC6.2.2 was the most virulent, causing 50% mortality at 3.37 days post-inoculation (Table 2). Similarly, high levels of pathogenicity and virulence were recorded with four *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.4.2, MIITAC6.2.2 and MIITAC1.1.5) and four *B. bassiana* isolates (BIITAC6.2.2, BIITAC8.1.5, BIITAC10.3.3 and BIITAC10.2.2), which were selected for dose–response experiments.

The values of lethal time of 50 and 90% mortality ($LT_{50}$ and $LT_{90}$) ranged 3.37–31.2 days and 12.5–34.2 days respectively for fungal isolates studied (Table 2). The least $LT_{50}$ values were obtained when adult BRB was inoculated with BIITAC6.2.2, MIITAC11.3.4 or MIITAC6.2.2. The isolate BIITAC6.2.2 had the lowest $LT_{90}$ (12.5 days).

### 3.3 | Dose–response

Survival analysis showed that there was no significant difference between doses, when *C. sordidus* was treated with BIITAC6.2.2 (log-rank test, $\chi^2 = 5.8$; $df = 3$; $p = .12$), BIITAC10.3.3 (log-rank test, $\chi^2 = 5.1$; $df = 3$; $p = .16$) and MIITAC11.3.4 (log-rank test, $\chi^2 = 5.96$; $df = 3$; $p = .11$). In contrast, survival rate was significantly lower at highest doses when *C. sordidus* was treated with *B. bassiana* isolates BIITAC8.1.5 (log-rank test, $\chi^2 = 32.5$; $df = 3$; $p < .0001$), BITTAC10.2.2 (log-rank test, $\chi^2 = 26.7$; $df = 3$; $p < .0001$) and *M. anisopliae* isolates MIITAC1.1.5 (log-rank test, $\chi^2 = 50.4$; $df = 3$; $p < .0001$), MIITAC6.2.2 (log-rank test, $\chi^2 = 8.7$; $df = 3$; $p = .03$), MIITAC6.4.2 (log-rank test, $\chi^2 = 33.3$; $df = 3$; $p < .0001$).

**TABLE 2** Pathogenicity and virulence of 13 *Metarhizium anisopliae* and *Beauveria bassiana* isolates to adult *Cosmopolites sordidus* at the dose of $3.2 \times 10^8$ conidia/ml

| Fungal Isolates | Mortality (%) | $LT_{50}$ and 95% FL (days) | $LT_{90}$ and 95% FL(days) |
|---|---|---|---|
| *Metarhizium anisopliae* | | | |
| MIITAC11.3.4 | 85.2 ± 9.70ab | 6.18 (3.41–8.60) | 34.1 (24.2–64.6) |
| MIITAC8.1.2 | 23.2 ± 6.48cd | [a] | [a] |
| MIITAC6.4.2 | 84.3 ± 4.63abc | 12.2 (10.82–13.58) | 34.2 (29.6–41.2) |
| MIITAC6.2.2 | 84.7 ± 3.50abc | 7.75 (6.07–9.30) | 32.9 (26.7–44.2) |
| MIITAC5.3.5 | 7.40 ± 7.40d | [a] | [a] |
| MIITAC1.1.5 | 92.6 ± 7.40ab | 10.4 (8.12–12.5) | 23.1 (19.0–31.1) |
| *Beauveria bassiana* | | | |
| BIITAC10.3.3 | 68.1 ± 15.70abc | 14.2 (9.83–19.0) | [a] |
| BIITAC10.2.2 | 76.9 ± 6.48abc | 15.3 (13.4–17.2) | [a] |
| BIITAC8.2.5 | 50.5 ± 6.01abcd | 31.2 (25.8–41.5) | [a] |
| BIITAC8.1.5 | 88.4 ± 6.43ab | 7.41 (5.43–9.21) | 28.76 (29.6–41.2) |
| BIITAC6.4.4 | 48.2 ± 24.28bcd | [a] | [a] |
| BIITAC6.2.2 | 96.3 ± 3.70a | 3.37 (1.82–4.78) | 12.5 (9.63–17.2) |
| BIITAC4.2.5 | 38.4 ± 3.24bcd | [a] | [a] |

*Note:* Mean mortality followed by same lower-case letters are not significantly different by Tukey's HSD multiple range test at $p < .05$. Lethal time 50 ($LT_{50}$) and 90 ($LT_{90}$) (days) ± 95% fiducial limit (FL).

[a]Mortality <50% or 90%.

Result of dose–response at 36 days after treatment showed that each fungal isolate had different lethal concentration ($LC_{50}$) value. The isolates MIITAC6.2.2 ($3.63 \times 10^3$conidia/ml), MIITAC6.4.2 ($3.78 \times 10^6$ conidia/ml), BIITAC8.1.5 ($4.88 \times 10^6$ conidia/ml), BIITAC10.3.3 ($5.09 \times 10^6$ conidia/ml) showed relatively low $LC_{50}$ values (Figures 1 and 2; Table 3). The *B. bassiana* isolate BIITAC6.2.2 had the lowest $LC_{90}$ value ($3.98 \times 10^3$ conidia/ml) (Figure 2 and Table 3). These six isolates were selected for pathogenicity test on eggs larvae and pupae of *C. sordidus*.

### 3.4 | Susceptibility of *Cosmopolites sordidus* eggs to entomopathogenic fungal isolates

While mycosis was recorded on both eggs and emerged larvae for all fungal isolates tested (Table 4). The entomopathogenic fungal isolates were significantly different regarding egg mycosis rates ($p = .002$), with only the three *M. anisopliae* isolates (MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2) being significantly different from control, but not significantly different from *B. bassiana* isolates. Emerged larvae from treated eggs were horizontally infected by fungi even as larvae were transferred on untreated food. Isolates were significantly different in the horizontal infection of larvae ($p < .001$) with the three isolates of *M. anisopliae* being significantly different from control while no difference was found between *B. bassiana* isolates and the control. Combined mycosis was significantly higher for the three *M. anisopliae's* isolates compared to control ($p < .001$).

### 3.5 | Susceptibility of *Cosmopolites sordidus* larvae to entomopathogenic fungal isolates

All isolates were pathogenic to the 5th instar larvae of banana weevil *C. sordidus*, with significant high mortalities in the six isolates tested ($p < .001$). Overall, *M. anisopliae* isolates showed equal or superior virulence compared with *B. bassiana* isolates. The *B. bassiana's* isolate BIITAC10.3.3 and *M. anisopliae* MIITAC11.3.4 were the most virulent isolates, causing 100.0 ± 0.00% mortalities on treated larvae (Table 5). The virulence in the other isolates was also high and

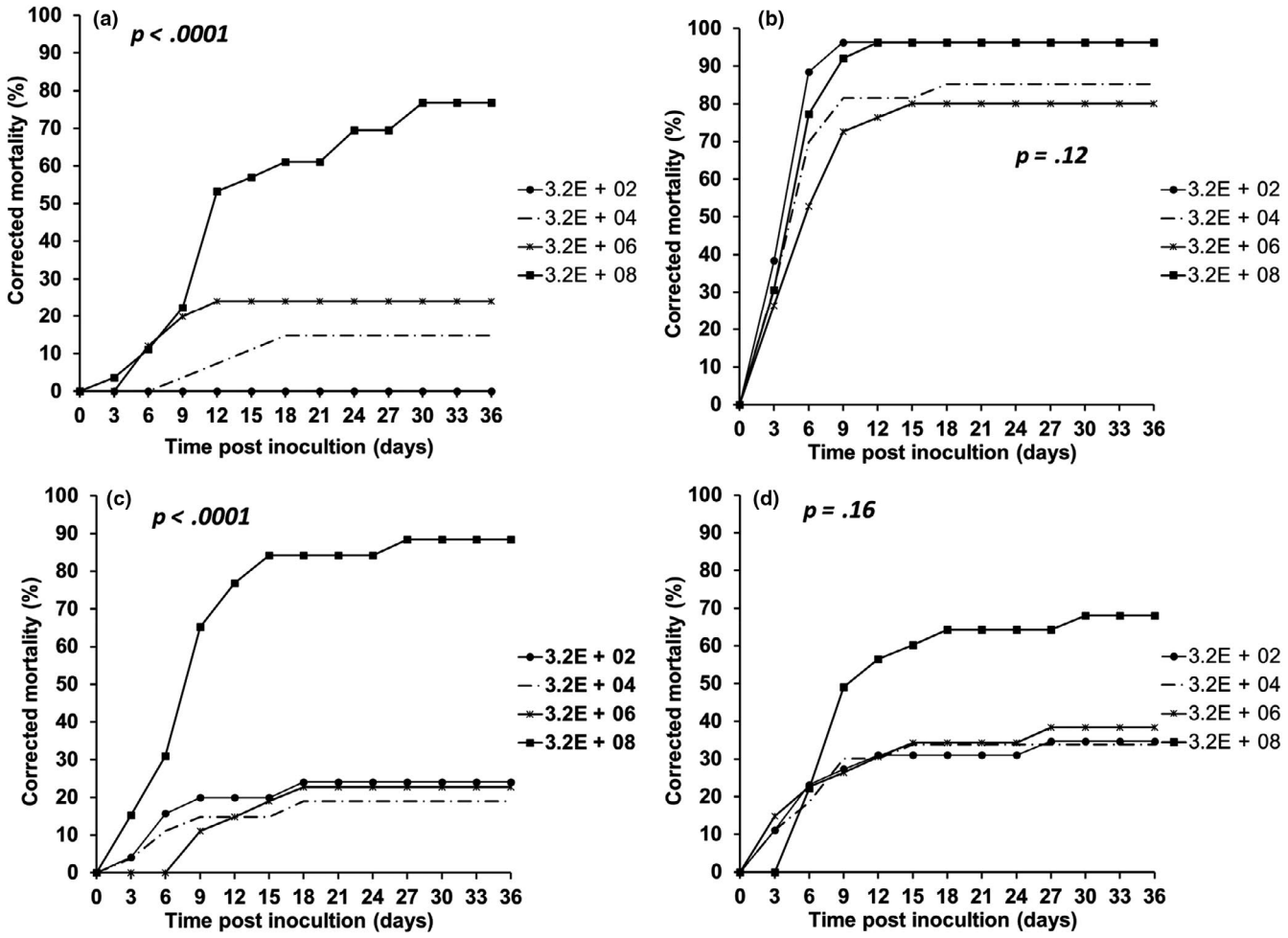


**FIGURE 1** Cumulative mortality of adults *Cosmopolites sordidus* treated with four concentrations of each of four *Beauveria bassiana* isolates; (a) BIITAC10.2.2; (b) BIITAC 6.2.2; (c) BIITAC8.1.5; (d) BIITAC10.3.3

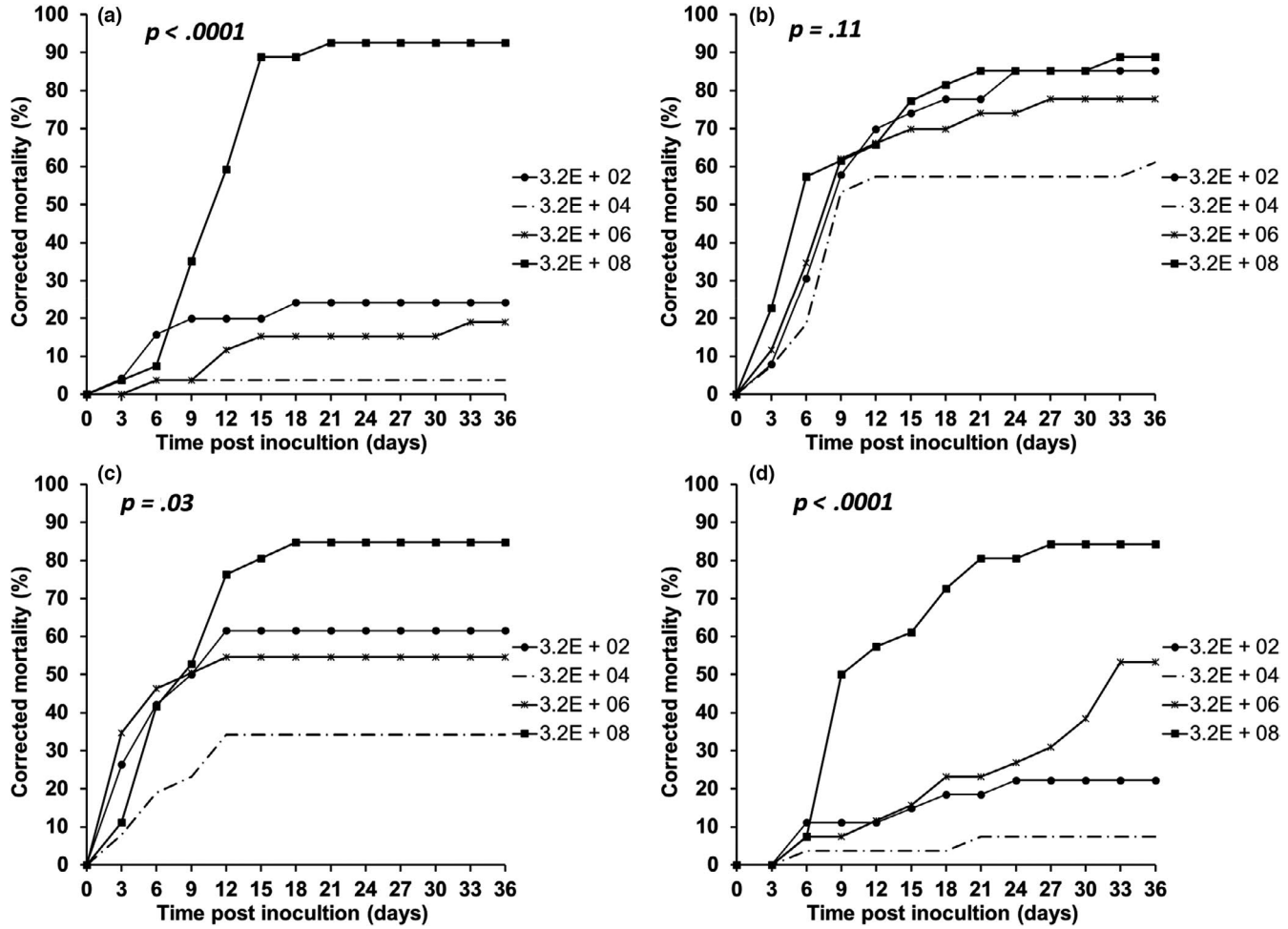


**FIGURE 2** Cumulative mortality of adults *Cosmopolites sordidus* treated with four concentrations of each of the four *Metarhizium anisopliae* isolates: (a) MIITAC1.1.5; (b) MIITAC11.3.4; (c) MIITAC6.2.2; (d) MIITAC6.4.2

**TABLE 3** Virulence of eight *Beauveria bassiana* and *Metarhizium anisopliae* isolates against adult *Cosmopolites sordidus*, 36 days' post-inoculation

| Fungal Isolates | $LC_{50}$ (conidia/ml) (95% FL) | Slopes | Chi-square |
|---|---|---|---|
| *Metarhizium anisopliae* | | | |
| MIITAC6.4.2 | $3.78 \times 10^6$ ($2.62 \times 10^4 \pm 1.66 \times 10^9$) | 0.33 ± 0.019 | 329.25 |
| MIITAC6.2.2 | $3.63 \times 10^{3a}$ | 0.12 ± 0.019 | 168.5 |
| MIITAC1.1.5 | $8.20 \times 10^{6a}$ | 0.32 ± 0.02 | 683 |
| *Beauveria bassiana* | | | |
| BIITAC10.3.3 | $5.09 \times 10^{6a}$ | 0.13 ± 0.017 | 196.68 |
| BIITAC10.2.2 | $1.98 \times 10^7$ ($3.06 \times 10^6 \pm 2.99 \times 10^8$) | 0.49 ± 0.03 | 146.6 |
| BIITAC8.1.5 | $4.88 \times 10^6$ ($4.85 \times 10^4 \pm 4.90 \times 10^{12}$) | 0.28 ± 0.018 | 315.77 |

[a]Unable to estimate lower and upper limits at 95% fiducial limit from the data.

ranged from 68.1 ± 9.21 to 95.8 ± 4.17%. The values of $LT_{50}$ and $LT_{90}$ ranged from 3.02 to 7.76 days and 7.33 to 9.08 days, respectively. The three *M. anisopliae* isolates and BIITAC10.3.3 had the shortest $LT_{50}$ and $LT_{90}$. Results of mycosis tests ($p < .001$) (Table 5) revealed approximatively the same trend as mortality rates.

### 3.6 | Susceptibility of *Cosmopolites sordidus* pupae to entomopathogenic fungal isolates

When the banana weevil's pupae were treated with the different entomopathogenic isolates, pupal mortality and mycosis rate

**TABLE 4** Effect of egg treatment with *Beauveria bassiana* and *Metarhizium anisopliae* on egg mycosis and emerged larvae survival (Mean ± SE)

| Isolates | Eggs mycosis (%) | Emerged larva mycosis (%)* | Combined mycosis (%) |
|---|---|---|---|
| *Beauveria bassiana* | | | |
| BIITAC10.3.3 | 10.0 ± 3.10ab | 50.0 ± 22.4abcd* | 26.0 ± 10.3bc |
| BIITAC6.2.2 | 6.00 ± 4.00ab | 14.7 ± 9.04cd | 12.0 ± 5.83c |
| BIITAC8.1.5 | 20.0 ± 10.5ab | 33.3 ± 14.9bcd | 30.0 ± 14.5bc |
| *Metarhizium anisopliae* | | | |
| MIITAC11.3.4 | 32.0 ± 10.2a | 100 ± 0.0a | 96.0 ± 4.00a |
| MIITAC6.2.2 | 26.0 ± 2.44a | 70.2 ± 12.5abc | 68.0 ± 6.70ab |
| MIITAC6.4.2 | 36.0 ± 9.27a | 80.0 ± 20.0ab | 68.0 ± 16.9ab |
| Control | 0b | 0d | 0c |
| | $F = 4.70$; $df = 6$; $p = .002$ | $F = 6.81$; $df = 6$; $p < .001$ | $F = 13.15$; $df = 6$; $p < .001$ |

*Larvae here were not treated but received the infection horizontally from eggs treated. Means followed by the same letter in the same column are not significantly different with Tukey HSD test, $\alpha = .05$.

**TABLE 5** Pathogenicity and virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates at $3.2 \times 10^8$ conidia/ml at against 5th instar larva of *Cosmopolitus sordidus*, 14 days' post-inoculation

| Fungal Isolates | Mortality (%) | $LT_{50}$ and 95% FL (days) | $LT_{90}$ and 95% FL (days) | Mycosis (%) |
|---|---|---|---|---|
| *Beauveria bassiana* | | | | |
| BIITAC10.3.3 | 100.00 ± 00a | 4.19 (3.50–4.82) | 9.08 (7.77–11.23) | 60.00 ± 8.94b |
| BIITAC6.2.2 | 70.56 ± 11.17bc | 7.76 (6.50–9.32) | * | 80.55 ± 9.04ab |
| BIITAC8.1.5 | 68.05 ± 9.21c | 6.07 (4.51–7.75) | * | 75.00 ± 12.00ab |
| *Metarhizium anisopliae* | | | | |
| MIITAC11.3.4 | 100.00 ± 00a | 4.10 (3.51–4.65) | 7.33 (6.41–8.81) | 100.00 ± 00a |
| MIITAC6.2.2 | 95.83 ± 4.17ab | 4.03 (3.14–4.83) | 8.96 (7.37–11.98) | 78.33 ± 8.33ab |
| MIITAC6.4.2 | 94.44 ± 5.55ab | 3.02 (1.88–3.95) | 7.38 (5.66–11.50) | 68.17 ± 4.17b |
| | $F = 5.85$; $df = 5$; $p < .0007$ | | | $F = 3.87$; $df = 5$; $p < .0008$ |

*Note:* *Mortality rate < 90 %.

Mean mortality and mycosis followed by same lower-case letters are not significantly different by Tukey's HSD at $p > .05$. Lethal time 50 ($LT_{50}$) and 90 ($LT_{90}$) (days) at 95% fiducial limit (FL).

differed significantly between isolates ($p = .002$ and <.0001 respectively). The most virulent isolate was BIITAC6.2.2. BIITAC10.3.3 did not cause any mycosis, while pupal mycosis by other isolates was high and similar. Treated pupae transmitted the fungal inoculum to the emerging adults. The mortality of emerged adult was significantly high when pupae were treated with the three *M. anisopliae* isolates and *B. bassiana's* isolate BIITAC8.1.5 ($p = .002$). Mycosis of subsequent adults was significantly high in the three *M. anisopliae* isolates ($p = .002$) (Table 6). However, combined mortality and combined mycosis rates were high for all isolates except BIITAC10.3.3 (Table 6).

## 4 | DISCUSSION

Biopesticides are important IPM tools and are safe alternatives to pesticides that increase every year in market of crop protection products (Olson, 2015). *Beauveria bassiana* and *M. anisopliae*, two entomopathogenic fungi mainly used as mycoinsecticides are known to be infectious to various agricultural pests including *C. sordidus*(Maina, Galadima, Gambo, & Zakaria, 2018). Discovering of new and indigenous isolates with high virulence and infectivity to all life stages of the target pest can give added value by reducing biopesticide's production cost and property right as well as increasing

**TABLE 6** Mortality and infection rates (Mean ± *SE*) of pupae and freshly emerged adults from pupae treated with *Beauveria bassiana* and *Metarhizium anisopliae* isolates

| Isolates | Pupal mortality (%) | Pupal mycosis (%) | Emerged adult mortality* (%) | Mycosis of adults (%) | Combined mortality (%) | Combined Mycosis (%) |
|---|---|---|---|---|---|---|
| *Beauveria bassiana* | | | | | | |
| BIITAC10.3.3 | 18.25 ± 7.08b | 0.00 ± 0.00b | 27.74 ± 9.93c | 12.50 ± 12.50bc | 39.30 ± 10.21b | 12.50 ± 12.50b |
| BIITAC6.2.2 | 73.41 ± 12.30a | 75.00 ± 9.37a | 38.90 ± 20.03bc | 45.83 ± 20.83ab | 95.24 ± 4.76a | 79.44 ± 7.22a |
| BIITAC8.1.5 | 45.33 ± 9.63ab | 49.67 ± 13.75a | 86.67 ± 8.16ab | 54.00 ± 12.88ab | 93.81 ± 3.81a | 51.52 ± 8.90a |
| *Metarhizium anisopliae* | | | | | | |
| MIITAC11.3.4 | 37.73 ± 8.87b | 78.33 ± 9.80a | 100.00 ± 0.00a | 85.83 ± 6.88a | 100 ± 00a | 77.18 ± 2.95a |
| MIITAC6.2.2 | 38.45 ± 8.60ab | 86.11 ± 10.90a | 90.83 ± 5.83a | 66.67 ± 11.38a | 94.76 ± 3.33a | 68.94 ± 8.86a |
| MIITAC6.4.2 | 41.90 ± 6.14ab | 73.61 ± 10.20a | 90.74 ± 9.26a | 88.90 ± 7.03a | 94.44 ± 2.78a | 77.85 ± 5.96a |
| | $F = 4.88$; $df = 5$; $p < .002$ | $F = 9.64$; $df = 5$; $p < .001$ | $F = 7.32$; $df = 5$; $p = .001$ | $F = 5.07$; $df = 5$; $p = .002$ | $F = 13.1$; $df = 5$; $p < .001$ | $F = 19.8$; $df = 5$; $p < .001$ |

*Note:* Means followed by the same letter in the same column are not significantly different with Tukey's HSD test, $p > 5\%$.
*Adults were horizontally infected as a result of pupal artificial infection.

their persistence in the system and significant reduction of conventional pesticides used in banana/ plantain plantations. Most studies on the efficacy of entomopathogenic fungi against *C. sordidus* however focused only on adult stage (Aby et al., 2010; Fogain, Messiaen, & Fouré, 2002; Lopes et al., 2011; Nankinga et al., 1999; Schoeman & Schoeman, 1999), and little is known about potential of indigenous entomopathogenic fungi of Cameroon against *C. sordidus*. The present study assessed Cameroonian indigenous isolates targeting both immature and mature life stages of *C. sordidus* in the scope of biopesticide development, commercialization and use in Central African region.

*Metarhizium anisopliae's* isolates showed higher germination rates compared to *B. bassiana's* isolates except for BIITAC10.2.2 and BIITAC8.1.5. Germination is a process that can be affected by many environmental abiotic factors as well as biotic parameters such as number of sub-culturing, genetic variability, thickness and/or plurality of membranes covering the conidia that influence the permeability of nutrients necessary for germ tubes formation and growth during germination phase. While a higher viability is important for biopesticide efficacy (Faria, Lopes, Souza, & Wraight, 2015), isolate with moderate viability should not be neglected during screening phase since germination can be improved during formulation process and by adding insect immature stage in the culturing media (Alejo et al., 2018; Mola & Afkari, 2012).

Our study also showed that all the fungi isolates were pathogenic and virulent to all stages of *C. sordidus*. However, the performance of isolates depended on life stages. Out of the 13 isolates studied, six isolates were highly pathogenic to adults while four and five were pathogenic to larva and pupa respectively. Unlike *M. anisopliae* where three isolates tested were highly virulent to all life stages, *B. bassiana* isolates' virulence varied significantly, with BIITAC10.3.3 being the only highly pathogenic isolate against larva and BIITAC6.2.2 and BIITAC8.1.5 for pupa. Compared to the results obtained for the same isolates by Mahot et al. (2019) on cocoa mirid *Sahlbergella singularis* (Haglun; Hemiptera: Miridae), the slopes in present study are lower, suggesting that the response of the BRB's populations exposed to fungi were heterogenous in their response compared to cocoa mirid populations used by Mahot et al. (2019). This results might be due to the fact that adults BRB used for virulence study were initially obtained from the field at unknown ages and acclimatized for the experimentation.

Previous efforts to control BRB using entomopathogenic fungi were mostly focus on using adult stage (Aby et al., 2010; Fancelli Dias et al., 2013; Godonou et al., 2000; González et al., 2018; Lopes et al., 2011; Omukoko, Maniania, Wesonga, Kahangi, & Wamocho, 2011; Tinzaara et al., 2015). Only few studies were interested in immature stages. Nganso, Fansi, and Okolle (2010) found that *B. bassiana* strain GHA (Botanigard) applied on corm in laboratory condition was not effective on adult, eggs and larva of *C. sordidus*. Kaaya et al. (1993) reported susceptibility of both larval and adult stages of *C. sordidus* to Kenyan, Thailand and UK isolates of *B. bassiana* and *M. anisopliae* while Godonou (1999) studied the effect of Ugandan and Kenyan isolates of *B. bassiana* on eggs, larvae and adult. Kaaya et al.(1993) obtained high pathogenicity and virulence against both larvae and adult ranging from 63% to 97% mortalities. Similarly to our findings, they reported that larvae were more susceptible than adult. Godonou (1999) reported low infectivity of fungi to adult stage (27% mortality) compared to immature stages (55% and 60% mortality on eggs and larvae respectively). While assessing the effect of *M. anisopliae* and *B. bassiana* against *Rhynchophorus ferrugineus* (Olivier; Coleoptera: Curculionidae), another cryptic weevil attacking palms, Yasin, Wakil, El-Shafie, Bedford, and Miller(2017) reported higher susceptibility of larval stage than adult as observed in our results on *C. sordidus*. This is, however, the first report of virulence of entomopathogenic fungi against *C. sordidus* pupae.

The successful infection process confirmed with sporulation on dead insects is the secondary source of inoculum generally involved in auto-dissemination and epizootic occurrence which helps in disease outbreak in the field and consequently reduces host population and limit insect pest outbreak (Vega et al., 2007). Infectivity of

immature stages, though known to be responsible of the damages caused to banana and plantain (Gold & Messiaen 2000), has been neglected due to difficulties in the direct application of entomopathogenic fungi against these cryptic instars, compared to adults. However, we hypothesize that epizootics in larvae in the banana corm will most likely result in wider spread of the disease within the corm, coupled with faster halting of damage to the plant compared to mortality of adult stage alone. The indigenous Cameroonian isolates hold therefore a high potential in contributing to environmentally friendly management of the BRB. This will even be of greater importance if they also work endophytically. Further studies are warranted to assess possible endophytic potential of these isolates on different banana cultivars and test the mentioned hypothesis on the performance of the isolates under field condition.

The pathogenicity and virulence of the different isolates tested were significantly different. Out of the 13 isolates screened, only six were found efficient against the BRB. These differences between isolates were found also within the same species. For instance, over six *M. anisopliae* isolates and seven isolates of *B. bassiana* tested, only three isolates in each fungal species were found efficient. This denotes differences between isolates belonging to same species as well as difference between both species. Belonging to the same fungal species is therefore not a guarantee for similar virulence level since difference between isolates can rely on different types and quantity of enzymes and metabolites (which are important virulence determinants) produced by specific strain (Abdelaziz et al., 2018). Similar results have been reported by Ruelas-Ayala, García-Gutiérrez, and Archuleta-Torres, (2013), Cheng et al. (2016) and Yasin et al. (2017) using *B. bassiana* and *M. anisopliae* isolates against other weevils like *Tenebrio molitor* (L.; Curculionidae: Coleoptera), *Sitophilus zeamais* (Motschulsky; Curculionidae: Coleoptera), *Curculio nucum* (L.; Curculionidae: Coleoptera) and *R. ferrugineus*, respectively. For instance, Cheng et al. (2016) while assessing four and two strains of *M. anisopliae* and *B. bassiana* respectively, found difference between isolates of *M. anisopliae* but not between isolates *B. bassiana*.

The six virulent indigenous isolates in the present study against the BRB were recently reported highly effective against cocoa mirid (Mahot et al., 2019). Both *M. anisopliae* and *B. bassiana* are known to be infectious to host of numerous insect orders including Orthopera, Lepidoptera, Dermaptera, Diptera and Coleoptera (Maina et al., 2018). Further studies will be carried out to assess the range of pests that could be controlled using these indigenous Cameroonian isolates and select suitable strains for each pest.

Earlier studies performed to control *C. sordidus* with *B. bassiana* and/or *M. anisopliae* isolates showed contrasting results. Kaaya et al. (1993) found that dry spores of an exotic isolate of *M. anisopliae* from UK was less effective against larvae and adult *C. sordidus* resulting in less than 63% mortality while Aby et al. (2010) reported that dry spores of a local isolate of *M. anisopliae* from Ivory Coast caused up to 100% mortality on adult *C. sordidus*. Our findings are similar to those of Aby et al. (2010) though we used fungal suspension and not dry conidia which can easily be attached on wet insect cuticle.

Pathogenicity test of *B. bassiana* isolates against BRB in previous studies varied from low to high virulence. On adults, mortalities varied from 63% to 97% (Kaaya et al., 1993), 6% to 96.7% (Lopes et al., 2011), 14% to 96% (Fancelli Dias et al., 2013) and 27.2% to 80.22% (González et al., 2018). There was limited effort to develop a biopesticide against BRB in central Africa. However, an exotic strain of *B. bassiana* caused low mortality of adult *C. sordidus* (Okolle et al., 2009). Further studies are required to assess the effect of environmental parameters like temperature, rainfall and relative humidity, photoperiod, UV light, pH, compatibility of isolates with biopesticide formulation ingredients in order to take the present findings to actual optimized development of environmentally friendly biopesticides based on the local Cameroonian isolates against BRB and other agricultural pests as well (Mahot et al., 2019).

Despite regular change of substrate (mashed corm), artificial inoculation of eggs and pupae caused infection and mortality in subsequent stages. This horizontal transmission contributed to high combined mortality and mycosis that varied among isolates. It was pronounced in the three *M. anisopliae* isolates while only one *B. bassiana* isolate resulted in horizontal transmission. This is the first such report of horizontal transmission of entomopathogenic fungi in *C. sordidus*. Entomopathogenic fungi are known to cause disease mostly through horizontal transmission (Godonou et al., 2000; Lopes et al., 2011; Omukoko et al., 2011; Schoeman & Schoeman, 1999). Horizontal transmission is not their primary mode of action, and only a few studies reported horizontal transmission through spray, immersion and food poisoning using entomopathogenic fungi (Asi, Bashir, Afzal, Zia, & Akram, 2013; Ekesi, Maniania, & Lux, 2002; Gindin et al., 2006; Gul, Freed, Akmal, & Malik, 2015). Our findings are, however, similar to those reported by Gindin et al. (2006) in which *M. anisoplie* killed horizontally neonate larva of *R. ferrugineus* when eggs were treated; and Asi et al. (2013) where *B. bassiana* and *M. anisopliae* reduced emergence of adult *Spodoptera litura* (Fabricius; Lepidoptera: Noctuidae) when pupal stage was treated. Horizontal transmission is an added value since it adds to mortality of the directly inoculated stage, and subsequently increasing total mortality. Further studies are warranted to understand the process and/or characteristics underlying the differences between isolate, particularly differences between *M. anisopliae* and *B. bassiana* in their potential to horizontally infect BRB.

Overall, 6 of the 13 screened indigenous Cameroonian isolates were found promising against the BRB; namely, *M. anisopliae* isolates MIITAC11.3.4, MIITAC6.2.2 and MIITAC6.4.2 and *B. bassiana* isolates BIITAC6.2.2, BIITAC8.1.5 and BIITAC10.3.3. Apart from causing high mortality of treated BRB life stages, the six isolates transmitted disease horizontally. Further studies on this horizontal transmission are needed to better understand the process and optimize the use of these isolates. Our study showed that six Cameroonian fungal isolates tested are pathogenic and virulent to multiple stages, an important attribute in disease epidemiology that increases efficacy against host populations. The output of our study also offers an unlimited opportunity, in Cameroon and Central Africa in general, to develop biopesticides against various

pests and in various cropping systems using local isolates. Further studies should test these virulent fungi isolates against non-target organisms to identify their host range, susceptibility to environmental parameters, persistence in field conditions and compatibility with different products used in biopesticide development will enable optimization, production and utilization of the indigenous Cameroonian isolate-based biopesticides to improve yield and livelihood of banana growers while promoting biodiversity and environmental safety. This will further improve income generation in agricultural value chains around climate smart and sustainable intensification of crop production.

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## CONFLICT OF INTEREST

No conflict of interest declared.

## AUTHOR CONTRIBUTION

GM, ZA, AFK and RH conceived research. GM conducted experiment. ZA, HCM, AFK, KKMF and RH provided research materials, tools and intellectual support during research execution. GM, KKMF and RH conducted statistical analyses. GM, ZA, HCM, AFK, KKMF and RH wrote the manuscript. RH and KKMF secured funding. All authors read and approved the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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