Title

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Laboratory assessment of virulence of Cameroonian isolates of *Beauveria bassiana* and *Metarhizium anisopliae* against mirid bugs *Sahlbergella singularis* Haglund (Hemiptera: Miridae)

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The brown cocoa mirid, *Sahlbergella singularis* (Hemiptera: Miridae), causes cocoa yield loss of about 30 % to 70 % in Cameroon. The pathogenicity of six indigenous isolates of *Beauveria bassiana* (BIITAC) and *Metarhizium anisopliae* (MIITAC) to the fourth and fifth nymphal stages of *S. singularis* was evaluated under laboratory conditions. Two methods of inoculation were tested at various conidial concentrations: (1) immersing the pod in 200 ml of the suspension for 3 min (ingestion method) and (2) immersing each insect in 1 ml of the suspension for 5 s (immersion method). Tween® 80 at 0.1 % (v/v) was used as a control. After 14 days from initial exposure to conidia, corrected mortality ranged from 35 to 100 % for immersion and from 16 to 94.3 % for ingestion. Mortalities due to fungi isolates were significantly different from that of their control ($P < 0.05$). The effect of immersion was more significant than that of ingestion, and mortalities increased with increasing spore concentration. Mycelial outgrowth and sporulation after seven days on some of the dead insects, kept on humidified filter paper in dark conditions, demonstrated that death was due to fungal infection (mycosis). Based on pathogenicity results, LC_{50}, LT_{50} and LT_{90} fungal outgrowth, *B. bassiana* isolates BIITAC10.3.3, BIITAC6.2.2, MIITAC6.2.2 and the *M. anisopliae* isolate MIITAC11.3.4 could be selected for their virulence, and advanced to field trials for the development of microbial control.

Key words: pathogenicity, entomopathogens, biocontrol, fungi.

INTRODUCTION

Mirid bugs, *Sahlbergella singularis* Haglund and *Distantiella theobroma* Distant (Hemiptera: Miridae), are the most damaging pest species of cocoa (*Theobroma cacao* L.) in West Africa (Lavabre 1977). In Cameroon, *S. singularis* is by far the most common species on cocoa (Babin 2009). Mirid bugs feed on pods and shoots. They induce cherelle wilt, contributing to young fruits' mortality and cause the drying up of leaves, branch tips, and the quick destruction of the cocoa canopy, which further results in a quick decline of farms when control measures are inadequate (Lavabre 1977; Anikwe 2010; Yédé *et al*. 2012; Mahob 2013). This pest can induce 30 % yield loss in cocoa in the first year and up to 80 % loss in two consecutive seasons if the farm is left unprotected (Anikwe 2010).

Chemical insecticides of the families organochlorines, organophosphates, pyrethroids, nicotinoids and carbamates are most commonly used to control the mirid bugs in Cameroon (Mahob *et al*. 2014). However, serious problems such as resistance, pest resurgence and elimination of economically beneficial insects, toxicity to humans and wildlife are often encountered (Padin *et al*. 2002; Hendrawan & Ibrahim 2006; Mahdneshin *et al*. 2009). Concerns over environmental issues related to the use of synthetic chemical pesticides have led to changes in strategy, and development of Integrated Pest Management (IPM) programmes, where biological control strategies are recommended. Indeed, the use of fungal biocontrol agents can potentially reduce the use of chemical insecticides and furthermore their residual effects. Entomopathogenic fungi are excellent candidates for biological control and are considered as promising alternatives, in attempts

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to improve the sustainability of crop protection.

Among the hundreds of entomopathogenic species of fungi reported from insects, *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hypocreales) and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycota: Hypocreales), are the most frequently used. *Beauveria bassiana* is one of the most effective entomopathogenic fungi, due to its cosmopolitan distribution (Bidochka *et al*. 1998), its ability to infect any life stage of its hosts, its wider host range than the other Deuteromycetes, its ability to infect almost all orders of insects and certain plant tissues (Roberts & Hajek 1992; Bing & Lewis 1992). It can be easily isolated from insect cadavers or from soils using simple media, as well as by baiting soils with insects (Meyling 2007). As opposed to *B. bassiana*, *M. anisopliae* is mainly found in sun-exposed habitats or agricultural soils (Bidochka *et al*. 1998).

These two entomopathogenic fungi have drawn attention as potential biocontrol agents for many important pests such as Thysanoptera, *e.g. Frankliniella occidentalis* (Pergande) (Ugine *et al*. 2005; Wang & Zheng 2012), Coleoptera, *e.g. Anthonomus signatus* (Say), *Otiorhynchus ovatus* (Linnaeus) (Sabbahi 2008), Orthoptera, *e.g. Uvarovisia zebra* (Uvarov) (Mohammadbeigi & Port 2013). Furthermore, their insecticidal potentials have been tested on some Hemiptera such as *Eurygaster integriceps* (Puton) (Abdulhai *et al*. 2010) and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Liu *et al*. 2002; Liu *et al*. 2003; Sabbahi 2008). Preliminary test of the effect of *Beauveria* isolates against *S. singularis* have been done (Mahot 2006). However, the beneficial effects of these two entomopathogenic fungi have never been documented against mirid pests of cocoa, one of the major export crops in Cameroon. Thus, considering the disadvantages of chemical control, there is an increasing need for alternative insect management strategies in agricultural systems.

This study aimed to evaluate the pathogenicity and virulence potential of some indigenous isolates of *B. bassiana* and *M. anisopliae* on the mirid, *S. singularis*, using direct and indirect methods of application in the laboratory.

MATERIAL AND METHODS

Insect rearing

Mirid bug nymphs were collected at Ngat (3°46′N 11°49′E, 705 m elevation) in the Centre Region of Cameroon and reared following Babin *et al*.'s (2008) approach. They were placed in plastic containers (25 × 25 × l.13 cm) in a room with the following controlled average characteristics (T = 25 °C, RH = 64.85 ± 9.73 %, photoperiod: 12L:12D). Ventilation of those plastic containers was provided through four rectangular holes (7 × 12 cm), one on each side and covered with muslin. Pods were replaced every 7–10 days, depending on the extent of feeding damage. The bottom of each container was lined with absorbent paper, to prevent water condensation. Nymph development was monitored daily. Newly emerged female mirids were collected and kept separately until sexual maturity (5–6 days) in ventilated plastic boxes (7 × 10 × 2 cm) containing three sections of young cocoa shoots. These cocoa shoots were changed every 2 days. Subsequently, a 1–2-day-old adult male was introduced into each box for mating. After 24 h, all adults were transferred to muslin cages and attached to cocoa pods in the field. After a minimum of 16 days, which represents the expected hatching time, muslin cages were checked daily to detect newly emerged nymphs. When one or more nymphs had been detected, the pods were cut from the tree and taken to the rearing room where they were kept on absorbent paper in plastic containers with holes for ventilation.

Fungal isolates

Beauveria bassiana and *M. anisopliae* were obtained from the Pathology Laboratory (IITA/ Cameroon). Isolates were named BIITAC 10.3.3, BIITAC 6.2.2, and BIITAC 8.1.5 for *B. bassiana* and MIITAC 11.3.4, MIITAC 6.2.2 and MIITAC 6.4.2 for *M. anisopliae*. BIITAC means *Beauveria* from IITA Cameroon and MIITAC means *Metarhizium* from the same institution. In previous work (Membang 2013) at the Pathology Laboratory aimed to isolate some potential entomopathogenic fungi, the abovementioned species were isolated from soil using the insect bait method (Meyling 2007). Soil sampled from banana farms in the Centre Region of Cameroon had been used. Larvae of banana weevils, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) were placed on 250 g of humidified soil and covered with black paper. After their death, fungal conidia appearing on cadavers were cultured and purified on Petri dishes containing potato dextrose agar (PDA) medium. The six indigenous isolates used to investigate their pathogenicity against *S. singularis* were screened

through laboratory pathogenicity on *C. sordidus* adult and larvae.

Bioassays

For bioassays, multiple concentrations of conidial suspensions of the different isolates of *B. bassiana* and *M. anisopliae* were prepared using a haemocytometer. Suspensions were prepared at 1×10^7, 1×10^8, 3.2×10^8 and 1×10^9 conidia/ml by scraping conidia from 21-day-old cultures and added in 0.1 % Tween 80 (Liu *et al*. 2003; Cherry *et al*. 2004; Mohammadbeigi *et al*. 2013). Water with 0.1 % Tween 80 was used as a control. Before using these suspensions, the viability of the conidia was evaluated. For each isolate, a sample of 50 μl was spread onto the surface of a Petri dish containing PDA with three replicates for each fungal isolate. Plates were incubated at 25 °C for 24 h. For each plate, four microscopic fields were delimited. In each of them, 100 conidia were counted under an optical microscope to reach the number of 400 viable conidia. Viable conidia were those that germinated with the germ tube at least longer than its diameter (Hywell-Jones & Gillespie 1990). The conidial germination percentage for each plate was calculated according to Nussenbaum (2013). Only the suspension in which conidia germination rate was above 90 % was used in further experiments. The insecticidal potential was evaluated directly, by immersing fourth and fifth instar nymphs of *S. singularis* in the conidial suspension, and indirectly by immersing a small cocoa pod used to feed nymphs.

Direct method

The experiments were arranged as a completely randomised design with treatments organised in four conidial concentrations, 1×10^7, 1×10^8, 3.2×10^8 and 1×10^9 conidia/ml and untreated control treatment of only water with 0.1 % Tween 80 for each isolate. Each treatment involved 35 fourth instar nymphs of the mirid divided in five replicates. The experiments were performed under laboratory conditions at 25 °C. Every single mirid was immersed in 1 ml of conidia suspension for 5 s. Seven insects treated by fungal suspension for each concentration and each isolate were transferred on filter paper in ventilated plastic containers ($19 \times 12 \times 4$ cm). The container was supplied with a small cocoa pod for insect feeding and placed in the screen house. The number of dead insects was recorded daily for 14 days and the feeding cocoa pod was renewed on the seventh day.

Indirect method or ingestion

Each cocoa pod serving as food for the mirids was immersed in 200 ml of conidia suspension for 3 min. In the control, pods were immersed in the same quantity of water having 0.1 % Tween 80 solution. After air drying, the treated pods were equally distributed in ventilated plastic containers, described above. Seven fourth instar larvae were released in each container, which represented one replication of each isolate and each concentration. The daily mortality was recorded as described above. Five replications were used for each isolate and each concentration.

Fungal sporulation on dead insects

Dead nymphs were immersed in 70 % ethanol for 15 s to sterilise their surface and rinsed in sterile distilled water for 10 s. Afterwards they were immersed in 1 % sodium hypochlorite for 1 min, then rinsed three times for 10 s with sterile distilled water. Insects were dried on sterile filter paper and under sterile conditions. They were placed in a humid Petri dish lined with moistened cotton wool and filter paper and incubated in the dark screen house for 7 days to allow mycelial outgrowth over the cadavers. *Beauveria bassiana* and *M. anisopliae* sporulation were respectively recognised by the white and green powdery muscardine on dead insects and this indicates that the insects died from a fungal infection.

Statistical analysis

Cumulative mortalities were corrected using Abbott's formula (Abbott 1925) and normality tested with Shapiro-Wilk test. Percentages were (arcsine square-root) x^2 transformed (Zar 1996) before analysis of variance (ANOVA). Paired mean comparisons among fungal isolate, concentrations and fungal species were made using Tukey HSD test. The time to 50 % and 90 % mortality (LT_{50} and LT_{90}) at the more efficient concentration and concentration for 50 % mortality (LC_{50}) on the 14th day post-treatment were determined using the probit analysis program. The software JMP 8.0.2 and SPSS 20.0 were used for all analyses.

RESULTS

Direct method

Isolates were all pathogenic to *S. singularis* at all concentrations, but had different levels of virulence. Average percentage of corrected mortalities at day 14 post-treatment ranged from 40–100 % with *B. bassiana* isolates and from 35–100 % with

M. anisopliae (Fig. 1). In the direct method (immersion method), mortalities were significantly affected by isolates ($F_{5,15} = 3.3$; $P = 0.0085$) and by concentrations ($F_{3,15} = 3.9$; $P = 0.0106$), then by the interaction isolates *versus* concentrations ($F_{15,15} = 6.4$; $P < 0.0001$). The effect of concentration, isolate and the two factors combined was significant at the level of 5 %. Isolate MIITAC11.3.4, at concentrations of 3.2×10^8 and 1×10^8 conidia/ml, had the same response with BIITAC10.3.3 at 1×10^9 and MIITAC6.2.2 at 1×10^8 conidia/ml. Results also showed that treatment with isolates MIITAC6.4.2 at 1×10^9 and 1×10^8 conidia/ml, BIITAC8.1.5 at 3.2×10^8 then BIITAC6.2.2 at 3.2×10^8 conidia/ml had the same effect (Fig. 1) on mirid mortality. Whatever the isolate, concentration response was significantly different with mortality rate except between 3.2×10^8 and 1×10^9 conidia/ml responses. However, there was a significant positive correlation between the corrected mortality and the fungal concentration (Pearson coefficient $r^2 = 0.57$, $\chi^2 = 3.2448$, d.f. = 22, $P = 0.004$, $Y = 2.403e - 08X + 7.299e + 01$). The mortality rate increased with increasing concentration. According to the only pathogenicity test through direct immersion method, all the six isolates could be retained, concentrations of 1×10^8, 3.2×10^8 and 1×10^9 conidia/ml were more effective than 1×10^7 conidia/ml on *S. singularis*.

The median lethal concentrations (LC_{50}) values were estimated for isolates of each fungus applied to the fourth and fifth instar larvae of *S. singularis*. Results indicated significant relationships ($P < 0.05$) between log dose and probit mortality. Based on the LC_{50}, the isolate BIITAC10.3.3 of *B. bassiana* with a value of 8.97×10^5 conidia/ml, the isolate MIITAC6.4.2 (3.69×10^6 conidia/ml) and to a lesser extend MIITAC11.3.4 (7.16×10^6 conidia/ml) of *M. anisopliae* had good insecticidal potentials compared with the others. The fungal suspension of BIITAC8.1.5 and MIITAC6.2.2 isolates had the least efficient LC_{50} (Table 1). The maximum daily

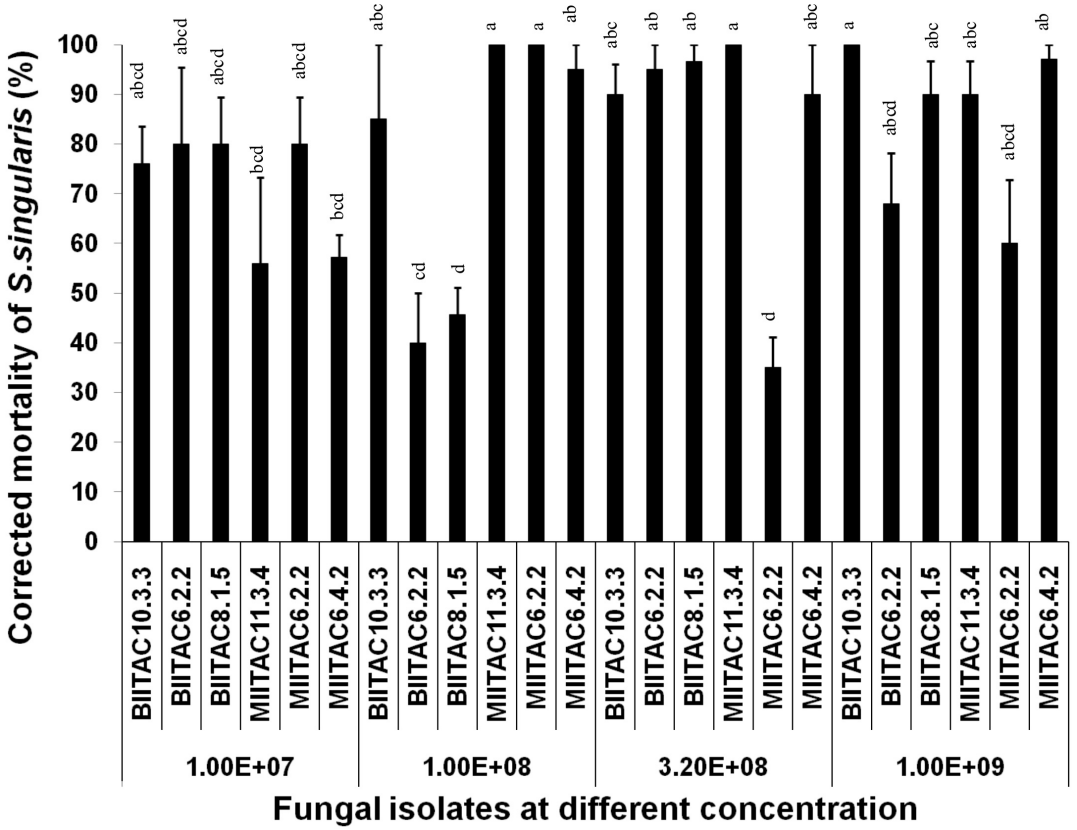

Fig. 1. Mean percentages of corrected mortalities of *Sahlbergella singularis* exposed by immersion to *Beauveria bassiana* and *Metarhizium anisopliae* isolates at 1×10^7; 1×10^8; 3.2×10^8 and 1×10^9 conidia/ml at day 14.

mortality caused by fungi was observed at day 4 for isolates BIITAC10.3.3, MIITAC11.3.4 and MIITAC6.2.2 at a concentration of 1×10^9 conidia/ml (not shown in table). The LT_{50} and LT_{90} values at the most significant concentration 3.2×10^8 conidia/ml for *B. bassiana* and *M. anisopliae* isolates were therefore calculated (Table 2). Among *B. bassiana* isolates, BIITAC10.3.3 gave LT_{50} and LT_{90} values of 4.2 and 8.7, respectively, while among the *M. anisopliae* isolates, MIITAC11.3.4 had the best LT_{50} and LT_{90} (Table 2). Based on these factors, BIITAC10.3.3 and MIITAC11.3.4 were acknowledged as the most virulent isolates.

Ingestion of fungi

Similar to the immersion method, all fungal isolates have also displayed a pathogenic potential by ingestion (Fig. 2). Average percentages of the corrected mortalities ranged from 6.7 to 94.3 % and from 16 to 94.3 % at day 14 respectively with *B. bassiana* and *M. anisopliae* isolates. The effects of concentrations ($F_{3,15} = 10.0$; $P < 0.0001$) and the effect of the interaction between isolates *versus* concentrations ($F_{15,15} = 3.5$; $P < 0.0001$) were significant on mortality response 14 days after exposure to contaminated residues. The highest mortality (94.3 %) caused by *B. bassiana* was obtained with BIITAC10.3.3 suspension at 3.2×10^8 conidia/ml, while the one due to *Metarhizium* was obtained with MIITAC6.2.2 at 1×10^8 conidia/ml (Fig. 2). Treatment BIITAC10.3.3 at 3.2×10^8, MIITAC6.2.2 at 1×10^8, BIITAC8.1.5 at 3.2×10^8 and MIITAC6.4.2 at 3.2×10^8 conidia/ml were more effective. The efficacy of those treatments is on a par with BIITAC6.2.2 at 3.2×10^8. Whatever the isolate, response of concentration 3.2×10^8 conidia/ml was significantly different from the others ($P < 0.0001$), then no significant difference has been found between concentrations 1×10^7, 1×10^8 and 1×10^9 conidia/ml. There was a significant and positive correlation between corrected mortality and concentration ($r^2 = 0.55$, $\chi^2 = 3.1177$, d.f. = 22, $P = 0.005$, $Y = 3.697e-08X + 5.251e+01$). In general, mortality rate increased with increasing conidial concentration. Based on the results of this bioassay, BIITAC10.3.3, BIITAC8.1.5, BIITAC6.2.2, MIITAC6.4.2 and MIITAC6.2.2 could be retained as promising isolates and a concentration of 3.2×10^8 conidia/ml should be chosen for next step (Fig. 2).

Table 1. Concentration–mortality response of *Sahlbergella singularis* nymphs exposed to *Beauveria bassiana* and *Metarhizium anisopliae* isolates by contact to and assessed after 14 days of observation.

Fungal isolates		LC_{50} (conidia/ml)	Probit parameter ± S.E.		χ^2-value
			Intercept	Slope	
Beauveria	BIITAC6.2.2	4.32×10^6	–3.54 ± 0.78	0.535 ± 0.98	24.92
	BIITAC8.1.5	1.15×10^7	–5.15 ± 0.78	0.729 ± 0.98	2.00
	BIITAC10.3.3	8.97×10^5	–3.49 ± 0.92	0.586 ± 0.12	7.185
Metarhizium	MIITAC11.3.4	7.16×10^6	–6.998 ± 0.96	1.021 ± 0.12	8.178
	MIITAC6.2.2	2.18×10^7	–8.678 ± 0.91	1.182 ± 0.12	5.895
	MIITAC6.4.2	3.69×10^6	–2.756 ± 0.74	0.420 ± 0.09	18.518

Table 2. Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates by contact to *Sahlbergella singularis* after 14 days post-treatment at 3.2×10^8 conidia/ml.

Fungal isolate	LT50	95 % confidence interval		LT_{90}	95 % confidence interval	
		Lower	Upper		Lower	Upper
BIITAC6.2.2	5.1	4.4	5.8	13.2	11.2	16. 6
BIITAC8.1.5	4.4	3.2	5.5	11.0	8.6	16.7
BIITAC10.3.3	4.2	3.0	5.2	8.7	6.9	12.6
MIITAC11.3.4	4.1	3.7	4.5	7.5	6.8	8.5
MIITAC6.2.2	4.7	4.1	5.2	12.6	11.0	15.1
MIITAC6.4.2	5.5	4.2	6.7	15.1	11.5	25.1

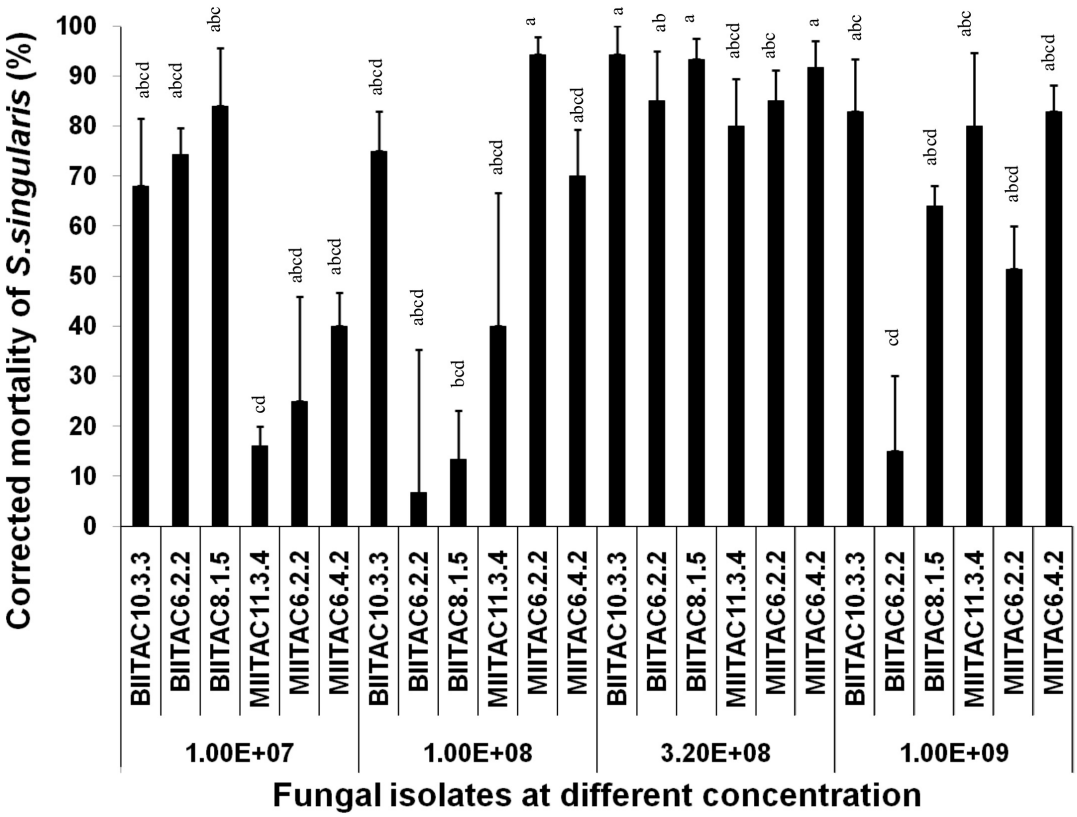

Fig. 2. Mean percentages of corrected mortalities of *Sahlbergella singularis* exposed by ingestion to *Beauveria bassiana* and *Metarhizium anisopliae* at 1×10^7; 1×10^8; 3.2×10^8 and 1×10^9 conidia/ml at day 14.

The concentration–mortality response was also evaluated for each isolate during the contamination by ingestion. Results indicated a significant relationship ($P < 0.05$) between log dose and probit mortality (Table 3). Among all *B. bassiana* isolates, BIITAC10.3.3 gave the smallest LC_{50} value (6.45×10^5 conidia/ml), thus it had the best insecticidal potential compared with BIITAC6.2.2 and BIITAC8.1.5. When taking into account the LC_{50} of *Metarhizium*, the best insecticidal potential could be attributed to the isolate MIITAC6.4.2 which recorded a LC_{50} value of 2.09×10^7 conidia/ml. Nevertheless MIITAC11.3.4 and MIITAC6.2.2 could also be considered as efficient entomopathogens as their LC_{50} values were so close to that of MIITAC6.4.2.

The regression analysis evaluated time–mortality response within the ingestion method of contamination. Between *B. bassiana* isolates, BIITAC6.2.2 had the smaller LT_{50} while BIITAC8.1.5 and BIITAC10.3.3, had the smaller LT_{90} (Table 4). The isolate MIITAC6.2.2 presented an intersting LT_{50} (4.0 days) and LT_{90} (10.8 days), respectively. The three isolates of *B. bassiana* and MIITAC6.2.2 could also be chosen according to their virulence on insect.

Fungal sporulation on dead insects

Insects that did not produce mycelia were recorded as other mortality, but cumulative mortality (mortality caused by fungal infection plus other mortality) until day 14 of the experiment was used in the analysis.

Results showed that both fungi can infect *S. singularis* (Fig. 3A, B) either by contact or by ingestion causing subsequent death of insects.

In general for *B. bassiana*, the immersion method could cause up to 100 % mortality with BIITAC10.3.3 at 1×10^9 conidia/ml, while for *M. anisopliae* mortalities reached 100 % with MIITAC11.3.4 at 3.2×10^8 and 1×10^8 conidia/ml, then followed by MIITAC6.2.2 at 1×10^8 (Fig. 1). The effect

Table 3. Concentration–mortality response of *Sahlbergella singularis* nymphs exposed to *Beauveria bassiana* and *Metarhizium anisopliae* isolates by ingestion.

Fungi isolate		LC_{50} (conidia/ml)	Probit parameter ± S.E.		χ^2-value
			Intercept	Slope	
Beauveria	BIITAC6.2.2	1.46×10^8	–8.490 ± 0.841	1.040 ± 0.103	33.876
	BIITAC8.1.5	5.71×10^7	–7.912 ± 0.814	1.020 ± 0.100	9.989
	BIITAC10.3.3	6.45×10^5	–2.266 ± 0.786	0.390 ± 0.098	3.427
Metarhizium	MIITAC11.3.4	9.47×10^7	–8.013 ± 0.818	1.005 ± 0.100	7.097
	MIITAC6.2.2	7.55×10^7	–10.49 ± 0.905	1.335 ± 0.112	47.471
	MIITAC6.4.2	2.09×10^7	–5.697 ± 0.772	0.778 ± 0.096	0.056

Table 4. LT_{50} and LT_{90} of *Beauveria bassiana* and *Metarhizium anisopliae* isolates applied by ingestion at 3.2×10^8 conidia/ml to *Sahlbergella singularis*.

Fungi isolate	LT_{50}	95 % confidence interval		LT_{90}	95 % confidence interval	
		Lower	Upper		Lower	Upper
BIITAC6.2.2	7.6	6.2	9.5	30.9	20.6	68.4
BIITAC8.1.5	9.6	9.2	10.0	19.0	17.4	21.3
BIITAC10.3.3	9.0	8.6	9.4	19.4	17.6	21.9
MIITAC11.3.4	11.3	9.4	15.7	24.7	17.2	63.1
MIITAC6.2.2	4.0	3.4	4.5	10.8	9.4	13.2
MIITAC6.4.2	7.8	7.4	8.2	18.0	16.4	20.2

obtained with the concentration of 3.2×10^8 conidia/ml was not significantly different from that of 1×10^9 conidia/ml whatever the isolate, except by the ingestion method. In almost all cases, the effect given by concentration value of 3.2×10^8 conidia/ml appeared to be the same as the 1×10^9 conidia/ml and in that sense, could be considered as more efficient. Results also showed that treatment effect by immersion was significantly different from ingesting an infected pod ($F_{1,15} = 22.2$, $P < 0.0001$). Mortality rates obtained when contaminating insect with fungal suspension through direct contact (immersion) at 3.2×10^8 conidia/ml were always slightly higher than those obtained by indirect contact method (ingestion).

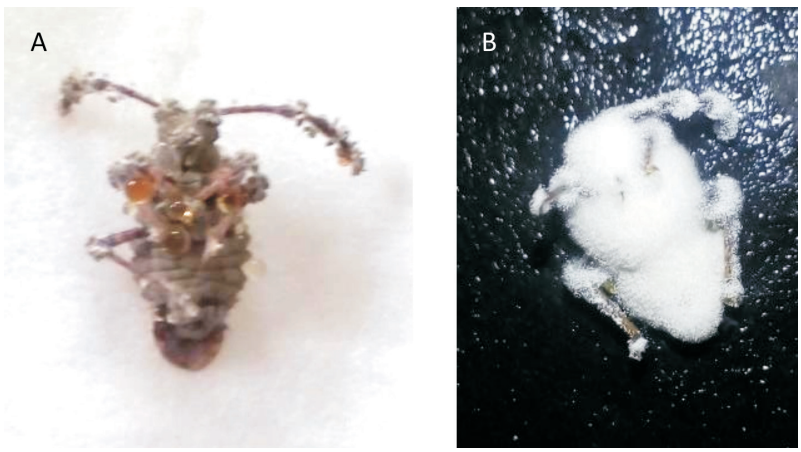

Fig. 3. *Sahlbergella singularis* infected by *Metarhizium anisopliae* (**A**) and *Beauveria bassiana* (**B**).

DISCUSSION

In nature, regulation of insect populations can be well achieved using key important factors, which are entomopathogenic fungi. With regard to cocoa, few studies have examined the harmful action of both *B. bassiana* and *M. anisopliae* against cocoa mirids (Tong-Kwee *et al*. 1989). Thus in this study, we have examined the entomopathogenic potential of several isolates of *B. bassiana* and by *M. anisopliae* against *S. singularis* nymphs.

The results of the present study demonstrated the ability of some isolates of *B. bassiana* and *M. anisopliae* to induce mortality of *S. singularis via* immersion and ingestion although the effectiveness varied depending on the conidial concentration of the fungi. When the fungal suspension of *B. bassiana* was applied by immersion of *S. singularis* at 1×10^7 conidia/ml, the induced mean mortalities ranged from 76.0–80.0 %. These results are different from those found by Abdulhai *et al*. (2010), who obtained mean mortalities from 86–100 % for *B. bassiana* isolates at 1×10^6 conidia/ml by immersion on *Eurygaster integriceps*, another Hemipteran. This difference could be supported either by the genotype of the strain involved, or by the difference in Hemipteran species. On the other hand, our isolates of *M. anisopliae* caused mortalities ranging from 60–97.1 % by immersion at 1×10^9 conidia/ml. This result could be compared to the ones of Khashaveh *et al*. (2011) who found 100 % of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) mortality 10 days after immersion in the *M. anisopliae* suspension at 1×10^9 conidia/ml. In previous laboratory studies using some local *B. bassiana* strains against *S. singularis*, through the ingestion method at 1×10^7 conidia/ml mortalities ranged from 35.7–100 % (Mahot 2006), which compares well with the results found in this study. This allows us to confirm that *B. bassiana* fungi could readily infect *S. singularis* and cause mortality through ingestion.

In addition, the highest mortality percentage generally occurred at highest concentration, and then decreased with a decline in inoculum concentration. Inoculum concentration is an important factor on the pathogenicity of entomopathogenic fungi (Demirci *et al*. 2011). In the Parker *et al*. (2003) study, *Isaria farinosa* (Holmskjold) Fries (Sordariomycetes: Hypocreales) was applied on pine litter and wheat plants at concentrations of 1×10^6 conidia/ml, 1×10^7 conidia/ml, and 1×10^8 conidia/ml and the percentage mortality of *E. integriceps* increased with increasing inoculum concentration. The same trend was observed in this study with the exception of some isolates (BIITAC6.2.2 and MIITAC6.2.2 by direct contact, and BIITAC8.1.5, BIITAC6.2.2 and MIITAC6.2.2 at 1×10^9 conidia/ml by ingestion), where the physiological stage of the insect could have been responsible for this deviation.

An important consideration in selecting a fungus strain is its virulence, which is the quantitative amount of death that a pathogen can incite in a group of insects (Inglis *et al*. 2001). Mortality caused by fungi, fungal sporulation on dead insects, the LC_{50}, LT_{50} and LT_{90} can be used to measure the level of virulence, when selecting beneficial fungal isolates.

The efficacy of fungi varied with isolates causing mortality from 6.7–100 %. Different isolates of the same species do not have equal potential for control of the same insect pest (Altre *et al*. 1999). Differences in potential of a strain's toxicity depended on physiological and enzymatic properties of each isolate (Leland *et al*. 2005). The fungal pathogenesis is a complex process and is dependent upon the attributes of both the pathogen and the host. The efficacy of isolates can also be attributed to their ability to germinate, to grow and to proliferate; when giving that germination, growth and proliferation within and outside a host are some stages that express the action of several entomopathogenic fungi (Sabbahi 2008; Mohammadbeigi 2012). Therefore, the outgrowth of fungi usually confirms that the insect is killed by fungal mycosis; it shows the fungal effectiveness and helps in the isolate's screening. During growth and proliferation, the isolates of *B. bassiana* and *M. anisopliae* rapidly colonise all tissues and organs while producing immune suppressor toxins that may damage haemocytes and cause insect paralysis and death (Flores-Villegas *et al*. 2016). These effects are possibly due to the ability of fungi to produce a collagenous coat of hyphal bodies that mask the recognition of β-1,3-glucans, the most powerful immune stimulant, by the insect's immune system (Sabbahi 2008; Flores-Villegas *et al*. 2016). Nevertheless, this effect is not very reliable as a test of virulence, given that insects may die from fungal mycosis and then be non-colonised by it because of competition by other saprophytic microorganisms (Inglis *et al*. 2012).

The lowest LC_{50} estimated by immersion method after 14 days of treatment in our study is 8.97×10^5 conidia/ml and 3.69×10^6 conidia/ml for the BITTAC10.3.3 and MITTAC6.4.2 isolates of *B. bassiana* and *M. anisopliae*, respectively. For the ingestion method, the lowest LC_{50} were estimated at 6.45×10^5 conidia/ml then at 2.09×10^7 conidia/ml, for these same isolates, respectively. These results stand in agreement with that of Liu *et al*. (2002) and Ziani (2008) who found that concentrations of 0.8×10^5 to 5.0×10^5 conidia/ml and 8.0×10^5 and 1.7×10^4 conidia/ml of some *B. bassiana* and *M. anisopliae* isolates were needed to cause 50 % mortality of *Lygus lineolaris* (Miridae). However, in their studies, between 8 and 6 days was necessary to reach this level, respectively, and less than ours, which was 14 days. This difference between our results may be related to the origins of fungal isolates, the type of insect hosts although both were Hemipteran. These same isolates of *B. bassiana* INRS-IP and INRS-CFL respectively killed 50 % of the nymphs in 4.77 and 5.03 days at 1×10^6 conidia/ml (Ziani 2008) while for our *Beauveria* isolates LT_{50} ranged from 4.1 to 5.1 days at concentration 3.2×10^8 conidia/ml by the immersion method. At the same conidial concentration used by the above-mentioned author, our LT_{50} could be lower or higher than that obtained. The expected difference between these LT_{50} values and ours could likely be attributed to the difference between the cuticle structures of insects. Cuticle types in various insects differ in their protein composition and level of sclerotisation (Charnley 2003). *S. singularis* cuticle could be less or more sclerotised than that of *L. lineolaris* and there is a need for this comparative study. The cuticle appears to influence all stages of the infection process: adhesion, germination and appersorium differentiation (Butt *et al*. 2001). Liu *et al*. (2002) also demonstrated the susceptibility of *L. lineolaris* nymphs to some fungal isolates. At 2×10^7 conidia/ml the values of LT_{50} were 2.5, 2.7, 3.4 and 4 days for *B. bassiana* isolates (ARSEF5665, ARSEF1394, ARSEF3769 and ARSEF0353, respectively) and 2.9 days for *M. anisopliae* isolate (ARSEF3540).

In conclusion, the importance of this study resides in the identification of virulent strains prior to field or large-scale use. This laboratory study provides preliminary evidence for the insecticidal potential and virulence of six indigenous *B. bassiana* and *M. anisopliae* isolates against *S. singularis* nymphs. Cameroonian entomopathogenic fungi are virulent by contact or by ingestion so the two methods are practical. When applying fungal biopesticide in the field, the target pest can be affected through direct contact on cuticle or by ingesting the infectious agent. Our results may help to improve the use of a biological control approach to control cocoa mirid in cocoa plantations and to contribute to IPM programmes. Cameroonian *B. bassiana* and *M. anisopliae* isolates are good candidates for further development as biological control agents for *S. singularis*.

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