

RESEARCH ARTICLE

Evaluation of the biocontrol potential of native *Metarhizium* (Hypocreales: Clavicipitaceae) species against the false codling moth (*Thaumatotibia leucotreta* Meyrick) on chilli peppers in Ghana

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The false codling moth (FCM, *Thaumatotibia leucotreta* Meyrick) is a major constraint on chilli pepper production and export in Ghana. Although chemical pesticides remain the main control strategy, their effectiveness is limited and export restrictions on synthetic residues highlight the need for sustainable alternatives. To address this challenge, the virulence of seven native *Metarhizium* isolates (UGKAP1, UGJKCS9, UGJKCS10, UGNAK1, UGAFMF20, UGAFMF8 and UGSUHC1) obtained from agricultural fields in Ghana against final instar FCMs at 1×10^8 conidia/mL was evaluated via conidial-sand assays, and compared with that of two USDA ARSEF isolates (*Beauveria bassiana* ARSEF 252 and *Metarhizium anisopliae* sensu lato ARSEF 4570). As abiotic environmental factors can affect fungal efficacy, the influence of humidity on the infectivity of four selected isolates (UGJKCS9, UGJKCS10, UGSUHC1 and UGAFMF20) was assessed. All seven native isolates caused > 80% pupal mortality within 21 days. The pupal mortality rates of ARSEF 252 and ARSEF 4570 were 49% and 57%, respectively. The most virulent isolate, UGJKCS9, exhibited an LC_{50} of 2.7×10^6 conidia/mL and an LT_{50} of 3 days. High pupal mortality (82–100% at 10^5 and 10^7 conidia/mL) occurred across all tested humidity levels (43%, 75% and 98% RH) for all four isolates. These results indicate that the tested isolates, particularly UGJKCS9, exhibit strong potential as sustainable alternative control agents for FCMs in Ghana, warranting further evaluation under field conditions for integration into chilli pepper pest management programmes.

INTRODUCTION

The chilli pepper (*Capsicum annum* L.) (Solanales: Solanaceae) is a key component of the daily diet in Ghana and the third most widely cultivated crop in the country, with annual production exceeding 100 000 metric tonnes (GSS 2014; MoFA-IFPRI 2020). Beyond their strong domestic demand, chilli peppers are amongst Ghana's major vegetable exports to the European Union (EU), where the demand, particularly for the Legon 18 variety, valued for its distinctive flavour and long shelf life, continues to increase (GEPA 2017). Consequently, the crop is grown throughout the year across all regions, with the Northern, Eastern and Volta regions being the major production hubs (MOFA-IFPRI 2020).

The false codling moth (FCM) (*Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae)) is a significant barrier to chilli pepper exports, as it is classified amongst the top 20 priority quarantine pests by the EU, Ghana's principal export destination for this crop (EUROPHYT 2014; Fening et al. 2020). Domestically, the FCM also imposes substantial production constraints. The adults lay their eggs on fruits. The neonate larvae burrow into the fruit, where they develop to the final (fifth) instar stage, feeding on the flesh and seeds and causing premature ripening, fruit drop and decay, leading to considerable yield losses (Adom et al. 2024a). The cycle continues with the final instar larvae exiting the fruit to pupate in the soil, followed by adults eclosing from the pupae to oviposit on new fruits. The EU banned chilli pepper exports from Ghana between 2015 and 2017 owing to repeated interceptions of infested produce (Fening et al. 2020). This embargo and the restriction of chilli peppers and two other FCM-infested vegetables (gourds and eggplants) led to an estimated export revenue loss of approximately US\$ 30 million (EPA Monitoring 2017). These regulatory challenges have significantly curtailed Ghana's chilli pepper export volumes and earnings. Export statistics from 2010 to 2014 indicated volumes ranging from approximately 984 to 1 080 metric tonnes, with earnings of US\$ 350 442 to 1 184 964 (GEPA 2021; GIRSA 2023). In contrast, from 2018 to 2021, exports dropped markedly, from US\$ 351 000 to US\$ 87 000 (GEPA 2017; GIRSA 2023).

Pyrethroids, organophosphates, neonicotinoids and oxadiazines are the classes of synthetic insecticides commonly used for FCM control in Ghana (Fening et al. 2016, 2017). However, their effectiveness is limited due to the short window for targeting the pest's concealed eggs and neonates on fruit surfaces. Moreover, the use of conventional pesticides faces strict regulatory scrutiny in export markets and raises environmental, health and non-target safety concerns (Wan et al. 2025).

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DATES

Received: 4 November 2025

Accepted: 30 January 2026

KEYWORDS

entomopathogenic fungi
humidity
mycopesticide
pathogenicity
virulence

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These limitations highlight the need for practical, sustainable and non-chemical management options. Whilst entomopathogenic bacterial-based products have proven effective against the above-ground life stages of the FCM in Ghana (Adom et al. 2024b), additional control agents targeting the soil-dwelling stages are required. This has prompted interest in native entomopathogenic fungi (EPF) as complementary biocontrol agents.

EPF are an important group of biological agents utilised worldwide to control arthropod pests (de Faria & Wraight 2007; Lacey et al. 2015; Maina et al. 2018; Hatting et al. 2019; Acheampong et al. 2023; Luke et al. 2023). These fungi, particularly the commonly commercialised species, *Beauveria bassiana* (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae), occur naturally in soils and have shown great promise as FCM control agents under laboratory and field conditions in some African countries, especially in the citrus industry in South Africa (Goble et al. 2011; Coombes et al. 2013, 2015, 2016). However, these microbial control agents are susceptible to abiotic environmental constraints, especially ultraviolet (UV) radiation, temperature and humidity (Acheampong et al. 2020a; Couceiro et al. 2021; Quesada-Moraga et al. 2024; Nyahe et al. 2025). The use of native EPF over similar commercial products from other countries is also important as their tolerance to environmental constraints can be dependent on their geoclimatic origins (Braga et al. 2001; Bidochka et al. 2002; Rangel et al. 2005; Fernandes et al. 2007, 2015; Bihal et al. 2023; Sharma et al. 2023). Furthermore, using native strains supports biodiversity conservation and may be significantly cheaper (avoiding import costs and regulatory barriers, adapted to local environments and reducing development and production expenses) and more easily accepted by local farmers (Rajula et al. 2021). Consequently, the search for EPF as biological control agents for the FCM was initiated in 2023.

One *Metarhizium* isolate (UGKAP1) obtained from a chilli pepper farm in Ghana, and six other Ghanaian *Metarhizium* isolates (UGJKCS9, UGJKCS10, UGAFMF8, UGAFMF20, UGNAKC1 and UGSUHC1) obtained from maize and cocoa farms (Table 1) have demonstrated significant *in vitro* infectivity against key insect pests in Ghana. Although these seven selected *Metarhizium* isolates are susceptible to UV radiation (Nyahe et al. 2025), it may not pose a significant environmental challenge for these isolates when used against FCMs, as the fungi would be applied to the soil to target the soil-dwelling stages (final instar larvae and pupae). Likewise, temperature may not be critical as soil temperatures within the top 10 cm, where these pre-pupal stages typically occur, are generally stable. In a previous study, temperature did not negatively affect EPF performance against FCMs, albeit under a different climate (South Africa) (Coombes et al. 2013, 2016). However, soil humidity could play a more critical role in field success. High relative humidity (RH) (above 90%) is essential for EPF germination, growth and infectivity (Luz & Fargues 1999; Shipp et al. 2003; Mishra et al. 2015; Sabri et al. 2022), and for subsequent sporulation and persistence in the environment (Arthurs et al. 2001; Roy et al. 2006; Jaronski 2010). Humidity requirements, however, can vary amongst isolates as some have demonstrated high infectivity even at moderate ambient RH (43–55%) (Michalaki et al. 2006; Athanassiou & Steenberg 2007; Athanassiou et al. 2017; Acheampong et al. 2020b). Understanding the specific humidity needs of each EPF isolate will be essential for selecting the most suitable candidate for development into a mycoinsecticide with reliable performance across Ghana's various agroecological zones. While EPF should be environmentally competent (Quesada-Moraga et al. 2024), there is also a need for them to retain their virulence against a targeted pest. These seven EPF have shown high efficacy against fruit flies and other key pests of crops in Ghana, including the larger grain borer (*Prostephanus truncatus* (Horn)) (Coleoptera: Bostrichidae), the maize weevil

Table 1. Isolation details and geographical origins of native and exotic entomopathogenic fungal isolates used in this study

Isolate ¹	Species	GenBank accession number ²	Farm type	Substrate	Year of isolation	GPS location coordinates
UGJKCS9	<i>Metarhizium anisopliae</i> s.l.	*	Cocoa	Soil	2024	5°15'55" N, 1°20'26" W
UGJKCS10	<i>Metarhizium</i> sp.	PQ778821	Cocoa	Soil	2024	5°15'54" N, 1°20'24" W
UGSUHC1	<i>Metarhizium</i> sp.	PQ800172-PQ800173	Cocoa	Soil	2023	6°03'31" N, 0°24'10" W
UGNAKC1	<i>Metarhizium</i> sp.	PQ781266	Cocoa	Soil	2023	6°04'35" N, 0°22'27" W
UGKAP1	<i>Metarhizium</i> sp.	PQ805341-PQ805342	Chilli pepper	Soil	2023	6°35'19" N, 0°49'42" W
UGAFMF8	<i>Metarhizium</i> sp.	PQ778958	Maize	Soil	2024	05°46'57" N, 0°36'31" E
UGAFMF20	<i>Metarhizium anisopliae</i> s.l.	Not applicable	Maize	Soil	2024	5°47'01" N, 0°36'38" E
ARSEF 252	<i>B. bassiana</i>	Unknown	Green house	<i>Leptinotarsa decemlineata</i> (Coleoptera: Chrysomelidae)	1978	1°16'50" W, 10°14'53" N
ARSEF 4570	<i>Metarhizium anisopliae</i> s.l.	Unknown	Macquarie Island	Soil	1993	0°01'35" W, 9°29'59" N

¹UG = University of Ghana; JUKCS = Jukwa Cocoa Sample; SUHC = Suhum Cocoa; NAKC1 = Nankese Cocoa; KAP = Kwaku Appiah Pepper; AFM = Ada Foah Maize. ²Molecular analyses based on the ITS1 and ITS4 regions. * *Metarhizium anisopliae* sensu lato.

(*Sitophilus zeamais* (Motschulsky)) and a cocoa mirid (*Distantiella theobroma* (Distant)) (Hemiptera: Miridae) (MA Acheampong, pers. comm.); however, the variability in pathogenicity between fungal strains does need to be determined. Thus in this study, the virulence of these seven *Metarhizium* isolates as control agents for FCMs was investigated and compared with that of two isolates from the USDA ARSEF culture collection (ARSEF 252 and 4570). In addition, the humidity required for infectivity was determined in four selected isolates (UGJKCS9, UGJKCS10, UGSUHC1 and UGAFMF20).

MATERIALS AND METHODS

Source and culture conditions of insects and fungal isolates

All final instar FCM larvae used in this study were obtained from the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, where a continuous colony is maintained on an FCM artificial larval diet (Moore et al. 2014). The seven indigenous *Metarhizium* isolates were sourced from the Entomopathology Laboratory at ARPPIS, where their dried conidia are preserved on Sabouraud dextrose agar (SDA) at 4 °C. These EPF were isolated from soils collected from chilli pepper, maize and cocoa farms in the Central, Eastern and Greater Accra regions of Ghana. *Galleria mellonella* L. (Lepidoptera: Pyralidae) was used as the bait insect following the protocol described by Goble et al. (2010) (Table 1). *Beauveria bassiana* ARSEF 252 and *M. anisopliae* s.l. ARSEF 4570 isolates were obtained from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF) of the US Plant, Soil and Nutrition Laboratory, Ithaca, New York, USA. All isolates were passed through fifth instar FCM larvae once prior to use, following the protocol described by Acheampong et al. (2020a). Conidia obtained from dead insects were subsequently cultured on SDA, stored at 4 °C and used as stock cultures for all assays.

Determination of the virulence of native *Metarhizium* isolates against the FCM

Pathogenicity assays

The pathogenicity of the native *Metarhizium* isolates against the FCM was assessed using conidial-sand bioassays adapted from Goble et al. (2010) and Coombes et al. (2015). Fungal cultures were grown on SDA (65 g/L) for 14 days at 25 ± 2 °C and 70 ± 5% RH under a 12 h photoperiod. Conidia were suspended in sterile distilled water containing 0.01% Tween 20 and adjusted to 1 × 10⁸ conidia/mL using a Neubauer haemocytometer. The germination rates of all isolates exceeded 80% after 24 h incubation. Briefly 50 µL conidial suspensions of each isolate at 1 × 10⁵ conidia/mL were plated on SDA and incubated under the same growing conditions described above. The percentage conidial germination (out of 300 conidia assessed) was evaluated after 24 h.

For each isolate, 10 final instar larvae were introduced into each of four replicate Petri plates containing 50 g of sterile sand (oven-dried at 70 °C for 6 h and autoclaved three times at 121 °C for 15 min). Each plate was inoculated with 5 mL of conidial suspension, while controls (four replicates) received sterile distilled water with 0.01% Tween 20. Treatments were arranged in a completely randomised design and maintained at 25 ± 2 °C and 70 ± 5% RH under a 12 h photoperiod for 7 days.

After exposure, the pupae were transferred into Petri plates containing untreated sterile sand. Adult emergence chambers were constructed by inverting 500 mL transparent plastic cups over the plates, each with a cotton-plugged hole moistened with sterile water. The plates were placed on trays containing sterilised perlite to maintain a high ambient humidity conducive to fungal infection.

Mortality was recorded daily until 10 days after the first adult emergence. All dead insects (individuals which did not move

following prodding with an entomological brush) in the fungal treated sand set-up, as well as those in the non-inoculated sterile sand, were removed daily. Mycosis was confirmed by surface-sterilising the cadavers in 0.5% sodium hypochlorite (3.5% active ingredient) and 70% ethanol for 2 min each, then incubating them on moistened filter paper in sterile Petri plates at 25 °C for 7 days. The proportion of cadavers that had fungal overgrowth (the same as the inoculated EPF, identified by examination of the sporulating structures under a compound microscope) represented the percentage of mycosis. The experiment was repeated twice using fresh fungal cultures.

Concentration–mortality response of FCMs to selected EPF

Based on the pathogenicity results, four *Metarhizium* isolates (UGJKCS9, UGJKCS10, UGAFMF20 and UGSUHC1) that produced high mortality and mycosis rates were selected for concentration–mortality trials. Conidial suspensions were prepared as described above and adjusted to 1 × 10⁵, 1 × 10⁶, 1 × 10⁷ and 1 × 10⁸ conidia/mL. The same bioassay procedures and incubation conditions were used as in the pathogenicity assays.

Exposure time response of FCMs to selected EPF

The relationship between exposure duration and FCM mortality was examined for the same four isolates (UGJKCS9, UGJKCS10, UGAFMF20 and UGSUHC1) using conidial-sand bioassays. Larvae were exposed to 1 × 10⁸ conidia/mL and monitored at six post-inoculation intervals: 12, 24, 48, 72, 96 and 120 h. Mortality and mycosis were recorded as previously described.

Humidity–mortality response of FCMs to selected EPF

Humidity–mortality assays were conducted to assess the effect of RH on the pathogenicity of the four *Metarhizium* isolates selected above, following the protocol of Acheampong et al. (2020b) with minor modifications. Target RH of 43%, 75% and 98% were generated using saturated solutions of K₂CO₃, NaCl and K₂SO₄, respectively (Greenspan 1977). The solutions were placed in 500 mL plastic containers within 5 L transparent chambers in an incubator (with no light exposure) and maintained at 27 °C. Humidity was monitored using a thermo-hygrometer (REED R6030) and remained within ± 1% of the target values for the 21-day experimental period.

Each isolate was tested at 1 × 10⁵ and 1 × 10⁷ conidia/mL. For each treatment, 10 fifth instar larvae were placed in four replicate Petri plates and sprayed with 2 mL of conidial suspension. After air-drying under a laminar flow hood for 1 h, each larva was transferred individually into a separate sterile 20 mL glass vial and the vials placed in the respective humidity chambers. Larval and pupal mortality were recorded daily. Adult mortality was not considered, as most pre-pupating larvae and pupae died within 14 days. Sporulation was observed directly on cadavers across treatments, hence surface sterilisation was not repeated. Each experiment was replicated twice using freshly prepared conidial suspensions.

Statistical analyses

FCM final instar larvae ready to pupate within a day were used in all assays; therefore, the number of dead larvae and pupae was combined as one insect stage, pupae. The few adults that died showed no mycosis and were therefore excluded from the analysis. Mortality, mycosis and adult emergence data in the pathogenicity assays were non-normal even after transformation; therefore, they were analysed using a generalised linear mixed model with a binomial distribution, implemented in the *glmmTMB* package (Brooks et al. 2017). This model provided the best fit based on the Akaike Information Criterion and the Likelihood Ratio or Wald chi-squared tests, outperforming generalised linear models (GLM) (Acheampong et al. 2020a).

Analysis of deviance (ANODEV) was followed by post hoc pairwise comparisons using the *emmeans* package (Lenth 2024) with Tukey's HSD adjustment ($\alpha = 0.05$).

For dose-response analyses, mortality data were fitted to generalised linear models and median lethal concentrations (LC_{50}), and times (LT_{50}) were estimated using the *dose.p* function in the *MASS* package (Venables & Ripley 2002). Pairwise comparisons of LC_{50} and LT_{50} values between fungal isolates were performed using lethal ratio tests (ecotox package; Wheeler et al. 2006).

Humidity-mortality data (cumulative larval and pupal death) were first combined for the two concentrations and analysed using GLMs with binomial error structures to determine the interaction effect. The data at each concentration were subsequently fitted to the same logistic regressions in the GLMs, followed by ANODEV and Tukey-adjusted pairwise comparisons using *emmeans*. All analyses were conducted in R v4.4.2 (R Core Team 2024).

RESULTS

Pathogenicity assays

Effects of EPF on adult FCM emergence

Adult FCM emergence differed significantly amongst the fungal treatments (LRT, $\chi^2 = 246.81$, $df = 9$, $p < 0.001$) and ranged from 3.3% to 18.3% for the seven native *Metarhizium* isolates (Figure 1). Two isolates, UGJKCS10 and UGAFMF20, reduced adult emergence to below 4%. However, their emergence rates did not differ significantly from the remaining native isolates, except for UGKAP1, which recorded slightly higher emergence (18.3%). All seven native isolates significantly reduced adult emergence compared to the two USDA isolates (ARSEF 252 and ARSEF 4570). The highest adult emergence occurred in the untreated control (95.3%), which was significantly higher than that in all fungal treatments (Figure 1).

Effects of EPF on pupal FCM mortality

Pupal mortality also varied significantly across treatments (LRT, $\chi^2 = 246.81$, $df = 9$, $p < 0.001$). All seven native *Metarhizium* isolates caused high pupal mortality (81.7–96.7%), with no significant differences amongst them, except for UGKAP1, which caused significantly lower mortality than UGJKCS9, UGJKCS10 and UGAFMF20 (Figure 2). The mortality caused by all seven native *Metarhizium* isolates was significantly higher than that caused by the ARSEF isolates. Mortality in the non-inoculated control remained below 5% (Figure 2).

Effects of EPF on pupal FCM mycosis

No mycosis was observed on the cadavers of the non-inoculated control group. Pupal mycosis in the fungal-treated sand differed amongst isolates (LRT, $\chi^2 = 80.60$, $df = 8$, $p < 0.001$) and ranged from 56.4% to 75.8% for the treatments with native isolates (Figure 3). The highest levels of mycosis were recorded in the UGSUHC1 and UGJKCS9 treatments, but these were not significantly different from those of the native isolate treatments, except for UGKAP1 which caused significantly less mycosis than UGSUHC1. The pupal mycosis of the two ARSEF isolate treatments did not exceed 45% and was significantly lower than that of the native isolate treatments, with the exception of the UGKAP1 group which did not differ statistically from the ARSEF isolate groups (Figure 3).

Concentration-mortality response of FCMs to selected EPF isolates

Four native isolates (UGJKCS9, UGJKCS10, UGAFMF20 and UGSUHC1) which caused high mortality and mycosis in the pathogenicity assay were tested at four conidial concentrations (1×10^5 , 1×10^6 , 1×10^7 and 1×10^8 conidia/mL) to determine the LC_{50} . UGJKCS9 exhibited the highest virulence, with the

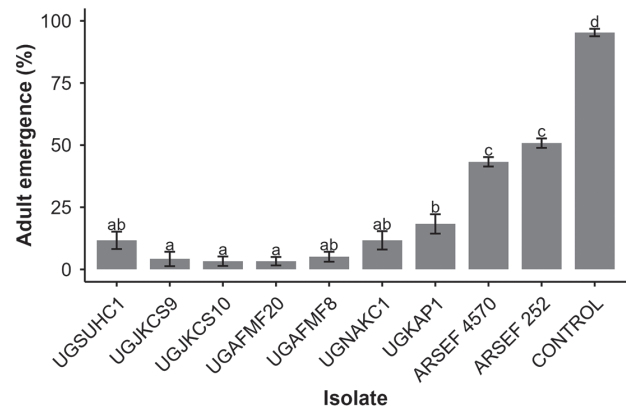


Figure 1. Mean percentage adult emergence of *Thaumatotibia leucotreta* fifth instar larvae treated with entomopathogenic fungal isolates at 10^8 conidia/mL and incubated for 21 days at $25 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH under a 12 h photoperiod. Error bars represent the standard errors of three independent experiments, each conducted with a fresh batch of conidia. Bars followed by the same lowercase letter are not significantly different ('emmeans' adjusted with Tukey's HSD test using $p > 0.05$).

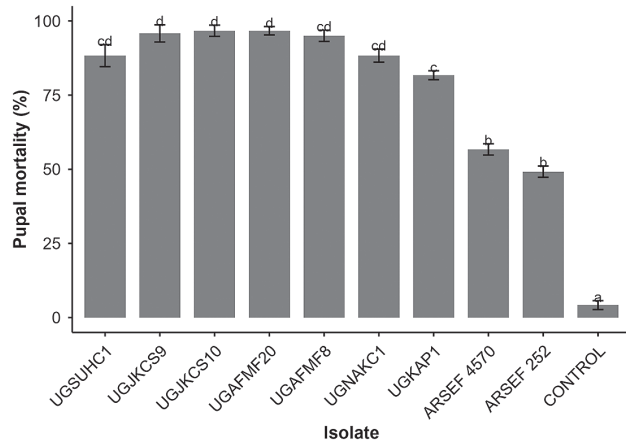


Figure 2. Mean percentage pupal mortality of *Thaumatotibia leucotreta* fifth instar larvae treated with entomopathogenic fungal isolates at 10^8 conidia/mL and incubated for 21 days at $25 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH under a 12 h photoperiod. Error bars represent the standard errors of three independent experiments, each conducted with a fresh batch of conidia. Bars followed by the same lowercase letter are not significantly different ('emmeans' adjusted with Tukey's HSD test using $p > 0.05$).

lowest LC_{50} (2.7×10^6 conidia/mL), followed by UGJKCS10 (5.8×10^6 conidia/mL), UGAFMF20 (8.1×10^6 conidia/mL) and UGSUHC1 (9.9×10^6 conidia/mL) (Figure 4). The lethal ratio test showed no significant differences in the LC_{50} values between isolates (Table 2).

Exposure time response of FCMs to selected EPF

The same four isolates were also evaluated for time-mortality relationships. Larvae were exposed to 1×10^8 conidia/mL and monitored at 12, 24, 48, 72, 96 and 120 h post-inoculation. The estimated LT_{50} values for all isolates were approximately 3 days, indicating similar rates of infection and mortality (Table 3). The lethal ratio test showed no significant differences in the LT_{50} values between isolates (Table 4).

Humidity-mortality response of FCMs to selected EPF

The three-way ANODEV showed that the interaction amongst treatment, concentration and humidity did not significantly affect pupal mortality (LRT, $\chi^2 = 6.88$, $df = 8$, $p = 0.549$). However, the two-way interaction between treatment \times humidity (LRT, $\chi^2 = 18.71$, $df = 8$, $p = 0.016$) was significant.

The interaction of fungal isolate and humidity induced consistently high mortality in all four *Metarhizium* isolate treatments at both conidial concentrations (Tables 5 and 6). At the lower conidial concentration (10^5 conidia/mL), mortality ranged from 81.7 to 94.2% and did not differ significantly amongst isolates at any humidity level (Table 5). At the higher conidial concentration (10^7 conidia/mL), mortality ranged from 88.3% to 100% and remained statistically similar across isolates at all humidity levels tested (Table 6). Control mortality remained low (5%) and was significantly lower than that in any of the fungal treatments at either conidial concentration (Tables 5 and 6).

DISCUSSION

To sustain production and meet export demand, microbial control agents are needed to complement other non-chemical strategies being developed to control FCMs on chilli peppers in

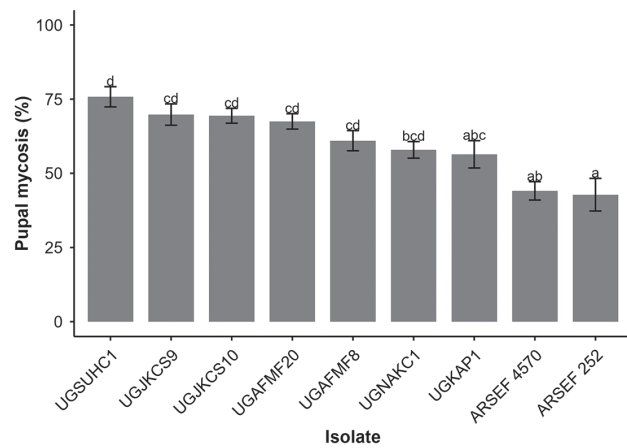


Figure 3. Mean percentage pupal mycosis of *Thaumatotibia leucotreta* fifth instar larvae treated with entomopathogenic fungal isolates at 10^8 conidia/mL and incubated for 21 days at $25 \pm 2^\circ\text{C}$, $70 \pm 5\%$ RH under a 12 h photoperiod. Error bars represent the standard errors of three independent experiments, each conducted with a fresh batch of conidia. Bars followed by the same lowercase letter are not significantly different ('emmeans' adjusted with Tukey's HSD test using $p > 0.05$).

Ghana. EPF were therefore identified as a possible solution to address this urgent need. The conidial-sand bioassays conducted in this study demonstrated that selected native *Metarhizium* isolates possess high pathogenic potential against FCMs on chilli peppers in Ghana. Exposure of pre-pupae FCMs to Ghanaian *Metarhizium* isolates induced high pupal mortality, significantly reducing adult emergence. These findings closely align with the work of Goble et al. (2011) and Coombes et al. (2015), who reported high mortality rates ($> 80\%$) of final instar FCM larvae following similar assays with South African *B. bassiana* and *M. anisopliae* isolates at 1×10^7 conidia/mL.

UGJKCS9, UGJKCS10 and UGAFMF20 exhibited the highest pathogenicity ($\sim 96\%$ within 21 days) amongst the tested isolates. Whilst the LT_{50} was approximately 3 days for all the tested isolates, the lowest LC_{50} (2.7×10^6 conidia/mL) was recorded for

Table 2. Pairwise comparisons of the LC_{50} values between the four *Metarhizium* isolates used in the concentration–response bioassays against *Thaumatotibia leucotreta* fifth instar larvae.

Isolate	Estimate ¹	z-ratio	p-value
UGJKCS9-UGJKCS10	-3.2×10^6	0.00	1.000
UGJKCS9-UGSUHC1	-7.3×10^6	0.00	1.000
UGJKCS9-UGAFMF20	-5.5×10^6	0.00	1.000
UGJKCS10-UGSUHC1	-4.1×10^6	0.00	1.000
UGJKCS10-UGAFMF20	-2.3×10^6	0.00	1.000
UGSUHC1-UGAFMF20	1.8×10^6	0.00	1.000

¹The estimate is the difference in the LC_{50} values for each comparison

Table 3. LT_{50} of *Thaumatotibia leucotreta* fifth instar larvae treated with native *Metarhizium* isolates at 10^8 conidia/mL for 24, 48, 72, 96 and 120 h at $25 \pm 2^\circ\text{C}$.

Isolate	LT_{50} (days \pm SE)
UGJKCS9	3.1 (0.3)
UGJKCS10	3.2 (0.3)
UGAFMF20	3.3 (0.3)
UGSUHC1	2.9 (0.3)

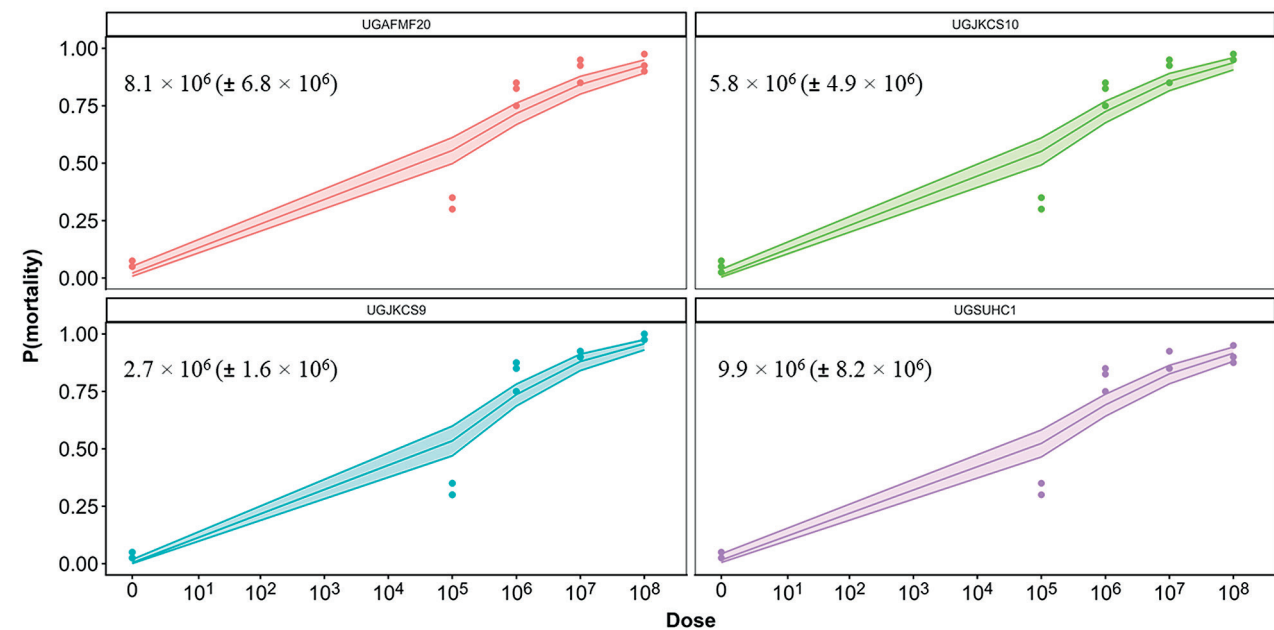


Figure 4. Probit regression dose–response curves for mortality of *Thaumatotibia leucotreta* pre-pupating larvae exposed to the log dose of four concentrations (conidia/mL) of the four most virulent tested *Metarhizium* isolates used in the dose–mortality assays. The central lines represent the average probability of mortality [P(mortality)], while the shaded regions show the 95% confidence intervals around the mean. Individual data points are displayed as dots. Each panel corresponds to a specific fungal isolate. The values shown are the median lethal concentrations ($LC_{50} \pm$ SE).

Table 4. Pairwise comparisons of the LT_{50} values between the four *Metarhizium* isolates used in the time–response bioassays against *Thaumatotibia leucotreta* fifth instar larvae.

Isolate	Estimate ¹	z-ratio	p-value
UGJKCS9-UGJKCS10	-0.13	0.07	0.941
UGJKCS9-UGSUHC1	0.25	0.17	0.868
UGJKCS9-UGAFMF20	-0.14	0.08	0.935
UGJKCS10-UGSUHC1	0.38	0.24	0.810
UGJKCS10-UGAFMF20	-0.01	0.01	0.994
UGSUHC1-UGAFMF20	-0.39	0.25	0.803

¹The estimate is the difference in the LT_{50} values for each comparison

Table 5. Pupal mortality of *Thaumatotibia leucotreta* fifth instar larvae exposed to various fungal isolates at 10^5 conidia/mL and three relative humidity levels for 21 days at 25 ± 2 °C.

Treatment	Mean ¹ % mortality (\pm SE) at the three humidity levels tested		
	43%	75%	98%
UGJKCS9	94.2 (1.5) b	85.0 (1.5) b	91.7 (1.1) b
UGJKCS10	90.8 (0.8) b	88.3 (1.1) b	85.8 (1.5) b
UGAFMF20	89.2 (0.8) b	90.0 (0.0) b	90.8 (0.8) b
UGSUHC1	81.7 (1.1) b	90.0 (0.0) b	85.0 (1.5) b
CONTROL	5.0 (1.5) a	5.0 (1.5) a	5.0 (1.5) a

¹Means with the same lowercase letter are not significantly different (Tukey's HSD test, $p > 0.05$).

Table 6. Pupal mortality of *Thaumatotibia leucotreta* fifth instar larvae exposed to various fungal isolates at 10^7 conidia/mL and three relative humidity levels for 21 days at 25 ± 2 °C.

Treatment	Mean ¹ % mortality (\pm SE) at the three-humidity level tested		
	43%	75%	98%
UGJKCS9	95.9 (1.5) b	88.3 (1.1) b	98.3 (1.1) b
UGJKCS10	96.7 (1.4) b	90 (0.0) b	92.5 (1.3) b
UGAFMF20	100 (0.0) b	92.7 (1.3) b	98.3 (1.1) b
UGSUHC1	92.5 (1.3) b	91.7 (1.1) b	100 (0.0) b
CONTROL	5.0 (1.5) a	5.0 (1.5) a	5.0 (1.50) a

¹Means with the same lowercase letter are not significantly different (Tukey's HSD test, $p > 0.05$).

UGJKCS9. Goble et al. (2011) reported a similar LC_{50} (2.1×10^6 conidia/mL) for a virulent *B. bassiana* isolate in their conidial-sand assays against the FCM. Likewise, Coombes et al. (2015) recorded an LC_{50} of 1.92×10^6 conidia/mL for a virulent *M. anisopliae* in a concentration–mortality response against the FCM. The variation in mortality amongst isolates may result from genetic and physiological differences that control virulence factors, such as conidial adhesion ability, enzymatic secretion (proteases, chitinases and lipases) and the capacity to overcome host immune defences (Charnley 2003; Leland et al. 2005; Nicoletti & Becchimanzi 2022; Ma et al. 2024).

Although adult mortality is not reported in this study, the few adults that died showed no mycosis. The absence of mycosis on adult cadavers indicates that the larval and pupal stages are the most susceptible to fungal infection, as supported by earlier studies on the FCM (Goble et al. 2011; Coombes et al. 2015) and other Lepidopteran insect pests (Omar et al. 2021; Louw et al. 2025). The sclerotisation of the adult cuticle provides greater resistance to infection (Carstens & Moore 2020). The high susceptibility of the subterranean stages of the targeted pest is also beneficial, as abiotic efficacy-impacting parameters (such as UV radiation and temperature) do not generally affect the performance of

EPF in the soil environment where they occur (Coombes et al. 2013, 2016). The potential control of the subterranean stages will ultimately reduce adult populations and crop infestation in the field, consequently reducing interceptions in trade.

The humidity–mortality response assay showed that the *Metarhizium* isolates induced consistently high pupal mortality across the tested humidity levels (43%, 75% and 98% RH). Interestingly, mycosis was observed on all cadavers across the humidity levels tested. This contradicts previous studies in which external sporulation on FCMs was only observed on cadavers under 98% humidity (Acheampong et al. 2020b). Ramoska (1984) also observed external sporulation on cadavers incubated at above 75% RH in similar studies against the clinch bug, *Blissus leucopterus* Say (Hemiptera: Lygaeidae). Comparable EPF infectivity at low ambient humidities has been reported by several authors (Michalaki et al. 2006; Athanassiou & Steenberg 2007; Athanassiou et al. 2017; Acheampong et al. 2020b). Such resilience is advantageous for field applications, especially in tropical and sub-tropical regions where relative humidity fluctuates throughout the growing season. This resilience could be possible because these native strains evolved with tolerance for varied humidity, another reason for exploring native strains. The humidities tested in the present study are reflective of ambient RH in Ghana; however, according to three-month RH datasets from two key pepper growing districts in the Volta region of Ghana (Peki and Adidome), where field trials are currently being conducted, RH ranged between 79 and 90%, averaging 85% in the major growing season for chilli peppers (July to September) this year (NASA POWER, 2025). This suggests that humidity may not limit efficacy if the virulence of the native *Metarhizium* isolates, particularly UGJKCS9, is replicated in the field. Their ability to remain infective under diverse humidity regimes could ensure consistent performance in both irrigated and rain-fed chilli pepper systems in Ghana.

Globally, there is a growing shift toward the adoption of microbial control agents in sustainable pest management approaches. EPF-based products have been incorporated into integrated pest management programmes in several countries to reduce chemical pesticide reliance (Maina et al. 2018; Hatting et al. 2019; Bihal et al. 2023; Chowdhury et al. 2023; Mesquita et al. 2023). The high pathogenicity of the native *Metarhizium* isolates warrants further evaluation under field conditions to determine their persistence, environmental tolerance, efficacy and compatibility with other biological control agents. The observed rapid mortality within a few days post-exposure also suggests their potential use for curative pest management through inundative application where immediate population suppression is desired. These native isolates also performed better than the ARSEF strains, which supports the need for environmentally competent strains. A difference of approximately 40% in pupal mortality was noted between the native and ARSEF strains. If these isolates are to be applied to the soil, the degree of formulation that may be required is likely to be low, which would be economical (Jaronski 2010, 2014, 2023). The fact that these isolates sporulate across all the tested humidity levels is also beneficial for persistence.

CONCLUSION

The results of the current study have demonstrated the potential of seven Ghanaian *Metarhizium* isolates as effective control agents against FCMs infesting chilli peppers. All isolates caused more than 80% pupal mortality within 21 days. The most virulent isolate, UGJKCS9, exhibited an LC_{50} of 2.7×10^6 conidia/mL and an LT_{50} of 3 days. High pupal mortality (82%–100% at 10^5 and 10^7 conidia/mL) was recorded across all tested humidity levels (43%, 75% and 98% RH). These findings suggest that the tested isolates, particularly UGJKCS9, hold strong promise as

an additional sustainable integrated pest management tool for managing FCMs in Ghana and warrant further testing in the field and subsequent commercialisation.

CONFLICT OF INTEREST

There are no conflicts of interest.

ACKNOWLEDGEMENTS

This work was carried out with the aid of a grant from UNESCO and the International Development Research Centre (IDRC), Canada (Grant Number 4500476458). The views expressed herein do not necessarily represent those of UNESCO-TWAS, OWSD (Organization for Women in Science for the Developing World), IDRC or its Board of Governors. The article processing fee was funded by OWSD. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHOR CONTRIBUTIONS

DA: Investigation, Writing – original draft, Writing – review & editing. VYE: Methodology, Supervision, Writing – review & editing, Validation. LA-A: Resources, Validation, Writing – review & editing. PASN: Investigation, Writing – review & editing. CAC: Methodology, Resources, Formal analysis, Writing – review & editing. MA: Resources, Writing – review & editing. KOF: Methodology, Supervision, Writing – review & editing, Validation. MKB: Methodology, Supervision, Writing – review & editing, Validation. MYO: Methodology, Supervision, Writing – review & editing, Validation. DNER: Resources, Writing – original draft. DS: Resources, Writing – original draft, Writing – review & editing. OFA: Resources, Formal analysis, Writing – review & editing. MAA: Conceptualisation, Funding acquisition, Methodology, Resources, Investigation, Supervision, Writing – original draft, Writing – review & editing, Visualisation, Project administration, Validation.

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