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ORIGINAL PAPER

Selection of fungal isolates for virulence against three aphid pest species of crucifers and okra

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Abstract Aphids are regarded as important pest problems of vegetable crops worldwide. Most vegetable growers in sub-Saharan Africa heavily rely on synthetic chemical insecticides for aphids' control. Fungus-based biopesticides are being considered as alternatives to chemical insecticides. This study evaluates virulence of five isolates of *Metarhizium anisopliae* and three of *Beauveria bassiana* against *Brevicoryne brassicae*, *Lipaphis pseudobrassicae*, and *Aphis gossypii*, and their thermotolerance and conidial yield as a prerequisite for strain selection. The study also evaluates performance of the best isolate in screenhouse experiment against target aphid species. Three isolates of *M. anisopliae* ICIPE30, ICIPE62, and ICIPE69 outperformed the others, causing mortality of 85–98 %, 83–97 %, and 73–77 %, in *B. brassicae, L. pseudobrassicae*, and *A. gossypii*, respectively, 7 days post-inoculation. Isolate ICIPE62 had the shortest LT_{50} values of 3.4, 2.5, and 2.6 days at 1×10^8 conidia ml^{-1}, and the lowest LC_{50} values of 7.3×10^5, 9.3×10^4, and 3.0×10^4 conidia ml^{-1} on day 7 against *A. gossypii, B. brassicae,* and *L. pseudobrassicae,* respectively. Furthermore, ICIPE62 produced more conidia on the surface of aphid cadavers than ICIPE30 and ICIPE69 and showed wider thermotolerance with optimum ranges between 25 and 30 °C. Application of conidia of ICIPE62 formulated in aqueous and emulsifiable formulations negatively affected aphid population growth rate on kale and okra plants compared to controls in screenhouse experiments. These results have demonstrated the potential of *M. anisopliae* isolate ICIPE62 in suppression of *A. gossypii, B. brassicae*, and *L. pseudobrassicae* populations and could therefore be considered as biopesticide candidate for the control of these target aphids.

Keywords Entomopathogenic · Fungi · Pathogenicity · Thermotolerance · Formulation

Key messages

- Several isolates of *B. bassiana* and *M. anisopliae* were screened for selection of isolate(s) that are virulent against three aphid pests infesting vegetables in sub-Saharan Africa.
- *M. anisopliae* isolate ICIPE62 outperformed the other isolates in terms of virulence against the three aphid pests conidial production and thermotolerance.
- Aqueous and emulsifiable formulations of *M. anisopliae* ICIPE62 provided acceptable control of the three aphid species in the screenhouse.

Introduction

Globally, aphids (Hemiptera: Aphididae) are regarded as one of the most important insect pests of vegetable crops causing direct feeding damage on plant sap, excretion of honeydew that may favor the growth of molds and

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transmission of plant viruses (Van Emden and Harrington 2007). In Africa, the cabbage aphid *Brevicoryne brassicae* (L.)—restricted to mid- and high-altitude agroecologies—and the turnip aphid *Lipaphis pseudobrassicae* (Davis)—restricted to mid- and low-land agroecologies—cause considerable yield losses of up to 100 % on many cruciferous crops (e.g., cabbage, kale) if not controlled (Nyambo and Löhr 2005; Sæthre et al. 2011; Waiganjo et al. 2011). The cabbage aphid is known to transmit 23 plant viruses, of which *Cauliflower mosaic virus* (CaMV) and *Turnip mosaic virus* occur in tropical Africa and can cause substantial reduction in cabbage production (Spence et al. 2007). In Kenya, survey of farmers perception on insect problems of kale and cabbage revealed that 89–97 % of the growers ranked aphids (*B. brassicae* and *L. pseudobrassicae*) as the major insect pest threat to the two crops (Oruku and Ndung'u 2001). In addition to these two aphid species, the melon/cotton aphid, *Aphis gossypii* (Glover), is also considered as one of the most serious pests on a broad array of vegetables in tropical Africa. For example in Kenya, yield losses due to *A. gossypii* on okra were estimated at 15–40 % (Sithanantham et al. 1997). *Aphis gossypii* can transmit more than 50 plant viruses causing symptoms that impair vegetable quality and yield.

In many African countries, the management of these aphid species relies heavily on application of synthetic chemical insecticides. Majority of vegetable growers who are smallholders with limited knowledge of pesticide use frequently apply cocktail of synthetic chemical insecticides (Williamson et al. 2008; Zalucki et al. 2012). This practice substantially elevates production costs, increases health risks of producers and consumers, and most often disrupts the activity of natural enemies that otherwise could contribute to keeping the aphids under control, in addition to the development of resistance to these chemicals (Aktar et al. 2009; Amoabeng et al. 2013). Therefore, exploring nonchemical alternatives for controlling aphids is fundamental to realizing sustainable vegetable production, especially among the smallholder farmers in the region.

Aphids are known to be attacked by a number of natural enemies including entomopathogenic fungi (EPF) which sometimes cause epizootics in their populations (Wraight and Hajek 2009). Among the EPF, Hypocreales of the anamorphic genera (*Beauveria, Metarhizium, Isaria,* and *Lecanicillium*) are best suited for development as biopesticides compared to Entomophthorales which are highly specialized and difficult to mass production. They involve parasitic, hemibiotrophic (i.e., phase in insect haemocoel), and saprophytic phases (Hesketh et al. 2010; Wraight and Hajek 2009) that are amenable to mass production on various organic substrates such as rice, maize, and sorghum among others. In Europe and North America, several commercial products based on *Metarhizium brunneum* (Petch), *Beauveria bassiana* (Bals.) Vuill., and *Isaria* spp. have been registered for aphid control. They include BotaniGard® and Naturalis-L® (*B. bassiana*-based products), Met52® (*M. brunneum*-based product), Preferal® (*Isaria* spp-based product), and Vertalec® (*Lecanicillium longisporum* R. Zare & W. Gams-based product) (Jandricic et al. 2014; Kabaluk et al. 2010; Ravensberg 2011). Similar products are not available on the African continent. In Africa, there is lack of guidance on the import and release of biocontrol agents and, thus, the decision not to import nonindigenous biocontrol agents because of fear of the potential impact of an unknown pest (Cherry and Gwynn 2007). It is, therefore, logical to identify virulent indigenous fungal isolates against aphids for further development as biocontrol agents by local private companies.

Since the ability of EPF to cause disease in insect populations is influenced by a number of factors including abiotic factors (Benz 1987; Hall and Papierok 1982; Inglis et al. 2001), the effect of environmental factor must be assessed in order to predict their efficacy in the field. Temperature, humidity, and solar radiation are among the abiotic factors that affect the EPF action (Inglis et al. 2001). Among these factors, temperature is considered as the most important as it affects the pathogen (conidial germination, growth), insect development, and virulence against the insects (Benz 1987; Fargues and Bon 2004; Maniania and Fargues 1992). The first objective of the present study is therefore to screen fungal isolates for selection of virulent ones against aphid species, *B. brassicae, L. pseudobrassicae*, and *A. gossypii.* The second objective is to assess the effect of constant temperatures on conidial germination, mycelial growth, and virulence of selected fungal isolates with the goal of selected fungal isolates to choose a desired candidate that is effective against the three target pests at a broad range of temperatures. We also evaluated the performance of two formulations of the best isolate against the three aphid species in the screenhouse.

Materials and methods

Insects

The stock cultures of aphids (*B. brassicae*, *L. pseudobrassicae*, and *A. gossypii)* came from field populations infesting cabbage, kale, and okra in vegetable production sites of Nyeri, Kenya (0°21′10.69″S 37°5′14.35″, 1878 m.a.s.l), and Nguruman, Kenya (1°48′22.1″S 36°03′41.2″E, 746 m.a.s.l). The aphids were reared on at least 3-week-old kale (var. 1000 headed) and okra (var. Pusa sawani) potted plants (4–5

leaves) in the insectary at 27–28 °C and photoperiod of 12:12 L:D. Laboratory-reared colonies were rejuvenated every 3 months by introducing field populations of the same aphid species to maintain genetic vigor. To obtain insects of the same developmental stage for use in bioassays, apterous adult aphids of each species were removed from laboratory-reared colonies using camel brush and maintained on fresh kale or okra leaves in 90-mm Petri dishes to larviposit for 24 h and adults were removed thereafter. The newly born nymphs were then transferred to fresh seedlings and maintained until adult stage ready for use in the bioassay.

Fungal isolates

Fungal isolates used in this study were obtained from the *icipe* Arthropod Germplasm Centre where they were stored at −80 °C. The origin of the fungal isolates and the year of their isolation are presented in Table 1. They were cultured on Sabouraud dextrose agar (SDA) and maintained at 26 ± 2 °C in darkness. Virulence of each fungal isolate was maintained by regular passages through the target insects. Conidia were harvested from 2- to 3-week-old sporulating cultures and suspended in 10 ml 0.05 % Triton X-100 in universal bottles containing glass beads (3 mm). The suspension was vortexed for 5 min at 100 rpm to break the conidial clumps and ensure a homogeneous suspension. Conidia were quantified using haemocytometer under light microscope. For viability test, concentration of 3×10^6 conidia ml^{-1} was prepared and 0.1 ml of the suspension was evenly spread on SDA and three sterile microscope cover slips were placed randomly on the surface of each inoculated plate. The plates were sealed with Parafilm and incubated under complete darkness at 26 ± 2 °C. Conidia germination was assessed after 18 h by counting 100 conidia beneath each coverslip under a light microscope.

Pathogenicity of fungal isolates against three aphid pests

Five isolates of *M. anisopliae* and three of *B. bassiana* were screened against the three aphid species. Twenty 2-day-old apterous adult aphids of each species were transferred on fresh leaf disks (ca. 80 mm diameter) of kale for *B. brassicae* and *L. pseudobrassicae,* and okra for *A. gossypii* in a 90-mm Petri dish. Insects were allowed to settle on the host plants and were then sprayed with 10 ml of 1×10^8 conidia ml^{-1} suspension using a Burgerjon's spray tower (Burgejon 1956). Air atomizing nozzle with a valve providing a constant airflow under 4 bar pressure fitted to the Burgerjon's spray tower resulting to suspension deposit of approximately 3.9×10^7 conidia cm^{-2}. Rotating plate fitted underneath of the tower also ensure even distribution of the suspension on the surface of the insects. The control groups were sprayed with sterile water containing 0.05 % Triton X-100. After treatment, embedded leaf disks containing aphids were allowed to dry (for approx. 5 min). The aphids were then transferred to fresh unsprayed surface-sterilized leaf disks (ca. 100 mm diameter). Prior to transferring treated insects, leaf disks were washed with tap water, surface sterilized with 0.01 % sodium hypochlorite (5 min) and thoroughly rinsed in distilled sterile water (5 min), and transferred to sterile laminar airflow hood and air-dried to remove excessive moisture. Both control and fungus-treated insects were transferred to a humid plastic box (40 × 120 mm) lined with moistened filter paper and incubated at 26 ± 2 °C. All the fungal isolates were bioassayed concurrently against the three aphid species and each treatment replicated four times over time. Treatments were arranged in a completely randomized design blocks. Aphid mortality was recorded daily for 7 days post-treatment. The dead insects were surface sterilized with 70 % alcohol and then rinsed thrice in sterile distilled water. They were kept separately in a

Table 1 List, origin and percentage germination of fungal isolates tested against three adult aphid species on SDA plates after 18 h at 26 ± 2 °C

Fungal species	Fungal isolate	Source of origin	Site (Country of origin)	Year of isolation	% germination ± SE[a]
B. bassiana	ICIPE 10	Soil	Mbita (Kenya)	2002	95.5 ± 0.3ab
	ICIPE 273	Soil	Mbita (Kenya)	2006	91.4 ± 2.1b
	ICIPE 279	Coleopteran larvae	Kericho (Kenya)	2005	93.1 ± 1.2b
M. anisopliae	ICIPE 18	Soil	Mbita (Kenya)	1989	98.1 ± 0.3a
	ICIPE 30	*Busseola fusca*	Kendubay (Kenya)	1989	98.9 ± 0.5a
	ICIPE 62	Soil	Matete (D.R. Congo)	1990	99.3 ± 0.1a
	ICIPE 69	Soil	Matete (D.R. Congo)	1990	99.8 ± 0.3a
	ICIPE 78	*Temnoschoita nigroplagiata*	Ungoe (Kenya)	1990	99.1 ± 0.3a

[a] Viability of conidia on SDA plates after 18 h at 26 ± 2 °C. Means within a column followed by the *same letter* are not significantly different by Tukey's HSD multiple range test at 5 % level

Petri dish lined with sterile moistened filter paper for fungal outgrowth to verify if their deaths were attributed to mycosis.

Concentration–mortality response

Metarhizium anisopliae isolates ICIPE 30, ICIPE 62, and ICIPE 69 had the lowest LT_{50} values against the three aphid species in the screening tests and were, therefore, selected for concentration–mortality response assays using the following concentrations: 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}. Suspensions were prepared as described above and different concentrations were obtained through serial dilutions. Cohorts of each adult apterous aphid species were placed on their respective host plant leaves (kale or okra) as described earlier and were sprayed with 10 ml of each concentration using Burgerjon spray tower. The control groups were sprayed with sterile distilled water containing 0.05 % Triton X-100. Twenty apterous adult aphids were used per replicate and per dose for each isolate and aphid species. Treated aphids were maintained as described above and mortality recorded daily for 7 days. Treatments were randomized and repeated three times. Dead insects were processed following the procedures described above.

Conidia production on aphid cadaver

To evaluate conidiation capacity on aphid cadavers killed by *M. anisopliae* ICIPE 30, ICIPE 62, and ICIPE 69 at 1×10^8 conidia ml^{-1}, three cadavers of each aphid species from each replicate were maintained for 3, 6, and 9 days under optimal conditions for the fungal isolates. Sporulated cadavers were dried at 30 ± 2 °C for 30 min and then individually transferred into 1 ml aliquots of 0.05 % Triton X-100 in tubes. The tubes were vortexed for 5 min to dislodge conidia and conidial concentration from each cadaver was determined using a hemocytometer (Niassy et al. 2012).

Effect of temperature on germination of selected fungal isolates

The three selected *M. anisopliae* isolates were further evaluated for the effect of constant temperatures on conidial germination. Conidia were harvested as described above and concentration of 3×10^6 conidia ml^{-1} was prepared. Conidial suspension (0.1 ml) was spread-plated on SDA plates and sterile microscopic cover slips placed on each plate. The plates were sealed with Parafilm membrane and later incubated at 10, 15, 20, 25, 30, and 35 °C in darkness. After 18 h post-inoculation, conidial germination was halted using 1 ml formaldehyde (0.5 %) and the plates were assessed for germination as described above. The experiment was replicated four times for each temperature and fungal isolate.

Effect of temperature on radial growth

To assess the effect of temperature on radial growth, 0.1 ml spore suspension of each isolate ICIPE 30, 62, and 69 titrated to 3×10^6 conidia ml^{-1} was evenly spread on SDA and allowed to grow for 3 days to obtain mycelial mats. Plugs (ca. 5 mm) of mycelium were cut from the plates using an 8-mm-diameter cork borer and placed upside down at the center of a 90-mm Petri dish containing sterile SDA. The plates were sealed with Parafilm membrane and incubated for 12 days in the conditions described above. Four replicate were used for each isolate and temperature combination. The radial growth was measured every other day using two cardinal diameters drawn on the bottom of each plate.

Effect of temperature on virulence

Thirty apterous adult aphids of each species were treated with 10 ml of conidial suspension titrated to 1×10^8 conidia ml^{-1} using Burgerjon spray tower and insects were handled as described earlier. *Aphis gossypii* and *L. pseudobrassicae* were incubated at 15, 20, 25, 30, and 35 °C, while *B. brassicae* at 10, 15, 20, 25 and 30 °C since it is largely a highland pest. All the treatment combinations were arranged in a complete randomized design with four replicates. Mortality was recorded daily for 5 days. Dead insects were placed on Petri dish lined with moist filter paper as described above. Mycosis was confirmed by examining the surface of the cadaver under a microscope as described earlier.

Screenhouse experiment

Based on the results from virulence, thermotolerance and conidial production on cadavers, *M. anisopliae* ICIPE 62 was selected for screenhouse experiments. Kale and okra were planted in plastic pots (15 × 20 cm) by direct seeding. Plants were thinned to one plant per pot when they were ca. 20 cm tall and transferred into sleeved cages (100 × 100 cm). Each caged plant was then artificially infested with 100 apterous adult aphids of uniform age and transferred into a screenhouse (5 × 10 m) thereafter. The prevailing temperature and HR in the screenhouse during the experiments ranged from 15 to 29 °C and 30 to 90 % RH, respectively, under 12:12 L:D photoperiod. The insects were allowed to multiply for 8 days and to ensure that they reach a stable age distribution (Banken 1996), basis on which different values for rates of increase can be

compared (Birch 1948). The initial aphid density (N_0) before fungus application was determined by destructive sampling of five plants for each species. For each aphid species, treatments were arranged in completely randomized design and replicated four times. Conidial suspension titrated to 1.0×10^8 conidia ml^{-1} was formulated in water (Triton X-100 + Water at the ratio of 0.05:99.95) and emulsifiable formulation (Triton X-100 + Corn Oil + Water at the ratio of 0.05:0.1:99.85). Nutrient agar (0.1 %), glycerin (0.1 %), and molasses (0.5 %) were added to each formulation as protectants to complete the formulations (Maniania 1993). Each infested plant was then sprayed with 20 ml of each formulation using a hand sprayer fitted with a hollow cone nozzle with a volume diameter droplet of 41 μm. Control cohorts were treated with either sterile water containing 0.05 % Triton X-100 or 0.1 % oil plus the protectant ingredients listed above. Treatments were performed late in the evening to lessen the adverse effect of ultraviolet radiation (Moore and Prior 1993). The efficacy of fungus application on aphids was based on mortality caused by the fungus on randomly selected aphid samples and aphid population growth rate (Birch 1948). Twenty apterous adult aphids were gently picked at random using a camel hair brush from treated plants and placed individually in screened transparent plastic vials (50 × 100 mm) and fed with surface-sterilized kale or okra leaf disks. Insects were maintained in a controlled environment room (26 ± 2 °C, 60 ± 5 % RH, and photoperiod of 12:12 L:D). Mortality was recorded daily for 7 days and dead insects were processed as described above. To determine aphid population growth rate on the potted plants following spray application of the fungus, all potted plants were removed from the cages after 7 days and all the aphids on the plants counted. The time interval of 7 days was chosen because it provided enough time for population growth to occur, but not enough time for the aphids to kill the plants.

Statistical analysis

Percent aphid mortality was corrected for control mortality using Abbott's formula (Abbott 1925) and arcsine square-root transformed (Gomez and Gomez 1984) before analysis of variance (ANOVA). Mean comparisons among aphid species, fungal species, and/or isolates were made using Tukey HSD test. Lethal time to 50 % mortality (LT_{50}) values were estimated with repeated measures logistic regression using generalized estimating equations (Stokes et al. 2000). This was carried out using Genmod of SAS procedure (SAS 1999a, b–2001). Lethal concentration to 50 % mortality (LC_{50}) and slope were estimated by probit analysis for correlation data (Throne et al. 1995) using SAS software. Tests of parallelism of probit regression lines for all isolates were performed using χ^2 statistics goodness-of-fit test.

Data on percent germination of fungal isolates were subjected to generalized linear (GLM) using binomial regression analysis. Whenever overdispersion was detected, data fitted to quasibinomial regression model. Radial growth data were fitted by regression analysis ($y = vt + b$), and then the linear regression slope (v), which indicated the growth rates (velocity in mm d^{-1}), used as the main parameter to evaluate the influence of temperature on fungal growth (Fargues et al. 1992; Ouedraogo et al. 1997; Yeo et al. 2003; Davidson et al. 2003). Means were separated using Tukey HSD test.

Furthermore, linear and nonlinear (Briere-1) models were used to estimate the effect of temperature on fungal growth. The linear model expressed as $y\ (t) = a + bt$ was used to estimate the relationship between relevant temperatures and growth rate of fungal isolates, where y is the rate of growth, t is ambient temperature, and intercept (a) and slope (b) as the model parameters. The minimum temperature (T_{min}) was determined using the inverse slope of the fitted linear regression line as the x-intercept ($-a/b$), and is the estimated lower temperature at which either no measurable growth occurs or zero (Campbell et al. 1974). On the other hand, the Briere-1 model was used for estimation of temperatures (minimum, maximum, and optimum) for fungal growth thresholds (Briere et al. 1999).

Count data on conidial production were checked for normality and homogeneity of variance using Shapiro–Wilk and Bartlett's tests before analysis. After normality test, data were fitted to GLM using negative binomial regression analysis. Negative binomial distribution was preferred for its appropriateness in handling the overdispersed conidial count data.

In the Screenhouse trial, the data on aphid numbers/plant were fitted to GLM model using negative binomial regression analysis. The final and initial aphid numbers/plant were compared using multiple comparisons based on the model parameter estimates. The aphid population growth rate was also determined as the instantaneous rate of increase (r_i) for each treatment using the equation:

$$r_i = \frac{\ln\left(N_t/N_0\right)}{t},$$

where N_0 is the initial number of aphids in the population, and N_t is the number of aphids in the population at the end of the time interval, t (in days) (Walthall and Stark 1997; Stark and Banks 2003). "Positive values of r_i indicate a growing population; when r_i is zero, the population is neither growing nor declining, and when r_i is negative, the population is on the decline and headed towards extinction" (Stark and Banks 2003). The data on r_i were subjected to ANOVA and means were compared using Tukey

HSD test. All data analyses were done using R v2.15.1 statistical software package (R Development Core Team 2011) except for LT_{50} and LC50.

Results

Pathogenicity of fungal isolates against aphid species

The percent germination for each isolate was greater than 90 % after 18 h incubation at 26 ± 2 °C and was considered as acceptable (Table 1). Control mortalities in the initial screening ranged from 15 to 20 % across the three aphid species. At a standard concentration of 1×10^8 conidia ml^{-1}, there was a significant difference between fungal species ($F = 65.2$, df = 1,69; $P < 0.0001$) and fungal isolates ($F = 14.48$, df = 7,69; $P < 0.0001$). There were also significant differences in interactions between fungal species and insect species ($F = 11.29$, df = 2, 69; $P < 0.0001$) and between fungal isolates and insect species ($F = 4.69$, df = 14,69; $P < 0.0001$) (Table 2). *M. anisopliae* isolates were more pathogenic to the aphid species than *B. bassiana* isolates, causing mortality ranging from 72.0 to 98.3 %, 74.3 to 96.8 %, and 61.9 to 77.0 % in *B. brassicae, L. pseudobrassicae*, and *A. gossypii,* respectively, 7 days post-treatment (Table 2). The lethal time required to achieve 50 % mortality (LT_{50}) among aphid species also varied with fungal isolates (Table 2). LT_{50} values varied between 2.5 and 5.5 days in *B. brassicae*, between 2.6 and 5.2 days in *L. pseudobrassicae*, and between 2.8 and 5.6 days in *A. gossypii. M. anisopliae* isolate ICIPE 62 had the shortest LT_{50} values against *A. gossypii*, *B. brassicae*, and *L. pseudobrassicae*, while *B. bassiana* isolate ICIPE 279 had the shortest against *A. gossypii.* The LT_{50} value for isolate ICIPE 279 was not computed against *L. pseudobrassicae* since mortality was less than 50 % after 7 days post-treatment (Table 2). Based on LT_{50} values across the three aphid species, *M. anisopliae* isolates ICIPE 30, ICIPE 62, and ICIPE 69 were selected for further studies.

Concentration-mortality response

Among the three isolates (ICIPE 30, ICIPE 62, and ICIPE 69) tested for lethal concentration 50 % mortality, isolate ICIPE 62 had the lowest LC_{50} values of 9.3×10^4, 3.0×10^4, and 7.3×10^5 conidia ml^{-1} to *B. brassicae, L. pseudobrassicae*, and *A. gossypii*, respectively, on day 7 (Table 3). Based upon the calculated potency ratio, both ICIPE 30 and 69 were less potent compared to ICIPE 62 against all the three aphid species (Table 3). Furthermore, the parallelism tests for the three isolates against each aphid species were as follows: *B. brassicae* ($\chi^2 = 1.03$, df = 2, $P > 0.05$), *L. pseudobrassicae* ($\chi^2 = 0.34$; df = 2; $P > 0.05$), and *A. gossypii* ($\chi^2 = 1.02$; df = 2; $P > 0.05$).

Conidial production

The conidial production significantly varied according to isolates and insect species ($\chi^2 = 35.13$, df = 4; $P < 0.0001$), isolates and days ($\chi^2 = 31.65$, df = 4; $P < 0.0001$), and isolates, insect species, and days ($\chi^2 = 85.69$, df = 8; $P < 0.0001$) (Fig. 1a–c). Across aphid species and fungal isolates, conidial production increased with increasing number of days. For example, the conidial production ranged from 4.9×10^5 to 3.9×10^6 conidia ml^{-1} on day 3, 3.2×10^6–

Table 2 Pathogenicity of isolates of *M. anisopliae* and *B. bassiana* to apterous adult *B. brassicae, L. pseudobrassicae*, and *A. gossypii* following exposure to 1×10^8 conidia ml^{-1} after 7 days: percentage mortality and LT_{50} values

Fungal species	Isolate	*Brevicoryne brassicae*		*Lipaphis pseudobrassicae*		*Aphis gossypii*	
		% Mortality ± SE	LT_{50} (days) (95 % FL)	% Mortality ± SE	LT_{50} (days) (95 % FL)	% Mortality ± SE	LT_{50} (days) (95 % FL)
M. anisopliae	ICIPE 18	77.5 ± 3.2bcA	3.8 (2.9–4.8)	74.3 ± 11.8bAB	4.6 (3.9–5.4)	61.9 ± 4.5bcB	5.8 (5.6–6.1)
	ICIPE 30	98.3 ± 1.7aA	3.6 (3.4–3.7)	96.8 ± 3.2aA	3.6 (3.3–4.0)	72.6 ± 6.0aB	4.4 (3.9–5.0)
	ICIPE 62	85.3 ± 2.2abB	2.5 (2.2–2.8)	92.3 ± 3.5abA	2.6 (1.6–3.7)	77.0 ± 7.8abB	3.4 (3.1–3.7)
	ICIPE 69	86.6 ± 2.0abA	3.2 (2.7–3.7)	82.6 ± 13.8abAB	2.8 (1.6–4.1)	74.1 ± 5.8abB	5.0 (4.3–5.7)
	ICIPE 78	72.0 ± 6.5bcB	3.9 (3.5–4.8)	95.5 ± 3.0aA	3.1 (2.7–6.3)	66.5 ± 8.1bcB	5.6 (4.7–6.4)
B. bassiana	ICIPE 10	68.5 ± 6.8cdA	5.4 (4.9–5.9)	64.3 ± 6.7bA	5.2 (4.5–6.0)	57.4 ± 1.8cdB	5.6 (5.2–6.0)
	ICIPE 273	52.0 ± 1.4 dB	5.5 (5.1–5.9)	61.0 ± 4.9bA	4.3 (3.8–4.9)	57.3 ± 1.9dAB	5.4 (4.7–6.0)
	ICIPE 279	75.9 ± 0.3bcA	5.3 (5.2–5.4)	32.8 ± 3.8cB	_	77.1 ± 7.7bcA	3.3 (2.9–3.6)

Means within a row followed by the same lower case and within column upper case letters do not differ significantly by Tukey's HSD multiple range test ($P = 0.05$). LT_{50} (in days) ± 95 % fiducially limit (FL). The LT_{50} value for isolate ICIPE 279 was not computed against *L. pseudobrassicae* since mortality was less than 50 % at 7 days post-treatment

Table 3 LC_{50} values for selected isolates of *M. anisopliae* and their relative potency of against apterous adult aphid pests at 7 days post-treatment

Aphid species	*M. anisopliae* isolates	LC_{50} (Conidia ml^{-1}) (95 % FL)[a]	Relative potency[b]	Slope[c] ± SE
B. brassicae	ICIPE 30	9.4×10^5 (3.3×10^5–2.7×10^6)	0.10	1.6 ± 0.2
	ICIPE 62	9.3×10^4 (2.8×10^4–2.7×10^5)	1.00	1.6 ± 0.2
	ICIPE 69	1.2×10^6 (4.2×10^5–3.4×10^6)	0.08	1.5 ± 0.2
L. pseudobrassicae	ICIPE 30	4.4×10^6 (1.4×10^6–1.6×10^7)	0.01	1.5 ± 0.2
	ICIPE 62	3.0×10^4 (6.4×10^3–1.2×10^5)	1.00	1.8 ± 0.2
	ICIPE 69	7.8×10^5 (2.3×10^5–2.5×10^6)	0.04	1.5 ± 0.2
A. gossypii	ICIPE 30	3.5×10^6 (1.0×10^6–1.3×10^7)	0.20	1.6 ± 0.2
	ICIPE 62	7.3×10^5 (2.1×10^5–2.5×10^6)	1.00	1.5 ± 0.2
	ICIPE 69	2.1×10^7 (5.7×10^6–1.0×10^8)	0.04	1.6 ± 0.2

[a] Values in the bracket represent 95 % Fiducial Limits (FL)

[b] LC_{50} value of ICIPE 62 divided by the LC_{50} value of a particular isolate

[c] Non-parallel probit models ± standard error (SE)

1.3×10^7 conidia ml^{-1} on day 6, and 8.8×10^6 –1.2×10^7 conidia ml^{-1} on day 9 post-treatment (Fig. 1a–c). On day 6 post-treatment, isolate ICIPE 62 produced higher number of conidia across aphid species compared to the other isolates. On day 9 post-infection, isolate ICIPE 62 outperfomed the other isolates, except ICIPE 30 that produced higher conidia (8.8×10^6 conidia ml^{-1}) on *A. gossypii* than the other isolates (Fig. 1a–c).

Effect of temperature on conidial germination

Conidia of the three isolates of *M. anisopliae* germinated at all the temperatures tested (ranging from 1.5 to 100 %), except at 10 °C where no germination was observed after 18 h post-incubation (Fig. 2). Fungal isolates ($F = 23.67$; df = 2, 57; $P < 0.0001$) and temperatures ($F = 595.72$; df = 4, 53; $P < 0.0001$) significantly affected conidial germination. For example, significant difference was observed in germination among the isolates at 15 °C ($F = 13.20$; df = 2,9; $P = 0.002$) and 35 °C ($F = 80.63$; df = 2,9; $P = 0.0001$). The interaction between temperatures and isolates were also significant ($F = 4.98$; df = 8, 45; $P < 0.001$). However, there was no significant difference among the fungal isolates at 20 °C ($F = 0.02$; df = 2, 9; $P = 0.9805$), 25 °C ($F = 2.35$; df = 2, 9; $P = 0.1511$), and 30 °C ($F = 1.23$; df = 2, 9; $P = 0.3363$). The optimal temperatures for conidial germination were observed at 25 and 30 °C for all the three isolates (Fig. 2).

Effect of temperature on fungal growth

The results showed significant differences in growth among the three fungal isolates at 15 °C ($F = 16.09$; df = 2, 9; $P = 0.0011$), 20 °C ($F = 55.37$; df = 2, 9; $P < 0.0001$), 25 °C ($F = 74.87$; df = 2, 9; $P < 0.0001$), 30 °C ($F = 102.64$; df = 2, 9; $P < 0.0001$), and 35 °C ($F = 22.49$; df = 2, 9; $P = 0.0003$). Growth was not significantly different among the isolates at 10 °C ($F = 3.20$; df = 2, 9; $P = 0.0894$) (Table 4). Estimated parameter values of the linear and nonlinear models are presented in Table 5. A positive linear relationship was observed between temperature and growth rates for all fungal isolates. Briere-1 models showed that optimum growth temperatures ranged between 25 and 30 °C for all isolates (Fig. 3; Table 5). The minimum temperature values generated by both linear regression and Briere-1 models were found to be comparable (Table 5).

Effect of temperature on virulence of *M. anisopliae* isolates to aphid species

Mortality of apterous adult *B. brassicae, L. pseudobrassicae*, and *A. gossypii* due to the three isolates of *M. anisopliae* at various constant temperatures is presented in Table 6. Apterous adult *B. brassicae* were unable to survive at 35 °C and was omitted from the analysis. Overall, the mortality of the three apterous adult aphid species was significantly affected by temperature ($F = 136.46$; df = 4, 121; $P < 0.0001$), fungal isolates ($F = 8.55$; df = 2, 121; $P = 0.0003$), and aphid species ($F = 38.10$; df = 2, 121; $P < 0.0001$). Significant interaction between fungal isolate and temperature ($F = 2.04$; df = 8, 121; $P = 0.0466$), isolates and aphid species ($F = 3.59$; df = 4, 121; $P = 0.0084$), temperature and aphid species ($F = 5.27$; df = 7, 121; $P < 0.0001$), and isolates, temperature and aphid species ($F = 2.85$; df = 14, 121; $P = 0.001$) were also observed. Across aphid species and fungal isolates, optimum temperature for virulence was found to be 25 °C

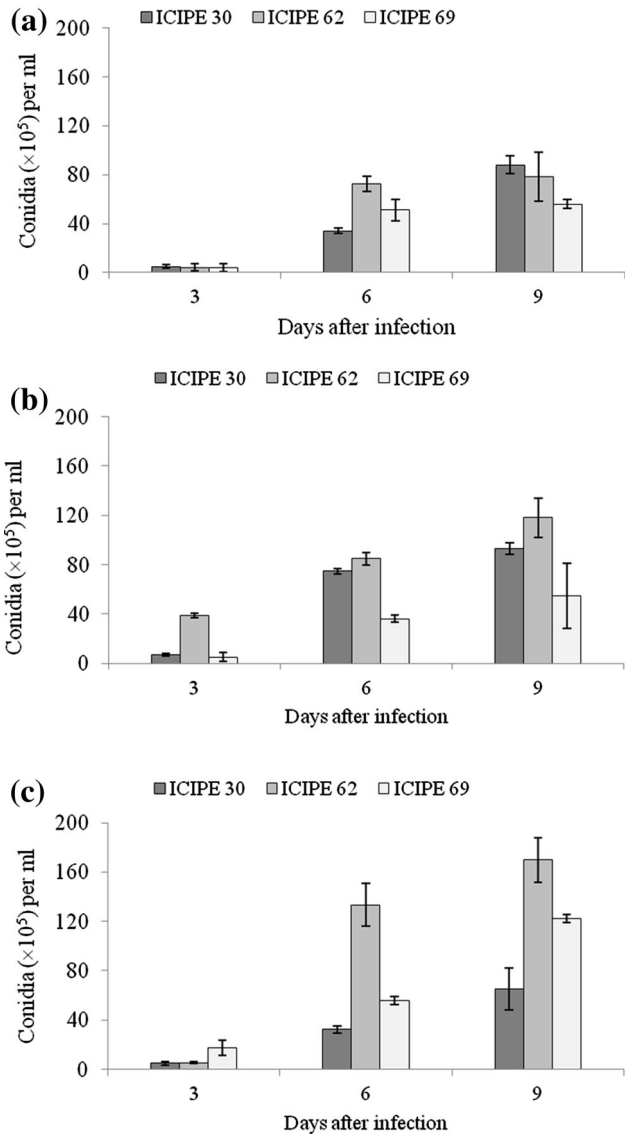

Fig. 1 Conidial production on three apterous adult aphid species: **a** *A. gossypii*, **b** *B. brassicae*, and **c** *L. pseudobrassicae* treated using 1×10^8 conidia ml^{-1} of three isolates of *Metarhizium anisopliae* at 3, 6, and 9 days after treatment. Data present mean ± SE at $P < 0.05$. *Bars* indicate SE at 95 % CI

(54.4–91.8 %) and 30 °C (73.4–93.7 %) although isolate ICIPE 69 caused higher mortality (81.7 %) of *B. brassicae* at 20 °C (Table 6). At 15 and 20 °C, the performance of each of the isolates in terms of virulence across aphid species was rather unique in that none of the isolates achieved comparable level of mortality on the test aphids. For example, isolate ICIPE 30 induced 25.1 and 34.5 % mortality of *A. gossypii* and *B. brassicae*, respectively, but performed poorly (10.4 %) on *L. pseudobrassicae* (Table 6). Similarly, at 20 °C, isolate ICIPE 62 caused 69.4 % mortality of *B. brassicae* and 63.3 % of *L. pseudobrassicae* but was less virulent to *A. gossypii* (25.0 %) at

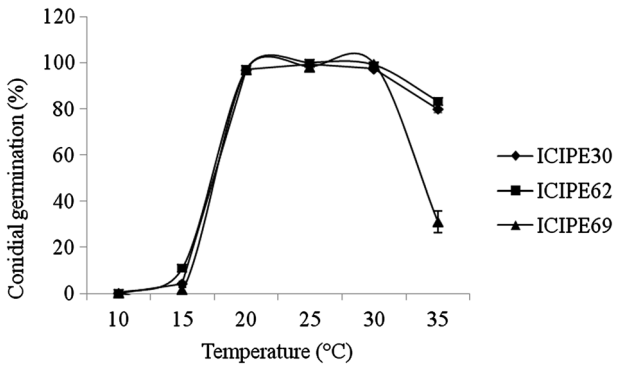

Fig. 2 Effect of temperature on conidial germination of selected isolates of *Metarhizium anisopliae*. *Bars* indicate SE at 95 % CI

the same temperature. However, at the optimum temperature of 25–30 °C, the three isolates were largely at par in terms of virulence to all the target aphid species (Table 6). The LT_{50} values decreased with increasing temperatures up to 30 °C. The values were not estimated at 15 °C for all the three isolates and at 35 °C for ICIPE 30 against *A. gossypii* because mortality was less than 50 % (Table 6). Generally, the shortest LT_{50} values were observed at the optimal temperatures of 25–30 °C.

Screenhouse experiment

Infection by *M. anisopliae* was observed in all the adult aphid species treated with fungal suspension and collected after spray application and processed in the laboratory. In *A. gossypii*, mortality in the control treatments was 13.8 and 17.5 % in aqueous and emulsifiable formulations, respectively. In fungus-treated cages, mortality was 72.7 and 93.8 % in aqueous and emulsifiable formulations, respectively (Fig. 4a). In *B. brassicae*, mortality in the control treatments was 18.8 and 20.0 % in the aqueous and emulsifiable formulations, respectively. In the fungal treatment, mortality was 89.4 and 95.0 % in the aqueous and emulsifiable formulation treatments, respectively (Fig. 4b). For *L. pseudobrassicae,* control mortality in the controls was 15.0 and 18.8 % in the aqueous and emulsifiable formulations, respectively, while in fungus treatments aphids incurred 90.8 and 85.2 % in the aqueous and emulsifiable formulations, respectively (Fig. 4c).

There was a significant interaction between aphid species and formulations ($P < 0.05$). Significant variation was also observed in conidia formulated as aqueous and emulsifiable with regard to mortality on *A. gossypii* ($P = 0.03$) (Fig. 4a). However, in the control treatments, there was no significant difference between aqueous and emulsifiable formulations across the three aphid species ($P \leq 0.05$) (Fig. 4a–c). Mortality induced by conidia formulated in emulsifiable and aqueous formulations on either

Table 4 Effect of temperature on growth rate day^{-1} of selected isolates of *M. anisopliae* cultured on SDA medium

Isolate	Mean growth rate (mm day^{-1}) ± SE					
	10 °C	15 °C	20 °C	25 °C	30 °C	35 °C
ICIPE30	0.47 ± 0.02aD	1.06 ± 0.03bC	2.03 ± 0.11cB	4.59 ± 0.13cA	4.46 ± 0.20cA	1.01 ± 0.11cC
ICIPE62	0.47 ± 0.02aE	1.44 ± 0.05aD	4.30 ± 0.14aC	8.39 ± 0.31aA	7.54 ± 0.18aB	1.86 ± 0.06aD
ICIPE69	0.58 ± 0.05aE	1.40 ± 0.11aD	3.15 ± 0.17bC	5.98 ± 0.13bA	5.37 ± 0.16bB	1.09 ± 0.06bD

Means within a column followed by the same lower case and within a row followed by the same upper case letters are not significantly different by Tukey's HSD multiple range test ($P = 0.05$)

Table 5 Parameter estimates and their approximate standard errors for linear and Brière-1 nonlinear model describing the relationship between temperature and growth of *M. anisopliae* isolates

Model	Parameters	Fungal isolates		
		ICIPE 30	ICIPE 62	ICIPE 69
Linear	a	−2.60 ± 0.462	−5.68 ± 0.742	−3.51 ± 0.481
	b	0.27 ± 0.03	0.53 ± 0.04	0.36 ± 0.03
	T_{min}	9.63 ± 0.87	10.66 ± 0.67	9.75 ± 0.69
	R^2	0.89	0.93	0.93
Briere-1	T_{min}	9.54 ± 0.98	9.96 ± 0.85	8.65 ± 0.94
	T_{max}	35.12 ± 0.06	35.12 ± 0.05	35.08 ± 0.04
	T_{opt}	29.24	29.30	29.08

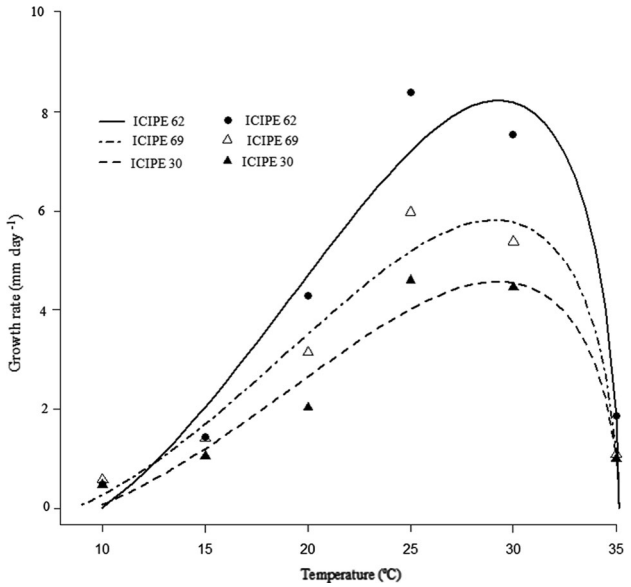

Fig. 3 Brière-1 model results (*curves*) describing the relationship between temperature and growth of three icipe fungi isolates. The observed growth values are plotted as points in the same graph

B. brassicae or *L. pseudobrassicae* were not significantly differ ($P < 0.05$) (Fig. 4b, c).

The average (±SD) initial aphid density (N_0) before fungus application was determined by destructive sampling of 5 plants for each species and the results were as follows: 341.5 ± 31.0 for *B. brassicae*, 509.3 ± 60.7 for *L. pseudobrassicae*, and 251.3 ± 54.7 for *A. gossypii*. The number of aphids per plant declined following application of aqueous and emulsifiable formulations, while it increased in the control treatments for all species (Fig. 5). There was, however, significant variation ($P = 0.002$) between the three aphid species with emulsifiable formulation of conidia. Significant reduction in the number of aphids/plant was observed on the plants sprayed with conidia formulated as aqueous ($P = 0.008$) and emulsifiable ($P < 0.001$) for *A. gossypii.* However, there was no significant difference between initial and final aphid numbers/plant in both oil alone ($P = 0.8$) and water alone as control treatments ($P = 0.05$). On the other hand, variation was observed in the number of aphids/plant in the water control and conidia formulated as emulsifiable ($P < 0.001$), water control and conidia formulated as aqueous ($P = 0.004$), and oil control and conidia formulated as emulsifiable ($P < 0.001$). However, there was no significant difference between conidia formulated as emulsifiable and aqueous ($P = 0.41$) (Fig. 5). The r_i values of aphid populations were negative following application of the two formulations of *M. anisopliae* isolate ICIPE 62, while they were positive in the control treatments (Fig. 6). Overall, both conidial formulations had significant effect on aphid population growth compared to the controls ($P < 0.05$) (Fig. 6a–c).

Table 6 Virulence of selected *M. anisopliae* isolates to apterous adult *B. brassicae, L. pseudobrassicae*, and *A. gossypii*: percent mortality (±SE) and LT_{50} values 5 days after exposure to 1×10^8 conidia ml^{-1} under different constant temperatures

Isolate	Temperature (°C)	*B. brassicae*		*L. pseudobrassicae*		*A. gossypii*	
		% Mortality ± SE	LT_{50} (day) (95 % FL)	% Mortality ± SE	LT_{50} (day) (95 % FL)	% Mortality ± SE	LT_{50} (day) (95 % FL)
ICIPE 30	15	34.5 ± 7.1cA	_	10.4 ± 2.5 dB	_	25.1 ± 3.5 cAB	_
	20	42.9 ± 3.7cA	_	40.2 ± 8.3cA	_	34.3 ± 9.3cA	_
	25	67.4 ± 7.5bB	4.9 (4.8–5.0)	82.1 ± 3.4aA	3.1 (3.0–3.2)	66.6 ± 2.7abB	3.9 (3.8–4.0)
	30	81.4 ± 3.0aA	3.8 (3.4–4.1)	80.3 ± 5.5aA	3.3 (2.9–3.7)	69.7 ± 4.0aB	3.7 (3.6–3.8)
	35	_	_	62.0 ± 7.4bA	4.1 (4.0–4.3)	36.8 ± 10.2bcB	_
ICIPE 62	15	33.3 ± 6.1cA	_	3.3 ± 2.4 dB	_	9.2 ± 7.4 dB	_
	20	69.4 ± 2.0bA	4.9 (4.3–5.6)	63.3 ± 11.1bcA	4.3 (3.6–5.1)	25.0 ± 5.3cB	_
	25	91.8 ± 3.9aA	3.5 (3.3–3.7)	77.0 ± 3.8bB	4.1 (3.9–4.2)	65.7 ± 4.6bB	4.8 (4.5–5.1)
	30	89.6 ± 4.3aA	2.6 (2.4–2.8)	89.7 ± 2.1aA	2.8 (2.5–3.1)	87.2 ± 1.9aA	3.0 (2.9–3.1)
	35	_	_	49.4 ± 3.3cA	_	56.3 ± 8.2bA	4.7 (4.1–4.9)
ICIPE 69	15	27.5 ± 8.4cA	_	5.6 ± 9.2fB	_	17.7 ± 2.9cA	_
	20	81.7 ± 3.9bA	5.3 (4.8–5.7)	44.9 ± 6.3eB	_	16.0 ± 0.7cC	_
	25	78.3 ± 7.5bA	2.8 (2.3–3.3)	70.0 ± 5.9bA	3.7 (3.5–3.8)	54.4 ± 3.9bB	4.6 (4.5–4.8)
	30	93.7 ± 2.3aA	2.3 (2.0–2.7)	83.9 ± 3.8aAB	3.1 (2.0–3.3)	73.4 ± 4.7aB	3.4 (3.0–3.7)
	35	_	_	57.3 ± 3.0dA	4.4 (4.1– 4.7)	60.6 ± 8.8abA	4.5 (4.3–4.7)

Means within a column followed by the same lower case and within a row followed by the same upper case letters are not significantly different by Tukey's HSD multiple range test ($P = 0.05$). Values presented here were subjected to Abbott's correction before analysis. Values in the bracket represent 95 % Fiducial Limits. Dash (–) means the LT_{50} value was not estimated for cumulative mortality less than 50 % at 5 days post-treatment, and also there are no data on *B. brassicae* at 35 °C

Discussion

All the fungal isolates tested were pathogenic to *B. brassicae*, *L. pseudobrassicae*, and *A. gossypii.* However, *M. anisopliae* isolates ICIPE 30, ICIPE 62, and ICIPE 69 were virulent to *B. brassicae* and *L. pseudobrassicae* as compared to *A. gossypii*. Isolates of *B. bassiana* were the least virulent against the three aphid species, except isolate ICIPE 279 which was virulent against *A. gossypii*. These results confirm the pathogenicity of *B. bassiana* and *M. anisopliae* toward aphids as reported by other workers. For example, mortality of 100 % was reported in third-instar nymphs of *Myzus persicae* (Sulz.) and *A. gossypii* by B. *bassiana* and *M. anisopliae* (Loureiro and Moino 2006). Similarly, Jandricic et al. (2014) identified a number of isolates of *Beauveria* and *Metarhizium* virulent to first-instar nymphs of *A. gossypii* and *M. persicae* following the screening of 44 fungal isolates and 4 commercially available strains. Variation in the virulence of fungal isolates has been reported on many groups of arthropods such as fruit flies (Dimbi et al. 2003), thrips (Ekesi et al. 1998; Niassy et al. 2012), two-spotted spider mite (Bugeme et al. 2009), red spider mite (Wekesa et al. 2005), and leafminer (Migiro et al. 2010). For example, *M. anisopliae* ICIPE 62, which was found to be virulent against the three species of aphids in the present study, was also reported to be virulent against *Ceratitis rosa* var. *fasciventris* (Karsch) and *C. cosyra* (Walker) (Diptera: Tephritidae) (Dimbi et al. 2003) and adult *Cylas puncticollis* Boheman (Coleoptera: Curculionidae) (Ondiaka et al. 2008) but less pathogenic to adult *Ceratitis capitata* (Weidemann) and *Tetranychus evansi* Baker and Pritchard (Acari: Tetranychidae) (Bugeme et al. 2008). Given the fast developmental period of aphids and the rather short window of opportunity for infection due to ecdysis, the speed of kill becomes essential. In addition to causing high mortalities, *M. anisopliae* isolates ICIPE 30, ICIPE 62, and ICIPE 69 had the shortest LT_{50} values compared to the other isolates against the three aphid species tested. The LC_{50} values varied with aphid species, with *A. gossypii* generally having the highest values, which is an indication that this species is less susceptible to fungal infection by isolates tested.

The identification of fungal isolates with proven efficacy against a broad range of aphid species should be a logical approach for the management of aphid on diverse crops, considering that most of them occupy the same ecological niche than strict specificity to one species. This assertion is in agreement with Jandricic et al. (2014) who stated that the ideal mycoinsecticide for aphid management should be more consistent, with broader activity against a variety of

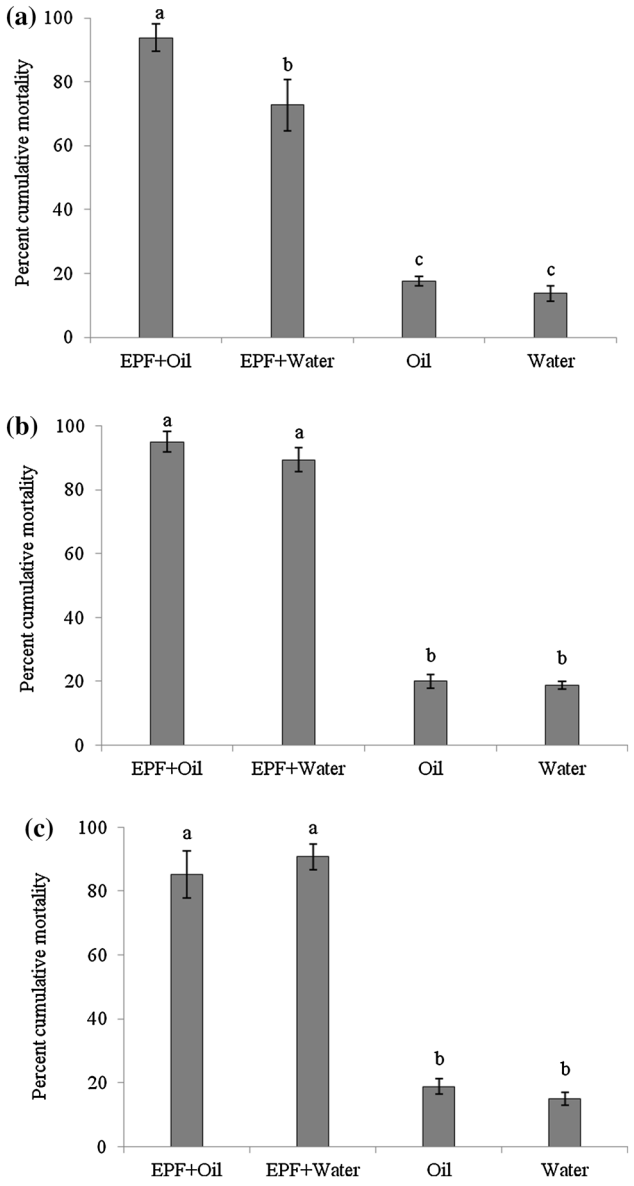

Fig. 4 Percent mortality of apterous adults of **a** *A. gossypii,* **b** *B. brassicae,* and **c** *L. pseudobrassicae* collected from screenhouse and processed in the laboratory 7 days post-treatment with conidia of *Metarhizium anisopliae* ICIPE 62 formulated in emulsifiable (EPF + Oil) and aqueous (EPF + Water) formulations, and oil alone and water alone. *Bars* indicate means SE at 95 % CI. Means followed by the same letter indicate no significant differences between treatments by Tukey's HSD multiple range test at P values of <0.05

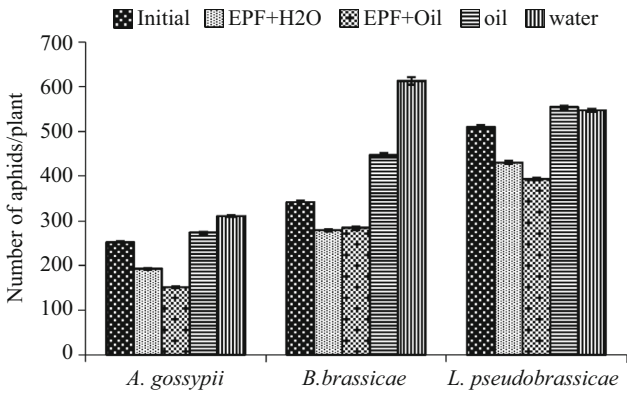

Fig. 5 Average number of aphids/plant before treatment (initial) and 7 days after treated with conidia of *Metarhizium anisopliae* ICIPE 62 formulated in aqueous (EPF + Water) and oil (EPF + Oil), oil alone, and water alone. *Bars* indicate means SE at 95 % CI

aphid species than the existing products that are relatively targeted at one species. In this regard, the three *M. anisopliae* isolates (ICIPE 30, ICIPE 62, and ICIPE 69) are potential candidates for development as biopesticides against the target aphid species compared to the other isolates tested in this study.

In addition to virulence, the three isolates of *M. anisopliae* were evaluated for conidial production on aphid cadavers, and isolate ICIPE 62 was proven to produce high number of conidia on the three aphid species. The conidiation potential of isolate ICIPE 62 on the three aphid species observed in the present study is similar to the one reported with *M. anisopliae* isolates on *M. persicae* reported by Shan and Feng (2010).

Conidial germination and mycelial growth of the isolates were observed at all temperature tested with the optimum temperature of growth occurring at 25 and 30 °C, which is in agreement with previous published reports (Bugeme et al. 2008; Dimbi et al. 2004; Ekesi et al. 1999; Fargues et al. 1992; Ouedraogo et al. 1997).

Mortality of the three apterous adult aphid species was significantly affected by temperature, fungal isolates, and aphid species. However, the optimal temperatures for virulence to the three aphid species were 25 and 30 °C which corresponded to optimal temperatures for germination and radial growth. While evaluating the effect of temperature on virulence of isolates of *B. bassiana* and *M. anisopliae* to *T. urticae,* Bugeme et al. (2009) observed that both fungal species were virulent at 25, 30, and 35 °C than at 20 °C. A correlation between optimum temperature for fungal growth and fungal infection has been reported by different authors (Bugeme et al. 2008; Ekesi et al. 1999; Maniania and Fargues 1992). Although fungal infection significantly slowed at temperature of 20 °C, this did not affect the overall cumulative mortality caused by isolates ICIPE 62 and 69. Thermotolerance of *M. anisopliae* isolate ICIPE 62 to broad temperature range compares favorably with prevailing temperatures under which the three aphid species thrive. *M. anisopliae* isolate ICIPE 62 is therefore suitable candidate for development as biopesticide for the control of the three species of aphids. Both aqueous and emulsifiable formulations of *M. anisopliae* isolate ICIPE 62 significantly reduced populations of *B. brassicae, L. pseudobrassicae*, and *A. gossypii*

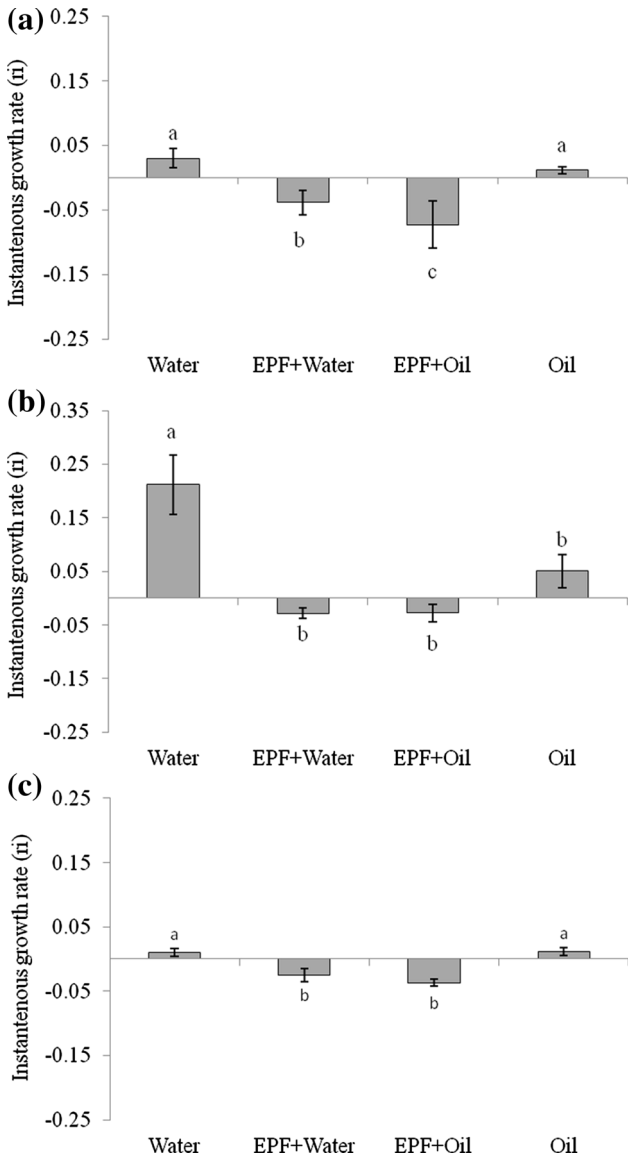

Fig. 6 Instantaneous rate of increase of three aphid species. **a** *A. gossypii,* **b** *B. brassicae*, and (c) *L. pseudobrassicae* 7 days after treated with conidia of *Metarhizium anisopliae* ICIPE 62 formulated in aqueous (EPF + Water) and oil (EPF + Oil), oil alone, and water alone. *Bars* indicate means SE at 95 % CI. Means followed by the *same letter* indicate no significant differences between treatments by Tukey's HSD multiple range test at P values of <0.05

in the screenhouse experiments. There were no significant between the two formulations, except in *A. gossypii* where emulsifiable formulation performed better. The mechanism underlying for the enhanced in efficacy of emulsifiable formulation on *A. gossypii* is probably due to less waxy layer on this particular aphid species which could promote conidia attachments. This is attributed to prevention of conidia from desiccation, increased adhesion, and spread of inoculum over the host cuticle and into crevices (Ibrahim et al. 1999).

Emulsifiable oil used as a control had no effect on population growth of the aphid species, indicating no toxic effect on the target aphids. This suggests a reduction in population growth from both adults' mortality and reduction in fecundity following the application of conidial formulations as previously reported by Jandricic et al. (2014). Among the two formulations, emulsifiable formulation performed better in terms of lower r_i values on *A. gossypii* compared to the conidia formulated as aqueous, but there was no significant difference in terms of this parameter on *B. brassicae* and *L. pseudobrassicae.* The lack of significant difference between the two formulations observed in the present study on *B. brassicae and L. pseudobrassicae* may be related to the waxy cuticle layer of the two aphid species. *Brevicoryne brassicae* and *L. pseudobrassicae* possess long, stilt-like legs and waxed cuticle that could minimize body contact with leaf surface reducing the likelihood of the aphids acquiring lethal dose of inoculum from treated leaf surfaces. Nevertheless, recent studies by Amnuaykanjanasin et al. (2013) showed that conidia may germinate and penetrate the aphid cuticle most efficiently on the inter-segmental membranes at the proximal end of the legs (close to the body's ventral surface) and this may have contributed to high impact of isolate ICIPE 62 on the highly waxed aphid species. A high proportion of the three aphid species collected from the treated plants were mycosed by *M. anisopliae,* which is an indication of the effects of fungal treatments.

This study has identified *M. anisopliae* isolate ICIPE 62 as potential candidate for further development as biopesticide for the control of the three aphid species based on the following desirable traits: virulence against the three target aphid pests, high conidial production, speed of kill (LT_{50}), lower LC_{50} values, and efficacy across a broad range of temperatures (Yeo et al. 2003). In addition, since aphids usually form large colonies on plant surfaces, the isolate of *M. anisopliae* ICIPE 62 profusely sporulated on dead aphids and could serve as source of inoculum for infection of healthy aphids which may lead to acute mortality. The potential of this isolate was further confirmed in screenhouse experiments against mixed stages of the targeted aphid species. This isolate could be therefore integrated with other control agents such as predators (coccinellids and syrphids) and parasitoids (*Diaeretiella rapae* (M'Cintosh) which have been observed attacking *B. brassicae* and *Aphidius colemani* Viereck attacking *A. gossypii*) (Bayissa et al. 2016; Ekesi unpublished data). Additional studies are underway to examine the interactions with these beneficial organisms. However, further field evaluation will be required to validate these results as part of an integrated management package for the aphid pests on crucifers and okra.

Author contribution

WB, SE, and SAM conceived and designed research. WB conducted the experiments. SE, NKM, SAM, and RH contributed reagents/materials/analysis tools. WB analyzed the data. WB, SE, NKM, SAM, GPK, and JMW wrote the manuscript. All authors read and approved the manuscript.

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