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Influence of Temperature on Virulence of Fungal Isolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the Two-Spotted Spider Mite *Tetranychus urticae*

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Abstract Twenty-three isolates of *Metarhizium anisopliae* (Metschnikoff) Sokorin and three isolates of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales: Clavicipitaceae) were assessed for their virulence against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Based on the screening results, nine isolates of *M. anisopliae* and two isolates of *B. bassiana* were tested for their virulence against young adult (1- to 2-day-old) female *T. urticae* at constant temperatures of 20, 25, 30 and 35°C. At all temperatures tested, all the fungal isolates were pathogenic to *T. urticae* but mortality varied with isolates and temperatures. Fungal isolates were more virulent at 25, 30 and 35°C than at 20°C. The lethal time to 50% mortality (LT_{50}) and lethal time to 90% mortality (LT_{90}) values decreased with increased temperature. There were no significant differences in virulence between fungal isolates at 30 and 35°C; however, significant differences were observed at 20 and 25°C.

Keywords *Beauveria bassiana* · *Metarhizium anisopliae* · Biological control · Temperature · *Tetranychus urticae* · Virulence

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Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is the most important mite pest of crops worldwide. It is polyphagous and has been recorded on more than 200 host plants, including ornamental plants (arborvitae, azalea, camellia, citrus, evergreens, hollies, ligustrum, pittosporum, pyracantha, roses and viburnum), fruit crops (blackberries, blueberries and strawberries), vegetables (tomatoes, beans, squash, eggplant and cucumber), as well as wild crops [1–3]. The mite feeds on the underside of leaves causing speckling, or in severe cases, premature leaf drop that result in yield loss or plant death [1, 2].

The management of *T. urticae* has mainly relied on the use of synthetic acaricides [1]. However, due to the excessive use of synthetic acaricides and the related problems of synthetic acaricide resistance

[4–6] and environmental contamination [7], there is an increased demand for alternatives that are sustainable and environment friendly. Several laboratory studies have reported predatory mites to be potential biological control agents of *T. urticae* [8–10]. In addition, biological control of *T. urticae* with phytoseiid predatory mites has been successful mainly in protected environments [1, 11, 12].

Entomopathogenic fungi are the most common pathogens associated with spider mites in the field [13, 14] and have been widely tested in the laboratory [15, 16]. They could be, therefore, used in biological control programmes against *T. urticae* either as a stand-alone solution to replace synthetic acaricides that are currently in use or as a component of integrated mite management. For instance, spray applications of *Beauveria bassiana* (Balsamo) Vuillemin, *Hirsutella thompsonii* Fisher, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Lecanicillium lecanii* (Zimm.) Zare & W. Gams (=*Verticillium lecanii*), and Naturalis-L (*B. bassiana*-based commercial biopesticide) (Ascomycota: Hypocreales: Clavicipitaceae) were reported to reduce *T. urticae* populations in tomato crop in glasshouse [16]. Naturalis-L was also found to be compatible with the predatory mite, *Phytoseiulus persimilis* Anthias-Henriot, in greenhouses [16].

Susceptibility of a host to a fungal pathogen is influenced by many factors including the host and pathogen properties as well as environmental factors [17, 18]. Temperature is considered as one of the environmental factors that influences the virulence of fungal isolates [19–21]. The present study aimed, therefore, at (1) evaluating the pathogenicity and (2) the effect of temperatures on virulence of selected isolates of *M. anisopliae* and *B. bassiana* against the two-spotted spider mite in order to select isolates with a broad temperature range.

Materials and Methods

Mite Culture

The *T. urticae* stock culture was established in the laboratory at ICIPE Headquarters, Nairobi, Kenya. The initial culture originated from mites was collected from rose plants in a screenhouse in Naivasha, Kenya, in 2004. The mites were reared on common bean, *Phaseolus vulgaris* L. variety GLP-2, at 25 ± 2°C, 60–70% RH and a photoperiod of 12:12 L:D. Quiescent deutonymphs were collected from the mite culture using a fine camel hairbrush and transferred to bean leaf discs. After two days, newly emerged adult female mites (1- to 2-day-old) were selected and used for the experiments.

Fungal Cultures

The 23 isolates of *M. anisopliae* and 3 isolates of *B. bassiana* used in this study were obtained from the ICIPE Arthropod Germplasm Centre (Table 1). Most of the isolates were selected at random and few others because of their virulence to other arthropod pests in previous studies. Conidia were harvested by scraping the surface of 3-week-old sporulating cultures grown on Sabouraud dextrose agar (SDA) in Petri dishes at 26 ± 2°C. Conidia were suspended in 20 ml sterile distilled water containing 0.05% Triton X-100. The conidial suspension was vortexed for 5 min to produce a homogenous conidial suspension. The viability of conidia was then determined by spread-plating 0.1 ml of the suspension (titrated to 3.0×10^6 conidia ml^{-1}) on SDA plates. A sterile microscope coverslip was placed on each plate. Plates were incubated at 26 ± 2°C and examined after 24 h. Percentage germination was determined by counting 100 spores for each plate. Four replicates were used for each isolate.

Bioassays

For pathogenicity tests, 10 ml of a standard concentration of 1.0×10^7 conidia ml^{-1} were sprayed on both sides of bean leaf discs (25 mm diameter) using a Burgerjon's spray tower [22] (INRA, Dijon, France), resulting in a deposit of approximately 3.8×10^6 conidia cm^{-2}. In the control, leaf discs were sprayed with sterile distilled water containing 0.05% Triton X-100. The leaf discs were then air dried in a laminar flow cabinet for 20 min and placed on wet cotton wool in Petri dishes (60 mm diameter). Twenty young adult female *T. urticae* (1- to 2-day-old) were then placed onto the treated bean leaf discs and maintained for 5 days, after which they were transferred to untreated leaf discs. The experiment was conducted at 26 ± 2°C in an incubator. Mortality was recorded daily for 10 days. Dead mites were transferred to Petri dishes lined with moist filter paper

Table 1 Fungal isolates used in the study and their percent viability

Species	Isolates	Year of isolation	Host/Substrate	Locality	Country	%Viability ± SE
Beauveria bassiana	ICIPE273	2004	Soil	Mbita	Kenya	91.5 ± 0.9
	ICIPE278	2005	*Cyclocephala* sp.	Kericho	Kenya	93.8 ± 0.6
	ICIPE279	1996	Soil	Mombasa	Kenya	96.3 ± 0.7
Metarhizium anisopliae	ICIPE7	1996	*Amblyoma variegatum*	Homabay	Kenya	92.5 ± 0.5
	ICIPE8	1990	*Galleria melonella*	Matete	DR Congo	92.6 ± 0.4
	ICIPE18	1989	Soil	Mbita	Kenya	92.0 ± 0.4
	ICIPE20	1989	Soil	Migori	Kenya	90.9 ± 1.0
	ICIPE21	1999	*Locusta gregaria*	Port-Sudan	Sudan	86.9 ± 1.0
	ICIPE24	2005	Soil	Embu	Kenya	93.2 ± 0.6
	ICIPE25	2005	Soil	Embu	Kenya	94.6 ± 0.4
	ICIPE30	1989	*Busseola fusca*	Mbita	Kenya	92.9 ± 0.7
	ICIPE41	1990	Soil	Lemba	DR Congo	94.4 ± 0.4
	ICIPE43	2005	Soil	Meru	Kenya	96.2 ± 0.5
	ICIPE48	2005	Unknown	Unknown	Kenya	93.6 ± 0.8
	ICIPE49	2005	Soil	Mount Kenya	Kenya	92.3 ± 0.5
	ICIPE51	1999	Sandy soil	Kitui	Kenya	91.3 ± 0.3
	ICIPE55	1999	Soil	Kitui	Kenya	92.0 ± 0.4
	ICIPE59	2005	Caterpillar	Nairobi	Kenya	88.5 ± 1.2
	ICIPE62	1990	Soil	Matete	DR Congo	90.2 ± 0.6
	ICIPE69	1990	Soil	Kinshasa	DR Congo	94.3 ± 0.4
	ICIPE78	1990	*Temnoschoita nigroplagiata*	Ungoye	Kenya	90.0 ± 0.5
	ICIPE84	2003	*Ornithacris turbida cavroisi*	Kaffraine	Senegal	94.0 ± 0.4
	ICIPE95	2005	Soil	Unknown	Kenya	90.7 ± 1.2
	ICIPE97	2005	Unknown	Unknown	Kenya	94.0 ± 0.4
	ICIPE315	2005	*Tetranychus urticae*	Mwea	Kenya	96.0 ± 0.4
	ICIPE316	2005	*Tetranychus* spp.	Kutus	Kenya	90.9 ± 0.9

to allow the growth of fungus on the surface of the cadaver. Mortality caused by fungus was confirmed by microscopic examination. Each treatment was replicated six times.

Based on the results from screening (mortality and LT_{50} values), 11 isolates were selected to evaluate the effect of constant temperature on their virulence against *T. urticae*. The procedure described above was used to infect the mites, except that mites were exposed at different constant temperature levels (20, 25, 30 and 35°C). The treatment was replicated four times.

Statistical Analysis

Mortality data were corrected for natural mortality in the controls using Abbott's formula [23] and arcsine transformed to normalise the data before analysis of variance (ANOVA) [24]. Means were separated by Student–Newman–Keuls test at $P = 0.05$. Lethal time to 50% mortality (LT_{50}) and the lethal time to 90% mortality (LT_{90}) were estimated with repeated measures logistic regression using generalised estimating equations (GEE) [25]. All analyses were carried out using GENMOD procedure of SAS [24]. The effect of temperature on germination, radial growth and mortality was analysed using ANOVA of SAS [24].

Results

In viability tests, 81–96% of spores germinated (Table 1). Mean mortality in the control was 11.5%

10 days after the treatment. In pathogenicity tests conducted at 26°C, all the fungal isolates were pathogenic to adult female *T. urticae*, causing mortality of between 36.5 and 100% (Table 2). There were, however, significant differences in mortality between fungal isolates ($F = 51.38$; df = 26,135; $P < 0.0001$) (Table 2). The lethal time to 50% mortality (LT_{50}) values ranged from 2.2 to 8.2 days, and the LT_{90} values ranged from 3.1 to 11.7 days.

Fungal isolates selected to study the effect of temperature on virulence against *T. urticae* were all also pathogenic to the host at all temperatures (e.g. 20, 25, 30 and 35°C), but mortality varied with isolates and temperatures (Table 3). For example, at 20°C significant difference was observed between *M. anisopliae* isolates ICIPE48 and ICIPE78 ($F = 2.17$; df = 10,33; $P = 0.0463$). At 25°C, there were significant differences between *M. anisopliae* isolates ICIPE78 and ICIPE49 and *B. bassiana* isolate ICIPE279 ($F = 3.36$; df = 10,33; $P = 0.0041$) (Table 3). However, there was no significant difference in virulence between fungal isolates at 30°C ($F = 1.87$; df = 10,33; $P = 0.0861$) and at 35°C ($F = 2.07$; df = 10,33; $P = 0.0574$) (Table 3). For all the isolates, mortality was lower at 20°C than at the other temperatures, except with isolates ICIPE7 and ICIPE78 where mortality was not significantly different at 20 and 25°C (Table 3). Most fungal isolates were

Table 2 Virulence of *B. bassiana* and *M. anisopliae* isolates against *T. urticae* exposed at 26 ± 2°C: Percent mortality and lethal time to mortality values

Species	Isolates	% Mortality (±SE)	LT_{50} (days) (95% fiducial limits)	LT_{90} (days) (95% fiducial limits)
Beauveria bassiana	ICIPE273	95.5 ± 1.6a	4.6 (3.9–5.6)	6.6 (5.5–7.9)
	ICIPE278	99.0 ± 1.0a	4.4 (3.8–5.1)	5.5 (4.8–6.3)
	ICIPE279	95.2 ± 3.9a	4.1 (3.6–4.7)	5.2 (4.5–6.0)
Metarhizium anisopliae	ICIPE7	100.0 ± 0.0a	2.6 (1.9–3.5)	3.4 (2.4–4.7)
	ICIPE8	98.3 ± 1.0a	2.6 (2.1–3.3)	3.5 (2.7–4.6)
	ICIPE18	85.6 ± 3.6ab	4.8 (4.2–5.4)	6.6 (5.9–7.5)
	ICIPE20	95.2 ± 6.1a	4.2 (3.6–4.9)	5.5 (4.5–6.6)
	ICIPE21	87.3 ± 3.9ab	4.8 (3.9–6.0)	6.5 (5.3–7.9)
	ICIPE24	99.1 ± 0.9a	5.0 (4.0–6.2)	6.6 (5.4–7.9)
	ICIPE25	100.0 ± 0.0a	6.0 (5.6–6.5)	7.1 (6.6–7.7)
	ICIPE30	63.4 ± 8.8cd	7.5 (6.4–8.9)	10.0 (8.2–12.3)
	ICIPE41	91.2 ± 3.7a	4.0 (3.5–4.6)	5.3 (4.6–6.2)
	ICIPE43	36.5 ± 8.1e	–	–
	ICIPE48	92.8 ± 3.0a	2.2 (1.8–2.8)	3.1 (2.4–3.9)
	ICIPE49	92.0 ± 4.4a	3.8 (3.3–4.4)	5.0 (4.3–5.8)
	ICIPE51	98.2 ± 1.1a	3.3 (3.1–3.6)	4.1 (3.8–4.5)
	ICIPE55	43.3 ± 13.0e	–	–
	ICIPE59	92.9 ± 3.8a	2.6 (2.1–3.3)	3.3 (2.7–4.0)
	ICIPE62	95.1 ± 2.8a	4.0 (3.7–4.4)	5.4 (4.9–5.8)
	ICIPE69	37.7 ± 12.7e	–	–
	ICIPE78	97.2 ± 1.9a	5.3 (4.8–5.8)	6.4 (5.8–7.1)
	ICIPE84	95.6 ± 1.6a	2.6 (2.1–3.3)	3.5 (2.7–4.5)
	ICIPE95	38.6 ± 8.1[e]	–	–
	ICIPE97	52.0 ± 6.2de	8.2 (7.3–9.3)	11.7 (10.9–12.5)
	ICIPE315	100.0 ± 0.0a	2.7 (2.5–2.9)	3.3 (3.1–3.6)
	ICIPE316	71.8 ± 4.1bc	7.2 (6.6–7.8)	9.1 (8.3–10.1)
	Control	11.5 ± 8.1f	–	–

Means with the same letter are not significantly different (Student–Newman–Keuls, $P = 0.05$)

Table 3 Effect of temperature on virulence of *M. anisopliae* and *B. bassiana* isolates against *T. urticae* 10 days post-treatment

Species	Isolates	% Mortality (Mean ± SE)			
		20°C	25°C	30°C	35°C
Beauveria bassiana	ICIPE278	54.7 ± 13.4abB	92.8 ± 2.7abA	89.7 ± 6.9aA	100aA
	ICIPE279	80.0 ± 7.2abB	98.5 ± 1.5aA	95.7 ± 2.8aA	100aA
Metarhizium anisopliae	ICIPE7	77.8 ± 2.8abB	84.4 ± 2.5abB	98.5 ± 1.5aA	97.1 ± 2.9aA
	ICIPE8	62.5 ± 11.0abB	91.4 ± 3.8abA	100aA	98.5 ± 1.5aA
	ICIPE25	54.0 ± 6.9abB	77.4 ± 10.4abA	88.9 ± 3.8aA	100aA
	ICIPE48	38.8 ± 11.4bC	77.1 ± 0.4abB	97.1 ± 1.7aA	100aA
	ICIPE49	57.8 ± 9.2abB	95.6 ± 2.8aA	91.5 ± 5.3aA	89.7 ± 4.4aA
	ICIPE62	57.8 ± 7.6abB	93.3 ± 2.5abA	100aA	91.1 ± 5.1aA
	ICIPE78	84.2 ± 5.6aAB	71.5 ± 8.3bB	100aA	97.2 ± 2.8aA
	ICIPE84	68.5 ± 8.1abB	93.0 ± 5.2abA	100aA	97.2 ± 2.8aA
	ICIPE315	58.1 ± 11.0abB	91.6 ± 2.8abA	93.4 ± 2.4aA	100aA

Means (±SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (Student–Newman–Keuls test, $P = 0.05$)

equally virulent at 25, 30 and 35°C, except isolates ICIPE7, ICIPE48 and ICIPE78 (Table 3). The LT_{50} values ranged from 6.3 to 10.9 days at 20°C, from 3.0 to 6.4 days at 25°C, from 1.7 to 4.0 days at 30°C and from 1.6 to 2.8 days at 35°C (Table 4). The LT_{90}, however, ranged from 8.2 to 18.5 days, from 5.1 to 9.2 days, from 2.1 to 5.6 days and from 2.1 to 3.5 days at 20, 25, 30 and 35°C, respectively (Table 4).

Discussion

Although all the 26 fungal isolates tested were pathogenic to *T. urticae*, there were significant variations amongst the isolates. Intraspecific variations in the pathogenic activity of isolates of *M. anisopliae* and *B. bassiana* have been reported in many arthropod pests [19, 26–28] including

Table 4 Effect of temperature on virulence of *B. bassiana* and *M. anisopliae* isolates against *T. urticae*: Lethal time to 50% and 90% mortality values (95% fiducial limits)

Isolates	20°C		25°C		30°C		35°C	
	LT_{50} (days)	LT_{90} (days)	LT_{50} (days)	LT_{90} (days)	LT_{50} (days)	LT_{90} (days)	LT_{50} (days)	LT_{90} (days)
B. bassiana								
ICIPE278	9.8 (7.6–12.6)	13.1 (9.5–18.1)	4.9 (4.5–5.3)	6.9 (6.3–7.6)	3.3 (2.8–4.0)	5.1 (4.8–5.3)	2.4 (2.1–2.9)	3.0 (2.6–3.4)
ICIPE279	6.9 (6.1–7.8)	8.9 (7.9–10.1)	3.8 (3.6–3.9)	5.1 (4.6–5.6)	1.7 (1.5–2.0)	2.1 (1.7–2.6)	2.1 (1.7–2.8)	2.8 (2.2–3.5)
M. anisopliae								
ICIPE7	7.1 (3.5–14.6)	9.0 (4.0–19.9)	5.4 (3.4–8.4)	7.5 (4.4–12.7)	2.6 (2.1–3.3)	3.5 (2.7–4.6)	2.8 (2.0–3.9)	3.5 (2.3–5.3)
ICIPE8	8.3 (6.8–10.1)	10.5 (8.2–13.3)	4.4 (3.0–6.5)	6.4 (4.8–8.6)	3.1 (2.9–3.4)	3.8 (3.5–4.0)	1.8 (1.1–2.9)	2.5 (1.6–3.8)
ICIPE25	8.4 (6.7–10.6)	13.2 (9.2–18.9)	3.0 (2.6–5.1)	5.3 (3.7–6.1)	2.5 (1.4–4.7)	2.7 (2.2–3.2)	2.2 (1.9–2.5)	2.6 (2.4–2.9)
ICIPE48	10.9 (7.6–15.6)	18.5 (11.6–29.4)	5.7 (5.2–6.2)	8.5 (8.0–8.9)	2.0 (1.7–2.2)	3.8 (1.8–7.9)	2.1 (1.9–2.4)	2.7 (2.4–3.1)
ICIPE49	8.4 (6.7–10.6)	12.3 (8.9–17.0)	5.0 (4.3–5.7)	6.9 (5.8–8.1)	4.0 (3.1–5.0)	5.6 (4.7–6.6)	1.6 (0.9–2.6)	2.1 (1.2–3.6)
ICIPE62	8.3 (6.6–10.5)	12.3 (9.6–15.7)	5.1 (4.7–5.7)	6.9 (6.4–7.5)	3.5 (3.4–3.6)	4.7 (4.3–5.1)	2.0 (1.6–2.6)	2.6 (2.0–3.6)
ICIPE78	6.3 (5.6–7.0)	8.2 (7.1–9.6)	6.4 (5.3–7.8)	9.2 (7.4–11.3)	2.7 (2.4–3.0)	3.6 (3.3–3.8)	2.2 (1.6–3.2)	2.9 (2.0–4.3)
ICIPE84	8.5 (7.0–10.3)	11.8 (10.2–13.8)	4.3 (3.5–5.2)	6.3 (5.0–8.0)	2.8 (2.6–3.1)	3.7 (3.3–4.1)	2.1 (1.8–2.4)	3.1 (2.7–3.6)
ICIPE315	7.5 (4.4–12.6)	12.7 (6.8–23.7)	4.4 (3.9–5.0)	6.5 (6.2–6.8)	3.3 (3.0–3.6)	4.6 (4.1–5.1)	2.7 (1.7–2.8)	3.4 (3.2–3.6)

T. urticae [15, 16, 29–31]. This emphasises the need of screening for strain selection [32].

All the 11 fungal isolates selected for temperature bioassays were pathogenic to *T. urticae* at all temperatures, but mortality varied with temperatures and fungal isolates. Our results are in agreement with many published reports on *B. bassiana* and *M. anisopliae* with other arthropod pests [19, 21]. Most fungal isolates were more pathogenic to *T. urticae* at 25, 30 and 35°C than at 20°C. Similar results have been reported in the legume flower thrips, *Megalurothrips sjostedti* (Trybom) [19], in second instar *Chilo partellus* (Swinhoe) larvae [20], and in three species of African tephritid fruit flies, *Ceratitis capitata* (Wiedemann), *C. cosyra* (Walker) and *C. fasciventris* (Bezzi) [21]. However, although virulence was less at 20°C than at 25, 30 and 35°C, some isolates were highly virulent to *T. urticae* from 20 to 35°C, causing more than 70% mortality (Table 3).

The selection of fungal isolates tolerant to the temperature range found in the target agricultural ecosystem is essential if pathogens have to be used in pest management programmes. The importance of temperature in fungal strain selection was stressed by Fargues et al. [33] and several other authors [19–21]. Since temperature not only affects the physiology of the fungus and host, but also the ability of the fungus to infect the host [20, 21], it should be taken into account in any biological control programme.

Since *B. bassiana* isolate ICIPE279 and *M. anisopliae* isolates ICIPE7, ICIPE78 and ICIPE84 were virulent against *T. urticae* over a broad temperature range, they are being considered for further studies to assess their potential as biological control agents of this pest.

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