Chapter 22
Effects and Persistence of Endophytic *Beauveria bassiana* in Tomato Varieties on Mite Density *Tetranychus evansi* in the Screenhouse

C. A. Omukoko, N. K. Maniania, V. W. Wekesa, and L. Turoop

Abstract The tomato red spider mite (RSM), *Tetranychus evansi* Baker and Pritchard, is an important exotic pest in the production of tomatoes and other solanaceous plants in Africa. Isolates of *Beauveria bassiana* ICIPE 273, ICIPE 279, ICIPE 283, ICIPE 10, and ICIPE 35 were previously tested for their endophytic activity in tomato in a screenhouse assay. ICIPE 35 was able to colonise leaves, stems and roots of the tomato varieties Cal J, Kilele and Anna F1, whereas the other four isolates were undetectable in all plant parts of the varieties tested, thus confirming no colonisation. Colonisation was assessed after every two-week period by plating the various plant parts on selective media. Persistence was examined by comparing colonisation from week 2, 4 and 6, since there was no colonisation detected after this time, for all the tested tomato varieties. There were no significant differences in levels of colonisation in the various parts of tomato varieties, i.e. stem ($F = 1.7$, $DF = 2$, $P = 0.186$), roots ($F = 2.0$, $DF = 2$, $P = 0.127$), and leaves ($F = 0.28$, $DF = 2$, $P = 0.752$). The density of *T. evansi* was lower in endophyte-colonised plants than the controls. In conclusion, this study revealed that *B. bassiana* can

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S. Niassy et al. (eds.), *Sustainable Management of Invasive Pests in Africa*,
Sustainability in Plant and Crop Protection 14,
https://doi.org/10.1007/978-3-030-41083-4_22

colonise and persist in the tested tomato varieties for a period of 6 weeks in the screenhouse and reduce adult RSM populations.

Keywords Endophytes · *Solanum lycopersicum* · Fungal Entomopathogens · Spider mites

22.1 Introduction

Tomato, *Solanum lycopersicum* Mill. (Solanales: Solanaceae), is a major vegetable crop, worldwide (Rice et al. 1987). It can be grown in backyards for home consumption, as well as in open fields and controlled environments for commercial purposes (van Dam et al. 2005). Tomato production in Kenya has been on the decline due to environmental stresses, declining soil fertility, poor crop management, and low quality seeds (KHDP 2008). Pest and disease pressures have also increased, reducing yields (FAO 2003).

Sustainable methods are being considered to manage pest populations in tomato crops. Among them there is the use of entomopathogenic fungi (Chandler et al. 2000; Maniania et al. 2008). These fungi also play additional roles in nature, such as endophytes, antagonists of plant pathogens, and possibly even plant growth-promoting agents (Vega et al. 2009).

Isolates of *Beauveria bassiana* have been tested against *Tetranychus evansi* and found to be highly virulent, suggesting a potential for their utilisation in the management of this pest (Wekesa et al. 2005). However, lack of an effective delivery system for entomopathogenic fungi limit their wide application. Hence, the need to develop an economically feasible mass-production and delivery system.

Beauveria bassiana has been detected inside tomato seedlings following seed treatment with the fungus. The endophytic association promoted growth and provided protection to tomato seedlings from damping-off disease (Ownley et al. 2004, 2008). The effectiveness of *B. bassiana* as an endophyte is determined by its survival and establishment within tomato plants.

Although *B. bassiana* is known to have the ability to exist in plants as an endophyte, there is a need to study its persistence in tomato plant tissues, so that the fungus can offer long-term protection against the *T. evansi* in tomatoes in screenhouses. This study, therefore, aimed at investigating the endophytic potential and persistence of ICIPE 35 isolate of *B. bassiana* in the Cal J, Kilele, and Anna tomato varieties in the screenhouse, studying the fungus effects on RSM density, and assessing the endophytic colonisation and persistence of *B. bassiana*.

22.2 Materials and Methods

22.2.1 Fungal Culture

From previous screening work done on five isolates of *B. bassiana*, ICIPE 273, ICIPE 279, ICIPE 283, ICIPE 10 and ICIPE 35, only ICIPE 35 was able to establish as an endophyte in tested tomato varieties. The isolates were obtained from the ICIPE Arthropod Germplasm Centre, (Nairobi, Kenya), and sub-cultured on Sabouraud Dextrose Agar (SDA) medium, amended with 0.05 g chloramphenicol antibiotics to minimise the bacterial contamination, and incubated for 3 weeks at 27 °C (Inglis et al. 1996). Conidia were gently scrapped from fungal cultures and suspended in 10 ml sterile distilled water in a 20-ml universal bottle containing 0.01% Tween-20 and glass beads. The suspension was vortexed for 5 min to produce a homogenous conidial distribution. From the stock solution, a concentration of 1×10^9 conidia ml^{-1} was prepared, since the standard concentration of 1×10^8 used in preliminary studies did not establish well in the tomato varieties. The viability of conidia was assessed prior to bioassays by spread-plating 0.1 mL of 3×10^6 conidia mL^{-1} onto 90-mm Petri dishes containing SDA (Goettel and Inglis 1997). The plates were incubated at 27 ± 2 °C and were examined after 16–20 h under a compound microscope (×40 magnifications). Conidia were considered as germinated when the germ tube was twice the diameter of the conidium. The experiment was replicated four times.

22.2.2 Tomato Plants

The tomato plants (*Lycopersicum esculentum* var. Cal-J, Kilele and Anna) that were used in this study were raised in the screenhouse at ICIPE. Cal-J is highly susceptible to red spider mites, but is commonly grown by farmers, while Kilele and Anna are popular new hybrids among farmers.

22.2.3 Red Spider Mite Culture

Tetranychus evansi was obtained from a regularly regenerated colony reared on tomato variety Cal-J (ICIPE) at 25 ± 2 °C, 60–70% R.H., and a photoperiod of 12:12 h (L:D). The initial culture originated using mites collected from Mwea Irrigation Scheme, Kenya, in 2001.

22.2.4 Seed Inoculation and Colonization

Seeds of Cal-J and two hybrid cultivars (Kilele and Anna) were surface-sterilised in 70% ethanol for 1 min and then in 1.5% sodium hypochlorite for 3 min. They were then washed three times with sterile distilled water and blot dried on sterile paper towels to remove the excess water. The last rinse water was plated out to assess the effectiveness of the surface sterilisation procedure. Inoculation was carried out by soaking seeds in conidial suspension (10 ml) of *B. bassiana* isolate ICIPE 35 at the concentration of 1×10^9 conidia ml^{-1} for 2 h. The seeds were hand stirred at a 30-min intervals until they were uniformly coated (Powell et al. 2009). Control seeds were soaked in sterile distilled water containing 0.01% Tween-20 for 2 h. The seeds were then removed and sown in pots filled with sterilised soil. There were 8 individual pots for each tomato variety for ICIPE 35 and for their respective controls. The pots containing the seedlings were grown in the screen house at 28 ± 2 °C and 70–80% R.H. for 6 weeks. The endophytic colonisation by *B. bassiana* on tomato plant was examined every 2 weeks after inoculation through destructive sampling. Plants were carefully removed from the pots, washed with tap water, and surface-disinfected by submersing in 70% ethanol for 15 s, followed by 3 min in 1.85% sodium hypochlorite, and rinsed 3 times in sterile distilled water. To determine the effectiveness of the disinfecting process, the final rinse water was plated on SDA, as described above. Plants were dried on sterile paper towels, and tissues from leaves, shoots and roots were cut into 4 mm^2 sections with a sterile scalpel, and about 5 of them were placed on Petri dishes containing SDA medium. Four plates were cultured for each tissue (roots, shoots and leaves) and for each treatment, including the control. They were allowed to sporulate for 2 weeks in the laboratory, and then the *B. bassiana* was identified, based on its morphological characteristics. For each plant part, the percentage of colonisation was calculated as the number of sections exhibiting *B. bassiana* outgrowth per total number of sections (Fisher and Petrini 1987).

22.2.5 Data Analysis

The colonisation frequency (CF) was calculated as described by Fisher and Petrini (1987). The proportions of fungal colonisation per plant part and persistence, which were expressed as percentages, were arcsine square root transformed before being subjected to ANOVA using the procedure generalised linear models of SAS software. Whenever treatment effects were significant, then means were compared using SNK (Student-Newman-Keuls). All tests were performed at 5% level of significance. Population counts of mites for the greenhouse experiment were log transformed to normalise data and transformed values were subjected to ANOVA. A Student's t-test was used to compare treatments and control. All data were analysed using the SAS statistical package ver. 8.

22.3 Results

22.3.1 *Endophytic Colonisation of* B. bassiana *on Tomato Varieties*

The *B. bassiana* isolate ICIPE 35 was able to colonise the roots, stems and leaves of all the tomato varieties in the screen house. However, there were no significant differences in levels of colonisation among parts of the three tomato varieties, i.e. stems (F = 1.7, DF = 2, p = 0.18), roots (F = 2.0, DF = 2, p = 0.12), and leaves (F = 0.28, DF = 2, p = 0.75) (Fig. 22.1). For example, in Anna, there was no significance difference in colonisation among the various plant parts, (leaves = 4.5%, stem = 5%, roots = 3.3%). Colonisation was slightly higher in the leaves of Cal J variety (5.8%), compared with the stem, (2.9%), and roots (3.3%). In Kilele, colonisation was highest in leaves (4.8%), followed by stem and roots (3.4 and 1%), respectively (Fig. 22.1). There was no colonisation of plant parts of the varieties tested as control.

There was no interaction between the variety and treatment for the various plant parts at 2, 4, 6, weeks after inoculation: roots (F = 2.3, df = 2, P = 0.10), stems (F = 1.8, df = 2, P = 0.17), and leaves (F = 0.34, df = 2, P = 0.71). Consequently, there was no interaction between the variety and the weeks: roots (F = 1.9, df = 4, P = 0.10), stems (F = 0.50, df = 4, P = 0.74), and leaves (F = 0.34, df = 4, P = 0.71). Although there was no interaction for the treatment and week for plant roots, (F = 1.7, df = 2, P = 0.18), there was a significant interaction for stems (F = 3.87, df = 2, P = 0.02) and leaves (F = 8.9, df = 2, P = 0.00). Despite the general decline

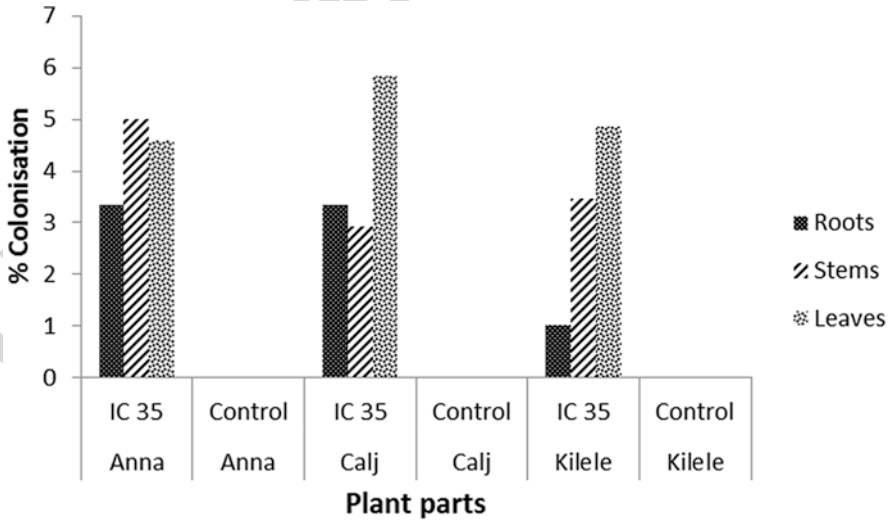

Fig. 22.1 Colonisation of different parts of tomato varieties by *Beauveria bassiana* isolate ICIPE 35

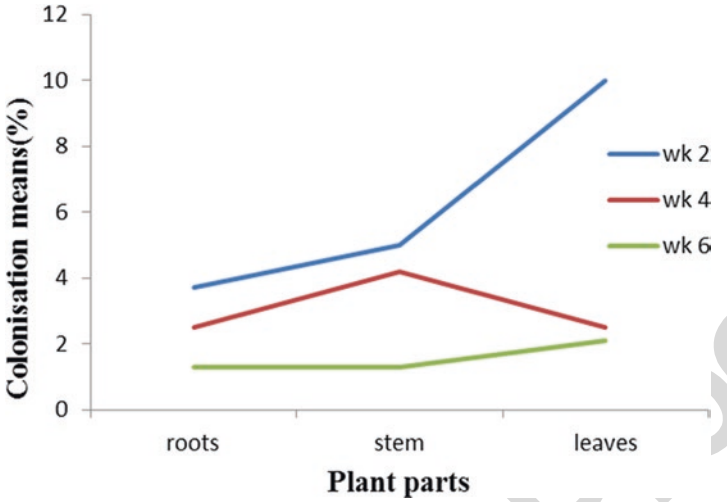

Fig. 22.2 Persistence of different parts of tomato varieties by *Beauveria bassiana* isolate ICIPE 35, 6 weeks after inoculation in the screen house

in percentage colonisation over time, the rate of decline was not different among the various plant parts for all the tested varieties, i.e. stem ($F = 1.7$, $df = 2$, $P = 0.18$), roots ($F = 2.0$, $df = 2$, $P = 0.12$), and leaves ($F = 0.28$, $df = 2$, $P = 0.75$).

The percentage for colonisation was generally high for week 2, as compared with weeks 4 and 6, although there were no significant differences for plant roots for weeks 2, 4 and 6. For stems, week 6 was significantly different from weeks 2 and 4, which were not different, while for the leaves, week 2 was significantly different from weeks 4 and 6, which were not different (Fig. 22.2).

The correlation between the top leaf and low leaf has an r value of 0.619, which is more than 0.05, and is a strong value. For top leaf and total mite, the value is 0.978, which is also a strong value. The correlation between the top leaf and leaf area has a weak value of 0.399. Generally, from the results above, as the leaf area increases, the mite density decreases, and vice versa (Fig. 22.3). Tomato plants inoculated with ICIPE 35 had less RSM, compared with the controls.

22.4 Discussion

Beauveria bassiana isolate ICIPE 35 was able to colonise the root, stem and leaves of Cal-J, Kilele and Anna tomato varieties, in the screenhouse. The levels of colonisation also differed among the various plant parts, likely due to the external environment or the biological differences within the plant tissues (Bayman et al. 1997). These results confirm those by Muvea et al. (2014), who found differences in the

		Correlations			
		topleaf	lowleaf	Tot_mite	l/area cm3
topleaf	Pearson Correlation	1	.619**	.978**	-.399**
	Sig. (2-tailed)		0	0	0
	N	181	158	181	181
lowleaf	Pearson Correlation	.619**	1	.748**	-.188*
	Sig. (2-tailed)	0		0	0.018
	N	158	158	158	158
Tot_mite	Pearson Correlation	.978**	.748**	1	-.393**
	Sig. (2-tailed)	0	0		0
	N	181	158	181	181
l/area cm3	Pearson Correlation	-.399**	-.188*	-.393**	1
	Sig. (2-tailed)	0	0.018	0	
	N	181	158	181	181

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Fig. 22.3 Correlations for top leaf and low leaf for adult mite populations

level of colonisation of different plant parts by fungal isolates. Similar results were also reported on French beans and Faba beans (Akutse et al. 2013), and coffee (Posada et al. 2007).

The fact that the fungus was found in several plant parts indicates that it was able to spread within the plant. This is important, especially if there is vertical transmission of the endophytes through the seeds, meaning that subsequent generations may not require seed dressing (Schardl et al. 2004). Colonisation of plants by entomopathogens can take several pathways, which all depend on the fungi and the plant in question. However, systemic spread has been advocated by most authors (Bing and Lewis 1991). This supports previous studies that attributed passive movement of the fungal hyphae within the xylem and vascular tissues, eliciting a systemic reaction (Bing and Lewis 1992).

The colonisation in the Anna variety was slightly higher in the stem than in leaves, while in Kilele and Cal J the leaves experienced a higher degree of colonisation, compared with the other parts. It is possible that the leaves provide a suitable environment for the establishment and survival of *B. bassiana*, as compared with the other plant parts in these two varieties (Fisher et al. 1992). Previous studies showed that most endophytic fungi are highly adapted to particular conditions present in a given plant organ (Carroll 1988; Fisher et al. 1992). This is likely due to micro-ecological and physiological conditions existing in the different tomato plant tissues, which confer varying survival degrees for *B. bassiana* (Bayman et al. 1997).

Although the various parts of the tomato varieties were colonised 2 weeks after inoculation, a decrease was noted by week 6, and by week 8, colonisation declined as it was not observed in any of the plant parts. The rate of decline was faster in leaves, followed by the stems, and lastly, the roots, which could probably be due to

no multiplication taking place within the plant rhizosphere, or inhibition of *B. bassiana* germination or growth (Quesada-Moraga et al. 2006). Similar results were observed in cocoa plants, where *B. bassiana* became established as an endophyte, but did not persist beyond 2 months (Posada and Vega 2005). Decline in colonisation of the various plant parts may also be caused by the plant response to the endophytic fungus, by other fungi that occur naturally within the plant, and also by the expansion of its tissues and parts (Posada et al. 2007).

The delivery of *B. bassiana* as endophyte bypasses the limitations imposed by their direct use, especially foliar spraying, where they are affected by UV radiation, varying conditions of temperature, and humidity, that frequently reduce conidial viability (Vega et al. 2008). Once established in the plant system, these endophytes provide plant protection against pests, reduce operation costs because no repeated applications are needed, and guarantee efficacy because the fungus is protected against abiotic factors.

The plant-defence ability exhibited by *B. bassiana* by reducing mite populations in tomato requires a greater understanding of the mechanisms that suppress herbivory, so as not compromise human and animal safety, as was the case with Epichloe, which protects grasses against nematodes but is also toxic to vertebrates (Schardl et al. 2004).

This study demonstrated that *B. bassiana* isolate ICIPE 35 can colonise tomato plants and persist up to 6 weeks in a screenhouse from seed inoculation. It also reduced the adult population of RSM. This can be used to complement existing control measures for controlling RSM in tomato crops.

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Abstract

Tuta absoluta is an invasive and devastating pest of tomato, native to Latin America. It was reported in Spain in 2006 and spread to Euro-Afro-Asian countries. In Sudan, it was reported in 2010. We studied the flying behaviour of *T. absoluta* to understand the times of insect activity and the role of crops in insect attraction, using a pheromone (Tua optima 0.8 mg) and baited water traps. The timing of male activity was monitored every 3 h from 6:00 pm to 9:00 am the next day. The height of flying was studied through installing four traps (from 0 to 150 cm). All traps used in these studies were put upwind of tomato and miscellaneous crops. Temperatures and wind velocities were recorded during the study period. The males activity started at photo phase and continued for almost 1 h thereafter, ending at almost 30.0 min after sunrise. The height of flying for 90% of males was at ground level. Traps deployed upwind of tomato crop captured 70% of males (n = 1466). The findings confirmed that males of *T. absoluta* were attracted by pheromone and tomato, and traps should be at the ground level, before photo phase.