Potential of Entomopathogenic Fungi in the Control of Economically Important Spider Mite Species *Tetranychus urticae* Koch and *Tetranychus evansi* Baker & Pritchard

David Mugisho Bugeme

A thesis submitted in fulfilment for the Degree of Doctor of Philosophy in Botany in the Jomo Kenyatta University of Agriculture and Technology

2008

ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato spider mite, *T. evansi* Baker & Pritchard (Acari: Tetranychidae), are among the most important pest of horticultural crops, such as tomato, beans, cur flowers, eggplants and several other vegetables. While *T. urticae* has been known as a worldwide pest of a wide range of horticultural crops both outdoors and in the greenhouses, the importance of *T. evansi* has dramatically increased during the last decade. In Africa, farmers largely rely on synthetic acaricides to control these two pests. However, due to problems related to the use of synthetic acaricides in controlling *T. urticae* and *T. evansi* (mite resistance and environmental contamination), the control of this pest is still a major problem for farmers and attract a strong attention in the world or researchers. Thus non chemical control measures are being developed as alternatives to synthetic acaricides for the control of the two pests. They include improved crop management, screening for resistance in commercial and wild tomato germplasm and biological control using predatory mites and entomopathogenic fungi.

The aim of this study is to investigate the potential of the entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycotina: Hypocreales), to control the two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), pests of horticultural crops.

Field surveys were carried out in Kerugoya, Kakamega, Machakos, Kitui, Makueni, Kajiado and Taita-Taveta Districts in order to prospect for new fungal isolates for use in the biological control of spider mites. One isolate of *B. bassiana* and three isolates of *M. anisopliae* were found

to be associated with spider mite species in the field in Kakamega and in Kerugoya districts, respectively, during the rainy season.

Twenty-six isolates of entomopathogenic mitosporic fungi *B. bassiana* and *M. anisopliae*, from the ICIPE (International Centre of Insect Physiology and Ecology) culture collection, were tested in the laboratory to determine their pathogenicity to adult *T. urticae* and *T. evansi*. All the fungal isolates were pathogenic to the two spider mite species, causing mortality ranging between 95.2 to 99.0% (*B. bassiana*) and 36.5 to 100% (*M. anisopliae*) in *T. urticae* and between 83.0 to 95.2% (*B. bassiana*) and 30.4 to 90.5 (*M. anisopliae*) in *T. evansi*. The lethal time to 50% mortality (LT_{50}) values varied from 3.0 to 8.3 days with *T. urticae* and from 4.7 to 8.2 days with and *T. evansi*. The radial growth of *B. bassiana* isolates was lower than *M. anisopliae* ones. The radial growth varied from 2 to 2.6 mm day^{-1} for *B. bassiana* isolates and from 3.3 to 5.8 mm day^{-1} for *M. anisopliae* isolates.

The effect of temperature on germination, radial growth and virulence of two isolates of *B. bassiana* and nine of *M. anisopliae* selected during the screening against *T. urticae* and *T. evansi*, was studied in the laboratory. Temperature had significant effects on germination, radial growth and virulence of the various isolates. Over 65.8% of conidia germinated at 20, 25 and 30 ºC while between 15.1 and 84.3% germinated at 35 ºC. Radial growth was slow at 20 and 35 ºC for all isolates, except *M. anisopliae* isolate ICIPE7 at 35 ºC. The optimum temperature for fungal germination was 25 and 30 ºC, while the optimum temperature for fungal radial growth was 30 ºC. All the *B. bassiana* and *M. anisopliae* isolates were virulent to both *T. urticae* and *T. evansi* at all the temperatures but the virulence varied with isolates and temperatures. Fungal isolates were more effective at 25, 30 and 35 ºC than at 20 ºC.

The susceptibility of *T. urticae* and *T. evansi* developmental stages to infection by *B. bassiana* and *M. anisopliae* was also evaluated in the laboratory. On one hand, the effect of *B. bassiana* (isolate ICIPE279) and the *M. anisopliae* (isolates ICIPE7, ICIPE78 and ICIPE84) were tested against *T. urticae* developmental stages, while on the other hand, the effect of *B. bassiana* (isolates ICIPE278 and ICIPE279) and *M. anisopliae* (isolates ICIPE78 and ICIPE84) were tested against *T. evansi* developmental stages. All stages of *T. urticae* and *T. evansi* were susceptible to infection by *B. bassiana* and *M. anisopliae*. An increment in the concentration reduced egg hatchability and increased mortality in motile stages. The lowest egg hatchability and the highest mortality occurred at the highest concentration of 1 x 10^7 conidia ml^{-1}. However mature stages (Deutonymphs and Adults) were more susceptible to fungal infection than the immature stages (Larvae and Protonymphs). The lethal concentration to 50% mortality (LC_{50}) values varied from 20.8 to 46.3 x 10^7, from 0.3 to 0.7 x 10^7, from 0.2 to 0.4 x 10^7 and from 0.06 to 0.2 x 10^7 conidia ml^{-1} in larvae, protonymphs, deutonymphs and adults, respectively in *T. urticae*, while they varied from 8 to 40.4, from 6.8 to 37.8, from 0.3 to 2.5 and from 0.1 to 0.3 x 10^7 conidia ml^{-1} in larvae, protonymphs, deutonymphs and adults, respectively, in *T. evansi*.

The compatibility between *B. bassiana* (isolate ICIPE279) and *M. anisopliae* (isolate ICIPE78) and synthetic acaricides (three insecticides and three fungicides) was studied in the laboratory. The effect of synthetic pesticides on fungal germination and fungal radial growth was evaluated for this purpose. All the synthetic fungicides inhibited the fungal germination, and are therefore not compatible with the two fungal isolates. The synthetic insecticides, however, showed a high compatibility between them and the fungal isolates, except in the case of *B. bassiana* and Malathion. The latter retarded the *B. bassiana* germination.

The effect of *M. anisopliae* (isolate ICIPE78) and Dynamec® (synthetic acaricide with abamectin as active ingredient) on the *T. urticae* population density and on the *T. urticae*-infested bean production parameters, in the greenhouse, was evaluated. There were significant differences in *T. urticae* population densities between the treatments at postal sampling dates post-spraying, in top and middle leaves. At 3 weeks post-spraying the mite densities were near zero in the treated leaves, compared to control (9.23 and 9.84 mites/cm^2 on top and middle leaves, respectively). At 5 weeks post-treatment, there were no more leaves in the control. There were also significant differences in the number of pods per plant, the number of seeds per pod and the dry weight of seeds per plant between the treatments. Yields were 10.5 and 10.8 more times than the control with fungal and acaricide treatments, respectively.

The effect of *M. anisopliae* (isolate ICIPE78) and the synthetic acaricide (Dynamec) on the population density of *T. urticae* and *T. evansi* in the field was assessed. The aqueous and emulsifiable fungal formulations reduced the population densities of *T. urticae* and *T. evansi* infesting bean and tomato plants, respectively. The two fungal formulations were as good control agents as the synthetic acaricide in controlling *T. urticae*. In tomato fields, however, the fungus in emulsifiable oil formulation provided a better control of *T. evansi* than the fungus in water formulation.

The results of this study underline the potential of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* as alternative to acaricides for *T. urticae* and *T. evansi* management.