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Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* fungi to *Tetranychus ludeni* (Acari: Tetranychidae)

Patogenicidade de fungos **Metarhizium anisopliae** *e* **Beauveria bassiana** *ao* **Tetranychus ludeni** *(Acari: Tetranychidae)*

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ABSTRACT: The use of acaricides is the main control method for *Tetranychus ludeni* (Acari: Tetranychidae) in horticultural crops. This mite has been recorded causing damage to sweet potato (*Ipomoea batatas* L.). The use of pathogenic fungi is an alternative to chemical control. The objective was to evaluate the pathogenicity of the fungi *Metarhizium anisopliae* (Meetch) Sorok, and *Beauveria bassiana* (Bals.) Vuill. to phytophagous mite *T. ludeni* in sweet potato plants. Conidial suspensions of *M. anisopliae* and *B. bassiana,* at concentrations of 10^6 and 10^7 con.mL^{-1} were applied to sweet potato leaves. After 24 hours, five females of *T. ludeni* newly emerged were released on the leaves. The bioassay was arranged in a completely randomized design with factorial arrangement 2 × 2 (two species of fungi and two concentrations) plus the control (distilled water), with 10 repetitions per treatment. The evaluation consisted of observing of three biological parameters of the mite: mortality, oviposition, and repellency, after 24, 48, 72, and 96 hours of contact with the fungi. The isolates of *M. anisopliae* cause high mortality rates of *T. ludeni* in laboratory. *Beauveria bassiana* has the potential to suppress future generations of mite, reducing its oviposition rate. Repellency behavior was not observed.

KEYWORDS: *Beauveria bassiana*; *Metarhizium anisopliae*; biological control; red mite.

RESUMO: O uso de acaricidas é o principal método de controle de *Tetranychus ludeni* (Acari: Tetranychidae) em cultivos hortícolas. Esse ácaro foi registrado causando danos em batata-doce (*Ipomoea batatas* (L.). A utilização de fungos patogênicos é uma alternativa ao controle químico. O objetivo foi avaliar a patogenicidade dos fungos *Metarhizium anisopliae* (Meetch) Sorok. e *Beauveria bassiana* (Bals.) Vuill. ao ácaro fitófago *T. ludeni* em batata-doce. Suspensões conidiais de *M. anisopliae* e *B. bassiana,* nas concentrações de 10^6 e 10^7 con.mL^{-1}, foram aplicadas sobre folhas de batata-doce. Após 24 horas, cinco fêmeas recém-emergidas foram liberadas sobre as folhas. O bioensaio foi inteiramente randomizado, com arranjo fatorial 2 × 2 (duas espécies de fungos e duas concentrações) e controle (água destilada), com 10 replicações por tratamento. A avaliação consistiu na observação de três parâmetros biológicos do ácaro: mortalidade, oviposição e repelência, após 24, 48, 72 e 96 horas de contato com os fungos. Os isolados de *M. anisopliae* causam altas taxas de mortalidade de *T. ludeni* em laboratório. *Beauveria bassiana* tem potencial para suprimir futuras gerações do ácaro, reduzindo a taxa de oviposição. Comportamento de repelência nos ácaros não foi observado.

PALAVRAS-CHAVE: *Beauveria bassiana*; *Metarhizium anisopliae*; controle biológico; ácaro vermelho.

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INTRODUCTION

Ipomoea batatas (L.) Lam. (Convolvulaceae), the sweet potato, is the seventh most important vegetable in the world (SONG et al., 2014), standing out for its easy cultivation, rusticity, adaptation to different soil types and climate, drought tolerance, and low production cost (ANDRADE JÚNIOR et al., 2012). Grown in practically all Brazilian regions, *I. batatas* is an excellent energy source due to its high carbohydrate and sugar content (MASSAROTO et al., 2013). It is used in human and animal food and has potential, as a raw material, to produce biofuels (ethanol) (SANTOS et al., 2010).

Pests may limit the production of *I. batatas*, reducing the quality and volume of roots (AZEVEDO et al., 2015). Phytophagous mites, such as *Tetranychus desertorum* (Banks), *Tetranychus ludeni* (Zacher), and *Tetranychus urticae* (Koch) (Acari: Tetranychidae) were reported for causing damage to *I. batatas* (MINEIRO et al., 2007; SOARES et al., 2012). Phytophagous mites cause damage when feeding on leaves, reducing their photosynthetic area (SOARES et al., 2012). *Tetranychus ludeni*, an arthropod polyphagous, occurs in the field most of the year. Leaves attacked by this mite initially have small yellowish spots, followed by necrotic spots, that take over the entire leaf, which later dries and falls (KAIMAL; RAMANI, 2011).

The use of acaricides is the main method of *T. ludeni* control in horticultural crops (KOUSIK et al., 2007), but it was already shown to be unfeasible, due to the high cost of pesticides in Brazil and the absence of selective and registered acaricides for the crop in the country (MAPA, 2016). The adverse effects of pesticides and restrictions on their application have encouraged the exploration of environmentally friendly pest control tactics (KHEDERI et al., 2014).

Pathogenic fungi, such as *Beauveria bassiana* (Balsamo) *Vuillemin* and *Metarhizium anisopliae* (Meetchnikoff) Sorokin, can regulate arthropod pest populations by penetrating their cuticles and destroying their tissues (KURTTI; KEYHANI, 2008; ROSSONI et al., 2014; COSTA et al., 2015). The ease of dispersal of these microorganisms in the field is one of the factors that fuel studies to investigate their use as biological control agents (MEYLING et al., 2009; COSTA et al., 2015).

The objective of the present study was to evaluate the pathogenicity of the fungi *M. anisopliae* and *B. bassiana* to the phytophagous mite *T. ludeni* in sweet potato, under laboratory conditions.

MATERIAL AND METHODS

The experiment was carried out at the Insect Biological Control laboratory of Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) in Diamantina - MG. *Tetranychus ludeni* was reared in conditions of humidity (65 ± 10%), temperature (25 ± 1°C), and photoperiod of 12 hours. Two hundred and fifty females of *T. ludeni* were used for the bioassay, and fifty females per treatment.

Ten leaf circles of 2 cm in diameter, per treatment, were cut from the sweet potato genotype BD 29, derived from the UFVJM germplasm bank. The leaf circles were surrounded with cotton, moistened with distilled water, and placed over nylon foam and in a tray. The foam was kept moist with the addition of distilled water (SOARES et al., 2012; ULLAH; LIM, 2015).

Suspensions containing fungal conidia were obtained from the commercial products Metarril® WP E9 and Boveril® WP PL 63 (Koppert Biological Systems). Conidia was suspended in an aqueous solution containing 0.01% Tween® 80 (ALMEIDA et al., 2006; ULLAH; LIM, 2015) at concentrations of 10^6 and 10^7 con.mL^{-1} for each isolate. Leaf circles were sprayed 100 µL of the suspensions of the isolates of *M. anisopliae* and *B. bassiana*, homogeneously.

After 24 hours, leaf circles were dry and conidia adhered to the plant's surface, and five freshly emerged *T. ludeni* females were released in each circle and kept at 25 ± 2°C, 70°C ± 10% relative humidity and 12 hour photophase. The bioassay was arranged in a completely randomized design with a 2 × 2 factorial arrangement (two fungal species and two conidia concentrations), and the control (distilled water) with 10 replications per treatment.

Tetranychus ludeni females in the leaf circles were observed under a stereomicroscope after 24, 48, 72, and 96 hours of contact with the fungi. Biological parameters evaluated were mortality, oviposition, and repellency. The mortality data of *T. ludeni* females were evaluated using the quadratic polynomial regression model, with significant curves with the Student's *t* test. The oviposition and repellency rates of the mites were compared using the Tukey test at 5% significance with free software R®.

RESULTS AND DISCUSSION

In the evaluation of the mortality percentage of *T. ludeni* (in sweet potato leaf samples), after a period of 96 hours after treatment with two suspensions containing *M. anisopliae* conidia plus one control, it was found that the higher conidia concentration in the suspension (10^7 con.mL^{-1}) of *M. anisopliae* caused a higher mortality percentage of *T. ludeni* females in relation to the treatment where fungus concentration was 10^6 con.mL^{-1} and the control treatment. In the last evaluation period (96 hours) was observed that the mortality percentage was 80% in the highest concentration. These values were 52 and 40% in the concentration of 106 con.mL^{-1} and in the control treatment, respectively (Fig. 1A). The mites treated with suspension containing the fungus *B. bassiana* showed an increase in mortality in all treatments compared to the control, and this variable showed similar mean values during the evaluation periods in the two concentrations tested. The maximum mortality of *T. ludeni* females observed after 96 hours was 30% (Fig. 1B).

According to TAMAI et al. (2002), the fungus *Metarhizium anisopliae* presents higher pathogenicity to *T. urticae* than *B. bassiana*. In reports by BUGEME et al. (2015), with *M. anisopliae* (isolated ICIPE78), the authors concluded that suspensions containing conidia of this fungus constitute an alternative to the use of acaricides to reduce *T. urticae* populations in common bean. The same authors also point out that *M. anisopliae* presents control similar to that obtained with acaricide abamectin in greenhouse and field.

SANJAYA et al. (2015) observed that different isolates of *B. bassiana*, *M. anisopliae*, and *Paecilomyces lilacinus*, at concentrations of 10^5, 10^6, 10^7 and 10^8 con.mL^{-1}, control *Tetranychus kanzi* Kishida (Acari: Tetranychidae) mite.

ULLAH; LIM (2015) showed an effect of different concentrations of *M. anisopliae* and *B. bassiana*funguson *T. urticae*, reporting mortality rates above 50% in all concentrations.The authors also found that the highest mortality rate, 98.31%, was reached by the *M. anisopliae* isolate, and applications of *B. bassiana* (10^8 con.mL^{-1}) reduced *T. urticae* damage by 94% in common bean.

In the paper by BARRETO et al. (2004), different concentrations of *B. bassiana* control up to 91% of the population of the green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), whereas isolates of *M. anisopliae* (10^8 con. mL^{-1}) caused 45% of mortality.

The high mortality values obtained with *M. anisopliae* isolates on the fourth day indicate a fast action of the pathogen on *T. ludeni* (Fig. 1A).

Mites in crops with high economic damage, such as ornamental crops and horticulture, require a rapid control. Isolates must be capable of causing high mortality, requiring less frequent spraying and reducing costs (TAMAI et al., 2002). The pathogenicity of acaropathogenic fungi can vary among mites, crops, and even cultivars (RIBEIRO et al., 2009; MORO et al., 2011).

In the present paper, the fungus *M. anisopliae* was effective to control *T. ludeni*. The *B. bassiana* fungus had a lower effectiveness in controlling the mite.

When evaluating the oviposition rate of *T. ludeni* females after treatment with different concentrations of *M. anisopliae* fungus conidia, the highest concentration of fungus conidia (10^7 con.mL^{-1}) provided the greatest reduction in this variable, differing from the other treatments (Fig. 2A).

The two *B. bassiana* conidia concentrations used to control *T. ludeni* were efficient in reducing oviposition of insect females, differing from the results found in the control treatment (Fig. 2B). Stressing factors, such as pathogens or toxic substances, may reduce the oviposition of phytophagous mites (SILVA et al., 2013), an effect considered important in IPM, because it reduces future generations of phytophagous. Sublethal effects of pathogenic fungi on target pests should be considered. *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) first-generation nymphs of adult descent treated with *B. bassiana* isolates are less successful in the molting process, and are not able to completely separate from the exuviae (TORRADO-LEÓN et al., 2006; WANG et al., 2014).

The effect of fungi on pest fecundity confirms their pathogenic potential as biological control agents and can be demonstrated by the difference in fecundity between infected and uninfected pests (WEKESA et al., 2006; SHI; FENG, 2009). SEYED-TALEBI et al. (2012) found that *T. urticae* longevity, oviposition period, and fecundity were lower in individuals treated with *B. bassiana*. Moreover, *B.bassiana* (GZGY-1-3) has toxic and sublethal effects on *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae),

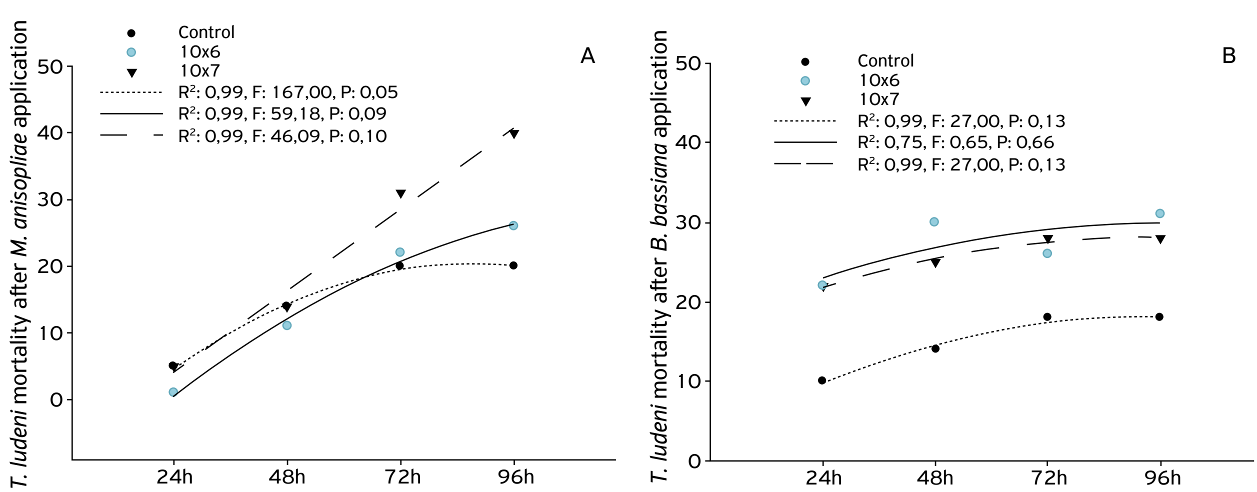

Figure 1. Mortality percentage of *Tetranychus ludeni* females after applying different concentrations of (A) *Metarhizium anisopliae*; and (B) *Beauveria bassiana*.

reducing reproductive success (ZHANG et al., 2015). Thrips that survive the action of the pathogen fungus have less reproductive success and aptitude than their offspring, which slows down population growth rates (LI et al., 2013). Sublethal effects caused by fungal infections were also observed in *Tetranychus evansi* Baker & Pritchard 1960 (Acari: Tetranychidae) (WEKESA et al., 2006) and *T. urticae* (ROSAS-ACEVEDO et al., 2003). Infections caused by *B. bassiana* and *M. anisopliae* caused mortality in *T. ludeni* females and reduced their fecundity. In the present study, there was a higher mortality of *T. ludeni* females when they were treated with conidia suspension containing *M. anisopliae* in relation to *B. bassiana* (Figs. 1A and 1B). However, treatment with *B. bassiana* provided lower oviposition rate of *T. ludeni* females than *M. anisopliae* (Figs. 2A and 2B), indicating differentiated efficiency of the two acaropathogenic fungi. *M. anisopliae* most clearly affected the pest mortality rate; and *B. bassiana* interfered in the reproduction of *T. ludeni* females.

The higher concentration of *M. anisopliae* conidia (10^7 con.mL^{-1}) promoted higher repellency rate of *T. ludeni* females, without differing from other treatments (Fig. 3A). The females of *T. ludeni* treated with the fungus *B. bassiana* showed no difference in the repellency rate between the different concentrations and the control (Fig. 3B).

Mites were not found adhered to the damp cotton layer, surrounding leaf circles, in any of the *M. anisopliae* and *B. bassiana* conidia concentrations, thus not demonstrating an escape behavior. Repellent behavior was not observed after 96 hours of contact with acaropathogenic fungi (Figs. 3A and 3B). In a study by BARRETO et al. (2004), isolates of *B. bassiana* at a concentration of 10^8 con.mL^{-1} caused repellency in *M. tanajoa* after 12 hours of evaluation. However, the escape percentage did not exceed 8%.

For greater efficiency in sweet potato Integrated Pest Management (IPM) programs, the use of the pathogens *B. bassiana* and *M. anisopliae*in mixture and with other natural control agents, such as predatory mites, should be investigated (CASTRO et al., 2014).

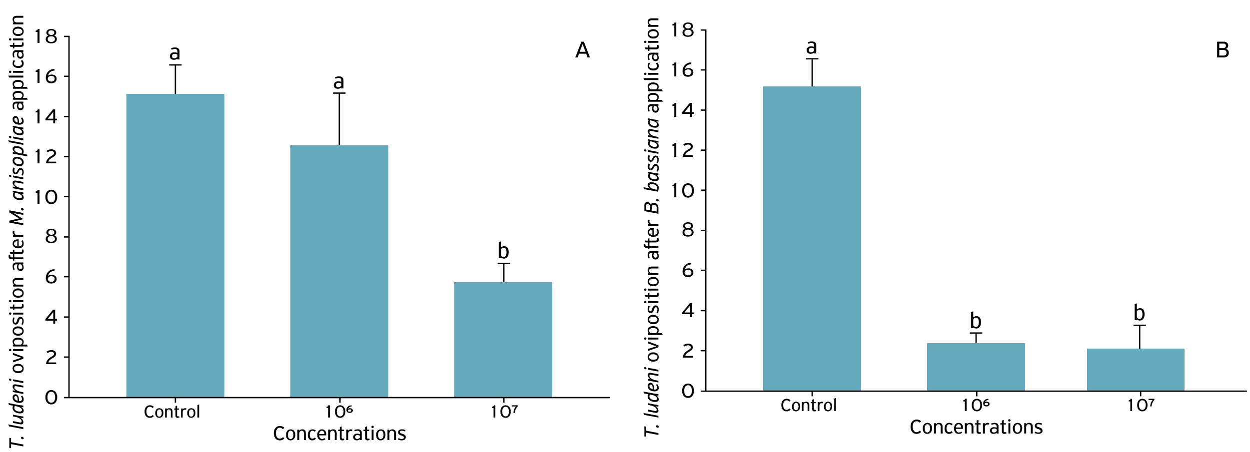

Figure 2. *Tetranychus ludeni* oviposition after applying different concentrations of (A) *Metarhizium anisopliae*; and (B) *Beauveria bassiana*. Means were compared with the Tukey test, $p \geq 0.05$.

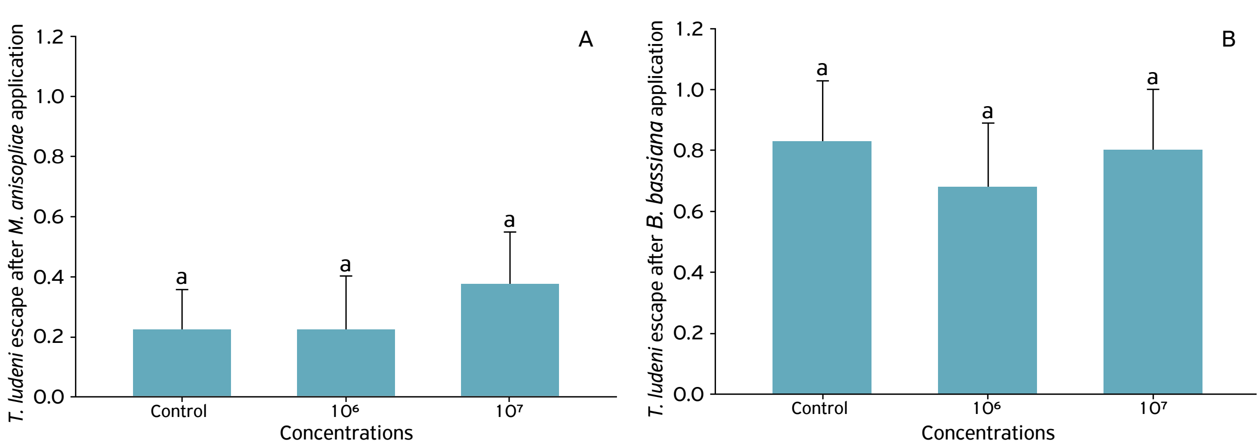

Figure 3. Effect of repellent of *Tetranychus ludeni* females after applying different concentrations of (A) *Metharhizium anisopliae*; and (B) *Beauveria bassiana*. Means were compared with the Tukey test, $p \geq 0.05$.

CONCLUSIONS

The *M. anisopliae* fungus has the potential to be used as a biological control agent, with a concentration of 10^7 con.mL^{-1}, efficient for population control of the *T. ludeni* phytophagous mite. The *B. bassiana* fungus has the potential to suppress future generations of the mite by reducing the oviposition rate at both concentrations tested.

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