RESEARCH ON THE PATHOGENICITY OF AUTOCHTHONOUS ENTOMOPATHOGENIC FUNGI IN TRIALEURODES VAPORARIORUM WESTWOOD

CERCETĂRI PRIVIND PATOGENICITATEA UNOR TULPINI FUNGICE ENTOMOPATOGENE AUTOHTONE ASUPRA *TRIALEURODES VAPORARIORUM* WESTWOOD

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Abstract. *Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae) belongs to whitefly species that cause significant damage to crops in protected areas and fields in Romania. Biological control with parasitoids and entomopathogenic fungi is an important alternative for the management of this pest. The susceptibility of the fourth larval stage of T. vaporariorum to 3 different strains of Beauveria sp. (Bals.) Vuill. and one strain of Metarhizium anisopliae (Metsch.) Sorok., isolated from natural outbreaks in Romania, was assessed. Conidial suspensions containing* 10^7*CFU/mL were applied directly on old nymphal stages of T. vaporariorum using a fine droplet spray nozzle, in a laboratory trial. The results of this study were compared with those obtained by using the commercial strain B. bassiana ATCC 74040 and they indicate that two autochthonous strains have potential as biological agents for the control of T. vaporariorum.*

Key words: whiteflies, *Trialeurodes*, *Beauveria*, *Metarhizium*

Rezumat. *Trialeurodes vaporariorum Westwood (Hemiptera: Aleyrodidae) este o specie de musculiță albă care provoacă daune semnificative culturilor din România, în câmp și spații protejate (sere sau solarii). Controlul biologic cu parazitoizi și fungi entomopatogenei reprezintă o alternativă importantă în managementul acestui dăunător. A fost evaluată susceptibilitatea celui de-al patrulea stadiu larvar al T. vaporariorum la infecția cu trei tulpini diferite de Beauveria sp. (Bals.) Vuill. și o tulpină de Metarhizium anisopliae (Metsch.) Sorok., izolate din focare naturale din România. Într-un studiu de laborator, indivizi de T. vaporariorum aflați în ultimul stadiu de dezvoltare au fost tratați cu suspensii conidiene conținând* 10^7 *CFU/mL, folosind un pulverizator cu picături fine. Rezultatele acestui studiu au fost comparate cu cele obținute prin utilizarea tulpinii comerciale B. bassiana ATCC 74040 și indică faptul că două tulpini autohtone au potențial de agenți de control biologic împotriva T. vaporariorum.*

Cuvinte cheie: musculița albă, *Trialeurodes*, *Beauveria*, *Metarhizium*

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INTRODUCTION

Globally, whiteflies are regarded as the primary pest of crops grown in greenhouses, being vectors of over 100 phytopathogenic viruses (Kanakala *et al.*, 2019). One of the most prevalent and commercially significant whitefly species is *T. vaporariorum*, the greenhouse whitefly, a cosmopolitan and polyphagous species (Helgesen and Tauber, 1974). *T. vaporariorum* adults and nymphs harm plants, directly and indirectly, in three different ways: (1) by sucking up plant sap - when whiteflies are in abundance, damage from sucking sap from plant tissue can be severe and plants may eventually collapse if whiteflies feed heavily, (2) by covering the plant with honeydew - the growth of sooty mold, which is feeding on sugary excretions and honeydew, interferes with normal photosynthetic processes. Furthermore, the harvested crop's quality may suffer. (3) as vectors for viruses: Lettuce infectious yellows virus (infectious yellows of lettuce), Potato yellow vein virus (yellow vein of potato), Tomato chlorosis virus (yellow leaf disorder of tomato) and Tomato infectious chlorosis virus (CABI, 2022). The potential of adults to spread plant viruses is the most dangerous.

Currently, pyrethroids, ketoenols, and neonicotinoids are used to control *T. vaporariorum* (Kapantaidaki *et al*., 2018) but many pests found in greenshouses, including whiteflies, have developed pesticide resistance (Pym *et al.*, 2019, Žanić *et al.*, 2021). The demand for a reduction in pesticide use and the requirement for residue-free food have resulted in an increasing interest in biological control systems for use in greenhouses. The use of fungal pathogens to manage insects, weeds, and plant diseases has been a very active area of research over the past 60 years (Charudattan, 2001, Faria and Wraight, 2001, Jackson *et al.*, 2010). The potential application of hyphomycete fungi as effective biocontrol agents against greenhouse whiteflies has encouraged the isolation of a variety of entomopathogenic fungi as *Aschersonia aleyrodis* Webber, *Lecanicillium lecanii* (Zimmermann) Zare & Gam, *Isaria fumosorosea* (Wize) Brown & Smith, *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok., according to studies conducted both in the lab and in the field (Fransen, 1990, Osborne and Landa, 1992, Wright, 1992, Pineda *et al*., 2007, Oreste *et al.*, 2015) and some of them have been formulated as commercial mycopesticides (Ravensberg, 2015). *B.bassiana*-based product Naturalis® is one of them. In the current study, strains of autochthonous entomopathogenic fungi isolated from natural outbreaks in Romania were examined for their potential utility as effective biocontrol agents against *T. vaporariorum*.

MATERIAL AND METHOD

Fungal isolates

The commercial product Naturalis® - manufactured by CBC-Biogard, Italy, formulation type *oil dispersion* (suspension of conidiospores in soybean oil), containing at least 2.3 × 10^7 CFU/mL (7.16% w/w) of *B. bassiana* ATCC 74040 strain - was chosen for testing as positive control.

Details on the fungal isolates isolated from natural outbreaks in Romania are presented in Table 1. The isolates were grown on potato dextrose agar (PDA) in slants and kept in the collection of the Research-Development Institute for Plant Protection from Romania (RDIPP), Department of Useful Organisms, at 4 °C.

Table 1

Details of entomopathogenic fungal isolates

Entomopathogenic fungi	Fungal strain	Host insect	Source
Beauveria bassiana	BbTd1	*Tanymecus dilaticollis* (adult)	RDIPP
B. bassiana	BbTd2	*Tanymecus dilaticollis* (adult)	RDIPP
B. pseudobassiana	BpPa	*Pyrrhocoris apterus* (adult)	RDIPP
Metarhizium anisopliae	MaA	*Anoxia villosa* (larva)	RDIPP

Each isolate have been cultivated for 12–16 days on PDA, at 25 ± 2 °C in the dark, prior to use. Conidia were harvested from sporulated colonies with a sterile inoculation shovel. Spore suspensions of each fungal isolate were prepared in a 0.02% water solution of Tween 80® and homogenized with a vortex mixer. The spores were counted using a Burker Turk hemocytometer and the spore suspension was diluted to the necessary concentration of 2 x 10^7 CFU/mL.

Insects

The whitefly population used in this study originated from a colony established on tomato plants in a greenhouse on the University of Agronomic Sciences and Veterinary Medicine of Bucharest campus. As critical feeding phase is more closely associated with reaching a critical weight than it is with either the beginning of adult development or the first peak in ecdysyteroid titers (Gelman and Hu, 2007), nymphs with oval case surrounded by a ring of upright waxy rods were collected for treatments (4th nymphal instars, fake pupa or puparium). With a brush, they were carefully removed from the leaves to be transferred to a colored cardboard, for a good visibility of the muscardine.

Tratment

The nymphs were placed in glass Petri dishes as described in Figure 1 and treated with 0.1 ml of aqueous formulations of spore suspension. The absorbent paper that was put in the Petri dishes absorbed the extra liquid.

On the fourth and seventh days after applying the fungi, all nymphs exhibiting symptoms of infection (muscardine) were counted in order to determine the efficacy of all tested strains. For the control group, the nymphs were counted as healthy if they completed development by undergoing adult eclosion.

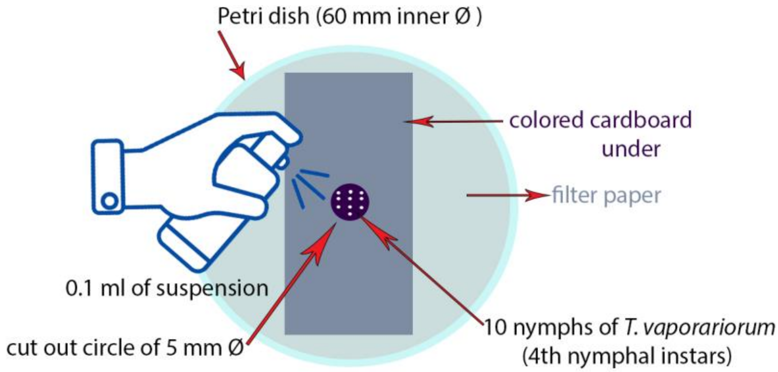

Fig. 1. Schematic diagram of the experiment

Conidial suspensions were sprayed on 4th nymphal instars of *T. vaporariorum.* The control group was sprayed with sterile water with 0.02% Tween® 80. Each strain was replicated five times and each replicate had ten nymphs.

RESULTS AND DISCUSSIONS

Cumulative mortality of *T. vaporariorum* nymphs treated with different fungal strains in the laboratory is shown in Figure 2. Natural mortality was 2.00 to 4 % after 7 days in the control.

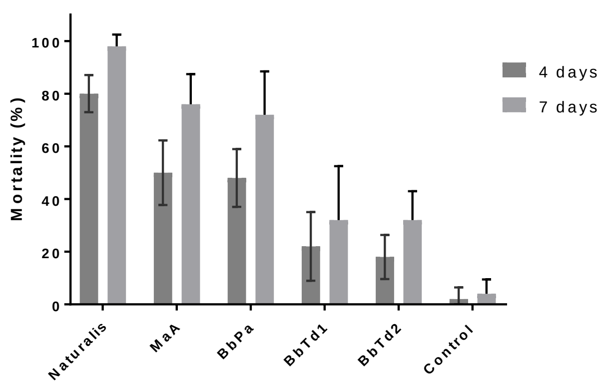

Fig. 2. Cumulative mortality of *Trialeurodes vaporariorum* nymphs treated with Naturalis®, *Metarhizium anisopliae*, *Beauveria pseudobassiana* and *Beauveria bassiana* at 4 and 7 days, post exposure.

At 4 days post treatments, the *T. vaporariorum* cumulative mortality in all experimental treatments except BbTd2 (p=0.0648) were statistically different from the control. The highest cumulative mortality (80%) was recorded in nymphs treated with the myco-insecticide Naturalis® and was significantly different from all of the other treatments. *Metarhizium* (MaA) and *B. pseudobassiana* (BbPa) treatments induced similar mortalities rates of 50 and 48%, respectively at 4 days post exposure. Not any significantly difference in mortality of *T. vaporariorum* caused by the two *B. bassiana* strains was found.

At 7 days post treatments, the *T. vaporariorum* mortality in all experimental treatments was statistically different from the untreated control ($F_{(5, 24)} = 37.55$, $p<0.0001$). The highest cumulative mortality (98%) was recorded in nymphs treated with the myco-insecticide Naturalis®. Subsequent comparisons among treatments indicated that mortality from Naturalis® was not statistically different from those from *Metarhizium* (p=0.113). Also, no difference in mortality was observed in nimphs treated with *M. anisopliae* (76%) and *B. pseudobassiana* (72%) (p=0.996). The lowest mortality rate was maintained lower at nymphs treated with both *B. bassiana strains*, not exceeding 35% at 7 days post treatment.

Commercial strains ATCC 74040 and GHA of *B. bassiana*, Apopka 97 and FE9901 of *I. fumosorosea*, Ve-6 of *Lecanicillium muscarium* Petch (formerly *Verticillium lecanii* Zimmerman) and Bipesco 5 and F52 of *Metarhizium brunneum* Petch (formerly *M. anisopliae* Metschnikoff) have been used successfully against whiteflies (Gonzales *et al*., 2016). It is known that, compared to aqueous formulations, oil formulations dramatically increase the isolates' effectiveness and pathogenicity against the nymphs (Paradza *et al*., 2021). The two indigenous strains seem to be a promising option to control *T. vaporariorum* and a proper formulation could increase their efficacy.

CONCLUSIONS

1. Whitefly mortality from all entomopathogenic fungal treatments ranged from moderate to high.

2. Nymphs treated with the commercial strain of *B. bassiana* had the highest cumulative mortality.

3. *M. anisopliae* and *B. pseudobassiana* isolates shown promising levels of pathogenicity to *T. vaporariorum* among the four autochthonous entomopathogenic fungi examined.

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