

Susceptibility of *Tetranychus urticae* Koch. (Acari: Tetranychidae) to Isolates of Entomopathogenic Fungus *Beauveria bassiana*

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SUMMARY

Bioassays with five isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin were conducted under laboratory conditions with a goal to estimate their virulence to the two-spotted spider mite *Tetranychus urticae* Koch. (Acari: Tetranychidae). Common bean plants with mites were treated by spraying conidial suspensions of isolates at concentrations of 10^6 , 10^7 and 10^8 conidia/ml. Lethal effects of the fungal isolates were evaluated as percentages of cumulative daily mortality due to mycoses, corrected for mortality in the control variant. Virulence of the isolates was estimated based on values of the median lethal time (LT_{50}) calculated by probit analysis for the variants treated with conidial suspensions at the concentration of 10^6 conidia/ml.

The two-spotted spider mite was found susceptible to the examined isolates of the entomopathogenic fungus *B. bassiana*.

Mycosis caused to *T. urticae* by the *B. bassiana* isolates 444 Bb and 445 Bb had fast lethal effect after treatment with conidial suspensions even at the concentration of 10^6 conidia/ml. The mean mortality values of host individuals were $83.78 \pm 3.62\%$ and $68.49 \pm 4.28\%$ on the first day, respectively, and up to 100% in both variants on the fourth day.

The isolates 426 Bb, 444 Bb and 445 Bb of *B. bassiana* were highly virulent to two-spotted spider mites with values of the median lethal time varied within overlapped narrow confidence intervals from 0.122 to 1.084 days (average value 0.162 days), from 0.117 to 1.398 days (average value 0.146 days) and from 0.106 to 1.162 days (average value 0.131 days), respectively. Significant differences regarding virulence of the three isolates at p-level < 0.05 could not be proved. The other two examined isolates were distinctly less virulent to *T. urticae* than these three *B. bassiana* isolates.

Keywords: Entomopathogenic fungi; *Beauveria bassiana*, Virulence; *Tetranychus urticae*

INTRODUCTION

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan and polyphagous species with great economic importance for crops in greenhouses and in the field. Conventional control of this pest includes acaricide treatments that could lead to undesirable side effects, such as death of non-target organisms (e.g. predators), development of pesticide-resistant races of two-spotted spider mite and residue concerns. Methods of integrated pest management are consistent with the consumers' demand to reduce health and environment risks. Screening for eligible biocontrol agents is a step in developing new or improving existing environment-friendly strategies offering an alternative to conventional pest control.

The fungal pathogens *Beauveria bassiana* (Bals.-Criv.) Vuillemin, *Metarhizium anisopliae* (Metsch.) Sorokin, *Lecanicillium* (= *Verticillium*) *lecanii* (Zimmermann) Zare and W Gams, *Isaria fumosorosea* Wize (= *Paecilomyces fumosoroseus*), *Isaria farinosa* (Holmsk.) Fries (= *Paecilomyces farinosus*), *Hirsutella thompsonii* Fisher, and *Neozygites floridana* Weiser and Muma often play an important role in decreasing native populations of phytophagous mites (Chandler et al., 2000; Van der Geest et al., 2000). Searching for microbial control agents in laboratory experiments it has been shown that some of the tested isolates of *B. bassiana* and *M. anisopliae* are highly virulent to the active stages of *T. urticae* (Alves et al., 2002a; Tamai et al., 2002; Chandler et al., 2005) and *T. evansi* Baker and Pritchard (Wekesa et al., 2005). According to Shi and Feng (2006, 2009) some of the tested isolates of *B. bassiana*, *Paecilomyces fumosoroseus* (Wize) Brown and Smith and *M. anisopliae* have high lethal effect on eggs and females of *T. urticae*. Chandler et al. (2005) found in a glasshouse experiment that *Beauveria bassiana* cultured from Naturalis-L (Troy Biosciences, USA) reduced the numbers of *T. urticae* adults, nymphs and eggs (98% reduction in all three cases) on glasshouse tomato crops.

Opportunities to exploit entomopathogenic fungi for biological control of mites were summarized by Chandler et al. (2000), Alves et al. (2002) and Maniania et al. (2008).

Maniania et al. (2008), reviewing the use of fungal pathogens in inundative, conservation and conventional biological control of *T. urticae* and *T. evansi*, concluded that there is a great potential for use of entomopathogenic fungi in conservation and conventional biological control programs. Successful use of entomopathogenic

fungi as microbial control agents of mites will ultimately depend on how well the strains are selected. For development of mycoacaricides based on entomopathogenic fungi in the Ascomycota, order Hypocreales, screening for more efficient strains is still necessary.

The current study was designed to determine the virulence of five Bulgarian isolates of the entomopathogenic fungus *B. bassiana* to the two-spotted spider mite *T. urticae*.

MATERIAL AND METHODS

Five fungal isolates of *B. bassiana*, applied in bioassays, were provided from a collection of entomopathogenic fungi maintained at the Department of Biological and Integrated Pest Control (Plant Protection Institute, Bulgaria). They were initially isolated into pure cultures from dead insects collected from natural pest populations found in different regions of Bulgaria. The isolate marked 417 Bb was obtained from a larva of *Hedya nubiferana* (Haw.) (Lepidoptera: Tortricidae) found in an apple orchard near Sofia in 2006; the isolates marked 444 Bb, 445 Bb and 447 Bb from larvae of *Cydia pomonella* L. (Lepidoptera: Tortricidae) found in apple orchards near the town of Plovdiv in 2007, and the isolate marked 426 Bb from a dead adult of *Ips sexdentatus* Boer. (Coleoptera: Scolytidae) found in pine bark in the Malashevska Mountain in 2006.

In order to prepare conidial suspensions for bioassay, fungal isolates were cultured on SDAY (Sabouraud dextrose agar with yeast extract) in tubes at $25 \pm 1^\circ\text{C}$ for 15 days. Conidia of each isolate were harvested by washing down with 10 ml sterile water per tube followed by decimal dilutions of the obtained conidial suspensions and determination of their concentrations by haemocytometer.

Bioassays with two-spotted spider mites were conducted under laboratory conditions ($23 \pm 1^\circ\text{C}$, photoperiod 12:12 L:D) with a host population reared on common bean plants (*Phaseolus vulgaris* L.) in pots. The plants with mites were treated by spraying the prepared conidial suspensions at the following concentrations: from 0.906×10^6 to 0.906×10^8 conidia/ml in variants with the fungal isolate 417 Bb; from 0.838×10^6 to 0.838×10^8 conidia/ml in variants with the isolate 426 Bb; from 0.590×10^6 to 0.590×10^8 conidia/ml in variants with 444 Bb; from 1.300×10^6 to 1.300×10^8 conidia/ml in variants with 445 Bb, and from 1.040×10^6 to 1.040×10^8 conidia/ml in variants with 447 Bb.

Control variants were treated with water. The experiments were proved in 3 replicates. Mite mortality was checked daily during five days. Leaves with dead mites were placed in a humid chamber to allow reproductive structures of the fungus to develop and to confirm that mycosis was the reason of death. Smears prepared from dead mites were stained by methylen blue and were observed under light microscope.

Lethal effects of the isolates were evaluated as percentages of cumulative daily mortality due to mycoses, corrected for mortality in the control variant according to Abbott's formula (1925). Virulence of the isolates was estimated based on median lethal time (LT₅₀) calculated by probit analysis (Finney, 1971) for the variants treated with conidial suspensions at the concentration of 10⁶ conidia/ml. Confidence intervals of varying LT₅₀ values were calculated at p-level < 0.05.

RESULTS AND DISCUSSION

Bioassay with five isolates of the entomopathogenic fungus *B. bassiana* showed that two-spotted spider mites with mycosis became red and later lost their turgor. Investigation of smears of mites' cadavers confirmed mycosis as the cause of death in variants treated with conidial suspensions of the fungal isolates.

The bioassay results showed that two-spotted spider mites were susceptible to the examined isolates of the entomopathogenic fungus *B. bassiana*. As shown in Figure 1, mites in the variants treated with conidial suspensions

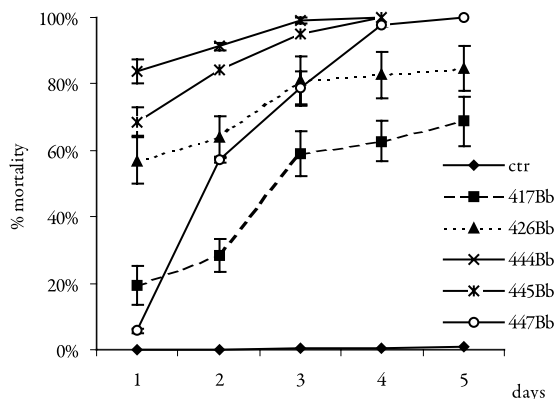


Figure 1. Lethal effect of isolates 417 Bb, 426 Bb, 444 Bb, 445 Bb and 447 Bb of *Beauveria bassiana* to *Tetranychus urticae* after treatment with conidial suspensions at concentration 10⁶ conidia/ml. (ctr – control variant, 0 conidia) Error bars: SD

of the isolates 444 Bb, 445 Bb and 426 Bb at concentration of 10⁶ conidia/ml developed mycosis with fast lethal effect. The mean mortality values for individuals on the first day were high - 83.78 ± 3.62%, 68.49 ± 4.28% and 56.88 ± 7.06%, respectively. Infections due to isolates 417 Bb and 447 Bb were less lethal - 19.30 ± 5.71% and 5.66 ± 0.76% mortality in the variants treated with conidial suspensions at concentration of 10⁶ conidia/ml. On the second day, mortality in the variants with isolates 444 Bb and 445 Bb increased to 91.36 ± 1.18% and 84.24 ± 0.53%, respectively, and than to 99.21 ± 0.79% and 94.99 ± 1.30% on the third day, and to 100% in both variants on the fourth day. The established mortality from fungal disease in the variants with isolates 447 Bb and 426 Bb was as follows: 57.05 ± 0.65% and 64.15 ± 6.03%, respectively, on the second day; 78.66 ± 4.96% and 80.88 ± 7.24% on the third day, and 97.60 ± 0.31% and 82.79 ± 7.01% on the fourth day. On the fifth day, lethal effect reached 100% in the variant with 447 Bb, and 84.87 ± 19.17% in the variant with 426 Bb. Mycosis caused by the isolate 417 Bb of *B. bassiana* had a less lethal effect - 68.89 ± 7.40% calculated for the fifth post-treatment day.

In our view, the fast lethal effect of the mycoses to *T. urticae* was due to toxic secondary metabolites and the pigment oosporein produced by fungal isolates. Growing the examined isolates 417 Bb, 426 Bb, 444 Bb, 445 Bb and 447 Bb of *B. bassiana* a red pigmentation of the media was also noticed.

The main characteristic that has an important role in the virulence of entomopathogenic fungi strains from the genera *Beauveria* and *Metarhizium* is the production of enzymes necessary for penetration through arthropod cuticle. The extracellular proteases are considered an important virulence factor in insect disease processes (St. Leger et al., 1986a, 1986b; 1988; Draganova, 1988; Bidochka and Khachatourians, 1990, 1994a, 1994b; Gupta et al., 1992; St. Leger, 1995). The extracellular chymoelastase (Pr 1) was noticed as the most important enzyme for overcoming the resistance of the insect integument (St. Leger et al., 1988; Bidochka and Khachatourians, 1994a, 1994b). Besides enzymes, many isolates of *Beauveria* species have been found to produce toxic compounds after invasion of the host haemolymph (Roberts, 1981). Some of the compounds were beauvericin, beauverolides, bassianolide, isarolides, pigments (bassianin, tenellin, oosporein) and oxalic acid (Roberts, 1981; Eyal et al., 1994; Strasser et al., 1998, 2000). Production of toxic metabolites was a step in mycosis development followed by death of the host.

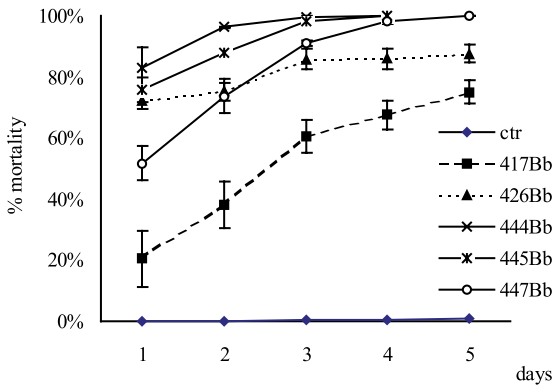


Figure 2. Lethal effect of isolates 417 Bb, 426 Bb, 444 Bb, 445 Bb and 447 Bb of *Beauveria bassiana* to *Tetranychus urticae* after treatment with conidial suspensions at concentration 10^7 conidia/ml. (ctr – control variant, 0 conidia) Error bars: SD

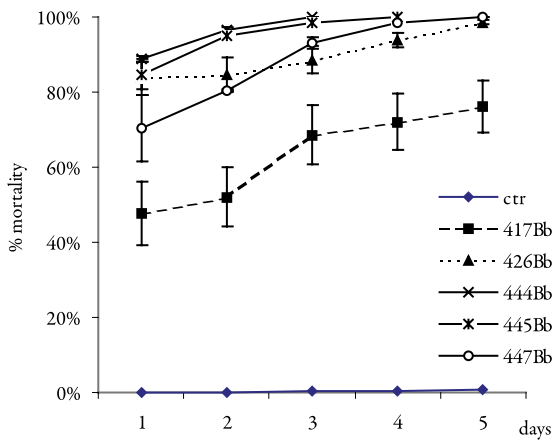


Figure 3. Lethal effect of isolates 417 Bb, 426 Bb, 444 Bb, 445 Bb and 447 Bb of *Beauveria bassiana* to *Tetranychus urticae* after treatment with conidial suspensions at concentration 10^8 conidia/ml. (ctr – control variant, 0 conidia) Error bars: SD

In the present bioassay, treatment with higher concentrations of conidial suspensions (10^7 and 10^8 conidia/ml) was found to result in higher mortality of the host but while lethal effect in more of the variants could be distinguished on the first day after treatment, significant differences among the mortalities in different variants could not be proved after the third day and later (Figure 2 and Figure 3).

So the lethal effects on the first day in variants treated with 10^7 conidia/ml of 444 Bb, 445 Bb, 426 Bb, 447 Bb and 417 Bb were: $82.92 \pm 6.90\%$, $75.76 \pm 3.96\%$, $72.40 \pm 3.05\%$, $51.79 \pm 5.62\%$ and $20.55 \pm 9.16\%$, respectively (Figure 2). On the third day they increased to $99.44 \pm 0.56\%$, $98.15 \pm 1.07\%$, $85.85 \pm 3.37\%$, $90.99 \pm 1.05\%$ and $60.53 \pm 6.66\%$, respectively. On the fifth day the isolates 444 Bb, 445 Bb and 447 Bb could not be distinguished by the lethal effect of mycoses but significant differences among the three isolates and the isolates 426 Bb and 417 Bb could be proved at p-level < 0.05.

The highest lethal effect of mycoses caused after treatment with conidial suspension at concentration of 10^8 conidia/ml was found on the first day in the variant with isolate 444 Bb ($88.93 \pm 0.74\%$), followed by 445 Bb ($84.80 \pm 3.86\%$), 426 Bb ($83.65 \pm 4.56\%$), 447 Bb ($70.38 \pm 8.72\%$) and the least lethal 417 Bb ($47.62 \pm 8.49\%$) (Figure 3). On the third day, mortality was over 88% in all variants with the exception of variant 417 Bb, where it was $68.57 \pm 5.31\%$.

Virulence of the examined isolates of *B. bassiana* to *T. urticae* was estimated based on values of the median lethal time (LT_{50}) calculated for the variants treated with conidial suspensions at the concentration of 10^6 conidia/ml (Table 1). The isolates 426 Bb, 444 Bb and 445 Bb were highly virulent to the two-spotted spider mite. Their values of the median lethal time varied within overlapped confidence intervals from 0.122 to 1.084 days (average value 0.162 days), from 0.117 to 1.398 days (average value 0.146 days) and from 0.106 to 1.162 days (average value 0.131 days), respectively.

Table 1. Virulence of isolates of the entomopathogenic fungus *Beauveria bassiana* to two spotted spider mite *Tetranychus urticae*

Isolates of <i>Beauveria bassiana</i>	Concentration of conidial suspensions (conidia/ml)	Median lethal time (LT_{50}) (days)		Regression coefficient ($b \pm S_{eb}$)	
		Average values	Confidence intervals*		
			from	to	
417 Bb	0.906×10^6	3.054	2.864	3.258	1.9878 ± 0.0552
426 Bb	0.838×10^6	0.162	0.122	1.084	1.3413 ± 0.0920
444 Bb	0.590×10^6	0.146	0.117	1.398	3.6677 ± 0.1458
445 Bb	1.300×10^6	0.131	0.106	1.162	3.7392 ± 0.1838
447 Bb	1.040×10^6	3.156	3.138	3.174	25.9976 ± 0.1128

*Confidence intervals were calculated at p-level < 0.05

Significant differences in the virulence of isolates 426 Bb, 444 Bb and 445 Bb (at p-level < 0.05) could not be proved. The isolates 417 Bb and 447 Bb were less virulent to *T. urticae*. The calculated p-level < 0.05 values of LT₅₀ for these variants were within overlapped confidence intervals from 2.864 to 3.258 days (average 3.054 days) and from 3.138 to 3.174 days (average 3.156 days), respectively. So the isolates 417 Bb and 447 Bb could not be distinguished for their virulence to *T. urticae* based on median lethal time but significant differences were proved between the two isolates and the other examined isolates. Calculated confidence intervals for the fifth fungal isolate were narrow, indicating an equalized susceptibility within the treated population of *T. urticae* to the studied *B. bassiana* isolates.

In conclusion, the two-spotted spider mite was found to be susceptible to the examined isolates of the entomopathogenic fungus *B. bassiana*. Mycosis caused to *T. urticae* by the isolates 444 Bb and 445 Bb had fast lethal effect after treatment with conidial suspensions even at the concentration of 10⁶ conidia/ml. The mean mortality values for host individuals on the first day were 83.78 ± 3.62% and 68.49 ± 4.28%, respectively, and on the fourth day up to 100% in both variants.

The isolates 426 Bb, 444 Bb and 445 Bb were highly virulent to two-spotted spider mites with values of the median lethal time varied within overlapped narrow confidence intervals from 0.122 to 1.084 days, from 0.117 to 1.398 days and from 0.106 to 1.162 days, respectively. Significant differences in the virulence of the three isolates at p-level < 0.05 could not be proved, unlike virulence between the isolates 417 Bb and 447 Bb.

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Osetljivost *Tetranychus urticae* Koch. (Acari: Tetranychidae) na izolate entomopatogene gljive *Beauveria bassiana*

REZIME

Pet izolata entomopatogene gljive *Beauveria bassiana* (Bals.-Criv.) Vuillemin ispitivano je u biotestu pod laboratorijskim uslovima sa ciljem da se proceni njihova virulentnost za grinje-paučinare *Tetranychus urticae* Koch. (Acari: Tetranychidae). Biljke pasulja sa grinjama tretirane su rasprskavanjem suspenzija izolata u koncentracijama od 10^6 , 10^7 i 10^8 konidija/ml. Letalno dejstvo izolata gljive ocenjivano je kao procenat kumulativne dnevne smrtnosti kao posledice mikoze, korigovan za smrtnost u kontroli. Virulentnost izolata je procenjena na osnovu vrednosti srednjeg letalnog vremena (LT_{50}) izračunatog probit analizom u varijantama sa suspenzijom konidija u koncentraciji od 10^6 konidija/ml.

Grinje-paučinari su pokazale osetljivost na ispitivane izolate entomopatogene gljive *B. bassiana*.

Mikoze koje su na *T. urticae* prouzrokovali izolati *B. bassiana* sa oznakom 444 Bb i 445 Bb pokazale su brzo letalno dejstvo nakon tretmana suspenzijama, čak i u koncentraciji od 10^6 konidija/ml. Srednje vrednosti smrtnosti grinja domaćina bile su respektivno $83,78 \pm 3,62\%$ i $68,49 \pm 4,28\%$ prvog dana, a četvrtog dana do 100% u obe varijante ogleđa.

Izolati *B. bassiana* sa oznakom 426 Bb, 444 Bb i 445 Bb bili su visokovirulentni za grinje-paučinare, a vrednosti srednjeg letalnog vremena varirale su uz preklapanje uskih intervala poverenja respektivno od 0,122 do 1,084 dana (prosečno 0,162 dana), od 0,117 do 1,398 dana (prosečno 0,146 dana) i od 0,106 do 1,162 dana (prosečno 0,131 dana). Nisu potvrđene značajne razlike u pogledu virulentnosti ova tri izolata na nivou $p < 0,05$. Manja virulentnost druga dva izolata za *T. urticae* bila je jasno izražena u odnosu na ova tri izolata *B. bassiana*.

Ključne reči: Entomopatogene gljive; *Beauveria bassiana*, virulentnost; *Tetranychus urticae*