ВИРУЛЕНТНОСТ НА ИЗОЛАТИ НА *BEAUVERIA BASSIANA* СПРЯМО ЛАРВИ НА *TROGODERMA VERSICOLOR* (COLEOPTERA: DERMESTIDAE)

VIRULENCE OF *BEAUVERIA BASSIANA* ISOLATES AGAINST LARVAE OF *TROGODERMA VERSICOLOR* (COLEOPTERA: DERMESTIDAE)

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Abstract

Trogoderma spp. including *Trogoderma versicolor* are important insect pests whose larvae damage different stored products and stuffs of both plant and animal origin. *Trogoderma versicolor* is a quarantine species for Bulgaria.

Bioassays were conducted with larvae from a laboratory population of *T. versicolor* with wheat varieties of foreign origin stored at the former Plant Protection Institute, Bulgaria.

The virulence of 5 isolates of the entomopathogenic fungus *Beauveria bassiana* was investigated in laboratory experiments. Active larvae (L_{2-4}) of *T. versicolor* were treated with conidial suspensions at concentrations 1-2x10^7 conidia/ml and the dead larvae were put into a humid chamber. The larval mortality was estimated as a percentage of the cumulative daily mortality due to mycosis, corrected with the control mortality. The virulence of the fungal isolates was evaluated according to the values of the median lethal time (LT_{50}), calculated by probit analysis.

The results showed that the larvae were susceptible to mycosis caused by the tested *B. bassiana* isolates except for the re-isolate 513reTgrBb. The larval mortality caused by isolate 687Bb was 1.11% on the 6th day after the treatment and it rose to 98.89% and 100% on the 7th and 10th day, respectively. Isolate 689Bb was the most virulent to the larvae with LT_{50} values 3.420 (2.994 – 3.905) days ($P < 0.05$).

Key words: *Beauveria bassiana,* larvae*,* median lethal time*, Trogoderma versicolor*, virulence.

INTRODUCTION

Larvae of dermistids from the genus *Trogoderma* (Coleoptera: Dermestidae) damage seeds of a great number of crops, products and stuffs from plant and animal origin. One of the representatives of the genus - the khapra beetle (*Trogoderma granarium* Everts), according to EPPO (2014) is a serious pest of stored products under hot dry conditions and complete destruction of grain and pulses may take place in a short time.The both species *T. granarium* and *Trogoderma versicolor* Creutz. are quarantine insects for Bulgaria.

Control of insect pest infestation in stored products in farmer stores and public ware houses is conducted by application of highly toxic insecticides and fumigants which according to Morallo-Rejesus and Rejesus (2011) has led to a crisis situation, including pest resurgence, insect resistance, and secondary pest outbreaks, environment contamination and hazards to human health.

In order to minimize or even to ovoid chemicals application alternative methods for control of stored product insects are searching for. Some of the alternatives include use of inert dusts such as silicon dioxide and diatomaceous earth (Obretenchev et al., 1998, 2005; Arthur, 2000; Athanassiou et al., 2005; Grieshop et al., 2012; Korunić, 2013), plant extracts and oils (Upadhyay and Ahmad, 2011), bioagents (parasitoids, entomopathogenic nematods, pathogens) singly or in combination with other lowrisk methods (Akbar et al., 2004; Alshamaa and Khaleda, 2006; Kavallieratos et al., 2006; Ahmedani et al., 2007; Lord, 2007; Michalaki et al., 2007; Khashaveh, 2011; Shafighi et al., 2014; Zare et al., 2014).

Reports on pathogens impact on *Trogoderma* spp. are few and they concern mainly to *T. granarium* (Alshamaa and Khaleda, 2006; Ahmedani et al., 2007; Khashaveh, 2011; Draganova et al., 2012; Moore et al., 2012; Zare et al., 2014).

Studies of Alshamaa and Khaleda (2006) and Ahmedani et al. (2007) show promising results of *Bacillus* strains and especially *Bacillus thuringiensis* Berliner and some of their commercial formulations for controlling *T. granarium* infestation in stored wheat.

According to Obretenchev et al. (2001), Vega and Kaya (2011) and Moore et al. (2012) the neogregarine *Mattesia trogodermae* Canning (Apicomplexa: Neogregarinida) was considered to have a great potential as a suppressive agent for dermestid beetles that are pests of stored products, including the destructive khapra beetle, *T. granarium*.

In laboratory experiments Khashaveh (2011) with Iranian isolates of *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota, Hypocreales) and Draganova et al. (2012) with Bulgarian isolates of *Beauveria bassiana* (Bals. – Criv.) Vuillemin (Ascomycota, Hypocreales) established that *T. granarium* larvae were susceptible to tested fungal isolates.

No literature data were found about bioassays with entomopathogenic fungi to *T. versicolor* larvae.

The aim of the study was to evaluate the virulence of five *B. bassiana* isolates to active larvae (L_{2-4}) of the larger cabinet beetle, *T. versicolor*, in laboratory bioassays.

MATERIALS AND METHODS

Active larvae (L_{2-4}) of the larger cabinet beetle used in bioassays were from a laboratory population of the pest originating from rust resistant wheat varieties of Plant Immunity Department (former Plant Protection Institute, Bulgaria). It was reared in thermostat at temperature 32°C and RH 75 ± 5% on wheat seeds variety Momchil with 14% moisture content (Kojanchikov, 1961).

Five isolates of the fungus *B. bassiana* used in the bioassay were from the collection of pure cultures of entomopathogenic fungi maintained on slopes of SDAY (Sabouraud dextrose agar with yeast extract) in tubes at 4 ± 1°C at the Department of Entomology (Institute of Soil Science, Agrotechnologies and Plant Protection, Bulgaria). Four isolates (686Bb, 687Bb, 688Bb и 698 Bb) were with an initial host *Tanymecus dilaticollis* Gyll. (Coleoptera: Curculionidae). Isolate 513reTgrBb was re-isolated from dead larvae of *T. granarium* after treatment with the same isolate.

Twenty larvae of *T. versicolor* in 4 replications pro variant were treated indirectly (Draganova & Staneva, 1988) with conidial suspensions at concentrations 1-2x10^7 conidia/ml and surfactant Twin-80 – 0.01%. The larvae in the control variant were treated with Twin-80 – 0.01%. The experiments were provided under laboratory conditions (25 ± 1°C, RH 75± 5%). Twenty wheat seeds variety Momchil were put into the Petri dishes for larval feeding. The dead larvae were placed into a humid chamber for fungal pathogen exhibition. The larval mortality was estimated as percentage of cumulative daily mortality due to mycoses, corrected with the mortality in the control following Schneider-Orelli's formula (Püntener, 1981). The virulence of the fungal isolates was evaluated according to the values of the median lethal time (LT_{50}), calculated by probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Conducted bioassays showed that active larvae (L_{2-4}) of *T. versicolor* were with different susceptibility to mycoses due to *B. bassiana* isolates. The observed fungal infections were with the typical time-mortality curves with the exception of the variant with the re-isolate 513reTgrBb (Fig. 1). The mortality increased slowly at the beginning of the disease - from 2.22% to 11.11% between the dpi (day post infection) 2 to dpi 4 in variants with isolates 689Bb and 688Bb and between the dpi 2 to dpi 9 in variants with isolates 686Bb and 687Bb. This is the period when the most susceptible individuals from the treated population die. Then mortality rose rapidly and it reached to high values at dpi 10 - up to 100% or 95.56% ± 12.03 in the variants treated with conidia of 687Bb and 689Bb, respectively.

Dynamics of studied mycoses could be explained with the mode of action of the fungal entomopathogens. The disease development starts with adhesion of fungal conidia to the insect cuticle. The biochemical interactions between fungal pathogens and their insect host epicuticle were studied by examining fungal hydrocarbon degrading ability by Pedrini et al. (2007). Authors revealed participation of a cytochrome P450 enzyme system in catabolism of very long chain hydrocarbons and virulence parameters. The next step in disease development is germination of propagules followed by penetration of germ tubes or infection pegs of the pathogen through the insect integument overcoming host defense by releasing extracellular cuticle-degrading enzymes and toxic substances (Pekrul and Grula, 1979; St. Leger et al., 1986a, b; Draganova, 1988; Bidochka and Khachatourians, 1990; St. Leger, 1995; Pedrini et al., 2007; Zare et al., 2014). Different extracellular enzymes are involved in the processes of penetration. According to studies of St. Leger et al. (1986a, b) proteases and esterases are produced first (<24 h), while chitinase and lipase activities appear substantially later (4–5 days) than enzymes of the proteolytic complex. Next steps are multiplication in the yeast phase (hyphal bodies) in the haemocoel, production of toxic metabolites and death of the host (Roberts, 1981). According to Strasser et al. (2000), Vey et al. (2001) and Xu et al. (2008) beauvericin, bassianolide, bassiatin, beauverolides and oosporein are the secondary metabolites with toxic action produced by *B. bassiana* which are involved in insect pathogenesis. As more amounts of extracellular cuticle-degrading enzymes and toxic substances will be released by the fungal isolate as higher will be the virulence and the mycosis will spread faster. Zare et al. (2014) found a strong positive correlation between the proteolytic activity and virulence of the isolates against the khapra beetle, *Trogoderma granarium*.

The current conducted bioassays showed that active larvae ($L_{2\text{-}4}$) of *T. versicolor* were the most susceptible to the fungal infection due to the isolate 687Bb and they were tolerant to the mycosis caused by the isolate 513reTgrBb. Mortality didn't reach even to 10% for 10 days after the treatment with conidial suspension of the re-isolate (Fig. 1).

In our opinion the isolate 513Bb lose it's virulence after transmission through the larvae of *T. granarium* and the newly obtained isolate 513reTgrBb was not virulent. In studies with active larvae of the khapra beetle (Draganova et al., 2012) the isolate 513Bb of *B. bassiana* caused mycosis with cumulative daily mortality 79.59% ± 5.84 on the 22nd day after the treatment with conidial suspension (2 x 10^8 conidia/ml).

Zare et al. (2014) found a wide variation in virulence of *B. bassiana sensu lato* isolates against *T. granarium*. The larval mortality of the khapra beetle ranged from 25 to 59% as a result of treatment with Iranian isolates BA and MITE, respectively. Such variations in virulence of different isolates of entomopathogenic fungi have been well documented in different species and seems to be caused by a number of factors such as insect host, host plant properties, host food, environmental conditions, etc.

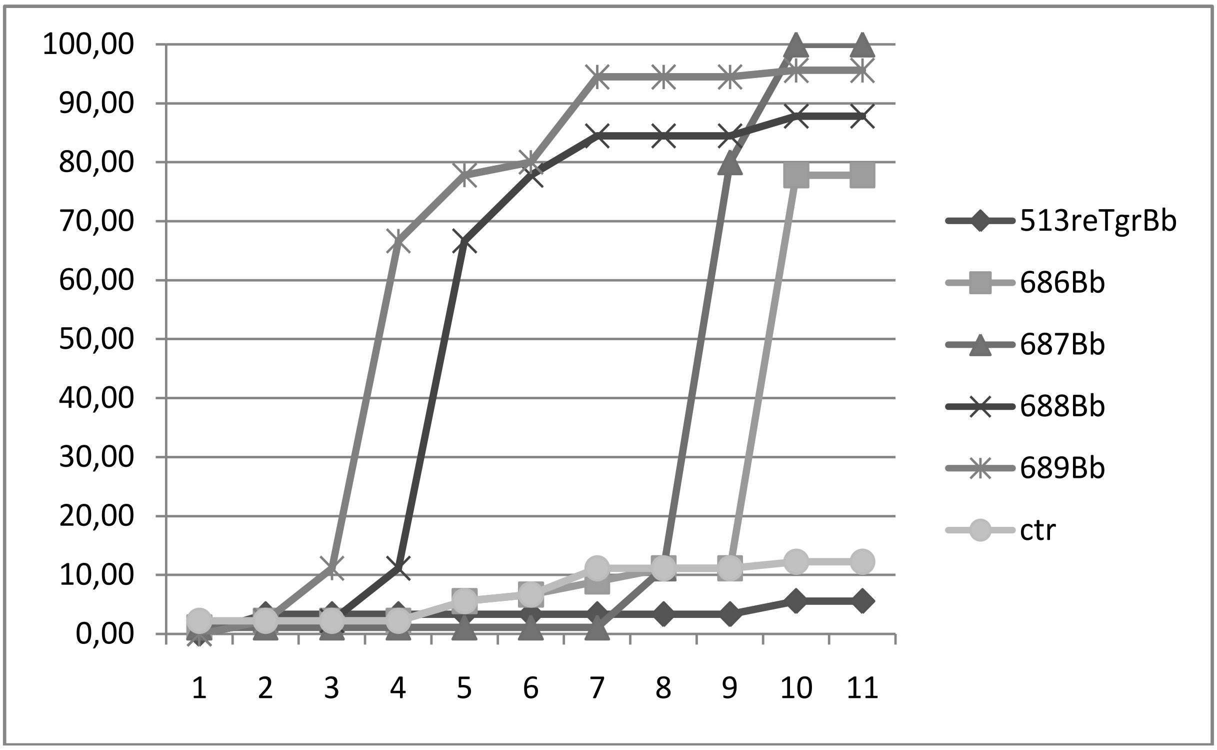

Fig. 1. *Lethal effect of mycoses to active larvae ($L_{2\text{-}4}$) of Trogoderma versicolor after treatment with conidial suspensions of B. bassiana isolates (% cumulative daily mortality corrected with control treatment mortality) (SE-ctr= ± 1.34; SE-686Bb= ± 8.86; SE-687Bb= ± 12.94; SE-688Bb= ± 12.02; SE-689Bb= ± 12.03; SE-513reBb= ± 0.44)*

Table 1. Virulence of isolates of the entomopathogenic fungus *Beauveria bassiana* to active larvae ($L_{2\text{-}4}$) of *Trogoderma versicolor*

Isolate of *Beauveria bassiana*	Inoculum (conidia/ml)	Values of the median lethal time (LT_{50})(days)			Coefficient of regression ($b \pm S_{\varepsilon b}$)
		Average values**	Confidence intervals*		
			from от	to до	
686Bb	0,69 x 10^8	10,440^d	9,548	11,42	6,0123 ± 0,3591
687Bb	0,97 x 10^8	6,699^c	6,534	6,868	18,5437 ± 0,6014
688Bb	1,19 x 10^8	5,065^b	4,707	5,451	4,3663 ± 0,3023
689Bb	0,56 x 10^8	3,420^a	2,994	3,905	2,5614 ± 0,2516

**Confidence intervals are calculated at P – level < 0.05.*

***Means followed by the different letters are significantly different (P < 0.05).*

Virulence of the examined by us *B. bassiana* isolates to active larvae ($L_{2\text{-}4}$) of *T. versicolor* was estimated according to the values of the median lethal time (Table 1) with the exception of the isolate 513reTgrBb. The isolate 689Bb was the most virulent with the lowest LT_{50}-values varying within confidence intervals 2.994 – 3.905 days (average value 3.420 days) calculated at P-level < 0.05. The isolate 686Bb was with the lowest virulence. The calculated values of the median lethal time for this isolate were 9.548 – 11.420 days with average value 10.440 days, respectively. The isolates 687Bb and 688Bb were with intermediate virulence to active larvae of the larger cabinet beetle. Differences among virulence of the examined isolates were significant and they were proved at P-level < 0.05. Calculated values of LT_{50} for all isolates were within confidence intervals which didn't overlap.

As no literature data were found about bioassays with entomopathogenic fungi to *T. versicolor* larvae no comparison could be made. The current studies are first not only for Bulgaria.

CONCLUSIONS

1. The conducted laboratory experiments showed that active larvae ($L_{2\text{-}4}$) of *T. versicolor* were with different susceptibility to mycoses caused by *B. bassiana* isolates with the exception of 513reTgrBb. The isolate 689Bb was with the highest virulence.

2. These first bioassays with active larvae of the larger cabinet beetle revealed that the isolate 689Bb of *B. bassiana* was promising as fungal bioagent in future.

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