ЛЕТАЛЕН ЕФЕКТ НА ИЗОЛАТИ НА ЕНТОМОПАТОГЕННИ ГЪБИ СПРЯМО ВЪЗРАСТНИ НАСЕКОМИ НА *TROGODERMA VERSICOLOR* (COLEOPTERA: DERMESTIDAE)

LETHAL EFFECT OF ISOLATES OF ENTOMOPATHOGENIC FUNGI ON ADULT INSECTS OF *TROGODERMA VERSICOLOR* (COLEOPTERA: DERMESTIDAE)

Славимира Драганова*, Евдокия Станева
Slavimira Draganova*, Evdokia Staneva

Институт по почвознание, агротехнологии и защита на растенията „Н. Пушкаров“ – София
Institute of Soil Science, Agrotechnologies and Plant Protection “N. Pushkarov” – Sofia

***E-mail: sdraganova19@gmail.com**

Abstract

Trogoderma granarium and *Trogoderma versicolor* are some of the most dangerous insects for stored products. Their larvae damage seeds of a great number of crops, products and materials of plant and animal origin. These species are quarantine pests for Bulgaria.

A laboratory population of *T. versicolor* was used in bioassays originating from samples of foreign wheat varieties stored in the Plant Protection Institute (Bulgaria). The lethal effect of mycoses caused by one *Metarhizium anisopliae* and ten *Beauveria bassiana* isolates to adult insects was studied under laboratory conditions. The insects were treated with conidial suspensions ($1x10^9$conidia/ml). The dead individuals were placed in a humid chamber. The lethal effect was evaluated as a percentage of the cumulative daily mortality due to mycosis after correction with the mortality in the control variant.

The highest lethal effect (100%) was recorded on the second day after the treatment with isolates 667Bb of *B. bassiana* and 619Ma of *M. anisopliae*, followed by mortalities of 97.56%, 95.13% and 93.90% caused by isolates 666Bb, 660Bb and 655Bb. The lowest lethal effect (24.39%–26.82%) was caused by 433Bb and 559Bb on the same day. Mortality caused by all isolates, except 563Bb, was 100% on the 5^{th} day.

This is the first report in Bulgaria on bioassays with fungal pathogens against *T. versicolor* adults.

Key words: adult insect*, Beauveria bassiana,* lethal effect*, Metarhizium anisopliae,Trogoderma versicolor.*

INTRODUCTION

Trogoderma granarium Everts and *Trogoderma versicolor* Creutz. (Coleoptera: Dermestidae) are quarantine species for Bulgaria. Their larvae damage grains and tremendous variety of products and materials from plant and animal origin. They considered to be insect pests with economic importance, especially *T. granarium*.

Different insecticides and fumigants are mainly used for stored product insects' control. Their long time application except negative effects on humans and environment leads to resistance increase in the populations of a great number of these insects (Chaudhry, 2000; Benhalima et al., 2004; Athie and Mills, 2005; Ahmedani et al., 2007; Obretenchev et al., 2011). According FAO resistant populations were observed in 80 countries and for 10% from all stored product insects ever since 1970–1973 (Mordkovich and Nestorov, 1983).

This is the reason to search for alternative methods and natural enemies (parasitoids, predators, entomopathogenic nematodes and pathogens) to control stored product insects (Moore et al., 2000; Lord, 2001; Michalaki et al., 2006; Athanassiou et al., 2008; Khashaveh et al., 2008; Rojht et al., 2010; Derbalah, 2012; Madkour et al., 2012). Reports on the occurrence and application of pathogens for control of *Trogoderma* species are scarce.

According to Obretenchev et al. (2001) the neogregarine *Mattesia trogodermae* Canning (Apicomplexa: Neogregarinida) and *Adelina* sp. (Apicomplexa: Sporozoa) were found in natural populations of *T. granarium* and *T. versicolor*. Treatments of populations of both insects with *M. trogodermae* resulted to 90% and 52% mortality, respectively. *Mattesia trogodermae* reduced also the fecundity and the life longevity of the adults. The authors suggested that the neogregarine has a potential to be used as a biological control agent of *T. versicolor* and *T. granarium*.

The entomopathogenic fungi *Beauveria bassiana* (Bals. – Criv.) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota, Hypocreales) are frequently found as causal agents of mycoses with large host range and they are the mostly used fungal species for biological control (Goettel et al., 2000; Zimmermann, 2007).

Wakil et al. (2014) in survey for naturally occurring entomopathogenic fungi on stored product pests in various geographic regions in Pakistan established 195 isolates from 24 different fungal species on 6 insect species, including *T. granarium*. *Beauveria bassiana*, *M. anisopliae*, *Purpureocillium lilacinum* (Thorn) Samson, and *Lecanicillium attenuatum* (Zare and Gams) were the most important Ascomycetes obtained but only some *Aspergillus* spp. and *Fusarium* spp. were isolated from dead *T. granarium* adults.

There are data in literature about application of *M. anisopliae* and *B. bassiana* isolates against the larvae of *T. granarium* (Draganova et al., 2012, 2013) but investigations on using of fungal isolates to adults are few (Draganova et al., 2012).

At the best of our knowledge there are no reports about bioassays with entomopathogenic fungi to *T. versicolor* adults.

The aim of the study was to evaluate the lethal effect of mycoses caused by *M. anisopliae* and *B. bassiana* isolates to adults of *T. versicolor* under laboratory conditions.

MATERIALS AND METHODS

One *M. anisopliae* and ten *B. bassiana* isolates used in the experiments were from the collection of entomopathogenic fungi maintained at the Department of Entomology (Institute of Soil Science, Agrotechnologies and Plant Protection, Bulgaria). Fungal isolates were initially isolated from dead insects collected from different agro-ecosystems and forests in Bulgaria (Table 1). Conidial suspensions applied in the experiments were prepared at concentration of $1x10^9$conidia/ml after serial dilution of aqueous suspensions with Twin-80 (0.01%) and enumeration of conidia in Bürker chamber. Twin-80 was used as a surfactant.

Experiments were carried out with adults from laboratory population of *T. versicolor. Trogoderma versicolor* were found in samples of rust resistant foreign wheat varieties kept in storage of the Department of Plant Immunity (former Plant Protection Institute, Bulgaria). *Trogoderma versicolor* was reared on wheat grain variety Momchil in thermostat at temperature 32°C and RH 70 ± 5%.

Three-five-days insects (by 30 adult individuals in 4 repetitions) were treated by contact with conidia of the fungal isolates placed on filter paper discs in Petri dishes (90 mm in diameter) with 1 ml of conidial suspensions ($1x10^9$conidia/ml) (Draganova and Staneva, 1990). After 24 h contamination with conidia insects were removed and placed in sterile Petri dishes. Insects in the control variant were

Table 1. Origin of the isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorok

Isolate and fungal species	Species of the initial insect host	
513Bb *B. bassiana*	*Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)	larva
433Bb, 434Bb *B. bassiana*	*Ips typographus* (L.) (Coleoptera: Curculionidae)	imago
653Bb, 655Bb *B. bassiana*	*Ips acuminatus* Gyll. (Coleoptera: Curculionidae)	imago
559Bb *B. bassiana*	*Dryocoetes autographus* Ratz. (Coleoptera: Curculionidae)	imago
563Bb *B. bassiana*	*Hylurgops palliates* Gyll. (Coleoptera: Curculionidae)	imago
660Bb *B. bassiana*	*Otiorhynchus balcanicus* Strl. (Coleoptera: Curculionidae)	imago
619Ma *M. anisopliae*	*Tanymecus dilaticollis* Gyll. (Coleoptera, Curculionidae)	imago
666Bb, 667Bb *B. bassiana*	*Tortrix viridana* L. (Lepidoptera: Tortricidae)	larva

treated with Twin-80 (0.01%) in sterilized water. Bioassays were conducted under laboratory conditions at temperature 23-25°C and RH 70 ± 5%. Insect mortality was checked daily for 5 days after contamination and dead insects were placed in a humid chamber for fungal pathogen exhibition. Lethal effect of the isolates was evaluated as percentages of cumulative daily mortality due to mycosis after correction with mortality in control variant following Schneider-Orelli's formula (Püntener, 1981). Differences among lethal effects of mycoses to *T. versicolor* adults in treatments with conidial suspensions of *M. anisopliae* and *B. bassiana* isolates were estimated by t-test for independent samples applying Descriptive statistics. Statistical analyses were performed using the software STATISTICA^{R} version 6.0 of Stat Soft Inc.

RESULTS AND DISCUSSION

Results of the conducted bioassays showed that adults of *T. versicolor* were susceptible to the tested *M. anisopliae* and *B. bassiana* isolates under laboratory conditions (Fig. 1). Very high initial effect 93.18% ± 1.43 was registered after the treatment with conidia of the isolate 655Bb followed by the lethal effect 88,64% ± 1.94 and 88,64% ± 2.22 in variants with isolates 660Bb and 619Ma, respectively.

High initial effect of some isolates could be explained with toxic action of secondary metabolites released in insect hosts during pathogen's development. This is a part of mode of action of such entomopathogenic fungi as *M. anisopliae* and *B. bassiana* (Roberts, 1981; Strasser et al., 2000; Vey et al., 2001; Skrobek & Butt, 2005; Zimmermann, 2007). According to Golo et al. (2014) destruxins are cyclic depsipeptides produced by many *Metarhizium* isolates that have long been assumed to contribute to virulence of these entomopathogenic fungi.

Toxic compounds of *Beauveria* species are mostly low molecular weight secondary metabolites, mainly cyclic peptides such as beauvericin and bassianolide, and the pigments bassianin and tenellin (Strasser et al., 2000; Vey et al., 2001). According to Vey et al. (2001) beauvericin is a specific cholesterol acyltransferase inhibitor and it can induce programmed cell death similar to apoptosis and it causes cytolysis to insects, murine and human cell lines. Toxicity is strain characteristics and it is important in risk assessment of microbial plant protection products (Zimmermann, 2007).

The highest lethal effect (100% mortality) was established at the 2nd day after the treatment with conidia of the fungal isolates 667Bb of *B. bassiana* and 619Ma of *M. anisopliae* (Fig. 2). Mycoses due to isolates 433Bb, 559Bb and 513Bb caused low mortality to the host – 24.39% ± 15.24, 26.82% ± 15.05 and 46.34% ± 13.48, respectively, on the same day.

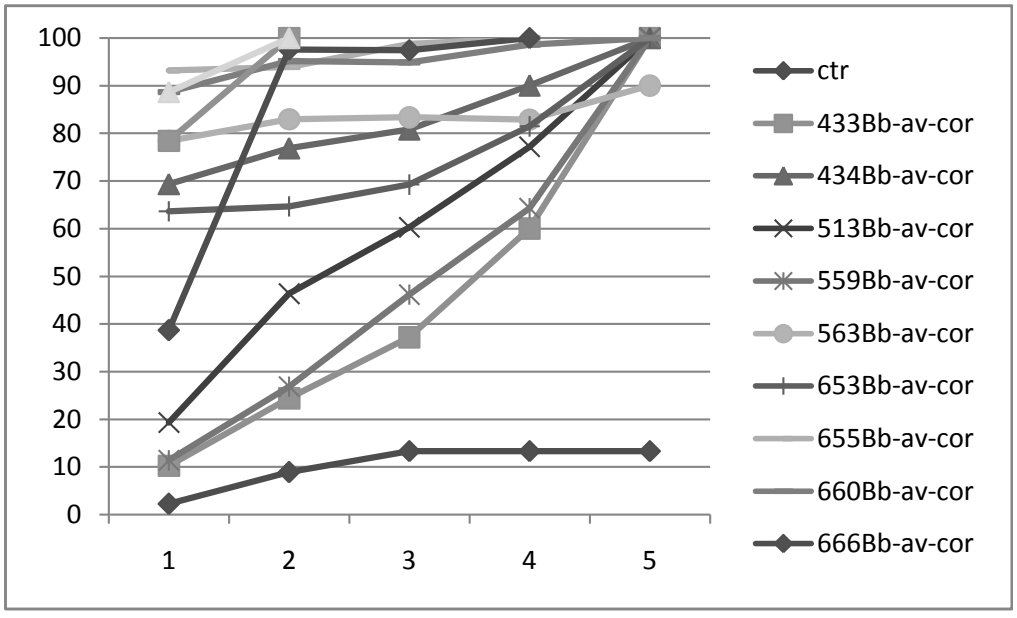

Fig. 1. *Dynamics of fungal infections 5 days after the treatment of Trogoderma versicolor adults with conidia of Metarhizium anisopliae and Beauveria bassiana isolates (%,cumulative daily mortality corrected with control treatment mortality) (SE-ctr= ± 2.18; SE-433Bb= ± 15.24; SE-434Bb= ± 5.21; SE-513Bb= ± 13.48; SE-559Bb= ± 15.05; SE-563Bb= ± 2.31; SE-653Bb= ± 6.52; SE-655Bb= ± 1.43; SE-660Bb= ± 1.94; SE-666Bb= ± 11.79; SE-667Bb= ± 4.22; SE-619Ma= ± 2.22)*

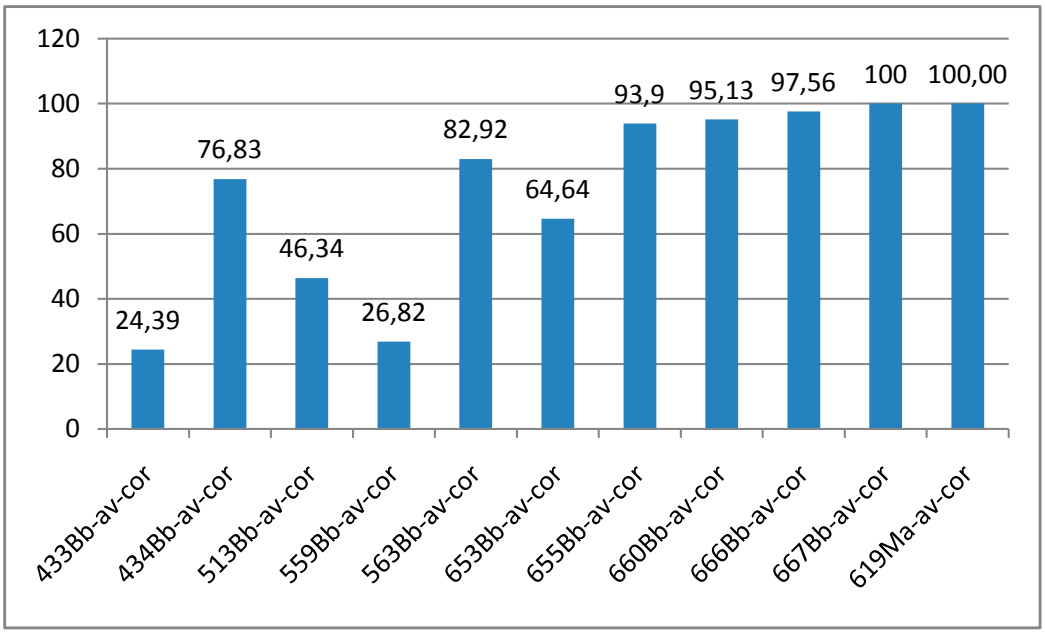

Fig. 2. *Lethal effect of mycoses to T. versicolor adults at the 2nd day after the treatment with conidia of the fungal isolates (%,cumulative daily mortality corrected with control treatment mortality) (SE-ctr= ± 2.18; SE-433Bb= ± 15.24; SE-434Bb= ± 5.21; SE-513Bb= ± 13.48; SE-559Bb= ± 15.05; SE-563Bb= ± 2.31; SE-653Bb= ± 6.52; SE-655Bb= ± 1.43; SE-660Bb= ± 1.94; SE-666Bb= ± 11.79; SE-667Bb= ± 4.22; SE-619Ma= ± 2.22)*

For 5 days, mortality by mycoses reached up to 100% in all treatments with an exception of the variant where insects were contaminated with conidia of the isolate 563Bb and where mortality increased to 90% ± 2.31 (Fig. 1).

To obtain fungal isolates with sharp and rapid lethal effect is with a great importance for the effective control of such dangerous stored product pest as *T. versicolor.*

Differences among susceptibility of *T. versicolor* adults to mycoses are seen in Table 2. It was proved at P<0.05 that differences between natural mortality in control variant and mortality due to fungal infections in variants treated with conidial suspensions of the isolates were significant. Significant differences were established between some other mortality means as well – in treatment with 433Bb vs. treatments with 655Bb, 660Bb, 667Bb, 619Ma; in treatment with 513Bb vs. treatments with 655Bb, 660Bb, 619Ma; in treatment with 559Bb vs. treatments with 655Bb, 660Bb, 667Bb, 619Ma; in treatment with 563Bb vs. treatments with 655Bb, 660Bb, 619Ma and in treatment with 653Bb vs. treatments with 655Bb, 660Bb, 619Ma.

The most promising results were obtained with isolates 655Bb, 660Bb, 667Bb and 619Ma. Statistical analyses didn't show significant differences between lethal effects in the fourth variants (P>0.05).

Because of lack of data on application of entomopathogenic fungi for control to *T. versicolor* comparison with the similar species *T. granarium* could be made. In previous studies Draganova et al., (2012) evaluated the virulence of *B. bassiana* isolates against adult insects and active larvae of *T. granarium.* Three of the fungal isolates in the cited experiments – 433Bb, 513Bb and 559Bb were the same as those used in the current study to the adult insects of *T. versicolor.* The highest cumulative daily mortality (100%) was obtained on dpi 15 and dpi 16 in the variants with isolates 559Bb and 433Bb. The same lethal effect was found to adults of *T. versicolor* but on the dpi 5. Comparison revealed that adults of *T. versicolor* were more susceptible to fungal infections than adults of *T. granarium.*

CONCLUSIONS

1. Conducted bioassays revealed that *T. versicolor* adults were susceptible to mycoses caused by *M. anisopliae* and *B. bassiana* isolates under laboratory conditions.

2. The most promising results were obtained applying isolates 655Bb, 660Bb, 667Bb and 619Ma - fungal infections that occurred in treated variants were with lethal effect over 90% on the second day. Statistical analyses didn't show significant differences between lethal effects in the fourth variants (P>0.05).

3. The present research is the first report in Bulgaria on the susceptibility of adults of *T. versicolor* to fungal pathogens.

Table 2. Significant differences between mean lethal effects of mycoses to *T. versicolor* adults in treatments with conidial suspensions (1x10^9 conidia/ml) of *M. anisopliae* and *B. bassiana* isolates for five-day period of study

Variants compared	Mean lethal effect Group 1	Mean lethal effect Group 2	t	df	p	F-ratio variances	p variances
433Bb vs. CTR	10,22	51,556	2,6841	8	0,027749	49,04353	0,002364
434Bb vs. CTR	10,22	84,888	13,2309	8	1,02E-06	5,720162	0,119679
513Bb vs. CTR	10,22	64	3,93758	8	0,004311	38,36154	0,003807
559Bb vs. CTR	10,22	54,442	2,90849	8	0,019635	47,77896	0,002487
563Bb vs. CTR	10,22	85,778	23,7811	8	1,04E-08	1,130036	0,908541
653Bb vs. CTR	10,22	78,222	9,88853	8	9,23E-06	8,978597	0,056232
655Bb vs. CTR	10,22	97,332	33,427	8	7E-10	2,309385	0,437482
660Bb vs. CTR	10,22	95,78	29,3611	8	1,96E-09	1,262971	0,826476
666Bb vs. CTR	10,22	87,112	6,4142	8	0,000206	29,32266	0,006382
667Bb vs. CTR	10,22	95,778	18,0114	8	9,26E-08	3,761196	0,227618
619Ma vs. CTR	10,22	97,778	28,1472	8	2,74E-09	1,041784	0,969308
BB_433 vs. BB_655	51,556	97,332	-2,98939	8	0,01735	113,2604	0,000457
BB_433 vs. BB_660	51,556	95,78	-2,87762	8	0,020586	61,94054	0,001499
BB_433 vs. BB_667	51,556	95,778	-2,79542	8	0,023362	13,03934	0,028995
BB_433 vs. MA_619	51,556	97,778	-3,00012	8	0,017069	47,0765	0,00256
BB_513 vs. BB_655	64	97,332	-2,45822	8	0,039428	88,59157	0,000742
BB_513 vs. BB_660	64	95,78	-2,333	8	0,047937	48,4495	0,002421
BB_513 vs. MA_619	64	97,778	-2,47179	8	0,038602	36,82294	0,00412
BB_559 vs. BB_655	54,442	97,332	-2,83742	8	0,021899	110,34	0,000481
BB_559 vs. BB_660	54,442	95,78	-2,72463	8	0,026062	60,34343	0,001577
BB_559 vs. BB_667	54,442	95,778	-2,64485	8	0,029491	12,70313	0,030398
BB_559 vs. MA_619	54,442	97,778	-2,84899	8	0,021512	45,86265	0,002693
BB_563 vs. BB_655	85,778	97,332	-4,24512	8	0,002818	2,609688	0,375436
BB_563 vs. BB_660	85,778	95,78	-3,31417	8	0,010632	1,427202	0,738717
BB_563 vs. MA_619	85,778	97,778	-3,74037	8	0,005702	1,084713	0,939081
BB_653 vs. BB_655	78,222	97,332	-2,86137	8	0,021107	20,73504	0,012311
BB_653 vs. BB_660	78,222	95,78	-2,58026	8	0,032603	11,33971	0,037275
BB_653 vs. MA_619	78,222	97,778	-2,83781	8	0,021886	8,618484	0,060359

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