Journal of Plant Protection Research ISSN 1427-4345

RAPID COMMUNICATION

Effect of fungus *Lecanicillium lecanii* and bacteria *Bacillus thuringiensis*, *Streptomyces avermitilis* on two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) and predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae)

Alexandra A. Zenkova[1], Ekaterina V. Grizanova[1], Irina V. Andreeva[1,2], Daria Y. Gerne[1], Elena I. Shatalova[1,3], Vera P. Cvetcova[1], Ivan M. Dubovskiy[1]*

[1] Department of Plant Protection, Novosibirsk State Agrarian University, Novosibirsk, Russia

[2] Laboratory for Biological Control of Phytophages and Phytopathogens, Siberian Federal Scientific Centre of Agro-Bio Technologies of the Russian Academy of Sciences, Novosibirsk, Russia

[3] Laboratory for Regulation Agricultural Animals and Plants Microbiocenosis, Siberian Federal Scientific Centre of Agro-Bio Technologies of the Russian Academy of Sciences, Novosibirsk, Russia

Vol. 60, No. 4: 415–419, 2020

DOI: 10.24425/jppr.2020.134917

Received: June 15, 2020
Accepted: September 4, 2020

*Corresponding address:
dubovskiy2000@yahoo.com

Abstract

The efficacy of the fungus *Lecanicillium lecanii* and two bacteria, *Bacillus thuringiensis* and *Streptomyces avermitilis* against the two-spotted spider mite *Tetranychus urticae* Koch and side effects on its predatory mite *Phytoseiulus persimilis* A.-H. was studied under laboratory conditions. Both *S. avermitilis* and *B. thuringiensis* based biopesticides resulted in maximum mortality rates of 90–100% and 91–99% for spider mite adults and larvae, respectively. The mortality of spider mite larvae under fungus *L. lecanii* treatment was around 60%. These bacteria and fungus also had toxic effects against *P. persimilis* on the same day of applying insecticides and releasing the predatory mite. The release of predatory mites one day post-treatment of plants with *L. lecanii* and 7 days post-treatment with *B. thuringiensis* or *S. avermitilis* did not negatively affect the survival of predators released. These findings support the potential use of entomopathogenic fungi and bacteria in combination with predatory mites in spider mite biocontrol.

Keywords: Avermectins, *Bacillus thuringiensis*, biocontrol, biopesticides, non-target effects

Introduction

The two-spotted spider mite *Tetranychus urticae* Koch. (Acari: Tetranychidae) is a serious pest of cucumber and more than 200 other plants, including eggplant, pepper, tomato, lemon, chrysanthemum, rose and carnation (Van Leeuwen *et al.* 2015). Greenhouse conditions allow wide application of biological agents for pest control. Several predatory mites, such as *Phytoseiulus persimilis* A.-H. (Acari: Phytoseiidae), have become useful for pest control in greenhouses (Cakmak *et al.* 2009). The modern concept of biological plant protection includes combining several biological control agents to suppress pest populations. This is due to synergistic effects achieved as a result of the effect of biological preparations on pests, the expansion of the spectrum of action of mixtures, and the ability of active bioagents (predators) to effectively suppress the residual number of the pest after the application of bioinsecticides (Tomilova *et al.* 2016; Dogan *et al.* 2017; Yaroslavtseva *et al.* 2017). Entomopathogenic fungi (EPF), such as *Metarhizium anisopliae*, *Beauveria* *bassiana, Isaria cateniannulata* and *Lecanicillium lecanii*, are often used to control *T. urticae* and other spider mites (Chandler *et al.* 2005; Maniania *et al.* 2008; Zhang *et al.* 2018). Interestingly fungal infection not only caused the death of *T. urticae* females but also significantly reduced the fecundity of surviving individuals (Shi and Feng 2009). Entomopathogenic fungi evolved to kill arthropods (insects, ticks and mites) and they may affect natural enemies of spider mites, including Phytoseiidae (Butt *et al.* 2016; Dogan *et al.* 2017). Different studies have been conducted to examine the possibility of combining the application of EPF with predatory mites. For example, the susceptibility of *Amblyseius swirskii* to *Beauveria bassiana* was found to vary and depended on the type of exposure (Midthassel *et al.* 2016). Additionally, acaricidal effects of fungi *B. bassiana, Metarhizium flavoviride, M. brunneum* and *L. lecanii* against both the two-spotted spider mite (Chandler *et al.* 2005) and predatory mites have been reported (Dogan *et al.* 2017). Moreover, a 100% mortality rate of *P. persimilis* infected by *B. bassiana* was shown in other studies, but without any data regarding the time of observation and features of fungal spore formation (Ludwig and Oetting 2001).

Biopesticides based on the bacterium *B. thuringiensis* (Bt) and metabolites of the bacterium *Streptomyces avermitilis* have also been used to control *T. urticae* in recent years (Chapman and Hoy 1991; Brown *et al.* 2017). It should be noted that Bt bacteria produce beta-exotoxins with high contact (percuticular) activity against arthropods (Liu *et al.* 2014) including effects on spider mites (Hall *et al.* 1971) and citrus red mites (*Panonychus citri*) (Krieg 1972). It is well known that bacteria *S. avermitilis* produce toxic metabolites (avermectins) with contact activity against mites (Lasota 1991). However, little is known about the nonspecific toxic effect of bacterial insecticides on predator mites.

The aims of this research were to determine the efficacy of bioinsecticides based on the fungus *L. lecanii* and the bacteria *B. thuringiensis* and *S. avermitilis* against the two-spotted spider mite *T. urticae* and its predatory mite *P. persimilis* and to determine the possibility of a combined treatment of these biological agents for suppressing two-spotted spider mite populations in greenhouses.

Materials and Methods

Rearing of *Tetranychus urticae* and *Phytoseiulus persimilis* mites

Laboratory populations of the two-spotted spider mite *T. urticae* and predatory mite *P. persimilis* were maintained in the laboratory at Novosibirsk State Agrarian University (Novosibirsk, Russia). The lines of both mite species were provided from a collection of laboratory populations of the All-Russian Institute of Plant Protection (Russia, St. Petersburg – Pushkin).

A culture of two-spotted spider mites was maintained on haricot (*Phaseolus vulgaris* L.) in the laboratory at 25 ± 1°C, 50% humidity and a photoperiod of 16 : 8 h (L : D). The predatory mite *P. persimilis* was bred on bean leaves pre-populated with spider mites in the laboratory under the same conditions as those for *T. urticae* but at 60–70% relative humidity.

Entomopathogenic fungus and bacteria: strains and preparation of inoculum

Biovert (suspension powder) – an insecticide based on *L. lecanii* Zimm. spores, strain B-80, 1×10^6 CFU · g^{-1} of media, industrial preparation by Sibbiofarm (Berdsk, Russia). Bitoxibacillin (BTB) (powder) – an insecticide based on spores, protein crystals (Cry toxins) and exotoxin of *B. thuringiensis* var. *thuringiensis* (BA-1500 EA · mg^{-1}, 20 billion spores · g^{-1}), industrial preparation by Sibbiofarm (Berdsk, Russia). Fitoverm (emulsion concentrate) – an insecticide based on a complex of natural avermectins produced by *S. avermitilis* (Aversectin C – 2 g · l^{-1}), industrial preparation by Farmbiomed (Moscow, Russia).

Bioassays

To determine toxicity in two-spotted spider and predatory mites, maximum concentrations of 1% Biovert, 1% BTB and 0.2% Fitoverm, allowed for use against spider mites, were used. The bioinsecticides were initially assayed against two-spotted spider mites in Petri dishes (90 mm in diameter) containing cut leaves (area 58.3 ± 3.4 square cm) at 25°C. Leaves were placed on a damp filter paper disc and kept constantly wet. The bioinsecticides were evaluated by spraying two-spotted spider mite-infested leaves using a hand sprayer (12 ± 1 µl per square cm). The experiment was carried out in five replicates per treatment (80–120 individuals per one Petri dish). The mortality rate of the two-spotted spider mites was monitored for 5 days for larvae (total per treatment: $n = 445$ per control; $n = 435$ per Fitoverm; $n = 598$ BTB; $n = 619$ Biovert) and 5 days for adults (total per treatment: $n = 456$ per control; $n = 364$ per Fitoverm; $n = 466$ BTB; $n = 490$ Biovert) post-bioinsecticide application. The predatory mite females (five adults per Petri dish, five replicates) were transferred to the bean leaves with *T. urticae* on the same day 10 min after bioinsecticide application as well as 1 and 7 days after bioinsecticide application. Control treatments involved spraying with water. The number of predatory mites and their offspring was monitored at 1, 3, 5 and 7 days post-bioinsecticide application.

Data analysis

Kaplan-Meier survival analysis followed by a log-rank test was applied to estimate differences in mortality dynamics of two-spotted spider mites. Data were checked for normality (Gaussian) using the Shapiro-Wilk test. Two-way ANOVA with Bonferroni multiple comparisons was applied to estimate differences in the mortality of predatory mites. Data were analysed using GraphPad Prism v8.0 (GraphPad Software Inc., USA).

Results and Discussion

Effect of fungal and bacterial preparations on spider mites

Both avermectins and Bt caused significant mortality in the two-spotted spider mite larvae at 99.3% (chi-sq: 823.6, $p < 0.001$) and 91.6% (chi-sq: 751, $p < 0.001$), respectively (Fig. 1A), on the 5th day after bioinsecticide application. The application of avermectins led to 100% (chi-sq: 787, $p < 0.001$) mortality of two-spotted spider mite adults on the 5th day post treatment (Fig. 1B). The Bt treatment resulted in 95% (chi-sq: 492, $p < 0.001$) mortality of spider mites on the 5th day after application.

Bioinsecticides based on bacterial Bt and avermectins exhibited high biological efficacy against the spider mites. It has been shown that a mixture of spores and a combination of Bt toxins have effects against *T. urticae* nymphs, resulting in high mortality and an increased mite development time (Alper *et al.* 2013). *Bacillus thuringiensis* var. *tenebrionis* is also highly toxic (90% mortality) to adult female spider mites (Chapman and Hoy 1991). The *B. thuringiensis* toxins were shown to have profound contact effects on the adults, the number of laid eggs, and the emerging larvae of citrus red mite and spider mites (Hall *et al.* 1971; Krieg 1972).

Additionally, the susceptibility of spider mites to avermectins has been demonstrated (Campos *et al.* 1996; Döker and Kazak 2019), whereby the resistance of spider mite populations to avermectins was correlated with the number of repeated treatments and the period of application. Furthermore, resistance was not detected in a nursery in which avermectins were applied less than six times a year and less than 30 times over 6 years (Campos *et al.* 1996).

The mortality rate of the two-spotted spider mite larvae was 65.8% on the 5th day after treatment by *L. lecanii* (chi-sq: 342, $p < 0.001$) (Fig. 1A). In contrast, *L. lecanii* did not cause mortality of the spider mite adults compared with the control (Fig. 1B).

Thus, we found that the efficacy of *L. lecanii* against the larvae of spider mites increased with time after application and resulted in a maximum mortality rate of 65% on the 5th day of the experiment. It should be noted that the mortality caused by this fungus has a long delay due to the fungal infection process. Overall, *Lecanicillium* entomopathogenic fungi are ecologically safe and have substantial potential for pest control (Dogan *et al.* 2017).

Effect of fungal and bacterial preparations on predatory mites

In the absence of negative influences from bioinsecticides, predatory mite females started to lay eggs in the control treatment. The first larvae emerged on the 3rd day, and a very large number of larvae and nymphs emerged on the 5th day (Fig. 2A–B).

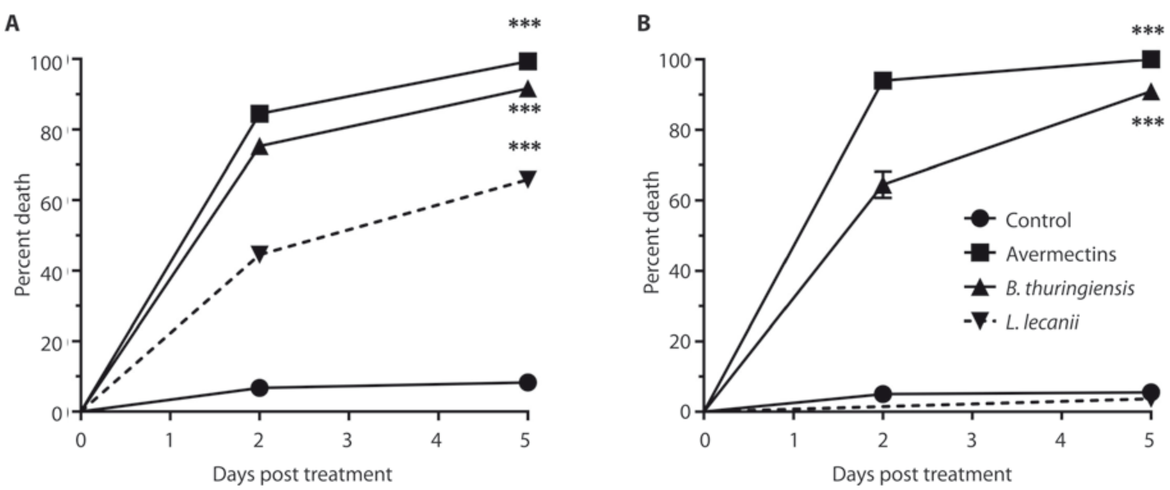

Fig. 1. Mortality rate of larvae (A) and adults (B) of the two-spotted spider mite *Tetranychus urticae* after treatment with avermectins (0.2%, Fitoverm), the bacterium *Bacillus thuringiensis* (1%, BTB) and the fungus *Lecanicillium lecanii* (1%, Biovert); ***$p < 0.001$ compared with the control

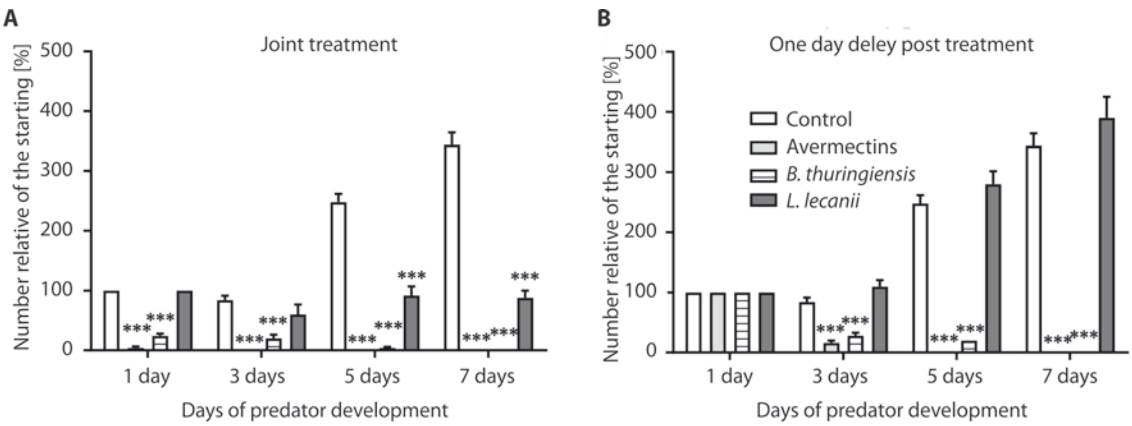

Fig. 2. Number of *Phytoseiulus persimilis* adults and their offspring at the release of predatory mites on the day of treatment with bioinsecticides (10 min after bioinsecticide application) (A) and 1 day post-treatment (B) with avermectins (0.2%, Fitoverm), the bacterium *Bacillus turingiensis* (1%, BTB) and the fungus *Lecanicillium lecanii* (1%, Biovert); $^{**}p < 0.01$; $^{***}p < 0.001$ compared with the control for the same day

Treatments with bioinsecticides and the time of post application had a significant effect on the predatory mite *P. persimilis* number released on the same day (10 min delay) when spraying with bacteria and fungi [treatments: F (3, 16) = 350.1; $p < 0.0001$; time: F (3, 48) = 42.42; $p < 0.0001$] and 1-day-delayed treatments [treatments: F (3, 15) = 175.3; $p < 0.0001$; time: F (3, 45) = 123.0; $p < 0.0001$].

Bioinsecticides based on Bt and avermectins were highly toxic to the predatory mite *P. persimilis* in the case of joint and 1-day-delayed treatments (Fig. 2A–B). We showed that the release of predatory mites 7 days after treatment with biopreparations did not cause their death. The abundance of predators was the same as that in the control (248 ± 13.56%), and on the 5th day of the experiment, abundance increased 232 ± 10.95% (Bt) and 220 ± 19.6% (avermectins) times compared to the initial number. It has been shown that *B. thuringiensis* var. *tenebrionis* was also moderately toxic to *Metaseiulus occidentalis* predatory mites (Chapman and Hoy 1991). The high toxicity of Bt and *S. avermitilis* can be related to bacterial toxins with contact activity, which may be active for a long time on leaves. It should be noted, that some strains of Bt produce toxin, thuringiensin, also known as β-exotoxin, a thermostable secondary metabolite. Thuringiensin has nonspecific insecticidal toxic effects by inhibition of DNA-dependent RNA polymerase in a wide range of insects, including species belonging to the orders Diptera, Coleoptera, Lepidoptera, Hymenoptera, Orthoptera, and Isoptera, and even several nematode species (Gohar and Perchat 2001; Liu *et al.* 2014).

The bioinsecticide based on *L. lecanii* was the least toxic to the predatory mites, with 40% of the released predatory mites being dead on the 3rd day of the experiment after joint treatment with both *P. persimilis* and the fungus (Fig. 2A). However, the surviving predatory mite females continued to feed and lay eggs, and on the 5th day of the experiment, the number of predatory mites began to increase again due to the emergence of larvae of the new generation. The number of predatory mites did not change on the subsequent days and was 3.9 times lower on the 7th day than in the control (Fig. 2A), in which the number of *P. persimilis* mites increased 3.4 times (Fig. 2A). With the release of the predatory mites 1 day post-treatment with *L. lecanii,* the total number of *P. persimilis,* including mites that emerged during the experiment, did not change significantly compared to the control (Fig. 2B).

It has been shown previously that entomopathogenic fungi such as *L. muscarium*, *M. brunneum*, *M. flavoviride* and *B. bassiana* can negatively affect the predatory mite *P. persimilis* (Donka *et al.* 2008). However, a delay in the contact of predatory mites with *B. bassiana* fungus can decrease the virulence of the pathogens (Ullah and Lim 2017). This result is probably because success in conidial adhesion is related to post-infection time (Butt *et al.* 2016).

This study shows that bacterial preparations (Bt and avermectins) caused high mortality of both spider mite larvae and adults. The *L. lecanii*-based preparation had a significant effect only on the larvae of the spider mite. Bacterial preparations also caused significant mortality of individuals of the predatory mite released both on the day of treatment and 1 day after treatment. In contrast to bacterial preparations, *L. lecanii* did not harm the predator if it was released 1 day after treatment. The release of predatory *P. persimilis* mites 7 days post-treatment of plants with Bt or avermectins did not negatively affect the adult predators released or their offspring. Thus, fungal and bacterial biological agents and *P. persimilis* have the potential to complement one another as part of an integrated spider mite management program in greenhouses.

The reported study was funded by Russian Foundation for Basic Research according to research projects No 19-016-00121, No 18-316-20007 and Russian Science Foundation № 19-16-00019 in part of Bt testing.

References

Alper M., Gunes H., Civelek H.S., Dursun O., Eskin A. 2013. Toxic effects of some native *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) isolates against *Tetranychus urticae* Koch (Acarina: Tetranychidae), *Ceroplastes rusci* L. (Homoptera: Coccidae) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Türkiye Entomoloji Bültenin 3 (2): 75–87.

Brown S., Kerns D.L., Gore J., Lorenz G., Stewart S. 2017. Susceptibility of twospotted spider mites (*Tetranychus urticae*) to abamectin in Midsouth cotton. Crop Protection 98: 179–183. DOI: https://doi.org/10.1016/j.cropro.2017.04.002

Butt T.M., Coates C.J., Dubovskiy I.M., Ratcliffe N.A. 2016. Entomopathogenic fungi: new insights into host-pathogen interactions. Advances in Genetics 94: 307–364. DOI: https://doi.org/10.1016/bs.adgen.2016.01.006

Cakmak I., Janssen A., Sabelis M.W., Baspinar H. 2009. Biological control of an acarine pest by single and multiple natural enemies. Biological Control 50 (1): 60–65. DOI: https://doi.org/10.1016/j.biocontrol.2009.02.006

Campos F., Krupa D.A., Dybas R.A. 1996. Susceptibility of populations of twospotted spider mites (Acari: Tetranychidae) from Florida, Holland, and the Canary Islands to abamectin and characterization of abamectin resistance. Journal of Economic Entomology 89 (3): 347–393. DOI: https://doi.org/10.1093/jee/89.3.594

Chandler D., Davidson G., Jacobson R.J. 2005. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. Biocontrol Science and Technology 15 (1): 37–54. DOI: https://doi.org/10.1080/09583150410001720617

Chapman M.H., Hoy M.A. 1991. Relative toxicity of *Bacillus thuringiensis* var. *tenebrionis* to the two-spotted spider mite (*Tetranychus urticae* Koch) and its predator *Metaseiulus occidentalis* (Nesbitt) (Acari, Tetranychidae and Phytoseiidae). Journal of Applied Entomology 111 (1–5): 147–154. DOI: https://doi.org/10.1111/j.1439-0418.1991.tb00305.x

Dogan Y.O., Hazir S., Yildiz A., Butt T.M., Cakmak I. 2017. Evaluation of entomopathogenic fungi for the control of *Tetranychus urticae* (Acari: Tetranychidae) and the effect of *Metarhizium brunneum* on the predatory mites (Acari: Phytoseiidae). Biological Control 111: 6–12. DOI: https://doi.org/10.1016/j.biocontrol.2017.05.001

Döker İ., Kazak C. 2019. Non-target effects of five acaricides on a native population of *Amblyseius swirskii* (Acari: Phytoseiidae). International Journal of Acarology 45 (1–2): 69–74. DOI: https://doi.org/10.1080/01647954.2018.1542457

Donka A., Sermann H., Buttner C. 2008. Effect of the entomopathogenic fungus *Lecanicillium muscariumon* the predatory mite *Phytoseiulus persimilis* as a non-target organism. Communications in Agricultural and Applied Biological Sciences 73 (3): 395–403.

Gohar M., Perchat S. 2001. Sample preparation for β-exotoxin determination in *Bacillus thuringiensis* cultures by reversed-phase high-performance liquid chromatography. Analytical Biochemistry 298 (1): 112–117. DOI: https://doi.org/10.1006/abio.2001.5373

Hall I.M., Hunter D.K., Arakawa K.Y. 1971. The effect of the β-exotoxin fraction of *Bacillus thuringiensis* on the citrus red mite. Journal of Invertebrate Pathology 18 (3): 359–362. DOI: https://doi.org/10.1016/0022-2011(71)90038-3

Krieg A. 1972. Über die Wirkung von *Bacillus thuringiensis*-Präparaten auf Spinnmilben (Tetranychidae). Anzeiger für Schädlingskunde und Pflanzenschutz 45: 169–171. DOI: https://doi.org/10.1007/BF01879428

Lasota J. 1991. Avermectins, a novel class of compounds: implications for use in arthropod pest control. Annual Review of Entomology 36: 91–117. DOI: https://doi.org/10.1146/annurev.ento.36.1.91

Liu X., Ruan L., Peng D., Li L., Sun M., Yu Z. 2014. Thuringiensin: a thermostable secondary metabolite from *Bacillus thuringiensis* with insecticidal activity against a wide range of insects. Toxins 6 (8): 2229–2238. DOI: https://doi.org/10.3390/toxins6082229

Ludwig S.W., Oetting R.D. 2001. Susceptibility of natural enemies to infection by *Beauveria bassiana* and impact of insecticides on *Ipheseius degenerans* (Acari: Phytoseiidae). Journal of Agricultural and Urban Entomology 18 (3): 169–178. DOI: https://doi.org/10.1080/019722499128484

Maniania N.K., Bugeme D.M., Wekesa V.W., Delalibera I., Knapp M. 2008. Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. Experimental and Applied Acarology 46: 259–274. DOI: https://doi.org/10.1007/s10493-008-9180-8

Midthassel A., Leather S.R., Wright D.J., Baxter I.H. 2016. Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents. BioControl 61 (4): 437–447. DOI: https://doi.org/10.1007/s10526-016-9718-3

Shi W.B., Feng M.G. 2009. Effect of fungal infection on reproductive potential and survival time of *Tetranychus urticae* (Acari: Tetranychidae). Experimental and Applied Acarology 48 (3): 229–237. DOI: https://doi.org/10.1007/s10493-009-9238-2

Tomilova O.G., Kryukov V.Y., Duisembekov B.A., Yaroslavtseva O.N., Tyurin M.V., Kryukova N.A., Skorokhod V., Dubovskiy I.M., Glupov V.V. 2016. Immune-physiological aspects of synergy between avermectins and the entomopathogenic fungus *Metarhizium robertsii* in Colorado potato beetle larvae. Journal of Invertebrate Pathology 96: 14–20. DOI: https://doi.org/10.1016/j.jip.2016.08.008

Ullah M.S., Lim U.T. 2017. Laboratory evaluation of the effect of *Beauveria bassiana* on the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae). Journal of Invertebrate Pathology 148: 102–109. DOI: https://doi.org/10.1016/j.jip.2017.06.006

Van Leeuwen T., Tirry L., Yamamoto A., Nauen R., Dermauw W. 2015. The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pesticide Biochemistry and Physiology 121: 12–21. DOI: https://doi.org/10.1016/j.pestbp.2014.12.009

Yaroslavtseva O.N., Dubovskiy I.M., Khodyrev V.P., Duisembekov B.A., Kryukov V.Y., Glupov V.V. 2017. Immunological mechanisms of synergy between fungus *Metarhizium robertsii* and bacteria *Bacillus thuringiensis* ssp. *morrisoni* on Colorado potato beetle larvae. Journal of Insect Physiology 96: 14–20. DOI: https://doi.org/10.1016/j.jinsphys.2016.10.004

Zhang X., Guo J.J., Zou X., Jin D.Ch. 2018. Pathogenic differences of the entomopathogenic fungus *Isaria cateniannulata* to the spider mite *Tetranychus urticae* (Trombidiformes: Tetranychidae) and its predator *Euseius nicholsi* (Mesostigmata: Phytoseiidae). Experimental and Applied Acarology 75: 69–84. DOI: https://doi.org/10.1007/s10493-018-0247-x