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# ACARICIDAL EFFECT OF SECONDARY METABOLITES FROM SYMBIOTIC BACTERIA *XENORHABDUS BOVIENII* AND *X. NEMATOPHILA* OF ENTOMOPATHOGENIC NEMATODES ON SPIDER MITE *TETRANYCHUS URTICAE* (TROMBIDIFORMES, TETRANYCHIDAE)

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In laboratory, the highest mortality rates of *Tetranychus urticae* after the use of metabolic products of symbiotic bacteria with a titer of $1 \times 10^7$ were observed in *Xenorhabdus bovienii* at 6–8 days post application (dpa) in the experiment with live and at 8 dpa of autoclaved culture (about 95%). In experiments with live and autoclaved culture with a titer of $1 \times 10^7$, the mortality mites at 8 dpa in *X. bovienii* was almost the same, but in *X. nematophila* it was slightly higher in autoclaved culture. At 8 dpa, the efficacy of the live and autoclaved metabolic products of *Xenorhabdus bovienii* and *X. nematophila* against the spider mite with a titer of $1 \times 10^5$ was about 1.4 times lower compared to the culture with a titer of $1 \times 10^7$. The relationship between the mortality of spider mites (%) and the exposure time (days) to bacterial metabolism products most reliably reflects by the polynomial dependence with the accuracy of approximation 0.93–1.0. In the greenhouse, the effectiveness of the bacterial metabolic products of *X. bovienii* against spider mite was highest in experiments with live culture with a titer of $1 \times 10^8$. In experiments with live culture of *X. bovienii* with a titer of 1 x $10^7$ (in vivo) the mortality rate of spider mites on leaves of shrub *Dracaena sanderiana* at 8 dpa increased from 84% on the ground floor to 90% on the second floor. The overall efficacy of the bacterial metabolic products of *X. bovienii* (in vivo, titer $1 \times 10^7$) against adults, larvae and nymphs of *T. urticae* on the leaves of perennial marsh grasses (*Potenderia cordata*, *Thalia geniculata* and *T. dealbata*) was about 98–99%.

**Keywords:** *Steinernema*, live bacterial culture, autoclaved culture, laboratory conditions, greenhouses, toxic secondary metabolites, efficiency

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## Introduction

Bacteria of the genus *Xenorhabdus* are symbionts of entomopathogenic nematodes (EPNs) in the genus *Steinernema*. The bacterium colonizes a specialized intestinal pocket within the infective stage of the third age nematode, which transports the bacteria between insects that are killed and consumed by the pair for reproduction. The infectious stage of the third-instar nematode, which lives in the soil without feeding, penetrates into the body of soil insects and regurgitates symbiotic bacteria that contribute to the death and digestion of the host's internal organs. Nematodes begin to feed on digested foods, turning into larvae of the fourth instar, then into males and females. Bacteria of the genus *Xenorhabdus* produce a large number of secondary metabolites that weaken the immune system of insects, causing their death, suppressing the development of other microorganisms. These include toxic peptides, amino acids, polypeptides, antimicrobial and antifungal substances with antibiotic properties (fabclavines, xenocoumacins and others). They have found wide application in plant protection against nematodes – parasites of stems and leaves, the root system of plants, their diseases and pests (Hazir et al., 2016; Dreyer et al., 2018; Eroglu et al., 2019; Abebew et al., 2022; Yüksel, 2022; Yüksel et al., 2022; Zhang et al., 2022). To protect plants from diseases and pests, three main forms of biological products containing toxic secondary metabolites of *Xenorhabdus* bacteria are used: culture fluids containing metabolites and of live (*in vivo*) or dead (*in vitro*) bacteria; cell-free culture supernatants. The cultivation of bacteria from stock cultures is initially carried out in Petri dishes on agar plates at 28°C for 24 h. One of the bacterial colonies is transferred to flasks containing sterile Tryptic Soy Broth and the flasks are incubated at 30°C and 150 rpm (revolution per minute) for 24 h. The density of bacterial culture cells is measured by a spectrophotometer. To extract the cell-free supernatants, the bacterial culture in the broth suspension is centrifuged at 20.000 revolutions per minute (rpm) for 15 min at 4°C in 50 ml Falcon tubes. The centrifuged supernatant solution is separated from the bacterial cells by passing through a 0.22 μm millipore filter. The filtrated solution is checked for the presence of bacterial cells by streaking onto NBTA agar (Hazir et al., 2016). Autoclaving at 121°C for 10 min do not influence the antibiotic activities of the cell-free cultures of *Xenorhabdus* (Fodor et al., 2010). Cell-free bacterial cultures and supernatants can be stored at 4°C for 2 weeks before use in experiments (Hazir et al., 2016). *Xenorhabdus nematophila*, *X. bovienii* and *X. szentirmaii* supernatants could be used as potential control agents against *T. urticae* (Incedayi et al., 2021). The effect of secondary metabolites of *Xenorhabdus* bacteria on plant diseases and pests has been studied mainly in laboratory conditions in Petri dishes and in pots.

*Tetranychus urticae* C.L. Koch, 1836 (two-spotted spider mite) is a widespread polyphagous, cosmopolitan species. It has been recorded from most countries in North, Central and South America, Europe, Asia, Africa and Australia. *T. urticae* infests about 1167 species host plants from 127 families, annuals, perennial grasses, shrubs and trees, wild and

cultivated both in field conditions and in greenhouses. The lower temperature threshold for it development is about 12°C and the upper limit for development is about 40°C, optimal temperature 26–30°C, air humidity 60–80%. The life cycle ranges from 8 days to 40 days. *T. urticae* is one of the most serious agricultural pests in the world. About 88 cultural host plants are infested by this pest, such as bean, soybean, cotton, cucumber, tomato, melon, peanut, vine, banana, papaya, corn, ornamental crops and others (Riahi et al., 2013; Migeon, Dorkeld, 2019). In most agricultural crops, the use of synthetic pesticides is the main method to control *T. urticae*. However, because environmental adverse effects of these pesticides, the development of pesticide resistance in the target pest, and potential impacts on biodiversity and Human health (Dermauw et al., 2013), alternative methods should be developed. *T. urticae* is very difficult to control with acaricides because most populations developed resistance to chemical groups after a few years of use (Cranham, Helle, 1985). In Turkey, Eroglu et al. (2019) were investigated the effects of secondary metabolites produced by 6 species symbiotic bacteria of the genera *Xenorhabdus* and *Photorhabdus* on different stages of *Tetranychus urticae* using cell-free bacterial supernatants in Petri dishes and in pot experiments. The number of living and dead individuals was recorded at 2, 5 and 7 day post application (dpa) of the cell-free bacterial supernants. Depending on the bacterial supernatant, mortality in the was less than 4% for eggs, 46–97% for larvae, 30–96% for protonymphs, 41–92% for deutonymphs, 92–100% for adult males and 46–93% for adult females.

The purpose of our research was to evaluate the effectiveness of exposure to secondary metabolites of live and autoclaved bacterial cultures of the genus *Xenorhabdus* in laboratory conditions and in greenhouses against *Tetranychus urticae* by spraying these cultures of experimental mite-infested plants.

## MATERIALS AND METHODS

The research was carried out in the Laboratory of Microbiology of the All-Russian Institute of Plant Protection and in the greenhouses of the Botanical Garden of the Institute of Botany of the Russian Academy of Sciences in March, April and May 2020 and 2021.

Cultures of symbiotic bacteria *Xenorhabdus bovienii* and *Xenorhabdus nematophila* were obtained indirectly from the nematodes by sampling the haemocoel of *Galleria mellonella* (L.) (Lepidoptera, Pyralidae) larvae of older ages infected by nematodes *Steinernema feltiae* strain RP18-91 and *Steinernema carpocapsae* strain "agriotes" and that stored in distilled water at 5–7°C within two weeks. The pathogenicity of the two forms of symbiotic bacteria the *X. bovienii* and *X. nematophila* was compared by estimation of $LD_{50}$ following intrahaemocoelic injection of *Galleria* larvae. The concentration of cells in shaken, 24 h broth cultures was estimated by use of a counting slide. Each culture was then serially diluted with sterile Ringer's solution (Akhurst, 1980).

The initial titer of $1 \times 10^8$ was taken as the maximum in the experiments. The concentration of bacterial cells with a titer of $1 \times 10^7$ and $1 \times 10^5$ was obtained by diluting the culture liquid with a titer of $1 \times 10^8$ with sterile water. Part of the resulting culture liquids were autoclaved at a tem-

perature of 121°C, pressure of 1 atmosphere for 30 min. As known, symbiotic bacteria of the genus *Xenorhabdus* produce both heat-labile and heat-stabile toxins, enzymes and antimicrobials. Their heat-stabile components are active after heat sterilization and can be used against different species of bacteria and pests (Inman, Holmes, 2012). In experiments against spider mites, live and autoclaved bacterial cultures were used in laboratory conditions and in greenhouses.

In the laboratory, the study of the effect of the products of the metabolism of symbiotic bacteria of entomopathogenic nematodes on the spider mite was carried out according to toxicological methods (Sukhoruchenko, Ivanova, 2013). The laboratory population of the spider mate was maintained in cages on bean plants (*Phaseolus vulgaris*) at a temperature of 22–24°C, relative humidity of 65–70%, photoperiod of L18: D6. The beans for the experiments were grown on water in glass jars with a volume of 0.5 l, closed with plastic lids with holes where the bean sprouts were inserted. Then the plants of bean with a height of 9–10 cm with roots and one leaf were placed in conical cones with a volume of 100 ml. Before the experiments, 20 or 25 female mites were placed on the leaf with a soft brush (depending on the size of the leaf) 2 hours before they are treated with bacterial preparations, so that the mites on the leaf began to feed. The number of mites on the leaf of bean in each experiment was the same. Then the plants with the mites were removed from the cone, carefully dipped in solutions of live or autoclaved bacterial culture with titers $1 \times 10^5$ or $1 \times 10^7$ for 3 seconds, allowed to drain excess moisture and placed back in the cones. Cones with mite-infested plants were placed on pallets with water to avoid their migration from one plant to another and kept under the above conditions of temperature, humidity and photoperiod. Control plants were dipped in water. The number of live and dead individuals of mite were recorded at 1, 4, 6 and 8 dpa (day post application) after reatment with bacterial preparations (Table 1). The replication of each experiment was 4-fold. Before the appearance of the larvae, the number of live females was counted on the leaf, and after the hatching of the larvae, the total number of individuals was calculated. In the control, female spider mites laid eggs in Petri dishes and the larvae hatched on the 5th-6th day. Mite mortality was determined taking into account changes in their number in the control according to the formula of Henderson and Tilton (1955): $E = 100 \times (1 – Oe \times Cc / On \times Cn)$, where: E – efficiency expressed as the percentage of pest population reduction adjusted for control;

Oe, Cc – the number of live individuals before processing in the experiment and in the control;

On, Cn – the number of live individuals after processing and in the control, respectively, by the accounting.

The effect of the live and autoclaved products of the metabolism of symbiotic bacteria *Xenorhabdus bovienii* of entomopathogenic nematode *Steinernema feltiae* with titers $1 \times 10^7$ and $1 \times 10^8$ was also tested against the spider mite in the greenhouses of the Botanical Garden of the Botanical Institute of the Russian Academy of Sciences (St Petersburg) 3.05–10.05.2020, 30.03–7.04 and 12.05–20.05 2021 (Tables 2, 3). In the Leningrad region, the spider mite develops in 8–10 generations per year. With a decrease in the duration of the daylight less than 16 hours fertilized female spider mites enter winter diapause, which is observed in St Petersburg since the beginning of August. Overwintered females appear on plants in early May when the air temperature rises above 12–14°C, feed and lay eggs among the cobwebs on the underside of the leaves. In other words, during the first period of experiments on plant protection from spider mite at the end of March – the first decade

of April, when an air temperature in greenhouses was of 19–21°C, overwintered females during the egg laying period were dominated. Females lay eggs for 15–20 days. The development from an egg to an adult takes about from 7 to 20 days, depending on the air temperature. During the second period of experiments (12–20.05) at an air temperature in greenhouses of 28–32°C, all stages of spider mite development (eggs, larvae, protonymphs, deutonymphs and adults) were presented. Spraying of experimental mite-infested plants was carried out with manual sprayers Marolex Profession, 5 l. The experimental plants were represented by trees (*Bolusanthus speciosus* (Fabaceae), *Ziziphus mauritiana* (Rhamnaceae)), shrub (*Dracaena sanderiana* (Asparagaceae)) and perennial marsh grasses (*Pontederia cordata* (Pontederiaceae), *Thalia geniculata* and *T. dealbata* (Marantaceae)). Experimental plants were sprayed in two experience options: with metabolic products of live and autoclaved culture of *X. bovienii* with titers $1 \times 10^8$ and $1 \times 10^7$ in 3-fold replication in each option. The consumption of bacterial cultural liquid for spraying of experimental plants in greenhouses was about 5 l at a titer of $1 \times 10^8$ and 10–15 l at a titer $1 \times 10^7$.

The numbers of spider mites were taken into account visually on the leaves of each experimental plant in ind./leaf in 10-fold replication before spraying and at 4, 6 and 8 dpa of spraying with bacterial preparations. The mortality of spider mites and the effectiveness of bacterial preparations against them were determined taking into account changes in their numbers in the control as well as in the laboratory conditions.

Statistical processing was performed in Microsoft Exel and Sigma Plot 12.0 programs. Biological efficiency was calculated using the Abbott's formula (Abbot, 1925), adjusted for control (Fleming, Retnakan, 1985).

**Table 1.** Effect of secondary metabolic products of *Xenorhabdus bovienii* on the mortality of *Tetranychus urticae* in laboratory conditions (25 females in 4-fold replication)

| Symbiotic bacterium (entomopathogenic nematode) | The titer of bacterial cells, n × ml$^{-1}$ | Bacterial culture | Mortality rate of mites after application of bacterial culture, % | | | |
|---|---|---|---|---|---|---|
| | | | 1 | 4 | 6 | 8 |
| *Xenorhabdus bovienii* (*Steinernema feltiae*) | $1 \times 10^7$ | Live (*in vivo*) | **48.0 ± 2.8** | **82.0 ± 2.0** | **94.5 ± 1.1** | **95.4 ± 0.7** |
| | $1 \times 10^5$ | | 2.0 ± 2.0 | 33.0 ± 1.9 | 38.6 ± 2.8 | 61.4 ± 3.6 |
| | $1 \times 10^7$ | Autoclaved at 121°C for 10 min | **57.0 ± 1.9** | **89.6 ± 1.2** | **90.0 ± 1.3** | **95.1 ± 0.2** |
| | $1 \times 10^5$ | | 26.0 ± 2.6 | 45.0 ± 1.9 | 60.7 ± 5.2 | 73.2 ± 2.9 |
| *Xenorhabdus nematophila* (*Steinernema carpocapsae*) | $1 \times 10^7$ | *In vivo* | **59.0 ± 1.9** | **80.0 ± 2.8** | **87.6 ± 1.8** | **85.6 ± 2.3** |
| | $1 \times 10^5$ | | 13.0 ± 2.5 | 40.0 ± 1.6 | 58.6 ± 1.2 | 62.4 ± 2.3 |
| | $1 \times 10^7$ | Autoclaved | **25.8 ± 2.3** | **81.0 ± 4.1** | **87.6 ± 2.6** | **91.3 ± 2.7** |
| | $1 \times 10^5$ | | 1.0 ± 1.0 | 36.0 ± 2.8 | 55.6 ± 2.0 | 72.6 ± 3.6 |
| $LSD_{0.05}$ (titer) | | | 7.8 | 10.2 | 11.3 | 12.8 |
| $LSD_{0.05}$ (species of *Xenorhabdus*, titer $1 \times 10^7$) | | | 6.5 | 4.2 | 1.6 | 2.8 |
| *T. urticae*, control, ind./leaf (dish) | | | 25 | 25 | 36.2 ± 0.4* | 133.8 ± 4.9* |

Notes. Air temperature 22–24°C, relative humidity 65–70%, photoperiod L18: D6.
1, 4, 6, 8 – day post application. *females and larvae.

**Table 2**. Effect of metabolic products of *Xenorhabdus bovienii* (titer 1 x $10^7$) of entomopathogenic nematode (*Steinernema feltiae*) on the mortality of females *Tetranychus urticae* in greenhouse conditions (leaves in 10-fold replication on plants in 3-fold replication)

| Dates, plants, bacterial culture, titer | | Number of adult mites before treatment, individuals/ leaf | Mortality rate of mites after application of bacterial culture, % | | |
|---|---|---|---|---|---|
| | | | 4 | 6 | 8 |
| 3–10.05.2020 | | | | | |
| *Bolusanthus speciosus*, *in vivo* | $1 \times 10^8$ | 20.0 ± 2.2 | 56.6 ± 2.5 | 84.4 ± 3.2 | 98.8 ± 0.8 |
| | $1 \times 10^7$ | 20.1 ± 2.1 | 41.7 ± 3.4 | 59.7 ± 2.2 | 88.6 ± 0.5 |
| *T. urticae*, control, ind./leaf | | 19.2 ± 1.4 | 20.0 ± 1.8 | 18.5 ± 1.6 | 19.4 ± 1.2 |
| *Ziziphus mauritiana*, autoclaved culture | $1 \times 10^8$ | 19.4 ± 1.5 | 39.1 ± 3.2 | 55.6 ± 5.0 | 90.8 ± 2.9 |
| | $1 \times 10^7$ | 21.3 ± 3.7 | 24.1 ± 3.5 | 46.5 ± 2.7 | 87.5 ± 2.8 |
| *T. urticae*, control, ind./leaf | | 18.7 ± 1.3 | 19.0 ± 1.6 | 21.5 ± 1.5 | 23.4 ± 1.2 |
| $LSD_{0.05}$ (*in vivo* and autoclaved culture) | | | 8.5 | 4.6 | 3.8 |
| $LSD_{0.05}$ (titer) | | | 6.8 | 7.5 | 4.2 |
| 30.03–8.04.2021 (females), $1 \times 10^7$, *in vivo* | | | | | |
| *Bolusanthus speciosus* (Fabaceae) | | 19.5 ± 1.6 | 55.1 ± 3.5 | 86.2 ± 4.3 | 99.0 ± 2.2 |
| *T. urticae*, control, ind./leaf | | 18.5 ± 1.2 | 23.2 ± 2.1 | 25.4 ± 2.3 | 28.6 ± 3.2 |
| *Ziziphus mauritiana* (Rhamnaceae) | | 22.3 ± 2.0 | 54.3 ± 3.0 | 85.6 ± 2.2 | 98.9 ± 0.6 |
| *T. urticae*, control, ind./leaf | | 19.0 ± 2.0 | 20.0 ± 1.8 | 21.5 ± 1.9 | 24.6 ± 1.8 |
| *Dracaena sanderiana* (Asparagaceae) | Ground floor | 20.1 ± 2.0 | 42.8 ± 3.1 | 59.7 ± 2.8 | 84.1 ± 1.5 |
| | First floor | 23.3 ± 2.1 | 51.9 ± 4.2 | 65.2 ± 3.1 | 88.0 ± 0.8 |
| | Second floor | 26.4 ± 2.3 | 54.5 ± 4.2 | 69.7 ± 3.8 | 90.2 ± 1.3 |
| *T. urticae*, control, ind./leaf | | 23.2 | 20.1 ± 1.4 | 16.6 ± 1.2 | 17.3 ± 1.5 |
| $LSD_{0.05}$ (*Dracaena sanderiana*) (floors) | | 2.5 | 6.3 | 4.5 | 2.8 |
| 30.03–8.04.2021 (females), $1 \times 10^7$, autoclaved culture | | | | | |
| *Pontederia cordata* (Potenderiaceae) | | 21.4 ± 2.1 | 43.6 ± 3.3 | 47.0 ± 2.4 | 87.8 ± 2.8 |
| *T. urticae*, control, ind./leaf | | 15.3 ± 1.5 | 16.8 ± 1.5 | 18.5 ± 1.2 | 18.9 ± 1.0 |
| *Thalia geniculata* (Marantaceae) | | 15.1 ± 0.7 | 42.7 ± 4.2 | 47.0 ± 4.4 | 81.4 ± 3.5 |
| *T. urticae*, control, ind./leaf | | 15.0 ± 0.9 | 16.5 ± 1.2 | 17.6 ± 0.8 | 18.9 ± 1.3 |
| *Thalia dealbata* | | 12.4 ± 0.8 | 40.3 ± 2.6 | 56.4 ± 3.5 | 90.1 ± 4.2 |
| *T. urticae*, control, ind./leaf | | 15.2 ± 1.1 | 16.3 ± 1.3 | 17.8 ± 1.0 | 19.7 ± 1.4 |
| $LSD_{0.05}$ (plants sprayed with bacterial culture) | | | 1.5 | 4.2 | 3.9 |

Notes. Peter the Great Botanical Garden, St. Petersburg, air temperature 19–21°C, relative humidity 85–90%. 4, 6, 8 – day post application.

**Statistical analyses.** Analysis of variance (ANOVA) was used to assess effect of trial to determine whether there were significant differences between the experiment repeats ($P \leq 0.05$). Data are presented as means ± standard error and least significant difference ($LSD_{0.05}$). Any difference between means larger than the LSD is considered a significant result.

## RESULTS

The effect of the live and autoclaved metabolic products of symbiotic bacteria *Xenorhabdus bovienii* and *X. nematophilus* entomopathogenic nematodes, respectively, *Steinernema feltiae*, and *S. carpocapsae* on the death (%) of the common spider mite (*Tetranychus urticae* Koch) depending on the titer of bacterial cells ($1 \times 10^8$, $1 \times 10^7$ and $1 \times 10^5$) and the exposure time (days). 1, 4, 6 and 8 dpa in laboratory and greenhouses have been investigated (Tables 1–3). In general, the mortality rate of mites with an increase in the titer of bacterial cells in the corresponding culture fluids, as well as with an increase in the exposure time (days). At the same time, the mortality rate of females and mite larvae significantly increased in two bacterial species (*X. bovienii* and *X. nematophilus*) with an increase in the exposure time (days).

In laboratory conditions, the highest mortality rates were observed in *X. bovienii* bacteria at 6 and 8 dpa with a titer of $1 \times 10^7$ culture fluid (about 95%). The mortality mites at 8 dpa in options with live and autoclaved culture fluid with a titer of $1 \times 10^7$ in *X. bovienii* was almost the same, but in *X. nematophilus* it was 5.7% higher in autoclaved culture fluid. At 8 dpa, the efficacy of the live and autoclaved metabolic products of the bacteria *Xenorhabdites bovienii* and *X. nematophilus* against the spider mite with a titer of $1 \times 10^5$ was lower compared to the culture with a titer of $1 \times 10^7$, respectively, by 1.3–1.6 and 1.3–1.4 times. Means of mortality rate of mites after application of bacterial culture (%) of *Xenorhabdus bovienii* and *X. nematophila* at titer $1 \times 10^7$ larger than the least significant difference is considered a significant result (Table 1). The relationship between the mortality of spider mites (%) and the exposure time (days) to bacterial metabolism products most reliably reflects mainly by the polynomial dependence with the accuracy of approximation 0.95–1.0 (Figs 1, 2). The maximum mortality of spider mites occurs the faster the higher the concentration of metabolic products of live cultures *X. bovienii* and *X. nematophila* in the preparation, in at a titer of $1 \times 10^7$ respectively on 8 and 6 dpa and autoclaved culture on 8 dpa in both species of bacteria, and at a titer of $1 \times 10^5$ more than 8 dpa later.

In the greenhouse in the first decade of May 2020, the effectiveness of the bacterial metabolic products of *X. bovienii* against spider mite was highest in experiments with live culture with a titer of $1 \times 10^8$ (Table 2). In experiments with an autoclaved culture with a titer of $1 \times 10^8$ at 8 dpa, it decreased by 8%, and with a titer of $1 \times 10^7$ by 1.1% compared with a live culture of *X. bovienii*. The average mortality rates of spider mites (%) after using live and autoclaved bacterial cultures of *X. bovienii*, with their titers of $1 \times 10^7$ and $1 \times 10^8$ exceed the least significant difference (LSD), which are considered a significant

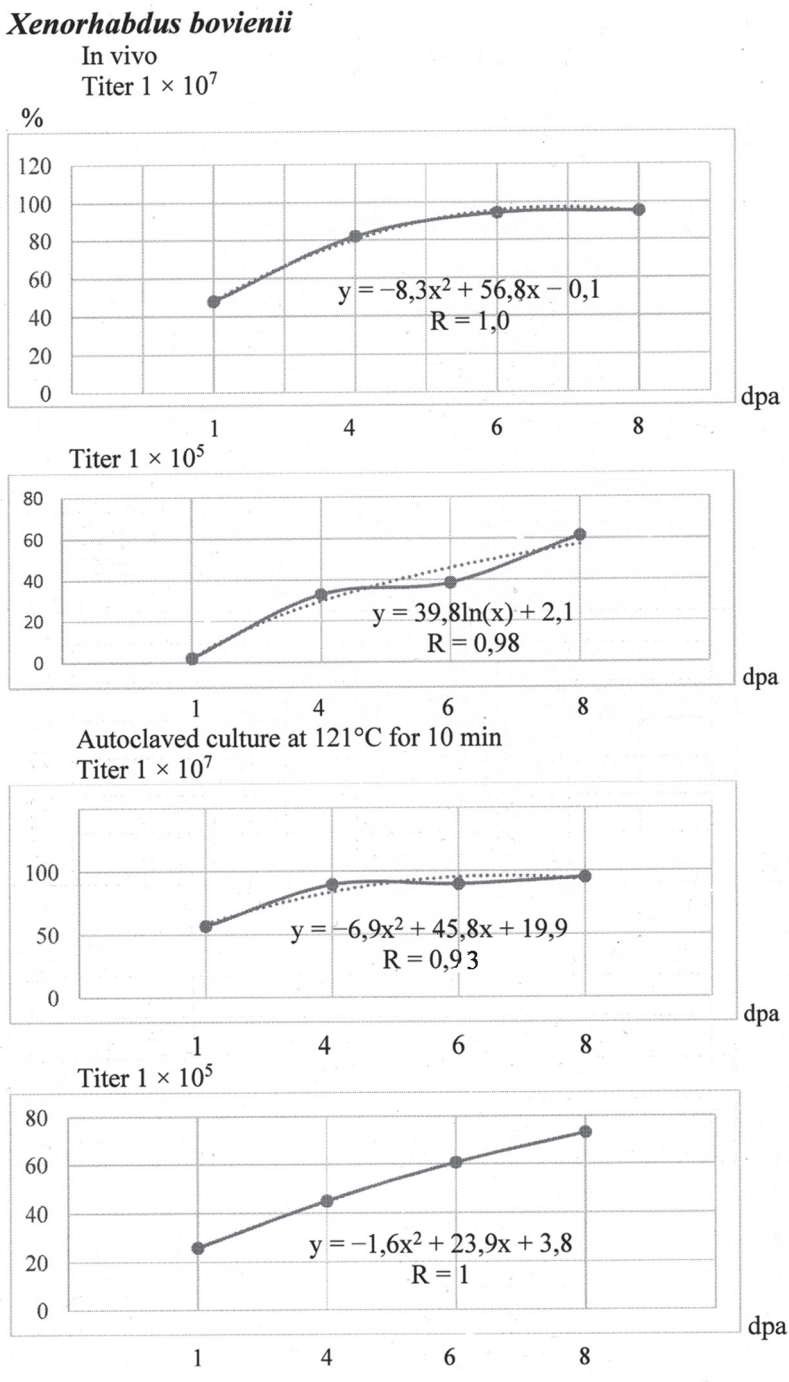


**Figure 1.** Efficiency of secondary metabolic products of *Xenorhabdus bovienii* on the mortality of *Tetranychus urticae* in laboratory conditions, %: dpa – day post application, R – the accuracy coefficient of the approximation.

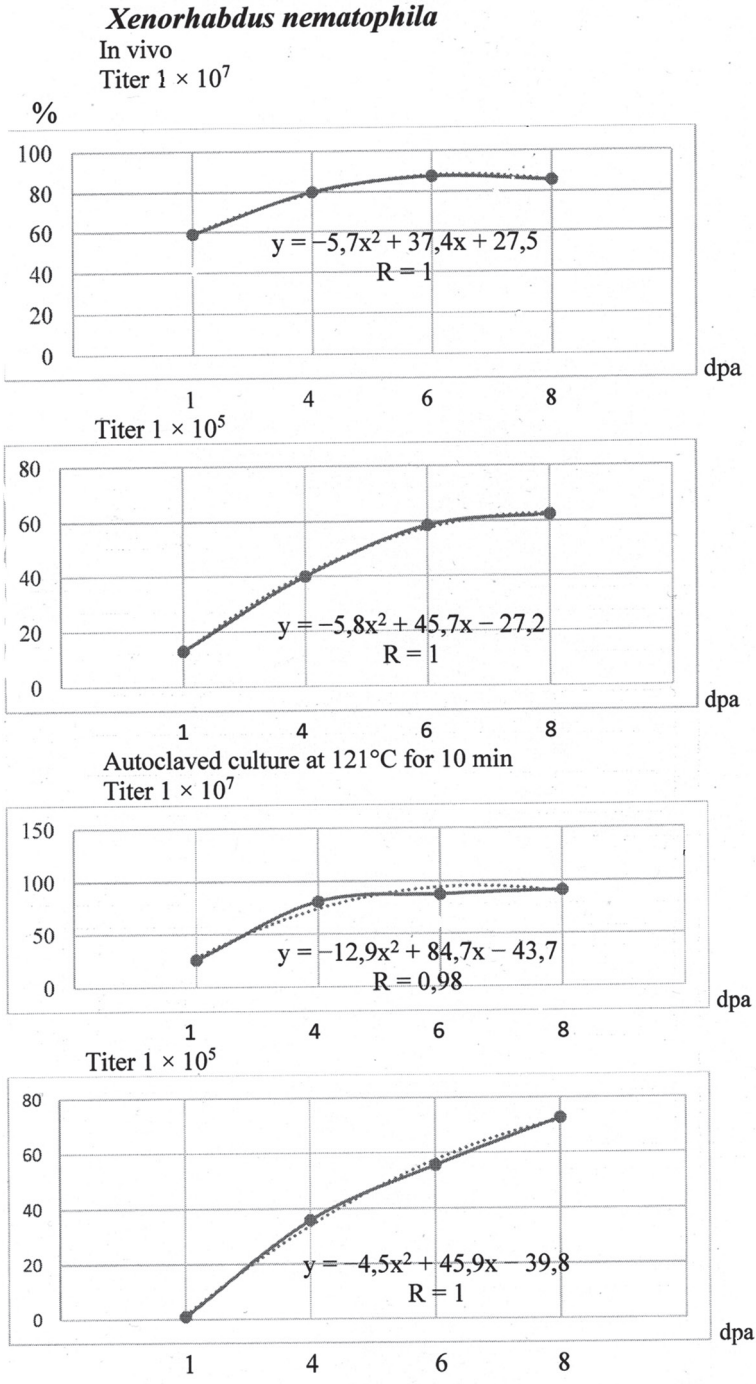


**Figure 2.** Efficiency of secondary metabolic products of *Xenorhabdus nematophila* on the mortality of *Tetranychus urticae* in laboratory conditions, %: dpa – day post application, R – the accuracy coefficient of the approximation.

result (Table 2). Similar experimental results were also obtained against overwintered females by the influence of the plant species on mite mortality at the end of March early April 2021. The floor arrangement of plants in the greenhouse also influenced the effectiveness of bacterial metabolism products against spider mites. In experiments with live culture fluid of *X. bovienii* with a titer of 1 x $10^7$, the mortality rate of spider mites on leaves of shrub *Dracaena sanderiana* at 8 dpa was about 84% on the ground floor, 88% on the first and 90% on the second floor (Table 2). In the second decade of May 2021 the overall efficacy of the bacterial metabolic products of *X. bovienii* (*in vivo*, titer 1 × $10^7$) against adults, larvae and nymphs of *T. urticae* on the leaves of perennial marsh grasses (*Potenderia cordata*, *Thalia geniculata* and *T. dealbata*) was about 98–99% (Table 3).

**Table 3.** Effect of metabolic products of *Xenorhabdus bovienii* (*in vivo*, titer 1 x $10^7$) of entomopathogenic nematode (*Steinernema feltiae*) on the mortality of *Tetranychus urticae* in greenhouse conditions (leaves in 10-fold replication on plants in 3-fold replication)

| Plant | Composition of spider mite populations, % | | Number of mites before treatment, ind./leaf | Mortality rate of mites after application of bacterial culture, % | | |
|---|---|---|---|---|---|---|
| | Adults | Larvae and nymphs | | 4 | 6 | 8 |
| *Pontederia cordata* | 50 | 50 | 6.7 ± 0.8 | 62.4 ± 4.9 | 87.8 ± 3.4 | 99.1 ± 0.9 |
| *Thalia dealbata* | 100 | 0 | 9.2 ± 0.9 | 58.3 ± 5.4 | 80.2 ± 3.5 | 98.3 ± 1.1 |
| *Thalia geniculata* | 60 | 40 | 9.2 ± 1.2 | 53.2 ± 5.0 | 73.8 ± 6.0 | 98.0 ± 1.4 |
| Average | 70 | 30 | 8.4 ± 1.0 | 58.0 ± 5.1 | 80.6 ± 4.3 | 98.5 ± 1.1 |

Notes. Peter the Great Botanical Garden, St. Petersburg, date of the experiment 12.05–20.05.2021, air temperature 28–32°C, relative humidity 85–90%. 4, 6, 8 – day post application.

## DISCUSSION

In the world practice, symbiotic bacterial-parasitic complexes of invasive larvae of the genus *Steinernema* and bacteria of the genus *Xenorhabdus* are widely used in the biological protection of agricultural crops mainly from soil insect pests and root-knot nematodes of the genus *Meloidogyne* in greenhouses and in the field (Lee, 2009; Lewis et al., 2001; Perez, Lewis, 2002; Lacey, Georgis, 2012 and others). Two biological preparations (Entonem and Nemabact) based on entomopathogenic nematodes and their symbiotic bacteria against insect pests were obtained and found practical application in Russia. They were included in the State Catalog of Pesticides and Agrochemicals Approved for Use on the territory of the Russian Federation (Kaplin, 2012).

Bacteria of the genus *Xenorhabdus*, when cultivated, form a large number of secondary mainly protein toxic metabolites against other microorganisms, soil nematodes, insects and spider mite *Tetranychus urticae*. Currently, live cultures are obtained in laboratories, as well as autoclaving culture at 121°C for 10 min and cell-free supernatants obtained by centrifuging cultures at 4°C for 15 min. They are widely tested in many countries in laboratories in Petri dishes and pots against pests and plant diseases. We have investigated the

effectiveness of exposure to secondary metabolites of live and autoclaved bacterial cultures of the genus *Xenorhabdus* in laboratory conditions and for the first time in greenhouses against *T. urticae* by spraying these cultures of experimental mite-infested plants.

## CONCLUSION

In our laboratory investigations, the highest mortality rates of spider mites were observed in  in experiments with *X. bovienii* at 8 dpa with a titer of $1 \times 10^7$ culture fluid (about 95%). The mortality mites at 8 dpa in experiments with live and autoclaved culture with a titer of $1 \times 10^7$ in *X. bovienii* was almost the same, but in *X. nematophila* it was slightly higher *in vitro*. After 8 dpa, the efficacy of the live and autoclaved metabolic products of *Xenorhabdites bovienii* and *X. nematophilus* against the spider mite with a titer of $1 \times 10^5$ was about 1.4 times lower compared to the culture with a titer of $1 \times 10^7$.

In the greenhouses, the effectiveness of the bacterial metabolic products of *X. bovienii* against spider mite was highest in experiments with live culture with a titer of $1 \times 10^8$. The efficiency of an autoclaved culture with a titer of $10^8$ was 8%, and with a titer of $10^7$ about 1% lower than in experiments with live culture. This was probably due to the negative effect of culture autoclaving at 121°C on the stability of some peptide secondary metabolites of the bacterium *Xenorhabdus bovienii*. In experiments with live culture of *X. bovienii* with a titer of 1 x $10^7$ (*in vivo*) the mortality rate of spider mites on leaves of shrub *Dracaena sanderiana* at 8 dpa increased from 84% on the ground floor to 88% on the first floor and to 90% on the second floor. The overall efficacy of the bacterial metabolic products of *X. bovienii* (*in vivo*, titer $1 \times 10^7$) against adults, larvae and nymphs of *T. urticae* on the leaves of perennial marsh grasses (*Potenderia cordata*, *Thalia geniculata* and *T. dealbata*) was about 98–99%.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.


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# АКАРИЦИДНОЕ ВЛИЯНИЕ ВТОРИЧНЫХ МЕТАБОЛИТОВ СИМБИОТИЧЕСКИХ БАКТЕРИЙ *XENORHABDUS BOVIENII* И *X. NEMATOPHILA* ЭНТОМОПАТОГЕННЫХ НЕМАТОД НА ПАУТИННОГО КЛЕЩА *TETRANYCHUS URTICAE* (TROMBIDIFORMES, TETRANYCHIDAE)

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**Ключевые слова:** *Steinernema*, живая бактериальная культура, автоклавированная культура, лабораторные условия, теплицы, токсичные вторичные метаболиты, эффективность

РЕЗЮМЕ

В лабораторных условиях самые высокие показатели смертности *Tetranychus urticae* после применения продуктов метаболизма симбиотических бактерий с титром $1 \times 10^7$ наблюдались у *Xenorhabdus bovienii* на 6–8-й день в опыте с живой и на 8-й день с автоклавированной культуральной жидкостью (около 95%). В экспериментах с живой и автоклавированной культурой с титром $1 \times 10^7$ смертность клещей на 8-й день после применения у *X. bovienii* была почти одинаковой, но у *X. nematophila* она была немного выше в опыте с автоклавированной культурой. Через 8 дней после применения продукты метаболизма живых и автоклавированных бактерий *X. bovienii* и *X. nematophila* против паутинного клеща с титром $1 \times 10^5$ были примерно в 1.4 раза менее эффективны, чем аналогичные продукты с титром $1 \times 10^7$. Взаимосвязь между смертностью паутинных клещей (%) и временем воздействия (дни) продуктов бактериального метаболизма наиболее достоверно отражает полиномиальная зависимость с точностью приближения 0.93–1.0. В теплицах эффективность продуктов бактериального метаболизма *X. bovienii* против паутинного клеща была самой высокой в экспериментах с живой культурой с титром $1 \times 10^8$. В экспериментах с живой культурой *X. bovienii* с титром $1 \times 10^7$ (*in vivo*) уровень смертности паутинных клещей на листьях кустарника *Dracaena sanderiana* на 8-й день после применения увеличился с 84% на первом этаже до 90% на третьем этаже. Общая эффективность продуктов бактериального метаболизма *X. bovienii* (*in vivo*, титр $1 \times 10^7$) против взрослых особей, личинок и нимф *T. urticae* на листьях многолетних болотных трав (*Potenderia cordata*, *Thalia geniculata* и *T. dealbata*) составила около 98%, *X. nematophila* – 99%.