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NEMATICIDAL ACTIVITY OF NEMATODE-SYMBIOTIC BACTERIA *XENORHABDUS BOVIENII* AND *X. NEMATOPHILA* AGAINST ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

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The lethal effects of metabolic products produced by the symbiotic bacteria *Xenorhabdus bovienii* from *Steinernema feltiae* and *X. nematophila* from *S. carpocapsae* were tested on *M. incognita* infective juveniles (J2). Treatments had cell titers of 2.5×10^9, 1.25×10^9 and 0.63×10^9 per ml at 20 °C, 23 °C and 26 °C. Exposure periods were 15 hr, 41 hr, 65 hr and 90 hr immediately after autoclaving and at 23°C, and exposure periods of 5 hr, 26 hr, 50 hr and 74 hr after storage for 21 days at 4 °C. The effectiveness of bacterial metabolic products immediately after preparation against *M. incognita* (J2) depended on the titer of bacterial cells, the temperature of the culture liquid, and the duration of its exposure to nematodes. Nematicidal activity of *X. bovienii* metabolic products was higher than that of *X. nematophila*. Mortality of *M. incognita* J2 was 92–93 % after 90-hr exposure to *X. bovienii* at 20 °C and cell titers of 2.5×10^9 and 1.25×10^9; also after 65 hr exposure at 23 °C, titer of 2.5×10^9 and 95–99 % at 26 °C and all tested titers. The efficacy of cultural liquid of *X. bovienii* metabolic products after storage at 4 °C for 21 days, after its 50 hr exposure to nematodes at 23 °C and cell titers of 2.5×10^9 and 1.25×10^9 and 74 hr exposure at all tested titers remained high at 97–100 %. The easiest way to control of plant pathogenic nematodes would be metabolic products of symbiotic bacteria of *Xenorhabdus*. Our results suggest that the active metabolites of symbiotic bacteria need to be identified for possible synthesis and use in the field.

Key words: entomopathogenic nematodes, symbiotic bacteria, metabolites, efficiency

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Among plant-parasite nematodes (PPNs) developing on plant roots, the most economically important are *Meloidogyne* spp. (root-knot nematodes) and cyst nematodes in the family Heteroderidae (Tylenchida). More than 100 species of root-knot nematodes have been described, for which more than 5000 species of host plants are known (Karssen, Moens,

2006; Uribe, 2008). Four *Meloidogyne* spp. (*M. incognita* Kofoid & White, *M. javanica* (Treub)*, M. arenaria* Chitwood and *M. hapla* Chitwood) are widespread. These four species account for 95 % of infestations on cultivated land and about 5 % of global crop loss. *M. incognita* accounts for 52 % of reports, *M. javanica* 31 %, *M. arenaria* 8 %, *M. hapla* 7 %, and other species 2 %, from agricultural land areas (Hadisoeganda, Sasser, 1982).

Five species of root-knot nematodes (*Meloidogyne incognita, M. javanica, M. arenaria, M. hapla* and *M. chitwoodi*) are common in the Russian Federation, but *M. incognita, M. javanica* and *M. arenaria* are found only in greenhouses. *Meloidogyne hapla* develops in the open ground and in greenhouses. Columbian root-knot nematode (*M. chitwoodi* Golden et al.) is the object of an external quarantine. About 60 % of greenhouses in Russia are contaminated with root-knot nematodes. *Meloidigyne incognita* almost everywhere damages vegetable and ornamental crops in greenhouses and its study is undoubtedly relevant.

Meloidogyne incognita is a cosmopolitan obligate plant-parasite and possesses a wide host range of over 230 plant genera and 3000 species including cotton, tobacco, legumes, vegetable crops, spices, and coffee in tropical and subtropical regions, in particular in the warmer areas (CABI Data Report, CABI, 2017). Estimates of crop losses due to *Meloidogyne* species, mainly *M. incognita* and *M. javanica*, have ranged from 18 % to 33 % for melon and from 24 % to 38 % for tomato (Sasser, 1979), and 25 % or more for potato (Mai et al., 1981). This species is also the main pest of vegetable and ornamental crops in greenhouses in temperate latitudes. In previous studies the minimum temperature threshold for development of *M. incognita* was found to be 10.1 °C. Infective J2 of *M. incognita* become active at soil temperatures of 16–20 °C (Vrain et al., 1978).

Organizational and economic, preventive, selection and seed-growing, physical, agrotechnical, biological, chemical and integrated methods are used in greenhouse management of root-knot nematodes. Among these approaches, the most economically justified and environmentally safe are biological methods.

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* Travassos (Steinernematidae) and *Heterorhabditis* Poinar (Heterorhabditidae) in the order Rhabditida include about 70 and 20 species, respectively. They are able to infect the post-embryonic stages of more than 1000 species of pests of agricultural and ornamental crops (Nickle, 1977). Two genera of entomopathogenic bacteria, *Xenorhabdus* Thomas and Poinar and *Photorhabdus* Boemare et al. (Morganellaceae, Enterobacterales), are intimately associated with EPNs. About 20 species of symbiotic gram-negative *Xenorhabdus* are associated with *Steinernema* spp. and 2 species of *Photorhabdus* are associated with *Heterorhabditis* spp. (Poinar, Thomas, 1967; Akhurst, Boemare, 1990; Nguyen et al., 2007). In the process of pathogenesis, bacteria and EPNs secrete proteolytic enzymes that can break down proteins as well as damage components of the host immune complex, causing host death.

In addition to insects, EPNs are also able to suppress the development of phytonematodes (Bird, Bird, 1986; Ishibashi et al., 1986; Lewis, Grewal, 2005; Molina et al., 2007; Kenney, Eleftherianos, 2016). Suppressive effects of EPNs have been observed on various

phytonematodes such as *Belonolaimus longicaudatus* Rau, *Criconemoides* spp. (Grewal et al., 1997) and *Globodera rostochiensis* (Wollenweber) Behrens (Perry et al., 1998). However, the most stable suppression was observed in species of *Meloidogyne* Göldi (Lewis et al., 2005). *Xenorhabdus* and *Photorhabdus* can be successfully used to regulate the density of root-knot nematodes. This effect was studied on culture filtrates of *Xenorhabdus nematophila* (Poinar et al.) and *X. bovienii* Akhurst and Boemare from *Steinernema carpocapsae* (Weiser) and *S. feltiae* Filipjev, respectively (Grewal et al., 1997). The culture fluids of these bacteria showed nematicidal properties, causing the death of 98–100 % of *M. incognita* infective juveniles (J2). The selective nematicidal ability of *Xenorhabdus* against root-knot nematodes has been confirmed by many researchers (Paul et al., 1981; Burman, 1982; Richardson et al., 1988; Pérez, Lewis, 2002). When tomato plants were immersed in culture liquid of *X. bovienii, M. incognita* egg production was suppressed and plants were taller compared to infected but untreated plants in the control (Pérez, Lewis, 2002; Kepenecki et al., 2016). Bowen and Ensign (1998) suggested that it would be appropriate to use toxins derived from liquid suspensions of the bacterial symbiont for plant protection. Antibiotic compounds produced by different species and strains of *Xenorhabdus* can differ significantly in quality indicators, which also depend on the nutrient medium composition, temperature and conditions of their cultivation. In the process of studying the nematicidal activity of metabolites produced by symbiotic *Xenorhabdus*, the prospects of work in this direction were enhanced (Hu et al., 1999; Nour El-Din et al., 2014).

The aim of our research was to study the nematicidal activity of the symbiotic bacteria *Xenorhabdus bovienii* (*S. carpocapsae*) and *X. nematophila* (*S. feltiae*), respectively, *in vitro* at different exposure times, temperatures and densities of bacterial cells in the nutrient medium and storage of cultural liquid, against *M. incognita* J2.

MATERIALS AND METHODS

The research was carried out in the Laboratory of Microbiology of the All-Russian Institute of Plant Protection. *Xenorhabdus nematophila* and *X. bovienii* were obtained from the cadavers of greater wax moth larvae (*Galleria mellonella* L.) infected with dauer juveniles of *Steinernema carpocapsae* strain "Agriotos" and *S. feltiae* strain RP18-91, respectively, and stored in distilled water at 5–7 °C. Ten mature larvae were placed in a Petri dish on two layers of filter paper, on the surface of which about 500 dauers were introduced in 1 ml of distilled water. After three days of incubation, the cadavers were superficially sterilized in 70 % ethanol for 2 min and placed for drying in a laminar air stream for 3 min. A drop of hemolymph was extracted from the larval proleg and transferred to Petri dishes on NBTA nutrient medium containing per liter: 0.5 g $NH_4H_2PO_4$; 0.5 g K_2HPO_4; 0.2 g $MgSO_4 \cdot 7\ H_2O$; 5 g NaCl; 5 g yeast extract; 12 g agar; 25 mg bromothymol blue and 40 mg dimethyltetrazolium chloride. Cultures were incubated at 26 °C. After 72 hr, one pure colony of green symbiotic bacteria was selected from colonies of the same size and morphology. The identification of the primary forms of symbiotic bacteria was performed by the method of Akhurst (1980). Subcultivation continued until bacterial colonies of the same size and morphology were obtained. The pathogenicity of isolates was confirmed by injection of bacterial cells into *G. mellonella* larvae and transfer

of hemolymph of infected larvae to NBTA in Petri dishes. Clean colonies of bacteria were transferred to tubes on slants with NBTA nutrient medium and grown for 3–4 days at 26 °C. A smear of bacteria was taken from the slant using a bacterial loop and transferred into flasks with 100 ml of nutrient broth and grown on a shaker at 150 revolutions per minute for 2 days at 26 °C before a titer of ~ 2.5×10^9 bacterial cells per ml. Beginning with the initial titer of 2.5×10^9 cells, lower concentrations of cells were obtained by doubling the culture liquid with sterile water to obtain titers of 1.25×10^9 and 0.63×10^9 cells per ml. The resulting culture liquids with titers of ~ 2.5×10^9, 1.25×10^9 and 0.63×10^9 cells per ml were autoclaved at a temperature of 121 °C, pressure of 1 atmosphere for 30 min and used to study the pathogenicity of the metabolic products of symbiotic bacteria against *M. incognita* J2. As known, *Xenorhabdus nematophila* and other symbiotic bacteria produce both heat-labile and heat-stabile toxins, enzymes and antimicrobials. Their heat-stabile components are active after heart sterilization and can be used against different species of bacteria and pests (Inman, Holmes, 2012).

Evaluation of the effectiveness of these inocula against J2 was tested immediately after preparation at temperatures of 20 °C, 23 °C and 26 °C and at 23 °C following their storage at 4 °C for 21 days (tables 1–3). Evaluation of the effectiveness of freshly obtained inocula was determined after 15, 41, 65 and 90 hr, and of stored inocula after 5, 26, 50 and 74 hr.

Table 1. Effect of metabolic products of *Xenorhabdus bovienii* on mortality of *Meloidogyne incognita* J2, in vitro

The titer of bacterial cells ($n \times ml^{-1}$)	The death of larvae at the exposure, hours (%)			
	15	41	65	90
		20 °C		
2.5×10^9	15.0 ± 0.7	28.3 ± 1.4	62.0 ± 1.2	**92.3 ± 1.1**
1.25×10^9	8.5 ± 0.6	19.8 ± 3.1	46.8 ± 2.5	**93.0 ± 2.5**
0.63×10^9	1.8 ± 0.6	5.0 ± 0.7	21.0 ± 3.1	65.0 ± 3.7
$LSD_{0.05}$ (titer)	2.6	7.8	9.3	10.4
$LSD_{0.05}$ (titer + control)	1.1	3.4	4.1	4.6
Control (water)	0 ± 0	0.75 ± 0.25	1.75 ± 1.25	3.00 ± 0.41
		23 °C		
2.5×10^9	22.2 ± 1.2	63.5 ± 1.7	**91.8 ± 2.8**	No data
1.25×10^9	18.2 ± 0.6	47.0 ± 1.4	82.0 ± 1.5	
0.63×10^9	6.2 ± 0.9	24.5 ± 2.4	56.5 ± 1.3	
$LSD_{0.05}$ (titer)	3.7	7.3	7.7	
$LSD_{0.05}$ (titer + control)	1.7	3.3	3.4	
Control (water)	1.25 ± 0.25	2.00 ± 0.41	2.25 ± 0.48	
		26 °C		
2.5×10^9	2.5 ± 1.0	47.0 ± 1.5	**99.5 ± 0.5**	No data
1.25×10^9	0.8 ± 0.5	37.0 ± 1.1	**98.8 ± 1.3**	
0.63×10^9	1.0 ± 0.6	15.0 ± 0.8	**97.5 ± 2.5**	
$LSD_{0.05}$ (titer)	2.9	4.5	6.4	
$LSD_{0.05}$ (titer + control)	2.8	4.3	2.9	
Control (water)	0.80 ± 0.25	1.25 ± 0.25	2.25 ± 0.25	

Table 2. Effect of metabolic products of *Xenorhabdus nematophila* on the mortality of *Meloidogyne incognita* J2, in vitro

Bacterial cell titer per ml	The death of larvae at the exposure, hours (%)			
	15	41	65	90
		20 °C		
2.5×10^9	11.8 ± 0.6	22.3 ± 1.7	47.8 ± 1.2	88.0 ± 2.8
1.25×10^9	11.3 ± 1.4	20.5 ± 0.9	24.8 ± 0.6	**94.0 ± 1.5**
0.63×10^9	1.3 ± 0.5	4.0 ± 0.4	8.3 ± 1.1	49.8 ± 3.8
$LSD_{0.05}$ (titer)	3.7	4.3	3.9	11.2
$LSD_{0.05}$ (titer + control)	1.6	1.9	1.8	4.6
Control (water)	0 ± 0	0.75 ± 0.25	1.75 ± 1.25	3.00 ± 0.41
		23 °C		
2.5×10^9	20.2 ± 1.1	57.8 ± 1.7	81.5 ± 1.3	
1.25×10^9	22.2 ± 0.6	28.8 ± 0.9	38.3 ± 1.3	
0.63×10^9	4.0 ± 0.7	11.8 ± 1.3	24.5 ± 2.5	No data
$LSD_{0.05}$ (titer)	3.3	5.1	7.0	
$LSD_{0.05}$ (titer + control)	1.5	2.3	3.1	
Control (water)	1.25 ± 0.25	2.00 ± 0.41	2.25 ± 0.48	
		26 °C		
2.5×10^9	1.5 ± 0.6	35.5 ± 1.6	**99.0 ± 0.6**	
1.25×10^9	1.8 ± 0.6	20.5 ± 1.0	**95.3 ± 2.8**	
0.63×10^9	0.8 ± 0.5	12.0 ± 1.3	82.0 ± 1.5	No data
$LSD_{0.05}$ (titer)	2.3	5.1	7.2	
$LSD_{0.05}$ (titer + control)	2.3	5.0	3.2	
Control (water)	0.80 ± 0.25	1.25 ±0.25	2.25 ± 0.25	

Table 3. Effect of temperature and storage duration (21 days at 4 °C) of metabolic products of symbiotic bacteria *Xenorhabdus bovienii* of entomopathogenic nematodes *Steinernema feltiae* SRP18-91 on death of invasive larvae of root-knot nematode *Meloidogyne incognita* at 23 °C, in vitro

The titer of bacterial cells ($n \times ml^{-1}$)	The death of larvae at the exposure, hours (%)			
	5	26	50	74
		No storage		
2.5×10^9	1.2 ± 0.6	55.0 ± 5.9	100.0 ± 0.0	100.0 ± 0.0
1.25×10^9	4.2 ± 1.3	54.8 ± 2.7	97.0 ± 2.7	100.0 ± 0.0
0.63×10^9	0	16.8 ± 1.7	76.3 ± 1.7	99.8 ± 0.3
$LSD_{0.05}$ (titer)	3.2	16.7	7.2	0.6
$LSD_{0.05}$ (titer + control)	1.4	7.4	3.2	1.2
		With storage (21 days at 4 °C)		
2.5×10^9	0.7 ± 0.8	36.0 ± 2.5	**94.5 ± 2.4**	**99.0 ± 0.6**
1.25×10^9	0	16.5 ± 2.1	82.5 ± 1.8	**97.3 ± 2.8**
0.63×10^9	0	13.3 ± 2.0	70.0 ± 3.9	**99.8 ± 0.3**
$LSD_{0.05}$ (titer)	1.7	8.7	11.1	6.4
$LSD_{0.05}$ (titer + control)	0.8	3.9	4.9	3.1
Control (water)	0	0.75 ± 0.25	2.75 ± 0.25	7.5 ± 1.2

Root-knot J2 were obtained from a pure culture of *M. incognita* propagated on tomato plants. Eggs were obtained by collecting galls from the affected tomato plants, washing them in water and grinding them in 0.5 % sodium hypochlorite solution. Eggs were placed in Baermann funnels for hatching and collection of J2 (Baermann, 1917).

Effects of bacterial metabolic products (three titers) on J2 were studied in Petri dishes. In the experiments, 2 ml of culture liquid was introduced into each Petri dish, while control dishes received 2 ml of tap water. Each dish received J2. All experiments were replicated four times. Density of live and dead J2 were determined. Alive larvae actively move in a liquid environment, the dead lie in the form of sticks and when they are touched with the tip of a preparaval needle, they remain lying without signs of activity, while live larvae always respond with activity when tactile action is applied to their body. Statistical treatment of the obtained data was carried out with Microsoft Excel and Sigma Plot 12.0 programs. Biological efficiency was calculated by Abbott's formula: $C = (A-B)*A^{-1}*100$ %, where A = J2 density before exposure, B = density of still alive larvae after exposure, and C = biological efficiency, if their natural mortality in the control did not exceed 5 %.

RESULTS

The metabolic products of symbiotic bacteria of the genus *Xenorhabdus* were obtained by culturing them using nutrient broth with NBTA for 2 days at 26 °C before a titer of ~ 2.5×10^9 bacterial cells per ml, followed by doubling the part of culture liquid with sterile water to titers of 1.25×10^9 and 0.63×10^9 cells per ml and autoclaving all received liquids.

Regression analysis of the efficiency of the metabolic products of *X. bovienii* and *X. nematophila* on the density of bacterial cells showed a linear or binomial dependence between them, with an R^2_1 of 0.48–0.97 and a correlation coefficient (r) of 0.70–0.98.

In laboratory conditions, the effectiveness of the bacterial metabolic products immediately after preparation against infective juveniles (J2) of root-knot nematode *Meloidogyne incognita* depended on the titer of bacterial cells, the temperature of the culture liquid, and the duration of its exposure to nematodes. In experiments with *X. bovienii* at titers of 0.63×10^9, 1.25×10^9, 2.5×10^9 bacterial cells per ml and on average, it was higher 1.4, 1.2, 1.05, and 1.2 times as in experiments with *X. nematophila*, respectively (tables 1–3).

Increasing the temperature and titer of the tested culture liquid contributed to faster growth of the efficacy of bacterial metabolic products against J2 of root-knot nematode, especially in experiments with *X. bovienii*. At 20 °C, the maximum increase of efficiency of metabolic products was observed in *X. bovienii* at all tested titers after 65 hr, in *X. nematophila* with titers of 0.63×10^9 and 1.25×10^9 cells per ml after 90 hr, and 2.5×10^9 after 65 hr. At 23 °C for all titers *X. bovienii* and *X. nematophila* showed the greatest increase of efficiency after 41 hr, and at 26 °C after less than 40 hr.

Comparison of efficiency of products of metabolism of bacteria at 23 °C, used against larvae of root-knot nematode directly after their production and after storage at 4 °C for 21 days showed that they differed slightly. At an exposure of 26 hr, the efficiency of the metabolic products of bacteria stored for 21 days was of 1.5–3.3 times, 50 hr was of 1.1–1.2 times lower, compared with the efficiency of their use immediately after production. However, at the exposure of 74 hr, they did not differ and amounted to 97–100 % regardless of the titer.

The results of the research indicate the possibility of using symbiotic bacteria of EPNs in the protection of vegetable crops in greenhouses against root-knot nematodes. *Xenorhabdus bovienii* appears to be especially promising. At a temperature of 20 °C and an exposure of 90 hr, J2 mortality was more than 90 %. Increasing temperatures above 20 °C may result in 99 % mortality after an exposure of 65 hr. Mortality of J2 was significantly increased by metabolites of two tested species of symbiotic bacteria at temperatures of 23 °C and 26 °C. The highest efficacy was observed with *X. bovienii* metabolites at 26 °C for all tested titers fluid (97.5–99.5 %).

DISCUSSION AND CONCLUSIONS

The entomopathogenic nematodes (EPN), *Steinernema carpocapsae* and *S. feltiae* are found on all continents of the Earth, except Antarctica (CABI Data Report. CABI, 2020). They are symbiotically associated with the bacteria *Xenorhabdus nematophila* and *X. bovienii.* These symbiotic bacterial-parasitic complexes are used as biological control agents against a wide variety of insect pests in agriculture and horticulture. Temperature is an important factor affecting both EPNs and their symbiotic bacteria. Optimum temperatures for infection and reproduction of *Steinernema carpocapsae* and *S. feltiae* is ranging from 22 °C to 28 °C and from 20 °C to 25 °C, respectively. Optimal culture temperature for both nematode species is 25 °C (Hirao, Ehlers, 2009). *Steinernema carpocapsae* is more sensitive to suboptimal temperature than *S. feltiae*. Development of *S. carpocapsae* does not occur at temperatures lower than 10 °C. Hazir *et al.* (2001) determined that the lowest temperature for *S. feltiae* infection and reproduction was 8 °C while the highest was 25 °C. *Steinernema feltiae* seems to be a better fit for temperatures expected in northern climates (Sharmila et al., 2018). In the northern part of Russia (Yakutia) *S. feltiae protense* is able to infect the insect host at a temperature of 6 °C, while the most optimal development of this nematode in the insect host occurs at 18 °C to 23 °C (Ivanova et al., 2001).

Suppressive effects of *Steinernema feltiae* (strain SN) and *S. riobrave* (Cabanillas et al.) (strain 7–12) applied as infective juveniles against *Meloidogyne partityla* Kleynhans, as well as application of the *S. feltiae* bacterial symbiont *Xenorhabdus bovienii*, were investigated in greenhouse trials (Shapiro-Ilan et al., 2006). Treatments were applied to pecan nut seedlings (*Carya illinoensis*) that were simultaneously infested with *M. partityla* eggs. Four months after initial treatment dry root weight was higher in the *S. feltiae*-infested host treatment than in the control (approximately 80 % increase).

Application of *Sterneinema pakistanese* Shahina et al. for suppression of *M. incognita* on tomato was investigated in Pakistan in a greenhouse at 27–35 °C. One-month-old tomato plants were transplanted into soil in 200-ml plastic pots. *Steinernema pakistanese* was applied at rates of 1250, 2500 or 5000 invasive juveniles per pot at the same time as *M. incognita*, at a rate of 1500 J2 per pot. Root systems were harvested 35 days after infestation. Egg mass densities per root system were decreased 29 %, 34 % and 59 % at the 1250, 2500 and 5000 *S. pakistanense* treatments, respectively. At the 2500 rate, of *S. pakistanese* root and shoot weight increased 12.5 % and 8.5 %, respectively (Khan, Javed, 2018).

The first report of the possible antagonistic relationship between plant-parasite nematodes (PPNs) and entomopathogenic nematodes (EPNs), resulting in reduced population densities of PPNs, was published by Ishibashi, Kondo (1986). One possible explanation was that both EPNs and PPNs were attracted to root tips, but as EPNs are larger and more active they could colonize them faster, preventing PPNs invasion and reproduction (Bird, Bird, 1986). A later hypothesis was that a compound produced by symbiotic bacteria living inside EPNs was toxic to PPNs (Lewis et al., 2001). Neither of these ideas (root surface competition, bacterial products) proved to be correct. Application of dead EPNs along with dead bacteria still reduced PPNs population density (Jagdale et al., 2002). The easiest way to control PPNs would be metabolic products of symbiotic bacteria of EPNs. Our results suggest that the active metabolites of symbiotic bacteria need to be identified for possible synthesis and use in the field.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Akhurst R.J. 1980. Morphological and functional dimorphism in *Xenorhabdus* spp. bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. Journal of General Microbiology 121 (2): 303–309. https://doi.org/10.1099/00221287-121-2-303

Akhurst R., Boemare N.E. 1990. Biology and taxonomy of *Xenorhabdus*. In: Gaugler R., Kaya H.K. (eds). Entomopathogenic Nematodes in Biological Control. Boca Raton, CRC Press, Florida, 75–87.

Baermann G. 1917. Eine einfache Methode zur Auffindung von Ankylostomum-(Nematoden) larven in Erdproben. Mededeel mit H. Geneesk. Laboratories Weltevreden, Feestbundel, Batavia: 41–47.

Bird A.F., Bird J. 1986. Observations on the use of insect parasitic nematodes as a means of biologica control of root-knot nematodes. International Journal for Parasitology 16 (5): 511–516. https://doi.org/10.1016/0020-7519(86)90086-X

Bowen D.J., Ensign J.C. 1998. Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium *Photorhabdus luminescens*. Applied and Environmental Microbiology 64 (8): 3029–3035.

Burman M. 1982. *Neoplectana carpocapsae*: A toxin production by axenic insect parasitic nematodes. Nematologica 28: 62–70.

CABI Data Report; CABI. 2017. *Meloidogyne incognita* (root-knot nematode). In: Invasive Species Compendium (Wallingford, UK: CAB International). Available online at: www.cabi.org/isc/datasheet/33245

CABI Data Report; CABI 2020. *Steinernema carpocapsae, Steinernema feltiae*. In: Invasive Species Compendium (Wallingford, UK: CAB International). Available online at: www.cabi.org/isc/datasheet/51706, 51707

Grewal P.S., Martin W.R., Miller R.W., Lewis E.E. 1997. Suppression of plant-parasitic nematode populations in turfgrass by application of entomopathogenic nematodes. Biocontrol Science and Technology 7: 393–399. https://doi.org/10.1080/09583159730802

Hadisoeganda W.W., Sasser J.N. 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars to root-knot nematodes based on host suitability. Plant Disease 66: 145–150.

Hazir S., Stock S.P., Kaya H.K., Koppenhöfer A.M., Keskin N. 2001. Developmental temperature effects on five geographic Isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). Journal of Invertebrate Pathology 77: 243–250. https://doi.org/10.1006/jipa.2001.5029

Hirao A., Ehlers R.-U. 2009. Effect of temperature on the development of *Steinernema carpocapsae* and *Steinernema feltiae* (Nematoda: Rhabditida) in liquid culture. Applied Microbiology and Biotechnology 84 (6): 1061–1067. https://doi.org/10.1007/s00253-009-2035-3

Hu K.J., Li J.X., Webster J.M. 1999. Nematicidal metabolites produced by *Photorhabdus luminescens* (Enterobacteriaceae), bacterial symbiont of entomopathogenic nematodes. Nematology 1: 457–469. https://doi.org/10.1163/156854199508469

Inman F.L., Holmes L. 2012. Effect of heat sterilization on the bioactivity of antibacterial metabolites secreted by *Xenorhabdus nematophila*. Pakistan Journal of Biological Sciences 15 (20): 997–1000. https://doi.org/10.3923/pjbs.2012.997.1000

Ishibashi N., Kondo E. 1986. *Steinernema feltiae* (DD-136) and *S. glaseri*: Persistence in soil and bark compost and their influence on native nematodes. Journal of Nematology 18: 310–316.

Ivanova T.S., Danilov L.G., Ivakhnenko O.A. 2001. New subspecies of entomopathogenic nematode *Steinernema feltiae protense* subsp. n. (Nematoda: Steinernematidae) from Yakutia. Parasitology 35 (4): 333–337. [In Russian]

Jagdale G.B., Somasehkar N., Grewal P.S., Klein M.G. 2002. Supression of plant-parasitic nematodes by application of live and dead infective juveniles of an entomopathogenic nematode, *Steinernema carpocapsae*, on boxwood (*Buxus* spp.). Biological Control 4: 42–49.

Karssen G., Moens M. 2006. Root-knot nematodes. In: Perry R.N., Moens M. (eds). Plant nematology. Wallingford, UK, CABI, 59–90.

Kenney E., Eleftherianos I. 2016. Entomopathogenic and plant pathogenic nematodes as opposing forces in agriculture. International Journal for Parasitology 46: 13–19. https://doi.org/10.1016/j.ijpara.2015.09.005

Kepenekci I., Hazir S., Lewis E.E. 2016. Evaluation of entomopathogenic nematodes and the supernatants of the in vitro culture medium of their mutualistic bacteria for the control of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria*. Pest Management Science 72 (2): 327–334. https://doi.org/10.1002/ps.3998

Khan Y.S., Javed N. 2018. Entomopathogenic nematodes survey, persistence in soil, reproductive potential and their effects on *Meloidogyne incognita*. Egyptian Journal of Agronematology 17 (2): 109–120.

Lewis E.E., Grewal P.S. 2005. Interactions with plant-parasitic nematodes. In: Grewal P.S., Ehlers R., Shapiro-Ilan D. (eds). Nematodes as biocontrol agents. New York, USA, CABI, 349–362.

Lewis E.E., Grewal P.S., Sardanelli S. 2001. Interactions between the *Steinernema feltiae–Xenorhabdus bovienii* Insect Pathogen Complex and the Root-Knot Nematode *Meloidogyne incognita*. Biological Control 21: 55–62. https://doi.org/10.1006/bcon.2001.0918

Mai W.F., Brodie B.B., Harrison M.B., Jatala P. 1981. Nematodes. In: Hooker W.J. (ed.). Compendium of Potato Diseases. American Phytopathological Society, 93–101.

Molina J.P., Dolinski C., Souza R.M., Lewis E.E. 2007. Effect of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on *Meloidogyne mayaguensis* Rammah and Hirschmann (Tylenchida: Meloidoginidae) infection in tomato plants. Journal of Nematology 39 (4): 338–342.

Nguyen K.B., Stuart R.J., Andalo V., Gozel U., Rogers M.E. 2007. *Steinernema texanum* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Texas, USA. Nematology 9 (3): 379–396.

Nick1e W.R. 1977. Taxonomy of nematodes that parasitize insects, and their use as biological conlrol agents. In: Romberger J.A. (ed.). Biosystematics in Agriculture. Symposium Proceeding (Beltsville symposia II in agricultural research, 9–11 May 1977), 37–51.

Nour El-Deen A., Cseh H.E., Darwesh H.Y. 2014. Evaluation of certain Hungarian plant extracts for their nematicidal properties against root-knot nematode, *Meloidogyne incognita* in-vitro. International Journal of Advanced Research 2 (8): 443–448.

Paul V.J., Frautschy S., Fenical W., Nealson K.H. 1981. Antibiotics in microbial ecology. Isolation and structure assignment of several new antibacterial compounds from the insect-symbiotic bacteria *Xenorhabdus* spp. Journal of Chemical Ecology 7: 589–597. https://doi.org/10.1007/BF00987707

Perez E.E., Lewis E.E. 2002. Use of entomopathogenic nematodes to suppress *Meloidogyne incognita* on greenhouse tomatoes. Journal of Nematology 34: 171–174.

Perry R.N., Hominick W.M., Beane J., Briscoe B. 1998. Effect of entomopathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*, on the potato cyst nematode, *Globodera rostochiensis*, in pot trials. Biocontrol Science and Technology 8: 175–180.

Poinar G.O., Thomas G.M. 1967. The nature of *Achromobacter nematophilus* as an insect pathogen. Journal of Invertebrate Pathology 9 (4): 510–514. https://doi.org/10.1016/0022-2011(67)90131-0

Richardson W.H., Schmidt T.M., Nealson K.H. 1988. Identification of an anthraquinone pigment and ahydroxystilbene antibiotic from *Xenorhabdus luminescens*. Applied and Environmental Microbiology 54: 1602–1605.

Sasser J.N. 1979. Economic importance of *Meloidogyne* in tropical countries. In: Lamberti F., Taylor C.E. (eds). Root-knot nematodes (*Meloidogyne* species). Systematics, Biology and Control. London, UK, Academic Press, 359–374.

Shapiro-Ilan D.I., Nyczepir A.P., Lewis E.E. 2006. Entomopathogenic nematodes and bacteria applications for control of the pecan root-knot nematode, *Meloidogyne partityla*, in the greenhouse. Journal of Nematology 38 (4): 449–454.

Sharmila R., Priya M.S., Subramanian S., Poornima K., Pandiyan M. 2018. Review on ecology of entomopathogenic nematodes. Journal of Entomology and Zoology Studies 6 (4): 1086–1093.

Vrain T. G., Barker K., Holtzman G. 1978. Influence of low temperature on rate of development of *Meloidogyne incognita* and *M. hapla* larvae. Journal of Nematology 10: 166–171.

Uribe G.E.M. 2008. Biodiversity of phytoparasitic nematodes associated with Musaceae and fruit crops in Colombia. Thesis submitted in fulfillment of the requirements for the degree of Doctor of Science (Biology). Ghent University, Belgium, 209 pp.

НЕМАТИЦИДНАЯ АКТИВНОСТЬ СИМБИОТИЧЕСКИХ БАКТЕРИЙ *XENORHABDUS BOVIENII* И *X. NEMATOPHILA* ПРОТИВ КОРНЕВОЙ ГАЛЛОВОЙ НЕМАТОДЫ *MELOIDOGYNE INCOGNITA*

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Ключевые слова: энтомопатогенные нематоды, симбиотические бактерии, метаболиты, эффективность

РЕЗЮМЕ

Эффективность продуктов метаболизма, продуцируемых симбиотическими бактериями *Xenorhabdus bovienii* (*Steinernema feltiae*) и *X. nematophila* (*S. carpocapsae*), против инвазионных личинок 2-го возраста *M. incognita* (J2) была испытана в лабораторных условиях при температуре 20 °C, 23 °C и 26 °C сразу после получения и автоклавирования с экспозицией 41, 65 и 90 ч, а также при температуре 23 °C после хранения в течение 21 дня при 4 °C с экспозицией 26, 50 и 74 ч с титрами бактериальных клеток 2.5×10^9, 1.25×10^9 и 0.63×10^9/мл. Эффективность продуктов метаболизма бактерий сразу после их получения против *M. incognita* (J2) зависела от титра бактериальных клеток, температуры культуральной жидкости и продолжительности ее воздействия на нематод. Нематицидная активность продуктов обмена веществ *X. bovienii* была выше, чем *X. nematophila*. В опытах с *X. bovienii* гибель *M. incognita* (J2) составляла 92–93 % после их 90-часового воздействия на нематод при 20 °C и титрах 2.5×10^9 и 1.25×10^9; 65-часового воздействия при 23 °C и титре 2.5×10^9; 98–99 %, при 26 °C и всех испытанных титрах. Эффективность культуральной жидкости *X. bovienii* после хранения при 4 °C в течение 21 дня при 23 °C после ее 50-часового воздействия на нематод и титрах 2.5×10^9 и 1.25×10^9 и после 74-часового воздействия при всех испытанных титрах составляла 97–100 %. Продукты метаболизма симбиотических бактерий рода *Xenorhabdus* против корневых галловых нематод показали высокую эффективность, они нуждаются в идентификации для возможного синтеза и использования в полевых условиях.