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Evaluation of the nematicidal effect of biodegradable polymer-based granules containing isothiocyanates on *Globodera rostochiensis*

A.A. Sukhanova[1], K.E. Zabolotnaya[1], A.A. Churakov[2], S.N. Syrtsov[1], E.V. Brilkova[1], A.N. Boyandin[1]
[1]*Reshetnev State University of Science and Technology, Krasnoyarsk, Russian Federation*
[2]*Krasnoyarsk State Agrarian University, Krasnoyarsk, Russian Federation*

Abstract

Background. Golden potato cyst nematode (*Globodera rostochiensis*, GPCN) is a dangerous quarantine pest of potato, and efficacy of usual nematicides is often limited. One is possible solutions is development of formulations with gradual and controlled release of active ingredients by their encapsulation into biodegradable carriers. Isothiocyanates (ITCs) have been previously shown as effective nematicides.

Purpose. The aim of this work was to develop and study granules composed of polycaprolactone (PCL) and bentonite loaded with ITCs (allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), and 2-phenylethyl isothiocyanate (PEITC)) against GPCN for soil application.

Materials and methods. Granules of poly-ε-caprolactone with bentonite containing ITCs as nematicide agents were obtained by extrusion. The encapsulation efficiency of ITCs was determined by chromatography. Efficacy of the granules against GPCN was assessed *in vitro* by mortality of nematodes and in laboratory soil ecosystem on potato plants of two varieties, 'Krasnoyarsky Ranniy' and 'Innovator', by yield assessment.

Results. *In vitro* experiments confirmed the efficacy of PCL/bentonite/isothiocyanate granules in inhibiting the development of second-stage juveniles (J2) of GPCN. The PCL/(PEITC/bentonite) granules achieved 100% nematode mortality within 48 hours, acting 1 to 2 times faster than those containing BITC or AITC. In soil experiments with potato plants, treatment with the PCL/(PEITC/bentonite) granules reduced the number of cysts on roots by 4.2–5.0 times relative to the control; a result was comparable to that of the commercial reference product 'Vydate® 5G'. Application of the PCL/(PEITC/bentonite) granules enhanced the yield of the 'Innovator' potato variety.

Keywords: polycaprolactone; bentonite; isothiocyanates; granules; extrusion; nematodes; potato varieties; nematicidal efficacy

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Оценка нематицидного действия биоразлагаемых полимерных гранул, содержащих изотиоцианаты, на *Globodera rostochiensis*

А.А. Суханова[1], К.Е. Заболотная[1], А.А. Чураков[2], С.Н. Сырцов[1], Е.В. Брилькова[1], А.Н. Бояндин[1]

[1]Сибирский государственный университет науки и технологий имени академика М. Ф. Решетнева, Красноярск, Российская Федерация

[2]Красноярский государственный аграрный университет, Красноярск, Russian Federation

Аннотация

Обоснование. Золотистая картофельная нематода (*Globodera rostochiensis*, ЗКН) – опасный карантинный вредитель картофеля, эффективность обычных нематицидов в отношении которого зачастую ограничена. Одним из возможных решений является разработка препаратов с постепенным высвобождением активных ингредиентов с помощью их инкапсулирование в биоразлагаемые носители. Изотиоцианаты (ИТЦ) ранее продемонстрировали свою эффективность в качестве нематицидов.

Цель. Целью данной работы была разработка и исследование гранул почвенного внесения, состоящих из поликапролактона (ПКЛ) и бентонита, нагруженных ИТЦ (аллилизотиоцианатом (АИТЦ), бензилизотиоцианатом (БИТЦ) и 2-фенилэтилизотиоцианатом (ФЭИТЦ)), для борьбы с ЗКН.

Материалы и методы. Гранулы ПКЛ с бентонитом, содержащие ИТЦ в качестве нематоцидных агентов, были получены методом экструзии. Эффективность инкапсулирования ИТЦ определяли хроматографически. Эффективность гранул против ЗКН оценивали *in vitro* по смертности нематод и в лабораторных условиях на растениях картофеля двух сортов – «Красноярский ранний» и «Инноватор», – по оценке урожайности.

Результаты. Эксперименты *in vitro* подтвердили эффективность гранул ПКЛ/бентонит/ИТЦ в подавлении развития личинок второй стадии (J2) ЗКН. Гранулы ПКЛ/(ФЭИТЦ/бентонит) обеспечивали 100%-ную гибель нематод в течение 48 часов, действуя до двух раз быстрее гранул, содержащих БИТЦ или АИТЦ. В почвенных экспериментах на картофеле обработка гранулами ПКЛ/(ФЭИТЦ/бентонит) снизила количество цист на корнях в 4,2–5,0 раза по сравнению с контролем; результат был сопоставим с применением коммерческого референтного препарата «Vydate® 5G». Применение гранул ПКЛ/(ФЭИТЦ/бентонит) повысило урожайность картофеля сорта «Инноватор».

Ключевые слова: поликапролактон; бентонит; изотиоцианаты; гранулы; экструзия; нематоды; картофель; нематицидная активность

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Introduction

Plant diseases caused by various phytopathogens, such as fungi, bacteria, and nematodes, represent one of the key factors significantly impacting crop yield. Specifically, the golden potato cyst nematode (*Globodera rostochiensis*, GPCN) is a dangerous quarantine pest of potato, capable of causing yield losses ranging from 19% to 90% [2]. The GPCN penetrates plant roots and feeds on cellular contents, disrupting the nutrient uptake process and leading to premature plant death. Despite the use of modern measures to prevent globoderosis, such as chemical treatment with nematicides and agrophytocenotic practices, their efficacy is often limited. Furthermore, they can harm soil microbiota, plants, and animals, leading to adverse environmental consequences [1; 4]. As a result, the use of several chemical nematicides, such as captafol, dazomet, and chloropicrin, has been banned in agriculture across developed countries [7].

Currently, increasing attention is being paid to the development of sustained-release formulations that provide gradual and controlled release of active ingredients. Several strategies for designing such systems have been described in the literature, including the encapsulation of chemical compounds within polymer matrices [5; 19; 21], the synthesis of novel active ingredients guided by the principles of green chemistry [3], and the development of nano- and microcapsules [10; 13]. In the development of such complex formulations, crucial factors include efficient methods for encapsulating active ingredients into biodegradable carriers, simplicity of manufacturing, low production cost, and ease of application. These characteristics include high cation exchange capacity, strong adsorption ability, high porosity, and a large specific surface area. For the development of complex nematicidal formulations, the use of bentonite – a natural clay with structural characteristics optimal for the controlled release of active substances – is widely considered. [9; 17; 23] Biodegradable polymers studied for such systems include polylactide [8], chitosan [16], polyhydroxybutyrate [21], gelatin [20], carboxymethyl cellulose [24], polycaprolactone (PCL) [13], among others. Among the listed materials, PCL, the polymer of 6-hydroxycaproic acid, stands out as a promising candidate due to its controllable degradation rates, low melting temperature, and adaptability to various processing methods.

Our previous studies have demonstrated the feasibility of producing PCL/montmorillonite-based granules using extrusion and solution-based technologies, which are used in various fields, including agriculture [19]. Based on the accumulated experience, the extrusion method was selected for this study. This technique proved to be technologically simpler, scalable, and, unlike solution-based methods, did not cause significant changes in the material's crystallinity upon clay addition (with values ranging from 14.2% to 27.7%). Isothiocyanates are among the promising active compounds for encapsulation. Isothiocyanates are sulfur-containing organic compounds extracted from plants of the Brassicaceae family (such as mustard and horseradish), which have been repeatedly shown to exhibit nematicidal activity [14; 15]. However, it should be noted that despite the experiments with various types of isothiocyanates presented in the literature, which are specific to nematode species [6; 12], information on their activity against *Globodera rostochiensis,* widespread in temperate climates, remains limited.

For potato, pre-emergence soil application of sustained-release formulations prevents nematode infection at the early stages of plant development. Pure ITCs have previously shown high efficiency against nematodes [12; 22]. Based on this, we aimed to evaluate the nematicidal efficacy of PCL/bentonite granules loaded with isothiocyanates (allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), and 2-phenylethyl isothiocyanate (PEITC)) on *Globodera rostochiensis* in vitro and in soil experiments with nematode-susceptible potato varieties.

Materials and methods

Materials. We used the biodegradable polymer polycaprolactone (PCL) from Sigma-Aldrich (USA) with a weight-average molecular weight (Mw) of

80 kDa, a crystallinity degree (Cx) of 52%, and a melting temperature (Tm) of 57 °C. Isothiocyanates (ITCs) – allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), and 2-phenylethyl isothiocyanate (PEITC) (≥98% purity) – were purchased from Zoranchem (China). Methanol was obtained from OOO AO Reakhim (Russia), and the emulsifier Tween-80 was supplied by OOO NPC Biokompas-C (Russia). Bentonite (Bentonite Company LLC, Moscow, Russia) with a montmorillonite (MMT) content of no less than 70% was used. The nematicidal preparation Vydate® 5G, containing the active substance oxamyl (N,N-dimethyl-2-methylcarbamoyloxyimino-2-(methylthio) acetamide) 50 g/kg, was produced by DuPont de Nemours, France.

Preparation of PCL granules with ITC complexes. Polymer granules based on PCL (Sigma-Aldrich, USA) and bentonite (50 wt % of the polymer) containing ITCs were obtained using a portable single-screw extruder (Bestfilaments, Russia) at a melting temperature of 110 °C and a screw speed of 24 rpm, followed by pelletization using a granulator (MCU, China). Encapsulation of ITCs in bentonite was performed according to the previously described method [18] with some modifications. ITCs (2 g) and bentonite (10 g) in a ratio of 1:5 were added to a solution containing Tween 80 (4 g) and methanol (60 ml). The mixture was stirred on a magnetic stirrer at 800 rpm until completely homogenized. Then, the mixture was dried at 67 °C and extruded with PCL using the method described above. The obtained PCL granules with the bentonite-phenethyl isothiocyanate complex were designated as PCL/(PEITC/bentonite); those with the bentonite-benzyl isothiocyanate complex were designated as PCL/(BITC/bentonite); and those with the bentonite-allyl isothiocyanate complex were designated as PCL/(AITC/bentonite).

Determination of ITC encapsulation efficiency. The encapsulation efficiency in the granules was determined using extraction followed by chromatography. One granule with a mass of 20 mg was dissolved in 1 mL of chloroform; and polymer subsequently precipitated using hexane. The supernatant, containing the ITCs, was obtained by centrifugation at 2500 rpm for 10 minutes. The ITC content in the resulting supernatant was determined by gas chromatography-mass spectrometry (GC-MS) using an Agilent 6890N/5975C system, operated by MSD ChemStation software (version E.02.02.1431). An Agilent VF-200ms column was installed. was used to operate the instrument. Helium was used as a carrier gas at 1.2 mL×min^{-1} flow rate. The sample was introduced in the splitless mode. The input device and interface temperatures were set to 220 °C and 230 °C, respectively. Operating temperature of the oven: 80 °C for 3 minutes, then heating to 220 °C at 10 °C×min^{-1} rate, holding the temperature for 5 minutes. Samples were ionized using electron impact (EI) at 70 eV. The peaks of the ITCs and their retention times were determined from external standard samples in full scan mode (with m/z ranging from 35 to 500). For construction of calibration curves using external standards and the determination of ITC concentrations in the samples, characteristic ions were selectively monitored: m/z 99 for AITC; m/z 91 and 149 for BITC; and m/z 91 and 163 for PEITC. For BITC and PEITC samples, spectra were acquired for both ions and converted into concentration values individually, after which the results were averaged. The encapsulation efficiency (EE) of the granules was calculated using the formula:

$$E_{dep}(\%) = \frac{C_{gd}}{C} \times 100,$$

where C_{gd} is the ITC content in the sample determined by gas chromatography (g/g), and C is the theoretical maximum ITC loading in the granules (g/g).

Determination of the LC$_{50}$ of ITCs for *Globodera rostochiensis*. To assess the toxicity of the isothiocyanates, solutions of the test compounds at a concentration of 0.0125 µL/mL, prepared using dimethyl sulfoxide (DMSO) as a solvent, were used. The experiments were conducted in Petri dishes, with four nematodes placed in each dish. At the first stage, 5 mL of potato root diffusate (obtained according to the method of Motlound et al. [11]) and dissected nematode cysts were added to the Petri dishes. These were then incubated at 25 °C in a dark room for 2–3 days until nematode hatching occurred. Then, 10 µL of the solutions containing the test compounds were added to each dish. Petri dishes containing only root diffusate, without any test compounds, were used as controls to assess natural mortality. The counting of live and dead individuals was performed after 5 and 24 hours using a Discovery Atto Polar digital microscope at 40x magnification (Figure 1, A).

Evaluation of the Efficacy of Isothiocyanates (ITCs) and PCL(ITC/Bentonite) Granules *in vitro* Against *Globodera rostochiensis*. Cysts, as well as live (motile) nematodes, were obtained using the method described above (Figure 1, B). Subsequently, once a sufficient number of live nematodes (n=10) were present in the Petri dish, PCL/(ITC/bentonite) granules were added (1, 2, and 3 granules; ITC concentration expressed as mg ITC per mg of granule) to assess their nematicidal activity. The duration of the experiment was 96 hours and varied depending on the specific ITC, with control time points every 24 hours. A nematode was considered dead if it was immobile and did not respond to a needle prick (Figure 1, C). Subsequently, the percentage of nematode mortality was calculated for all groups for each 24-hour period, and the obtained data were analyzed.

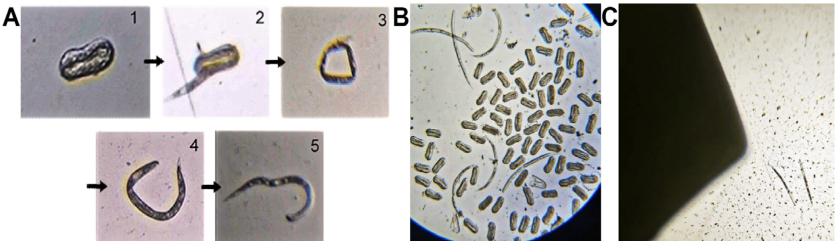

Fig. 1. A. The process of nematode hatching from an egg over 2–3 days: 1 – nematode egg; 2, 3, 4 – nematode uncoiling; 5 – J2 stage nematode. B. The contents of a cyst after mechanical dissection (prior to the start of the experiment). C. A granule and non-viable nematodes (post-experiment).

Evaluation of the efficacy of PCL/(PEITC/Bentonite) granules in laboratory soil ecosystem experiments on potato plants. The nematicidal activity of the granules was investigated at a research station of Krasnoyarsk State Agrarian University in the Vetluzhanka microdistrict, the city of Krasnoyarsk (coordinates: 56.026720, 92.740338). The experimental groups were divided into the following treatments: 1) Control – intact plants; 2) Plants infected with nematodes (Gro); 3) the commercial nematicide ‘Vydate’; 4) Experimental PCL/PEITC granules – (PCL/(PEITC/bentonite)); 5) Liquid form of PEITC (PEITC).

The vegetation experiment was established in 5 L containers filled with the substrate ‘Agrobalt S’, which is based on high-moor peat with a particle size of 0–20 mm. The solution has a slightly acidic reaction (acidity pH H_2O 5.5–6.6), is enriched with nutrients (nitrogen and phosphorus content of not less than 150 mg/L each, potassium 250 mg/L, and trace elements). The number of replications was 4, with five containers per replication. The treatments were arranged randomly. Two potato varieties susceptible to the golden nematode were used: ‘Krasnoyarsky Ranniy’ and ‘Innovator’. For the experiment, virus-free microplants grown on Murashige and Skoog (MS) medium were used. Plant maintenance consisted of watering as needed and the routine application of fungicides to all treatments to protect against late blight and avoid premature foliage death. To protect against insect vectors of viruses, insecticide treatment was applied. The initial insecticide application was carried out on July 20 with Karate Zeon (CS) at a rate of 0.2 L/Ha. The volume of spray solution applied was 200 L/Ha. On August 6th, spraying was conducted with a tank mixture of Revus Top, SC (0.6 L/Ha) and Karate Zeon, CS (0.2 L/Ha), at a spray volume of 200 L/Ha. The final treatment was performed on August 19th with Revus Top, SC (0.6 L/Ha). The experiment was assessed on October 1st.

The substrate was inoculated with the pathogen by introducing 30 g of a peat-sand mixture, containing at least 6 cysts per 1 g, into the planting zone of the microplants in each container. Depending on the experimental treatment, the following were added to the planting containers: 18 g of PCL/(PEITC/bentonite) granules, 0.105 g of Vydate (calculated at a rate of 20 kg per hectare potato planting norm), or 200 μL of pure PEITC. In the Gro treatment, only the carrier (with nematodes) was used. The control group consisted of the ‘Agrobalt S’ peat substrate without the parasite and without nematicidal preparations.

The experiment utilized the species *Globodera rostochiensis* Wollenweber, 1923 – the golden potato cyst nematode, pathotype Ro1, which is highly pathogenic and is a quarantine organism in the Russian Federation.

The research was conducted on the varieties ‘Innovator’ and ‘Krasnoyarsky Ranniy’, which are approved for use in Eastern Siberia. The ‘Innovator’ variety, bred by HZPC HOLLAND B.V., is classified as mid-early and is suitable for French fry production. The tuber is yellow, elongated-oval in shape, with shallow eyes and light- yellow flesh. It holds one of the leading positions in the country for cultivation for processing.

The ‘Krasnoyarsky Ranniy’ variety was developed by A.G. Lorkh Federal Research Center for Potato in collaboration with Krasnoyarsk State Agrarian University. It is an early-maturing variety, classified as a table potato. It is highly valued in Western and Eastern Siberia for its excellent taste, high marketability, and good storability. The tubers of this variety are yellow with light yellow flesh and eyes ranging from small to medium in size. An assessment of the local weather patterns at the research site and the specific weather conditions during the growing season was also conducted. Upon completion of the experiment, a final assessment and count were performed.

Statistical Analysis of the Results. The data were statistically processed using StatSoft STATISTICA 13. To determine the statistical significance of differences between the experimental treatments for individual indicators, one-way and two-way analysis of variance were employed, followed by a comparison of individual means using Duncan’s multiple range test. Spearman’s rank correlation was used to calculate correlation coefficients for data distributions that did not conform to normality. Factor analysis was applied to study the relationships between the investigated parameters, using R. Cattell’s scree test criterion to determine the number of factors.

Fig. 2. The appearance of the granules: A – PCL/(AITC/bentonite), B – PCL/(BITC/bentonite), C - PCL/(PEITC/bentonite)).

Table 1.

Encapsulation efficiency of the granules

Granule	Efficiency, %	Concentration, mg/mg
PCL/(AITC/bentonite)	0,2±0,02	0,0003±0,00006
PCL/(AITC/bentonite)	25,3±4,7	0,02±0,002
PCL/(PEITC/bentonite)	32,5±3,2	0,03±0,002

Results and discussion

Characteristics of PCL/(Bentonite/PEITC) granules

Filaments of PCL with bentonite and isothiocyanates (PCL/(AITC/bentonite), PCL/(BITC/bentonite), PCL/(PEITC/bentonite)) were obtained by extrusion. The resulting filaments were cut into granules approximately 10 mm in length (weighing about 20 mg) and 1.5–2.0 mm in diameter (Figure 2).

According to Table 1, the lowest encapsulation efficiency was observed for the granules (PCL/(AITC/bentonite)). This may be attributed to the lower molecular weight of AITC (99.15 g/mol) compared to BITC (149.22 g/mol) and PEITC (163.24 g/mol), and consequently, to its higher volatility.

Determination of the lethal concentration of ITCs for *Globodera rostochiensis*

To investigate the nematicidal effect of pure ITCs on *G. rostochiensis* nematodes, the first step involved obtaining a sufficient quantity of live nematodes. It was established that after mechanical damage to the cysts and placing nematode eggs in a root diffusate solution (5 ml in a Petri dish), their hatching occured within 2–3 days.

A characteristic sign of nematode viability is their S-shaped movement and cyclical coiling/uncoiling, while actual spatial displacement is minimal. In contrast, dead nematodes become less curved and more elongated/straight due to the loss of muscle tone and movement (Figure 3, A).

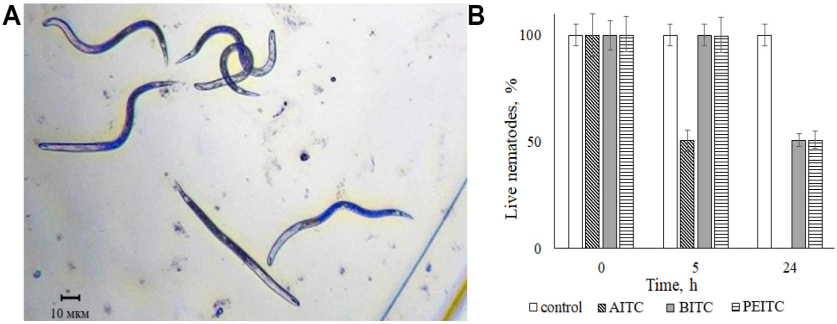

Fig. 3. A: nematodes under the microscope. B: a graph for LC_{50} determination.

The toxicity assessment of pure ITCs against nematodes to determine the lethal concentration (LC_{50}) was conducted using ITC solutions in DMSO at a concentration of 0.0125 µL/mL, as this concentration was established as the minimum effective dose in prior studies on the nematicidal activity of ITCs against *Globodera pallida* [22]. AITC demonstrated the highest nematicidal activity among the ITC solutions (Figure 3, B). In the group treated with AITC, 50% nematode mortality was observed after 5 hours. In contrast, in the Petri dishes containing PEITC and BITC solutions, the number of dead nematodes reached 50% only after 24 hours. In the control group, all nematodes remained viable throughout this period.

Evaluation of the efficacy of PCL/(ITC/Bentonite) granules *in vitro* against *Globodera rostochiensis*

The nematicidal effect of the obtained ITC-loaded granules was investigated in vitro by incubating them with nematodes in Petri dishes containing a root diffusate solution (5 mL) for 96 hours. The concentration of the active substance (ITC) in 1, 2, and 3 granules was as follows: 0.03, 0.06, and 0.09 mg/mg for PEITC; 0.02, 0.04, and 0.06 mg/mg for BITC; and 0.0003, 0.0006, and 0.0009 mg/mg for AITC.

A 24-hour incubation with a single PCL/(PEITC/bentonite) granule in Petri dishes resulted in 20% nematode mortality. When the number of granules of the same composition was increased to 2 and 3, nematode mortality reached 40% and 70%, respectively. After 48 hours of incubation with the maximum number of PCL/(PEITC/bentonite) granules, 100% nematode mortality was achieved. Simultaneously, incubation with 1–2 granules resulted in 60% to 80% mortality, respectively (Figure 4). After 72 hours, 100% nematode mortality was achieved, regardless of the number of PCL/(PEITC/bentonite) granules used.

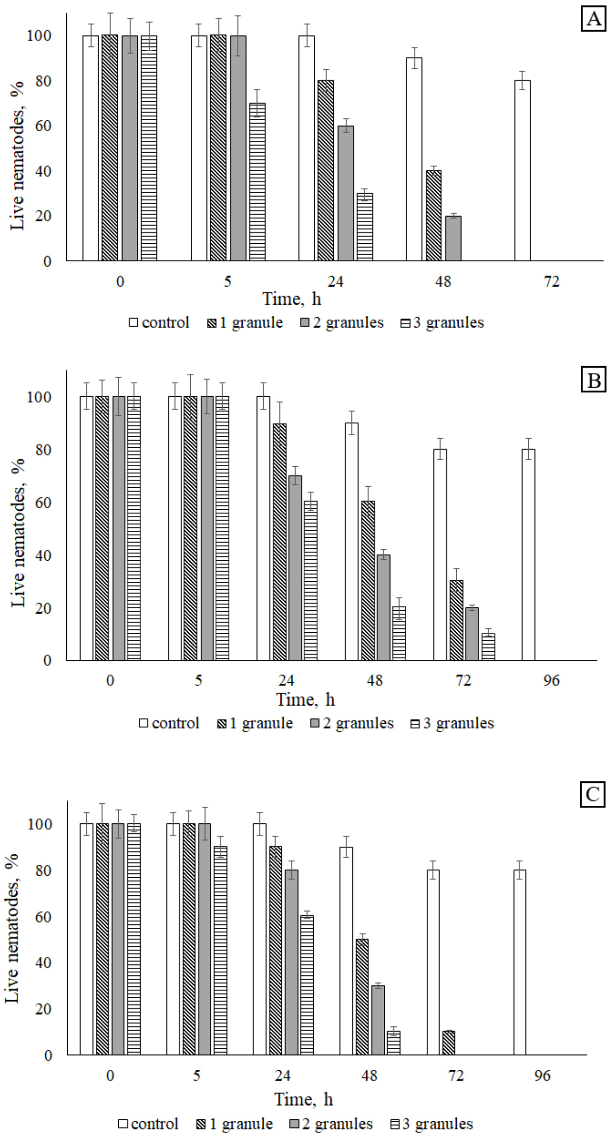

Fig. 4. Amount of live nematodes (%) relative to the number of ITC-loaded granules over time. A – granules with PCL/(AITC/bentonite); B – PCL/(BITC/bentonite); C – PCL/(PEITC/bentonite).

When PCL/(BITC/bentonite) granules were incubated with nematodes in Petri dishes for the first 24 hours, comparable results to those of the PCL/(PEITC/bentonite) granules were obtained. After 24 hours, nematode mortality reached 10% and 20% in Petri dishes containing 1 and 2 PCL/(BITC/bentonite) granules, respectively. However, after 48 hours, nematode mortality increased to 50%, 70%, and 90% upon incubation with 1, 2, and 3 PCL/(BITC/bentonite) granules, respectively. On the third day, in this experimental group, nematode mortality reached 100%. However, upon incubation with a single PCL/(BITC/bentonite) granule, the mortality rate remained at 90%. The less pronounced nematicidal effect of the PCL/(BITC/bentonite) granules, compared to the PCL/(PEITC/bentonite) granules, can be explained by the concentration of the active ingredient itself. The BITC content in the PCL/(BITC/bentonite) granules was 0.02 mg/mg.

Table 2.

Productivity parameters of the 'Krasnoyarsky Ranniy' variety across experimental treatments (mean per planting container)

Treatment	Plant height, cm	Number of stems	Number of tubers	Stem mass, g	Tuber mass, g	Mass per tuber, g	Number of cysts
Control	55,38	1,50	2,25	79,06	88,13	47,19	0,00
Gro	50,81	1,19	1,75	70,63	85,00	50,70	60,56
Vydate	68,13	1,19	1,81	76,25	81,25	56,15	17,94
PCL/(PEITC/bentonite)	51,94	1,25	1,94	54,06	85,00	43,59	17,38
PEITC	34,71	1,29	1,57	77,86	25,71	16,43	64,43
Total	54,41	1,28	1,90	70,77	79,01	46,16	27,96

Table 3.

Productivity parameters of the 'Innovator' variety across experimental treatments (mean per planting container)

Treatment	Plant height, cm	Number of stems	Number of tubers	Stem mass, g	Tuber mass, g	Mass per tuber, g	Number of cysts
Control	40,13	1,40	2,27	65,00	49,33	32,25	0,00
Gro	28,27	1,07	1,53	49,00	38,00	18,38	73,00
Vydate	35,00	1,08	1,08	58,85	32,31	22,88	12,23
PCL/(PEITC/bentonite)	39,92	1,08	3,08	107,08	61,25	20,35	12,00
PEITC	28,78	1,00	1,00	44,44	17,22	11,39	60,78
Total	34,67	1,14	1,83	65,00	40,94	21,93	30,39

Despite the comparable results of the AITC granules to the other two types of granules, PCL/(PEITC/bentonite) and PCL/(BITC/bentonite), on the first day, the nematode mortality after incubation with PCL/(AITC/bentonite) granules for three days was still only 70%, 80%, and 90% (for 1, 2, and 3 granules, respectively). Indeed, in our earlier LC_{50} assessment, we established that AITC was the most effective among the ITCs, exhibiting the most pronounced effect on the nematodes. However, due to its high volatility – particularly during the extrusion process – its content in the granules remains extremely low (0.0003 mg/mg in 1 granule) compared to the initial volume for encapsulation. Therefore, for further research, PCL/bentonite granules containing PEITC were selected.

Evaluation of the efficacy of PCL/(PEITC/Bentonite) granules in laboratory soil ecosystems with potato plants

The efficacy of PCL/(PEITC/bentonite)-based granules against *Globodera rostochiensis* was investigated in soil experiments using two potato varieties. Tables 2 and 3 present the results of the assessment of the influence of the developed granules and comparison groups on plant productivity parameters.

The minimum productivity parameters for both potato varieties were obtained in the nematode-infected treatment (Gro) and the liquid PEITC treatment. In the latter case, the liquid PEITC treatment did not have a significant effect on nematode cyst formation. Plant growth inhibition was observed, caused by both the action of the parasite and the potential toxic effect of the compound on the microplants at the initial stage of their growth. In the control group, both varieties exhibited some of the best plant development parameters. This confirms the crop losses caused by parasite infection.

Table 4.

Effect size measures of the studied treatments on the observed parameters of potato plants (p – statistical significance of the effect)

Parameter	Treatment	p	Variety	p	Treatment*Variety	p	Error	p
Plant height	**14,2**	**0,000**	**27,3**	**0,000**	**7,3**	**0,000**	51,3	0,002
Stem mass	**2,62**	**0**	0,5	0,439	**10,4**	0,404	86,5	**0,006**
Mass per tuber	**7,0**	**0,000**	**12,4**	**0,028**	2,7	0,017	77,9	0,371
Number of cysts	**76,7**	**0,000**	0,0	**0,000**	1,3	0,864	22,0	0,123
Number of tubers	**12,7**	**0,000**	0,074	0,985	**6,58**	0,042	80,60	**0,042**

The treatments with the commercial preparation ‘Vydate’ and the experimental PCL/(PEITC/bentonite) granules demonstrated a high nematicidal effect. A slight reduction in productivity may be caused by the toxic effect of the commercial preparation and minor nematode damage.

According to the results of the two-way analysis of variance (ANOVA) (Table 4), individual tuber mass was genetically determined and also depended on the nematicide treatment applied. Only the application of the treatments had a significant influence on the number of cysts (effect strength 76.7%,). Plant height was influenced not only by the genetic characteristics of the varieties and the protection treatments, but also by the interaction between genotype and treatment.

It was established that cyst formation on potato roots had a weak negative effect on all studied parameters of plant growth and development. The average tuber mass showed a moderate positive correlation with plant height. It is also worth noting the unusually high positive correlation between total tuber mass and average tuber mass. This is due to the small number of tubers formed in each experimental pot.

Based on the calculation of the t-test for independent samples, a significant difference from the control was established for treatment 2 (nematode infection) in plant height ($t = 2.19$, $p = 0.03$) and number of cysts ($t = -15.78$, $p < 0.001$). For treatment 3 (Vydate), the parameter "number of tubers per plant" significantly exceeded the control value ($t = 2.208$, $p = 0.003$). For the fourth treatment (PCL/(PEITC/bentonite)), no significant difference from the control was detected. For the fifth treatment (PEITC), significant differences were established for plant height ($t = 4.595$, $p < 0.001$), tuber mass ($t = 4.21$, $p < 0.001$), mass per tuber ($t = 3.946$, $p < 0.001$), and number of tubers ($t = 2.209$, $p = 0.003$). Since the control group was not inoculated with nematodes, all treatment variants differed statistically significantly in the number of cysts on the roots. Therefore, to determine the significant differences between the preparative forms, it is advisable to perform an analysis of variance (ANOVA) without including the control treatment.

According to the results of the two-way analysis of variance (ANOVA), a statistically significant genotype × treatment interaction was detected for the number of tubers per plant ($F = 4.941$, $p = 0.003$), stem mass ($F = 4.368$, $p = 0.006$), and plant height ($F = 4.886$, $p = 0.003$, Figure 5). Among the treatments using nematicidal formulations (without the control), an analysis of variance (ANOVA) with Scheffé's test for plant height identified 'Vydate' as superior.

Due to the detection of significant variety × nematicide interaction effects, a one-way analysis of variance (ANOVA) was subsequently performed. For the 'Krasnoyarsky Ranniy' variety, Scheffé's test revealed significant effects for the Vydate treatment compared to the nematode-infected control (Gro) in terms of plant height ($p = 0.019$, Figure 5), as well as for the number of cysts in both the 'Vydate' ($p < 0.001$) and PCL/(PEITC/bentonite) ($p < 0.001$) treatments. For the remaining treatments and the other investigated parameters, the obtained differences fell within the margin of experimental error and are attributed to other factors not studied in this experiment.

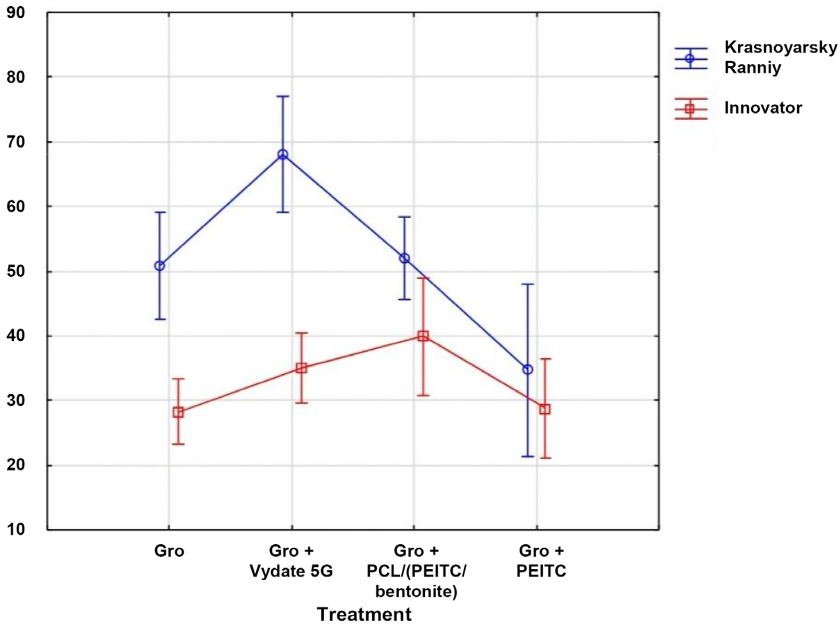

Fig. 5. Plant height (cm) across different experimental treatments. Vertical bars represent the 95% confidence intervals for the mean.

The 'Innovator' variety exhibited a different interaction between the studied factors. The influence of the experimental preparation PCL/(PEITC/bentonite) had a highly significant effect on stem mass (p 0.030) and the number of tubers per plant (p 0.003). The commercial preparation 'Vydate' (p 0.000), as well as the developed PCL/(FEITC/bentonite) preparation (p 0.000), exhibited a substantial nematicidal effect reducing the number of cysts on roots to the minimum value observed in the experiment (Figure 6). No significant difference in tuber mass per plant was found among the treatments, except for the pair PCL/(PEITC/bentonite) – PEITC (p 0.036). The lowest values for all productivity parameters were observed in the PEITC treatment. This may be attributed to the inhibitory effect of the compound on the microplants and its high initial concentration upon application.

According to the Friedman rank-based analysis of variance, the greatest effect on the yield structure components of the 'Krasnoyarsky Ranniy' variety (Table 5) was exerted by 'Vydate' and PCL/(PEITC/bentonite). However, for this genotype, the effect of the preparations did not have a statistically significant impact on the entire set of studied parameters. For the 'Innovator' variety, the experimental preparation PCL/(PEITC/bentonite) played the leading role in shaping productivity and its components. Vydate ranked second in terms of efficacy (Table 6). The calculation of pairwise correlation coefficients for the mean ranks (Table 7) showed no significant relationships between the 'Krasnoyarsky Ranniy' and 'Innovator' varieties. Similar results were obtained when calculating rank correlation coefficients using Spearman's method (Table 8).

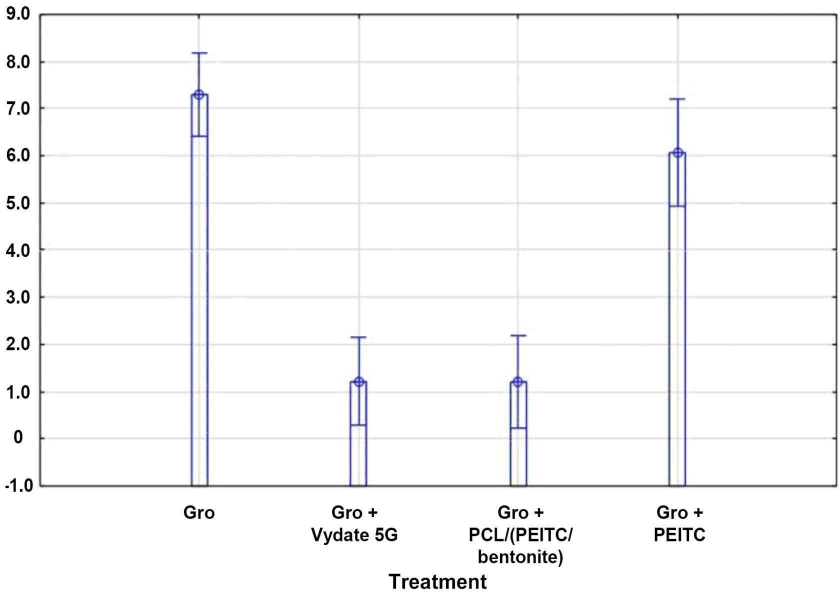

Fig. 6. Number of golden potato cyst nematodes on the roots of the 'Innovator' variety across experimental treatments at harvest. Current effect: F (3.45) = 46.319, p=0.000. Vertical bars represent the 95% confidence intervals for the mean.

Table 5.

Results of the analysis of variance for the 'Krasnoyarsky Ranniy' variety data, χ^2 (N = 7, df = 4) = 4.23, p = 0.376

Variable	Mean rank	Sum of ranks	Mean	Std.dev.
Control	4,00	28,00	39,07	37,93
Gro	2,71	19,00	45,81	32,53
Vydate	3,14	22,00	43,24	35,23
PCL/(PEITC/bentonite)	2,71	19,00	36,45	30,96
PEITC	2,43	17,00	31,71	29,76

Table 6.

Results of the analysis of variance for the 'Innovator' variety data, χ^2 (N = 7, df = 4) = 11.89 p = 0.018

Variable	Mean rank	Sum of ranks	Mean	Std.dev.
Control	4,00	28,00	27,20	26,26
Gro	2,86	20,00	23,35	20,78
Vydate	4,00	28,00	34,97	38,34
PCL/(PEITC/bentonite)	2,57	18,00	29,89	26,01
PEITC	1,57	11,00	23,52	22,56

Table 7.

Correlation coefficients for mean ranks, n = 5, significance level < 0.05

Variable	Krasnoyarsky Ranniy	Innovator
Krasnoyarsky Ranniy	1,000	0,644
Innovator	0,644	1,000

Table 8.

Spearman's correlation coefficients for mean ranks, significance level < 0.05

Variable	Krasnoyarsky Ranniy	Innovator
Krasnoyarsky Ranniy	1,000	0,711
Innovator	0,711	1,000

Table 9.

Factor analysis (Varimax raw) of the set of traits. Factor loadings > 0.700 are marked

Variable	Factor 1	Factor 2	Factor 3
Plant height	0,677	-0,068	0,484
Stem mass	0,486	0,510	0,286
Tuber mass	**0,812**	0,300	0,231
Mass per tuber	**0,936**	-0,051	-0,025
Number of cysts	-0,153	-0,122	**-0,868**
Number of stems	0,072	**0,843**	-0,073
Number of tubers	-0,036	**0,733**	0,484
Total variance	2,262	1,622	1,365
Proportion of total variance	0,323	0,231	0,195

The absence of a statistically significant difference between the genotypes indicates that the obtained results are due to the impact of the applied treatments. Moreover, the efficacy of the preparation will vary depending on the potato variety. For the 'Innovator' variety, preference should be given to the experimental formulation PCL/(PEITC/bentonite).

Factor analysis was applied to study the relationships between the investigated parameters. Based on the primary analysis, three factors with eigenvalues close to or greater than one, were selected for further work. The results are presented in Table 9.

It was established that Factor 1 is associated with such potato plant development parameters as total tuber mass per plant and average mass per tuber. Two parameters were also attributed to Factor 2: the number of stems and tubers per plant. Factor 3 was separately identified with a single trait – the number of nematode cysts on the roots at harvest time. The extracted factors account for 74.9% of the variance. The first factor can be described as “tuber size”. Since potato stems and tubers are directly connected through stolon formation, the second factor can be labeled as “stolon number.” The third factor describes nematode infestation.

Based on the conducted research, it can be stated that there is a variety-specific response of plants to the applied preparations and nematode infection. For the ‘Krasnoyarsky Ranniy’ variety, a substantial effect of the ‘Vydate’ preparation was established on plant height. For the ‘Innovator’ variety, this treatment had a significant influence on stem mass. The experimental preparation PCL/(PEITC/bentonite) had a positive effect on the number of tubers per plant in the ‘Innovator’ variety.

The predominant effect in terms of nematicidal activity was observed for the ‘Vydate’ preparation and the experimental one (PCL/(PEITC/bentonite)). The general view of the plants at harvest is shown in Figure 7.

Conclusion

1. Granules based on polycaprolactone (PCL) and bentonite, loaded with isothiocyanates – allyl isothiocyanate (AITC), benzyl isothiocyanate (BITC), and 2-phenylethyl isothiocyanate (PEITC) – were designed for soil application against the potato cyst nematode.

2. *In vitro* tests confirmed that granules containing PEITC possess a more pronounced nematicidal effect compared to those with BITC and AITC, suppressing the development of second-stage juveniles (J2) of the nematode *Globodera rostochiensis*.

3. In soil experiments, treatment with the granules promotes a reduction in the number of cysts on the roots and is comparable in efficacy to the commercial preparation ‘Vydate’.

4. The application of PCL/(PEITC/bentonite) granules has a positive effect on the yield of the ‘Innovator’ potato variety. The obtained granules are safer than chemical nematicides and could be used as an alternative means for controlling potato cyst nematode in agriculture.

Fig. 7. General view of plants at harvest for the experimental treatments: 1 – 'Vydate', 2 – Control, 3 – Gro, 4 – PCL/(PEITC/bentonite), 5 – PEITC.

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AUTHOR CONTRIBUTIONS

Anna A. Sukhanova, Anatoly N. Boyandin: development of the scientific concept, drafting of the manuscript.

Kristina E. Zabolotnaya: conducting experiments with nematodes, processing the results.

Andrei A. Churakov: conducting the field experiment, processing the results.

Sergei N. Syrtsov, Elena V. Brilkova: preparing the granules, processing the results.

ВКЛАД АВТОРОВ

Суханова А.А., Бояндин А.Н.: разработка концепции научной работы, подготовка текста статьи.

Заболотная К.Е.: проведение экспериментов с нематодами, обработка результатов.

Чураков А.А.: проведение полевого эксперимента, обработка результатов.

Сырцов С.Н., Брилькова Е.В.: изготовление гранул, обработка результатов.

DATA ABOUT THE AUTHORS

Anna A. Sukhanova, PhD in Biology, Senior Researcher
Reshetnev State University of Science and Technology
31, Krasnoyarsky Rabochy Ave., Krasnoyarsk, 660037, Russian Federation
shumilova.ann@mail.ru
SPIN-code: 8540-6543
ORCID: https://orcid.org/0000-0002-5830-1450
ResearcherID: N-6778-2016
Scopus Author ID: 55916360000

Kristina E. Zabolotnaya, Junior Researcher
Reshetnev State University of Science and Technology
31, Krasnoyarsky Rabochy Ave., Krasnoyarsk, 660037, Russian Federation
zabolotnaya1999@bk.ru
ORCID: https://orcid.org/0000-0001-8788-1012

Andrei A. Churakov, Candidate of Agricultural Sciences, Head of the Center for Selection and Seed Production
Krasnoyarsk State Agrarian University
90, Mira Ave., Krasnoyarsk, 660049, Russian Federation
andchurakov@gmail.com
SPIN-code: 5184-9372
ORCID: https://orcid.org/0000-0002-3150-5541
Scopus Author ID: 57214220568

Sergei N. Syrtsov, Researcher
Reshetnev State University of Science and Technology
31, Krasnoyarsky Rabochy Ave., Krasnoyarsk, 660037, Russian Federation
kaideil@list.ru
SPIN-code: 1826-0855
Scopus Author ID: 54413114300

Elena V. Brilkova, Researcher
Reshetnev State University of Science and Technology
31, Krasnoyarsky Rabochy Ave., Krasnoyarsk, 660037, Russian Federation
evmorbril@mail.ru

Anatoly N. Boyandin, PhD in Biology, Senior Researcher
Reshetnev State University of Science and Technology

31, Krasnoyarsky Rabochy Ave., Krasnoyarsk, 660037, Russian Federation
boyandin@biopolymer.pro
SPIN-code: 7990-1174
ORCID: https://orcid.org/0000-0002-9190-2792
ResearcherID: H-1641-2016
Scopus Author ID: 6507584996

ДАННЫЕ ОБ АВТОРАХ

Анна А. Суханова, канд. биол. наук, старший научный сотрудник
Сибирский государственный университет науки и технологий имени академика М. Ф. Решетнева
пр-кт им. газеты Красноярский рабочий, 31, г. Красноярск, 660037, Российская Федерация
shumilova.ann@mail.ru

Кристина Е. Заболотная, младший научный сотрудник
Сибирский государственный университет науки и технологий имени академика М. Ф. Решетнева
пр-кт им. газеты Красноярский рабочий, 31, г. Красноярск, 660037, Российская Федерация
zabolotnaya1999@bk.ru

Андрей А. Чураков, канд. с.-х. наук, руководитель центра селекции и семеноводства
Красноярский государственный аграрный университет
пр. Мира, 90, г. Красноярск, 660049, Российская Федерация
andchurakov@gmail.com

Сергей Н. Сырцов, научный сотрудник
Сибирский государственный университет науки и технологий имени академика М. Ф. Решетнева
пр-кт им. газеты Красноярский рабочий, 31, г. Красноярск, 660037, Российская Федерация
kaideil@list.ru

Елена В. Брилькова, научный сотрудник
Сибирский государственный университет науки и технологий имени академика М. Ф. Решетнева

пр-кт им. газеты Красноярский рабочий, 31, г. Красноярск, 660037, Российская Федерация
evmorbril@mail.ru

Анатолий Н. Бояндин, канд. биол. наук, старший научный сотрудник
Сибирский государственный университет науки и технологий имени академика М. Ф. Решетнева
пр-кт им. газеты Красноярский рабочий, 31, г. Красноярск, 660037, Российская Федерация
boyandin@biopolymer.pro

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