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BIOLOGICAL PROTECTION OF SUGAR BEET SOWINGS FROM BEET NEMATODE

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Goal. *To identify the efficiency of the bionematicide Clariva 156, FS (spores of the bacterium Pasteuria nishizawae, strain Pn 1, 0.02 l/seed unit) against beet nematode in sugar beet sowings.* ***Methods.*** *Laboratory, field. The research was carried out in Vinnytsia region in the years 2017—2019. Small-scale experiments were established in a natural invasive background. To determine the number of beet nematodes, soil samples were taken and analyzed before sowing and before harvesting sugar beet, as well as after the development of the first generation of the parasite. The accounting of sugar beet yield was carried out by weighing all root crops from each plot and counted per hectare of sowing. The sugar content of roots was determined on the on-stream line Venema by the method of cold-water digestion.* ***Results.*** *The use of bionematicide Clariva 156, FS allowed to reduce the number of the first generation of beet nematode by 27.7—35.3% (1.5 times) compared to the density of its population before sowing sugar beet. It is noted that the decrease in the number of parasites in the soil in the first half of the growing season had a positive effect on crop productivity. In particular, in the treatments where the seeds were treated with the biological preparation Clariva 156, FS, root yield increased by 2.3—12.6 t/ha, and the sugar collection increased by 0.5—1.9 t/ha, compared to the treatments without nematicide.* ***Conclusions.*** *Given the widespread and significant damage caused by the beet nematode to sugar beet sowings, the problem of protecting this crop from heteroderosis is still relevant. Its solution begins with a nematological survey of fields and ends with the introduction of an integrated control system, an important element of which is the use of biological methods. The obtained results prove that treatment of sugar beet seeds with bionematicide Clariva 156, FS gives the chance to reduce number of the first generation of beet nematode in sugar beet sowings up to 35%.*

beet cyst nematode; sugar beet; bionematicide; efficiency of action; yield

In many countries of the world, heteroderosis is considered the most dangerous disease that affects the root system of both cultivated plants, primarily sugar beets, and many types of weeds. The causative agent of this disease is the beet cyst-forming nematode *Heterodera schachtii* Schmidt, 1871 — one of the most dangerous parasites that lives in the soil, and its source of nutrition and environment for reproduction and development are only living cells of the plant organism [1—5].

It is currently established that in Ukraine the beet nematode has been detected in 18 regions, and sugar beet yield losses due to heteroderosis can reach 50—70%, in some cases there is complete death of plants (Figs. 1, 2). In particular, the fields with the largest number of *H. schachtii* in the soil are located in Kyiv, Vinnytsia, Cherkasy, Sumy, Chernihiv and Kharkiv regions [4—8]. In addition, it was found that this parasite occurs not only in industrial fields, but also in small private areas, where it parasitizes on fodder and table beets (Fig. 3, 4) [7, 8]. Scientists note that the increase in the harmfulness of the beet nematode in sugar beet crops may be due to several reasons. The main ones are the violation of crop rotation and shortening the time of return of beets and other host plants to the previous place, the lack of systematic nematological field sur-

Fig. 1. Harmfulness of beet nematode in sugar beet sowings (photo L. Suslyk)

Fig. 2. Affected by beet nematode sugar beet root with white females of the parasite (photo K. Kalatur)

veys and effective nematicides [7, 8]. The first and second reasons can be eliminated by following the recommended crop rotation or by introducing special anti-nematode crop rotations with the inclusion of crops «hostile» to the beet nematode, by inspecting the fields in autumn or spring for the presence of the parasite in soil samples. But the use of nematicides has a negative impact on the environment, useful flora, fauna and human health [8, 9]. Therefore, they are prohibited in many countries of the world, in particular in Ukraine. Considering this situation and the constant growth of environmental requirements for the cultivation of any agricultural crop, scientists suggest using alternative environmentally friendly, but at the same time effective methods of protection against the beet nematode. In particular, use to treat seeds of sugar beet nematicides of biological origin [10, 11].

Goal — to identify the efficiency of the bionematicide Clariva 156, FS (spores of the bacterium *Pasteuria nishizawae*, strain Pn 1, 0.02 l/seed unit) against beet nematode in sugar beet sowings.

Materials and methods of research. The research was conducted in the years 2017—2019 in Vinnytsia region (Uladovo-Lyulinetska research and breeding station (ULRBS, Kalinov district), Khmilnyk (Khmilnytskyi district), Verbivka village (Lypovetskyi district). Laid small plots on a natural invasive background. Plot size — 13.5 m^2, placement — randomized, repeatability of the experiment — four times.

To determine the number of beet nematode, soil samples were taken before sowing and before sugar beet harvesting, as well as after the development of the first generation of the parasite.

In the laboratory, the density of the beet nematode population in the soil was determined by the flotation method by the number of cysts, eggs and larvae isolated from 100 cm^3 of soil. To do this, the soil samples were thoroughly mixed, sieved through a sieve with a hole diameter of 2 mm and dried in air to air-dry state. Next, a soil with a volume of 100 cm^3 was poured into a beaker with a capacity of 1 liter and filled with 2/3—3/4 with water. The soil was stirred with a glass rod for 2—3 minutes, then the contents of the glass were allowed to settle for 5 minutes until the precipitate. The upper layer of water with surfaced cysts and organic particles was poured on a sieve with a hole diameter of 0.1—0.2 mm. This procedure was repeated three times, adding water to a glass. The sieve precipitate was washed with a rubber pear into a funnel with a in filter. After filtering, the filter was removed from the funnel and examined under an MBS-9 microscope to count cysts. The cysts found on the filter were transferred to a drop of water on a glass slide and counted.

Determination of the completeness of beet nematode cysts was performed by counting the number of larvae and eggs in them. To do this, cysts isolated from the soil with a scalpel or dissecting needle were collected from the filter on the edge of the slide in a drop of water, covered with another slide and squeezed both slides with your fingers. They were then separated and both ends with crushed cysts were rinsed in a 200 ml glass beaker in 100 ml of water. Using a pipette with a capacity of 10 ml, the suspension

a *b*

Fig. 3. Symptoms of the lesion beet nematode of table (a) and fodder (b) beets sowings (photo K. Kalatur)

Fig. 4. Cysts of beet nematode (photo K. Kalatur)

was purged and immediately taken 1 ml in the counting chamber. This operation was repeated 3—4 times. Larvae and eggs were counted at a magnification of 1:4 in each replicate [12].

The accounting of sugar beet yield was carried out by weighing all root crops from each plot and counted per hectare of sowing [13]. The sugar content of roots was determined on the on-stream line Venema by the method of cold-water digestion [14].

Results and discussion. Today, the bionematicide Clariva 156, FS (0.02 l/s.u.), has been developed and successfully tested in the USA and some European countries, the active substance of which is the spores of *Pasteuria nishizawae* strain Pn 1 [15, 16]. The mechanism of action of this drug is very simple. After sowing, the spores of the bacteria get into the soil, where they create a protective zone around the germinating beet seeds (3—5 cm). At the same time, the larvae of the beet nematode, which emerged from the cyst, begin to move in the direction of the young root and find themselves in a protective zone, where spores of *P. nishizawae* are attached to their cuticle. Later, a spore tube is formed from the spores, which quickly germinates inside the body of the larva. Affected larvae stop moving, can not enter the plant, feed and reproduce. Eventually, the bacteria destroy their internal organs and they die. Gradually, the dead bodies of the larvae decompose, and the bacterial spores inside get back into the soil, and their development cycle starts all over again [15—17].

Currently, the effectiveness of bionematicide Clariva 156, FS against sugar beet heteroderosis and its positive effect on crop productivity is confirmed by the results of studies conducted in fields with medium and high population density of beet nematode in the soil (Table 1).

It was found that despite the different presowing numbers of nematodes in the experimental fields (from 291 to 1966 eggs + larvae/100 cm³ of soil) the use of biological product Clariva 156, FS negatively affected the development of the first generation of *H. schachtii*. Thus, the largest reduction in the nematode population occurred in the research field of ULRBS — almost 1.5 times, or 35.3% compared to its number before sowing beets. Similar results were obtained in other experimental fields, where the use of bionematicide Clariva 156, FS allowed to reduce the number of the first generation of beet nematode by 27.7—29.2%, or 1.4 times. It should be noted that in the control variant, where the seeds were not treated with the drug, the population density of the nematode in the first month of the sugar beet growing season increased by an average of 1.2 times.

Analysis of soil samples taken before beet harvest showed a multiple increase in the number of parasites in all variants of the experiment. The results of the research are consistent with other experiments that have shown that bionematicide Clariva 156, FS effectively protects plants only in the first 30 days after the beginning of the growing season. Scientists suggest that by the end of the growing season, beets form a significant root system that extends beyond the protective zone created by spores of the bacterium *P. nishizawae*, and beet nematode larvae can easily penetrate the plant [15]. It is also necessary to take into account the biological features of *H. schachtii* — the ability to develop in several generations per year. By developing the second and third generation, this pathogen can increase its number in the soil several times and thus eliminate the negative impact of the drug on the first generation [4, 7, 8]. However, scientists hope that under favorable environmental conditions, the spores of the bacterium *P. nishizawae* will be able to multiply in the soil and provide a longer period of plant protection against heteroderosis. In addition, currently scientists recommend combining several elements of biological control of the number of beet nematode — to carry out treatment with bionematicide Clariva 156, FS seeds hybrids of sugar beets, tolerant to heteroderosis [16]. Such double protection will increase the efficiency of application of various components of the biomethod and will allow to restrain the mass re-

1. The efficiency of the bionematicide Clariva 156, FS for the treatment of sugar beet seeds against beet nematode (2017—2019)

№	Experiment variant	Number of beet nematode, eggs + larvae/100 cm³ of soil			Efficiency of nematicide action against the first generation of nematode, %
		before sowing sugar beet	after development of the first generation of nematode	before sugar beet harvesting	
Uladovo-Lyulinets research and breeding station (ULRBS) (production field, Kalinov district, Vinnytsia region)					
1.	Control — seeds not treated with nematicide	1966	2536	5547	–
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	1880	1342	6572	28.6
Uladovo-Lyulinets research and breeding Station (ULRBS) (research field, Kalinov district, Vinnytsia region)					
1.	Control — seeds not treated with nematicide	1655	2224	12359	–
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	875	566	7288	35.3
Khmilnyk (Khmilnytskyi district, Vinnytsia region)					
1.	Control — seeds not treated with nematicide	498	597	826	–
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	291	206	517	29.2
Verbivka village (Lypovets district, Vinnytsia region)					
1.	Control — seeds not treated with nematicide	1713	1863	3706	–
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	1577	1140	3670	27.7

production of *H. schachtii* throughout the beet growing season.

Despite the restoration of the population density of the beet nematode in the second half of the sugar beet growing season, the use of bionematicide Clariva 156, FS allowed to protect young plants in the most vulnerable initial stages of their growth and development. It is noted that the reducing the number of parasite in the soil in the first half of the growing season not only contributed to the preservation of beet yields in fields infected with beet nematode, but also provided a significant increase in productivity. In particular, in the variants of the experiment, where the seeds were treated with the biological preparation Clariva 156, FS, the yield of sugar beets increased by 2.3—12.6 t/ha, and the sugar collection increased by 0.5—1.9 t/ha, compared to the variant where nematicide was not used (Table 2).

The research was conducted within the framework of the Scientific Research Program 27 «Creation of competitive sugar beet hybrids and development of technological measures to realize their biological potential» according to task 27.00.03. 01 F «Establish the biological basis of screening signs of sugar beet resistance to the beet nematode».

CONCLUSIONS

Given the widespread and significant damage caused by the beet nematode to sugar beet sowings, the problem of protecting this crop from heteroderosis is still relevant. Its solution begins with a nematological survey of fields and ends with the introduction of an integrated control system, an important element of which is the use of biological methods. The obtained results prove that treatment of sugar beet seeds with bionematicide Clariva 156, FS gives the chance to reduce number of the first generation of beet nematode in sugar beet sowings up to 35%.

2. Productivity of sugar beets for seed treatment with bionematicide Clariva 156, FS against beet nematode (2017–2019)

№	Experiment variant	Indicators of sugar beet productivity		
		yield, t/ha	sugar content, %	sugar collection, t/ha
Uladovo-Lyulinets research and breeding station (ULRBS) (production field, Kalinov district, Vinnytsia region)				
1.	Control — seeds not treated with nematicide	31.7	16.8	5.3
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	34.0	17.0	5.8
Uladovo-Lyulinets research and breeding Station (ULRBS) (research field, Kalinov district, Vinnytsia region)				
1.	Control — seeds not treated with nematicide	13.6	12.8	1.7
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	26.2	13.7	3.6
Khmilnyk (Khmilnytskyi district, Vinnytsia region)				
1.	Control — seeds not treated with nematicide	32.8	17.3	5.7
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	37.6	17.3	6.5
Verbivka village (Lypovets district, Vinnytsia region)				
1.	Control — seeds not treated with nematicide	30.4	16.5	5.0
2.	Seeds treated with nematicide Clariva 156, FS (0.02 l/s.u.)	40.0	17.0	6.8

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Біологічний захист посівів буряків цукрових від бурякової нематоди

Мета. *Визначити ефективність дії біонематоциду Кларіва 156, ТН (спори бактерії Pasteuria nishizawae, штам Pn 1, 0,02 л/п.о.) проти бурякової нематоди в посівах буряків цукрових.* **Методи.** *Лабораторно-польовий. Досліджували впродовж 2017—2019 рр., у Вінницькій області. Закладали дрібноділянкові досліди на природньому інвазійному фоні. Для визначення чисельності бурякової нематоди відбирали та аналізували зразки ґрунту до сівби та перед збиранням буряків цукрових, а також після розвитку першого покоління паразита. Облік врожаю буряків цукрових здійснювали шляхом зважування всіх коренеплодів з кожної ділянки і перераховували на гектар посіву. Цукристість коренеплодів буряків цукрових визначали на поточній лінії «Венема» методом холодного водного дигерування.* **Результати.** *Встановлено, що застосування біонематоциду Кларіва 156, ТН дозволило знизити чисельність першого покоління бурякової нематоди на 27,7—35,3%, (в 1,5 раза) порівняно з щільністю її популяції до сівби буряків цукрових. Відзначено, що зменшення чисельності паразита в ґрунті у першій половині вегетації позитивно вплинуло на показники продуктивності культури. Зокрема, на варіантах досліду, де насіння обробили біологічним препаратом Кларіва, врожайність буряків цукрових збільшилася на 2,3—12,6 т/га, а збір цукру зріс на 0,5—1,9 т/га, порівняно з варіантом, де нематоцид не застосовували.* **Висновки.** *Зважаючи на широке розповсюдження та значну шкоду, яку спричиняє бурякова нематода посівам буряків цукрових, проблема захисту цієї культури від гетеродерозу не втрачає актуальності. Вирішення її починається з нематологічного обстеження полів і закінчується запровадженням системи інтегрованого захисту, важливим елементом якої є застосування біологічного методу. Одержані результати досліджень переконують, що використання для обробки насіння буряків цукрових біонематоциду Кларіва 156, ТН дає можливість зменшити чисельність першого покоління бурякової нематоди в посівах культури до 35%.*

бурякова цистоутворююча нематода; буряки цукрові; біонематоцид; ефективність дії; урожайність

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