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THE EFFECT OF LIQUID FILTERING PARAMETERS ON INSECTICIDAL NEMATODES SURVIVAL (VIABILITY) - BIOLOGICAL PLANT PROTECTION PRODUCT

Summary

The described research dealt with the viability of insecticidal nematodes contained in the spray liquid flowing through filters used in spraying machines. Steinernema feltiae entomopathogenic nematodes were used in the research. The liquid was filtered using two filters mounted in spraying machines with 50 and 100 mesh number. Concentration of nematodes in the liquid was: 1 million and 2 million in 1 dm^3 water. The liquid flowed at three pressure values: 1x10^5, 2×10^5 and 4×10^5 Pa. Significance of nematodes loss with the following liquid flow was observed: through 50 mesh filter (at pressure 4×10^5 Pa) and through 100 mesh filter (at all flow pressure values).
***Key words**: organic farming, entomopathogenic nematodes, sprayer, filters*

WPŁYW PARAMETRÓW FILTROWANIA CIECZY NA PRZEŻYWALNOŚĆ ZAWARTYCH W NIEJ OWADOBÓJCZYCH NICIENI - BIOLOGICZNEGO CZYNNIKA OCHRONY ROŚLIN

Streszczenie

Opisano badania nad zmianami przeżywalności owadobójczych nicieni zawartych w cieczy opryskowej przepływającej przez filtry stosowane w opryskiwaczach. Do badań użyto nicieni owadobójczych gatunku Steinernema feltiae. Ciecz filtrowana była za pomocą dwóch filtrów montowanych w rozpylaczach o liczbie mesh 50 i 100. Koncentracja nicieni w cieczy wynosiła: 1 mln i 2 mln sztuk w 1 dm^3 wody. Przepływ cieczy odbywał się przy trzech wielkościach ciśnienia: 1x10^5, 2×10^5 i 4×10^5 Pa. Stwierdzono istotność strat nicieni przy przepływie cieczy: przez filtr mesh 50 (przy ciśnieniu 4×10^5 Pa) i przez filtr mesh 100 (przy wszystkich wartościach ciśnienia przepływu). Wyniki pokazały, że na straty nicieni istotny wpływ miały: numer siatki filtra i ciśnienie cieczy wewnątrz instalacji.
***Słowa kluczowe**: rolnictwo ekologiczne, owadobójcze nicienie, opryskiwacz, filtry*

1. Introduction

As a biological plants protection agent, etomopathogenic nematodes gained recognition for their lack of impact on both the natural environment and humans. Thanks to technology facilitating mass production, infectious larvae of insecticidal nematodes are a commonly available agent which is quite affordable in use. The formulation is also easy to apply, it just needs to be mixed with the right amount of water and applied into soil or onto leaves using spraying machines. The current research indicates that some of the spraying machines' elements might contribute to death of the nematodes. The spraying machine installation parts that, according to the research, cause the greatest losses in nematodes are: hydraulic mixer, filters and nozzles. The nematodes destruction might result from the spraying process parameter and physical phenomena that affect the liquid flowing through the spraying machine installation, such as: high liquid pressure and heating up of the liquid [1, 5, 8, 15].

The resting-invasive larvae of *Steinernematidae* and *Heterorhabditidae* nematodes are used in biological plants protection. Their digestive tract contains bacteria of *Xenorabdus* or *Photorabdus* species. The bacteria freed by the nematodes after penetrating the host's body cause paralysis and death. Nematodes larvae are highly active when looking for victims. They move freely through the soil. Their body is adapted to surviving in difficult environmental conditions. One of the crucial features that help it adapt to predation is the ability to identify the victim and a quick reaction. Entomopathogenic nematodes species are used to eliminate: fungus gnat larvae, otiorrynchus, sitonae, beetles (e.g. potato beetles), dipteran larvae (e.g. carrot fly), butterfly caterpillars (e.g. cabbage moth, cabbage butterfly, winter moth), as well as Western flower thrips, preying in greenhouses and storage rooms. *Pfasmarhabditis hermaphrodita* species nematodes, used for eliminating slugs, are also available on the market. [4, 12, 13, 14].

Biopreparations with nematodes are also used in forestry. The research shows that *Steinernema carpocapsae* and *Steinernema feltiae* species can be used to control large pine weevil larvae (*Hylobius abietis*), which is the largest forest plantations pest feeding on young plants [3, 17]

The presence of filters in the spraying machine installation is vital for protecting specific installation elements against damage caused by contaminated working liquid. Producers of plants protection agents that contain nematodes recommend removing all filters from spraying machines as their presence may allegedly cause losses in nematodes during application. Flowing through the tiny holes of the filter under the pressure of the liquid nematodes can be damaged or may clog the filtrating surface [6, 9, 10, 11, 18], even though this was not confirmed by research [2].

Selection of equipment and liquid pressure for applying entomopathogenic nematodes are not the only factors influencing effectiveness of the process. What is also important is selecting optimum intensity of discharge of the liquid from the spraying machine, which may influence the number of times the liquid flows through the installation [2].

2. Aim of the study

The aim of the study was to investigate the effect of filtering holes density in the filter and the pressure of the liquid flowing through the filter to losses of selected insecticide nematode species mixed with this liquid

3. Research methodology

3.1 Research material

Steinernema – System preparation produced by Biobest Belgium N.V. (Fig. 1) was used as the research material. Invasive larvae of *Steinernema feltiae* nematodes species were its active ingredient.

3.2 Test bench

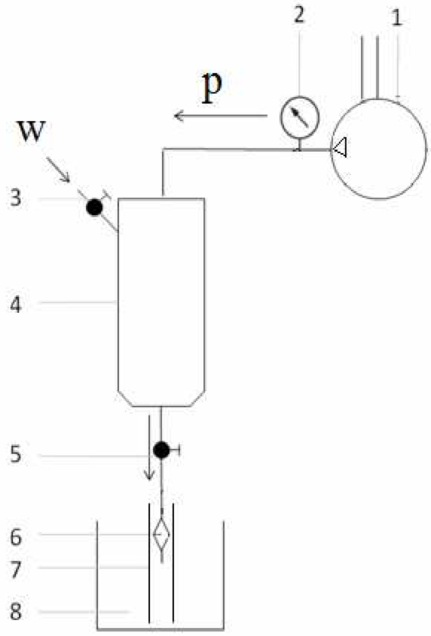
A test bench was constructed for the experiments. An outline of the installation is presented in Fig. 2.

Source: own work / Źródło: opracowanie własne

Fig. 1. Steinernema - System product containing Steinernema feltiae entomopathogenic nematodes
Rys. 1. Produkt Steinernema- System zawierający owadobójcze nicienie Steinernema feltiae

The main element of the construction included the container (4) with capacity of 4,8 l, made of a steel pipe (length: 380 mm) with square cross-section (120 mm). The top part of the container was closed with a lid made of steel sheet welded to the pipe. A brass pressure coupler was welded to the central part of the lid, used to connect the hose supplying compressed air. Compressed air from the compressor was used to create pressure of the liquid in the container. A gate (w) in the form of a tube, closed with a globe valve (3) with diameter of ½ inch was welded to one of the side walls in the top part at a 45° angle.

The bottom of the container was made of externally convex sheet, welded to the sides of the container. Such a shape of the bottom was supposed to guarantee the outflow of the entire liquid volume, preventing minor amount of the liquid from remaining at the bottom of the container. A discharge tube 85 mm long, ended with a globe valve, was welded to the central part of the container bottom. A flexible tube in the form of an armored plastic hose Ø 1,8 mm, 115 mm long, tightened with a metal band, was mounted on the other side of the globe valve. A screw coupler made of plastic was fixed to the hose, used for mounting the examined filter (6). The length of the entire discharge tube with the filter was 350 mm. On the outside the filter and the cord were inserted into an additional plastic protective cord, placed vertically at the bottom of the container, whose job it was to have the liquid maintained around the filter. Having flowed through the filter, the liquid flowed through the top part of the cover cord into the container.



Source: own work / Źródło: opracowanie własne

Fig. 2. Diagram presenting the test bench construction: 1- compressor, 2- manometer, 3 - valve opening outflow of the liquid from the container, 4 - container, 5 - ball valve, 6 - filter, 7- pipe covering up the filter, 8 - vessel
Rys. 2. Schemat budowy instalacji badawczej: 1- kompresor, 2 - manometr, 3 - zawór otwierający wypływ cieczy ze zbiornika, 4 - zbiornik, 5 - zawór kulowy, 6 - filtr, 7 - przewód osłaniający filtr, 8 - naczynie

Two individual atomizer filters TeeJet ®: 55215-50 and 55215-100 were used in the tests (Fig. 3). Individual nozzle filters are an important element of the spraying machine liquid system as they constitute the last stage of filtering the working liquid before it passes through the atomizers' apertures.

The filters were equipped with a stainless-steel net, placed in a finned plastic body. The filtering surface was of the following dimensions: length -18 mm, diameter - 9 mm. The filters had different density of the steel net holes, expressed in the mesh number. Red filter 55215-50 – mesh number 50, green filter 55215-100 – mesh number 100.

Source: own work / Źródło: opracowanie własne

Fig. 3. Individual nozzle filters: 55215-100 and 55215-50
Rys. 3. Indywidualne filtry rozpylaczy: 55215-100 i 55215-50

Three factors were variable components in the tests: I - concentration of infectious nematodes larvae in the liquid, II - filter net hole density expressed with the mesh number and III - the pressure at which the liquid flowed through the filter. Liquid with nematode concentration of 1 mln pcs. x dm^{-3} and 2 mln pcs. x dm^{-3} flowed through mesh 50 and mesh 100 filters at three working pressure values for each filter: 1x 10^5 Pa, 2x 10^5 Pa, 4x 10^5 Pa.

Absolute viability (survival) was expressed as the number of live nematodes found in equal measurement amounts of the liquid, as well as the change in the absolute viability of the nematodes as the difference between the absolute viability in the liquid prior to flowing through the test installation, and the absolute viability in the liquid after flowing through the installation.

Significance of the change in absolute viability was calculated using t – Student statistics determined for the assumed significance level α=0,05.

In order to show which filtration parameter had the greatest impact on the nematode losses, a multifactor analysis of variance was conducted. What was calculated it was the ratio between the number of nematodes in the measurement samples from liquid samples collected after flowing through the filter – and the arithmetic mean of results of samples collected from the liquid prior to filtration as control samples. Results presented in this way were respectively divided into groups according to factors and their levels.

3.3. Research

Two water slurries were prepared for the tests (temp. 18-20°C) with the Steinernema – System preparation, each of 10 dm^3 volume. The liquid containing the nematodes was intensively mixed in order to prevent the nematodes from settling at the bottom of the container, and to obtain an even concentration of the nematodes in the liquid. Two samples of 0,03 dm^3 were collected from each dm^3 of the liquid after stirring, in order to determine the initial absolute viability of the nematodes.

The liquid with nematodes was poured into the container through gate - w. Next valve – 3 was closed, the compressor was turned on. The required pressure of air in the container was determined using the control valve mounted in the compressor. Having obtained the right pressure, valve 5 was opened, causing the liquid with nematodes to flow through the filter.

After the liquid flowed through the test installation, two samples were collected from it in order to determine the absolute viability. After each test, the containers and filters were thoroughly cleaned. Samples with liquid for analysis of nematodes viability were numbered and placed in the refrigerator for 24 hours. It was assumed that during that time the wounded nematodes larvae would cease to live. After 24 hours, after stirring, measurement samples were collected from each sample for analysis under a stereoscopic microscope. Six measurement samples were collected from each liquid sample for optical evaluation of the number of nematodes. In the samples of liquid with concentration of nematodes 1 mln pcs. x dm^{-3}, the measurement samples volume was 0,050 cm^3, while in those with concentration of 2 mln pcs. x dm^{-3} it was 0,025 cm^3. Because of the mobility of live nematodes, it was very easy to tell the live organisms from the dead ones. In case of doubt as to whether a given nematode was alive or not, its body was pinched with a needle and it was observed whether it reacted with movement.

4. Results

Table 1 presents analysis of the test results. On basis of the t – Student significance test it was concluded which of the calculated changes in the absolute viability of nematodes in the liquid were significant.

The variance analysis table (Table 2) presents amounts organized by factors, calculated on basis of analysis of variance formulas [7, 16]. The significance of operation of a factor or factors' correlation was calculated using the F- Snedecor's test according to the following relation: if $F_{calc.} > F_{crit.}$ – influence of the factor significant for the adopted significance level α=0,05.

Table 1. Analysis of the test results 55215 - 50 filter, significance of change in the absolute viability of the nematodes
Tab. 1. Analiza wyników badań filtra 55215 - 50, istotność zmiany przeżywalności bezwzględnej nicieni

Filter 55215 – 50								
	Pressure [Pa]	Average viability from liquid samples taken before / after filtration		Standard deviation [S]	Variance [S^2]	Change in viability	Value |t|	The significance of changing nematode viability
Concentration of nematodes in suspension: 1mln pcs. x dm^{-3}	1x10^5	$\bar{x}_1$	50,67	2,23	4,964	0,33	0,447	Insignificant
		$\bar{x}_2$	50,33	1,80	3,245			
	2×10^5	$\bar{x}_1$	56,58	1,76	3,089	0,83	1,421	Insignificant
		$\bar{x}_2$	55,75	1,83	3,351			
	4×10^5	$\bar{x}_1$	64,33	2,60	6,749	6,08	7,158	Significant
		$\bar{x}_2$	58,25	1,61	2,597			
Concentration of nematodes in suspension: 2 mln pcs. x dm^{-}	1x10^5	$\bar{x}_1$	55,83	1,78	3,154	0,92	1,707	Insignificant
		$\bar{x}_2$	54,92	1,66	2,751			
	2×10^5	$\bar{x}_1$	60,00	1,23	1,521	1	1,986	Insignificant
		$\bar{x}_2$	59,00	2,00	4,017			
	4×10^5	$\bar{x}_1$	63,33	1,43	2,039	9,17	24,091	Significant
		$\bar{x}_2$	54,17	1,46	2,146			

Source: own work / Źródło: opracowanie własne

Table 2. Analysis of test results 55215 - 100 filter, significance of change in the absolute viability of the nematodes
Tab. 2. Analiza wyników badań filtra 55215 - 100, istotność zmiany przeżywalności bezwzględnej nicieni

Filter 55215 – 100								
	Pressure [Pa]	Average viability from liquid samples taken before / after filtration		Standard deviation [S]	Variance [S^2]	Change in viability	Value |t|	The significance of changing nematode viability
Concentration of nematodes in suspension: 1 mln pcs. x dm^{-3}	$1x10^5$	$\bar{x}_1$	58,92	1,94	3,760	3,92	5,026	Significant
		$\bar{x}_2$	55	2,19	4,810			
	2×10^5	$\bar{x}_1$	57,00	2,17	4,711	6,42	5,750	Significant
		$\bar{x}_2$	50,58	2,75	7,561			
	4×10^5	$\bar{x}_1$	59,67	2,30	5,295	9,5	7,939	Significant
		$\bar{x}_2$	50,17	2,80	7,865			
Concentration of nematodes in suspension: 2 mln pcs. x dm^{-3}	$1x10^5$	$\bar{x}_1$	61,92	1,71	2,917	5,42	13,71	Significant
		$\bar{x}_2$	56,50	1,20	1,430			
	2×10^5	$\bar{x}_1$	58,33	2,82	7,972	7,17	5,077	Significant
		$\bar{x}_2$	51,17	2,75	7,551			
	4×10^5	$\bar{x}_1$	62,67	1,92	3,725	12,42	29,887	Significant
		$\bar{x}_2$	50,25	0,92	0,845			

Source: own work / Źródło: opracowanie własne

Table 3. Multifactor analysis of variance to determine the significance of influence of factors and their correlation on the nematode viability
Tab. 3. Wieloczynnikowa analiza wariancji dla stwierdzenia istotności wpływu czynników i ich korelacji na przeżywalność nicieni

Type of factor	Sum of squares	Number of degrees of freedom	Average squared	*F*- calculated	Significance of factor influence or correlation of factors
I - Pressure	0,293	2	0,146	11,407	Significant
II – Filter type	0,205	1	0,205	15,976	Significant
Factor **I** *x* **II**	0,013	2	0,006	0,505	Insignificant
III – Nematode concentration	0,017	1	0,017	1,356	Insignificant
Factor **I** *x* **III**	0,010	2	0,005	0,374	Insignificant
Factor **II** *x* **III**	0,000	1	0,000	0,006	Insignificant
Factor **I** *x* **II** *x* **III**	0,001	2	0,000	0,036	Insignificant

Source: own work / Źródło: opracowanie własne

5. Conclusions

1. The results show that the liquid filtering parameters that had significant influence on losses of nematodes were the filter mesh number and working pressure at which the liquid flew through the test installation.
2. It was concluded that the flow of liquid containing insecticidal nematodes through the filter with 50 mesh number, at the pressure of $1x10^5$ Pa and 2×10^5 Pa did not cause significant changes in the organisms viability, while liquid flow rate with pressure of 4×105 Pa caused considerable nematodes loss.
3. Flow of the slurry with Stei-nernema feltiae species nematodes through the filter with 100 mesh number at the range of working pressure from $1x10^5$ Pa to 4×10^5 Pa caused considerable losses of nematodes.
4. No significant influence of entomopathogenic nematodes concentration in the liquid onto nematodes viability during liquid flow through 50 and 100 mesh filters was observed.

6. References

[1] Chojnacki J.: Szacowanie spadku przeżywalności owadobójczych nicieni w instalacji opryskiwacza. Journal of Research and Applications in Agricultural Engineering, 2011, 56(3).

[2] Chojnacki J.: Wpływ filtra siatkowego zamontowanego w instalacji opryskiwacza na straty owadobójczych nicieni. Journal of Research and Applications in Agricultural Engineering, 2012, 57(3).

[3] Dillon A.B., Ward D., Downes M.J., Griffin C.T.: Suppression of the large pine weevil *Hylobius abietis* (L.)(*Coleoptera: Curculionidae*) in pine stumps by entomopathogenic nematodes with different foraging strategies. Biological Control, 2006, 38.

[4] Dzięgielewska M., Erlichowski T.: Wykorzystanie nicieni owadobójczych do biologicznego zwalczania szkodników glebowych w uprawach rolniczych. Ziemniak Polski, 2010, 4.

[5] Fife J.P., Ozkan H.E., Derksen R.C., Grewal P.S., Krause C.R.: Viability of a biological pest control agent through hydraulic nozzles. Trans. ASAE, 2005, 48.

[6] Garcia L.C., Raetano C.G., Leite L.G.: Application technology for the entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* sp. *(Rhabditida: Heterorhabditidae* and *Steinernematidae)* to control *Spodoptera frugiperda* (Smith) *(Lepidoptera: Noctuidae)* in corn. Neotrop. Entomol., 2008, 37.

[7] Greń J.: Modele i zadania statystyki matematycznej. PWN, 1967.

[8] Grewal P.S.: Formulation and application technology. Entomopathogenic nematology. CABI Publishing, 2002.

[9] Hołownicki R.: Technika opryskiwania roślin dla praktyków. Plantpress, Kraków, 2006.

[10] Kamionka J.: Technika Ochrony Roślin, Instytut Technologiczno-Przyrodniczy, Mazowiecki Ośrodek Badawczy w Kłudzienku, Falenty-Kłudzienko, 2014.

[11] Klein M.G., Georgis R.: Application techniques for entomopathogenic nematodes. Proceedings of the 6th International Colloquium on Invertebrate Pathology and Microbial Control, France, 1994.
[12] Kowalska J., Pruszyński S.: Metody i środki proponowane do ochrony roślin w uprawach ekologicznych. Instytut Ochrony Roślin, Poznań, 2007.
[13] Kowalska J.: Wzajemne powiązania pomiędzy nicieniami owadobójczymi, owadami i bakteriami oraz ich wykorzystanie w praktyce. Wiadomości Parazytologiczne, 2006, 52(2).
[14] Makulec G., Franczak R., Ilieva-Makulec K.: Nicienie glebowe na polu i odłogu - zagęszczenie i różnorodność. Studia Ecologiae et Bioethicae, 2011, 2.
[15] Nilsson U., Gripwall E.: Influence of application technique on the viability of the biological control agents *Vertcillium lecanii* and *Steinernema feltiae.* Crop Protection, 1999, 18(1).
[16] Pawłowski Z.: Statystyka matematyczna. PWN, Warszawa, 1976.
[17] Skrzecz I.: Tempo rozprzestrzeniania się nowych inwazyjnych szkodników i patogenów drzew w ekosystemach leśnych w Europie na podstawie modelowania komputerowego w latach 2014-2100. Leśne Prace Badawcze, 2014, 75(3).
[18] Tomalak M.: Ekspertyza: Czynniki biologiczne dostępne w ochronie upraw ekologicznych przed szkodnikami. Zakład Biologicznych Metod i Kwarantanny, Instytut Ochrony Roślin, Państwowy Instytut Badawczy, Poznań, 2009.

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