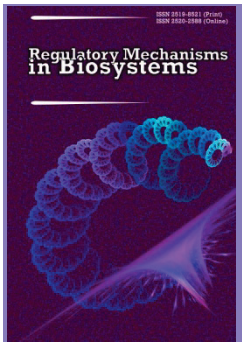

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Impact of mineral fertilizers, growth stimulators, pH regulators, vitamins and pigment supplements on the vitality of entomopathogenic nematodes of Steinernematidae and Heterorhabditidae families

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Parasites of agricultural crops cause significant losses of quality and decrease in the profitability of agricultural production. Complex measures against pests are aimed both at termination of parasites and prevention of repeated infection of plants. One of the most progressive ways of protecting plants against harmful insects is the use of entomopathogenic nematodes. This method is most expedient in the conditions of organic arable farming. The results of our study reveal the impact of the fertilizers in various concentrations on the vitality of nematode larvae (L_3) of four species (*Steinernema feltiae*, *S. kraussei*, *S. carpocapsae* and *Heterorhabditis bacteriophora*) and the possibility of their combined application for the treatment of plants. Mineral fertilizers and stimulators of growth of plants in 1% concentration insignificantly reduced the vitality of larvae, allowing them to be applied at the same time. We determined the tolerance of invasive larvae to pH for *S. carpocapsae* equaling pH = 0.9–13.4; optimum values of pH without reliable increase in the mortality during 24 h – pH = 1.3–12.8. Increase in the vitality of larvae (L_3) of entomopathogenic nematodes was studied. The survivability of the cultures of larvae increased during the use of solutions of vitamins C, B_1, B_6, B_{12}. We determined the influence of 21 pigment colourings on larvae (L_3), the lowest effect on the vitality of nematode larvae was exerted by pigment bases Abrikos (7.0–10.8% mortality during 24 h), Zolotoi Pesok (6.0–11.8%), Pudra Ananasa (7.7–13.4%), and complex DMAE (7.6–17.4%). The results we obtained allow development of recommendations for agriculturalists for combined use of entomopathogenic nematodes with various substances and also improving the vitality of invasive nematodes.

Keywords: *Steinernema feltiae*; *S. kraussei*; *S. carpocapsae*; *Heterorhabditis bacteriophora*; insect pests; insecticides; range of tolerance.

Introduction

Insect pests of agricultural crops inflict significant economic damage on agricultural businesses. According to the data of FAO, global economic losses in the agricultural sector caused by biological pests in the period of 2005–2015 accounted for US $ 9.5 billion. Prevention of significant loses of yield is possible if one takes an integrated approach to the protection of plants (Dara, 2019). Application of chemical methods of plant protection in agriculture has a negative effect on the environment, polluting water, soil and air (Benhalima et al., 2004; Pimentel et al., 2009). The result of using pesticides is death of useful biota, development of resistance to them in pests. Large-scale and low-controlled use of chemical insecticides in agricultural territories is accompanied by changes in the composition of the structure of the complex of pests, which contributes to the transformation of secondary (potential) pests into economically-significant pests.

Currently, many countries are developing and introducing ecologically clean methods of reducing the number of pests (Koul & Walia, 2009). The most efficient biological methods are the ones that include the use of bacteria, fungi, viruses and entomopathogenic nematodes (Martynov, 2017). During planning of modern biological methods of protection of agricultural crops, it is necessary to take into account risks which may emerge when applying one or the other methods. More and more often insect pests are being combatted using their natural enemies – parasites, predators or disease pathogens (Khachatourians, 1986; Lasey et al., 2001). Over the two recent decades, a large amount of biological preparations for protection of plants comprises entomopathogenic nematodes. The first mentions about the nematode-caused diseases of insects could be found back in the XVII century (Nickle, 1984), but only in the XX century has the serious interest in the study of practical approaches to the use of nematodes in regulation of the number of pests of agricultural crops emerged. Over the recent years studies on this group of parasites have noticeably intensified. Since 1990, 23 species of entomopathogenic nematodes were described – this was over 70% of the total amount of the discovered species of this type.

Nematodes can infect a large number of species of insects, infecting them in different stages of the development, except the egg; they are adapted to long dwelling in soil; safe for plants, warm-blooded animals and humans. One of the important peculiarities of nematode parasites is their use against the stages of insects' development which are related to their presence in the soil, in pathways of stems, shoots and tree trunks, i.e. when there is no opportunity of applying any other effective methods of protection (Burnell & Stock, 2000). Nematodes are amenable to mass production and require no specialized equipment – they are compatible with the standard agrotechnical equipment, including various sprinklers and irrigation systems. The survey of entomopathogenic nematodes in more than 60 countries of the world suggests that for the regulation of the number of agricultural pests the most appropriate are the species of two families – Steinernematidae and Heterorhabditidae. There are 32 known species of entomopathogenic nematodes, 25 of which belong to the Stei-

nemematidae family (*Steinernema* genus – 24 species and *Neosteinernema* genus – 1 species) and 7 species to the Heterorhabditidae family (*Heterorhabditis* genus). One of the commonest species is *Steinernema carpocapsae*, the representatives of which are found in all the continents of the world, except Antarctica (Weiser, 1955; Poinar, 1967; Turco et al., 1971; Stanszek, 1974a; Pye & Burman, 1978; Akhurst, 1980; Georgis & Hague, 1981; Poinar, 1986, 1990). *Steinernema feltiae* is a less common species, it is found in Australia (Poinar, 1990), New Zealand (Wouts, 1982; Wright & Jackson, 1988), Tasmania (Poinar, 1986), Africa (Poinar, 1992) and Eurasia (Mracek et al., 1982; Vainio & Hokkanen, 1993; Pollitt et al., 1994). Nematodes of the species *Heterorhabditis bacteriophora* occur in Australia, Italy (Akhurst, 1987), Argentina and the USA (Creighton, 1985; Poinar, 1990; Poinar & Georgis, 1990). The remaining species have a limited range.

Over recent years, the tests of nematodes as insecticides are often accompanied by contradictary data on their efficacy. The reason for this may be the simplified approach to the process of their application for the regulation of number of pests without taking into account the peculiarities of biology and ecology of separate species and strains. Absence of systemic approaches to the study on the activity of entomopathogenic nematodes depending on the conditions of the environment, underdevelopment of effective ways and technologies and application of nematodes, underlie the necessity for further scientific research in this direction.

The objective of the article was studying the effect of mineral fertilezers, growth stimulators, acids, alkali, vitamins and additive pigment on entomopathogenic nematodes of the Steinernematidae and Heterorhabditidae families for the optimization of the technology of their use in agriculture.

Materials and methods

The studies were conducted during 2018–2019. Aqueous cultures of nematode larvae (L_3) of four species (*Steinernema feltiae*, *S. kraussei*, *S. carpocapsae* and *Heterorhabditis bacteriophora*) were put in plastic test tubes of 1.5 mL capacity in the amount of 0.1 mL (50–70 specimens), adding to them 1 mL of the tested substance of a certain concentration. The exposure lasted for 24 h. The studied samples were kept at the temperature of 22 ºC.

At the first stage of the experiment the nematode larvae were exposed to fertilizers in five concentrations of 1%, 0.1%, 0.01%, 0.001%, 0.0001%. In the experiment, we used 7 fertilizers of various groups: ammonium nitrate (NH_4NO_3), ammonium sulfate $(NH_4)_2SO_4$, superphosphate ($Ca(H_2PO_4)_2 \times H_2O + CaSO_4$), Nitrophoska ($N_{16}$:$P_{16}$:$K_{16}$), monopotassium phosphate (P_2O_5 – 50%, K_2O – 33%), magnesium sulfate (MgO – 16%, SO_3 – 32%) and succinic acid (HOOC-CH_2-CH_2-COOH).

At the second stage, we determined the impact of acids and alkali on the survivability of *S. carpocapsae* larvae using 11 concentrations, equaling 20%, 10%, 5%, 2.5%, 1.25%, 0.63%, 0.31%, 0.16%, 0.08%, 0.04%, 0.02%. In the experiment, we used solutions of five acids – hydrochloric acid (HCl), sulfuric acid (H_2SO_4), acetic acid ($C_2H_4O_2$), citric acid ($C_6H_8O_7$), salicylic acid ($C_7H_6O_3$) and solutions of sodium bicarbonate ($NaHCO_3$) and sodium hydroxide (NaOH).

The third stage of the study focused on the effect of vitamins on the survivability of nematode larvae of *S. feltiae*, *S. kraussei*, *S. carpocapsae* and *H. bacteriophora*. Solutions of vitamins of thiamine hydrochloride (B_1), pyridoxine hydrochloride (B_6), cyanocobalamin (B_{12}) and ascorbic acid (C) were studied in the concentrations of 50, 25, 12.5, 6.25 and 3.12 mg/mL, and also complex vitamin preparations Undevit and Komplevit were evaluated according to the concentration of vitamin B_1 – 3000, 600, 120, 24, 4.8 μg/mL.

The final stage was the study on the effect of 1% solutions of 21 colourings (Henné neutre, Rose oxide, Brazilian ginseng powder, Ayurveda powder, Pigment base farfor, sodium alginate, red ochre, *Centella asiatica* powder, Apricot mica, pigment base Bezhevyi Voshod, plant pigment Kashtan, pigment bases Abrikos, Zolotoi Pesok, Kofe, SLSA, xantane, Aristoflex, pineapple powder, DMAE complex, cocamidopropyl betaine).

The results were statistically analyzed in Statistica 8.0 software (Statsoft Inc., USA). The data in Tables 3–7 are presented as x ± SD.

Results

In all the three studied species of nematodes, mortality did not exceed 25% from 24 h exposure to 0.1% solution of ammonium nitrate (Fig. 1). Solution in the concentration of 1% killed 30% of *S. feltiae*, 54% of *S. kraussei* and 63% of *H. bacteriophora*. The impact of ammonium sulfate (Fig. 2) had similar intensity as ammonium nitrate: this type of mineral fertilizer killed no more than 28% of nematodes in the concentration of 0.1% for 24 h. Ammonium sulfate in 1% concentration caused death to 32% of *S. feltiae*, 51% of *S. kraussei* and 54% of *H. bacteriophora*.

Magnesium sulfate in 1% concentration during 24 h exposure killed 17–20% of invasive nematode larvae, for the same period 0.1% concentration caused death to only 6–10% of the worms (Fig. 3). Monopotassium phosphate KH_2PO_4 (Fig. 4) in 0.1% concentration killed 6–10%, and at 1% – only 17–20% of the larvae. Superphosphate was much more active against the studied species of nematodes (Fig. 5): in 24 h the 1% concentration of this fertilizer led to death of 44–68% of the larvae, 0.1% – 12% of *S. feltiae*, 17% of *S. kraussei* and 46% of *H. bacteriophora*. The complex mineral fertilizer Nitrophoska (Fig. 6) caused a slight increase in the mortality of larvae of *S. feltiae* (up to 4% and 8% in aqueous solution respectively) and *S. kraussei* (up to 5% and 8% in 0.1% and 1% concentrations). *H. bacteriophora* increased the death rate from Nitrophoska more efficiently: up to 7% and 17% in 0.1% and 1% concentrations. Succinic acid (Fig. 7) even in 1% concentration exhibited low increase in the mortality of nematode larvae: up to 6% in *S. feltiae* and 7% in *S. kraussei* and *H. bacteriophora*.

To determine acid osmotic tolerance on the example of *S. carpocapsae* larvae, we used solutions of acids (hydrochloric, sulfuric, acetic acid, citric, salicylic), sodium bicarbonate and sodium hydroxide in 11 concentrations, in 6-fold replication.

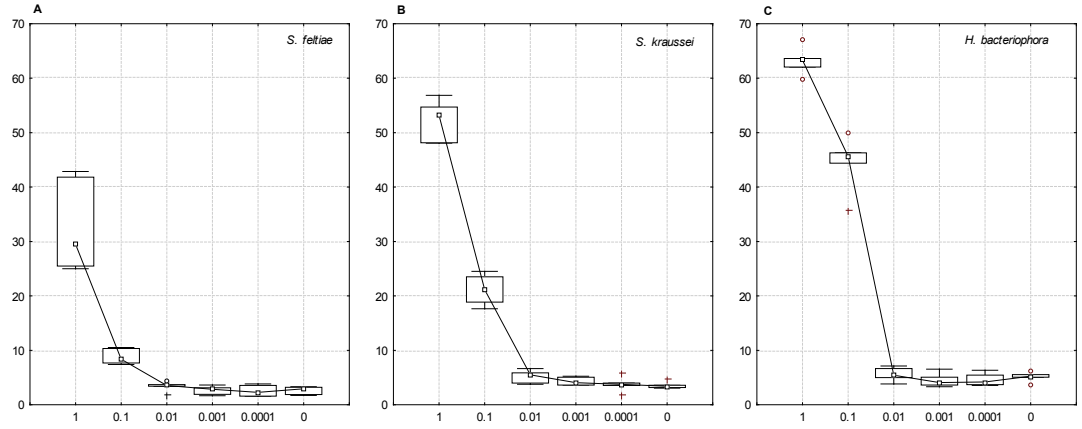

Fig. 1. Impact of on the invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (c): abscissa axis – concentration of substance (%); 0 – control (0% of active substance); ordinate axis – mortality of nematode larvae during 24 h experiment (%); n = 6

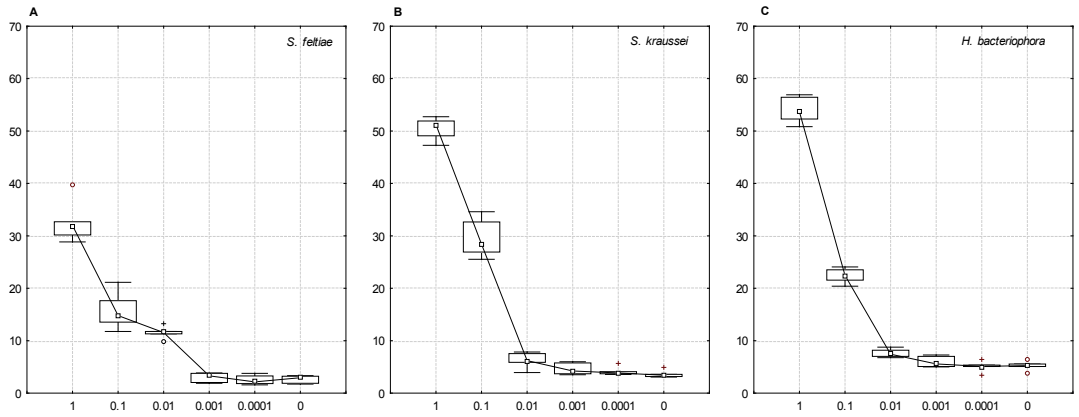

Fig. 2. Impact of $(NH_4)_2SO_4$ on invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (*c*): for indications see Fig. 1

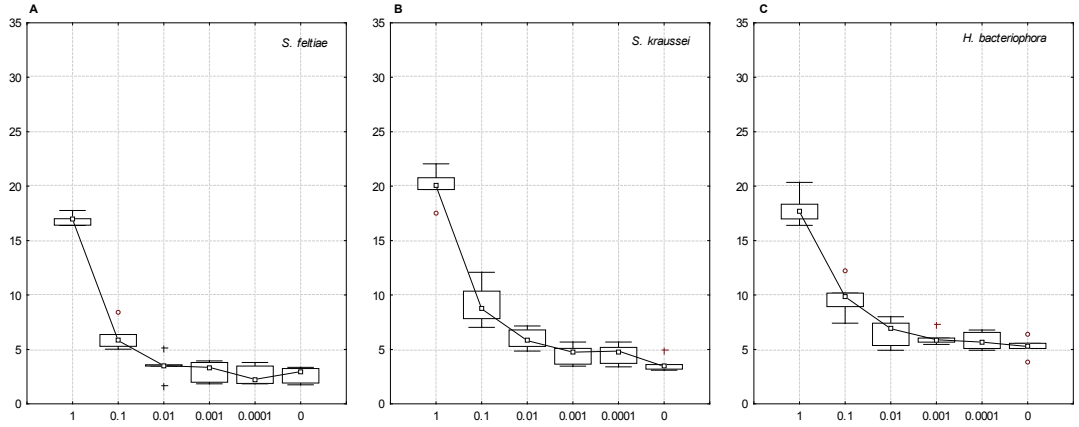

Fig. 3. Impact of $MgSO_4$ on invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (c): for indications see Fig. 1

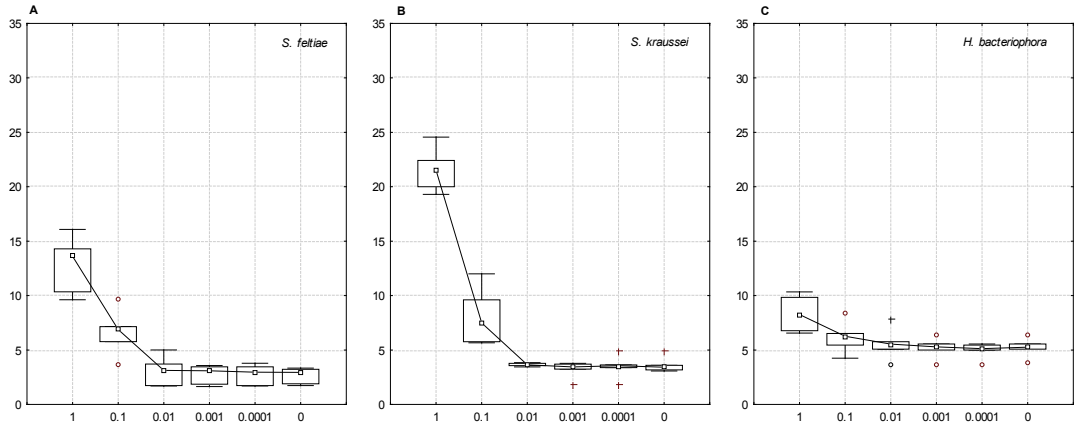

Fig. 4. Impact of KH_2PO_4 on invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (*c*): for indications see Fig. 1

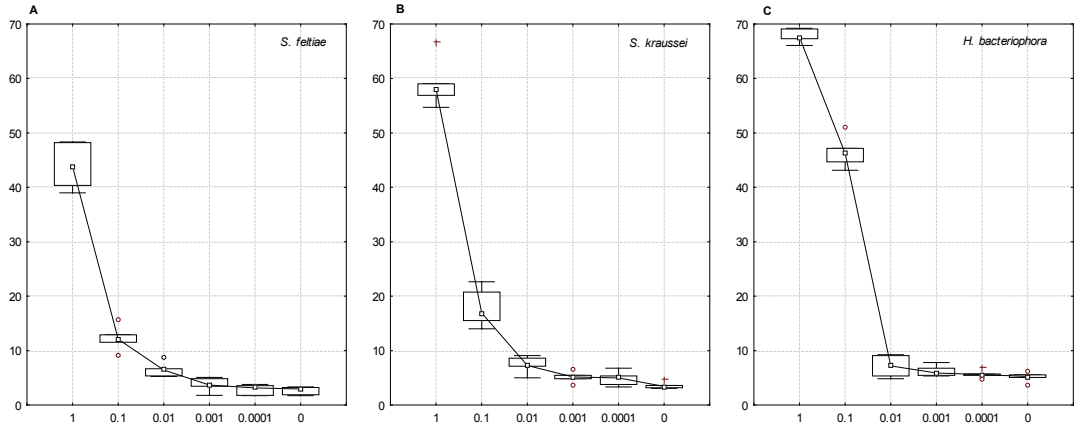

Fig. 5. Impact of superphosphate $(CaH_2PO_4)_2 \times H_2O + 2CaSO_4 \times 2H_2O$ on invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (*c*): for indications see Fig. 1

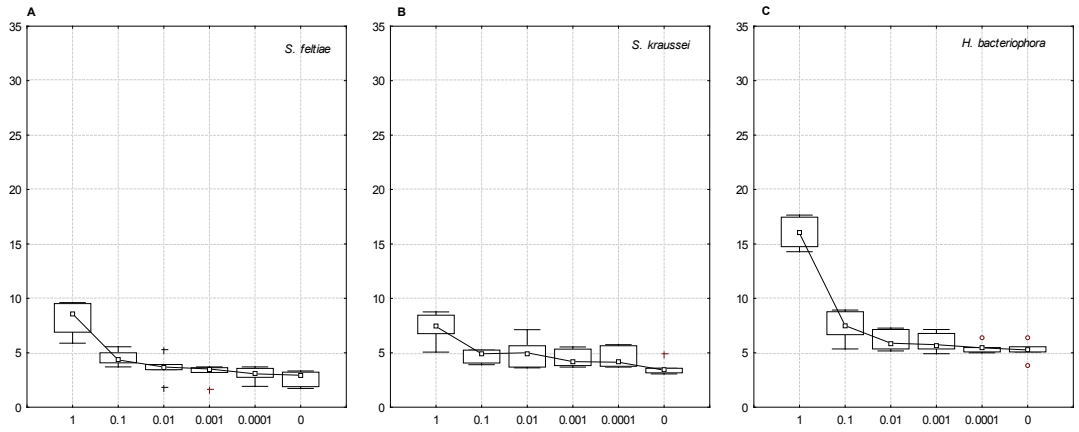

Fig. 6. Impact of Nitrophoska on invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (*c*): see Fig. 1

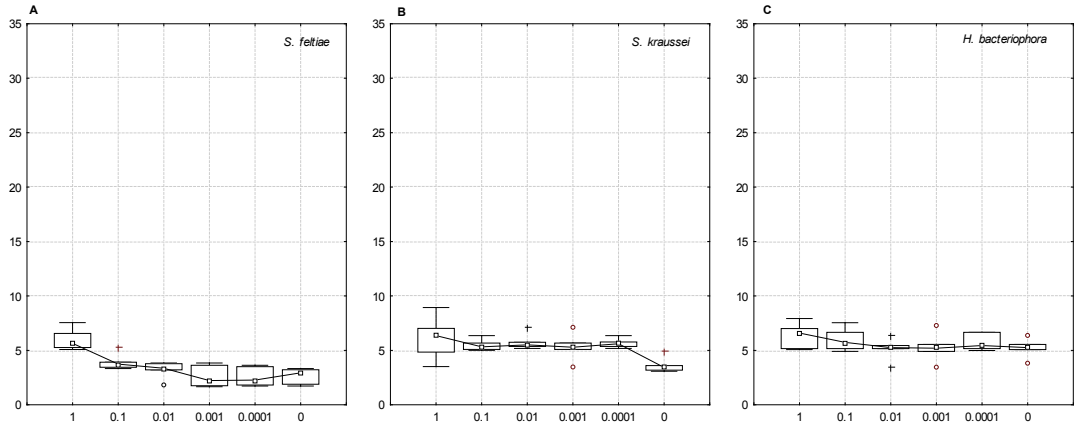

Fig. 7. Impact of succinic acid on invasive larvae of *Steinernema feltiae* (*a*), *S. kraussei* (*b*) and *Heterorhabditis bacteriophora* (*c*): see Fig. 1

Table 1

Mortality of *Steinernema carpocapsae* L_3 exposed to various solutions of substances (%) for the period of 24 h experiment

Name of substance	Concentration of substance, %											
	20.00	10.00	5.00	2.50	1.25	0.63	0.31	0.16	0.08	0.04	0.02	0.00
hydrochloric acid	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	4.5
sulfuric acid	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	4.5
acetic acid	100.0	100.0	100.0	100.0	96.6	43.6	7.0	3.5	3.4	3.7	3.4	4.5
citric acid	100.0	100.0	100.0	92.7	48.0	15.1	5.6	5.3	5.2	5.0	6.8	4.5
salicylic acid	–	–	–	–	–	–	–	5.2	6.9	7.9	5.2	4.5
sodium bicarbonate	91.8	50.9	15.1	5.2	3.4	3.5	7.3	5.3	5.0	3.6	3.6	4.5
sodium hydroxide	100.0	100.0	100.0	100.0	60.3	22.4	8.5	5.3	3.4	5.2	4.0	4.5

Note: "–" – highest possible concentration of substance of salicylic acid 0.18% in 20 ºC.

Table 2

The main characteristics of vitality of *Steinernema carpocapsae* L_3 exposed to solutions of various substances (%) for the period of 24 h experiment

Name of substance	Formula	LC_{50}, %	pH(LC_{50})	Optimum concentrations without reliable change in the mortality, %	Optimum value of pH
hydrochloric acid	HCl	<0.02	–	–	–
sulfuric acid	H_2SO_4	<0.02	–	–	–
acetic acid	$C_2H_4O_2$	0.68	0.9	0–0.31	1.3–7.0
citric acid	$C_6H_8O_7$	1.30	1.2	0–0.31	1.8–7.0
salicylic acid	$C_7H_6O_3$	–	–	0–0.18	1.9–7.0
sodium bicarbonate	$NaHCO_3$	9.50	10.2	0–2.50	7.0–9.9
sodium hydroxide	NaOH	1.05	13.4	0–0.31	7.0–12.8

Morphologically the acids in high concentrations do not deform the larvae, they fixate them practically with no alterations in the shape. Solution of sodium hydroxide in high concentrations completely deformed the larvae, leaving their parts as body fragments. In the solution of sodium bicarbonate, the larvae created indusia and in 20% and 10% solutions they became inactive and bent into spirals.

Optimum neutral values of pH acidity for the survival of *S. carpocapsae* larvae equal 7.0. Mortality of larvae accounted for 100% in the lowest of the studied (0.02%) concentration of hydrochloric and sulfuric acids. The action of acetic acid towards *S. carpocapsae* was weaker: compared to the control group, the mortality of larvae reliably did not change in up to 0.16–0.31% concentrations, and 1.25% concentration of acetic acid caused death to 96.6% of the larvae. LC_{50} for L_3 of *S. carpocapsae* exposed to acetic acid equaled 0.68%. While exposed to citric acid, this species was characterized by even wider tolerance range: mortality of larvae in 0.31% concentration reliably did not differ from the control group, and reached 92.7% (LC_{50} = 1.30%) in 2.50% concentration. Salicylic acid is hard to dissolve in water at room temperature, and the vitality of nematodes did not change even in the highest, 0.2% concentration. LC_{50} for sodium bicarbonate was 9.5% of the larvae of *S. carpocapsae* over 24 h reliably had no changes in vitality in the concentrations of $NaHCO_3$ of up to 2.5%. LC_{50} for sodium hydroxide was lower – 1.05%: the mortality of larvae reliably did not change in 0.31% concentration of NaOH. Over 24 h, tolerance of invasive larvae of *S. carpocapsae* to pH

(Table 2) was pH = 0.9–13.4; optimum values of pH without reliable increase in mortality were – pH = 1.3–12.8.

To determine the concentration of vitamin preparations needed to increase the vitality of larvae of entomopathogenic nematodes, we tested four species of parasitic worms – *S. feltiae*, *S. kraussei*, *S. carpocapsae* and *H. bacteriophora*. Over the laboratory experiment, we determined that addition of ascorbic acid (Table 3) to the cultivation medium of the nematode larvae reliably worsened their vitality (on average up to 26.4%) compared with the control (14.6%). And by contrast, addition of thiamine hydrochloride (B_1), pyridoxine hydrochloride (B_6) or cyanocobalamin (B_{12}) to the growth medium reduced the mortality of *S. carpocapsae* by 1.70–1.78 times (Table 4). Effective concentrations of water-soluble vitamins, in which increase in the vitality of nematode larvae occurred were determined as follows: 6–50 mg/mL for thiamine hydrochloride (B_1), 25–50 mg/mL for pyridoxine hydrochloride (B_6), and 0.1–0.2 mg/mL for cyanocobalamin (B_{12}). Addition of complex vitamin preparations Undevit and Komplevit to the growth medium of nematodes (Tables 5, 6) reliably decreased the extent of their survivability. Each of the preparations includes over ten components, each of which could easily negatively affect the vitality of nematode larvae.

Among the studied pigment colourings, the lowest effect on the vitality of invasive nematode larvae was exerted by pigment bases Abrikos (7.0–10.8% mortality per 24 h), Zolotoi Pesok (6.0–11.8%), Pudra Ananasa (7.7–13.4%), complex DMAE (7.6–17.4 %). According to the percentage of mortality of larvae, the indicated pigment colourings reliably do not differ from non-stained preparations (5.8–9.3%). The other analyzed colourings led to higher mortality of nematode larvae, therefore their application is not expedient (Table 7).

Table 3

Mortality (%) of invasive larvae of *Steinernema carpocapsae* exposed to solution of vitamins (x ± SD, n = 7) for 24 h experiment

Substance	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.12 mg/mL
Vitamin C	26.4 ± 6.7	14.0 ± 4.5	14.5 ± 6.1	13.1 ± 3.1	14.2 ± 4.7
Vitamin B_1	8.3 ± 1.6	7.4 ± 2.3	8.0 ± 2.1	9.9 ± 1.6	13.6 ± 3.5
Vitamin B_6	8.2 ± 3.1	7.4 ± 3.4	11.7 ± 3.5	12.4 ± 5.8	13.5 ± 4.9

Note: in absence of the effect of the vitamin in the control, the mortality of larvae in the medium equaled 14.6 ± 5.4%.

Table 4

Mortality (%) of invasive larvae of *Steinernema carpocapsae* exposed to solution of vitamins (x ± SD, n = 7) for 24 h experiment

Substance	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL
Vitamin B_{12}	8.6 ± 2.3	10.7 ± 3.9	13.4 ± 4.1	14.9 ± 5.3	15.3 ± 5.0

Note: in absence of the effect of the vitamin in the control, the mortality of larvae in the medium equaled 14.6 ± 5.4%.

Table 5

Mortality (%) of invasive nematode larvae in solution of Undevit preparation (x ± SD, n = 7) for 24 h experiment

Nematode	Concentration of preparation (by concentration of vitamin B_1, µg/mL)					Control
	400	80	16	3.20	0.64	0.00
Heterorhabditis bacteriophora	100.0 ± 0.0	91.6 ± 3.9	21.4 ± 2.4	10.7 ± 1.2	11.1 ± 1.8	9.3 ± 1.1
Steinernema feltiae	99.3 ± 0.8	71.4 ± 2.8	9.4 ± 1.5	4.2 ± 0.8	3.2 ± 0.7	2.5 ± 0.9
Steinernema kraussei	99.0 ± 0.8	80.2 ± 3.8	12.5 ± 0.9	6.3 ± 0.9	4.8 ± 0.7	4.9 ± 0.9

Note: Undevit preparation contains the following amount of elements per one dragée: vitamin A – 3300 MO, E – 10 mg, B_1 – 2 mg, B_2 – 2 mg, B_6 – 3 mg, B_{12} – 2 µg, C – 75 mg, PP – 20 mg, folic acid (vitamin B_9) – 0.07 mg, pantothenic acid (vitamin B_5) – 3 mg, rutin (vitamin P) – 10 mg, additional substances – wheat flour, starch syrup, talc, light liquid paraffin, sugar, yellow wax, flavoring Mint aroma.

Table 6

Mortality (%) of invasive nematode larvae in Komplevit preparation (x ± SD, n = 7) for 24 h experiment

Nematode	Concentration of preparation (according to concentration of vitamin B_1, µg/mL)					Control
	3000	600	120	24	4.8	0
Heterorhabditis bacteriophora	37.1 ± 1.9	14.1 ± 1.4	9.9 ± 1.3	9.2 ± 1.9	9.6 ± 2.2	9.3 ± 1.1
Steinernema feltiae	37.9 ± 3.3	8.5 ± 2.1	4.2 ± 0.8	4.1 ± 0.8	3.8 ± 0.7	2.5 ± 0.9
Steinernema kraussei	37.4 ± 3.2	13.0 ± 2.0	5.3 ± 1.2	6.3 ± 1.5	5.7 ± 1.2	4.9 ± 0.9

Note: Komplevit preparation contains the following amount of elements per a capsule: vitamins B_1 – 15 mg, B_2 – 15 mg, B_6 – 10 mg, B_{12} – 2 µg, C – 100 mg, PP – 50 mg, folic acid (vitamin B_9) – 0,25 mg, pantothenic acid (vitamin B_5) – 25 mg, additional substances – lactose monohydrate, colloidal silicon dioxide, potato starch, magnesium stearate or calcium stearate.

Table 7

Mortality (%) of L_3 of *S. feltiae*, *S. kraussei* and *Heterorhabditis bacteriophora* in 1% solution of colourings (x ± SD, n = 5) for 24 h experiment

Substance, concentration	*S. feltiae*	*S. kraussei*	*H. bacteriophora*
Control (without addition of colourings)	5.8 ± 0.8	9.3 ± 1.7	7.3 ± 1.3
Henné neutre	92.2 ± 0.7	92.0 ± 2.9	90.5 ± 0.9
Rose oxide	92.4 ± 1.8	90.3 ± 1.7	86.2 ± 1.0
Yellow oxide	58.4 ± 4.1	56.1 ± 4.5	50.0 ± 0.9
Brazilian ginseng powder	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Ayurveda powder	51.5 ± 3.5	48.1 ± 0.1	51.7 ± 0.0
Pigment base Farfor	100.0 ± 0.0	99.1 ± 0.9	99.0 ± 1.0
Sodium alginate	24.1 ± 1.9	41.3 ± 10.4	21.7 ± 0.6
Red ochre	81.7 ± 2.5	79.6 ± 0.4	84.2 ± 3.5
Centella asiatica powder	100.0 ± 0.0	99.1 ± 0.9	100.0 ± 0.0
Apricot mica	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Plant pigment Kashtan	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Pigment base Abrikos	7.0 ± 1.8	9.8 ± 3.1	10.8 ± 0.8
Zolotoi Pesok	6.0 ± 1.0	6.9 ± 0.1	11.8 ± 1.6
Pigment base Kofe	76.8 ± 3.2	77.0 ± 3.2	75.5 ± 3.7
Pigment base Bezhevyi Voshod	71.2 ± 3.6	76.1 ± 1.2	69.2 ± 2.5
SLSA	52.8 ± 0.9	52.8 ± 1.1	55.1 ± 4.2
Xantane	25.7 ± 1.6	23.4 ± 3.0	34.2 ± 2.6
Aristoflex	93.8 ± 0.9	93.9 ± 2.7	98.2 ± 1.8
Pineapple powder	7.7 ± 1.1	8.6 ± 3.5	13.4 ± 1.6
DMAE complex	12.3 ± 3.3	7.6 ± 2.6	17.4 ± 1.6
Cocamidopropyl betaine, 45%	19.0 ± 1.7	21.8 ± 1.5	29.8 ± 1.8

Discussion

One of the disadvantages of using nematode larvae against agricultural pests is that the procedure of introduction requires fulfillment of certain technological regimes of influence on plants, additional financial and human resources (Gaugler et al., 1997; Millar & Barbercheck, 2001; Boemare, 2002). Our studies indicate the possibility of combined use of nematode preparations and mineral fertilizers and growth stimulators for plants, thus reducing the negative effect of agricultural measures on the plant organisms, and also reducing economic costs on growing plant production. The data we obtained indicate that short term use of mineral fertilizers and growth stimulators for plants in the concentration of 0.1% causes low decrease in the vitality of invasive larvae of three species of entomopathogenic nematodes.

Some species of nematodes of the Steinernematidae and Heterorhabditidae families feed on various species of invertebrate hosts, which could be attributed to certain characteristics of osmotic pressure and acidity (alkalinity) of hemolymph of these arthropods (Koppenhöfer & Kaya, 1996; Kaya & Campbell, 2000; Eng et al., 2005). Alkalinity and osmotic pressure can also be due to optimum conditions for the development of symbiont bacteria of the intestines of nematodes (Kaya & Koppenhöfer, 1996; Boemare, 2002; Martens et al., 2003), which in particular create the pathogenic effect on phytophage insects. Nematodes, similarly to other invertebrates, have certain optima of tolerance to acids (alkali) of aqueous environment and its osmotic pressure which differ in different species of worms (Wright et al., 2000; Boyko & Brygadyrenko, 2019a). However, currently, these characteristics of the environment have not been used for the optimization of the technology of use of nematode larvae in the invasive stages of development. As a result of the conducted work, we determined the range of tolerance of invasive stage of *S. carpocapsae* to pH, possibility of combined use of larvae of this nematode in solutions of organic acids and alkali to increase the period of their vitality. Decrease in mortality of third age larvae in the 24 h experiment by 1.0–1.2% was observed while using them with solutions of acetic acid in the concentration of 0.16% or sodium bicarbonate in the concentration of 1.25%, or sodium hydroxide in 0.08%. Our studies allow increasing the survivability of larvae on plants depending on the conditions of open land or greenhouse conditions. General increase in the level of survivability of larvae of *S. carpocapsae* on plants in the conditions of both open land and greenhouses using these substances was 23% over 24-48 h after their use, which significantly increases the effectiveness of nematode preparations against phytophage pests of agricultural crops.

The efficiency of using particular species of nematodes depends on the action of a number of factors of their existence (Bedding et al., 1993; Stuart & Gaugler, 1994). Nematodes, if their invasive larvae survive for the use against insect pests, lose their vitality. To increase it, we recommend using vitamin preparations. The range of tolerance of invasive stages of *S. carpocapsae*, *S. feltiae*, S. *kraussei* and *H. bacteriophora* to vitamins was determined, as well as the possibility of using solutions of ascorbic acid, thiamine hydrochloride (B_1), pyridoxine hydrochloride (B_6) or cyanocobalamin (B_{12}) for the improvement of vitality of nematode preparations in the concentrations of 6–50, 25–50, 25–50 and 0.1–0.2 mg/mL respectively. This method, compared to other methods, allows one to increase the degree of survivability of nematode larvae during maintenance and transport without great financial costs.

Different species of entomopathogenic nematodes are used against certain species of insect pests (Koppenhöfer & Kaya, 1996; Kaya & Campbell, 2000; Eng et al., 2005). Starting from the stage when agricultural farm purchases live invasive nematode larvae to the stage of sprinkling them in the agrocenose, several stages of preparation of technogenic solutions take place. If in ecologically clean agricultural production, several species of entomopathogenic nematodes effective against different species of insect pests are used at the same time, confusion with preparations can occur. Therefore, at the stage of using these bioinsecticides, it is practical to stain nematocidal preparations with colourings which would not cause death to larvae in the period of several to twenty four hours (Boyko & Brygadyrenko, 2017, 2018, 2019b). Our work reveals the results of the experiments with different food colourings (21 colourings) in order to develop recommendations for farms to use them for staining nematode larvae of *S. feltiae*, *S. kraussei* and *H. bacteriophora*. As a result, we composed a list of four pigment additives which according to the percentage of mortality of larvae reliably do not differ from preparations without colouring, allowing improvement of the preparative forms of the invasive stages of development of several species of nematodes for their simultaneous use.

Conclusions

We determined the influence of mineral additives, growth stimulators for plants, acids and alkali, and colourings on survivability of entomopathogenic nematodes. The ways of increasing the duration of life of larvae (L_3) by introducing vitamins to their culture are presented. The results of the studies can help decrease the economic costs of the processing of plants.

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