Vestnik Zoologii, **53**(4): 285–296, 2019
DOI 10.2478/vzoo-2019-0028

sciendo

UDC 595.132:574.4(477)

OCCURRENCE OF ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEIENERNEMATIDAE,HETERORHABDITIDAE) FROM AGRICULTURAL ECOSYSTEMS IN FOREST (POLISSYA) AND FOREST-STEPPE NATURAL ZONES OF UKRAINE

D. D. Sigareva[1], A. M. Kovtun[1*], V. V. Kornyushin[2]

[1]Institute of Plant Protection, NAAS of Ukraine,
vul.Vasylkivska, 33, Kyiv, 03022 Ukraine
[2]Schmalhausen Institute of Zoology, NAS of Ukraine,
vul. B. Khmelnytskogo, 15, Kyiv, 01030 Ukraine
**Corresponding author*
E-mail: andrii_kovtun@ukr.net

Occurrence of Entomopathogenic Nematodes (Rhabditida, Steienernematidae, Heterorhabditidae) from Agricultural Ecosystems in Forest (Polissya) and Forest-Steppe Natural Zones of Ukraine. Sigareva, D. D., Kovtun, A. M., Kornyushin, V. V. — A survey for entomopathogenic nematodes (EPNs) of the Steienernematidae and Heterorhabditidae in soils of different crop types of agricultural lands (household plots, commercial fields) in the forest (Polissya) and forest-steppe (Lisostep) zones of Ukraine was carried out in spring, summer and autumn months from 2016 to 2018. In total, 205 soil samples and 92 live-traps were processed. In addition, 98 samples of soil-living larvae and adults of insect crop pests, including cockchafer beetles, click beetle larvae, darkling beetle larvae, caterpillars of the scoops were collected. It is shown that the EPNs distribution and frequency of occurrence depends on the natural geographical features (regional characteristics) and habitat types. EPNs were found in 46 (15.4 %) out 297 samples. The incidence (% of samples) of the entomopathogenic nematodes, and their diversity varied depending on the location-based sampling, the type of agrocenosis, and the predominant agricultural plant species or typical species-edificators. The proportion of entomopathogenic nematodes (*Steinernema* spp., *Heterorhabditis* spp.) recovery from regions of Lisostep zone was 16.9 %, and 13.6 % from regions of Polissya zone. The highest number among all positive samples was recorded from the apple trees, *Malus domestica* Borkh., 1803 (10 samples). The least number of positive samples (one sample) was obtained from sweet cherry trees, *Prúnus cérasus* L., 1753, junipers, *Juniperus communis* L., 1753, alfalfa, *Medicago sativa* L., 1753, common beans, *Phaseolus vulgaris* L., 1753, and common pumpkin, *Cucurbita pepo* L., 1753. The steinernematid nematodes were noticeably dominant over heterorhabditid nematodes: 60.8 % vs 39.2 % respectively. *Steinernema* spp. is widespread in different regions and plots, whereas *Heterorhabditis* spp. are common in fruit orchards and coniferous decorative perennial plantings.
Key words: entomopathogenic nematodes, Steienernematidae, Heterorhabditidae, agricultural ecosystems, Polissya, Lisostep, Ukraine.

Introduction

The nematode parasitism in insects is a phenomenon interesting from the biological and significant from the economical points of view (Steinhaus, 1952; Polozhentsev, 1956). More than 1,000 species of nematodes are known to be adapted to the existence in the insect body at all stages of their development, dwelling in various organs and tissues in the form of eggs, larvae, or adults (Zlotin, 1989).

Among the entire ecological group of insect-specific parasitic nematodes two families, namely Steienernematidae Chitwood et Chitwood, 1937 (= Neoaplectanidae Sobolev, 1953) and Heterorhabditidae Poinar, 1976, also known as 'entomopathogenic nematodes' deserve special attention (Spiridonov, 2001). Presently, the family Steienernematidae contains two genera: *Steinernema* Travassos, 1927 (about 95 valid species) and *Neosteinernema* Nguyen & Smart, 1994, which is represented by only one type species *N. longicurvicauda* Nguyen & Smart, 1994. The Heterorhabditidae also contains two genera: a recent genus *Heterorhabditis* Poinar, 1976 (= *Chromonema* Khan, Brooks & Hirschman, 1976) (comprises 16 valid species) and a fossil genus † *Proheterorhabditis* Poinar, 2011 with the species † *P. burmanicus* Poinar, 2011, from the Early Cretaceous (Poinar, 2011). Steinernematid and heterorhabditid nematodes are found on every continent except Antarctica (Griffin et al., 1990), and in almost all natural zones of the Earth at different latitudes/altitudes above sea level (reviewed by Hominick, 2002).

The representatives of the Steienernematidae and Heterorhabditidae have similar life histories. This is, first of all, the presence of complex life cycles with distinct life stages separated in time and space (Wang & Bedding, 1996). Characteristic and biologically logical in the ontogenesis is that infective juveniles (dauer larvae) are the only free-living stage in the soil for several months; adult stages are found only inside the cadavers of insect hosts (including larvae or nymphs, pupae, and adults) (Burnell & Stock, 2000). One of the most characteristic features of entomopathogenic nematodes is their mutualistic association with the gram-negative bacteria of the genera *Xenorhabdus* Thomas, Poinar, 1979 emend. Thomas, Poinar, 1983 (obligatory symbionts of *Steinernema* species) and *Photorhabdus* Boemare et al., 1993 (obligatory symbionts of the *Heterorhabditis* species) (γ-proteobacteria: Enterobacteriaceae) (Adams et al., 2006). The pathogenicity of the entomopathogenic nematodes is related directly to these bacteria (Ryss et al., 2011).The entomopathogenic nematodes from the families Steienernematidae and Heterorhabditidae practically does not show specificity with regard to the type of insect victim.

The general concept of plant protection against insect pests is nowadays changing gradually. The main idea for insect control is not a total destruction of harmful insects, but only a control of their populations. Greater emphasis is placed on the restoration, preservation, and maintenance, as far as possible, of self-regulation of ecosystems and more specifically of certain driving forces in them that suppress populations of some pests. Such a goal can only be achieved by a set of appropriate methods and techniques selected on the deep-level understanding of basic processes in various biocenoses and agrocenoses as well (Djadechko et al., 2001). Entomopathogenic nematodes, therefore, particularly correspond to this concept called integrated plant protection. Integrated protection involves using as much as possible the selective means of pest control. Entomopathogenic nematodes (in literal meaning) allow to control pests without affecting, or affecting at minimal extent other organisms of biocenosis. These nematodes are not toxic to humans, warm-blooded animals and plants (Gaugler & Kaya, 1990).

In Ukraine, investigations on the distribution of entomopathogenic nematodes from the families Steinernematidae and Heterorhabditidae were performed only recently. Entomopathogenic nematodes were first recorded on the territory of Ukraine in 1952. A nematode sample was isolated from sugar beet weevil (*Bothynoderes punctiventris* Germ., 1824) larvae collected on sugar beet fields from Veselopodilsky research and breeding station in Poltava Region. It was described as belonging to a new species *Neoplectana bothynoderi* Kiryanova et Puchkova, 1955 (later synonymized with *Steinernema feltiae* (Filipjev, 1934)) by Ye. S. Kiryanova and L. V. Puchkova (Kiryanova & Puchkova, 1955).

The most comprehensive studies of entomopathogenic nematodes in the agrocenoses of field crops were performed in the laboratory of nematology, the Institute of Plant Protection of the National Agrarian Academy of Sciences of Ukraine. The fauna of entomopathogenic nematodes in the soil ecosystems of nature reserves was investigated at the I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine.

Nowadays in Ukraine, three species of the genus *Steinernema*: *S. carpocapsae* (Weiser, 1955) Wouts et al., 1982, *S. feltiae* (Filipjev, 1934) Wouts et al., 1982 and *S. arenarium* (Artyukhovsky, 1967) Wouts et al., 1982, and one of the genus *Heterorhabditis*: *H. bacteriophora* Poinar, 1976 were recorded (Stefanovska, 2007; Yakovlev, Olenenko, 2009; Kornyushyn, 2009; Sigharjova et al., 2010; Sigharjova et al., 2012; Kharchenko et al., 2012; Yakovlev, Kharchenko, 2013; Yakovlev et al., 2014; Yakovlev, 2017; Yakovlev et al., 2017).

Material and methods

The work is based on the materials sampled on commercial arable land and orchards, kitchen gardens and household plots (including dacha gardening), during spring, summer and fall of 2016–2018 in 21 localities in total from 16 districts into 6 regions. The Zhytomyr Region comprised 3 districts: Ovruch District (Ovruch), Lyubarskyi District (Bicheva village), Popilnya District (Harlievka village, Krasnogirka village, and

Vasilivka village). The Kyiv Region comprised 3 districts: Borodyanka District (Kozintsy village), Kyiv City (Kitaevo tract), Baryshivka District (Baryshivka). The Chernihiv Region comprised 3 districts: Kozeletskyi District (Town of Ostér and Morivsk village), Chernihiv District (Boromyky village), Mena District (Kisilivka village). The Cherkasy Region comprised 3 districts: Zhashkiv District (Skybin village and Khizhnya village), Talnivskyi District (Sokolivochka village), Korsun-Shevchenkivskyi District (Korsun-Shevchenkivskyi). The Khmelnytskyi Region comprised 3 districts: Volokhskyi District (Bokiivka village and Ozhigovtsi village), Teofipol District (Borshchivka village), Gorodotskyi District (Kumaniv village), Khmelnytskyi Region, and the Vinnytsia Region comprised Khmilnytskyi District (Semky village), which located in Polissya and Lisostep agro-ecological zones of the Ukraine (table 1).

Ukrainian Polissya is a natural region located in within the zone of mixed forests of the East European plain, extending west-east for 750 km. The average part of cultivated land in Polissya is more than 30 %, including up to 90 % of the land areas with mid-podzolic soil having the best water-physical properties and greatest fertility (Syrotenko, Chernov, 2000).

The Forest-steppe zone is extending from the Pre-Carpathian region to the Mid-Russian uplands for approximately 1100 km. It occupies one-third of Ukraine's territory. Characteristic of forest-steppe is the alternation of forest islands and areas of more herbaceous vegetation. The average part of cultivated land in Forest-steppe zone is about 75 %; and up to 90 % on the Left-bank part of Ukraine (East to the Dnipro River) (Syrotenko, Chernov, 2000).

Soil samples were taken, live-traps were embedded in the topsoil, and some soil-dwelling insect pests and also target insects *Galleria mellonella* L., 1758 (Lepidoptera, Pyralidae) were collected and analyzed for the presence of nematode infections. Total number of processed samples was 297, of which 46 contained entomopathogenic nematodes.

Soil samples in agricultural land were taken randomly using a shovel: for the formation of a composite sample (volume of 500 cm^3), 5 single point samples were collected from the area of 4 m^2 in the top 15–30 cm of soil (for field crops) or 40 cm and in a radius of 1 m around the trunks of individual trees and bushes (for fruit orchard, decorative perennial plantings, etc.) (Orozco et al., 2014). In addition, soil live-traps were embedded in the top of soil using larvae of *Galleria mellonella*, which were cultured in a thermostat at 27–30 °C on a wax raw

Table 1. Frequency and number of positive samples of *Steinernema* spp. and *Heterorhabditis* spp. in agrocenoses of separate regions of Ukraine

No.	Geographic regions	Total N*analyzed samples	Total *n* EPN-positive samples		
			Present N (*% OI*)	Proportion of genus of (% of all EPN-positive samples) of	
				St. spp.	*Het.* spp.
			Polissya Zone		
1.	Chernihiv	91	11 (12.0)	10 (90.9)	1 (9.1)
2.	Zhytomyr	28	7 (25.0)	5 (71.4)	2 (28.6)
3.	Kyiv	13	0 (0.0)	0 (0.0)	0 (0.0)
Total No. of Polissya		**132**	**18 (13.6)**	**15 (83.3)**	**3 (16.7)**
			Forest-Steppe Zone		
1.	Kyiv	64	28 (43.7)	13 (46.4)	15 (53.6)
2.	Khmelnytskyi	49	0 (0.0)	0 (0.0)	0 (0.0)
3.	Cherkasy	30	0 (0.0)	0 (0.0)	0 (0.0)
4.	Vinnytsia	22	0 (0.0)	0 (0.0)	0 (0.0)
Total No. of Forest-Steppe		**165**	**28 (16.9)**	**13 (46.4)**	**15 (53.6)**
Polissya + Forest-Steppe		297	46 (15.4)	28 (60.8)	18 (39.2)

*Soil and live-traps samples. *St.* — *Steinernema*; *Het.* — *Heterorhabditis.*

material in the laboratory. Two specimens of *G. mellonella* last instar larvae (with the weight of 0.20 ± 0.03 g) were placed into a steel spherical capsule (Ø 45 mm) of long handle tea infuser (L = 150 mm) and embedded at 10-cm depth in the soil (for field crops) or 20–30 cm depth in the soil and at 1 m from the tree trunk (for tree plantations). After 5–6 days, all live insect were excluded from further analysis; unalive insects were removed for lab analysis. In total 98 larvae and imago samples of soil-living insect pests: 35 click beetle larvae (Coleoptera, Elateridae), 10 darkling beetle larvae (Coleoptera, Tenebrionidae), 44 imago and larval stages (known as white grubs) cockchafer beetles (also referred to as maybug) (Coleoptera, Scarabaeoidea, Melolonthinae), 9 caterpillars of the scoops (also: cutworm) (Lepidoptera, Noctuidae) were analyzed. All the insects were collected manually. To detect small insects and larvae, a universal manual analysis of soil samples was used (Dunaev, 1997).

Isolation of entomopathogenic nematodes from soil samples was carried out by biotesting method using *G. mellonella* larvae under laboratory conditions (Bedding & Akhurst, 1975; Orozco et al., 2014). We calculated the Occurence Index (% OI) of entomopathogenic nematodes for each geographic location and type of agrocenoses. The index % OI was determined as the ratio of the number of EPN-positive samples to the total number of investigated samples:

$$\% \text{ OI} = n / N \times 100 \ (\%),$$

where % OI is the occurrence index of; n = number of positive specimens having EPNs; and N = the total number of collected and tested samples.

In addition, we calculated the ratio (shares) of Steienernematidae and Heterorhabditidae in positive samples from a specific region, thereby determining the taxonomic structure of the fauna of entomopathogenic nematodes of the region.

A correct diagnosis of insects (*Galleria* larvae) infected with entomopathogenic nematodes among others types disease-causing organisms (pathogens or parasites) was carried out on the basis of a macroscopic analysis (with the naked eye and/or by using magnifying glass).The main focus was on typical pathological symptoms from nematode infection which include a change in the color of the outer surfaces, or the epicuticle, the size and shape of the body, and the absence of any specific smell of rotting in the nematode-killed test object larvae, etc.

The isolation of entomopathogenic nematodes from insect cadavers was carried out by 2 methods: (1) White trap method (White, 1927) in a dark place (or in low diffuse light) at room temperature (22–24 °C) for third larval stage (L3) of the nematodes (infective juveniles (IJs) or dauer (enduring) juveniles); and (2) the method of helminthological dissection (Lazarevskaya, 1962) of hosts under a stereoscopic microscope MBS-9 for adult stage (males and females) of entomopathogenic nematodes. All discovered nematode isolates were stored separately in the form of water suspension in 250 ml conic flasks in the refrigerator at the temperature of 4 °C in saline containing 0.001 % formalin (at a concentration of 1000–3000 IJs/ml).

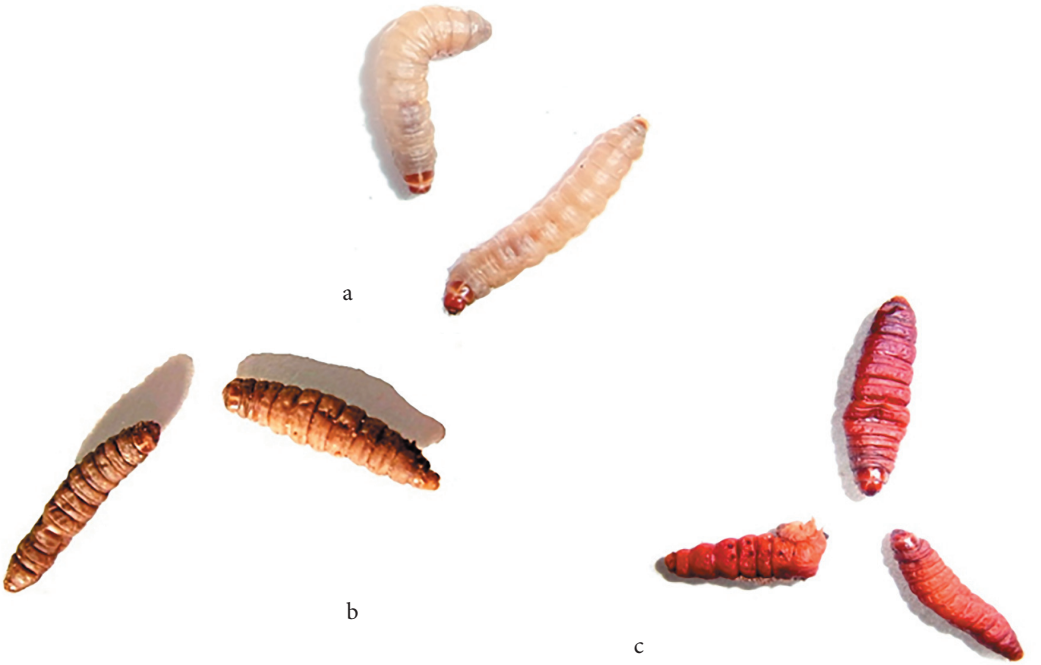

Fig. 1. Manifestation of EPN-infections on greater wax moth, *Galleria mellonella*: a — uninfected larvae; b — larvae infected with *Steinernema* sp. (isolate DD-5); c — larvae infected with *Heterorhabditis* sp. (isolate DD-8).

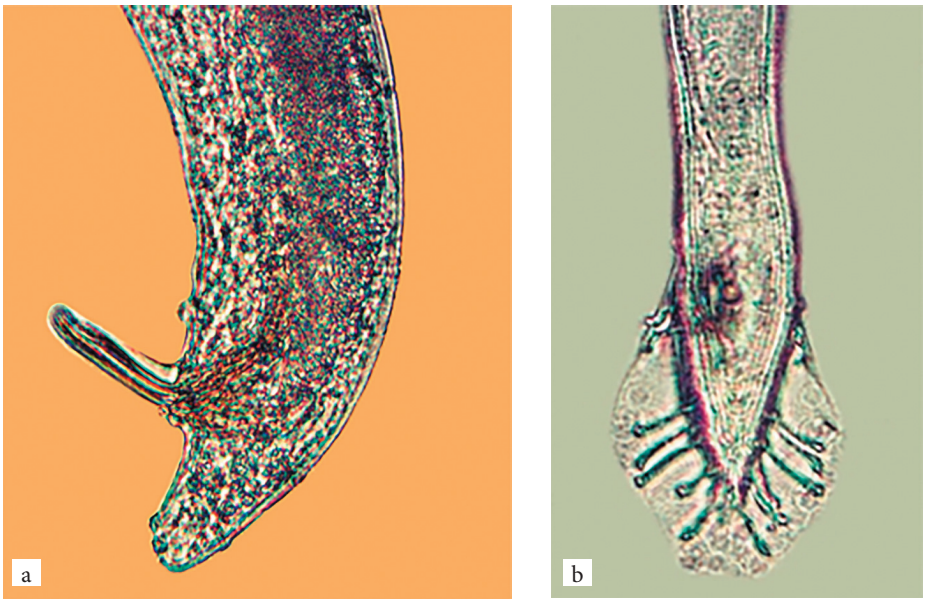

Fig. 2. Posterior end of male: a — *Steinernema* sp., isolate DD-5 (lateral view); b — *Heterorhabditis* sp., isolate DD-8 (ventral view). ×400.

Photographs were made with the digital camera "Olimpus SH-21" mounted at stereoscopic microscope MBS-9, and with the digital camera ToupCam SCMOS03000KPA mounted at the compound microscope Carl Zeiss Primo Star, using ToupView (3.7) software.

The identification of entomopathogenic nematodes up to the generic level was performed based on the biological and morphological features: the change in the color of the outer surfaces of the body of *Galleria* larvae influenced of the nematode-bacterium complexes (fig. 1), and the characteristics of the copulative apparatus of males (in particular, the presence/absence of bursa) (fig. 2) (Spiridonov, 2001). The monotonous change in color from grayish-white (intact larvae) (fig. 1, a) to saturated brown, or yellowish, is characteristic for insect larvae affected by *Steinernema* species (fig. 1, b); and to red-raspberry for those affected by *Heterorhabditis* species (fig. 1, c). The males without bursa were assigned to *Steinernema* (fig. 2, a), those with bursa to *Heterorhabditis* (fig. 2, b).

Results

We found 46 (15.4 %) out of 297 positive samples (soil and live-traps samples) containing entomopathogenic nematodes, isolates belonging to the genera *Heterorhabditis* and *Steinernema* were detected (table 1). Based on the morphology of the isolated entomopathogenic nematodes (infective larvae, adult males and females of 1st and 2nd generation), and their morphometric characteristics, we assign them to three species: *Steinernema carpocapsae*, *Steinernema* sp. (representative of the "*glaseri*" group) and *Heterorhabditis bacteriophora*.

None of the insect pests collected in the topsoil layer appeared to be infected with nematodes. All of the studied insects were previously reported as hosts of entomopathogenic nematodes (Steienernematidae and Heterorhabditidae) (Filipev, 1934; Artyukhovskiy, 1967; Veremchuk, 1969; Kozodoy, 1984).

In this paper, we present a generalized evaluation of distribution of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp. in the different regions of Ukraine, and their occurrence in different agrocenoses of the study regions. According to our survey, the frequency of occurrence of these nematodes, the diversity of nematodes in agroecosystems (at the generic level) varied depending on the geographic location (e. g., areas, regions, etc.), the type of agrocenosis (row crop, fruit orchard, decorative landscaping perennial plantings), and on the dominant plant species (or species-edificators).

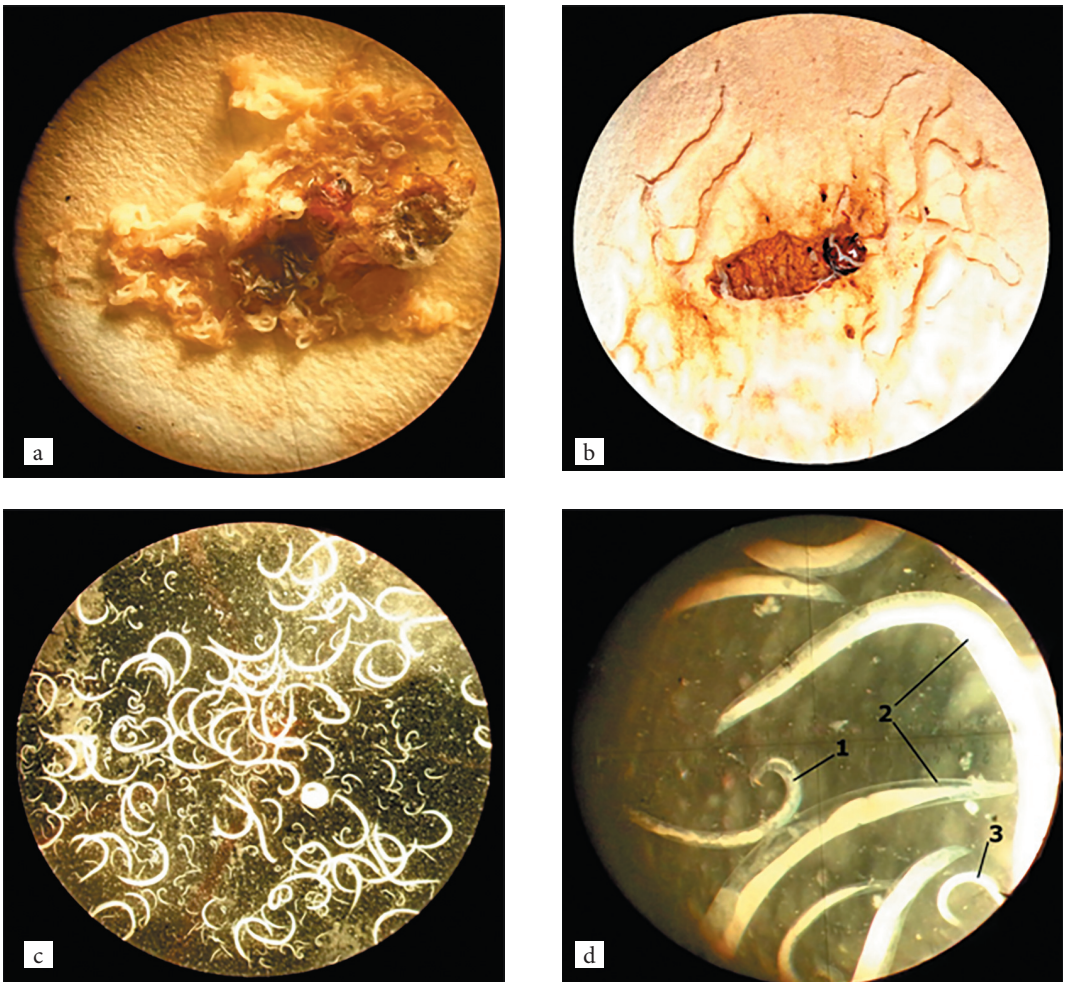

Fig. 3. Wax moth larvae (*Galleria mellonella*) on White traps after EPNs infection: a — rupture in the cuticle of the insect host (*Galleria mellonella*) and exit of EPNs; b — active larval migration through *G. mellonella* directly into White's trap (positive hydrotropism); c — general view of L3 juveniles, adult females and adult males of *Steinernema* sp. (×25); d — male (1), female (2), and third-stage infective juveniles (3) of *Steinernema* sp. (×87.5).

Percentage of positive samples (% OI) in the areas of the forest-steppe zone of Ukraine was slightly higher than in the Polissya zone: 16.9 % and 13.6 %, respectively. The main inhabited localities where entomopathogenic nematodes were detected were those in northern and north-eastern part Polissya and north-eastern part of the forest-steppe: Town of Oster and Morivsk village (Kozelets District), Boromyky village (Chernihiv District), Kisilivka village (Mena District) of Chernihiv Region; Ovruch (Ovruch District) of Zhytomyr Region, and Baryshivka (Baryshivka District) of Kyiv Region. Among those regions, the relative frequency of occurrence of entomopathogenic nematodes was uneven, and ranged from a low of 12.0 % in Chernihiv Region to a high of 43.7 % in Kyiv Region (table 1).

Extremely long arid spring and summer periods of the years 2016 and 2017 could have affected the presence of soil-inhabiting entomopathogenic nematodes in soil samples, especially in the forest-steppe zone. The laboratory tests of soil samples from field crops in Khmelnytskyi, Vinnytsya and Cherkasy Regions (101 samples in total) showed no entomopathogenic nematodes (table 1).

In general, perennial plantations were found to be 4.8 times more populated with entomopathogenic nematodes than field crops — 12.7 % vs 2.6 %. One possible reason for this

discrepancy, in our opinion, is specific features of microclimate. For example, a dense cover with foliage created by plants in fruit orchard, inhibit a fluctuation of temperature and relative humidity, thereby providing more optimal conditions for the normal prevalence of entomopathogenic nematodes in soil habitat. In addition, perennial plantations are characterized by a richer arthropod diversity. Most agricultural insect pests, which can provide a population recovery of entomopathogenic nematodes, seek for a shelter commonly in dense stand of trees, that generally increases the chances for detecting of entomopathogenic nematodes. In contrast, over-cultivating of sown fields has a negative effect on the entomopathogenic nematodes because it exposes them to solar radiation and ultraviolet light. In addition, an intensive use of pesticides and chemical fertilizers is a highly negative factor suppressing and limiting the development of insect and, therefore, prevent formation of the sustainable 'host-parasite' relationships between insects and nematodes. This is particularly inherent to industrial intensive farming.

The highest number of positive samples was found in soil of plantings of perennial crops (38 samples), in particular, where no chemicals are used for old fruit trees. That was true of almost all plants the Rosaceae (stone fruit and pome fruit crops), berries (mulberry family, Moraceae; oleaster family, Elaeagnaceae), and nut plants (walnut family, Juglandaceae). Also, entomopathogenic nematodes were found in plantations of some coniferous decorative plants (Cupressaceae). To a lesser extent, these nematodes occurred in land planted with row crops (8 samples), including legumes (Fabaceae), cucurbits (Cucurbitaceae), knotweed (Polygonaceae), and aster family (Asteraceae). In soils from under apple trees, *Malus domestica* Borkh., 1803 the highest number of samples containing entomopathogenic nematodes (10) among total 46 EPN-positive samples was found, whereas the least number of positive samples (one sample) was obtained from under cherry trees, *Prúnus cérasus* L., 1753, juniper bushes, *Juniperus communis* L., 1753, alfalfa, *Medicago sativa* L., 1753, common beans, *Phaseolus vulgaris* L., 1753, and common pumpkin, *Cucurbita pepo* L., 1753 (table 2).

The analyses of soil samples collected from commercial soybean fields (*Glycine max* (L.) Merr., 1917), bread wheat fields (*Triticum aestivum* subsp. *aestivum* L., 1753) and sugar beet fields (*Beta vulgaris* subsp. *vulgaris* L., 1753) at the four Regions of Ukraine (Zhytomyr Region, Cherkasy Region, Khmelnytskyi Region and Vinnytsia Region) did not reveal any entomopathogenic nematodes (table 2). It seems possible, that the absence of entomopathogenic nematodes in soil is due to the pesticide treatments applied in a given crop fields that suppresses entomofauna.

According to our observation, the steinernematid nematodes were noticeably predominating over the heterorhabditids (ratio 1.5 to 1) (table 1). Comparison of our results to literature data (reviewed by Hominick, 2002) made it possible to conclude that such a trend persists in many countries of the world. It should be mentioned that populations of *Steinernema* spp. were widespread in different regions and agro-ecosystems, whereas *Heterorhabditis* spp. occurred exclusively in fruit orchards and coniferous decorative perennial plantings. Obviously, heterorhabditid nematodes are more demanding of micro-environment and require high soil humidity and relatively stable temperature regimes, especially in winter. Such conditions are provided in soil of foliar habitats that are covered by leaf litter protecting nematodes from adverse effects of UV.

It is important to note that in addition to nematode infection (fig. 1, 2), the death of test insects was found to be caused by several other types of pathogenic microorganisms such as fungi, bacteria and parasites in the soil and live traps. Those were fungal infections (white muscardine caused *Beauveria bassiana* (Bals.-Criv.) Vuill, 1912; green muscardine caused *Metarhizium anisopliae* (Metschn.) Sorokin, 1883), various bacterial diseases (bacterioses), and also natural infection by endoparasitic dipteran larvae (Tachinidae). Occasionally, mixed infections caused by two types of pathogens, e. g., muscaridine disease with nematodosis or nematodosis with entomophagous insects, were observed.

Table 2. Number of positive samples[1] for both genera of entomopathogenic nematodes (*Steinernema*+ *Heterorhabditis* spp.) in each type of crop within Polissya (N = 132 samples) and forest-steppe (N = 165 samples) in 2016–2018

Crop	Regions							Total
	Polissya			Forest-steppe				
	Chern.	Zhyt.	Kyiv	Khmel.	Vin.	Kyiv	Cherk.	
Fruit orchard, decorative perennial plantings								
Malus domestica	4/22*	1/2	0/1	–#	–	5/6	–	10/31
Pyrus communis	0/2	1/2	–	–	–	1/2	–	2/6
Prunus domestica	1/5	2/2	–	–	–	1/4	–	4/11
Prúnu scérasus	0/5	1/2	–	–	–	0/2	–	1/9
Prúnu sarmeníaca	–	–	–	–	–	4/11	–	4/11
Prunus avium	–	–	0/1	–	–	0/10	–	0/11
Prúnus pérsica	–	2/2	–	–	–	–	–	2/2
Juglans regia	–	–	–	–	–	3/4	–	3/4
Móru snígra	–	–	–	–	–	3/6	–	3/6
Hippophaë rhamnoides	–	–	–	–	–	6/7	–	6/7
Viburnum opulus	–	–	–	–	–	0/2	–	0/2
Juniperus communis	–	–	–	–	–	1/2	–	1/2
Thújaoccidentális	–	–	–	–	–	2/2	–	2/2
Annual row and field crops								
Medicago sativa	1/4	–	–	–	–	–	–	1/4
Phaseolus vulgaris	–	–	–	–	–	1/3	–	1/3
Glycine max	–	0/6	–	0/13	0/22	–	–	0/41
Cucurbita pepo	–	–	–	–	–	1/3	–	1/3
Fagopyrum esculentum	2/47	–	–	–	–	–	–	2/47
Helianthus annuus	3/3	–	–	–	–	–	–	3/3
Avena sativa	0/3	–	–	–	–	–	–	0/3
Triticum aestivum	–	–	–	0/36	–	–	0/26	0/62
Solanum tuberosum	–	–	0/11	–	–	–	–	0/11
Beta vulgaris	–	0/12	–	–	–	–	0/4	0/16

Note.[1]— soil and trap samples.*— the number of samples: EPN — positive/total of analyzed. #— no samples.

Discussion

The literature data about the distribution peculiarities of entomopathogenic nematodes remains controversial in many respects. A part of researchers tend to the judgment that entomopathogenic nematodes (Steienernematidae, Heterorhabditidae) are more often observed in natural ecosystems, not changed by human activities. According to other researchers, these nematodes mainly inhabit artificial ecosystems altered by men (for example, cultivated field or agro-ecosystem). Also quite often they are detected in urban, suburban, and country soils: in plantings of street trees, park vegetation, and roadside soils, etc. (reviewed by Hominick, 2002: 135).

However, despite the fact that the entomopathogenic nematodes had been first found in dead insects, it was later revealed that level of nematode infections in natural populations of insects is not too high. According to the literature, the chance of finding insects infected with entomophatogenic nematodes is as small as 3 % (Orozco et al., 2014), unless epizooty takes place in insects (Akhurst et al., 1992) or massive sample size of the insects is examined (Nielsen & Philipsen, 2003).

The most recent study of Yakovlev (2017) on the distribution of entomopathogenic nematodes in the ecosystems of nature protected areas in Ukraine (national parks, nature reserves,

landscape reserves) showed that these nematodes are characterized by a lower frequency of occurrence in comparison with our results. Entomopathogenic nematodes were isolated from 13 soil samples (< 3 % of the total number of samples), two species from the family Steienernematidae of the genus *Steinernema* (*S. feltiae* and *S. arenarium*) were recovered, whereas *Heterorhabditis* species were not found. Identification of nematode isolates was confirmed by molecular analysis. Those species of the genus *Steinernema* were found in the Zakarpattia Region, Kyiv Region, Cherkasy Region, Kherson Region, Zaporizhzhia Region, and in Crimea.

Worth mentioning is recent large-scale comparative studies focused on the isolation and identification of entomopathogenic nematodes from various biocenoses, in particular agricultural fields in Ukraine (Sigharjova et al., 2010, 2012). The material was collected from different regions of the country (Kyiv, Vinnytsia, Khmelnytskyi, Sumy, Zaporizhzhya, Mykolaiv and Zakarpattia Rergions), and Autonomous Republic of Crimea. Overall, the percentage of EPN-positive soil samples was about 13.6 % of the total of 747 soil samples, according to the authors. All the isolates of entomopathogenic nematodes were identified as *S. feltiae*, *S. carpocapsae*, and *H. bacteriophora* (Sigharjova et al., 2012). 354 soil samples were analyzed from artificial ecosystems of aforementioned regions of Ukraine, out of which 80 (22.6 %) contained entomopathogenic nematodes. Entomopathogenic nematodes prefer more cultivated habitats (arable land soils) than soils from gardens: 27.9 % vs 17.7 % of positive samples. Soil sampling and soil-testing for nematodes taken from pine seedlings, garden plantings of walnut trees, peach and plum in some areas of the country did not detect entomopathogenic nematodes. The results of Sigharjova et al. (2010; 2012) somewhat do not coincide with our data, which indicate the presence of entomopathogenic nematodes in the gardens with the above-mentioned fruit plantations (walnut, peach, plum trees) under the conditions of the Polissya and forest-steppe zone.

Dzięgielewska (2012) showed that in the orchards without chemical treatments the higher occurrence and diversity of entomopathogenic nematodes is observed, compared to orchards exposed to chemical treatments. In addition, there are many records (Shulong et al., 2003; Negrisoli et al., 2008; Bezruchenok, 2010), which indicate a significant negative impact of some of the most commonly used pesticides (e. g., thiophanate-methyl, thiamethoxam, imidacloprid, aldicarb, carbofuran, chlorpyrifos, heptenophos) directly on the viability and biological activity of entomopathogenic nematodes.

In Crimea, only 22 out of 393 soil samples (5.6 %) contained entomopathogenic nematodes (Sigharjova et al., 2010). The largest percentage of positive samples was recorded from fruit and berry plantations (orchards and vineyards) — 8.2 % and 5.4 % respectively; the smallest percentage in forest park areas (ornamental plants, forest vegetation) — 4.9 %. Soil samples collected from mulberry plantings, juniper saplings, and sunflower fields did not contain entomopathogenic nematodes. These data are different from our results, perhaps because the surveys of Sigharjova et al. (2010) covered the hot, dry arid steppe and mountainous regions of Crimea, in contrast to our nematode surveys conducted in agro-ecological conditions of Polissya and forest-steppe zones.

It should be noted that in the forest-steppe and Polissia zones, as well as in the Carpathians, the species of the genus *Steinernema* predominated (97.5 % of all positive samples). The highest frequencies of occurrence of *Steinernema* species were on field crops (Sigharjova et al., 2010). On the contrary, in Crimea region the studies more often recorded the species of the genus *Heterorhabditis* (86.4 % of all positive samples), which were mostly isolated from orchards, vineyards and ornamental plots.

Our data demonstrate that entomopathogenic nematodes (Nematoda, Rhabditida, Steienernematidae et Heterorhabditidae) are an integral component of the agro-ecosystems of Ukraine. On the other hand, the prevalence of these nematodes in agro-ecosystems is effected by a number of factors, such as biotic (for example, target insect hosts); abiotic environmental factors (temperature, variation in light, moisture and edaphic factors in various seasons, microclimate in general); and also anthropogenic influence.

Conclusion

In the soils of agrocenoses of different crops we found three species of the entomopathogenic nematodes: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *Steinernema* sp. which may be attributed to the "*glaseri*" group s. l. using some morphological features. The occurrence index (OI) of these nematodes in agrocenoses was rather high, 15.48 % (46 out of the 297 samples). *Steinernema* species were isolated more often (OI 9.42 %) than *Heterorhabditis* species (OI 6.06 %). Their share among all 46 positive samples is respectively 60.8 % and 39.2 %.

The occurrence index of entomopathogenic nematodes in the soils of certain agrocenoses varied depending on the features of a regional climate, influence of weather conditions and season, the type of agrocenosis (row crop fields, fruit orchards, decorative landscaping perennial plantings), and agro-technical measures carried out in accordance with the technology of cultivating the plant species-edificators. The extent of occurrence of the entomopathogenic nematodes in soils of some areas can be quite high at 25.0–43.75 % of all investigated samples.

Entomopathogenic nematodes were somewhat more common in the agrocenoses of the forest-steppe zone than in farms located in Polissya zone, with a frequency of 16.9 % and 13.6 %, respectively. At the same time, steinernematid nematodes proportion reached 83.3%, in Polissya and only 46.4 % in the forest-steppe zone it was. The proportion of heterorhabditids was 16.7 % and 53.6 %, respectively. Thus, heterorhabditid nematodes were much more common in the soils of the agrocenoses of the forest-steppe zone (southern part of the Kyiv Region).

A fruit and berry plantations and ornamental plants are more inhabited by entomopathogenic nematodes than field crops. OI-values are 36.54 % and 3.15 %, respectively, and the proportion of positive samples is 12.7 % and 2.6 %. The most important factor is anthropogenic pressing, such as plowing of the soil, application of fertilizers and pesticides, spraying of chemical defoliants which are stronger at many field crops, especially at technical crops. These agrotechnical measures negatively affect the infective juveniles (= dauer larave) of nematodes and their insect hosts.

The distribution of the entomopathogenic nematodes varied to some extent depending of the predominant agricultural plant species. The highest number of positive samples was recorded from the apple trees, plum trees and somewhat less from the apricot trees, peach trees, mulberry trees, walnut trees. Entomopathogenic nematodes were not found only in plantations of two crops such as cherry trees and rowan trees from all thirteen species of fruit and ornamental crops. At the same time, these nematodes were found only in five of the ten field crops examined. It is sunflower, which completely shades the soil during early stages of vegetation. In the same way, entomopathogenic nematodes were found in soils under legumes (alfalfa and common beans), which in the final stages of vegetation form a dense cover of the stems, as well as on crops of buckwheat and common pumpkin, where their frequency were relatively low. No positive samples were found in the soil of such crops as common wheat, oats, soybean, potatoes and sugar beet.

Nematodes of the genus *Steinernema* are common in agrocenoses of both types, whereas *Heterorhabditis* occur exclusively in soil of fruit orchards and coniferous decorative perennial plantings, OI — is 39.2 %. Probably, these nematodes are more susceptible to the environmental influences (e. g., soil moisture, temperature regimes) on the free-living stages of the lifecycle, especially in winter. Such conditions are provided in soil covered by leaf litter, which protects against the effects of UV.

Entomopathogenic nematodes (*Steinernema*, *Heterorhabditis*) were not found in the collected samples of insects (immature stages and larvae of beetles, butterfly of caterpillars).

References

Adams, B. J., Fodor, A., Koppenhöfer, H. S., Stackebrandt, E., Stock, S. P., Klein, M. G. 2006. Biodiversity and systematics of nematode–bacterium entomopathogens. *Biological Control*, **37**, 32–49.

Akhurst, R., Bedding, R. A., Bull, R. M., Smith, D. R. 1992. An epizootic of *Heterorhabditis* spp. (Heterorhabditidae: Nematoda) in sugar cane scarabaeids (Coleoptera). *Fundamental & Applied Nematology*, **15** (1), 71–73.

Artyukhovskiy, A. K. 1967. *Neoaplectana arenaria*nov. sp. (Steinernematidae, Nematoda) — vozbuditel nematodoza mayskogo zhuka v Voronezhskoy obl. [*Neoaplectana arenaria*nov. sp. (Steinernematidae, Nematoda) as causative agent of the nematode disease of the May bug in the Voronezh region]. *Tr. Voronezhsk. gos. zapovdenika*, 15, 94–100.

Bedding, R. A., Akhurst, R. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. *Nematologica*, **21** (1), 109–110.

Bezruchenok, N. N. 2010. Vliyanie insektitsidov na entomopatogennye nematody [Effect of insecticides on entomopathogenic nematodes].*Vestnik Polesskogo gosudarstvennogo universiteta. Seriya obshchestvennykh i gumanitarnykh nauk* [Bulletin of Palesky state university. Series in social sciences and humanities], 1, 67–71.

Burnell, A. M., Stock, S. P. 2000. *Heterorhabditis, Steinernema* and their bacterial symbionts: lethal pathogens of insects. *Nematology*, **2** (1), 31–42.

Djadechko, M. P. 2001. *Biologhichnyj zakhyst roslyn* [Biological plant protection]. Bila Cerkva [In Ukrainian].

Dunaev, Ye. A. 1997. *Metody ekologo-entomologicheskikh issledovaniy* [Methods for ecological and entomological research]. Moscow City Station of Young Naturalists, Moscow, 1–44 [In Russian)].

Dzięgielewska, M. 2012. Occurrence of entomopathogenic nematodes of the family Steinernematidae and Heterorhabditidae in orchards chemically protected and unprotected. *Progress in plant protection*, **52** (2), 415–420.

Filipev, I.N. 1934.*Nematody vrednye i poleznye v selskom khozyaystve* [Harmful and useful nematodes in agriculture]. Ogiz, Selkhozgiz, Moscow, 1–440 [In Russian].

Gaugler, R., Kaya, H. 1990. *Entomopathogenic nematodes in biological control*. USA, CRC PressInc, 1–365.

Griffin, C. T., Downes, M. J., Block, W. 1990. Tests of Antarctic soils for insect parasitic nematodes. *Antarctic Science*, **2** (3), 221–222.

Hominick, W. M. 2002. Biogeography. *In*: Gaugler, R., ed. *Entomopathogenic Nematology*. CABI Publishing, Wallingford, 115–143.

Kharchenko, V., Sigareva, D., Galagan, T., Olenenko, V. 2012. Study of entomopathogenic nematodes from genera *Steinernema* and *Heterorhabditis* in Ukraine. Abstracts of the XI European Multicolloquium of Parasitology. Cluj-Napoca, July 25–29, 506–507.

Kiryanova, Ye. S., Puchkova, L. V. 1955. Novyy parazit sveklovichnogo dovgonosika*Neoaplectana bothynoderi* Kirjanova et Putschkova, sp. n. (Nematodes) [Anew parasite of sugar beetweevil *Neoaplectana bothynoderi* Kirjanova et Putschkova, sp. n. (Nematodes)]. *Trudy Zoologicheskogo instituta Akademii nauk SSSR* [Proceedings of the Zoological Institute of the USSR Academy of Sciences], **18**, 53–62.

Kornjushyn, V. A. 2009. Entomopatoghenni nematody jak perspektyvni aghenty biologhichnogho metodu borotjby zi shkidnykamy siljsjkoghospodarsjkykh kuljtur [Entompathogenic nematodes as promising agents for biological method of crop pest control]Proceedings of the *Ekologhija. Ljudyna. Suspiljstvo*: *XII Mizhnarodna naukovo-praktychna konferencija studentiv, aspirantiv ta molodykh vchenykh (Ukraine, Kyiv, May 13–17, 2009)*, Ekologhija. Ljudyna. Suspiljstvo, Kyiv, 42–43.

Kozodoy, Ye. M. 1984. Novaya entomopatogennaya nematode *Neoaplectana anomaly* sp. n. (Rhabditida, Steinernematidae) I nablyudeniya za ee biologiey [New entomopathogenic nematode *Neoaplectana anomaly* sp. n. (Rhabditida, Steinernematidae) and observation for its biology]. *Zoological Journal*, **11** (63), 1605–1609.

Lazarevskaya, S. L. 1962.K metodike izucheniya nematode nasekomykh [To the studying method of insect nematodes]. *Trudy GELAN SSSR* [Transactions of the Helminthology Laboratory, Academy of Sciences of the USSR], **12**, 43–51.

Negrisoli, A. S. Jr., Carla, R. C. Barbosa, A. M. Jr. 2008. Avaliacaoda compatibilidade de produtos fitossanitarios com nematoides entomopatogenicos (Rhabditida: Steinernematidae, Heterorhabditidae) utilizando o protocolo modificado da IOBC/WPRS. *Nematologia Brasileira*, **32** (2), 111–116.

Nielsen, O., Philipsen, H. 2003. Danish surveys on insectsnaturally infected with entomopathogenic nematodes. *Insect Pathogens and Insect Parasitic Nematodes, IOBC/WPRS Bulletin*, **26**, 131–136.

Orozco, R. A., Lee, M. M., Stock, S. P. 2014. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). *Journal of Visualized Experiments,* **89**, 1–8.

Poinar, Jr, G. O. 2011. The evolutionary history of nematodes as revealed in stone, amber and mummies. *In*: Hunt, D. J., Perry, R. N., eds. *Nematology Monographs and Perspectives. Vol. 9*. Brill, Leiden, 1–429.

Polozhentsev, P. A. 1956. Chervi — parazity nasekomykh [Worms as parasites of insects]. *Priroda* [Nature], 12, 102–104.

Ryss, A. Yu., Kulinich, O. A., Turitsin, V. S., Mazurin, Ye. S. 2011. Mutualisticheskie kompleksy nematod i bakteriy, assotsiirovanye s nasekomymi [Mutualistic complexes of nematodes and bacteria associated with insects]. *Entomologicheskoe obozrenie* [Entomological Review], **90** (3), 662–671.

Steinhaus, E. A. 1952.*Patologiya nasekomykh* [Insect pathology]. Inostrannaya literature, Moscow, 1–839 (In Russian).

Shulong Chen, Xiuying Han, Moens, M. 2003. Effect of chlorpyrifos on infectivity and survival of *Steinernema feltiae*. *Russian Journal of Nematology*, **11** (1), 1–6.

Sigharjova, D. D., Olenenko, V. V., Ghracyanova, N. V. 2010. Chastota vyjavlennja entomopatoghennykh nematod rodyn Steinernematidae ta Heterorhabditidae v biocenozakh Ukrajiny [Detection the frequency of occurrence of the entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae in biocenoses of Ukraine]. *Karantyn i zakhyst roslyn*, **3** (164), 19–21.

Sigharjova, D. D., Olenenko, V. V., Ghracyanova, N. V. 2012. Vydovyj sklad entomopatoghennykh nematod rodiv *Steinernema* ta *Heterorhabditis*, vyjavlenykh na terytoriji Ukrajiny [Species of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis*, found on the territory of Ukraine]. *In*: Sergeeva, Yu. A., ed. *Biologicheskaya zashchita rasteniy na puti innovatsiy*. Editing-Publishing Commission of EPS IBCO,Chernivci-Bojany, 63–66 (Information Bulletin No. 43).

Spiridonov S. E. 2001. Entomoparaziticheskie i entomopatogennye nematody [Entomoparasitic and entomopathogenic nematodes]. *Patogenny nasekomykh: strukturnye i funktsionalnye aspekty* [Pathogens of insects: structural and functional aspects]. Kruglyy god, Moscow, 428–474.

Stefanovska, T. R. 2007. Efektyvnistj rozmnozhennja entomopatoghennykh nematod *Steinernema carpocapsae* ta *Heterorhabditis bacteriophora* na lychynkakh *Galleria melonella* L. ta *Tenebrio molitor* L. [The efficency of rearing entomopathogenic nematodes *Steinernema carpocapsae* and *Heterhorabditis bacteriophora*-on*Galleria melonella* L. and *Tenebrio molitor* L. larvae]. *Naukovi dopovidi NAU* (electronic journal), 2, (7}, 1–11. Available at:http://www.nbuv.gov.ua/e-Journals/nd/2007–2/07stratm.pdf (accessed 20 January 2017).

Syrotenko A. J., Chernov, B. O. 2000. *Gheoghrafija Ukrajiny* [Geography of Ukraine]. Blaghovist, Kyiv, 1–256 [In Ukrainian]

Veremchuk G. V. 1969. Novye vidy entomopatogennykh nematod roda *Neoaplectana* (Rhabditida, Steinernematidae) iz shchelkunov [New species of entomopathogenic nematodes of the genus *Neoaplectana* (Rhabditida, Steinernematidae) from click beetle]. *Mater.nauch. konfer. VOG*, **1**, 44–50.

Wang, J., Bedding, R. A. 1996. Population development of *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* in the larvae of *Galleria mellonella.Fundamental & Applied Nematology,* **19** (4), 363–367.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. *Science*, **66**, 302–303.

Yakovlev, Ye. B. 2017. *Entomopatoghenni nematody (Nematoda: Rhabditida) v pryrodnykh ekosystemakh Ukrajiny [Entomopathogenic nematodes (Nematoda: Rhabditida) in the Natural Ecosystems of Ukraine]*. PhD Thesis, Schmalhausen Institute of Zoology NAS of Ukraine, Kyiv, 1–131 [In Ukrainian].

Yakovlev, Ye. B., Kharchenko, V. O. 2013. Entomopatoghenni nematody pryrodnykh ekosystem Ukrajiny [Entomopathogenic nematodes of natural ecosystems of Ukraine]. Proceedings of the *XV Konferencija Ukrajinsjkogho naukovogho tovarystva parazytologhiv (Ukraine, Chernivtsi, October 15–18, 2013).* Eds. Akimov, I. A., Kornjushyn, V. V., Volkov, R. A., Cherevatov, F., Korol, E. M., Kuzjmin, Yu. I., Kuzjmina, T. A., Lisitsyna, O. I., Ghrebin, O. B., Pavlikovsjka, T. M., Didyk, Yu. M., Kharchenko, V. O. Ukrajinsjke naukove tovarystvo parazytologhiv, Kyiv, 1–111.

Yakovlev, Ye. B., Kharchenko, V. A., Mračeк, Z. 2014. Findings of entomopathogenic nematodes (Rhabditida, Steinernematidae) in nature reserves in Ukraine.*Vestnik Zoologii*, **48** (3), 203–210.

Yakovlev, Ye., Nermut, J., Puza, V., Kharchenko, V., Mracek, Z. 2017. New record of *Steinernema arenarium* (Artyukhovsky) (Rhabditida: Steienernematidae) from Ukraine and a note on its distribution. *Acta Parasitologica*, **62** (2), 255–264.

Yakovlev, Ye. B., Olenenko V. V. 2009. Do fauny entomopatoghennykh nematod rodyn Steinernematidae ta Heterorhabditidae (Nematoda: Rhabditida) Centraljnogho lisostepu Ukrajiny [The fauna of the entomopathogenic nematodes of Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) of the Central Lisostep of Ukraine*]. Proceedings of the Zoologhichna nauka u suchasnomu suspiljstvi*: *Materialy Vseukrajinsjkoji naukovoji konferenciji, prysvjacheni 175-richchju zasnuvannja kafedry zoologhiji (Ukraine, Kyiv, 2009).* Fitosociocentr, Kyiv, 516–520.

Zlotin, A. Z. 1989. *Tekhnicheskaya entomologiya* [Technical entomology]. Naukova Dumka, Kiev, 1–183 [In Russian].

Received 19 September 2018
Accepted 4 March 2019