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Frequency of occurrence and identification of nematodes among entomopathogenic organisms in agrocoenoses of Ukraine

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abstract

The research objects were entomopathogenic organisms (Nematoda, Fungi, Bacteria, and Insecta) collected in agrocoenoses in different regions of Ukraine during 2016–2018 and 2020–2021. The following research materials were used: soil samples, soil live-traps, specimens of Galleria mellonella L., 1758 (Lepidoptera: Pyralidae) and potential host insects (Coleoptera: Elateridae, Tenebrionidae, Melolonthinae; Lepidoptera: Noctuidae). We analysed 312 samples (220 soil + 92 live-trap samples) and >100 specimens of potential host insects. Our data demonstrate that in soils of agrocoenoses, favourable conditions are created for the dispersal of entomopathogenic organisms. We report the frequency of occurrence of insect-pathogenic nematodes in agrocoenoses of Ukraine, and describe their identification and the specifics of pathology they cause to the insect Galleria mellonella in the context of other entomopathogenic organisms. The frequency of occurrence (% of samples) of entomopathogenic nematodes (genus Steinernema Travassos, 1927 and genus Heterorhabditis Poinar, 1976) in agrocoenoses was the highest among other organisms that caused infectious and parasitic diseases of insects and were found in 15% of the samples. Three species of entomopathogenic nematodes—Steinernema carpocapsae (Weiser, 1955) Wouts et al., 1982, Steinernema ex gr. 'glaseri', and Heterorhabditis bacteriophora Poinar, 1976—have been identified. We have found 8% of samples to contain fungal disease-causing agents (genera Beauveria Vuill., 1912, Metarhizium Sorokin, 1879, and Akanthomyces Lebert, 1858). The frequency of occurrence (%) of all remaining causative agents of infectious and parasitic diseases of insects, namely bacterial diseases and myiasis (infection of a fly larva) (Diptera: Tachinidae) were 3% and 2%, respectively. A mixed infection was detected in 2.5% of the total number of analysed samples; nematodoses-mycoses mixed infections were most often recorded. We have recorded the phenomenon of hyperparasitism with nematodosis-entomosis co-infection inside dead G. mellonella larvae for the first time.

Частота трапляння та ідентифікація нематод серед ентомопатогенних організмів у агроценозах України

Андрій Ковтун, Світлана Петренко

Резюме. Об'єктом досліджень були ентомопатогенні організми (Nematoda, Fungi, Bacteria, Insecta), виявлені в агроценозах різних регіонів України протягом 2016–2018, 2020–2021 років. Матеріалом для досліджень слугували проби ґрунту, ґрунтові «живі» пастки, зразки тест-комах *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) та потенційних комах-хазяїв різних видів (Coleoptera: Elateridae, Tenebrionidae, Melolonthinae; Lepidoptera: Noctuidae). Упродовж досліджень проаналізовано 312 проб (220 ґрунтових проб та 92 «живих» пасток), більше 100 екземплярів потенційних комах-хазяїв. Встановлено, що у ґрунтах агроценозів створюються сприятливі умови для розселення ентомопатогенних організмів. Охарактеризовано частоту трапляння нематод, що є патогенами комах в агроценозах України, їх ідентифікацію та особливості прояву патологічних ознак на комасі *Galleria mellonella* та інших ентомопатогенних організмів. Частота трапляння (% проб) ентомопатогенних нематод (збудники — нематоди з родів *Steinernema* Travassos, 1927 та *Heterorhabditis* Poinar, 1976) в агроценозах була найвищою — 15% серед усіх інших ентомопатогенних організмів, що зумовлювали інфекційні та паразитарні захворювання комах. Виявлені ізоляти ентомопатогенних нематод віднесено до трьох видів — *Steinernema carpocapsae* (Weiser, 1955) Wouts et al., 1982, *Steinernema* ex gr. *glaseri*, та *Heterorhabditis bacteriophora* Poinar, 1976. Дещо нижче (8%) припадало на грибкові патогени, що призводили до різних мікозів (збудники — *Beauveria* Vuill., 1912, *Metarhizium* Sorokin, 1879 та *Akanthomyces* Lebert, 1858). Частота трапляння (%) решти збудників інфекційних та паразитарних захворювань комах — бактерій (бактеріози) та личинки паразитичних мух (Diptera: Tachinidae) (ентомози, міази) сягала 3% та 2% відповідно. Мікст-інфекція виявлена у 2,5% від загальної кількості досліджених проб, найчастіше реєструвалися нематодози із мікозами. Уперше зареєстровано явище гіперпаразитизму у тілі загиблих личинок *G. mellonella* при змішаній нематодозно-ентомозній інфекції.

Ключові слова: частота трапляння, ентомопатогенні нематоди, патологія комах, агроценози.

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Introduction

The composition of the heterotrophic part of any biocoenosis is highly diverse and dynamic. In many cases, certain species that are part of the zoocoenosis and microbiocoenosis of an ecosystem form active parasitic complexes—interaction of the predator–prey and parasite–host types, which positively affect the stability and diversity of biological species of biocoenoses [Wall *et al.* 2015]. Entomopathogenic organisms plays an important role in the soil environment of ecosystems, leading to various infectious and parasitic diseases of helminth (nematodoses), fungal (mycoses), bacterial (bacterioses), viral (viroses), and protozoan (protozoan diseases) etiology [Padiy 2001; Deka *et al.* 2021].

Insect diseases have a significant impact on population dynamics in nature and culture. In nature, insect diseases often have a rather complex etiology, as they are often caused by several pathogens (i.e. mixed infection). This contributes to the faster development of epizootic processes that cover large areas, affect behaviour and physiology, and lead to profound changes in the host body [Telenga 1955; Zlotin 1989; Spescha *et al.* 2023; Puza & Tarasco 2023].

The parasitism of helminths, in particular nematodes, in insects is an interesting biological and economically important phenomenon. Worm infection in insects is less common than in vertebrates and humans. This ecological group of nematodes has adapted to exist in the body of insects, causing nematode infections (i.e. nematodoses) [Polozhencev 1956; Spiridonov 2001]. The body of nematodes is transparent, filamentous or spindle-shaped, tapering to one or both ends, round in cross-section, covered with a dense cuticle, and varies in length from several tens of micrometers to 35 cm. In total, there are more than 1000 species of entomoparasitic nematodes that are somehow associated with insects—from casual relations to parasitic forms that parasitize them at all stages of their development from egg to adult, while concentrating in various organs and tissues in the form of eggs, larvae,

or adults. They affect insects from more than 100 families of 21 orders, including the vast majority of beetles, lepidopterans, dipterans, and others [Zlotin 1989].

Representatives of two families of rhabditid worms (Nematoda: Rhabditida)—Steienernematidae and Heterorhabditidae—have the greatest impact among the entire ecological group of entomonematodes on reducing the number of insects [Spiridonov 2001]. Due to their obligate mutualistic relationship with intestinal symbiont bacteria of the family Enterobacteriaceae Rahn, 1937, they are able to kill insects and develop in their bodies, which allows them to be used in practical purposes as a biological method of plant protection against economically important insect pests.

For a long time, these nematodes have remained poorly studied due to the complexity of their detection and diagnosis, which is related to the specifics of their biology. Entomopathogenic nematodes (Steienernematidae and Heterorhabditidae) are characterised by a secretive lifestyle as the development of infective juveniles (dauer larvae) and adults is separated in space and time, and therefore either only infective juveniles are found in soil or their adult stages are found inside larvae and pupae of insect hosts. Accordingly, the first findings of entomopathogenic nematodes were associated with the analysis of the causes of death of various insects, their dissection and subsequent isolation from the cadavers of victims. The researchers began to examine soils to isolate infective juveniles only after the life cycles of their development were clearly established [Poinar & Grewal 2012].

In nature, the chance of finding insects infected with nematodes are on average less than 3%, unless a widespread natural epizootic occurs in insects with a significant extent of infestation or a large sample size of the insects is examined [Orozco *et al.* 2014]. One striking example and confirmation of this is the survey by Nielsen & Philipsen [2003], which was conducted in agrocoenoses in Denmark to obtain information on the possible impact of entomopathogenic nematodes on pest populations, mainly on the cabbage root fly *Delia radicum* L., 1758 (Diptera: Anthomyiidae). Over the course of two years, more than 6000 insect specimens were collected and analysed, of which 4000 specimens belonged to *D. radicum*. The results of the study showed that 69 specimens of different insect species were infected with entomopathogenic nematodes (Steienernematidae), including only 4 specimens of *Delia radicum*.

So far, four species of entomopathogenic nematodes (Steienernematidae and Heterorhabditidae) have been recorded in Ukraine: *Steinernema carpocapsae* (Weiser, 1955) Wouts et al. 1982, *Steinernema feltiae* (Filipjev, 1934) Wouts et al. 1982, *Steinernema arenarium* (Artyukhovsky, 1967) Wouts et al. 1982, and *Heterorhabditis bacteriophora* Poinar, 1976 [Stefanovska 2007; Anonymous 2012; Yakovlev *et al.* 2014; Sigareva *et al.* 2019]. All findings of entomopathogenic nematodes were associated with the analysis of soil samples collected in different biocoenoses in Ukraine. No entomological surveys with subsequent determination of the causes of death and selection of dead individuals based on signs of damage typical for entomopathogenic nematodes have been conducted, and this information remains a 'white spot' in agricultural entomology and nematology in Ukraine.

The aim of this research is to study the frequency of occurrence of entomopathogenic nematodes in agrocoenoses of Ukraine, their identification and specifics of pathology they cause to the insect *Galleria mellonella* in the context of other entomopathogenic organisms that cause lethal infectious and parasitic diseases of insects.

Materials and Methods

The study is based on the materials of fragmentary surveys of agrocoenoses (annual row and field crops—cereals and legumes, technical crops, vegetable, as well as perennial plants of orchards and decorative plantations) in the summer–autumn period of 2016–2018 and 2020–2021. The surveyed agrocoenoses were located within the main soil-climatic zones of Ukraine (administrative regions, i.e. oblasts): Zhytomyr, Kyiv, Chernihiv, Cherkasy, Khmelnytskyi, Vinnytsia, and Odesa) (Fig. 1).

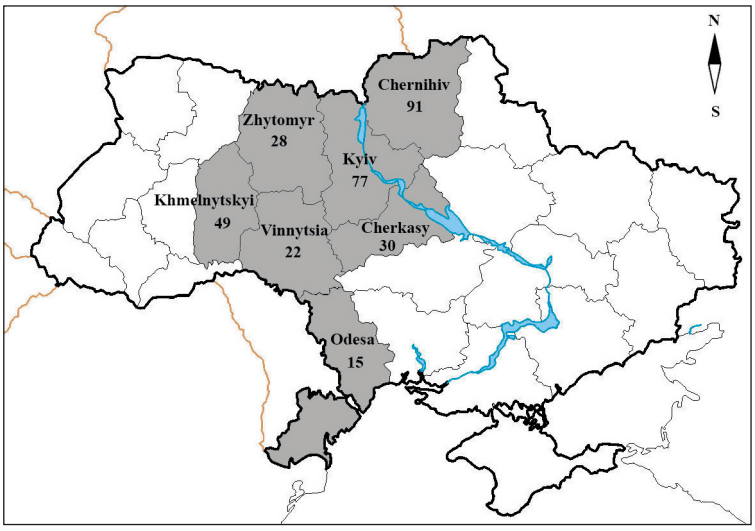

Fig. 1. Map of soil sample collection and soil live-traps. The numbers indicate the number of analysed samples.

Рис. 1. Карта-схема відбору ґрунтових проб та закладання «живих» пасток. Цифри — кількість відібраних проб.

The material for the study included soil samples, soil live-traps, test insect *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) specimens and potential host insects of various species (Coleoptera: Elateridae, Tenebrionidae, Melolonthinae; Lepidoptera: Noctuidae), as well as entomopathogenic organisms (Nematoda, Fungi, Bacteria, and Insecta) that cause lethal infectious and parasitic diseases of insects.

Soil samples for the study were collected randomly using a shovel: for the formation of a composite sample (volume of approximately 500 cm^3), 5 single point samples were collected from an 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 orchard and decorative perennial plants) [Orozco *et al.* 2014]. In addition, soil live-traps in the form of metal spherical capsules (tea ball strainers) with *G. mellonella* larvae at the final instar stage (weighing 0.20 ± 0.03 g) were embedded in the topsoil (10–30 cm). *G. mellonella* were cultured in a thermostat at 27–30 °C on a natural wax raw material in the laboratory. After 5–6 days, the test insects were dug up for further analysis in the laboratory. We also collected sick and dead soil insects, mainly crop pests (Coleoptera: Elateridae, Tenebrionidae, Melolonthinae; Lepidoptera: Noctuidae) with further determination of the cause of death. In total, 312 samples (220 soil samples and 92 soil live-traps) were processed, and more than 100 specimens of potential host insects were analysed.

The isolation of entomopathogens from the collected soil samples/soil live-traps was carried out by the biotesting method using susceptible to microbial infections greater wax moth larvae (*Galleria mellonella*) under laboratory conditions [Orozco *et al.* 2014; Correa *et al.* 2022]. Once the insects had died, their cadavers were either placed in a White trap [White 1927] to isolate entomopathogenic nematodes or immediately processed following classic dissection techniques [Lazarevskaya 1962] under an MBS-9 stereoscopic microscope and tissues were scrutinised for pathogens.

The differentiation of the detected infectious and parasitic diseases caused by a variety of etiological agents was carried out according to the macroscopic pathological changes (using a magnifying glass [10×] or stereoscopic microscope MBS–9) observed in the infected and dead larvae of the genus *Galleria* during the penetration into the body and subsequent development of agents [Shtejnhauz 1952]. The main pathologies revealed include the following: changes in the colour of the outer surface (or epicuticle); changes in the size, shape, and consistency of the body; presence of a specific smell of the test insects; auxiliary signs related to behavioural features (e.g. mobility, feeding); posture changes; and other changes.

The identification of isolated entomopathogens (at genus/species level) was carried out by the appearance of *G. mellonella* test insects killed by them after infection, as well as by the features of

morphological characteristics of entomopathogens using light microscopy (Carl Zeiss Primo Star microscope, 100×–1000×). Different special keys were used for the identification of entomopathogens, with special attention paid to nematode identification. The external cuticular structure, trophic-sensory part of the body, trophic-genital part of the body, and caudal part of the body were examined for species identification of entomopathogenic nematodes, along with different morphometric parameters of adult generations (primarily males, ♂) and infective third-stage larvae (L3), using special keys for entomopathogenic nematodes (Steienernematidae and Heterorhabditidae) [Nguyen & Smart 1996; Nguyen *et al.* 2007]. The following abbreviations and ratios were used: n, number of specimens measured; L, body length; W, maximum body width; EP, distance from the anterior end to the secretory-excretory pore; ES, pharynx length; TL, tail length; ABW, anal body width; SL, spicule length; and GL, gubernaculum length. Additionally, four indices were calculated: D% = (EP/ES) × 100; E% = (EP/TL) × 100; GS% = (GL/SL) × 100; SW% = (SL/ABW) × 100. The study of the species composition and frequency of occurrence of entomopathogens was conducted at the Laboratory of Nematology of the Institute of Plant Protection, National Academy of Agrarian Sciences of Ukraine, and in the Department of Horticulture, Viticulture, Biology, and Chemistry of the Faculty of Agrobiotechnology of Odesa State Agrarian University.

The frequency of occurrence (F, %) of entomopathogenic organisms was calculated as the ratio of the number of positive samples (containing entomopathogenic species) to the total number of samples collected:

$$F = n / N \times 100\ (\%),$$

where *F* is the frequency index; *n* is the number of positive samples with entomopathogens; and *N* is the total number of samples collected.

To describe the general quantitative patterns, we used standard statistical measures: *M*, mean value; *min*, minimum value; and *max*, maximum value.

The individual stages of the study were recorded by photographing with a digital camera Olympus SH-21 connected to a stereomicroscope MBS-9.

Results and Discussion

The study has revealed that of the 312 samples analysed, 30.5% were positive for the presence of various entomopathogenic organisms that cause infectious and parasitic diseases of insects in the form of mono- and mixed infections (Fig. 2–3). Nematode infections have the highest rate (15%, see Fig. 2) and are caused by representatives of the genera *Steinernema* Travassos, 1927 and *Heterorhabditis* Poinar, 1976 (Rhabditida: Steienernematidae and Heterorhabditidae). It is known that the symptoms observed in insects when they are infected by entomopathogenic organisms depend on the species of the parasite (pathogen), and on the developmental stage, age, and species of the host insect.

The main obvious signs of nematode infection in *G. mellonella* larvae were as follows: a characteristic change in the colour of the outer surfaces of the body of infected larvae to red-raspberry (typical for *Heterorhabditis* spp.) or various gradations of grey, or yellowish (typical for *Steinernema* spp.); changes in the size and shape of insect cadavers (infected individuals slightly increase in volume, the bodies swelled and acquired a soft, elastic rubber-like consistency as the internal organs under the influence of symbiont bacteria turned into a cloudy liquid; and the absence of any specific smell in the nematode-killed insect larvae (Fig. 3).

The identified isolates of entomopathogenic nematodes from agrocoenoses of different zones and regions of the country, based on the study of morphological and morphometric parameters (Table 1–2), were assigned to three species: *Steinernema carpocapsae*, *Steinernema* ex gr. '*glaseri*', and *Heterorhabditis bacteriophora*. The species *S. carpocapsae* is a common and widespread, while the other two are less common. It was found that entomopathogenic nematodes exhibit considerable variability in many of the analysed morphological characters and morphometric indices (see Table 1–2),

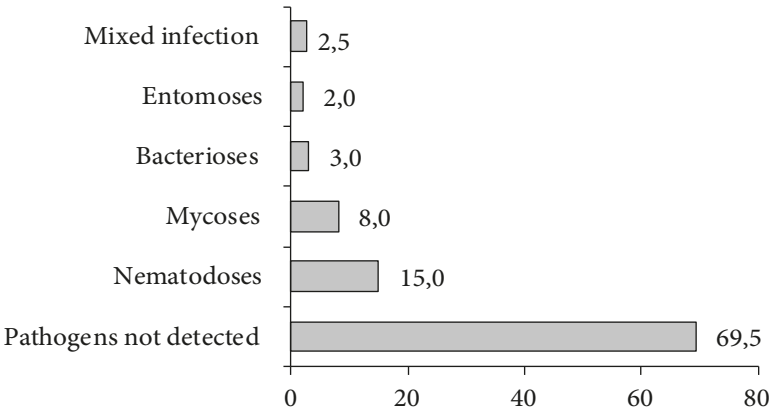

Fig. 2. The ratio of various causative agents of infectious and parasitic diseases among the larvae of *Galleria mellonella* in the form of mono- and mixed infection (%) found in the soil samples and live-traps (from agricultural soils of Ukraine in 2016–2018, 2020–2021).

Рис. 2. Співвідношення інфекційних та паразитарних збудників захворювань серед личинок *Galleria mellonella* у формі моно- і змішаної (мікст) інфекції (%) виявлених у пробах ґрунту та «живих» пастках (із ґрунтів агроценозів України 2016–2018, 2020–2021 років).

which complicated the process of identification of individual isolates. In particular, this applies to *Steinernema* ex gr. '*glaseri*'. According to the preliminary morphological characters and morphometric parameters of infective third-stage larvae (L3), we assigned this isolate to the morphological group '*glaseri*'. Currently, there are five such groups (*glaseri*, *feltiae*, *intermedium*, *carpocapsae*, and *bicornutum*), which show the relationship between certain close species of entomopathogenic nematodes of the genus *Steinernema*, and are based on the body length of infective third-stage larvae (L3) [Nguyen *et al.* 2007].

In addition to nematode infection, numerous cases of mycoses were diagnosed in the samples (8% of all the examined samples), which are caused by the following fungal pathogens (Fig. 2–3): the causative agent of white muscardine disease—species of the genus *Beauveri*a Vuill, 1912 (Hypocreales: Clavicipitaceae) (see: Fig. 3, *1–3*); the causative agent of green muscardine disease—species of the genus *Metarhizium* Sorokin, 1879 (Hypocreales: Clavicipitaceae) and the genus *Akanthomyces* Lebert, 1858 (Hypocreales: Cordycipitaceae). In cases of mycoses, the bodies of insects germinated with filamentous hyphae of the fungus, thus becoming dense (mummified) and fragile, and are difficult to be separated from the substrate. Externally, depending on the pathogen, insect cadavers were covered with a white or green spore coating (see: Fig. 3). Among the mycoses, the most widespread are entomophthorosis caused by members of the family Entomophthoraceae and muscardinosis caused by species of the genus *Bauveria* Vuillemin, 1912; less common are mycoses caused by fungi of the genus *Cordyceps* Fries, 1818 [Pérez-González *et al.* 2014; Tkaczuk *et al.* 2016; Korosi *et al.* 2019; Mantzoukas *et al.* 2022].

The analysis of the appearance of some dead test insects made it possible to reveal the bacterial nature of the diseases (bacterioses), which accounted for 3% of all the examined samples (see: Fig. 2–3). In case of bacterioses, freshly killed insect larvae became lethargic and their bodies became slightly darker. The hypodermal cells of the dead insects remained dense, as the skin did not break, and the cadavers had a specific smell of rotting [Abdelgaffar *et al.* 2022].

In addition, during the research period, we recorded cases of natural infection with entomoses (called miases) caused by the parasitisation of endoparasitic dipteran larvae (Diptera: Tachinidae), which account for 2% of the total number of samples examined (Fig. 2).

Occasionally, mixed infection was observed, caused by several pathogens, such as nematodes and fungi, or nematodes and endoparasitic fly larvae. Mixed infection was detected in 2.5% of the total number of samples examined (see: Fig. 2), with the highest rate of infection of the insect *G. mellonella* with nematodoses-mycoses (called muscardinoses). As it was already mentioned, mixed infection is quite common among insect pathologies, which leads to deeper changes in the victim's body, affects the development and number of the offspring, and contributes to the faster development of epizootics [Diachenko & Padiy 2001; Spescha *et al.* 2023; Puza & Tarasco 2023]. According to our observations,

in the case of entomophagy–nematode parasitic association in nematodosis with entomosis (miasis), we can speak of the phenomenon of hyperparasitism in natural conditions, since the larvae of the parasitic dipteran that infected *G. mellonella*, in turn, were infected with entomopathogenic nematodes in the body of the dead insects. Such case of a mixed infection is not mentioned in the recent scientific literature at all. This fact is of considerable scientific and practical interest, since, for example, it allows for a more rational combined use of both groups of biological pest control agents to increase their insecticidal effectiveness.

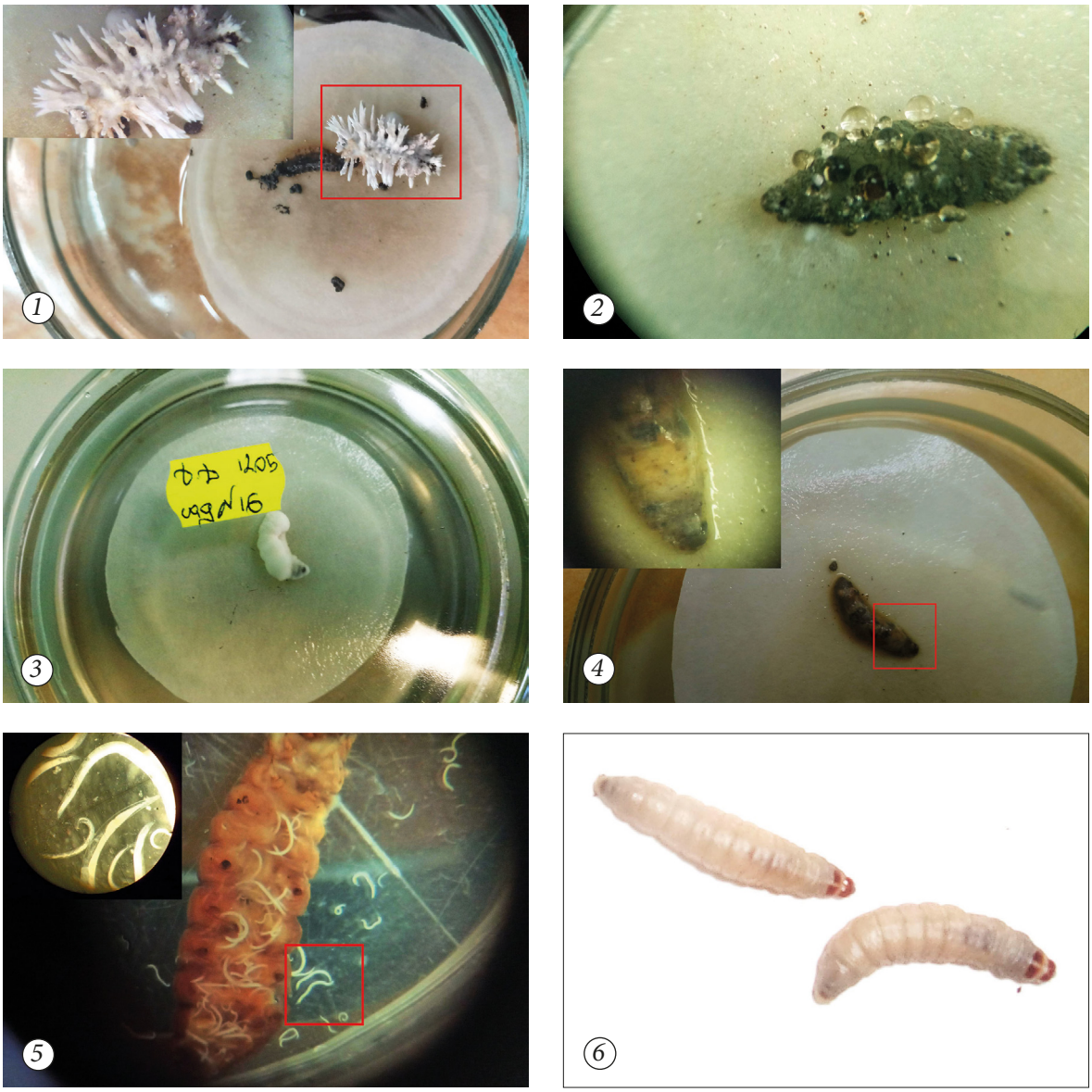

Fig. 3. Manifestation of different infectious and parasitic pathologies in larvae of the insect *Galleria mellonella*: (*1-3*) mycoses caused by *Akanthomyces* sp. (*1*), *Metarhizium* sp. (*2*), and *Beauveria* sp. (*3*); (*4*) bacteriosis; (*5*) nematodosis caused by *Heterorhabditis* sp.; (*6*) uninfected larvae of *G. mellonella* (control). Photo by the authors.

Рис. 3. Прояв різних інфекційних та паразитарних патологій на личинках тест-комахи *Galleria mellonella*: (*1-3*) мікози: (*1*) збудник *Akanthomyces* sp., (*2*) збудник *Metarhizium* sp., (*3*) збудник *Beauveria* sp.; (*4*) бактеріоз; (*5*) нематодоз (збудник *Heterorhabditis* sp.); (*6*) не уражені особини *G. mellonella* (контроль). Оригінал.

Table 1. **Comparative morphometric characters of infective juveniles and first generation males among the populations of** *Steinernema carpocapsae* **from agrocoenoses of different zones and regions of Ukraine***

Таблиця 1. **Порівняльна морфометрична характеристика інвазійних личинок та самців першого покоління серед популяцій** *Steinernema carpocapsae* **із агроценозів різних зон та регіонів України***

Character	Polissia		Forest–steppe
	Zhytomyr Oblast (strain Ovr–1)	Chernihiv Oblast (strain Mor–1)	Kyiv Oblast (strain DD–5)
Infective juveniles (n = 10)			
L	530 (516–548)	522 (445–550)	527 (513–552)
W	24 (21–27)	22 (19–26)	23.5 (21.3–26.4)
EP	36.2 (32.4–39)	31.6 (30–37.4)	33 (30–36)
ES	105 (102–108)	100 (96–110)	107 (102–113)
TL	50.5 (47.5–55)	46 (44–51)	48.5 (46–59)
ABW	25 (17–36)	28 (19–34)	28.6 (19–32)
D%	35 (34–38)	31 (30–37)	30 (26–32)
E%	70 (64–78)	69 (60–77)	65 (60–72)
First generation males (n = 10)			
L	1282 (1074–1378)	1510 (1110–1766)	1293 (1124–1424)
W	170 (140–182)	135 (78–168)	113 (96–126)
TL	21.5 (15–27)	25 (18–30)	19 (16–20,4)
ABW	59 (44–73)	52 (44–73)	62 (44–73)
SL	67 (66–68)	67.9 (66–72)	61,3 (57.6–63)
GL	42 (39–44)	45 (42–54)	42 (38–45)
EP	66 (64–84)	69 (60–73)	60 (55–65)
ES	165 (140–196)	168 (138–189)	141 (125–148)
D%	45 (40–50)	41 (32–49)	39 (37–41)
E%	33 (27–38)	29 (22–39)	25 (24–26)
GS%	60 (57–71)	67 (60–75)	71 (63–84)
SW%	122 (110–136)	130 (114–140)	98 (91–113)

* All measurements are given in μm as M (min–max).

* Вимірювання наведено в мкм і представлено у формі: M (min–max).

Table 2. **Comparative morphometric characters of infective juveniles and second generation males among the populations of** *Heterorhabditis bacteriophora* **from different zones and regions of Ukraine***

Таблиця 2. **Порівняльна морфометрична характеристика інвазійних личинок та самців другого покоління серед популяцій** *Heterorhabditis bacteriophora* **із різних зон та регіонів України***

Character	Polissia		Forest–steppe
	Zhytomyr Oblast (strain Ovr–2)	Chernihiv Oblast (strain Ost–4)	Kyiv Oblast (strain DD–14)
Infective juveniles (n = 10)			
L	550 (506–610)	512 (504–520)	600 (520–660)
W	21.4 (19–24)	20.3 (19–22)	23 (21–25)
EP	100 (82–106)	89 (86.4–90)	105 (89–108)
ES	117 (110–120)	109 (106–112)	116 (108–120)
TL	85 (80–90)	90 (80–95)	94.4 (78–114)
ABW	16 (14–19)	14 (13–17)	14 (13–16)
D%	84 (82–89)	81 (79–85)	93 (70–100)
E%	110 (100–120)	100 (90–110)	108 (100–117)
Second generation males (n = 10)			
L	968 (890–1026)	850 (770–940)	925 (895–1018)
W	44.7 (42–48)	47.5 (45–48)	57.5 (39.5–97)
TL	22 (18–24)	27 (24–31)	35.5 (31–42.3)
ABW	23.2 (18.3–26.7)	21 (13–25.5)	23.1 (19–25.4)
SL	38.3 (37–43)	42.6 (37–48)	41.2 (33.8–46.5)
GL	22.7 (19–24)	24,3 (23–26)	21.5 (18.3–23.9)
EP	134 (107–162)	136.4 (115.5–169.0)	97.6 (84.5–119.7)
ES	100 (89–102)	103 (98–107)	115.3 (101.4–139.4)
D%	118.4 (104.8–150.7)	86.8 (77.9–107.6)	92 (77.9–107.6)
E%	448 (334–611)	489 (402–585)	470 (394–570)
GS%	52.2 (43.7–57.7)	4.8 (43–61)	49.8 (42.3–65)
SW%	179.8 (150.2–220.4)	180.6 (117.6–230.8)	180.6 (117.6–230.8)

* All measurements are given in μm as M (min–max).

* Вимірювання наведено в мкм і представлено у формі: M (min-max).

It should also be emphasised that when examining specimens of dead *G. mellonella* larvae, we have found the following facts: in two cases, no entomopathogenic nematodes were found inside the body of *Galleria* individuals despite clear signs of infection. In particular, the appearance of the dead insects (red-raspberry colour of the outer surfaces) and the typical appearance of the remains of the internal tissues and organs of the insect, which were decomposed by symbiont bacteria, clearly indicated nematodosis (etiological agent—*Heterorhabditis* sp.). The absence of entomopathogenic nematodes in these cases, in our opinion, can be explained by the fact that a protective immune response was triggered in these insects to the invasion of the entomopathogen, namely the encapsulation of nematodes with their subsequent melanisation [Ebrahimi *et al.* 2011]. Nevertheless, the entomopathogenic nematode infective juveniles, after penetrating the insect body, managed to inoculate symbiotic bacteria into the haemocoel before encapsulation occurred. It is highly likely that the success of overcoming the protective barrier can be explained by the small number of nematodes penetrating the host body and their low pathogenicity.

The results of our surveys of various agrocoenoses show that, in general, perennial agricultural plantations are more populated with entomopathogenic organisms than field crops— 82.7% vs 17.3% of all positive samples, respectively. In our opinion, one possible explanation of this difference is that perennial plantations are characterised by a richer arthropod diversity, which directly ensure the recovery of entomopathogen populations (entomopathogenic nematodes in particular).

Equally relevant and complex is the issue of the frequency of infection of pests by entomopathogenic nematodes and their host insect specificity in biocoenoses. Despite the fact that the first findings of entomopathogenic nematodes were obtained from dead insects, it was later found that the level of insect infection in natural populations is not high.

Laboratory analysis of soil-living insect pests collected and captured in nature by us for their infection with entomopathogens did not reveal any infections. We believe that this is primarily due to the small sample size of the insects related to the fact that we did not conduct entomological surveys (insect surveys) in agrocoenoses, but simply selected sick and dead insects that came into our field of view (mostly well-marked, large in size), with the subsequent establishment of the causes of death. In different surveyed areas, the composition of harmful insects was quite diverse. The most numerous among them were as follows: click beetle larvae (Coleoptera: Elateridae), darkling beetle larvae (Coleoptera: Tenebrionidae), and imago and larvae (known as white grubs) of cockchafer beetles (Coleoptera: Scarabaeoidea: Melolonthinae). In addition to the above insects, caterpillars of the scoops (also referred to as cutworm) (Lepidoptera: Noctuidae), some crickets (Orthoptera: Gryllidae), ants (Hymenoptera: Formicidae), fly pupae (Diptera), and others were found in the topsoil layer. All of the studied insects, with some exceptions, according to the literature [Artyukhovskiy 1967; Veremchuk 1969], could potentially be hosts of entomopathogenic nematodes.

Conclusions

The results of the research indicate a rather significant level of occurrence of entomopathogenic nematodes in Ukrainian agrocoenoses compared to other groups of entomopathogenic organisms.

The detected isolates of entomopathogenic nematodes were assigned to three species, such as *Steinernema carpocapsae*, *Steinernema* ex gr. '*glaseri*', and *Heterorhabditis bacteriophora*. The species *S. carpocapsae* is common and widespread, while the other two are less common and rare.

For the first time, the phenomenon of hyperparasitism in the body of dead insects of the species *Galleria mellonella* was recorded in the detected mixed infection, namely entomophagy–nematode parasitic association. Of considerable scientific and practical interest is the fact that we have identified in agrocoenoses different subpopulations of entomopathogenic nematodes from the genera *Steinernema* and *Heterorhabditis*, which are promising bioagents for controlling harmful insect species.

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