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PARASITIC NEMATOFAUNA IN PEA CROPS (*Pisum sativum* L.) UNDER THE IMPACT OF THE UNSTABLE ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The results of the present research estimated the efficiency of the phytosanitary helmintological control in peas grown in open field and elucidated the helmintological parasitic impact, establishing the range of the invasive nematofauna, the frequency and the abundance of the associations. As a result of the phytosanitary research and the analysis of the helmintological impact on pea plants, it was found that the parasitic nematode complexes consisted of 14 species included in 4 families: Aphelenchidae, Hoplolaimidae, Tylenchidae, Heteroderidae, of the order Tylenchida, class Nematoda, distributed according to the investigated areas and classified according to the spectrum of trophic specialization in 5 groups. A larger number of species was detected in the Center area (14 species), as compared to the North area (8 species). It was found that the values varied from 7 to 30%, the damage being caused mainly by invasive associations of parasite nematodes of the genera: Ditylenchus, Pratylenchus, Heterodera, Meloidogyne, Helicotylenchus, Aphelenchus. The results of the phytosanitary monitoring contributed to elucidating the degree of nematological damage and brought new evidence in favor of applying sustainable pest control measures suitable for fabaceae agrocenoses.

Key words*: nematodes, helmintological control, pea sectors, parasitic impact, trophic specialization.*

INTRODUCTION

Cultivating peas for grains, seed material and fodder solves three strategic problems: increasing seed production, vegetable protein production and improving soil fertility, being 2-3 times richer in vegetable protein and having the advantage that it can be sown and harvested extra early (Starodub & Gheorghiev, 2013; Starodub et al., 2015). They are sown as a first priority under the environmental conditions of the Republic of Moldova, when the soil has sufficient moisture and allows sowing, usually in early March, but delayed sowing leads to significant production losses. Pea plantations for grain and fodder represent 8% of the arable land in the Republic of Moldova. They are annually invaded by over 40 harmful species, which are also associated with invasive nematode complexes that trigger helminthiasis, which motivates specific annual monitoring to control the populations and their parasitic impact in the cultivation process of this crop (Nesterov, 1997; Poiras et al., 2016; Iurcu-Străistaru et al., 2019). Annually, according to the new institutional project 2024-2027, we have conducted nematological research, including biodiversity estimates, morpho-ecological assessments and analyses of the structure of complexes of parasitic fauna, affecting various field crops, including peas.

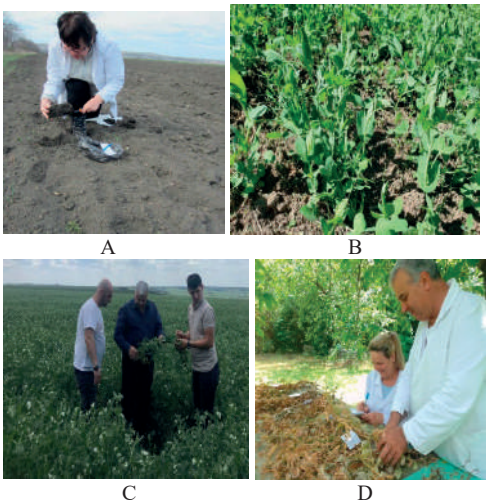
In the Republic of Moldova, helminthological research and monitoring of the biodiversity and structure of parasitic nematode complexes, detected in the agrocenoses of field crops cultivated in state-owned and private enterprises, were initiated and conducted by specialists in phytohelminthology and parasitology, renowned at the national level and recognized internationally, such as: Petru Nesterov (1979-1980); Maria Melnic, Dumitru Erhan, Ștefan Rusu (2014); Larisa Poirasa (1996-2016).

Currently, taking into account the specifics of areas with contrasting and unstable climates, we aim at investigating invasive nematode

complexes from the families *Heteroderidae Hoplolaimidae, Tylenchidae*, affecting pea plants (*Pisum sativum* L.), in the context of applying new modern cultivation technologies, comparatively by areas, production associations, plantations and purpose of production. Based on current events and the estimated purpose, the goal of our research has been to establish the structure and diversity of invasive helminth species from the families: *Heteroderidae*, *Hoplolaimidae* and *Tylenchidae,* associated with parasitic forms affecting pea (*Pisum sativum* L.), determining the parasitic impact by comparative analyses of frequency and abundance indices, in various production and experimental sectors, in the dynamics of phenological stages.

MATERIALS AND METHODS

The proposed program included specific, helminthological investigations in field crop plantations (legumes, winter cereals, technical crops), where samples of soil and plants affected by helminths were taken and surveys and periodic records were made comparatively across various investigated sectors, mainly in the Central area as compared with the Northern area.

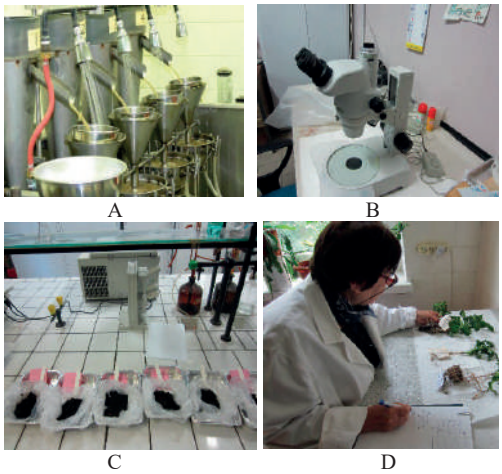


A B C D

Figure 1. Surveys with soil sampling and analysis of plants collected during the growing season (Criuleni district, March-June 2023-2024). A - Taking samples before sowing; B - Pea - vegetative stage; C - Field surveys - flowering stage; D - Plant analysis - ripening stage

To establish the areas affected by helminthiasis, over 6 field trips, in 10 localities, 12 sectors, from 4 administrative districts in the Central area, were undertaken, where over 200 samples of soil and diseased plant organs were collected and analyzed (Figure 1).

Subsequently, indices of parasitic impact were determined, such as: species diversity, population density, frequency of attack (F%), intensity of attack (I%), estimation scales were used following phytosanitary control, field and laboratory records were made.



A B C D

Figure 2. Logistics and helminthological extraction analyses and microscopic establishment of morpho-taxonomic indices of nematodes extracted from analyzed samples: A - instalația Baermann funnel; B - binocularul fotonic; C - Analysis of soil samples; D - visual analysis of samples

The collected samples were subsequently analyzed in the laboratory, according to the classical and new methods, with some modifications depending on the specifics of the nematode genera. Through specific techniques, nematodes were extracted from the soil and affected organs, applying the classic flotation-decantation method (Baermann funnel), with some methodological adjustments, with subsequent fixation in 4% formalin, at temperature of 60°C, for morpho-taxonomic studies, reflected in figure 2. The fixed material was analyzed under a microscope, establishing the population size, trophic groups, as well as other parasitic indices in the investigated soil and plants. Methodological and logistical support was offered by the Parasitology and Helminthology Laboratory, with methodology

according to Парамонов, 1970; Nesterov P., 1978-1988; Melnic M., Erhan D., Rusu Ş., 2014.; Poiras L. et al., 2016; Iurcu-Straistaru E. et al., 2018-2023. The taxonomic units were determined using nematological identification guidelines according to: Decker, 1972; Baldwin & Moens, 2004; Perry & Moens, 2006; Decramer & Hunt, 2006; Siddiqi, 2010; Sasanelli et al., 2018.

Based on phytopathological research, the frequency of attack **(F %)** and the intensity of development **(I%)** were established. The frequency of attack **(F%)** represents the relative proportion of attacked plants or plant organs **(n)** compared to the total number of analyzed plants or organs **(N)** and is calculated using a standard formula. The intensity of attack **(I %)** represents the extent, as a percentage, to which a plant or organ is affected. The following formula is used to calculate the intensity of attack:

$$I\% = \frac{(n_1 . 1) + (n_2 . 2) + (n_3 . 3) + (n_4 . 4)}{N \times 4},$$

where: *n1, n2, n3, n4* - the number of plants or organs attacked at the respective grade; N - the total number of plants or organs examined; 4 - the maximum grade on the scale used (Îndrumări metodice pentru testarea produselor chimice şi biologice de protecţie a plantelor în Republica Moldova, 2012).

RESULTS AND DISCUSSIONS

Biological control of pea plantations affected by helminths was verified in various private households and production associations in the North and Center of the Republic of Moldova and in the administrative districts of Criuleni, Anenii Noi, Nisporeni, located in the South-East and South-West, where winter cereals, corn, sunflower, fodder crops, etc. are also intensively cultivated. Peas are cultivated on large and significant areas, in the sectors of the Central area, but in the North the areas are limited, instead, soybean plantations for grains and fodder predominate. For this reason, our research was focused mainly on sectors of the Central area, making detailed surveys and analyses.

Phytosanitary surveys were conducted monthly, in the period March-June, 2023-2024, which were characterized by relatively warm and humid weather conditions, which had an advantageous influence on the development of plants including but also of parasitic nematode complexes. The average frequency and intensity of helminthic attack were established, reaching values up to 25-30%. Abundant precipitation and higher temperatures have determined the severity of helminthiasis in association with various disastrous diseases such as: ascochytosis, fusariosis, which appeared early on the roots and stems during the vegetative phase. Simultaneously, on the preventively marked samples the presence of helminthic diseases was detected, the values of intensity and frequency of attack were analyzed comparatively, and the structure and predominance of certain genera and species with specific pathogenic invasive impact were highlighted. In all the investigated sectors, helminthic diseases and infestations were reported through local outbreaks, with retarded plants, in association with wilted, partially necrotic ones in variegated colors, presented in Figure 3.



Figure 3. Plants affected by mixed helminthic diseases associated with downy mildew and powdery mildew, on all plant organs, in the pod formation phase; A - plant samples affected by multiple pests productive sector May-June 2024 Criuleni district; B - sector affected locally by helminths in a productive field; C - plants affected severely by multiple pests; D - visual analysis of plants affected by helminths on roots; experimental sector of "Al. Ciubotaru" NBGI, 2024

The periods with abundant rainfall and unstable daytime temperatures, in the months of April-June, caused an increase in the damage severity and in the numerical density of the pest populations, reaching average values in the Central area - 15-250 individuals/100 g/soil. It was found that, in all the investigated sectors, there were extensive nematode complexes that produce helminthiasis, in certain sectors affecting the plants severely. This is due to the adaptive resistance capacities in the soil. Meanwhile, nematodes form specialized associations in these crops, caused by non-compliance with maintenance techniques. Such conditions favored the increase in the degree of parasitic helminthological impact, affecting plants in the stages of leaf formation, until the formation of pods. During this period, the reported helminth symptoms remain clearly accentuated and extensive, the visual symptoms including chlorosis, a reduced number of mature leaves, dwarf leaves, roots seriously affected by necrosis and rot caused by helminths. The species of pathogenic fungi in the soil estimated in ascending comparative values, by phenological stages, vary from 5% in the stage of 2-5 leaves up to 30% in the fruit development stage, presented in Table 1.

Table 1. The estimation of phytosanitary indices by comparative values of helminthic parasitic impact in various phenological stages of pea plants grown in the districts of the Central area of RM (March-June, 2024)

Central area and pilot districts	26 March, stage of 2-5 developed leaves			25 April, stage of 10-15 developed leaves			27 May, flowering stage			26 June, fruit development - ripening stage		
	D. (100 g soil)	F (%)	I (%)	D (100 g soil)	F (%)	I (%)	D (100 g soil)	F (%)	I (%)	D (100 g soil)	F (%)	I (%)
Nisporeni district, Vărzărești village	5-17	7-10	5-7	17-23	9-12	7-10	25-35	12-15	8-11	180	25-28	22-25
Criuleni district, Pașcani village	7-15	5-9	3-5	14-18	8-9	6-8	20-28	10-13	9-12	250	27-30	24-27
Criuleni district, Bălăbănești village	8-16	8-12	5-7	10-15	7-10	6-9	28-33	13-18	10-13	220	25-28	22-25
Anenii Noi district, Mereni village	6-14	5-8	2-5	13-16	10-14	8-12	23-30	9-14	7-9	190	20-25	18-22

Legend: D. – density of nematode populations per 100 grams of soil; F (%) – frequency of attack; I (%) – intensity of attack.



A C

Figure 4. development stages, poly-invasively affected by helminthiasis and other diseases associated with specific pathogens, May - June 2024, Nisporeni district. A - productive sector affected by helminths; B - pea plantations in the budding-fruit

Such investigations were carried out for the first time in the Republic of Moldova on legume crops, including peas, as this research was conducted in another technologically intensive format and in the new private system, related to establishing the diversity and structure of nematode complexes, aspects of parasitic impact, trophic specialization, strategically adapted specialization, with the estimation of the most invasive forms in this early-spring crop. The investigations carried out consistently reflect in the seasonal dynamics, during growth stages, of the parasitic helminthic impact associated with specific diseases affecting peas, such as: downy mildew - pathogen *Peronospora pisi* Syd., powdery mildew - pathogen *Erysiphe pisi* Diet., blight - pathogen *Ascochyta pisi* Sacc., wilt - pathogen *Fusarium* spp., triggered by some problems in the cultivation techniques and environmental factors. Pea plantations affected by the above-mentioned diseases, in the budding-fruit development stages, are presented in Figure 4 A, B. These analyses and results are useful for adjusting short and long-term forecasts and are useful for applying an efficient integrated

protection management for peas, namely by reducing the damage caused by pest invasions, the degree of infestation, the resistance in critical phases etc., aimed at production associations, experimental research sectors and private households. In this regard, by analyzing the samples collected from soil and affected plants in the laboratory, the structure and taxonomic diversity of invasive nematode complexes was determined in the dynamics of plant growth and development stages, in the presence of helminth associations accumulated in the soil planted with peas, the growing season March-June 2024, presented in Table 2. The analyses presented in Table 2 estimate the structure, diversity and density of the nematode populations, established at genus level, and the level of parasitic impact and infestation, up to 3 points (250 individuals per 100 grams/soil), detected from the first stages of plant development and increasing each month, according to the biological development cycle. In the detected complexes, the individuals belonged to 8 genera, namely: *Ditylenchus* spp., *Helycotilechus* spp., *Tylenchus* spp., *Fylenchus* spp, *Nothotylenchus* spp., *Heterodera* spp., of the order Tylenchida, with diverse trophic specialization (endo- ecto- and semi-endoparasites), migratory and sedentary.

Table 2. Diversity and structure of nematode complexes, accumulated in the soil planted with peas (March-June, 2024) in the dynamics of phenological stages

Detected genera	Trophic group	Date of collecting and analyzing soil samples (100 g) from the plant's rhizosphere			
		25.03	25.04	25.05	30.06
1. *Ditylenchus* sp.,	Migratory	+	+	++	++
2. Prathylenchus sp.,	endoparasites	-	+	++	+++
3. *Helycotilechus* sp.,	Ectoparasites	-	+	+	++
4. Tylenchus sp.,		-	+	+	++
5. Fylenchus sp.,		-	+	++	++
6. Nothotylenchus sp.		-		+	
7. *Meloidogyne* sp.,	Cyst and gall-inducing	+	+	++	+++
8. Heterodera sp.,	semi-endoparasites	-	+	++	++

Legend: - no individuals; + from 30 to 100 individuals are present; ++ from 100 to 200 individuals are present; +++ over 200 individuals are present.

All the estimated genera were found practically in all the investigated sectors, but the most invasive, with high frequency and abundance, were the forms belonging to the genera *Meloidogyne* and *Prathylenchus*, from the families Tylenchidae and Heteroderidae. These highlighted complexes represent a significant danger to pea plants, because they are associated with various species of insects and diseases, and are also vectors of microbial infections in the soil.

CONCLUSIONS

As a result of the phytosanitary and helminthological monitoring, carried out in 4 production associations from 4 districts, mainly belonging to the Central area of the Republic of Moldova, in plantations of peas grown for grains (*Pisum sativum* L.), the diversity and structure of invasive nematode complexes in the dynamics of phenological stages were established for the first time, detecting the presence of the most dangerous forms associated in 8 genera, such as: *Ditylenchus* spp., *Pratylenchus* spp., *Meloidogyne* spp., *Helycotilechus* spp., *Tylenchus* spp., *Fylenchus* spp., *Nothotylenchus* spp., *Heterodera* spp., which belong to the families Heteroderydae, Aphelenchidae Tylenchidae, Hoplolaimidae, Neotylenchidae, order Tylenchida, with diverse trophic specialization (endo- ecto- and semi-endoparasites), migratory and sedentary.

The more abundant and frequent pests were the forms of migratory endoparasitic nematodes of the Tylenchidae family and the gall-inducing forms belonging to the Heteroderidae family, class Nematoda.

After establishing the indices of the population density and frequency and intensity of attack, it was found that the nematodes that appear early, at the seed germination stage, the complexes belonging to the genera *Pratylenchus* and *Meloidogyne*, detected in the rhizosphere of young pea plants, had an abundance of 100 to

250 individuals (adult forms, eggs, invasive larvae).

Helminthic diseases were reported as local outbreaks, with a frequency of 5 to 30% with an average attack intensity of 7-25%. These highlighted complexes represent a significant danger to pea plants, because they are also associated with species of lepidopteran and coleopteran insects in the soil, various diseases, causing unfavorable conditions for obtaining biological and agricultural harvests.

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