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HELMINTOLOGICAL BIOLOGICAL CONTROL IN SOYBEAN (*Glycine max* L.*)* UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

An important step in the process of increasing the productivity and quality of soybean (Glycine max L. Merr.), grown under the conditions of the Republic of Moldova, is the phytosanitary control on the parasitic helminthological fauna in order to apply the necessary protection measures. The investigations were carried out in 10 administrative districts of the North-Central–South East areas, 10 localities, on 15 soybean sectors from various areas cultivated with fabaceae, where over 200 soil samples were collected, for further analysis in the laboratory. As a result of the helminthological investigations, the degree of impact was established, being represented by the number density of 50-250 individuals 100 g/soil, with an abundance of 10-25%, in spring. The indices of frequency and intensity of helminthologic impact were estimated at higher values (10-30%) in late spring-summer, differing among plantations. The complexes of phyto-nematode parasites consisted of 22 species, of 8 families and 2 orders. The endoparasites of the Pratylenchidae family, genus Pratylenchus predomonated, followed by the ectoparasites and semiendoparasites of the Hoplolaimida, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae families, differentiated by areas and environmental conditions.

Key words*: soybean, nematodes community, phytosanitary monitoring, abundance, trophic groups, diversity of species, helminthological diseases.*

INTRODUCTION

The cultivation of legumes, including soybeans as a source of grains, seeding material and feed, solves three strategic problems: increasing seed production, increasing plant protein production and improving soil fertility, being 2-3 times richer in plant protein as compared to cereals grown in crop rotation systems (Starodub, 2013; Starodub, 2015). The estimation of the annual sustainability of the invasive impact on plantations of fabaceae species, during the growing season, would play a significant role. These crops are attacked by more than 70 species of harmful organisms, the parasitic nematode complexes, causing plant-specific helminthosis, also fall into this group as invasive pests (Paramonov, 1970; Decker, 1972; Nesterov, 1988). Annually, according to the new institutional project 2023-2027, we conduct nematological research, including bioecological, morpho-taxonomic and invasion estimates, regarding the study of parasitic fauna, affecting various phytotechnical crops. This research program also includes soybean (*Glycine max* L. Merr.), widely cultivated, due to its industrial, food, fodder, technological significance, with its advantages of high ecological plasticity, major adaptability to environmental conditions in various climatic zones (Nesterov, 1997; Sasanelli et al., 2018; Iurcu-Straistaru et al., 2019).

In the Republic of Moldova, soybeans are grown for food, zootechnical, chemical, curative-pharmaceutical, agrotechnical and ecological uses, because the plants have high capacities of resistance and tolerance to some species of harmful organisms, they easily fit into modern high-performing agricultural systems, organic farming, due to the features allowing them to fit into optimal crop rotation systems and modern technologies. Despite

these advantages, soybean cultication still requires a lot of attention and efforts in terms of increasing productivity through the permanent implementation of phytosanitary biological control, with the application of integrated protection elements, which motivated us to initiate a specific study on the nematological parasitic impact (Iurcu-Straistaru et al., 2019).

Soybean plantations represent 10% of the arable surface and are annually affected by more than 80 harmful species, which include the parasitic nematode complexes capable of causing significant damage and which are the reason for the application of measures to control their number and parasitic impact in the cultivation process (Nesterov, 1988; Starodub, 2013; Starodub, 2015).

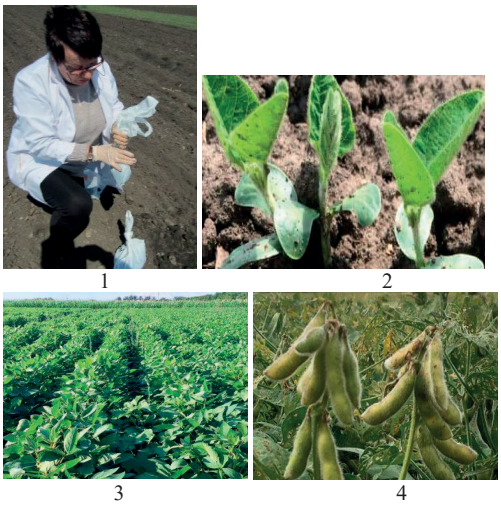
In the Republic of Moldova, helminthological research on the monitoring of biodiversity and the complex structure of parasitic phytonematode populations, detected in leguminous agrocenoses, was initiated in the period 1988-2016 (Nesterov, 1988; 1997; Iurcu-Straistaru et al., 2019). Taking into account the predominance of the areas with a contrasting climate, we have set as a research objective, the biological parasitic control of fabaceae agrocenoses, with diverse agronomic characteristics and the establishment of the degree of parasitic impact with the trophically specialized parasitic nematode complexes and the estimated adaptation potential depending on the environmental factors (Baldwin, 2004; Decramer, 2006). Considering the above, the current work presents some results of the research on helminthological surveys, which established the extent of parasitic invasions, the intensity of attack, the relevance of the numerical abundance, the taxonomic diversity of parasitic nematode species detected in soybean plantations, for effective application in the adaptation of some elements of prevention and integrated protection (Iurcu-Straistaru, 2019).

The purpose of the research: Investigations on the complexes of invasive nematodes of the families *Aphelenchidae, Hoplolaimidae, Tylenchidae, Heteroderidae, Trichodoridae, Longidoridae* associated with parasitic forms of the sub-class *Adenophorea* affecting the soybean species *Glycine max* L. Merr., in the context of the new modern technologies of comparative cultivation by area, production associations, private sectors.

Objectives: Establishing the structure and diversity of invasive helminth species of the families: *Hoplolaimidae*, *Tylenchidae, Heteroderidae*, associated with parasitic forms of the sub-class *Adenophorea* affecting the soybean species (*Glycine max* L. Merr.), determining the parasitic impact, through comparative analyzes of the frequency and abundance indices, conducted in production and experimental sectors, in various phenological phases.

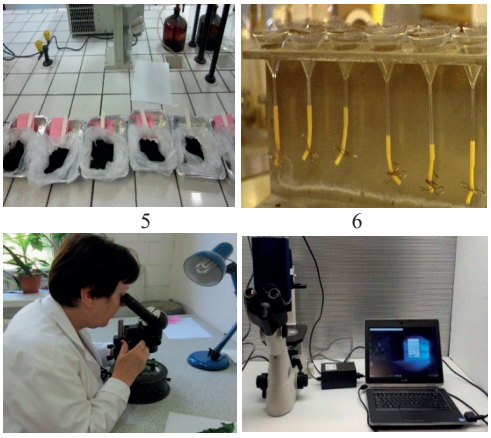
MATERIALS AND METHODS

In order to achieve the proposed goal and objectives, specific, helminthological investigations were done in the agrocenoses of phytotechnical plants (legumes, autumn cereals, technical crops), through field surveys and periodic records, taking samples of soil and plants affected by helminths, comparing various investigated sectors, from the North and Center area of the country. In order to establish the surfaces affected by the species that cause helminthiasis, more than 10 sectors, 6 delegations from 4 districts, were evaluated, by collecting and analyzing 200 samples of soil and infested plants.



Figures 1-4. Field phytosanitary surveys in the dynamics of plant development phases: 1-sowing; 2-seedling emergence; 3-formation of the aerial part; 4-fruiting; taking soil samples for laboratory analyses, April-July

Figures 1-4 estimate the phytosanitary surveys carried out periodically in the dynamics of plant growth and development, covering the main plant development phases (spring - summer) presented in Figures 5-8.
Consecutively, indices were established to estimate the parasitic impact, such as: ***pest density, frequency of attack (F.), intensity of attack (I%), estimation scales*** used following the phytosanitary control.
The collected samples were later analyzed in the laboratory, according to the classical and current methods, with some modifications depending on the specific traits of the nematode genera. Through specific techniques, the nematodes were extracted from the soil and affected organs, applying the classical flotation-decantation method, with some methodological adjustments of extraction, with subsequent fixation in 4% formalin, at the temperature of 60°C, for morpho-taxonomic studies.



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Figures 5-8. Helminthological extraction analyzes and microscopic determination of morpho-taxonomic indices of nematodes extracted from soil and affected soybean plant samples, April-June, 2024

The fixed material was analyzed microscopically, establishing the abundance, the trophic spectrum, as well as other parasitic indices in soil and investigated plants. Methodical and logistical support was offered by the Laboratory of Parasitology and Helminthology, with methodical applications according to Decker H (1972), Paramonov A. (1970), Nesterov P.I. (1988-1997), Sasanelli N. et al. (2018), Iurcu-Straistaru E. et al. (2019). The taxonomic units were identified with the help of species determination guidelines written by taxonomists specialized in nematology: Perry & Moens (2006), Siddiqi (2018) and setting the permanent preparations and the subsequent determination of the taxonomic position, with specific modifications and adaptations with classifications of the trophic spectrum, were done according to Baldwin, (2004), Perry (2006).

RESULTS AND DISCUSSIONS

In order to establish the soybean areas affected by invasive nematode species, various private households and production associations from the North, Central and South-East areas of the Republic of Moldova were monitored, resulting in the establishment of helminthosis outbreaks, numerical density indices, the frequency of attack, the intensity of attack, the trophic specialization and the dominance of certain species. Phytosanitary surveys were done in the spring-summer season (March-June) of 2023-2024; the weather being relatively warm and humid had an advantageous influence on the evolution of parasitic nematode complexes and their phytoparasitic impact on soybean plants reached the average frequency of 10-25% in the North area, 7-15% in the Center area, 15-30% in the South-East area. Indices of a higher intensity of phytohelminthic attack prevailed in the South-East area, through the evidence of the sectors in the districts of Căușini, Ștefan Vodă, recording by 10-15% more as compared to the North and Center areas (5-15%), being favored by the abundant rainfall and higher temperatures, a fact that determined the extent of phytohelminthosis in association with fusarium contamination on the roots and stems during the formation of mature leaves and the formation of flower buds. These results indicate that, the structure of the parasitic nematode complexes being made up of several species with polyphagous trophic specialization, which provide a better biological reserve, both for the year 2024 and for the next year depending on the favorable environmental and soil conditions, for the intensive reproduction of parasitic nematode complexes, and manifestations of significant invasions and attacks on plants and for successive cultivation.

Practically in all the sectors checked, helminthic diseases and infestations were detected as sporadic outbreaks, resulting in plants with retarded growth, in association with wilted, partially necrotic ones, in variegated colors.

Periods of low precipitation in April-June and unstable daytime temperatures, caused an increase in the damaging potential and an increase in the numerical density reaching average values, per areas, of: Northern area 80-250 individuals/100 g soil, Central area 50-180 individuals/100 g Soil, South-East zone 60-120 individuals/100 g soil, that is 30-45% more severely attacked sectors of the North zone as compared with the Center and South-East zone (Table 1). According to the cultivation pattern, relatively, the soybean sectors of the Central area are more scattered, divided in small areas, besides, dry periods with extreme diurnal variable temperatures frequently prevail, thus, these facts diminish the capacity for biological reproduction by decreasing the number of invasive nematodes in the rhizosphere of soybean plants.

Thus, it was found that in all the investigated sectors of the North-Center-South-East zones, the extensiveness of the nematode complexes that cause phytohelminthosis was significant; in certain sectors, the helminthic impact was severe because of the adaptive capacities of resistance in the soil. Meanwhile, nematodes form specialized associations with these crops, under the influence of the neglect of specific agrotechnological measures in regulating the number and reducing the degree of infestation and parasitism.

Table 1. The distribution of parasitic nematode complexes in soybean plantations, according to trophic spectrum, in the agrocenoses investigated in 2023-2024

Indices of trophic-phytoparasitic specialization	North zone	Central zone	South-Eastern zone
Migratory endoparasites	**7**	**5**	**4**
Semi-endoparasites	**6**	**6**	**5**
Migratory ectoparasites	**4**	**4**	**4**
Ectoparasites – nutrients of absorbing hairs	**5**	**5**	**5**
Sedentary ectoparasites	**1**	**1**	**1**
Numerical density individuals/100 g/soil	80-250	50-180	60-120
Total number of phytoparasitic species detected	**22**	**21**	**19**

Based on the establishment of the structure and taxonomic diversity of the nematode complexes parasitic on the soybean crop, as well as the spectrum of trophic classification, Table 1 and Figure 9, a and b, estimate the indices of numerical specialization, comparatively by areas, with the differentiation of trophic specialization groups, with insignificant variations depending on the sector, biotope and area. Sedentary ectoparasites are an exception, which have practically been reported with no specific pathogenic impact; the other groups pose a danger to crops such as autumn wheat, as parasitic agents specific to field crops and the respective areas.

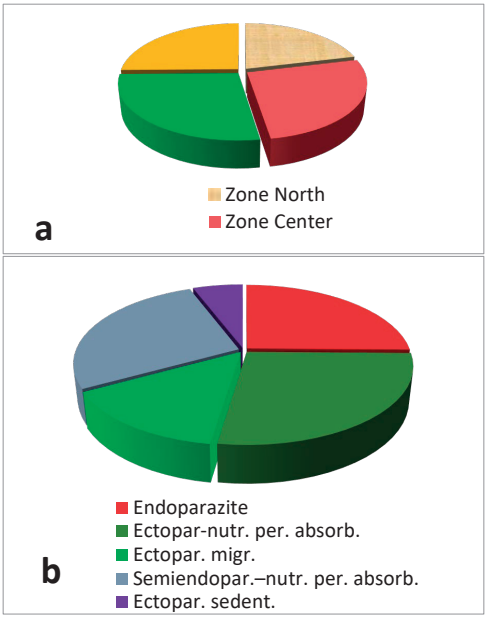

Figure 9, a and b. The rate of distribution of species frequency in parasitic phytonematode complexes affecting soybean plantations, according to trophic specialization groups, 2023-2024

Figure 9a, reflects the distribution of the number of species of parasitic phytonematodes detected in areas with almost equivalent variations, but we would like to mention the bigger green space in the chart that presents the South-East area, followed by the Central area and fewer species in the Northern area. Figure 9b estimates the graphical distribution of the number of detected species according to the spectrum of trophic specialization. In all the investigated areas, the group of ectoparasites is

predominant, followed by semi-endoparasites specializing in the area of absorbing hairs, and then - by endoparasites and migratory ectoparasites. Thus, with regard to the structure of the populations of phytonematodes parasitic on the soybean crop, the same species are found frequently, with diverse distribution in the areas and sectors investigated. The largest share is represented by the associated species that form specialized complexes of the genera: *Pratylenchus, Tylenchus, Aglenchus, Rotylechus, Helicotylenchus, Paratylenchus, Tylenchorhynchus, Ditylenchus, Merlinius, Criconemoides* together with the mycophagous species and those with phyto-parasitic specialization, specific to cereal crops of the genus *Afelenchoides,* which reached a numerical density of 50-250 individuals/100 g soil, an intensity of infection on average 7-30% of the total investigated plantations, depending on the period and the plant growth phase.

Analyzing the structure of parasitic nematode species, reflected in Table 2 and figure 9 a and b, a share of 44% to 100% of the total nematode populations was determined. Practically in all samples analyzed from all investigated sectors, endoparasitic species of the genus *Pratylechus* predominated with an abundance of 20-22%, the presence of the following species being detected: *P. penetran, P. pratensis, P. neglectus, P. subpenetrans*. Other species associated with these complexes were those with semi-endoparasitic specialization and ectoparasites of the genus *Paratylenchus: P. nanus, P. crenatus, P. microdorus*, genus *Helicotylenchus: H. multicinatus, H. dipystera, H. vulgaris*, including other ectoparasitic species of the genus *Tylechorynchus (T. cylindrucus, T. elangans), Criconemoides elengatus, Tylenchus davainei, Nothotylenchus acris* and species of the genus *Afelenchoides* (Decker H., 1972; Siddiqi, M.R., 2000; Sasanelli et al., 2018). These species and genera belong to the families: *Tylenchidae, Aphelenchidae Hoplolaimidae, Heteroderidae, Trichodoridae, Longidoridae,* including the genus *Afelenchoides,* of the sub-class *Adenophorea,* detected on the species *Glycine max* (L.) Merr., in the context of the new modern technologies of cultivation analyzed comparatively by area, production associations and private sectors [Iurcu-Straistaru et al. 2019].

Table 2. Taxonomical structure of parasitic nematode complexes detected in soybean plantations, comparatively by zones, Republic of Moldova, 2023-2024

The taxonomic units detected	Northern Zone		Central Zone		Southern Zone	
	Briceni	Ocnița	Criuleni	Ialoveni	Cimișlia	Basarabeasca
Pratylenchus hamatus	+++	+++	+++	+++	+++	+++
Pratylenchus pratensis	++	+++	++	++	+++	+++
Pratylenchus subpenetrans	++	++	+++	+++	+++	++
Pratylenchus neglectus	++	++	++	++	++	++
Tylenchus davainei	+	+	+	++	++	++
Filenchus filiformis	+	+	+	+	+	+
Filenchus polyhypnus	-	+	-	-	+	+
Aglenchus agricola	+	+	+	+	+	+
Aglenchus costatus	+	+	+	+	+	-
Helicotylenchus dihystera	++	+	-	+	++	-
Helicotylenchus multicenetus	+	-	+	-	-	+
Ditylenchus myceliophagus	+	+	+	+	-	+
Ditylenchus dipsaci	+	+	-	+	+	+
Rotylenchus robustus	+	+	-	-	+	+
Paratylenchus nanus	++	++	++	++	++	++
Paratylenchus hamatus	-	+	++	++	++	+
Bitylenchus dubius	+	+	+	+	+	+
Nothotylenchus acris	+	-	+	+	+	-
Merlinius dubius	+	+	+	+	+	+
Tylenchorhynchus elegans	+	+	+	+	+	+
Heterodera shachtii	++	++	+	+	+	+
Longidorella parva	+	+	+	-	+	-
Total number of parasite species - **22**	**20**	**22**	**19**	**19**	**21**	**18**

Legend: - no individuals **(0 points)**; + from 50 to 100 individuals **(1 point)**; ++ from 100 to 150 individuals **(2 points)**; +++ from 150 to 250 individuals **(3 points)**; ++++ over 250 individuals **(4 points**).

The results of the taxonomic analysis carried out on soil samples and soybean plants estimate the presence of 22 species of nematodes with parasitic trophic specialization, which form the complexes specialized on soybean crops, during the years 2023-2024, on average 18-22 species, in the investigated districts and areas, a fact that determines the equivalent abundance and frequent presence of species in the formation of specialized nematode complexes throughout the investigated agrocenotic territory (Table 2).

CONCLUSIONS

The results of the helminthological parasitic surveys conducted in soybean plantations estimate the presence of helminthological diseases in comparative proportions per plant development phase in values of 7-30%, as well as the abundance of 22 species of parasitic nematodes, with the numerical density of populations reaching 50-250 individuals/100 gr. soil, under the conditions of the Northern, Central and South-Eastern zones of the Republic of Moldova.

In soybean plantations, the taxonomic units and the frequency of species, their trophic classification and distribution in the investigated areas and sectors were determined, and the largest share comes from the genera: *Pratylenchus, Tylenchus, Aglenchus, Rotylechus, Helicotylenchus, Paratylenchus, Tylenchorhynchus, Ditylenchus, Merlinius, Criconemoides*. these genera belong to the families: *Tylenchidae, Aphelenchidae Hoplolaimidae, Heteroderidae, Trichodoridae, Longidoridae,* including the species of the genus *Afelenchoides*, of the sub-class *Adenophorea,* detected on soybean (*Glycine max* L. Merr.) plants, in the context of the new cultivation technologies applied, comparatively by zones, production associations and private sectors.

The most predominant species were endoparasites from the *Pratylenchidae* family, genus *Pratylenchus*, *P. pratensis, P. penetrans, P. negletus* followed by the ectoparasites and semiendoparasites species from the *Hoplolaimida, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae* families, differentiated by areas and environmental conditions.

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