

RESEARCHES ON THE NEMATODE *Ditylenchus dipsaci* IN *Allium cepa* CROPS UNDER MONOCULTURE CONDITIONS IN THE CENTRAL DEVELOPMENT REGION OF THE REPUBLIC OF MOLDOVA

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Abstract. During the vegetation period, in *Allium cepa* L. (onion) crops under monoculture conditions, outbreaks of the phytoparasitic nematode species - *Ditylenchus dipsaci* (Kühn, 1857) (Tylenchida: Anguinidae), with an extensiveness of 30 - 40%, have been detected. It was calculated that the density of the nematode *D. dipsaci* in such bulbs constituted a total of 34.12×10^3 specimens/gram of infested tissue, of which the highest percentage - 42%, belongs to *D. dipsaci* larvae in different stages of development (L2; L3; L4). During the storage period, the density of nematodes *D. dipsaci* was - 2.8×10^3 specimens/gram of infested tissue, with a major percentage distribution of *D. dipsaci* specimens of - 46% for saprophytic nematode species. The vegetal tissue of *Allium cepa* bulbs infested in the initial stage only by *D. dipsaci* populations contains remains of empty, dissociated vegetal cells. The pectocellulose membranes, as well as the parenchymal intercellular tissue are completely macerated. Biochemical analyses have shown that, in the same infested plant tissue, the dry mass of the bulbs infested with the nematode *D. dipsaci* decreases compared to the control (uninfested bulbs) with 2.7% and of the leaves with 0.6%. At the same time, it was observed that, in the infested plants, the amount of water increases, both in the bulbs of *A. cepa* and in their leaves.

Keywords: phytoparasitic nematodes, *Ditylenchus dipsaci*, *Allium cepa*, biochemical analysis.

Rezumat. Cercetări privind nematodul *Ditylenchus dipsaci* la culturile *Allium cepa* în condiții de monocultură în Regiunea de Dezvoltare Centru a Republicii Moldova. În perioada de vegetație, la culturile *Allium cepa* L. (ceapa) în condiții de monocultură, au fost depistate focare ale speciei de nematozi fitoparaziți - *Ditylenchus dipsaci* Kühn, 1857 (Tylenchida: Anguinidae), cu o extensivitate de 30 - 40%. S-a determinat că densitatea nematozilor *D. dipsaci* în asemenea bulbi a constituit $34,12 \times 10^3$ exemplare/gram țesut infestat, dintre care cel mai mare procent - 42%, le revine larvelor *D. dipsaci* în diferite stadii de dezvoltare (L2; L3 ; L4). În perioada de depozitare, densitatea nematozilor *D. dipsaci* a constituit - $2,8 \times 10^3$ exemplare/gram tesut infestat, cu o distribuie procentuală majora a exemplarelor *D. dipsaci* de - 46% pentru speciile de nematozi saprofiți. În țesutul vegetal al bulbilor *Allium cepa* infestați în stadiul inițial doar de populațiile *D. dipsaci*, se conțin rămășițe ale celulelor vegetale goale, disociate. Membranele pectocelulozice, precum și țesutul intercelular parenchimatic sunt completamente macerate. Analizele biochimice au demonstrat că, în asemenea țesut vegetal infestat masa uscată a bulbilor infestați de *D. dipsaci* se micșorează în comparație cu martorul (bulbi neinfestați) cu 2,7%, iar a frunzelor - cu 0,6%. Paralel s-a observat că, în plantele infestate are loc majorarea cantității de apă, atât în bulbii de *A. cepa* cât și în frunzele acestora.

Cuvinte cheie: nematode fitoparazite, *Ditylenchus dipsaci*, *Allium cepa*, analize biochimice.

INTRODUCTION

Onion, (*Allium cepa* L.), is one of the most consumed and grown vegetable crops worldwide. Onion bulb, with its characteristic flavor, is the third most essential horticultural spice with a substantial commercial value. There are over 400 known species of *Allium cepa* in the world, but from this diversity of vegetable crops, only 6 species are cultivated, the most widespread of which is the common onion - *Allium cepa* L. (BOTNARI & BRÎNZILA, 2001). A total of 37 varieties have been included in the *Catalog of Plant Varieties of the Republic of Moldova* for 2022. Currently, local onion production fully meets the needs of the domestic market, providing in addition significant quantities for export (BOTNARI, 2011).

Most varieties of the genus *Allium* are not resistant to pests. In the bulbs of these crops, both in field and storage conditions, the nematodes *Ditylenchus dipsaci* penetrate and settle, followed by numerous species of saprophytic nematodes, bacteria, fungi, mites, etc.

According to *European and Mediterranean Plant Protection Organization* (EPPO) 2025, phytoparasitic nematodes *Ditylenchus dipsaci* is known to infect over 500 different plant species, including weeds. According to *Food and Agriculture Organization* (FAO) data, *D. dipsaci sensu lato*, or stem nematode, attacks more than 1 200 species of wild and cultivated plants. Moreover, the EPPO has placed *D. dipsaci* as no. 174 on the A2 list of phytosanitary categorization, which is distributed locally in EPPO countries, and it is regulated as a quarantine pest (EPPO, 1997). Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. Morphological, biochemical, molecular and karyological analyses of different populations and races of *D. dipsaci* have suggested that it is a complex of at least 30 host races, with limited host ranges. (JESZKE et al., 2013). The damage caused often compromises production. Crop losses differ depending on the initial nematode infection level in host plants. STURHAN & BRZESKI (1991) reported crop losses of 60%–80% in heavily infected fields. MENNAN (2001) reported that *D. dipsaci* caused a 65% yield loss in onion in the Suluova district of Amasya Province. In Brazil, outbreaks of *D. dipsaci* affect 100% of the *A. sativum* harvest (CHARCHAR et al, 2003). Outbreaks of 54.9% invasion have been observed in onion areas in Turkey, with *D. dipsaci* being common in 13 provinces of the surveyed 5 regions (MENNAN, 2005; YAVUZASLANOGLU et al, 2019). Similarly, in southern Italy, the greatest crop losses in g. *Allium*

crops are caused by *D. dipsaci* (GRECO, 1993; 2001). In the Russian Federation this species has been detected in 120 regions, the maximum density in *A. sativum* plants being observed at temperatures of 20-22°C, and the extent of invasion increases from north (4%) to south (7%) (SHUBINA, 2004).

According to *Food and Agriculture Organization* annually publishes statistical data on annual global crop losses caused by pests, diseases and weeds. These are still estimated at 35-40% of potential agricultural production, or 54-60% of actual production.

The species *Ditylenchus dipsaci* is a migratory endoparasite, distributed worldwide especially in temperate regions, lives in aerial parts of plants (stems, leaves and flowers), but also attacks bulbs, tubers and rhizomes. It is on the list of quarantine organisms in many countries. Although *D. dipsaci* primarily infects onions and garlic, it has a wide host range including peas, celery, strawberries, beetroot, vegetable marrow, pumpkin, rhubarb, ornamental bulbs (hyacinth, daffodil and tulip), oats, rye and some weeds, and is an economic pest because infected crops are unmarketable. Over 30 physiological races of the nematode are known, some being host-specific and others polyphagous (SOUSA et al., 2003; SIKORA & GRECO, 2005; STURHAN & BRZESKI, 1991).

All stages of species *D. dipsaci* are infective and enter plants through stomata or wounds, releasing enzymes that soften cell walls and facilitate feeding on parenchymatous cells of the cortex. The feeding nematode withdraws the cell contents through its stylet, and surrounding cells begin to divide and enlarge, leading to malformation of the plant tissue. Reproduction occurs by amphimixis, and population growth can be very rapid; a female *D. dipsaci* lays approximately 460 eggs during a season, and 5 generations can develop under optimal conditions when the temperature is in the range of 15–20°C, J2 hatch in 2 days and, within 4–5 days, have developed into females, which live for more than 10 weeks (STURHAN & BRZESKI, 1991; DUNCAN & MOENS, 2006). In the conditions of the Republic of Moldova, during a season there are an average of 4-5 generations of *D. dipsaci* (NESTEROV, 1997). Rapid population growth can lead to severe crop damage, even when the initial population density is low. As the nematode population increases, symptoms, including peppery growth, swollen and twisted stems, and bulb discoloration, become visible, and secondary pathogens such as bacteria and fungi can enter.

According to our previous research, in the Republic of Moldova the nematode *D. dipsaci* is found in *Allium sativum* crops, on individual lots, the extensiveness often being 78-100%, which led to total crop losses (MELNIC et al., 2020, MELNIC & GLIGA, 2024). There is a direct correlation between the density of the nematode and the value of total losses recorded during the storage of onion crops. In the present study, data on the stem nematode *Ditylenchus dipsaci* in *Allium cepa* plants, grown under monoculture conditions, are reported.

MATERIAL AND METHODS

The research was carried out in the Laboratory of Parasitology and Helminthology, Institute of Zoology, Moldova State University. The object of study - the stem nematode *Ditylenchus dipsaci* Kühn, 1857 and other specific parasitic and saprophytic species for *Allium cepa* crops. Plant samples have been collected by the itinerary method from individual households in the Central Development Region of the Republic of Moldova. The modified Baermann method (NESTEROV, 1979) was applied to extract the nematodes, with an exposure time of 24 hours, at room temperature. To calculate the intensity of the nematode *D. dipsaci*, onion bulbs with obvious external symptoms of ditylenchosis, caused by *D. dipsaci*, were selected. In assessing the nematode attack, the plant samples have been weighed in advance to establish the ratio between the number of extracted nematodes and the amount of analyzed material, and their density was determined applying De Gisse chamber. The amounts of dry mass and water have been determined according to the Ermacova method.

RESULTS

During the vegetation period late May - early June (biological phase of technical ripening), in individual households from the Central Development Region of the Republic of Moldova, outbreaks of the phytoparasitic nematode species, *Ditylenchus dipsaci* with an extensivity of 30 - 40%, were detected (Fig. 1).

As a result of the research, it was found that, on individual plots in the surveyed households, the main parasite of *Allium cepa* culture, both during the vegetation period and during storage, is the nematode *Ditylenchus dipsaci*, also known as the stem and bulb nematode. During the growing season, it is characteristic for strongly attacked bulbs to crack in the disc area (Figs. 1; 6; 7). During the harvest period, *Allium cepa* bulbs affected by ditylenchosis splits open. Often, various neoformations appear in infested bulbs due to excessive development. These features are similar and for the garlic crops (*Allium sativum*). Plants with advanced ditylenchosis due to the roots detaching from the bulb disk, are easily pulled out.

The results of the laboratory analyses, carried out during the vegetation period, demonstrated that, in the plant tissue of infested plants in the initial stages of ditylenchosis, a monotypic primary population of the plant tissue occurs only with populations of the obligate parasite of this crop – *Ditylenchus dipsaci* (females, males, larvae, eggs).

The density being higher in the protective leaves of the bulbs, which often remain in the soil during the harvesting process and pose a danger in the case of repeated planting on the same lot. The soil for nematodes is the path through which they move from infested plants to healthy ones, which occurs at temperature values ranging from 12-14°C. Being a polyphagous species, the nematode can exist for a fairly long time – 3-5 years, on various weeds, which have also been called reservoir plants.



a



b



c



d

Figure 1. a,b,c - *Allium cepa* plants and bulbs heavily infested with *D. dipsaci*,
d - pure culture of *D. dipsaci* populations (original).

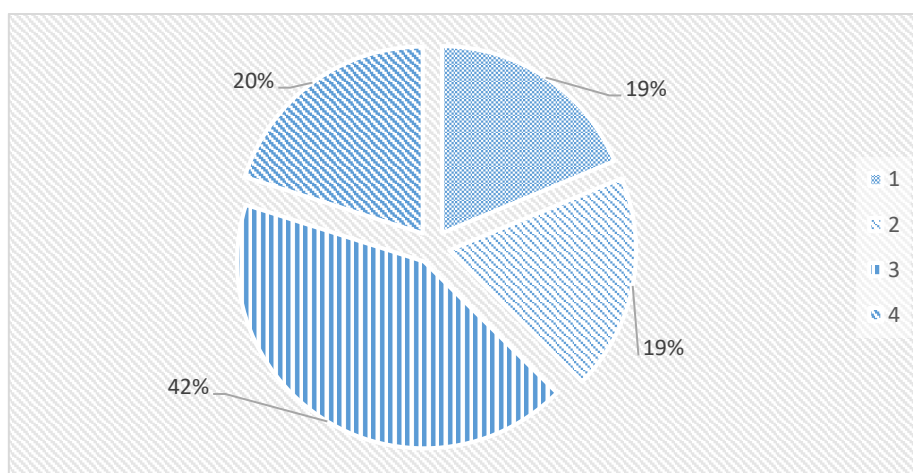


Figure 2. Distribution of *D. dipsaci* specimens, extracted from onion bulbs infested only with this species (harvest period): males (1); females (2); larvae (3); eggs (4).

The researches, have shown, that the density of individuals in *A. cepa* crops in the analyzed samples, in according to De Grisse chamber, varied up to 34×10^3 specimens/gram of infested tissue. It was established that in the analyzed onion bulbs (Fig. 1), only populations of a single species of parasitic nematodes *D. dipsaci* are present. Their percentage distribution in heavily infested onion bulbs is as follows: males and females account for 19% each, the highest percentage is for larvae in different stages of development (L2; L3; L4) – 42%, and laid eggs – 20% (Fig. 2).

In the course of our experiments, it has been observed that in *A. cepa* bulbs, infested in the initial stage only by *D. dipsaci* populations (females, males, juvenile forms L2 - L4, eggs) (vegetation period), changes in the plant tissue occur. Under the stereoscopic microscope, remains of empty, dissociated plant cells were observed (Fig. 3). The pectocellulose membranes, as well as the parenchymatous intercellular tissue, are completely macerated.

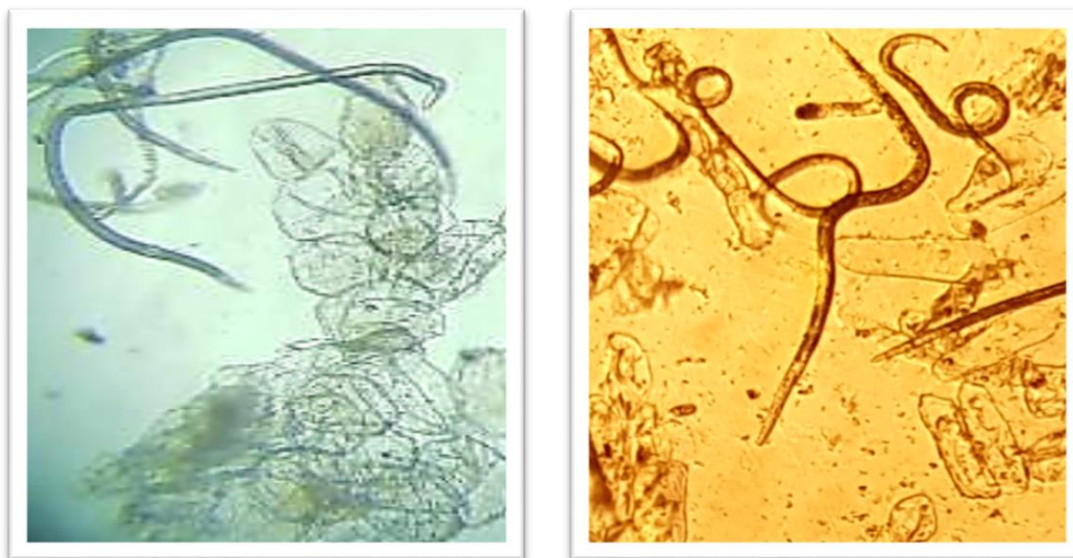


Figure 3. The fragments of *Allium cepa* plant tissue infested with parasitic nematode *Ditylenchus dipsaci* (original).

By biochemical methods (ERMACOVA, 1987) the amount of dry matter in various vegetative organs such as bulbs and leaves of *A. cepa* plants infested only by the nematode *D. dipsaci* have been determined, compared to the control (uninfested plants) (MELNIC, 2008).

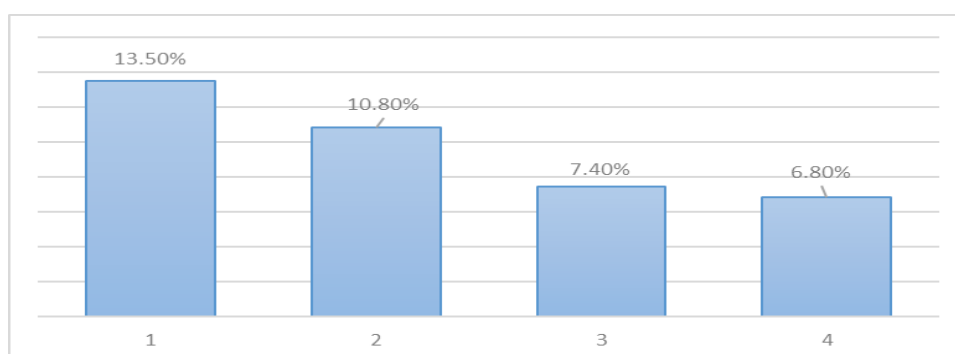


Figure 4. Distribution of dry matter in *A. cepa* plants: 1 - uninfested bulbs; 2 - infested by *D. dipsaci*; 3 - uninfested leaves; 4 - infested by *D. dipsaci*

It was established that the amount of dry matter of varieties *A. cepa* cultivated in the Republic of Moldova constitutes 13.5% in bulbs and 7.4% in leaves. We have been observed that the dry mass of bulbs infested by *D. dipsaci* decreases compared to the control (uninfested bulbs) with 2.7%, and of infested leaves, compared to uninfested ones - with 0.6%. At the same time, the amount of water increases, both in *A. cepa* bulbs and in their leaves (Figs. 4; 5).

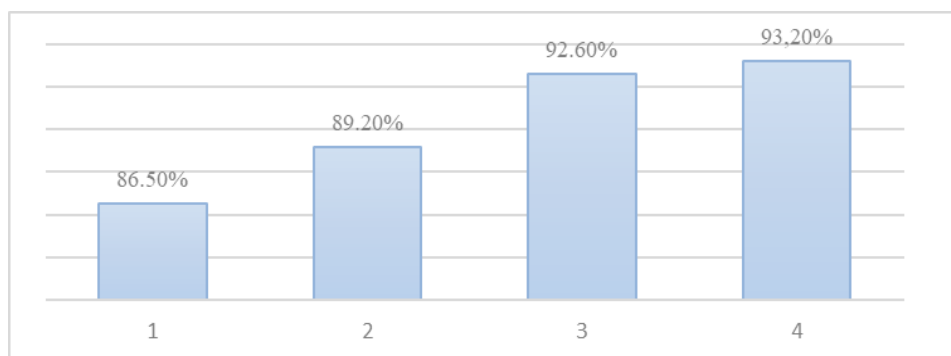


Figure 5. Distribution of water content in *Allium cepa* plants: 1 - uninfested bulbs; 2 - infested bulbs by *D. dipsaci*; 3 - uninfested leaves; 4 - infested leaves by *D. dipsaci*.

Storage period. During the harvest period, onion bulbs infested in the early stages of dithylenchosis are practically indistinguishable from healthy ones and are unconsciously introduced by humans into storage warehouses. It is necessary to mention that phytonematode *D. dipsaci* parasitizes *Allium* crops throughout the year, both during the vegetation period and during storage, especially when the rules of permanent ventilation and maintenance of constant temperature are not respected. The *Allium cepa* bulbs, infested in advanced stages of dithylenchosis (Figs. 6; 7), collected from storages, were also investigated. Nematode populations are transmitted from one bulb to another. The disease of dithylenchosis progresses rapidly, forming clusters of tens and hundreds of thousands of individuals of different ages, which move from the heavily infested plant tissue to the surface of the bulb, where a sticky substrate is formed, similar to fungal infections. Most nematodes (L4) survive in a state of suspended animation for quite a long time. In laboratory experiments, cases have been observed when nematodes fall into suspended animation.



a



b

Figure 6. *Allium cepa* crops: a - uninfested crop; b - infested bulbs (100%), after 2-3 months of storage. Original



a



b

Figure 7. a - *Allium cepa* bulb infested with *D. dipsaci* (initial phase);
b - bulb infested with *D. dipsaci* (advanced phase of dithylenchosis)
in association with saprophytic nematodes, microorganisms, mites (storage period). Original

The results of the analyses have shown that in such bulbs the parasitic nematodes *D. dipsaci* are replaced by saprophytic nematodes, most of which are bacterivorous in their mode of nutrition. The parasitic nematodes associate with the saprophytes, with microorganisms and with mites. Among the mites, the most widespread is the species *Rhizoglyphus echinopus*.

It was calculated that the percentage distribution of nematodes in bulbs infested in advanced stages of dithylenchosis (collected from storage) occurs as follows: total – 2.8×10^3 specimens/gram, of which 43% belong to *D. dipsaci* specimens: males - 25%; females - 18%; larvae of different species - 11%, and the highest percentage to saprophytic nematode species (mature forms) - 46%, most of which are from the order Rhabditida, which informs us that microorganisms are present in such bulbs (Fig. 8).

It was also observed that in the investigated bulbs in advanced stages of dithylenchosis (Fig. 6) mites are very frequent, most of them from the genus *Rhizoglyphus*. Such bulbs are in the process of total transformation into waste, which leads to losses of the harvests in the warehouses.

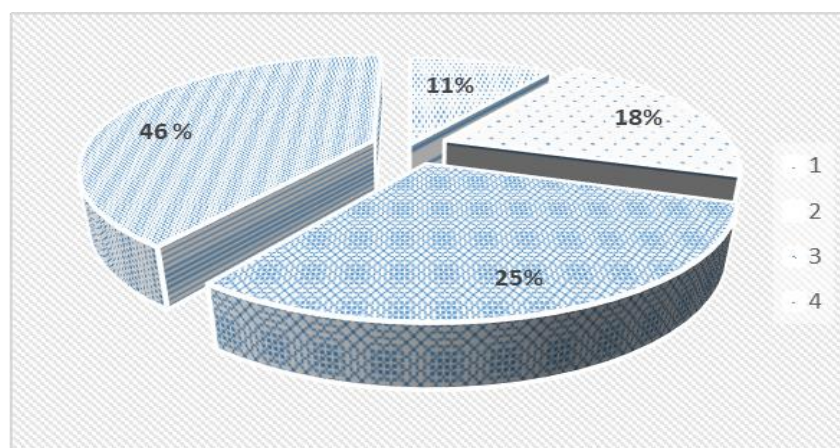


Figure 8. Percentage distribution of *D. dipsaci* specimens, extracted from onion bulbs infested in advanced stages of dithylenchosis: males; females; larvae, different species; saprophytic nematodes.

DISCUSSIONS

The species *Ditylenchus dipsaci* is a quarantine object and one of the most representative cases of migratory endoparasitic species, which seriously affect the cultivated varieties of *Allium sativum*, *Allium cepa*, *Allium porrum* etc., as well as the wild ones (MELNIC, 2008; MELNIC & BIVOL, 2012; MELNIC et al., 2020; BAZARBECOV, 2003). In the process of nutrition the parasitic nematode *D. dipsaci* absorbs through the lumen of the stylet the cytoplasmic content - simple hydrocarbons, proteins, amino acids, peptides, lipids, organic acids, fatty acids, etc. However, the main role in the interrelations with the host plant belongs to the secretions of the esophageal glands of the nematodes, which are eliminated in the parasitized plant, parallel to the nutrition process. By biochemical methods it was established that *D. dipsaci* (parasite of crops of the genus *Allium*) eliminates enzymes, which destroy cellulose fibers (cellulases) and pectin (pectinases) (BUMBU, 1998; 2009; MELNIC, 2008). The presence of cellulases and pectinases eliminated by species of the genus *Ditylenchus* - *D. dipsaci*, *D. destructor*, in plant tissue, causes maceration of the parenchymal intercellular tissue of the parasitized host plant, as well as the pectocellulose membranes (cell walls) of the cells, as we have observed. Due to this fact, phytoparasitic nematodes have the possibility of moving both through the intercellular and intracellular space (Fig. 3), while being in the process of nutrition.

It is necessary to mention that, obligate plant parasites, including nematodes of crops of the genus *Allium* - *D. dipsaci*, feed exclusively only on the contents of living plant cells. In the process of nutrition, parasitic species penetrate into the cells that are absorbed through the lumen of the stylet, leaving empty, dissociated cells in the parasitized portions.

In the process of nutrition, phytoparasitic nematodes absorb through the lumen of the stylet the cytoplasmic contents - simple hydrocarbons, proteins, amino acids, peptides, lipids, organic acids, fatty acids, etc. Through the lumen of the stylet, intracellular microinclusions are also absorbed - ribosomes, lysosomes, dictyosomes, with a smaller diameter than that of the stylet capillary, which freely pass through the stylet into the esophagus. However, the main role in the interrelations with the host plant belongs to the secretions of the esophageal glands of the nematodes, which are eliminated in parallel.

Some authors (GOFFART & EINIGE, 1964; SHUBINA, 1972) observed that, in infested plants with nematodes *D. dipsaci*, the dry mass and polysaccharide content decreases compared to uninfested ones. The pectolytic and cellulolytic enzymes eliminated by *D. dipsaci* species are of particular importance, since they exert an action on the polymers that make up the membranes of plant cells and the parenchymal intercellular tissue, which allows the parasite to penetrate into plants (MELNIC, 2008; BUMBU & MELNIC, 1975). Other authors (RIEDEL & MAY, 1971) found that in the vegetable tissue of onion, attacked by species *D. dipsaci*, the enzymes endo-polygalacturonase and endo-polymethyl-transaminase are contained.

During the multi-year research (MELNIC & GLIGA, 2024) it was observed that during the vegetation period the phytonematode *D. dipsaci* primarily attacks the protective leaves of both the bulb and the newly formed garlic cloves. Here, an intense reproduction of the parasite takes place, forming clusters of tens of thousands individuals. From the protective leaves the nematode moves to the growth cone and the root rudiments of the cloves. After harvesting, the seed material, as a rule, is stored in warehouses with a low temperature regime of +1 - +3°C, when the females of *D. dipsaci* stop laying eggs, the relative air humidity is 75-80% and permanent ventilation. From the root rudiments of the bulbs, *D. dipsaci* moves to the foliar ones, which leads to their total destruction. It is important to note that under such conditions the expansion of the invasion occurs through the movement of *D. dipsaci* populations of different ages from one bulb to another.

During the harvest period, *A. cepa* bulbs infested in the early stages of dithylenchosis are difficult to distinguish from healthy ones and are unconsciously introduced by humans into storage warehouses. In case of non-compliance with

the low temperature regime and relative air humidity, single specimens of the parasite, remaining on the bulbs, falling into favorable conditions, they penetrate the bulbs, where they mass reproduction. In order to avoid the spread of the phytoparasite nematode *D. dipsaci*, it is strictly necessary to use healthy seed material, free from nematodes, for planting in polyculture conditions. To apply anthelmintic rotations for a period of 3-4 years, with the introduction of crops resistant to this parasite - potatoes, tomatoes, eggplants, corn etc.

CONCLUSIONS

During the growing season, in *Allium cepa* crops under monoculture conditions, outbreaks of phytoparasitic nematode species - *Ditylenchus dipsaci*, with an extensivity of 30-40%, were detected.

It was determined that, during the harvest period, in the plant tissue of infested plants in the initial stages of ditylenchosis, a monotypic primary population occurs only with populations of the obligate parasite of this crop – *Ditylenchus dipsaci* (females, males, larvae, eggs). It was calculated that the density of species *D. dipsaci* in such bulbs amounted to a total of 34.12×10^3 specimens/gram of infested tissue, of which the highest percentage - 42%, belongs to larvae in different stages of development (L2; L3; L4).

During the storage period, the density of phytoparasitic nematodes *D. dipsaci* was - 2.8×10^3 specimens/gram of infested tissue, with a percentage distribution of *D. dipsaci* specimens: males - 25%; females - 18%, larvae of different species - 11% and saprophytic nematode species (mature forms) - 46%, most of which are from the order Rhabditida, which proves that microorganisms are present in such bulbs.

It has been observed that the plant tissue of *Allium cepa* bulbs infested in the initial stage only by *D. dipsaci* populations contains remains of empty, dissociated plant cells. The pectocellulose membranes, as well as the parenchymatous intercellular tissue, are completely macerated.

It was established that during the vegetation period the amount of dry matter of *Allium cepa* varieties cultivated in the Republic of Moldova constitutes 13.5% in bulbs and 7.4% in leaves. The dry mass of bulbs infested with *D. dipsaci* decreases compared to the control with 2.7%, and of leaves with 0.6%. At the same time, it was observed that in infested plants there is an increase in the amount of water, both in *A. cepa* bulbs and in their leaves.

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