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Biodiversity of Nematofauna of Earthworm Casts

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***Abstract* – The main goal of the investigation was to study the nematodes' species composition in cattle manure and in biohumus for comparing them.**

54 forms of nematodes were registered and up to 44% of them were identified. Among them, 40% of bacterivores, 20% - plant feeders, three species of predators and four species of fungivorous nematodes. In the repeated tests of vermicompost the species composition of nematodes has changed: appeared the species that were not found during the early stages of research. Also, should be noted that 35% of nematodes were found in the manure before the experiment began.

Extraction of the nematodes was made by flotation method.

Another goal of the study: comparison of the breeding rates of two different earthworm populations - *Lumbricus rubellus* and *Eisenia fetida*.

The earthworm life activity considerably depends on food composition. After the hay adding in the manure, which was the main component of earthworms feed, the growing number of Californian Red Wriggles doubled, and the number of the Red earthworm population continued to increase sequentially.

Investigations were carried out from May to October. During the experiments we used the rape cake as one of the fodders for earthworms.

The laboratory analysis of the chemical composition of soil, biohumus, cattle manure was made in the Institute of Earth Sciences of Ilia State University and the chemical composition of the rapeseed (*B. naps*) by Sun West Food Laboratory Ltd, using MOIL-01 and MPROT-02 methods.

***Keywords* – Soil, Fertility, Vermicompost, Life Activity, Taxonomic Structures.**

I. INTRODUCTION

As the soil fertility mainly depends on the vital activity of soil invertebrates, the study of their lifestyle and relationships is too important. A problem of soil fertility and its perfection obviously acquires more and more seriousness day by day, so every study in this area is very significant. One of the best ways to enrich and restore soil fertility worldwide is considered the vermicomposting and Vermitechnologies. The possibility of applying biological methods to protect plants from phytopathogens has been studied for many years. Biological plant protection is constantly evolving: new more perspective, organisms for biological control of plant pathogens appear [1], [2]. However, there are many organisms, whose role in the biological control is still underestimated; among them are earthworms - producers of a unique organic fertilizer - vermicompost [3]. Despite the fact that earthworms, their lifestyle and importance for the environment have long been studied by scientists of different countries and still arise a great interest. Considering that the soil fertility depends not only on plant covering, but also mainly depends on the vital activity of soil invertebrates, the study of their lifestyle and relationships are very important. Based on the numerous cases of poisoning the population caused by excessive use of nitrate fertilizers and various chemicals, scientists decided to draw attention to the seriousness of the problem and inform the society about the importance of implementing vermitechnology to get ecologically pure and safe bio products.

The vermitechnologies and vermiculture are at least a century old, but now the problem is revived the worldwide because of diverse ecological objectives such as waste management, soil detoxification, regeneration and sustainable agriculture. Earthworms in the soil improve water movement, nutrient dynamics; their function is to be an aerator, the destructor of plant residues, chemical and biological stimulator. Earthworms secrete enzymes and celluloses in their digestive tract that make rapid biochemical transformation of cells and protein-containing compounds into such organic matters, which restore and enrich the soil structure, and plant growth. They are not essential to all healthy soil systems, but their presence is usually an indicator of a healthy system. Earthworms perform several beneficial functions. All this will enable to start a long term production of bio humus in "conscious" peasants' farms and its intensive usage. Furthermore, the high-efficiency natural organic fertilizer obtained with earthworm activity is a warranty of a good harvest and provides ecologically pure product.

It should be noted, that habitat of earthworms - soil also contains enzymes. Enzymes emerge from the organisms existing in the soil (e.g. Earthworms and other soil inhabitants, microorganisms) and plants playing a great role in soil fertility. Most of the enzymes are distinguished by their high activity in soil and excrements of earthworms [4]. The complex of micro arthropods is constituted not more than 1% of humic acids of litter substances, which do not move to the lower horizons without the participation of soil invertebrates.

Nowadays humanity has faced an acute environmental problem how to save the Earth. Taking care of the Earth is not just a responsibility - it's a privilege. Hundreds tons of biodegradable organic waste are being generated in cities and towns in the country, creating disposal problems. This waste can be converted into valuable compost applying vermicomposting technology. Such approach reduces pollution, provides good substitutes for chemical fertilizers and makes a less harmful impact on the environment. A great attention should be paid to the production of ecologically pure food and increase of agricultural crop productivity using organic fertilizers to protect soil and crops from contamination with nitrates, phosphates and other mineral fertilizers.

Ecology is that branch of science concerned with the interrelations between organisms and their surrounding medium. The most important ecological factors to which phytonematodes must adapt themselves in natural conditions are: climate, weather, type of soil and composition, including soil biocenose, and terminal points of plants which form temporary or permanent habitations or permanent habitations or sources of food for the worms. The individual conditions of the surrounding medium do not act in isolation on the nematode but rather in concert. Furthermore, these conditions are themselves interrelated and hence any ecological action is perforce heterogeneous.

Scientists concerned with the interrelations between organisms and their surrounding medium, they also are conducting observations and studies relations between nematodes and earthworms, even found and described new species of nematodes. In this respect it can be noted the paper by E. Kvavadze and I. Eliava, who gave the description of new species of nematodes *Dicelis ivericus* Kvavadze et Eliava, 1975 from the Georgian endemic amphibious earthworm *Allolobophora iverica* Kvavadze, 1973 [5]. The individual conditions of the surrounding medium do not act on the nematode but they have a rather concerted action with temperature regime, soil composition and moisture. So, all these conditions are themselves interrelated and hence any ecological action is perforce heterogeneous [6]. The relationships between nematodes and other soil organisms are highly variable. The various products of exchange decay of matter in microorganisms may possibly exert a suppressive and a stimulatory effect on nematodes. Moreover, several studies have actually shown a decrease in nematode populations in the presence of earthworms [7].

The roundworms or nematodes, after protozoa, are the richest in number and species diversity of soil animal group. Biology of various species is quite different, depends on the climatic zones, the soil usage and changes of their number and composition.

Unlike mites and springtails the free-living nematodes, directly aren't involved in the decomposition of plant residues. However, their nutrition and metabolism has the influence on soil processes. The food of nematodes consists of 50% of protein and the remaining 50% of equal parts of carbohydrates and fats. That is one of the reasons to include the nematodes in the plant material decomposition process. For plants, it is a favorable uniform distribution of nitrogen in the soil. The body tissue nematodes themselves are also an important reserve of nitrogen that is constantly released at dying off animals caused by rapid change of generations and may be involved in the formation of humic acids. Finally, it's good for plants.

II. Materials and Methods

In view of the fact that vermitechnology make their first steps in Georgia any research in this regard is very significant.

The main goal of this investigation was to study the species composition of nematodes in the cow manure and in the biohumus, depending on the structure and food composition (Table 1).

Table 1: The Taxonomic & Ecological Structure of Nematodes in the Substrates (Manure) and Earthworms Casts (Vermicompost)

№	SPECIES	MANURE	VERMICOMPOST		ECOLOGICAL GROUP
			I Test	II Test	
1	Acrobeloides tricornis			+	**BACTERIAL FEEDERS**
2	Acrobeloides sp.			+	
3	Alaimus sp.	+			
4	Anaplectus granulosus	+		+	
5	Caenorhabditis elegans	+			
6	Cephalobus nanus	+		+	
7	Cephalobus parvus	+	+	+	
8	Cephalobus persegnis		+		
9	Cephalobus sp.		+		
10	Cruzinema sp.	+			
11	Diplogaster sp.			+	
12	Eucephalobus mucronatus		+	+	
13	E. striatus			+	
14	Eucephalobus sp.			+	
15	Panagrolaimus rigidus			+	
16	Plectus annulatus			+	
17	Pl. armatus	+			
18	Pl. elongatus		+	+	
19	Pl. parietinus		+		

20	Pl. parvus		+		
21	Plectus sp.	+	+		
22	Rhabditis sp.a	+			
23	Rhabditis sp.b			+	
24	Tripyla glomerans		+		
25	Aporcelaimellus adriani		+		**OMNIVORES**
26	A. kikereensis	+			
27	A. obtusicaudatus	+	+		
28	A. crigeri		+		
29	Aporcelaimellus sp.	+	+		
30	Enchodelus hopedorus		+		
31	Eudorylaimus acutus			+	
32	E. acuticauda			+	
33	E. carteri			+	
34	E. leucarti			+	
35	Eudorylaimus sp.			+	
36	Mesodorylaimus abberans			+	
37	Anatonchus ginglimodontus			+	**PREDATORS**
38	A. tridentatus			+	
39	Coomansus parvus	+		+	
40	Mononchus sp.	+			
41	Filenchus filiformis		+		**FUNGAL FEEDERS**
42	Filenchus sp.	+	+		
43	Tylencholaimellus striatus		+		
44	Tylencholaimellus sp.		+		
45	Ditylenchus misellus		+		**PLANT FEEDERS**
46	Ditylenchus sp.			+	
47	Lelenchus sp.	+			
48	Psilenchus minor			+	
49	Psilenchus sp.		+		
50	Psilenchus sp. a	+			
51	Tylenchus davainei	+		+	
52	T. filiformis	+			
53	Tylenchus sp.a		+		
54	Tylenchus sp. b		+		

The investigation was carried out in the laboratory conditions. A standard method was applied for vermicomposting: the initial number of earthworms was - *Lumbricus rubellus* - 60 and *Eisenia fetida* - 25 per container, the earthworms were placed into plastic containers with an area of $0.2m^2$ a basic substrate for worms feeding was cow dung without impurities. The cow dung and hay were collected on private farms, cow dung was not fresh. All containers were maintained in the laboratory at 24°C. The repeated tests to specify the presence of nematodes in the earthworm casts were held after 3 months.

The other goal of this study was to compare the earthworms breeding rates of two different populations, local - Red earthworm (*Lumbricus rubellus* Hoffmeister 1843) and Californian Red Wigglers (*Eisenia fetida*, Savigny 1826). These populations were used to observe the recycling and vermicomposting process of organic waste in vitro.

To study the breeding rate of earthworms and the dependence of their vital activity from fodder the hay was added to the cow dung. While comparing the breeding rates the earthworms were counted manually. During this time the boxes were kept in an open air at a temperature 30-35°C. The observations were carried out within 6 months from May to October. During the experiments we tried the rape cake as one of the fodders for the earthworm feeding.

The laboratory analysis of the chemical composition of soil, biohumus and manure was made in the Institute of Earth Sciences of Ilia State University and the chemical composition of rapeseed (*Brassica napus*) by SunWest Food Laboratory Ltd, using MOIL-01 and MPROT-02 methods.

The nematofauna of the biohumus and cow manure, which was the main substrate for earthworm feeding, was studied. The extraction of the nematodes from the substrates was made by the flotation method (Berman modified method) [8] at room temperature. In each case three samples approximately fifty grams of substrate (fresh weight) were taken from each container with earthworms for nematode extraction. The nematodes from compost and manure extracted through sieves covered with cotton and water. The extraction process usually lasts for 48 hours.

The purpose of our study has not been to trace variability of the nematode number. All the methods used to control the nematodes - both chemical and environmental - cannot lead to its total destruction, but can reduce its population to a degree, that damage becomes insignificant. But even if this is managed to succeed, it does not mean that we can rest on our laurels. One has only to disrupt the technology of farming, or make a mistake, as the number of nematodes has instantly increased on several times.

III. Results and Discussion

An interaction between Nematodes and Earthworms was studied by several scientists [7]−[10]. There are some data on the effect of vermicompost to improve plant resistance to pests, in particular to soil nematodes [11]. The most of the authors showed a reduction in the presence of nematodes influenced by earthworms. It should be noted that this effect is not due to a direct effect on the parasite, but is associated with the expression in the leaves of the genes encoding enzymes: lipoxygenase, phospholipase D and cysteine protease.

As a result of the application of vermicompost a significant suppression of populations of pathogenic microorganisms, nematodes and insect pests was found. In the presence of earthworms the lesion of rice by nematodes significantly was decreased by 82% [11]−[15]. In time of vermicompost application the agricultural crops productivity increases 30-70%. Also it has the capacity to speed up the germination and the development of different agricultural crops and ensures their protection against diseases and pests [13].

The taxonomic structures of the studied species were recorded in the table below.

It is known that the trophic relations and allocation according to ecological groups have a special importance for the ecological characteristics of nematodes. The investigations showed that the fauna of nematodes in the earthworms casts are much more diverse than in the cattle manure and mostly represented by the bacteria feeder - 24 species, omnivores - 13 species, including the other ecological groups: two families of fungus feeder nematodes - by *Tylenchidae* and *Leptonchidae*, 3 species of predator nematodes and 9 species of the plant feeder nematodes. Furthermore, it should be noted carefully that nematodes have not been detected in the gut of earthworms.

Some other researchers noted that microorganisms found in the different feed substrates before ingestion by the earthworms were not found in the casts [16]. In our trials the repeat tests of vermicompost showed that the species composition of nematodes has a bit changed and were detected some other species of nematodes which were not found in early stages of the research. Namely, all species of predatory species were found in repeat experiment and only one species of predator nematodes - *Coomansus parvus* was revealed later in the food (cattle manure) of earthworms. In other ecological groups nematode species composition is more diverse (Table 1). It should also be noted that unlike predatory forms of nematode fungi feeder species were identified only in the first test, while in the repeated experiment they have not been recorded. As in the case with predatory this also only one species of nematodes - *Filenchus sp*. has been registered in the cattle manure, the main food of earthworms.

According to the survey of the fauna it can be concluded that the various samples of nematodes taxonomic structure did not significantly differ from each other.

It's known that earthworms have many predators, including: mites, centipedes, earwigs and others among them - nematodes, but in our case only three predacious species of nematodes were found. The presence of other species of free-living nematodes in retesting of vermicompost may be due to direct ingestion and consequent effects on the fertility, viability and germination of cysts present in casts.

The benefit of compost is that we get fertilized plants with highly resistant to pests and diseases. The disease resistance is partly due to the fact that the microscopic fungi living in the compost produced antibiotics. They suppress the activity of pathogenic bacteria in the soil. Antibiotics absorbed by the roots fall into the above ground part and protect it from infection.

It was proved experimentally that, even a slight addition of vermicompost fertilizer in the soil for plants and significantly accelerated the germination, growth, flowering and ripening of the crop, regardless of their own nutrient storage plants [12].

The fungi static activity of vermicompost was also proved in experimental conditions [17], [18]. The compost is a home to mushroom predators with long sticky filaments, which pierce the depth of compost and become a trap for the nematodes. The filaments form rings which hold them falling into nematodes and gradually digest them. Therefore, the compost is considered to be the best means of nematodes. The predacious fungi, which are completely filled with adherent hyphaes compost and prey nematodes, can significantly reduce the quantity of phytohelminthes, which allows us to assume that the faulty closed humus (long-term) and open (short-term) ground area can be applied. The study showed that, with introduced of organic fertilizer into the soil, the amount of predatory fungi, much more than if use of mineral fertilizers. Unfortunately, the study of predacious fungi presence in the biohumus was not the purpose of our work. Taking into consideration the above mentioned nematodes are phytophagous and carnivorous. Carnivore nematodes devour their relatives – herbivorous nematodes, and even though they are not completely destroyed, they significantly reduce their number. On beds fertilized with compost, you cannot be afraid of strong root damage by nematodes.

The other goal of the experiments was to compare earthworms breeding rates of these two populations - local Red earthworm (*Lumbricus rubellus* Hoffmeister, 1843) and Californian Red Wigglers (*Eisenia fetida* Savigny, 1826) for recycling and vermicomposting of organic waste.

In this case the Figure 1 presents the process of earthworm hatching in Lab condition and below the Fig. 2, clearly shows the breeding rates of both populations.

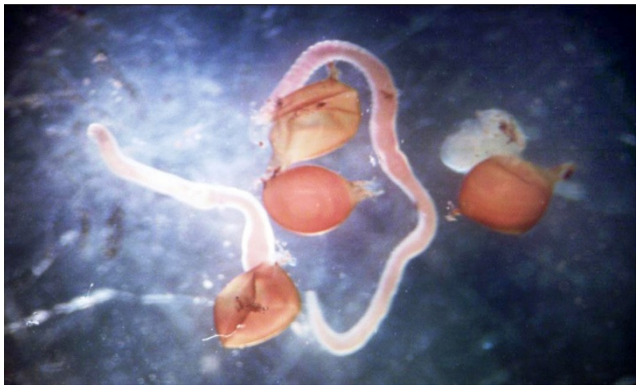

Fig.1.The Hutching of the Earthworms in the Experimental trials

The amount of accountability carried out monthly and approximately on the same date. For worms feeding cattle manure was used in a pure form and as a mixture with hay - in August. As a result the Californian worms number increased - almost doubled while the growth of Georgian population's number was continual and sequential. The similar results were described by other authors [19] when the *Eisenia andrei* showed higher growth rates in mixed cultures, while the growth rate of *L. rubellus* decreased slightly in mixed cultures as compared to pure cultures.

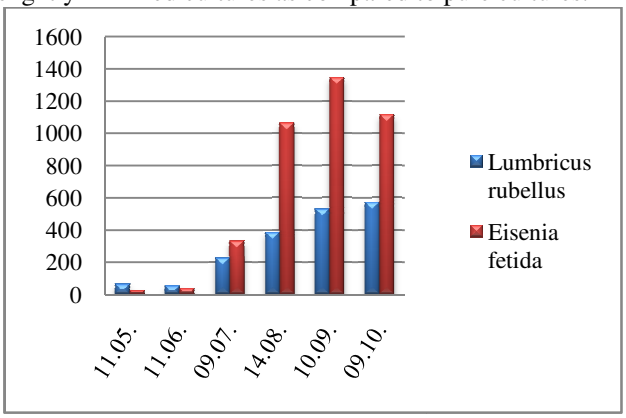

Fig.2. The comparison of earthworms breeding rates in two - local Red earthworm (*Lumbricus rubellus* Hoffmeister, 1843) and Californian Red Wigglers – (*Eisenia fetida*, Savigny, 1826) populations

The most important ecological factors to which phytonematodes must adapt themselves in natural conditions are: climate, weather, type of soil and composition, including soil biocenose and terminal points of plants which form temporary or permanent habitations or permanent habitations or sources of food for the worms, the fodder composition and quality are of the same importance. It is known that all these factors have a significant influence on the vital activity of earthworms [20].

The results of the chemical composition of soil, biohumus and cattle manure laboratory analyses showed in the Table 2.

Table 2: The chemical composition of various substances

№	Titles	Humus	N	P_2O_5	K_2O
1	Vermicompost	25,3	1,47	1,18	1,04
2	Cattle dung	20,3	0,5	0,25	0,6
3	Soil	5,62	0,21	0,37	1,25

The contents of the organic matters in biohumus are 5 times more than its content in the soil and 5 per cent higher than in manure. The content of nitrogen is approximately three times and the content of phosphate - 4.5 times, higher than the manure breeding. The results clearly indicate the importance of the earthworms' role in enriching of biohumus.

IV. Conclusion

The investigations showed that 54 forms of the nematodes are registered and up to 32 species are identified. There were discovered more than 20 species (about 45%) of nematodes mainly belonging to a group of bacteriovores, about 10 species of plant feeder and four species of fungus feeder nematodes.

The most of them are the geographical ubiquitous and so they may exist in every type of habitats. According to the survey of the fauna it can be concluded that the various samples of nematodes taxonomic structure did not significantly differ from each other. As investigations showed the contents of the organic matters in biohumus is 5 times more than its content in the soil and 5 per cent higher than in manure.

In summary, it should be said, that the soil effective treatment could be performed with well-made mature compost. Therefore, the risk of nematode mass propagation is minimized there, where a ground is fertilized with compost. Approximately the same significance has earthworm's casts and organic mulch. The experiments in the lab conditions showed that the live activity of the earthworm population considerably depends on food composition. After the addition of hay in the manure, which was the main component of earthworms' food, the number of Californian Red Wigglers growth doubled, and the number of the Georgian population continued to increase sequentially (see Fig. 2). The experiments on using rape cake as earthworms' food showed that they had not eaten rapeseed. In our opinion the main reason of this is the high amount calories - 419.16 and the oil content - 15, 32% of rape.

The experiments clearly demonstrated the vital activity and the role of earthworms in the enrichment of organic fertilizers that will allow to conducting the recommendation conversations with farmers about the cost-effectiveness and benefits of using vermicompost in the processing of the soil for sowing.

A selection of the most active population allows to offer to local farmers more accessible and active population of earthworms to produce biohumus for the soil enrichment, enhancing and improve productivity of yield, as well as the best way to increase soil fertility and biohumus production and the recycling of organic waste. Moreover,

all these will improve the soil quality and raise the level of crop yield.

This work should draw additional attention of local farmers and producers to Vermitechnologies and its importance in solving the problems to renew the soil and in the recycling process of organic residues.

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