THE MICROBIOTA DYNAMICS OF A CAMBIC CHERNOZEM IN EZARENI FARM, THE RESEARCH SITE FROM IASI

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Abstract

The soil micromycetes are important biological agents for soil structure and an active compartment of soil organic matter. The main objective of this study was to isolate and identify the main genres of saprophytes and parasites micromycetes that colonize the experimental land of Ezareni farm. The analysed soil type is a poorly degraded cambic chernozem. The research was conducted during the autumn of 2012 - autumn of 2013.
Nine genres of saprophytes and parasites micromycetes were isolated from the soil samples. The results of the analyses performed on soil fungal genera indicated the presence of *Sporotrichum*, *Trichoderma*, *Mucor*, *Zygorhynchus*, *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizopus* and *Alternaria*. From the isolated micromycetes the greatest preponderance was held by *Penicillum*, the medium concentrations were held by *Fusarium*, *Sporotrichum*, *Alternaria*, *Aspergillus*, *Rhizopus* and the reduced ones were held by *Zygorhynchus*, *Mucor* and *Trichoderma*. The relatively high number of isolated fungal genera and their increased levels illustrate the fact that the soil type could be considered a good environment for developing micromycetes.

Key words: micromycetes, saprophytes, parasites, soil, cambic chernozem

The research on a cambic chernozem microbiota dynamics took place in the period of 2012-2013. The mycological analyses were conducted in the laboratory of Microbiology of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi. The soil samples represented by cambic chernozem were taken from the farmland Ezareni of the Didactic Station of Iasi.

The soil type that is under study is a clay loam cambic chernozem, formed on loess, with a slightly acidic pH comprised between 6.6 and 6.8; the humus content is from 2.73 to 2.93% with a medium content of N (0.17 g/100 g of soil), P_2O_5 (12.6 g/100 mg of soil) and K_2O (20,2 mg/100 g of soil). The area is characterized by average annual temperatures of 9.6^oC, annual rainfalls of 517.8 mm and air relative humidity of 69% (Ulea E., et al, 2012). A soil profile was performed in the field for a complex analysis of the soil type. The obtained data show a soil morphology of type Ap, Atp, Am, AB, Bv1, Bv2, Bv3k Cca1, Cca2, II Ck.

Saprophytic and parasitic fungal genera have been identified in the surface layers of the soil (the sampling depth is 8-10 cm). The situation can be explained by the fact that in this soil layer large quantities of organic substances used as nutrients by the fungus are to be found. The soil microflora plays a crucial role in agricultural ecosystems as actively participating in the processes of degradation and mineralization of soil organic matter and on the formation and stabilization of soil aggregates.

MATERIAL AND METHOD

In order to determine the micromycetes per 1 g of soil the culture method in Petri dishes was used. The biological material used to perform the analyses on determining the saprophytic and parasitic colonies of micromycetes from soil was taken from Ezareni farm, Iaşi.

The soil samples were gathered from a depth of 8 to 10 cm by using a metal spatula respecting classical aseptic conditions and then transferred to paper bags. The soil dilutions were prepared according to the method of successive dilutions, in sterile water, using a dilution factor in rate of 10 (dilution 10^{-1}, 10^{-2} etc). In aseptic conditions, from the obtained dilutions there were made seedings by introducing a ml of dilution in each Petri dish. On the inoculum from the Petri plate 18 ml of PDA with rose Bengal medium was poured at a temperature of 45°C so that the agar could be fluid and the temperature would not destroy the inoculated microorganisms.

To determine the number of fungal the Petri dishes were incubated in a thermostat at 28ºC for 5

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days. After incubation in the thermostat, the colonies were counted by the naked eye and in the cases of higher densities the Wolfhügel plate was used.

RESULTS AND DISCUSSION

As we expected, the results of mycological tests carried out on chernozem cambic indicate the presence of saprophytic and parasitic fungal genera in the soil type. Determined fungal genera and their systematic classification is shown in Table 1. The most numerous types (8 in number) of saprophytes and parasites fungal genera were determined from the soil samples taken in the autumn of 2012. In the following spring there was an abatement regarding the number of micromycetes genera (4 in number) and in the fall of 2013 we could determine five fungal genera.

Table 1

The systematic classification of isolated micromycetes genera and phylum ratio in the analised soil

The systematic classification of the isolated saprophytic and parasitic fungal genera					Colonies average/dish			Phylum ratio in the soil
Phylum	Class	Order	Family	Genus	A. 2012	S. 2013	A. 2013	
Zygomycota	*Zygomycetes*	*Mucorales*	*Mucoraceae*	*Mucor*	0,2	-	0,8	33,4%
				Zygorhynchus	0,2	-	-	
				Rhizopus	0,2	0,2	-	
Ascomycota	*Plectomycetes*	*Eurotiales*	*Eurotiaceae*	*Aspergillus*	2	0,2	-	55,5%
				Penicillium	5,2	0,8	0,4	
	Ascomycetes	*Hypocreales*	*Hypocreaceae*	*Fusarium*	0,8	0,2	2,6	
				Trichoderma	0,2	-	0,8	
		Pleosporales	*Pleosporaceae*	*Alternaria*	-	-	2,6	
Deuteromycota	*Hyphomycetes*	*Moniliales*	*Moniliaceae*	*Sporotrichum*	0,8	-	-	11,1%
TOTAL number of colonies/dish dilution 10^{-4}					9,6	1,4	7,2	

The genera *Mucor*, *Rhizopus*, *Zygorhynchus* were determined from phylum *Zygomycota*; the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Alternaria* were determined from phylum *Ascomycota* while the genus *Sporotrichum* was determined from *Deuteromycota* phylum.

Our obtained data, after comparing the results, suggest that the phylum *Ascomycota* amongst the isolated fungal genera, has a high share in the type of analysed soil (55.5%), followed by phylum *Zygomycota* with a share of 33.4% while phylum *Deuteromycota* presents the lowest percentage, 11.1% *(tab. 1)*.

Based on the CFU/1 g soil (number of cells per one gram of dry soil) values, the saprophytic and parasitic micromycetes variation was analysed during the study that took place in the period autumn 2012 - spring 2013 - autumn 2013 *(fig. 1)*. The comparative analysis of results from the autumn of 2012 showed that the highest values were with genus *Penicillium* 5.2 x 10^4 CFU/1 g soil, followed by *Aspergillus* genus with 2 x 10^4 CFU/1 g soil. Genera as *Fusarium* and *Sporotrichum* recorded values that are equal to 0.8 x 10^4 CFU/1 g soil, and genus *Mucor*, *Zygorhynchus*, *Rhizopus* and *Trichoderma* have the lowest values regarding the number of CFU/1 g soil (0.2 x 10^4).

The total number of CFU/1 g soil was 9.6 x 10^4 and this value was the highest throughout the study and was reported in the autumn of 2012.

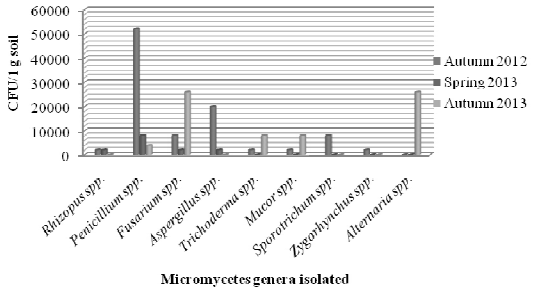

Figure 1 **Variation in biological micromycetes activity during the study, 2012-2013**

The spring research indicates values of CFU/1 g soil for genus *Penicillium* of 0.8 x 104; these values are much lower as compared with data from the autumn of 2012. Genera *Rhizopus*, *Aspergillus* and *Fusarium* amounted to 0.2 x 10^4 CFU/1 g soil. The total number of CFU/1 g soil showed the lowest value in the spring of 2013 (1.4 x 10^4). The fact that micromycetes develop in soils with pH values in the range 4-5 and the recorded value of the pH of the soil in the spring was 7.3 may explain why their activity is reduced. The fact that micromycetes develop in soils on pH values in the range 4-5 and the recorded value of the soil pH

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The analyses were repeated in the autumn of 2013 and the results led to a twist of situation. The total number of CFU/1 g soil was 7.2 x 10^4. Genera *Fusarium* and *Alternaria* predominated in the soil sample of cambic chernozem with 2.6 x 10^4 CFU/1 g soil each. Genera *Mucor* and *Trichoderma* showed values of 0.8 x 10^4 CFU/1 g soil for each while genus *Penicillium* recorded the lowest value on the duration of the study, 0.4 x 10^4 CFU/1 g soil.

Seasonal variations of fungal community is caused by temperature variations, changes in soil solution reaction (Lauber C., et al, 2008), fact demonstrated also by our study. Elevated CFU/1 g soil values in both autumns may be explained by large water quantities in the soil and the presence of roots remnants that are easily decomposed during this period of year.

The conducted research on frequency and spectrum of saprophytic and parasitic fungal genera shows different proportions in the analysed soil type.

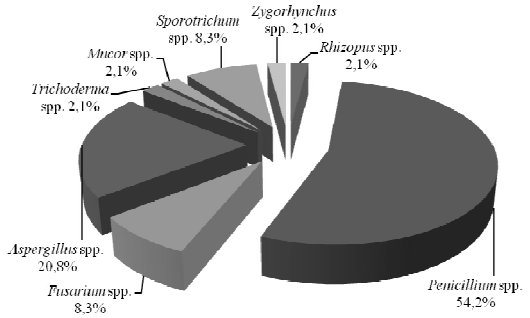

Figure 2 **Saprophytic and parasitic micromycetes genera isolated in the autumn of 2012**

The mycological analyzes from the autumn of 2012 indicate the highest percentage for the genus *Penicillium* (54.2%), followed remotely by genus *Aspergillus* (20.8%). Genera *Fusarium* and *Sporotrichum* are presented in equal percentage (8.3%) and genres such as *Trichoderma*, *Mucor*, *Rhizopus* and *Zygorhynchus* present the lowest values (2.1%).

Fertilizers were used on the soil where the sample was taken from. The use of mineral fertilizers in arable soils modifies the soil pH that influences the soil micromycetes; fertilizers increase the total number of microfungi (Rousk, J., et al, 2010) and this may explain the high percentage of certain genera of fungal in soil sample.

The study from the spring of 2013 indicates a rate of 57.1% for the genus *Penicillium* and an equal percentage of 14.3% for the genera *Fusarium*, *Rhizopus* and *Aspergillus (fig. 3)*.

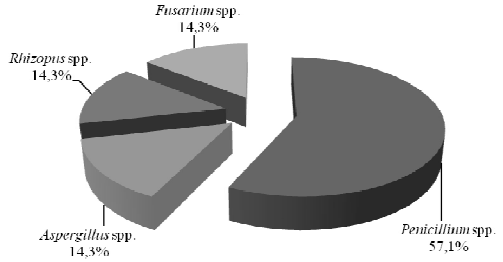

Figure 3 **Saprophytic and parasitic micromycetes genera isolated in the spring of 2013**

After we had processed soil samples and compared the results from the autumn of 2013, we observed equal percentage for both *Fusarium* and *Alternaria* genus (36.1%) while genera *Trichoderma* and *Rhizopus* showed frequency values of 11.1% each.

In the first two stages of the study genus *Penicillium* frequency predominated in the soil; then it was surprising to find that in autumn of 2013 it presented a value of 5.6% *(fig. 4)*.

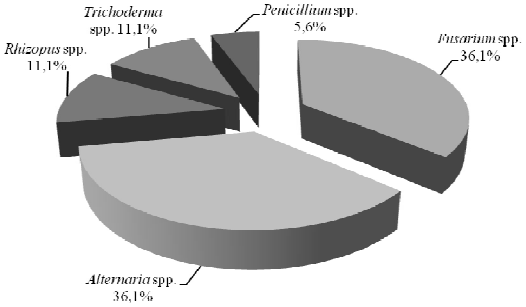

Figure 4 **Saprophytic and parasitic micromycetes genera isolated in the autumn of 2013**

The study carried out by Wheeler in 1991, showed that the genera *Penicillium*, *Fusarium* and *Aspergillus* grew in soil pH values between 2-11 and temperatures of 20, 30 and 37°C, fact that was confirmed also by our research.

Depending on the CFU/1 g soil values of the recorded fungal genera we identified the predominant genera in the cambic chernozem during the study conducted in 2012-2013. The activity values of fungal genera taxonomic assigned in the phylum *Ascomycota* are significantly higher while the genera from phylum *Zygomycota* reach medium average values of activity in soil; the values of genera from phylum *Deuteromycota*, according to the study we conducted, are minimum *(fig. 5)*.

The research conducted by Durowade in 2008 on some soils showed that micromycetes from phylum *Ascomycota* were present in a large number of genera in the analysed soil samples. The results of our study confirm Durowade's results.

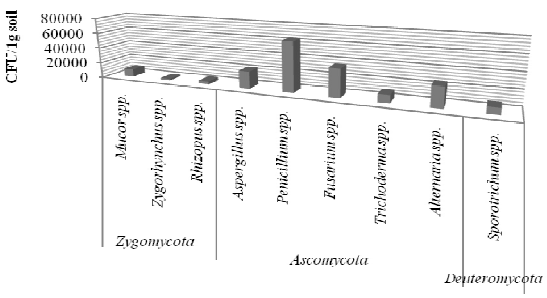

Figure 5 **Prominence of saprophytic and parasitic micromycetes genera dominated cambic chernozem**

Experimental data show that during our research the most intense activity is recorded by genus *Penicillium* with 6.4 x 10^4 CFU/1g soil *(fig. 5)*. A lower activity was noticed for the genus *Fusarium* 3.6 x 10^4 CFU/1 g soil, followed remotely by genera *Alternaria* (2.6 x 10^4) and *Aspergillus* (2.2 x 10^4). The present study indicates reduced activity in cambic chernozem for the genera *Mucor* and *Trichoderma* with 1 x 10^4 CFU/1 g soil. However, the lowest values are posed by genera *Sporotrichum* 0.8 x 10^4 CFU/1 g soil, *Rhizopus* 0.4 x 10^4 CFU/1 g soil and *Zygorhynchus* 0.2 x 10^4 CFU/1 g soil.

Micromycetes present an important role in the influence on pesticides, having the ability to degrade and quickly remove toxic substances. The detoxifying metabolism or catabolism occurs when soil microorganisms are using pesticides as a source of C and energy.

CONCLUSIONS

The observations carried out on cambic chernozem led to the conclusion that this type of soil is a favorable environment for the development of saprophytic and parasitic micromycetes.

Three genera: *Mucor*, *Rhizopus* and *Zygorhynchus* were determined from phylum *Zygomycota*; five genera: *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Alternaria* were determined from phylum *Ascomycota* and one genus *Sporotrichum* was determined from phylum *Deuteromycota*.

Our results showed a seasonal variation of micromycetes which can be characterized by a maximum in the autumn of 2012 and a minimum in the spring 2013. Seasonal variation affects primarily the superficial layers of the soil decreasing in depth. Seasonal variations of microbiological processes are caused by changes in soil solution reaction, the amount of organic matter in the soil, rainfalls and temperature variations. The most intense activity was recorded by genus *Penicillium*, followed by genus *Fusarium*, *Alternaria* and *Aspergillus* while the lowest activity was noticed with the genera *Rhizopus* and *Zygorhynchus*.

Our experimental data reveal that the intense activity of saprophytic and parasitic micromycetes occurs in the 0-10 cm depth of cambic chernozem.

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