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PRELIMINARY RESEARCH ON SOIL MICROFLORA AND MACROFAUNA IN THE EXPERIMENTAL FIELD MOARA DOMNEASCĂ

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Abstract

Microorganisms and insects that living in the soil are an important component of it. These components play an important role in supporting and growing plant communities. Microflora and macrofauna exercise processes that influence the physical-chemical, biological and agricultural characteristics of the soil. In order to determine the species of microorganisms per 1 g of soil by the Petri dish culture method on different culture medium: potato-glucose-agar (PGA), dichloran-rose bengal chloramphenicol (DRBC) agar and dichloran 18% glycerol (DG18) agar decimal dilutions were performed beforehand. The results were compared with those obtained by the soil washing method. Bacteria from the genus Pseudomonas, yeasts, fungi from the genera Penicillium, Fusarium, Rhizopus, Aspergillus and Sclerotinia and one species each of coleoptera and lepidoptera were detected along with Lumbricus terrestris.

Key words*: soil microorganisms, bacteria, fungi, culture medium, macrofauna.*

INTRODUCTION

Healthy soils are essential for sustainable agriculture. Agriculture has a recognized impact on soil microorganisms. Soils constitute the vital environment for numerous microorganisms. Microbial activity is very important to improve soil health (Toor and Adnan, 2020).

In the soil there are both microorganisms, which improve the state of soil fertility and contribute to the growth of plants, microorganisms called biofertilizers (nitrogen-fixing bacteria), as well as pathogenic microorganisms for plants that can infect plants through the roots. Ecosystem functioning is largely regulated by soil microbial dynamics (Kennedy and Smith, 1995).

Soil holds millions of microbes which takes part in the improvement of soil fertility and favors plant growth (Gougoulias et al., 2014).

Soil physical and chemical properties depend on quantity and quality of soil organic matter, pH and conditions of redox potential. All of these significantly influence the structure and dynamics of the soil microorganisms (Lombard et al., 2011).

Also, the composition of microorganisms is strongly influenced by various environmental factors, such as climate, soil properties and water (Lau and Lennon, 2012; Wipf et al., 2021; Cotuna et al., 2022).

There are many microorganisms that help plants grow, through which they cope with climate change and agricultural land degradation (Antoszewski et al., 2022).

Among bacteria: *Acinetobacter* sp. and *Pseudomonas putida* ensure to corn increased tolerance to Cu, enhanced chlorophyll content and increased Cu concentration in tissues through IAA (the phytohormone indole-3-acetic acid) synthesis, production of siderophores and solubilization of Cu and P (Rojas-Tapias et al., 2014); *Arthrobacter* sp., and *Bacillus megaterium* to tomato enhanced seed germination ratio, seedling length, and dry and fresh weight under salt stress (Fan et al., 2016); Also to tomatoes, *Pseudomonas putida* ensure increased plant height, stem diameter, radical volume, dry biomass, and fruit yield through production of IAA (Hernández-Montiel et al., 2017) and *Burkholderia tropica* increased yield through N-fixation and P solubilization (Bernabeu et al., 2015); *Azospirillum lipoferum* to wheat improved germination, plant growth, higher chlorophyll content, and improved membrane stability under salt stress; increased production of soluble protein, and sugars under salt stress;

Serratia proteamaculans, *Pseudomonas putida* and *Pseudomonas aeruginosa* reduction the effect of salt stress and ensures enhanced plant height, root length, and yield, and higher chlorophyll content through ACC (1-aminocyclopropane-1-carboxylate) deaminase (an immediate precursor of ethylene) production (Mazhar et al., 2015); *Streptomyces* sp. to alfalfa ensures protection against root-lesion nematode-*Pratylenchus penetrans* (Samac and Kinkel, 2001). Among fungus: *Alternaria solani* IA300 to sweet bell peppers ensure enhanced number of leaves, flowers, dry, and fresh weight (Mauricio-Castillo et al., 2020); *Aspergillus niger* 9-p to bean ensure increased biomass through production of IAA, ACC deaminase, siderophores, protease, amylase, pectinase, xylanase, and P solubilization (Galeano et al., 2021); *Aspergillus fumigatus* to soybean reduction the effect of salt stress and ensures enhanced biomass, leaf area, chlorophyll content, and photosynthetic rate, increased isoflavones, proline, SA (salicylic acid), and JA (jasmonic acid) content and lower ABA (abscisic acid) content through GAs (Gibberellins-diterpenoid phytohormones) production (Khan et al., 2011); Also to soybean, *Fusarium verticillioides* and *Humicola* sp. reduction the effect of salt stress and ensures increased shoot length, protein content, carotenoid, salicylic acid, and enhanced SOD (superoxide dismutase) activity, decreased ABA level and lipid peroxidation (Radhakrishnan et al., 2015); *Penicillium bilaii* to pea ensure increased root dry weight, length, and P content in the shoot (Vessey and Heisinger, 2011); *Penicillium* sp. to hop clover (*Medicago lupulina*), lentil and wheat ensure enhanced shoot growth and dry weight, and increased P uptake (Wakelin et al., 2007); *Trichoderma hamatum*, *T. harzianum* and *T. viride* to common freesia ensure accelerated flowering and enhanced development of lateral inflorescence shoots, increased K, Fe, Mn, and Zn uptake (Janowska et al., 2020); *Trichoderma viride* to rapeseed ensure enhanced biomass, lateral roots development, germination ratio and changes in microbial composition (Znajewska et al., 2018).

Insects make up the most numerous group of organisms on earth, around 66% of all animal species (Zhang, 2011).

Herbivorous insects damage 18% of world agricultural production (Losey and Vaughan, 2006).

Despite this damage less than 0.5 percentage of the total number of the known insect species are considered pests (Kim, 1993).

Although insects are mostly perceived as pests, they are the key components in diverse ecosystems. Of these, the coleoptera it contributes to the loosening of the soil, through the galleries created, and to the shredding of plant material; increase nitrogen, phosphorous and humus from the soil (Zală, 2015).

Insects larvae clean up dead plant matter and break it down for further decomposition by microbes (Jankielsohn, 2018).

MATERIALS AND METHODS

The soil samples were collected from experimental field Moara Domnească-Ilfov (44°29′33″N 26°15′20″E) in early November 2021 from a plot sown with winter wheat, which had maize as the preceding crop The experimental fields are located on preluvosol-reddish soft (reddish-brown) type soil.

The reddish preluvosol (according to the Romanian Soil Taxonomy System) is part of the luvisols class, which presents a mollic A horizon (Am) followed by an intermediate argic horizon (Bt) having colors with values above 3.5 (wet) at least on the faces of the structural aggregates, starting from the upper part and degree of saturation in bases (V) over 53% (Mihalache, 2006).

The climate in the Moara Domnească Farm area falls under the Köppen-Geiger classification system (Peel et al., 2007) in the formula D.f.a.x.

For the determination of microorganisms, were taken 5 samples from the cultivated plot (one from each of the 4 edges and one from the center), with the help of the agrochemical probe. At each point, a sample was taken from the depth of 0-20 cm. Samples were collected in sterile plastic bags. For the agrochemical characterization, 2 soil/parcel samples were taken at a depth of 0-20 cm.

The determination of the species of microorganisms per 1 g of soil was carried out by the culture method in Petri plates on different culture media: potato-glucose-agar

(PGA), dichloran-rose bengal chloramphenicol (DRBC) agar and dichloran 18% glycerol (DG18) agar. To determine the microorganisms species we used decimal dilutions 10^{-1} … 10^{-7} (Waksman, 1927 cited by Zală, 2021; Constantinescu, 1974, cited by Manole and Ciocoiu, 2011) (Figure 1).

Figure 1. Preparation of decimal dilutions 10^{-1} …10^{-7}

From the dilutions performed, seeding were made in Petri plates with a diameter of 7 mm (Figure 2.).

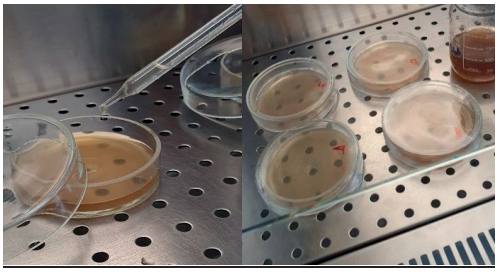

Figure 2. Inoculation of samples on PGA culture medium

Isolation of fungi from the soil was carried out by the soil washing method, which consisted of placing one gram of soil in an Erlenmayer beaker containing 200 ml of sterile water, shaking, tilting the flask at an angle of 45 degrees for one minute to settle the particles, it replaces the water, and the operation is repeated several times until the soil particles are dispersed in the sterile water and inoculated on the culture medium (Watson, 1960, cited by Constantinescu, 1974).

The simple microscopic preparation was performed with the help of a scalpel and consisted of detaching a small amount of the fruiting bodies formed on the surface of the culture media, placing it in the drop of water on the blade and lamella coating (Figure 3.).

Figure 3. Making the microscopic preparation from the white-fluffy area and the yeast area

The microscopic preparations were visualized with a Panthera S (Motic) microscope.

Soil pH was determined in a 1:2.5 aqueous suspension (Figure 4.), by the potentiometric method, with a Hanna pH-meter (Madjar et al., 2019).

Figure 4. Indication of the pH values of the soil samples

The pits for determining the numerical density of pests in the soil had a square shape, with a side of 0.5 m (0.5 x 0.5 = 0.25 sq m) and a depth of 20 cm. The equal distribution of the 5 gopis was in the form of a checkerboard. The soil from a pit was washed away and the insects were placed in a jar (Istrate and Roșca, 2009).

RESULTS AND DISCUSSIONS

On the potato-glucose-agar (PGA) culture medium, in the Petri dishes where samples were taken from the washed soil, more and more varied colonies of microorganisms developed (Figure 5.), unlike the dishes in which there were samples resulting from dilutions (Figure 6.).

The fungi were predominant in the first 20 cm of the soil.

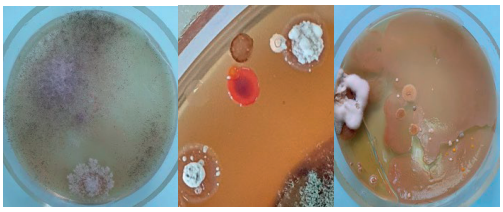

Figure 5. Highlighting the colonies of microorganisms on the PGA culture medium, in the Petri dishes in which samples were taken from the washed soil

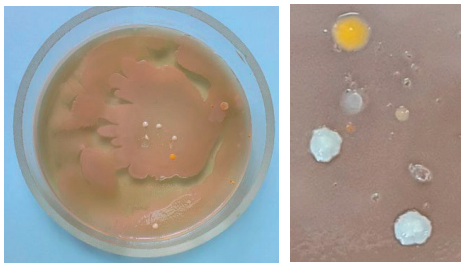

Figure 6. Highlighting the colonies of microorganisms on the PGA culture medium, in the Petri dishes in which the samples resulting from the dilutions were taken

It is known that the optimal pH values for the growth and development of fungi are: 4.5-6.5. (Child et al., 1973).

We identified fungi from 3 genera: *Rhizopus* (rarefied cotton-like mycelium-to be observed in Figure 5., the Petri dish on the left; and the presence of sporangiophores - Figure 7, left), *Fusarium* (fungal colony, white and dense - to be observed in Figure 3., the Petri dish on the left side; and the presence of mycelial hyphae, micro- and macroconidia - Figure 7, right) and *Penicillium* (dense greenish mycelial mass - to be observed in Figure 5, in the right corner of the central Petri dish).

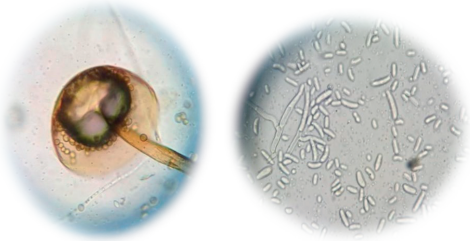

Figure 7. *Rhizopus* spp: sporangiophore, sporangium and sporangiospores (left); *Fusarium* spp: mycelial hyphae, micro- and macroconidia (right) (40x)

On PGA (Figure 3, the Petri dish on the right side), DRBC and DG18 (Figure 8) culture mediums colonies of yeasts of the genus *Saccharomyces* are flat, smooth, moist, shiny and cream-colored.

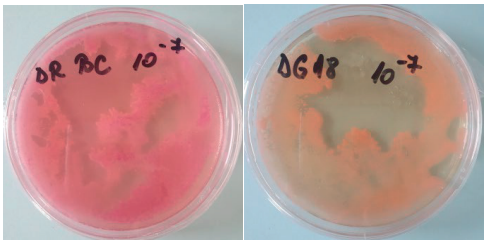

Figure 8. Colonies of *Saccharomyces* spp.

On DRBC and DG18 culture media in the Petri dishes with samples from the washed soil numerous colonies of some species of fungi from the genera *Fusarium* and *Aspergillus* have developed (Figure 9.). As stated by Jackson (1975) cited by Rangaswami and Bagyaraj (2005) the fungi of these genera are part of the mycotic microorganisms most frequently found in the soil.

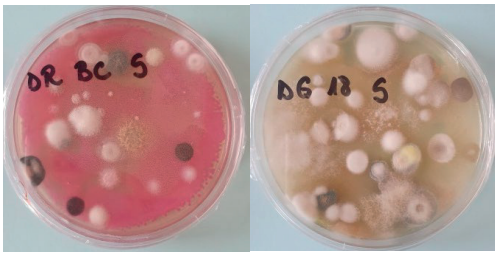

Figure 9. Colonies of *Fusarium* and *Aspergillus* spp.

The presence of bacteria on the PGA culture medium (Figures 5 and 6) was evidenced by the appearance of pigmented colonies in yellow: from the genus *Xanthomonas* and orange-red: from the genus *Corynebacterium* and white: from the genus *Streptomyces* (Schaad et al., 2001).

Regarding the number of colonies grown on the three culture media, in the case of the readings of the dishes with washed soil (Table 1), we note that *Fusarium* spp. recorded the highest number of colonies (13.5) on the DG18 culture media; *Aspergillus* spp. had the highest number of colonies (5.75) on DRBC culture medium; fungi of the genera *Rhizopus* and *Penicillium*, as well as bacteria of the genera *Xanthomonas* and *Corynebacterium* were reported only on the CGA culture medium. Yeasts of the genus

Saccharomyces and bacteria of the genus *Streptomyces* were reported on all three culture media; *Streptomyces* being found in greater numbers (2.5) on the DRBC culture medium. Regarding the number of colonies, in the case of the readings of the dishes with 10^{-7} dilutions, we found the presence of species from the genera *Fusarium* and *Aspergillus* only on the PGA culture medium, while *Saccharomyces* spp. was reported in all culture media.

Table 1. Colony count obtained from wash (W) soil or 10^{-7} dilutions/culture medium

Species	Culture medium					
	PGA		DRBC		DG18	
	W	10^{-7}	W	10^{-7}	W	10^{-7}
Fusarium spp.	5.25	1.25	11.5	-	13.5	-
Aspergillus spp.	2.25	0.5	5.75	-	5.5	-
Penicillium spp.	3.75	-	-	-	-	-
Rhizopus spp.	6.25	-	-	-	-	-
Saccharomyces spp.	+	+	+	+	+	+
Xanthomonas spp.	1.75	-	-	-	-	-
Corynebacterium spp.	1.25	-	-	-	-	-
Streptomyces spp.	1.75	-	2.5	-	0.75	-

Were also detected 2 sclerotia of the *Sclerotinia sclerotiorum* fungus.

After carrying out the five soil surveys, 19 specimens of the common frame-*Lumbricus terrestris* were also detected. They had an average length of 20.7 cm and an average number of 141 segments.

The insect species detected in the 20 cm deep soil layer were: black maize beetle (*Pentodon idiota*) and corn earworm (*Helicoverpa armigera*). Regarding the numerical density of the *Pentodon idiota* species, we identified 6 larvae/m^2 and 1.6 adults/m^2. Regarding the species *Helicoverpa armigera*, we detected a number of 3.2 pupae/m^2. The captured insect species were detected in the Moara Domnească fields and in the context of other research (Roșca and Istrate, 2004).

CONCLUSIONS

The soil is populated by various groups of living organisms. Among them, fungi and bacteria belong to the soil microflora.

The surface layer of the soil contains a large number of microorganisms, because it is well supplied with oxygen and nutrients.

The growth of microorganisms is influenced by the culture medium,

the largest number of species (8) being recorded on the PGA culture medium. Only 4 species develop on DRBC and DG 18 culture media.

Only two species of insects have been detected, a coleoptera: black maize beetle, whose adults feed on stems right at the surface of the soil while the larvae feed on the underground portions of the plant, including the roots; and a lepidopteran: the corn earworm, whose larvae feed on the silk and grains in the milk-wax phase.

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