

PARASITIC AND SAPROPHYTIC MICROMYCETES IN SOIL OF GREENHOUSES WITH TOMATOES(I)

MICROMICETE PARAZITE ȘI SAPROFITE DIN SOLUL SERELOR CU TOMATE

MIROIU CARMEN

Vaslui Phytosanitary Unit, Husi Centre

***Abstract.** The work presents the result of investigations made upon micromycetes soil in greenhouses cultivated with tomatoes. Were been recorded, at various dilutions, between 8800 and 18000 conidia/g soil. The author presents the diagnostic at five species of *Penicillium*, two species of *Aspergillus*, and one species of *Cladosporium*, *Scopulariopsis*, *Verticillium*, *Alternaria*, *Rhizopus* and *Ceratocystis*.*

The spectrum of the micromycetes from soil varies very much in the cultivated lots, because interfere the influence of the working made for planting and the plants influence during their growth. Towards the previous situation of planting, it can be seen the alteration of the micromycetes spectrum both in quantity and in variability of isolated species.

MATERIAL AND METHOD

After tomatoes have been planted, have been gathered tests from soil which were brought to lab for analyses. In order to determine the micromycetes from soil, the petries were sterilized in which was put Czapek medium which has following composition: NaNO₃ - 3 g ; K₂ HPO₄ - 1 g ; MgSO₄ .7 H₂O - 0,5 g ; KCl - 0,5 g ; FeSO₄ .7H₂O - 0,01 g ; Sucroze - 30 g; Agar - 15 g; distilled water -1000 ml. The petries were sterilized. The seeding was made on 20-th June 2007 and have been Thermostated at 22°C. Two dilutions have been made: 10⁻⁴ and 10⁻⁶ , for this were used five petries for each dilution and test. After the colonies of micromycetes have appeared, there were photographed on 25-th June 2007.

RESULTS AND DISCUSSIONS

After counting the colonies, we found the following results:

- at test I, at dilution 10⁻⁴ at V-1- have been registered an average of 88,00 germs /g soil and at dilution 10⁻⁶ at V-1 an average 32,000 germs /g soil;
- at test II, at dilution 10⁻⁴ at V-2- an average of 52,000 germs micotici/g soil and at dilution -6 V-2- an average of 18,000 germeni micotici/g sol.

In Petri vassels have been identified the following micromycetes:

Test I

- 1 0-4 V-1R – 1 = 16 colonies:

8 colonies *Penicillium coryophilum*
 1 colony *Cladosporium lignicolum*
 1 colony *Mucor sp.*
 1 colony *Scopulariopsis brevicaulis*
 2 colony *Aspergillus fumigatus*
 1 colony *Micelii sterile*
 $R - 2 = 6$ colonies:
 4 colonies *Penicillium brevi-compactum*
 2 colonies *Penicillium coryophilum*
 $R - 3 = 8$ colonies:
 5 colonies *Penicillium coryophilum*
 2 colonies *Mucor sp*
 1 colony *Micelii sterile*
 $R - 4 = 7$ colonies:
 3 colonies *Penicillium brevi-compactum*
 1 colony *Penicillium coryophilum*
 1 colony *Penicillium rugulosum*
 1 colony *Cladosporium lignicolum*
 1 colony *Micelii sterile*
 $R - 5 = 7$ colonies:
 2 colonies *Penicillium brevi-compactum*
 3 colonies *Penicillium coryophilum*
 1 colony *Penicillium rugulosum*
 1 colony *Micelii sterile*
 10-6 V-1
 $R - 1 = 4$ colonies:
 2 colonies *Verticillium lateritium*
 2 colonies *Alternaria humicola*
 $R - 2 = 3$ colonies:
 1 colony *Penicillium coryophilum*
 2 colonies *Rhizopus nigricans*
 $R - 3 = 4$ colonies:
 1 colony *Penicillium coryophilum*
 1 colony *Penicillium rugulosum*
 2 colonies *Verticillium lateritium*
 $R - 4 = 2$ colonies:
 1 colony *Penicillium brevi-compactum*
 1 colony *Ceratocystis paradoxa.*
 $R - 5 = 3$ colonies:
 3 colonies *Penicillium frequetans*

Test II

 10-4 V-2R - 1 = 7 colonies:
 1 colony *Alternaria tenuis*

1 colony *Aspergillus terreus*
 4 colonies *Cladosporium lignicolum*
 1 colony *Penicillium rugulosum*
 R – 2 = 6 colonies:
 1 colony *Aspergillus funiculosus*
 3 colonies *Penicillium brevi-compactum*
 2 colonies *Verticillium lateritium*
 R – 3 = 4 colonies:
 2 colonies *Penicillium rugulosum*
 2 colonies *Micelii sterile*
 R – 4 = 4 colonies:
 3 colonies *Penicillium brevi-compactum*
 1 colony *Humicola grisea*
 R – 5 = 4 colonies:
 1 colony *Penicillium brevi-compactum*
 2 colonies *Penicillium ochraceum*
 1 colony *Cladosporium lignicolum*
 10-6 V-2 R - 1 = 1 colony:
 1 colony *Penicillium coryophilum*
 R - 2 = 2 colonies:
Penicillium rugulosum + *Paecilomyces marqandii*
 R – 3 = 1 colony:
 1 colony *Penicillium coryophilum*
 R – 4 = 3 colonies:
 3 colonies *Penicillium coryophilum*
 R – 5 = 1 colony:
 1 colony *Micelii sterile*

The analyses of the tests from the soil in June, prove the fact that the presence of high temperatures and of bigger plants made that the spectrum of micromycetes from soil to diversify and so have been isolated: 5 species of *Penicillium*, 2 species of *Aspergillus* and 1 specie of *Cladosporium*, *Scopulariopsis*, *Verticillium*, *Alternaria*, *Rhizopus* and *Ceratocystis*.

In the soil tests have been observed 5 species of *Penicillium*: *Penicillium coryophilum*, *Penicillium brevi-compactum*, *Penicillium rugulosum* and *Penicillium frequetans*, species which have been described in the previous work and *Penicillium ochraceum*.

In the following we will diagnose only the new species observed in the soil tests analyzed in June 2007.

1. ***Penicillium ochraceum*** (Bainier) Thom, The *Penicillia*, p. 309-310 (1930); Gilman, A manual of Soil Fungi, p. 272 (1959);

The colonies developed on medium Czapek agar reach 3 cm in diameter after 10 days. They have the surface more or little floccos and 2-3 mm high. At first the colonies are velvety, and then floccos, with white margins and wrinkles. On the superior side appear drops and the other side is yellow to olive in colour.

The conidiophores have $150 \times 4 \mu\text{m}$ and thick walles. The heads form colums. The conidiophores have branches of $15-17,5 \times 3 \mu\text{m}$, metulas of $10 \times 2,5 \mu\text{m}$ and fialides of $8-10 \mu\text{m}$. The conidies are subglobose of $3 \mu\text{m}$ with thick walles.

Micromyceta is qnoted only once in the country be Racoviță A and colab from Transylvania in 1969 from salame, and we qnote it first from soil.

Have been pointed 2 species of *Aspergillus*: *Aspergillus funiculosus* and *Aspergillus terreus*. Because the full description of this species *Aspergillus funiculosus* has been made prewionsly we will give the full data only for the other species.

2. *Aspergillus terreus* Thom, Thom and Church, Ann. J. Botany V, p. 85-86 (1918); Gilman, A Manual of Soil fungi, p. 225 (1959); Raper and Fennell, The genus *Aspergillus*, p. 568, fig. 128 (1965).

The colony developed on Czapek medium agar has a pink colour with shades of cinamon bark and yellow brown on the other side. The conidiophores give the fluffy aspect of the surface of the colony. Conidiophores have $150 - 200 \times 5 \mu\text{m}$, by pliant and ends with a bladder of de $12,5 - 15 \mu\text{m}$ in diameter, which support, a two row of fialides at end. The first layer of fialides has $7 - 9 \times 2 - 2,5 \mu\text{m}$, and those exterior has $5 - 7 \times 2 \mu\text{m}$. These are compact and eliminate the conidiouses of elliptical to globose, de $2 - 2,5 \mu\text{m}$ with smooth surface.

Micromyceta has been pointed before on tanned skin by Ioniță and colab. in 1976 and from soil by Niță L. and Balinschi Irina in 1966, Lucia Turcu in 1971, 1974 și 1975 from Cluj and Viorica Iacob from Iași in 1973.

3. *Cladosporium lignicolum* Corda, Icon. I, fig. 2067, p. 14 (1837); Massee, Brit. Fung. Fl. III, p. 394 (1885); Gilman, A Manual of Soil Fungi, p. 334 (1959).

Micromyceta develops on Czapek medium agar a black colony, circular, with the central zone bulging, of $1 - 1,5 \text{ cm}$ in diameter. The conidiophores are short, branched and sustain conidioues ellipsoidal put in short chains or only by two. Their colour is brown- black of $8-10 \times 5 \mu\text{m}$. Micromyceta is new for România .

4. *Scopulariopsis brevicaulis* (Sacc.) Bainier, Bull. Trimest. Soc. Mycol. Fr. XXIII, (1907); Ellis, Dematiaceous Hyphomycetes, p. 327, fig. 224 A (1971).

On Czapek medium agar, after five days of thermostated, the colony is withe , with short conidiophores of $10 - 15 \mu\text{m}$. At the beginning, conidioues are withe-beije and then become slight brown. They are put in short chains, the shape is almost spheric, smooth at the beginning, then become slight and have $7 \times 8 \mu\text{m}$.

Micromyceta is cosmopolit, has been observed on defferent mediums: on frescoes (Alice Săvulescu, 1960), on paper (Alice Săvulescu, 1971), on tanned skin (Viorica Lazăr, 1967 and Alice Săvulescu 1970) walls of the coves (Lörenti, 1966) and soil Lucia Turcu, 1974,1975).

We mention it from the soil of greenhouses cultivated with tomatoes.

5. *Alternaria alternata* (Fr.) Keissler, Beih. Bot. Zbl., 29, p. 434 (1912); Ellis, Dematiaceous Hyphomycetes, p. 465, fig. 330 (1971).

Micromyceta develops colonies brown-black, covered by conidiophores which are short, brown septate. The conidiophores appear in chains, they are appearance of blackberry, brown-black, very varied as dimension and has 30-36 x 15 µm.

In literature is pointed on plants and leftovers of the plants in the soil.

6. *Verticillium lateritium* Berkeley, Cooke, Brit. Fgi., p. 635 (1871); Gilman, A Manual of Soil Fungi, p. 304 (1959).

Micromyceta develops on Czapek medium agar circular colonies, hemispheric, of red-brown at maturity but delimited by a white zone. The conidiophores are very fine, with rare branches that sustain long filaments of 7,5 x 2 µm which broadcast many conidiophores ovate, of 2-3 x 2 µm, smooth.

The micromyceta is found by Negru Al. on different vegetables cultivated (1966, 1972), by C. Sandu-Ville şi colab. on dahlia and by researcher from Cluj which has been determined the fungus of soil, Lörinczi (1970) and Lucia Turcu (1974, 1975). We point this fungus from the soil the greenhouse cultivated with tomatoes.

7. *Rizophus nigricans* Ehrenb., Nova Acta Acad. Leop. X, p. 198 (1820); Sacc., Syll. Fung. VII, p. 212 (1888);

Micromyceta develops very quickly a colony made of hyaline hyphae, very fine on which appear hyaline sporangioforous that sustain the sporangial heads of black colour. The sporangioforous that are 1-1,5 cm appear by two and have a diameter of 25-40 µm. The columella has 70 x 90 µm and sustains the round or oval spores of 10 x 7,5 µm.

The fungus is cosmopolitan and we find it very often on different layers from the soil.

8. *Ceratocystis paradoxa* (Dade) C. Moreau, Rev., Mycol., XVII, p. 22 (1952); Ellis, Dematiaceous Hyphomycetes, p. 31, fig. 5A (1971).

Micelian colony is brown at the maturity become black. The conidiophores are short of 50 x 5 µm. On the micelian hyphae appeared the arthroconidiophores are light brown, with double walls, longer than wider of 12-17,5 x 5-6 µm. The conidial stadium belongs to Thielaviopsis.

The fungus is quoted in the bibliography from the part of dead plants cultivated in the tropical area. In our country is quoted only one species *Ceratocystis longilostellata* from the roots of the oak. In the soil of the greenhouses this year have been registered high temperatures which favored the appearance of micromycete.

9. *Humicola grisea* Traaen, Nyt. Mag. Naturvid., 52, p. 31 (1914); Gilman, A Manual of Soil Fungi, p. 60, fig. 29B (1971).

The fungus forms at the surface of the medium hyphae septate, hyaline of 4 µm in diameter. The micelian colony covers a mass of yellow-brown conidiophores. The conidiophores have short side branches that sustain the conidiophores with double walls and a granular surface of 9 - 15 µm.

Micromyceta has been quoted before from soil by Viorica Iacob din sol de at Podu Iloaiei - Iași, in 1973 and by Ioachimescu Dinulescu Mariana from rotten wood in mines in 1978.

10. *Paecilomyces marquandii* (Masse) Hughes, Studies in microfungi, Mxcol. Pap. 38,1-8 (1951); Domsch K., Gams W., Pilze aus Agrar, p. 93 (1970).

In order to determine micromyceta, Petri vassels have been refrigerated. After 3 monts the colonies of *Penicillium rugulosum* have been covered by a micelium of beije colour that belongs to this micromycete. Differently from the fructifies of the penicillium or spicaria, the appearance of branches and fialides is irregular. Singular fialides or grouped eliminate conidioues which are spheric of 2 -2,5 μm . Micromyceta is new for România.

We attach the photos and the microscopic lamellas have been included in micologic Herbal of Moldavia „C. Sandu-Ville”.

CONCLUSIONS

1.The study of micromycetas from soil is very useful taking into account the fact that follows the plantation of rasads which at first phases of development , are extremely sensible.

2.The presence of parasitic micromycetas has an economic implication because it can compromise the culture and so a new disinfection of the soil is needed and of course another replantation which modifiens the period of development of plants, delayning the valorification of obtained production.

3.The analyses of the tests from soil harvested in June 2007 proves the fact that the presence of high temperatures and of more developed plants made that the spectrum of micromycetas from soil to modify both in quantily and of variability of isolated species. It is noticed the presence of new species for România or qnoted for the first time in the soil cultivated with tomatoes like: *Cladosporium lignicolum*, *Paecilomyces marquandii*, *Ceratocystis paradoxa*, *Verticillium lateritium*, *Scopulariopsis brevicaulis*, *Penicillium ochraceum*.

4.Out of the sum of izolated species, 16,2% represent micromycetes new for our country or qnoted for the first time in soil.

REFERENCES

1. Bontea Vera, 1985 - *Ciuperci parazite și saprofite din România*, Ed. Acad. R.S.R.
2. Constantinescu O., 1974 - *Metode și tehnici în Micologie*, Ed. CERES, București.
3. Domsch K., Gams W., 1970, - *Pilze aus Agrarböden*, VEB-Gustav Fischer Jena
4. Ellis M.B., 1971 - *Dematiaceous Hyphomycetes*, England.
5. Gilman J.C., 1957 - *A Manual of Soil Fungi*, Iowa , U.S.A.
6. Lindau G. 1910 - *Fungi Imperfecti*, Leipzig, Germania
7. Migula W., 1913 - *Kryptogamen Flora*, Germania
8. Raper K., C. Thom and Dor. Fennell, 1968 – *A Manual of the Penicillia*, London.
9. Raper K., Doprothy Fennell, 1965 - *The genus Aspergillus*, Baltimore.