# ANTIFUNGAL INDICES OF SOME STRAINSOF MICROMYCETES AGAINST MYCOTIC INFECTIONS OF *APIS MELIFERA*

**N. STARCIUC, VERONICA BUGNEAC**

State Agrarian University of Moldova, Faculty of Veterinary Medicine, MD 2049, str. Mircesti 44, Chisinau, Republic of Moldova
E-mail: n.starciuc@uasm.md

**Summary**

The propose of study had served 21 strains of micromycetes from the National Collection of Non-pathogenic Microorganisms from Institute of Microbiology, Academy of science of Moldova and two pathogenic strains – *Aspergillus flavus* and *Aspergillus niger*. The tested strains were isolated from soil samples taken from the central zone of Moldova. As nutrient medium for isolation, of micromycetes served malt agar, Czapek, Sabouraud-Amidono and ammonium. Isolation of strains was performed in thermostat at temperature 28$^{o}$C, during 14 days. As a result of screening were selected two strains SP.62 and SP. 97 with potential antifungal increased against *Aspergillus flavus* pathogen with higher antifungal actions against petrified offspring of bees. The diameter of inhibition zone *of Aspergillus flavus* was on the strain SP. 62 - 30 mm, and of the strain p. SP. 97 - 35 mm.

**Key words** starins, micograma, însemination, screening, micromycetes.

Micromycetes are microscopic fungus. They are widespread in environment, live in air, water, soil, in habitation of people, etc. Separately each fungus is invisible with the naked eye, but on surfaces we can observe them in the form of a mold – spots or raids with a certain smell and color. These are the same microorganisms, as well as bacteria, they only differ in more difficult structure (1,4).

There are different classifications of micromycetes. Some scientists refer to them yeast, which are widely used in the food industry, others consider them a separate type of microorganisms. The famous representative of micromycetes is mold fungus – penicillium. Thus, many types of microscopic fungus are widely used by the person in the food industry, economy and medicine. But there are also other micromycetes which do a lot of harm to environment and with the person tries to fight in every possible way.

According to many authors filamentous fungi are very common ones in nature, with a major concentration in soil, especially in the top layer of the soil which ensures conditions for growth and survival. Fungal spores are found frequently in surface of plants, in the digestive tract, especially the herbivores in the food industry bio decomposition activity is unwelcome because the fungal causing loss of seed foods.

As a side effect is the formation of mycotoxins by some molds that foods become unusable. The molds are the aerobic microorganisms therefore require the presence of oxygen for growth in air or dissolved oxygen in liquid medium. Molds

processes are possible if there is empty and are produced by species of the genus *Penicillium, Aspergillus, Phyalophora*, etc. Molds can be formed on the surface of the juice and may release coloring substances or degraded natural pigments the juice or other natural products. *Micromycetes* supports the great amounts of tanning substances, often associated with woody vegetation, realizing their debris degradation. For this reason, bring their mushrooms in the formation of organic soils of the party at the same time, having a special role within trophic chains ( 3).

Currently *Micromycetes* are widely used in producing of biologically active substances used in the production of additives for stimulating the immune system, and some of them as products with antibacterial effect. The importance of pharmaceutical products obtained with the help of microorganisms can mention: antibiotics, amino acids and enzymes with therapeutic role, hormones, vaccines, interferon, interleukins, bacteriocine, alkaloids, and vitamins. Of substances derived from filamentous fungal are vitamin B and ergosterol-provitamine D.

Taking into account the above mentioned the purpose of study served 21 strains of *Micromycetes* from the National Collection of Non-pathogenic Microorganisms from Institute of Microbiology, Republic of Moldova. The objectives of the investigations was a screening of some strains of micromycetes from different regions of the soil with antifungal action against pathogenic fungals which causes mycotic diseases in bees; appreciation of inhibition properties of some fungal strains producers of pathogenic strains toward 2-*Aspergillus flavus, Aspergillus niger*; establishing the possibility of use of fungal strains with antifungal action in some diseases in honey bees (2, 5).

## Materials and methods

The study materials served 21 strains of *Micromycetes* from the National Collection of Nonpathogenic Microorganisms. Tested strains were isolated from soil samples taken from the central zone of Moldova. As a test crop were used 2 strains of fungi: *Aspergillus flavus* and *Aspergillus niger*, pathogens of Asspergilosis (petrified offspring) to bees, which were isolated from samples taken from a hive of bees. Antimicrobial properties of micromycetes have been studied according to the diffusion method by using Agar blocks (2, 3). The method is based on the ability of diffusion of metabolites produced microorganisms studied in depth of agar and of the action of the active substance diffusion area on the test-crop. To perform the experiment of fungal strains to be tested were grown for 4 days on malt agar medium at a temperature of 28-30°C. The diameter of the zone of inhibition of infection agent was measured after 4 days of cultivation at a temperature of 28-30 °C on the malt-agar strains pathogenic on Petri plates in volume were introduced with agar blocks of tested micromycetes. Morpho-cultural study of fungal strains with antifungal activity increased potential has been conducted examining the visual and microscopic strains grown on four nutrient medium: Malt agar; Czapek;

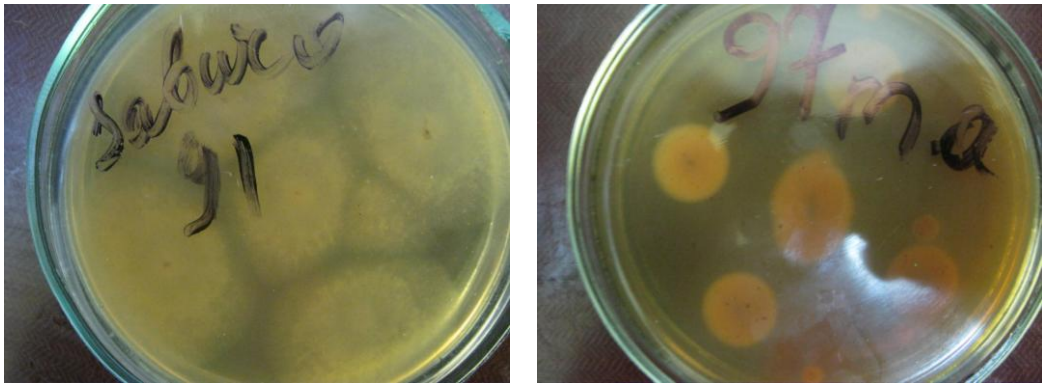
Sabouraud - amidono and ammonium (4). The investigations were carried out after 4; 7 and 14 days of cultivation at temperature 28-30 ° C (5).

## Results and discussions

This study was conducted in the laboratory of non-pathogenic micro-organisms of Academy of science of Moldova. In the images below are presented the stages of research (fig. 1 a, b).

a) b)

Fig.1 a) Collection of the pathological material from bee family, b) pleasing of the blokes of micromycetes on environmental mediums

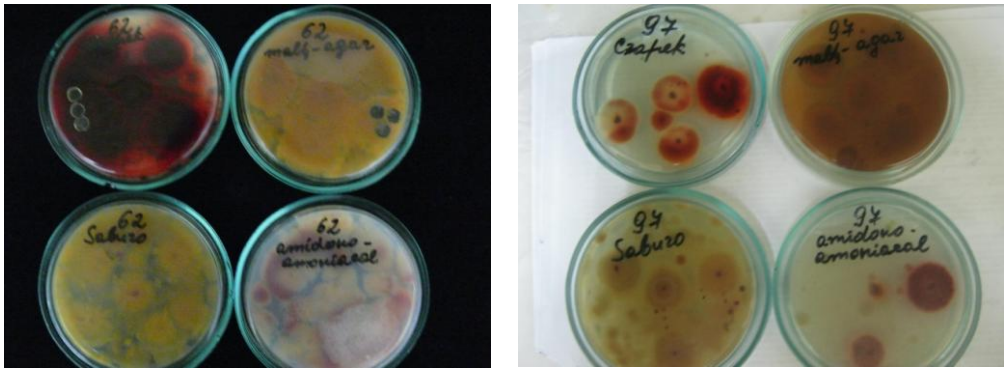


a) b)

Fig. 2 a) Fungal colonies on Saburo environment;
b) fungal colonies on malt agar environment

The pathologic material for laboratory investigation was collected from bee family of the pandan of the Academy of Sciences of Moldova (fig.1, a) and sended in laboratory for investigation (fig. 1, b). For isolation of micromyceters was used the malt agar; Czapek; Sabouraud-amidono and ammonium nutrient mediums.

The diameter of fungal colony (fig.2 a) has -1.4 – 1.5 cm, with green-dark color and irregular shape, wavy profile, with a white border up to 3 cm. In the situation of inoculation on the malt agart, the diameter of the colony was 2.4 – 2.5 cm, with dark green color, very smooth and with round or free-form mycelium and flat profile, greenish pink toward to the center.



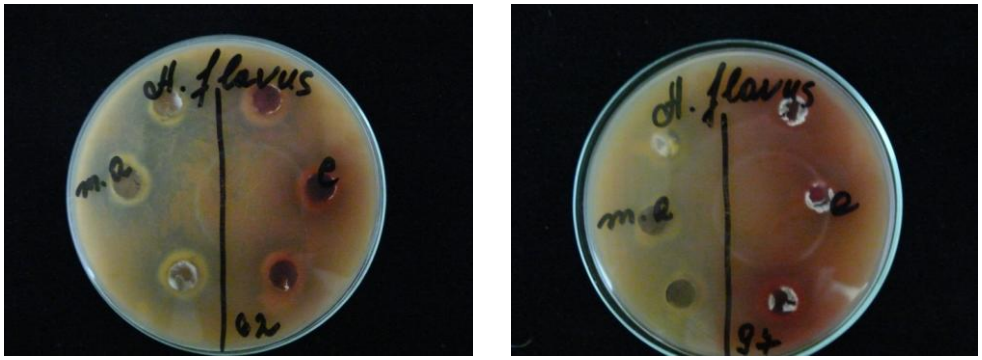

a) b)

Fig. 3. Colony appearance of p. SP. 62 and p. SP 97 after 14 days of cultivation in different environments: a) p. SP. 62; b) p. SP 97

Diameter size of the colony was 2.5 – 3.0 cm, with dark – green color, very sporulated placement, with round or oval shape. On rivers side, more intense color is pink. The morphological structure of colonies are presented with fluffy colonies that differ by type of nutrient medium and period if incubation.

The strain p.sp.62 is growing and developing very well on all four tested cultivation medium. For this strain, malt-agar and Czapek environments are more favorable for cultivation as it grows, with faster sporulation. On these cultivatiing media the maximum diameter of the colony was 3.5 – 4.0 cm, as well as an increased sporulation. Taking into account that the malt-agar and Czapek media are more conducive to growth these experiments were carried out in order to determine the antifungal activity of the strain grown on these culture media. The strain p. SP 97 colony diameter was - 2,3 -2,5 cm, with dark green, rough, irregular shape, flat profile, wavy edge. Border yellow - red with diameter 1cm.

As shown in fig. 3 a) and b) it can confirm that, in order to obtain exzomethabolitics proprieties to combat the pathogen *Aspergillus flavus*, antifungal strains p.sp. 62 and p.sp. 97 have grown better on malt-agar medium.




a) b)

Fig. 4. Areas of inhibition zones to the pathogen *Aspergillus flavus* of the action of exomethabolits of micromycetes: a) - p.sp.62 and b) - p.sp.97.

In fig. 4 a) and b) there are the results on the inhibition of the growth of colonies of pathogen *Aspergillus flavus* development under the action of blocks of micromycetes. The diameter of the blocks of culture of micromycetes had the varies of inhibition zones of micromycete strain p. SP 62 up 25.8 to 4.2 cm, and of the micromycete strain p. SP 97 up 22, 0 to 3,2cm .

## Conclusions

Following the screening performed in 21 fungal strains were selected two cultures of micromicetes (p.sp.62 and p.sp. 97) which demonstrated the increasing antifungal potential on Aspergillus flavus, pathogens of aspergillosis (petrified brood) in bee.

Diameter of inhibition area on pathogen *Aspergillus flavus* under the action of micromycete exometabolits of strain p.SP. 62 was around 30 mm and of strain p. SP.97 – 35 mm.

The optimal cultivating media for growing strains p.SP.62 and p. SP. 97 in order to obtain enhance exometabolits with antifungal properties is malt-agar medium.

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