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The materials on citrus mycobiota research

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A B S T R A C T

In this paper the results on citrus fruit mycobiota research are presented. The following citrus varieties are widely spread in the humid subtropical climate zone of Western Georgia, on the Black Sea coast: lemon, mandarin, orange and grapefruit. The favorable climatic conditions for fruit development promote the development of various fungal diseases as well, including the diseases of the fruit. The aim of the present research was to study the mycobiota of citrus fruits and to determine the causal agent of disease – the pathogenic fungi. For the identification of fruit mycobiota the isolation of fungi in pure culture from the lesion fruit were performed in the test-tubes and onto the Petri dishes. The identified by us 15 species of fungi include the following: *Rhizopus nigricans* E., *Fusarium lateritium* Nees, *F. limonii* Br., *Penicillium italicum* Wehmer, *P. Digitatum* Sacc., *Aspergillus niger van. Tiegh., Trichoderma lignorum* Tode, *Botrytis cinerea* Pers., *Alternaria citri* Pierce, *Colletotrichum gloeosporioides* Penz., *Sphaceloma fawcettii* Jenk. *(Elsinoe fawcettii* Bitancourt &Jenk.*)*, *Phoma citricarpa* Mc. Alpine, *Ph. limonis* Thum and *Phomopsis citri* Faw. The Fungus *Aternaria citri* differs from above listed by the frequency of occurrence. Their diagnosis are provided below.

Keywords: *Citrus fruit, Fungal diseases, Mycobiota, Morphology, Identification. Diagnosis.*

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Introduction

Citriculture is one of the most important, leading commercial industries of agriculture of Georgia. Citrus plants belong to the subtropical fruit tree cultures. Mandarin ranks the first in Georgia by the spreading and producing products, lemon – the second and orange – the third. Citrus fruits are distinguished with nutritional value, dietary, medicinal properties and palatable taste. They contain a large number of different vitamins (C, D, B, PP) carotene (provitamin A), sugars, organic acids, pectin, mineral salts. Citrus fruits are used as raw and as well as processed in the form of fruit jam, candied fruit, marmalade, soft drinks, compote, and they are widely used as well as in confectionery, pharmacology, perfumery (fruit skin, leaves, flowers) and culinary (for production of essences, essential oils, pectin).

The favorable climatic conditions for the development of citruses on the Black Sea coast (humid climate of Western Georgia, periodic frosty winters) promote the development of diseases, which should explain the abundance of mycobiota's representatives in the Black Sea coastline to a number of agricultural crops, including citrus plants.

Numerous researches were conducted on mycobiota of citrus fruit crops in Georgia. N. Voronikhin detected 26 species of fungi of citrus fruits even in 1937 [1].

P. Nagorni with co-authors has conducted a thorough study of citrus mycobiota. They have identified 38 species of fungi, which are spread on citrus branches, leaves and fruit [2].

L. Tsereteli and N. Chanturia have noted 30 species of fungi on citrus fruits [3].

K. Gikashvili has revealed the following genus of fungi on citrus fruits *Phytophthora, Sphaceloma, Colletotrichum* and *Botrytis* [4].

L. Beradze with the co-authors has mentioned the following types of fungi on citrus fruit: *Elsinoe fawcettii, Glomerella cinguata, Botryotinia fuckeliana, Phytophthora citrophthora* and *Alternaria citri* [5].

Foreign researchers have identified different types of fungi on citrus fruits: *Colletotrichum gloeosporioides, Penicillium italicum, P. digitatum,*

Aternaria citri, Mycosphaerella citri, Diaporthe citri, Elsinoe fawcettii, Elsinoe australis, Septoria sp., Aspergillus sp., Phytophthora palmivora, Sclerotinia sclerotiorum, Botryris cinerea, Botryosphaeria rhodina, Trichoderma sp. and etc. [6, 7].

Objectives and Methods

The citrus fruits (lemon, mandarin, orange), lesion by fungal diseases represented the research object.

The aim of the present research was to study the mycobiota of citrus fruits and to determine the causal agent of disease – the pathogenic fungi. Laboratory investigations were conducted in the LEPL Laboratory of the Ministry of Agriculture of Georgia.

For the identification of fruit mycobiota the isolation of fungi in pure culture from the lesion fruit were performed in the test-tubes and onto the Petri dishes. The Wort Agar was used as or the growth and development of fungi. The cultivation of fungi occured in an incubator at 20-25^0 C temperature. The microscopic analyses of isolated cultures was performed every 3-5 days, prior to the development of fruiting bodies of fungi. For the identification of fungi were used the corresponding Guides of Fungi [8-14].

Results and Discussion

As a result of our studies, a total of 15 species of fungi is identified from the lesion citrus fruits, belonging to the following classes - *Zygomycetes, Ascomycetes and Deuteromycetes* and orders - *Mucorales* or molds, *Dothydiales, Hyphales*, *Melaconiales* and *Sphaeropsidales*. Their diagnoses are given below.

Rhizopus nigricans E.

Mandarin peel with watery spots, covered with white plaque, were placed in the Petri dishes. This white plaque later turned dark gray. The colony is fast-growing, with loose-felty texture, olive-brown-gray in color, hyphaes and stolons have developed, which are differentiated as rhizoids and stylosporangiophores. Rhizoids are branched and dark brown in color. Stylosporangiophores deployed in stolon's nodes in groups in number of 2-5 are almost black; stylosporangiophores within the size of 500-3000×10-35 μm, stylosporangium 80-150 μm in diameter, columella 50-120 μm in diameter. The sporangiospore (Fig.1) ellipsoidal-spherical shaped, 4-12×4-10 μm in diameter [8].

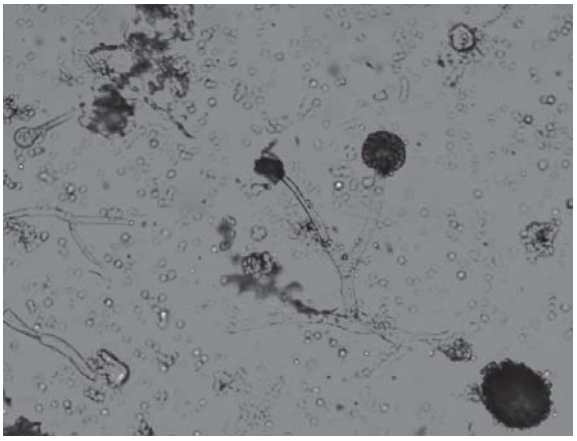

Fig. 1. *Sporangium and sporangiospores of Rhizopus nigricans E. (Mag. X20)*

Fusarium lateritium Nees.

Following inoculation of lesion mandarin fruit peel on the Wort Agar growth media, the tall, aerial, reddish-pink multicellular mycelium was developed, divided by septa. Macroconidia - sickle-shaped, colorless, multicellular, number of septa 2-5; spores (Fig.2) are within the size of 30-45.6×3.8-5.7 μm [9].

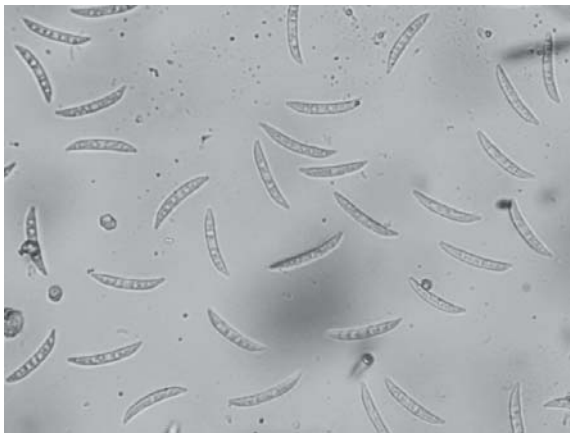

Fig. 2. *Spores of Fusarium lateritium Nees. (Mag. X40)*

Fusarium limoii Br.

Following inoculation of lesion mandarin and lemon fruits in tube on Wort Agar the low, cotton-like, whitish-pinkish multicellular mycelium has developed, spores sickle-shaped, colorless, multicellular, number of septa 1-3; spores are within the size of 12.5-30.4×3.4-4.9 μm [9].

Penicillium italicum Wehmer.

The white mycelium was developed on mandarin fruit peel following incubation on a wet-chamber, afterwards on which the blue, loose plaque appeared - the fruiting bodies of fungi. The fruit tissue is softened, watery. The microscopic analysis has shown that, mycelium is branched, colorless, with well-defined septa; conidiophores are of the various forms and standing in upright position, at the end of which one or two tiers of sterigmata are developed, on which the chain of spores are (Fig.3) created. The spores are colorless, single-celled, and roundish, within the size of 4.2-5.5×2.5-3 μm [10].

Fig. 3. *Conidiophores and spores of Penicillium italicum Wehmer (Mag. X40)*

Penicillium digitatum Sacc.

The white mycelium was developed on mandarin fruit peel following incubation in a wet- chamber, afterwards the dark yellowish-green color plaque appeared on warty spots, which is edged with the wide, white lace. Conidiophores are branched, at the tip of the branches sterigmata are observed located in ring. Conidia of an ovoid shape is seen, some of them cylindrical, single celled, colorless, within the size of 6.2-8×4.5-5 μm [10].

Aspergillus niger van.Tiegh.

The softened, mandarin and lemon peels, with watery spots, on which at first white and afterwards black loose plaque developed, were placed on Potato-Dextrose Agar (PDA) in the Petri dishes. After 5 days of incubation at 25^0 C the colony growth till 43-60 mm was observed. The mycelium surface was velvety and smooth, on that conidiophores have developed within the size of 200-400×7-10 μm. They are prolonged, divided by septa, clavate, with spherical thickened section part on the tip 20-50 μm in diameter, covered with radially constructed pro phialide of 6-100 μm long and phialide of 20-30 μm long. On phialide one after another single celled brown conidia (Fig.4) developed in chains 3-5 μm in diameter. The taller conidia is of the chain, the bigger is it in size, intensely colored and matured [7].

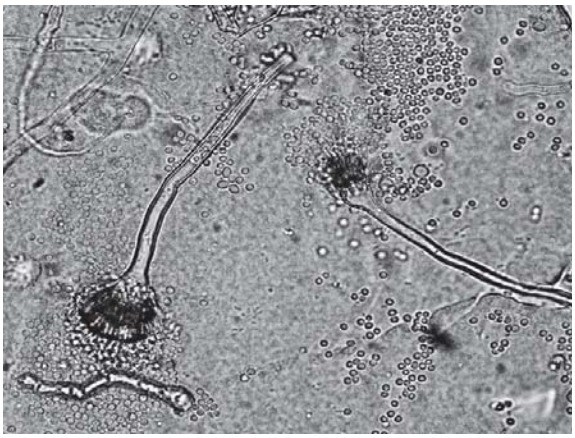

Fig. 4. *Conidia of Aspergillus niger van. Tiegh. (Mag. X20)*

Trichoderma lignorum Tode

Following inoculation of affected mandarin fruit peel spots in tube on the Wort Agar, the dark yellowish-green color mycelium was developed, that is low, felt like, wavy, multicellular; the conidiophores (Fig.5) are highly branched, at the tips of which the heads appeared, created from conidiospores. The conidium are spherical, dark yellow-green color, single celled, within the size of 3.7×4.5 μm [10].

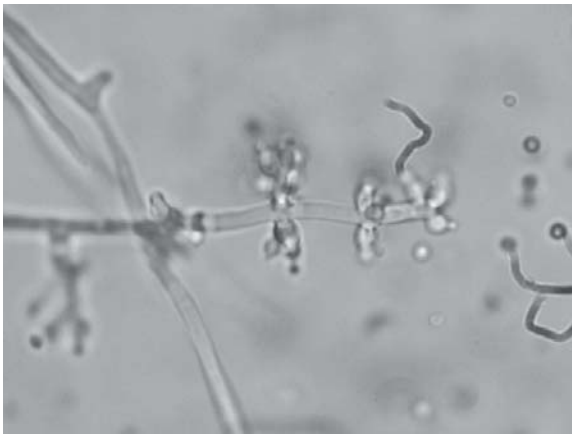

Fig. 5. *Conidiophores and phialides of Trichoderma lignorum Tode (Mag. X40)*

Botrytis cinerea Pers.

Mandarin and lemon peels, with the red-brown lesions, were placed in the Petri dishes. Following some days greyish mycelium appeared on spots with the well developed dense, felty texture. Conidiophores (Fig.6) are branched tree-like, in upright position, on their top rows the conidia are developed in groups of 8-10 pieces. The spores are ellipsoidal-spherical, colorless, 4-11×6-18 μm in diameter [7].

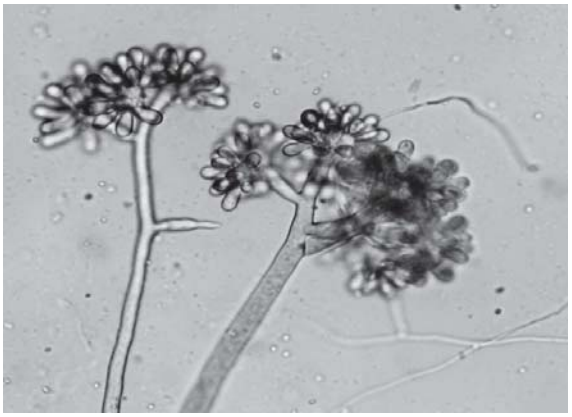

Fig. 6. *Conidiophores and spores of Botrytis cinerea Pers. (Mag. X40)*

Alternaria citri Pierce

Following inoculation of lesion mandarin and lemon fruits on the Wort Agar the dark grayish-blackish, low mycelium developed. The fruiting bodies of fungi are developed on the tube walls in the form of the black spots. Conidiophores are brown, short; spores (Fig.7) prolonged, bottle-shaped, brown, with transverse and longitudinal septa. The number of longitudinal septa is 1-3, transverse - 2-6, spores are within the size of 15.2-49.4×11.4-12 μm [11].

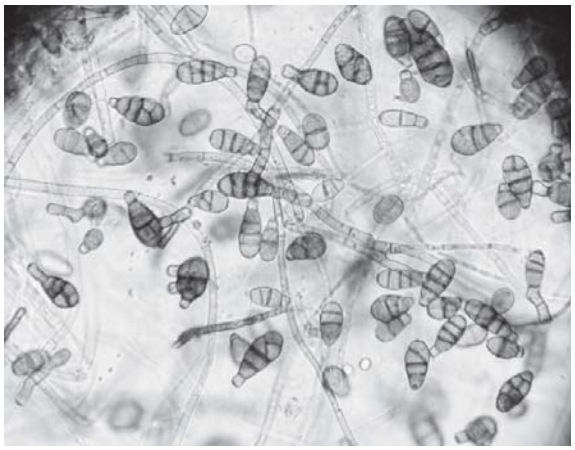

Fig. 7. *Spores of Alternaria citri Pierce (Mag. X40)*

Colletotrichum gloeosporioides Penz.

By the two weeks of incubation in a wet-chamber the mandarin fruit became covered with whitish mycelium. The warty spots of orange color (the fruiting bodies of fungi - acervuli) developed on the peel. Spores (Fig.8) are single celled, ellipsoidal, colorless, within the size of 10.2-20×5-6 μm [12].

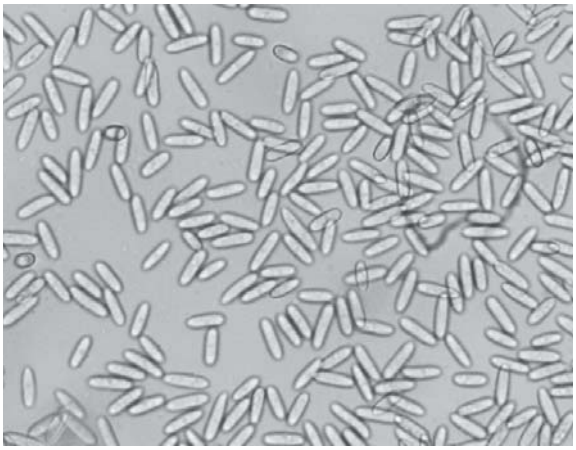

Fig. 8. *Spores of Colletotrichum gloeosporioides Penz. (Mag. X40)*

Sphaceloma fawcettii Jenk. (anamorph); *Elsinoe fawcettii* Bitancourt &Jenk. (telemorph)

Mandarin and lemon fruits peels, with watery spots, were placed on agar medium in the Petri dishes. Colony is slow growing, from pink to violet. Ascomata is pad-like, ellipsoidal, dark colored, pseudoparenchimal, multichambered, thickness - up to 80-120 μm, up to 20 asci in each chamber. An ascus spherical in shape or egg shaped, 12-16 μm, is enclosed in a double wall, inner wall is thickened to the tip, with 8 ascospores, which are transparent, ellipsoidal or prolonged-ellipsoidal, from two to four cells. Typically, is stretched in the mid region of septa, 10-12×5-6 μm.

The acervuli inner epidermal or sub-epidermal (half epidermal), pseudoparenchimal. The conidiophores cells are generated from the upper pseudoparenchimal cells or pale brown phialide conidiophores, which consist of 2-4 septa, up to 12-22×3-4 μm in size. The mycelium is colorless, poorly developed, septated, short branched. Spores are colorless, single celled, ellipsoidal, bulging, 5-7×2-5 μm [7; 13].

Phoma limonis Thum.

For the 10th days of incubation in a wet-chamber, the white, aerial mycelium was developed, which entirely covered the fruit. The dark black spots are developed on peel – pycnidia, that represent the fruiting bodies of fungi. Pycnidia are 70-210 μm in diameter. Spores are colorless, single celled, round, within the size of 1.9-3.8X1.5-3 μm [12].

Phoma citricarpa Mc. Alpine

The various black spots were developed on the peel of lemon - pycnidia, which represent the fruiting bodies of fungi. The pycnidia (Fig.9) are dark brown, rounded, with well defined porus, with 70-250 μm in diameter. The spores are single celled, colorless, ovoid, within the size of 7.5-13×5-7 μm [14].

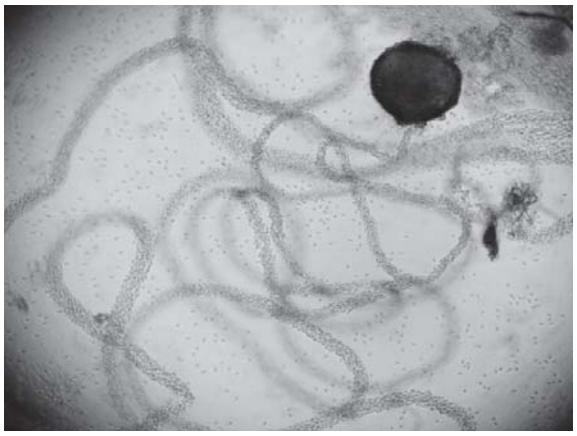

Fig. 9. *Pycnidia and spores of Phoma citricarpa Mc. Alpine (Mag. X20)*

Phomopsis citri Faw.

Following inoculation of lesion mandarin fruit on the Wort Agar the low, white, cotton like, multicellular mycelium developed. The fruiting bodies of fungi - pycnidia are ovoid, with well defined porus, 100-350 μm in diameter. The pycniospores (α conidia) are spindle-shaped, colorless, single celled, within the size of 3.7-11.4×2.6-3.8 μm (Fig.10). Stylospores (β conidia) are thread-like, hook-shaped, colorless, within the size of 18-25 μm [15].

Conclusion

The diseases of citrus fruit (lemon, mandarin, orange) are widespread in the humid subtropical climate zone of Western Georgia, on the Black Sea coast and are characterized by a great harmfulness.

15 species of fungi are identified from the citrus fruit, affected by fungal disease, which belong to the class of *Zygomycetes, Ascomycetes* and *Deuteromycetes*; *Mucorales* or to the order of molds, *Dothydiales, Hyphales*, *Melaconiales* and *Sphaeropsidales*: *Rhizopus nigricans* E., *Fusarium lateritium* Nees, *F. limonii* Br., *Penicillium italicum* Wehmer., *P. digitatum* Sacc., *Aspergillus niger van. Tiegh., Trichoderma lignorum* Tode, *Botrytis cinerea* Pers., *Alternaria citri* Pierce, *Colletotrichum gloeosporioides* Penz., *Sphaceloma fawcettii* Jenk. *(Elsinoe fawcettii* Bitancourt &Jenk.*)*, *Phoma citricarpa* Mc. Alpine, *Ph. limonis* Thum and *Phomopsis citri* Faw. From the above stated, the fungus *Alternaria citri* Pierce differs by the frequency of occurrence.

Fig. 10. *Spores (α and β) of Phomopsis citri Faw. (Mag. X40)*

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