Asian Journal of Research in Botany

2(1): 1-13, 2019; Article no.AJRIB.47924

The Maloideae (*Rosaceae*) Structural and Functional Features Determining Passive Immunity to Mycosis

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Authors' contributions

This work was carried out in collaboration among all authors. Author TKK designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ASV performed the statistical analysis and managed the analyses of the study. Author OOB managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
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Complete Peer review History: http://www.sdiarticle3.com/review-history/47924

Original Research Article

Received 03 January 2019
Accepted 20 March 2019
Published 26 March 2019

ABSTRACT

The defeat of the fruits of fungal diseases is currently an important issue of plant science and is also of great economic importance. With the help of microscopic methods the leaves and fruits surface tissues of plants of four genera of the *Maloideae* subfamily were screened: *Malus* Mill., *Pyrus* L., *Cydonia* Mill., *Mespilus* L. and attempts were made to explain the dependence of mycosis damage on microstructural features. The species composition of fungi that cause damage to the *Maloideae* leaves and fruits in the Russia southern regions is analyzed. It is established that among pathogens with different types of parasitism there are common excitants, as well as highly specialized responses as on *Mespilus germanica*. Higher resistance to the complex of fungal diseases, in comparison with apple and pear, was found in quince and medlar. This stability at the initial stage of the pathological process is associated with structural features such as micromorphology of the fruits and stomata cuticle in the abaxial epidermis of leaves. The leaves stomatal openings of medlar are narrow with raised outgrowths, on the surface of the fruit – the layered structure of the cuticular

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layer. Quince has a continuous cuticular cover. Compared with *Malus* and *Pyrus*, *Cydonia* and *Mespilus* also have a large (30 % or more) polyphenol content in the pericarp of outer layer cells. In addition to the general specific differences in the microstructure of the integumentary tissues and the content of polyphenols affecting the resistance to pathogens at the stage of their penetration, general patterns of leaf surface formation, such as hypostomacy, anomocytic stomata, folded microrelief of the cuticular surface, and the presence of single and multicellular trichomes are noted. Epidermal cells from the 4 genera examined contained most of the condensed polyphenols compared to the hypodermal cells.

Keywords: Maloideae; pathogenic fungi; resistance; ultrastructure; polyphenols.

1. INTRODUCTION

Maloideae, or *Pomoideae* is one of the subfamilies of *Rosaceae*, which includes the most ancient cultivated representatives of angiosperms. *Rosaceae* are widespread in many ecological and geographical zones andinclude such famous fruit plants as pear (*Pyrus* L.), apple (*Malus* Mill.), quince (*Cydonia* Mill.), medlar (*Mespilus*L.) and others. Their main feature is the fruit, overgrown with a modified hypanthium – prefabricated leaflets or drupaceous modified carpels, called in a broad sense ‘apples’ [1,2]. The large (juicy) part of the fruit is formed due to the succulentization of the tissues of the pericarp. A fruit is typical for apple, pear and quince, and the drupaceous apple is for the medlar germanica (*Mespilus germanica*).

According to practical importance in world production, including in Russia, the apple tree takes the leading place among fruit crops, the second is the pear tree. The enormous consumer value of the fruits of these crops is widely known, as they are extensively used in the food, medical, perfume and other industries. Along with vitamin-bearing qualities, the special value of apple and pear fruits is due to good antioxidant properties [2]. Despite the huge number of varieties currently available, there is a need to improve the assortment, since during the long selection process many of the valuable traits that their wild relatives were lost.

Among the promising cultivated plants is common quince (*Cydonia oblonga* Mill.). It is quite flexible to the conditions of growth, especially soil, as it can grow successfully on different types of soils (alluvial, chernozemic, etc.). Also, quince has a high resistance to various stressors: it is light-demanding, heat-resistant, salt-tolerant, fast-growing, durable and fruitful. *C. oblonga* fruits are distinguished by high quality, being, rich in vitamins, organic acids, microelements, pectin and other substances. Some researchers note that quince, to a lesser extent than other fruit crops from the subfamily *Maloideae*is damaged by pests and diseases [3]. Despite the fact that quince is a promising fruit crop in its characteristics, especially for the southern regions of Russia, until now it has not received due attention, and industrial plantations do not exist yet.

To the group of promising fruit plants of the fruit (*Maloideae*) it is possible to include a medlar germanica (*Mespilus germanica*), which is represented by two ecotypes: xerophilous, confined to open habitats, and mesophilic, that grows in forests [1,4]. In Russia, it is widespread in the mountain ecosystems of the southern regions, especially in the North Caucasus – from the lower zone to the upper limits of arboreal vegetation (300–2000 m above sea level), as well as in the Crimea and adjacent territories. However, there are no industrial plantations. The value of the medlar fruit is known since ancient times. As a vitamin and medicinal plant, it entered the culture of many countries of the Near and Middle Asia, the Eastern Mediterranean and Western Europe. Many biologically active substances have been found in the vegetative and reproductive organs of *M. germanica*, in particular, triterpenoids, tannins, phenolic carboxylic acids, flavonoids, catechins, organic acids and their derivatives, aliphatic aldehydes, higher fatty acids, significant amounts of fatty oil in seeds [5,6].

The development of the *Maloideae* of various habitats and the increased adaptability of representatives are based primarily on the development of structural and physiological-biochemical features that also play an important role in resistance to phytopathogenic organisms. For research in this respect, the representatives of the above four genera were taken as model objects.

Anatomical and morphological features of passive immunity, or horizontal stability, include the specificity of the deposition of wax deposits and the thickness of the cuticle of integumentary tissues, the structure of stomata, and a number of other features [7]. Information on the structure of superficial tissues (epidermis and hypoderma) accumulated by some *Maloideae* representatives for the vegetative and reproductive organs, as well as for their mycobiota, is fragmentary [8,9,10]. There is insufficient information on the polyfunctionality of the epidermal tissue, the specificity of the microrelief of the leaf epidermis (the formation of cuticular folds, microtens in the stomata and trichomes, peristomatic rings, cork tissue and lentils on the fruits). Their functional significance is not entirely clear to this day. In the literature there are disagreements and assumptions about the mechanism of opening and closing stomata, and also the interaction of the stomata with the surface structures of the leaf [7,10]. The role of the ultrastructure of the cuticle of leaves and fruits in passive immunity for many pathogens also remains controversial, and direct dependence on the type of structure and overall thickness is not revealed.

The chemical factors of passive immunity include, first, the presence or absence in the plants of substances necessary for the life of the pathogen, and secondly, the presence of substances that inhibit pathogen growth (phytoncides, or phytoantipypsins). The leading role in the formation of the latter belongs to the synthesis and dynamics of various phenolic compounds, ranging from relatively simple phenols to complex polyphenolic composites that are toxic to many phytopathogenic fungi [11]. According to modern concepts of the significance of the features of the structure of the surface and the subcellular structures of plants in protection from phytopathogens, the synthesis and dynamics of changes in phytohormones[12] plays a major role. At the same time, the character and distribution of lipophilic components, the specificity of the formation and localization of polyphenolics and terpenoids, as well as playing important protective functions against the attack of fungal pathogens in the surface tissues have not been adequately studied. It is shown that the fitontsidyeffect of plants on specialized parasites is generally poorly expressed. In most cases, it affects microorganisms that do not affect this plant. Phytoncidal activity is not the same, it varies depending on the grade and age of the plant, as well as on the time of day, the phase of development, weather conditions and other factors.

Thus, despite the availability of separate information from biology and disease affection of the most valuable fruit representatives of the Apple Subfamily, no attempts have yet been made to fully study their mechanisms of resistance.

In connection with the above, the purpose of this work was to conduct a comprehensive search of micromorphological, anatomical and histochemical, and also ultrastructural and physiological-biochemical features of plants of 4 genera of the Apple Subfamily, and an evaluation of these indicators as markers of passive immunity to phytopathogenic fungi.

2. MATERIALS AND METHODS

The objects of research (botanical and phytopathological) were the leaves and fruits of plants of different apple varieties (*Malus domestica*), pears (*Pyruscommunis*) collected from the experimental sites of the North Caucasian Research Institute of Mountain and Foothill Gardening, as well as common quince (*Cydonia oblonga* Mill.) from plantings of private plots, and a wild medlargermanica (*Mespilus germanica*), which grows in high altitude zones in the North Caucasus. Leaves and fruits for research were selected from the middle part of the crown of model trees. To diagnose plant diseases and identify microbiological agents, the wet chamber methods, was used with isolation of pathogens into a universal artificial nutrient medium (potato glucose agar) followed by microscopy of infectious structures.

Histochemical studies of the fruits were carried out on an AxioImager D1 microscope (CarlZeiss, Germany) in transmitted light. Sections 50 µm thick were made using a microtome with a vibrating blade (Thermo Scientific, Microm HM 650V). To detect condensed polyphenols, the sections were treated with potassium dichromate ($K_2Cr_2O_7$) [13,14]. Microphotographs were obtained with an AxioCamMRc camera (CarlZeiss, Germany); images were processed using the ZEN lite 2012 program (CarlZeiss, Germany).

The microstructure of the surface of leaves and fruits was studied with the help of a *scanning electron microscope* (SEM) in high vacuum mode and with gold deposition. Autofluorescence of the surface structures of leaves and fruits was examined using the confocal laser scanning microscope «Olympus FV1000D» under excitation with light 405, 473, 560 nm [13].

Electron microscopic (TEM) *studies* of the polyphenol f in the cover outer tissues was carried out according to a modified procedure. The material was fixed with glutaraldehyde (0.1 M phosphate buffer pH = 7.2) and 1 % osmium tetroxide solution. Then the samples were dried in a series of alcohols and acetones of increasing concentration and poured into Epon-812. Ultrathin sections were made on ultramicrotome LKB-III-8801A. The sections were contrasted with a 2 % aqueous solution of uranyl acetate (37°C) and lead citrate by Reynolds [7].

3. RESULTS AND DISCUSSION

Based on the results of phytopathological studies in various plantings in the southern region of Russia, as well as analysis of data available in previous literature [9,15], a summary table of the main fungal diseases of the leaves and fruits of the Maloideae subfamily was compiled (Table 1).

On all species of the fruit crops under investigation, the leaves and shoots were damaged by rust (*Gymnosporangium* sp.) and powdery mildew (*Podosphaera* sp.) caused by phytopathogenic fungi that are biotrophic obligatory, highly specialized parasites, (Table 1). These plant genera are systematically affected by a number of pathogens related to typical facultative parasites andnecrothrophs, usually with a broad phylogenetic specialization. Monilial fruit rot (*Moniliafructigena* Pers.), gray rot of flowers and fruits (*Botrytis cinerea* Pers.), black cancer (*Sphaeropsismalorum* Peck.) and anthracnose, or bitter rot of fruits (*Colletotrichum fructigenum* (Berk.) Vassil.) occur on fruits of all studied cultures (apple, pear, quince and medlar). Alternaria (*Alternaria* spp.), present during the vegetation period affects not only their fruits, but also leaves. All four plant species are affected by host specific species of fungi of the genus *Phyllosticta*, causing phyllosticosis, or a brown leaf spot (P. *mali* Pr.et Del., P. *pirina*Sacc., P. *cydoniae* var. *cydoniicola* (Allesch.) Cif. and P. *mespili*Sacc.) Brown spot caused by *Entomosporium maculatum* Lév. f. *maculate*Kleb. occurs on the pear and quince, and the medlar is attacked by a species specialized only in this culture Entomosporium (*Diplocarpon*) *mespili*Sacc. Thefungus *Ascochytapiricola* Sacc. parasitizes only the leaves of pear and apple, while a closely related species of A. *mespili* West. infectsmedlar. Quince pathogens of this genus have not been described.

Table 1. Fungal diseases of leaves and fruits and their agents in representatives of the subfamily Apple (*Maloideae*)

Apple (*Malus* Mill.)	**Pear (*Pirus* L.)**	**Quince (C*ydonia* Mill.)**	**Medlar (*Mespilus* L.)**
1a$_1$, 2b, 4d, 5e, 8g$_{1,2,3}$, 10h$_1$, 12j$_1$, 14l$_1$, 15m$_1$, 16n, 21o$_5$, 22o$_6$, 23p, 24s	1a$_2$, 2b,4d, 5e, 6f$_1$, 8g$_3$, 10h$_2$, 11i$_1$, 12j$_1$, 14l$_2$, 15m$_1$, 16n, 21o$_5$, 22o$_6$, 23p, 24s	3c, 4d, 6f$_1$, 8g$_4$, 11i$_2$, 14l$_3$, 15m$_2$, 16n, 17o$_1$, 22o$_6$, 23p,24s	4d, 7f$_2$, 9g$_5$, 10h$_3$, 11i$_3$, 12j$_2$, 13k, 14l$_3$, 15m$_2$, 16n, 18o$_2$, 19o$_3$, 20o$_4$, 22o$_6$, 23p, 24s

1 – Scab (a$_1$ – Fusicladium dendriticum (Wallr.) Fuck., ascigerous stage of Venturia inaequalis (Cooke) Wint, a$_2$ – F. Pirinum Fuck., ascigerous stage V. pirina Aderh.); 2 – Olive mold (b –Cladosporium herbarum Link., Alternaria tenius Nees.); 3 – Anthracnose (c – Cylindrosporium cydoniae (Mont.) Schoschiaschwili (= GloeosporiumcydoniaeMont.)); 4 – Anthracnose, bitter rot of fruit (d – Colletotrichum fructigenum (Berk.) Vassil. (= Gloeosporium fructigenum Berk.) ascigerous stage – Glomerella cingulata (Ston.) Sp. Et Schr.); 5 – Sooty blotch (e – Leptothyrium pomiSacc.); 6 – Brown spot (f$_1$ – Entomosporium maculatumLév. f. maculate Kleb. ascigerous stage Fabraea maculata (Lév.) Atk.); 7 – Dark brown spot (f$_2$ – E. mespili Sacc.); 8 – Phallosticosis, brown spot (g$_1$ – Phyllosticta mali Pr.et Del.; g$_2$ – P. Briardi Sacc; g$_3$ – P. Pirina Sacc; g$_4$ – P. Cydoniae var.cydoniicola (Allesch.) Cif.); 9 – Phallostictosis, brown spotting (g$_5$ – P. Mespili Sacc.); 10 – Spotting (h$_1$ – Hendersonia mali Trum, h$_2$ – H. Piricola Sacc, h$_3$ – H. Mespili West.); 11 – Septoria, white spot (i$_1$ – Septoria piricola Desm.; i$_2$ – S. Cydoniicola Trum; i$_3$ – S. Mespili Sacc.); 12 – Ascochitis (j$_1$ – Ascochyta piricola Sacc, j$_2$ – A. Mespili West.); 13 – Brown spot (k – Asteroma mespili Rob., Et Desm.); 14 – Rust (l$_1$ – Gymnosporangium juniperinum (L.) Mart. = G. Tremelloides Hartig, l$_2$ – G. sabinae (Dicks) Wint, l$_3$ – G. confusumPlowr.); 15 – Powdery mildew (m$_1$ – Podosphaera leucotricha Salm; m$_2$ – P. oxyacanthae DB.); 16 – Gray rot of flowers and fruits (n – Botrytis cinerea Pers.); 17 – Moniliosis, leaf spot (o$_1$ – Monilia cydoniae Schell.); 18 – Light brown spot (o$_2$ – M. Linhartiana Sacc.); 19 – Yellow spot (o$_3$ – M. foliicola Woronich.); 20 – Brown spot (o$_4$ – M. Necans Ferr.); 21 – Monilial burn, moniliolysis (o$_5$ – M. laxaHer. = M. cinereaBonord); 22 – Fruit rot, moniliasis (o$_6$ – Monilia fructigenaPers.); 23 – Black cancer (p – Sphaeropsis malorum Peck.), 24 – (s – Alternaria spp).

The analysis of the species composition of mycosis agents of representatives of the Apple subfamily indicates t *M. germanica* is the most highly specialized pathogens belonging to the group of facultative parasites and facultative saprotrophs. This may be due to the physiological differences of these fungi that determine their different responses when colonizing the surface tissues of plants and further development of mycelium and sporulation.

Undoubtedly, the degree of disease prevention to representatives of this subfamily strongly depends on the varietal characteristics and conditions of their growth. But in general, according to our long-term observations, quince is more resistant to the complex of fungal diseases, as is medlar, whose wild forms and most varieties are practically not affected by diseases of fungal etiology.

According to our observations, the features of the structure of integumentary tissues are of great importance during the first stages of the pathological process. Study of *Maloideae* leaves with SEM showed a number of common morphogenetic characteristics of their surface. these include hypostomacy (the stomata is located only on the abaxial surface of the leaf) and the anomocyte type of stomata (the cells adjacent to the stomata do not differ in form from the epidermal ones themselves), the folded surface microrelief (Figs. 1 and 2), and the presence of single and multicellular trichomes. In this case, the abaxial and adaxial surfaces of the leaf epidermis have different specific features as viewed in microrelieftht are attributed to the functional load. Due to its stability, including in different conditions of plant growth, the features of the microrelief of the leaf surface can be considered as diagnostic features and used not only in plant systematics and taxonomy, but also as possible markers of resistance to pathogens at the stage of their penetration.

The adaxial surfaces of the investigated representatives of the subfamily *Maloideae* are characterized by a different morphology and degree of development of the cuticular folds (Fig. 1).

However, for all four genera there is typically a rather weak development of epicuticular wax, so they are strongly affected by agents that easily penetrate directly through the integumentary tissues. Pathogens include the causal agents of powdery mildew (*Podosphaera* sp.) and rust (*Gymnosporangium* sp.), which are widespread and quite harmful to these plants (Table 1). Infection with many other fungi occurs mainly through mechanical damage to the adaxial surface.

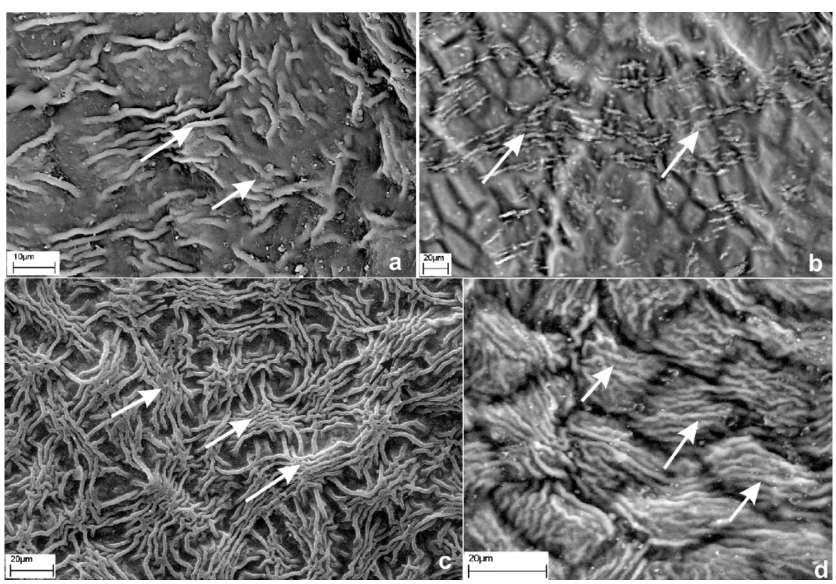

Fig. 1. The microrelief of the adaxial epidermis of the apple subfamily (*Maloideae*) leaves
(a – Malus Mill.; b – Pyrus L.; c – Cydonia Miil.; d – MespilusL. Arrows indicate the different structure (height and location features) of the cuticular folds)

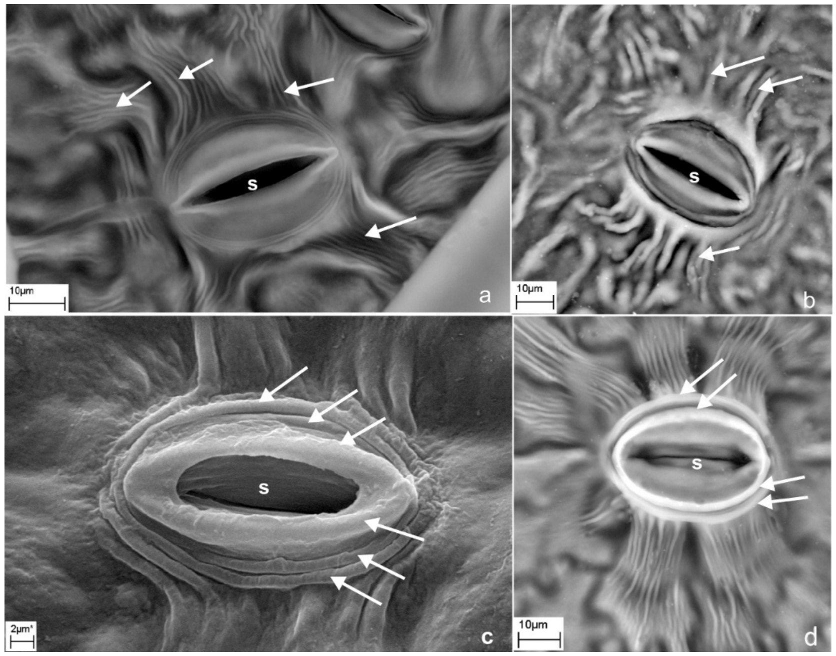

Fig. 2. Cuticular microstrands and peristomatic rings on the abaxial epidermis of the apple subfamily

(Maloideae) leaves: a – Malus Mill.*; b – Pyrus* L.*; c – Cydonia* Miil.*; d – Mespilus*L. *Designation: s – stomata. Arrows indicate: cuticular microstrands (one arrow) and peristomatic rings (two arrows)*

For the abaxial epidermis of the representatives of *Maloideae*, different micromorphology of the cuticular folds responsible for the regulation of stomatal movements is characteristic (Fig. 2). For example, on the leaves of quince and medlar, the folds in the stomata are arranged in the form of clearly pronounced high peristomatic rings encircling the closing cells (Fig. 2 c, d). On the leaves of the apple and pear they are less pronounced, and numerous radial microstrandsdiverge in all directionsfrom them, often extending over the borders of the actual epidermal cells (Fig. 2 a, b).

The height of the cuticular folds changes the character of wettability of the leaf surface [7]. Water drops only touch the upper edges of the cuticular ridges due to high surface tension and therefore easily slide down from the epidermis. It can be assumed that sporadic blowing out or washing away by rain of spores (conidia) of phytopathogenic fungi, occurs, at which point they cannot firmly hold onto the folds of the cuticle before the germination of the hypha germ begins. The nature of the surface of *Cydonia* leaves and, to a lesser extent, *Mespilus*, contribute to increased resistance to fungal pathogens at the stage of their entry onto the surface of the affected organ, preventing further penetration.

The structure of stomata and the number of them on the leaf are also of great importance if the pathogen penetrates through them into the tissues. Narrow gaps of the stomata (lenticles) with their rarer location delay the infection of plants with fungal and bacterial pathogens. The peculiarity of the structure of stomata in the representatives of the genus *Mespilus* – narrow stomatal gaps with raised outgrowths above the surface (Fig. 2 d), most likely complicates penetration by pathogens.

In representatives of the genus *Pyrus*, the availability of fungal penetration through the stomata is due to the absence of these features and to the depth of the stomata in the epidermal tissue (Fig. 2 b). In this case, a funnel is formed, which protects spores from rapid removal by air or water streams under natural plant growth conditions.

It is also important to note that the penetration of pathogenic fungi in plants of the genus *Pyrus*in many cases occurs through the spaces between the guard cells and proper epidermal cells (Fig. 3 a, b) rather than the traditional methods through the stomatal slit, as in *Malus* (Fig. 3 e, f). Penetration of pathogenic fungi into leaf tissue can also be carried out through t fallen trichomes, as, for example, we noted for the genus *Mespilus* (Fig. 3 c, d).

When studying the surface of the tissues of fruits of representatives of this subfamily, we found that in apple and pear they are covered with a continuous layer of cuticle with a thickness of 13.7 ± 2.7 and 11.5 ± 1.6 μm, respectively. The fruits of quince also have a continuous cuticular cover on their surface that is 80% larger than that of apple and pear (22.6 ± 4.0 μm). Unlike the listed representatives of Maloideae, the fruits of the medlargermanica (*M. germanica*) do not have such a continuous cuticular layer in their mature state. Morphologically it is represented by separate areas remaining between the crests of the microrelief of the embryonic surface. If you take this feature into account, then the medlar barrier that prevents the penetration of pathogens should be less effective than that of apple, pear and especially quince. But the structural and functional difference of the cuticular layer in the genus *Mespilus* is its peel surface, the properties of which contribute to the loss of adherence of spores of pathogens at this stage of their germination and penetration into tissues. Also in this genus *Mespilus,* the thick-walled, suberized cells, limit the penetration and localization of microorganisms in the surface tissues of the host plant. This, in many respects, explains the high level of resistance of Medlar germanica fruit to various fungal diseases during the growing season. Stomata of quince on fruits are larger and more numerous than in other cultures (from Fig. 4 d). However, the fruits of this culture are less often affected by mycosis. It can be assumed that some gaseous compounds located directly in the under stomatal cavity are inhibitory to pathogens.

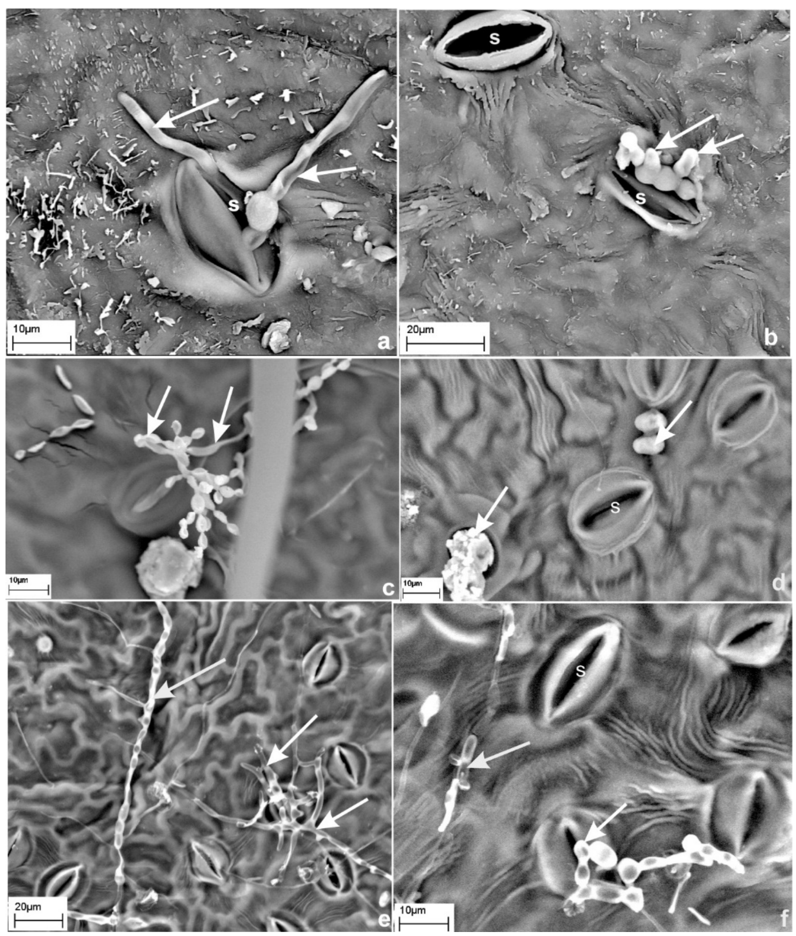

Fig. 3. The surface of the abaxial epidermis of apple subfamily (*Maloideae*) leaves with various pathogenic fungi

(a, b – Cydonia oblonga Mill.*; c, d – Mespilus germanica* L.*; e, f – Malus* Mill.*, mycelium of the fungus in the stomatal gap. Arrows indicate hyphae of fungi)*

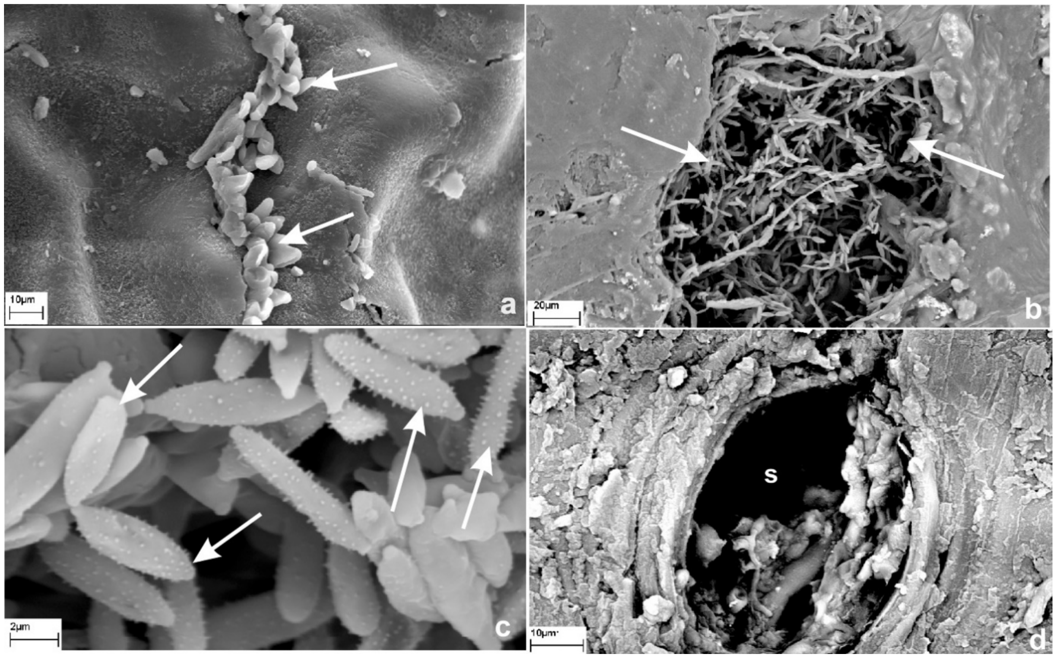

Fig. 4. Fragments of the surface of the fruit of apple subfamily (*Maloideae*) with the infectious structures of fungi in SEM

*(a – conidia Fusicladium dendriticum (*Wallr.*) Fuck. In microcracks of the cuticle Malus* Mill*; b, c – conidia of F. pirinum Fuck in Pyrus* L *lentils; d – stomata with mycelium on the surface of the fruit Cydonia* Mill. *Arrows indicate conidia of the fungus)*

The study of regularities in the distribution of phytopathogenic fungi on the surface of leaves and fruits, as well as the ways of pathogen penetration into internal tissues, in our opinion relates the features of the microstructure of the integumentary tissues with disease and partially explain the mechanism of the formation of resistance.

When examining the cuticle surface of apple fruit using SEM, microcracks were visible with conidia of scab fungi emerging from them–were noted, as well as numerous lenticles (especially in *Pyrus*), often filled with conidia of various fungi (Fig. 4 b, c).

Hyphae of a pathogenic fungus, presumably of the genus *Fusicladium*, were also found in the lenticule cavity by the method of confocal microscopy (Fig. 5 a, b). Moreover, the hyphal cell walls produced an intense autofluorescence in the blue part of the spectrum. Due to this, they were clearly distinguished among the cells of the host tissue of these structures.

It has been established that flavonoids and flavonoid glycosides of quercetin and anthocyanins are widely represented in the fruits of the plants of the Apple subfamily, as well as polyphenolic compounds – catechin and epicatechin [16]. Phenolic compounds with antioxidant activity are present in large numbers in plant tissues as precursors of lignin and other complex flavonoids (polyphenols) [17,18,19]. They have roles as growth regulators, pigments andstructural elements of the cell wall. Phenols are stress metabolites, the synthesis of which increases dramatically when wounding or infecteion with pathogens occurs [20,21]. These compounds are almost all fungitoxic. Many flavonoids, even with minimal effect on the growth of phytopathogenic microbes, are able to inhibit the production of mycotoxins from certain fungi, for example, aspergillus species *Aspergillus flavus* and *A. parasiticus*, that are dangerous to human health and animals [22,23,24,25]. Due to their high inhibitory activity and potential value as food products, biflavonoids may be promising candidates for the role of inhibitors of aflatoxin genesis [26].

As a result of histochemical study of the surface tissues of apple, pear, quince and medlar fruits, it was found out that the common feature of all studied representatives of 4 genera of the Maloideae subfamily is the presence of condensed polyphenols in epidermal cells and hypodermis (Fig. 6 a2–d2). These compounds are readily detected by microscopy when staining transverse sections of the pericarp with potassium dichromate. If flobaphenes are present in the cells – products of oxidation of tanning substances related to polyphenols, they become saturated dark brownish- reddish brown in color. Control cells that did not contain condensed polyphenols in sufficient quantities had a pale brown hue after staining (Fig. 6).

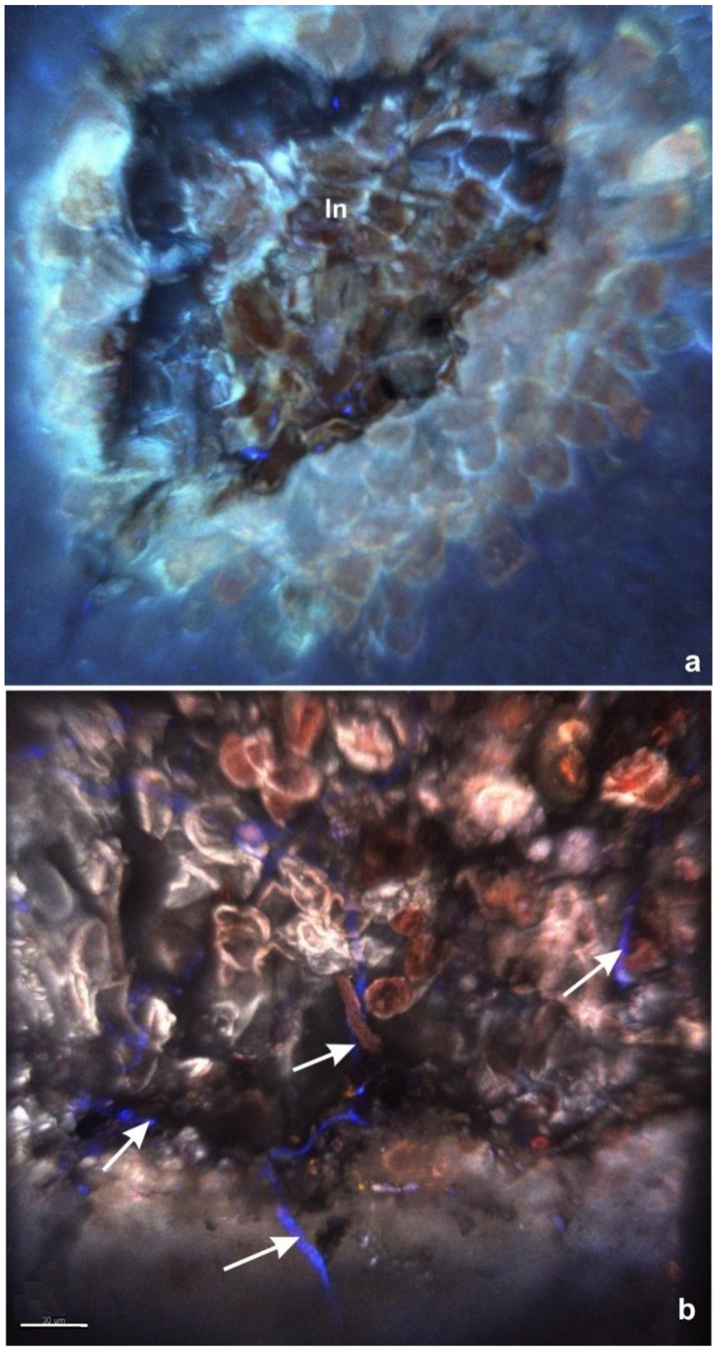

Fig. 5. Fragments of the surface of the fruit *Malus* Mill. with lentils in a confocal microscope
(a – general view; b – lenticular cavity with hyphae of the fungus in an enlarged form. Designation: In – lenticels. Arrows indicate the hyphae of the fungus)

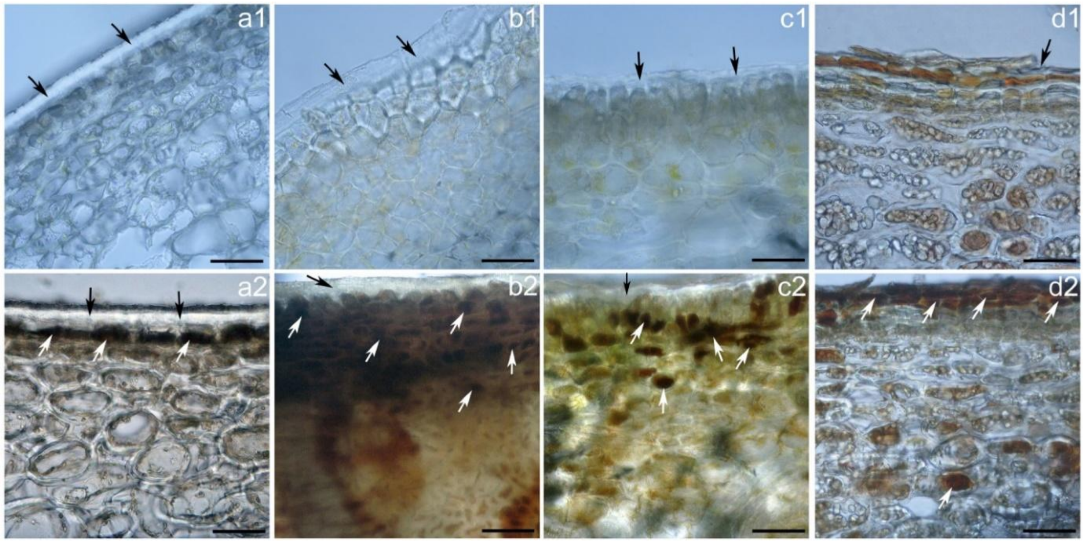

Fig. 6. Sections of apple subfamily fruits (*Maloideae*)

(a – Malus Mill.*; b – Pyrus* L.*; c – Cydonia* Miil.*; d – Mespilus* L. *(1) – control (not stained); (2) – stained with $K_2Cr_2O_7$. Black arrows indicate the cuticle on the surface of the epidermal cells, white – the cells with polyphenols. Bar = 100 µm)*

According to our data, epidermal cells contained most of the condensed polyphenols, which is one of the chemical factors of field (horizontal) resistance to phytopathogenic fungi. Moreover, in the deeper layers of the cells of the pericarp, especially the hypoderm, the number of cells with condensed polyphenols is significantly less than in the epidermis (Fig. 7). The exception was the pear fruit of some varieties, which, on average, had 10 % more cells with polyphenols in the hypodermis.

We found that in a medlar, more than 80% of the area of a mature fruit is covered with plug-like cells containing condensed polyphenols. This may be a compensatory mechanism due to the absence of a continuous cuticular cover. Thus, in a medlar passive immunity can be caused by at least two factors: first, a high concentration of polyphenols in the surface tissues, and also by the peculiarities of the exfoliated cuticle layer and the suberinized cells beneath it.

The presence of condensed polyphenols in the cells of the epidermis and the hypoderma of the Apple fruit was also revealed by transmission electron microscopy (TEM), since in this method the fixation of the material with osmium oxide (OsO_4) allows us to visualize complexes of proteins with polyphenols in the form of black electronically dense material (EDM). The presence of electronically dense material in the ovary cells observed in the transmission electron microscope is consistent with the results obtained in the histochemical study of the pericarp tissue (when staining with potassium dichromate) and indicates the identity of the observed structures and their relationships in representatives of the Apple subfamily (Fig. 8).

As can be seen on the TEM micrographs, most of the cells in the outer layer of the pericarp, the medlar (*Mespilus*) are completely clogged with polyphenols, unlike the apple tree (*Malus*), in which the polyphenols are deposited only in the form of separate rounded formations – tannoglobules [14]. In medlar, fruit cells of the epidermis and hypoderma also contained a significant amount of condensed polyphenols.

It was established that representatives of the genera *Malus, Pyrus, Cydonia* and *Mespilus* share common pathogens, as well as having highly specialized ones, especailly on *Mespelus germanica*. In comparison with apple and pear, resistance to a complex of fungal diseases is established in quince and medlar. Stability in the penetration of phytopathogens, at the initial stage of the pathological process is associated with the morphology of stomata in the abaxial epidermis of leaves, features of the layered structure of the cuticle, the presence of suberinized cells of the medlar fruit, a powerful continuous cuticular cover in quince, and a large polyphenol content in the cells of the outer layer of their pericarp.

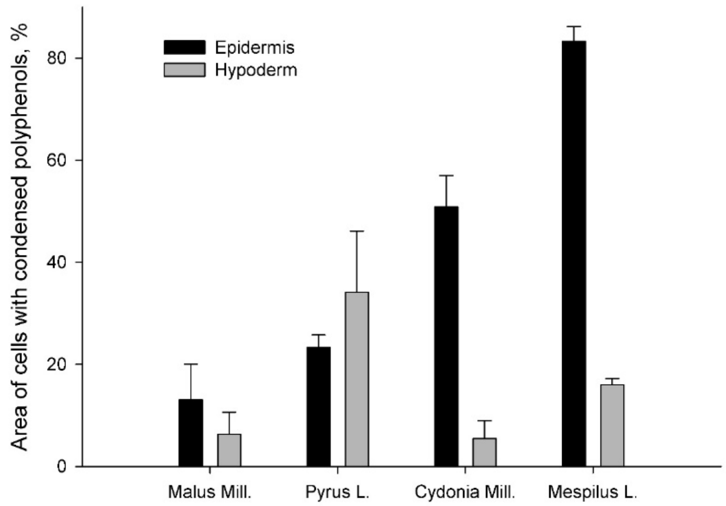

Fig. 7. The percentage of the area of cells containing condensed polyphenols from the area of the entire outer layer of apple subfamily fruits (*Maloideae*)
(The mean values of measurements of at least 50 micrographs and their standard deviations are given)

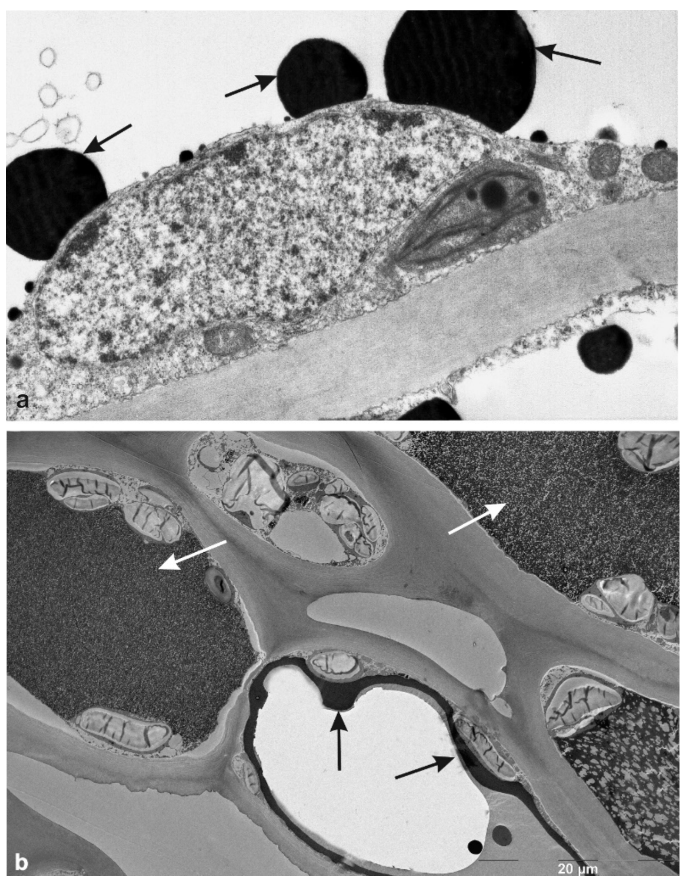

Fig. 8. Fragments of cells of the outer layer of apple subfamily fruits (*Maloideae*) with polyphenols in TEM in the form of electron dense material (EDM)
(a – apple (Malus Mill.*); b – medlar (Mespilus* L.*). Arrows indicate EDM)*

Using representatives of the *Maloideae* subfamily as models, structural features of the epidermis, such as hypostomacy, anomocytic stomata, folded microrelief, the presence of single and multicellular trichomes, were revealed by different electron microscopic methods, as well as specific differences in the abaxial and adaxial surface of the leaves andultrasculpture of the fruit surface. In the genera studied, the content of condensed polyphenols in the epidermis and hypoderm of the ovules was different. In our opinion, the established genera-specific microstructural and functional features of integumentary tissues determine the different degree of passive immunity to mycosis within the subfamily *Maloideae*.

Information on the correlation between microstructure characteristics of the surface tissues, their functional state and damage by fungal pathogens obtained with the use of electron microscopy methods can be useful and important in carrying out selection work on the breeding of resistant varieties. These data are also of practical interest for assessing the physiological and phytosanitary status of vegetating plants, as well as their production during storage, for determining the rates and stages of development of pathogens. Timely monitoring of the surface of plants with modern microscopic methods will help to identify the primary signs of infection and determine the timing of preventive protective measures, as well as reduce the toxic load and adverse effects on the environment by reducing the use of pesticides.

4. CONCLUSION

Thus to function of passive immunity in Maloideae fruits, the most important role belongs to the surface layer of the cells – the epidermis. Its cells form a sufficient powerful cuticle on the surface, which may well be a mechanical barrier to penetration of pathogens, and also contain most of all condensed polyphenols, which are one of the chemical factors of field (horizontal) resistance to phytopathogenic fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tsitsin NV. Atlas of medicinal plants of the USSR. Medical literature; 1962.
2. Kamelin RV. Rose family (*Rosaceae*). Barnaul: Op. Com. Altai pages; 2006.
3. Baskakova VL. Creation of varieties of quince for industrial gardening. Collection of scientific works of GRBS. 2017;1:98-102.
4. Strasburger E. Botanica. Moscow: Pub. «Academy»; 2007.
5. Stanimirović SG, Stanimirović D, Nastić DR. Identivicaton des acidesorganiqueset saccharides dans le fruits et la feuille de Neflier (*Mespilus germanica*). Bull. Soc. Chim. Beograd. 1963;28(5-6):327-335.
6. Glew RH, Ayaz FA, Vanderjagt DJ, Millson M, Dris R, Niskanen R. A research note mineral composition of medlar (*Mespilus germanica*) fruit at different stages of maturity. Journal of Food Quality. 2003;26:441-447.
7. Kumakhova TH, Melikyan AP. Ultrastructure of the cuticle of fruits of different varieties of apple *Malus domestica* (*Rosaceae*). J Botanical Journal. 1989;74(3):328-332.
8. Kumakhova TH. Some features of the anatomy of *Malus domestica* fruits (*Rosaceae*) depending on the height of cultivation in the mountains. J Botanical Journal. 2003;88(6):75-92.
9. Shkalikov VA, Dyakov YuT, Smirnov AN. Immunity of plants. Moscow KolosS; 2005.
10. Pautov AA, Yakovleva OV, SapachYuO. Structure and functions of peristomatic rings in the epidermis of the leaf of flowering plants. Proceed of the XII Cong. of the RBO «Fundamental and Applied Problems of Botany at the Beginning of the 21st Century». 2008;1:67-69.
11. Upadyshev MT. The role of phenolic compounds in the processes of life of garden plants. Moscow: Publishing House of SMEs; 2008.
12. Kumakhova TH, Skorobogatova IV. Conjugation of ultrastructural changes and the hormonal status in the cells of the pericarp *Malus domestica* (*Rosaceae*) during aging. J Izvestia TSHA. 2017;4:40-56.
13. Marin M, Jasnić N, Lakušić D, Duletić-Laušević S, Ascensão L. The micromorphological, histochemical and confocal analysis of satureja Subspicata Bartl. ex Vis. glandular trichomes. Archives of Biological Sciences. 2010;62(4):1143-1149.
    DOI:https://doi.org/10.2298/ABS1004137G

14. Kumachova TKh, Voronkov AS, Orlova YuV, Zhirov VK. Tannosomes in the pericarp cells of *Maloideae* (*Rosaceae*). Doklady Biol. Sciences. 2018;482(6):214-218. DOI:https://doi.org/10.1134/S0012496618050149
15. Sorokopudov VN, Kuklina AG, Fedulova YuA. Diseases of rare orchards in the European part of Russia. Proceed. of the Int. Conf. «Epidemics of Plant Diseases: Monitoring, Forecasting, Control», Bolshie Vyazemy, Moscow Region. 2017;8:152-158.
16. Budantsev AL. Vegetable resources of Russia: Wild flowering plants, their component composition and biological activity. Families Actinidiaceae – Malvaceae, Euforbiaceae – Haloragaceae SPb. and Moscow: Assoc. of Scien. Pub. KMK; 2009.
17. Solovchenko A, Schmitz-Eiberger M. Significance of skin flavonoids for UV-B-protection in apple fruits. Journal of Experimental Botany. 2003;54(389):1977-1984. DOI: https://doi.org/10.1093/jxb/erg199
18. Merzlyak MN, Solovchenko AE, Smagin AI Gitelson AA. Apple flavonols during fruit adaptation to solar radiation: Spectral features and technique for non-destructive assessment. J. Plant Physiol. 2005;162: 151-160. DOI:https://doi.org/10.1016/j.jplph.2004.07.002
19. Assumpçãoa CF, Hermesa VS, Pagnoa C, Castagna A, Mannucci A, Sgherri C, Pinzino C, Ranieri A, Flôres SH, Rios AO. Phenolic enrichment in apple skin following post-harvest fruit UV-B treatment. Postharvest Biology and Technology. 2018;138:37-45. DOI:https://doi.org/10.1016/j.postharvbio.2017.12.010
20. Menden K, Hahn M, Deising H. Morphogenesis and mechanisms of penetration of plant pathogenic fungi. J Ann. Rev. Phytopathol. 1996;34:367-386.
21. Khokhryakov MK, Dorozrakova TL, Stepanov KM, Letova MF. The determinant of plant diseases. 3-Ed., Rev. St. Petersburg: Lan; 2003.
22. Mabrouk SS, El-Shayeb NMA. Inhibition of aflatoxin production in *Aspergillus flavus* by natural coumarins and chromones. World J. Microbiol. Biotechnol. 1992;8:60-62.
23. Duarte J, Lugnier C, Torres AI, Perez-Vizcaino F, Zarzuelo A, Tamargo J. Effects of visnagin on cyclic nucleotide phosphodiesterases and their role in its inhibitory effects on vascular smooth muscle contraction. J Gen. Pharmacol. 1998;32:71-74.
24. Goncalez E, Felicio JD, Pinto MM. Biflavonoids inhibit the production of aflatoxin by *Aspergillus flavus*. Brazil. J. Med. Biol. Res. 2001;34:1453-1456.
25. Dzhavakhiya VG, Voinova TM, Popletaeva SB, Statsyuk NV, Mikityuk OD, Nazarova TA. Some natural and synthetic compounds that block the biosynthesis of aflatoxin B1 and melanin in Aspergillus flavus. J Agricul. Bio. 2016;51:533-542. DOI:https://doi.org/10.15389/agrobiology.2016.4.533eng
26. De Luca C, Passi S, Fabbri AA, Fanelli C.Ergosterol oxidation may be considered a signal for fungal growth and aflatoxin production in Aspergillus parasiticus. J Food Addit. Contam.1995;12:445-50.

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Peer-review history:
The peer review history for this paper can be accessed here:
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