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Species of the *Rhododendron* L. genus on the coast of Adjara and the antimicrobial activity of their leaf extracts against phytopathogens

Metreveli Mariam[1], Kandelaki Mariam[2], Jabnidze Nana[1], Meskhidze Avtandil[1], Mepharishvili Galina[1], Muradashvili Maka[1], Gorgiladze Lamziri[1]

[1] Institute of Phytopathology and Biodiversity of Batumi Shota Rustaveli State University, Georgia.

[2] Batumi Botanical Garden, Georgia.

Abstract

Based on the Institute of Phytopathology and Biodiversity of Batumi Shota Rustaveli State University, studied the antimicrobial action of the extracts obtained from the leaves of the species: *Rhododendron japonicum* (A.Gray) Suringer*, Rhododendron arborescens* (Pursh.) Torr*, Rhododendron brachycarpum* D.Don ex G.Don.*, Rhododendron macrosepalum* Maxim., *Rhododendron dalavayi* Franch.*,* on the example of fungicidal and fungistatic actions. The experiments were mainly conducted during periods of active vegetation. It has been determined: extracts obtained from *Rhododendron dalavayi* and *Rhododendron brachycarpum* are characterized by very high antimicrobial action. The highest fungicidal activity was observed in the case of dilution of ethanol extract 1:1, 1:2, in the case of fairly high dilutions of 1:4and 1:8, in the rest of the cases there was a clearly expressed fungistatic activity; in the case of aqueous extracts (tinctures), high fungicidal action was detected in relation with fungi *Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia theae, Fusarium moniliforme,* whereas fungistatic – in relation with the fungi *Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme.* The rest of the species of Rhododendron under study are characterized by weakly expressed fungistatic activity. The high antimicrobial activity of the extracts of the leaves of *Rhododendron dalavayi* and *Rhododendron brachycarpum* is of interest for the purpose of practical use.

Keywords: *antimicrobial activity, Rhododendron, fungicidal action, fungistatic action.*

Introduction

The genus Rhododendron (*Rhododendron* L.) is distinguished by its diversity over the world, which can be explained by its ancient origins. Six species of the genus *Rhododendron* L. grow wild in Georgia, they are *Rhododendron ponticum* L.*, Rhododendron smirnowii* Trautv. ex Regel.*, Rhododendron ungernii* Trautv. ex Regel., *Rhododendron caucasicum* Pall., *Rhododendron luteum* Sweet., *Rhododendron × sochadzeae* Kharadze & Davlian., except the last one, all these species are significant representatives of Ajarian flora (1-2).

Rhododendron ponticum L. and *Rhododendron luteum* Sweet. are widely spread in foothills, slopes and gorges under the conditions of the humid subtropical climate of Ajara littoral. Representing the species for the creation of the sub-forest of the Colchian forest, till today, they are massively spread not only in the conditions of highlands but also in coastal areas. Foreign species of rhododendron are available only in the collection of the Batumi Botanical Garden (BBG). Up to 20 introduced species plus four others from local flora: *Rhododendron ponticum, Rhododendron smirnowii, Rhododendron ungernii, Rhododendron luteum* grow in the Batumi Botanical Garden. Among the said local species, *Rhododendron Ungernii* Trautw. and *Rh. Smirnovii* Trautw., are endemic to Ajara-Lazeti (1-2).

The representatives of the genus *Rhododendron* L. are hardwood evergreen, semi-evergreen, deciduous species, breeds and forms distinguished by being highly decorative with original flowers and habitus. They are important cultures not only for open soil but also rooms, interior design and containers. Some species of rhododendron are characterized by containing high content of bioactive substances in aboveground organs. Bearing medicinal qualities, they are often used for healing cardiovascular systems, rheumatic diseases, vegetative neurosis, epilepsy, chronic colitis, etc. (3-9).

On the coast of Adjara, the greatest variety of species of the genus Rhododendron L. is found in the collection of deciduous plants of the Batumi Botanical Garden. Here there are rare, introduced single species that are not fully studied. The study of bioecological, biochemical and other features of poorly studied species of the genus Rhododendron L. in the soil and climatic conditions of the Batumi Botanical Garden is relevant and necessary for their further reproduction and rational consumption.

Organic substances released into the environment as a result of the activity of plant cells, or in most cases their complexes, are characterized by antiviral and antimicrobial properties. Interesting in this direction and research species of Rhododendrons.

Materials and Methods

On the basis of the Institute of Phytopathology and Biodiversity of Batumi Shota Rustaveli State University, we studied the antimicrobial effect of extracts obtained from the leaf of the studied species: *Rhododendron japonicum* (A.Gray) Suringer*, Rhododendron arborescens* (Pursh.) Torr*, Rhododendron brachycarpum* D.Don ex G.Don.*, Rhododendron macrosepalum* Maxim., *Rhododendron dalavayi* Franch.*,* for example, fungicidal and fungistatic action.

The experiments were mainly conducted during the periods of active vegetation.

For the determination of fungicidal activities the following pathogenic fungi: *Phytophtora infestans, Alternaria alternata, Alternaria solani, Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme, Pestalotia theae, Fusarium moniliforme,* causing important cultural plant diseases: Potato – *Phytophthora, Alternariosis;* Hazelnut – Pink Rot, *Trichotillocytosis;* Tomato – *Alternaria* Leaf Spot, *Fusarium;* Blueberry - Brown Leaf Spot, Tea – Leaf Spot, were applied for the experiment. The effect of the extract activity was determined according to the interruption of the fungus development.

The determination method of the fungicidal activity in agarised feeding areas was used in order to determine antimicrobial sensitivity of extracts of leaf in vitro conditions. Water extracts (tincture) made of leaf were prepared for the research and Ethanol (40 %) extracts with various dilutions

including the identification of fungistatical and minimal fungicidal concentration. Instead of leaf extracts, sterile water as a control option was used during the experiment. Sowing the fungi and their consistent cultivation were conducted within agarised feeding areas containing the extracts of research plants. Outcome analysis was carried out by the development quality of the fungus. 2% Potato Glucose Agar was used as a feeding area. Water extracts of leaf were prepared as follows: liquid extract of the plant was gained from newly-picked leaf cleaned with distilled and sterile water, 20 cm^3 boiled water was poured on 5 gr minced raw materials and left during 40 minutes in a water bath till boiling point. Then the received extract was cooled down and filtered with a sterile filter paper. 20 cm^3 melted potato Agar was added to the plants extract prepared in 2 cm^3 and immediately poured in sterile petri dishes. Spore suspension of the following fungi: *Phytophtora infestans, Alternaria alternata, Alternaria solani, Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme, Pestalotia theae, Fusarium moniliforme,* was placed on the surface of cooled Agar by an injection. They were cultivated during 3 days at 25^0C. The Ethanol extracts are gained by leaving (1:5) the raw materials in Ethyl alcohol (40%) during 7 days. The extracts were prepared with different concentrations: 1:1, 1:2, 1:4, 1:8. Average rate was counted according to the results (10-14).

Fungal pathogens were locally separated from diseased plants; strains from the Institute Phytopathology and Biodiversity collection were also used.

Results and Discussion

During the experiment, significant results were obtained especially in two species of rhododendrons:

Based on three times repeated studies about fungicidal activity of water and Ethanol extracts gained from *Rhododendron delavayi* and *Rh. Brachycarpum* lives extracts, on the development of phytopathogen fungi, it was detected, that the extracts prepared from the plant material collected in August are characterized with higher fungicidal and fungistatical activity.

Concerning the water extracts (tincture), the strongest fungicidal activity was shown toward the following pathogen fungi: *Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia theae, Fusarium moniliforme.* In this case, the growth of the fungus mycelium was completely stopped, while the mycelium of the following fungi: *Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme,* appeared difficult to grow or their development was interrupted, fungistatical activity was revealed.

Regarding the Ethanol extracts, the highest fungicidal activity according to the conditions of both experiments, was revealed in 1:1, 1:2 diluted extracts, good result was reached in case of 1:4 and 1:8 dilutions, lysis zones were clearly shown during the experiment completed by the diffusion method. In other dilution cases, fungistatic activity was revealed except the fungus *Trichothecium roseum*, weak fungicidal activity was shown when the pathogens were placed in an agarised feeding area, although there was no fungistatic activity while conducting the diffusion testing (Tabl. 1;2).

As for the control option, the fungi pathogens were characterized by good development.

Table 1 Fungicidal activity of water and ethanol extracts of leaves of *Rhododendron brachycarpum*

№	Phytopathogen fungus	The growth of fungus strains in the case of various diluted Ethanol extracts, water extracts and the control option						
		Various diluted Ethanol extracts					Water extracts (tincture)	Control option
		1:1	1:2	1:4	1:8	1:12		
1	*Phytophtora infestans*	_	_	±	±	±	_	+
2	*Alternaria alternata*	_	_	_	±	±	_	+
3	*Alternaria solani*	_	_	_	±	±	_	+
4	*Trichothecium roseum*	_	±	±	±	±	±	+
5	*Pestalotia coryli*	_	_	_	±	±	±	+
6	*Pestalotia theae*	_	_	±	±	±	_	+
7	*Fusarium moniliforme*	_	_	_	±	±	±	+

Note: „+“ - Growth of the fungus mycelium; „-“ - Termination of the growth of the fungus mycelium; „±“ - Interruption of the growth of the fungus mycelium.

Table 2. Fungicidal activity of water and ethanol extracts of leaves of *Rhododendron delavayi*

№	Phytopathogen fungus	The growth of fungus strains in case of various diluted Ethanol extracts, water extracts and the control option						
		Various diluted Ethanol extracts					Water extracts (tincture)	Control option
		1:1	1:2	1:4	1:8	1:12		
1	*Phytophtora infestans*	_	_	_	±	±	_	+
2	*Alternaria alternata*	_	_	_	_	±	_	+
3	*Alternaria solani*	_	_	_	±	±	_	+
4	*Trichothecium roseum*	_	±	±	±	±	±	+
5	*Pestalotia coryli*	_	_	_	±	±	±	+
6	*Pestalotia theae*	_	_	_	±	±	_	+
7	*Fusarium moniliforme*	_	_	_	±	±	±	+

Note: „+“ - Growth of the fungus mycelium; „-“ - Termination of the growth of the fungus mycelium; „±“ - Interruption of the growth of the fungus mycelium.

Conclusion

As a result of studying the antimicrobial action of extracts obtained from the leaves of *Rhododendron japonicum, Rhododendron arborescens, Rhododendron brachycarpum*, *Rhododendron macrosepalum, Rhododendron dalavayi,* on the example of fungicidal and fungistatic actions, it has been determined: extracts obtained from *Rhododendron dalavayi* and *Rhododendron brachycarpum* are characterized by very high antimicrobial action on the example of fungicidal and fungistatic activity.

The highest fungicidal activity was observed in the case of dilution of ethanol extract 1:1, 1:2, in the case of fairly high dilutions of 1:4and 1:8, in the rest of the cases there was a clearly expressed fungistatic activity; in the case of aqueous extracts (tinctures), high fungicidal action was detected in relation with fungi *Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia theae, Fusarium moniliforme*, whereas fungistatic – in relation with the fungi *Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme.* The rest of the species of Rhododendron under study are characterized by weakly expressed fungistatic activity.

Rhododendron L. გვარის სახეობები აჭარის ზღვისპირეთში და მათი ფოთლის ექსტრაქტების ანტიმიკრობული აქტივობა ფიტოპათოგენების მიმართ

მარიამ მეტრეველი[1], მარიამ კანდელაკი[2], ნანა ჯაბნიძე[1], ავთანდილ მესხიძე[1], გალინა მეფარიშვილი[1], მაკა მურადაშვილი[1], ლამზირი გორგილაძე[1]

[1] ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტის ფიტოპათოლოგიისა და ბიომრავალფეროვნების ინსტიტუტი, საქართველო. [2] ბათუმის ბოტანიკური ბაღი, საქართველო.

აბსტრაქტი

ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტის ფიტოპათოლოგიისა და ბიომრავალფეროვნების ინსტიტუტის ბაზაზე შესწავლილია სახეობების: *Rhododendron japonicum* (A.Gray) Suringer*, Rhododendron arborescens* (Pursh.) Torr*, Rhododendron brachycarpum* D.Don ex G.Don.*, Rhododendron macrosepalum* Maxim., *Rhododendron dalavayi* Franch.*,* ფოთლებიდან მიღებული ექსტრაქტების ანტიმიკრობული მოქმედება, კერძოდ, ფუნგისტატიკური და ფუნგიციდური მოქმედება. ექსპერიმენტი ძირითადად ტარდებოდა აქტიური ვეგეტაციის პერიოდში. დადგენილია, რომ *Rhododendron dalavayi*-ის და *Rhododendron brachycarpum*-ის ექსტრაქტები ხასიათდება ძალიან მაღალი ანტიმიკრობული მოქმედებით. ყველაზე მაღალი ფუნგიციდური აქტივობა დაფიქსირდა ეთანოლიანი ექსტრაქტის 1:1, 1:2 განზავების შემთხვევაში, საკმაოდ მაღალი 1:4 და 1:8 განზავების შემთხვევაში, დანარჩენ შემთხვევებში გამოვლინდა აშკარად გამოხატული ფუნგისტატიკური აქტივობა. წყლიანი ექსტრაქტების შემთხვევაში მაღალი ფუნგიციდური მოქმედება გამოვლინდა სოკოვანი ფიტოპათოგენების: *Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia theae, Fusarium moniliforme*, მიმართ, ხოლო ფუნგისტატიკური, *Trichothecium roseum, Fustanaria* ფიტოპათოგენებთან მიმართებაში. შესწავლილი როდოდენდრონის დანარჩენ სახეობებს ახასიათებს სუსტად გამოხატული ფუნგისტატიკური აქტივობა.

Rhododendron dalavayi-ისა და *Rhododendron brachycarpum*-ის ფოთლების ექსტრაქტების მაღალი ანტიმიკრობული აქტივობა საინტერესოა პრაქტიკული გამოყენების თვალსაზრისით.

საკვანძო სიტყვები: ანტიმიკრობული მოქმედება, როდოდენდრონი, ფუნგიციდური მოქმედება, ფუნგისტატიკური მოქმედება.

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