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RESEARCH ARTICLE

SCREENING OF ADJARA SEASIDE'S DENDRON PLANT EXTRACTSON *IN-VITRO* GROWTH OF *RALSTONIA SOLANACEARUM*

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ABSTRACT

The article discusses the results of screening for antibacterial activity of Dendron plant extracts growing in humid subtropical zone of Western Georgia, Ajara. Phythopatogenic bacteria, an important, quarantinable microorganism *Ralstonia solanacearum*, giving economic loss, was selected as the test-culture. We used the strains collected in 2012-2014, which were isolated from different bacterial recipient plants (potato, tomato, pepper). 7 strong and 12 medium of antibacterial activity out of the studied 60 species have been identified.

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INTRODUCTION

Bacteriosis of plants is still a difficult and complex challenge for agriculture in many countries. In this context, bacterial diseases of potato, tomato and other species of Solanaceae fam. caused by Phythopatogenic bacterium *Ralstonia solanacearum*, which result in great economic loss and reduce the quality of the harvest deteriorating its quality as well are especially remarkable. The systematic position this bacteria is expressed as follows: class-Proteobacteria; sub-class – proteobacteria; series –Burkholderiales; family – Ralstoniaceae; genus - *Ralstonia* (Smith, 1896). *Ralstonia solanacearum* is a gram-negative, aerobic rod-shaped bacterium with the size of 0,5-0.7x1.5-2mm. Moving forms have polar flagellum. The Optimal temperature for growth is 28-32°C, the temperature may be lower -27°C for some forms (Denny et al., 2001). The Bacteria *R. solanacearum* is quarantinable microorganism and

is included in so-called A2 list by European Foundation for Plant Pathology (EPPO) as a quarantinable object of limited distribution (EPPO, Bulletin, 2004). Phythopatogen is also included in the list of bioterrorism agents of agriculture (Directive 2000/29/EC of 8 May 2000). It should be noted that many cases of bacterial decay of tomato and potato caused by *R. solanacearum* were also registered in Georgia (Muradashvili et al., 2014). Currently, it is a quarantinable pathogen of limited distribution in our country as well.

Biological means still occupy a small part in the set of measures against bacterial decay of plants caused by *R. Solanacearum*. Various agro-technical methods, physical and chemical treatment are mainly used that often leads to environmental pollution and pose a threat to human health. At the same time, the number of bacterial strains resistant to widely used antibiotics increases significantly (Sundin et al., 1996) that, often makes the measures ineffective. Methods against bacteriosis should be economically sound and ecologically appropriate in accordance with modern requirements.

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In the last decade interest to development and introduction of biological methods against plant diseases based on using bacteria-antagonists, specific enzymes, phytoncides and other biologically active agents (Islam, 1990) has increased. That is why this study aims to identify antimicrobial properties of native and exotic Adjara seaside's Dendron plant against *R. Solanacearum*. Therefore, the aim of our study was the screening of plant leaves extracts for antibacterial activity towards *Ralstonia solanacearum*.

MATERIALS AND METHODS

60 species of deciduous and evergreen native and exotic Dendron plants growing in humid subtropical climate of Ajara, Western Georgia have been selected for screening for antibacterial activity, in particular, *Bignonia*, *Viburnum*, *Rhododendron*, *Manihot*, *Corylopsis*, *Hamamelis*, *Liquidambar*, *Loropetalum*, *Distylium*, *Parrotiopsis*, *Parrotia*, *Fortunearia*, *Illicium*, *Iuglans*, *Cinnamomum*, *Machilus*, *Neolitzea*, *Magnolia*, *Cocculus*, *Dorifora*, *Eucalyptus*, *Camellia*, *Myrtus*, *Pittosporum*, *Laurocerasus*, *Buxus*. These species are mostly part of a rich collection of Dendron plant of Batumi Botanical Garden, many of them have already been widely and successfully planted in decorative horticulture, gardens, parks and other green facilities of seaside Ajara. It should also be noted that 9 out of the studied species are in the Red List (IUCN): *Abies nordmanniana*, *Corylopsis pauciflora*, *Ginkgo biloba*, *Iuglans regia*, *Liquidambar styraciflua*, *Magnolia delavayi*, *Magnolia officinalis*, *Taxus baccata*. Samples to analyse was taken in Spring, Summer and Autumn. The extracts were obtained by squeezing the well-crushed leaves.

The study used Georgian strains of *R. solanacearum* collected in 2012-2014 isolated from different bacterial recipient plants (potato, tomato, pepper) and identified with standard bacteriological methods cultivating on semi-selective areas (Kelman's 2,3,5-triphenyl tetrazolium chloride (TZC) and modified SMSA areas) 24-48 hour incubation at 28°C diagnostics (Elphinstone et al., 2005). They were diagnosed in the laboratory of molecular biology of the UK Food and Environment Research Agency using Real-time PCR.

We obtained liquid extract from the leaves of freshly picked test plant leaves (100g), cleaned them with distilled or sterile water, cut into very small pieces with sterile scissors and crushed in mechanical mortar adding 100 mg sterile water. Then centrifugation (500 rpm) was performed for 10 minutes and supernatant was used in our study. The method of disc-diffusion was used to identify antimicrobial susceptibility of plants *in vitro* conditions. Indicators of susceptibility was estimated in accordance with the Regulations of "European Committee on Antimicrobial Susceptibility Testing" (EUCAST), according to which, for example, the strains for IMP are considered to be resistant, if the diameter of inhibitory zone is <17 mm, and are considered susceptible if the diameter is ≥22 mm. Then 4.5 mg of 0.7% semi-liquid agar is added to the test-culture and poured into a bowl. After it is solid, we drop 10 µl of test extract (Dudonne et al., 2011). The test was conducted three times per plant considering one control (we used sterile water instead of the extract). Experimental Petri cups were placed at 28°C. Monitoring was carried out within 24-48 hours.

RESULTS AND DISCUSSION

The tests were mainly conducted within the active and completed vegetation period of evergreen and deciduous Dendron plants, particularly, in the second half of May, July-August and September-October. As a result of experiment, part of plant extracts could not affect the growth of *R. solanacearum*, while another part have positive results. It is noteworthy that the tests carried out in August gave positive results. We obtained strong and medium types of antibacterial activity by depression of diameter of growth of bacterial culture, areas of lysis produced by the action of plant extracts (Table 1) strong types of antibacterial activity: *Parrotia persica* (DC.)C. A. Mey, *Hamamelis japonica* Sieb. et Zucc., *Hamamelis virginiana* L., *Hamamelis mollis* Oliv., *Myrtus communis*, *Liquidambar styraciflua* L., *Corylopsis pauciflora* Sieb. et Zucc., *Buxus colchica* Pojark.; 2) medium types of antibacterial activity : *Abies nordmanniana* (Stev.) Spach., *Iuglans regia* L., *Taxus baccata* L., *Parrotiopsis jaquemontiana* (Decne.) Rehd., *Corylopsis sinensis*Hemsl., *Pittosporum floribundum* Wight. et Arn., *Liquidambar formosana*Hance., *Corylopsis spicata*Sieb. etZucc., *Eucalyptus cinerea* F. Muell.

Table 1. The results of screening of anti-bacterial activity of Ajara Seaside's Dendron plants

No	Specie	Living form	Depression diameter of <i>R. solanacearum</i> growth (mm)	Quality of anti-bacterial activity
1	<i>Abies nordmanniana</i>	Evergreen tree	10-15	medium
2	<i>Buxus colchica</i>	Evergreen bush, tree	18-20	high
3	<i>Ginkgo biloba</i> L.	Deciduous tree	10-15	medium
4	<i>Corylopsis pauciflora</i>	Deciduous bush	20-30	high
5	<i>Corylopsis sinensis</i>	Deciduous bush	10-15	medium
6	<i>Corylopsis spicata</i>	Deciduous bush	12-15	medium
7	<i>Eucalyptus cinerea</i>	Deciduous tree	10-15	medium
8	<i>Hamamelis japonica</i>	Deciduous bush	20-25	high
9	<i>Hamamelis mollis</i>	Deciduous bush	20-25	high
10	<i>Hamamelis virginiana</i>	Deciduous bush	20-25	high
11	<i>Iuglans regia</i>	Deciduous tree	13-15	medium
12	<i>Laurocerasus officinalis</i>	Evergreen bush	10-15	medium
13	<i>Liquidambar formosana</i>	Deciduoustree	10-15	medium
14	<i>Liquidambar styraciflua</i>	Deciduous tree	20-25	high
15	<i>Myrtus communis</i>	Evergreen bush	10-15	medium
16	<i>Parrotiapersica</i>	Deciduous tree	20-25	high
17	<i>Parrotiopsis jaquemontiana</i>	Deciduous bush	10-15	medium
18	<i>Pittosporum floribundum</i>	Evergreen tree	10-15	high
19	<i>Taxusbaccata</i>	Evergreen tree	15-18	medium



Figure 1. Sensitive bacteria to plant extracts Depression zone of *R. solanacearum* growth

etBenth., *Laurocerasus officinalis* M. Roem., *Ginkgo biloba* L. Thus, based on the results of screening for antibacterial activity of *in-vitro* plants, we can say, that the mentioned plant extract can be successfully used against bacteriosis caused by *R. Solanacearum*. This study is an innovation and requires further research to determine which agent causes growth depression of bacteria, that can be used to produce bacteriocide in the future.

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