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Antimicrobial Activity of the Himalayan Cedar (*Cedrus Deodara* Loud.) in Seasonal Dynamics

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The Himalayan Cedar (*Cedrus deodara* Loud.) is the best drought and moisture-tolerant tree-plant bearing -25°C frost and any type of ubdesirable conditions. That's why it is widely used in landscape gardening and decorative gardening in the whole Georgia. It is especially widely spread in Georgia littoral, growing and developing ideally. The research aimed in studying the environmental healing qualities of the Himalayan Cedar. For this purpose, at this stage of the research, antimicrobial qualities of the extracts taken from raw needle in seasonal dynamics on the example of protistocide, fungicidal and fungistatical activity were studied in vitro conditions. Porridge-type mass made of cedar needles, water (tincture) and Ethanol extracts with different dilutions were used as experimental materials. In order to determine the protistocidal activity, so-called the simplest unicellular laboratory organism – Paramecium caudatum was used as an experimental microorganism according to Tokin's classical method. For the determination of fungicidal and fungistatical activities, the following phytopathogenic fungi were included in the experiment in order to determine the fungicidal activity in agarised feeding areas according to Disk-diffusion method. It is worth of mentioning, that the nature of antimicrobial activity of the extract was determined by the type of its separation, dilution quality and plant development phases. Based on the experiments, it was identified, that the extracts gained from the needles of the Himalayan Cedar are characterized with quite high antimicrobial activity and this quality together with lots of other values must be considered in green landscape planning of contemporary urban environment and for the other purposes too. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Cedar, extract, antimicrobial, protistocidic, fungicidal, fungistatical

Himalayan Cedar, *Cedrus deodara* Loud., is a coniferous plant, one of the species from the genus *Cedrus* Trew. of the family *Pinaceae* Lind. It is native to Pakistan, naturally grows in East Asia, North-western part of Himalayas, mountains of Afghanistan, Pakistan, India, Nepal. It is a fast-growing species, reaching up to 50 m tall, with a trunk up to 3 m in diameter. It occurs at 3600 m altitude, growing in a mixture with spruces, pine and fir-trees. The Himalayan cedar has exceptionally handsome wide conic forms, branches grow horizontally. It has reddish-brownish strong, firm and at the same time soft and aromatic bark. Beautiful needles are clustered as

cones, up to 40 soft needles in a cone, 5 cm long, needles live 3-6 years, then fall. Handsome, 15-20 cm long cones need 1-1,5 years to ripen up. After two-three years of maturing, the cones disintegrate in separate scales and fall off. The cones are upright on the branches. Seeds are white in color, 16-17 cm long with big light brown and resinous wings. They can live 1000 years and survive up to 3000 years in ideal conditions. Nowadays, the Himalayan cedar is included in the Red List of IUCN, has been categorized as – *Least Concern* (LC).

Because of valuable bark and extremely valuable "Cedar oil", it has been widely used in various ways for many centuries since BC till today, in naturally spreading areas. In India, it is considered to be "a divine tree". Its name is derived from "Deodara", a Sanskrit word – "Devaradu" meaning "the forest of the Gods". It is extremely popular in Kashmir and Punjab states. The Himalayan Cedar is also called "the pearl of Kashmir" [1-3].

Compared with the other species of cedar, the Himalayan cedar gets on better with new environmental conditions, it is the best drought and moisture-tolerant tree-plant bearing – 25°C frost and any type of ubdesirable conditions. That's why it is widely used in landscape gardening and decorative gardening not only in Ajara littoral, but also the whole Georgia. It is especially widely spread in Georgia littoral, growing and developing ideally [1-3].

We got interested in healing properties of the Himalayan cedar, as its organic substances or mostly its complex appear in the environment from plant cells activities, characterized with antiviral or antibacterial qualities, being very important for cities and densly populated areas.

For this purpose, antimicrobial activity of extracts taken from raw needles of the Himalayan cedar was determined at this stage of the research, based on the examples of protistocidal, fungicidal and fungistatical activities.

Experiments were basically conducted in active and finished vegetation periods, research materials were collected in Spring, Summer and Autumn, in particular, the 2nd half of May, July-August and September-October.

Materials and methods. In order to determine the protistocidal activity, so-called the simplest unicellular laboratory organism – *Paramecium caudatum* was used as an experimental micro-organism. The experiment was conducted in accordance with a famous classical method by Boris Tokin [4], about researching herbal, basically volatile compounds with suppressive and destructive effect on phytoncides and microorganisms and appearing in needles and leaves as the most influencing plant organs on the environment. The total destruction time of the infusoria by the activity of a plant leaf extract determines the protistocidal activity. The infusorias were grown within a feeding area made of hay broth. An average sample was taken from the leaves parted by chromated scissors, crushed in a pan and then 1gr experimental material was taken from the crushed materials and placed in special miniature glass cells. The object glass with a feeder droplet containing the infusorias was placed on the cell, where the droplet appeared in the environment of volatile compounds of experimental materials. The effect of phytoncide as an indicator of antimicrobial activity of the plant material was depicted in time by minutes, from the destruction of the first infusoria to the destruction of the last one.

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 [5] in agarised feeding areas was used in order to determine antimicrobial sensitivity of extracts of cedar needles in vitro conditions. Water extracts (tincture) made of cedar needles were prepared for the research and Ethanol (40%) extracts with various dilutions including the identification of fungistatical and minimal fungicidal concentration. Instead of herbal 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. Disc-diffusion method [6,7], was also applied, 100-100 mkl experimental extract was dripped in a small deepened part of the firm feeding area and then placed in the fridge during 16-18 hours at 6-9°C in order to diffuse the extracts in Agar layers. After that, they were left in thermostat during 24-28 hours at 28-30°C. The quality of antimicrobial activity, in particular, fungicidal and fungistatical activity was discussed according to the zone in the diffusion area of the extracts, where the growth of strains is inhibited or weakened, depicted in millimeters or by measuring the diameters of grown fungi colonies. Indicators of susceptibility was estimated in accordance with the Regulations of the ,,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 of inhibitory zone is ≥22 mm [6]. The test was conducted three times per plant considering one control (we used sterile water instead of the extract). 2% Potato Glucose Agar was used as a feeding area. Water extracts of cedar needles were prepared as follows: liquid extract of the plant was gained from newly-picked cedar needles 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°C. 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.

The works are done at the Institute of Phytopathology and Biodiversity of the Batumi Shota Rustaveli State University. Fungal pathogens were locally separated from diseased plants; strains from the institute collection were also used. Cedar needles were collected from 50-60 years old Cedar trees located in central squares and parks of Batumi.

Results and discussion. While conducting the experiment for the phytocinde activity on the extract taken from cedar needles, the total destruction (death) of the infusoria occurred after a certain period preserving its full structure, which means that its lysis didn't happen immediately after its death. Although the destruction of the infusoria was obvious in the microscope, its basic structures – nucleus, lashes, etc. were preserved. Their structure gets firmer and it's clearly visible, that after a certain period, approximately 4-5 hours, they start self-destruction process – the destruction of proteins and other compounds. In our other

experiments while working on the extracts of various plants, there are cases, when the infusoria gets parted into small pieces and dies. When there was no plant extract in a hanging droplet, the death of infusoria wasn't recorded. The "behavior" of the infusoria in the feeder droplet was observed through the microscope, which was moving quietly during hours until the droplet got fully dry.

Based on several repeated experiments in each period, the result was the following:

The total destruction time of the infusoria in minutes recorded in August is: 3.30 minutes, 3.30 minutes, 5.00 minutes, 5.35 minutes, 5.40 minutes, 3.40 minutes, 4.20 minutes, 3.40 minutes, 3.10 minutes.

The total destruction time of the infusoria in minutes recorded in Spring is: 6.05 minutes, 6.05 minutes, 6.30 minutes, 6.35 minutes, 6.35 minutes, 7.10 minutes, 7.30 minutes, 7.50 minutes.

The total destruction time of the infusoria in minutes recorded in Spring is: 9.30 minutes, 9.30 minutes, 10.00 minutes, 11.15 minutes, 9.50 minutes, 10.20 minutes, 9.40 minutes, 10.10 minutes.

Based on three times repeated studies about fungicidal activity of water and Ethanol extracts

Table 1. Fungicidal activity of water and Ethanol extracts of *Cedrus* while testing the fungal spore suspension in agarised feeding area

№	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*	–	–	–	±	±	±	+

Table 2. Fungicidal activity of Ethanol extracts and water extracts of *Cedrus deodara* while testing by diffusion method

№	Phytopathogen fungus	Inhibition zone diameter (mm) of the growth of fungi strains						
		Various diluted Ethanol extracts					Water extracts (tincture)	Control option
		1:1	1:2	1:4	1:8	1:12		
1	*Phytophtora infestans*	25-30	23-28	22-25	20-22	15-18	25-28	+
2	*Alternaria alternata*	28-32	28-30	25-28	23-24	17-19	27-30	+
3	*Alternaria solani*	26-28	25-27	22-26	23-25	20-22	25-30	+
4	*Trichothecium roseum*	25-28	25-28	22-23	17-19	15-17	20-22	+
5	*Pestalotia coryli*	30-35	28-30	25-28	20-22	18-20	25-28	+
6	*Pestalotia theae*	30-33	28-30	24-29	20-22	18-20	25-30	+
7	*Fusarium moniliforme*	28-30	26-29	25-28	21-22	16-18	16-18	+

Based on the experiments carried out in three periods, it was concluded the phytoncide intensity is increased from Spring to Summer and decreased from Summer to Autumn. According to the experiments carried out in Spring, cedar extract is characterized with high phytocinde activity, while in Autumn it has average phytocinde activity and the strongest phytocinde activity is revealed in August.

gained from cedar needles, 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. It is noteworthy, that the results appeared almost the same, but the strongest fungicidal activity was revealed during diffusion method, when the diameter of the lysis zone of the following fungi strains: *Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia coryli, Pestalotia theae,* was up to 22mm (Tables 1,2).

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 agarised feeding area, although there was no fungistatical activity while conducting the diffusion testing (Tables 1,2).

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

Conclusion. On the basis of our experiments, we can conclude, that the extracts gained from the needles of the Himalayan Cedar are characterized with quite high Antimicrobial activity on the example of protistocide, fungicidal and fungistatical activity. It is worth of mentioning, that the nature of antimicrobial activity of the extract was determined by the type of its separation, dilution quality and plant development phases.

The extract gained from cedar needles is distinguished by the strongest protistocide activity in August, enough high in May and average in October.

The highest fungicidal activity was revealed in case of 1:1, 1:2 diluted Ethanol alcohol, enough high with 1:4 and 1:8 dilutions and in all other cases fungistatic activity was obvious; Related to the water extracts (tincture), fungicidal activity was revealed toward the following fungi *Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia theae, Fusarium moniliforme,* and fungistatical activity was revealed toward the following fungi *Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme,* Fungus mycelium was actively grown in the control option.

ბოტანიკა

ჰიმალაიური კედრის (*Cedrus deodara* Loud.) ანტიმიკრობული თვისებები სეზონურ დინამიკაში

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*ფიტოპათოლოგიისა და ბიომრავალფეროვნების ინსტიტუტი, ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტი, ბათუმი, საქართველო

(წარმოდგენილია აკადემიის წევრის ვ. პაპუნიძის მიერ)

შესწავლილ იქნა in vitro პირობებში, ჰიმალაიური კედრის (*Cedrus deodara* Loud.) ანტიმიკრობული თვისებები სეზონურ დინამიკაში პროტისტოციდური, ფუნგიციდური და ფუნგისტატიკური აქტივობის მაგალითზე. საანალიზო მასალად გამოყენებული იყო კედრის წიწვებიდან დამზადებული ფაფისებრი მასა, წყლიანი (ნაყენი) და ეთანოლიანი სხვადასხვა განზავების ექსტრაქტები. პროტისტოციდური მოქმედების განსაზღვრის შემთხვევაში საექსპერიმენტო მიკროორგანიზმის სახით გამოყენებული იყო ე.წ. ლაბორატორიული უმარტივესი ერთუჯრედიანი ორგანიზმი – ინფუზორია ქალამანა (Paramecium caudatum). ფუნგიციდური და ფუნგისტატიკური მოქმედების განსაზღვრის მიზნით ცდაში ჩართული იყო მნიშვნელოვანი კულტურული მცენარეების დაავადებები: კარტოფილის – ფიტოფტოროზი, ალტერნარიოზი; თხილის – ვარდისფერი სიდამპლე, ტრიხოტეციოზი; პომიდვრის – ალტერნარიოზული სილაქავე, ფუზარიოზი; ლურჯი მოცვის – ყავისფერი სილაქავე, ჩაის – ფოთლის სილაქავე – გამომწვევი პათოგენი სოკოები: Phytophtora infestans, Alternaria alternata, Alternaria solani, Trichothecium roseum, Pestalotia coryli, Pestalotia theae, Fusarium moniliforme. ცდები ჩატარდა აქტიური და დასრულებული ვეგეტაციის ვადებში, საანალიზო მასალა აღებულ იქნა გაზაფხულის, ზაფხულისა და შემოდგომის პერიოდებში, კერძოდ, მაისის მეორე ნახევარში, ივლის-აგვისტოსა და სექტემბერ-ოქტომბერში. ექსტრაქტის ანტიმიკრობული მოქმედების ხასიათი დამოკიდებული იყო ექსტრაქტის გამოყოფის ტიპზე, მისი განზავების ხარისხზე, მცენარის განვითარების ფაზაზე. კედრის წიწვებიდან მიღებულ ექსტრაქტს (ნაყენს) ყველაზე ძლიერი პროტისტოციდური აქტივობა ახასიათებს აგვისტოს თვეში, საკმაოდ მაღალი მაისში, საშუალო ოქტომბერში. ასევე, კედრის წიწვებიდან დამზადებული წყლიანი (ნაყენი) და ეთანოლიანი ექსტრაქტების მაღალი ანტიმიკრობული აქტივობა აგვისტოში ვლინდება. ყველაზე მაღალი ფუნგიციდური აქტივობა გამოვლინდა ეთანოლიანი ექსტრაქტის 1:1, 1:2 განზავების შემთხვევაში, საკმაოდ მაღალი 1:4 და 1:8 განზავების შემთხვევაში, დანარჩენ შემთხვევაში ადგილი ჰქონდა ნათლად გამოხატულ ფუნგისტატიკურ აქტივობას; წყლიანი ექსტრაქტების (ნაყენი) შემთხვევაში მაღალი ფუნგიციდური მოქმედება გამოვლინდა Phytophtora infestans, Alternaria alternata, Alternaria solani, Pestalotia theae, Fusarium moniliforme, სოკოებთან მიმართებაში, ხოლო ფუნგისტატიკური, Trichothecium roseum, Pestalotia coryli, Fusarium moniliforme, სოკოებთან მიმართებაში. საკონტროლო ვარიანტში სოკოს მიცელიუმი აქტიურად განვითარდა. ჰიმალაიური

კედრის წიწვებიდან მიღებული ექსტრაქტების საკმაოდ მაღალი ანტიმიკრობული მოქმედება კედრის სხვა უამრავ ღირსებასთან ერთად, გასათვალისწინებელია თანამედროვე ურბანული გარემოს მწვანე ლანდშაფტების დაგეგმარების დროს, ასევე, სხვა დანიშნულებითაც.

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