DETECTION OF THE PATHOGEN OF VIRAL DISEASE IN SAMBUCUS NIGRA PLANTS

For the first time viral disease of elderberry (Sambucus nigra L.) was detected in Ukraine. Symptomatology of the disease and morphological properties of the virus are studied. Based on scientific literature data, screening of viruses that can infect elderberry plants in Ukraine is conducted. Antigens of PVY, PVM, SMV, AMV, and BYMV in elderberry plants with symptoms of viral disease were not detected.

Key words: elderberry, Sambucus spp., viral diseases.

Introduction. Elderberry (Sambucus nigra L., family Adoxaceae) is widely used in scientific and traditional medicine [Chekman]. Extracts of elder berry flowers are part of complex drugs as "Novo Passit", "Sinupret", "Atima", etc [27]. It is known that the therapeutical activity of elderberry fruit is second only to chokeberry, which superior elderberry fruit for antioxidant effect. Biologically active compounds of elderberry fruit, flowers, leaves have antiviral, antibacterial, anti-inflammatory, analgesic, immunomodulatory and antiproliferative effects. Traditional medicine recommends taking elderberry fruit with damage to the mucosa of the stomach, liver and pancreas. Tincture of the flowers and leaves of black elderberry has anti-inflammatory, antioxidant and hepatoprotective activity [29].

Sambucus nigra L. plants are sensitive to atmospheric drought. Elderberry propagated mostly by seeds. Elderberry flowers contain up to 82 mg% ascorbic acid, glycoside sambunigrin, rutin, essential oils, organic acids, anthocyanins, phenolic compounds, coumarin, triterpenoids, micro elements, etc [27]. It is known that the content of ascorbic acid and essential oil in the raw material depends on the illumination of elderberry growth place. Ascorbic acid content in the raw material collected at cutting down was higher by 20% and amounted to 75-82 mg% compared with raw materials collected from plants undergrowth. Essential oil content indicators were also higher – by 10-15%. Essential oil content within the same version of the raw material varies depending on the lighting stage, from which were collected materials. Essential oil content can vary from 0.03% to 0.14% depending on the placement of flowers on the plant. Wild elderberry plants are good spring and summer honey. One flower provides 0.16 mg nectar containing 23% sugar. One hectare of continuous planting in open, well-lit areas allocates about 85 kg of nectar. The fruits of elderberry have a unique sweet-sour flavor and fresh not edible. But collected at the stage of full ripeness used as industrial raw materials for processing and manufacture of confectionery products, juices, in wine production, textile industry etc.

On the chemical composition and content of biologically active substances in medicinal plants significantly affect pests and diseases, including viral [17]. It is known that various elderberry species infected by viruses that affect the metabolism of plants, reduce productivity and can degrade the quality of medicinal raw materials.

Viral diseases of elderberry plants first described back in 1925 [15]. Many viruses are known to cause detrimental symptoms in both American and European elderberry including members of the family Bromoviridae: Cucumber mosaic virus [19]; Secoviridae: Arabis mosaic virus, Cherry leaf roll virus, Strawberry latent ringspot virus, Tobacco mosaic virus, Tomato black ring virus, Tomato ringspot virus [5, 6, 10, 14, 19, 20, 21, 22, 24, 28, 30]; Virgaviridae: Tobacco mosaic virus [18], and Tombusviridae: Elderberry latent virus, Tobacco necrosis virus, Tomato bushy stunt virus [8, 9, 19, 25].

Most reports of elderberry infecting are about Cherry leaf roll virus and carlaviruses. Blueberry scorch virus (BScV), Elderberry symptomless virus (EISBV) and several other putative members of the genus Carlavirus (family Betaflexiviridae) have also been reported in elderberry [1, 3, 4, 11, 26]. There is report about infecting of Sambucus canadensis plants by filamentous virus which similar in morphological features to carlaviruses [9]. Subsequently, the virus was detected in the Netherlands and was named Elderberry virus A [26]. Recent studies of elderberry samples (Sambucus spp.) from Missouri (USA) showed infecting of these plants with two different viruses, which also belong to the genus Carlavirus [12]. Five novel carlaviruses tentatively named as Elderberry virus A–E (EVA–EIVE, respectively) were discovered [7, 8]. Elderberry carlaviruses group 1 (EVA, EIVB, EIVD) and group 2 (EIVC and EIVE) appear to have emerged from two distinct lineages, containing closely related viruses that infect the same host, indicative of sympatric speciation [Ho et al, 2016]. This, in addition to the recombination analysis, imply that elderberry, along with hop, phlox and potato (respectively infected by Hop latent virus and Hop mosaic virus; Phlox virus B and Phlox virus S; Potato virus P and Potato virus S), are major contributors of the carlaviruses evolution.

Despite the considerable amount of the studies of elderberry viruses in the world and particularly in Europe, such investigations in Ukraine haven't been conducted.

That's why the aim of the research was to obtain the Sambucus nigra plants on the presence of viral diseases.

Materials and methods. For diagnostics of viruses in the plants applied the methods of visual diagnostics, ELISA and transmission electronic microscopy (EM). Contrasting has been made with 2% solution of phosphorus – tungstic modification) using commercial test-systems of firm LOEWE (Germany). The results of reaction registered on the JEM 1230 (JEOL, Japan).

Detection and identification of viruses has been carried out with enzyme-linked immunosorbent assay (DAS-modification) using commercial test-systems of firm LOEWE (Germany). The results of reaction registered on the termo Labsystems Opiis MR(THE USA) with Dynex Revelation Quicklink software at lengths of waves of 405/630 нм. All samples showing values three times higher than the negative controls are assumed as virus positive.

The extinction values (the optical density) of the samples were processed by statistical analysis of Student's criterion, quoted by Lidanski [13]. The confidential intervals were at a significance rate of P ≤ 0.05 of Student's criterion.

Results and discussion. Under observations of wild elderberry plants in Poltava (2015-2016) and Kyiv (2016) regions we detected plants with chlorotic symptoms (a, b) and rolling of leaf tops (c) and twisting up the edges of the leaves (Fig.1).

Number of affected plants accounted for over 20% of surveyed wild elderberry.

It should be noted that analysis of world scientific literature on elderberry viruses showed that the most common symptom is chlorotic mottling ("blotching") and induced by many viruses [3, 20, 22]. But the chlorotic foliage symptom is not found in any report.

Filamentous virions 650±50 × 12 nm were found in the elderberry leaves conducting the transmission electron microscopy method. It was marked higher concentration of virions in plants with leaf rolling symptom compared with chlorotic. In addition, earlier PVM and PVY were identified by us in tomatoes with leaf rolling symptoms for the same agroecological conditions (in Poltava and Kiev regions) [16]. Such morphology is characteristic for viruses from the genus Potyvirus (Potyviridae) and Carlaviruses (Betallexviridae).

Thus, literature data indicate circulation of many viruses in elderberry plants, and other shrubs of this family. Elderberry viral disease was for the first time founded by us also in Ukraine that is potentially dangerous in epidemiological aspect. Shrubs are reservoirs of viruses and contribute to the viruses wintering and future spreading to economically important crops in this region.

Conclusions.
1. For the first time viral disease of elderberry (Sambucus nigra L.) was detected in Ukraine.

2. Symptomatology of the disease and morphological properties of the virus are studied.

3. Based on scientific literature data, screening of viruses that can infect elderberry plants in Ukraine is conducted. Antigens of PVM, PVM, SMV, AMV, and BYMV in elderberry plants with symptoms of viral disease were not detected.

References

dae). It is known that these genuses have a large number of representatives. So in our research, we settled on poty- and carlaviruses that are wide spread in Ukraine and have a wide range of host plants.

Based on the our results and on the data of other scientists, we tested elderberry plants with mentioned above symptoms on the presence of carlaviruses (Potato virus M), potyviruses (Potato virus Y, Soybean mosaic virus, Bean yellow mosaic virus) and Alfalfa mosaic virus. Alfalfa mosaic virus was detected in guelder rose (Viburnum), belonging to the same family with the elderberry [3, 23].

According to the ELISA results antigens of PYV, PVM, SMV, AMV, and BYMV in the tested elderberry samples were not found (tabl.).

<table>
<thead>
<tr>
<th>antiserum</th>
<th>sample</th>
<th>chlorotic foliage symptoms</th>
<th>leaf rolling</th>
<th>Positive control (commercial)</th>
<th>Negative control (sap of healthy elderberry plants)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVY</td>
<td>0.039±0.006</td>
<td>0.040±0.005</td>
<td>1.560±0.009</td>
<td>0.042±0.003</td>
<td></td>
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<tr>
<td>PVM</td>
<td>0.038±0.006</td>
<td>0.042±0.005</td>
<td>1.42±0.005</td>
<td>0.039±0.003</td>
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<tr>
<td>SMV</td>
<td>0.042±0.004</td>
<td>0.046±0.001</td>
<td>1.39±0.015</td>
<td>0.046±0.003</td>
<td></td>
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<tr>
<td>AMV</td>
<td>0.024±0.005</td>
<td>0.024±0.005</td>
<td>1.04±0.012</td>
<td>0.034±0.005</td>
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<tr>
<td>BYMV</td>
<td>0.049±0.005</td>
<td>0.041±0.001</td>
<td>1.80±0.007</td>
<td>0.042±0.004</td>
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Виправлення збудників вірусного захворювання у рослин бузини чорної

Вперше в Україні виявлено вірусне захворювання рослин бузини чорної (Sambucus nigra L.). Досліджено симптоматику хвороби та методи діагностування. Розроблено схеми боротьби з захворюванням. Виведено та впроваджено в практику нові сорти бузини.
INTRODUCTION

Herpes simplex virus type 1 (HSV-1) is member of the Alphaherpesvirinae subfamily within the Herpesviridae virus family [1]. HSV-1 is a common infection in developed countries where rates of seropositivity usually exceed 50%. In both humans and experimental animals, primary infection of skin or mucosa results in the local replication of virus, infection of sensory nerve ending, and spread via retrograde axonal transport to the ganglia of the peripheral nervous system (PNS) where a productive infection of neurons ensues. Although infectious virus is eventually cleared, a latent infection is established in neurons of the PNS ganglia [1, 2]. In humans, HSV-1 is a common cause of sporadic viral encephalitis with mortality rates reaching 20-30% despite treatment [2]. Also the virus plays an important role in human infectious pathology, causing diseases such as keratoconjunctivitis, stomatitis gingivitis, congenital herpes and others [2].

The problem of finding effective antiviral drugs caused high morbidity and wide spread of viral infections accompanied by the development of protracted and chronic forms of severe consequences. In clinical practice for treating these diseases most frequently use nucleosides, modified in heterocyclic, phosphate or carbohydrate fragment of the molecule. Today discovered many anti-herpetic drugs. However, acyclovir and other acyclic nucleosides in it is purpose and mechanism of action inhibit only those herpesvirus actively replicate, so the virus will prevent a latent state, is one of the problems of treatment of HSV-1. Another issue that complicates treatment herpesvirus is the development of viral resistance to abnormal nucleosides. There are many compounds are promising system in vitro, but only a few remain active in vivo.

In response to a viral infection in the body is activation of cytokines that modulate the overall immune response. In this regard, one of the methods of treatment of viral infections is the use of various drugs – interferon inducers that stimulate the production of interferon in the body, providing enhancement antiviral response [3, 4]. Interferon-gamma (IFN-γ) is a cytokine that plays physiologically important roles in promoting innate and adaptive immune responses. The absence of IFN-γ production or cellular responsiveness in humans and experimental animals significantly predisposes the host to viral infection, a result that validates the physiologic importance of this cytokine in preventing infectious disease [4]. Recently, an additional role for IFN-γ in preventing development of primary and transplanted tumors has been identified. Focusing on the data implicating IFN gamma as a critical immune system component that regulates antiviral immune response is important question for research [4, 6, 7]. Interleukin (IL)-4 and IL-2 are lymphokines synthesized primarily by activated T helper lymphocytes, and both are important regulators for development of T helper subsets (Th1-like vs. Th2-like) [8, 9]. Th1 cells are involved in cellular immunity (delayed type hypersensitivity and cellular cytotoxicity) and produce IL-2, tumor necrosis factor (TNF)-β, and IFN-γ. Th2 cells are involved in humoral (antibody-mediated) immunity and produce IL-4, IL-5 and IL-10 [10]. IL-4 is an important regulator of isotype switching, inducing IgE production in B lymphocytes and can exhibit anti-inflammatory effects [10, 11, 12]. IL-2 is important for in vitro growth of cytotoxic T cell (CTL) lines and can enhance NK cell and B cell responses [13, 14]. The IFN-γ production is the most rapid reaction in response to a virus infecting cells, as determined immunomodulatory potential nucleoside compounds at the level of IFN-γ and two pro-and anti-inflammatory cytokine IL-2 and IL-4 [15].

The purpose of this study was to investigate of antiviral activity fluorinated nucleoside G8 and G9 compounds (2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole) in in vivo models and determine their immunomodulatory potential. Shown significant inhibition of virus reproduction under the influence of the compounds at concentrations of 0.4 and 0.5 mg/kg, which was more effective of acyclovir. Protection ratio amounted to 80%. Increasing level of IFN-γ production is the most rapid reaction in response to a virus infecting cells, as determined immunomodulatory potential nucleoside compounds at the level of IFN-γ and two pro-and anti-inflammatory cytokine IL-2 and IL-4 [15].

MATERIALS AND METHODS

Herpes simplex virus type 1 (HSV-1, strain US1), obtained from the Institute of antiviral chemotherapy, The Center for Clinical and Theoretical Medicine (Germany). The compounds under study were G8 and G9 (they are the 2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole). They were provided by the Institute of Organic Chemistry of Ukraine. The substance of acyclovir was used as a reference compound. Their structural formulas are given on table 1.