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 Pasit", "Sinupret", "Atma", etc [27]. It is known that the therapeutic 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 phase. 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, Cucumber curly top virus, Tobacco ringspot virus, Tobacco black ring virus, Tomato ringspot virus [5, 6, 10, 14, 19, 20, 21, 22, 24, 28, 30]; Virgaviroidae: 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 (BiScVv), Elderberry symptomless virus (EBSV) 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 on morphological features to carlaviruses [9]. Subsequently, the virus was detected in the Netherland 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 carlavirus group 1 (EVA, EIBV, 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 acid. Virions are investigated using electron microscope 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 LOE-WE (Germany). The results of reaction registered on the reader Termo Labsystems Opsis MR(THIUSA) 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).

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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 Potyivirus (Potyviridae) and Carlavirus (Betaflexvirinae).

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.

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The problem of finding effective antiviral drugs caused high morbidity and wide spread of viral infections. The purpose of this study was to investigate of antiherpetic 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 and IL-2 in serum of animals, indicated available immunomodulatory effect fluorinated nucleoside compounds. Our studies indicated that there is antiherpetic, immunomodulatory activity of fluorine containing triazole and there is need in in-depth study of the mechanisms of this process.

Key words: HSV-1, fluorinated nucleoside, antiherpetic activity.

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, stomato 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 of fluorine containing triazole and there is need to in-depth study of the mechanisms of this process. The substance of polyfloralkyl-1,2,3-triazoles). They were provided by the D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kiev, Ukraine.

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.