

TESTING OF VIRAL INFECTIONS OF SOME MEDICINAL PLANTS OF FAMILIES ASTERACEAE AND LAMIACEAE

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A monitoring and testing of viral infections of some medicinal plants of the family Asteraceae and Labiatae in the steppe zone of Ukraine. Methods of visual diagnostics, biological testing and electron microscopy set impression viruses herbs Echinacea purpurea, lofanta anise and mint on industrial plantations. Infectious disease detected nature of Echinacea proven by a triad Koch and viral etiology - biological testing method and electron microscopy. The possibility of using hybrid sanberri (Solánium retrofléxum) as an indicator plant virus diseases of medicinal plants.

Keywords: *viral diseases, Echinacea purpurea, lofant anise, peppermint, visual diagnostics, biological testing, electron microscopy*

Protection of plants in medicinal plant occupies a prominent place among the measures to increase the production of medicinal plants. Medicinal plant enables sustainable resource base growing needs of the pharmaceutical industry and reduce the cost of medicinal plants. Artificial cultivation of medicinal plants - The most effective way of rare and endangered medicinal plants. In the culture of medicinal plants are usually damaged by a variety of pests, pathogens affected fungal, bacterial and viral etiology. Reliably protect them from viral infections may only set of measures defined species composition of virus carriers and their bio-ecological characteristics.

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To prevent the spread of infection and disease reduce harm to the economically acceptable level must comply with prevention at all stages of the process, including the use of healthy seed. However, information on the distribution and species diversity of viruses that strike medicinal plants in Ukraine, is insufficient [3]. The study of the etiology of diseases will allow to develop effective methods of protecting plants and harvest and preserve the quality of raw materials for phyto pharmaceuticals.

Because the diagnosis of diseases of plants only visual signs are not always accurate, this method can be limited only if the characteristic symptoms of the virus corresponds to it. In practice often happens that one and the same or very similar signs of damage caused by various factors [7].

Therefore, to prove (or disprove) the preliminary diagnosis of the disease with the external review is necessary to apply additional methods. One is biological testing. In particular, this method was found differences tomato mosaic virus isolates (TMV), isolated from plantain plants grown in different regions of Ukraine [12]. Biological testing is also used to confirm the viral etiology of diseases of black currants and raspberries [10].

The aim of research - to monitor viruses that strike some medicinal plants of the family Asteraceae and Lamiaceae in the steppe zone of Ukraine, and learn some of their biological properties.

Materials and methods. The method of inoculation by juice of diseased plants suitable for transmission to healthy plants only those viruses that can spread through contact-mechanically [5]. For juicing used leaves of diseased plants with clear symptoms. Finely cut leaves were ground in a mortar with a small amount of water and abrasive (ground glass, carborundum, fine sand, etc.). Then with pounded mass separate juice through a double layer of cheesecloth and rubbed it in a healthy plant leaf finger, cotton swab or spatula. Inoculated plants were placed in transparent insulators and observed the emergence over time of symptoms.

Infectious nature of diseases identified by the method set indicator plants (biological testing). As infectious material used herbs juice of patients with family Asteraceae (Echinacea) and Labiatae (lofanta, mint). Sample of plant material (5.10 g) crushed and ground in a mortar with the addition of 0.1 M phosphate buffer pH 7,0 in the ratio 1: 2. The resulting homogenate was filtered.

To prevent the inactivation of viruses in the environment for extracting added reducing agent (β - mercaptoethanol). On the leaves of plants indicator applied silicon carbide powder and conducted inoculated virusovmism material (juice of patients experimental plants). Excess of inoculum washed with water, then plant indicators were kept overnight in a shaded place and then growing them in a greenhouse [4, 6]. Control plants were used as uninfected, "inoculated" virus-free buffer. Repeatability - five experiments.

Biological testing performed by mechanical inoculation indicator plants in phase 4-6 true leaves. Indicator plant served *Datura stramonium* L., *D. metel* L., *Nicotiana tabacum* L. varieties immune, *N. glutinosa*, *Gomphrena globosa* L., *Solanum nigrum* L., *Chenopodium album*, *Chenopodium Quino* Willd., *Chenopodium amaranticolor* Coste et Reyen. , *Phaseolus vulgaris* L. Pinto grade and sanberri (*Solanum retrofléxum*).

Morphology of virus particles was studied by electron microscopy. Negative staining of purified virus preparations was performed 2% solution of phosphorus tungstic acid for 2 min [8]. Drugs studied electron microscope JEM-1230 (JEOL, Japan) and EM-125 (Sumy, Ukraine). Prevalence was determined by [11].

Results of research. An examination of crops of medicinal plants in Poltava and Kiev regions we found a high prevalence of Echinacea plants (70-80%) with the degree of lesion score of 5 (on a 6-point scale). In budding observed symptoms of chlorotic mosaic, leaf deformation plate as twisting, bulges and tuberosity (Fig.1).



Fig. 1. Field crops echinacea in budding symptoms of viral infection

Gradually, light green leaves chlorotic in a phase early flowering passed into a yellow spot, which occupied almost the entire leaf at the end of flowering phase culture. In the flowering phase revealed severe symptoms of viral infection on plants Echinacea (Fig. 2).



Fig. 2. Severe symptoms of viral infection on the leaves of Echinacea in bloom phase

Properties viruses studied by biological testing Indicator plant. Among the indicators used to hit the juice of the plant Echinacea patients reacted D. stramonium and Chenopodium Quino. On the 20th day after inoculation on leaves Ch. Quino local necrosis appeared light brown with dark brown halo diameter of 2-3 mm (Fig. 3).



Fig. 3. Necrotic reaction on leaves *Chenopodium Quino* infected sap of infected plants *Echinacea* (right – control).

The results of our testing of biological coincide with very limited published data [3]. The authors also found small local necrosis of about 1 mm on plants *Chenopodium hybridum*, infected leaves juice of *Echinacea*.

Plants *Datura stramonium* on the 14th day of unreacted emergence of systemic reactions such as yellow-green mosaic leaf blade, yellowing and curling of young leaves.

Another objective of our work was the establishment of an infectious nature of the disease by a triad Koch. To do this, provided: 1) transmission from infected to healthy field samples *Echinacea* plants (artificial inoculation of healthy plant sap of plants infected in the laboratory); 2) transmission from artificially affected the plant *Echinacea-indykotor* *Chenopodium quinova* (inoculation indicator *echinacea* juice); 3) reverse transfer of infection from plant samples indicator healthy *Echinacea* (*Echinacea* plant sap inoculation *Chenopodium quinova*). Identity initial symptoms of plants from the field, artificially inoculated in the laboratory and inoculated juice plant indicator shows the infectious nature of the disease we found *Echinacea* for triad Koch (Fig. 4).



Fig. 4. Symptoms destruction plant Echinacea juice inoculated Chenopodium quinova during transmission by triad Koch (top), control healthy plants (bottom)

By electron microscopy in leaves were found echinacea rod viral particles of $40 \text{ nm} \pm 10$ and $100 \pm 10 \text{ nm} \times 17 \text{ nm}$ with a clear channel (Fig. 5).

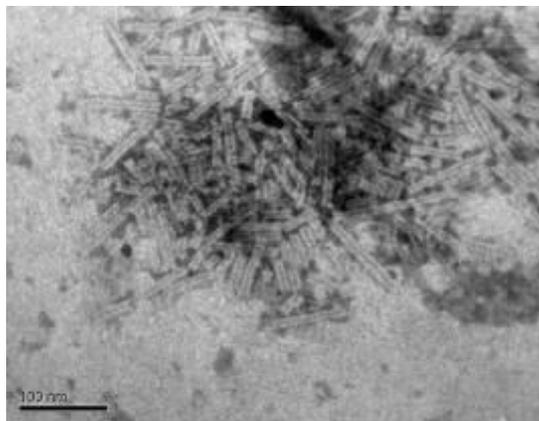


Fig. 5. electron viruses detected in leaves of *Echinacea purpurea* (L.) Moench, JEM-1230 with the prefix

Thus, the biological testing method was first brought us the nature of infectious diseases of plants Echinacea for triad Koch. The method of electron microscopy established viral etiology of the disease.

During surveys of plantations of lofant grade blue giant revealed the presence of diseases and selected plants, symptoms which are typical of viral

infection. The disease is manifested on the leaves of all layers in the form of diffuse chlorotic mosaic, which occupied almost all of leaf lamina (Fig. 6, 7).



Fig. 6. Plants of lofant grade blue giant phase in early flowering with symptoms of viral infection, 2012



Fig. 7. Detail of diseased plants with mosaic symptoms on leaves of lofant

In young leaves mosaic patches merged, resulting plate had a light yellow color. In older leaves chlorotic spots interspersed with green areas, forming a characteristic mosaic patterns. The color of the veins remain green. In plants with the described symptoms were found spherical virus particles with a diameter of 110 ± 10 nm [1, 2]. For us morphology recorded viruses like viruses genus Tospovirus family Bunyaviridae, which are spherical virions of different sizes: 50, 80 and 120 nm [14]. Members of this genus affect a wide range of single and dicotyledonous plants that do not have explicit specialization and transmitted thrips, which may explain their presence on plants lofanta.

With further monitoring the infestation of plants lofanta observed even

brighter severe mosaic, that the symptoms become more severe.

Among the investigated indicators on plants inoculated with the sap of diseased plants reacted lofant plant *Datura stramonium* advent of the 25-day local necrosis and yellowing leaves (Fig. 8). Note that looks dope on the symptoms were similar to those observed in lofant.



Fig. 8. yellowing and necrosis appearance on the leaves of plants *Datura stramonium* after inoculation juice from diseased plants lofanta (left – research, right – control)

Fast-growing demand of the domestic industry in the essential oil of mint in recent years almost completely satisfied by supplies it from abroad, indicating the urgent need for rapid recovery of volatile oil industry in our country. This can be achieved as the restoration of areas occupied by this culture, and because of the intensification of production and improving the technology of its cultivation. However, a major problem in drug crop is viral infection. We know that peppermint plants affected by viruses from at least 17 different families [13].

An examination plantations mint in the Poltava region plants with symptoms of chlorotic mosaic attenuation, small leaf deformation plate (Fig. 9).

In addition, there was stunting of infected plants. By electron microscopy preparations made from material aboveground plant parts mint marked filamentous virus particles size of $520 \pm 20 \times 11$ nm (Fig. 10).



Fig. 9. The symptoms of viral diseases in plants *Mentha piperita* L. grade Chornolysta

In [9] in mint roots Prylutska 6 and Krasnodar 2 also revealed viral particles of different sizes. In the roots of infected plants *Mentha piperita* grade Chornolysta in our studies of viruses found.

ELISA method (test system firm LOEWE, Germany) we tested herbal mint examples of containing HVK and other viruses described in the literature on culture mint. The result was negative - antigens Potato virus X-recorded. Not found also in mint antigen described by researchers from other countries (ArMV, PVY, TRV, INSV, TSWV, AMV) and viruses spread in Ukraine (CMV, TuMV, CGMMV, PVM, PepMV, TAV).

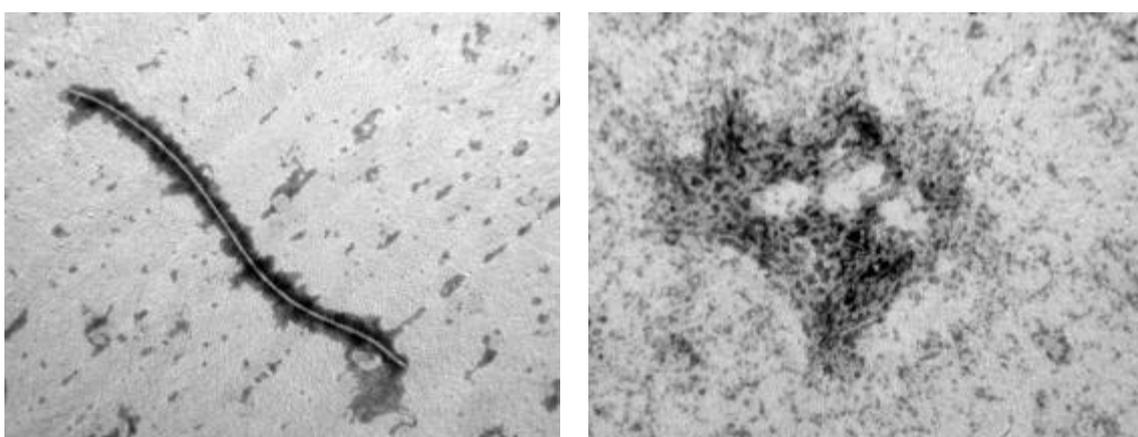


Fig. 10. The electron viruses detected in leaves of mint varieties Chornolysta, increasing $\times 30\ 000$

Among the studied plant indicator reaction to inoculation patient mint juice plants showed sanberri (*Solanum retrofléxum*) emergence pryzhylkovoyi yellow

mosaic 35 days after infection (Fig. 11). This kind of hybrid nightshade for biological testing viruses medicinal plants used by us for the first time.



Fig. 11. Leaf sanberri (*Solánum retrofléxum*) with symptoms of viral infection (left) after inoculation juice with mint diseased plants

In grade 6 Prylutska mint, which were grown in the Crimea, detected by ELISA STM, X, B-potato viruses [9].

Thus, according to the literature data for the 1961-2011 biennium. 19 describes the world of viruses that strike mint. In Ukraine now recorded only three viruses mint [9], two of which (X and Y viruses potatoes) by anyone in the world diagnosed, including our study. Therefore, to prevent the spread of viral diseases of medicinal plants should be carried out constant monitoring of lesions mint plants.

Conclusions

1. A monitoring and testing of viral infections of some medicinal plants of the family Asteraceae and Labiatae in the steppe zone of Ukraine.

2. Defeat viruses herbs echinacea, peppermint and lofanta in the field revealed the results of visual detection of symptoms of typical of viral diseases, biological testing affected juice samples Indicator plant virus infections, as well as identifying rod, and Whiskers spherical virions by electron microscopy.

3. Infectious diseases of the fixed nature of Echinacea proven by a triad Koch and viral etiology - biological testing method and electron microscopy.

4. Hybrid sanberri (*Solanum retrofléxum*) can be used as an indicator plant for identification of viral plant mint.

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ТЕСТУВАННЯ ВІРУСНИХ ІНФЕКЦІЙ ДЕЯКИХ ЛІКАРСЬКИХ РОСЛИН ІЗ РОДИН *ASTERACEAE* ТА *LAMIACEAE*

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*Проведено моніторинг і тестування вірусних інфекцій окремих лікарських рослин родин айстрових та губоцвітих у Лісостеповій зоні України. Методами візуальної діагностики, біологічного тестування й електронної мікроскопії встановлено ураження вірусами лікарських рослин ехінацеї пурпурової, лофанту анісового та м'ята перцевої на виробничих плантаціях. Інфекційна природа виявленого захворювання ехінацеї доведена за триадою Коха, а вірусна етіологія – методом біологічного тестування й електронної мікроскопії. Встановлено можливість використання гібрида санберрі (*Solanum retrofléxum*) як рослини-індикатора вірусних хвороб лікарських рослин.*

Ключові слова: вірусні хвороби, ехінацея пурпурова, лофант анісовий, м'ята перцева, візуальна діагностика, біологічне тестування, електронна мікроскопія

ТЕСТИРОВАНИЕ ВИРУСНЫХ ИНФЕКЦИЙ НЕКОТОРЫХ ЛЕКАРСТВЕННЫХ РАСТЕНИЙ ИЗ СЕМЕЙСТВ *ASTERACEAE* И *LAMIACEAE*

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*Проведены мониторинг и тестирование вирусных инфекций отдельных лекарственных растений семейств астровых и губоцветных в лесостепной зоне Украины. Методами визуальной диагностики, биологического тестирования и электронной микроскопии установлено поражение вирусами лекарственных растений эхинацеи пурпурной, мяты перечной и лофанта анисового на производственных плантациях. Инфекционная природа выявленного заболевания эхинацеи доказана триадой Коха, а вирусная этиология – методом биологического тестирования и электронной микроскопии. Установлена возможность использования гибрида санберри (*Solanum retrofléxum*) в качестве растения-индикатора вирусных болезней лекарственных растений.*

Ключевые слова: вирусные болезни, эхинацея пурпурная, лофант анисовый, мята перечная, визуальная диагностика, биологическое тестирование, электронная микроскопия