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HEAVY METALS CONTENT IN VIRUS INFECTED PURPLE CONEFLOWER PLANTS

It was revealed that purple coneflower plants infected Tomato spotted wilt virus. It was investigated that concentration of nine microelements (As, V, Sb, Cr, Fe, Ba, Sr, B, Mn) in purple coneflower plants under viral infection is higher than in healthy plants. Content of seven metals (Cd, Ni, Mo, Ti, Al, Zn i Al) was less than in the control samples. Differences for other elements (Pb, Hg, Cu, Co, Li) are not educed. It is necessary to notice that content of high-toxic elements (As, V, Sb, Cr, Fe) in the infected plants exceeded maximum allowable concentrations (MAC) in 1.2, 7, 2.3, 2.5 and 3.4 times respectively, unlike control samples in which concentration of these metals was within the limits of norm.

Key words: plant viruses, Tomato spotted wilt virus, purple coneflower, heavy metals.

Introduction: It is known that medical plants contain many important microelements which are necessary for the proper functioning of human organism. On the other side, except the positive effect of microelements, is also negative: there is a list of elements the concentration of that in foods is subject to hard control. Ability of medical plants to accumulate heavy metals from an environment is studied in the last ten years widely enough and does not cause doubts [4, 5, 6, 11, 14, 15, 18, 25, 26], that stipulates actuality of researches in this direction.

For today following classification of microelements is developed in relation to their operating on the human organism: microelements which matter in the human feed (Co, Cr, Ce, F, Fe, I, Mo, Mn, Ni, Se, Si, V, Zn); microelements which have a toxicological value (As, Be, Cd, Co, Cr, F, Hg, Mn, Mo, Ni, Pb, Pd, Se, Sn, Ti, V, Zn) [20]. It is needed to notice, that 10 from the transferred elements is taken in both groups. Thus, it is not always possible to set a difference between vitally necessary and toxic metals. All metals can show toxicity, if they enter organism in a surplus amount.

However, there are metals which show the expressed toxicological properties at the lowest concentrations and do not execute which that was not by a useful function – mercury, cadmium, lead, arsenic. In this connection, the common commission of FAO and WHO in obedience to a food code (*Codex Alimentarius Europaeus*) included mercury, cadmium, lead, arsenic, copper, strontium, zinc, iron in the number of components, content of which is controlled at the international trading in the products of feed. In Russia and CIS 7 elements (furnace, nickel, chrome, aluminium, fluorine, iodine, tin) are controlled. Some other metals can be controlled at presence of the certificates [16]. Content of these metals must be regulated and in medical plants because for today there are a few data about the degree of metals transition in to medicamental forms that is made from raw material of these plants [13, 17, 22]. In addition, information about element composition of medical plants is needed both for estimation of pharmacological properties of preparations from them and for standardization, development of analytically-normative documentation on a medical digester, from that get the plant-based preparations [12, 19, 26].

Mechanisms of viruses influence on heavy metals content in the plants are not found out. Data about determination of their concentration in virus infected herbs that are grown on territory of Ukraine are not present.

Aim of the research was to investigate heavy metals concentration in purple coneflower plants infected with viruses.

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) [23].

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 rider Termo Labsystems Opsis MR(THE USA) with Dynex Revelation Quicklink software at lengths of waves of 405/630 нм. For reliable took on values that exceeded negative control in three times [1].

Studying of the concentration microelements in plants taken in the flowering stage (both with symptoms of the disease and symptomless samples) has been carried out by mass spectrometry method using ISP-MC X-Series 2 (Termo Fisher Scientific) [8].

As raw material from echinacea is used in an untilled kind – in the plant-based preparations, infusions and biologically active additives (BAA), the analysis of data on determination of heavy metals concentration was carried out by comparison with: 1) level of maximum allowable concentrations (MAC) that is regulated by medical and biological requirements and sanitary norms of food quality № 5061-89 [21]; 2) possible norms that is regulated by State Pharmacopoeia of Ukraine (SPU) – not more than 0,1% [12]; 3) 'Hygienic Requirements for Foodstuff Safety and Nutritional Value' (SanPiN 2.3.2.1078-01) that establish hygienic requirements for substances and materials that come into contact with foodstuffs [24]; 4). WHO norms [10].

Results and discussion. Under monitoring of purple coneflower plantations (*Echinacea purpurea* (L.) Moench.) we revealed plants with yellow spotted symptoms on the leaves (Fig. 1).

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Figure. 1. Purple coneflower plants with symptoms of yellow spotted on the leaves (control – at the left)

Spherical viral particles 100±20 nm in diameter were detected in the sap of the sick coneflower plants (Fig.2).

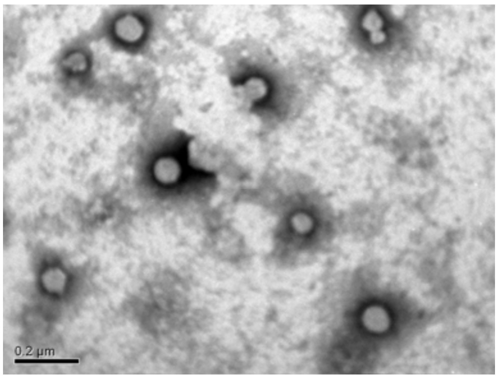


Figure. 2. Electron microscopy picture of the virions detected in the purple coneflower leaves

Viral particles presented on the Fig. 2 are similar to the viruses of the genus *Tospovirus* family *Bunyaviridae* in their morphology and size. It is known that tospoviruses are spherical virions with diameter 80-120 nm and have a wide circle of sensitive plants [9]. The typical member of the genus is *Tomato spotted wilt virus* (TSWV). It is necessary to mark that TSWV and also *Impatiens necrotic spot virus* (INSV) were already detected in the purple coneflower plants in Bulgaria and Lithuania [2,3,7].

Having regard to said early, the plants of Echinacea were tested by us in the presence of TSWV and INSV. ELISA results showed a presence of TSWV antigens in the purple coneflower plants (Fig. 3).

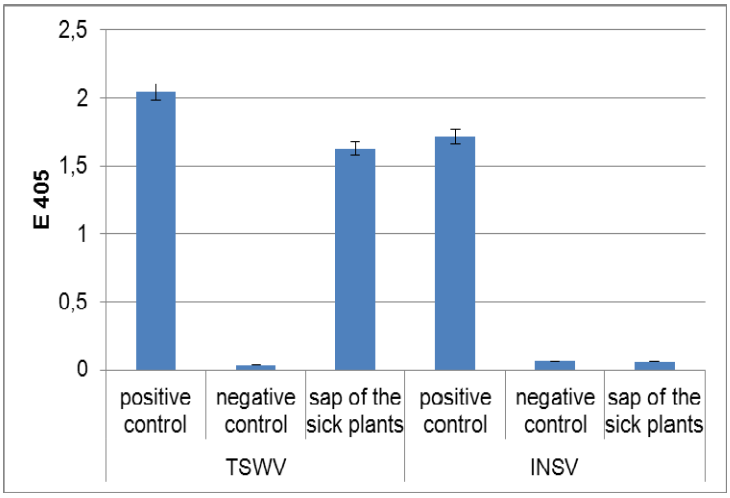


Figure 3. Content of the TSWV and INSV antigens in purple coneflower plants

Some debate exists as to exactly what constitutes a "heavy metal" and which elements should properly be classified as such. Some authors have based the definition on atomic weight, others point to those metals with a specific gravity of greater than 4.0, or greater than 5.0. The actinides may or may not be included. Most recently, the term "heavy metal" has been used as a general term for those metals and semimetals with potential human or environmental toxicity. That's why analysis of the results on research of heavy metals concentration was carried out in relation to their toxicity for the human organism. So, all investigated metal were divided into three groups: high-toxic, "mildly toxic" and low toxic metals.

The results of our research have not revealed differences in the content of such heavy metals as Pb, Hg, Cu between healthy and virus infected samples. Norms regulated by medical and biological requirements 5061-89 (MBR), SPU and WHO are not is not exceeded (table 1).

Table **1. Concentration of heavy metals in the purple coneflower plants, mg/kg** p≤0,01

Metal	Virus infected plants	Healthy plants	MBR 5061-89	SanPiN 2. 3.2.107801	WHO
Pb	0,48	0,52	0,5	5,0	10,0
Cd	0	0,09	0,03	1,0	3,0
As	0,25	0,06	0,2	3,0	-
Hg	0	0	0,02	1,0	-
Cu	4,50	4,81	5,0	-	-
Zn	11,12	27,68	10,0	-	-

However, it is necessary to notice that concentration of such high-toxic element as As in the Echinacea infected with viruses was higher in 4,5 times in comparison with healthy plants (table 1). Content of Cd in healthy plants was 0,085, that exceeds MAC in 2,8 times, unlike sick plants in which this element is not educed. A tendency to the reduce of concentration at a viral infection is marked for Zn (in 2,5 times), thus in the healthy plants MAC is exceeded in 2,7 times. On the SanPiN requirements, that estimate biologically active additives, content of all four metals (Pb, Cd, As, Hg) and in healthy, and in the virus infected samples of coneflower was within the limits of norm.

Except described 6 elements, Tl, V, Be, Ni and Bi are high-toxic metals too. Our research educed that the concentration of vanadium in all samples had exceeded MAC (0,5 mg/kg) and in TSWV-infected plants been in 1,3 times higher, than in healthy (Fig. 4).

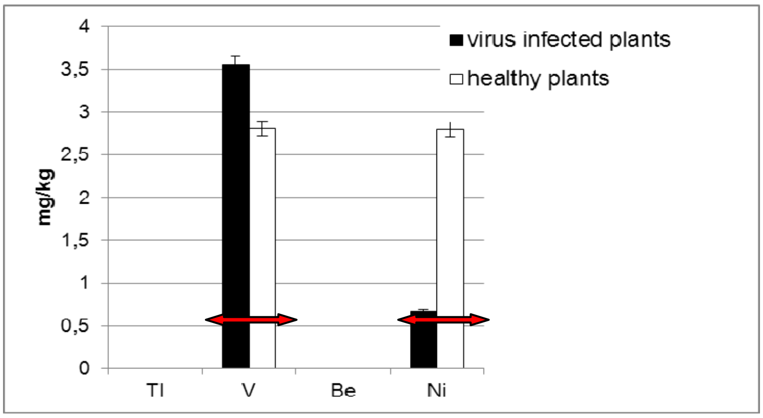
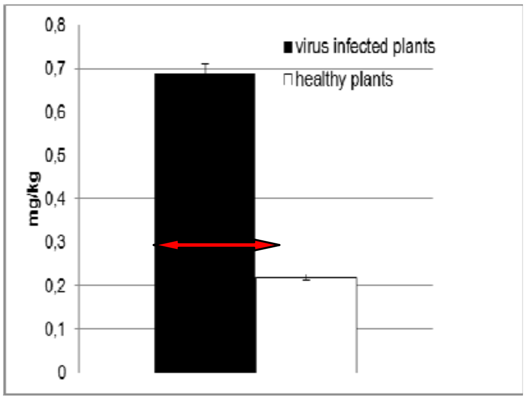


Figure 4. Concentration of high-toxic metals in purple coneflower plants (MAC is marked a pointer)

Thallium and beryllium are educed neither in sick plants nor in control (fig. 4). Concentration of nickel in infected plants was on verge of MAC (0,5 mg/kg), and here in healthy exceeded it in 5,6 (!) times. The same conformity revealed by us and for Bi. Its to law is a concentration was in 3 times higher in healthy plants than in sick plants and presented 0,65 mg/kg. The accumulation of such potentially dangerous metals even in healthy plants testifies to the necessity of an increase control of their content in plant raw material.

Concentration of four so-called "mildly toxic" for a man metals (Sb, Cr, Mn, Ba) in the coneflower plants under viral infection was higher, than in control (fig. 5, 6). Thus MAC for Sb and Cr is exceeded in 2,5 times (fig. 5).



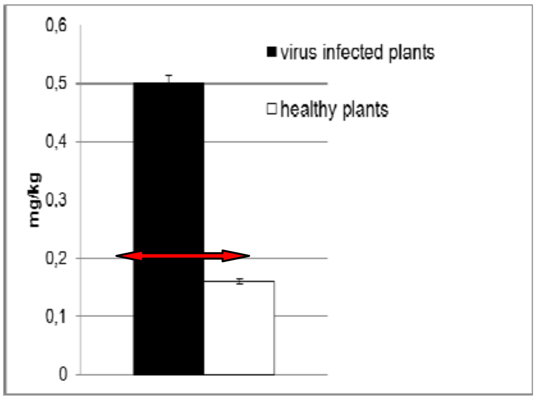


Figure 5. Concentration of Sb (at the left) and Cr (at the right) in purple coneflower plants (MAC is marked a pointer)

Increase of concentration under viral infection is marked by us and for such metals as a barium (Ba) and manganese (Mn) – in 1,3 and 1,2 times accordingly (fig. 6).

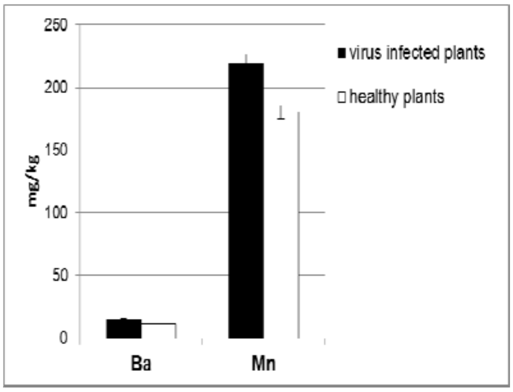


Figure 6. Concentration of Ba and Mn in purple coneflower plants

Content of iron that is subject to hard control in food, in healthy coneflower plants exceeded MAC in 2 times, in virus infected – in 3,4 times. In addition, this index exceeded in 1,7 times possible norms regulated SPU (Fig. 7).

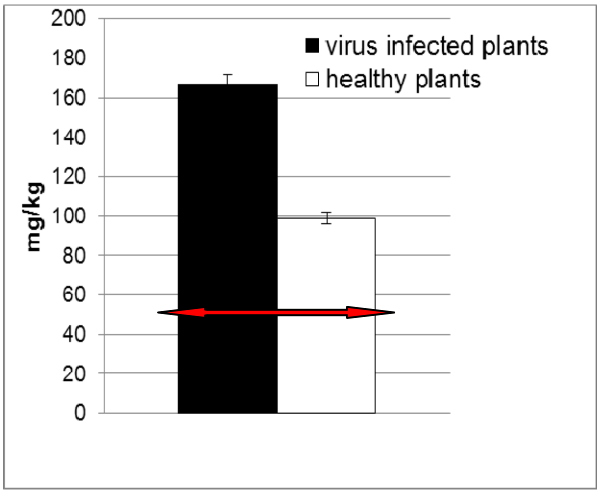


Figure. 7. Content of iron in the purple coneflower plants (MAC is marked a pointer)

Investigation of concentration of "low toxic metals" is shown that content of Sr and B was higher in TSWV-infected coneflower in 1,3 and 1,6 times in comparison with healthy plants (table 2).

***Table* 2. Concentration of "low toxic metals" in purple coneflower plants, mg/kg $p \le 0,01$**

Metal	Virus infected plants	Healthy plants
B	253,4	156,6
Sr	150,5	116,7
Li	2,9	2,9
Ti	11,0	16,1
Al	35,7	62,1

Content of lithium in the coneflower plants infected with TSWV did not differ from such in the control. And the concentration of Ti and Al was lower in sick plants in 1,5 and 1,7 times accordingly (table 2).

Conclusions. Thus, it was investigated that viral infection substantially effects on the microelements content in the purple coneflower plants. It is necessary to notice that the conducted research found out the tendency of accumulation of some heavy metals in these plants under TSWV infection in amounts which exceed MAC in food products. Although these indexes were within the limits of norms, regulated State Pharmacopeia of Ukraine and SanPiN. But presently and until now there is not clear information about passing of heavy metals to the medical forms. Thus, our studies demonstrated the obvious negative role of viruses in the production of high-quality medical raw material and to the necessity of the viral of the monitoring of medical plants with further development of protective methods from the detected viruses.

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ВМІСТ ВАЖКИХ МЕТАЛІВ У ВІРУСІНФІКОВАНИХ РОСЛИНАХ ЕХІНАЦЕЇ ПУРПУРОВОЇ

Виявлено, що рослини ехінацеї пурпурової уражені вірусом плямистого в'янення томатів. Встановлено, що із 21-го проаналізованого мікроелемента концентрація дев'яти (As, V, Sb, Cr, Fe, Ba, Sr, B, Mn) у вірусінфікованих рослинах ехінацеї пурпурової є вищою, ніж у контролі, семи (Cd, Ni, Mo, Ti, Al, Zn і Al) – нижчою, по інших (Pb, Hg, Cu, Co, Li) – різниці не виявлено. Показано, що вміст високотоксичних елементів (As, V, Sb, Cr, Fe) у рослинах, уражених вірусом, перевищував гранично допустимі концентрації (ГДК) у 1.2, 7, 2.3, 2.5 та 3.4 рази відповідно, на відміну від контрольних зразків, у яких концентрація вказаних мікроелементів була у межах норми.

Ключові слова: фітовіруси, вірус плямистого в'янення томатів, ехінацея пурпурова, важкі метали

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СОДРЖАНИЕ ТЯЖЕЛЫХ МЕТАЛЛОВ И ВИРУСИНФИЦИРОВАННЫХ РАСТЕНИЯХ ЭХИНАЦЕИ ПУРПУРНОЙ

Обнаружено, что растения эхинацеи пурпурной поражены вирусом пятнистого увядания томата. Установлено, что из 21-го проанализированного микроэлемента концентрация девяти (As, V, Sb, Cr, Fe, Ba, Sr, B, Mn) в вирусинфицированной эхинацее пурпурной выше, чем в здоровых растениях, семи – (Cd, Ni, Mo, Ti, Al, Zn і Al) – ниже, по другим – (Pb, Hg, Cu, Co, Li) – разницы не выявлено. Показано, что количественное содержание высокотоксических элементов (As, V, Sb, Cr, Fe) в пораженных вирусом растениях превышало гранично-допустимые концентрации в 1.2, 7, 2.3, 2.5 и 3.4 раза соответственно, в отличие от контрольных образцов, в которых концентрация указанных металлов была в пределах нормы.

Ключевые слова: фитовирусы, вирус пятнистого увядания томата, эхинацея пурпурная, тяжелые металлы.

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MANTAINING THE STATUS OF UKRAINE AS A POLIO-FREE TERRITORY

The article presents data on polio immunization coverage in Ukraine by age group during 2009-2012 and the results of investigating immunity to polioviruses in various population groups during 2009-2011.Considering the current poliomyelitis situation in the world and the possibility of wild poliovirus importation from endemic countries, continued monitoring of herd immunity is necessary for Ukraine to maintain its status as a polio-free territory.

Key words: poliomyelitis, Ukraine, immunity.

Introduction. Poliomyelitis is an acute infectious disease affecting the nervous system, primarily the grey matter of the spinal cord [1, 2]. Poliovirus, the causative agent of poliomyelitis, is a human enterovirus and member of the family of *Picornaviridae*.

Poliomyelitis has appeared in epidemic form, become endemic on a global scale, and been reduced to near-elimination, all within the span of documented medical history [1]. Global expansion of polio immunization resulted in a reduction of paralytic disease from an estimated annual pre-vaccine level of at least 600,000 cases to fewer than 1,000 cases in 2000 [3]. Indigenous wild poliovirus type 2 was eradicated in 1999, but unbroken localized circulation of poliovirus types 1 continues in 3 countries in Asia and Africa

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