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# *TOMATO SPOTTED WILT VIRUS* ON GIANT HYSSOP AND COMMON VALERIAN IN UKRAINE AND BULGARIA

B. DIKOVA[1], L. MISHCHENKO[2], A. DUNICH[2] and A. DASHCHENKO[3]
[1]*Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", BG - 1331 Sofia, Bulgaria*
[2]*Taras Shevchenko" National University of Kyiv, Kyiv, 01601, Ukraine*
[3] *National University of Life and Environmental Sciences of Ukraine, 03041 Kyiv, Ukraine*

## Abstract

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Giant hyssop, *Lophanthus anisatus* Benth., cultivated in Ukraine, has been found for the first time naturally infected by *Tomato spotted wilt virus*. Common research of TSWV-infection of medicinal plants in Ukraine and Bulgaria are carried out. It was investigated that TSWV infects giant hyssop in Ukraine and common valerian in co-infection with AMV, CMV, TMV, PVY or BBWV in Bulgaria. Symptoms of the diseases and size of TSWV isolates are determined. Characteristic symptom of TSWV infection in giant hyssop was yellow mosaic on the leaves, in common valerian - yellow irregular spots on the leaves. Recommendations about TSWV disease management on commercial plantations of medicinal plants in ecological conditions of Ukraine and Bulgaria are submitted in article.

*Key words: tomato spotted wilt virus*, TSWV, medicinal plants, common valerian (*Valeriana officinalis* L.), giant hyssop (*Lophanthus anisatus* Benth)

*List of abbreviations:* AMV – *Alfalfa mosaic virus*; ArMV – *Arabis mosaic virus*; BBWV – *Broad bean wilt virus*; CMV – *Cucumber mosaic virus*; ELISA – enzyme-linked immunosorbent assay; INSV – *Impatiens necrotic spot virus*; OD – optical density; PVY – *Potato virus Y*; TAV – *Tomato aspermy virus*; TMV – *Tobacco mosaic virus*; TSWV – *Tomato spotted wilt virus*

## Introduction

Analysis of world scientific literature about composition of viruses infecting medicinal plants testifies that almost all of them are polyphagous. It is the viruses that have very broad host range and effective ways of distribution and transmission (vectors, plants-reservoirs, and contact between infected and healthy plants). These viruses are not specific for this crops that makes them more dangerous. Because it complicates prediction and an assessment of risk of emergence of epiphytoties in these regions, so as there is appearance of a new viruses in the same agrocenosis each year. In valerian plants TSWV, AMV, BBWV and CMV were identified in Bulgaria (Kovatchevsky, 1976; Dikova, 2006; Dikova, 2009; Dikova, 2011) and BBWV, CMV, TSWV – in Italy (Bellardi et al., 1998; Bellardi et al., 1999). TSWV was identified on *Leuzea ncarthamoides* (Willd.) DC or *Rhaponticum carthamoides* (Willd) Iljin with test plants, DAS-ELISA and RT-PCR for the first time in Bulgaria and in the world (Dikova et al., 2013).

Information about viral infections of giant hyssop in the world scientific literature is very poorly. Only one paper was founded by us that devoted this question. Bruni R. with colleagues has been found infected giant hyssop, *Agastache anethiodora* Britton, cultivated at the Herb Garden of Casola Valsenio, Italy, by *Cucumber mosaic virus* (CMV) (Bruni et al., 2007). Characteristic symptoms on the leaves were chlorotic or yellow mosaic, ring and line patterns and malformation, followed by yellowing and stunting of the entire plant. CMV was mechanically transmitted to species of the families *Chenopodiaceae* and *Solanaceae* and identified by applying PAS–ELISA and RT–PCR techniques (Bruni et al., 2007).

E-mail: b.dikova@abv.bg, korenevochka@mail.ru, lmishchenko@ukr.net

Except significant effect of viruses on yield and general links of plants metabolism, it was proved that these pathogens decrease content of biologically active substances in medicinal plants. So, the infection of *A. anethiodora* by CMV was found to induce significant reduction in the yield of essential oil and several changes in the relative composition of the main components: pulegone, menthone, **iso**-menthone, methyl chavicole and limonene (Bruni et al., 2007). Bellardi et al. shown that CMV decrease content of some lipophilic compounds in purple coneflower plants (Bellardi et al., 2001c). CMV infection leads to decreasing of concentration of the hydrocinnamic acids sum in coneflower plants, sometimes to the level below than norms regulated the State Pharmacopoeia (Koreneva, 2009). Sum of caftaric and cichoric acids and content of polysaccharides was significantly reduced too (Mishchenko et al., 2011; Mishchenko et al., 2013).

Negative influence on quality of medicinal raw materials is shown and for some other viruses. So, it was revealed that BBWV decrease concentration of some compounds of essential oil in *Thymus vulgaris* (Bellardi et al., 2001a) and *Salvia sclarea* (Bellardi, 2001b; Hudaib et al., 2001). It was found that the AMV infection decrease essential oil secretion in *Lavandula* plants (Bruni et al., 2006), *Peppermint stunt virus* – in mint plants (Crowe et al., 1995).

Identification and circulation of such harmful viruses on commercial plantations for medicinal crops demands monitoring of viruses with following development of disease management for these plants.

The aim of the research is identification of infected with virus common valerian - *Valeriana officinalis* L. in Bulgaria and giant hyssop - *Lophanthus anisatus* Benth. (anise hyssop, *Agastache foeniculum* (Pursh) Kuntze = *Agastache anethiodora* Britton) in Ukraine.

## Materials and Methods

Visual diagnostics method, ELISA and transmission electronic microscopy method (EM) were applied for diagnostics of viruses in the plants. Valerian plants with viral symptoms were taken in plantations of the trial field of Institute of Roses, Aromatic and Medicinal Cultures (IRAMC) near Kazanlak, Bulgaria. *Lophanthus anisatus* plants cv. Syniy veleten were monitored on plantations of Research station of medicinal plants, Institute of agroecology and nature using of National Agricultural Academy of Ukraine, Ukraine.

Morphology of virus particles was determined by transmission electron microscopy method. Contrasting has been made with 2% solution of phosphorus-tungstic acid. Virions are investigated using electron microscope JEM 1230 (JEOL, Japan) (Koreneva, 2009).

Detection and identification of viruses has been carried out by enzyme-linked immunosorbent assay, ELISA (DAS-modification) using commercial test systems of firm LOEWE (Germany) (Clark and Adams, 1977). In Bulgaria the valerian samples (except to TSWV) were analyzed by applying the kits of other viruses: *Broad bean wilt virus* I (BBWVI), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV) and *Potato virus* Y (PVY). The extinction values were measured on spectrophotometer SUMAL PE, Karl Zeiss, Jena, Germany at length of the wave 405 nm. Negative controls were samples of symptomless healthy plants and positive controls infected indicator plants with TSWV and as well as the positive controls from the kit. All samples showing values two and a half times higher than the negative controls were assumed as virus positive namely virus carriers. The extinction values (the optical density) of the samples were processed by statistical analysis of Student's criterion, quoted by Lidanski (1988). The confidential intervals were at a significance rate of $P \leq 0.05$ of Student's criterion. Average extinction values of optical density were calculated as well as standard deviation.

In Ukraine *Lophanthus anisatus* plants were tested on the presence of antigens of TSWV (*Tomato spotted wilt virus*), CMV (*Cucumber mosaic virus*), AMV (*Alfalfa mosaic virus*), ArMV (*Arabis mosaic virus*), TAV (*Tomato aspermy virus*), and INSV (*Impatiens necrotic spot virus*). The results of reaction registered on the rider (Termo Labsystems Opsis, USA) with Dynex Revelation Quicklink software at lengths of waves of 405/630 nm. All samples showing values three times higher than the negative controls are assumed as virus positive namely virus carriers.

## Results

Twenty one samples of *V. officinalis* were analyzed for TSWV presence in Bulgaria. It was investigated that valerian were infected by TSWV in mixed infection (Figures 1 and 2).

It was revealed that TSWV was present only in five common valerian plants (23.8%) growth in Bulgaria (Figure 1). TSWV was in mixed infection with BBWVI, CMV, TMV or PVY in these five plant samples (Figure 2). *Alfalfa mosaic virus* (AMV) was earlier established on common valerian in Bulgaria (Dikova, 2006).

Confidence interval at significance $p \leq 0.05$ by Student's criterion for the positive extinction values to TSWV of the samples was 0.395 ± 0.076 OD (Optical density). Confidence interval at significance $p \leq 0.05$ for the negative extinction values to TSWV of the samples was 0.124 ± 0.021 OD.

TSWV was established in leaves, shown yellow or bright white irregular spots. Some of these spots turned into necrotic (Figure 3).

Also many colonies of thrips in the racemes of the common valerian plants were observed in plantations of IRAMC, as on the plants with symptoms and as well as on the symptomless.

In 2009-2011 *Lophanthus anisatus* Benth. plants (giant hyssop) with symptoms of light-yellow mosaic were revealed in production plantations in Ukraine. On the young leaves mosaic mottle met, as a result leaves had bright yellow color. Chlorotic mottling on leaves of the bottom circles alternated with green sites, forming the mosaic patterns (Figure 4).

Infected plants developed slower. Sometimes inflorescences did not appear in infected hyssop plants (Figure 5).

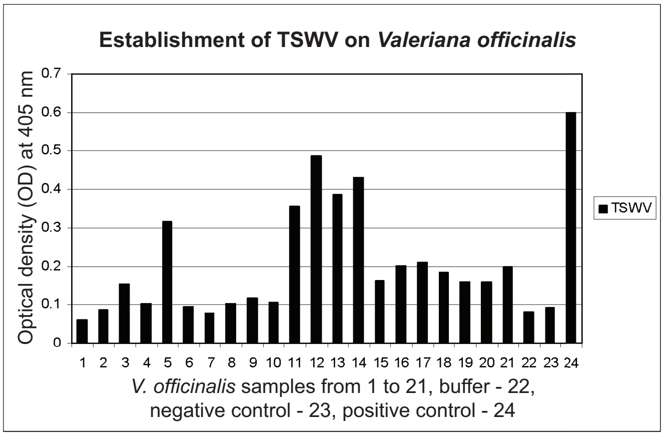


**Fig. 1. Establishment of *Tomato spotted wilt virus* (TSWV) on *Valeriana officinalis* with DAS-ELISA**

Note: Cut off was the boundary value that surved as a threshold of all extinction values over two and a half times higher than the negative controls i.e. a threshold for all positive reacted of viruses samples of the common valerian plants

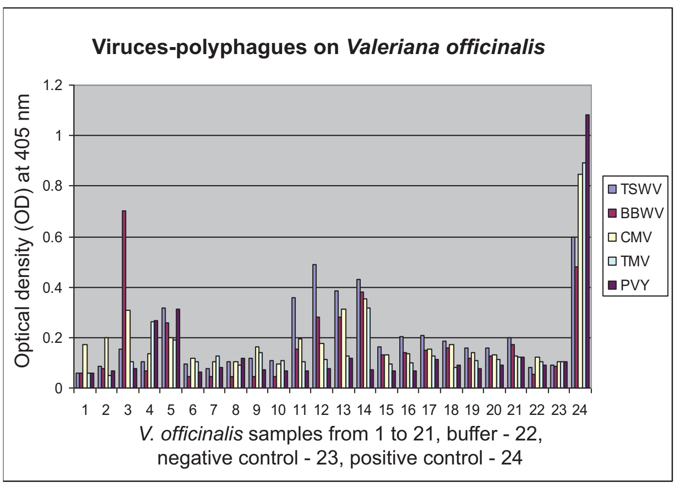


**Fig. 2. Establishment of some viruses – polyphagues on *Valeriana officinalis* with DAS-ELISA**

Note – Antiserum to *Broad bean wilt virus I* (BBWV I) is used

**Fig. 3. Leaves of *Valeriana officinalis* with symptoms of bright white elongated and irregular spots caused by the mixed infection of TSWV and two or three viruses**

**Fig. 4. Yellow mosaic symptoms on the leaves of *Lophanthus anisatus* cv. Syniy veleten**

**Fig. 5. Symptoms of viral infection on *Lophanthus anisatus* cv. Syniy veleten (left); healthy plants (right)**

Spherical viral particles 110 ± 10 nm in diameter were detected in *Lophanthus* leaves (Figure 6).

Viral particles presented on the Figure 6 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 (Virus Taxonomy, 2012). The typical member of the genus is *Tomato spotted wilt virus* (TSWV), serologically relative – *Impatiens necrotic spot virus.*

Based on other scientists' data and on our results we tested giant hyssop plants on the presence of TSWV and a few viruses (Figure 7).

So, the sherical virions in giant hyssop were identified as TSWV (Figure 6). ELISA test demonstrated that CMV, AMV, ArMV, INSV, TAV are not presented in tested samples. It is first report about TSWV infection of giant hyssop plants (Figure 7).

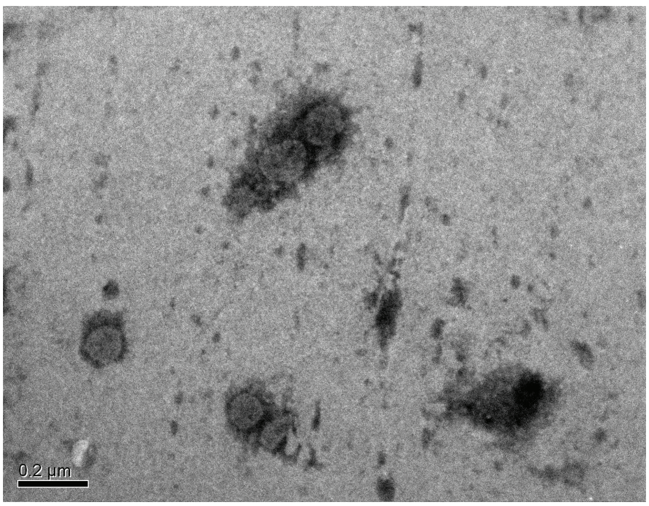


**Fig. 6. Spherical virions detected in the *Lophanthus anisatus* leaves**

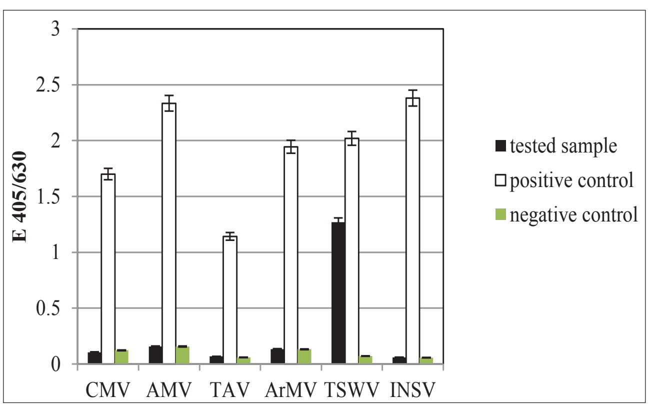


**Fig. 7. Viruses detection by ELISA**

This virus is revealed on a giant hyssop in Ukraine for the first time, and in Bulgaria the virus circulates on common valerian already not first year. Therefore in both countries the efficient system of protection of these medicinal cultures is necessary. Despite the use of these plants for the production of dietary supplements and herbal remedies that are used by man, in the system of protection of these cultures may not be harmful chemical compounds. Therefore the most optimal and safe plant protection measures of TSWV are: monitoring plantations for thrips and TSWV; elimination of plants serving as reservoirs of virus and isolation of medicinal crop from them; thrips treatment. TSWV reservoirs are: solanaceous (tobacco, potatoes, tomatoes, eggplant, capsicum), leguminous (liupin, pea, beans) species of *Asteraceae* family susceptible to TSWV: dahlia, chrysanthemum, gerbera, calendula, lettuce. We recommend growing up medicinal crops separately from tomatoes and dahlia that are the main hosts of the virus. Also it is impossible to land nearby an onion as it is a place of a tobacco thrips location. The weeds-reservoirs for TSWV are: *Amaranthus spinosus, Arctium lappa, Bidens pilosa, Chenopodium album, Ch. murale, Tropaeolum majus, Capsella bursa-pastoris, Melilotus officinalis, Solanum nigrum, Stellaria media, Sonchus oleraceus.* Dikova (2013) has established TSWV on large spread weed from *Asteraceae* family *Cirsium arvense.*

## Discussion

*Tomato spotted wilt virus*, family *Bunyaviridae,* causes serious diseases of many economically important plants. TSWV is now ranked at 2$^{nd}$ place of the top ten plant viruses based on scientific/economic importance (Scholthof et al., 2011). A distinguishing feature of TSWV is the large number of species it can infect: from 150 reported in 1968, to 650 in 1994 and to about 1100 species from more than 80 families in 2003 (Peters, 2003). Increase in quantity of plants sensitive to TSWV is observed in recent years too. It is explained by some features of epidemiology of this virus. There are innumerable infected weeds that serve as reservoirs for primary infection (Tutina et al., 2012). These plant sources perpetuate TSWV are important habitats for thrips vectors and serve as foci for subsequent movement and infection of susceptible crop plants (Groves, 2002). TSWV is transmitted in a persistent and propagative manner exclusively by thrips *Frankliniella occidentalis, F. bispinosa, F. cephalica, F. gemina. F. fusca, F. intonsa, F. schultzei, F. setosus* and *Thrips tabaci* (Ullman et al., 2002; Virus Taxonomy, 2012). Therefore in the paper we submitted recommendations about protection of medicinal cultures from TSWV, which fundamental unit is removal of plants which serve as reservoirs of infection (the list of these species of plants is provided).

It is known that TSWV produces a variety of disease symptoms including a range of chlorotic, necrotic, stunting and enation symptoms in all parts of the plant. Symptoms of TSWV infection is depending from plant species and virus strains. In our paper symptoms of TSWV infection on giant hyssop and common valerian plants are in details described because it is important for diagnostics and well-timed identification of disease.

Our data on infected of medicinal plants with TSWV coincide with results of other scientists. In particular, circulation of TSWV on plantations of medical cultures is set in a few countries of Europe. In Lithuania mixed infection of TSWV and INSV were revealed in purple coneflower plants (Samuitienė et al., 2003). In Hungary TSWV affect purple coneflower plants in co-infection with CMV, TMV and PVY (Horvath et al., 2006). In Italy TSWV identified in valerian plants (Bellardi et al., 1999) and mint (Grieco et al., 2000). Later *Tomato spotted wilt virus* were founded at fist time in Bulgaria in purple coneflower and common valerian plants (Dikova, 2011; Dikova et al., 2013). In Ukraine disease caused TSWV was monitored on production plantation of purple coneflower (Dunich, Mishchenko, 2013). However, it should be noted that identification of TSWV in giant hyssop plants is the first in Ukraine and the world.

## Conclusions

For the first time TSWV is revealed on giant hyssop plants. Results of the research showed TSWV circulation on the common valerian in Bulgaria the third year in a row. It was established that in valerian TWSV is presented in mixed infection with CMV, TMV, PVY or BBWV.

- It was investigated that TSWV isolates from giant have a spherical form and diameter about 110±10 nm, which are characteristic for classical TSWV strains.
- In the paper the detailed description of symptoms of TSWV disease for both cultures and the recommendation about protection against this virus in Bulgaria and Ukraine are given.

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