ESSENTIAL OIL-BEARING AND MEDICINAL PLANTS – NEW HOSTS OF TOMATO SPOTTED WILT VIRUS IN BULGARIA

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ABSTRACT

Tomato spotted wilt virus (TSWV) was established in the following essential oil-bearing and medicinal plants: *Centranthus rubber* (L.) DC. – red valerian (Valerianaceae family); *Glaucium flavum* Crantz. – yellow horned poppy (Papaveraceae family); *Melissa officinalis* L. – lemon balm; *Mentha piperita* L. – peppermint; *Mentha spicata* L. – spearmint; *Stachys officinalis* L. – betony (Lamiaceae family) and the weed *Cirsium arvense* (L.) Scop. – thistle (Asteraceae family). These TSWV hosts were newly reported in Bulgaria. The means for control of TSWV vectors – thripses and weeds – reservoirs of virus infection were discussed.

Key words: *Tomato spotted wilt virus, essential oil-bearing (aromatic) and medicinal (drug) plants*

Introduction

Tomato spotted wilt virus (TSWV) is one of the ten most widespread plant viruses in the world. It causes diseases in vegetables, flower and field crops and last, but not least, essential oil-bearing and medicinal plants. Virus diseases, caused by TSWV decrease the yield and deteriorate the quality of the production of all crops. This is also true for essential oil-bearing and medicinal plants that are the raw material for the pharmaceutical, food-processing, perfume and cosmetics industry.

Lemon balm *Melissa officinalis* (L.), pepper mint- *Mentha piperita* and spear mint - *Mentha spicata* (L.) are known for their extensive use in Bulgaria and all over the world. Red valerian - *Centranthus rubber* (L.) DC, yellow horned poppy - *Glaucium flavum* Crantz. and betony - *Stachys officinalis* L. are less known. *C. rubber* is cultivated as a flower, for consumption in soups and salads and as a curative plant like valerian. *G. flavum* contains the alkaloid glaucine and has antiphlogistic and bronchial clearing effect. *S. officinalis* contains essential oil with antibacterial properties.

Some of the following species: *C. rubber*, *G. flavum*, *M. officinalis*, *M. piperita*, *M. spicata* and *S. officinalis* were proven as TSWV hosts in the international phytovirological literature. *C. rubber* is a natural host of TSWV according to Hausbeck et al. (1992). The TSWV hosts in other species were species of the same genus and/or family as those we analyzed. TSWV was found in Australia on one species of betony *Stachys arvensis* and two species of poppy – *Papaver nudicaule* and *P. rhoeas* (Büchen-Osmond, 2002). *Papaver dubium* (Grieco et al., 2000) and *Papaver rhoeas* (Chatzivassiliou et al., 1998) were naturally infected with TSWV. According to Stobbs et al. (1992) TSWV was transmitted with thripses of the species *Frankliniella occidentalis* on *Papaver* sp. Cho et al. (1986) established natural infection of the same virus on *Stachys* sp. *M. officinalis* is cultivated in large areas in Bulgaria and is an important medicinal culture for our country. A natural TSWV infection in lemon balm was proved by Chatzivassiliou et al. (1998) and on *Mentha* sp. according to Marchoux et al. (2000). TSWV was established on *Mentha piperita* by Seither et al. (1991), on *M. arvensis* and on the weed *Cirsium arvense* by Grieco et al. (2000).

The objective of this study was the establishment of naturally TSWV infected hosts, which are new to Bulgaria among the essential oil-bearing and medicinal plants.

Material and methods

We analyzed samples of essential oil-bearing and medicinal plants of different families: *Melissa officinalis, Mentha piperita, Mentha spicata* and *Stachys officinalis* (Lamiaceae family); *Glaucium flavum* (Papaveraceae family); *Centranthus rubber* (Valerianaceae family) and the weed *Cirsium arvense* (Asteraceae family). The samples with and without symptoms of virus diseases, predominantly TSWV were collected from plantations in the trial fields of the Institute of Roses Essential and Medicinal Cultures (IREMC) near Kazanlak, Bulgaria. Each sample from a single plant was analyzed by ELISA method variant DAS-ELISA (Clark and Adams, 1977) with kits, purchased from the German company LOEWE, Biochemica. Except for TSWV the samples were tested for *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) because the last two were often found in mixed infection with TSWV. The extinction values were measured on spectrophotometer SUMAL PE, Karl Zeiss, Jena, Germany at a wave length of 405 nm. All samples, having shown extinctions two and a half times higher than the negative control were considered as virus positive or virus carriers. The negative controls were samples from symptomless healthy plants or negative control from the kits and the positive controls were infected with TSWV indicator (test) plants as well as the positive controls from the kits.

The extinction values (optical density) of the samples were processed by a statistical analysis of Student's criterion, quoted by Lidanski (1988). The average extinction values of optical density for all tested samples as well as standard deviations were calculated. The optical density (OD) or extinction means the logarithm of the ratio between intensity of fallen and passed light via the solutions. The confidential intervals were at a significance rate of $P \leq 0.05$ of Student's criterion. The confidential intervals for the positive and the negative extinction values of the plant samples were presented in Table 1. The indicator method of Noordam (1973) was used for some of the samples, that showed symptoms, resembling TSWV symptoms. TSWV indicator plants *Nicotiana rustica* and *Petunia hybrida* were infected with infectious materials from two neighbor pepper mint plants.

Results and discussion

Tomato spotted wilt virus (TSWV) was established by DAS-ELISA in 26 (35%) of all 74 samples of aromatic and medicinal plants tested (Table 1). The same virus was proven in three samples of the common weed *C. arvense*. TSWV was found in comparatively high virus concentration in essential oil-bearing and medicinal cultures. The average extinction values of samples with positive response to DAS-ELISA were from 0.5 OD to over 1.0 OD (Table 1). The high virus concentration proved the presence of TSWV in lemon balm, two species of mint, red valerian, yellow horned poppy, betony and thistle.

Cucumber mosaic virus (CMV) and *Tobacco mosaic virus* (TMV) were present in mixed infection in most of the samples with positive reaction to TSWV (Table 2). *C. rubber, C. arvense, G. flavum, M. officinalis, M. piperita, M. spicata* and *S. officinalis* plants were infected in mixed infection by TSWV and CMV. Some plants from the species; *C. rubber, C. arvense M. piperita* and *M. spicata* were infected in mixed infection by TSWV, CMV and TMV or TSWV and TMV. When CMV and TMV multiply in high virus concentration, they manifest conspicuous mosaic symptoms on the leaves of the plants. No similar mosaic symptoms were established on the aromatic and medicinal plant species, listed in Table 1. CMV and TMV were multiplied in low, or rarely, average virus concentration in the same aromatic and drug plants together with TSWV and did not cause typical symptoms. Only symptoms of TSWV caused diseases were observed and established by DAS-ELISA. We observed chlorotic spotting on the leaves of pepper mint that spread over the entire laminas at the lower levels of the leaves with resulting necrotic symptoms (Figure 1). Complete chlorosis on leaf laminas were observed on the yellow horned hoppy, naturally infected with TSWV (Figures 2 and 3). Sectorial spottings, like large chlorotic areas were observed in lemon

balm, red valerian, betony and the wild plant thistle, naturally infected with TSWV (Figures 4, 5, 6, 7 and 8).

TSWV originating from pepper mint was diagnosed both by DAS-ELISA and on test plants. Chlorotic spots, turning necrotic, were observed as systemic reaction on *N. rustica* (Figure 9) and local necrotic lesions on *P. hybrida* (Figures 10).

TSWV as a cause of virus diseases is among the economically most important viruses in essential oil-bearing and medicinal plants because their magnificent flowers and racemes are the preferable places for inhabitance of the TSWV vectors – thripses. These insects, were observed several times in the racemes of red valerian. Most often they belonged to the species *Frankliniella occidentalis*. This enables TSWV transmission to neighboring fields with lemon balm, peppermint, spearmint, yellow horned poppy and betony. Thistle plants (*C. arvense*) in fields with red valerian and betony were infected with TSWV. We recommend destruction of this weed species by all means. We should the watch out for the appearance of thripses and any single specimens observed must be destroyed. It would be too late for control after the pests have multiplied into the racemes. The fields, sown or planted with aromatic and drug plants must have space isolation of at least 1000 meters from the fields with vegetable and flower crops.

Conclusion

Tomato spotted wilt virus (TSWV) was established on important essential oil-bearing (aromatic) and medicinal (drug) crops in Bulgaria: lemon balm (*Melissa officinalis*), two species mint (*Mentha piperita* and *M. spicata*), yellow horned poppy (*Glacium flavum*), red valerian (*Centranthus rubber*) and betony (*Stachys officinalis*. They were newly reported TSWV hosts for Bulgaria. The same virus was established in thistle plants (*Cirsium arvense*) as well in plantations with red valerian and betony. The control of diseases, caused by TSWV on aromatic and drug plants should be organized in three prevention directions: space isolation of essential oil-bearing and medicinal plants from vegetable and flower crops; elimination of TSWV vectors – thripses and destruction of weeds – reservoirs of virus infection in and around the plantations.

Table 1 Establishment of TSWV on essential oil-bearing and medicinal plants

Family and species	Total number of tested plants	Plants with TSWV	Optical density OD, confidential intervals for the positive extinction values	Optical density OD, confidential intervals for the negative extinction values
Asteraceae *Cirsium arvense* (L.) Scop. - thistle	3	3	0.918 ± 0.310	0
Lamiaceae *Melissa officinalis* L. – lemon balm	21	5	1.039 ± 0.392	0.080 ± 0.028
Mentha piperita L. – peppermint	23	3	0.769 ± 0.439	0.111 ± 0.017
Mentha spicata L. - spearmint	7	3	0.506 ± 0.212	0.140 ± 0.010
Stachys officinalis L. - betony	5	5	1.068 ± 0.137	0
Papaveraceae *Glaucium flavum* Crantz. – yellow horned poppy	6	6	0.888 ± 0.221	0
Valerianaceae *Centranthus rubber* (L.) DC. – red valerian	12	4	0.597 ± 0.183	0.122 ± 0.011

0 – no samples with measured negative controls for TSWV.
k- control negative for TSWV – 0. 124 OD
k+ control positive for TSWV – 0. 544 OD
PBS-T buffer with 0.2 PVP (Polyvinylpirolidone) and 0.02 OV (Ovoalbumin)– 0.092 OD

Table 2 Comparison between average extinction values for viruses, established on aromatic and medicinal plants

Family and species	Average extinctions for TSWV	Average extinctions for CMV	Average extinctions for TMV
Asteraceae *Cirsium arvense* (L.) Scop. - thistle	0.918	0.442	0.440
Lamiaceae *Melissa officinalis* L. – lemon balm	1.039	0.348	0.327
Mentha piperita L. – peppermint	0.769	0.632	0.284
Mentha spicata L. - spearmint	0.506	0.495	0.310
Stachys officinalis L. - betony	1.068	0.409	0.825
Papaveraceae *Glaucium flavum* Crantz. – yellow horned poppy	0.888	0.268	0.293
Valerianaceae *Centranthus rubber* (L.) DC. – red valerian	0.597	0.262	0.484

k- control negative for TSWV – 0. 124 OD
k+ control positive for TSWV – 0. 544 OD
PBS-T buffer with PVP and OV – 0.092
k- control negative for CMV – 0.077
k+ control positive for CMV – 0.767
PBS-T buffer with PVP and OV - 0.052
k- control negative for TMV – 0.110
k+ control positive for TMV – 1.137
PBS-T buffer with PVP and OV – 0.087

Figure 1. *Mentha piperita* – to the right - two leaves in which TSWV was established with symptoms of chlorotic spots, turning to necrotic on large areas.

Figure 2. *Glaucium flavum* –chlorotic leaf to the right, in which TSWV was estsblished to the left – symptomless leaf
Figure 3. Plantation of *Glaucium flavum*

Figure 4. *Melissa officinalis* with chlorotic spotting of top leaves, in which TSWV was proven
Figure 5. Plantation of *Melissa officinalis*

Figure 6. *Centranthus rubber*-to the right: leaf with chlorotic spotting turning to necrotic, in which TSWV was proven

Figure 7. *Stachys officinalis* – leaves with chlorotic and necrotic spotting, in which TSWV was proven

Figure 8. *Cirsium arvense* – leaves with chlorotic and nerotic spotting, in which TSWV was proven

Figure 9. Systemic symptoms on *Nicotiana rustica*, test plant for TSWV (artificial infection with TSWV)

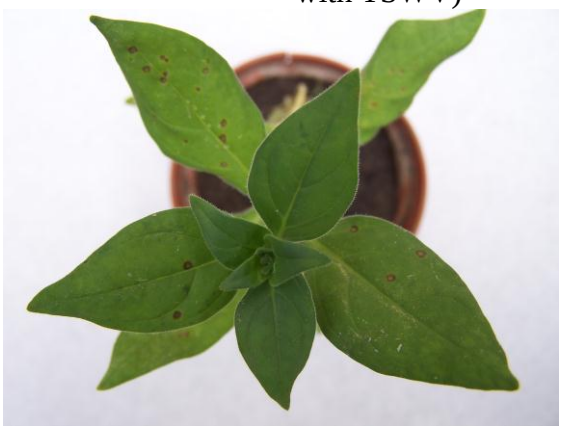

Figure 10. Local necrotic lesions, caused by TSWV on test plant *Petunia hybrida*. (artificial infection with TSWV)

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