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TOMATO SPOTTED WILT VIRUS ON SOME MEDICINAL AND ESSENTIAL OIL-BEARING PLANTS IN BULGARIA

B. DIKOVA
Plant Protection Institute, BG - 2230 Kostinbrod, Bulgaria

Abstract

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In the period spring – autumn 2009 – 2010 samples of 18 medicinal and essential oil-bearing (aromatic) species were analyzed for *tomato spotted wilt virus (TSWV)* infection. They were collected from the territory of the Institute of Roses, Essential and Medical Cultures (IREMC) near Kazanluk, Bulgaria and one private garden.

TSWV was established by serological ELISA method (DAS - ELISA) on *Althaea officinalis* L. – marshmallow, *Artemisia absinthium* L. – wormwood, *Echinacea purpurea* (L.) Moench. – purple coneflower, *Foeniculum vulgare,*L., - fennel*, Inula helenium* L. – white elecampane or horseheal, *Leuzea carthamoides* (Willd) DC. or *Rhaponticum carthamoides* (Willd.) Iljin – maral root, *Nepeta cataria* L. – catmint, *Ocimum basilicum* L. - basil, *Salvia officinalis* L. – garden sage, *Salvia sclarea* L. – clary sage, *Thymus vulgaris* L. – thyme and *Valeriana officinalis* L. – valerian.

TSWV was identified in *Leuzea carthamoides* except by DAS – ELISA and by indicator method with the following test plants: *Chenopodium quinoa, Cucumis sativus* cv. Delikates, *Datura stramonium, Nicotiana glutinosa, Nicotiana rustica, Nicotiana tabacum* cv. Samsun NN, *Petunia hybrida* and *Tropaelum majus*.

TSWV was not established on *Coriandrum sativum,* L.*, Levisticum officinale* (L.) Koch.*., Matricaria hamomilla* L., *Melissa officinalis* L. *Mentha spicata* L. and *Origanum heracleoticum* L. in that study.

This is the first report for *TSWV* isolation and identification from exceptionally important as imuno and energetic stimulator medicinal plant species *Leuzea carthamoides.*

Key words: *TSWV*, DAS – ELISA, medicinal plants, *Leuzea carthamoides*

Introduction

Tomato spotted wilt virus (TSWV) is wide spread plant virus and it has large host range between cultured and wild plant species (Parella et al., 2003).

Tomato spotted wilt virus (TSWV) was proven in a range of host medicinal and essential–oil bearing (aromatic) plants as well as some ornamental and vegetable plants, such as *Chrysanthemum* sp.*, Coriandrum sativum, Mentha piperita, Mentha spicata, Nepeta cataria, Ocimum basilicum, Salvia* sp.*, Valeriana officinalis* and *Verbena officinalis* (Bellardi et al., 1999; Best, 1968; Gardner et al., 1935; Ghotbi et al., 2005; Green and Skotland, 1993; Hamasaki et al., 1994; Knyaseva et al., 1996;

e-mail: b.dikova@abv.bg

Milbrath, 1939; Parrella et al., 2003; Roggero et al., 1998; Samuitiene et al., 2003; Sether et al., 1991). *TSWV* was established only on *Calendula officinalis* L. – marigold from the medicinal plants in Bulgaria (Ivancheva – Gabrovska, 1965).

The objective of this study was the establishment of *tomato spotted wilt virus (TSWV)* on medicinal and essential oil-bearing plants and isolation and identification of the same virus from *Leuzea carthamoides* (Willd.) DC.

Material and Methods

In the period spring – autumn 2009 - 2010 were noticed 18 plant species with conspicuous symptoms of virus diseases, some of them resembled *TSWV* symptoms. The plant species were tested for different viruses and one of them was *TSWV*. A special attention was paid to *TSWV* from *Leuzea carthamoides* (Willd.) DC., isolated in 2009 and identified on test (indicator) plants in 2010 according to Ie (1970).

We analyzed samples of medicinal and essential oil-bearing (aromatic) plants, with symptoms of virus diseases, collected from plantations in the trial fields of the Institute of Roses, Essential and Medical Cultures (IREMC) and one private garden near Kazanluk, Bulgaria. They were *Althaea officinalis* L. – marshmallow, *Arthemisia absinthium* L. – wormwood, *Coriandrum sativum* L. - coriander, *Echinacea purpurea* L. (Moench.) – purple coneflower, *Foeniculum vulgare* L. - fennel, *Inula helenium* L. – white elecampane or horseheal, *Leuzea carthamoides* (Willd.) DC., or *Rhaponticum carthamoides* (Willd.) Iljin – maral root, *Levisticum officinale* (L.) Koch. - lovage , *Matricaria chamomilla* L. - camomile, *Melissa officinalis* L. - lemon balm, *Mentha spicata* L.- spear mint, *Nepeta cataria* L. – catmint, *Ocimum basilicum* L. – basil, *Origanum heracleoticum* (L.) Engl. - oregano, *Salvia officinalis* L. – garden sage, *Salvia sclarea* L. – clary sage, *Thymus vulgaris* L. – thyme and *Valeriana officinalis* L. – valerian. Each sample of medicinal and essential oil-bearing plant species as well as test plants was analyzed by ELISA method (DAS – ELISA); (Clark and Adams, 1977) with a kit purchased from the German company LOEWE, Biochemica. The samples were from spotted young or middle age leaves of the tested plants. The extinction values were measured using a spectrophotometer SUMAL PE, Jena, Germany. All samples with values two and a half times higher than the negative controls were assumed as virus positive. Negative controls were samples of symptomless healthy plants and positive controls – *TSWV* infected indicator plants as well as the positive control from the kit. The extinction values of the samples were processed by statistical analysis of Student's criterion, quoted by Lidanski (1988) at a significance rate of $P \leq 0.05$. The confidence intervals of the positive and negative extinction values for the samples were given in Table 1.

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The *TSWV* isolate from *Leuzea carthamoides* (Willd.) DC. was also studied on indicator plants, using the indicator method (Noordam, 1973).

Results and Discussion

The results for *TSWV* establishment in different medicinal and essential oil-bearing plant species by DAS – ELISA are presented in Table 1. *TSWV* was established in twelve plant species. They were: *A. officinalis* L., *A. absinthium* L., *E. purpurea* (L.) Moench, *F. vulgare L, I. helenium* L., *L. carthamoides* (Willd) D.C., *N. cataria* L., *O. basilicum* L., *S. officinalis* L., *S. sclarea* L., *T. vulgaris* L. and *V. officinalis* L. The plants that were TSWV carriers (positive samples) were 36 (32.7 %) from 110 the total number of tested samples, belonging to the twelfth species. The plants TSWV carriers were 24.3 % to the total number of all 148 tested samples from the eighteenth analyzed species. *TSWV* was not established on *Coriandrum sativum* L., *Levis-*

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

Plants species	Total number of samples	Samples with *TSWV*	Optical density (OD) Confidential interval, obtained after analysis of the positive extinction values for TSWV	Optical density (OD) Confidential intervals, obtained after analysis the negative extinction values for *TSWV*
Althaea officinalis L.	5	4	0.390* ± 0.162**	0.100* ± 0.01**
Artemisia absinthium L.	8	2	0.395 ± 0.062	0.098 ± 0.027
Echinacea purpurea (L.) M.	11	3	0.333 ± 0.071	0.056 ± 0.013
Foenicilum vulgare L.	22	9	0.419 ± 0.068	0.089 ± 0.022
Inula helenium L.	6	2	0.265 ± 0.194	0.120 ± 0.056
Leuzea carthamoides W.D.C.	17	2	0.472 ± 0.210	0.113 ± 0.015
Nepeta cataria L.	3	2	0.286 ± 0.092	0.104***
Ocimum basilicum L.	5	1	0.231***	0.076 ± 0.023
Salvia officinalis L.	6	3	0.303 ± 0.026	0.119 ± 0.091
Salvia sclarea L.	7	5	0.386 ± 0.117	0.124 ± 0.011
Thymus vulgaris L.	8	2	0.276 ± 0.123	0.115 ± 0.041
Valeriana officinalis L.	12	1	0.256	0.087 ± 0.015

Remark - Positive control for TSWV, purchased from the company LOEWE was zero. 715 OD

Legend:

* - average arithmetic valus of extinction values for the samples with and without TSWV

** - standart deviation

*** - extinction values for one sample in case it is the only one containing TSWV

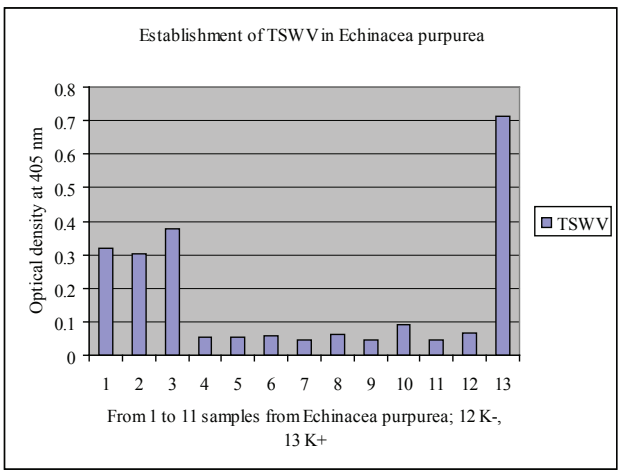

Fig. 1. Results from establishment TSWV in fennel (*Foeniculum vulgare*) plants

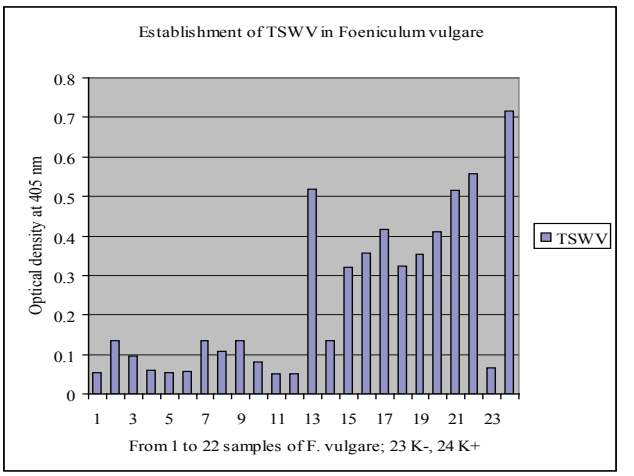

Fig. 2. Results from establishment TSWV in purple coneflower (*Echinacea purpurea*) plants

ticum officinale (L.) Koch, *Matricaria hamomilla* L., *Melissa officinalis* L. *Mentha spicata* L. and *Origanum heracleoticum* L. to date.

Our results confirmed the data for the *TSWV* presence in *Echinacea purpurea*, *Nepeta cataria*, *Ocimum basilicum, Salvia* sp. (in our case *Salvia officinalis* and *Salvia sclarea*) and *Valeriana officinalis* (Bellardi et al., 1999; Gardner et al., 1935; Hamasaki et al., 1994; Milbrath, 1939; Samuitiene et al., 2003).

According to Cho et al. (1986) *TSWV* was proven on celery, but not on *Foeniculum vulgare*. Parrella et al. (2003), quoting Marchoux et al. (unpublished), reported that *TSWV* was experimentally transmitted on fennel and this host showed positive reaction in ELISA test. In our study *TSWV* was present in 9 from 22 *Foeniculum vulgare* plants as natural infections. Fennel was cultivated as annual culture and may be for that reason *TSWV* was present in the samples with numbers from 13 to 22, harvested in autumn, but not in samples from 1 to 12, harvested in spring (Figure 1).

TSWV was wide spread on vegetative propagated and perennial plants (Adkins and Baker, 2005). Some of medicinal and essential oil – bearing (aromatic) plants in that study were such

Table 2
The presence of TSWV and TMV in different leaves of annual shoot of *Nicotiana tabacum* cv Samsun NN from 12.05.2010

Samples of different leaves	Symptoms	Optical density (OD) - Extinction values for viruses at 405 nm	
		TSWV	TMV
*L*eaf from uninoculated Samsun NN sedlling	Symptomless	0.72	0.112
Old leaf	Mosaic and white necrotic figures	0.564	0.060
Old leaf	Light green spots along the veins	0.754	0.066
Middle leaf	Mosaic and white necrotic figures	0.900	0.064
Middle leaf	Symptomless	0.065	0.093
Young (Upper) leaf	Light green spots, deformation	0.713	0.070

Table 3
The presence of TSWV and TMV in indicator plants, inoculated with an isolate of *Leuzea carthamoides*

Indicator plants species	Optical density (OD) - Extinction values for viruses at 405 nm	
	TSWV	TMV
Datura stramonium L.	0.216	0.047
Nicotiana rustica L.	0.354	0.044
Nicotiana tabacum cv. L.Samsun NN	0.324	0.042
Tropaelum majus L.	0.253	0.042

Legend:
TSWV - tomato spotted wilt virus
TMV - tobacco mosaic virus

plants: *Echinacea purpurea*, *Leuzea carthamoides*, *Nepeta cataria*, *Salvia officinalis*, *Salvia sclarea*, *Valeriana officinalis*). *TSWV* concentration in plants from *Echinacea purpurea* was considerable in spring (samples from 1 to 3) then in autumn (samples from 4 to 11), because this virus increased

Table 4

Reaction of indicator plants to *tomato spotted wilt virus (TSWV)* - an isolate of *Leuzea carthamoides*

Family/Indicator plants species	Descriptions of symptoms	Results from tests of indicator plants by DAS - ELISA	
		OD for TSWV samples*	OD for K-**
CHENOPODIACEAE *Chenopodium quinoa* L.	Local chllorotic lesions	0.245; 0.246	0.031
CUCURBITACEAE *Cucumis sativus* L. cv. Delikates	Local chllorotic lesions on cotyledons	0.534	0.065
SOLANACEAE *Datura stramonium* L.	Systemic chlorotic spots, mosaic, ring spots, line pattern proceeding to necrotic	0.465	0.058
SOLANACEAE *Nicotiana glutinosa* L.	local chlorotic lesions, systemic chlorotic spots proceeding to necrotic, Deformation	0.685	0.089
SOLANACEAE *Nicotiana rustica* L.	Local necrotic lesions or latent infection, systemic chlorotic spots, ring spots, line pattern proceeding to necrotic	0.431;0.486;1.709	0.042
SOLANACEAE *Nicotiana tabacum* L. cv Samsun NN	Local necrotic lesions rarely latent infection, systemic chlorotic spots, ring spots, line pattern proceeding to necrotic	1.554; 0.373	0.033
SOLANACEAE *Petunia hybrida* L.	Local necrotic lesions 3-5 days after inoculation; not systemic reaction	-	-
TROPAEOLACEAE *Tropaeolum majus* L.	local latent infection; Systemic chlorotic spots, typical mosaic, ring spots,	1.898; 1.847	0.04

Legend *OD - Optical density - extinction values at 405 nm;
** k - negative controls for *TSWV;*
Positive control for *TSWV* was zero. 585 OD
- not tested

Fig. 3. Systemic necrotic figures on Samsun NN caused by Leuzea isolate of TSWV

Fig. 4. Local necrotic spots on *P. hybrida* caused by Leuzea isolate of TSWV

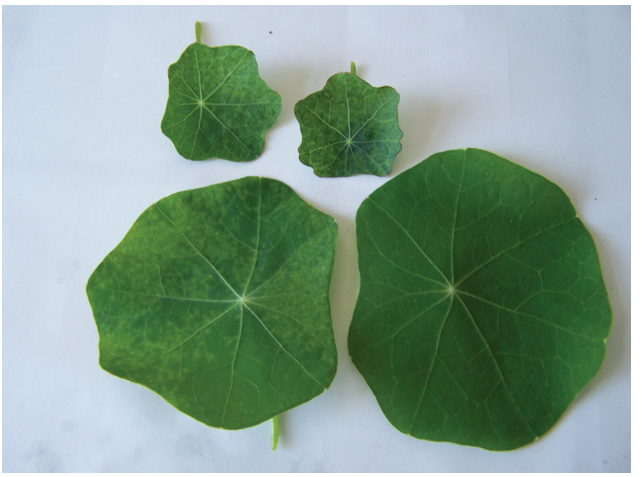

Fig. 5. Systemic chlorotic spots on *T. majus* caused by Leuzea isolate of TSWV; on the right – symptomless leaf

Fig. 6. Systemic ring spot and figures on *N. rusticacaused* by Leuzea isolate of TSWV

itself in rootages of purple coneflower during over wintering (Figure 2). *TSWV* was established on three *Echinacea purpurea* plants, but on one of them symptoms were typical for this virus. These symptoms were chlorotic–necrotic dark red - brown ring spots.

Tomato spotted wilt virus (TSWV), originating from one plant *Leuzea carthamoides* from a private garden near Kazanluk with severe mosaic symptoms on the leaves was identified and isolated in 2010. *Tobacco mosaic virus (TMV)* was identified in the same *Leuzea carthamoides* plant in 2009 (Dikova et al., 2010). More probably, *TMV* and *TSWV* have been present in mixed infection in the *Leuzea* leaves with severe mosaic symptoms but the second virus was in low virus concentration in 2009. So *TMV* was identified by the local reaction of the indicator plants *Datura stramonium, Nicotiana glutinosa* and *Nicotiana tabacum* cv. Samsun NN (Dikova et al., 2010). These plant species reacted systemically to a *TSWV* isolate of *Leuzea carthamoides* in 2010. In early spring

2010, we noticed systemic reaction on the leaves of vegetative grown annual shoot of *N. tabacum* cv. Samsun NN, infected directly from the plant *Leuzea carthamoides* in the preceding year. *TSWV* probably increased its virus concentration after over wintering 2009-2010. The extinction value for TSWV accounted by DAS – ELISA in material of the annual Samsun NN shoot was 1.554 optical densities (OD) from 23.04.2010. The indicator plants for *TSWV* inoculated with the *Leuzea carthamoides*'s isolate were studied after tests for *TMV* content (Tables 2 and 3).

TMV was missing in the leaves from different floors of the annual shoot, grown on the stem of the Samsun NN plant and in the indicator plants, inoculated directly from the annual shoot of *N. tabacum* cv. Samsun NN.

The results from these tests are presented in Tables 2 and 3.

The lack of *TMV* in the Samsun NN annual shoot and in the inoculated with its infectious material indicator plants permitted us to study a *TSWV* isolate of *Leuzea carthamoides.* According to Ie (1970) the following species are diagnostic species for *TSWV*: *Cucumis sativus* L.*, Nicotiana tabacum* L cv. Samsun NN*, Petunia hybrida* L. and *Tropaeolum majus* L. They showed similar reaction in our study as well (Figures 3 to 5). The isolate of *TSWV* from *Leuzea carthamoides* manifested necrotic ring spot and other necrotic figures (Figures 3 and 6).

TSWV is the second virus, except *TMV,* isolated from the exceptionally important as immune and energetic stimulator medicinal plant species *Leuzea carthamoides*.

Tomato spotted wilt virus (TSWV) as infection material of *Valeriana officinalis* infected *Datura stramonium*. Systemic reaction such as mosaic symptoms appeared on *D. stramonium* leaves and the extinction value for *TSWV*, tested by DAS - ELISA was 0. 653 OD.

The same virus in the sample of *Thymus vulgaris* had infected the indicator plant *Nicotiana rustica* that reacted with systemic chlorotic spots. The extinction value for *TSWV*, tested by DAS – ELISA, was 0.349 OD. *Thymus vulgaris* is unreported host for *TSWV* to date.

Conclusions

Tomato spotted wilt virus (TSWV) was established on twelve species of important medicinal and essential–oil bearing (aromatic) plants in the present study for the first time in Bulgaria.

The plants TSWV carriers were 24.3 % to the total number of all 148 tested samples from the eighteenth analyzed species.

TSWV was isolated and identified from *Leuzea carthamoides* for the first time in the world.

There is a difference between annual (*F. vulgare*) and perennial (*E. purpurea*) medicinal species about their TSWV status in spring and in autumn.

References

Adkins, S. and C. A. Baker, 2005. *Tomato spotted wilt virus* Identified in Desert Rose in Florida. *Plant Disease,* **89**: 526.

Bellardi, M. G., V. Vicchi, P. Roggero, G. Dellavalle and V. Lisa, 1999. *Valeriana officinalis* – a new host of tomato spotted wilt tospovirus [Emilia – Romagna – Liguria]. *Informatore Fitopatologico*, **49**: 47 – 49.

Best, R. J., 1968. Tomato spotted wilt virus .pp. 65 – 146. In Smith, K. M. &, M. A. Lauffer: Advances in Virus Research, vol. 13, *Academic Press*, New York.

Cho, J. J., R. F. L. Mau, D. Gonsalves and W. C. Mitchell, 1986. Reservoir weed hosts of tomato spotted wilt virus. *Plant Disease*, **70**: 1014 – 1017.

Clark, M. and A. Adams, 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.,* **34**: 475 – 483.

Dikova, B., A. Djourmanski and H. Lambev, 2010. Isolation of Viruses (Polyphagues) from Some Im-

portant Medicinal Plants in Bulgaria. *J. of Balkan Ecology*, **13**: 33 - 45.

Gardner, M. W., C. M. Tomkins and O. C.Whipple, 1935. Spotted wilt of truck crops and ornamental plants. *Phytopathology*, **25**: 17.

Ghotbi, T., N. Shahraein and S. Winter, 2005. Occurrence of *Tospoviruses* in ornamental and weed species in Markazi and Tehran provinces in Iran. *Plant Dis*., **89**: 425 – 429.

Green, R. J. and C. B. Skotland, 1993. Diseases of Mint (*Mentha piperita*) L., *M. cardiaca* Rake, *M. spicata* L. and *M. arvensis* L.). *APS net*, **3**: 23.

Hamasaki, R., H. R. Valenzuela, D. M.Tsuda and J. Uchida, 1994. Fresh basil production. Guidelines for Hawaii.Research Extension Series 154. 630 U S ISSN 0271 – 9916, December 1994. Hawaii Institute of Tropical Agriculture and Human Resources, Honolulu, Hawaii.

Ie, T. S., 1970. C. M. I./ A. A. B. Descriptions of Plant Viruses № 39.

Ivancheva – Gabrovska, T., 1965. *Tomato spotted wilt (Lycopersicum virus 3 Smith)* on tobacco in Bulgaria. PhD Thesis. Plant Protection Institute, Kostinbrod, Bulgaria (Bg).

Knyaseva, N. A., A. O. Sakussilo, L. F. Didenko and A. L. Boyko, 1996. Some property of the virus, isolated from sunflower. *Biopolymers and Sells*, **12**: 72 – 78.

Lidanski, T., 1988. Statistical Methods in Biology and Agriculture. *Zemizdat*, Sofia (Bg).

Milbrath, J. A., 1939. *Tomato tip – blight virus*. P*hytopathology*, **29**: 156 – 168.

Noordam, D., 1973. Identification of plant viruses. Wageningen PUDOC.

Parrella, G., P. Gognolous, K. Gebre – Selassie, C. Vovlas and G. Marchoux, 2003. An Update of the Host Range of Tomato Spotted wilt virus. *Journal of Plant Pathology*, **85**: 227 – 264 (4, Special issue).

Roggero, P., G. Dellavalle, V. Lisa, 1998. *Tomato spotted wilt tospovirus* in two cultivars of *Chrysanthemum frutescens* in Liguria. ISMEA 1999060874 *Colture Protette*, 0390-0444, **27**: 67 – 68.

Samuitiene, M., M. Navalinskiene and E. Jackeviciene, 2003. Detection of *Tospovirus* infection in ornamental plants by DAS – ELISA. *Vagos*, **57**: 38 – 42.

Sether, D. M., J. D. De Angelis and P. A. Rossignol, 1991. First report of *tomato spotted wilt virus* in peppermint (Mentha x piperita). *Plant Dis*., **75**: 644.

Sether, D. M., and J. D. De Angelis, 1992. *Tomato spotted wilt virus* host list and bibliography. Special Report 888, February 1992, Oregon State University, Department of Entomology.

Zitter, T. A., M. L. Daughtrey and J. P. Sanderson, 1989. Vegetable crops. *Tomato Spotted Wilt Virus*. Department of Plant Pathology, Cooperative extension, New York State Cornell University, Ithaca, NY14853, Fact Sheet Page: 735.90.

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