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Cucumber Mosaic Virus on Aromatic and Medicinal Plants of *Lamiaceae* and *Asteraceae* Families

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Abstract

The occurrence of Cucumber mosaic virus (CMV) and the related disease symptoms on aromatic and medicinal plants of *Lamiaceae* and *Asteraceae* families were studied in 2004-2015. The best essential oil plant species belong to these families. Virus identification was done by the serological method DAS ELISA and indicator method of test plants for CMV. The results for CMV detection on fifteen most popular species of the *Lamiaceae* and eight of the *Asteraceae* families were presented. CMV was found on ten of all aromatic and medicinal plant species in over fifty percent of the analyzed plants of each of them. These species were: *Agastache foeniculum, Melissa officinalis, Ocimum basilicum, Origanum heracleoticum, Salvia officinalis, Salvia sclarea, Satureja hortensis, Thymus officinalis* (*Lamiaceae*), *Inula helenium, Serratula coronata* (*Asteraceae*). The control of CMV caused viroses during the cultivation process is crucial for obtaining high yield of prime quality.

Key words: Cucumber mosaic virus on species of *Lamiaceae* and *Asteraceae.*

Резюме

В периода 2004-2015 г. бе проучено разпространението на Cucumber mosaic virus (CMV) и на причиняваните от него симптоми на заболявания при ароматни и медицински растения от семейства *Lamiaceae* и *Asteraceae*, към които спадат най-перспективните за добив на етерично масло растителни видове. Идентифицирането на вируса бе извършено чрез серологичен метод DAS-ELISA и чрез индикаторен метод на тестови за CMV растения. Представени са резултати от установяване на CMV по растения от 15 широко застъпени за отглеждане видове, принадлежащи към *Lamiaceae* и 8 вида – към *Asteraceae.* При 10 от видовете, CMV присъства в над 50% от анализираните от всеки вид растения. Тези видове са: *Agastache foeniculum, Melissa officinalis, Ocimum basilicum, Origanum heracleoticum, Salvia officinalis, Salvia sclarea, Satureja hortensis, Thymus officinalis* (*Lamiaceae*), *Inula helenium, Serratula coronata* (*Asteraceae*). Висок и качествен добив би се получил, ако в процеса на отглеждане се обърне специално внимание върху контрола на причиняваната от CMV вироза.

Introduction

The aromatic and medicinal (curative) plants of the *Lamiaceae* and *Asteraceae* families account for the largest share of essential oil production in the world. The world production of essential oils at the beginning of the 21st century was 11785.8 tons at the value of USD 211.634 million. The species of *Lamiaceae* family produced 10396.2 tons of essential oils, valued at USD 129.195 million (Yankulov, 2000). The species of the following families are the richest in essential oil in the temperate climatic zone, which includes Bulgaria: *Lamiaceae* – 187, *Asteraceae* – 177, *Apiaceae* – 170, *Rosaceae* – 58, *Brassicaceae* – 35 and *Cupressaceae* – 35 (Yankulov, 2000).

Cucumber mosaic virus (CMV) is among the five most common viruses in the world that cause diseases on vegetables, flowers, some field and medicinal cultures. According to Zitter and Murphy (2009), more than 1200 species of over 100 families of monocotyledon and dicotyledonous plants, including vegetables, ornamental, woody and semi woody plants, were CMV hosts. Plant viruses, including CMV, decrease the yield and deteriorate the quality of production (Bellardi *et al*., 2006a,

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2006b; Dikova *et al*., 2013). The CMV caused virus infection has a negative effect on the quality of root extracts from *Echinacea purpurea* L. Moench (Bellardi *et al*., 2001).

Kovachevsky (1965) found CMV in *Hyssopus officinalis* and *Ocimum basilicum* (*Lamiaceae*) as well as *Calendula officinalis* (*Asteraceae*) in Bulgaria. CMV was proven in *Lavandula angustifolia* Mill. (Kobylko *et al*., 2008); *Echinacea purpurea* (L). Moench. (Dikova, 2009; Dikova *et al.,* 2013); *Ocimum sanctum* L., *Salvia officinalis* L. and *Calendula officinalis* (Seth and Raychaudhuri, 1972); *Salvia sclarea* L. (Pisi and Vicci, 1989) and *Mentha* sp., (Sevik, 2012).

The objective of the study was to identify the essential oil-bearing (aromatic) and medicinal (curative) plants that acted as natural hosts of Cucumber mosaic virus (CMV) in Bulgaria.

Material and methods

Essential oil-bearing (aromatic) and medicinal (curative) plants, belonging to *Lamiaceae* and *Asteraceae* families, were analyzed in the period 2004-2015. Samples of single plants or tufts, taken from the trial fields of the Institute of Roses, Essential and Medical cultures (IREMC) near Kazanlak, showed symptoms of the virus disease. Each sample (as well CMV test plants) was analyzed by the ELISA method (DAS – ELISA); (Clark and Adams, 1977) with a kit, purchased from the German company LOEWE, Biochemica. The extinction values were measured using a spectrophotometer SUMAL PE, Karl Zeiss, Jena, Germany. All samples showing values two and a half times higher than the negative controls were assumed as virus positive. The negative controls were samples of symptomless healthy plants and the positive - CMV infected indicator plants as well as the positive control from the kit.

The extinction values (optical density) of the samples were processed by statistical analysis of Student's criterion, quoted by Lidanski (1988). The average extinction values of optical density were calculated as well as the standard deviation. The confidence intervals had a significance rate of $p \leq 0.05$ according to Student's criterion. The confidence intervals of the positive and negative extinction values of the samples are given in Tables 1 and 2. The indicator method of Noordam (1973) was used for the identification of CMV isolates from

Board I. Symptoms of Cucumber mosaic virus (CMV) on aromatic and medicinal plants

Fig. 1. Symptoms of mosaic on *Nepeta cataria*, on the right – symptomless leaf

Fig. 2. Symptoms of mosaic on *Ocimum basilicum* plant

Fig. 3. Symptoms of mosaic and chlorosis on *Satureja hortensis* tuft

Fig. 4. Symptoms of severe mosaic on *Echinacea purpurea* leaves, on the left – symptomless leaf

aromatic and medicinal plants. Besides the serological method DAS-ELISA that allowed for the analysis of a large number of plant species, we also applied the method of test plant infection. The so called indicator method was used for the following CMV infected species: *Chenopodium quinoa* and *Vigna unguiculata* as test plants reacting with local lesions and *Cucumis sativus*, *Nicotiana glutinosa Nicotiana tabacum* as test plants reacting with systemic symptoms according to Franski *et al*. (1979) and Palukaitis and Garcia-Arenal (2003) (Board II, Fig.4 and Board III, Fig.1 and 2).

Results

The symptoms caused by Cucumber mosaic virus (CMV) on the leaves of *Nepeta cataria* (Fig. 1*), Ocimum basilicum* (Fig. 2*), Satureja hortensis* (Fig. 3*)* and *Echinacea purpurea* (Fig. 4*)* were as follows: light to dark green mosaic spots or entirely chlorotic young leaves as on *Satureja hortensis* (Fig. 3; Board 1*).*

CMV infected a large number of plants of each species of *Lamiaceae* family – more than 1/3 and 1/2 of all tested plants (Table 1).

Table 1. CMV in aromatic and curative plant species of *Lamiaceae* family.

Plant species	Total number of samples	Samples with CMV	%	Extinction values (Optical density - OD)	
				Positive extinction values	Negative extinction values
Agastache foeniculum (Purch.) Kuntze - blue giant hyssop	8	4	50.00	0.503 ± 0.109	0.120 ± 0.055
Hyssopus officinalis L. - hyssop	8	3	37.5	0.553 ± 0.220	0.136 ± 0.030
Lavandula angustifolia Mill. - lavender	25	9	36.00	0.666 ± 0.324	0.085 ± 0.024
Melissa officinalis L. - lemon balm	25	16	64.00%	0.278 ± 0.052	0.082 ± 0.017
Mentha piperita L - peppermint	17	7	41.1	0.467 ± 0.176	0.162 ± 0.011
Mentha spicata L - spearmint	13	6	46.15	0.418 ± 0.146	0.124 ± 0.023
Monarda fistulosa L. - bee balm	12	2	16.67	0.767 ± 0.097	0.084 ± 0.025
Nepeta cataria L. - catmint	22	8	36.36	0.472 ± 0.128	0.125 ± 0.022
Nepeta racemosa L. - Persian catmint	17	6	35.29	0.400 ± 0.059	0.124 ± 0.013
Ocimum basilicum L. - basil	18	9	50.00	1.466 ± 0.544	0.106 ± 0.035
Origanum heracleoticum (L.) Letsw. - Greek oregano	11	7	63.64	0.573 ± 0.246	0.080 ± 0.010
Salvia officinalis L. - garden sage	19	10	52.63	0.635 ± 0.206	0.161 ± 0.025
Salvia sclarea L. clary sage	38	19	50.00	0.616 ± 0.141	0.097 ± 0.010
Satureja hortensis L. summer savory	9	5	55.56	0.391 ± 0.146	0.100 ± 0.050
Thymus vulgaris L. - thyme	14	10	71.43	0.417 ± 0.131	0.090 ± 0.034

The high percentage of CMV infected plants was characteristic of almost all species, belonging to *Lamiaceae* family with one exception for *Monarda fistulosa* (Table 1). Of all 256 plants of *Lamiaceae family,* CMV was found in 121 (47.27 %) (Table 1). Some of the *Lamiaceae* species: *Hyssopus officinalis, Monarda fistulosa, Origanum vulgare* ssp. hirtum (Link.) are perspective cultures, recommended to the farmers for 2016 by IREMC. CMV was established in high percentage in the species of both *Lamiaceae* and *Asteraceae* family, except for *Artemisia absinthium* and *Calendula officinalis*, where the infected plants were less than 30% (Table 2). Of a total of 198 analyzed plants of the *Asteraceae* family, eighty three (41.92 %) were CMV carriers.

The rate of CMV infection was 50% or over 50% in ten of the 23 species of both families - eight plant species of *Lamiaceae* and two of *Asteraceae* family. They belonged to the following species: *Agastache foeniculum Melissa officinalis, Ocimum basilicum, Origanum heracleoticum, Salvia officinalis, Salvia sclarea, Satureja hortensis, Thymus officinalis* (*Lamiaceae*), *Inula helenium* and *Serratula coronata* (*Asteraceae*). CMV monoinfection in aromatic and medicinal plant species is not a frequent phenomenon. Usually, CMV is in mixed infection with one or more plant viruses, common for these cultures. Thus, both CMV and Tomato spotted wilt virus (TSWV) monoinfections were found in plants of *Serratula coronata* species as well as mixed infection of both viruses (Figs. 5, 6 and 7; Board 2*)*.

Indicator plant species *Chenopodium quinoa* and *Vigna unguiculata* reacted to CMV infection with local chlorotic, turning to necrotic, lesions and were suitable for diagnosis of CMV isolates from aromatic and medicinal species (Table 3).

The identification and maintenance of multiplying CMV isolates was carried out on indicator plants that responded with systemic symptoms of this virus, namely *Cucumis sativus, Nicotiana tabacum* and *Nicotiana glutinosa* (Table 3 and Figs. 8, 9, 10).

Moreover, the species *Cucumis sativus* served to differentiate the two viruses – CMV и To-

Table 2. CMV in aromatic and medicinal plant species of *Asteraceae* family

Plant species	Total number of samples	Samples with TSWV	%	Extinction values (Optical density, OD)	
				Positive extinction values	Negative extinction values
Artemisia absinthium L. - wormwood	11	3	27.27	0.858 ± 0.560	0.129 ± 0.044
Calendula officinalis L.- pot marigold	12	3	25.00	0.297 ± 0.059	0.087 ± 0.023
Echinacea purpurea (L.) Moench. - purple coneflower	53	24	45.28	1.121 ± 0.290	0.121 ± 0.011
Inula helenium L. white elecampane	8	4	50.00	0.349 ± 0.137	0.110 ± 0.076
Leuzea carthamoides (Willd.) DC - maral root	56	20	35.71	0.419 ± 0.099	0.103 ±0.01
Matricaria chamomilla L. - chamomile	9	4	44.44	0.422 ± 0.401	0.117 ± 0.047
Silybum marianum (L.) Gaerth. - milk thistle	27	11	40.74	0.420 ± 0.120	0.118 ± 0.025
Serratula coronata L. - sickle moon	22	14	64.00	0.299 ± 0.023	0.105 ±0.036

Board II. Mixed CMV and TSWV infection

Fig. 5. Leaves with chlorotic spots and mosaic on *Serratula coronata* plant

Fig. 6. Large bright yellow areas, caused by TSWV on *Serratula coronata* plant

Fig. 7. Dwarfed plant *Serratula coronata with mixed infection of both viruses – CMV and TSWV, on the right - healthy S. coronata*

Fig. 8. Symptoms of CMV on *Cucumis sativus* cv. Delikates- on the left, lack of symptoms on C. sativus cv. Delikates, inoculated with TSWV – on the right

Board III. Symptoms of CMV on test (indicator) plants

Fig. 9. Systemic symptoms on the leaves of *Nicotiana glutinosa* plant

Fig. 10. Systemic symptoms on some leaves of *Nicotiana tabacum* cv. Samsun NN

mato spotted wilt virus (TSWV) that infected the medicinal species *Serratula coronata* in mixed infection (Board II, Figs. 7 and 8). CMV caused systemic mosaic symptoms (Fig.8, left), while TSWV caused no systemic symptoms on the leaves of *Cucumis sativus* (Fig. 8, right).

Discussion

The symptoms of mosaic, spotting, chlorosis and plant dwarfing due to stem and branch shortening affected the yield of drugs in terms of quantity and quality both in CMV monoinfection and in mixed infection with TSWV as well as other virus-

Table 3. Data on the reaction of indicator plants and some host plants to the isolate 11/09 of Cucumber mosaic virus (CMV), originating from *Echinacea purpurea*

Indicator (test) plant	Description of symptoms on leaves	Optical density (OD) at 405 nm (extinction values) for CMV
Chenopodium quinoa	Chlorotic local lesions, gradating to necrotic	*
Cucumis sativus cv. Delikates	Chlorotic local lesions, chlorotic systemic spots and mosaic	2.005
Nicotiana tabacum cv. Samsun NN	Latent local infection, chlorotic systemic spots, mosaic, deformation	1.738, 0.628**
Nicotiana glutinosa	Chlorotic local lesions, chlorotic systemic spots, mosaic, blisters, deformation	1.957, 2.011, 1.248, 2.097 0.683
Vigna unguiculata	Necrotic local lesions	*
Capsicum annuum cv. Sivria	Chlorotic systemic spots, mosaic	1.458
Lycopersicon esculenthum cv. Ideal	Chlorotic systemic spots, mosaic, narrow strand leaves	1.549
Negative control for CMV (K-)	Young symptomless leaf from cucumber	0.063
Positive control for CMV (K+)	Chlorotic systemic spots, mosaic	0.847

*- indicator plants were not tested with antiserum to the corresponding virus
**- more than one test of the indicator plants

es (Dikova, 2010). The yield of leaves, seeds and roots of *Echinacea purpurea* from spotted plants was twice to several times lower in comparison with symptomless plants (Dikova *et al*., 2013). CMV infection in the above aromatic and medicinal plants should be kept under control as they could become a serious source of infection for the vegetables and flowers, cultivated nearby. It is necessary to effect strict control of the CMV vectors, namely, aphids, especially those of the species *Myzus persicae* Sulz during the vegetative season. Spatial isolation from vegetable and flower gardens would be necessary as well.

Conclusion

Cucumber mosaic virus (CMV) was one of the major viral pathogens – agents of diseases in more than half of the individual plants of aromatic and medicinal species, namely 8 species of *Lamiaceae* and 2 species of *Asteraceae* families. They were as follows: *Agastache foeniculum, Melissa officinalis, Ocimum basilicum, Origanum heracleoticum, Salvia officinalis, Salvia sclarea, Satureja hortensis, Thymus officinalis* (*Lamiaceae*) and *Inula helenium, Serratula coronata (Asteraceae)*. The percentage of infection was lower in 7 species of *Lamiaceae* (*Hyssopus officinalis, Lavandula angustifolia, Mentha piperita, Mentha spicata, Monarda fistulosa, Nepeta cataria, and Nepeta racemosa*) and 6 species of *Asteraceae* (*Artemisia absinthium, Calendula officinalis, Echinacea purpurea, Leuzea carthamoides Matricaria chamomilla, Silybum marianum*).

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