Influence of viral pathogens on the market appearance of pepper fruits

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Abstract

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Diagnostic trials for detecting most widespread viruses on pepper fruits were carried out in the Department of Plant Protection of the Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", Sofia, for the period 2012-2017 by ELISA method (DAS-ELISA). Samples from pepper fruits with symptoms of virus diseases were collected from markets near towns Sofia and Plovdiv. Symptoms of virus diseases on pepper fruits were described and the influence of the damages upon the yield was commented. The samples were tested by DAS-ELISA with antisera for seven viral pathogens: *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY) and *Broad bean wilt virus* (BBWV). These plant viruses are widespread on large numbers of agricultural crops and were very important for pepper. *Tomato spotted wilt virus* (TSWV) was established as the basic and most widespread and as an agent responsible for the injuries on pepper fruits. The indicator method with some diagnostic test plants for TSWV, CMV and TMV (ToMV) was used for identification of these viruses. It was proven that the infectious character of TSWV from one year to the other, i.e. in duration of the infection over 8 months, was preserved by freezing through -20°C, as in the flesh of the spotted and deformed pepper fruits, as well as in pepper isolates of TSWV, multiplied in indicator plants (most often on *Nicotiana tabacum* cv. Samsun NN).

Keywords: viruses; pepper; *Tomato spotted wilt virus*; DAS-ELISA

Влияние на вирусни патогени върху пазарния вид на плодове от пипер

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Резюме

Диагностичните анализи за установяване на вирусни патогени, причинители на болестни симптоми по плодове от пипер, бяха проведени в Института по почвознание, агротехнологии и защита на растенията „Н. Пушкаров" чрез серологичния метод ELISA (DAS-ELISA). Плодове от пипер със симптоми на вирусни болести бяха събрани от пазари и търговски вериги от София и Пловдив през периода 2012-2017 г. Описани са симптомите и е обсъдено влиянието на повредите. Проби от напетнените и деформирани плодове от пипер бяха тестирани чрез DAS-ELISA с антисеруми срещу седем вирусни патогена, разпространени в пиперовите насаждения: *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY) and *Broad bean wilt virus* (BBWV). Беше установено, че вирусът на доматената бронзовост – *Tomato spotted wilt virus* (TSWV), е основният и най-разпространен причинител на повреди по плодовете на пипер. Инди-

каторният метод на някои диагностични тестови растения за TSWV, CMV, и TMV (ToMV) бе използван за идентифициране на тези вируси. Доказано бе, че инфекциозността на TSWV от една година за друга, т.е. в срок повече от 8 месеца, се запазва чрез замразяване при -20°C, както в месото на напетнените и деформирани пиперови плодове, така и в пиперови изолати на TSWV намножени на индикаторни растения (най-често на *Nicotiana tabacum* cv. Samsun NN).

Ключови думи: вируси; пипер; вирус на доматената бронзовост; DAS-ELISA

INTRODUCTION

The pepper plants are hosts of a large number of plant viruses – polyphagues, i.e. viruses causing diseases on different agricultural crops. These viruses are: *Alfalfa mosaic virus* (AMV), *Broad bean wilt virus* (BBWV), *Cucumber mosaic virus* (CMV), *Potato virus Y* (PVY), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV) and *Tomato spotted wilt virus* (TSWV) (Kovachevsky et al., 1995; Svoboda and Svobodová-Leišová, 2012). A lot of these viruses were established on pepper crops in Bulgaria (Hristova and Maneva, 2009; Kostova et al., 2003; Dikova, 2014).

Tomato spotted wilt virus (TSWV), family *Bunyaviridae* causes economically important diseases on tomatoes and peppers, decreasing their yield. Parella et al. (2003) have reported significant losses in production of tomato and pepper caused by TSWV, as and its large host range. Dikova (2010, 2011, 2015) has established that TSWV host range includes different plant species, as essential oil-bearing and medicinal plants and they are also included.

TSWV is wide spread in the Balkan peninsula. It was found recently in Serbia (Dekić et al., 2008), Turkey (Kamberoglu, 2011), Montenegro (Zindović et al., 2011) and Bulgaria (Dikova, 2014), but it was well known since last century.

The objective of the research was the establishment of viruses, affecting one aspect of the quality, specially the market appearance of pepper fruits.

MATERIAL AND METHODS

Samples from fruits with symptoms of virus diseases were collected from markets in the vicinity of Sofia and Plovdiv in the period of 2012-2017 as well as several samples from the Maritza Vegetable Crops Research Institute (MVCRI), Bulgaria. The samples were tested by the serological ELISA method – DAS-ELISA, according to Clark and Adams (1977) and by the indicator method using test plants, according to Kovatchevsky et al. (1995) and Noordam (1973). Kits, purchased from the German company LOEWE, Biochemica, were used for the following seven viruses: *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV), *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY) and *Broad bean wilt virus I* (BBWV I). 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, namely, virus carriers. Samples of symptomless healthy plants were used as negative controls, while infected indicator plants were used as positive controls along with the positive controls from the kits. The controls: negative, positive controls are presented on the end of each chart (Figures from 9 to 15). The pepper fruit samples and the controls: negative-uninoculated test plants and positive – inoculated and infected test plants with TSWV and the other viruses are presented on the abscisses of all charts on Figures from 9 to 15. Optical density (OD) is presented on the ordinates. Samples from pepper fruits with symptoms were used also for the inoculations of test plants mainly as diagnostic and propagation indicator plants from the species *Nicotiana tabacum* cv. Samsun NN (Ie, 1970) and the plants from *Capsicum annuum* cv. Sofiyska kapia, as very favourable.

But for the identification of TSWV, CMV and ToMV we used some diagnostic for these viruses indicator plants from the species and according to Descriptions of Plant Viruses. Indicator plant tests were carried out each year from 2012 to 2017 in parallel with the serological DAS-ELISA tests or after DAS-ELISA tests with the reacted positively on the viruses (TSWV, ToMV and CMV) pepper fruits.

For diagnostics of some of viruses in the pepper fruits we used following diagnostic indicator test plants according to the main viral pathogens: for TSWV - *Nicotiana tabacum* cv. Samsun NN, *Cucumis sativus* sv. Levina or cv. Bistrenski and Petunia hybrida (Ie, 1970; Kormelink, 2005); for CMV - *Cucumis sativus* sv. Levina or cv. Bistrenski and *Cucurbita pepo* var. giramonia cv. Isobilna (Palukatis and Garcia-Arenal, 2003); for TMV (ToMV) *Nicotiana tabacum* cv. Samsun and Samsun NN (Zaitlin, 2000). The symptoms, resulting from the response to the viral infection were observed after 3-5 days for the local lesions of the test plants and a fortnight – for the systemic spots and line patterns.

The testing of the samples, originated from the spotted pepper fruits by indicator plants *Nicotiana tabacum* cv. Samsun NN was carried out by the following manner - indicator plants were inoculated with raw saps from grinded with buffer flashes from the spotted pepper fruits № 4, № 7, № 8 and № 9 (Table 1).

Experiments for preservation of TSWV from one year for the next year were carried out and the data were done, because TSWV infected materials were very necessary for our studies about antiphytoviral effect of different substances on this virus. Samples approximately per 1 g from spotted by viruses (most often TSWV) pepper fruits – red long flashy pepper (kapia) were tested by DAS-ELISA each year from 2012 to 2017 and the results for TSWV existence were given on Figures from 9 to 15. After that each red pepper fruit was packed in filter paper and nylon envelope, labeled and frozen in -20°C. The samples from such frozen fruits were tested again by DAS-ELISA after 7-8 months by the reason of our necessity to prepare infected by TSWV indicator plants, i. e. our necessity to provide for the viral multiplication in propagative indicator plants (*Capsicum annuum* cv. Sofiyska kapia and *Nicotiana tabacum* cv. Samsun NN).

RESULTS AND DISCUSSION

The symptoms, provoked by the virus diseases, caused by TSWV on the pepper fruits were chlorotic spots or chlorotic rings (Board I, Figure 1). Chlorotic spots and rings often turned to necrotic rings and irregular patterns (Board I; Figure 2) that deteriorated the market appearance of different varieties of pepper fruits: kapia (Board I, Figure 1) and sivria (Board I, Figure 2). Symptoms typical for TSWV were created by the artificial inoculations on pepper seedlings - *Capsicum annuum* cv. Sofiyska kapia (Board I, Figure 3) and on indicator test plant *Nicotiana tabacum* cv. Samsun NN and showed on Board I, Figure 4. TSWV symptoms are shown on Figures 5 and 6. Local necrotic lesions, caused by

Table 1. Results from DAS-ELISA for the spotted pepper fruits (long fleshy pepper - kapia) in which practically only TSWV existed

№ of the sample from the respective spotted pepper fruit	Testing by indicator plant *Nicotiana tabacum* cv. Samsun NN	Extinction values (Optical Density - OD) from DAS ELISA				
		TSWV	ToMV	AMV	PVY	CMV
№ 4	Systemic necrotic line pattern	1.733	0.106	0.09	0.077	0.159
№ 7	Systemic necrotic line pattern	1.893	0.106	0.073	0.107	0.173
№ 8	Systemic necrotic line pattern	2.103	0.104	0.077	0.102	0.125
№ 9	Systemic necrotic line pattern	2.066	0.084	0.068	0.068	0.165
Negative control	Lack of symptoms	0.091	0.082	0.105	0.122	0.104
Positive control	Systemic necrotic line pattern	0.647	0.849	0.748	1.235	1.165

Legend: Negative control – uninoculated plant of *Nicotiana tabacum* cv. Samsun NN; Positive control – inoculated plant with TSWV, ToMV, AMV, CMV and PVY plants of *Nicotiana tabacum* cv. Samsun NN

Board I. Symptoms of *Tomato spotted wilt virus* (TSWV)

Figure 1. Symptoms of TSWV on red pepper fruit – long flashy pepper (red kapia)

Figure 2. Symptoms of TSWV on green pepper fruit – two fruits on the left; symptomless fruit – on the right

Figure 3. Symptoms of TSWV on pepper - *Capsicum annuum* plant cv. Sofiyska kapia

Figure 4. Symptoms of TSWV on test plant *Nicotiana tabacum* cv. Samsun NN

Figure 5. Local necrotic lesions, caused by TSWV (pepper fruit isolate from 2013) on *Petunia hybrida*

Figure 6. The difference between CMV and TSWV on *Cucumis sativus* cv. Bistrenski: on the left – systemic symptoms of CMV (an isolate from 2012); on the right - lack of systemic symptoms on the plant, inoculated with TSWV (pepper fruit isolate from 2013)

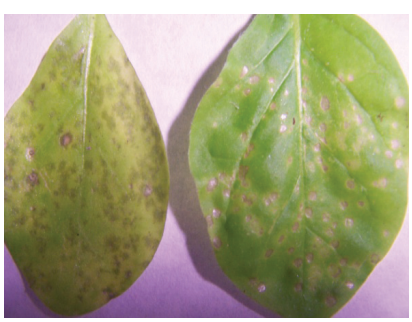

Figure 7. Local lesions on *N. tabacum* cv. Samsun NN leaves, caused by ToMV

Figure 8. Systemic necrotic line pattern on *N. tabacum* cv. Samsun NN leaves, caused by TSWV

TSWV were visible on the leaves of the diagnostic for TSWV indicator test plant *Petunia hybrida* (Figure 5) and lack of any systemic symptoms on *Cucumis sativus* cv. Bistrenski (Figure 6). The difference between TSWV symptoms on *P. hybrida* from one hand and CMV or TMV (ToMV) symptoms from the other hand was that the second symptoms were really systemic even after the first passage. Situation was similar with TSWV and CMV symptoms on *Cucumis sativus* cv. Bistrenski or cv. Levina. The cucumber plants reacted on CMV with visible systemic mosaic (Figure 6, on the left). TSWV caused a few chlorotic/necrotic sporadic lesions on the cucumber cotyledons, without any systemic symptoms (Figure 6, on the right). The difference between TSWV symptoms on one hand and TMV/ToMV symptoms on the other hand on *Nicotiana tabacum* cv. Samsun NN was visibly shown on Figures 7 and 8. TMV/ToMV caused only local lesions without systemic symptoms, but TSWV caused local latent infection or sporadic local necrotic lesions and systemic necrotic line pattern, named "Bronzing".

The description of the appearance of the symptoms, caused by TSWV were confirmed by the TSWV isolates, originated from such pepper fruits in samples 4, 7, 8 and 9 (Figure 15) in which practically only TSWV existed. Each year we have dissociated such TSWV isolates, that originated from spotted pepper fruits with positive results for TSWV by DAS-ELISA and with negative results for ToMV, CMV and others (Table 1).

The following tests by DAS-ELISA and by indicator test plants showed that only *Tomato spotted wilt virus* was responsible for the damages on pepper fruits. The results from DAS-ELISA are presented as charts in Figures from 9 to 15 for the period 2012-2017.

Tomato spotted wilt virus (TSWV) was found in the majority of pepper fruits, tested in 2012 in high or moderate viral concentration from over 0.5 to over 2.0 OD (optical density) (Figures 9 and 10). *Tomato spotted wilt virus* was responsible for the damages on all five pepper fruits from Sofia market net in 2012, because the optical density for TSWV was on the average 0.6 OD. All five viruses – BBWV, CMV, TMV, ToMV and PVY, were presented by negative extinction values with only one exception for PVY (extinction value 0.225 OD) (Figure 9).

TSWV was present in all pepper fruits from Sofia market net, while *Potato virus Y* (PVY) was only present in one pepper fruit (Figure 9). TSWV existed in high viral concentration in pepper samples from Plovdiv market net in the samples 1, 2 and 4 (Figure 10) and in low or moderate viral concentration in fruits originating from MVCRI - samples from 5 to 9 (Figure 10). *Tomato mosaic virus* (ToMV) was second after TSWV, found in six fruits. *Cucumber mosaic virus* (CMV) and *Tobacco mosaic virus* (TMV) were found only in one fruit as PVY. Viruses CMV, TMV and PVY were present only in separate pepper fruits in low viral concentration (Figures 9 and 10).

The results from 2013 showed that TSWV was the major in the pepper fruits again and probably the major causing spotting on these fruits. It was found in moderate viral concentration in 7 fruits, while ToMV was found only in 3 fruits, CMV - in 2 fruits and BBWV (*Broad bean wilt virus*) - in 1 fruit (Figure 11).

TSWV existed in all seventeen red long flashy pepper fruits 2014, being in very high viral concentration in twelve of them - 2.0 to 3.0 OD. The results for TSWV were the consecutive confirmation that TSWV was the major viral pathogen, provoking the injuries as chlorotic and nectotic spots, rings and variegated deformations. ToMV was present in eight of 17 pepper fruits and CMV – in five of them (Figure 12). ToMV was the second viral pathogen after TSWV, which existed in high viral concentration in the pepper fruits, tested in 2014 (but only in 4 of them from a total of 17) (Figure 12). Therefore ToMV was responsible for the body injuries only on separate fruits from the batch, tested in 2014. CMV, like ToMV, can be responsible for the injuries of only separate fruits of the batch in 2014. CMV was found in 5 pepper fruits of 17, but only in 3 of a total pepper fruits in high viral concentration – over 1.5 OD (Figure 12).

The results from 2015 and 2016 were only for the two most spread viruses: TSWV and ToMV (Figures 13 and 14). Our opinion was confirmed by the results for both viruses – TSWV and ToMV in the damaged pepper fruits in 2015 and 2016. TSWV was found either in the majority or even all of the injured pepper fruits from the batches. TSWV was present in high viral concentration in most of the pepper fruits: over 1.5 OD in 2015 and over 2.0 in 2016 (Figures 13 and 14). ToMV was not found in the same pepper fruits. The extinction values showed low viral concentration vs. the negative control and

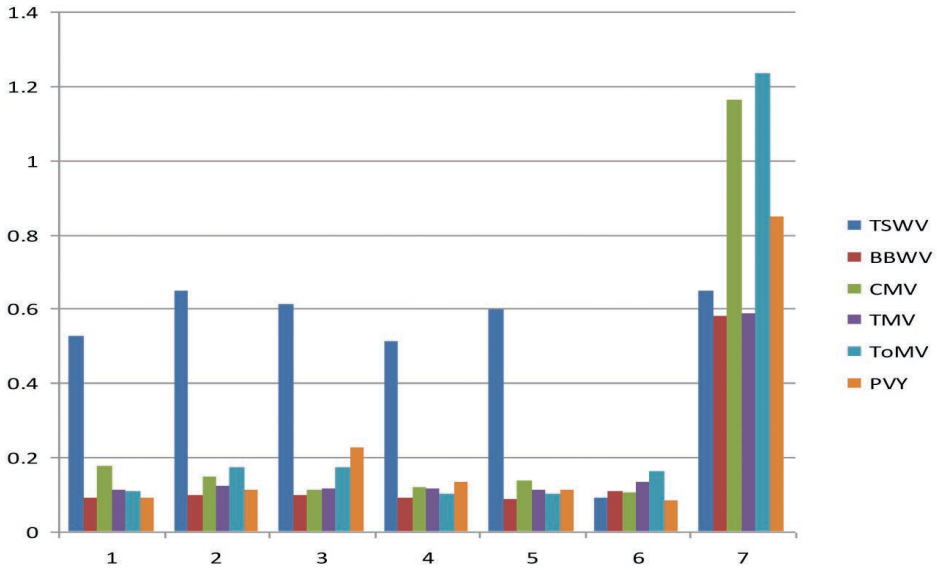

Figure 9. Results of DAS-ELISA tests of pepper fruits with injuries resembling symptoms of virus diseases in 2012 – pepper fruits from Sofia market net

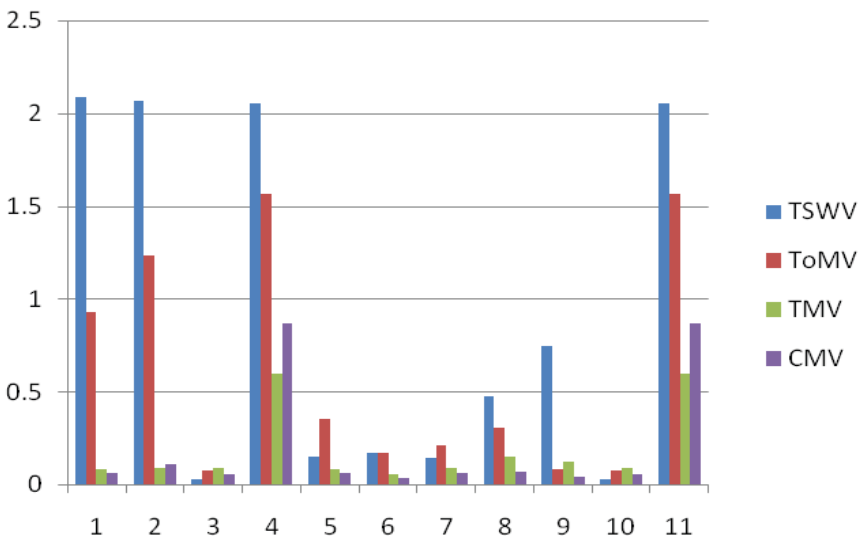

Figure 10. Results of DAS-ELISA tests of pepper fruits with injuries resembling symptoms of virus diseases in 2012 – pepper fruits from Plovdiv market net and MVCRI

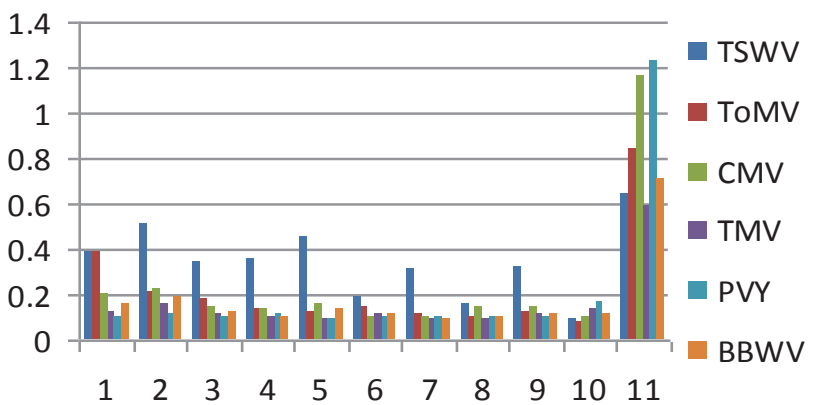

Figure 11. Results of DAS-ELISA tests of pepper fruits from Sofia market net with injuries resembling symptoms of virus diseases in 2013

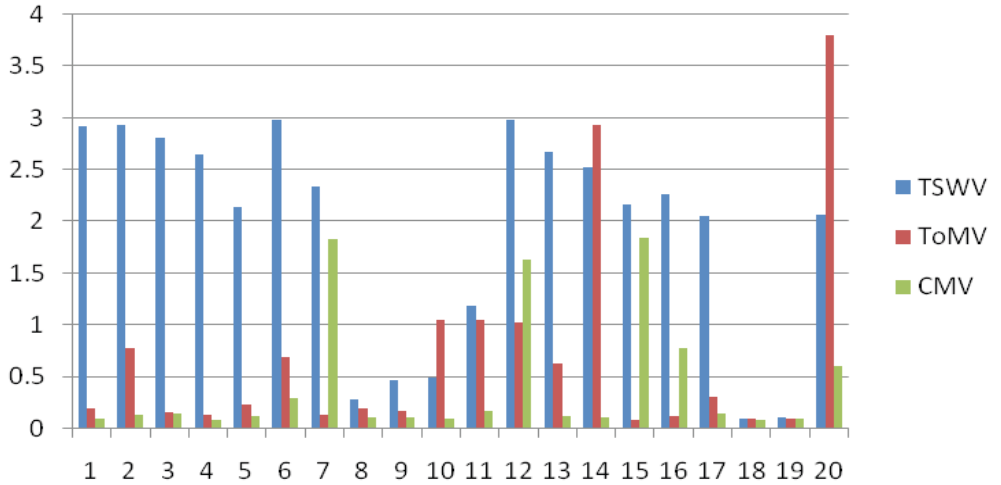

Figure 12. Results of DAS-ELISA tests of pepper fruits from Sofia market net with injuries resembling symptoms of virus diseases in 2014

therefore ToMV was absent in the injured fruits from 2015 and 2016 years (Figures 13 and 14).

Several viruses (TSWV, ToMV, AMV, PVY and CMV) were tested in the pepper fruits again in 2017 (Figure 15).

The last confirmation that TSWV was the causal agent for the injuries of the pepper fruits was received in 2017. The chart on Figure 15 shows that TSWV existed in high viral concentration – over 1.5-2.0 OD in all eleven pepper fruits cv. red long flashy pepper, while ToMV was found in only one fruit in high viral concentration over 1.5 OD (Figure 15). Our results were in accordance with the data of Salamon et al. (2016) about the damaging symptoms, caused by TSWV on the leaves and fruits of different pepper varieties – "kapia", "sivria", "dolma". In our study AMV, CMV and PVY were present in only separate pepper fruits in low viral concentration below 0.5 OD. AMV and PVY existed in only one fruit each and CMV – in 4 fruits. As an exception CMV was in significant viral concentration 1.0 OD only in one fruit (Figure 15).

Potato virus Y (PVY), Potyviridae family, is a very important viral pathogen with regard to potato production. It was found on some important farm crops but it made a sporadic presence in tested pepper fruits with symptoms of virus disease. PVY was present in separate pepper fruits in 2012 (Figure 5) and 2017 (Figure 15). The following viruses had a similar status: BBWV, CMV and TMV (Figures 9, 10, 15).

Some of the pepper fruits were with mixed infection of several viruses, but the majority of the fruits from the market batches were in the individual infection caused by TSWV. The following samples from the spotted pepper fruits were with mixed infection by several viruses in the years from 2012 to 2017: sample 3 - TSWV and PVY (Figure 9); sample 4 - TSWV, ToMV, TMV and CMV; samples 1, 2 – TSWV and ToMV (Figure 10); sample 2 – TSWV, ToMV, CMV and BBWVI (Figure 11); samples 2, 13 – TSWV and ToMV; samples 6, 12 – TSWV, ToMV and CMV; samples 15, 16 – TSWV and CMV (Figure 12); sample 1 - TSWV, ToMV and CMV; sample 2 – TSWV, ToMV, AMV and CMV, sample 10 – TSWV and CMV; sample 11 – TSWV, PVY and CMV (Figure 15).

The visual difference between symptomless and TSWV spotted pepper fruits can be seen on Board II, Figures from 16 to 19. Pepper fruits from cv. red long flashy pepper – "kapia", of one and the same batch are shown on Figure 16 and 17. The symptomless and practically healthy four fruits were with a total weight 688 g (Figure 10), but the TSWV spotted and deformed fruits were with a total weight 283 g, i.e. 2.4 times lower in comparison with the symptomless fruits (Figure 17). The TSWV spotted fruits from of the variety of "chili" pepper were less in quantity and worse in quality in comparison with the symptomless fruit (Figure 18, on the left). The same situation was true for the TSWV spotted fruits of "sivria" pepper – the spotted sivria fruits were smaller, deformed and with unmarket-

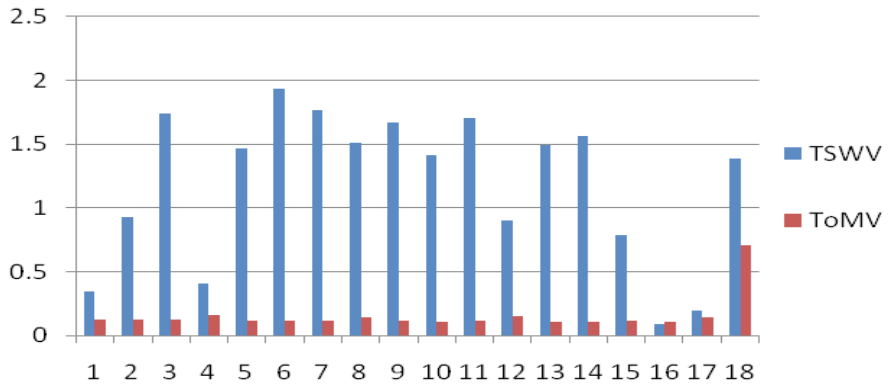

Figure 13. Results from testing by DAS-ELISA of pepper fruits with injuries resembling symptoms of virus diseases – pepper fruits from Sofia market net in a batch from 2015

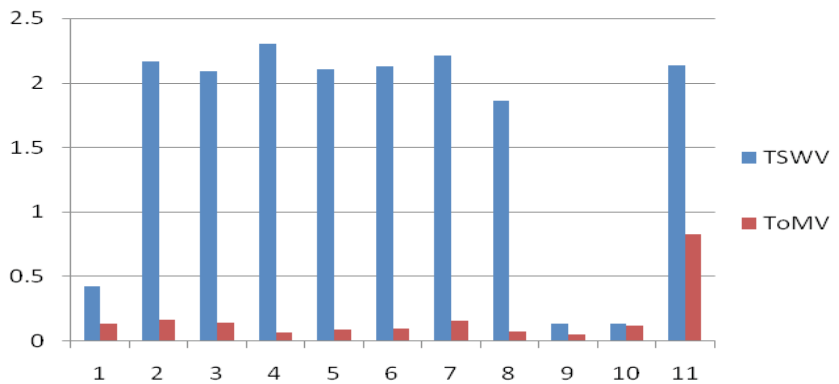

Figure 14. Results from testing by DAS-ELISA of pepper fruits with injuries resembling symptoms of virus diseases – pepper fruits from Sofia market net in a batch from 2016

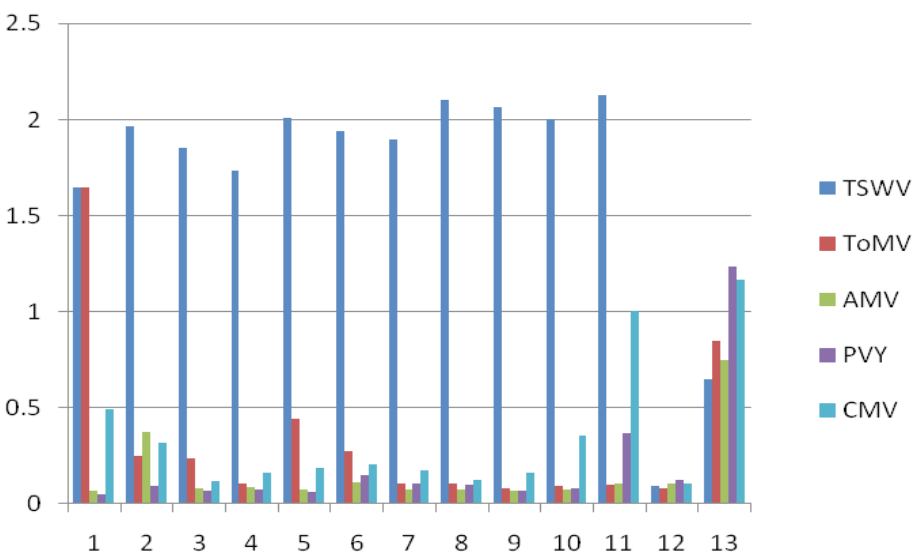

Figure 15. Results of DAS-ELISA of pepper fruits from one batch of Sofia market net with symptoms of virus disease in 2017

able necrosis in comparison with the symptomless one (Figure 19, on the right). Therefore the disease, caused by TSWV deteriorated the market appearance of the market pepper fruits, affecting their quantity and quality. It is possible that such properties as taste, preservation and conservation have been worsened.

The data from 51 spotted and deformed by TSWV pepper fruits showed that the most prevalent in our study was TSWV – in 45 fruits (88%), the second was ToMV – in 22 fruits (43%), the third was CMV – in 13 fruits (26%); (Figures 9, 10, 11, 12 and 15). We have concluded in the determination of the situation of TSWV, ToMV and CMV only Figures 9, 10, 11, 12 and 15 respectively with large number of the tested viruses. The tested pepper fruits from all charts were 74, but the fruits from Figures 9, 10, 11, 12 and 15 were 51. The other viruses: AMV, BBWV, PVY, TMV had sporadic spread in the spotted and deformed fruits from market bathes. Really the presented charts don't show the results for all tested viruses, but only some of them, as AMV (Figure 15), BBWV (Figures 9, 11), CMV (Figures 9, 10, 11, 12, 15), PVY (Figures 9, 11, 15), TMV (Figures 9, 10, 11), ToMV (Figures from 9 to 15) and TSWV (Figures from 9 to 15). The charts for the 23 tested pepper fruits in 2015 (Figure 13) and 2016 (Figure 14) showed that TSWV existed in all 23 fruits in high viral concentration, where as ToMV was absent.

Tomato spotted wilt virus (TSWV) was the main viral pathogen responsible for the injuries as spots and deformations on pepper fruits, belonging to different market batches. The results from the preservation of such fruits in refrigerator UNIFROST at -20°C were given in Table 2.

As shown in the Table 2, the viral concentration of TSWV, expressed by the extinction values for the data "after preservation" in the fridge by deep freezing -20°C were near four times lower in comparison with the data "before preservation" in the

Board II

Figure 16. Symptomless pepper fruits of red long flashy pepper (red kapia)

Figure 17. Spotted and deformed pepper fruits of red long flashy pepper (red kapia) in which TSWV was present in high viral concentration

Figure 18. Chillis pepper – two TSWV spotted fruits (on the right) that are smaller than the symptomless fruit (on the left)

Figure 19. Three TSWV spotted and deformed fruits from variety sivria (on the left) in comparison with the symptomless fruit (on the right)

Table 2. Results from DAS-ELISA for the preservation of TSWV in spotted pepper fruits and plants from *Capsicum annuum* cv. Sofiyska kapia and *Nicotiana tabacum* cv. Samsun NN

Description of the sample	Years of testing	Results from DAS-ELISA		Results from indicator plant tests on *N. tabacum* cv. Samsun NN (infected/ inoculated plants)	
		Before preservation, testing from	After preservation, testing from	Before preservation, testing from	After preservation, testing from
Red long fleshy pepper (red kapia)	2012-2013	*N. tabacum* cv. Samsun NN - 2.063 OD	*N. tabacum* cv. Samsun NN - 0.828 OD	*N. tabacum* cv. Samsun NN - 4/4	*N. tabacum* cv. Samsun NN - 2/4
Nicotiana tabacum cv. Samsun NN, infected in 2012 by a spotted fruit (red kapia)	2012-2013	on *Capsicum annuum* cv. Sof. kapia - 2.197 OD	on *Capsicum annuum* cv. Sof. kapia - 0.647 OD	on *Capsicum annuum* cv. Sof. kapia - 4/4	on *Capsicum annuum* cv. Sof. kapia - 1/4
Red long fleshy pepper (red kapia)	2013-2014	Concentric spot from fresh fruit of red kapia - 2.128 OD	Concentric spot from frozen fruit of red kapia - 0.783 OD	*N. tabacum* cv. Samsun NN - 4/4	*N. tabacum* cv. Samsun NN - 1/4
Red long fleshy pepper (red kapia)	2014-2015	Concentric spot from fresh fruit of red kapia - 2.131 OD	Concentric spot from frozen fruit of red kapia - 1.602 OD	*N. tabacum* cv. Samsun NN - 4/4	*N. tabacum* cv. Samsun NN - 2/4
Red long fleshy pepper (red kapia)	2015-2016	Concentric spot from fresh fruit of red kapia - 2.176 OD	Concentric spot from frozen fruit of red kapia - 1.596 OD	*N. tabacum* cv. Samsun NN - 3/3	*N. tabacum* cv. Samsun NN - 2/3
Red long fleshy pepper (red kapia)	2016-2017	Concentric spot from fresh fruit of red kapia - 2.064 OD	Concentric spot from fresh fruit of red kapia - 1.310 OD	*N. tabacum* cv. Samsun NN - 3/3	*N. tabacum* cv. Samsun NN - 1/3

investigations in 2012 and 2013. We have recemed and tested by DAS-ELISA and by the reaction of TSWV on *N. tabacum* cv. Samsun NN a large number of spotted market pepper fruits especially from the variety "red kapia" in 2015, 2016 and 2017. The average samples from these fruits have shown high viral concentration as well as in fresh and in frozen kind (Table 2). We needed a large quantity of TSWV infected materials for different tests and the way for preservation them from one year to the other was to create their refrigeration. We established that the plants of the propagated indicator species *Nicotiana tabacum* cv. Samsun NN and *Capsicum annuum* cv. Sofiyska kapia as well as the raw flashy materials from spotted pepper fruits were with proven TSWV infection and were susceptible for preservation by deep freezing.

DISCUSSION

The situation with pepper plant samples from the cultivated crops in the fields and samples from pepper fruit market batches are different. For example *Potato virus Y* (PVY) is a very important viral pathogen for the pepper field crops in Czech Republic in the study carried out with 375 leaf samples, tested by ELISA in five years (Svoboda and Svobodová-Leišová, 2012). In our study with pepper fruits from different market bathes TSWV was found as the most prevalent, but PVY made a sporadic presence in tested pepper fruits with symptoms of virus disease. PVY was present in separate pepper fruits in 2012 and 2017. According to Svoboda and Svobodová-Leišová (2012), CMV was the second most widespread after PVY. According our

data CMV existed in 26% from the injured pepper fruits.

Tomato spotted wilt virus (TSWV) is very difficult for the experiments, because it is inactivated in raw sap samples soon after their preparation. The preservation in liquid nitrogen is recommended (Kostova et al., 2003). But it is possible that aggressive strains of TSWV could be appeared on pepper in the last decade and this virus could be able to accumulate and store in high viral concentration in pepper fruits.

CONCLUSIONS

The damages and injuries, caused by virus diseases on pepper fruits, account for the deteriorating of their market appearance, probably concern their quantity and quality and such properties as taste, preservation and conservation.

The major viral pathogen which provoked the damages on the pepper fruits was *Tomato spotted wilt virus* (TSWV).

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