ESTABLISHMENT OF VIRUS PATHOGENS ON THE MEDICINAL PLANT *SALVIA SCLAREA*

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ABSTRACT

The medicinal plant species *Salvia sclarea* (L.) – clary sage is important for the production of raw materials (drugs) necessary for the pharmaceutical and food industry. The virus diseases decreased the yield of leaves (herba) and racemes, because yellow spotted and dwarfed plants (i.e. plants with symptoms of viroses) were often observed in the clary sage plantations. The research was carried out at the Plant Protection Division of the Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Poushkarov", Sofia, Bulgaria. The clary sage samples were collected in the trial fields of the Institute of Rose, Essential and Medicinal cultures near Kazanlak, Bulgaria. Six plant viruses, causing diseases on *S. sclarea* were established in the period 2008 - 2013. They were: *Alfalfa mosaic virus*-AMV, *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) *Broad bean wilt virus* (BBWV) and *Potato virus Y* (PVY). These viruses were identified by ELISA method (variant DAS-ELISA) and some of them by the indicator method on suitable test plants. Indicator test plants were used for two of the viruses – CMV and TSWV. The viruses, identified as pathogens for clary sage are also pathogens for many other agricultural crops, so *S. sclarea* could be considered as an important host of different viruses – polyphagues.

Key words: *Salvia sclarea, plant viruses, AMV, BBWV, CMV, TSWV TMV and PVY*

Introduction

Salvia sclarea (L.) – clary sage *Lamiaceae* family is cultivated as a biennial medicinal plant and for use as essential oil containing flavoring. The distillated essential oil is widely used for perfumes and as an aromatic tool for flavoring wines and liqueurs. It is largely used in aromatherapy for lightening anxiety, fright and insomnia.

The medicinal plant species *S. sclarea* is important for the pharmaceutical industry. The virus infections cause diseases that reduce plant yields and deterioration of essential oils and other curative substances' quality. Kovachevsky (1976) used the name musk horse basil for *S. sclarea*. According to the same author, the infection of this medicinal plant species with *Alfalfa mosaic virus* (AMV) causes intensive yellow mosaic, especially on the young leaves. AMV on clary sage in Italy was proven by Bellardi et al. (1999). *Cucumber mosaic virus* (CMV) was established by Pisi and Vicchi (1989) in Italy. *Tomato spotted wilt virus* (TSWV) was proven by Marchoux et al. (2000) in France. AMV, CMV, TSWV and *Tobacco mosaic virus* (TMV) were found in Bulgaria by Dikova (2010, 2011, 2012, and 2013). *Broad bean wilt virus* (BBWV) and *Potato virus Y* (PVY) were established by Bellardi et al. (2001d and 2001b). PVY more exactly the strain $PVY^{N/NTN}$ is spread on vegetable crops and its reservoirs are weeds – *Chenopodium album, Solanum nigrum, Xanthium strumarium* (Petrov, 2012). These weeds are spread in clary sage plantations too.

The objective of the study is the establishment of diseases, caused by widespread virus pathogens on *S. sclarea.*

Material and methods

Samples from clary sage leaves with symptoms of virus diseases were collected from the trial fields of the Institute of Roses, Essential and Medicinal Cultures (IREMC) near Kazanlak, Bulgaria, in the period 2008-2013. The samples were tested by the serological ELISA method – variant DAS-ELISA (Clark and Adams, 1977) and by the indicator method, using test plants (Noordam, 1973). Kits, purchased from the German company LOEWE, Biochemica were used for the following

viruses: *Cucumber mosaic virus* (CMV), *Alfalfa mosaic virus* (AMV), *Broad bean wilt virus* (BBWV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) and *Potato virus Y* (PVY). The extinction values were measured using a spectrophotometer SUMAL PE, Karl Zeiss, Jena, Germany. All samples with extinction 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 – indicator plants infected with the respective viruses, as well as some negative and positive controls from the kits of the company LOEWE. Optical density (OD) or extinction is a logarithm of the ratio between intensity (If) of fallen and passed (Ie) light via the solutions: OD = lg (If / Ie). The optical density was shown by the measured extinction values for the samples. Cut off was the boundary value that served as a threshold of all extinction values over two and a half times higher than the negative controls, i.e. a threshold for all clary sage samples with positive reaction to the viruses.

Results and discussion

The leaves with symptoms of virus diseases – yellow spotting, covered parts or the entire leaf laminas (Board 1, Figure 1) distinguish from the symptomless leaves (Board 1, Figures 2 and 3). The spotting was yellow around the nerves in the low and the middle leaves. We observed light green spotting in the upper leaves, accompanied by crinkling of the top leaves (Board 1, Figure 4). Sometimes the spotting of viral origin is slight and difficult to observe (Board 1, Figure 5 – on the left). Symptoms, characteristic of specific viruses, e.g. bronzing of old leaves caused by TSWV, were observed (Board 1, Figure 6). As is seen on Figure 7 and of Table 1, AMV was present in 40% of the analyzed plants in high viral concentration near to 1.0 and over 1.0 optical density (OD). Regardless of the low percentage of spreading, AMV concentration in clary sage plants was high, which probably contributed to its frequent establishment on this essential oil-bearing plant (Kovachevsky, 1975; Bellardi et al., 1999). The percentage of the CMV infected clary sage plants was the highest in comparison with the other tested viruses – 69% in moderate and high viral concentration – over 0.6 to 2.0 OD (Figure 8 and Table 1). TMV was next with 57% spreading on clary sage plants in a moderate viral concentration of 0.4 to over 0.8 OD (Figure 9, Table 1). TSWV came third after CMV and TMV with 45% spreading in clary sage plants in law viral concentration over 0.2 OD and in moderate viral concentration over 0.8 OD (Figure 10 and Table 1). BBWV showed the lowest percentage - only 13% in comparison with the other analyzed viruses (Figure 11, Table 1). BBWV was found in sample 9 in a viral concentration of 0.7 OD. The lowest percentage of BBWV infected clary sage plants could be explained with the smaller number of hosts as sources of infection by means of aphids as vectors of this virus. PVY was established in only two tested samples of *S. sclarea* plants. PVY was found in two of four samples (Figure 12, Table 1). Additional investigations are necessary to prove whether PVY was present in 50% of the clary sage plants (Table 1). PVY – a strain $PVY^{N/NTN}$ was most widespread in vegetable crops in Bulgaria (Petrov and Lyubenova, 2011). Probably this strain could be also present in essential oil-bearing and medicinal plants in particular in *S. sclarea.* Two of the six viruses with mixed infection were isolated and identified both by ELISA and the indicator method on suitable test plants in the autumn of 2010. So *Cucumber mosaic virus* (CMV) caused systemic mosaic symptoms on the indicator plants – *Cucumis sativus* cv. Levina, *Cucurbita pepo* cv. Beli edri and *Nicotiana tabacum* cv. Samsun NN (Figure 13). *Tomato spotted wilt virus* (TSWV) infected the test plant *N. tabacum* cv. Samsun NN and caused systemic chlorotic, turned into necrotic spotting on middle and top leaves (Figure 14). The spotting character for TSWV was accompanied with severe deformation (crinkling) of the top Samsun NN leaves. The proven viruses as pathogens for clary sage are pathogens for many agricultural cultures too, so *S. sclarea* could be considered as an important host of different viruses – polyphagues.

Conclusion

Six plant viruses, causing diseases on *S. sclarea* were established in the period from 2008 to 2013 in Bulgaria. They were: *Alfalfa mosaic virus*-AMV, *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) *Broad bean wilt virus* (BBWV) and *Potato virus Y* (PVY). These viruses were identified by ELISA method (variant DAS-ELISA) and some of them (CMV and TSWV) by the indicator method on suitable test plants. These viral pathogens on clary sage are pathogens for many agricultural cultures, too. So *S. sclarea* could be considered as an important host of different viruses – polyphagues.

Board I. Symptoms of virus diseases on *Salvia sclarea*

Figure 1. *Salvia sclarea* leaves, spotted by virus diseases

Figure 2. Young plantation of *Salvia sclarea* without symptoms of virus diseases on the leaves

Figure 3. Symptomless leaves of *Salvia sclarea*

Figure 4. Entire plant of Salvia sclarea with leaves, severely spotted by virus diseases and deformed top leaves and flowers

Figure 5. *Salvia sclarea* leaf, slightly spotted by virus diseases – on the left; Symptomless leaf of the same plant species- on the right

Figure 6 Three old leaves with TSWV bronze spotting – on the left, top and bottom Symptomless leaf –on the right

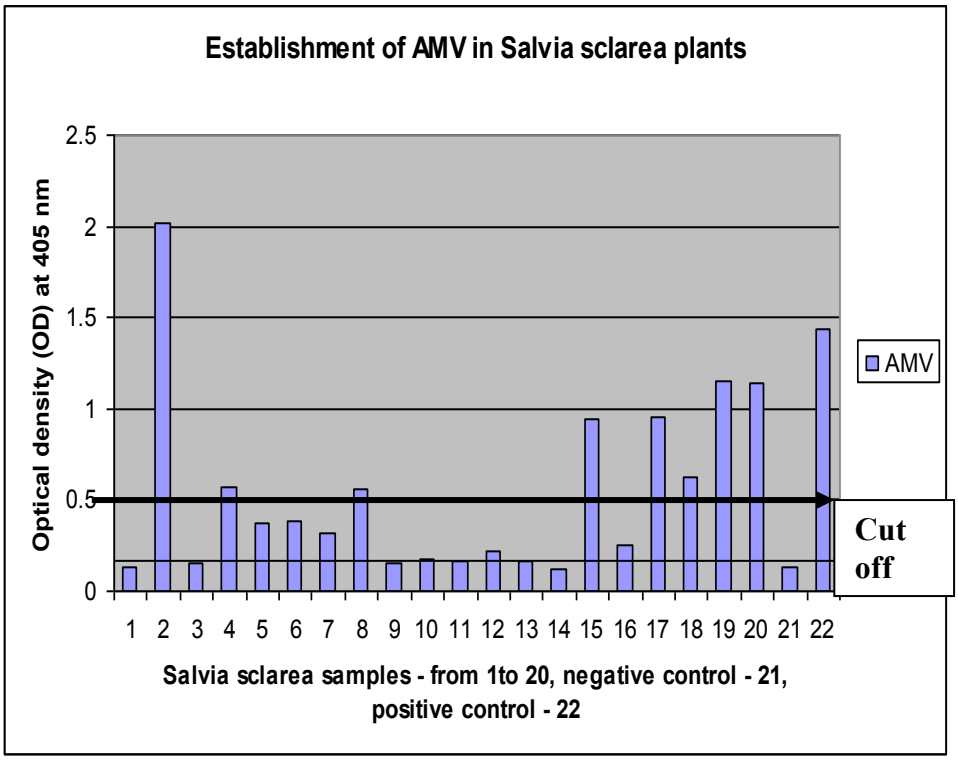

Fig.7. Establishment of *Alfalfa mosaic virus* (AMV) on *S. sclarea*

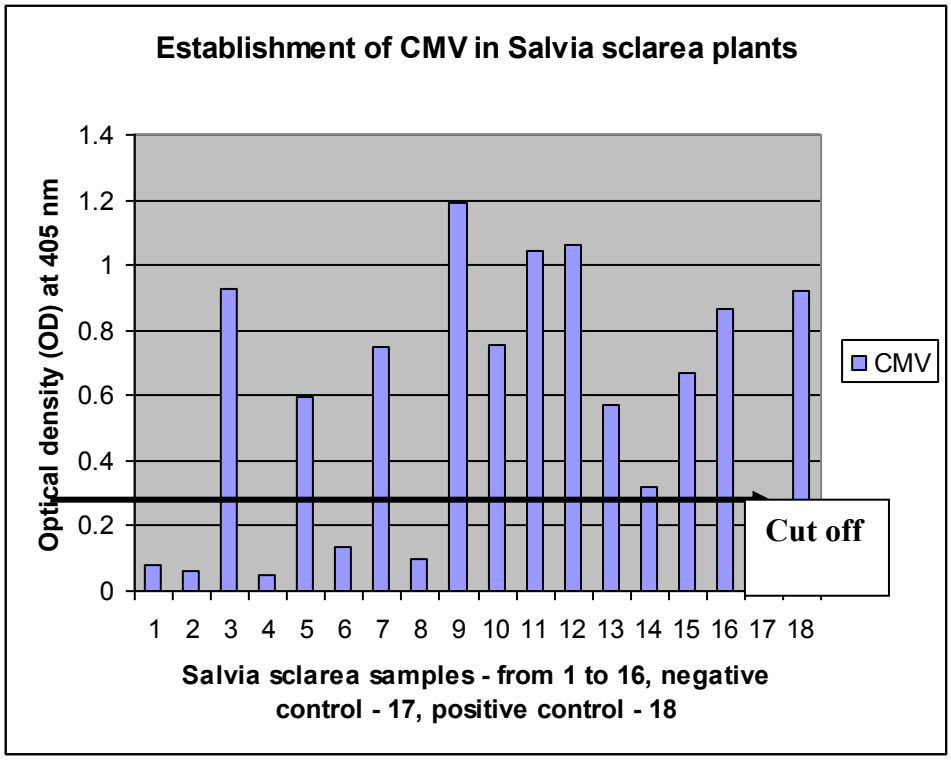

Fig. 8. Establishment of *Cucumber mosaic virus* (CMV) on *S. sclarea*

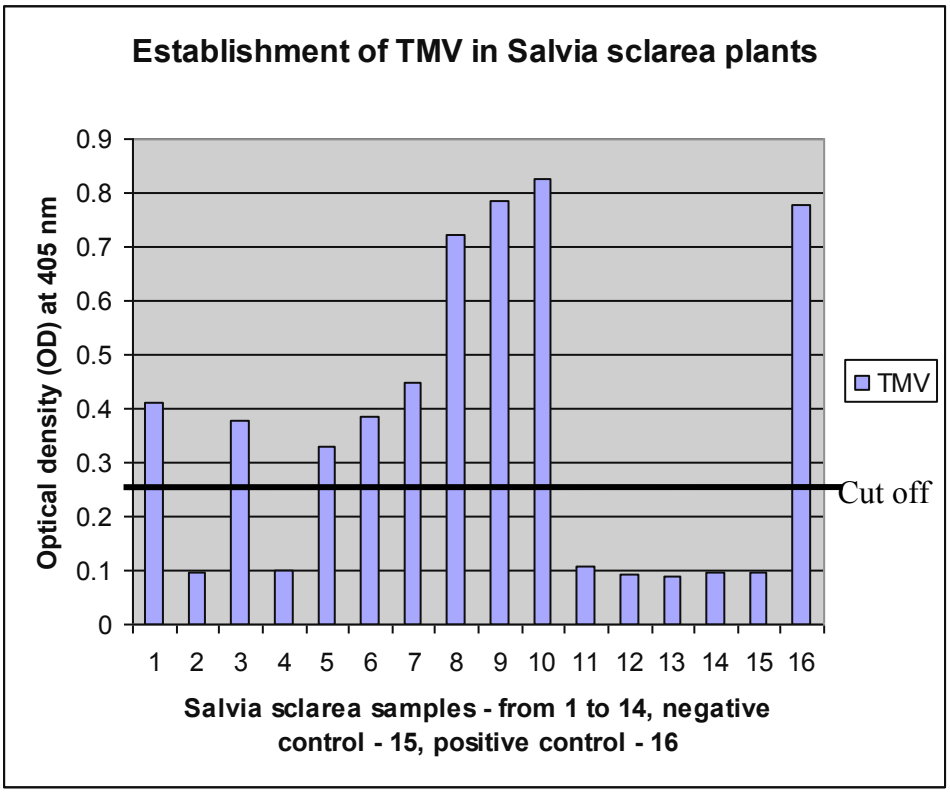

Fig. 9. Establishment of *Tobacco mosaic virus* (TMV) on *S. sclarea*

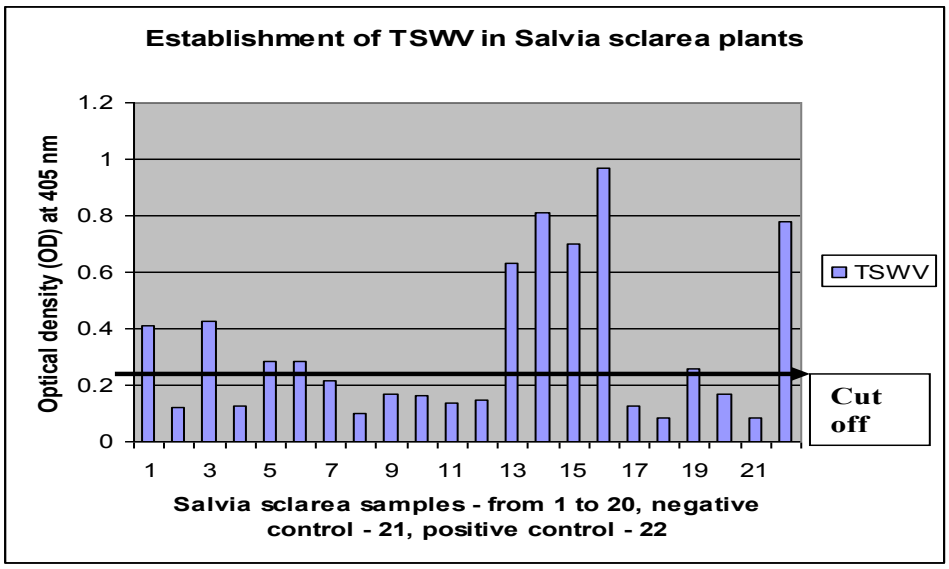

Fig.10. Establishment of *Tomato spotted wilt virus* (TSWV) on *S. sclarea*

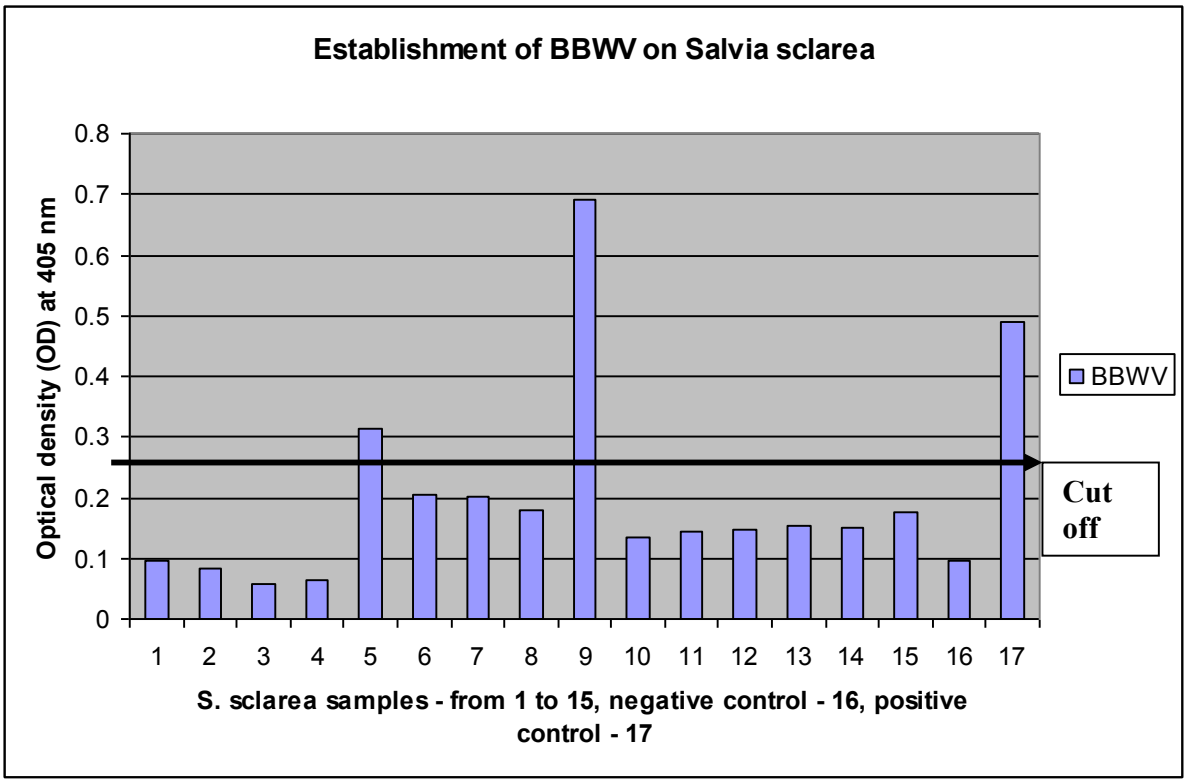

Fig. 11. Establishment of *Broad bean wilt virus* (BBWV) on *S. sclarea*

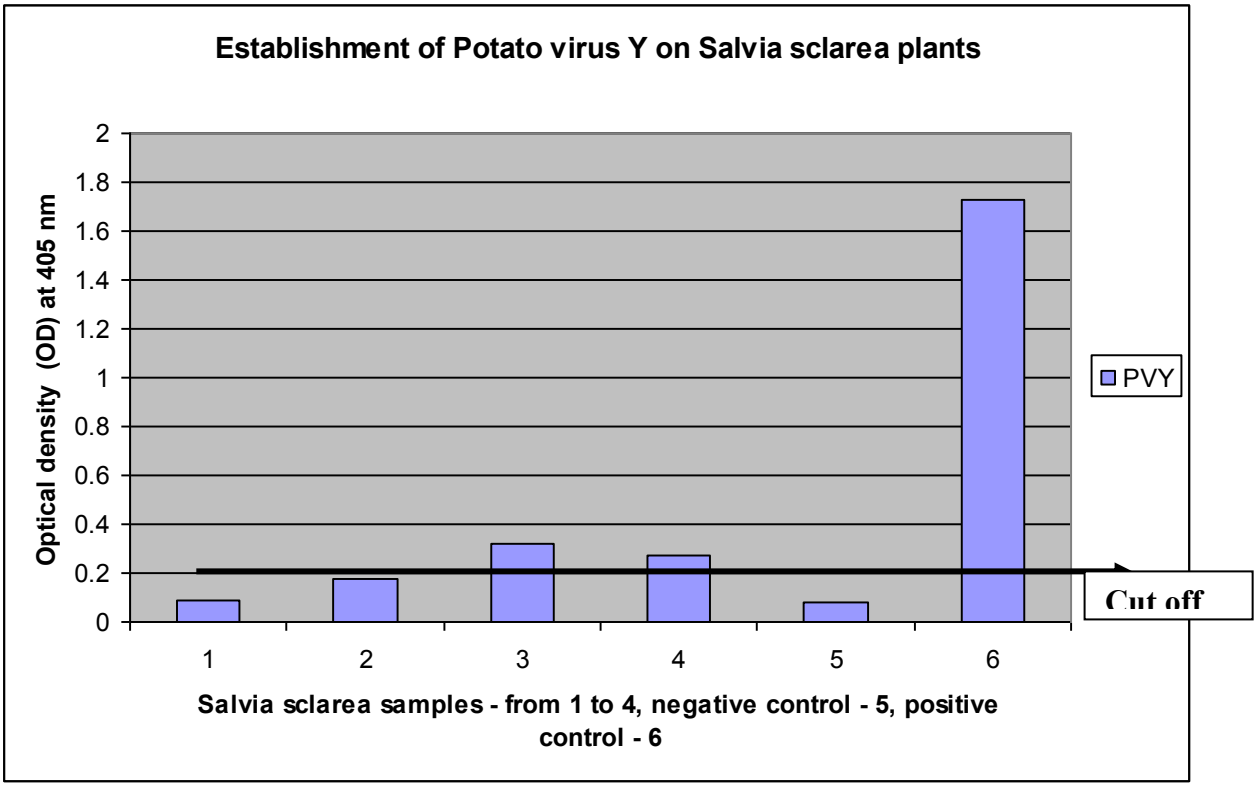

Fig.12. Establishment of *Potato virus Y* (PVY) on *S. sclarea*

Table 1. Establishment of viruses on *Salvia sclarea*

Name of virus	Total number of the analyzed plants	Plants with virus	Percentage of the total number of the tested plants
Alfalfa mosaic virus (AMV)	20	8	40
Cucumber mosaic virus (CMV)	16	11	69
Tobacco mosaic virus (TMV)	14	8	57
Tomato spotted wilt virus (TSWV)	20	9	45
Broad bean wilt virus (BBWV)	15	2	13
Potato virus Y (PVY)	4	2	50

Board II. Symptoms of viruses, isolated from *Salvia sclarea* on test (indicator) plants

Figure 13. Symptoms of mosaic, caused by *Cucumber mosaic virus* (CMV) from left to right on leaves of Nicotiana tabacum cv. Samcun NN, Cucumis sativus cv. Levina, Cucurbita pepo cv. Beli edri.

Figure 14. Symptoms of *Tomato spotted wilt virus* (TSWV) on three tobacco Samcun NN leaves – left; symptomless leaf – on the right

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