

Influence of Virus Diseases on the Yield of the Medicinal Plant *Echinacea purpurea*

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Abstract

Symptoms of virus diseases on the leaves of the medicinal plant species *Echinacea purpurea* (L.) Moench. – purple coneflower were observed in the trial fields of the Institute of Rose, Essential and Medicinal Cultures (IREMC) near Kazanlak and private gardens in Kazanlak, Bulgaria. Virus diseases, caused by the following viruses were established on *E. purpurea*: *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) and *Potato virus Y* (PVY). These viral pathogens existed in *Echinacea* plants in individual or most commonly in mixed infections. The symptoms caused by viral pathogens were spotting (chlorotic spots of different shape and size) on the leaves, sometimes typical mosaic, reduction in leaf size and flower head diameter and especially decrease in the height of the stems, called dwarfing. The results of the study have shown, that the most widely spread were CMV, AMV, TMV - over 45 % and TSWV – nearly 30 %. Clear differences were detected in the yields of roots, seeds and leaves between Group I of symptomless *Echinacea* plants (practically healthy without symptoms or damage) and the spotted *Echinacea* plants (Group II – of spotted and dead plants and Group III - of spotted, but vital plants, in which the viruses were established. The spotted *Echinacea* plants were shorter (dwarfed) in comparison with the symptomless plants with fewer leaves and flower heads. The yield of the seeds, leaves and roots of the spotted plants was twice or several times lower in comparison with the symptomless plants

Keywords: influence on the yield of *Echinacea purpurea* plants, caused by AMV, CMV, TMV, TSWV.

Резюме

Симптомите на вирусни болести по листата на медицинското растение *Echinacea purpurea* (L.) Moench. бяха наблюдавани в Института по Розата, етеричномаслените и медицински култури (ИРЕМК) в Казанлък и частни градини около Казанлък, България. Вирусни болести, причинени от следните вируси: *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV) и *Potato virus Y* (PVY) бяха установени на *E. purpurea*. Вирусните патогени заразяват ехинацевите растения по-често като смесена инфекция от няколко вируса и по-рядко единична инфекция от един вирус. Вирусните патогени причиняват симптоми на напетняване (хлоротични петна с различна форма и големина), понякога типична мозайка, намаляване размера на листните петури и диаметъра на съцветията и специално намаляване височината на стъблата, наречено вджуджаване. Резултатите от проучването показват, че най-широко разпространени са CMV, AMV и TMV – над 45 % в насаждение и TSWV – почти 30 %. Отчетливи разлики в добивите на корени, листа и семена от ехинацевите растения бяха доказани в разпределените по групи растения: Първа група – безсимптомни (практически здрави растения - без вируси или с латентна инфекция, без проява на симптоми), Втора група – напетнени от вирусни патогени растения - изсъхващи и загиващи и Трета група – напетнени от вирусни патогени растения, но жизнеспособни. Напетнените ехинацевите растения бяха с намален хабитус (вджуджени) и с намален брой листа и съцветия в сравнение с безсимптомните. Добивът на семена, корени и листа при напетнените растения беше от два до няколко пъти по-нисък в сравнение с безсимптомните ехинацевите растения.

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Introduction

Virus diseases reduce yields and deteriorated the quality of the agricultural crops. The results of the research showed that the economically important purple coneflower (*Echinacea purpurea*) viruses in Bulgaria are *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV) *Tomato spotted wilt virus* (TSWV) (Dikova, 2011; 2012; 2016; Dikova *et al.*, 2010; 2013). In Ukraine, it was found that the purple coneflower plants were infected with TSWV – in 2012, PVY – in 2013, and TRV – in 2014 (Dunich and Mishchenko, 2015; Mishchenko *et al.*, 2015). CMV, PVY, TMV и TSWV in a mixed infection in only one purple coneflower plant were established by Horvath *et al.* (2006) in Hungary. The most harmful is CMV, which has circulated on the *E. purpurea* plants for several years, because *Echinacea* plants are cultivated for harvesting as a perennial crop.

E. purpurea (L.) Moench.), the most powerful immunostimulating agent among the medicinal plants is widespread in natural habitats in the North of the USA and southern Canada. The soil and climatic conditions in Bulgaria are very suitable for cultivation of this medicinal plant species. Apart from the reduced quantities of the root, leaf and seed yields, *Echinacea* plants affected by viral pathogens have pathogen-induced changes in the chemical composition most probably reflected on the immunostimulating properties of this medicinal crop. According to Bellardi *et al.*, (2001) the concentration of Germacrene D and of some alkylamide components in root extracts of *Echinacea* plants, infected by CMV is significantly decreased in comparison with the root extracts of healthy plants.

The objective of the research was to establish the influence of virus diseases on the yield of the medicinal plant species *E. purpurea* (L.) Moench.

Material and Methods

E. purpurea plants were analyzed in the Department “Plant Protection” in the Institute of Soil Science, Agrotechnologies and Plant Protection (ISSAPP) “Nikola Poushkarov”, Sofia, Bulgaria. The methods of analysis were ELISA (DAS-ELISA) (Clark *et al.*, 1977) and the indicator method using indicator test plants (Kovachevsky *et al.*, 1985). Samples of *Echinacea* plants were collected from the trial fields of the Institute of Rose, Essential and Medicinal Cultures (IREMC), near Kazanlak, Bulgaria and from private gardens

in Kazanlak. Some of the *Echinacea* plants were with symptoms of virus diseases while others were symptomless. Each sample from an individual *Echinacea* plant was analyzed with DAS-ELISA kits, purchased from LOEWE, Biochemica (Germany) for the following viruses: *A. mosaic virus* (AMV), *C. mosaic virus* (CMV), *T. mosaic virus* (TMV), *T. spotted wilt virus* (TSWV) and *Potato virus Y* (PVY). DAS-ELISA tests were carried out according to detailed descriptions in other publications (Dikova, 2012; 2016). The indicator method using differential indicator (test) plants, such as *Nicotiana tabacum* cv. Samsun NN, which was a differential indicator for TSWV, ToMV and CMV is very suitable for visual detection of each of these plant viruses (Dikova, 2016; 2019). In addition to DAS-ELISA some viruses, such as TSWV, ToMV and CMV were also identified by the reaction of the indicator (test) plants: *Nicotiana tabacum*, cv. Samsun NN.

The negative influence of the disease-causing viral pathogens, affecting the quantity and quality of the yield of roots, leaves and seeds from these plants was studied in a three- year *Echinacea* plantation in a private garden near IREMC, Kazanlak. The data for each of these 7 *Echinacea* plants were presented in another paper (Dikova *et al.*, 2013). The present study recapitulates the initial data on the following groups of plants: Group I included two symptomless *Echinacea* plants; Group II were three spotted and perishing plants and Group III were two spotted, but vital *Echinacea* plants without drying.

Results and Discussion

Symptoms of virus diseases were often observed in private gardens, adjacent to vegetable gardens and viral pathogens were afterwards established by DAS-ELISA and the indicator method. The viral pathogens caused damage to the *Echinacea* plants such as stunted plant habitus (mainly height and width of the stems), decrease in leaf laminae and the diameter of the flower heads. Typical symptoms caused by CMV and TSWV viruses on purple coneflower plants originally were spotting, mosaic symptoms (Fig. 1) and dwarfing – decreasing the habitus of the plants. The difference between the symptomless *Echinacea* plants (Fig. 2) and the spotted virus - infected plants (Fig. 3) can be determined by some of these biometrical indices. TSWV caused typical symptoms as like light yellow to bright yellow circular or irregular spots on the middle and lower stages of leaves that subsequently turned into necrotic spots.



Fig. 1. *E. purpurea* leaves with symptoms of mosaic, caused by CMV.



Fig. 2. Symptomless *E. purpurea* plant



Fig. 3. Tuft of *E. purpurea* plants -spotted and dwarfed plants by viral pathogens



Fig. 4. Root sprout, used for propagation and preparation of seedlings



Fig. 5. *E. purpurea* seedlings-spotted by viral pathogens



Fig. 6. *E. purpurea* plant tuft with symptoms of spotted and dried leaves by artificial CMV/ TSWV infection

The mixed infection of several viral pathogens caused dwarfing of some plants (Fig.3) and as a consequence decreased quantity and quality of leaves (herba) and flowers. Symptoms (typical mosaic symptoms) of single infection with *Cucumber mosaic virus* (CMV) on the *Echinacea* leaves are shown on Fig. 1. The purple coneflower plants are often propagated by root sprouts (Fig. 4) and then the incidence of the virus diseases in the adult plants is certain (Fig. 5).

The results on the viruses established in *E. purpurea* plants by DAS – ELISA are presented in Table 1.

CMV was established by DAS-ELISA as well as by the reaction of the indicator (test) plant *N. tabacum* cv. Samsun NN (Fig. 7) and TSWV

was proven by the reaction of the *N. tabacum* cv. Samsun NN too (Fig. 8).

The yield of seeds, obtained from the symptomless *E. purpurea* plants (Group I) was over 20 times higher in comparison with the yields of Groups II and III – plants with symptoms of virus diseases (Table 2). Group I were practically healthy without symptoms and without viruses *Echinacea* plants, or viral pathogens were in very low concentrations and caused latent infection. The yield of roots in Group I – the symptomless (practically healthy) *Echinacea* plants was 11 times higher in comparison with the yield in Group II of spotted and dead plants and 4 times higher in comparison with the yield of Group III of spotted, but vital plants (Table 2). The yield of leaves (herba) from

Table 1. Data about economically important viral pathogens established on *E. purpurea* by DAS-ELISA

Name of viral pathogen	Number of analyzed plants	Number of plants with viruses	% diseased plants in comparison with all tested plants
<i>Cucumber mosaic virus</i> (CMV)	53	24	45.5
<i>Alfalfa mosaic virus</i> (AMV)	36	20	55.5
<i>Tomato mosaic virus</i> (TMV)	12	6	50.0
<i>Tomato spotted wilt virus</i> (TSWV)	11	3	27.3
<i>Potato virus Y</i> (PVY)	32	1	3.13



Fig. 7. Systemic symptoms of CMV on differential test plant *N. tabacum* cv. Samsun NN



Fig. 8. Systemic symptoms of TSWV on differential test plant *N. tabacum* cv. Samsun NN

Table 2. Comparison between the yield of roots, leaves and seeds from symptomless (practically healthy) *E. purpurea* group of plants and virus-spotted *E. purpurea* groups of plants, and biometrical indices influenced by virus diseases

Yield	Yield in g per groups of <i>Echinacea</i> plants			Average yield in g per groups of <i>Echinacea</i> plants		
	Group I – symptomless (practically healthy) plants	Group II – spotted by viruses perishing plants	Group III – spotted by viruses vital plants	Group I – symptomless (practically healthy) plants	Group II – spotted by viruses perishing plants	Group III – spotted by viruses vital plants
Seeds in group	42	3	1	21	1	0.5
Leaves (herba) in group	875	0	195	438	0	98
Roots in group	1565	212	355	783	71	178
Stem height in cm	164	167	125	82	56	63
Number of rosette leaves	39	24	19	20	8	10
Number of stem leaves	390	124	77	195	41	39
Number of flower heads	70	32	32	35	11	16

dead and perishing *Echinacea* plants was zero, and the yield of spotted and vital plants was fourfold lower in comparison with the symptomless plants (Table 2). The biometrical indices in Group I of symptomless plants were higher in comparison with the biometrical indices of the plants in the groups with symptoms of virus diseases. The stem height of Group I of symptomless plants was 26 cm taller in comparison with Group II of spotted and dead plants and 19 cm taller than in Group III of spotted, but vital plants (Table 2). The number of the stem leaves in Group I of symptomless plants was 4 times higher in comparison with Group II of spotted and dead plants and 5 times higher in comparison with Group III of spotted, but vital plants (Table 2).

The flower yield (flower heads) in Group I of symptomless plants was 2,9 times higher in comparison with Group II of spotted and dead plants and twice higher in comparison with Group III of spotted, but vital plants (Table 2).

Wilting and drying, caused by *C. mosaic virus* (CMV) of adult vegetable plants were proven on the field and in greenhouses (Kovachevsky *et al.*, 1985). Perishing *Echinacea* plants with mosaic symptoms, caused by CMV on the leaves were established in *Echinacea* tufts too (Dikova *et al.*, 2013).

The means of virus control in *Echinacea purpurea* crops are preventions of viral invasion by insect pest control of transmitters: aphids for AMV,

CMV and PVY and of thrips for TSWV after investigations of the plantations. TMV control includes phyto-sanitary means against this mechanically transmitted virus.

Conclusion

The results from the studies of the symptoms on virus diseases, caused by CMV, AMV, TSWV, TMV and PVY on the yield showed that the viral pathogens caused serious injuries on the *E. purpurea* plants that has worsened their yield of roots, seeds and leaves in terms of quantity. ELISA method (DAS-ELISA) permitted the analysis of a large number of *E. purpurea* samples for several viral pathogens simultaneously. The indicator method using a differential indicator (test) plant, such as *N. tabacum* cv. Samsun NN, which was a differential indicator for TSWV, TMV and CMV is very suitable for visual detection of each of these plant viruses (Dikova, 2016).

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