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Effective use of the elements of organic farming in medicinal herb cultivation to increase plant resistance the viruses on an example of purple coneflower

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Abstract

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Regardless of its quantity and quality, soil organic matter can have significant effects on soil and plant nutrient content. There are only a few studies focusing on soil properties and viral disease incidence which investigate the contribution of soil or tissue nutrient contents to disease-suppressive effects. But none of them refers to purple coneflower viruses and nutritional elements of this crop. The aim of our study was investigation of infecting of purple coneflower with viruses under conditions of organic farming. An effective use of organic farming element in medicinal plants cultivation on the example of infection of purple coneflower of 1st and 2d year of vegetation with viruses is revealed. The research results showed that the economically important purple coneflower viruses in Bulgaria are AMV, CMV, TSWV, TMV and PVY. In Ukraine, it was found that the purple coneflower plants are infected with TSWV – in 2012, PVY – in 2013, and TRV – in 2014. The most harmful is CMV, which circulated on the *Echinacea purpurea* plants for 8 years. One of the most widespread viruses on the purple coneflower culture in both Ukraine and Bulgaria is CMV. Some properties of isolates of this virus were investigated. Significant yield reducing of coneflower under viral infection is shown. The absence of viral diseases and an increase in the productivity of coneflower plants grown under organic farming conditions as compared to control plants was demonstrated. The necessity of optimal supply of plants with nutrients is shown especially when the soil humus content is no less than 5.2%. This is the first report about the reduction of the infection of *Echinacea purpurea* plants with viruses under conditions of organic farming.

Keywords: medicinal plants; purple coneflower; effectiveness; organic farming; viral diseases
Abbreviations: AMV – *Alfalfa mosaic virus*, CMV – *Cucumber mosaic virus*, PVY – *Potato virus Y,* TMV – *Tobacco mosaic virus*, TRV – *Tobacco rattle virus*, TSWV – *Tomato spotted wilt virus*, ELISA – enzyme-linked immunosorbent assay

Introduction

After the problem of nuclear disarmament the problem of producing quality and safe food for human ranks second. Particularly important component of health preservation is quality plant raw materials for the production of therapeutic and prophylactic phytopreparations. Therefore, the ecologization of farming, along with economic indicators, becomes the main criterion for effectiveness evaluation. In Ukraine, organic farming is officially recognized. In September 2013, the Verkhovna Rada adopted the law "On the production and circulation of organic agricultural products and raw materials". In Ukraine in recent years, areas occupied by organic farming remain unchanged and amount to about 270 thousand hectares. Annual cost of the products of organic origin reaches 5.1 million euros. At the same time, according to the estimates of the Food and Agriculture Organization of the United Nations (FAO), the potential of organic farming in Ukraine is one of the largest in the world, given the natural productivity of the soil.

According to the requirements of organic farming, both in world practice and in Ukraine, cultivation of medicinal plants is most common in small-sized farms, which are unable to compete with powerful producers of traditional agricultural crops. In its turn, for many pharmaceutical companies that specialize in the production of medicinal products of plant origin, it is important to ensure production of raw materials which are ecologically clean and safe for the consumer. Products from such raw materials promote the sale of medicines in the domestic and foreign markets. It is known that more than 40% of medicines are made from plant material (Chekman, 2006). Medicinal plants are also employed in organic gardening.

Today, the natural reserves of many species of wild medicinal plants are sharply reduced due to the intensive impact of technogenic factors such as putting in crop production of natural lands, deforestation, as well as violations of rules and timing of harvesting. For this reason, most of medicinal plants in Ukraine and Bulgaria are grown in culture. This is one of the traditional branches of plant growing. Cultivation of medicinal plants is directly dependent on pests and infectious diseases of fungal, bacterial and viral etiology (Bellardi et al., 2001; Mishchenko et al., 2009; Dikova et al., 2010; Dikova et al., 2013; Glushchenko, 2013), which lead to an economically perceptible decline in crop productivity and deterioration in product quality (Bellardi et al., 2001; Dikova et al., 2013; Glushchenko, 2013; Mishchenko et al., 2015). A special problem of protecting medicinal plants from viral infections is their deficient study in the world, as well as the presence of only few reports of viral diseases of individual medicinal crops in Ukraine (Mishchenko et al., 2009; Dunich and Mishchenko, 2015; Mishchenko et al., 2015) and Bulgaria (Dikova et al., 2010; Dikova et al., 2013; Dikova et al., 2016). Monitoring of the infection of medicinal plants with viral diseases has shown that the purple coneflower plants suffers most from them (Dunich and Mishchenko, 2015; Mishchenko et al., 2015).

An analysis of the literature data showed that in the world purple coneflower (*Echinacea purpurea*) is infected by 10 viruses (Dunich and Mishchenko, 2015; Mishchenko et al., 2015). In Ukraine, the infection of purple coneflower with viral infections was proved by the methods of visual diagnostics, enzyme-linked immunosorbent assay, electron microscopy and polymerase chain reaction. Annual increasing of viral load on plants, variety and severity of symptoms were noted (Dunich and Mishchenko, 2015; Mishchenko et al., 2015). Almost all viruses revealed in purple coneflower are considered to be harmful and economically important. Additionally, some of them (CMV, TSWV, PVY) are in the top 10 of the most economically important plant viruses in the world and occupy the first places. Our researches (Dunich and Mishchenko, 2015; Mishchenko et al., 2015) revealed several new, previously not described and not identified viruses. In Bulgaria, the following viruses in mixed infection were revealed on *Echinacea purpurea*: AMV, CMV, PVY, TMV and TSWV (Dikova, 2011, 2012; Dikova et al., 2010, 2013, 2016).

We drew attention to the fact that fewer infected plants were noted by us on soils fertilized with organic manures. Since coneflower does not have its specialized viruses, its infection depends on the physiological state of plants and optimal provision of soil with nutrients and humus. In recent years, organic farming is becoming more widespread. Soil is one of the main objects of attention in organic agriculture. Technologies for improving soil fertility are called to balance soils physical, chemical and biological characteristics. The generally recognized components of organic farming in the world are crop rotation, cultivation of fodder crops, using of green fertilizers, recycling of waste from plants and animals, energy saving agrotechnologies and dosed application of the necessary minerals (Glushchenko et al., 2016).

The leader of organic agricultural production in Ukraine is the agrarian company "Agroecology" (Shyshaky district of Poltava region). This company is certified in accordance with the requirements of the EU Directive No. 834/2007, which provides state regulation in the field of organic products in the EU member countries, and has conditioned the demand for its products in Ukraine and in many countries of Europe (Organic production; Antonets et al., 2010; 2013). For about 40 years on the fields of agrarian company "Agro-

ecology" pesticides and mineral fertilizers are not used. Using of organic fertilizers (humus, siderates, etc.) provided an annual increase in humus content. According to the data of 2015, the average humus content in the soils of this farm is 5.2%. For comparison, Poltava black soils (Chernozems) today have a humus content of 2.6 to 3.6%. Authors found that in conditions of long-term maintenance of the organic farming system, the threat of damage to the yield of cereal crops through the infection with viral diseases is reduced (Antonets and Mishchenko, 2016). The most widespread and economically important in the Poltava region viruses are *Wheat streak mosaic virus* and *Barley yellow dwarf virus*, affecting winter wheat (Mishchenko, 2009).

Although quantity and quality of soil organic matter can have dramatic impacts on soil and plant nutrient content there are only a few studies which focus on soil properties and viral disease incidence which investigate the contribution of soil or tissue nutrient contents to disease-suppressive effects. But none of them refers to purple coneflower viruses and nutritional elements of this culture.

The aim of our study was investigation of infecting of purple coneflower with viruses under conditions of organic farming.

Materials and Methods

Sample collection in Bulgaria was conducted on the two plantations near Kazanlak city – field near Kazanlak and plantation on experimental field of the Institute of Rose, Essential and Medical Cultures near Kazanlak (IREMK). The experiments were conducted in 2016-2017 in the farm Mercury in Poltava region on typical medium-loam black soil (Chernozem). The control was purple coneflower plants (*Echinacea purpurea* (L.) Moench.) cv. Charivnytsia, which were grown in agrotechnical short-rotation shift of crops for more than 7 years using traditional technologies. Content of micro- and macroelements and humus in the soil were determined by the method of Rinkis and Nollerdorf (1982). 1H HCl was used for extraction of micro- and macroelements. The humus content in the control was 2.6%. In other conditions of the same farm, the elements of organic farming technology were employed. Coneflower seeds of the indicated variety were sown in May 2016. The predecessor was vegetable crop. Earlier on this field for almost half a century (since 1960) there was a pasture with mixed grasses (Samorodov and Pospelov, 1999). In the past eight years, this field has been alternating with crops of alfalfa, cereals, vegetables, black steam. The content of humus in the soil of this field in 2015 was 5.7%. In this area, mineral fertilizers and chemical plant protection preparations were not ever used. In the first two years of coneflower vegetation (2016 and 2017), on the experimental field, manual weeding and loosening of inter-rows was carried out.

Phenological observations and visual inspections of coneflower plants for their infection by viral diseases were conducted regularly (Mishchenko et al., 2015). Identification of viruses has been carried out using the enzyme-linked immunosorbent assay, ELISA (DAS modification), using commercial test systems of Loewe firm, Germany (Clark and Adams, 1977). Leaf samples from healthy plants served as negative controls. Positive controls were commercial (Loewe, Germany). The results were recorded on Termo Labsystems Opsis MR reader (USA) with Dynex Revelation Quicklink software in Ukraine and using a spectrophotometer DTX 880 Detector in Bulgaria at wavelengths of 405 nm. Samples were considered positive when their absorbance values at 405 nm were at least three times higher those of negative controls.

Morphology of virus particles was determined by transmission electron microscopy method. Contrasting has been made with 2% solution of phosphorus-tungstic acid. Virions are investigated using electron microscope JEM 1400 (JEOL, Japan).

The indicator method of Noordam (1973) was used for the identification of CMV isolates from purple coneflower plants. *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 were used.

Statistical analysis of experimental data was carried out by the method of variance analysis (Dospekhov, 1985; Lidanski, 1988) using built-in statistical functions of the application software Microsoft Office.

Results and Discussion

Our inspections of purple coneflower plants during the growing seasons of the first and second years of cultivation showed that in the control coneflower plants were affected with viral diseases up to 15-40%. There were symptoms of mosaics, yellow mottling, corrugation and wrinkling of the leaves (Fig. 1).

Similar symptoms were also observed on the coneflower plants of the third and subsequent years of vegetation. On the plants of five to seven years of cultivation, the symptoms became more severe.

No better situation was observed with viral diseases of purple coneflower in Bulgaria. Monitoring inspections showed that AMV, CMV, TMV and TSWV were spread of plants and caused symptoms of yellow spotting, mottle and

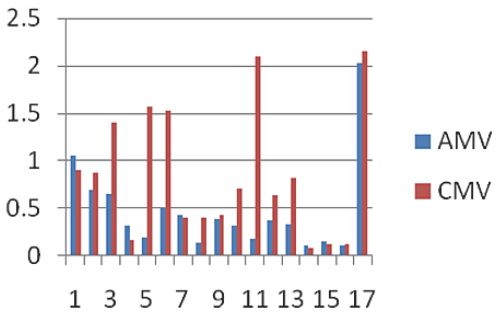
A

B

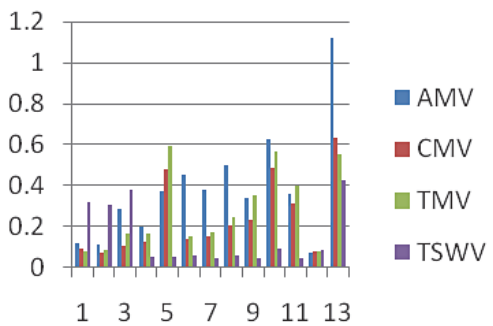
C

D

Fig. 1. Symptoms of viral infections on *Echinacea purpurea* plants: *a* – cv. Charivnytsia, Ukraine, yellow mottling; wrinkling, corrugation, leaves deformation, 2nd year of the vegetation in the flowering stage; *b* – chlorotic mosaic on the coneflower leaves of 1st year cultivation, in the center – healthy leaves; *c* – *Echinacea purpurea* leaves, naturally infected with CMV in Bulgaria, at the left – healthy leaf; *d* – *E. purpurea* plants in Bulgaria in mixed infection with AMV, CMV, TMV and TSWV.



A



B

Fig. 2. Establishment of viruses in *Echinacea purpurea* plants in Bulgaria by DAS-ELISA: *a* – in the vegetable garden in 2009, *b* – in the trial field of the IREMK, Kazanlak in 2010

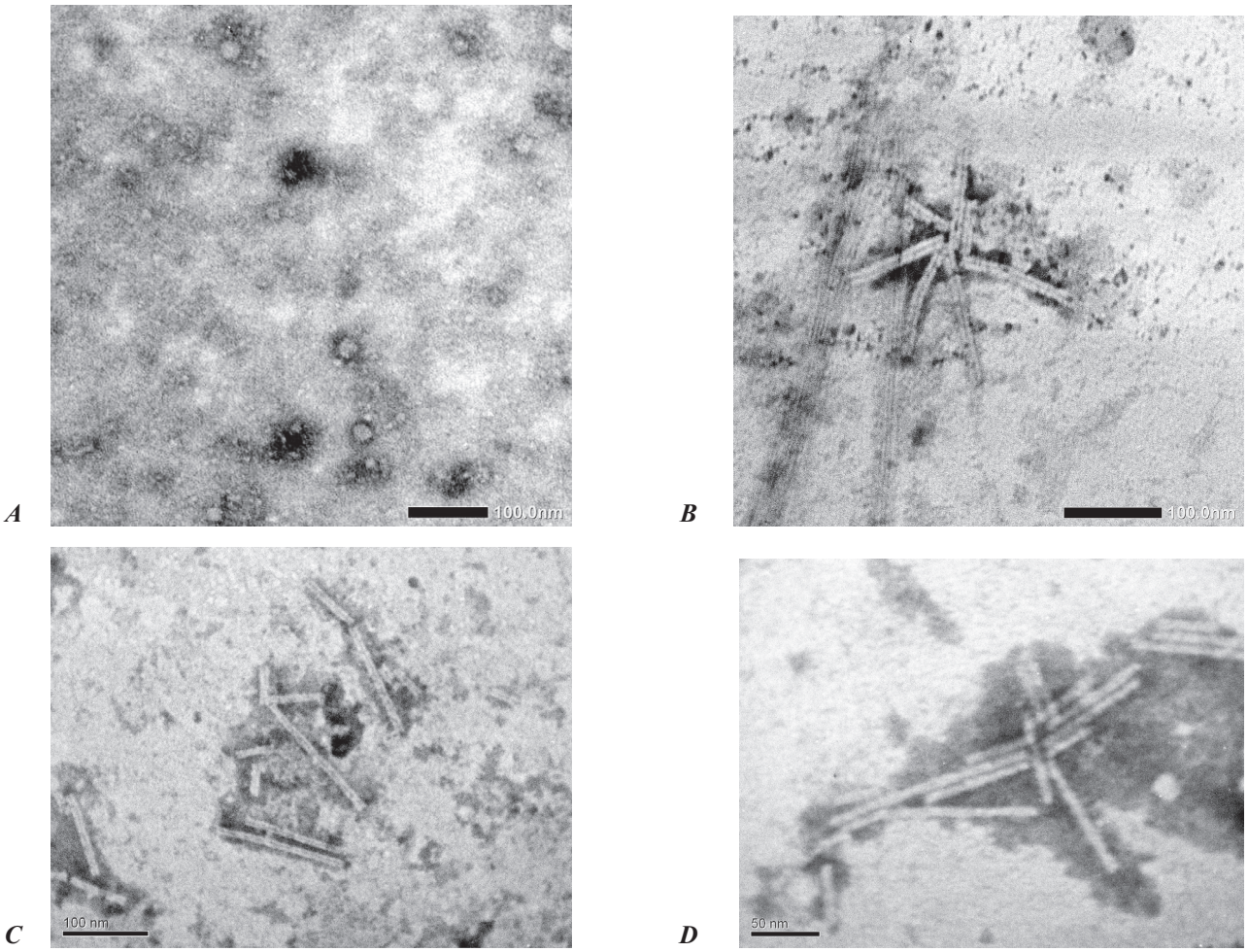


Fig. 3. Viral particles detected in *Echinacea purpurea* leaves; *a* – CMV; *b, c, d* – unidentified rod-shaped virions

Fig. 4. CMV Symptoms on test plants (artificial inoculation with sap of virus infected coneflower plants): *a* – *Nicotiana tabacum* cv. Samsun NN; *b* – necrosis on the *Chenopodium quinoa*

A

B

mosaic on leaves (Fig. 1d). Of great importance is the fact that there are identical symptoms of chlorotic mosaic in Bulgaria and Ukraine (Fig. 1b, 1c).

The research results showed that economically important purple coneflower viruses in Bulgaria are those that are carried by aphids – AMV and CMV, thrips – TSWV, and also TMV, which is transmitted mechanically. In Ukraine, it was found that the purple coneflower plants are infected with TSWV – in 2012, PVY – in 2013, and TRV – in 2014. The most harmful is CMV, which circulated on the plants of *Echinacea purpurea* for 8 years (Dunich and Mishchenko, 2015).

The condition of two purple coneflower crops near Kazanlak, Bulgaria, was studied in 2009 and 2010. The first Bulgarian *Echinacea* plantation was in homestead garden, occupying an area with alluvial-delluvial soil, near to a small river, going down the Balkan as one of the feeders of Tundja river. Many purple coneflower plants showed symptoms of virus diseases in 2009. These symptoms included chlorotic (yellow) spotting and severe mosaic and deformations of the leaves (Fig. 1c). The presence of CMV was proven in 13 positive samples, belonging to 13 *Echinacea* plants – virus carriers (87%) from all 15 tested plants (Fig. 2a).

CMV was in high concentration in 9 plants – from 0.639 to 2.097 OD and in very high viral concentration – in 4 plants – from 1.5 to over 2.0 OD. AMV was identified in 10 *Echinacea* plants (67%) from the tested 15 plants. AMV was in high viral concentration in 3 plants from 0.651 OD to 1.051 OD (Fig. 2a). These results showed that the spatial isolation (1-2 km) of the purple coneflower plants from vegetable gardens is absolutely necessary and the combined cultivation of *Echinacea* and tomato, cucumber, spinach and other crops is not favorable. CMV and AMV were aphid transmitted. The aphids migrated on different vegetables and on the purple coneflower plants and so the viruses were transmitted.

The second *Echinacea* plantation was found in the experimental field of the Institute of Rose, Essential and Medical Cultures (IREMK) near Kazanlak and was investigated in 2010. Different species of aromatic and medicinal plants were in the neighborhood of *Echinacea* plants. The soils in the trial field of IREMK are lye-cinnamonic (maroon). They are acid (pH 5.2-5.4), poor, water permeable and gravel with content of nitrogen (16.9-21.8 mg of 100 g soil) and phosphorus (12.8-17.6 mg of 100 g soil). The humus content is 2.03% (Nedkov, 1989).The results were obtained for eleven *Echinacea* plants for four economically important viral pathogens: *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV) and *Tomato spotted wilt virus* (TSWV). The number of the AMV infected plants was the largest – 8 (73%), TMV infected – 4 plants (36%), CMV and TSWV infected – each in 3 plants (27%) (Fig. 2b). The concentration of all four viruses was not high, but moderate from 0.3 OD to 0.6 OD. This concentration was in accordance with moderate spotting, observed on the plants. AMV was the most spread on the *Echinacea* plantation in IREMK, because it was revealed on other aromatic and medicinal plants in 2010 which were AMV hosts. AMV was established in *Lavandula angustifolia* (Miln.), *Ruta graveolens* (L.), as and alfalfa, finding near the trial field of IREMK.

The results of many years of research have shown that CMV is one of the most widespread viruses on the purple coneflower culture in both Ukraine and Bulgaria. Some properties of isolates of this virus were investigated (Fig. 3, 4).

In Ukraine, in the experimental (organic) variant, the wrinkling of the leaves was noted only on singular plants (about 1.5%). Yellow ring mottling, mosaics were absent until the late autumn. In this experimental variant with a late sowing (May 28), we marked the budding of individual plants. However, low temperatures in October 2016 prevented the beginning of the flowering season in the first year of vegetation (Fig. 5a). Inspections of the purple coneflower plants of the first year of vegetation, which were grown in the experiment with elements of organic farming, showed no symptoms of viral infections (Fig. 5a). Similar results (absence of viral symptoms) were obtained in the spring of 2017. Thus, purple coneflower plants of the 2nd year of cultivation, grown in organic farming, also had no symptoms of viral infections (Fig. 5 b, c, d).

Specific antibodies against viruses that are already detected in the coneflower plants (PVY, CMV, TRV, TMV, and TSWV) were used in ELISA. These antibodies were selected according to the scientific literature reports and electron microscopy data, published by us earlier (Dunich and Mishchenko, 2015; Mishchenko et al., 2015). Also, plants were tested for viruses that could potentially infect coneflower plants – PLRV and TAV. ELISA results showed absence of antigens to the listed viruses in purple coneflower plants, grown under the conditions of Mercury farm with elements of organic farming (Fig. 6).

The absence of various ring mottling symptoms and viruses in the purple coneflower plants under organic farming can be explained by the optimal supply of nutrients. This is evidenced, first of all, by the humus content, which was 5.7% compared to the 2.6% in the control (Mishchenko et al., 2017) (Table 1).

Results of the Table 1 indicate that the content of NH_4^+ and NO_3^- ions is almost twice higher in the experiment compared to the control, but the content of phosphorus is the

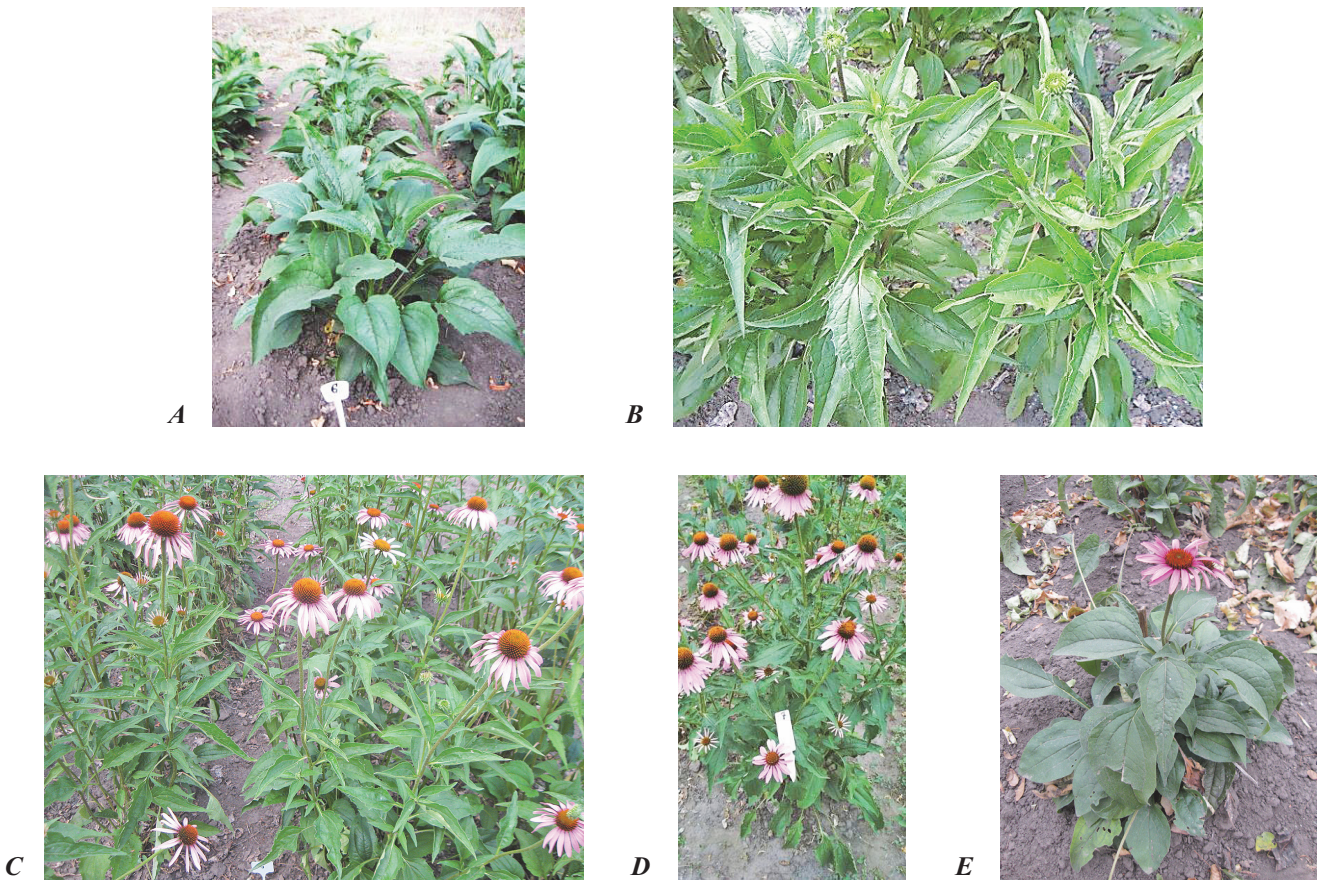


Fig. 5. Healthy purple coneflower plants cv. Charivnytsia under organic farming conditions: *a* – 1st year of cultivation, October 2016; *b* – 2nd year of cultivation, budding phase, May 25, 2017; *c* – 2nd year of cultivation, flowering phase, 6 July, 2017 *d* – 2nd year of cultivation, flowering phase, 23 July, 2017; *e* – October, 2017

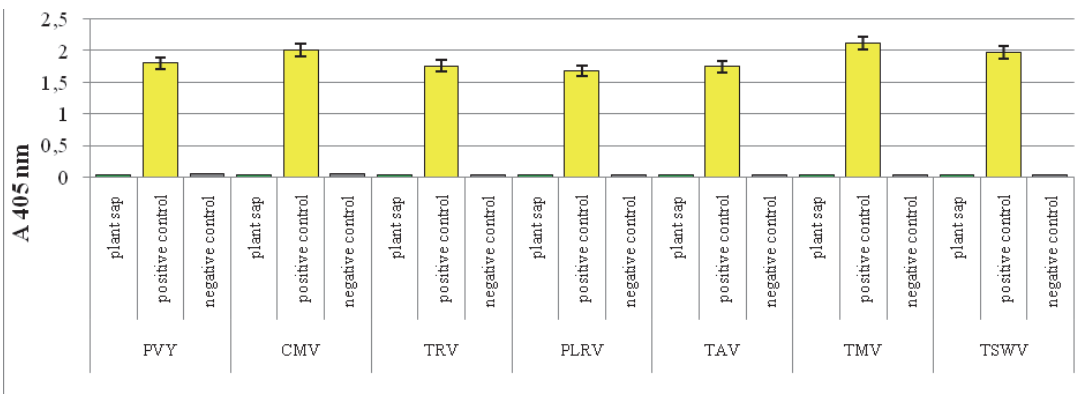


Fig. 6. Content of viral antigens in purple coneflower plants cv. Charivnytsia, grown under organic farming conditions, 16.06.2017

Table 1
Content of the nutrients, humus and pH of the soil

Options	Chemical elements, mg/ 1 kg of dry soil										Humus, %
	NH_4^+	NO_3^-	P	K	Ca	Mg	Fe	S	Mn	pH	
Organic farming	60.0	7,5	109,1	261,4	6997,2	1240,4	137,5	25,6	140,0	6.70	5.7
Control	30.0	3,8	109,0	550,5	8163,4	2032,0	125,1	37.5	120.0	7.45	2.6

same. The potassium content is almost two times lower in the experiment, and calcium, magnesium and sulfur content is decreased too in comparison with the control. It is connected with the harvesting and time of soil samples collection. Iron and manganese content is increased in the experiment. But the listed biogenic elements are in the needed (optimal) amount for plant nutrition on black soils (chernozems). The soil reaction is neutral and slightly alkaline (pH 6.70-7.45). The main factor was humus, which provided the resistance of plants against viruses in the experimental field with elements of organic farming.

It is also known that organic farming ensures the balance of the entomofauna. Natural conditions are created to reduce the number of vectors of plant viruses, which leads to a decrease in the level of agricultural crops disease (Krasylovets, 2013; Korniychuk et al., 2014). It has also been found that soil properties (organic farming) have a significant impact on the diversity of the species of medicinal plants in the Lemon verbena ecosystem in Greece (Solomou et al., 2017). Our previous studies have shown that chemical elements play an important role in increasing the resistance of wheat plants against viruses (Mishchenko, 2009). Viral disease symptoms, the infective potential of viral particles and the susceptibility of the host to virus were shown to be influenced by the nutrition status of the host plant. Therefore, nutrients can affect disease incidence by increasing plant resistance, improving plant growth (allowing the plant to escape the disease) and influencing the pathogen's environment (Dordas, 2008).

According to Dikova et al. (2010, 2013) the yield of *Echinacea* roots from the plants with symptoms of virus diseases was 3-4 times lower in comparison with the yield of the symptomless plants. The yield of leaves (herba) of the spotted plants was from 2 to 5 times lower and seeds were from 2 to 20 times lower in comparison with the symptomless plants.

The yield of the underground mass and roots of *Echinacea* plants with virus-like symptoms (yellow spotting) decreases two or more times. Symptoms of viral infections (chlorosis, mosaic mottling) cause the reduction of the photosynthetic apparatus, a typical picture of the aging of chloroplasts is observed, which is accompanied by a decrease in the intensity of photosynthesis, which leads to a significant decrease in the crop yield and grain quality deterioration (Boiko et al., 1997).

There are reports that bone meal, or cow and horse manure was the best organic manures in fertilizing *Echinacea* (Hobbs, 1989). In Iran it was showed that vermicompost without using of chemical fertilizers leads to the high dry weights of coneflower stem, flower and root (Nia et al., 2015). T.S.C. Li showed that pig compost and biological wastes increased the weight of dry and fresh coneflower leaves (Li, 1998).

Thus, the research results pertaining to *Echinacea* plants with symptoms of virus diseases from two plantations, spread of different fields around Kazanlak showed that one or other virus was uppermost (predominating) and was depend on the neighboring hosts – CMV in the vegetable garden and AMV in the trial field of IREMK. The aphids – vectors of AMV and CMV and the plant hosts – sources of the viral infection in nearness to the *Echinacea* plantations furthered the virus development of both crops. The results about the establishment of virus diseases in two random *Echinacea* plantations in Bulgaria showed that these diseases would be exerted influence upon the quantity and quality of the yield. When the *Echinacea* plants are cultivated on poor, water permeable and gravel soils with low humus content, all possible measures should be taken for the increasing of the humus content and the safe bio-production of drugs.

So, purple coneflower plants were grown on the experiment field with elements of organic farming, namely: near a half of century it was a pasture, than the field was occupied by alfalfa about 5 years, after that – winter wheat, vegetables with humus for plowing, winter wheat, black steam. In such a five-field rotation, we obtained virus-free *Echinacea* plants. In the control field (agrotechnical short-rotation crop rotation), but without humus, where its content was only 2.6%, and in Bulgaria 2.05%, we noted severe symptoms of viral infections, and presence of the CMV, PVY, TSWV confirmed by ELISA and electron microscopy. The research results have shown the perspective of further research and introduction of the principles of organic farming in medicinal plants cultivation. This is the first report about the reduction of the infestation of *Echinacea purpurea* plants with viruses under conditions of organic farming.

It is important to provide a balanced nutrition and at the time when the nutrient can be most effective for disease control and also for higher yield. Not only the application of the fertilizer can affect the disease development, but also anything that affects soil environment such as pH modification through lime application, tillage, optimal seedbed preparation, moisture control (irrigation or drainage), crop rotation, cover crops, green manures, manures and intercropping. It is known that practices such as addition of sphagnum peat, green manures and animal manures have been shown to produce suppressive soils on which pathogens do not establish or persist and do not affect the crop plants (Dordas, 2008).

Conclusions

For the bio-production of the aromatic and medicinal cultures (in particular *Echinacea* plants) it is necessary to use all manners, methods and means for increasing soil productivity. The results of the cultivation of purple coneflower in Ukraine showed that the yield is high on the soil with high humus content and applying of crop-rotations with intensive cultures, such as wheat and balancing fertilizations. Thus, our research on growing of purple coneflower under different farming systems has established the absence of the main severe symptoms of viral diseases, namely, yellow ring mottling and mosaic under conditions of organic farming in Poltava region. The cultivation of purple coneflower plants under conditions of organic farming (with a complete optimal supply of nutrients, and first of all, with soil organic matter content at least 5.2%) is one of the effective ways to increase the plant's resistance to viral diseases.

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