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EPIDEMIOLOGICAL PATTERNS, PREVALENCE AND SEASONAL DYNAMICS OF DIFFERENT VIRUSES IN SUSCEPTIBLE CUCURBIT CROPS

SUMMARY

Vegetables represent valuable food source and are cultivated worldwide. Cucurbits are of great importance for farmers, and viruses endanger their profitable production. Monitoring of vegetable viruses in Ukraine showed that cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus (WMV) were the most harmful and common viruses in reference regions. This work was focused on establishing sources of virus introduction and/or maintenance in a population of susceptible crops, as well as on assessing relative changes in virus-infected symptomatic plants during the vegetative season in open field conditions. Samples were collected based on visual symptoms on leaves and fruits. For virus indication, commercial DAS-ELISA kits for CMV, ZYMV, and WMV were used (Loewe, Germany). Following this work, epidemiological patterns for viruses infecting vegetable crops based on their biological properties were discovered. Seed transmission was confirmed as an important way of spreading of ZYMV and CMV, and often neglected source of viruses in the ecosystems requiring special control for pathogens with wide host range (CMV). CMV was the commonest virus found in symptomatic plants (21%) followed by ZYMV (14%) and WMV (8%). A large portion (18%) of mixed virus infection was typically induced in Ukraine by the following virus groups: CMV/ZYMV, CMV/WMV, ZYMV/WMV, and rarely CMV/ZYMV/WMV. Field experiments showed steep decline of healthy plants from the end of May until the end of August, in parallel to an increase of virus-infected cucurbits. CMV was detected starting from the beginning of the experiment. Similarly, WMV was also found during the duration of screening with sharp increase of diseased plants by the end of the season. ZYMV was detected only in July with subsequent decrease in the number of ZYMV-positive

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samples as opposed to WMV. Artificial ecosystems were demonstrated as important factors maintaining populations of viruses infecting vegetable crops.

Keywords: cucumber mosaic virus, zucchini yellow mosaic virus, watermelon mosaic virus, cucurbit, Ukraine.

INTRODUCTION

In Ukraine, vegetable crops are considered strategically important as they account for a very significant amount of gross plant production and value reaching ~20%. The areas used for production of these crops and production volumes (including cultivation in glasshouse conditions) increase annually. In addition, many vegetables (tomato, melon, squashes, cucumber, etc.) traditionally form an important article for international trade (http://minagro.gov.ua/).

Viruses are the major cause of losses both in terms of yield quantity (15-100% decrease, ~20% on average) and quality of plant production (loss of marketable appearance/shortening of the storage period with resulting sharp decrease of commercial value). Since viral diseases of plants are incurable, the means of their control are mostly limited to routine diagnostics of production plants and prevention of virus spread on the fields through cultural practices, vector control and use of resistant cultivars when they are available.

Cucurbits and other vegetable crops are known hosts for several dozens of viral pathogens, and the larger part of these is transmitted by flying vectors – mostly aphids. Knowing profile of viruses and their respective vectors, as well as other means of their transmission, for a given ecosystem and cultivation technology is the absolute must-do for introducing efficient control measures for such diseases.

During the recent years, vegetable viruses attain growing attention. Several major factors are deemed to contribute to wider profile of viruses appearing in the ecosystems. The most important ones are global warming leading to migration of flying vectors, and especially aphids, to earlier unfavorable (colder) regions coupled with global trade involving plant and seed material together with their pests.

In Ukraine, several previously absent new highly damaging vegetable viruses have been described including zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) on zucchini, squash and pumpkin plants; pepper mild mottle virus (PMMoV) and pepino mosaic virus (PeMV) on tomatoes and sweet pepper; turnip mosaic virus (TuMV) on many wild-growing and domesticated brassicas; onion yellow dwarf virus and leek yellow stripe virus on various onions (Tsvigun *et al*., 2016; Shevchenko *et al*., 2018a; Snihur *et al*., 2019; Sherevera *et al*., 2019). The phylogeny of several viral pathogens infecting fruits and vegetables in Ukraine was also studied in detail showing that both known and novel isolates circulate in the region (Budzanivska *et al*., 2016; Shevchenko *et al*., 2018b; Kutsenko and Budzanivska, 2020), confirming the significant role of interregional plant transportation in virus spread and evolution.

CMV is a representative of *Bromoviridae* family and has icosahedral particles with tripartite genome (King *et al*., 2012). CMV was first described nearly 90 years ago and remains a devastating pathogen with extremely wide host range (>16 plant families) endangering production of a vast majority of vegetables including tomato, spinach, and cucurbits all over the world (CABI Datasheet – Cucumber mosaic virus). CMV is efficiently transmitted by aphids in a non-persistent manner, by mechanical inoculation, and by seed.

On the other hand, ZYMV and WMV belong to the large *Potyviridae* family, were first isolated in 1960-70-ies and then identified in all continents within a decade (CABI Datasheet – Zucchini yellow mosaic virus; CABI Datasheet – Watermelon mosaic virus). They represent a major constraint in the world-wide production of cucurbits, from cucumbers to watermelons. Contrary to ZYMV, which is naturally restricted mainly to cucurbits, WMV has wider host range. Both viruses induce persistent symptoms (yellow and green leaf mosaic, fruit mosaic, spotting and deformation). The viruses can cause massive damage (to total loss) to the crops (especially in co-infection) and prevent their growth in certain areas (Gal-On, 2007). ZYMV and WMV are mainly transmitted by aphids non-persistently, mechanically from plant to plant, as well as with planting material. ZYMV was also confirmed seed-born in zucchini, squash, and holl-less seeded oil pumpkin, which could have contributed to its rapid spread worldwide (Tobias and Palkovics, 2003).

Ukraine is one of the largest European countries enjoying strategic position between the eastern EU states and Black Sea/Middle East region. Our previous work showed that cucumber mosaic virus (CMV), ZYMV and WMV were the commonest viruses infecting cucurbits in Ukraine, where ZYMV and WMV were first detected in 2006 both in mono- and mixed infection, whereas CMV was known for decades but was officially ‘reconfirmed’ back in 2015 (Rudneva *et al*., 2006; Shevchenko *et al*., 2015).

This paper describes comparative analysis of viruses infecting cucurbits which are truly devastating for profitable production of these crops in Ukraine, in order to establish their epidemiological patterns, prevalence and seasonal dynamics in susceptible crops to recommend simple and reliable means for controlling virus spread.

MATERIAL AND METHODS

Plant sampling was conducted during vegetative seasons (summer) of 2016-2019 in open field conditions on plots annually used for cultivation of various cucurbit crops predominantly including squash, zucchini, and pumpkin in central part of Ukraine (Poltava region) considered an important area for commercial cultivation of seasonal cucurbits. Cucurbit plants were visually examined; samples (leaves and fruits) were collected from plants with virus-like symptoms.

Cucurbit plants (squash, zucchini, etc.) were visually examined; approx. 260 samples (leaves and fruits) were collected from plants with virus-like symptoms.

Collected samples were tested for CMV, ZYMV, and WMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Loewe (Germany) and following the manufacturer's recommendations. Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Greiner Bio-One, Germany) were coated with virus-specific broad-spectrum polyclonal antibodies (1:200) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of virus(es) in the samples was detected in 200 µL homogenate by virus-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Thermo Labsystems Opsis MR microtitre plate reader (USA). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

For transmission electron microscopy (TEM), copper grids (Sigma, USA) were coated with chloroform-dissolved 0.2% polyvinyl formaldehyde (Serva, Germany) and dried overnight on filter paper at room temperature. The samples deposited onto grids were stained with 2.5% uranyl acetate and 0.02 N lead citrate (Serva, Germany), and examined using JEM 1400 (JEOL, Japan) transmission electron microscope. The samples were photographed at a magnification of 5,000-60,000x (Mendgen, 1991).

RESULTS AND DISCUSSION

On sampled cucurbits (zucchini, squash, and pumpkin) virus-like symptoms typically included leaf mosaic and yellowing, leaf/fruit mottling and deformation, plant stunting, etc. Typical symptoms for mono-infected plants are shown on Fig.1.

Using DAS-ELISA, symptomatic plant samples were subsequently screened for CMV, ZYMV, and WMV. Overall, 61% of symptomatic plants were shown positive for one or more viruses whereas 39% were negative. CMV has been detected in large part of the collected plant samples (21%) in every cucurbit species tested. This was followed by ZYMV monoinfection (14%) and WMV monoinfection (8%) found in pumpkin, squash, and zucchini. A large portion (18%) of mixed virus infection was typically induced by the following virus groups: ZYMV/WMV (6%), CMV/ZYMV (5%), CMV/WMV (5%), and rarely CMV/ZYMV/WMV (2%) (Fig.2).

Figure 1. Virus-like symptoms on sampled plants in field conditions later confirmed as virus-infected: A – leaf yellowing/mosaic/deformation and plant stunting of CMV-positive pumpkin, B – yellowing and ringspots on fruit of ZYMV-positive squash, C – dark green veinal mosaic of leaves of WMV-positive squash

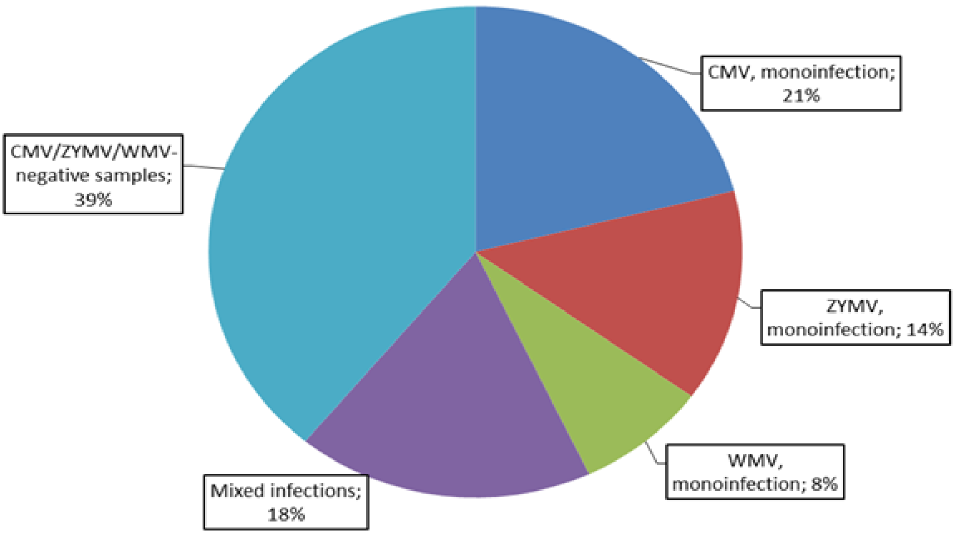


Figure 2. Double-antibody enzyme-linked immunosorbent assay for the detection of cucumber mosaic virus, zucchini mosaic virus and watermelon mosaic virus in mono- and mixed infection in cucurbits in Ukraine

In line with ELISA results, transmission electron microscopy of plant sap confirmed the presence of spherical (~25-35 nm in diameter) and filamentous virions (720-850 x 16 nm) typical for cucumoviruses and potyviruses, respectively (Fig.3).

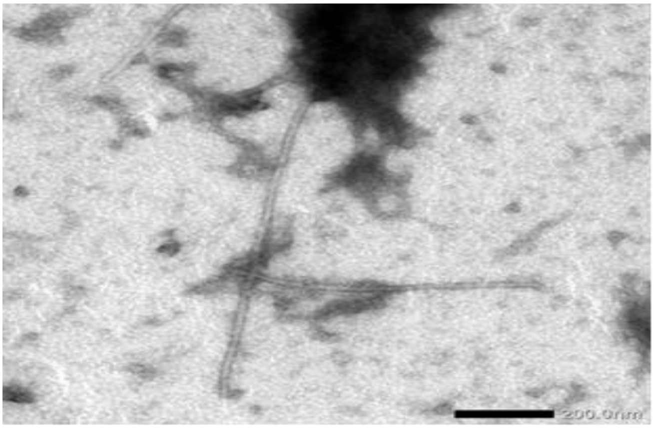
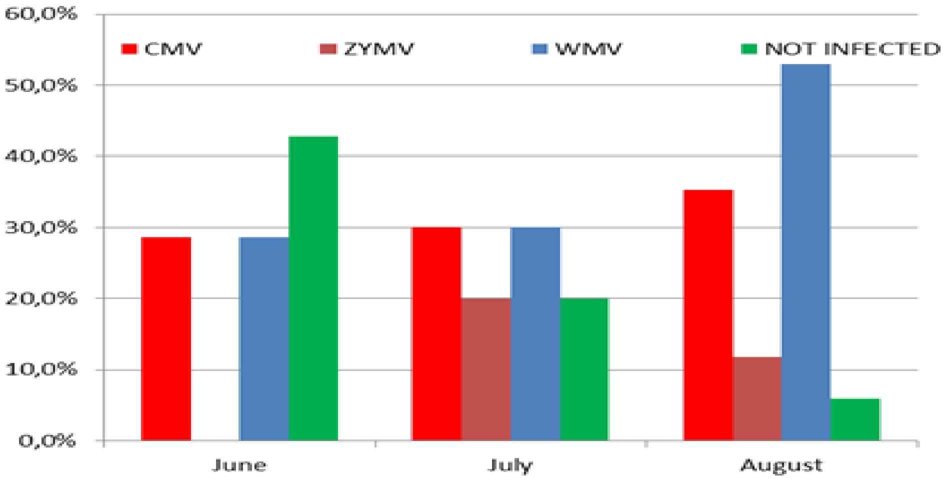


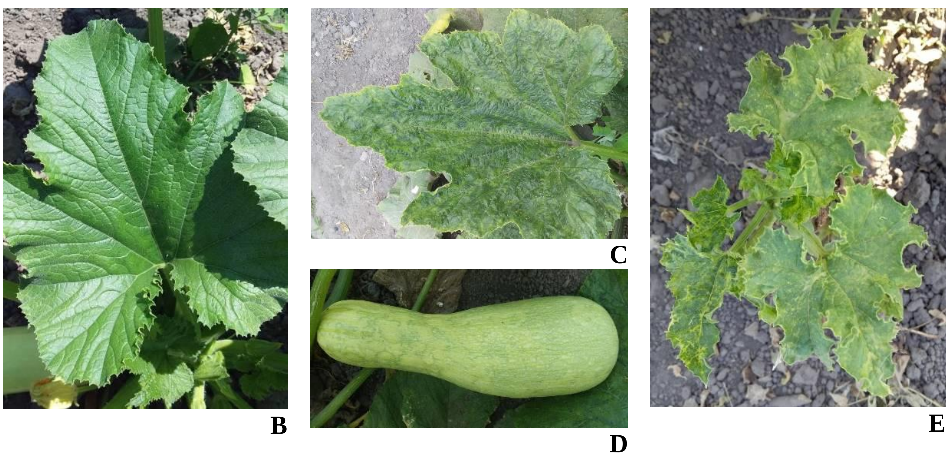
Figure 3. Electron micrograph of partly purified sap of CMV/ZYMV-positive squash showing small icosahedral CMV particles and filamentous ZYMV particles. Scale bar corresponds to 200 nm

The pattern of virus occurrence and prevalence (Fig.2) follows general knowledge on these viruses (King *et al*., 2012; Tsvigun *et al*., 2016; Shevchenko *et al*., 2018b) and probably reflects their biological and epidemiological properties (namely host range, means of transmission and stability in the environment) rather than plant insusceptibility/resistance to virus infection. In this context, CMV benefits from a very wide host range, diverse transmission routes including seed transmission (absent for WMV and most of ZYMV isolates), and highly stable virions. ZYMV and WMV, on the contrary, have significantly narrower host range and do not demonstrate notable stability in the environment.

Surprisingly high level of mixed infections can be explained differently and needs more analysis. However, the symbiotic interactions between CMV, ZYMV and WMV are probably unavoidable in artificial ecosystems, especially if these are routinely used for cultivation of the same crops annually allowing virus accumulation in the environment (plan debris, concomitant weeds and substrate). Expectedly, ZYMV and WMV co-infection was most frequent (6%) followed by CMV/ZYMV and CMV/WMV (5% each), and occasionally by CMV/ZYMV/WMV (2%) – the latter rare combination inducing more severe symptoms on infected plants (Fig.4E).

According to available literature data, CMV, ZYMV and WMV are often found in co-infection. For example, CMV mostly interfere with another virus(es) including WMV and ZYMV (Sano and Kojima, 1989; Barbosa *et al.*, 2016). In contrast, WMV and ZYMV frequently co-occur in cucurbits (Desbiez *et al*., 2020) and share the same aphid vectors. Interestingly, ZYMV replication rates did not depend on the type of infection (mono- or mixed with WMV) but ZYMV enhanced aphid recruitment to infected plants. However, WMV did not much affect plant-vector relationships, while its strains were shown to accumulate to significantly lower levels in the presence of ZYMV. Despite poor in-plant competition with ZYMV, WMV readily transmitted from mixed infections (Salvaudon *et al.*, 2013). The line of evidence suggests that lower occurrence of WMV shown in this study may be conditioned by its wider host range (ability to survive in neighbour weeds thus avoiding the competition) and antagonism with ZYMV.





June **July** **August**

Figure 4. Seasonal dynamics of CMV, ZYMV and WMV in susceptible cucurbit crops as percentage of infected plants on experimental plot (A) and intensification of visual disease symptoms on such plants (B-E) during summer months of 3 consequential years: B – healthy-looking leaf of WMV-infected squash, C – dark green veinal leaf mosaic of ZYMV-infected squash, D – irregularly shaped ringspots on fruit of ZYMV-infected squash, E – severe leaf mosaic, yellowing and stunting of CMV/ZYMV/WMV-infected squash

Following this lead, an experimental plot annually used for cultivation of various cucurbit crops was regularly screened for CMV, ZYMV and WMV to elucidate their seasonal dynamics during vegetative period (typically June-August in open field conditions of central Ukraine (Fig.4).

From Figure 4 it follows that the percentage of healthy plants (green color) steeply declined from the beginning of June until the end of August, in parallel to an increase of virus-infected cucurbits. CMV was detected starting from the beginning of the experiment, with gradual increase of CMV-positive plants during the experiment. Similarly, WMV was also found during the whole duration of screening with sharp elevation of diseased plants by the end of the season. However, ZYMV was first detected only in July with subsequent decrease in the number of ZYMV-positive samples as opposed to WMV.

Expectedly, visual virus-induced symptoms became more pronounced with time, from healthy-looking leaves (Fig.4B) to severely diseased plants showing complex and systemic symptoms, especially in case of mixed infection (Fig.4E).

The results suggest long-term persistence of CMV and possibly WMV in the ecosystem where these pathogens may circulate off-season by infecting other available non-cucurbit hosts. Nevertheless, ZYMV has the narrowest host range and obviously requires susceptible cucurbits for massive multiplication occurring mostly in the midsummer after crop sawing and mass aphid infestation in May-June. Such narrow and disadvantageous ‘window of opportunity’ for ZYMV may be compensated by its active spread by vectors in the midseason which may be sufficient for maintaining virus population.

Altogether, obtained results clearly demonstrate the correlation between epidemiological properties and seasonal dynamics of cucurbit viruses in susceptible crops, and underline the importance of routine control of planting material and cultivated crops on a regular basis which remain highly efficient measures in preventing the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

CONCLUSIONS

Visual and serological screening of cucurbit crops showed that 61% of symptomatic plants were positive for one or more viruses, where CMV has been detected in 21% of samples of every species tested followed by ZYMV (14%) and WMV (8%) found in pumpkin, squash, and zucchini. A large portion (18%) of mixed virus infection was typically induced by ZYMV/WMV (6%), CMV/ZYMV (5%), CMV/WMV (5%), and rarely CMV/ZYMV/WMV (2%). The pattern of virus occurrence and prevalence reflects their biological and epidemiological properties. The outcomes of studying seasonal dynamics of CMV, ZYMV and WMV in susceptible crops suggest long-term persistence of CMV and possibly WMV in the ecosystem where these pathogens may circulate off-season as opposed to ZYMV which obviously requires susceptible cucurbits for massive multiplication. Obtained data confirm that CMV, ZYMV and WMV remain damaging vegetable viruses in Ukraine, and raise questions of their proper control.

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REFERENCES

Barbosa G. da S., Lima J.A. de A., Queiroz M.A. de, Dias R. de C.S., Lima C.S. (2016): Identification and effects of mixed infection of potyvirus isolates with cucumber mosaic virus in cucurbits. Revista Caatinga, 29(4), pp. 1028-1035.

Budzanivska I.G., Shevchenko T.P., Shevchenko O.V., Polishchuk V.P. (2016): Phylogenetic analysis of Ukrainian isolates of RNA viruses of plants. Acta Microbiologica Bulgarica, 32(3), pp. 10-19.

CABI Datasheet – Cucumber mosaic virus: https://www.cabi.org/isc/datasheet/16970#REF-DDB-138961. Accessed on 30/07/2020.

CABI Datasheet – Watermelon mosaic virus: https://www.cabi.org/isc/datasheet/56821. Accessed on 30/07/2020.

CABI Datasheet – Zucchini yellow mosaic virus: https://www.cabi.org/isc/datasheet/57657. Accessed on 30/07/2020.

Clark M.F., Adams A. M. (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. The Journal of General Virology 34, pp. 475-483.

Desbiez C., Wipf-Scheibel C., Millot P., Berthier K., Girardot G., Gognalons P., Hirsch J., Moury B., Nozeran K., Piry S., Schoeny A., Verdin E. (2020): Distribution and evolution of the major viruses infecting cucurbitaceous and solanaceous crops in the French Mediterranean area. Virus Research, doi: 10.1016/j.virusres.2020.198042

Gal-On A. (2007): Zucchini yellow mosaic virus: insect transmission and pathogenicity – the tails of two proteins. Mol. Plant Pathol., 8(2), pp. 139-150.

King A.M.Q., Adams M.J., Carstens E.B., Lefkowitz E.J. (eds.) (2012): Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press.

Kutsenko O., Budzanivska I. (2020): Distribution and transmission of Plum pox virus in Ukraine. Agriculture and Forestry, 66(1), pp. 49-56.

Mendgen K. (1991): Electron Microscopy of Plant Pathogens. Edited by D.E. Lesemann. Springer,. 336 p.

Rudneva T.O., Shevchenko T.P., Budzanivska I.G., Shevchenko O.V., Polischuk V.P. (2006): Virus diseases of Cucurbitaceae plants on the territory of Ukraine. Rasteniev'dni Nauki, 43(6), pp. 508-510.

Salvaudon L., De Moraes C.M., Mescher M.C. (2013): Outcomes of co-infection by two potyviruses: implications for the evolution of manipulative strategies. Proc R Soc B, 280, http://dx.doi.org/10.1098/rspb.2012.2959

Sano Y., Kojima M. (1989): Increase in cucumber mosaic virus concentration in Japanese radish plants coinfected with turnip mosaic virus. Ann. Phytopathol. Soc. Jpn., 55, pp. 296-302.

Sherevera K., Shevchenko T., Snihur H., Budzanivska I., Shevchenko O. (2019): First report of Leek yellow stripe virus on Allium sativum L. in Ukraine. Agriculture & Forestry, 65(4), pp. 47-53.

Shevchenko O., Yasaka R., Tymchyshyn O., Shevchenko T., Ohshima K. (2018a): First evidence of the occurrence of Turnip mosaic virus in Ukraine and molecular characterization of its isolate. Journal of Phytopathology, 166 (6), pp. 429-437.

Shevchenko T.P., Tymchyshyn O.V., AlDalain E., Bysov A.S., Budzanivska I.G., Shevchenko O.V., Polishchuk V.P. (2015): The first evidence of subgroup IB isolates of Cucumber mosaic virus in Ukraine. Biopolymers and Cell, 31(1), pp. 57-62.

Shevchenko T.P., Tymchyshyn O.V., Kosenko I.A., Budzanivska I.G., Shevchenko O.V., Polishchuk V.P. (2018b): Molecular characterization and phylogenetic analysis of Ukrainian isolates of Cucumber mosaic virus based on the partial sequences of three genes. Biopolymers and Cell, 34(1), pp. 32-40.

Snihur H., Shevchenko T., Sherevera K., Budzanivska I., Shevchenko O. (2019): First report of onion yellow dwarf virus in Ukraine. Journal of Plant Pathology, 101(4), pp. 1283–1283.

Tobias I., Palkovics L. (2003): Characterization of Hungarian isolates of Zucchini yellow mosaic virus (ZYMV, Potyvirus) transmitted by seeds of Cucurbita pepo var Styriaca. Pest Management Science, 59(4), pp. 493-497.

Tsvigun V.O., Rudneva T.O., Shevchenko T.P., Budzanivska I.G., Polishchuk V.P. (2016): Strain attribution of Ukrainian isolates of Zucchini yellow mosaic virus and their occurrence in Ukraine. Biopolymers and Cell, 32(3), pp. 235-241.