

Determination of Watermelon Mosaic Virus (Wmv-2) By Serological Methods and Biological Index Studies in Some Cucurbits in UŞAK

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ABSTRACT: The detection and distribution of watermelon mosaic virus (WMV-2), which causes severe damages to Cucumber, *Cucumis sativus* L. Pumpkin, *Cucurbita moschata* L. and Edible squash, *Cucurbita pepo* L. (Cucurbitales: Cucurbitaceae) plants in Uşak province and its districts were investigated in this study. For this, cucurbit plants showing the symptoms of WMV virus were sampled in 2017-2018 and analyzed by DAS-ELISA method. Biological indexing studies were also conducted. According to the results obtained, 31 (51.31%) of the 76 samples taken in 2017, 67 (67.67%) of the 99 samples taken in 2018, and 106 (60.57%) of the 175 samples taken in both years were tested positive for the virus. As a result of mechanical inoculation studies, indicator plants showing signs of virus contamination were also tested by DAS-ELISA. The infection of the virus was confirmed by finding the positive results in cucumber, watermelon and tobacco among the tested plants. Serological and mechanical inoculation analyzes were parallel. The presence and distribution of WMV-2 in cucurbit growing areas in Uşak province were determined for the first time with this study.

KEYWORDS: Cucurbitaceae, DAS-ELISA, mechanical inoculation, Uşak, WMV-2

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I. INTRODUCTION

The cucurbit crops (Cucurbitaceae), including many cultivated and wild varieties, especially Cucumber, *Cucumis sativus* L., Pumpkin, *Cucurbita moschata* L, Edible squash *Cucurbita pepo* L., Melon, *Cucumis melo* L., Watermelon, *Citrullus lanatus* (Thumb.) Matsum. & Naka and Gherkin, *Cucumis anguria* L. (Cucurbitales: Cucurbitaceae) have been widely cultivated for years in Turkey as well as worldwide. Being rich in vitamins and minerals, this plant community serves as a nutritional source for both humans and animals. As an annual crop, it can also be a good choice for crop rotation. There are many different squash varieties, but the most important of these in terms of agricultural production globally is pumpkin and edible squash. Apart from squash, the cucumber is grown in almost every part of the world, though it is native to tropical climates. Cucumbers are not only eaten fresh or consumed as pickle, but are increasingly used in the cosmetic industry.

According to FAO data (2018), squash, pumpkin and gourd production reached 27.643.932 tons in the world. Cucumber and gherkin production were 75.219.440 tons in the same year [1]. Although cucurbit production has an important place in Turkey's agriculture, it has been observed that the production of squash, pumpkin and gourd (616.777 tons) and cucumber (1.848.273 tons) has gradually decreased in the recent years in the Uşak province [2]. There are many factors affecting production, but virus diseases cause serious economic losses as they prevent normal plant development. In addition, the lack of chemical control for the prevention of viral diseases is an important problem [3]. One of the highly damaging viruses infecting cucurbits in our country is watermelon mosaic virus (WMV-2), which was first detected by Nogay and Yorgancı in 1984 [4]. Being a member of the Potyvirus group, this virus has at least 38 species belonging to 19 genera from the order Aphididae, which is transmitted in non-persistent manner and can spread easily and effectively. Symptoms of this virus have been observed in many production areas in the region where the study was conducted. However, a detailed study on this virus in cucurbit plants has not been done before in Uşak Province. Therefore, this research was conducted to determine the presence and distribution of WMV-2 in pumpkin, edible squash and cucumber in Uşak center and its districts.

II. MATERIAL AND METHODS

The main material of this study was the plant samples taken from 175 plants that were grown in the agricultural areas where cucurbits are cultivated in Uşak center and its districts. The samples were taken from the leaves, stem, fruit and flower parts of plants showing symptoms such as dark-green and green mosaic appearance, mottling, vein banding, blistering, curling, asymmetry, shrinkage, wrinkling and deformation in the leaves, stunting and short internodes in the body, distortion, shrinkage and mottling in dark green and yellow

tones in the fruits [5, 6]. Samples collected according to the guided sampling method were first placed in polyethylene bags and labelled and then brought to the laboratory in an icebox.

In laboratory studies, antiserum set, various chemicals and other materials were used to analyze suspicious plant samples. Serological analyzes were performed by Clark and Adams [7]'s method by DAS-ELISA and according to the procedures specified by Bioreba AG company. The tests were carried out in duplicate and absorbance values at 405 nm wavelength were obtained using the M201 model (Hong Kong, China) spectrophotometer.

Biological indexing studies were carried out in the climated room. In these studies, different indicator plants, including, *C. melo*, *C. sativus*, *C. moschata*, *C. pepo*, *C. lanatus*, *Solanum lycopersicum* L., *Vigna unguiculata* L., *Nicotiana rustica* L. and *Nicotiana tabaccum* L. (Solanales: Solanaceae), as well as sterilized peat, sand, soil and pots were used. Test plants used in biological indexing studies were grown in the climated room at Uşak University, Scientific Analysis and Technological Application and Research Center at 4000-6000 Lux light intensity, 16/8 hours light / dark period, 60-70% humidity and 25°C temperature. The indicator plant seeds were planted separately in each pot and when they became 2-4 leaves, 3 controls were inoculated with 3 repetitions. In addition, indicator plants showing virus symptoms were tested serologically. Inoculated plants were identified.

III. RESULTS

In the field studies, symptoms such as chlorosis, banding in the veins, blistering, curl, shrinkage, thread, asymmetry of the leaves, stunted shoots and superficial protrusions, deformation, mottling and shrinkage of the fruits and similar virus symptoms in some cucurbit plants were observed (Figure 1).



Figure 1. Deformity symptoms on squash (left) and cucumber leaves (right)

In serological tests carried out in laboratory studies, 105 (60.00%) of 175 cucumber plant samples were found to be WMV-2 positive (Figure 1).

Looking at the virus infection rates of cucurbit samples collected from the research area in 2017, the highest infection rate on a plant basis was found in squash plants (70,00%), followed by pumpkin (58.82%) and cucumber (22.72%). In 2018, again the highest infection rate was noted in squash (88.63%), followed by 52.63% in cucumber and 47.05% in pumpkin plants (Table 1).

Table 1. Distribution and disease incidence rates (%) in different cucurbits varieties, tested in 2017 and 2018 in Uşak province.

Plant species	Tested (2017)	Positive	Infection rate (%)	Tested (2018)	Positive	Infection rate (%)
Pumpkin	34	20	58,82	17	8	47,05
Squash	20	14	70,00	44	39	88,63
Cucumber	22	4	18,18	38	20	52,63
Total	76	38	50,00	99	67	67,67

When examining the distribution and total disease incidence rates (%) of cucurbit samples tested in 2017-2018 in Uşak province, viruses were found in 105 (60%) of 175 samples tested in two years. In total, it was observed that the highest incidence was in squash with 82.81%, followed by 54.90 % in pumpkin and 40.00 % in cucumber (Figure 1).

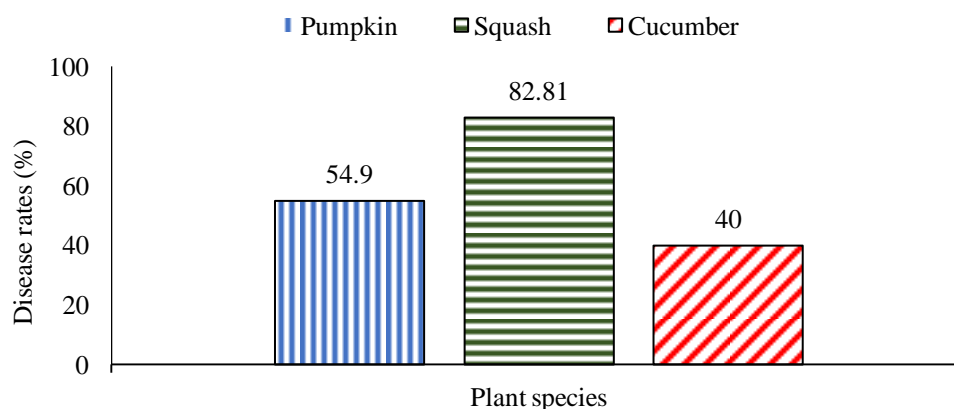


Figure 1. Plant distribution and total disease rates of cucurbit samples tested in Uşak Province in 2017-2018 years (%)

Considering the distribution and the total disease rates (%) of cucurbit samples taken from the different districts and center of Uşak province in 2017 and 2018, Eşme was found to be the most infected district with a total incidence rate of 69.56%.

Table 2. Distribution and disease incidence rates of cucurbit samples tested in 2017 and 2018 in Uşak province (%)

Location	Tested samples (2017)	Positive	Infection rate (%)	Tested samples (2018)	Positive	Infection rate (%)	Tested samples (Total)	Positive (Total)	Total Infection rate (%)
Uşak Center	15	8	53,33	26	18	69,23	41	26	63,41
Banaz	11	6	54,54	18	14	77,77	29	20	68,96
Eşme	8	4	50,00	15	12	80,00	23	16	69,56
Karahallı	12	6	50,00	9	5	55,55	21	9	42,85
Sivaslı	18	9	50,00	17	9	52,94	35	18	51,42
Ulubey	12	5	41,66	14	9	64,28	26	14	53,84
Total	76	38	50,00	99	67	67,67	175	105	60,00

Various test plants were grown under controlled conditions for biological indexing studies. These plants were mechanically inoculated from infected plant samples, which were recorded positive as a result of serological tests. Inoculated test plants showing virus symptoms were also tested serologically and the test results were found to be WMV-2 positive in *C. sativus*, *C. lanatus* and *N. tabaccum* plants.

IV. DISCUSSION

It is almost impossible to make diseases and pests free vegetables production, especially in open areas. A study conducted in the Uşak province, where the production of squash and cucumber has decreased gradually in recent years, symptoms of viral diseases have been found in the areas where cucurbit plants are grown. Signs of WMV-2 infection such as shrinkage, asymmetry, mosaic appearance, dark-light green spots, yellowing and deformation were observed in the plants leaves.

For this reason, plant samples collected from many production areas in Uşak and its districts in 2017 and 2018 were tested by DAS-ELISA method to identify WMV-2. As a result of the tests, it was determined that, of the total samples taken in 2017 and 2018, 82.81% of squash, 54.90% of pumpkin and 40% of cucumbers were infected with the virus. Test results of the samples taken from various surveys showed differences in the rate of contamination by years. While the virus was found in 50.00% of the samples taken in 2017, 67.67% of the total samples taken in 2018 were recorded as contaminated. Studies conducted by many researchers, also show the variation in the rates and prevalence of the disease caused by the virus [8, 9, 10]. Various factors can influence the infection rates of the virus in different geographical regions. Disease symptoms may vary according to the type of the virus, vector and host plant variety, environmental and climatic conditions. In

addition, as stated in many different research studies, the most important reason for the higher prevalence of virus diseases in water-rich production areas is thought to be related to the high occurrence rate of moisture-loving vector insects in these areas, such as virus-carrying aphids and thrips [6, 11, 17]. Likewise, the study conducted in Uşak and its districts confirmed high aphid populations in areas with high infection rates. Results obtained in our research are confirmed by some other studies where similar symptoms and results were observed in cucurbits [12].

It has been observed that there was no weed control in many fields, where the plant samples were collected. Insufficient control practices for vector insects and many other reasons cause the virus to be transported to different areas and spread the disease to different regions far from the origin, thereby affecting the contamination rate and economic loss [10]. Watermelon mosaic virus (WMV), which is very common in countries with temperate climates such as the Mediterranean climate [14], was first isolated from a watermelon in 1965 in Turkey [15]. Viral infections in crops have been detected in many countries, especially in watermelon and other cucurbit plants, and the result of isolation studies reports WMV as the source of infection [16 - 18]. In a study conducted in Antalya, DAS-ELISA test results revealed that 79 pumpkin samples were contaminated with ZYMV (21.5%) and WMV (59.4%) [13]. Similar results have been reported in many survey studies conducted in different years by different researchers [8, 19]. To meet the food demand of the increasing human population in the world, it is extremely important to control the viruses that prevent higher yields per unit area. Producers need to receive training and assistance from experts on plant protection methods in controlling virus diseases that negatively affect both the regional and national economy.

The presence and distribution of WMV-2 in cucurbit growing areas in Uşak province were determined for the first time with this study.

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REFERENCES

- [1]. Internet: Food and Agriculture Organization of United Nations, 2018, <http://www.fao.org/faostat/en/#data/QC>
- [2]. Internet: Turkish Statistical Institute, Plant Production data, 2018, <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr>.
- [3]. Thomas, W., 1971. Watermelon Mosaic Virus, a Disease of Cucurbits in New Zealand. *New Zealand Journal of Agricultural Research* 14, 235–241
- [4]. Nogay, A., Yorgancı, U., 1984. "Purification and Particle Morphology of TMV, CMV and ZYMV Isolated From Various Cultivated Crops Grown Along the Mediterranean Coast of Turkey. 1: The Identification of Viruses Infecting Cucurbits in Marmara Region", *J. Turkish Phytopathol.*, 14 : 9-28.
- [5]. Hewitt, W.B., Gifford, E. M., 1956. Symptoms for Identifying Fanleaf in Dormant Grapevines. *The Bulletin Department of Agriculture State of California*, Vol Xlv, Number 3.
- [6]. Bovey, R., 1965. Identification of Viruses in Clonally Propagated Plants Having One or More Viruses. *Proc. Conf. On Virus and Vector on Perennial Hosts with Special Reference to Vitis*, 223-227.
- [7]. Clark, M. F., Adams, A. N., 1977, "Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of Plant Viruses", *Journal of General Virology*, 34: 475-483.
- [8]. Şevik, M.A., Arlı-Sökmen, M., 2003, "Viruses Infecting Cucurbits in Samsun, Turkey", *Plant Dis.*, 87: 341-344.
- [9]. Masika, F. B., Kisekka, R., Alicai T., Tugume, A. K., 2017, "Incidence of viruses and virus-like diseases of watermelons and pumpkins in Uganda, a hitherto none investigated pathosystem", *African Journal of Agricultural Research*, 12, (3), 177-191.
- [10]. Kamberoglu, M. A., Caliskan, A. F., Desbiez, C., 2016, "Current Status of Some Cucurbit Viruses in Çukurova Region (Adana and Mersin Provinces) of Turkey and Molecular Characterization of Zucchini Yellow Mosaic Virus Isolates", *Romanian Biotechnological Letters*, 21 (4), 2016.
- [11]. Kızmaz, M. Z., 2014, "Determination of Prevalence, Rates and Factors of Viral Diseases in Cucurbit Production Areas of Diyarbakır and Mardin Provinces ", Master Thesis, Dicle University, Institute of Science, Department of Plant Protection, Diyarbakır, 51.
- [12]. Demski, J. W., Sumner, D. R., 1979, "Spread of Watermelon Mosaic Virus in Georgia Grown Squash", *Research Bulletin* (University of Georgia. College of Agriculture. Experiment Stations), 234.
- [13]. Topkaya, Ş., Ertunç, F., 2012, "Current status of virus infections in cucurbit plantations in Ankara and Antalya provinces, Cucurbitaceae 2012", *Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (eds. Sari, Solmaz and Aras) Antalya (Turkey), 759-762.
- [14]. Ullman, E. D., Cho, J. J. German, T. L., 1991, "Occurrence and Distribution of Cucurbit Viruses in the Hawaiian Islands", *Plant Dis.*, 75: 367-370.
- [15]. Webb, R. E., Scott, H. A., 1965, "Isolation and Identification of Watermelon Mosaic Viruses 1 and 2", *Phytopathology*, 55:895-900.
- [16]. Gibbs, A.J., Mackenzie, A.M., Wel, K.J., Gibbs, M.J., 2008, "The Potyviruses of Australia", *Arch Virol.*, 153: 1411-1420.
- [17]. Alonso-Prados, L.J., Luis-Arteaga, M., Alvarez, J.M., Moriones, E., Batlle, A., Lavina, A., Garcia-Arenal, F., Fraile, A., 2003, "Epidemics of Aphid-transmitted Viruses in Melon Crops in Spain", *European Journal of Plant Pathology.*, 109: 129-138.
- [18]. Nameth, S. T., Dodds, J. A., Paulus, A. O. 1986, "A New Potyvirus Associated with Severe Disease of Cantaloupe (*Cucumis melo*) in Southern California", *Phytopath.*, 73:793.
- [19]. Yılmaz, M.A., Lecoq, H., Abak, K., Baloğlu, S., Sarı, N., 1992, "The Harmful Viruses in Cucurbit Vegetable Species in Turkey", First National Congress of Horticultural Turkey, Izmir, Volume 2, 439-442.