

# Detection of *Zucchini Yellow Mosaic virus* from Cucurbits in Burdur Province, Turkey

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**Abstract**—During the 2015 growing season, 94 samples were collected from zucchini, muskmelon, watermelon and cucumber plants showing virus-like symptoms in Burdur province of Turkey. Presence of Zucchini yellow mosaic virus (ZYMV) was investigated using Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) method. Plants showed different types of symptoms; mosaic, blistering of leaf lamina, leaf yellowing, leaf deformation, stunting and fruit deformation. As a result of DAS-ELISA test, it was found that 9.5 % of leaf samples with ZYMV.

**Keywords**—*cucurbits; Zucchini yellow mosaic virus; DAS-ELISA*

## I. INTRODUCTION

Cucurbit plays an important role in human nutrition because of ingredient of vitamin and other dietary substances [1]. Cucurbit crops are very sensitive to viral infection. Over 35 viruses from Cucurbitaceae family have been reported [2]. Plant viral diseases are major limiting factors for successful cultivation causing major yield losses in agricultural crops worldwide. Chemical agents such as fungicides and bactericides are not effective for controlling virus diseases [3]. Plant virus management strategies are mostly aimed at eradicating the source of infection to prevent it from reaching the crop and interfering with the movement of vectors to prevent the spread of the disease [4]. Reference [5] have stated that there are about 59 viruses that cause diseases in cucurbits. The viruses that are observed in cucurbit crops which cause significant losses in efficiency are as follows; Zucchini yellow mosaic virus (ZYMV), Watermelon mosaic virus (WMV), Papaya ringspot virus (PRSV), Cucumber mosaic virus (CMV) and Squash mosaic virus (SqMV). ZYMV is a member of the genus Potyvirus and exists as a long flexuous virus particle containing a single-stranded RNA genome [6]. It was first reported in Italy in 1981 [7]. ZYMV is transmitted by certain species of aphids, by plant sap containing the virus and through infected seeds [8]. In studies on viral diseases in cucurbits conducted in Turkey, ZYMV have been detected [3; 9; 10; 11; 12; 13]. In this study, DAS-ELISA method was used for the identification of ZYMV in cucurbit growing areas in Burdur province in Turkey. However this is the first time that is was determined of ZYMV on cucurbit growing areas in Burdur provinces, Turkey.

## II. MATERIALS AND METHODS

### A. Plant Materials

The main material of this study consisted of 94 leaf samples totally from Burdur showing signs of the disease and thus suspected to be infected with the virus. The samples were collected from cucurbit production areas (squash, muskmelon, watermelon and cucumber) in June-September of 2015. During the field surveys, virus-like symptoms including yellowing, mosaic pattern of light and dark green, yellow spotting, malformation symptoms on the leaves, ring-spots or line patterns on leaves or fruit were observed and symptoms are photographed. The collected samples were labeled in polyethylene bags, brought to the laboratory in ice boxes and kept in a freezer (-20°C) until the necessary tests were made.

### B. Serological Test Method (DAS-ELISA)

ZYMV DAS-ELISA Agdia (Inc. Elkhart, IN) kit was used in the study. The application was performed according to the method prepared by the manufacturer. Accordingly, 100 µl IgG 1:1000 diluted in coating plate was added to each well of the ELISA plate and kept at 4° C overnight. Then, ELISA plates were washed with washing buffer. Washing was repeated three times. Each well was filled with 100 µL plant extracts obtained by 1/10 dilution with extraction buffer and kept at 4° C overnight. The washing was repeated the next day. Following the washing process, conjugated antibodies were diluted in conjugated buffer in a 1:1000 ratio and 100 µl dilution was added to each well and kept at 37° C for 4 hours. Following the washing process, 100 µL of substrate which was prepared as 1 mg/mL in substrate solution was added and kept at room temperature. The samples with values at least twice the value of the negative control value according to the absorbance at 405 nm were accepted as positive [14].

## III. RESULTS AND DISCUSSIONS

In survey studies, leaf samples were collected from five different locations (Çeltikçi, Bağsaray, Bucak, Ağlasun, Yazır) in Burdur provinces in 2015 year. The symptoms of virus in different cucurbits include stunting, leaf and fruit deformation, blistering, discoloration of the leaves, mosaic, yellowing, mottling,

ring-spot. Photographs of these plants are presented in Fig. 1 and Fig. 2. The symptoms were similar to those obtained in previous studies and support the suggestion that the virus may be ZYMV [12; 15; 16].

ELISA tests were performed in order to determine the presence of ZYMV in the leaf samples taken from 94 plants with virus infection symptoms. Among the tested samples, 9 samples gave positive ZYMV reactions. One watermelon, one zucchini and one cucumber samples collected from Çeltikçi location, two zucchini samples collected from Bucak location, four zucchini samples collected from Bağsaray location which tested positive for ZYMV by DAS-ELISA. Samples collected from Ağlasun and Yazır locations were not found to be infected with ZYMV (Table 1.). ZYMV infections have also been detected in studies carried out in other regions in Turkey [9; 11; 12; 17].



Fig. 1. Mosaic patterns, interveinal yellowing and deformations on leaves.



Fig. 2. Blistering on fruit

The positive reactions for the targeted virus obtained in serological tests performed for the identification of the virus indicated the presence of ZYMV in the plant. ELISA tests have been widely used by researchers for the determination of viruses in cucurbits. This method is preferred due to its speed, sensitivity, affordability and reliability [16; 18; 19].

With this study, the existence of ZYMV in cucurbit fields at Burdur province have been found out using serological method. No study has been carried out previously in the region to detect ZYMV. This study is the first.

TABLE I. SAMPLES TESTED, PLACE WHERE SAMPLES TAKEN, NUMBER OF INFECTED SAMPLES DETERMINED BY DAS-ELISA TEST, PERCENTAGE OF INFECTION.

Locations	ZYMV		
	Total Samples	ZYMV Infected Samples	Percentage of infection (%)
Çeltikçi	38	3	7.89
Bağsaray	24	4	16.66
Bucak	9	2	22.22
Ağlasun	8	-	-
Yazır	15	-	-
TOTAL	94	9	9.5%

#### REFERENCES

- [1] G. N. Agrios, "Plant Diseases Caused by Viruses," In Plant Pathology, pp. 479-556, 1997.
- [2] T.A. Zitter, D.A. Hopkins and C.F. Thomas, "Compendium of Cucurbit Diseases," APS Pres, VI+120p, 1996.
- [3] M.A. Yılmaz, and R.F. Davis, "Purification ve Particle Morphology of TMV, CMV, ve ZYMV Isolated from Various Cultivated Crops Grown Along the Mediterranean Coast of Turkey," Journal of Turkish Phytopathology, vol. 13(1), pp. 29-38, 1984.
- [4] R.E.F. Matthews, Plant Virology, 3rd ed., Academic Press., London.,1991.
- [5] H. Lecoq, and C. Desbiez, "Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture," Advances in Virus Research, 84, pp.67-126. 2012.
- [6] J. Komata, M. Melzer, and S. Nelson, "Zucchini yellow mosaic virus," College of Tropical Agriculture and Human resources, Plant Disease, vol. PD-104, pp. 1-5, 2014.
- [7] V. Lisa, G. Boccoardo, G. D'agostino, G. Delavalle, and M. D'aquilio, "Characterization Of A Potyvirus That Causes Zucchini Yellow Mosaic Virus," Phytopathology, 71, pp. 667-672, 1981.
- [8] C. Schrijnwerkers, N. Huijberts, L. Bos and L. Neth, "Zucchini yellow mosaic virus: two outbreaks in the Netherlands and seed transmissibility," Journal of Plant Pathology, vol. 97, pp.187-191, 1991.
- [9] M.A. Şevik, and M.A. Sökmen, "Viruses infecting cucurbits in Samsun, Turkey," Plant Disease, vol. 87, pp. 341-344, 2003.
- [10] M.A. Yılmaz, K. Abak, H. Lecoq, S. Baloğlu, N. Sarı, S. Kesici, M. Özasan, and M.E. Güldür "Control of Zucchini yellow mosaic virus (ZYMV) in cucurbits by

ZYMV-WK strain," 9th Congress of the Mediterranean Phythopathological Union, Kuşadası-Aydın, Turkey, pp. 353-356, 1994.

[11] N. Yardımcı, and S. Korkmaz, "Studies on Spread and Identification of Zucchini Yellow Mosaic Virus Disease in the North-West Mediterranean Region of Turkey by Biological Indexing and Double-Stranded RNA Analysis," *Plant Pathology Journal*, vol. 3(1), pp. 1-4, 2004.

[12] M. Özaslan, T. Aytekin, B. Baş, I.H. Kılıç, I.D. Afacan, and S. Dağ, "Virus diseases of Cucurbits in Gaziantep- Turkey," *Plant Pathology Journal*, vol. 5(1), pp. 24-27, 2006.

[13] A. Karamanlı, "Survey of Cucumber Mosaic Virus (CMV) and Zucchini Yellow Mosaic Virus (ZYMV) in Turkish Republic of Northern Cyprus in cucurbits growth fields," Çukurova University, Institute of Natural and Applied Sciences, Plant Protection Department, Msc Thesis, 2007.

[14] M.A. Şevik, and A. Balkaya, "Seed transmissibility of viruses in winter Squash Landraces collected from the Black Sea Region of Turkey," *Plant Protection Science*, vol. 51(4), pp. 195-199, 2015.

[15] A.H. Malik, S. Mansoor, S. Iram, R.W. Briddon, and Y. Zafar, "Severe disease of melon in North West frontier province is associated with simultaneous infection of two RNA Viruses," *Pakistan Journal of Botany*, vol. 42(1), pp. 361-367, 2010.

[16] S. Safara, J. Hayati, M.R. Ardakani, M.K. Habibi, "Occurrence, Distribution and Biological Variability of Zucchini yellow mosaic virus in Cucurbits of Khuzestan Province, South West of Iran," *International Journal of Plant Biology*, vol. 2, e6, pp. 19-23, 2011.

[17] M. Özer, H.M. Sipahioğlu, M. Usta and H. Fidan, "Cloning and sequencing of coat protein gene of Zucchini yellow mosaic virus isolated from squash and muskmelon in Turkey," *Turkish Journal of Biology*, vol. 36, pp. 423-429, 2012.

[18] M.F. Clark, and A.N. Adams, "Characteristic of microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses," *Journal of General Virology*, 34, pp. 475-483, 1977.

[19] M.A. Al-Saleh, M.A. Amer, I.M. Al-Shahwan, O.A. Abdalla, and M.A. Zakri, "Characterization of different isolates of Zucchini yellow mosaic virus from cucurbits in Saudi Arabia", *African Journal of Microbiology Research*, vol 8 (19), pp. 1987-1994, 2014.