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Detection and incidence of cucurbit viruses in Kırşehir province, Türkiye

Türkiye'nin Kırşehir ilinde kabakgil virüslerinin tespiti ve yaygınlığı

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ABSTRACT

This study aimed to determine the status and prevalence of viral diseases in cucurbit production areas in Kırşehir province. During the 2023 production season, surveys were conducted in the districts of Center, Kaman, and Mucur in Kırşehir, where cucurbit production is intensive, and a total of 149 squash, pumpkin, cucumber, and melon leaf samples were collected from plants exhibiting virus-like symptoms in the region. Leaf samples were tested for the common vegetable viruses *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), and *Tomato spotted wilt virus* (TSWV) using the DAS-ELISA method.

According to the results obtained, CMV, TSWV, and ToMV are the most common viruses in cucurbit production areas of Kırşehir province, detected at rates of 52.3%, 26.2%, and 24.2%, respectively. TMV and ZYMV have been detected at lower rates in cucurbit plants. In addition, mixed infections involving double, triple, and quadruple infections of the viruses studied were identified in 28.9% of the samples. When assessing the damage caused by viral infections to cucurbit plants, it was observed that the most damage to squash and pumpkin leaves were caused by mixed TMV+TSWV infections, to cucumber leaves by TSWV, and to melon leaves by CMV.

The results showed that the five viruses studied are quite widespread in the cucurbit production areas of Kırşehir and could significantly limit production. These findings, presented for the first time in Kırşehir, emphasize the importance of using healthy propagation materials, integrated disease management, resistant/tolerant varieties in cultivation, and vector control to reduce virus-induced yield and quality losses in cucurbit production in the region.

INTRODUCTION

Cucurbits such as squash (*Cucurbita pepo* L.), pumpkin (*Cucurbita moschata* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus*) and melon (*Cucumis melo* L.) in the Cucurbitaceae family, whose native land is the American continent (Balkaya et al. 2021) are the most important species produced economically (Düzeltir and Yanmaz 2004, Günay 2005). In addition to being an important kind of vegetables in human nutrition, they are also very beneficial for health because of the vitamins, minerals and antioxidants they content (Günay 2005). 2022 world production data shows that Türkiye ranked 2nd after China (159.479.778 tons) with a total cucurbit production of 7.664.858 tons. Türkiye was respectively followed by India, Russia and the USA (FAO 2024). As of 2023, Türkiye's cucurbit production was 7.193.522 tons, of which 3.147.921 tons was watermelon, 1.871.712 tons was cucumber, 1.403.214 tons was melon, 726.594 tons was squash + pumpkin, and 44.081 tons was ajur. Central Anatolia statistical region produced 208.843 tons of cucurbits and Kırşehir ranked 7th among the provinces in the Central Anatolia region with a production of 8.427 tons. Cucurbit production in Kırşehir province was 2.959 tons of melon, 908 tons of cucumber and 221 tons of squash + pumpkin (TUIK 2024).

Fungal, bacterial and viral diseases are among the factors that reduce yield and quality in vegetable cultivation areas. Along with over 60 viruses that are known to cause diseases worldwide in cucurbits alone, a new virus is detected every day (Lecoq and Desbiez 2012, Romay et al. 2014). Due to the absence of any chemical control against plant virus diseases, their control becomes difficult, and viruses cause significant yield losses (Çandar and Gümüş 2012). The incidence and disease severity caused by viruses vary according to the strain of the virus, host plant, vector, environmental conditions and location (Yılmaz et al. 1992, Zitter et al. 1996). *Zucchini yellow mosaic virus* (ZYMV) (Caciagli 2008) and *Cucumber mosaic virus* (CMV) (Kosaka and Fukunishi 1997) are among the most important viral diseases of cucurbits, but *Tobacco mosaic virus* (TMV) (Lueloff and Hudelson 2024) and *Tomato mosaic virus* (ToMV) (Xu et al. 2021), which have a very large host range, also cause infections in cucurbit crops. *Tomato spotted wilt virus* (TSWV), which is known to cause natural infection mostly in plants belonging to the Solanaceae family but also infects plants such as cucumber, cowpea, cauliflower and broccoli, was firstly reported of its natural infection in watermelon plants in Georgia. It was recorded that necrotic ring spots and veinal necrosis symptoms were observed on the leaves of this watermelon plant taken from a tobacco field (Pappu et al. 1998).

The symptoms observed in cucurbit plants affected by ZYMV, which causes serious infections in cucurbits grown

worldwide, especially in subtropical and tropical climates (Lecoq and Katis 2014) and is responsible for yield losses up to 80% (Rao and Reddy 2020), are mosaic, deformation, blistering, chlorosis on the leaves, and general stunting of the plant. In severe systemic infections, the affected fruits develop deformations and irregular spotting on the fruit surface, and the fruits become unmarketable (Coutts et al. 2011). On the other hand, CMV causes a wide range of symptoms and disease severity on cucurbits plants, but almost all cucurbits are susceptible to the virus. In early infections of summer squash with CMV, severe epinasty (downward bending of the petiole and leaf blade) and shrinking of the leaf blade are common symptoms. In early infected cucurbit plants (such as squash, pumpkin, melon, cucumber) with CMV, severe stunting and leaf deformations are observed, while the fruits become unmarketable due to severe deformation of the fruits, marked wrinkling and rugose symptoms (Zitter and Murphy 2009).

Many studies have been conducted to determine the presence and prevalence of CMV, ZYMV, TMV, ToMV and TSWV which cause diseases in cucurbit crops in various countries of the world (Abou-Jawdah et al. 2000, Alishiri et al. 2013, Asghar et al. 2008, Davis 1986, Listihani et al. 2018, Pappu et al. 1998, Qing et al. 2010), and in many provinces of Türkiye (Altınay 2017, Kamberoğlu et al. 2016, Karabıyık and Yeşil 2021, Karanfil 2021, Karanfil and Korkmaz 2021, Kaya and Erkan 2011, Kızmaz et al. 2016, Korkmaz et al. 2018, Sevik and Arli-Sokmen 2003, Yeşil 2014, Yeşil 2021). However, there is no study to determine the status of virus diseases in cucurbit production areas of Kırşehir province. In order to effectively control plant virus diseases, the causal agent of the disease must be correctly identified (Rubio et al. 2020). For this reason, surveys were carried out in Kaman, Mucur and Central districts where cucurbit cultivation is intensive in the province during the production period of 2023. The samples collected from surveys were tested by serological methods after symptomatological examinations both in the field and in the laboratory. In addition, the prevalence of these 5 viruses was calculated on the basis of host and districts, and infection rates were revealed. Also, it was tried to determine which infections cause more damage to cucurbit leaves using a leaf imaging analysis system. Our study is the first record to determine the presence, prevalence and status of viral diseases in Kırşehir cucurbit production areas.

MATERIALS AND METHODS

Sample collection and plant material

Surveys were carried out in Kaman, Mucur and Merkez districts where cucurbit cultivation is intensively cultivated in Kırşehir province during the production period of

2023. In the field studies, a total of 149 leaf samples were collected from cucurbit plants exhibiting symptoms similar to TMV, CMV, ToMV, TSWV and ZYMV. The collected leaf samples were numbered and information such as the collecting location, plant species and symptoms of the plant were recorded. The number of samples obtained from the districts was determined based on cultivated area (da) and production quantities (tons) and the mentioned data are shown in Table 1.

specific for TMV (Art. No. 190475 complete reagent set 480), ToMV (Art. No. 152675 complete reagent set 480), TSWV (Art. No. 190175 complete reagent set 480), CMV (Art. No. 160675 complete reagent set 480), and ZYMV (Art. No. 161275 complete reagent set 480) according to the protocol of BIOREBA-AG (Switzerland) (Bioreba 2017, Clark and Adams 1977). Firstly, the cucurbitaceous plant leaf samples were extracted according to the manufacturing protocol and stored at +4°C until the tests were performed.

Table 1. Cucurbit (squash + pumpkin, cucumber, melon) production quantities by district and number of samples collected

Districts	Squash + Pumpkin			Cucumber			Melon		
	Cultivated Area (Decares)	Production (tons)	Numbers of Sample	Cultivated Area (Decares)	Production (tons)	Numbers of Sample	Cultivated Area (Decares)	Production (tons)	Numbers of Sample
Center	645	98	25	231	212	22	1.350	2.228	41
Kaman	-	-	8	118	203	20	28	84	6
Mucur	338	50	16	30	45	8	85	85	3
Total	983	148	49	379	460	50	1.463	2.397	50

Note. Cultivated area (decares) and production amount (tons) data are from TUIK for the year of 2023

50 cucumber, 50 melon and 49 squash + pumpkin leaf samples obtained from the districts of Kırşehir province constituted the material of the study and the samples were placed in polyethylene bags and brought to the laboratory with cold chain (icebox).

Symptomatological investigations and measurement of damaged leaf area (%)

Cucurbit leaf samples transferred to the laboratory in an icebox were first examined symptomatologically and the symptoms observed were recorded in a notebook. Then, the damaged leaf areas (%) of the samples were measured using the WinDIAS Leaf Image Analysis System to determine the status of infections caused by viruses likely to be present in cucurbits. Cucurbit leaf samples, for which symptomatological examinations and measurements were completed, were stored at +4°C for a while until the DAS-ELISA tests were performed, and then refrigerated at -20°C.

Serological test (DAS-ELISA) method

Cucumber, melon and squash + pumpkin leaf samples were tested by Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) using antibodies

In the first step of tests, ELISA plates were coated with 100 µl of IgGs diluted in 1:1000 in coating buffer and incubated at 34°C for 4 hours. After the plates were washed 3 times at 3 min intervals with washing buffer and dried, 100 µl each of the plant sample extracts, extraction buffer (as buffer blank), negative and positive controls were loaded to duplicate wells of ELISA plates and incubated at 4°C overnight. Following washing and drying, 100 µl of the conjugated antibodies diluted 1:1000 in conjugate buffer were added to the wells of plates and incubated at 34°C for 4 hours. Afterward 3 additional washing and drying, 100 µl of substrate buffer prepared with pNPP (para-nitrophenylphosphate) dissolved in substrate buffer to a final concentration of 1 mg/ml was loaded into each well. In order to avoid false yellowing of the substrate by light, the plates covered with substrate buffer were incubated in a dark environment at room temperature (20-25°C) for at least half an hour until the positive control showed yellow color (Clark and Adams, 1977, Bioreba 2017).

After the substrate step, the plates were visually monitored according to the color formation in the wells and spectrophotometrically at 405 nm wavelength. Spectrophotometric measurements were performed by

determining the OD values on an ELISA Reader twice at 60 and 120 min after the substrate step and the cut-off value for each test was calculated according to the formula in the company's data analysis descriptions. Mean OD values above the cut-off value were considered positive (Bioreba 2014).

Statistical analysis

DAS-ELISA test results, symptoms observed in plant samples collected from the field, and diseased leaf area (%) values were evaluated together to determine the damage caused by viruses to plant leaves. Statistical analyses were performed using SPSS software version 22.0 (IBM Corp., Armonk, NY, USA). Analysis of variance (ANOVA) was conducted to determine the significance of differences among virus infections, and mean comparisons were made using Tukey's HSD test at $p < 0.05$.

RESULTS

Results from surveys and symptomatological examinations

During the surveys, 49 squash + pumpkin, 50 cucumber and 50 melon plant leaf samples with and without virus-like symptoms were collected from cucurbit production areas of Kırşehir province, and the symptoms were recorded both in the field (Figure 1a, Figure 1b) and noted during laboratory examinations.



Figure 1. Symptoms recorded during surveys in cucurbit production areas a) vein banding symptoms on pumpkin leaves b) yellow spots on cucumber leaves

Symptomatological examinations revealed chlorosis, mosaic, blister mosaic, rugose, vein banding, deformation, shoestring on leaves and shortening of internodes in squash and pumpkin plants (Figure 2).



Figure 2. Symptoms recorded on squash + pumpkin leaves a) vein banding b) blistering mosaic, rugosity and leaf deformation c) shoestring

The common symptoms recorded in cucumber plants were yellowing, mosaic, blister mosaic, rugose on leaves and leaf deformity (Figure 3).

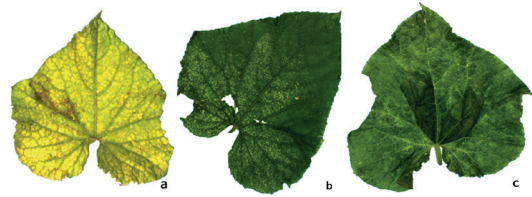


Figure 3. Symptoms recorded on cucumber leaves a) chlorosis and vein banding b) yellow spots c) blistering mosaic, rugosity and leaf deformation

Finally, yellowing, mosaic, blistering, rugose, vein banding on leaves and leaf deformity were the most common symptoms in melon plants. In addition to these symptoms, shortening of internodes was also observed in some plants (Figure 4).



Figure 4. Symptoms recorded on melon leaves a) vein banding b) blistering mosaic and vein banding c) blistering mosaic, rugosity and vein banding

Results of DAS-ELISA tests

Within the scope of the study, a total of 149 leaf samples collected from cucurbit production areas in Mucur, Kaman and Central districts of Kırşehir province were tested by DAS-ELISA using anti sera specific for TMV, CMV, ToMV, TSWV and ZYMV. As a result of the tests, single and mixed infections with various viruses were detected in 118 of 149 samples (79.2%). CMV single infections were most common in cucurbit plants (46 leaf samples) with an infection rate of 30.9%. On the other hand, TSWV in 15 leaf samples (10%), ToMV in 10 (6.7%) and ZYMV in 4 (2.7%) were determined single infections respectively, while none of the cucurbit samples had TMV single infections. When the status of mixed infections in cucurbit plants is evaluated, CMV+TMV (6.7%) in 10 samples, CMV+ZYMV+ToMV+TSWV (5.4%) in 8 samples, TMV+TSWV (4.7%), CMV+ToMV in 5 samples (3.4%), CMV+ToMV+TSWV in 5 samples (3.4%), CMV+ZYMV+ToMV in 4 samples (2.7%), ToMV+TSWV in 2 samples (1.3%) and ZYMV+ToMV+TSWV in 2 samples (1.3%) (Table 2).

Table 2. Virus infections detected in cucurbit leaf samples and their infection rates (%) according to the DAS-ELISA test results

Detected Virus Infections	Squash + Pumpkin		Cucumber		Melon		Total	
	N.S.*	IR%**	N.S.	IR	N.S.	IR%	N.S.	IR%
TMV	0	0	0	0	0	0	0	0
CMV	6	12.2	10	20	30	60	46	30.9
ToMV	0	0	10	20	0	0	10	6.7
TSWV	10	20.4	5	10	0	0	15	10
ZYMV	4	8.2	0	0	0	0	4	2.7
TMV+TSWV	2	4.1	5	10	0	0	7	4.7
CMV+TMV	0	0	0	0	10	20	10	6.7
CMV+ToMV	0	0	5	10	0	0	5	3.4
ToMV+TSWV	2	4.1	0	0	0	0	2	1.3
CMV+ToMV+TSWV	0	0	5	10	0	0	5	3.4
CMV+ZYMV+ToMV	4	8.2	0	0	0	0	4	2.7
ZYMV+ToMV+TSWV	2	4.1	0	0	0	0	2	1.3
CMV+ZYMV+ToMV+TSWV	8	16.3	0	0	0	0	8	5.4
Total Infected Plant Samples	38	77.6	40	80	40	80	118	79.2
Total Negative Plant Samples	11	22.4	10	20	10	20	31	20.8
Tested Plant Samples	49		50		50		149	

*NS: Number of infected samples

** IR%: Infection rate (%)

Thirty-eight of the 49 leaf samples obtained from squash + pumpkin plants were determined to be infected by the investigated viruses and the infection rate was 77.6%. TSWV in 10 leaf samples (20.4%), CMV in 6 (12.2%) and ZYMV in 4 (8.2%) as single infections; CMV+ZYMV+ToMV+TSWV in 8 (16.3%), CMV+ZYMV+ToMV in 4 (8.2%), TMV+TSWV in 2 (4.1%), ToMV+TSWV in 2 (4.1%) and ZYMV+ToMV+TSWV in 2 (4.1%) as mixed infections were detected by DAS-ELISA tests (Table 2). TSWV detected in 24 squash + pumpkin plants as single or mixed infections with 49% occurrence rate was the most common virus. It was followed by CMV and ZYMV with 36.7%, ToMV with 32.7%, and TMV, which was only detected in case of mixed infections, with 4.1% (Figure 5a).

As a result of the DAS-ELISA test on a total of 50 cucumber leaf samples, 40 samples (80%) were found to be infected with various viruses. CMV and ToMV in 10 leaf samples each with 20% infection rate and TSWV in 5 (10%) in case of single infections; TMV+TSWV in 5 (10%), CMV+ToMV in 5 (10%) and CMV+ToMV+TSWV (10%) in mixed infections were detected (Table 2). CMV and ToMV, detected in 20 cucumber plants each in single and mixed infections, were the two most common viruses with an infection rate of 40%, followed by TSWV with 30%. The relative incidence of TMV, which was found in 5 samples as only mixed infections and was not found in any sample as a single infection, was 10% (Figure 5b).

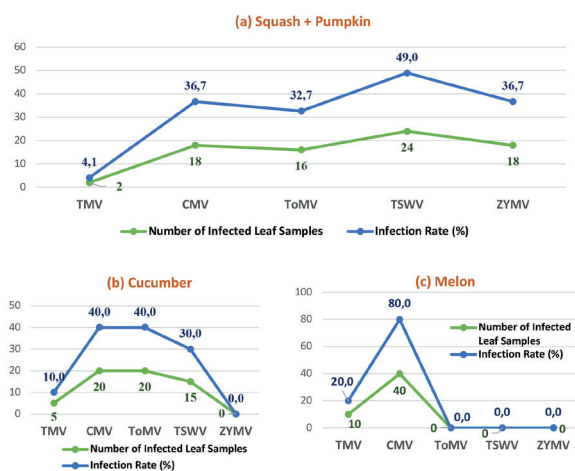


Figure 5. Infection rates of TMV, ToMV, CMV and TSWV infections detected in a) squash + pumpkin b) cucumber c) melon leaf samples

40 of total 50 melon leaf samples (80%) tested by DAS-ELISA, were determined to be infected with the tested viruses. CMV (60%) was detected in 30 melon samples as single infections, while CMV+TMV (20%) mixed infections were detected in 10 samples (Table 2). CMV was the most common virus (80%) in 40 melon samples in cases of single and mixed infections, while TMV was found in 10 plants in mixed infections at a rate of 20% (Figure 5c). Finally, ZYMV was not detected in any of the cucumber and melon samples collected from Kırşehir province, while ZYMV infection was found to be quite prevalent (36.7%) in squash + pumpkin leaf samples (Figure 5).

The relative incidence rates of virus infections detected in squash + pumpkin leaf samples collected from production areas in Kırşehir province are presented in Table 3. Based on this, CMV was the most common virus in Mucur district with an infection rate of 74.1%, followed by ToMV with 51.9%, ZYMV with 44.4%, and TSWV with 29.6%. No TMV infection was detected in any of the samples collected from the Mucur district, while the virus infection rate in the district was determined to be 81.5%. The infection rate of viruses investigated in Kaman district was 82.4%, and ToMV was the most common virus in the district with an infection rate of 47.1%. ToMV was followed by CMV with 41.2%, TSWV with 35.3%, ZYMV with 11.8%, and TMV with 5.9%, respectively. CMV was the most widespread virus in the Central district with a relative incidence rate of 50%, followed by TSWV with 21.6%, TMV with 17.1%, ToMV with 6.8%, and ZYMV with 2.3%. When the cucurbit samples collected from the districts of Kırşehir were evaluated all together, CMV was the most widespread viral infection, detected in 78 (52.3%) of the 149 leaf samples, followed by TSWV with %26.2 (39 samples), ToMV with %24.2 (36 samples), ZYMV with %12.1 (18 samples), and TMV with %11.4 (17 samples) (Table 3).

Effect of viral infections on diseased leaf area (%)

The diseased leaf area of infected cucurbit plants was measured using the WinDIAS Leaf Image Analysis System (Figure 6), and the diseased leaf area values of leaf samples with the same single or mixed infections were averaged

Table 3. Status of virus infections in cucurbit plant by district

Districts	Numbers of Infected Samples and Infection Rates (%)					Total Infected	Total Tested	Relative Incidence Rates (%)
	TMV	CMV	ToMV	TSWV	ZYMV			
Mucur	0	20	14	8	12	22	27	81.5
	0%	74.1%	51.9%	29.6%	44.4%			
Kaman	2	14	16	12	4	28	34	82.4
	5.9%	41.2%	47.1%	35.3%	11.8%			
Center	15	44	6	19	2	68	88	77.3
	17.1%	50%	6.8%	21.6%	2.3%			
Total IR (%)*	17	78	36	39	18	118	149	79.2
	11.4%	52.3%	24.2%	26.2%	12.1%			

*IR: Infection rate (%)

(Table 4). Accordingly, the TMV+TSWV mixed infection caused the most damage ($42.73 \pm 1.84\%$) in squash + pumpkin leaves. Severe disease symptoms such as chlorosis, blistering mosaic, leaf deformation, vein banding, and shoestring were observed in leaf samples with mixed TMV and TSWV infections (2 plants) (Figure 6a) (Table 4). This was followed by TSWV with $39.62 \pm 2.58\%$, CMV with $24.10 \pm 1.69\%$, CMV+ZYMV+ToMV+TSWV with $23.46 \pm 0.85\%$, ZYMV+ToMV+TSWV with $20.30 \pm 1.25\%$, CMV+ZYMV+ToMV with $19.82 \pm 0.83\%$, ZYMV with $10.92 \pm 0.39\%$ and ToMV+TSWV with $10.79 \pm 0.15\%$ (Table 4).

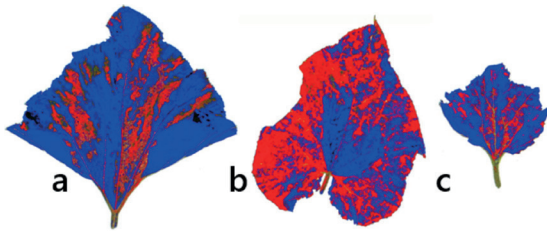


Figure 6. Images of diseased leaf areas recorded with the WinDIAS Leaf Image Analysis System for a) squash + pumpkin b) cucumber c) melon leaf samples

On the other hand, TSWV single infections caused the most damage in cucumber leaves, with the diseased leaf area of $55.54 \pm 1.76\%$ (Table 4). Severe mosaic and rugose symptoms observed in the five cucumber leaves infected with only TSWV (Figure 6b). In cucumber samples, the most damaging virus infections after TSWV infections in terms of mean diseased leaf area were TMV+TSWV ($42.65 \pm 3.63\%$), CMV+ToMV ($33.34 \pm 2.76\%$), CMV ($31.25 \pm 4.65\%$), ToMV ($24.13 \pm 3.23\%$), and CMV+ToMV+TSWV ($23.01 \pm 0.98\%$) (Table 4).

Finally, according to mean diseased leaf area data, the most damaging virus infection to melon plants was CMV ($17.65 \pm 0.94\%$), while CMV+TMV infections caused $10.56 \pm 0.67\%$ damage. While blistering mosaic, rugosity, and leaf deformation were observed in 10 of the CMV-infected melon leaf samples; chlorosis, mosaic, vein banding, and shortening of internodes were recorded in 20 samples (Table 4, Figure 6c).

DISCUSSION

When the recent years' cucurbit production data in the world and Türkiye are examined, it is seen that there has been a significant increase, and our country ranks second in the world in terms of cucurbit production in 2022 (FAO 2024). Cucurbits play an important role in vegetable production in the province of Kırşehir, located in the Central Anatolia

statistical region, and the province's cucurbit production areas are increasing day by day (TUIK 2024). Viral diseases are among the most important factors limiting agricultural production (Çandar and Gümüş 2012), and new viruses causing infection in cucurbit plants are being identified all the time (Lecoq and Desbiez 2012, Romay et al. 2014). This study was conducted to assess the status of virus infections observed in cucurbit production areas in Kırşehir province. The results provide important insights into the epidemiology of viruses causing infection in cucurbits in general of Türkiye and the situation in Kırşehir, specially. As a result of the study, the presence of TMV, CMV, ToMV, TSWV, and ZYMV in cucurbit plants collected from production areas in Kırşehir province was revealed for the first time. It has been determined that these viruses cause significant damage to squash, pumpkin, cucumber, and melon plants, as measured by the percentage of diseased leaf area, and that they can cause significant yield losses in plants.

Significant parallelisms and some differences have been identified between studies investigating viruses causing infection in cucurbit plants in Türkiye and the findings obtained from this study conducted in the districts of Mucur, Kaman, and Center in the province of Kırşehir. The virus infection rates detected in Kırşehir with our study reflect the variability observed in other cucurbit production areas in Türkiye. For example, the rate of CMV infections in the provinces of Çanakkale, Balıkesir, and Bursa in the South Marmara Region was found to be 13.8% (Karanfil and Korkmaz 2021), a comprehensive study conducted in the province of Tokat detected various infections of the viruses investigated in 74% of cucumbers, 72.4% of melons, 47.7% of watermelons, and 31.7% of cucumbers (Korkmaz et al. 2018). In Eskişehir, although the mean virus incidence rate was determined to be 4.13%, the total rate of single or multiple infections reached a high level of 73.78% (Karabıyık and Yeşil 2021). In particular, it has been reported that virus infections occur at a very high rate of 88.32% on average in edible seed pumpkin production areas in the Central Anatolia Region, specifically in the provinces of Kayseri, Yozgat, Nevşehir, Niğde, Eskişehir, and Aksaray (Yeşil 2021). In our study conducted in Kırşehir, located in the Central Anatolia Region, the virus infection rate was found to be 77.6% in squash, 80% in cucumber, and 80% in melon, and the total virus infection rate was found to be high at 79.2%, similar to the study conducted by Yeşil (2021).

CMV was identified as the most prevalent virus in Kırşehir cucurbit production areas, with a rate of 52.3%. This situation is consistent with studies exhibiting that CMV is a widespread problem in cucurbit crops in provinces such as South Marmara (Karanfil and Korkmaz 2021), Izmir,

Table 4. Virus infection detected in cucurbit samples by DAS-ELISA, recorded symptoms, and mean diseased leaf area (%) of samples

Determined Virus Infection	Squash + Pumpkin Leaf Samples			Cucumber Leaf Samples			Melon Leaf Samples		
	Infected	Symptoms	MDLA (%) (Mean ± SE)	Infected	Symptoms	MDLA (%) (Mean ± SE)	Infected	Symptoms	MDLA (%) (Mean ± SE)
CMV	4	CH	24.10±1.69 bc	5	NS	31.25±4.65 c	10	BM, R, LD	17.65±0.94 a
	2	CH, BM, LD, VB, SS		5	BM		20	CH, M, VB, SI	
ToMV	-	-	-	6	LD	24.13±3.23 d	-	-	-
	-	-	-	4	CH, M		-	-	-
TSWV	4	CH, VB	39.62±2.58 ab	5	M, R	55.54±1.76 a	-	-	-
	2	BM		-	-		-		
	4	CH, BM, LD, VB, SS		-	-		-		
ZYMV	2	CH, M	10.92±0.39 d	-	-	-	-	-	-
	2	LD, SI		-	-	-	-	-	
TMV+TSWV	2	CH, BM, LD, VB, SS	42.73±1.84 a	5	CH, VB	42.65±3.13 ab	-	-	-
CMV+TMV	-	-	-	-	-	-	10	BM, R, LD	10.56±0.67 b
ToMV+TSWV	2	BM, VB	10.79±0.15 d	-	-	-	-	-	-
CMV+ToMV	-	-	-	5	M, LD	33.34±2.76 bc	-	-	-
CMV+ToMV+ TSWV	-	-	-	5	M	23.01±0.98 c	-	-	-
CMV+ZYMV+ ToMV	4	BM, VB	19.82±0.83 c	-	-	-	-	-	-
ZYMV+ToMV+ TSWV	2	M, VB	20.30±1.25 c	-	-	-	-	-	-
CMV+ZYMV+ ToMV+ TSWV	8	BM	23.46±0.85 bc	-	-	-	-	-	-

Note. “-”-“: Undetermined virus infections; MDLA: Mean diseased leaf area; CH: Chlorosis on leaves; BM: Blister Mosaic; LD: Leaf Distortion; VB: Vein Banding; SS: Shoe-String; NS: No symptom; R: Rugose; M: Mosaic; SI: Shortening of the internodes. Values are means ± SE (n = 5–10). Means followed by the same letter are not significantly different (Tukey HSD, p < 0.05).

Aydin, Manisa, Balıkesir (Kaya and Erkan 2011), and Tokat (Korkmaz et al. 2018). CMV's wide host range, its effective transmission by aphids in production areas, and its potential for seed transmission (Karanfil and Korkmaz 2021, Palukaitis and Garcia-Arenal 2003) are among the most important reasons for its widespread occurrence in production areas. The severity of disease caused by CMV in cultivated plants varies depending on the plant species, the strain of the virus, the vector density, and climatic environmental conditions. For this reason, it has been reported to frequently cause epidemics around the world and in various regions of Türkiye (Güller et al. 2024a, Usta et al. 2020). Climatic conditions and the adaptation of the virus to its host affect epidemics and the prevalence of viral infections, as well as the phylogenetic diversity and strain or variant generation capabilities of virus isolates (Güller et al. 2024b, Usta et al. 2020). Indeed, studies on the phylogenetic diversity of CMV in cucurbit plants from our country have determined that isolates from Diyarbakır province cluster in Subgroup IA (Güller et al. 2024a), isolates from Iğdır (Güller et al. 2024b) and 2 isolates from Bingöl in Subgroup IB (Güller and Usta 2020).

ZYMV was one of the two most common virus with *Watermelon mosaic potyvirus* (WMV) (an other members of *Potyviridae*) on edible seed pumpkin plants in Konya (Yeşil 2014, 2021); on watermelon, squash, cucumber plants in Tokat (Korkmaz et al. 2018); on squash, cucumber, watermelon plants in Diyarbakır and Mardin (Kızılmaz et al. 2016); on cucumber, melon, squash, pumpkin, watermelon plants in Samsun (Sevik and Arli-Sokmen 2003); on pumpkin in Bingöl (Güller and Usta 2019) and on native edible seed pumpkin genotypes in Eskisehir (Karabıyık and Yeşil 2021). In these studies, CMV infections have followed these two viruses. Also, natural infections of WMV in bottle gourd and snake melon were firstly reported in Türkiye by Güller et al. (2024c). On the other hand, the rate of ZYMV infections detected only in squash and pumpkin plants in Kırşehir province was determined to be 12.1%. ZYMV, which is mechanically transmitted by plant sap and non-persistently by aphids (Güller and Usta 2019, Kamberoğlu et al. 2016), has not reached the same high infection rate as in the studies mentioned in Kırşehir province.

ToMV and TSWV which are widespread in *Solanaceae* plants such as tomato and pepper, are also known to infect cucurbit plants (Pappu et al. 1998, Xu et al. 2021) and have the same seed-borne transmission characteristic, were detected at significant rates of 24.2% and 26.2% in cucurbit production areas of Kırşehir province, respectively. This situation, when considered together with CMV being the most prevalent virus in the cucurbit production areas of Kırşehir province,

indicates that viruses capable of being transmitted via seeds are spreading more effectively in the region. ToMV was serologically detected for the first time in cucurbit crops in Tokat province of Türkiye and it has been reported that it rarely causes infection in cucurbit crops (Korkmaz et al. 2018). The first natural infection of TSWV, known to infect cucumber plants, was reported on watermelon plants in Georgia (Pappu et al. 1998) and on winter squash in Serbia (Vučurović et al. 2012). In Türkiye, the first report of TSWV in cucumber plants was recorded in 2022 (Erilmez 2022), and no reports on squash and melon plants have been found in literature reviews. On the other hand, TSWV was detected in 24 squash + pumpkin and 15 cucumber plant leaf samples from Kırşehir province, while the virus was not determined in melon samples. Finally, in our study TMV infections which were found at low levels (11.4%) similar to Tokat (Korkmaz et al. 2018) and South Marmara (Karanfil and Korkmaz 2021) squash and pumpkin, were detected in squash, pumpkin, cucumber, and melon leaf samples in mixed infections with other viruses.

In the cucurbit production areas of Kırşehir province, single infections of TMV, ToMV, CMV, TSWV and ZYMV, as well as dual and multiple virus infections, have been widely detected. Previous studies have reported that mixed infections of WMV and ZYMV are frequently observed, and it has been noted that mixed infections cause more severe symptoms in plants and lead to higher yield losses due to synergistic effects (Yeşil 2021). Symptoms observed and recorded in cucurbit plants such as mosaic, blister mosaic, yellowing, shoestring, leaf deformation, shortening of internodes, and stunting of plants, correspond to the typical symptoms of cucurbit virus diseases in our country and around the world (Coutts et al. 2011, Kaya and Erkan 2011, Yeşil 2014, Zitter and Murphy 2009). However, it should be considered that similar symptoms can be caused by different viral diseases or even abiotic factors (Karanfil and Korkmaz 2021). Another unique aspect of our study was the mean diseased leaf area (%) data. When these data were evaluated, it was calculated that TSWV infections caused damage to squash and pumpkin plant leaves at a rate of 39.6% and to cucumber plant leaves at a rate of 55.5%. In TMV+TSWV infections in both squash + pumpkin and cucumber, the mean diseased leaf area was 42.7%. Symptoms of chlorosis, necrosis, mottling, and leaf deformation in the leaves of plants infected with viruses reduce photosynthetic activity by inhibiting chloroplast development and decreasing chlorophyll content. This situation causes disruption in the host plant's carbohydrate and hormone metabolism (especially auxin hormone) and results in significant yield losses (Ertunç 2020).

Serological methods used in the diagnosis of plant viruses, particularly DAS-ELISA, are widely preferred due to their rapid and reliable results (Güner and Yorgancı 2006). On the other hand, molecular methods are used for the more specific detection and characterization of plant viruses (Back and Mulholland 2024). The effectiveness of the DAS-ELISA method in detecting viral infections in Kırşehir will provide a basis for developing accurate diagnoses and control strategies. In our study, the virus types detected in samples collected from cucurbit production areas in Kırşehir and the overall infection rates show similarities and differences when compared to the situation across Türkiye. The incidence of viruses such as CMV, ToMV and TSWV, which are highly transmitted by seeds, is a significant risk factor in cucurbit production. In fact, a study by Paylan and Çandar (2020) found that CMV infections were most prevalent (13.5%) in cucurbit seeds. The parallels between our study and research conducted on cucurbit seeds once again emphasize the importance of using healthy seeds in cultivation for cucurbit producers in Kırşehir. Additionally, implementation of effective vector control in the region to control viruses, taking hygiene measures in cultivation, and the use of virus-resistant/tolerant varieties should be encouraged. The adaptation of virus-resistant or tolerant cucurbit varieties to the region and the promotion of their using in the region is critical for long-term sustainable production (Yaşar et al. 2025).

In conclusion, this study constitutes a valuable reference point for the region's agriculture as it is the first comprehensive study on cucurbit viruses in Kırşehir province. One of the most significant limitations of the study is that the molecular characterization of the identified viruses was not performed. Therefore, future studies should focus on the molecular characterization of the identified viruses and their virulence, virus-vector and virus-host relationships, the development of virus-resistant/tolerant cucurbit varieties adapted to the region, and the development of integrated control methods. In this way, economic losses caused by viral infections in squash production in Kırşehir will be minimized, ensuring the sustainability of agriculture in the region.

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Author's Contributions

The authors' contributions to the study are as follows:

Literature Search: Çandar, A., Şavkan, A.N. Sample Collection: Başak, H., Çandar, A., Aydın, A., Şavkan, A.N. Sample Preparation for Tests and Symptomatological Observations: Çandar, A., Güngör, R., Şavkan, A.N., Aydın, A. Laboratory Analysis: Çandar, A.; Writing, Review and Editing: Başak, H., Çandar, A., Aydın, A.

ÖZET

Bu çalışmada Kırşehir ili kabakgil üretim alanlarında virüs hastalıklarının durumunun ve yaygınlığının belirlenmesi amaçlanmıştır. 2023 yılı üretim döneminde kabakgil üretiminin yoğun olarak yapıldığı Kırşehir'in Merkez, Kaman ve Mucur ilçelerinde sörveyler yapılmış ve bölgeden virüs benzeri belirtiler gösteren bitkilerden toplam 149 adet kabak, hıyar ve kavun yaprak örnekleri toplanmıştır. Yaprak örnekleri yaygın sebze virüslerinden olan - *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV) ve *Tomato spotted wilt virus* (TSWV) enfeksiyonları açısından DAS-ELISA yöntemiyle test edilmiştir.

Elde edilen bulgulara göre Kırşehir ili kabakgil üretim alanlarında CMV, TSWV ve ToMV en yaygın virüsler olup sırasıyla %52.3, %26.2 ve %24.2 oranında saptanmıştır. Kabakgil bitkilerinde TMV ve ZYMV ise daha düşük oranda belirlenmiştir. Ayrıca örneklerin %28.9'unda araştırılan virüslerin ikili, üçlü ve dörtlü karışık enfeksiyonları belirlenmiştir. Kabakgil bitkilerine virüs enfeksiyonlarının verdiği zararlar değerlendirildiğinde, kabak yapraklarına en fazla zararı TMV+TSWV karışık enfeksiyonunun, hıyar yapraklarına TSWV'nin ve kavun yapraklarına ise CMV'nin verdiği gözlenmiştir.

Sonuçlar araştırılan 5 virüsün Kırşehir kabakgil üretim alanlarında oldukça yaygın olduğunu ve üretimi ciddi oranda sınırlandırabileceğini göstermiştir. Kırşehir açısından ilk kez ortaya koyulan bu bulgular, bölgedeki kabakgil üretiminde virüs kaynaklı verim ve kalite kayıplarının azaltılması için temiz tohumluk kullanımı, entegre hastalık yönetimi, yetiştiricilikte dayanıklı/toleranslı çeşit kullanımı ve vektör kontrolünün önemini vurgulamaktadır.

Anahtar kelimeler: *Cucumber mosaic virus* (CMV), DAS-ELISA, Kabakgil virüsleri, *Tomato mosaic virus* (ToMV), *Tomato spotted wilt virus* (TSWV), *Zucchini yellow mosaic virus* (ZYMV)

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