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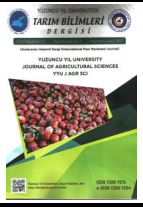

Araştırma Makalesi

Some Virus Diseases of Edible Seed Squash (*Cucurbita pepo* L.) in Aksaray Province, Turkey

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Abstract: Cucurbits (the Cucurbitaceae family) consist of 119 genera and 825 species and are cultivated worldwide, especially in tropical and subtropical regions. Squash (*Cucurbita pepo* L.), a member of this family, is one of the most cultivated and important vegetable species worldwide. As well as, squash is grown for fresh consuming; its seeds are used as a snack in some Mediterranean countries, Austria, Germany, China and Hungary like in Turkey. One of the most destructive diseases on seed squash is virus diseases in Aksaray province. In this study, it was aimed to determine the virus infections in main squash planting areas in Aksaray. For that purpose, totally 153 plant samples which showing widespread virus symptoms such as curling, mosaic, blistering, mottling, shoe-string, distortion, and stunting were collected from seed squash plants in 2014 and tested by DAS-ELISA method. According to the results of the DAS-ELISA 84.96 % of the samples were infected with *Cucumber mosaic cucumovirus* (CMV), *Papaya ring spot potyvirus* - watermelon strain (PRSV-W), *Zucchini yellow mosaic potyvirus* (ZYMV), *Squash mosaic comovirus* (SqMV) and *Watermelon mosaic potyvirus - 2* (WMV-2). The prevalent virus was ZYMV with the ratio of 66.01 % and followed by WMV-2 with 57.51 %. Besides, double and multiple virus infections were determined commonly in the samples such as, ZYMV+WMV-2, ZYMV+CMV, WMV-2+CMV, ZYMV+SqMV, ZYMV+WMV-2+PRSV-W, and ZYMV+WMV-2+SqMV. Especially, ZYMV+WMV-2 mixed infections were the most common. The infection of *Cucumber green mottle mosaic tobamovirus* (CGMMV) wasn't determined in the survey area.

Aksaray İli Çerezlik Kabak (*Cucurbita pepo* L.) Ekim Alanlarında Görülen Bazı Virüs Hastalıkları

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Anahtar kelimeler
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ZYMV

Öz: Kabakgiller (Cucurbitaceae) familyası, öncelikle tropik ve subtropikal bölgelerde dağılmış 119 cins ve 825 tür içerir. Bu familyanın bir üyesi olan kabak (*Cucurbita pepo* L.), dünya çapında en fazla yetiştirilen ve önemli sebze türlerinden bir tanesidir. Kabak çekirdekleri, Türkiye gibi bazı Akdeniz ülkelerinde ve Almanya, Macaristan, Avusturya ve Çin'de çerez olarak kullanılmaktadır. Aksaray çerezlik kabak ekim alanlarında yetiştirilen çerezlik kabaklarda (*C. pepo* L.) özellikle viral hastalıklar yıkıcı olmaktadır. Bu çalışmada, Aksaray çerezlik kabak ekim alanlarında sorun olan viral hastalıkların belirlenmesi amaçlanmıştır. 2014 yılında yapılan arazi çalışmalarında, mozaik, kıvırcıklaşma, beneklenme, iplik yapraklılık, bodurluk, yaprak ve meyvelerde şekil bozukluğu belirtileri gösteren bitkilerden toplam olarak 153 adet örnek toplanmıştır. Bu örneklerdeki virüsler, DAS-ELISA yöntemi ile belirlenmiştir. DAS-ELISA sonuçlarına göre; toplanan bitki örneklerinin %84.96'sının Zucchini

Sarı Mozaik Virüsü (ZYMV), Karpuz Mozaik Virüsü-2 (WMV-2), Hıyar Mozaik Virüsü (CMV), Papaya Halkalı Leke Virüsü-karpuz straini (PRSV-W) ve Kabak Mozaik Virüsü (SqMV) ile bulaşık olduğu saptanmıştır. ZYMV, çalışmanın gerçekleştirildiği üretim alanlarında %66.01 yaygınlık oranı ile en yaygın virüs olarak saptanmıştır. Bu virüsü WMV-2 (57.51%) takip etmiştir. Aynı zamanda, kabak örneklerinde ZYMV+WMV-2, ZYMV+CMV, WMV-2+CMV, ZYMV+SqMV, ZYMV+WMV-2+PRSV-W ve ZYMV+WMV-2+SqMV gibi karışık enfeksiyonlar da oldukça yaygın olarak gözlenmiştir. Özellikle, ZYMV+WMV-2 karışık enfeksiyonları bunların arasında en yaygın olarak saptanmıştır. Laboratuar çalışmaları sonucunda, üretim alanlarından toplanan bitki örneklerinde Hıyar Yeşil Beneklilik Mozaik Virüsü (CGMMV)'ne rastlanmamıştır.

1. Introduction

Squash seeds are one of the most nutritionally rich vegetable by-products out there, having a high content of unsaturated fat, protein, beta carotene, vitamin C, vitamin B1, fiber, iron, calcium, and potassium. These seeds were originally a main food for countries like China, United States, India, and Mexico and recently whole world has realized to the health benefits of these seeds. Although edible seed squash has been grown in Turkey for many years, there has been a rapid increase in the production area and quantity since 2004. As a result, in the year of 2017, edible seed squash production has reached 41 326 tons in about 65 000 ha production area (TÜİK, 2018). In our country, the most important reason for the increase in the production of edible seed squash is that this plant can be grown in both arid and irrigated field conditions. This plant is seen as an economic alternative product that can grow especially in arid conditions. For squash growing, one of the most important problems is virus diseases. It's hard to estimates or calculates amount of yield losses in crops due to virus diseases. According to the different calculations, 3-5% of overall cultivated vegetable crops are lost because of virus diseases, but these losses can be sometimes very high, where pest control is inadequate, particularly in developing countries (Caciagli, 2010). Viruses can cause important economic losses in the world for cucurbit growing. Indeed, on cucurbits, more than 35 different species have been determined as pathogen (Provvidenti, 1996). These pathogens cause complicated and dynamically varying problems. Edible seed squash is one of the most common vegetable crops which is grown in Aksaray province in Turkey. It occupied 4 495 ha in Aksaray in 2017, with a predicted production of 3 977 tons (Anonymous, 2018). Previous studies from different parts of Turkey have reported different viruses such as *Zucchini yellow mosaic potyvirus* (ZYMV), *Squash mosaic comovirus* (SqMV) and *Cucumber mosaic cucumovirus* (CMV, *Cucurbit aphid borne yellows polerovirus* (CABYV), *Papaya ring spot potyvirus* - watermelon strain (PRSV-W), *Watermelon mosaic potyvirus* - 2 (WMV-2), *Cucumber vein yellowing ipomovirus* (CVYV), *Tomato ring spot nepovirus* (TRSV), *Melon mosaic virus* (MMV), and *Tomato black ring nepovirus* (TBRV) in the plants of family Cucurbitaceae. (Kurcman, 1977; Davis and Yılmaz, 1984; Erdiller and Ertunç, 1988; Yılmaz et al., 1991; Yılmaz et al., 1992; Fidan, 1995; Gümüş et al., 2001; Çağlar et al., 2004).

ZYMV, WMV-2, and PRSV-W are typical members of *Potyvirus* genus (Potyviridae), so they have flexuous filamentous particles, and single stranded positive sense RNA genome. *Potyviruses* can be transmitted efficiently by mechanical inoculation and vector aphid species. ZYMV can be transmitted with seed to a low level, while PRSV-W and WMV-2 are not transmitted with seeds (Lisa and Lecoq, 1984). CMV is a polyhedral shaped virus that is member of the genus *Cucumovirus* in the Bromoviridae family and has a three-part genome consisting of ssRNA with positive polarity. This virus can be transmitted by seeds of some hosts, aphids and mechanically (Kaper and Waterworth, 1981). SqMV, which has a positive sense ssRNA genome, belongs to the genus *Comovirus* (Secoviridae). The particle of the virus is hexagonal formed of isometric subunits and can be transmitted by insect vectors, seed and mechanically (Campbell, 1971). CGMMV is a member of the *Tobamovirus* genus in the Virgaviridae family and is an important viral pathogen of cucurbit crops worldwide. The virus has rod shaped particles and single stranded positive sense RNA genome. CGMMV can be transmitted by foliage contact, handling of plants during cultivation, soil contamination, or through cucurbit seeds; no biological vector is known (Dombrovsky et al., 2017).

In this study, one year of surveys were carried out for determining the incidence and distribution of viruses (CGMMV, CMV, PRSV-W, WMV-2, SqMV, ZYMV) infecting edible seed squash crops grown in Aksaray province.

2. Materials and Methods

2.1. Collecting of virus infected squash leaves

Surveys were conducted by collecting symptomatic squash leaf samples from main squash growing fields in 6 different districts (Center, Ortaköy, Gülağaç, Eskil, Ağaçören and Sarıyahşi) of Aksaray province during July through September in 2014 (Table 1). In order to samples to represent Aksaray province, more than % 1 of total edible seed squash growing areas (1 280 da) of the province were surveyed. For this purpose, 29 edible seed squash fields were visited in the province. In these studies, the number of collected samples from each field was determined according to the amount of surface area of the field. So that, at least 5 samples were collected from the fields which have up to 50 da and 8 samples were collected from the fields which have more than 50 da growing areas. The samples were picked from plants which showed virus diseases symptoms like blistering-distortion, mottling, vein clearing, mosaic, yellowing, shoe-string, or stunting and fruit discoloration and deformation. In each field, it was tried to take samples from plants with different symptoms. Five different leaves from each plant showing symptoms of virus diseases were taken as a sample. The samples were tested to determine for the infections of CMV, ZYMV, WMV-2, SqMV, CGMMV and PRSV-W. They were put in plastic bags, and kept in a deep-freezer (-20°C) until test.

2.2. Testing by DAS-ELISA

For determining the virus infections (CGMMV, PRSV-W, CMV, WMV-2, SqMV, and ZYMV) on the squash leaf samples, DAS-ELISA (Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assay) test method was used (Clark and Adams, 1977). The antibodies were provided from commercial companies and utilized with respect to the instructions of them. Before the homogenization of the leaf samples, microplates were coated with virus IgG that were diluted in carbonate buffer (pH 9.6), and incubated for 4 h at 37°C. The squash leaf samples were grinded in a mortar with the addition of the sample extraction phosphate buffer solution at a ratio of 1:10 (PBS, pH 7.4). After washing the microplates with washing buffer (PBST) thrice, the extracted samples were added to wells and incubated overnight in a refrigerator (+4°C). Alkaline phosphatase (APP) conjugated antibody diluted in conjugate buffer (pH 7.4) was added after washing the plates, and incubated for 4 h at 37°C. Substrate buffer (pH 9.8) with *Para*-nitrophenylphosphate (*P*-NPP) was added to each well and then, incubated for 30 to 90 min. at dark and room temperature (Fig. 1). Absorbance values were determined at 405 nm by Anthos 2010 Microplate Reader (Biochrom Ltd., Cambridge, UK). Test was assessed as positive when the average absorbance value of tested sample was greater than two times of healthy (uninfected) control (Yeşil and Ertunç, 2012).

2.3. Determining infection rates of the viruses

Numbers of infected plant samples for each virus were determined by DAS-ELISA tests. Infection rates of each virus were calculated by simple proportion. Therefore, for each virus, numbers of sum of single, double and multiple virus infected plant samples were divided to numbers of total tested samples then the results were multiplied with 100. In this way, infection rates of each virus were calculated as percentage.

Table 1. Surveyed districts and number of collected plant samples

Districts	Number of collected samples	Number of surveyed fields	Total areas of the fields (da)
Aksaray (Center district)	34	6	297.5
Ortaköy	35	7	267.5
Gülağaç	34	6	287.5
Eskil	20	4	150
Ağaçören	15	3	142.5
Sarıyahşi	15	3	135
Total	**153**	**29**	**1 280**

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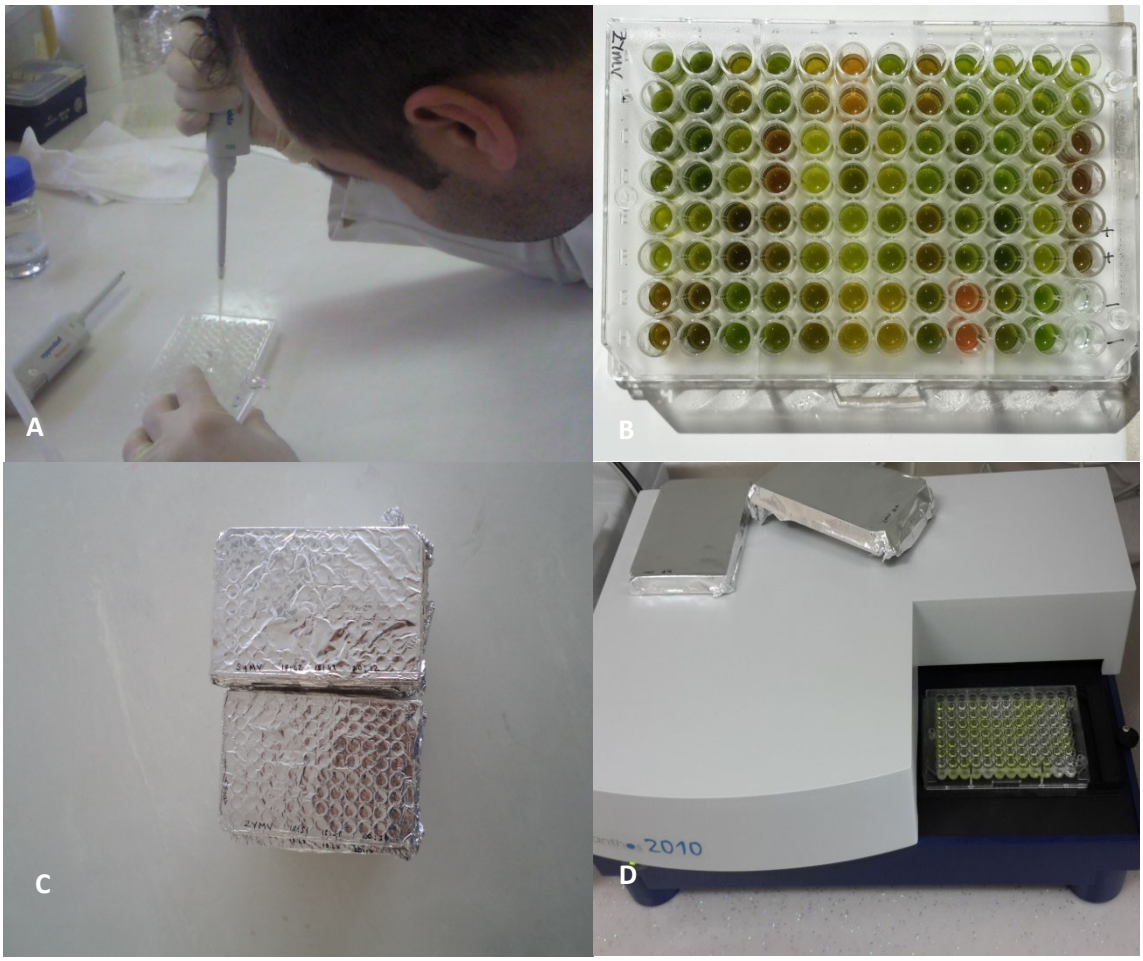

Figure 1. Steps of DAS-ELISA tests. A: Coating wells with virus IgG. B: Extracted samples were added to wells and incubated overnight in a refrigerator (+4°C). C: Substrate (*P*-NPP) added microplates, incubated for 30 to 90 min. at dark and room temperature. D: End of the incubation, the positive wells got yellow and absorbance values were determined by ELISA reader.

3. Results

In this study, totally 153 edible seed squash leaf samples were tested by DAS-ELISA. The incidences of the different viruses which infect squash are given in Tables 2 and 3. They obviously show that ZYMV and WMV-2 are the most common squash viruses in the survey area. According to the results of the DAS-ELISA 84.96 % of the samples were infected with PRSV-W, ZYMV, CMV, WMV-2, and SqMV. ZYMV was the most common virus in the survey area with the ratio of 66.01 %.

The second important virus disease in the research area was WMV-2; it was detected on the samples at the ratio of 57.51 %. They are followed by CMV, SqMV and PRSV-W, 7.18, 5.22 and 2.61% in all tested samples, respectively. None of the tested squash samples were given positive reaction to CGMMV (Table 2). On 73 of all the samples were determined double virus infections. Mixed infections of WMV-2 + ZYMV were the most frequently detected ones in the samples with the ratio 37.25 % (Table 3). Double infections with ZYMV + CMV, WMV-2 + CMV and SqMV + ZYMV were detected as 3.26, 2.61 and 1.96 %, respectively. Triple infections involving different combinations with all viruses except of CGMMV were detected in only 3 samples. Only one squash sample was simultaneously infected with ZYMV+WMV-2+SqMV+PRSV-W. Among the surveyed districts, the highest incidences of the viruses were detected in Aksaray-Center with the ratio of 91.17%. ZYMV was the most commonly detected in samples from Eskil (70%), Ortaköy (68.57%) and Ağaçören (66.66%). As for WMV-2, CMV, and SqMV were frequently detected in samples from Sarıyahşi (73.33%), Gülağaç (11.76%), and Eskil (10%), respectively. PRSV-W infections were only determined on three and one samples of Eskil (15%) and Gülağaç (2.94%), respectively.

Table 2. According to DAS-ELISA tests results, the number of single virus infections

District	No. Tested	Healty	CMV	SqMV	WMV-2	PRSV-W	ZYMV	CGMMV
Aksaray (Center)	34	3	0	1	7	0	10	0
Ortaköy	35	5	0	0	4	0	8	0
Gülağaç	34	4	1	0	6	0	6	0
Eskil	20	5	0	0	1	0	3	0
Ağaçören	15	4	0	0	1	0	2	0
Sarıyahşi	15	2	0	0	2	0	1	0
Total	**153**	**23**	**1**	**1**	**21**	**0**	**30**	**0**

Table 3. According to DAS-ELISA tests results, the number of double and multiple virus infections

	Double virus infections						Multiple virus infections			
District	WMV-2 +ZYMV	WMV-2+ CMV	ZYMV+ CMV	ZYMV+ PRSV-W	WMV-2 +SqMV	ZYMV +SqMV	ZYMV+ WMV-2 CMV	ZYMV+ WMV-2 PRSV-W	ZYMV+ WMV-2+ SqMV	ZYMV+ WMV-2+ SqMV+ PRSV-W
Aksaray (Center)	11	1	0	0	0	1	0	0	0	0
Ortaköy	13	1	2	0	1	0	1	0	0	0
Gülağaç	11	2	1	1	0	1	0	0	1	0
Eskil	7	0	0	1	0	1	0	1	0	1
Ağaçören	7	0	1	0	0	0	0	0	0	0
Sarıyahşi	8	0	1	0	1	0	0	0	0	0
Total	**57**	**4**	**5**	**2**	**2**	**3**	**1**	**1**	**1**	**1**

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4. Discussion and Conclusion

Edible seed squash is economically important in Aksaray province, but have a high incidence of virus-like symptoms. During the surveys, different symptoms were observed such as leaf deformations (crinkle, blistering, shoe-string, etc.), different chlorotic patterns on leaves (mosaic, ring spot, oak leaf, etc.), fruit deformations and growth reductions on squash plants (Fig.2). Also, symptoms of mineral deficiencies like growth reductions, wilting and yellowing were observed in some fields. The observed diseases symptoms in this study were similar to the symptoms previously reported from virus-infected cucurbits fields worldwide (Provvidenti, 1996; Luis-Arteaga et al., 1998; Yuki et al., 2000; Massumi et al., 2007; Malandraki et al., 2014). The occurrence and incidence of viruses on cucurbit plants have been determined in different parts of Turkey. The infection of CMV, CABYV, ZYMV, PRSV-W, WMV-2, SqMV, and ToMV has been reported in previous studies (Davis and Yılmaz, 1984; Erdiller and Ertunç, 1988; Yılmaz et al., 1991; Fidan, 1995; Çağlar et al., 2004; Köklü and Yılmaz, 2006; Yeşil and Ertunç, 2012; Yeşil, 2014; Şevik and Balkaya, 2015; Korkmaz et al., 2018). But there is few reports on virus diseases of edible seed squash plants (Yeşil and Ertunç,

2012; Yeşil, 2014). With this study, ZYMV and WMV-2 were determined as the most prevalent viruses in research area. They are effectively transmitted by either infected sap or vector aphids. These two viruses have been accepted as the most common viruses of cucurbits in the world (Al-Ali et al., 2013). Similarly, in a study which was carried out in different provinces of Turkey by Yılmaz et al. (1992), ZYMV and WMV-2 were the most widespread viruses among the tested viruses (ZYMV, WMV-2, CMV, CABYV and PRSV-W). The similar results were reported by Kızmaz et al. (2016). These researchers were conducted a survey in cucurbit fields of Mardin and Diyarbakır provinces and they reported that the incidences of WMV-2 (60.00%), CMV (43.13%), ZYMV (39.38%), PRSV-W (21.25%) and CABYV (16.25%). Also, a survey was carried out in Konya province, 334 edible seed squash leaf samples were tested by DAS-ELISA, and ZYMV, WMV-2 and CMV were determined on the samples with the ratios of 60.18%, 52.99% and 13.77%, respectively (Yeşil 2014). The similar results were reported by the Özaslan et al. (2006); they carried out a survey study to determine infections of cucurbit viruses in Gaziantep province of Turkey. They reported that ZYMV was the most common virus species on cucurbit plants and, also, the infections of CMV and *Potato potyvirus Y* (PVY) on cucurbits are common. To reveal viruses of cucurbits in Tokat province, a survey performed by Korkmaz et al. (2018). Totally 146 squash plant samples were tested by DAS-ELISA and they found WMV-2 (38.35%), ZYMV (26.71%), ToMV (*Tomato mosaic tobamovirus*) (22.53%), CMV (13.01%) and PRSV-W (5.47%) infections on the samples. Also, they didn't determine any infection of TMV (*Tobacco mosaic tobamovirus*), SqMV and PVY.

Also, mixed virus infections were determined on 77 of the plant samples with this study (Table 3). In previous studies, mixed virus infections on cucurbits were reported (Yuki et al., 2000; Yeşil and Ertunç, 2012; Yeşil, 2014).

Some of the cucurbit viruses can be transmitted by seeds such as CMV, ZYMV, SqMV, TRSV, and CGMMV. A research was performed to detect the presence of seed borne viruses in pumpkin seed lots collected from Samsun, Sinop, and Bolu provinces during 2013-2014. According to the results of this research, the seed samples were only infected with ZYMV (12.5%) and CMV (4.1%). Moreover, any infection wasn't determined on the seeds of SqMV, TRSV, and CGMMV. With another study about determining seed infections of some viruses in major cucurbit growing areas in Konya, Karaman and Aksaray provinces of Turkey during 2009 and 2010. The results of this study showed that 8.7% of seed samples were infected with ZYMV (4.3%), WMV-2 (3.3%) and CMV (1.1%). PRSV-W, SqMV and CGMMV were not determined in any of the tested samples and were not present in the tested cucurbit seeds lots (Yeşil and Ertunç, 2016). As can be seen in the above mentioned studies, the reason of occurring frequently infections on cucurbits by the viruses such as ZYMV, WMV-2 and CMV may be infected seeds.

In the present study, WMV-2 and ZYMV were detected as most common viruses. In previous studies, similar viruses were detected in different incidences (Erdiller and Ertunç, 1988; Yılmaz et al., 1991; Fidan, 1995; Köklü and Yılmaz, 2006; Yeşil and Ertunç, 2012; Yeşil, 2014; Şevik and Balkaya, 2015; Korkmaz et al., 2018). It may be two main reasons for this. Firstly, although other cucurbit crops are grown only in irrigated fields, edible seed squash plant can be grown in either irrigated or semi-arid conditions. The second reason is regional differences. It's normal that, different viruses infect to the same plant species in different environmental conditions. Because differentiations in environmental conditions determine significantly some factors which effect virus epidemiology. These factors are populations and varieties of weed and vector species in or near fields, plant species which are grown in adjacent fields and plant vitality.

Figure 2. Virus diseases symptoms on edible seed squash plants. A: Leaf deformations and stunting caused by ZYMV. B: Severely leaf deformations and mosaic symptoms because of ZYMV+CMV double infection. C: Leaf deformations and vein banding symptoms on squash leaf and blisters on fruit (D) because of ZYMV+WMV-2 double infections.

The presences of ZYMV, WMV-2, CMV, PRSV-W, and SqMV on edible seed squash were detected in Aksaray. The results showed that one of the most important problems in squash growing in the province is virus infections. Because, during the survey studies, symptoms of virus diseases were observed almost each edible seed squash fields in the province. According to the results of this study, for reducing or eradication of virus diseases in squash production areas in Aksaray province and can be produced more yielded and more quality edible squash seeds the following suggestions must be regarded. First of all, healthy, pathogen-free and certified seed should be used.

For controlling virus diseases efficiently, it is very important to know about transmission ways and infection sources of the viruses. It's known that, except of SqMV, all viruses detected in our study were spread by mechanical inoculation and aphids (Kaper and Waterworth, 1981). Unfortunately, squash growers in the province neither know symptoms of virus diseases nor know transmission ways of the viruses from plant to plant. Therefore, they are not able to efficiently control virus diseases as they can't prevent the spread of viruses by vector aphid species.

Also, some of the common weed species in squash growing areas have a great importance in the epidemiology of virus diseases because they role as reservoir plants for virus diseases (Zitter, 2002; Yeşil and Ertunç, 2015). For preventing virus infection of cucurbits, weeds must be controlled.

As well in other plant crops production, in squash production cultural practices are very important. If all conditions which are necessary for growing healthy plant can be obtained, possibility of chance of phytopathological problems occurrence will be minimum. Therefore, cultural practices such as tilling, planting, fertilizing and irrigation should be done properly.

The plants which show virus diseases symptoms should be eradicated as soon as seen. Since, they act as infection sources for later infections.

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