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Identification, Distribution and Incidence of Important Tomato and Cucurbits Viruses in the Southeast of Iran

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ABSTRACT

Tomato and Cucurbits viruses diseases are the major production constraints in worldwide. A survey was conducted to determine the incidence some of the most important viruses infecting tomato and Cucurbits naturally in the major growing areas in the southeast of Iran. A total of 962 symptomatic leaf samples (542 tomato and 420 cucurbits) were surveyed for virus infection by Enzyme-linked Immunosorbent Assay methods (ELISA) viz, double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and triple antibody sandwich ELISA (TAS-ELISA). This survey revealed infection of cucurbits to ZYMV, WMV-2, CMV and CGMMV and tomato to ToMV, TSWV, TYLCV and PVY° and CMV in Southeast of Iran. TYLCV with incidence of 28.9% was the most important restriction factor in greenhouse and field-grown tomato. ZYMV ZYMV and CMV-2 was identified in all four cucurbits and prevalence of ZYMV (19.7%) is much stronger than the one of CMV-2 (8.8%). The results showed a relation between TYLCV infection and sowing date for greenhouse-grown tomato. Infections were less in early sowing date (End of July) than late sowing (First of February on forward). To our knowledge, this is the first report of serological detection of PVY° on tomato in Iran and ZYMV and CGMMV on cucurbits in Southeast of Iran.

Key words: DAS-ELISA, incidence, TAS-ELISA, tomato yellow leaf curl virus, zucchini yellow mosaic virus

INTRODUCTION

Cucurbits and tomato are grown in fields and specially greenhouses in most area of Southeast of Iran. Viruses have always been a major cause of reduced quantity and quality of the worldwide tomato and Cucurbits crop (Shirazi *et al*., 2010; Vahdat *et al*., 2008). There are some reports for virus diseases on cucurbits and tomato in the southeast of Iran (Hajimorad *et al*., 1996; Massumi *et al*., 2007). However, there is no competitive survey to detect agents of viral diseases and those distributions.

Two virus viz, Potato Virus Y (PVY) and Tomato Mosaic Virus (ToMV) were found to be widespread in Mauritius and PVY° is the prevalent race on tomato (Ganoo and Saumtally, 1998). Tomato Yellow Leaf Curl Virus (TYLCV) is one of the most devastating viral diseases of cultivated tomato in tropical and subtropical regions worldwide and losses of up to 100% are frequent

(Moriones and Navas-Castillo, 2000). In Iran, TYLCV was reported from Sistan and Baluchestan province (Behjatnia *et al*., 2004; Vahdat *et al*., 2008). Also southern provinces of Iran and there is prevalent in field-grown tomato in Sistan and Baluchestan, Kerman, Hormozgan and Khuzestan provinces (Hajimorad *et al*., 1996). TYLCV has a broader host range than other whitefly-transmitted geminiviruses which includes tomato, pepper, cucumber and some weed species such as *Malva* sp., *Trigonella* sp., *Daucus* sp. and *Melilotus officinalis* (Shirazi *et al*., 2010). Surveys in Iran was confirmed for detection of Cucumber Mosaic Virus (CMV), Beet Curly Top Virus (BCTV), Tomato Yellow Leaf Curl Virus (TYLV), Potato Virus Y (PVY), Tomato Spotted Wilt Virus (TSWV), Tomato Ring Spot Virus (TRSV), Arabis Mosaic Virus (ArMV), Tobacco Mosaic Virus (TMV) and Tomato Mosaic Virus (ToMV) in Tomato (*Lycopersicum esculentun*) from agricultural regions in southeast and central parts of Iran. The BCTV, ToMV, TSWV and TMV were detected in 8.6, 6.5, 5 and 3.5% of samples tested, respectively. TBSV, TcSV, TAV, TSV was not detected in all samples tested (Massumi *et al*., 2005). In USA, the estimated losses caused by whitefly-transmitted geminiviruses reach about 20% of tomato production but in the Dominican Republic, Cuba, Mexico, Guatemala, Honduras, Nicaragua, Costa Rica, Venezuela and Brazil the damage is much greater, ranging between 30 and 100% of the yield. The loss in The Dominican Republic during 1989-1995 was estimated at $50 million (Polston and Anderson, 1997). In African countries, a total of 11,694,000 t of tomatoes were produced on an area of 602,744 ha with an average yield of 19.4 t ha^{-1}. As most of the production is in open fields, plants are exposed to infection by TYLCV, which results in severe epidemics of the disease (personal observations). In 1988, losses from this disease in Egypt were estimated at 32,000 ha (Nakhla *et al*., 1993).

Cucurbits are major vegetable crops in Iran, ranking first in economic value, second in yield and third in acreage (Bananej and Vahdat, 2008). Among the diseases affecting cucurbits, viral diseases can result in lethal syndromes and yellowing phenotypes and are very difficult to manage (Shang *et al*., 2009). More than 35 viruses are detected on Cucurbits (Provvidenti, 1996). Identification and distribution of Seven Viruses Infecting Greenhouse-Grown Cucurbits in Iran showed that CMV and ZYMV were the viruses most frequently detected, accounting for 21.2 and 18% of the infected plants, respectively. WMV-2 was detected with 4.3% incidence in 15 regions and TSWV with 1.25% incidence only in 2 regions. CuNV, SqMV and PRSV-W were not detected in any samples (Massumi *et al*., 2007). ZYMV is a potyvirus with a worldwide distribution that cause serious economic losses in many cucurbits. This virus can transmit by *Aphis fabae*, *A. gossypii*, *A. craccivora* and *Myzus persicae* in Iran (Shaabanian *et al*., 2005).

Limited information is available on the occurrence and the relative importance of viruses infecting tomatoes and Cucurbits in this major tomato growing area in southeastern of Iran. In the present study, the incidence and distribution of Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Bushy Stunt Virus (TBSV), Tomato Mosaic Virus (ToMV), Tomato Spotted Wilt Virus (TSWV), Cucumber Mosaic Virus (CMV), Eggplant Mottled Dwarf Virus (EMDV), Potato Virus X (PVX), Potato Virus Y (PVY) and its races like PVY^{n}, PVY°, PVY^{c} on tomato, and Zucchini Yellow Mosaic Virus (ZYMV), Watermelon Mosaic Virus-2 (WMV-2), Cucumber Green Mottle Mosaic Virus (CGMMV), CMV, TSWV and EMDV on Cucurbits was documented.

MATERIALS AND METHODS

Surveys and sample collection: Samples including 542 tomato and 420 cucurbits were collected from field and greenhouses of five agricultural region in Southeast of Iran (Zahedan, Khash, Saravan, Iranshahr and Chabahar) during 2010 (Fig. 1). In each region, tomato and cucurbits

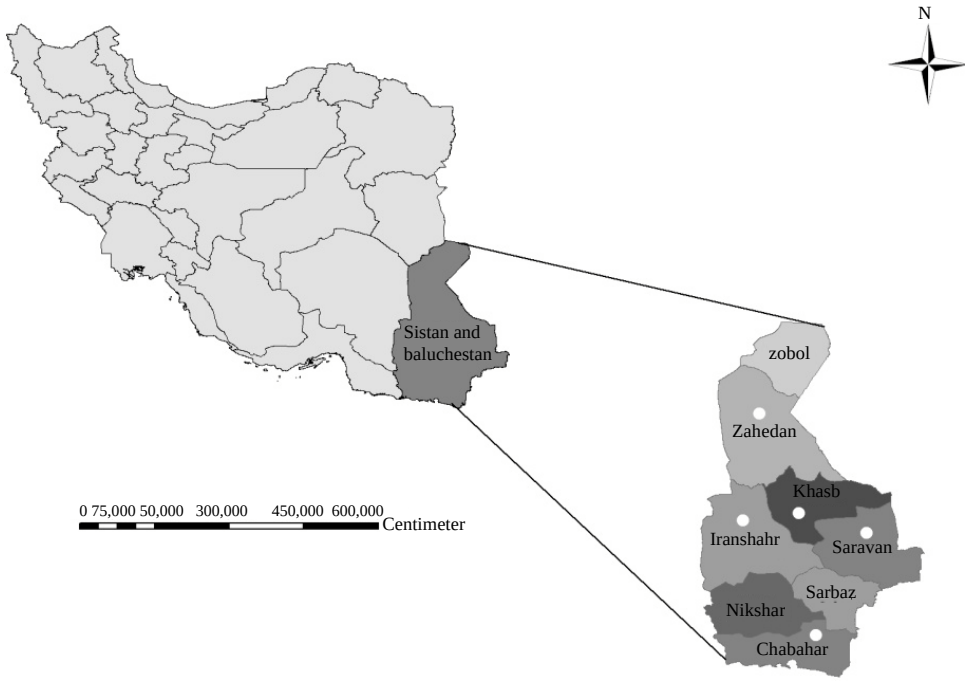

Fig. 1: Map of Iran showing sistan and baluchestan province in which surveys were conducted. Site of samples collecting; Zahedan, Khash, Saravan, Iranshahr and Chabahar

such as cucumber, squash, watermelon and melon were grown in fields and greenhouses, surveyed for virus infection. Surveys were preferably conducted during a mid-developmental stage of the plant. Plants were randomly evaluated based on symptoms thought to be caused by virus infection such as mosaic, necrosis, chlorosis, veinclearing, mottle and/or leaf malformation and fruits that were smaller and malformed and showed abnormal discoloration on their surface. Young leaves from some symptomatic plants was placed separately in a plastic bag and kept on ice until surveyed.

Serological method and virus identification: Tomato and cucurbit leaf samples were tested by the standard DAS-ELISA according to Clark and Adams (1977). IgGs and alkaline phosphatase conjugated IgGs were used for all viruses, exception of TSWV and TYLCV that was determined by using triple antibody sandwich ELISA (TAS-ELISA). All Antisera used in this study was prepared as Table 1 and 2.

The incidence of EMDV was determined by DAS-ELISA but 2% PVP and 0.1% 2-Mercaptoethanol are added in buffer samples. For detection of PVY races (PVY°, PVY^{c} and PVY^{n}), PVY$^{d/o}$, PVY^{c} and PVY^{n} conjugates was used. Results that showed positive to test both of PVY$^{d/o}$ and PVY^{c} confirmed PVY^{c} infection, but if was positive for PVY$^{d/o}$ while was negative for PVY^{c} confirmed PVY° infection.

Triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) was carried out for detection of TSWV and TYLCV by use of the sample grinding buffer and inclusion of a blocking step using Phosphate-buffered Saline (PBS) containing 0.05% Tween 20 with 0.2% skim milk powder for 30 min at room temperature. Clarified extracts (200 μL/well) were added to microtiter plates percolated with the polyclonal antibody and stored overnight at 4°C. The MAb and conjugate

Table 1: Properties and references of virus antisera and conjugates were used in DAS-ELISA and TAS-ELISA in tomato leaf samples

Virus	Abrasive	Reference of kit preparation
Tomato spotted wilt virus	TSWV-0105 MAb:0106/1+0116/1	Com. DSMZ
Eggplant mottle dwarf virus	EMDV-P/Ir	Dr. Lokhart and Dr. Danesh in university of Minesota
Cucumber mosaic virus	CMV-0475	Com. DSMZ
Cucumber green mottle mosaic virus	CGMMV-0190	Com. DSMZ
Zucchini yellow mosaic virus	ZYMV-0234	Com. DSMZ
Watermelon mosaic virus	WMV-0203	Com. DSMZ

Table 2: Properties and references of virus antisera and conjugates were used in DAS-ELISA and TAS-ELISA in cucurbits leaf samples

Virus	Abrasive	Reference of kit preparation
Tomato mosaic virus	ToMV-0104	Com. DSMZ
Tomato bushy stunt virus	TBSV-0098	Com. DSMZ
Tomato spotted wilt virus	TSWV-0105MAb:0106/1+ 0116/1	Com. DSMZ
Tomato yellow leaf curl virus	TYLCV-0588MAb:0546	Com. DSMZ
Tomato yellow leaf curl virus	TYLCV-0588MAb:0546/4	Com. DSMZ
Potato virus Y	PVY	Dr. Salazar in international research center of Prou
Potato virus Yn	PVYn	Dr. Salazar in international research center of Prou
Potato virus Yc/o	PVYc/o	Dr. Salazar in international research center of Prou
Potato virus Yc	PVYc	Dr. Salazar in international research center of Prou
Potato virus X	PVX-126	Com. DSMZ
Eggplant mottle dwarf virus	EMDV-P/Ir	Dr. Lokhart and Dr. Danesh in Miosota university
Cucumber mosaic virus	CMV-0475	Com. DSMZ

were used at a dilution of 1:1000 and incubated at 4 and 37°C for 3 h, respectively. The substrate p-nitrophenyl phosphate (PNPP) was used at 0.6 mg mL^{-1}. Samples with absorbance values (at 405 nm) greater than three times the mean of the absorbance of the healthy controls were considered positive. Reaction for both DAS-ELISA and TAS-ELISA were measured spectrophotometrically at 405 nm using an EL808 ELISA reader (Bio-Tek Instrument). Positive and negative controls were included in all tests for each virus. A sample was considered virus-positive if the Optical Density (OD) exceeded the mean plus two standard deviations of the OD of the healthy controls.

Relation between date culture and disease incidence: Some properties of culture such as date culture, greenhouse or field growing and the first time observation of viral disease symptoms by farmers registered. This information is used to consider ecological status, disease progression and to predict relation between disease incidence and date culture. In order to detect of distribution and host range of viral agents that cause disease in tomato and cucurbits, results of DAS-ELISA and TAS-ELISA tests was registered.

RESULTS

Field and greenhouse symptoms: Infected plants exhibited a variety of symptoms, consist of a more or less prominent upward curling of leaflet margins, reduction of leaflet area and yellowing of young leaves, together with stunting and flower abortion (Fig. 2). Infection results in a general decrease of plant growth and reduced yields. If plants are infected during early growth, the production is almost entirely lost.

Fig. 2(a-f): Symptoms of the disease, (a) watermelon infected by Watermelon Mosaic Virus (WMV), (b) symptom of ToMV on tomato and mosaic that caused by WMV-2 on watermelon (c) Mosaic, leaf malformation and mottling on young leaves and fruits of SqMV infected squash, (d) Dwarf and leaf curl on field growing tomato that caused by TYLCV, (e) Healthy (right) and infected tomato (left) in greenhouse growing that infected by TYLCV, (f) Mosaic, leaf malformation and mottling is clearly symptoms of SqMV on infected squash

The severe growth reduction of the plants, the typical yellowing and curling of the leaves due to TYLCV infection is easily detected by farmers, even not being familiar with those symptoms (Fig. 2, d and e). EMDV can produce the same symptoms like TYLCV, such as dwarfing, reduction of leaflet area together with flower abortion on tomato (Jafari *et al*., 2007). Distinctive symptom of TYLCV is curling of the leaves especially on tomato greenhouse varieties (Fig. 2e) that can not produced by EMDV. Bushy stunt may be a common symptom for TYLCV and TBSV. In this survey, EMDV and TBSV on tomato was not detected. This finding confirmed that TYLCV was one of the tomato infecting viruses, inducing the most obvious symptoms and there was a very good correlation between symptoms and disease incidence of TYLCV on tomato. This virus vectored by the whitefly *B. tabaci*, it is expected that the introduction of TYLCV in a new environment is detected soon after the first infection of tomato plants. Race PVY° of PVY was detected in some open fields grown tomato. Infected plants exhibit mild mosaic, leaf crinkling symptom, not readily distinguishable from ToMV symptoms (Fig. 2b).

Identification and distribution of viruses in cucurbit samples: A 420 symptomatic leaf samples that taken from melon, squash, watermelon and cucumber in field and greenhouses of sistan and baluchestan province was analyzed. Cucurbit viruses that identified were involved

Table 3: Occurrence of viruses on tomato in different regions of southeast Iran

	Viruses										
Region	ToMV	TBSV	TSWV	TYLCV	PVY	PVYn	PVYc/o	PVYc	PVX	EMDV	CMV
Zahedan	8/105[a]	0/105	0/105	35/105	0/105	0/105	0/105	0/105	0/105	0/105	12/105
Khash	13/115	0/115	5/115	42/115	8/115	0/115	0/115	0/115	0/115	0/115	18/115
Saravan	22/140	0/140	6/140	48/140	5/140	0/140	7/140	0/140	0/140	0/140	0/140
Iran-shahr	0/95	0/95	0/95	18/95	4/95	0/95	0/95	0/95	0/95	0/95	23/95
Chabahar	0/87	0/87	0/87	14/87	0/87	0/87	0/87	0/87	0/87	0/87	0/87
Total	43/542	0/542	11/542	157/542	17/542	0/542	7/542	0/542	0/542	0/542	53/542
Percent	7.9	0.0	2.0	28.9	3.1	0.0	1.2	0.0	0.0	0.0	9.7

[a]Number of infected plants/number of plants sampled

Table 4: Occurrence of viruses on cucurbits in different regions of southeast Iran

	Viruses					
Region	TSWV	EMDV	CMV	CGMMV	ZYMV	WMV-2
Zahedan	0/80[a]	0/80	8/80	0/80	22/80	7/80
Khash	0/95	0/95	11/95	7/95	21/95	11/95
Saravan	0/110	0/110	7/110	6/110	29/110	14/110
Iran-shahr	0/75	0/75	13/75	0/75	7/75	3/75
Chabahar	0/60	0/60	0/60	0/60	4/60	2/60
Total	0/420	0/420	39/420	13/420	83/420	37/420
Percent	0	0	9.2	3.0	19.7	8.8

[a]Number of infected plants/number of plants sampled

Table 5: Host rang of viral agents were detected in field cucurbits in southeast of Iran

	Cucumber	Squash	Melon	Watermelon
TSWV	-	-	-	-
EMDV	-	-	-	-
CMV	+	-	-	-
CGMMV	-	+	-	-
ZYMV	+	+	+	+
WMV-2	+	+	+	+

+: Detected, -: Not detected

CGMMV, ZYMV, CMV and WMV-2 (Table 5). ZYMV and CMV-2 were the most frequently found viruses, accounting for 19.7 and 9.2%, respectively in all areas surveyed (Table 4). Whereas, incidence of WMV (8.8%) and CGMMV (3%) were rather rare in those cucurbits. TSWV and EMDV in all four cucurbit species, CGMMV in Cucumber, Melon and Watermelon were not detected in any of the samples tested (Table 5).

Identification and distribution of viruses in tomato samples: A survey encompassing the major tomato growing regions of siatan and baluchestan province of Iran was conducted to determine the incidence and relative importance of ToMV, TBSV, TSWV, TYLCV, PVY, PVYn, PVYc/o, PVYc, PVX, EMDV and CMV in tomato fields and greenhouses. Of 542 tomato leaf samples tested, ToMV was found in 43 samples (7.9% incidence), TSWV in 11 (2%), TYLCV in 157 (28.9%), PVY in 17 (3.1%), $PVY^{c/o}$ in 7 (1.2%), CMV in 53 (9.7%) and TBSV, PVY^{n}, PVY^{c}, PVX and EMDV

were not detected in any of the samples tested. TYLCV was the most frequently found in all of the regions surveyed (Table 3). PVY$^{d/o}$ was detected only in saravan region.

DISCUSSION

Viral diseases are a restriction factor to produce cucurbit in worldwide since yet there are no efficient chemical treatments that protect plant from virus infection (Ullman *et al*., 1991; Ozaslan *et al*., 2006). it is usually difficult to give definitive diagnosis based of symptoms since symptoms induced by these viruses vary depending on the host, environmental condition and individual virus infecting plant (Ozaslan *et al*., 2006). Serological techniques such as ELISA are more convention and inexpensive than existing technique, although they require some specialized equipment (Vinayarani *et al*., 2011). ZYMV, WMV, CGMMV, WmCSV, CMV, Cucumber yellow stunting disorder virus (CYSDV), PRSV-W, SqMV, Ourmia melon virus (OuMV), CABYV and CVYV have been reported from field-grown cucurbit crops in Iran (Danesh, 1969; Ebrahim-Nesbat, 1972; Ebrahim-Nesbat, 1974; Rahimian and Izadpanah, 1978; Keshavarz and Izadpanah, 2004). This survey revealed that ZYMV is the most common virus causing severe symptoms and yield loss in open fields of cucurbits in Southeast of Iran (19.7%). ZYMV were detected in different cucurbit crop in different part of Iran (Safaeizadeh, 2008). Similar study was performed in Gaziantep-Turkey and 40% ZMYV, 36% CMV and 5% PVY of 56 samples were determined (Ozaslan *et al*., 2006).

Severe infection of field-grown squash is major reason for most farmers in Sistan and Baluchestan province no longer grow summer and fall squash. ZYMV, WMV-2 and CMV are the prevalent viruses in fields and greenhouses of Iran (Massumi *et al*., 2007). WMV-2 and ZYMV from *potyvirus* genus are more economical and prevalent viruses of cucurbits in Baluchestan of Iran. The widespread occurrence of viruses showed that the Iranian local varieties are very susceptible and Results confirmed the management of ZYMV in fields that grown squash is necessary.

Integrated management is necessary to control aphid-born viruses specially ZYMV in the squash and whiteflies in the tomato grown-greenhouses and fields. To use Resistant cucurbit cultivars to viral disease agents considerably will be useful in siatan and baluchestan province. Out of several methods that have been trying to control virus diseases, the protection of vector transmission is widely recognized (Bhyan *et al*., 2007). Presumably, the isolation of vector in greenhouse is an essential factor for disease incidence. It is seems that, because of weather is cold in the beginning of plant growth, condition for activity and infection of vector *B. tabaci* is unsuitable. Activity of vector is contemporary with fruiting in the first of spring that the damage of virus is low. However, an important option for disease control would be the use of cultivars with genetic resistance to the virus. It was concluded that planting variety marketer and controlling the disease especially at 3-4 leaf and flower bud stages could form important components of integrated pest management programme of TSWV (Rapando *et al*., 2006). To our knowledge, this is the first report of PVY° infecting tomato and ZYMV and CGMMV on cucurbits for the first time in Southeast of Iran and investigated the geographical distribution.

About 25 different viruses are reported on tomato worldwide. Some of the most important viruses infecting tomatoes naturally are: CMV, ToMV, TYLCV, TSWV, BCTV, TMV and PVY. Many others, including TBSV and Arabis mosaic virus ArMV are also damaging to tomato. TYLCV was a serious virus disease, can infect at any growth stages of the plant which exhibits various prominent symptoms such as upward curling of leaf margins, stunting, reduction of leaf size at the top of the plant, corrugated leaf, shortening of internodes and severe reduction in fruit yield, has

become prevalent in tomato fields all over the Middle East (Makkouk and Laterrot, 1983; Farid Uddin *et al*., 2004). The Results confirmed that TYLCV not only is the most important viral disease but also is the most restriction agent of tomato culture in Sistan and Baluchestan province. TYLCV causes economic losses upto 100% in tomato crop in many tropical and subtropical regions, and is spreading towards new areas (Pico *et al*., 1996). In some regions, the impact of the virus disease has been so severe that some farmers were forced to shift from tomato to eggplant or other crops (Hajimorad *et al*., 1996). The incidence of CMV, PVX, PVY, ToMV and Tomato Yellow Top Virus (TYTV) in tomato fields in Pakistan, neighboring the southeastern region of Iran was reported by Ali and Hassan (2002).

TYLCV has previously been reported from Sistan and Baluchestan province (Hajimorad *et al*., 1996). Almost total fields of Baluchestan that are grown tomato can be infected TYLCV (28.9%). TYLCV infected tomato grown under field conditions in various areas of Sistan and Baluchestan province showed that incidence of disease was high (28.9%) and infected plants can not able to produce any product in the end of season. Behjatnia *et al*. (2004) have recently isolated a Tomato Leaf Curl Iran Virus (TLCIRV) which causes leaf curling disease symptoms in tomato fields in Iranshahr region.

Unlike, the fields, the infection of tomato to TYLCV was not the same in greenhouse. Our study of relation between date of culture and disease incidence of tomato viruses in Southeast of Iran showed a significant relation in greenhouse-grown tomato, unlike field-grown tomato. Severe and low infection was observed in July-August and January-February 2010, respectively. The TYLCV was transmitted by *B. tabaci* (Cohen and Harpaz, 1964) to tomato and jimsonweed and since these vectors are found in most regions of Iran, the viruses may become a serious threat for cucurbit crops (Bananej and Vahdat, 2008).

Unlike, Babaie and Izadpanah (2002) that could detect EMDV on tomato and cucumber in Charmahal and Bakhtiari province, that it's whether is cold, we couldn't detect EMDV on cucurbits and tomato in Sistan and Baluchestan province. These two areas have different climates and it seems that because of warm weather in sistan and baluchestan province that is unsuitable for EMDV and its vector. Also the absence of positive reactions may be due to infection of the plants with other viruses or an as yet unidentified virus present in the southeastern of Iran.

CONCLUSION

Results of the present study showed that virus infections should be of special concern in the Southeast of Iran. They may become a serious threat to the production of tomato as a major winter crop in the fields and greenhouses, where a great proportion of the Iranian tomato crop is produced annually during the cooler seasons. Disease incidence was different in the end of season in greenhouse that it depend the management and especially environment conditions such as temperature.

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