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BIOLOGICAL, SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF EGYPTIAN *Zucchini yellow mosaic virus* ISOLATE INFECTING SQUASH PLANTS IN FAYOUM GOVERNORATE

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ABSTRACT: Zucchini yellow mosaic virus (ZYMV) is one of the most important viruses and is responsible for significant losses in cucurbit crops worldwide. In Egypt, it causes serious economic losses in squash especially in spring season. During 2014-2015, samples were collected from squash fields in different Provinces, Fayoum Governorate. In Fayoum, ZYMV-naturally infected squash plants exhibited severe mosaic and blisters, leaves deformation, blisters on the fruits and discoloration hardening of flesh, as well as external fruit cracks. Percentages of infection in squash cv. Eskandrani, ranged between 20-35% and 70-78% in mid-March and May, respectively. Meanwhile, it ranged between 15-25% in September and 62% in the end of November. The virus was isolated from naturally-infected squash plants serologically depending on enzyme-linked immunosorbant assay (ELISA), and biologically by mechanical inoculation to different species belonging four families *i.e.*, Amarnthaceae, Chenopodiaceae, Cucurbitaceae, Solanacea. In virus vector relationship, transmission rates recorded 84.6, 86.6, 93.3, 90, and 91.6%, respectively for squash cvs. Alia, Asma, Eskandrani, Mabrouka and Safa. In this context, one insect vector (*Myzus persicae*) proved its ability to transmit ZYMV. Electron microscopy (EM) of partially purified preparation method revealed flexuous particles approximately 750X15 nm. Serologically, TBIA proved to be effective in detecting ZYMV in the infected samples and reliable technique for the virus detection in squash leaf midrib and petioles. Electrophoresis analysis of the reverse transcription polymerase chain reaction (RT-PCR) amplification revealed that the primers amplified a product size of 458bp for the RNA extracted from ZYMV-infected tissues.

Key words: Myzus persicae, polymerase chain reaction (PCR), RT-PCR, zucchini yellow mosaic virus, ZYMV.

INTRODUCTION

Cucurbitaceous crops comprise a large and diverse group of crops. Cucurbits are warm-season crops that are cultivated and harvested over spring, summer, and autumn seasons. They constitute an important part of a diverse and nutritious diet throughout the world, which are used as salad and pickled (cucumber), cooked (all gourds and squashes), candied, or preserved (ash gourd) vegetables or as dessert fruits (muskmelon and watermelon). Also, cucurbits are used as fiber source and for utensil preparations, decorations, and ceremonial and medicinal purposes (**McGrath, 2004; Sharma and Gaur, 2015**). All cucurbitaceous crops are susceptible to several viruses *i.e.*, *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic*

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virus (SqMV) (**Mansilla *et al.*, 2013; Chinnaraja *et al.*, 2016**), *Watermelon mosaic virus* (WMV) and *Tobacco ring spot virus* and they also infect certain non-cucurbitaceous weeds and wild cucurbits.

Zucchini yellow mosaic virus (ZYMV) is a member of the genus *Potyvirus*, the largest group among plant viruses, and is the major limiting factor for production of cucurbits worldwide. The disease is characterized by pronounced reduction in plant growth, yellow mosaic and distortion of leaves, and malformation of fruit (**Simmons *et al.*, 2008; Lecoq *et al.*, 2014; Khalifa *et al.*, 2015**). ZYMV can result in yellowing and stunting of the plant, as well as severe leaf and fruit deformities that can reduce yields up to 94%. The economic significance of this crop pathogen is enormous. Understanding the epidemiology and evolution of ZYMV is therefore central to controlling this devastating crop disease (**Simmons *et al.*, 2011**). This virus is transmitted through contact between plants, seeds and mechanically (**Coutts *et al.*, 2011; Coutts *et al.*, 2013; Tymchyshyn *et al.*, 2017**). Also, a large number (26) of aphid species has been shown to be capable of transmitting the virus, although two species, *Myzus persicae* and *Aphis gossypii*, have the highest reported transmission efficiencies (41% and 35%, respectively) (**Katis *et al.*, 2006; Pinto *et al.*, 2008; El-Borollosy, 2015**). ZYMV particles are flexuous, 750 nm in length, and contain a monopartite genome consisting of a positive-sense ssRNA with a 5′ genome-linked protein (VPg) (**Anthony-Johnson *et al.*, 2013**). Most *potyvirus* proteins are multifunctional and the P3 proteins are among the most variable proteins in *potyvirus* genomes and are considered key virulence factors (**Tymchyshyn *et al.*, 2017**). Several methods were used for laboratory diagnosis ZYMV included serological and nucleic acid-based techniques utilizing polymerase chain reaction (PCR) (**Hosseini *et al.*, 2007; Zheng *et al.*, 2010; Amer, 2015; Ghanem *et al.*, 2016**). Therefore, the purpose of this research was focused on biological, serological and molecular characterization of ZYMV isolate from Fayoum Governorate.

MATERIALS AND METHODS

Biological Studies

Transmission test was carried out with squash (*Cucurbita pepo* cv. Eskandrani) plants grown in 30 cm pots, under greenhouse conditions, at Plant Pathology Dept., Fac. Agric., Cairo Univ. and Plant Pathology Research Institute, ARC, Giza.

Mechanical transmission and host range study

In early spring (mid-March), field inspection of squash plants were done in Abou Khlaf, Menshat Abdullah, Zawia, Edwa and Azab provinces belonged to Fayoum Governorate. In order to identify and detect ZYMV, visual inspection and samples were collected during 2014 and 2015 growing seasons from squash fields (*Cucurbita pepo*), Fayoum Governorate. In this region, squashes are planted during early March and harvested from May. Infected squash plants exhibited symptoms of mosaic, leaves deformation, blistering of leaves and fruits collected and kept in ice chests for transportation to the laboratory. Young leaves from some symptomatic plants were collected at random. Each plant sample was labelled and kept separately in a plastic bag at 4ºC until analyzed. Percentages of infection were calculated and confirmed by ELISA test.

ZYMV was mechanically inoculated on hosts including certain diagnostic hosts belonging to Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Leguminosae and Solanacae families. Five plants were tested from each species. The experiment was repeated three times. Symptoms were examined periodically through a period of 45 days. Back inoculation was performed onto *Chenopodium amaranticolor* as a local lesions host. The virus was then transmitted mechanically onto squash plants cv. Eskandrani as a source for virus propagation. Potassium phosphate buffer (0.1 M), pH 7.2 was used in mechanical inoculation. Inoculated plants were maintained in the greenhouse at daily temperatures of about 25ºC.

Aphid transmission

In the surveyed squash fields, *Myzus persicae* is the most existence and spread aphid vectors

during 2-years experimentation. Aphid transmission using *Myzus persicae* was conducted as described by **El-Borollosy (2015)**. Aphid species were collected from the same squash field and identified in Econ. and Pest. Dept., Fac. Agric., Cairo Univ. Aphid's samples were reared on turnip plants as a virus-free culture, then tested biologically and serologically to be confirmed. Non-viruliferous insects were used for transmission experiments and reared on turnip plants under cadges. After starvation for 1 hr., groups of 5 insects were placed for 15 min acquisition access feeding (AAF), on ZYMV-infected squash plants cv. Eskandrani which were inoculated 3 weeks previously. Viruliferous insects (5 aphids/plant) were transferred on healthy squash seedlings of cvs. Alia, Asma, Eskandrani, Mabrouka and Safa (carrying the two true leaves) for 1 hr., inoculation access feeding (IAF) and then sprayed with insecticide, then kept in an insect-proof cages. To determine specificity of vector in virus transmission, one insect (*M. persicae*) was used to transmit the virus from plant to plant in separated experiment. After 2weeks, plants were inspected for ZYMV-infection and results were confirmed using ELISA test and rates of transmission were calculated as follows:

$$\text{Rates of transmission (\%)} = \frac{\text{No. of infected}}{\text{No. of total tested plants}} \times 100$$

Physical Studies Using Electron Microscopy (EM)

Carbon coated grid was dipped in partially purified virus for 5min, then stained with 2% sodium phosphotungstate (PTA) and air dried. Examination was carried out using an electron microscope JEOL (JEM-1400 TEM, Japan) at the candidate magnification at Faculty of Agric., Cairo Univ. Images were captured using CCD camera Model AMT. Virus examination was done in Research Park (FARP), TEM Lab., Fac. Agric., Cairo Univ.

Serological Detection of ZYMV

Serological tests of enzyme linked immunosorbent assay (DAS-ELISA) and tissue blot immuno-printing assay (TBIA) were done for ZYMV detection as described by **Ghanem (2003) and Ghanem *et al.* (2016)**, respectively. DAS-ELISA was conducted using polyclonal antiserum (ZYMV- Pab) to ZYMV. ELISA Kit containing ZYMV-Pab. was supplied by Agdia, India.

Concerning tissue blot immuno-printing assay (TBIA) on nitrocellulose membrane (NCM), the protocol was performed to detect ZYMV-infection and to confirm ELISA detection. This procedure was done to determine the distribution of ZYMV-antigen in infected tissue sections (petioles and midrib) according to **Ghanem *et al.* (2016)**. Preparations were treated with equal volumes of TBST buffer containing 0.01 M Tris-HCl, 0.05% Tween-20 and 0.15 M NaCl, pH 8.0 in TBIA test; chromogenic substances were used for color development. Nitroblue tetrazolium (NBT)-5-bromo-4-chloro-3-indolyl phosphate (BCIP) complex was used for purple-color development in positive reaction compared with green color for negative reaction.

Molecular Detection Using Reverse-Transcriptase Polymerase Chain Reaction

In this procedure, reverse-transcriptase polymerase chain reaction (RT-PCR) was done according to **Hosseini *et al.* (2007)**. Total RNA from ZYMV-infected plants was extracted using a phenol/ chloroform protocol. Three µl of RNA were submitted to reverse transcription in a final volume of 20 µl, using 2 µl PCR buffer 10x (0.5M Tris-HCl, 0.7 M KCl, 0.1 M $MgCl_2$, pH 8), 1µl DTT (100 mmol/µl), 1µl dNTPs (10 mmol/µl), 0.5µl RNase inhibitors enzymes (10 mmol/µl) and 2µl Reverse-DAG primer (100 pmol/µl) (5´- GCG TGG CAA TGA CAT–`3 nucleotide position 8735-8749 on sequence L 31350) for one hour at 42ºC with 0.5 µl MMLV reverse transcriptase (200mmol/µl). Five µl of the RT reactions were used for PCR using a 5 µl PCR buffer 10 x, 2µl $MgCl_2$, 1µl dNTPs (10 mmol/µl), 0.5µl Taq polymerase (5 unit/µl), 1µl Reverse-DAG (100pmol) and 1µl Forward-DAG (100pmol) (5´- ATT TGC GCT GCG ATG-`3/: 8291-8305 on sequence L 31350) oligonucleotides encompassing the N-terminal part of the coat protein coding region and the C-terminal part of the polymerase (NIb) (primers designed by **Desbiez *et al.*, 2002**). PCR reactions were performed by a first denaturation of the samples at 94ºC for 3 minutes followed

by 35 cycles at 94ºC for 30 seconds, 43ºC for 30 seconds and 72ºC for 30 seconds and a final elongation step at 72ºC for 7 min. PCR products and DNA ladder were analyzed by electrophoresis through agarose gel (1%). Gel was visualized and photographed with UV-illuminator.

RESULTS AND DISCUSSION

Zucchini yellow mosaic virus (ZYMV) is an aphid-transmitted virus that causes devastating damage and a major constraint to cucurbit crops in all production areas worldwide included Egypt.

Biological Studies

Symptomatology, virus isolation and host range studies

Diagnosis of ZYMV was essentially based on field inspection followed by ELISA screening indicated that the naturally infected plants were infested with *Aphis gossypii*. ZYMV-naturally infected squash plants exhibited severe mosaic and blisters, filiform, leaves deformation, blisters on the fruits and discoloration hardening of flesh as well as, and external fruit cracks (Figs. 1A, B and C; 2A, B). Squash plants showing symptoms indicative of ZYMV-infection were observed in both of commercial fields on Alia, Asma, Eskandrani and Safa, Mabrouka cultivars, as well as in the greenhouse on the mechanically-inoculated plants, compared with healthy leaf which depicted as a control. The obtained results indicated that all the aforementioned squash cultivars currently available were susceptible to ZYMV and confirmed by ELISA test. Such collective symptoms have previously been described for ZYMV-infection on cucurbit crops worldwide (**Zhao *et al.*, 2003; Müller *et al.*, 2006; El-Hoseny *et al.*, 2010; Sharma and Gaur, 2015; Wang and Li, 2017**).

Concerning naturally infected squash cv. Eskandrani with ZYMV, in spring season, percentages of infection ranged between 20-35% and 70-78% in mid-March and May, respectively. Meanwhile, the percentages of infection ranged between 15-25% in September and 62% in the end of November. These results revealed that the incidence and spread of aphid in the early spring increased percentages of infection which reflect on the yield losses and fruit discoloration as well as deformation due to ZYMV-infections. In the end of the two seasons, the lowest percentage of infection in November compared with that of May due to the existence and heavily infestation with whitefly (*Bemesia tabaci*), which reduce aphid population. Similar results with significant yield losses due to ZYMV infections have been reported ranging from 50 to 94% in Germany (**Müller *et al.*, 2006**); up to 80% in Western Australia (**Coutts *et al.*, 2011**) and 19.7- 84.9% in Ivory-Coast (**Kone *et al.*, 2017**).

The virus was sap and aphid-transmitted from squash cv. Eskandrani to other cultivars and other plant species as well as back inoculated with high efficiency. The host range of ZYMV included members within the families: Amarnthaceae, Chenopodiaceae, Cucurbitaceae, Solanacea. Results presented in Table 1 show that Cucurbita pepo, Cucumber sativus and Luffa aegyptiaca reacted with systemic symptoms, compared to the other tested plant species which reacted with local lesion symptoms (Table 1). Further, mechanically inoculated test plant *Chenopodium amaranticolor* reacted with local lesions symptom (Fig. 3). Obtained results show that there is little variation in the host range among ZVMV isolates spread globally and these reactions could be useful in differentiation among the *Potyviruses* that infect cucurbit crops. Symptoms and host range of the virus under study are consistent with reports of ZYMV in several countries (**Xu *et al.*, 2004; Glasa *et al.*, 2007; Massumi *et al.*, 2011; Hasiów-Jaroszewska *et al.*, 2013**).

Concerning virus vector relationship, ZYMV is transmitted efficiently in a non-persistent manner by *M. persicae*. All the aphid-inoculated squash cultivars *i.e.*, Alia, Asma, Eskandrani, Mabrouka and Safa were reacted with systemic symptoms. Transmission rates recorded 84.6, 86.6, 93.3, 90, and 91.6%, respectively for Alia, Asma, Eskandrani, Mabrouka and Safa. Results in Table 2 reveal that the highest rate of aphid transmission recorded in cv. Eskandrani (93.3%) compared with the lowest one (84.6%) for cv. Asma. Also, the results indicates that one insect vector (*M. persicae*) able to transmit ZYMV (Fig. 4). This result reveals the occurrence, ability and specificity of *M. persicae* in ZYMV transmission, as well as its possible role as a vector in the epidemiology of the disease. Moreover, our observations in Fayoum, reveal

Table 1. Reaction of host range plant species to ZYMV- infection

Family	Tested plant	Symptoms
Amaranthaceae	*Amaranthus reroflexous*	CLL
Cucurbitaceae	*Cucurbita pepo*	SM, LD,Y,B
	Cucumber sativus	SM, LD, Y
	Luffa aegyptiaca	SM, LD
Chenopodaceae	*Ch. amaranticolor*	CLL
	Chenopodium quinoa	CLL
	Solanum nigrum	CLL
	Celosia spicota	NR
Solanaceae	*Datura stramonium*	NLL
	Nicotiana glutinosa	NR
	Gompherena globosa	NR

B= Blisters on fruit, LD= Leaf deformation, CLL= Local lesions
Y= Yellowing, SM= Systemic mosaic, NLL=Necrotic local lesions
and NR= No reaction

Table 2. Transmission rates form ZYMV-infected plants by *M. persicae* to different squash cultivars

Cultivar	No. of infected/tested plants	Rates of transmission (%)	Cultivar reaction
Alia	11/13	84.6	MM, B
Asma	13/15	86.6	MM, B
Eskandrani	14/15	93.3	SM, B, Mal
Mabrouka	9/10	90	SM, B
Safa	11/12	91.6	SM, B, Mal

MM= Mild mosaic, SM= Severe mosaic, Mal= Malformation

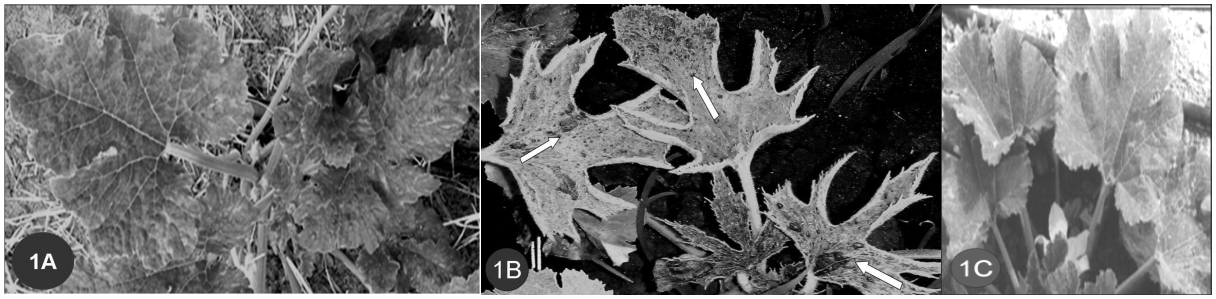

Fig. 1. ZYMV-naturally infected squash cv. Eskandrani exhibiting severe mosaic (A) and blisters, filiform, leaves deformation (B), compared with healthy control (C)

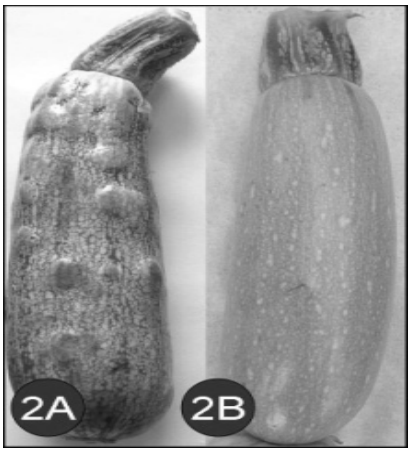

Fig. 2. ZYMV-developed blisters symptom on the fruits of naturally infected squash cv. Eskandrani in Fayoum

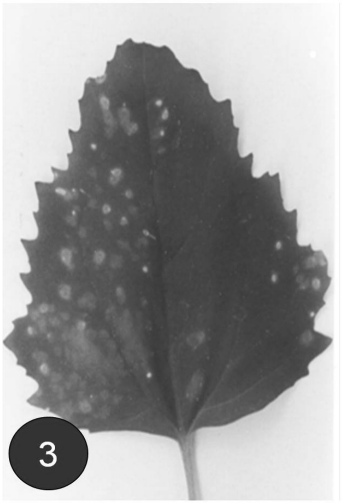

Fig. 3. Chlorotic local lesions symptom produced on *Ch. amaranticolor*, artificially inoculated by ZYMV

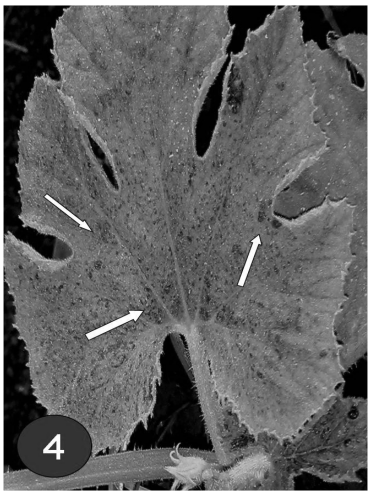

Fig. 4. In virus-vector relationship experiment, ZYMV-developed blisters symptom illustrating the ability and specificity of one insect aphid M. persicae in ZYMV transmission

that ZYMV epidemics are sporadic but occasionally very severe and causes serious economic losses especially in squash in the spring season, compared with the other cucurbit crops such watermelon and cucumber. Symptoms induced on plants infected by aphid transmission were similar to those induced by mechanical inoculation of the virus on squash plants. These results are consistent with those reported by **Pinto *et al.* (2008)** who stated that *M. persicae* transmitted ZYMV at rate of 67%. Furthermore, **El-Borollosy (2015)** revealed that the most efficient vector for Cucumber mosaic virus was *A. gossypii* and for ZYMV was *M. persicae* achieving 95% and 100%, respectively. Nevertheless, **Lecoq *et al.* (2014)** in France, found that ZYMV epidemics are sporadic but occasionally very severe compared with WMV, same genus (*Potyvirus*) which causes regular and early epidemics as well as factors influencing ZYMV epidemiology are still poorly understood. Additionally, **Provvidenti *et al.* (1984), Prendeville *et al.* (2012) and Romay *et al.* (2014)** explained that the epidemiology in tropical and subtropical regions, because cucurbits can be cultivated all year and several aphid vectors can transmit ZYMV from one crop to the next one and alternatively wild cucurbits can be efficient virus reservoirs. Moreover, **Coutts *et al.* (2013)** showed that ZYMV can spread from infected to healthy cucurbit plants by leaf contact (rubbing and crushing) and on blades contaminated with sap from ZYMV-infected leaves. They also, proved important new information on virus epidemiology.

Physical Studies Using Electron Microscopy (EM)

Electron microscopic examination of leaf dip preparations revealed the presence of long, filamentous flexuous virus particles measuring 750X15 nm which are characteristic of the family *Potyviridae* (Fig. 5). These results confirmed by those obtained by **Hosseini *et al.* (2007); Khalifa *et al.* (2015) and Wang and Li (2017)**.

Serological Detection of ZYMV using TBIA

Squash plants showing symptoms indicative of ZYMV were observed in both of commercial fields and in greenhouse on cv. Eskandarani. Symptomatic plants were stunted, and failed to produce normal fruits. ZYMV-infected squash in the field on cv. Eskandarani showing mosaic, Blisters, leaf malformation. TBIA was done to detect virus distribution within stem tissue in cv. Eskandarani grown either in the field or in the mechanically inoculated plants grown in greenhouse. Obtained results either indicated color formation in diseased plants in naturally infected cultivars of squash or mechanically inoculated in greenhouse (Fig. 6). No color formation (remained green) was observed in the control healthy squash tissue.

Similar results have been observed by **Ghanem (2003)** working on Okra leaf curl virus and **Fath-Allah *et al.* (2011)** working on ZYMV. TBIA technique proved to be sensitive in ZYMV detection. The obtained results are in agreement with those obtained by **Ajlan *et al.* (2007), Abou-Jawdah *et al.* (2008) and Ghanem *et al.* (2016)** who mentioned that TBIA was the most sensitive serological method used because it allowed CYSDV detection within 5-6 days post-inoculation. Also, they found that TBIA is recommended for rapid detection and comparison of CYSDV movement between resistant and susceptible cucumber accessions.

Molecular Detection Using Reverse-Transcriptase Polymerase Chain Reaction

Results presented in Fig. 7 show that RT-PCR resulted in amplification product size of 458 bp in the RNA extracted from ZYMV-infected squash. The obtained results are in harmony with that described by **Hosseini *et al.* (2007)** and **Zheng *et al.* (2010)** who successfully detected ZYMV by RT-PCR.

Conclusion

Zucchini yellow mosaic virus (ZYMV) is an aphid-borne virus that causes yield losses and fruit quality defects in all cucurbit crops. It infects all cultivated cucurbit types including cucumber, pumpkin, squash, watermelon, weeds and ornamental plants as well as its transmission by different ways. Therefore, to minimize ZYMV spread in cucurbit crops integrated pest management (IPM) approaches must be used as follows:

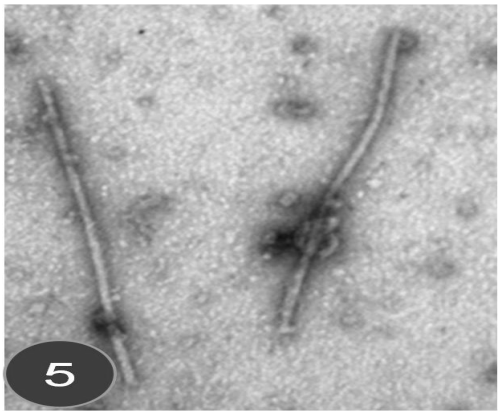

Fig. 5. Electron micrographs showing flexuous filamentous particles, measuring 750 × 15 nm.

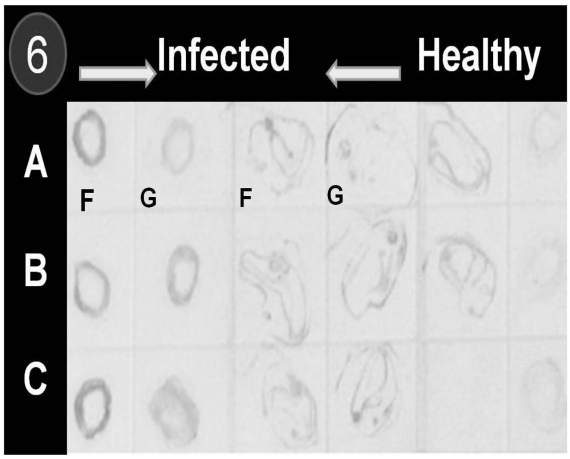

Fig.6. TBIA detection of ZYMV in the leaves of squash plants cv. Eskandrani. (A) leaf petiole and midrib of infected plants grown in the field (F) and greenhouse (G), respectively. (B) leaf petiole and midrib of mechanically inoculated plants and grown in the greenhouse. (C) leaf petiole and midrib of aphid inoculated plants grown in greenhouse, compared with healthy leaf petiole and midrib

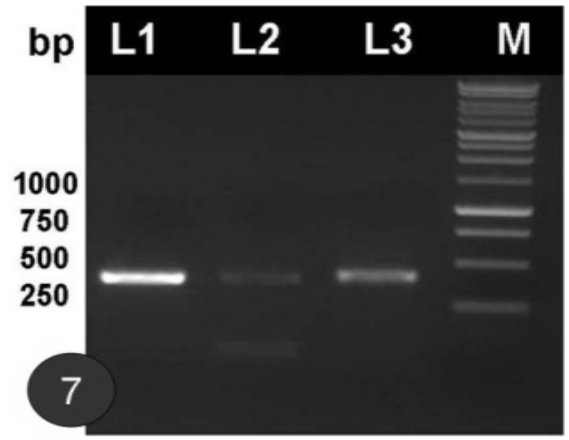

Fig.7. Electrophoretic analysis of the RT- PCR amplification revealed the primers amplified products of 458 bp for the coat protein, M = marker, Lanes 1-3, L1 = Infected leaf cv. Eskandrani, L2 = Healthy leaf cv. Eskandrani and L3 = Infected leaf petiole cv. Eskandrani

* Eradication reservoir abundant weeds and old cucurbit crops after the final harvest because they are considered as a major factor for ZYMV transmission in early season cucurbit crops and play an important role in virus epidemiology.
* Prevention culturing of ornamental species including begonia and *Malva* sp. due to its potentiality as an alternative hosts (as mentioned by **Lecoq *et al.*, 2014**).
* Cleaning footbaths and to wash equipment and machinery using 10% Clorox.
* Removing virus sources (cucurbit plants exhibiting virus symptoms) within the crop may help to reduce virus spread within the same field.
* Prevention moving workers, equipment and machinery to minimize virus spread from older crops to young ones.
* Planting certified cucurbits seed lots to reduce primary infection in early season.
* Culturing virus tolerant or resistant cucurbit varieties when available.
* Chemical control using alternative pesticides and plant extracts as well as repellents to reduce aphid population.

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التشخيص البيولوجي والسيرولوجي والجزيئي لعزلة فيروس موزايك الزوكيني الأصفر الذي يصيب نباتات الكوسة في محافظة الفيوم

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استهدف البحث دراسة طرق مختلفة لتوصيف والكشف عن وجود فيروس موزايك الزوكيني الأصفر الذي يسبب تدهور محاصيل القرعيات خاصة الكوسة نتيجة تأثيره على المحصول كماً وكيفاً، حيث يؤدي إلى فقد المحصول بالإضافة إلى تشوه الثمار الناتجة مما يقلل من قيمتها التسويقية، تركزت الدراسة فى محافظة الفيوم بمراكزها المختلفة مناطق زراعة الكوسة، تم حصر الحقول المزروعة بخمسة أصناف حيث أظهرت النباتات المشتبه إصابتها بالفيروس أعراض الموزايك شديد وبثور (فقافيق) تشوه الأوراق بالإضافة إلى ظهور بثور (فقافيق) على الثمار، تم الحصر في الفترة من ٢٠١٤-٢٠١٥ بالتفتيش الدوري لمشاهدة الأعراض متبوعاً بالاختبارات السيرولوجية وذلك في زراعات الأصناف المختلفة من الكوسة في مراكز محافظة الفيوم، وسجلت نسبة الإصابة في نباتات الكوسة صنف اسكندراني تراوحت ما بين ٢٠-٣٥%، ٧٠-٧٨% من منتصف مارس إلى مايو على التوالي، بينما تراوحت ما بين ١٥-٢٥% إلى ٦٢% في سبتمبر ونهاية نوفمبر على التوالي، وقد تم عزل وتعريف الفيروس بيولوجياً (بالنقل الميكانيكي- الحشري)، سيرولوجياً (اختبارات الإليزا–والبصمة النسيجية على أغشية النيتروسيليولوز"TBIA")، أيضاً فُحصت جزيئات الفيروس بالمجهر الإليكتروني بالإضافة إلى الاختبارات المعتمدة على الحمض النووي (اختبار عديد البلمرة المتسلسل[PCR])، أثبتت نتائج الحقن الميكانيكي انتقال الفيروس إلى العوائل التابعة للعائلة القرعية، وبخصوص علاقة انتقال الفيروس بالحشرة الناقلة (مَنْ الخوخ *M. persicae*) سجلت نتائج النقل الحشري معدلات ٨٤,٦، ٨٦,٦، ٩٣,٣، ٩٠,٠ و٩١,٦% على التوالي في الأصناف الآتية: عاليا، أسما، إسكندراني، مبروكة، صفا، أيضاً تم استخدام حشرة واحدة فقط وحاملة للفيروس وأثبتت كفاءتها في نقل الفيروس بعد تغذيها على النبات السليم، وأظهر الفحص بالميكروسكوب الإليكتروني لتحضير شبه منقى من الفيروس تواجد جزئيات خيطية طولها وعرضها ٧٥٠×١٥ نانوميتر. تم أيضاً الكشف بنجاح عن وجود أنتيجينات الفيروس في أنسجة نباتات الكوسة مُصابة صنف إسكندراني في كل من ورقة، عنق الورقة بواسطة اختبارالبصمة النسيجية والذي أظهر أن الأنسجة المصابة تلونت باللون الوردي بينما تظل السليمة باللون الأخضر، أيضاً تم الكشف عن الفيروس في نباتات الكوسة المصابة صنف إسكندراني بواسطة اختبار عديد البلمرة المُتسلسل باستخدام البادئات، وتم الحصول على شظايا الحمض النووي أطواله ٤٥٨ زوج من القواعد النيتروجينية والتي دلت علي أنه فيروس موزايك الزوكيني الأصفر المعروف عالمياً عند استخدام نفس البادئات، واستنتاجاً لما سبق إلحاق البحث بالعديد من التوصيات كوسائل للمكافحة المتكاملة لتقليل انتشار الفيروس بالإضافة لتقليل تعداد الناقل الأمر الذي ينعكس على تقليل نسبة الإصابة.

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