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Biological and serological characteristics of Zucchini yellow mosaic virus isolated from Cucurbita pepo from Egypt

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***ABSTRACT*: Zucchini yellow mosaic virus (ZYMV) is one of the most destructive viruses which cause significant economic losses in many cucurbits crops in Egypt. The purpose of this study was to use biological and molecular techniques to characterize zucchini yellow mosaic virus (ZYMV) strain isolated from open fields of Sharqia Governorate. ZYMV was isolated from infected zucchini plants showing virus like symptoms, samples were collected from open fields of Sharqia Governorate In different seasons (2019-2020). The fruit samples of zucchini exhibited severe mosaic, blisters, yellowing and malformation. The isolated virus was detected biologically to different species belonging six families as *(Cucurbitaceae, Chenopodiaceae, Leguminoseae, Graminaceae, Solanaceae and Asteracea)* by mechanical inoculation, serologically by DAS-ELISA (Double antibody sandwich enzyme linked immunosorbent assay) and also the stability of ZYMV was studied, found that Thermal inactivation point (TIP), Dilution end point (DEP) and Longevity in vitro (LIV)] were 65ºC, 10-4, and 96 hours, respectively. Purified virus sample was negatively stained and examined under electron microscopy (EM) revealed flexuous filamentous particles approximately (680-840) nm long and (12-14) nm in diameter. ZYMV was molecularly identified by the reverse transcription polymerase chain reaction (RT-PCR), the size of the potyvirus P1 gene was shown to be about 600 bp using sodium dodecyl sulphate–polyacrylamide gel electrophoresis. ZYMV P1 gene was quite similar, with a nucleotide sequence similarity of 98 % between several ZYMV previously isolated.**

KEYWORDS: Zucchini Yellow Mosaic virus (ZYMV), DAS-ELISA, TIP, DEP, LIV, ReverseTtranscription Polymerase chain reaction (RT-PCR).

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I. INTRODUCTION

ZYMV was first isolated in Italy in 1973 and later was found in Egypt **(Provvidenti et al., 1984; Desbiez and Lecoq, 1997)**. Also observed in France, where it was named as Muskmelon yellow stunt potyvirus (MYSV) **(Lecoq et al., 1981 and 1983).**ZYMV was reported in many African countries including Algeria, Egypt, Madagascar, Mauritius, Morocco, Reunion, Swaziland and Tunisia **(Radwan et al., 2007).** ZYMV present all over the world, Asia, Europe, Africa, Middle East, South as well as North America where cucurbits are grown and dominate in all environmental condition **(Asad et al., 2019**). ZYMV belongs to the genus Potyvirus, family Potyviridae **(Ashfaq et al., 2021),** Like other potyviruses RNA genome (≈9 600 nucleotides) encapsidated in a flexuous filamentous particles 750 nm in length and 11nm in diameter genome with viral protein covalently linked on 5′-terminal and poly (A) tail on 3′-terminal ends which codes for a polyprotein that is proteolytically processed into ten smaller mature proteins; P1 (protease), HC (helper component/protease) , HC (helper component/protease), P3, 6K1, CI (cylindrical inclusion), 6K2, NIa (nuclear inclusion a), VPg (viral protein linked genome), NIb (nuclear inclusion b) and CP (coat protein**)(Maghamnia et al., 2018).** The Egyptian isolates of ZYMV showed symptoms closely resembling those caused by European isolates of this virus and the American strain, ZYMVCT **(provvidenti et al., 1984).** In Egypt, it was first described in 1983 (**El-fallal et al., 2015).** ZYMV was isolated from squash plants by several researchers in different areas in Egypt **(Abd El-Aziz et al., 2020; Kheder et al., 2017; Nasr-Eldin et al., 2016 and El-fallal et al., 2015).** ZYMV is transmitted in a non-persistent manner by approximately 60 species of aphid especially Aphis gossypii, Myzus persica also can be transmitted mechanically but not through seed **(Umar et al., 2016).** Aphid transmits ZYMV in non-persistent manner. In high disease incidence areas population rate of Myzus persicae was little higher

then Aphis gossypii. Previous studies supported that Myzus persicae transmit virus more efficiently then Aphis gossypii **(Asad et al., 2019).** Seed transmission occurs occasionally at low levels in squash and zucchini (*Cucurbita pepo*) **(Yeşil, 2020),** and Delica type butternut squash (*Cucurbita maxima)* but has not been reported to occur in melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita moschata*), or cucumber (*Cucumis sativus*) **(Coutts et al., 2011). Coutts et al., (2013)** shows 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. Contact transmission can occur when healthy and infected plants intertwine or leaves rub together through wind movement. Several methods were used to identify and characterize ZYMV in squash and other cucurbit crops. Bioassay, on test plants belong to *Cucurbitaceae* and other families, as well as double antibody sandwich using ZYMV specific antibodies were widely used for this objective **(Al-Ani et al., 2011).** The symptoms of ZYMV infection on leaves are severe vein banding, yellowing, severe mosaic, deformation, leaf malformation, blistering and reduced size, while the fruits are stunted, twisted and deformed, resulting in reduced yield making the plants unmarketable **(Nasr-Eldin et al., 2016).** Fruit symptoms of pumpkin, zucchini, squash, watermelon and cucumber include mottled skin, different colouring and knobbly areas that cause deformation **(mondal et al., 2020).** Infected plants produce a range of symptoms while individual cells contain cylindrical inclusions (pinwheels) as described by **(Edwardson and Christie 1978). (Singh et al. 2003)** reported that the dilution end point of the virus (DEP) was 10-3, thermal inactivation point (TIP) between 50-55°C and longevity in vitro (LIV) was found 48 hours. The DEP of the virus was 10-3, TIP between 50 and 55 °C and LIV was 48 h **(Viswanathan et al., 2017). EL-Shafi, (2006**) showed that the Physical properties of ZYMV the virus was sap transmissible, its thermal inactivation point is 65°C,dilution end point is 10-3 and longevity in vitro is 24h at room temperature. **Kaldis et al., (2018**), found that, ZYMV was detected and Characterized in Zucchini plants by bioassay and Enzyme-Linked Immunosorbent Assay (ELISA) with specific antiserum ZYMV. According to **Ghanem, (2003); Ghanem et al., (2016), Kheder et al., (2017),** serological tests of enzyme linked immunosorbent assay (DAS-ELISA) and tissue blot immuno-printing assay (TBIA) were done for ZYMV detection. DAS-ELISA was managed using polyclonal antiserum to ZYMV. According to **Hossein et al., (2007) and Kheder et al., (2017)** molecular detection using reverse-transcriptase polymerase chain reaction (RT-PCR) was done to detect ZYMV. The aim of this study was to isolate, identify and partially characterize the biological, serological and molecular to the most important virus infecting cucurbits in Sharqia governorate in Egypt.

II. MATERIALS AND METHODS

Sample collection: Survey was conducted in zucchini growing areas at many sites of Sharqia governorate were collected from different locations of Sharqia Governorate (Zagazig, Belbis, burdayn, al-adliyyah and inshas).during 2019 – 2021 growing seasons. Samples of Zucchini plants showing distinct viral symptoms in the form of severe mosaic, mottling, blisters, crinkle, yellowing and malformation (approximately 150 samples). The samples were stored at - 20˚C until use.

Mechanical inoculation and host range:

Virus inoculation: Leaves from systemically infected zucchini plants were homogenized in 0.02 M phosphatase buffer pH=7.2 (1 g/5ml). The homogenate was filtered through double layer of muslin and the filtrate was used as virus inoculum. The inoculum was gently rubbed on the upper leaf surface of test and host plants previously dusted by carborandum (400- 600 mesh) at the cotyledons leaves according to (**Yarwood,. 1955).** The inoculated plants were maintained in the glasshouse and the plants were monitored weekly for symptoms development.Twenty-nine host plant species belonging to 6 families *(Cucurbitaceae, Chenopodiaceae, Leguminoseae, Gramineae , Solanaceae and Asteraceae)***,** Table (1) were mechanically inoculated with virus isolate prepared from infected *Chenopodium murale* using (ten plants of each host) . Control plants were inoculated with buffer only. The inoculated plants were kept under an insect proof in greenhouse conditions at 25-30 °C and observed daily for three to five weeks after inoculation for symptoms development **(Lisa et al., 1981; Damayanti et al., 2022** and **Hammad et al., 2022)**.

Stability of ZYMV:

Thermal inactivation point (TIP), Dilution end point (DEP) and Longevity *in vitro* (LIV)] were performed according to **Amel et al., (2014)**. Infectious crude sap of ZYMV infected zucchini (*Cucurpita pepo cv*. Eskandarani) leaves was used to determine the stability of ZYMV. The healthy Zucchini plants (*C. pepo cv*. Eskandarani) were mechanically inoculated with infectious crude sap of ZYMV as systemic host and kept under a greenhouse conditions.

Thermal Inactivation Point (TIP): The infectious sap was distributed in 2 ml aliquots in Eppendorf tubes. Thermal inactivation point of the virus isolate was carried out by exposing the tubes containing the infectious sap at certain temperatures (15℃ to 70℃ intervals 5℃) in water bath for 10 minutes. Tubes were then rapidly cooled by dipping them in cold water. Each treated infectious sap and untreated one was then inoculated on 10 healthy zucchini plants (*C. pepo cv*. Eskandarani)**.**

Longevity *in vitro* (LIV): Two ml of infectious crude sap in Eppendorf tubes were kept at room temperature (15℃) for 1-6 days and covered with a drop of benzene as a preservative material. Every 6 hours one tube used for inoculate 10 healthy plants of zucchini (*C. pepo cv*. Eskandarani).

Dilution End Point (DEP): The infectious sap the virus isolate was serially diluted with sterile distilled water starting from 10^{-1} to 10^{-9}. Each dilution of infectious sap and undiluted infectious sap was then inoculated on 10 healthy Zucchini plants (*C. pepo cv*. Eskandarani)

Serological detection of ZYMV:

For the detection of Zucchini yellow mosaic virus in infected fruit samples , DAS-ELISA (Double antibody sandwich enzyme linked immunosorbent assay) was performed according to the manufacturer's instructions (Bioreba, Switzerland; Sediag, France).Two wells were used per sample. All buffers, polyclonal antibodies, and positive and negative controls were provided by the manufacturers. Plant fruit samples were ground in extraction buffer (1:10 w/v), and extracts were added to ELISA plates coated with a specific antiserum and incubated overnight at 4 °C. After washing, the enzyme conjugate was added, and plates were incubated at 30 °C for 5 h. After washing once more, the substrate (p-nitrophenyl phosphate, AppliChem, Germany) was added to wells, and then, plates were incubated at room temperature in the dark for 60–120 min. After incubation, absorbance values were determined at 405 nm by Anthos 2010 Microplate Reader (Biochrom Ltd., Cambridge, UK). A positive test result was obtained when the average absorbance value of the tested sample was greater than two times according to **(Clark and Adams 1977).**

Physical examination of virus particles (Electron microscope):

Negative staining was performed on carbon coated copper grids (400-mesh). Crude sap of infected zucchini leaves was applied on top of the grid for five min; therefore the grid was washed in 0.06 M phosphate buffer (pH 6.5). Depositions on the grid were then stained with 2%-phosphotungstic acid in phosphate buffer (pH 6.5) for two min. The grids were air-dried and observed in the TEM according to **(Zechmann et al., 1953).** (JEOL-JEM-2015 Electron microscope) in (electron microscope unit at Faculty of Agriculture Mansoura University).

Molecular detection using reverse-transcriptase polymerase chain reaction (RT-PCR):

Total RNA was extracted from ZYMV-infected leaves and positive in an ELISA test using the RNeasyÒ Plant Mini Kit (Qiagen) according to (**Glasa et al., 2007**). Primers were constructed to amplify a portion of the P1 coding region based on available ZYMV sequences on database. In this procedure, oligonucleotide primers (forward primer: 5′ AGTGGCACCTGGCCACATGGC3′and reverse primer 5′ CATCTCAGTGTGCCGCATTCG 3′) were designed to amplify a fragment of the P1 (protease). RT-PCR was performed in a two-step format using the extracted total RNA. PCR amplifications were performed using the proofreading TaKaRa Ex Taq TM polymerase (Takara Bio, Inc.) and under the following cycling conditions (Techne Genius, Merck): initial denaturation at 94°C for 5 min, 35 cycles of 94°C/1 min, 54°C/45 s, 72°C/1 min, and final extension at 72°C for 10 min. The PCR product products were separated by electrophoresis at 100 V in a 1.5% agarose gel (1x TAE buffer) stained with 0.5µg/ml ethidium bromide. The fragment sizes were determined using a Gelpilot 100 bp Ladder (Qiagen GmbH, Germany). The results were analyzed using associated software. The PCR products were gel-purified (QIAexII gel extraction kit, Qiagen) and directly sequenced using a MegaBACE TM 1000 DNA Analysis System (Amersham Biosciences) by priming the sequencing reaction with the same oligonucleotides as used for PCR. Partial sequences were compared to the sequences available in the Genbank database (http://www.ncbi.nlm. nih.gov). The nucleotide sequences have been deposited in the GenBank database under accession number OP056334.

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III. RESULTS

Isolation of Zucchini yellow mosaic virus (ZYMV):

The collected samples of naturally infected Zucchini plants from different locations at Sharqia Governorate showing distinct viral symptoms in the form of severe mosaic, mottling, blisters, crinkle, yellowing and malformation (Fig.1).

Host range and symptoms:

Twenty nine plant species belonging to different families were mechanically inoculated with the isolated zucchini yellow mosaic virus. The host range and the reaction of different plants are listed in Table (1).The host plants reacted with ZYMV can be divided into the following groups.

Hosts showing local lesions symptoms necrotic local lesions were shown on leaves of *Cheopodium murale* and *Vigna unguiculata* cv. black eyed cowpea. Therefore chlorotic local lesions were appeared on leaves of *Datura metale, Datura stromonium, Cheopodium amaranticolor, C. quinoa and Vigna unguiculata cv.* red eyed cowpea **Fig. (2).**

Hosts showing systemic symptoms, most species belonging to the family cucurbitaceae developed systemic symptoms, *Cucurbita pepo, C. moschata, Cucumis melo, Citrullus lanatus, Cucumis sativus and Luffa acutangula.* These results to ZYMV were similar to results described by **(Al-Shahwan, 1990; Lisa et al., 1981; Lisa and Lecoq, 1984 ; Provvidenti, 1984 and Wong,1992)** Fig. (3).

Hosts showing no symptoms No symptoms were observed on plants of, *chenodium album, Pisum sativum, Vicia faba, Trigonella foenum-graceum,Trifoleum alexandrium, Zea mays, Triticum aestivum, Nicotiana tabacum, N.glutinosa, N. rostica, Calendula arvensis* which mechanically inoculated by virus sap.

Fig. 1. Different types of natural infection symptoms on zuchinni fruits showing mosaic, mottling, blisters, crinkle, yellowing and malformation.

Table (1) Response of plant species to Zucchini yellow mosaic virus under glasshouse conditions:

Families	plant species	Symptoms
Cucurbitaceae	*Cucurbita pepo cv. Zucchin*	SM, LMf, VC, M
	Cucurbita moschata 'Butternut	SM, LMf, VC, M,
	Cucumis sativus	YLL, Mm, LMF
	Cucumis melo flexuosus	YLL, LMf, M
	Cucumis melo var. reticulatus	YLL, LMf, VC
	Cucumis melo var. cantalupensis	YLL, LMf, M
	Citrullus lanatus	LMf ,YLL, VC
	Luffa aegyptiaca	LMf, VC, YLL
Chenopodiaceae		NLL
	Chenopodium murale	CLL
	C. amaranticolor	CLL
	C. quinoa	NS
	C. album	NS
Leguminoseae	*Phaseolus vulgaris cv. Red Kidney.*	SM
	p. vulgaris cv. white Kidney.	CLL
	Vigna unguiculata cv. Red eyed cowpea	NLL
	V. unguiculata cv. black eyed cowpea.	NS
	Trigonella foenum-graecum	NS
	Trifolium alexandrinum	NS
	Vicia faba	NS
Graminaceae	*Zea mays*	NS
	Triticum aestivum	NS
Solanaceae		
	Dautura metel	CLL
	D. stramonium	CLL
	Nicotiana tabacum	NS
	N. glutinosa	NS
	N. rostica	NS
	Solanum tuberosum	LC
Asteraceae	*Calendula arvensis*	NS

CLL = Chlorotic local lesion, NLL = Necrotic local lesion, M = Mosaic, SM =Severe mosaic, LMf = Leaf Malformation, VC = Vein clearing, YLL= Yellowing, Mmo= Mild mottling, Smo = Severe mottling, LC= Leaf curling, NS = No symptoms.

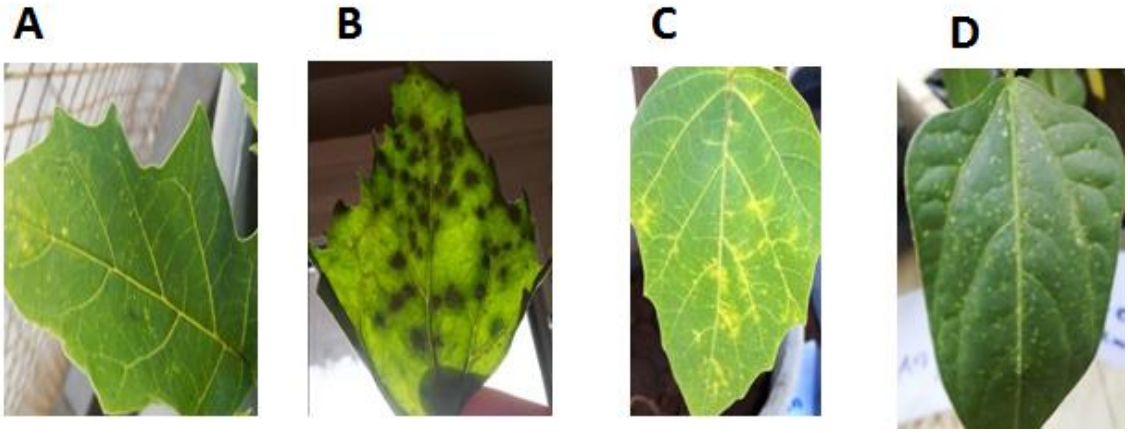

Fig. 2. Hosts showing local lesions symptoms *Datura stromonium and D. metal showing chlorotic local lesions* while *Chenopodium murale and Vigna unguiculata cv. black eyed cowpea* Showing necrotic local lesions.

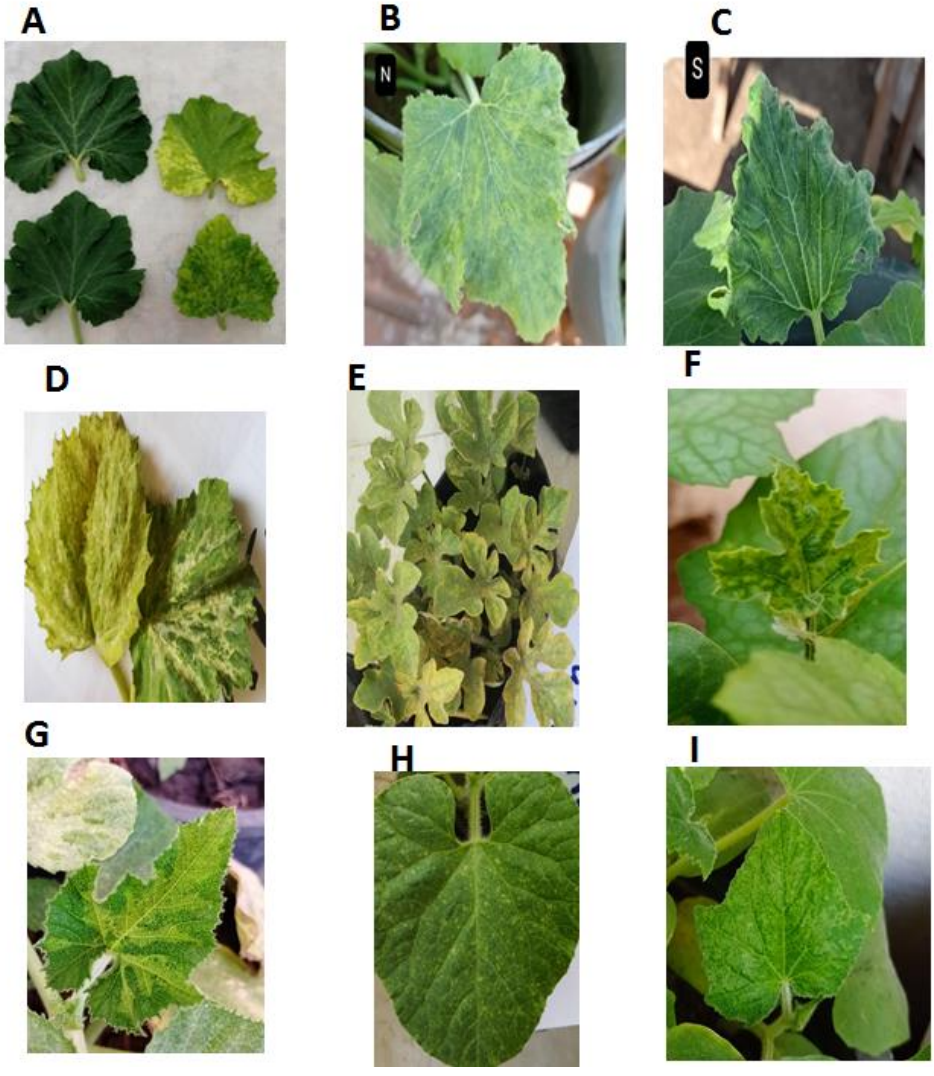

Fig. (3) Hosts showing systemic symptoms (A,B,C and D) Zucchini *(cucurpita pepo, L)* showing severe mosaic, yellowing, crinkle, vein clearing, blisters and leaf malformation. (E) *citrulls lantus,(F) Luffa acutangula,(G) C. moschata,(H) Cucumis melo and (I) Cucumis sativus.*

Stability of zucchini yellow mosaic virus (ZYMV) isolate:

Thermal inactivation point (TIP): The result of TIP revealed that ZYMV isolate was completely inactivated in crude sap when heated to 65°C for 10 minutes with relative virus activity 0% compared with un-heated one 100% (Table 2) and Fig.(4).

Dilution end point (DEP): the result of DEP showed that ZYMV isolate has DEP 10^{-4} and was completely inactivated when diluted up to 10^{-5} at room temperature with relative activity compared with undiluted crude sap 100%. These were represented in Table (2) and Fig. (5).

Longevity in vitro (LIV): The result showed that ZYMV isolate was completely inactivated after 96 hours when kept at room temperature with relative activity compared with un stored one100%. The results were represented in Table (2) and Fig. (6).

Table(2): Assesment of Zucchinin yellow mosaic virus (ZYMV) isolate stability in infectious sap.

TIP			DEP			LIV		
Temp. ◦C	No of infected plants**	Infectivity***	Dilutions	No of infected plants	infectivity	Aging (hr)	No of infected plants	infectivity
Untreated infectious sap*	10	100%	Untreated infectious sap*	10	100%	Untreated infectious sap*	10	100%
15	10	100%	10^{-1}	9	90%	0	10	100%
20	10	100%	10^{-2}	7	70%	6	7	70%
25	9	90%	10^{-3}	4	40%	12	5	50%
30	8	80%	10^{-4}	2	20%	18	3	30%
35	8	80%	10^{-5}	0	0%	24	3	30%
40	6	60%	10^{-6}	0	0%	48	2	20%
45	3	30%	10^{-7}	0	0%	72	1	10%
50	3	30%	10^{-8}	0	0%	96	0	0%
55	1	10%	10^{-9}	0	0%	120	0	0%
60	1	10%				144	0	0%
65	0	0%						
70	0	0%						

TIP= Thermal inactivation point, DEP= Dilution end point, LIV= Longevity in vitro.

*untreated infectious sap kept under lab temperature.

*** % infectivity $= \frac{C-T}{C} \times 100$

Serological detection of ZYMV using Double antibody sandwich ELISA (DAS-ELISA**):**

The virus antigen was serologically precipitated against specific polyclonal IgG-ZYMV by (DAS-ELISA) assay **table (3).** It was found that the **Double antibody sandwich ELISA (**DAS-ELISA**)** sensitive to detect ZYMV in all infected samples. A yellow color was developed with infected zucchini in the positive reaction, whereas extracts from healthy plants remain colorless in the negative reactions

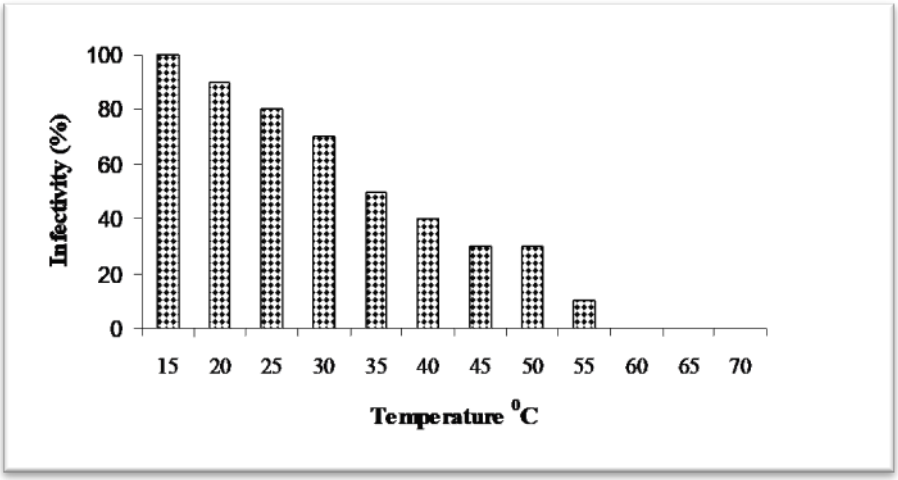

Fig.(4): Histogram showing the (TIP) stability of ZYMV isolate.

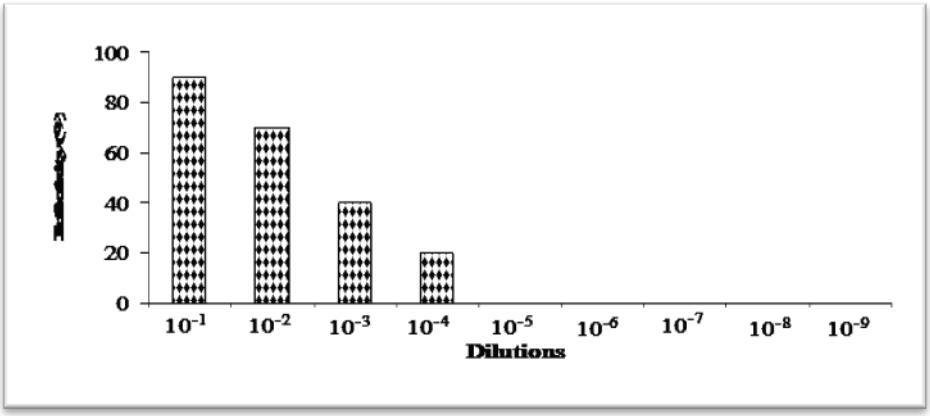

Fig.(5): Histogram showing the (DEP) stability of ZYMV isolate.

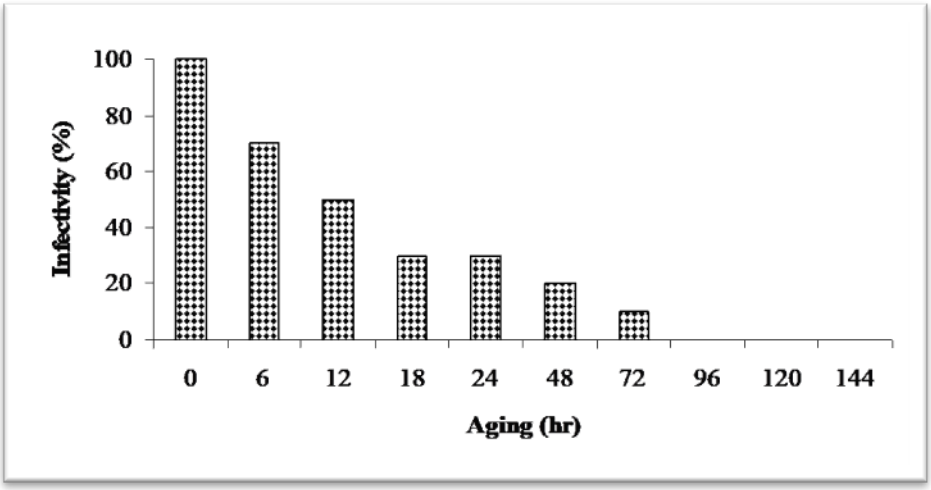

Fig.(6): Histogram showing the (LIV) stability of ZYMV isolate.

Table (3) Serological detection of ZYMV naturally infected zucchini plants by (DAS-ELISA):

Blank	Negative control	Positive control	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6
0.113	**0.589**	**2.176**	**1.623**	**1.211**	**2.101**	**2.363**	**0.627**	**1.438**

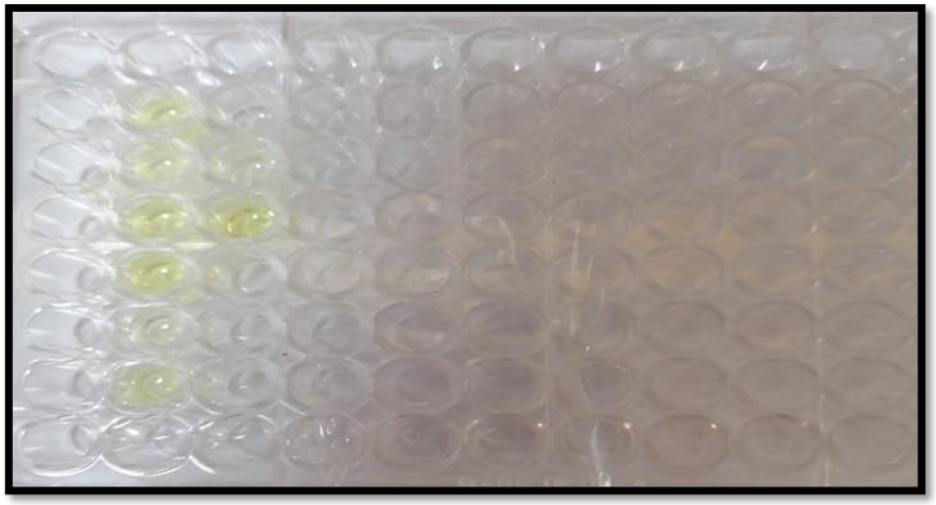

Fig.7. Serological detection of ZYMV naturally infected zucchini plants by (DAS-ELISA).

Electron microscope identification of ZYMV isolate:

Transmission electron microscope examination of partially purified preparation of infected zucchini leaves stained with phosphotungstic acid showed the flexible filamentous particles of ZYMV.

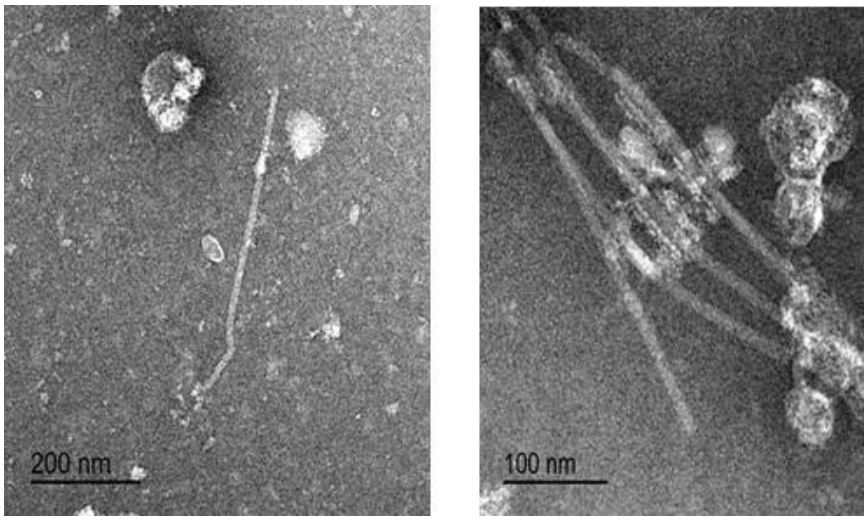

Fig.(8): Electron micrograph showing flexible filamentous particles of ZYMV isolate negatively stained with phosphotungstic acid with magnification power 20000x.

5. Molecular identification of ZYMV isolate:

Polymerase chain reaction (PCR):

The total viral RNA extracted from mechanically infected zucchini leaves was reverse transcribed by reverse transcriptase and the reverse primer of the p1 gene of *Zucchini Yellow Mosaic Virus*. The complementary DNA resulted was amplified using the forward and reverse primers of ZYMV. Gel electrophoresis of total RNA of infected zucchini leaves. The size of the product DNA band was approximately 600 bp (Fig.9). **Safaeizadeh, (2010)** and **Yakoubi et al., (2008)** used the same technique to isolate ZYMV and reported that there was no specific product obtained from healthy material and no bands were found when the PCR assay was performed without the initial RT step.

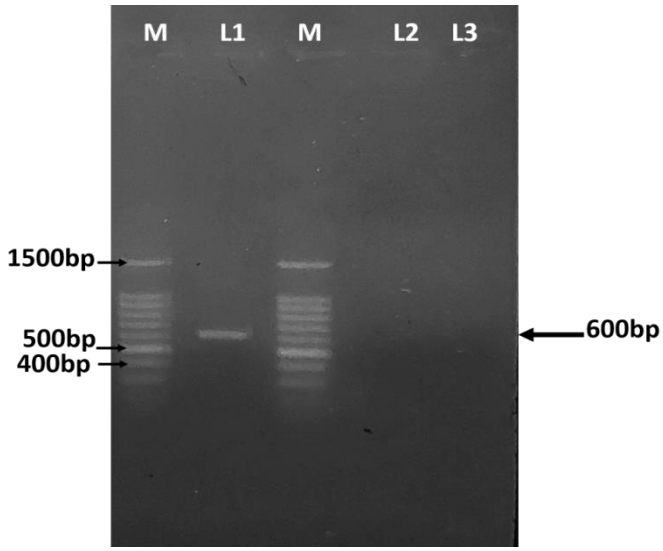

Fig. (9) Agarose gel electrophoresis patterns of RT-PCR products of ZYMV isolate.
M: 100 bp DNA ladder (Biomatik).**L1:** Sample of the naturally infected squash plant. **L2 and L3:** Negative Squash plant (Healthy).

V –DISCUSSION

In Egypt cucurbits are major vegetable crops, annual cultivated area of squash (*Cucurbit pepo L*) **(Chomicki et al., 2020).** *Cucurbita pepo* is an economically important crop. Its production reached 25 million tons in 2014, with nearly two million cultivated hectares. The zucchini group rank among the highest-valued vegetables worldwide **(Montero-Pau et al., 2021).** Zucchini is popular among people on a diet due to its low amount of calories. This is due to its high water content (approximately 96 %), but zucchinis still have high nutritious value because of their significant amount of potassium, folate, and vitamin A **(Enneb et al., 2020).** Zucchini yellow mosaic virus (ZYMV) is one of the most important viruses affect cucurbit production. It causes destructive diseases to a large variety of economically important cucurbit plants including zucchini **(Derbalah et al., 2022).** In Egypt, according to (**Kheder et al., 2017**); ZYMV infections caused significant losses in yield from about 20 to 78%. Also, **Coutts et al., (2011)** mentioned that the yield decreased 50 to 94% in Western Australia and in Germany yield losses recorded up to 80% according to (**Müller et al., 2006**). Based on our results from this study, the isolated virus from zucchini plants showed symptoms similar to which described by (**Desbiez and Lecoq, 1997**) stated that, In zucchini (*Cucurbita pepo*) leaves develop a yellow mosaic and become severely blistered and Fruits are distorted with prominent lumps and in yellow fruit cultivars, fruits may stay green with glossy yellow blisters. ZYMV is one of the most destructive viruses of cucurbits in EGYPT. Also, **(Prieto et al., 2001**) found that the zucchini samples showed yellow mosaic and severe leaf blistering, with distorted fruits forming prominent bumps. Melons cv. Honey dew exhibited vein clearing, yellow mosaic, and deformed leaves. Watermelon samples showed mottle, mosaic, and leaf polymorphism. Symptoms in zucchini varied from mottle to severe mosaic. Samples were also confirmed by ELISA using polyclonal antisera against ZYMV (Zucchini yellow mosaic virus). These results showed that DAS-ELISA

developed in this study is suitable for detection of ZYMV in collected samples, where the results either give yellow color in diseased plants in naturally infected cultivars of zucchini (**Fig.7**). No color formation (remained colorless) was observed in the control healthy zucchini plants. Serological diagnosis of viruses is suitable and easy to handle (**Ashfaq et al., 2021**). According to (**Yuki et al., 2000**) DAS- ELISA is using worldwide for identification of plant viruses. The stability of ZYMV was studied, found that Thermal inactivation point (TIP), Dilution end point (DEP) and Longevity *in vitro* (LIV)] were 65ºC, 10-4, and 96 hours, respectively. **(Singh et al., 2003)** reported that the dilution end point of the virus (DEP) was 10^{-3}, thermal inactivation point (TIP) between 50-55°C and longevity in vitro (LIV) was found 48 hours. **(Amel et al., 2014)** found that stability of the isolated virus in vitro was found as 55 o C for TIP, 10-4 for DEP and LIV 4 days**.** The DEP of the virus was 10-3, TIP between 50 and 55 °C and LIV was 48 h **(Viswanathan et al., 2017)**. **(EL-SHAFI, 2006)**, found that ZYMV its thermal inactivation point is 65°C,dilution end point is 10-3 and longevity in vitro is 24h at room temperature. According to **(Hammad et al., 2022),** Dilution end point, thermal inactivation point and longevity in vitro of the studied virus were found to be 10-3, 60 ºC and 24 hours, respectively. Electron microscopic examination of the isolated virus sap negatively stained revealed the presence of long, filamentous flexuous virus particles measuring (680-780) nm long and (12-15)nm in diameter which are characteristic of the family Potyviridae (**Fig. 8).** Our results confirmed by those obtained by (**Hosseini et al., 2007);** (**Usher et al., 2012**), **(El-fallal et al., 2015)**, (**Wang and Li, 2017**) and (**Kheder et al., 2017**).Our SDS-PAGE pattern showed the presence of a protein, The size of the product DNA band was approximately 600 bp **(Fig. 9**). The obtained results are in similar that described by (**Glasa et al., 2007**).

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