Egyptian Journal of Phytopathology, Vol. 53, No.2, pp 212 - 231 (2025)
DOI: 10.21608/EJP.2025.425970.1158

ORIGINAL ARTICLE

Serological, Electron Microscopy, and Molecular Identification of *Bean Yellow Mosaic Virus* Naturally Infecting Canna Plants

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Received: 22 September 2025 / **Accepted**: 3 November 2025 / **Published online**: 4 November 2025

ABSTRACT

Bean yellow mosaic potyvirus (BYMV) is a highly destructive virus that inflicts significant economic damage on many important plants worldwide. It naturally infects numerous species of legumes as well as some non-leguminous plants, such as canna plants. The current study aimed to isolate and identify BYMV from naturally infected Canna plants and to produce BYMV polyclonal antibodies. The naturally infected Canna plants exhibiting typical streak mosaic patterns on the infected leaves, along with black spots grown in nurseries in the Alexandria governorate of Egypt, were collected. The BYMV virus was detected in infected Canna leaves sap by indirect ELISA and confirmed by RT-PCR via Cp-gene with an expected size of 525bp. The nucleotide sequence of BYMV-EG2 Cp-gene isolate recorded in NCBI under (Acc. No. PQ836641.1). BYMV isolate was transmitted in a non-persistent manner by *Acyrthosiphon pisum*, *Aphis fabae*, *A. nerii* and *Brevicoryne brassicae*, with average transmission rates of 66.7, 75, 33.3, and 50 % respectively. The partially purified virus using polyethylene glycol (PEG) 6000 and ultra-centrifugation have purity $A_{260/280}$ ratio was 1.17, and the $A_{max/min}$ ratio was 0.85. The virus yield was 13.8 mg/100 g of fresh weight infected faba bean leaves. The immunogenicity of the virus isolate produced an antiserum titer was to $1:1.28x10^5$ determined by indirect ELISA. The transmission electron microscopy revealed that circular inclusions were formed by the extension of pinwheel circles in Canna leaves. The TEM highlighted flexible rod-shaped particles of BYMV measuring approximately 740 nm in length by the negatively stained method with phosphotungstic acid.

Keywords: DBIA; Indirect ELISA; Polyclonal antibodies; RT-PCR; TBIA; TEM

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INTRODUCTION

Canna indica is a mono-coat leaf and belongs to a monotypic genus, being the sole flowering species in the family *Cannaceae* (El-Zahara and El-Tony, 2021 and Fu *et al*., 2022). It's a common perennial ornamental plant known for its spectacular and distinctive large, bright flowers, making it one of the most remarkable plants in the world (Mitrofanova *et al*., 2018). It is widely available in wholesale and retail market areas, found in residential and commercial areas, also enhancing the beauty of landscape design in public gardens (Zsiláné-André *et al*., 2019). *Bean yellow mosaic virus* (BYMV) is classified within the Potyviridae family, the second-largest family of plant viruses (Nigam *et al*., 2019). BYMV is a species of the genus Potyvirus, which encompasses a wide variety of plant viruses that diminish most crops, yields and quality, leading to significant financial losses globally (Gadhave *et al*., 2020). These viruses cause devastating severe damage to economically important crops (Mrkvová *et al*., 2024, and Choi *et al*., 2025). BYMV is a non-enveloped, filamentous virus (genus: Potyvirus, family: Potyviridae) that has a characteristic modal "length of 740 nm and a breadth of 12-15 nm". Also, it is with "0.0% fat, 5% nucleic acid and 95% protein", the virion is composed mainly of a monopartite positive-sense single-stranded RNA (ssRNA) genome (Mrkvová *et al*., 2024). BYMV is a highly destructive virus that inflicts significant economic damage on many important plants worldwide (Attia *et al*., 2024). It naturally infects numerous species of legumes (Omar, 2021), as well as some non-leguminous plants, such as canna plants (Kumari *et al*., 2015, and 2022). Many aphid species spread these viruses either non-persistently or persistently "non-circulative or circulative" (Moya-Ruiz *et al*., 2023). BYMV of faba beans under Egyptian field conditions include mosaic patterns, "light mosaic, spots, yellowing, bending, and rolling of leaves". Ultimately, these symptoms hinder plant growth by reducing leaf area and negatively impacting flower production (Attia *et al*., 2024). BYMV caused infections in *C. indica* plants and presenting a variety of symptoms on their leaves, "including yellow mosaic, striations, extreme discolouration, veins necrosis, black spots, and streak mosaic " (Wijayasekara *et al*., 2018, and Kumari *et al*., 2022). Molecular and phylogenetic analyses based on partial genomic sequencing can provide biased results of the BYMV CP gene (Attia *et al.*, 2024). The objectives of this study are: (i) to detect and isolate BYMV affecting the *C. indica* plants, (ii) to identify the isolated virus using biological, serological methods, as well as transmission electron microscopy, and molecular techniques, (iii) to produce polyclonal antisera of BYMV isolate.

MATERIALS AND METHODS

Collection of Canna leaf samples and isolation of BYMV

Continuous monitoring was conducted on *C. indica* plants grown at various nurseries in the Alexandria Governorate. Naturally infected leave samples exhibiting virus like symptoms were collected in the Summer of 2024 in plastic bags from each region. The virus was detected by indirect ELISA using BYMV polyclonal antibodies and subsequently confirmed by RT-PCR. Infected leaves ELISA positive was utilized for virus isolation by single local lesions assay on *Chenopodium amaranticolor* leaves as mentioned (Kuhn 1964). The isolated virus was propagated in Faba bean plants through mechanical inoculation, serving as the source of the virus (Attia *et al.*, 2024).

Detection of BYMV Biological assay

The infectious saps of naturally infected *C. indica* leaves were serially diluted in 0.1 M phosphate buffer (pH 7.0) at dilutions of 1:10, $1:10^{-2}$, $1:10^{-3}$, $1:10^{-4}$ and $1:10^{-5}$. These dilutions were then used to mechanically inoculate C. *amaranticolor* a indicator host for local lesion assays (Younes *et al.*, 2021).

Determination of insect virus transmission

Four different species of aphids were collected from naturally infested Canna plants. These aphid species were specified by Prof. Dr H. Hamza, Entomology Dept., Faculty of Agriculture, Alexandria University. The identified aphid species (*i.e.*, *Acyrthosiphon pisum*, *Aphis fabae*, *A. nerii* Boyer, and *Brevicoryne brassicae*) were examined for their ability to transmit isolated BYMV. After an hour of starvation, apterous aphids were permitted to feed for 5, 30 and 60 minutes on BYMV-infected faba bean leaves. Subsequently, they were transferred to virus-free fit faba bean plantlets at a rate of ten aphids/plantlet (ten replicate plants for each of the aphid species), where they were allowed a five-minute inoculation feeding period. insecticide ultimately employed, with the Common name "Malathion" to eliminate the aphids, Manufacturer “Agrochem Co”, formulation " 57 EC" at a rate "75 mL/ 3.785 L" (Sultana *et al*., 2024, and Beekman, 2025). The treated plants were kept under insect-proof cages that were impervious to insects, and the development of their symptoms was meticulously closely monitored. In each case, in every instance, the percentage of recorded transmission was noted (Abd El-Aziz, 2020b).

Determination of sap mechanical virus transmission

Mechanically inoculated plants with BYMV were serially diluted in 0.1 M phosphate buffer (pH 7.0) at dilutions of 1:10, 1:10^{-2}, 1:10^{-3}, 1:10^{-4} and 1:10^{-5}. These dilutions were then used to mechanically inoculate *C. amaranticolor*, a test host for local lesion assays (Younes *et al.*, 2021).

Determination of diagnostic hosts and symptomology

The isolated virus was tentatively identified using sundry diagnostic hosts, including "*Chenopodium amaranticolor*, *Gomphrena globosa*, *Phaseolus vulgaris*, *Pisum sativum* and *Vicia faba"* for tentative characteristic symptoms of the BYMV virus. Ten plantlets of every examined plant species were mechanically inoculated with BYMV. After which, they were maintained under greenhouse stipulations. Plants were checked every day for the manifestation of symptoms for three weeks. Back-inoculation to the indicator host *C. amaranticolor* was employed to assess latent infections in inoculated plants that did not display any signs of disease (Moury and Desbiez, 2020).

Purification of isolated BYMV

Isolated BYMV was partially purified using the technique qualified by *Kheder et al.*, (2002) with minor adjustments made through Younes *et al.*, (2021). This purification process is illustrated and explained (Fig. 1).

Evaluation of BYMV purification method by biological assay

The Purified BYMV was serially diluted in 0.1 M phosphate buffer (pH 7.0) at dilutions of 1:10, 1:10^{-2}, 1:10^{-3}, 1:10^{-4} and 1:10^{-5}. These dilutions were then used to mechanically inoculate *C. amaranticolor*, a test host for local lesion assays (Zeid, 2016).

Determination of the ultraviolet absorption spectrum of the purified BYMV

Using "an Ultraviolet Jon Way 6405 UV/VIS spectrophotometer", the absorption spectra of the purified suspension of the isolated BYMV were recorded at wavelengths ranging from 220 to 340 nm in 10-nm intervals (Noordam, 1973). The *UV spectrum* along with the "$A_{260/280}$, $A_{280/260}$" and $A_{max/min}$ ratios were predestined. The virus yield was calculated using the absorbance "value at 260 nm and the extinction coefficient of 2.8 for the potyvirus group (Abd El-Aziz, 2020a). The virus purity was calculated at $A_{260/280}$ ratio.

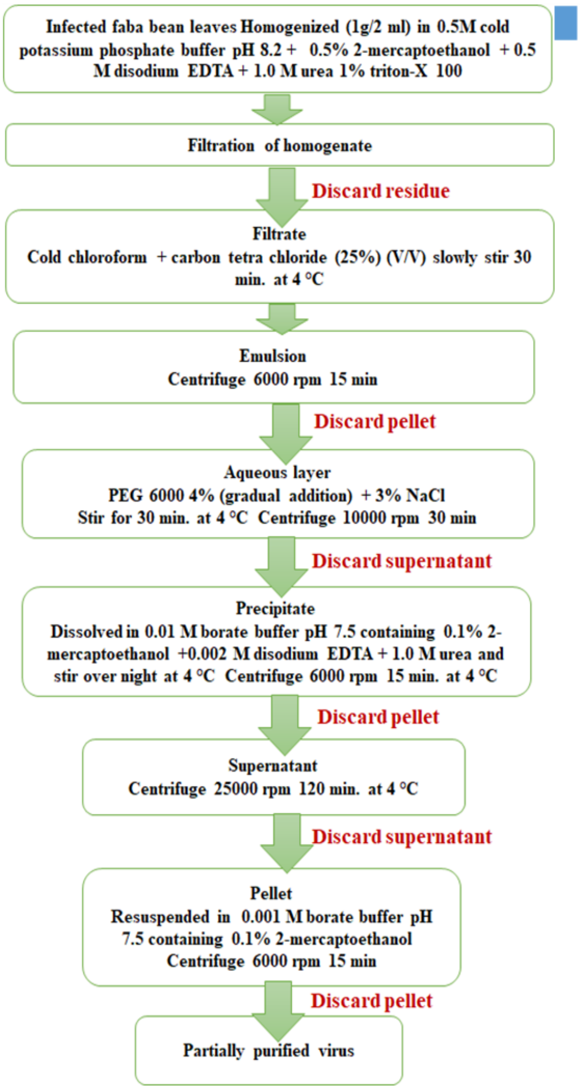

Fig. 1. Diagrammatic outline of BYMV purification by infected *V. faba* leaves

Production of BYMV polyclonal antibodies

The schedule provided by Kawanna (2007) was followed to prepare an antiserum against BYMV, with minor adjustments made by Abd El-Aziz, (2020b). Four white New Zealand rabbit, two rabbits to collect normal serum and two rabbits were immunized with the prepared purified virus. The production of the antiserum involved five weekly intramuscular injections of 2.4 mg/ml of virus emulsified with an equal volume of “Freund's adjuvant (Difco Lab, U.S.A.)” over four weeks. The initial injection contained complete adjuvant, and the subsequent injection contained incomplete adjuvant thereafter. Bleeding the rabbit was performed using marginal ear veins two weeks after the last immune serum injection. The blood was allowed to clot for two hours at room temperature and then refrigerated overnight. High centrifugation “at 10000 g/30 min”, was employed to clarify the separated antiserum. The antiserum was then divided into aliquots and stored in a freezer until needed for various serological tests. Indirect ELISA was used to determine the antiserum titer (Abd El-Aziz, 2024).

Evaluation of the produced antisera

Determination of antisera titer

Serial dilutions of produced antisera were diluted at ½ , 1/4 , 1/8 , 1/16 , 1/32 , 1/64 , 1/132 1/264 , 1/528 and 1/1056 were tested against purified BYMV by indirect ELISA for the serological reactions, as reported by Fegla *et al.* (2001b).

Determination of the dilution end point (DEP) of BYMV infectious sap.

Serial dilutions of BYMV infectious sap (infected faba bean leaves), beginning from 10^{-1} to 10^{-5}. were tested against antiserum at 1:500 by indirect (ELISA) and DBIA to determine DEP of BYMV infectious sap as reported by Fegla *et al*. (2001b). The infected samples were collected 18 days after inoculation with BYMV (Abd El-Aziz, 2019b).

Detection of BYMV by serological methods

The source of antiserum used in this study was BYMV, which was kindly provided by Prof. Dr Hosny Aly Younes, Plant Pathology, Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University.

Tissue blot immunoassay (TBIA)

Tissue blot immunoassay (TBIA), as described by Lin *et al.,* (1990), with slight modifications by Fegla *et al.*, (2001a), was checked to serologically detect the presence of the virus in samples of faba beans that appeared healthy, and ostensibly infected. TBIA method, was applied to the tissue of rolled leaves and stems. Razor blades were used to cut both healthy and infected samples. After previously dipped in 0.05M carbonate buffer (pH 9.6), the exposed cut edges were pressed onto a nitrocellulose membrane (NCM 0.45 nm, BIO-Rod Laboratories, Richmond, CA). There was no colour development in the negative reaction, as reported by Abd El-Aziz (2019).

Dot blot immunoassay (DBIA)

DBIA, as characterized by Fegla *et al.* (2000) optimized the concept, and Abd El-Aziz *et al.* (2019) applied it in their research. A pencil was used to draw a grid consisting of one cm squares on a sheet of nitrocellulose membrane (NCM 0.45 nm, BIO-Rad Laboratories, Richmond, CA). The sheet was then cut to the would size to accommodate the number of samples required in an individual test. Following the procedure outlined by (Awad and El-Helaly, 2017) dilutions of extracted sap from healthy and infected plant leaves were prepared at ratios of 1:10, 1:100, 1:1000, 1:10000 and 1:100000 in carbonate buffer. According to Younes *et al.,* (2021), the blots developed a purple color, indicating a positive DBIA reaction, while a negative reaction resulted in no color change or green color.

Indirect ELISA

Indirect ELISA was carried out as described by Abd El-Aziz and Younes (2019). Tested *C. indica* samples were ground in a coating buffer and followed the detection steps (Abd El-Aziz, 2019). The analyzed *C. indica* samples were crushed in a coated buffer and then put through the detection procedures. Fifty microliters of 3M NaOH were added to inhibit the enzyme activity. Multi Skan Ex ELISA Reader absorbance measurements at 405 nm were used to express ELISA values (Abd El-Aziz, 2019a). If the optical density of a tested sample was more than two times of the healthy control sample, the sample was deemed positive. In each test set, wells that lacked antigen (coating only the coating buffer) were included as blanks (Khalil *et al.,* 2022).

Molecular Identification of isolated virus by RT-PCR

Total RNA was isolated from naturally infected Canna plants using the modified CTAB protocol according to Ortiz *et al.*, (2006). Viral RNA was subsequently extracted, and reverse transcription polymerase chain reaction (RT-PCR) was performed to confirm the presence of BYMV Briefly, 0.05 g of Canna leaves was ground in a mortar with liquid nitrogen ($N_{2)}$ to a fine powder and then transferred to a microfuge tube containing 500µl of cetyl trimethyl ammonium bromide (CTAB) buffer (2 % CTAB; 100 mM Tris-HCl, pH 8.0; 20 mM EDTA; 1.4 M NaCl), with 2 % *β*-mercaptoethanol added just before use. The Samples were incubated for 30 minutes at 65 °C. The Suspensions were extracted twice with chloroform/isoamyl alcohol (24:1). After adding 10 M LiCl, the RNA was precipitated for one hour at -20°C. Subsequently, washed twice with 70% ethanol. The pellet was dried on a sterile bench, and then re-suspended in 30 µl of RNase-free water and kept at – 80°C until used for testing. Complementary DNA (cDNA) was synthesized using 2µl of total RNA and 1.5 µl of BYMV-specific primers for the coat protein gene, P1984 (5'CAA GGT GAG TGG ACA ATG ATG G-3') and P1985 (5'-GAG AGA ATG ATA CAC ATA CTG AA-3'). These primers amplify a segment of the coat protein gene, specifically 417 nucleotides corresponding to the carboxyl end of the capsid protein gene, and 108 nucleotides of the 3' non-coding region (3'NCR) of the BYMV genome, approximately 525bp as described by (Ortiz *et al.,* 2006). Following the manufacturer's instructions, complementary DNA (cDNA) was prepared using M-MLV (Moloney Murine Leukemia Virus) reversed transcriptase (Sigma - Product Code M1302). In order to complete the PCR reaction, the sample was first denatured at 94 °C for three minutes. This was followed by 35 cycles consisting of 30 seconds at 94 °C, one minute at 52°C, and one minute at 72 °C, with the final elongation step at 72 °C for ten minutes. The PCR-amplified product was analyzed using 1% agarose gel electrophoresis in 1X TAE buffer, stained with EZ-View (Biomatik). The amplified band was visualized under UV illumination.

Nucleotide sequence analysis

The QIA Quick PCR purification kit was utilized to purify the RT-PCR amplicons of the BYMV isolate. These amplicons were then directly sequenced in both directions at "Macrogene Korea" and analyzed using an automated DNA sequence "ABI 3700 capillary sequencer, Macrogene, Korea". DNAMAN sequence analysis software (Lynnon Bio-Soft. Quebee, Canada) version 7 was utilized to organize and analyze the obtained sequences. The sequences were then subjected to a BLAST$^{®}$ search against the "National Center for Biotechnology Information (NCBI)" available sequence Database (http://blast.ncbi.nlm.nih.gov), before being submitted to GenBank. The nucleotide sequences of 20 BYMV isolates from various geographical locations, which were entered into the GenBank database, were compared. The molecular evolutionary genetics analysis (MEGA11) program (Tamura *et al.,* 2021), was utilized to conduct a phylogenetic analysis. The neighbour-joining method, accompanied by bootstrap analysis with 1000 repetitions, was employed to generate the phylogenetic tree with closely related potyvirus, *Clover Yellow Vein Virus* (CYVV), with GenBank accession number (NC_003536), which was used as an outgroup (Aseel *et al*., 2019).

Transmission electron microscope

a- Detection of virus morphology

For five minutes, copper grids coated with Formavar were floated on droplets of purified virus preparations, then stained with 2% phosphotungstic acid (pH 7), and examined using a JEM-1400 TEM–Japan. (Hamza *et al.*, 2018).

b- Tissue samples of *Canna indica*

Tissue samples, consisting of small pieces of mid-vein blades with adjacent mesophyll tissues, were excised from both virus-infected and healthy canna leaves. These samples were fixed in a solution of 2 % paraformaldehyde and 2 % glutaraldehyde in 0.05 M cacodylate buffer at (pH 7) for two hours at room temperature under vacuum conditions. The tissues were rinsed several times with the same buffer, then post-fixed for two hours in 1 % osmium tetroxide (OsO_4), and subsequently bulk-prestained overnight at 4°C. in 0.5 % aqueous uranyl acetate (Younes, 2003). Following an ethanol series for dehydration, the tissues were embedded in Spurr's epoxy resin and cut into 1 µm dense sections using a glass knife in an LKB ultramicrotome. Before being examined using a Transmission Electron Microscope (TEM) model JEOL – JSM-1400 PLUS, thin sections were double-stained with 2 % aqueous uranyl acetate for 10 minutes and lead citrate for 2 minutes (Xie and Hong, 2022).

RESULTS

The natural source of BYMV

The naturally infected *C. indica* leaves collected from various nurseries in Alexandria Governorate, Egypt, exhibited symptoms including streak mosaic on the infected *C. indica* Leaves. and black spots, which confirmed serological and molecular as shown in the Figure 2, was used as source of BYMV.

Mode of transmissions

Mechanical transmission

The isolated BYMV was with a transfer efficiency of 90%, BYMV was easily mechanically transmitted from Canna leaves to faba bean plants using a 0.1 M phosphate buffer (pH 7.0) as well as to diagnostic hosts. It was Mechanically inoculated, was serially diluted in 0.1 M phosphate buffer (pH 7.0) at dilutions of 1:10, 1:10^{-2}, 1:10^{-3}, 1:10^{-4} and 1:10^{-5}to *C. amaranticolor* a test host for local lesion assays

Fig. 2. Symptoms of BYMV on naturally infected *C. indica* plant showing typical streak mosaic (orange arrows) and black spots

Aphid transmissions

Four aphid species *Acyrthosiphon pisum*, *Aphis fabae*, *A. nerii* Boyer, and *Brevicoryne brassicae* were found to transmit BYMV non-persistently. When five aphids were applied to each tested plant faba bean the average transmission rates were 66.7, 75, 33.3, and 50%, respectively (Table 1).

Diagnostic hosts and symptomology

BYMV caused mottled mosaic symptoms on *Vicia faba*, mild mosaic on *P. vulgaris* leaves, mosaic, blisters, and deformation on *P. sativum*, as well as mosaic and yellowing on *V. unguiculate* leaves (Fig. 4). Following inoculation with BYMV, symptoms of red local lesions on *Ch. amaranticolor* and red-necrotic local lesions on *G. glbosa* appeared (Fig.5). The reactions of various hosts to infection with the BYMV isolate are presented in Table 2.

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Table 1. Transmission of BYMV by aphid species.

Transmission of BYMV by aphid species		
Aphis species	**Transmission**	
	Rate*	**Percentages**
Acyrthosiphon pisum	08/12	66.7
Aphis fabae	10/12	75.0
A. nerii	04/12	33.3
Brevicoryne brassicae	06/12	50.0

* No. of infected plants/No. of tested plants, five aphids per plant were used

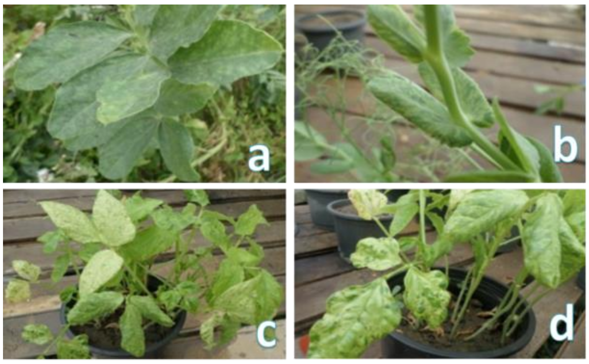

Fig. 4. Diagnostic plants Infected with *Bean yellow mosaic virus*-like symptoms showing, **a)** mottling mosaic on *V. faba*, **b)** mosaic, blisters and deformation on *P. sativum*, **c)** mild mosaic on *P. vulgaris* leaves and **d)** Mosaic and yellowing on *V. unguiculate* leaves.

Fig. 5. Photographs of diagnostic plants a- *G. glbosa* inoculated with BYMV isolate observed Red-necrotic local lesion ocal lesion b- C. *amaranticolor* and b) Red local lesions on *C. amaranticolor*

Table 2. Reactions of different hosts to infection with BYMV isolate

Reactions of different hosts to infection with BYMV isolate		
Family	**Scientific name**	**Symptoms BYMV isolate**
Amarnthaceae	*Gomphrena globosa*	red-necrotic local lesions (RNLL)
Apocynaceae	*Vinca rosa*	-*
Cannaceae	*Canna indica*	systemic mosaic (SM), black spots (BS), vein streaking (VStr)
Chenopodiaceae	*Chenopodium amaranticolor*	red local lesions (Rll)
Cucurbitaceae	*Cucurbita pepo* L.	-
Fabaceae	*Arachis hypogaea* L.	Mottle (Mt)
	Glycine max L.	SM
	Medicago sativa L.	SM and VStr
	Phaseolus vulgaris L.	SM, systemic yellow mosaic (SYM) and leaf curling (LCu)
	Pisum sativum L.	SM, Mt and VStr
	Vicia faba L.	SM, Mt, vein cleaning (VC), mosaic (M), and SYM
	Vigna unguiculate	SM and Mt
Solanaceae	*Datura metal* L.	-
	Nicotiana glutinosa L.	-
	Petunia X hybrida Vilum	chlorotic local lesion (CLL)

* (-) = no symptoms

Ultraviolet spectra of purified BYMV

The absorption spectrum of the purified virus isolate, determined through Jon Way 6405 UV/VIS spectrophotometer was typical for nucleoprotein (Fig. 6). The A 260/280 ratio was 1.17, while the max/min ratio was 0.85. The concentration of the virus in the preparation was estimated using an extinction coefficient of $E^{260}_{0.1\%} = 2.8$. The yield of the purified virus was about 13.8 mg/100g of fresh weight of *V. faba* leaves. The ultraviolet absorption spectrum of the collected virus exhibited a minimum at 240 nm and a maximum at 260 nm. When the purified virus was tested biologically on *C. amaranticolor* leaves, numerous local lesions were observed.

BYMV antiserum production

An antiserum against the BYMV isolate was produced. The titer of BYMV isolate antiserum was determined using an indirect ELISA. Positive ELISA values were obtained at dilutions of up to 1:1.28x10^5 and not at 1:2.56 x10^5 (Table 3).

Antiserum titer of purified BYMV:

The antisera titer was 1/512, tested against the purified BYMV with indirect ELISA (Table 4). Positive ELISA value was obtained at 0.826, and a negative ELISA value was obtained at 0.126.

Dilution end point (DEP) of purified BYMV

The DEP of purified BYMV was 1:10^6 tested against ***a***ntisera BYMV with indirect ELISA (Table 5).

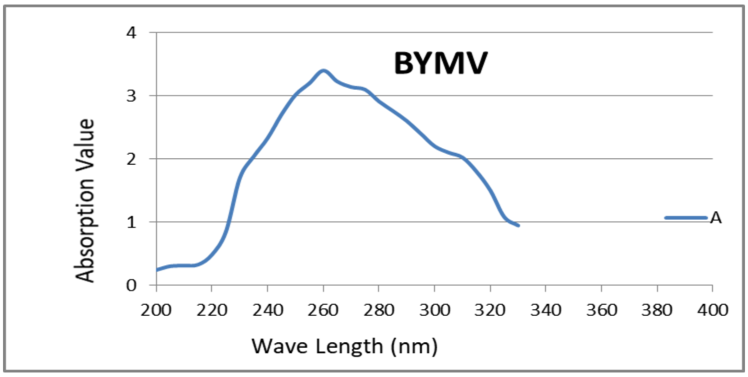

Fig. 6. Ultraviolet absorption spectrum of the purified BYMV isolate.

Table 3. Indirect ELISA optical density (E 405 nm) of extract of BYMV-infected faba bean plants in various dilutions of BYMV antiserum*

Optical density of the extract of BYMV-infected faba bean		
Antiserum dilution	**Healthy**	**Infected**
1:5 X 10^2	0.383**	0.842
1:10^3	0.236	0.637
1:2 X 10^3	0.182	0.398
1:4 X 10^3	0.134	0.325
1:8 X 10^3	0.099	0.261
1:1.6 X 10^4	0.083	0.202
1:3.2 X 10^4	0.074	0.198
1:6.4 X 10^4	0.052	0.178
1:1.28 X 10^5	0.033	0.120
1:2.56 X10^5	0.024	0.043

*The experiment was repeated twice, and the ELISA optical density at 405 nm are average of two replicates for each dilution.

**Optical density of at least double that of the healthy control was considered positive.

Table 4. Titer antiserum of BYMV isolate using an indirect ELISA and comparative between serological methods.

Antiserum Dilutions	Indirect ELISA	DBIA		TBIA	
		Stem	Leave	Stem	Leave
½	0.875	++	+++	+++	++
¼	0.742	++	+++	+++	++
1/8	0.692	++	+++	+++	++
1/16	0.525	++	+++	+++	++
1/32	0.461	++	+++	+++	++
1/64	0.402	++	+++	+++	++
1/128	0.398	++	+++	+++	++
1/256	0.348	++	+++	+++	++
1/512	0.292	++	++	+++	++
1/ 1024	0.212	-	-	-	-
1/2048	0.042				

+ve ELISA= 0.826, -ve ELISA= 0.126

Table 5. Dilution end point (DEP) of purified BYMV with indirect ELISA.

BYMV dilutions	Indirect ELISA optical density
1:10	0.685
1:10^2	0.624
1:10^3	0.582
1:10^4	0.512
1:10^5	0.465
1:10^6	0.312
1:10^7	0.184
1:10^8	0.145

+ve ELISA= 0.826, -ve ELISA= 0.126

Comparative of Serological methods for BYMV detection

Collected samples for testing were initially used for TBIA, followed by the necessary preparations for DBIA and indirect ELISA (Table 6)..

Serological detection by tissue blot immunoassay (TBIA)

TBIA was used to detect BYMV isolate in infected *V. faba* plants following mechanical inoculation, and the results were compared with those from healthy plants. The findings indicated that BYMV antiserum at a dilution of 1:500 was capable of detecting the virus in infected plant tissue (leaves and stems) after inoculation by TBIA on a nitrocellulose membrane (Fig. 7 & Table 6).

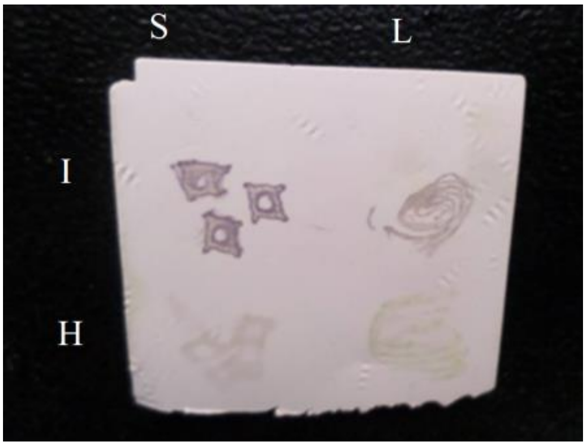

Fig. 7. TBIA used for the detection of BYMV in infected leaves and stems of *V. faba* on nitrocellulose membrane, where L: leaves, S: stems, I: infected, H: healthy.

Dot blot immunoassay (DBIA) and indirect ELISA

DBIA was used to detect the isolated BYMV in infected *V. faba* plants at serial dilutions (1: 10, 1: 10^2, 1: 10^3, 1: 10^4 and 1: 10^5), following mechanical inoculation. The dilutions were prepared in carbonate buffer (pH 9.6) using sap from BYMV-infected plants. Healthy plants were also tested. The results indicated that BYMV antiserum at a dilution of 1:500 successfully detected the virus in infected sap at a dilution of 1: 10^4 n using indirect ELISA (Table 4). Additionally, DBIA on a nitrocellulose membrane was detected at the same dilution (Fig. 8 & Table 7).

Table 7. Indirect ELISA for BYMV isolate in serial dilutions of infected faba bean plant sap

Dilutions	Healthy	Infected
1:10	0.285	0.724
1:10^2	0.195	0.645
1:10^3	0.147	0.482
1:10^4	0.124	0.356
1:10^5	0.117	0.196

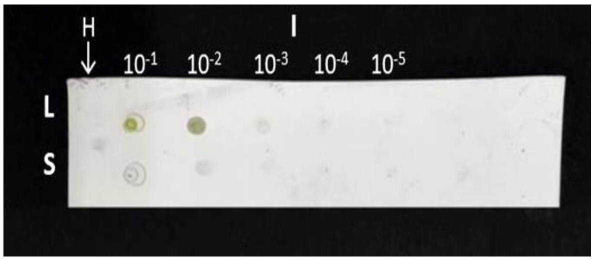

Fig. 8. DBIA is used for the detection of BYMV in infected leaves and stems of *V. faba* on nitrocellulose membrane. Where; L: leaves, S: stems, I: infected, H: healthy.

Biological assay

Ch. amaranticolor was mechanically inoculated with Serial dilutions of faba bean plants infected with the BYMV isolate, including dilutions of 1:10, 1:10^{-2}, 1:10^{-3}, 1:10^{-4} and 1:10^{-5} in inoculation 0.1 M phosphate buffer at pH 7.0 up to a dilution of 1: 10^{-4}, red local lesions were observed to appear (Table 8).

Table 8. The dilution end point of BYMV isolate infectivity test on *Ch. Amaranticolor.*

Dilutions of infected plant sap	Mean* of observed RLL
1:10	47
1:10^2	24
1:10^3	12
1:10^4	03
1:10^5	-

**each treatment containing 10* Chenopodium amaranticolor *leaves and replicated twice.*

Extraction of viral RNA and RT-PCR

To ensure the integrity and purity of the RNA suitable for the RT-PCR reaction, the modified CTAB methodology was utilized in the total RNA extraction process. This method produced high-quality RNA, and the presence of BYMV was successfully confirmed by RT-PCR. The BYMV coat protein (cp) gene was detected using RT-PCR in naturally infected Canna plants exhibiting yellow mosaic pattern symptoms on their leaves. The P1984 and P1985 primers amplified a fragment of approximately 525 bp for the Egyptian isolate, which includes the carboxyl end of the capsid protein gene and the 3’ non-coding region (3’NCR) of the virus. The investigation using 1.5% agarose gel electrophoresis analysis revealed one specific PCR amplification product corresponding to the expected 525 bp band, which was visible under UV light, as shown in Fig. 9.

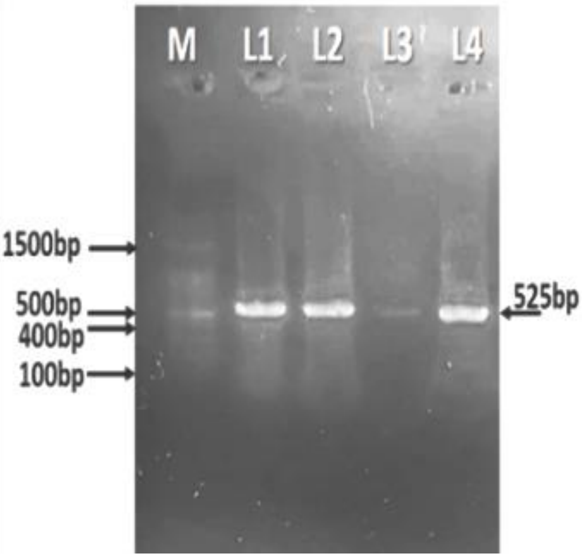

Fig. 9. EZ-View-stained agarose gel showing reverse transcription polymerase chain reaction (RT–PCR) products amplified from infected *C. indica*, Lane 1, *Bean yellow mosaic potyvirus* (BYMV) infected Faba bean plants; lanes 2, 3, and 4 infected *C. indica*; molecular weight marker 100 bp ladder (Biomatik-USA).

Nucleotide sequence analysis

After being assembled and analyzed using MEGA 11, the 525 bp nucleotide sequences of the BYMV isolate BYMV-EG2 were compared with 22 nucleotide sequences in NCBI database and deposited under accession number (PQ836641.1) have been deposited in the GenBank. According to the nucleotide sequence analysis of BYMV- EG2, isolates Kh-B2250 from Iran and MH180 from Spain exhibited the highest percentage identity of 99.6% and 99.4 % respectively. Isolate LJu25 and Sle19 demonstrated a 99 % identity as shown in (Fig. 10 and Table 9). Additionally, the sequence results indicated that the identity was approximately 98.8 % with the Spanish isolate BH41, 98.6 % with the Mexican isolate CP1-MEX, 97.9 % with the Japanese isolates No.4 and B-33, 97.7 % with the Iranian isolate Kh-B2252, and 97.5 % with the isolates Hangzhou BYMV-Eg and E-24N, from China, Egypt and Japan respectively. Moreover, nucleotide sequence analysis revealed that the identity ranged from 96.7 to 90.4 % when compared to sequences from other countries. While, results indicated a divergence in nucleotide sequence between the BYMV-EG2 isolate and the closely related potyvirus, *Clover Yellow Vein Virus* (CYVV, GenBank accession number NC 003536.1), which exceeded 75.8 % identity.

A phylogenetic analysis was conducted using maximum likelihood (ML), which was selected as the best-fitting nucleotide substitution model based on the Tamura-Nei Model implemented in MEGA 11. Isolates are identified by their GenBank accession number, name, and country of origin (Fig. 11). The BYMV-EG2 Egyptian isolate sequenced in this study is highlighted and marked with a bold square. A closely related potyvirus, *Clover Yellow Vein Virus* (CYVV, GenBank accession number NC 003536.1), was used as an outgroup. Scale bars indicate genetic distances of 0.05.

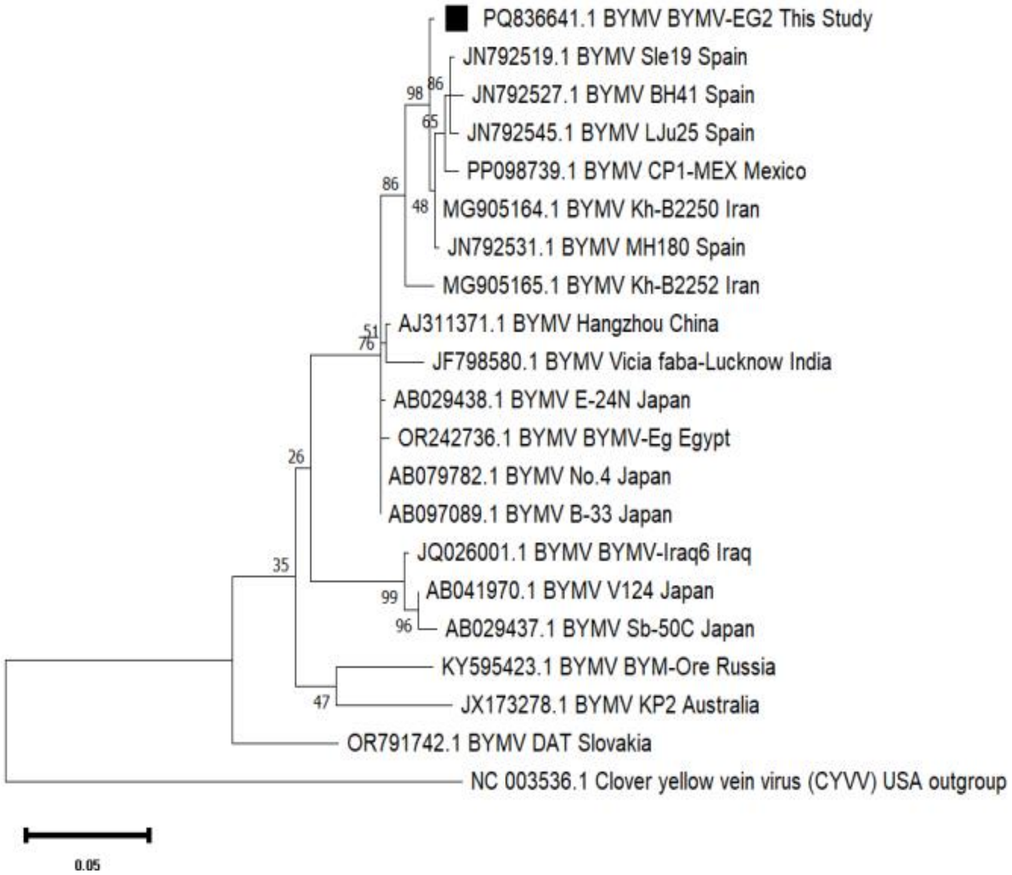

Fig. 10. A phylogenetic tree generated from a part of the coat protein gene of the Egyptian isolate of BYMV (GenBank accession number PQ836641.1) with twenty BYMV isolates available in the GenBank database.

Table 9. Comparison of the ***Cp-gene*** nucleotide sequences of BYMV Egypt isolate in the present study (PQ836641.1) with sequences of BYMV isolates available from other countries the available in the GenBank database.

	Accession number*	BYMV Isolate	Country	Host plant	% Id**
1	PQ836641.1	BYMV-EG2	Egypt	*Canna* spp.	
2	MG905164.1	Kh-B2250	Iran	Phaseolus vulgaris	99.6%
3	JN792531.1	MH180	Spain	broad bean	99.4%
4	JN792545.1	LJu25	Spain	bean	99.0%
5	JN792519.1	Sle19	Spain	lentil	99.0%
6	JN792527.1	BH41	Spain	broad bean	98.8%
7	PP098739.1	CP1-MEX	Mexico	Vicia faba	98.6%
8	AB079782.1	No.4	Japan	Gentian	97.9%
9	AB097089.1	B-33	Japan	Gentian	97.9%
10	MG905165.1	Kh-B2252	Iran	Phaseolus vulgaris	97.7%
11	AJ311371.1	Hangzhou	China	Not available	97.5%
12	OR242736.1	BYMV-Eg	Egypt	broad bean	97.5%
13	AB029438.1	E-24N	Japan	broad bean	97.5%
14	JF798580.1	Vicia faba-Lucknow	India	Vicia faba	96.7%
15	AB041970.1	V124	Japan	Gladiolus	92.6%
16	JQ026001.1	BYMV-Iraq6	Iraq	broad bean	92.6%
17	AB029437.1	Sb-50C	Japan	broad bean	91.8%
18	KY595423.1	BYM-Ore	Russia	*Canna* spp.	90.3%
19	OR791742.1	DAT	Slovakia	Trifolium pratense	90.4%
20	JX173278.1	KP2	Australia	Diuris magnifica	90.4%
21	NC003536.1	No.30	USA	Clover	75.8%

*BYMV-isolates can be directly queried with any protein sequence in the GenBank database by accession number pressing, **Id: Identity and the highest identity value is indicated in bold.

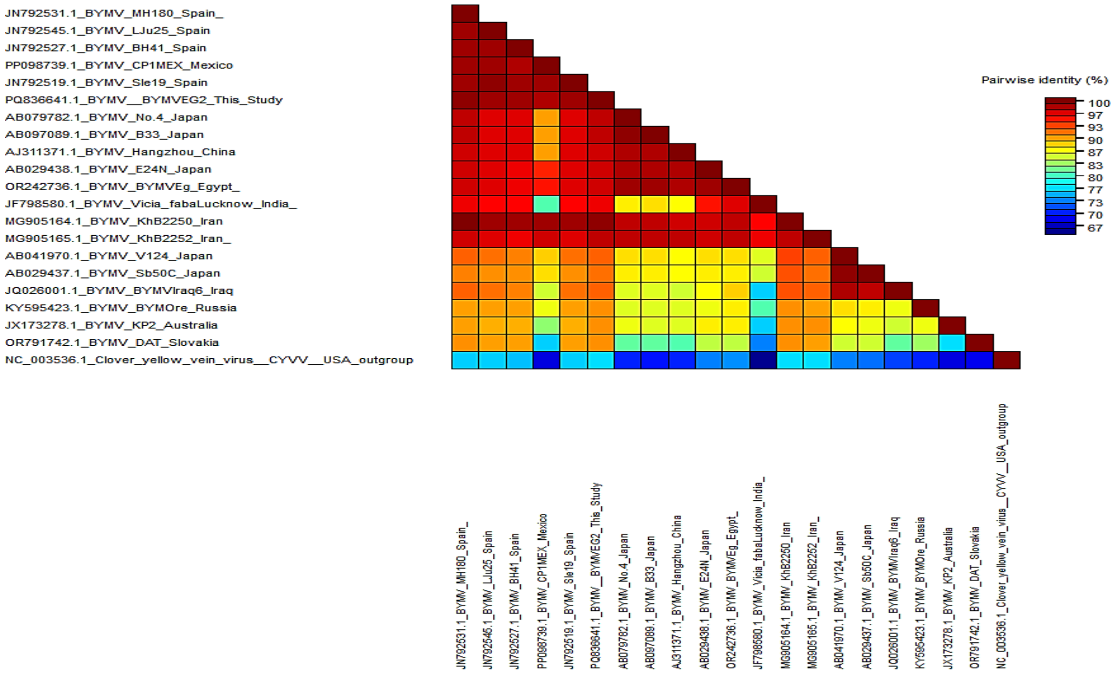

Fig. 11. Comparative sequence analysis based on the coat protein gene of BYMV isolate BYMV-EG2 (GenBank accession number PQ836641.1) with the available sequences of BYMV isolates from other countries in the GenBank database.

Transmission electron microscopy

Electron microscopic examination of ultrathin sections of *C. indica* leaf infected with BYMV revealed the presence of prominent cytoplasmic inclusions. The induced inclusions were composed of bundles, cylinders, tubes and pinwheel figures. The pinwheels were formed from thin circles oriented around a central core with frequently thicker walled scroll-like circles (Figure, 12, a). Electron micrographs indicate that circular inclusions were formed by the extension of the pinwheel circles. Transmission electron microscope Examination of a partially purified preparation from infected *C. indica*. Leaves negatively stained with phosphotungstic acid, using a transmission electron microscope revealed flexible rod-shaped particles of BYMV with helical symmetry measuring 740 nm (Figure 12,b).

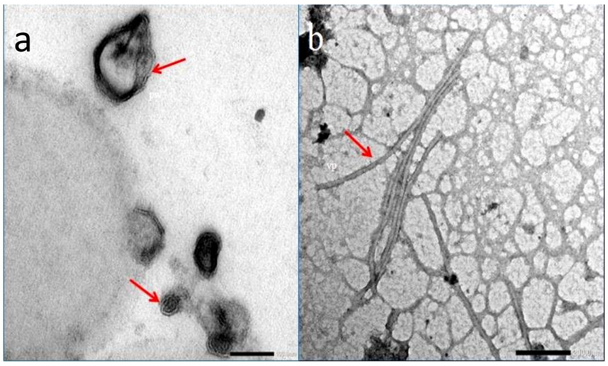

Fig. 12. Photo Electron micrographs were done by TEM, a): Ultra-thin section in BYMV infected Canna leaf showing pinwheel inclusions (pw) (red arrows), circular inclusion (scroll-like), represents a longitudinal section through cylindrical inclusions in cytoplasm, b): showing rod flexible filamentous BYMV particles (vp) negatively stained with 2% phosphotungstic acid with magnification power 40000x.

DISCUSSION

The *Bean Yellow Mosaic virus* (BYMV) affects a variety of commercially, socially, and economically important crops, including *Canna* species, from the *Cannaceae* family (Kumari *et al.*, 2015, and 2022). Among its species is *Canna indica*, a stunning, ornamental, herbaceous, herbaceous and aromatic plant (Pandey and Bhandari, 2021). BYMV has emerged as the most prevalent virus in recent years, causing typical streak mosaic and black spot symptoms characterized by bright yellowing in various plant species. This virus has been isolated from several locations in Egypt (Awad and El-Helaly, 2017). BYMV typically causes severe economic damage to the crops it infects, such as beans (Younes *et al.*, 2021, and El Gamal *et al.*, 2022). Leaves of the affected *Canna indica* by BYMV displayed prominent veinal streaking and severe mosaic symptoms (Kumari *et al.*, 2015 and Li *et al.*, 2019).

According to our research, BYMV affects the mono-coat leaves of *Canna* sp., resulting in the appearance of black spots. These align harmony with those reported by Chauhan *et al.* (2015), and Kumari *et al.* (2022), Those plants are cultivated in different regions of Egypt (Attia *et al.*, 2024).

Many species of aphids are known to naturally transmit the plant viruses (El Gamal *et al.*, 2022). Dangerous pests, aphids are small, sap-sucking insects that can cause significant harm to agriculture, primarily by transmitting economically important plant viruses (Beekman, 2025). Leading to a high rate of viral infections in host species (Elsharkawy *et al.*, 2021; El Gamal *et al.*, 2022), including *Canna* sp. (Kumari *et al.*, 2022), and faba bean (Attia *et al.*, 2024). BYMV could be transmitted easily by mechanical inoculation, and also by aphids in a non-persistent mode (Younes *et al.*, 2021). The spread of BYMV in certain regions of Egypt is significantly influenced by alternative hosts and vectors (Attia *et al.*, 2024).

Egypt cultivates a significant amount of *P. vulgaris* L. (Ahmed *et al.*, 2020) a summer crop that is susceptible to both natural and artificial infections of the BYMV (Younes *et al.*, 2021). BYMV could be propagated from overlapping winter hosts to summer hosts (Ahmed *et al.*, 2020) and can return to winter faba bean, which serves as a propagative host when there is a high population of aphid vectors (Younes *et al.*, 2021). Finding an approach that is sensitive, user-friendly, straightforward, reliable, and cost-effective for identifying the virus in various plant sections is crucial (Khalil *et al.*, 2022). The current study evaluated the sensitivities of indirect ELISA (Abd El-Aziz and Younes, 2019), TBIA (Jaber *et al.*, 2025) and DBIA to detection of BYMV as reported by Devi ***et al.*** (2024). One of the most important factors in the effective implementation of viral control strategies is the availability of sensitive and efficient detection techniques (Devi *et al.*, 2024). These methods encompass a variety of techniques that were evaluated, including immunoassays using the polyclonal reagent from the BYMV isolate, biological assays, and visual inspections (Younes *et al.*, 2018). Following a series of serial dilutions of virus concentration, an evaluation was conducted (Attia *et al*., 2024).

Findings indicated that TBIA could successfully identify the isolated virus in infected stems and leaves; our results are consistent with those obtained by Devi *et al.* (2024), and Jaber *et al*. (2025). The DBIA detected BYMV after a 10^4 dilution, demonstrating sensitivity comparable to indirect ELISA (Younes *et al.*, 2021). Generally, these findings contradict those of Fegla *et al.*, (2001), who found that indirect ELISA was more sensitive than DBIA for detecting *Potato virus Y potyvirus* (PVY) and *Cucumber mosaic cucumovirus* (CMV). Since TBIA was established to be less expensive, could be done in almost four hours without compromising instability, required advanced facilities, and was sufficiently sensitive to detect the virus in all infected plants, many researchers have utilized it for surveys, diagnosis, and virus detection in various plant tissues (Devi *et al.*, 2024, and Jaber *et al*., 2025). Although DBIA was utilized in this investigation, it was initially described by Powell (1987) and subsequently refined by Fegla *et al.*, (2000), which enhanced its sensitivity in virus identification by a detection ten replicate was used in the present study. According to Devi *et al.*, (2024), and Jaber *et al*. (2025) The sensitivity of the method was nearly conformable to that of TBIA.

The current investigation demonstrated the advantages of DBIA in detecting significantly lower amounts of virus due to a small sample volume of 2 µl, compared to the 100 µl required for ELISA. This finding is consistent with the work of Devi et al. (2024). Based on the significantly reduced levels of the virus in the sample, increasing the sample size could enhance the sensitivity of the DBIA (Younes *et al.,* 2018 and 2021). However, numerous researchers have conducted comparisons between various serological techniques (Younes 2021 and Devi *et al.*, 2024).

Moreover, the molecular characterization of the virus that infects *C. indica* confirmed the identification of BYMV (Kumari *et al.*, 2015, and 2022). Using BYMV-specific primers for the coat protein gene, which amplify a segment of 417 nucleotides corresponding to the carboxyl end of the capsid protein gene and 108 nucleotides of the 3’ non-coding region (3’NCR) of the BYMV genome, the PCR-amplified product was of the expected size (~525 bp), as described by (Ortiz *et al.*, 2006, and Kumari *et al.*, 2015). The final PCR product was directly sequenced and deposited in GenBank (BYMV-EG2 isolate, acc. number PQ836641.1), sequence comparison and phylogenetic analysis of the BYMV-EG2 isolate revealed the highest percentage identity of 99.6% and 99.4% with the isolates Kh-B2250 from Iran and MH180 from Spain respectively. Furthermore, 97.5% identity with Hangzhou, BYMV-Eg and E-24N from China, Egypt, and Japan respectively, moreover, nucleotide sequence analysis showed identity ranged from 96.7% to 90.4% with other countries (Hasan *et al.*, 2024), one specific amplification PCR product of the expected 525 bp band was detected through 1.5% agarose gel electrophoresis and was visible under UV light. These findings align with previous research conducted by Ortiz *et al.* (2006), which reported a similar amplification of the coat protein gene region in BYMV-infected plants.

Biological assays were conducted as described by Younes *et al.* (2021) using serial dilutions of mechanically inoculated faba bean plant leaves infected with the BYMV isolate. Red local lesions were observed on inoculated *Ch. amaranticolor* at dilutions of up to 10^{-4}, similar to the findings of Kawanna (2007) and Zeid (2016).

In several studies focused on purification of filamentous plant viruses, particularly those belonging to the potyviridae family, various procedures have been performed to help prevent aggregation. However, none of these methods have proven to be universally applicable (Younes *et al.*, 2021). Our purification procedure closely resembles the method previously reported for BYMV (Kawanna, 2007) with little modifications.

The purification of BYMV was conducted to determine the photometric characteristics of the purified virus and to prepare the specific antisera (Younes *et al*., 2021). The yield of BYMV, along with its specific photometric data such as A260/280 and A mix/min, falls within the range of BYMV (Kawanna, 2007). However, the slight differences in absorbance ratios, as well as the yield of our samples compared to theirs, may be attributed to the purification method used, specifically precipitation with PEG (6000), such as ultracentrifugation. BYMV isolate was purified, and several properties of the virus particles were determined. Additionally, a specific antiserum was prepared by injecting the rabbits with the purified virus. The titer of the antiserum acquired for BYMV was 1:1.28 $x10^{5;}$ analogous results were acquired by Kawanna (2007).

Indirect ELISA was employed to determine the antiserum titer (Khalil *et al.*, 2022). Positive ELISA results were obtained at dilutions up to 1:1.28 $x10^{5}$, but not at 1:2.56 $x10^{5}$.

Electron microscopy of plant cells infected by the studied viruses revealed two distinct types of cell inclusions induced by infection, although some minor virus-specific differences were also noted. The BYMV strain produced long, "relaxed" pinwheel inclusions, while those induced by PVY were shorter, "tighter," and curved (Moghal and Francki, 1981). The significance of these observations for the identification and classification of potyviruses is discussed (Hamza *et a*l., 2018). The cylindrical inclusions appeared in longitudinal sections as bundles of thin plates associated with thick-walled tubes. No inclusions were observed in healthy tissues (Lecoq *et al.*, 2001). BYMV particles are typically long, flexuous, non-enveloped filaments, and in purified virus preparations, exhibit a distinct modal length of 740 nm (El Gamal *et al.*, 2022). The cytoplasm of infected Canna cells contains inclusion bodies that resemble pinwheels and scrolls, indicating that the virus belongs to the Potyviridae family. These findings were confirmed by (Kumari *et al.*, 2015, and 2022). All Bean Yellow Mosaic Virus particles were found to be approximately 750 nm in size.

CONCLUSION

This study demonstrated the feasibility of isolating Bean Yellow Mosaic virus from the ornamental plant Canna (*Canna* sp.) and identifying it through serological and molecular biology techniques. The isolated virus was propagated on faba bean plants for purification via ultracentrifugation and the production of a specific polyclonal antiserum. Pinwheel inclusion bodies formed by the virus in Canna plants were visualized, along with the shape and length of the virus particles in the purified plant juice. The virus was transmitted by aphids in a non-persistent manner and was also transmitted to compatible hosts of economically important crops. Serological identification of the virus was achieved using indirect ELISA, TBIA, and DBIA methods. Additionally, it was identified through RT-PCR and registered in the GenBank, where it was compared and related to existing isolates. Future work should focus on genome-wide characterization of Egyptian BYMV isolates and their epidemiological impact on ornamental crops.

ACKNOWLEDGMENT

The authors briefly acknowledge those who helped them in conducting this research, mainly Prof. Dr Hosny Aly Younes, Plant Pathology, Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University. The authors would like to thank Prof. Dr Hedaya Hamza, Entomology Dept., Faculty of Agriculture, Alexandria University.

AUTHOR CONTRIBUTIONS

Conceptualization, M. H. Abd El-Aziz; T. F. M. Abdel-Rahman and A. A. Kheder; methodology, M. H. Abd El-Aziz; M. A. El-Abhar; A. Zeid; T. F. M. Abdel-Rahman and A. A. Kheder; software, M. H. Abd El-Aziz; M. A. El-Abhar; T. F. M. Abdel-Rahman and A. A. Kheder; validation, M. H. Abd El-Aziz; M. A. El-Abhar; A. Zeid; T. F. M. Abdel-Rahman and A. A. Kheder; formal analysis, M. H. Abd El-Aziz; M. A. El-Abhar and A. A. Kheder; investigation, M. H. Abd El-Aziz; M. A. El-Abhar; A. Zeid; T. F. M. Abdel-Rahman and A. A. Kheder; resources, M. H. Abd El-Aziz; A. Zeid and A. A. Kheder; data curation, M. H. Abd El-Aziz; M. A. El-Abhar; A. Zeid; T. F. M. Abdel-Rahman and A. A. Kheder; writing—original draft preparation, M. H. Abd El-Aziz; T. F. M. Abdel-Rahman and A. A. Kheder; writing—review and editing, M. H. Abd El-Aziz; M. A. El-Abhar; A. Zeid; T. F. M. Abdel-Rahman and A. A. Kheder; visualization, M. H. Abd El-Aziz; and A. A. Kheder; supervision, M. H. Abd El-Aziz; and A. A. Kheder; project administration, M. H. Abd El-Aziz and A. Zeid; All authors have read and agreed to the published version of the manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study.

FUNDING

This research was supported by the Virus and Phytoplasma Research Department, Plant Pathology Research Institute, Agricultural Research Centre (ARC).

REFERENCES

Abd El-Aziz, M.H. (2019). Three modern serological methods to detect plant viruses. Journal of Plant Science and Phytopathology. 3: 101-106. DOI: 10.29328/journal.jpsp.1001039

Abd El-Aziz, M.H. and Younes, H.A. (2019). Serological and molecular characterization of two seed born cowpea mosaic comovirus isolates affecting cowpea plants (*Vigna unguiculata* L.) in northern Egypt. Journal of Plant Science and Phytopathology, 3: 086-094. DOI: 10.29328/journal.jpsp.1001037

Abd El-Aziz, M.H. (2020a). The Importance of Potato virus Y Potyvirus. Journal of Plant Science and Phytopathology. 4: 009-015 DOI: 10.29328/journal.jpsp.1001044

Abd El-Aziz, M.H. (2020b). Natural infection of squash fruits (Cucurbita pepo) by Zucchini Yellow Mosaic potyvirus (ZYMV) in Alexandria governorate. *Journal of Plant Science and Phytopathology*, 4: 028-032. DOI: 10.29328/journal.jpsp.1001047

Abd El-Aziz, M.H. (2024). Cowpea (chapter 15) pages: 127-130. *(Viral Diseases of Field and Horticultural*

Crops) Academic Press. [DOI: 10.1016/B978-0-323-90899-3.00036-7](https://doi.org/10.1016/B978-0-323-90899-3.00036-7)

Ahmed, R.S., Hmed, A.A., Alnaggar, A.M., Dawoud, R.A., Elshaarawy, R.F.M. and Mahmoud R.S. (2020). Mitigating effects of Bean Yellow Mosaic Virus infection in *faba bean* using new carboxymethyl chitosan-titania nanobiocomposites. International Journal of Biological Macromolecules, (163):1261-1275. DOI: 10.1016/j.ijbiomac.2020.07.066

Aseel, D. G., Abd El-Aziz, M. H. and Hafez, E. E. (2019). Detection of new isolate of Grapevine fan leaf virus naturally infecting Grapevine Plants in Egypt using Real Time-qPCR. *Novel Research in Microbiology Journal*, 3(6): 493-501. DOI: 10.21608/nrmj.2019.66742

Attia, S.A.M., Kheder, A.A., Ahmed, E.A., Farrag, A.A. and Darwish, H.S.A. (2024). Evaluation of *Clitoria ternatea* flower extracts as a novel method to manage *Bean yellow mosaic virus* (BYMV) infecting *Faba Bean*. *Egyptian Journal of Agricultural Research*. 102: 680 - 697. DOI: 10.21608/EJAR.2024.301936.1556

Beekman, M. M. (2025). Aphids out of control: Heritable resistance mechanisms and consequences for pest control. [internal PhD, WU, Wageningen University]. Wageningen University. https://doi.org/10.18174/680993

Chauhan, R.P., Wijayasekara, D., Webb, M.A. and Verchot, J. (2015). A reliable and rapid multiplex RT-PCR assay for detection of two potyviruses and a pararetrovirus infecting Canna plants. *Plant Dis*., 99: 1695-1703. DOI: 10.1094/PDIS-02-15-0225-RE

Choi, S., Kwon, S.H., Kwon, G.S., Choi, H.S., Seo, H.H., Kim, Y.S., Lee, J.H., Cho, W.K. and Moh, S.H. (2025). Role of *Bean Yellow Mosaic Virus* P1 and HC-Pro in enhancing gene expression and suppressing RNA silencing in *Nicotiana benthamiana*. *Life*. 15: 472. h DOI: 10.3390/ life15030472

Devi, B.M., Guruprasath, S., Balu, P., Chattopadhyay, A., Thilagar, S.S., Dhanabalan, K.V., Choudhary, M., Moparthi, S. and Jailani, A.A.K. (2024). Dissecting diagnostic and management strategies for plant viral diseases: what next? *Agriculture, 14*(2): 284. DOI: 10.3390/agriculture14020284

El Gamal, A.Y., Atia, M.M., El Sayed, T., Abou-Zaid, M.I. and Tohamy, M.R. (2022). Antiviral activity of chitosan nanoparticles for controlling plant-infecting viruses (118): 1-9. DOI: 10.17159/sajs.2022/10693

Elsharkawy, M.M., Sidaros, S.A., Elkewey, S.A.E.R., ElAidy, N.A.E. and Elkhiat, G.M. (2021). Suppression of Bean Yellow Mosaic Virus by plant extracts in *Faba Bean* plants. *Organic and Medicinal Chem IJ.*, 11:(1), 555805. DOI: 10.19080/OMCIJ.2021.11.555805

El-Zahara, F., and El-Tony, H. (2021). Phylogenetic relationship among different varieties and species of Canna using molecular markers. *Scientific J. Flowers & Ornamental Plants*, 8: 165-180. DOI: 10.21608/sjfop.2021.163280

Fegla, G.I., El-Samra, I.A. Younes, H.A. And Abd El-Aziz, M.H. (2000). Optimization 0f Dot Immunobinding Assay (DIA) for detection Of *Tomato Mosaic* Virus (ToMV). *Journal of The Advances In Agric. Res*., 5:1495-1506.

Fegla, G.I., El-Samra, I.A., Younes, H.A. and Abd El-Aziz, M.H. (2001a). Comparative studies for detection of *Tomato mosaic tobamovirus* (TOMV), *Cucumber mosaic cucumovirus* (CMV) and *Potato Y potyviruses* (PVY). Adv. Agric. Res. 6: 239–254.

Fegla, G.I., El-Samra, I.A Younes, H.A. and Abd El-Aziz, M.H. (2001b). Plain and filter papers as solid carriers for detection of three plant viruses by dot and tissue blot immunoassays. *Adv. Agric. Res*. 6: 755 – 761.

Fegla, G.I., Younes, H.A., Abdelmonem, A.M. and Rasmi, M.R. (2003). Incidence of some seed-borne viruses affecting faba bean in Alexandria

Governorate. *Journal of the Advances in Agric. Res*., 8:461-472.

Fu, Y.H., Jiang, S.R., Zou, M.L., Xiao, J.J., Yang, L., Luo, C.F., Rao, P., Wang, W.Q., Ou, Z.G., Liu, F.Z. and Xia, Z.Q. (2022). High-quality reference genome sequences of two Cannaceae species provide insights into the evolution of Cannaceae. *Frontiers in Plant Science*, 13:955904. DOI: 10.3389/fpls.2022.955904

Gadhave, K.R., Gautam, S., Rasmussen, D.A. and Srinivasan, R. (2020). Aphid transmission of *Potyvirus*: the largest plant-infecting RNA virus genus. *Viruses*. 12(7):773. DOI: 10.3390/v12070773

Hamza, K.A., Abd El-Aziz, M.H., Behiry, S.I. And Younes, H.A. (2018). Isolation and purification of *potato virus y* isolate infecting potato (*Solanum tuberosum* l.) in al-nubaria region. *Middle East Journal of Agriculture Research*, 7(04): 1201-1207.

Hasan, S.A., Khadhum, M.K., Hanoon, M.B. and Abdullah, R.M. (2024). Genetic analysis of the phenotypic and molecular correlations among the RAPD-PCR markers in Peanut (*Arachis hypogaea* L.). *SABRAO Journal of Breeding & Genetics,* 56(3): DOI: 10.54910/sabrao2024.56.3.19

Jaber, H.A., Younis, H.S., Jiheel, W.R., Abdullah, R.M. and Hasan, S.A. (2025). Genetic evaluation study of *Fava bean Vicia faba* L. under the influence of the transfer and diagnosis of the Bean Yellow Mosaic Virus in several areas of kirkuk Governorate. *Natural and Engineering Sciences*. 10 (1): 151-161. DOI: 10.28978/nesciences.1642299

Kawanna, M.A.I. (2007). Viral diseases of faba bean in northern Egypt. Ph. Thesis. Fac. Agric. Alexandria University. 157 pp.

Khalil, M.S., Abd El-Aziz, M.H. and Selim, R.E. (2022). Physiological and morphological response of tomato plants to nano-chitosan used against bio-stress induced by root-knot nematode (*Meloidogyne incognita*) and *Tobacco mosaic tobamovirus* (TMV). *European Journal of Plant Pathology*, 163: 799-812. DOI: 10.1007/s10658-022-02516-8

Kheder, M.A., El-Kady, M., EL-Said, H.M., Atia, M.M.M. and Khattab, E.H. (2002). Production of specific antiserum for bean yellow mosaic virus and broad bean stain virus. Zagazig J. Argic., Res . 29: 1629-1648.

Kumari, A., Kaur, C., Kumar, S., Raj, S.K., Roy, R.K. and Nautiyal, C.S. (2015). First report of bean yellow mosaic virus causing a mosaic disease of Canna sp. in India. Plant Dis 99:897. https:// doi. org/ 10. 1094/PDIS- 01- 15- 0001- PDN

Kumari, A., Kumar, S., Chauhan, P.S. and Raj, S.K. (2022). Elimination of coexisting canna yellow mottle virus, bean yellow mosaic virus and cucumber mosaic virus from Canna generalis cv. black knight through in vitro chemotherapy of rhizome explants. New Disease Reports 12(10): DOI:10.1007/s13205-022-03330-z

Lecoq, H., Dafalla, G., Desbiez, C., Wipf-Scheibel, C., Delécolle, B., Lanina, T., Ullah, Z. and Grumet, R. (2001). Biological and molecular characterization of Moroccan *watermelon mosaic virus* and a potyvirus isolate from Eastern Sudan. *Plant Dis*. 85:547-552. DOI: 10.1094/PDIS.2001.85.5.547

Li, Y., Xia, F., Wang, Y., Yan, C., Jia, A. and Zhang, Y 2019). Characterization of a highly divergent *Sugarcane mosaic virus* from *Canna indica* L. by deep sequencing. *BMC Microbiol* 19, 260 .DOI: 10.1186/s12866-019-1636-y

Mitrofanova, I.V., Alexander, M., Zakub, A. and Mitrofanova, O. (2018). Viruses infecting main ornamental plants: An overview. *Ornamental Horticulture* 24(2):95-102. DOI: 10.14295/oh.v24i2.1199

Moghal, S.M. and Francki, R.I. (1981). Towards a system for the identification and classification of potyviruses. II. Virus particle length, symptomatology, and cytopathology of six distinct viruses. *Virology*. 112: 210-6. DOI: 10.1016/0042-6822(81)90626-7. PMID: 18635068

Moury, B. and Desbiez, C. (2020). Host range evolution of potyviruses: a global phylogenetic analysis. *Viruses*, 12(1): 11. DOI: 10.3390/v12010111

Moya-Ruiz, C.D., Gómez, P. and Juárez, M. (2023). Occurrence, Distribution, and Management of Aphid-Transmitted Viruses in Cucurbits in Spain. *Pathogens*, 12(3):422. DOI: 10.3390/pathogens12030422

Mrkvová, M., Kemenczeiová, J., Achs, A., Alaxin, P., Predajˇna, L., Šoltys, K., Šubr, Z. and Glasa, M. (2024). Molecular characteristics and biological properties of *Bean Yellow Mosaic Virus* isolates from Slovakia. *Horticulturae*. 10: 262. DOI: 10.3390/ horticulturae10030262

Nigam, D., Tourrette, K., Souza, P.F.N. and Garcia-Ruiz, H. (2019). Genome wide variation in potyviruses. *Frontiers in Plant Science*. 10:1439. DOI: 10.3389/fpls.2019.01439

Noordam, D. (1973). Identification of plant viruses, methods and experiments. center for Agricultural publishing and Documentation, Wageningen Netherlands, 207 pp. CABI Record Number: 19731308855

Omar, S.A.M. (2021). The importance of *Faba Bean* (*Vicia faba* L.) diseases in Egypt. In Mitigating Environmental Stresses for Agricultural Sustainability in Egypt; *Springer: Berlin, Germany*. pp. 371–388. DOI: 10.1007/978-3-030-64323-2_13

Ortiz, V., Navarro, E., Castro, S., Carazo, G., and Romero, J. (2006). Incidence and transmission of Faba bean necrotic yellows virus (FBNYV) in Spain. *Span. J. Agric. Res.* 4:255–260. DOI: 10.5424/sjar/2006043-200

Pandey, S. and Bhandari, M. (2021). Hidden potential of *Canna indica*-anamazing ornamental herb. J. Tech. Res. Sci, 112-118. (Special Issue). DOI: 10.30780/specialissue-ICAASET021/020

Sultana, N., Soma, M.A. and Rashid, M.A. (2022). Analgesic, hypoglycemic and antidiarrheal activities screening of *Canna indica* L. *Bangladesh Pharm J.* 25:137–142. DOI: 10.3329/bpj.v25i2.60965

Sultana, M.S., Azad, M.A.K., Shimizu, N., Rana, G.M.M. and Yeasmin, M.S. (2024). Efficacy of aqueous extracts from three plants in controlling cabbage aphid [*Brevicoryne brassicae* L. (Hemiptera: Aphididae)] on field-grown cabbage (*Brassica oleraceae* L.). *Technology in Horticulture* 4: e025 DIO: 10.48130/tihort-0024-0022

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38:3022-3027. DOI: 10.1093/molbev/msab120

Wijayasekara, D., Hoyt, P., Gimondo, A., Dunn, B., Thapa, A., Jones, H. and Verchot, J. (2018). Molecular characterization of two badnavirus genomes associated with Canna yellow mottle disease. Virus Res, 2(243):19-24. DOI: 10.1016/j.virusres.2017.10.001. Epub 2017 Oct 5. PMID: 28988983

Xie, L., and Hong, J. (2022). Transmission electron microscopic methods for plant virology. *Plant Virology: Methods and Protocols*, 297–317. DOI: 10.1007/978-1-0716-1835-6_28

Younes, H. A. A. (2003). Natural infection of luffa (*Luffa aegyptiaca*. Mill) with *Zucchini Yellow Mosaic Virus* in Egypt. *Journal of Advanced Agricultural Research*. 8: 227–239.

Younes, H.A., Aseel, D.G. and Abd El-Aziz, M.H. (2018). Identification and purification of *cowpea mosaic comovirus* isolated from infected cowpea (*vigna unguiculata* l.) In northern Egypt. *International Journal of Agriculture and Environmental Research* (IJAER). 4 (4): 206 – 212.

Younes, H.A., Abd El-Aziz, M.H. and Zeid, A.H. (2021). Detection of *Bean yellow mosaic potyvirus* in infected faba bean plants (*Vicia faba* L.) from Northern Egypt. *Archives of Phytopathology and Plant Protection*. 54(19-20) 1633-1648. DOI: 10.1080/03235408.2021.1932285

Zeid, A. H. (2016). Biological and molecular studies on some viruses affecting faba bean. Ph. Thesis. Fac. Agric. (Saba - Basha) Alexandria University. 136 pp.

Zsiláné-André, A., Vámos, A., Molnár, B., Szabolcs, A. and Holb, I.J. (2019). Effect of six pre-storage rhizome treatments on rhizome vitality and seasonal growth characteristics of three Canna × generalis cultivars. *Scientia Horticulturae* 254 (3): 155-162 155-162. DOI: 10.1016/j.scienta.2019.04.076