Polyclonal antibodies production against *Broad bean mottle virus* affecting faba bean (*Vicia faba* L.) plants in Egypt

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ABSTRACT:

An isolate of BBMV was obtained from naturally infected faba bean plants growing in the experimental fields of Giza Agricultural Research Station showing mottle and distortion of leaves. The identity of the virus was confirmed biologically, serologically and by light and electron microscopy. It was found that BBMV reacted systemically with 12 species belonging to *Fabaceae* and locally with 2 species belonging to *Chenopodiace*. It was transmitted mechanically and by *Sitona lineate*. It was detected serologically by indirect ELISA using BBMV specific antiserum. Light microscopy of epidermal strips of faba bean infected-leaves revealed amorphous cytoplasmic inclusions (X-bodies). The UV absorption spectrum of the purified virus had a maximum at 260 nm and a minimum at 245 nm. The ratios of Amax/Amin and A260/280 were 1.39 and 1.57, respectively. Yield of the purified BBMV preparation was about 2.5 mg/100g of infected tissue. Electron micrograph of purified virus preparation revealed isometric shaped particles of with mean diameter of 26 nm typical of Bromoviruses. Polyclonal antibodies raised against BBMV have maximum titer of 1:1024 from the first bleeding. IgG and IgG conjugate were 1.0 µg/ ml and 1:1000, respectively. The produced antiserum was evaluated by DBIA and TBIA using infected plant.

Key words: *Broad bean mottle virus*, Host range, Inclusion bodies, Transmission, Purification, Electron microscopy, Immunization, ELISA reagents, DBIA and TBIA.

INTRODUCTION:

Faba bean (*Vicia faba* L.) plants are important food legume in Egypt and other countries of the world. They contain high percentage of proteins, carbohydrates, vitamins and mineral salts which are essential for human nutrition (Hassan, 1996).

The production of faba bean is greatly reduced as a result of infection with virus diseases. The losses vary from one year to another

depending on location, host variety, the virus and the environmental factors (Mattews, 1991).

Faba bean plants are liable to infection by more than 30 viruses (Thornberry, 1966). The most common viruses are *Broad bean mottle virus* (BBMV), *Broad bean stain virus* (BBSV), *Broad bean true mosaic virus* (*BBTMV*), *Broad bean wilt virus* (BBWV), *Bean common mosaic virus* (BCMV), *Bean leaf roll virus* (BLRV), *Bean yellow mosaic virus* (BYMV), *Faba bean necrotic yellows virus* (FBNYV), *Pea seed borne mosaic virus* (PSbMV), *Pea enation mosaic virus* (PEMV), *Alfalfa mosaic virus* (AAMV) and *Chickpea chlorotic dwarf virus* (CCDV) (Salama,1998; Khattab, 2002, Fegla *et al*., 2003; Leur *et al*., 2003 and El-Hammady *et al*, 2004).

This study aimed to detect the most serious viral diseases on Legumes, to investigate the method of purification, production of antisera and application of serological assays using the prepared antiserum specific to the isolated virus.

MATERIAL AND METHODS

1. Source of samples:

The virus under study was isolated from naturally infected faba bean showing distincted viral sympotoms included mottle and distortion of leaves. The infected plants was obtained from Agricultural Research Experimental Station (ARES) Egypt, Giza.

2. Isolation and propagation of the isolated virus:

The infected faba bean samples were examined by ELISA test as described by Clark and Adams (1977) using 5 specific antisera against some faba bean viruses, BBMV, BBSV, BBTMV, BBWV and BCMV. Antisera were kindly provided by Dr.Safaa Kumari, Virology Lab; Germplasm Program. ICARDA, Aleppo, Syria. Samples representing distincted symptoms of BBMV isolate was reacted positively with only BBMV antiserum. The local lesion technique (Kuhn, 1964) was carried out for biological purification of BBMV using *Chenopodium amaranticolor* L. *as* local lesion host plant for BBMV isolate.The virus isolate was inoculated mechanically then propagated in faba bean cv.Giza 843 and kept in greenhouse for virus source.

3. Identification of the isolated virus:

a. Host range and symptomatology:

Forty three plant species and cultivars belonging to eight different families were mechanically inoculated by BBMV isolate. The inoculum was prepared by homogenizing infected leaves of *Vicia faba* cv. Giza 843 in 0.01 M phosphate buffer, pH 7.2. The inoculated plants were kept in the glasshouse at 20-30C° and examined daily for external symptoms.

b. Modes of transmission:

1. Insect transmission:

The green peach aphids (*Myzes persicae* Sultz), black bean aphid (*Aphis faba* Scop), cowpea aphid *(Aphis craccivora* Kich*)* and the beetles (*Sitona lineata*) were checked for the ability to transmit BBMV. This experiment was carried out by the method as described by Noordam (1973).

2. Seed transmission:

Seed transmission was carried out as described by Khattab (2002) using faba bean cv.Giza 843.

3. Serological diagnosis:

Infected and healthy faba bean plants were tested by indirect ELISA for the presence of BBMV using specific antiserum as mentioned before .

4. Light microscopy:

Cytochemical techniques were done using light microscopy for the determination of inclusion bodies in epidermal strips of infected faba bean leaves cv. Giza 716 at 15,21 and27 days of inoculations with BBMV. The leaves were treated with 5% Triton x-100 for 10 minutes to disrupt the plastids and facilitate the observation of the inclusions (Mazia *et al.*1953).

5. Purification of BBMV:

The virus was purified from faba bean cv Giza 843 as described by Alconero *et al*. (1986). UV – absorption spectra of the supernatants containing virus isolate were measured to evaluated the purity and virus concentration was estimated spectrophotomeatrically using an extinction coefficient of 5.4 with Spectronic 2000 Spectrophotomete.r

Electron microscopy:

The purified preparation of BBMV was negatively staining as described by Noordam (1973) and examined by transmission electron microscope of Zagazig Univ**.**

6. Polyclonal antibodies production:

a. Rabbit immunization:

Two New Zealand white rabbits, each one weighted 4kg were used for production of antiserum against the purified preparation of the virus (BBMV). A total of 10mg. purified virus was emulsified with an equal volume of Freund's incomplete adjuvant at 10 days intervals. Intramuscular injection was performed in the right and the left hand thighs, respectively using a sterile 20-22-gauge needle (Hampton *etal*,1990).

b. Rabbit bleeding:

Rabbits were bleed 10, 20 and 30 days after the last injection from the right ear. The blood was collected, left to coagulate at 37 ^{o}C in an incubator for 1-2 hr, and then kept at 4 ^{o}C overnight (Khattab, 2002).

c. Separation of antiserum:

Antiserum was separated through centrifugation at 4000 rpm for 20 min. The antiserum was

collected and stored at 4 ^{o}C (Hampton *etal*,1990).

d. Determination of antiserum titer:

Antiserum titer was measured with the indirect ELISA technique as described by Clark and Adams(1977). Clarified sap of virus infected leaves as well as healthy faba bean plants was diluted at 1/10, using phosphate buffer, pH7.2 contained 0.85% NaCL. The produced antiserum was diluted with the serum buffer (PBST+2% PVP + 0.2% Egg albumin powder, to 1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1:512, 1/1024 and 1/2048).

e. Purification of the immunogamma globulins (IgG):

Gamma globulins were purified using the caprylic acid method as described by Steinbuch and Audran (1969).

f. Preparation of IgG enzyme conjugate:

IgG was conjugated with alkaline phosphatase (AP) according to the method described by Clark and Adams (1977).

7. Determination of antigen dilution end point:

Clarified sap of faba bean leaves infected with BBMV was diluted with phosphate buffer, pH7.2 to 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280 and 1/2560 to determine the dilution end point of BBMV by using the produced ELISA reagents .

7. Immunological detection of BBMV by DBIA and TBIA:

Dot blot and tissue blot immuno binding assay (DBIA) and tissue blot immune binding assay (TBIA)on nitrocellulose membranes described by Line *et al* (1990) were used for BBMV detection.

RESULTS

1. Isolation and propagation of the isolated virus:

The isolated virus was identified on the bases of host range, symotomatology, modes of transmission, serological diagnosis, inclusion bodies and morphology of virus particles as described bellow:

2. Virus identification:

2-a. Host range and symptomatology :

Reaction of forty three plant species and cultivars belonging to eight different families to virus infection is shown in Table (1) and Figs. (1, 2, 3,4,5 and 6). It is obvious from Table (1) that species and cultivars tested can be classified into three general groups:

(i) Plants reacted only with local sympotoms: *Chenopdium amaranticolor*, *C. quinoa* and *C. album*

(ii) Plants reacted only with systemic sympotoms: *Phseolus vulgaris* cv.Proteco *Pisum sativum* cv. Master B, cv.Sugar sweet, cv. Lincolin, cv. Little marvel and *Vicia faba* cv. Sakha2, cv. Egypt2 ,cv. Giza843, cv. Giza716, cv. Giza2, cv. Egypt1

(iii) Plants that were inoculated, but from which BBMV could not be recovered either by back inoculation onto the indicator host plant or by ELISA test include the following:

Glycine max L, *Lupinus termis forsk* ,*Medicago sativa* L., *Arachis hypogaea* L ,*Arachis hypogaea* L., *Trifolium alexadrinum* L,Gompherina gloposa L., *Zinnia elegans, Zea mays* L, *Hordeum vulgare,Triticum aestivum* ,*Cucurbita maxima* L.,*Cucumis sativus* L., *Cucurbita pepo*, *Cucumis melo*, *Datura metal* L., *D.stramonium*L., *C.mural Beta*, *vulgaris*, *Spinacia oleraceae L*., *Zinnia elegans*, *Allium cepae* and *Allium sativum* .

Host plant tested	Reaction to inoculation with BBMV	
	symptoms	ELISA reading
1. Family: *Fabacea*		
Glycine max L.	0	0.350-
Lupinus termis forsk	0	0.255-
Medicago sativa L.	0	0. 021-
Phseolus vulgaris cv.Proteco	SIY	0.960+
Pisum sativum cv. Master B	SIY	0.566+
cv.Little marvel	SIY-DE	0.655+
cv.Lincolin	SIY-DE	0.690+
cv.Sugar sweet	SIY-DE	0.695+
Lens esculenta	0	0.231-
Vicia faba cv. sakha2	SBM-DE	0.998+
cv. Giza843	SVC	0.999+
cv. Giza716	SBM-DE	0.995+
cv. Giza2	SBM	0.970+
cv. Egypt1	SBM-DE	0.997+
cv. Egypt2	SBM	0.960+
Vigna sinensis Savi cv.Blackeye	M	0.743+
Arachis hypogaea L.	0	0.404-
Trifolium alexadrinumL	0	0.102-
2.Fam.: *Amaranthaceae*		
Gompherina gloposa L.	0	0.099-
3.Fam.: *Graminaceae*		
Zea mays L.	0	0.099-
Hordeum vulgare	0	0.102-
Triticum aestivum	0	0.107-
4.Fam.:Cucurbitaceae		
Cucurbita maxima L.	0	0.100-
Cucumis sativus L.	0	0.130-

Cucurbita pepo	0	0.122-
Cucumis melo	0	0.224-
Table 1. Continued		
Host plant tested	Reaction to inoculation with BBMV	
	symptoms	ELISA Reading
5. Fam.: *Solanaceae*		
Datura metal L.	0	0.356-
*D.stramonium*L.	0	0.332-
Nicotiana glutinosa L.	0	0.403-
N. rustica L.	0	0.443-
N. clevelendii	0	0.212-
N.tabaccum cv. White burely	0	0.400-
Lycopersicon esculentum Mill	0	0.440-
Petunia hybrida Villm	0	0.322-
6.Fam.:*Chenopodiaceae*		
Chenopdium amaranticolor	CLL	0.899+
C. quinoa L.	NLL	0.985+
C. album	NLL	0.512+
C.mural	0	0.333-
Beta vulgaris	0	0.445-
Spinacia oleraceae L.	0	0.433 –
7.Family:*Compositae*		
Zinnia elegans	0	0.130-
8.Fam.:*Alliaceae*		
Allium cepae	0	0.137-
Allium sativum	0	0.136-

ELISA reactions were considered positive when the A405 values were greater than twice of healthy control.

Table (1). Host range and symptomatology of BBMV

A **B**

Fig.(1): Blotchy mottle on *Vicia faba* CV.Giza617

A **B**

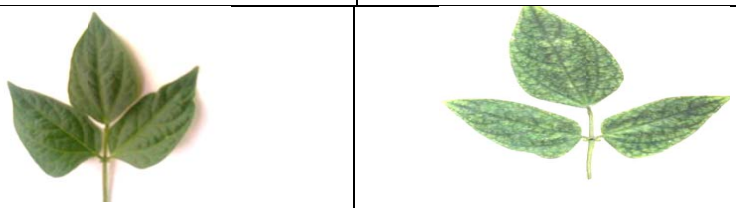
Fig.(2): Vein clearing on *Vicia faba* cv. Giza 843

A **B**

Fig.(3): Interveinal yellowing and leaflets deformation on *Phaseolus vulgaris* cv.Proteco

A **B**

Fig.(4): Chlorotic local lesion on *Chenopodium amaranticolor*

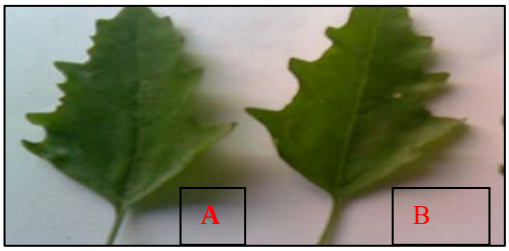


A **B**

Fig .(5): Mottling on *Vigna sinensis* cv. Kaha1

0=N0 reaction
SIM=Systemic Interveinl Mottle
- = Negative Reaction
M=Mottling
SIY=Systemic Interveinal Yellowing
NLL=Necrotic Local Lesion
SVC=SystemicVein Chlorosis

CLL= Chlorotic Local Lesion
DE= Deformation
+ = Positive
SBM= SystemicBlotch Mottle
* Healthy **c**ontrol of direct ELISA absorbance at 405nm = 0.244
M= Mottle



F**ig.(6):** Necrotic local lesion on *Chenopodium album* A:Healthy leaf, B: Infected leaf

2-b. Modes of transmission:

1. Insect transmission:

The three aphids, *Myzes persicae* Sultz, *Aphis faba* Scop, *Aphis craccivora* Kich and Sitona *lineate* were checked for the ability to transmit the BBMV isolate. The results obtained (Table 2) showed that the BBMV was transmitted only by *Sitona lineata* with 7%. Infection was confirmed by back inoculation to *C. amaranticolor*.

2. Seed transmission:

Results showed that, BBMV was not transmitted through seeds of faba bean cvs. Giza 843, Giza 716 and Egypt 1.

3. Serological diagnosis:

Positive reaction was obtained only between sap of infected leaves of faba and BBMV specific antiserum using indirect ELISA technique.

4. Light microscopy:

Amorphous cytoplasm inclusions induced by BBMV were observed with light microscop in infected epidermal stripes of *Vicia faba* leaves Giza 716 at 15, 21 and 27 days after inoculation (Figs 8,9 and 10) but have never been observed in epidermal stripe of healthy leaves (Figs. 7).

5-Purification of BBMV:

Following the purification method used one zone was found in the sucrose density-gradient columns, this zone was found infectious when tested on the indicator host plant. Concerning the UV absorption spectra of the purified virus preparation, Fig. (11) revealed typical nucleoproteins with a minimum at 245nm and a maximum at 260 nm. The ratios of A260/A280 and A max/A min were 1.57 and 1.39, respectively (not corrected for light scattering). The yield of the purified virus was about 2.5mg/100g infected faba bean leaves.

Electron microscopy:

Examination of purified virus preparation revealed the presence of isometric virus particles with mean diameter of 26 nm (Fig.12).

6. ELISA reagent production:

a. Determination of antiserum titer:

The polyclonal antibodies were obtained from rabbit bleedings taken 10, 20 and 30 days after the last injection, the titer values of antisera against BBMV were 1:1024, 1:256 and 1:128 for the first, second, and third bleeding, respectively (Table 3). Antiserum titer against BBMV

obtained from the first bleeding (1: 1024) was considered the best one and was used in the subsequent experiments.

b. Purification of imuno gam globulins:

Immunogamglobulin against BBMV was purified from the antisera and its concentration was adjusted to 1mg/ml (A280nm=1.4).

c. Concentration of IGg and IGg conjugate :

Results in Table (4) indicated that concetrations of IGg and IGg conjugated with AP were 1.0 µg/ ml and 1/1000, respectively could be used for antigen detection in faba bean extracts.

Table (2) Transmission of BBMV by four different insect species

Insects	No. of inf.pl. No. of inoc.pl.	%infection
Aphis faba	0/40	0
Mysus persicae	0 /40	0
Aphis crassivora	0/40	0
Sitona lineata	3/40	7

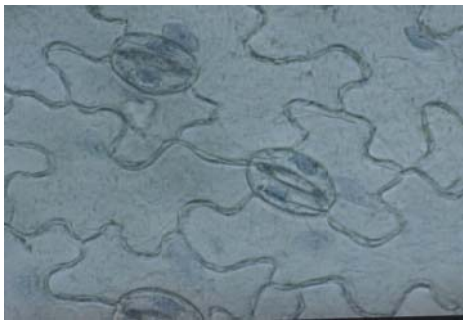


Fig.(7)

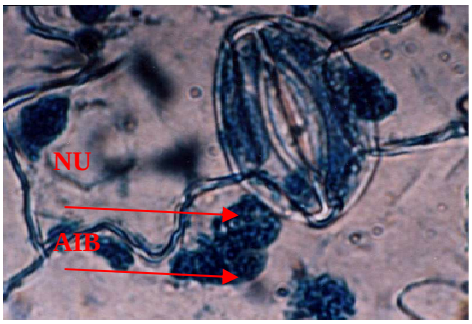


Fig.(8)

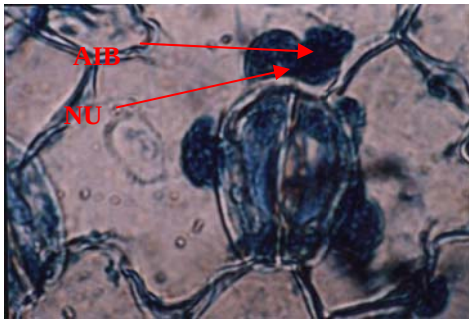


Fig.9

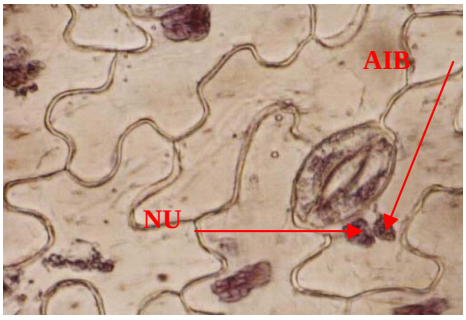


Fig.10

Fig.(7): Light microscopy of epidermal stripes of healthy leaf of *Vicia faba* cv. Giza 716 Magnification(x-200) NU:Nucleus

Figs. (8, 9and 10): Light microscopy of epidermal stripe of *Vicia faba* cv. Giza 716 leaf infected with BBMV, 15, 21 and 27 days, respectively post–inoculation showing amorphous inclusion (X-400). NU: Nucleus, AIB: Amorphous inclusion bodies

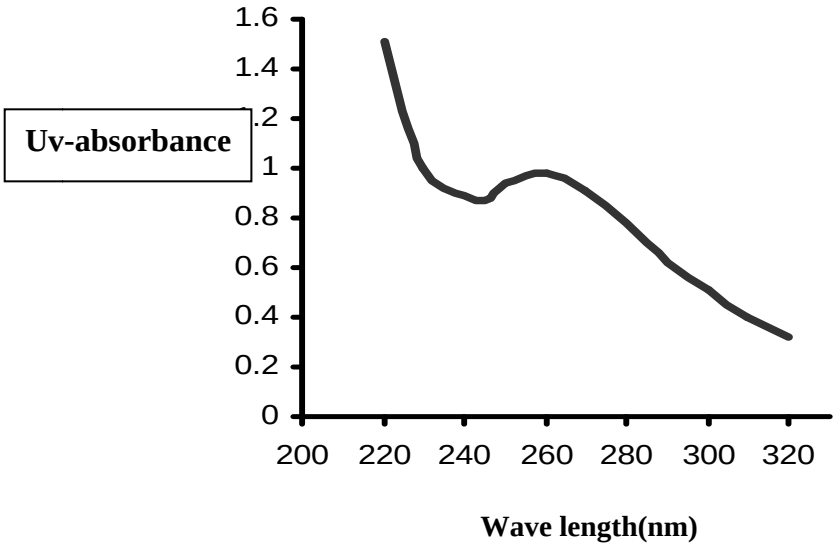


Fig.(11): UV-absorption spectrum of purified preparation of BBMV.

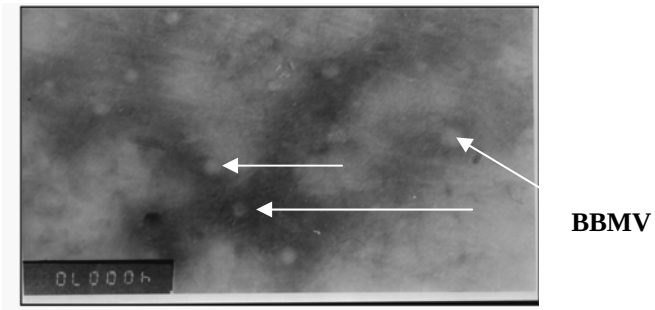


Fig.(12): Electron micrograph of purified BBMV preparation negatively stained with 2%phosphotungestic acid showing isometric virus particles.(X-40000).

d: Determination of antigen dilution end point:

Data presented in **Table (5)** showed that BBMV can be readily detected by direct ELISA in infected plants up to 1/640 for PSbMV when IGg and IGg conjugate produced were used.

7: Immunological detection of BBMV TBIA and DBIA on nitrocellulose membranes:

BBMV was readily detected immunologically using DBIA and TBIA (Fig 13). Positive reaction was indicated by development purplish color; whereas in negative reaction tissues from healthy plants remained green.

Table(3). Titer determination of antiserum against BBMV collected at 10 day intervals after rabbit immunization using indirect ELISA.

Dilution	10 days		20 days		30 days	
	Absorbance at 405nm for					
	I	H	I	H	I	H
1/1	2.99	1.051	1.704	0.245	**0.689-=b**	0.200
1/2	2.734	0.856	1.450	0.200	0.546	0.195
1/4	2.730	0.744	0.773	0.182	0.347	0.180
1/8	2.204	0.691	0.770	0.178	0. 338	0. 175
1/16	2.201	0.690	0.721	0.162	0.334	0. 160
1/32	1.997	0.633	0. 621	0.158	0.326	0. 155
1/64	1.957	0.686	0. 5340	0.155	0. 224	0.110
1/128	1.713	0.693	0. 330	0. 154	**0. 223**	**0.110**
1/256	1.595	0.609	**0. 320**	**0.149**	0.215	0.109
1/512	1.411	0.531	0. 248	0.139	0.210	0. 136
1/1024	**1.036**	**0.360**	0.245	0.135	0.200	0. 130
1/2048	0.690	0.358	0.230	0.130	0.190	0.125

Table (4): Schematic diagram of checker for determination of approximate working dilution of IgG and IGg conjugate to BBMV for ELISA test.

Con. of IgG µg/ml	Dilution of IgG conjugate							
	1:250		1:500		1:1000		1:2000	
	Absorbance at 405nm for							
	I	H		H	I	H	I	H
4.0	0.999	0.315	0.870	0.250	0.787	0.220	0.399	0.200
	0.979	0.300	0.872	0.240	0.773	0.225	0.350	0.199
2.0	0.870	0.203	0.785	0.198	0.690	0.200	0.301	0.180
	0.879	0.201	0.781	0.193	0.679	0.199	0.298	0.185
1.0	0.625	0.198	0.510	0.180	0.431	0.170	0.298	0.150
	0.615	0.192	0.520	0.183	0.390	0.160	0.289	0.151
0.5	0.404	0.203	0.301	0.182	0.109	0.063	0.101	0.055
	0.420	0.222	0.300	0.194	0.105	0.055	0.100	0.053

Table(5). Determination of BBMV(antigen) dilution end point.

Antigen dilution	ELISA reading at 405nm for	
	I	H
1/5	2.567+	1.015
1/10	1.993+	0.9 96
1/20	1.922+	0.890
1/40	1.712 +	0.730
1/80	0.950 +	0.4 01
1/160	0.890 +	0.400
1/320	**0.785 +**	**0.3 80**
1/640	0.640 -	0.370
1/1280	0.506 -	0.260
1/2560	0.475 -	0.240

- Reaging after one hour ,incubation with the substrate.
- **I= Infected plant H= Healthy plant**

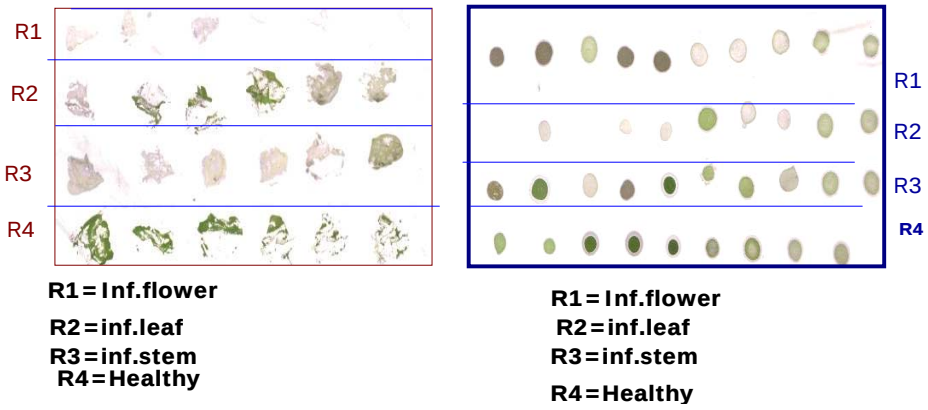


Fig.(13): Detection of BBMV by TBIA(left) and DBIA (right).

DISCUSSION

Faba bean (*Vicia faba* L.) is considered one of the most economically important leguminous crops cultivated in different regions not only in Egypt but also in other countries of the world. It suffers from the infection by several viruses such as *Broad bean true mosaic virus* (EL-Banna, 1979) and *Broad bean mottle Bromovirus* (Makkouk *et al*., 1988, EL-Afifi and EL-Dougdoug, 1997 and Efaisha, 2005).

In the present work, BBMV was isolated from naturally infected faba bean plants collected from Giza Governorate.

The isolated virus was identified on the bases of symptomatology, host range, modes of transmission, inclusion bodies, serological reaction, and particle morphology.

*Chenopodium amaranticolor*L., *Chenopodium quino* and *Chenopodium album* produced chlorotic or necrotic local lesions when rub inoculated whereas it produced systemic symptoms on the inoculated, plants of *Vicia faba* L. , *Pisum sativum* L. cultivars tested, *Phasolus vulgaris cv.* .Proteco and *Vigna sinensis* cv. Blackeye . These results are to be in the line with those of other workers (Makkouk *et al* 1988, EL-Afifi and EL-Douddouge, 1997 and Efaisha, 2005).

The environmental conditions under which plants grown before inoculation, and during the development of disease can have profound effect on the course of infection. A

plant that is highly susceptible to a given virus under one set of conditions may be completely resistance under another (Matthews, 1991).

Virus reservoirs and alternative hosts play an important role in virus spread and epidemiology. BBMV was easily transmitted mechanically by infectious sap. This explains the widespread of this virus. These results are in full agreement with those reported by Thornberry (1966), Chang *et al*.(1989), EL-Afifi and EL-Dougdoug(1997) and Efaisha(2005).

Three aphid species were checked for their ability to transmit the isolated virus. The three aphid species *Aphis faba, Myzus persicae* and *Aphis crassivora* were failed to transmit BBMV but the virus was transmitted by the beetle (*Sitona lineata*) at the rate of 6-7%. These results are in agreement with those of Sequeria and Borges(1989); Makkouk and Kumari(1995) and Efaisha(2005). While EL-Afifi and EL-Dougdoug (1997) found that BBMV was transmitted by *A. faba*. In this work BBMV was not transmitted through faba bean seeds. The same result was obtained by Sequeira and Borges (1989) and Brunt *etal* .(1996) but was transmitted in accordance with results of Makkouk *et al*. (1988) and Fortass & Bos (1992),they mentioned that BBMV was transmitted through faba bean seeds at the rate of 1.37% and 1.2.%respectively.Variations in viral seed transmission rates would be expected due to the results coming from interactions involving host genotype, age of infection, viral isolate characteristics, plant vigor, nutrition, ambient, temperature and possible virus-pathotype interactions (Hampton, 1982 and Kohnen *et al*., 1995)

Light microscopy is still important in the study of cytological abnormalities as much grater areas of tissue can be scanned for presence or absence of inclusion bodies (Matthews, 1991). In the present work amorphous cytoplasmic inclusions induced by BBMV was observed in infected epidermal strip of faba bean leaf15, 21and 27 days after inoculation.They persisted throughout the priod of observation (30 day), but incidence was highest from 7-21 days after inoculation, with a peak of 15 days. These results are in conformity with those obtained by Edwardson *et al*. (1984); Makkouk *et al.* 1988; Chang *et al*. (1989) and Calder and Ingerfeld (1990). Cytopathological effect of virus infection illustrated by Franck *et al*. (1985) showed that many viruses have no detectable cytological effects on nuclei; others give rise to intranuclear inclusions of various sorts and might affect the nucleus, even though they appear not to replicate in this organelle.

The X body is usually present in early stage of infection but may be absent in older infection (Matthews 2002).When plant pathologists have become involved in immunology the goal generally is to generate an antibody probe which will significantly identify a target antigen in the assay(Van Regenmortal, 1982). One of the most important objectives of this work is to produce ELISA reagents specific to BBMV.

One virus zone was collected from sucrose gradient columns. Ultraviolet spectrum of purified virus preparation showed that the minimum and the maximum absorbance were 245 and 260 nm, respectively. The absorbance ratios of A max/Amin and A260/A280 were 1.39 and 1.57, respectively. The yield of purified BBMV preparations was 2.5 mg/100g of infected faba bean leaves. The same results were obtained by Makkouk *et al*. (1987); Sequeria and Borges (1989) and Efaisha (2005).

Data concerning electron microscopy of purified BBMV preparation revealed that virus particles are isometric with diameter of 26 nm. This results agreed with those described by Gibbs (1972); Makkouk *et al*.

(1988); Brunt *et al*. (1996) and EL-Afifi and EL–Dougdoug (1997) they mentioned that the virus is a member of the *Bromoviridae* which are characterized with this shape and diameter.

Good results depend very much on optimizing the extraction method and the staining procedure for the particular virus and host plant (Christie *etal.* 1987).

In the present work, polyclonal antibodies raised against BBMV were prepared. The rabbits were bled three times 10, 20 and 30 days after the last injection. The titer of antiserum against BBMV were1:1024, 1:256 and 1:128 for the first, second and the third bleeding, respectively as determined by indirect ELISA technique. However, scince the caprylic acid method is simpler and less time consuming (Steinbuch and Audran, 1969, Khattab, 2002 and El-Kady *et al* ,2012), it was adopted for the isolation of BBMV immunoglobulins.

The concentration of IgG and IgG conjugate with alkaline phosphatase were 1.0 µg/ml and 1:1000 for BBMV using direct ELISA technique. This agrees with the results obtained by Khattab (2002) and El - kady (2004,2007 and 2012).. Results from direct ELISA test clearly showed that the IgG and IgG conjugate can be readily applied for virus detection in infected faba bean plant extracts. The obtained data indicated that the dilution end point of BBMV was 1:640 determined by DA-ELISA using IgG and IgG conjugate producted against BBMV. Khattab (2002) found that IgG and IgG conjugate can be readily applied for virus detection in infected faba bean extracts at dilution 1:1600 for BYMV and 1:800 for BBSV by DAS-ELISA test. Also El-Kady *et al*. (2012) found that IgG and IgG conjugate can be readily applied for *Pepper mottle virus* detection at dilution up to 1:800 in pepper extracts.

Since the production of ELISA reagents specific to BBMV one of the main objectives of this work to cover the continuous increasing needs for virus detection and for production of virus free materials, they has been readily used for many aspects in plant virus research. ELISA method is very economical in the use of reactants adapts to quantitative measurements. It can be applied to viruses of various morphological types in both purified preparations and crude extracts. It is very sensitive, detecting concentrations as low as 1-10mg/ml (Van Regenemortel,1982, Clark & Bar-Joseph, 1984 and Matthews 1991). One of the aims of the present work was the application of the prepared antiserum for detection of the virus under study in faba bean plants. In these respect techniques of tissue blotting and dot blot on nitrocellulose membranes were applied to detect BBMV in infected faba bean plants by using antiserum produced. In the tissue blotting technique, the specific antigens are immunologically localized with enzyme labeled antibodies on nitrocellose membranes. Although this method is similar in principal to dot blot immunoassay of antigens on various membrane supports, the tissue blotting technique does not require mechanical disruption of tissues for the extraction of antigen or reqired different buffers for each antigen. Both techniques have been found to have much higher sensitivity for the detection of BBMV. These results in agreement with those recorded by Dijkstra and De - jager (1998), Efaisha (2005) and EL - Kady(2012).

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