Purification, serology and prevalence of *Broad bean stain* Comovirus (BBSV) and *Cowpea aphid borne mosaic Potyvirus* (CABMV).

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Abstract

BBSV and CABMV were purified from faba bean and cowpea plants, respectively using two different methods. The UV absorption spectrum had maxima and minima at 260 and245 nm, respectively for the two viruses. The absorbance ratios of $A_{max/min}$ and $A_{260/280}$ of the combined lower bands of purified BBSV preparations were 1.19 and 1.62, respectively. Infected faba bean plants yielded up to0.48 mg/100g tissue. The corresponding data for CABMV were 1.20&1.22 and its yield was 3.52 mg/100g of cowpea leaves. The maximum antiserum titer against either BBSV or CABMV was 1:512. The dilution end point of BBSV in faba bean sap was 1:320 using indirect ELISA, whereas it was 1:280 for CABMV. Both of BBSV and CABMV were detected in stems and all flower parts (sepals, petals, pistils and anthers) in intact seeds and all seed parts (seed coats, cotyledons and embryos) of immature seeds obtained from infected faba been and cowpea plants. Both of the viruses were not detected in seed coat of ripened seeds and in roots of infected plants. BBSV was detected in stained seeds more than un-stained seeds obtained from infected faba bean plants.

Keywords: *Broad bean stain Comovirus* (BBSV) and *Cowpea aphid borne mosaic* (CABMV), antiserum production, indirect-ELISA, Dot-ELISA

Introduction

Faba bean (*Vica faba* L.) and cowpea (*Vigna unguiculata* L.) are important food legumes 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).**

In 2004 about 240854 feddans were cultivated with faba bean which produced about 330451.6 tons whereas, the area cultivated with cowpea was about 18000 feddans which produced about 36000 tons (statistical and Agricultural Economic Institute, **Ministry of Agriculture, 2004).**

The production of either faba bean or cowpea is greatly reduced by virus diseases. The losses of yield were varied from season to another depending on kind of viruses, host cultivar, location and environmental factors **(Frison, 1988; Fortass and Bos, 1991 and Bashir *et al.*, 2002)**

BBSVand CABMV cause severe diseases wherever susceptible crops are grown, because both viruses are transmitted through seeds **(Bashir and Hampton, 1996 and Khatab,2002).** The virus transmitted by seed provides the initial inoculums and insects are responsible for the secondary spread of the diseases under field conditions **(Yossef, 1999 and Bashir *et al.*, 2002).**

The present work was designed to study

1- Purification of BBSV and CABMV
2- Production of their specific antisera.
3- Detection of BBSV and CABMV by serological methods.
4- Prevalence of the virus in different plant organs.

Materials and Methods

1. Serological studies:

1.1. Virus purification:

1.1.a. Purification of BBSV:

BBSV was purified according to the method described by **Azzam and Makkouk (1986)** with some modifications, 100 grams of infected faba bean cv. Improved Giza 3 leaves showing distinct symptoms, 4 weeks after virus inoculation were harvested and pulverized in liquid nitrogen. The pulverized tissues were homogenized with a blender in 300 ml of 0.5 M potassium phosphate buffer, containing 0.5 M ethylene diamine tetra acetic acid (EDTA), 0.5% sodium sulfite (Na_2SO_3) and 0.01 M diethyldithiocarbamate (DIECA), pH 8.5. The homogenate was filtered through two layers of cheesecloth and subjected to low-speed centrifugation (LSC)8000 rpm for 10 min . at 4°C in a Beckman J-21 C centrifuge using JA-20 rotor. The supernatant obtained was stirred with 25% chloroform plus 25% carbon tetra chloride (CCl4) for 30 min., and the aqueous phase was separated by (LSC). The virus was

precipitated from the supernatant with 6% polyethylene glycol (PEG 6000) and 0.3 M sodium chloride (NaCl) by stirring overnight at 4°C. The pellets were collected by 20 min. centrifuged at 10.000 rpm at 4 °C and suspended in 70 ml of 0.1 M phosphate buffer, pH 7.5, containing 0.1% 2-mercaptoethanol (2-ME) and left overnight at 4°C with slowly stirring, then centrifuged for 10 min. at 8000 rpm at 4°C to eliminate any non-soluble materials followed by centrifugation for 90 min. at 30.000 rpm at 4°C in a Beckman L8-80 M Ultracentrifuge using rotor 80 Ti. The pellets were resuspended in 0.01 M potassium phosphate buffer, pH 7.5, layered onto of 10-40% sucrose gradient prepared in 0.01 M potassium phosphate buffer, pH 7.5, and then centrifuged for 2 hours at 28.000 rpm in Beckman SW60 rotor at 4°C. Gradient columns were stored overnight at 8°C prior to use. The virus zones were collected with a bent tip hypodermal needle and syringe, diluted 1:1 with 0.001 M potassium phosphate buffer, pH 7.5, without additives, then concentrated by centrifugation for 90 min. at 36.000 rpm .Final pellets were suspended in 1 ml of 0.001 M potassium phosphate buffer, stirring overnight. After clarification, the UV- absorption spectrum of the supernatants containing the purified virus isolate was estimated spectrophotometrically using an extinction coefficient of 8.2 with a spectronic 200 spectrophotometer. Infectivity was tested on leaves of *C. quinoa*. The virus was lyoph - lized and stored at – 30 °C until used. CABMV was purified according to the method given by **Lima *et al.* (1979)** with some modifications. One hundred grams of infected cowpea cv. Fetriaat leaves showing typical mosaic symptoms were collected ,18 days after mechanical inoculation by CABMV and homogenized in a blender with 0.5 M potassium phosphate buffer, pH 7.2, containing 0.5%. Na_2SO_3, 0.5 M urea, 0.5% 2-mercaptoethanol, and 0.05 M EDTA (2ml buffer/g tissues). The homogenized extract was strained through two layers of cheesecloth. Crude extract was emulsified intensively for 5 min. with chloroform (2:1, v/v) and n-butanol (8%, v/v) and stirred (30 min/4°C.) followed by low speed centrifugation (LSC) 8000 rpm for 10 minutes at 4°C in a Beckman J-21C centrifuge using JA-20 rotor. Virus from clarified sap was precipitated from the supernatant with 6% PEG and 2% NaCl followed by stirring for 90 min. at 4 °C. The precipitated virus was collected by centrifugation at 8000 rpm for 20 min. The pellet was emulsified with 0.02 M potassium phosphate buffer containing 0.1% 2-mercaptoethanol, 1.0 M urea and 0.05 M EDTA and stirring at 4 °C overnight followed by LSC at

8000 rpm for 10 min. The upper phase was subjected to one cycle of differential centrifugation (high speed at 35,000 rpm in Beckman L8-80M Ultracentrifuge using rotor 80 Ti. and low speed at 8000 rpm for 10 min.). Two ml of the resultant virus suspensions were layered onto 10-40 % sucrose gradient and centrifuged 2.5 hr at 28000 rpm at 4 °C in Beckman SW 60 rotor. Gradient columns were stored overnight at 8 °C prior to use. The virus zones were collected with a bent tip hypodermal needle and syringe, diluted 1:1 with 0.02 M potassium phosphate buffer, pH 8.2 then concentrated by centrifugation for 90 min. at 35000 rpm. Final pellet was resuspended in 0.02 M Tris-HCL buffer, pH 8.2 and stirring overnight. After clarification, the UV- absorption spectrum of the supernatants containing the purified virus isolate was estimated spectrophotometrically using an extinction coefficient of 2.4 with a spectronic 200 spectrophotometer .Infectivity was tested on leaves of *C. quinoa*. The virus was lyophilized and stored at – 30 until used.

Electron microscopy

A drop of purified virus preparation was placed on Formavar-coated grids (300 mesh). Grids were then dried with filter paper and air, then stained with 2% unranyl acetate for 2 min.. Excess of stain was removed with filter paper. After air drying , grids were examined with Ajoal 100S x 11 electron microscope, in the Electron Microscope Unit, Faculty of Science, Zagazig University, Zagazig.

1.2. Antiserum production of BBSV and CABMV

1.2.a. Rabbit immunization:

Four New Zealand white rabbits about 4.5 k.g each were used for antiserum production (2 rabbits for each virus isolate). A total of 7 mg purified CABMV and 4 mg purified BBSV were used for injections **(Table 1).** For intramuscular and subcutaneous injections purified virus was emulsified with an equal volume of Freund's incomplete adjuvant. Intramuscular injection was performed in the right and the left hand thighs, respectively using a 5 ml disposable syringe and the virus was diluted with phosphate buffer, pH 7.5.

Table (1): The rabbit immunization scheme used for antiserum production against BBSV and CABMV.

Injection No.	Route of injection	Dosage of injection	
		Purified BBSV	Purified CABMV
1	Subcutaneous	0.5	0.5
2	Intramuscular	0.5	0.5
3	Subcutaneous	0.5	1
4	Intramuscular	0.5	1
5	Subcutaneous	1	2
6	Intramuscular	1	2
Total		4	7

1.2.b. Rabbit bleeding:

Rabbits were bled 10, 20 and 30 days after the last injection from the right ear. The blood was left to coagulate for 2-3 hrs at 37°C then kept at 4 °C overnight. Antiserum was separated through centrifugation at 4000 rpm/15 min. The obtained antiserum stored at -20 °C until used for titer determination and other serological studies.

1.3. Determination of antiserum titer:

Antiserum titer was measured with the indirect ELISA technique as described by Clark and Adams(1977) .Clarified sap of infected and control leaves were diluted at 1/5, 1/10, using phosphate buffer, pH 7.2, contained 0.85% Nacl.

On the other hand, antisera prepared against either BBSV or CABMV were diluted with the serum buffer, 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. The reaction was done between infected clarified extract and its induced antiserum by indirect ELISA test.

1.4. Determination of antigen dilution end point:

Specific antisera for BBSV and CABMV were used for determination of antigen dilution end point of BBSV and CABMV, respectively. using indirect ELISA technique.

Clarified sap of faba bean leaves infected with BBSV and cowpea leaves infected with CABMV were diluted with phosphate buffer pH 7.2 to 1/5, 1/10,1/20, 1/40, 1/80, 1/160, 1/320, 1/460, 1/1280 and 1/2560.

1.5. Immunological detection of BBSV and CABMV by:

Dot blot immunoassay (DBIA) and tissue blot immunoassay (TBIA) which were described by Lin *et al.* (1990) were used for

detection of both BBSV and CABMV

2. Prevalence of the virus in different plant organs:

2.a. Prevalence of the virus in root, stem and flowers:

Flowers, stems and roots from faba bean cultivar Improved Giza 3 infected with BBSV and cowpea cultivar Cream No.7 infected with CABMV, were collected and the flowers were aspectically separated to their morphological organs, viz. sepals, petals ,anthers, and pistils. Each organ was homogenized in phosphate buffer saline in polyethylene small bags. Each extract was used as antigen using Indirect ELISA technique.

2.b. Prevalence of the virus in seeds and seed parts

For detection of seed-borne viruses, seeds were obtained from pods harvested at two stages: green stage and over ripened (dry) stage from CABMV- infected cowpea plants, whereas three stages: upper, medium and lower pods were taken from BBSV-infected faba bean plants. Seeds from pods of each stage were divided into two replicates (15 seeds for each one) and were taken at random selection. The seeds aspectically dissected into three parts: seed coats, cotyledons and embryos.

The different parts of seeds were washed several times by rinsing in distilled water for 15 min. The dissected seed portions were homogenized separately in phosphate buffer saline (PBS) by pressure-crushing in polyethylene bags. The bags were then shaken for one hour at room temperature. Each extract was used as antigen using indirect ELISA technique.

Results and Discussion

1. Serological studies:

1.1. Virus purification:

BBSV and CABMV were purified with a high degree of purity following the purification procedures used. After sucrose density gradient centrifugation one very faint upper and two distinct lower bands2.3 and 3.5 cm. respectively below the meniscus of sucrose density-gradient columns. BBSV particles were detected. Concerning CABMV, one band was detected 2cm below the meniscus of the tubes .The UV absorption spectrum had maxima and minima at 260and 245 nm respectively for the two viruses. The absorbance ratios of $A_{max/.min}$ and $A_{260/280}$ of purified BBSV preparations were 1.19 and 1.62.refpectively.BBSV infected faba bean plants yielded up to 0.48 mg/ 100g tissue. The corresponding Figures(1,2) for CABMV, absorbance ratios were 1.20&1.22.respectively and infected cowpea plants yielded 3.25mg/100g tissue .Similar results were reported by **Gibbs *et al* .(1968), Makkouk *et al.* (1987)** and **Khatab (2002)**for BBSV and **Awad (1988)** for CABMV.

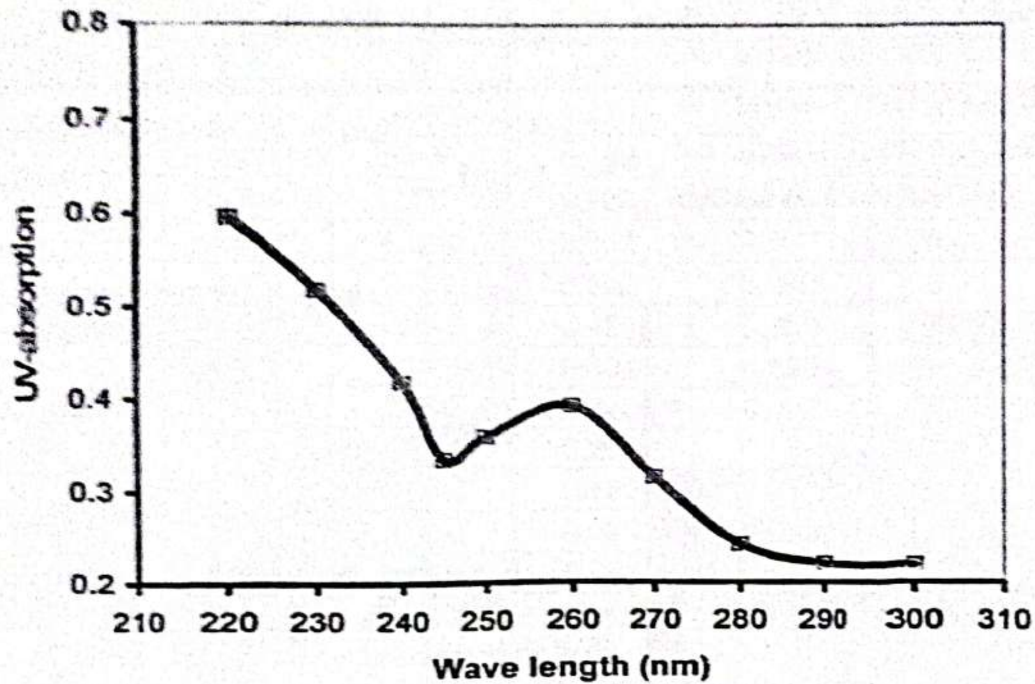

Fig. (1): Ultraviolet absorption spectrum of purified BBSV.

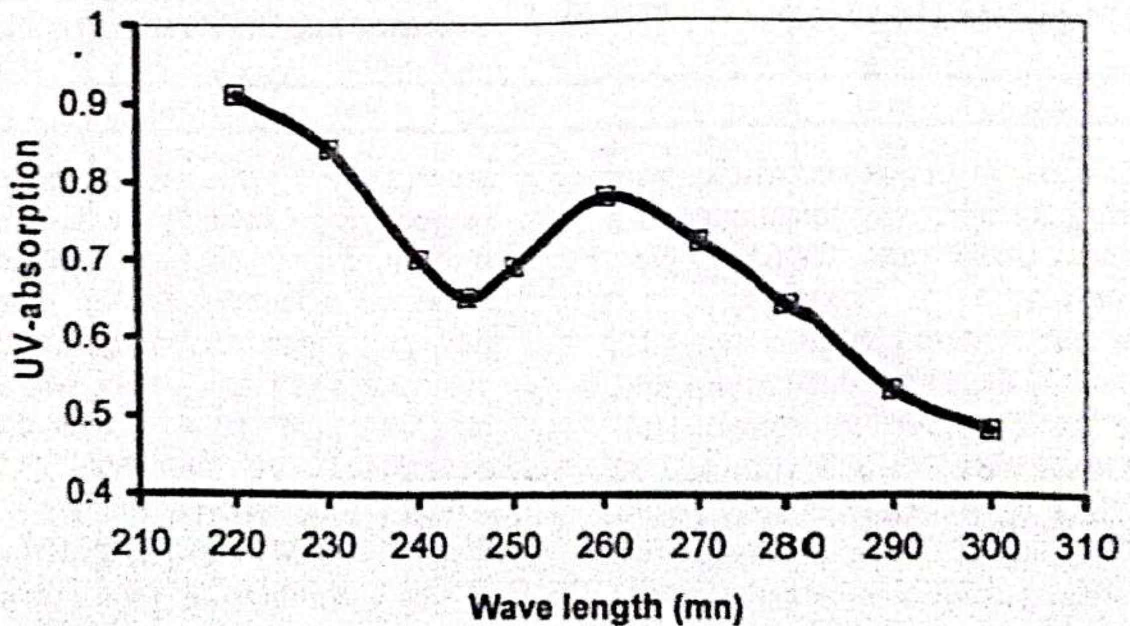

Fig. (2): Ultraviolet absorption spectrum of purified CABMV.

Electron microscope

Examination of purified BBSV preparations revealed isometric virus particles 28 nm. in diameter. Our result figs.(3,4)is similar in this respect to those reported by **Brunt *et al.* (1996) and Khatab (2002).** On the other hand, the purified CABMV preparations revealed flexuous filamentous particles 750 nm. in length. Similar results obtained by **Tolba (1977); Chang *et al* (1983) and Awad (1988).**

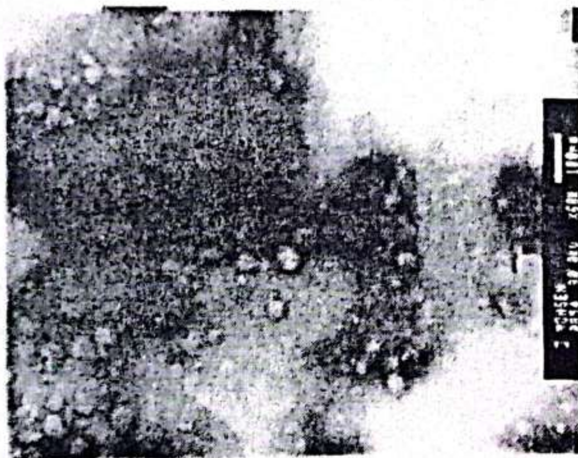

Fig. (3): Electron micrograph of purified BBSV negatively stained with uranyl acetate. (Magnification X-40.000).

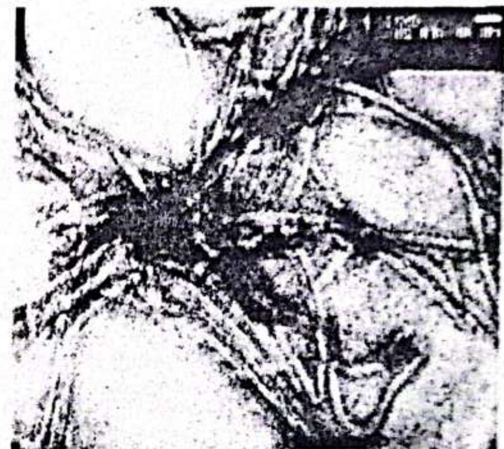

Fig. (4): Electron micrograph of purified CABMV negatively stained with uranyl acetate.(Magnification X-60.000).

1.2. Titer of produced antiserum

In this study, polyclonal antibodies against BBSV and CABMV were prepared.

The rabbits were bled three times 10, 20 and 30 days after the last injection. The titer of antiserum against BBSV **(Table2)** were 1:512, 1:512 and 1:32 for the first, second and third bleeding, respectively as determined by indirect ELISA technique. Whereas, the titer of antiserum against CABMV were 1:512, 1:512 and 1:32 for the first, the second and the third bleeding, respectively **(Table3)** as determined by indirect ELISA technique. However Khattab, (2002) reported that polyclonal antibodies produced against BYMV and BBSV had titers of 2048 and 1024.respestively as determined by indirect ELISA. **Awad (1988)** reported that antiserum titer of CABMV was 1/4096 for the second bleeding using microprecipitin test. Titers vary depending on serological test and antigen dilution.

Table (2): Antiserum titer against BBSV collected at 10 days intervals after rabbit immunization using indirect -ELISA

Antiserum dilution	10 days		20 days		30 days	
	Inf.	H	Inf.	H	Inf.	H
1/1	0.989	0.450	0.879	0.425	0.99	0.44
1/2	0.890	0.400	0.799	0.390	0.85	0.39
1/4	0.764	0.322	0.700	0.320	0.78	0.30
1/8	0.690	0.234	0.622	0.300	0.65	0.25
1/16	0.599	0.199	0.502	0.290	0.50	0.19
1/32	0.499	0.197	0.488	0.200	**0.42**	**0.20**
1/64	0.490	0.089	0.401	0.180	0.39	0.32
1/128	0.382	0.078	0.370	0.098	0.25	0.24
1/256	0.269	0.069	0.269	0.082	0.19	0.20
1/512	**0.199**	**0.065**	**0.160**	**0.066**	0.11	0.09
1/1024	0.109	0.064	0.089	0.050	0.08	0.07
1/2048	0.052	0.039	0.048	0.040	0.04	0.05

Reading after 30 min incubation with the substrate. Reading greater than twice the A_{405} value of healthy controls were considered positive.
Inf. = Infected plant. H. = Healthy plant.

Table (3): Antiserum titer against CABMV collected at 10 days intervals after rabbit immunization using indirect -ELISA

Antiserum dilution	10 days		20 days		30 days	
	Inf.	H	Inf.	H	Inf.	H
1/1	0.899	0.449	0.812	0.400	0.85	0.40
1/2	0.790	0.320	0.705	0.301	0.79	0.36
1/4	0.655	0.299	0.690	0.259	0.63	0.29
1/8	0.549	0.220	0.490	0.200	0.56	0.23
1/16	0.450	0.200	0.340	0.150	0.48	0.22
1/32	0.360	0.129	0.280	0.098	**0.63**	**0.19**
1/64	0.290	0.100	0.192	0.089	0.27	0.17
1/128	0.200	0.090	0.120	0.059	0.18	0.15
1/256	0.150	0.070	0.990	0.440	0.09	0.07
1/512	**0.100**	**0.045**	**0.650**	**0.320**	0.08	0.06
1/1024	0.050	0.052	0.440	0.400	0.07	0.05
1/2048	0.030	0.045	0.350	0.340	0.05	0.04

Reading after 30 min incubation with the substrate. Reading greater than twice the A_{405} value of healthy controls were considered positive.
Inf. = Infected plant. H. = Healthy plant.

1.3. Determination of antigen dilution end point

The obtained data indicate that, the dilution end point of BBSV in infected faba bean extracts was 1:320 as determined by Indirect-ELISA using the produced antiserum against BBSV **(Table4)**. In case of CABMV the dilution end point in infected cowpea extracts was 1:1280 **(Table5)** as determined by indirect-ELISA using the produced antiserum against CABMV. Results obtained by **Makkouk *et al.* (1987)** indicated that, the dilution end point seems the dilution end point of BBSV in faba bean seeds was 1:400. In addition. The data reported by , **Khatab (2002)** showed 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 direct ELISA test. She also reported that the virus was detected infected extracts at delutions up to 1:16000.

Table (4): Determination of BBSV dilution end point by indirect -ELISA using the produced antiserum.

Dilution of tissue extract	Indirect-ELISA	
	A_{405} values	
	Inf.	H
1/5	0.825	0.289
1/10	0.744	0.205
1/20	0.650	0.188
1/40	0.523	0.120
1/80	0.447	0.101
1/160	0.335	0.150
1/320	0.243	0.110
1/640	0.100	0.088
1/1280	0.067	0.068
1/2560	0.045	0.044

Reading after one hour and half incubation with the substrate.
Reading greater than twice the A_{405} value of healthy controls were considered positive.
Inf. = Infected plant. H. = Healthy plant.

Table (5): Determination of CABMV dilution end point by indirect - ELISA using the produced antiserum.

Dilution of tissue extract	Indirect-ELISA A_{405} values	
	Inf.	H
1/5	0.995	0.420
1/10	0.889	0.380
1/20	0.790	0.299
1/40	0.698	0.189
1/80	0.588	0.102
1/160	0.490	0.098
1/320	0.379	0.087
1/640	0.245	0.079
1/1280	**0.123**	**0.055**
1/2560	0.073	0.069

Reading after one hour and half incubation with the substrate.
Reading greater than twice the A_{405} value of healthy controls were considered positive.
Inf. = Infected plant. H. = Healthy plant.

Immunological detection of BBSV and CABMV by dot blot immunoassay (DBIA) and tissue blotting immunoassay (TBIA)

Dot blot immunoassay (DBIA) and tissue blotting immunoassay (TBIA) were found to be sensitive enough to detect BBSV and CABMV in all parts of infected faba bean plants and cowpea plants, respectively **(Figs. 5 and 6)**. A purplish blue color was obatined from tissues in the positive reactions, whereas extracts from healthy plants remain green in negative reactions. The same results obtained by **Makkouk *et al.* (1987) and Khatab (2002)** for BBSV and **Bhat *et al.* (1999) and Chaicharoen *et al.* (2003)** for CABMV.

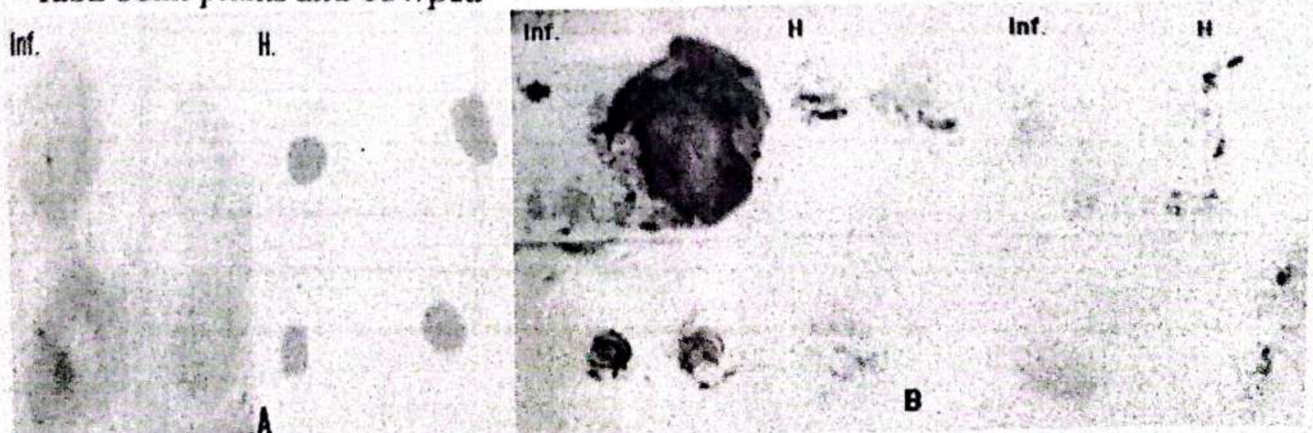

Fig. (5): Detection of BBSV by Dot Blot Immunoassay (A) and Tissue Blotting Immunoassay (B). Inf. : Infected samples H.: Healthy samples

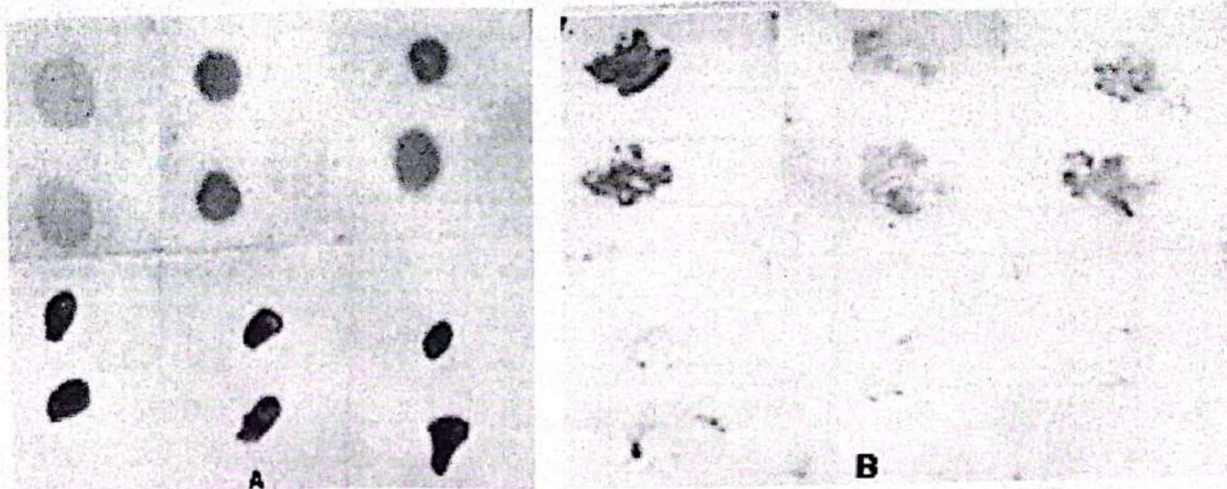

Fig. (6): Detection of CABMV by Dot Blot Immunoassay (A) and Tissue Blotting Immunoassay (B). Inf. : Infected samples H.: Healthy samples

2. Prevalence of the virus in different plant organs.

2.1. Prevalence of the virus in root, stem and flowers

In the present study, BBSV and CABMV were detected in stems and different flower parts (sepals, petals, pistils and anthers) but they were not detected in roots of faba bean and cowpea plant respectively **(Table6).** These results resemble those reported by **Vorra and Cockbain (1977)** for BBSVand **Morsy (1979) and Awad (1988)** for CABMV.

Table (6):prevalence of CABMV and BBSV in different organs of Cowpea and Faba bean plants using indirect ELISA.

Plant organs	CABMV in Cowpea	BBSV in Faba bean
	%	%
1- flower		
Sepals	40	20
Petals	80	70
Anthers	80	90
Pistils	70	70
2- Stem	60	40
3- Root	0	0

Prevalence of the virus in seeds and seed parts using indirect - ELISA.

Data in **Table (7)** indicate that BBSV was detected in intact seeds and all seed parts (seed coats, cotyledons and embryos) of immature seeds obtained from lower, medium and upper pods in faba bean cvs. Nubaria 1 , Sakha 2 , Giza 461, Egypt 1 and Improved Giza 3 using indirect ELISA. BBSV was also detected in intact seed, cotyledons and embryo of fully ripened seeds obtained from lower, medium and upper pods of all tested faba bean cultivars. **Leoyd *et al* (1965)** reported that BBSV produces a stain (called Eveshan stain or dark discoloration) in the form of peripheral brown necrosis of the seed coat. The BBSV was not detected in the seed coat of the ripened seeds obtained from lower and medium pods of all faba bean cvs. Tested and from lower, medium and upper pods of cvs Egypt 1. The percentage of BBSV detection was increased gradually from lower to medium and then to upper pods. These results resemble those reported by **vorra and Cockbain(1977).**

Results in **Table (8)** showed that by using indirect -ELISA, the average percentages of BBSV detection were 16.65, 11.15, 10.99, 12.17 and 12.5% in seeds of faba bean cvs. Nubaria 1, Sakha 2, Giza 461, Egypt 1 and improved Giza 3, respectively. The highest percentage of BBSV detection was obtained using seeds of Nubaria cultivar (16.65) while the lowest percentage was noticed in Giza 461 (10.99). Also according to ELISA value, BBSV was detected in stained seeds more than unstained seeds in all faba bean cultivars tested. On the other hand **Ghazalla (1998)** concluded that there was no clear relationship between the external symptoms on seed coat and seed infection and almost all abnormal characters of fada bean seeds. The average percentage of BBSV detection in stained seeds of faba bean cv. Nubaria 1 ,Sakha 2, Giza 461, Egypt 1 and Improved Giza 3 were 20.00, 13.00, 13.3, 15 and 16.33, compared with 11.30, 9.30, 8.67, 9.33 and 8.67 for unstained seeds of the same cultivars, respectively.

Table(7): Detection of BBSV in different parts of seeds in five faba bean cultivars using indirect -ELISA.

Faba bean cultivars	Pods position	Percentage of BBSV detection in							
		Intact seed		seed coat		cotyledons		embryo	
		Immature	ripened	Immature	ripened	Immature	ripened	Immature	ripened
Nubaria 1	lower pods	18	15	10	0	11	12	15	18
	medium pods	20	16	12	0	13	15	16	20

	upper pods	25	18	15	2	17	18	19	23
Sakha 2	lower pods	14	10	9	0	9	11	12	15
	medium pods	16	11	10	0	11	13	15	18
	upper pods	19	17	12	1	13	16	17	20
Giza 461	lower pods	13	9	9	0	10	12	11	15
	medium pods	15	13	13	0	13	14	13	17
	upper pods	18	15	15	1	16	18	16	19
Egypt 1	lower pods	11	8	10	0	9	10	12	16
	medium pods	13	11	12	0	11	13	13	18
	upper pods	17	14	14	0	15	18	15	20
Improved Giza 3	lower pods	10	7	10	0	9	11	13	15
	medium pods	12	10	13	0	10	13	15	17
	upper pods	16	12	15	1	14	15	17	20

Table (8): Percentage of seed transmission of BBSV in five cultivars of faba bean seeds using Indirect-ELISA.

Faba bean cultivars	Pods position	Percentage of seed transmission of BBSV using ELISA (%)		General mean
		Stained seeds	Unstained seeds	
Nubaria 1	lower pods	17	10	
	medium pods	19	11	
	upper pods	24	13	
	Mean	20	11.3	16.65
Sakha 2	lower pods	11	6	
	medium pods	13	8	
	upper pods	15	14	
	Mean	13	9.3	11.15
Giza 461	lower pods	10	6	
	medium pods	13	8	
	upper pods	17	12	
	Mean	13.3	8.67	10.99
Egypt 1	lower pods	12	7	
	medium pods	15	9	
	upper pods	18	12	
	Mean	15	9.33	12.17
Improved Giza 3	lower pods	13	6	
	medium pods	16	8	
	upper pods	20	12	
	Mean	16.33	8.67	12.50

Using indirect ELISA, CABMV was reliable detected in intact seeds and all seed parts (seed coat, cotyledons and embryo) of immature seeds of cowpea plants cvs. Kafr EL Sheikh 1, Fetriaat, Cream No. 7, Black eye and Azmerley Table(9). Whereas it was not detected in seed coat of ripened seed of the same cowpea cv. cultivars and in roots of cowpea cv.cream NO.7 These results are in harmony with those obtained by **Morsy (1979) and Awad (1988).**

Table (9): Detection of CABMV in different parts of seeds in Five cowpea cultivars using indirect -ELISA.

Cowpea cultivars	Seed	Seed parts			
		ELISA on Intact seed	ELISA on seed coat	ELISA on cotyledons	ELISA on embryo
Kafr El-Sheikh 1	Immature	6/100	3/100	5/100	7/100
	Ripened	5/100	0/100	7/100	8/100
Fetriaat	Immature	8/100	4/100	7/100	9/100
	Ripened	6/100	0/100	8/100	10/100
Cream No.7	Immature	7/100	5/100	6/100	7/100
	Ripened	5/100	0/100	7/100	8/100
Black eye	Immature	5/100	2/100	4/100	5/100
	Ripened	4/100	0/100	5/100	6/100
Azmerly	Immature	8/100	1/100	5/100	7/100
	Ripened	7/100	0/100	7/100	8/100

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دراسات على التنقية والسيرولوجى والكشف عن فيروسى

تلون بذور الفول وموزيك اللوبيا المحمول بالبذرة

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** قسم بحوث الفيروس و الفيتوبلازما مركز البحوث الزراعية-الجيزة

لقد تم استخدام طريقتان مختلفتان لتنقية فيروسان تلون بذور الفول وموزيك اللوبيا المنقول بالمن من نباتات الفول واللوبيا المصابة على التوالي.
وجد أن أقصى طيف امتصاص لأشعة فوق البنفسجية عند 260 نانوميتر وكان اقل طيف لامتصاص عند 245 نانوميتر لكلا الفيروسين.
وجد أن نسبة أقصى امتصاص لأدنى امتصاص لطيف الأشعة فوق البنفسجية كانت 1.19 بينما كانت نسبة 280/260 هي 1.62 وكان محصول الفيروس 0.48 ملجم لكل 100جم من أوراق الفول المصابة بفيروس تلون بذور الفول
وجد أن نسبة أقصى امتصاص لأدنى امتصاص لطيف الأشعة فوق البنفسجية كانت 1.2 بينما كانت نسبة 280/260 هي 1.22 وكان محصول الفيروس 3.52 ملجم لكل 100جم من أوراق الفول المصابة بفيروس موزيك اللوبيا المنقول بالمن
تم إنتاج مصل مضاد لفيروس تلون بذور الفول وكذلك مصل مضاد لفيروس موزيك اللوبيا المنقول بالمن ووجد أن تركيز هذان المصلان كان 512:1 لكلا الفيروسين وذلك بعد 10أيام للفيروس الاول وبعد 20 يوم للفيروس الثانى من اخر حقنة.
وجد أن نقطة التخفيف النهائية لعصير الفول المصاب بفيروس تلون بذور الفول كانت1: 320 بينما كانت بالنسبة لفيروس موزيك اللوبيا المنقول بالمن1 :1280
لقد تم استخدام تكنيكات Dot-Blot Immunoassay (DBIA) and
Tissue Blotting immunoassay (TBIA) في الكشف عن فيروسات تلون بذور الفول وموزيك اللوبيا المنقول بالمن فى النباتات المصابة.

وبالنسبة لوجود الفيروس فى الأجزاء النباتية المختلفة فقد ثبت وجود كلا الفيروسان فى السيقان وأجزاء الأزهار (السبلات والبتلات والقلم والمبيض) ولكنهم لم يظهرا فى الجذور.
ظهر فيروس تلون بذور الفول في القرون السفلى والوسطى والعليا وفى جميع أجزاء البذرة (جنين-فلقات-قصرة) بالنسبة للبذور الغير ناضجة بينما لم يظهر فى القصرة بالنسبة للبذور الناضجة.
وجد أن نسبة النقل خلال البذور الملونة والناتجة من نباتات فول مصابة بفيروس تلون بذور الفول كانت اكبر من مثيلتها الغير ملونة.
ظهر فيروس موزيك اللوبيا المحمول بالمن في جميع أجزاء البذرة الغير ناضجة بينما لم يظهر في القصرة بالبذور الناضجة.