

Incidence of some Seed-borne Viruses Affecting faba bean in Alexandria Governorate

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ABSTRACT

Field survey was conducted during two growing seasons 1998/1999 and 1999/2000 to study the prevalence and distribution of some seed-borne viruses infecting faba bean in some locations at Alexandria Governorate. The incidence of mosaic disease symptoms was ranged from 11-64% during the two growing seasons. Five viruses namely: broad bean mottle virus (BBMV), broad bean true mosaic virus (BBTMV), broad bean stain virus (BBSV), pea seed borne mosaic virus (PsbMV) and bean yellow mosaic virus (BYMV) were detected using indirect ELISA. BYMV was the most prevalent (88.54%) followed by PsbMV (61.73%) which could be singly presented or in combinations with other viruses. BBMV, BBTMV and BBSV were not found singly in any of the infected samples. Samples of *Beta vulgaris* and *Melilotus* sp. growing naturally in faba bean fields were also collected and examined by indirect ELISA. PsbMV+BYMV were found in samples of *Beta vulgaris* while samples of *Melilotus* sp were infected with the above mentioned viruses except BBSV. Results of comparison between indirect ELISA and tissue-blot immunoassay (TBIA) for virus detection in 28 infected faba bean samples showed that not all infected samples with a particular virus (es) revealed by TBIA were detected with ELISA. TBIA was more sensitive, more practical, cheaper and does not require sophisticated facilities than ELISA.

INTRODUCTION

Seed borne viruses are known to infect faba bean plants under field conditions. These viruses are broad bean mottle virus (BBMV), broad bean true mosaic virus (BBTMV), broad bean stain virus (BBSV), pea seed borne mosaic virus (PsbMV) and bean yellow mosaic virus (BYMV) (El-Hammady *et al.*, 2002 and Makkouk *et al.*, 1988)

Survey of faba bean plantations for viruses were conducted in many Arab countries (Fortass and bos, 1991; Makkouk *et al.*, 1987; Makkouk *et al.*, 1988; Mouhanna *et al.*, 1994 and Najjar *et al.*, 2000) as well as in Egypt (El-Affi and El-DougDoug, 1997; El-Hammady *et al.*, 2002 and Makkouk *et al.*, 1994).

Details studies on faba bean viruses were not carried out at Alexandria Governorate. The objective of this work is to determine the distribution and prevalence of faba bean seed transmitted viruses in different locations at Alexandria Governorate using indirect ELISA. Comparison between indirect ELISA and TBIA for virus detection was also conducted.

MATERIALS AND METHODS

Survey studies were conducted during the growing seasons of 1998/1999 and 1999/2000. The most common symptoms detected in the field were different types of mosaic, leaf crinkling, reduction of the leaf blade and stunting of plants. Evaluation of disease incidence was based on external symptoms and serological diagnosis. External symptoms were evaluated in the fields, while serological diagnosis was carried out using indirect ELISA test.

245 samples during the growing season of 1998/1999 and 113 samples at 1999/2000 with different mosaic symptoms were separately collected in plastic bags at random from diseased faba bean plants from different locations at Alexandria Governorate. The samples were indexed by indirect ELISA against BBMV, BBTMV, BBSV, PsbMV and BYMV antisera.

Also, mosaic disease incidence was estimated at random by counting the number of plants showing mosaic symptoms out of 100 consecutive plants in each of four patches selected, at random, from each inspected field.

Samples of wild chard (*Beta vulgaris*) and *Melilotus* sp. growing naturally in faba bean fields and showing virus-like symptoms were also collected.

Serological reaction:

a) Source of antisera:

The used antisera for broad bean mottle virus (BBMV), broad bean true mosaic virus (BBTMV), broad bean stain virus (BBSV), pea seed borne mosaic virus (PsbMV) and bean yellow mosaic virus (BYMV) were kindly supplied by antiserum-Bank, Institute of Seed Pathology for developing countries, Denmark.

b) Indirect ELISA:

Indirect ELISA was carried out as described by Fegla *et al.* (1997), extracts from infected and healthy faba bean plants were diluted with coating buffer (0.05 M carbonate, pH 9.6) to 1:10. Wells were coated with antigens by adding 100 μ l to the bottom of the well and incubated for 3 hours at 37°C or overnight at 4°C. The plates were rinsed three times by flooding wells with PBST for 3 minutes each.

Antisera requiring cross-adsorption were diluted 1:500 with filtered extract from healthy tissues diluted 1:20 in serum buffer (PBS-Tween 20 containing 2% soluble polyvinylpyrrolidone, 0.2% BSA), and incubated for 45 min. at 37°C. The precipitate, which had formed, was removed by centrifugation for 10 min. at 5000 rpm. 100 μ l aliquots from the diluted antisera were added to each well, after which the plates were incubated at 37°C for 2 hours or at 4°C overnight, then washed as before.

Goat anti-rabbit gamma globulin conjugated to alkaline phosphatase was diluted 1:1500 in serum buffer, and 100 μ l were added to each well, followed by one hour incubation at 37°C, then washed as before.

100 μ l of the enzyme substrate, 0.5 mg/ml paranitrophenyl phosphate

in 10% diethanolamine buffer, pH 9.8 were added to each well and incubated at room temperature (25°C) for about 30 minutes. The enzyme activity was stopped by adding 50 µl of 3 M NaOH. The ELISA values measured by Multiskan-MS ELISA reader were expressed as absorbency at 405 nm and absorbency values of at least double that of the healthy control were considered positive.

c) Comparison between indirect ELISA and TBIA:

Comparison between indirect ELISA and TBIA for virus detection in 28 faba bean samples and 8 *Melilotus* sp. samples showing mosaic symptoms, collected from faba bean fields, was conducted.

TBIA as described by Lin *et al.* (1990) and Abdel-Salam (1997) was used. Sections were cut from fresh tissues of stems from healthy and infected broad bean plants by hand with a new razor blade for each sample, and exposed cut edges were pressed onto nitrocellulose membrane, then blocked with blocking buffer (2% bovine serum albumin and 2% triton X-100 solution in tris buffer saline (TBS) pH 7.5), gently agitated for one hour at room temperature. The membrane was removed from the blocking solution, dipped in distilled water and transferred to the virus antisera (the five antisera tested were diluted 1:500 in TBST). The solution contained clarified plant sap (1g healthy plant leaf tissue per 10 ml of TBST buffer triturated and clarified by centrifugation at 8000 rpm for 20 min). The membrane was removed from the first antibodies solutions, dipped in distilled water, and washed twice by agitation for 10 min. TBST. The membranes were dipped in distilled water, and transferred to 1:1000 dilutions of goat anti-rabbit IgG conjugated to alkaline phosphatase in TBST and gently agitated for one hour.

Finally, the membranes were removed from the second antibody dilution, dipped in distilled water, washed twice in TBST for 10 min each. The 5-bromo -4-chloro -3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT) substrate solution was made during the final washing in which membranes were incubated for colour development. After colour development, the reaction was stopped by washing the nitrocellulose membrane in 0.01M Tris-HCl containing 0.05M EDTA, pH 7.5.

The positive reaction of TBIA was indicated by the development of purple colour on the blots. The negative reaction developed no colour or green colour.

RESULTS

Survey studies:

Data in tables (1&2) showed that incidence of mosaic disease was from 11-64% and from 14-54% during the first and second growing season respectively.

245 samples of infected faba bean plants were collected at the first growing season. Obtained data presented in tables (1 and 3) showed that

BYMV was the most prevalent virus being detected in 225 samples followed by PsbMV in 165 samples and then BBSV in 63 samples, which in turn was followed by BBTMV in 47 samples and finally by BBMV in 46 samples representing 91.8%, 67.3%, 25.7%, 19.2% and 18.8% respectively from the whole previously inspected samples.

Regarding the single infections, it was found that out of 225 samples infected with BYMV, 73 samples were singly infected and out of 165 samples infected with PsbMV only one sample was infected singly. No samples were infected singly with BBMV, BBTMV or BBSV.

As for double infection, the combination between PsbMV+BYMV was the most prevalent (76 samples), followed by BBSV+PsbMV (16 samples) and the BBSV+BYMV (4 samples). No other combinations were found in any doubly infected samples. Triple infection was only found between BBSV+PsbMV+BYMV (25 samples). Tetra infection were found between BBMV+BBTMV+PsbMV+BYMV (29 samples), BBMV+BBSV+PsbMV+BYMV (one sample) and BBTMV+BBSV+PsbMV+BYMV (14 samples). 3 samples were found to be infected by the five tested viruses. No samples were infected by any other combinations. Three samples showed negative results with the tested virus antisera.

At the second growing season, 113 infected samples were collected. The incidence was less to some extent. Data presented in tables (2 and 4) showed that BYMV had the highest frequency (92 samples, 81.4%), followed by PsbMV (56 samples, 49.5%), then BBMV (9 samples, 7.9%), BBMV (7 samples, 6.2%) and finally BBSV (5 samples, 7.91%).

With respect to the single infections, 40 samples were infected by BYMV and 6 by PsbMV. No single infection with BBMV, BBTMV or BBSV was detected. Data concerning double infection revealed that combination between PsbMV+BYMV was the most prevalent one being occurred in 42 samples followed by BBTMV+BYMV in 2 samples.

Triple infection BBMV+PsbMV+BYMV was shown in one sample and BBTMV +sbMV+BYMV in 3 samples. Four samples were infected by tetra infection: BBMV+BBTMV+PsbMV+BYMV. No other infection combinations were detected. 13 samples, indexed viruses were not detected.

Two weed i.e., (*Beta vulgaris* and *Melilotus* sp.) were indexed. *Beta vulgaris* was infected with PsbMV+BYMV, while some samples of *Melilotus* sp was singly infected with BYMV, and others had mixed infection with BBMV+PsbMv+BYMV or BBMV+BBTMV+PsbMv+BYMV.

Comparison between indirect ELISA and TBIA:

Comparison between indirect ELISA and TBIA for virus detection in 28 samples of faba bean showing mosaic symptoms was conducted. Data presented in Table (5) and Fig. (1) revealed that not all infected samples with a particular virus (es) showed by TBIA were detected with ELISA. For example,

samples No. 5 and 25 were found to be infected with BYMV in TBIA while ELISA showed negative results. In addition, samples No. 11,16 and 22 revealed infection with BBSV and PsbMV in TBIA whereas ELISA detected only PsbMV in these samples. On the other hand, sample No9, when tested for BYMV, gave negative results with TBIA and positive results with ELISA.

All samples of *Melilotus* sp. gave positive reactions with BYMV antiserum by indirect ELISA and TBIA.

Table 1. Occurrence and relative prevalence of seed-borne viruses infecting faba bean in some faba bean producing areas in Alexandria governorate during the growing season 1998/1999

Location	Mosaic disease incidence	No. of tested samples	No of samples found infected with					
			BBMV	BBTMV	BBSV	PsbMV	BYMV	unknown
<u>Elsabahia</u>								
(farm of fac. of agric.)	27	57	4	4	8	10	54	3
Maamora	31	59	6	6	6	26	59	-
<u>Abis:</u>								
Village 2	60	48	33	33	4	48	48	-
Village 10	64	28	2	2	27	28	28	-
(farm of fac. of agric.-	11	53	1	2	18	53	36	-
Saba Basha								
Total		245	46	47	63	165	225	3

Table 2. Occurrence and relative prevalence of seed-borne viruses infected faba bean in some faba bean producing areas in Alexandria governorate during the growing season 1999/2000

Location	Mosaic disease incidence	No. of tested samples	No of samples found infected with					
			BBMV	BBTMV	BBSV	PsbMV	BYMV	unknown
<u>Elsabahia</u>								
(farm of fac. of agric.)	18	28	-	-	-	7	22	3
Maamora	-	-	-	-	-	-	-	-
<u>Abis:</u>		32	2	2	1	10	25	3
Village 2	54							
Village 10	35	37	3	3	3	31	37	-
(farm of fac. of agric.-	14	16	2	4	1	8	8	4
Saba Basha								
Total		113	7	9	5	56	92	10

Table 3. Viruses causing single and mixed infections in samples collected from naturally infected faba bean plants in fields distributed in some locations of Alexandria during growing season of 1998/1999

Viruses	El-Sabahia		Maamora		Abis				
	No. of samples	%	No. of samples	%	Village 2		Village 10		Saba B
					No. of samples	%	No. of samples	%	No. of samples
BBMV	0	0	0	0	0	0	0	0	0
BBTMV	0	0	0	0	0	0	0	0	0
BBSV	0	0	0	0	0	0	0	0	0
PsbMV	0	0	0	0	0	0	0	0	1
BYMV	40	70	33	56	0	0	0	0	0
BBSV+PsbMV	0	0	0	0	0	0	0	0	16
BBSV+BYMV	4	7	0	0	0	0	0	0	0
PsbMV+BYMV	6	11	20	34	15	31	1	4	34
BBSV+PsbMV+BYMV	0	0	0	0	0	0	25	89	0
BBMV+BBTMV+PsbMV+BYMV	0	0	0	0	29	61	0	0	0
BBTMV+BBSV+PsbMV+BYMV	4	7	6	10	4	8	0	0	0
BBMV+BBTMV+BBSV+PsbMV+BYMV	0	0	0	0	0	0	2	7	1
Unknown	3	5	0	0	0	0	0	0	1
Total	57		59		48		28		53

Table 4. Viruses causing single and mixed infections in samples collected from naturally infected faba bean plants in fields distributed in some locations of Alexandria during growing season of 1999/2000

Viruses	El-Sabahia		Maamora		Abis					
	No. of samples	%	No. of samples	%	Village 2		Village 10		Saba B	
					No. of samples	%	No. of samples	%	No. of samples	%
BBMV	0	0	0	0	0	0	0	0	0	0
BBTMV	0	0	0	0	0	0	0	0	0	0
BBSV	0	0	0	0	0	0	0	0	0	0
PsbMV	3	11	0	0	3	10	0	0	0	0
BYMV	18	64	0	0	16	50	6	16	0	0
BBMV+BBSV	0	0	0	0	1	3	0	0	1	0
BBTMV+BYMV	0	0	0	0	2	6	0	0	0	0
BBSV+PsbMV	0	0	0	0	0	0	0	0	0	0
BBSV+BYMV	0	0	0	0	0	0	0	0	0	0
PsbMV+BYMV	4	14	0	0	6	19	28	76	4	2
BBMV+BBSV+BYMV	0	0	0	0	3	9	0	0	0	0
BBTMV+PsbMV+BYMV	0	0	0	0	0	0	0	0	3	1
BBSV+PsbMV+BYMV	0	0	0	0	0	0	0	1	6	0
BBMV+BBTMV+PsbMV+BYMV	0	0	0	0	0	0	3	8	7	4
BBMV+BBTMV+BBSV+PsbMV+BYMV	0	0	0	0	1	3	0	0	0	0
Unknown	3	11	0	0	0	0	0	0	0	0
Total	28				32		37		16	

Table 5. Incidence of seed-borne viruses detected by TBIA and indirect ELISA in 28 samples of infected faba bean samples

Sample No.	BBMV		BBTMV		BBSV		PsbMV		BYMV	
	TBIA	ELISA	TBIA	ELISA	TBIA	ELISA	TBIA	ELISA	TBIA	ELISA
1	-	0.044	-	0.063	-	0.020	-	0.041	+	0.230
2	-	0.049	-	0.051	-	0.013	-	0.064	+	0.394
3	-	0.032	-	0.055	-	0.014	-	0.034	-	0.020
4	-	0.037	-	0.056	-	0.017	-	0.036	+	0.132
5	-	0.044	-	0.067	-	0.025	-	0.045	+	0.049
6	-	0.065	-	0.066	-	0.019	-	0.079	+	0.580
7	-	0.051	-	0.063	-	0.024	-	0.042	+	0.257
8	-	0.062	-	0.077	-	0.022	-	0.057	+	0.319
9	-	0.032	-	0.046	-	0.017	-	0.034	-	0.268
10	-	0.052	-	0.050	-	0.017	-	0.041	+	0.326
11	-	0.026	-	0.041	+	0.015	+	0.255	-	0.014
12	-	0.035	-	0.039	-	0.013	-	0.032	+	0.197
13	-	0.033	-	0.043	-	0.018	-	0.028	+	0.283
14	-	0.032	-	0.044	-	0.017	-	0.029	+	0.191
15	-	0.030	-	0.048	-	0.016	-	0.031	+	0.188
16	-	0.019	-	0.042	+	0.012	+	0.534	-	0.015
17	-	0.035	-	0.042	-	0.014	-	0.033	+	0.352
18	-	0.028	-	0.042	-	0.013	-	0.042	+	0.309
19	-	0.041	-	0.046	-	0.012	-	0.043	+	0.331
20	-	0.031	-	0.040	-	0.010	-	0.035	+	0.222
21	-	0.026	-	0.036	-	0.013	-	0.026	+	0.138
22	-	0.023	-	0.034	+	0.013	+	0.595	-	0.009
23	-	0.028	-	0.044	-	0.012	-	0.045	+	0.523
24	-	0.025	-	0.032	-	0.011	-	0.019	+	0.083
25	-	0.032	-	0.067	-	0.008	-	0.024	+	0.037
26	-	0.040	-	0.064	-	0.005	-	0.030	+	0.226
27	-	0.023	-	0.054	-	0.009	-	0.017	-	0.024
28	-	0.020	-	0.047	-	0.007	-	0.018	-	0.030
control		0.043		0.053		0.017		0.042		0.025

+ = infected

- = not infected

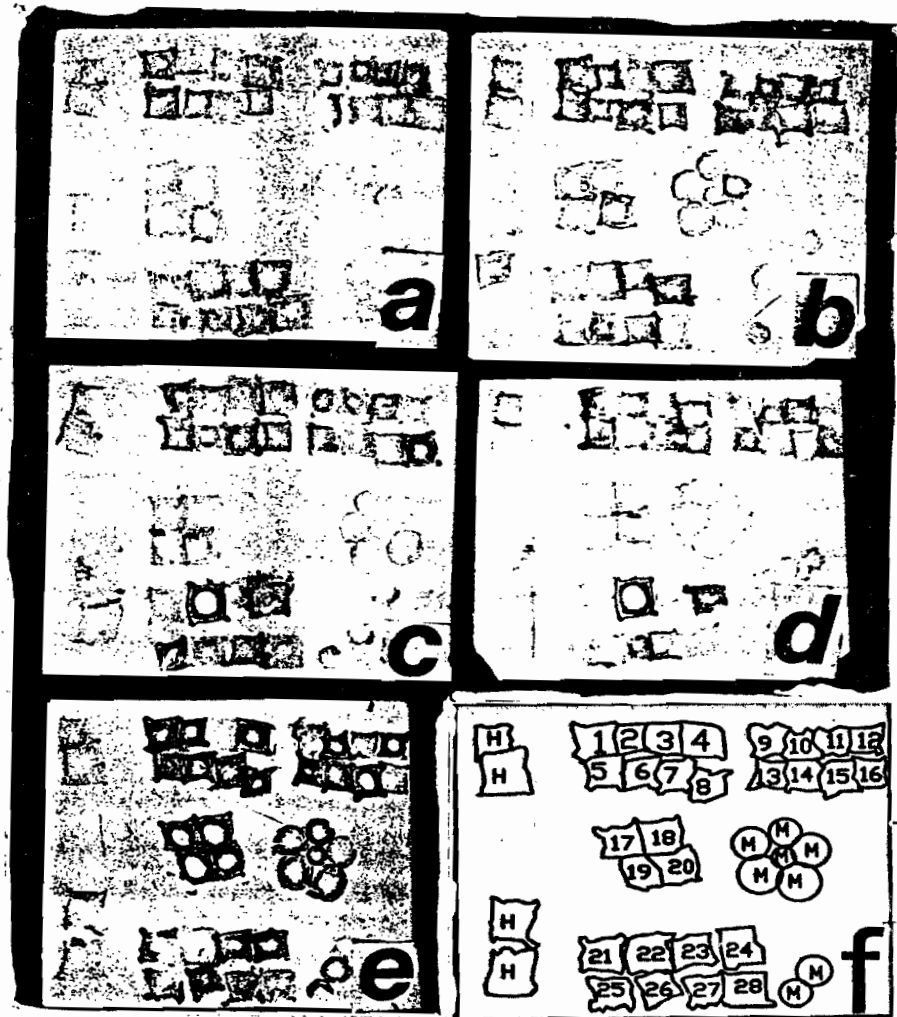


Fig 1: Detection of viruses in 28 faba bean samples showing mosaic symptoms using TBIA. A-BBMV antiserum b- BBTMV antiserum c- BBSV antiserum d- PsbMV antiserum e- BYMV antiserum f- drawing showing the 28 faba bean samples distribution H- healthy faba bean plants M- *Melilotus* sp

DISCUSSION

Field surveys were conducted during two growing seasons to study the prevalence and distribution of some seed-borne viruses infecting faba bean in some locations along Alexandria governorate. Five viruses namely: BBMV, BBTMV, BBSV, PsbMV and BYMV were detected using indirect ELISA. These viruses were reported in other countries (Fortass and bos, 1991; Makkouk *et al.*, 1987; Makkouk *et al.*, 1988; Mouhanna *et al.*, 1994 and Najar *et al.*, 2000) and in Egypt (El-Affifi and El-DougDoug, 1997; El-Hammady *et al.*, 2002 and Makkouk *et al.*, 1994).

BYMV was the most prevalent followed by PsbMV which could be presented singly or in combinations with other viruses and with each other. BBMV, BBTMV and BBSV didn't found singly in any infected samples. These results were in contrary with that of El Hammady *et al.*, 2002 who mentioned that BBMV and BBTMV were the most prevalent viruses in their survey. This difference may be due to different climatic conditions between Alexandria and other governorates they surveyed and also presence of aphids which transmit the BYMV and PsbMV.

The incidence of mosaic disease symptoms ranged from 11-64% during the two season survey. The high incidence in village 2 and village 10 could be attributed to the fact that farmers of these villages used their own seeds while the low incidence in the farms of faculties of agriculture (Saba-Basha and Shatby) may due to the use for certified seeds.

Some seed-borne viruses were isolated from naturally infected wild chard (*Beta vulgaris*) and *Melilotus* sp indicating that these weeds act as additional reservoirs for these viruses. The same conclusion was reached by El-Hammady *et al.* (2002) for *Beta vulgaris maritima*

Results of comparison between indirect ELISA and Tissue-blot immunoassay (TBIA) for virus detection in 28 infected faba bean samples showed that not all infected samples with a particular virus (es) revealed by TBIA were detected with ELISA. Our results also showed that TBIA is relatively more sensitive in virus detection than indirect ELISA. Such results agreed with those of Makouk and Kumari (1996) who detected easily ten viruses affecting legumes by TBIA, and Fegla *et al.*, (2000) who compared between TBIA and indirect ELISA for detection of AMV in 21 days old alfalfa seedlings of two alfalfa cultivars, they mentioned that TBIA is more practical, cheaper and doesn't require sophisticated facilities than regular ELISA.

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الملخص العربي

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أجرى حصر لعدة مزارع فول بمناطق مختلفة بمحافظة الإسكندرية لعدد من الفيروسات المحمولة بالبذرة وذلك خلال موسمي ١٩٩٨/١٩٩٩ و ١٩٩٩/٢٠٠٠. تراوحت نسبة الإصابة بأعراض التبرقش من ١١-٦٤% . جمعت ٢٤٥ عينة فى السنة الأولى و١١٣ عينة فى السنة الثانية مصابة بأعراض التبرقش وعرفت للفيروسات وذلك باستخدام اختبارات الاليزا غير المباشرة فكانت خمس فيروسات هى: فيروس موزايك الفول ، فيروس الموزايك الحقيقى للفول ، فيروس تلون بذور الفول ، فيروس موزايك البسلة المحمول بالبذرة و فيروس الموزايك الأصفر للفاصوليا . كان فيروس الموزايك الأصفر فى الفاصوليا الأكثر انتشاراً ، يليه فيروس موزايك البسلة المحمول بالبذرة ثم فيروس تلون بذور الفول و فيروس موزايك الفول و فيروس الموزايك الحقيقى للفول. كانت نسبة الإصابة الفردية بفيروس الموزايك الأصفر فى الفاصوليا الأكثر ترددا (٨٨,٥٤%) يليه فيروس موزايك البسلة المحمول بالبذرة (٦١,٧٣%) بينما لم يعثر على أى إصابة فردية بفيروس موزايك الفول أو فيروس الموزايك الحقيقى للفول أو فيروس تلون بذور الفول. عرفت الفيروسات التى على نباتات سلق برى وحنندقو تنمو طبيعيا فى حقول الفول فوجد أن السلق البرى يصاب بفيروس الموزايك الأصفر فى الفاصوليا و فيروس موزايك البسلة المحمول بالبذرة أما الحنندقو فيصاب بجميع الفيروسات المختبرة ما عدا فيروس تلون بذور الفول. أظهرت نتائج المقارنة بين طريقتى الاليزا غير المباشرة وبصمة النسيج فى الكشف عن الفيروسات فى ٢٨ عينة فول مصابة فوجد أنه ليس كل الفيروسات المعروفة فى العينات المصابة بطريقة البصمة النسيجية تظهرها طريقة الاليزا غير المباشرة ، كما بينت النتائج أن طريقة البصمة النسيجية أكثر حساسية ، أقل تكلفة وليسط وأسهل فى إجرائها عن طريقة الاليزا غير المباشرة.