STUDIES ON FABA BEAN MOSAIC
CAUSED BY BEAN YELLOW MOSAIC VIRUS

By
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Fac. Agric., Banha Univ..

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ABSTRACT

This study was conducted at the Laboratory, Greenhouse, and Farm of Faculty of Agriculture, Moshtohor, Banha University and the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, during two successive spring surveys (2003/2004 – 2004/2005).

The aims of this study are surveyed the viruses affecting faba bean crop at Qalyoubia Governorate, identified the collected infected samples via ELISA, determine the disease incidence and severity. On the basis of specific virus symptoms, estimated the viruses frequencies, subsequently, determine the dominant faba bean viruses at the surveyed fields. Completely, isolated and identified the dominant virus using symptomatology, host range, mode of transmissions, physical properties, serological diagnosis, particle properties (inclusion bodies, via light microscope and virus-particle dimension via transmission electron microscope). Study the response of some cultivated faba bean cultivars to artificial infection with bean yellow mosaic virus under greenhouse conditions. Spraying faba bean crop with six systemic resistant inducers medicinal plant extracts as natural antiviral agents. Modified the serological reaction (SDS-double diffusion test) by replace agarose with Gelrite and sodium azide with Kombucha.

Key words:

BYMV, Faba bean, Antiviral proteins, Cultivars, EM, Serology, Gelrite, Kombucha
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ARABIC SUMMARY ......................................................................................................................
1- INTRODUCTION

Faba bean (*Vicia faba* L., Fabaceae) considered the most important nutritive popular food crop in the world and Egypt. It plays a major role in the Egyptian diet as a source of protein. Faba bean crop rich in protein (protein content ranges from 26 to 41%) and the supply of essential amino acids (*Fernández et al.*, 1996). It has many uses, as human food in developing countries and as animal feed, mainly for pigs, horses, poultry and pigeons in industrialized countries. It can be used as a vegetable, green or dried, fresh or canned.

In Egypt, faba bean cultivated area was declined from 380,800 feddan in 1992 to 147,520 feddan in 1993 due to virus diseases (Anon., 2001).

The statistical data recorded in year book, 2005, of Statistical Institute, ARC, Giza, Egypt, showed that faba bean cultivated area was declined from 333,693 feddan (produced 2,835,358 Ardab) in 2001 to 240,854 feddan (produced 2,132,171 Ardab) in 2004.

Faba bean is susceptible to number of viruses that cause substantial yield losses in this crop in Europe, Middle East, the Sudan, and North America (*Bailiss and Senanayake, 1984*). Many viruses, belonging different viral families, causing serious diseases in faba bean crop as follows: Alfalfa mosaic alfamovirus (AMV), Bean yellow mosaic potyvirus (BYMV), Bean leaf roll luteovirus (BLRV), Beet western yellows luteovirus (BWYV), Broad bean mottle bromovirus (BBMV), Broad bean stain comovirus (BBSV), Broad bean true mosaic luteovirus (BBTMV), Broad bean wilt fabavirus (BBWV, BBWV-1, BBWV-2), Chickpea chlorotic dwarf geminivirus (CpCDV), Cucumber mosaic cucumovirus (CMV), Broad bean true mosaic comovirus (BBTMV), Faba bean necrotic yellows nanovirus (FBNYV), Pea seed-borne mosaic potyvirus (PSbMV), pea early browning tobravirus (PEBV), pea enation mosaic enamovirus (PEMV), Tomato spotted wilt tospovirus (TSWV) and Soybean dwarf virus (SbDV).

Bean yellow mosaic potyvirus (BYMV) is a common disease of legumes and other hosts, found worldwide (*Bos, 1970*). It is reduced seed yield in faba bean particularly when plants were infected at the pre- and mid-bloom stage (*Bailiss and Senananyake, 1984*). Moreover, BYMV infection reduced yield (Kg/ha), protein content and *in vitro* protein digestibility (IVPD) but increased tannin content (mg/100
ml) (Babiker et al., 1995). It is the most dominant one all over the world in the faba bean (Vicia faba L.) fields causing the considered losses in the grain yield. This virus was isolated, either alone or combined with other viruses, from faba bean in many countries as follows: In Austria, Egypt, Ethiopia, German, Hungary, India, Italy, Lebanon, Mexico, Morocco, Poland, Portugal, Spain, The Sudan, The United Kingdom, Tunisia, Western Australia, Yemen.

Mosaic with bright yellowing symptoms suggestive due to bean yellow mosaic potyvirus were the most frequently found – and likely most damaging- virus in the faba bean crop in Qalyoubia Governorate.

Viruses are responsible for considerable losses in crop productivity and quality. Several conventional strategies to control virus infection have been explored but without much success. In many of recent approaches involving viral components, the induced resistance is very specific to a particular strain or group of viruses (Gholizadeh et al., 2004).

The present study aims to investigate the faba viruses (especially BYMV) with some details and design a small integrated program to control or reduce the faba virus-infection.

The work schemes were:

1- Survey for faba bean viruses at Qalyoubia governorate.
2- Isolation and identification of faba viruses and estimate their incidence and severity.
3- Selected the dominant isolated virus to study:
   - Symptomatology and host range.
   - Transmission methods (via; mechanical, seeds, insects, etc..).
   - Physical properties.
   - Serological diagnosis using double diffusion and ELISA tests.
   - Cytopathological inclusions.
   - Particle dimensions using TEM.
4- Study the response of some faba bean cultivars to infection with the isolated virus under greenhouse conditions.
5- Evaluate the induced systemic resistance activities of some medicinal plant extracts against faba bean viruses.
2- REVIEW OF LITERATURE

Survey for faba bean viruses:

Surveys, all over the world, were conducted to identify viruses infecting the faba bean (Vicia faba) crop and assessed their incidence and geographical distribution.

In Egypt, a total 88 faba bean fields was surveyed from Middle Egypt (Menia and Beni Suef), Delta Region (Qalyoubia, Menoufia, Sharkia, Gharbia, Dakahlia, Kafr-El Sheikh, Behaira and Noubaria) and Fayoum Governorate (Fayoum district, Tamia, Ebshowi and Senoris), total of 1760 samples of faba bean were collected. The total samples were tested by ELISA in the virology Lab against the different antiserum and found 24.7% from the total samples of faba bean diseased by FBNYV and 41% diseased by BYMV and 3.5% diseased by BBWV. Generally, the major virus problem on faba bean in Egypt was BYMV (Rizkalla, 2002).

In Egypt, serological tests showed that BYMV, an aphid-transmitted and seed-borne virus that was identified in 89% of samples tested, was the most common virus. In most of the fields surveyed, BYMV symptoms were noted to occur at high levels (80–100% infection) (Makkouk et al., 2003).

In Egypt, laboratory tests by DAS-ELISA of 1414 samples with symptoms suggestive of virus infection collected during the 1993 first survey and 1069 similar samples collected in the second survey showed that faba bean necrotic yellows virus (FBNYV) was the most frequently encountered virus (50.6%), followed by bean yellow mosaic potyvirus (BYMV) (24.5%) and broad bean wilt fabavirus (BBWV) (4.6%). Other viruses such as bean leaf roll luteovirus (BLRV), cucumber mosaic cucumovirus (CMV) and alfalfa mosaic alfamovirus (AMV) were less frequently detected (less than 1%). During 1994, FBNYV was again the most common, where it was detected in 62.1% of the 1166 samples tested followed by BYMV (31.2%), AMV (2.5%), BBWV (2%), BLRV (1.7%) and PSbMV (1.1%) (Makkouk et al., 1994).

A collaborative effort between ICARDA scientists and NARS colleagues from Yemen, Egypt, Sudan, and Ethiopia during survey conducted in 1996. Faba bean fields visited were in the governorates of Sana’a, Hajjah, Al-Mahweet, Dhamar, Al-Beida, and Ibb. Disease-symptom data were recorded for each field, and diseased samples were collected for diagnosis. Nearly 15% of the fields visited had a virus disease incidence of
10% or higher. Laboratory tests were conducted at the ELISA laboratory of the Yemeni–German Plant Protection Project at Sana’a using the recently developed tissue-blot immunoassay technique. The most common virus disease on faba bean in Yemen was bean yellow mosaic potyvirus, followed by alfalfa mosaic alfamovirus. The results will help in targeting faba bean improvement efforts against these virus diseases (ICARDA, 1997).

In Poland, Blaszczak and Weber (1978) presented results of experiments in 1972-75 on the effect of bean yellow mosaic virus (pea strain), broad bean common mosaic, white clover mosaic and cucumber mosaic viruses on the growth and seed yield of these hosts.

In Italy, Russo and Rana (1978) isolated bean yellow mosaic and broad bean wilt viruses from globe artichoke plants showing vein yellowing, yellow flecking and line pattern of leaves and identified on the basis of reactions of differential hosts, light and electron microscopy and serology. Both were transmitted non-persistently by aphids. The 2 isolates seem to be different from the strains of these viruses found on broad bean in Apulia.

In Morocco, Fischer (1979) isolated broad bean stain and bean yellow mosaic, economically the most important, was present in 90% of samples. Broad bean wilt and broad bean mottle viruses cause severe disease but their distribution is limited. Alfalfa mosaic and pea early browning viruses are rare in broad bean. The various diseases could not be distinguished from field symptoms but test plant reactions and electron microscopy allowed rapid and conclusive identification. Since both major viruses are seed-transmitted, control methods should concentrate on a seed certification scheme.

Schmidt et al. (1981) identified 18 viruses (52 virus and host combinations) on pea, bean (Phaseolus), soybean, broad bean and lupin during a survey using serology, electron microscopy and test plants. The most important viruses were bean yellow mosaic, pea enation mosaic, pea leaf roll, bean common mosaic and occasionally cucumber mosaic, alfalfa mosaic and broad bean true mosaic.

In Germany, Schmidt (1981) found that, among the 16 viruses identified during 1970-79, bean yellow mosaic isolated from all legume crops, except resistant pea varieties and 5 strains were differentiated. Broad bean true mosaic and broad bean stain were infrequent, mainly on faba bean.
In Portugal, Borges (1982) distinguished, on the basis of host range, behaviour in sap, serology and morphology, between bean yellow mosaic virus and broad bean mottle virus in the faba bean crops.

In the United Kingdom, Bailiss and Senanayake (1984) reported that Bean yellow mosaic, true broad bean mosaic and bean (pea) leaf roll viruses, often prevalent in UK, reduced seed yield in faba bean particularly when plants were infected at the pre- and mid-bloom stage. EAMV and BYMV, but not BLRV, delayed senescence and increased branching on glasshouse-grown plants so that more inflorescences were produced on diseased plants; most of the additional flower buds necrosed.

In Germany, Schmidt (1984) surveyed 63 localities during 1973-80 and showed that the average virus incidence on faba beans to be 22.4% (bean yellow mosaic virus 13.3%, pea enation mosaic virus 5.1%, bean leaf roll virus 3.6% and true broad bean mosaic virus and others 0.5%) and the health index to be 83 (healthy = 100).

In Mexico, Alvarez et al. (1990) tested the interactions of bean yellow mosaic potyvirus, R. solani and F. solani caused greater damage on faba bean plants (variety Criolla) grown in the greenhouse than those of one of the fungi with BYMV. Of each of the pathogens tested separately, the greatest damage was caused by R. solani, followed by F. solani and BYMV.

In India, Bhardwaj et al. (1993) found that, on the basis of host range, behaviour in sap and serology, a mosaic disease of faba bean caused by a member of the potyviruses in Himachal Pradesh, India.

In Morocco, Fortass (1993) conducted a survey covering the main areas where faba bean (Vicia faba L.) is grown in Morocco in 1988 and 1990. From the 240 leaf samples collected on the basis of symptoms suggestive of virus infection from 52 fields, the following viruses were detected by means of electron microscopy, biological indexing, and serology, and their incidence and geographical distribution were assessed: alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), broad bean mottle virus (BBMV), broad bean stain virus (BBSV), broad bean true mosaic virus (BBTMV), pea early browning virus (PEBV), pea enation mosaic virus (PEMV), pea seed-borne mosaic virus (PSbMV), and luteoviruses.

In Hungary, Simay and Beczner (1993) collected a samples during 1982-87 yielded isolates of bean leaf roll luteovirus (identified by symptoms and transmission), broad bean stain comovirus (identified serologically) and bean yellow mosaic potyvirus,
alfalfa mosaic alfamovirus, cucumber mosaic cucumovirus, broad bean true mosaic comovirus and pea enation mosaic enamovirus, identified by serology and hosts.

In the Sudan, Makkouk et al. (1995) conducted a survey of faba beans for virus infection, during February 1994, showed that bean yellow mosaic potyvirus occurred commonly in faba bean crop.

In Western Australia, serials of surveys were conducted to determine the faba bean virus infections. Latham and Jones (1999) sampled thirty three faba bean (100 random shoot tip samples/crop). Samples were sent to the laboratory in South Perth and tested by ELISA with a general potyvirus antiserum, and antisera specific to AMV, BYMV, CMV and PSbMV. Four viruses, BYMV, CMV, AMV and PSbMV, infecting faba bean crops, with BYMV reaching 31% plant infection. Latham and Jones (2000) found that samples from four out of 32 of the faba bean crops reacted with a general potyvirus monoclonal antibody. Upon further investigation three of these proved to be infected with BYMV at infection levels of 11-31% and one was infected with PSbMV (2% of plants).

In Yemen, Makkouk et al. (1998) conducted a survey for viruses affecting faba bean during 1996. Six viruses were found to infect this crop naturally; alfalfa mosaic alfamovirus (AMV), bean yellow mosaic potyvirus (BYMV), pea seed-borne mosaic potyvirus (PSbMV), leaf roll virus (BLRV), faba bean necrotic yellow nanovirus (FBNYV) and chickpea chlorotic dwarf geminivirus CCDV).

In Tunisia, Najar et al. (2003) collected a total of 292 faba bean samples with symptoms of viral infection (leaf rolling, yellowing, and mosaic) from faba bean (Vicia faba L.) in six regions (Beja, Bizerte, Cap-bon, Le Kef, Siliana, and Zaghouan) during survey performed in April 2003. The samples were tested at the virology laboratory of the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, for 11 viruses using the tissue-blot immunoassay procedure. Serological tests showed
that BBMV, a beetle-transmitted and seedborne virus identified in 23.3% (68 samples) of the samples tested, was the most common. BLRV, FBNYV, BWYV, BYMV, SbDV, and PSbMV were detected in 56, 33, 31, 10, 5, and 1 sample(s) of 292 samples tested, respectively.

In Ethiopia, Bekele et al. (2005) undertaken a field surveys to identify the viral diseases affecting faba bean in two regions of Ethiopia during the 2003/2004 and 2004/2005 growing seasons, respectively. The survey covered 48 randomly faba bean in the Amhara region, and 29 faba bean in the Oromia region. Virus disease incidence was determined by laboratory testing of 100-200 randomly-collected samples from each field against the antisera of 12 legume viruses. Serological tests indicated that the most important viruses in the Amhara region were Faba bean necrotic yellows virus (FBNYV), Bean yellow mosaic virus (BYMV), Pea seed-borne mosaic virus (PSbMV) and the luteoviruses [e.g. Beet western yellows virus (BWYV), Bean leaf roll virus (BLRV), Soybean dwarf virus (SbDV)].

In Spain, Ortiz et al. (2006) detected the virus by TAS-ELISA in faba bean ‘Muchamiel’ in the Murcia region, Spain. They noticed that the Spanish FBNYV was 93.75% identical to two previously sequenced isolates of FBNYV from Syria and Egypt. Mixed infections of FBNYV and Tomato spotted wilt virus (TSWV), Bean leaf roll virus (BLRV) and Bean yellow mosaic virus (BYMV) were commonly observed. The necrotic symptoms developed on the leaf borders were more pronounced in these mixed infections.

In Spain, also, broad bean (Vicia faba L.) plants showing symptoms suggestive of viral infection, such as stunting, leaf roll, mosaic, chlorosis, necrosis, and yellowing, were observed. A 4-year field survey showed the presence of five viruses: bean leaf roll luteovirus (BLRV), beet western yellows luteovirus (BWYV), bean yellow mosaic potyvirus (BYMV), tomato spotted wilt tospovirus (TSWV), and cucumber mosaic cucumovirus (CMV). Of the 250 samples assayed, 93 were positive for BYMV, 21 for BLRV, 10 for BWYV, 30 for TSWV, and 2 for CMV. BYMV was distributed in all regions (Fresno et al., 1997).

A field experiment on French bean (Phaseolus vulgaris L.) with six sowing dates of fifteen days interval was carried out during 1994 and recorded the natural incidence of bean yellow mosaic virus disease (aphid borne) under Assam condition. The lowest disease incidence (26.04 percent) was observed in the crop sown at September 1 and
highest disease incidence (79.50 percent) was recorded for November 15 sowing. The natural disease incidence was found 28.25, 39.06, 51.04 and 56.25 percent in crop sown on September 15, October 1, 15 and November 1, respectively. The time taken for the appearance of initial and final disease incidence in the field was found to differ in respect of different sowing dates. The initial disease symptoms appear within 28 days after sowing, in crop sown on October 1, 15 and November 15, and 35 days after sowing on September 1, 15 and November 1 (Permey et al., 1997).

Depending on the previous literature cited and our survey results, Bean yellow mosaic potyvirus was the dominant one at all faba bean surveyed fields. So, this virus will reviewed in details as follows:

**Bean Yellow Mosaic Potyvirus (BYMV).**

- **Name, Synonyms and Lineage**

  Synonym(s): bean virus 2, canna mosaic virus, gladiolus mosaic virus, gloriosa stripe mosaic virus.

  ICTV approved acronym: BYMV. Virus is an ICTV approved species of the genus *Potyvirus*; family *Potyviridae* (ICTVdB, 2006).

**1-Isolation of bean yellow mosaic potyvirus:**

Bean yellow mosaic potyviruses (BYMV) showed be the most dominant one all over the world in the faba bean (*Vicia faba* L.) fields causing the considered losses in the grain yield. This virus was isolated, either alone or combined with other viruses, from faba bean in many countries as follows:

In Lebanon, Makkouk et al. (1982) observed that, in fields with 1-5% plants showing mild to severe mottle or mosaic, only 25% were infected with BYMV. Identification was based on host range, aphid transmission, serology and electron microscopy.

In India, Verma et al. (1999) found that bean yellow mosaic potyvirus (BYMV) naturally infecting *Vicia faba* in Aligarh.

In Italy, Parrella and Castellano (2002) identified the virus as an isolate of bean yellow mosaic virus (BYMV) by biological and serological tests. The same antiserum clearly decorated virus particles from leaf dips. This is the first record of BYMV infection in *Passiflora caerulea*. 
Arneodo et al. (2005) isolated Bean yellow mosaic virus (BYMV) from gladiolus (Gladiolus x hortulanus, cv. Rose Supreme) leaves showed virus-like symptoms, which included leaf chlorotic mosaic and flower colour breaking. The isolated virus was identified by electron microscopy, serology and reverse transcription - polymerase chain reaction.

A virus disease in Masdevallia orchids was reported in Germany affecting orchids imported from the USA; the virus was identified as bean yellow mosaic virus (BYMV) on the basis of host range and serological reactions. Isolation of BYMV has also been reported from Calanthe orchids in Japan (Hammond and Lawson, 1988 and Lesemann and Koenig, 1985).

2-Host range and symptomatology.

Bean yellow mosaic virus (BYMV) infects a wide range of leguminous and non-leguminous plants. In Western Australia, BYMV is a serious pathogen of cultivated lupins, especially narrow leafed and yellow lupins (Lupinus angustifolius, L. luteus) (Wylie and Jones, 1997).

Eid (1983) reported that weeds play a part in the epidemiology of the diseases caused by these viruses, field-collected seedlings of various weed species were mechanically inoculated with one or other of the viruses. Only seedlings of Medicago polymorpha (hispida), Melilotus indica and M. messanensis (sicula) showed any symptoms of BYMV.

On the basis of symptoms, Fortass et al. (1991) isolated suggesting BYMV infected faba-bean plants from Sudan, Syria, and Netherlands and identified as BYMV isolates especially adapted to faba bean. All of them were weakly pathogenic to Phaseolus bean with the exception of SV205 cv., assuming an intermediate position between Phaseolus-bean isolates, with low pathogenicity to faba bean, and faba-bean isolates, usually having low pathogenicity to Phaseolus bean. Strains of BYMV are thus hard to delimit.

Sasaya et al. (1997) collected bean yellow mosaic virus (BYMV) isolate from different host plant species and locations in Japan. BYMV isolate, grouped on the basis of host reactions on Chenopodium amaranticolor, C. quinoa, Nicotiana clevelandii, N. benthamiana, Vicia faba, and Trifolium repens, corresponded to two serotypes
determined by double-antibody sandwich– and triple-antibody sandwich–enzyme-linked immunosorbent assay using three polyclonal and nine monoclonal antibodies.

**Gillaspie et al. (1998)** found that bean yellow mosaic potyvirus (BYMV) do not infect Perfected Wales pea and it produce mosaic, distortion, and necrosis on white lupine. BYMV produced local latent infection of *N. tabacum* and produced mosaic with distortion on *N. benthamiana*.

**Cheng and Jones (1999)** found a new strain of bean yellow mosaic virus (BYMV), a non-necrotic strain, in south-west Western Australia. It differs from the original necrotic strain of BYMV in that it does not kill *Lupinus angustifolius* (narrow-leafed lupin) plants. BYMV causes symptoms of mottle and stunting, or dead growing points, fleshy expanded leaves, and stunting.

**Cheng and Jones (2000)** observed that the six isolates from *Vicia faba* (faba bean) all caused non-necrotic reactions in *L. angustifolius* cv. Danja. These and two necrotic isolates readily infected five genotypes of *V. faba* always causing severe symptoms. However, three non-necrotic isolates from *L. angustifolius* and a further necrotic isolate were poorly infectious on *V. faba* in which they generally induced mild symptoms.

**McKirdy et al. (2000)** studied the host range of isolate MI of bean yellow mosaic virus (BYMV). The most susceptible and sensitive were *Biserrula pelecinus, Trifolium cherleri, T. incarnatum*, and *T. spumosum*. *Ornithopus sativus* was resistant but sensitive, whereas *Hedysarum coronarium* was highly resistant. *H. coronarium* was not infected when manually inoculated repeatedly with 3 different BYMV isolates. Overall, the most susceptible and sensitive crop legume species were *Lens culinaris* (most genotypes), *Lathyrus cicera, L. ochrus*, and *Vicia narbonensis*. *Lathyrus sativus* (3 genotypes only), *V. sativa* (4 genotypes), *Cicer arietinum, Pisum sativum*, and *V. faba* were resistant to isolate MI, and *Lens culinaris* ILL7163 was highly resistant.

**Parrella and Castellano (2002)** observed that, in spring 2000, chlorotic spots and light mottling in the leaves of a blue passionflower plant (*Passiflora caerulea*, family *Passifloraceae*), growing in a private garden of Napoli (southern Italy).
Bellardi and Bianchi (2003) found that, during summer 2002, some plants of Aegopodium podagraria were affected by BYMV which caused yellow and/or necrotic rings on leaves.

Recently, Skelton et al. (2006) isolated and identified bean yellow mosaic virus from Dactylorhiza foliosa (is a hardy orchid species, native to the Island of Madeira) showed symptoms of chlorotic mottle and streaking. Finally the virus was transmitted to two indicator species by mechanical inoculation. Leaf symptoms were observed on Chenopodium quinoa (chlorotic local lesions) and Nicotiana benthamiana (distortion and mosaic). ELISA testing of indicator plants with symptoms confirmed the presence of BYMV.

Bean yellow mosaic has a wide host range in legumes and can readily overwinter in perennial legume crops (e.g., alfalfa, clovers) or weeds (vetch). It also commonly infects gladiolus. The diagnostic symptom of bean yellow mosaic is the bright yellow to green mosaic or mottle appearance of infected leaves, which becomes most apparent on leaves as they become older. Infected leaves also show varying degrees of leaf distortion, down cupping, and wrinkling. Plants infected at a young age may show stunted growth. The striking yellow mosaic symptoms differentiate bean yellow mosaic infections from those of bean common mosaic, which causes light and dark green mosaic patterns of infected leaves (Orellana and Fan, 1978).

The virus infected members of families leguminosae, chenopodiaceae, solanaceae, iridiaceae and compostitae. With a view to identify the causal virus of widely occurring mosaic disease of broad bean, investigations were carried out. Infected plants showed peculiar vein clearing, severe mosaic of broad bean leading to curling and rolling of leaves in addition to reduction in their number and size (Vaid, 1988).

Most commonly used maintenance and propagation host species are Pisum sativum, Vicia faba, Phaseolus vulgaris, Nicotiana clevelandii. Gomphrena globosa reacted as necrotic local lesions; not systemic, Chenopodium amaranticolor, C. quinoa as necrotic local lesions with red border; some systemic chlorotic vein banding and spots, Phaseolus
vulgaris as necrotic or chlorotic local lesions; systemic mosaic, *Pisum sativum* as Perfection types not infected; systemic mosaic in others, *Vicia faba* - mild systemic mosaic. Meanwhile, diagnostic host was insusceptible host species *e.g. Cucumis sativus, Trifolium repens* Scott strain (ICTVdB, 2006).

3- Mode of Transmission:

A- Mechanical transmission:

Koenig (1976) reported that, an isolate of bean yellow mosaic virus from *Gladiolus nanus* showed some peculiarities in its mechanical transmissibility from one host to another. The virus was easily transmitted from *Nicotiana clevelandii* to *Nicotiana clevelandii*, but only with difficulties from *N. clevelandii* to *Vicia faba*. From *V. faba* the virus was easily transmitted to *V. faba* and to *N. clevelandii*. A greatly increased infectivity for *V. faba* was retained after one passage on *N. clevelandii* following a passage on *V. faba*, it was lost, however, after several passages on *N. clevelandii*. Since the "adaptation" to *V. faba* was thus reversible and since single lesion isolates showed the same behavior, a selection of preexisting strains from the original inoculum cannot be the explanation for the observed phenomenon. The most likely explanation is that in *V. faba* variants are produced (induced or spontaneously) and that those variants which are especially well adapted to this host are propagated preferentially. After transfer to *N. clevelandii* other new variants may develop which gradually dilute out those with a high infectivity for *V. faba*.

Lesemann and Koenig (1985) reported that BYMV could be mechanically transmitted to a number of test plants including *Nicotiana clevelandii* and *Vicia faba* which were infected systemically.

Dafallah and Hussein (1994) reported that bean yellow mosaic potyvirus was the most common mechanically transmitted virus detected on faba beans.

Verma et al. (1999) reported that BYMV had a restricted host range and was transmitted mechanically.

Parrella and Castellano (2002) found that BYMV was readily transmitted by inoculation of sap to a range of herbaceous hosts and to healthy seedlings of *Passiflora caerulea*, reproducing the field syndrome.
Raj et al. (2002) inoculated crude sap mechanically on a number of plant species of different families, and inoculated plants were observed for one month for the appearance of symptoms.

Bean yellow mosaic virus showed to be mechanical transmissible readily with the infectious sap (Bos, 1970; Vaid, 1988 and ICTVdB, 2006).

B- Aphid transmission:

Economically important faba bean insect pests include aphids that cause direct feeding damage and transmit plant viruses (e.g., Aphis fabae Scopoli, A. craccivora Koch, Acrithosiphon pisum (Harris), and Megoura viciea Buckton) (Hemiptera: Aphididae), as well as leafhoppers, thrips, moth larvae, leaf mining fly larvae, seed beetles and weevils (Nuessly et al., 2004).

Orellana and Fan (1978) reported that Bean yellow mosaic transmitted by over 20 species of aphids (e.g., the pea, green peach, and black bean aphids). Beans become infected when virus-carrying aphids move into bean fields. Transmission of the virus occurs within seconds once aphids begin feeding on the crop. Aphids can efficiently spread the virus within a field, resulting in high rates of infection. The virus is not known to be seed-transmitted in beans.

Borges (1982) noticed that a virus causing broad bean mosaic was transmitted by Myzus persicae and Aphis fabae and was sap transmissible to Lathyrus ochrus, lupin, pea, clover (Trifolium pratense and T. subterraneum), Chenopodium amaranthicolor, C. quinoa and some bean (Phaseolus vulgaris) cultivars.

Eid (1983) observed that bean yellow mosaic virus (BYMV) is transmitted by Aphis craccivora Koch and Myzus persicae (Sulz.) and cause severe damage to faba bean (Vicia faba) in Egypt.

Nooh (1985) reported that BYMV was transmitted by Aphis gossypii in a non persistent manner. Hammond and Lawson (1988) demonstrated that green peach aphids (Myzus persicae) are able to transmit BYMV from infected Masdevallia to healthy plants of Nicotiana benthamiana. Vaid (1988) reported that BYMV was also transmitted by aphid species viz., Myzus persicae, Brevicoryne brassicae and Aphis fabae.
Karl et al. (1989) noticed that the main aphid transmitted virus of faba bean is bean yellow mosaic potyvirus (transmitted by several species including A. pisum, Myzus persicae and Aphis fabae).

Schmidt and Karl (1989) found that lupin aphid (Macrosiphum albifrons) transmit a faba bean strain (Vf Wu 3/75) of bean yellow mosaic potyvirus from mechanically infected faba bean plants to healthy faba bean [Vicia faba] plants. The average transmission rate was 16.4%. In comparison, the average rate of transmission of the virus by Myzus persicae was 90.0%.

Bhardwaj et al. (1993) found that the virus was transmitted by Myzus persicae, Brevicoryne brassicae and Aphis fabae through sap to some members of the Leguminosae [Fabaceae], Chenopodiaceae, Solanaceae, Compositae [Asteraceae] and Iridiaceae.

Knoxfield (1999) reported that BYMV is spread by several species of aphid including, the Cowpea Aphid Acyrthosiphon pisum, the Green Peach Aphid Myzus persicae and the Black Bean Aphid Aphis fabae. The virus does not persist for long in the aphid. To spread the virus the aphids must acquire it from an infected plant in a short feeding time (minutes) and transfer it to a healthy plant within one hour.

Verma et al. (1999) reported that four aphid species (Aphis fabae, A. gossypii, Brevicoryne brassicae and Myzus persicae) transmitted BYMV non-persistently.

Cheng and Jones (2000) reported that a collection of 51 bean yellow mosaic virus (BYMV) isolates was transmitted from infected Trifolium subterraneum (subterranean clover) to Lupinus angustifolius (narrow-leafed lupin) by Myzus persicae (green peach aphid).

Wylie et al. (2002) compared an isolate of bean yellow mosaic virus (BYMV) not transmitted by aphids (NAT) with the aphid-transmissible isolate (MI) from which it was derived. Loss of aphid transmissibility in isolate BYMV(MI)-NAT was most likely caused by this mutation. Systemic movement and accumulation of the virus in infected plants were not affected by the mutation.
Hampton et al. (2005) observed that colonies of *Myzus persicae* (Sulzer), *Acyrthosiphon pisum* (Harris), and *Aphis fabae* Scopoli associated with virus spread were established in an insectary and shown to vector this virus.

ICTVdB (2006) recorded that virus is transmitted by arthropods, by insects of the order Hemiptera, family Aphididae; more than 20 species including *Acyrthosiphon pisum, Macrosiphum euphorbiae, Myzus persicae, Aphis fabae*. Virus is transmitted in a non-persistent manner.

C- Seed transmission:

The virus is not known to be seed-transmitted in beans (Orellana and Fan, 1978). Also, not through the seeds of soybean (Nooh, 1985). Bean yellow mosaic virus was not transmitted by faba bean seeds (Vaid, 1988). Fidan and Yorganci (1990) inoculated healthy seedlings of faba bean with 13 isolates (8 viruses), and found seed transmission was not demonstrated for alfalfa mosaic virus or bean yellow mosaic potyvirus. Meanwhile, BYMV was transmitted by 3% through *Phaseolus vulgaris* seeds (ICTVdB, 2006).

BYMV is seed transmission with 0.4% rate. This seed-borne strain of BYMV is thought to be different from the normal BYMV strains found in Western Australia, which are not seed-borne in lupins or faba bean (Latham and Jones, 1999).

Fiedorow (1981) noticed that only 0.2% of seeds originating from faba bean plants infected with BYMV gave infected seedlings.

Haack (1990) examined faba bean seeds from different origins, BYMV was detected at very low rates. Results indicate a need for an improved diagnostic test for BYMV.

El-Dougdoug et al. (1999) found that the percentage of seed-borne BYMV using a local lesion host was 0, 3 and 3.2% for smooth, crinkled, and mottled seeds of faba bean (*Vicia faba* var. Giza 674), respectively.

McKirdy et al. (2000) tested seedlings for seed transmission of BYMV, germination on moist paper towels before testing usually proved more effective than
growing in soil in the glasshouse. Low rates of seed transmission of BYMV (0.03–1%) were detected in 9 alternative pasture or forage and 3 alternative crop legume species.

4- Physical properties.

BYMV was inactivated between 55 to 66°C and between the dilutions of $10^{-4}$ to $10^{-5}$. The virus was viable up to 24 but not 36 hours at room temperature (Noooh, 1985).

Borges (1982) observed that the thermal inactivation point was 66 to 68°C, dilution end point $10^{-3}$ - $10^{-4}$ and longevity in faba bean sap 5-7 days.

Thermal Inactivation Point (TIP), Dilution End Point (DEP), and Longevity in vitro (LIV) was recorded 60-65°C, $10^{-4}$-$10^{-6}$ and 3 days at room temperature and 5 days under refrigeration, respectively (Vaid, 1988).

Bhardwaj et al. (1993) mentioned that BYMV had a thermal inactivation point between 60 and 65°C, dilution end point between $10^{-4}$ and $10^{-5}$, and longevity in faba bean sap of 3 and 5 days at room and refrigeration temperature, respectively.

Verma et al. (1999) recorded that BYMV withstood heating upto 55°C for 10 min, dilutions up to $10^{-3}$ and it had a longevity in crude sap at room temperature (25±5°C) of 30 hours and at 4°C for 56 hours.

The thermal inactivation point (TIP) is at 65°C. The longevity in vitro (LIV) is 2-7 days. Although the titer is dependent on the host, the decimal exponent (DEX) of the dilution end point is usually around 3-5 (ICTVdB, 2006).

5. Cytopathological study.

Weintraub and Ragetli (1966) revealed, in the sections of Vicia faba leaves infected with bean yellow mosaic virus, that the presence of three forms of abnormal virus inclusions associated with cytoplasm rich in ribosomes and endoplasmic reticulum. The crystals were also found in the nucleus and nucleolus. The organelles most altered from normal were the mitochondria, the matrices of which became very opaque, while their cristae assumed an electron transparent block-shaped appearance.

Borges (1982) demonstrated that ultra structural characteristics of infected faba bean cells indicate that the virus is identical to bean yellow mosaic virus.

Vaid (1988) reported that cytopathological examination of bean yellow mosaic virus-infected faba bean cells showed presence of pinwheels, scrolls, lamellae tubes as potyvirus cytoplasmic inclusions evidence.
Bellardi and Bianchi (2003) found that examination of ultra thin sections of small symptomatic leaf fragments by electron microscopy revealed the presence of cylindrical inclusions, such as pinwheels with more or less curved long arms and laminated aggregates, typical of subdivision II of Potyviruses.


Makkouk et al. (1982) reported that negative staining of infected leaf extracts revealed flexuous particles 750-800 nm. In immune electron microscopy virus particles were heavily decorated with BYMV antiserum, less with watermelon mosaic virus-2 antiserum and very weakly if at all with WMV-1 antiserum.

In Japan, Uyeda et al. (1982) isolated bean yellow mosaic virus from French beans (Phaseolus vulgaris) caused severe stem and tip necrosis in French bean and faba bean. The purified virus was a flexuous rod 760 nm long.

Vaid (1988) found that electron micrographs showed flexuous virus particles of 790 x 12 nm diameter.

Parrella and Castellano (2002) reported that electron microscope observations of leaf dips from naturally infected plant showed a virus with filamentous particles ca 750 nm in length.

Bellardi and Bianchi (2003) showed that electron microscopic observations of leaf sap (leaf-dip preparations, stained with uranyl acetate and phosphotungstic acid) showed the presence of filamentous virus particles ca. 760 nm in length.

Flexuous rod particles approx. 750 x 11 nm were observed in the electron microscope. The virus was identified as a strain of BYMV (Verma et al., 1999).

Recently, Skelton et al. (2006) reported that subsequent examination of Bean yellow mosaic virus isolated from Dactylorhiza foliosa (is a hardy orchid) by transmission electron microscopy revealed the presence of potyvirus-like particles, measuring ca. 750 nm in length.

ICTVdB (2006) recorded that BYMV virions consist of a capsid. Virus capsid is not enveloped. Capsid/nucleocapsid is elongated with helical symmetry. The capsid is filamentous, flexuous with a clear modal length with a length of 750 nm (longer in presence of divalent cations) and a width of 12-15 nm. Axial canal is indistinct. Basic helix is obvious. Pitch of helix is 3.4 nm.
7. Serological diagnostics.

A- Gel double diffusion (SDS-immunodiffusion) test:

Moghal and Francki (1976) studied antigenic relationships of six distinct potyviruses by immunodiffusion tests using highly purified sonicated virus preparations and anti-intact virus sera devoid of detectable antibodies to host-plant antigens. Three variants of bean yellow mosaic virus (BYMV) including BYMV sensu stricto and two variants of pea mosaic virus (PMV and SPMV) were shown to be antigenically very similar and also relatively closely related to lettuce mosaic virus (LMV). Distant antigenic relationships were detected between the BYMV variants and bean common mosaic virus (BCMV); between BCMV and passion fruit woodiness virus (PWV); and between PWV and potato virus Y (PVY).

Makkouk et al. (1982) stated that Bean yellow mosaic potyvirus was reacted in SDS-immunodiffusion tests with BYMV antiserum.

Parrella and Castellano (2002) reported that in the agar gel SDS-immunodiffusion tests, crude sap from naturally infected plant formed clear precipitin lines with an antiserum to BYMV.

B- Enzyme-Linked Immunosorbent Assay (ELISA):

Nooh (1985) reported that the virus causing MACS-13 soybean mosaic was found serologically related to BYMV but not to SMV, PMV, CAMV, CMMV and SWBDMV. The virus was also not found to be serologically related to SMV and CAMV in ELISA.

Hammond and Lawson (1988) improved the virus detection using appropriate monoclonal antibodies in the indirect ELISA, but best results were obtained with monoclonal antibodies in a dot-blot ELISA on nitrocellulose membranes. The lack of sensitivity of these tests was due to low antigen titer, as the orchid isolates of BYMV and TuMV were readily detected in sap of bioassay plants, and no inhibitory effect of healthy orchid sap was detected.

Gillaspie et al. (1998) observed that indirect-enzyme-linked immunosorbent assay tests with a general potyvirus monoclonal antibody and BYMV and white lupine
mosaic virus (WLMV) polyclonal antisera were strongly positive. Tests of the Sesbania virus against a monoclonal antibody panel suggest that it is not BYMV or any of the previously described subgroup members, but is a member of the BYMV subgroup. This is the first report of a seed-borne BYMV-like virus of Sesbania spp.

Cheng and Jones (2000) found that all necrotic and non-necrotic isolates reacted with BYMV antiserum in ELISA but only two cross-reacted with antiserum to clover yellow vein virus (CYVV). When selected necrotic and non-necrotic isolates were inoculated to differential hosts, all behaved like BYMV and not CYVV. When three isolates of each type were transmitted to 11 other cool season grain legume species, except in Cicer arietinum (chickpea), there were no necrotic reactions, but symptom severity varied with the isolate and species inoculated. The two isolates that caused necrosis in C. arietinum did not do so in L. angustifolius.

Bellardi and Bianchi (2003) identified the virus as an isolate of Bean yellow mosaic virus (BYMV) by serological tests including protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA), immunosorbent electron microscopy (ISEM) and gold-labeling antibody decorations (GLAD).

Recently, Skelton et al. (2006) tested the sample of Dactylorhiza foliosa (is a hardy orchid) by ELISA for several viruses which are known to infect orchids including Tomato spotted wilt virus, Impatiens necrotic spot virus, Cymbidium mosaic virus, Odontoglossum ring spot virus and Bean yellow mosaic virus. Of these viruses, the sample tested was positive only for Bean yellow mosaic virus (BYMV), using a polyclonal-based DAS-ELISA kit (Loewe Biochemica, Germany).

8- Induced systemic resistance against Bean yellow mosaic potyvirus (BYMV) using some medicinal plant extracts:

Potyviruses are very largely a problem of outdoor cultivation even an unscreened glasshouse offers a surprising degree or protection against the introduction and subsequent epidemic spread. As with many other viruses, control depends upon planting healthy material and preventing
the introduction of virus from outside sources. The production and distribution of virus-free planting material has formed the basis for effective control measures in a wide variety of vegetatively propagated crops. Where existing stocks still free from virus cannot be found, the techniques of thermotherapy and meristem-tip culture have been successfully applied, and foundation clones established free from potyviruses and other pathogens (Hollings and Brunt, 1981).

Induction of such systemic resistance by substances from higher plants was first demonstrated by Verma and Mukherjee (1975) in Nicotiana glutinoza after inoculation with TMV and in N. tabaccum after infection with the tobacco ring spot virus previously treated with extract from brinjal (Capsicum melongena) leaves applied 24 hours before virus inoculation. Since then a number of plants have been reported to possess systemic resistance inducing substances, all of which are proteinaceous.

Isolated a single chain ribosome-inactivating proteins from some medicinal plants (Clerodendrum inerme, Dianthus caryophyllus, Gelonium multiflorum, Momordica charantia, Phytolacca americana, Saponaria officinalis, Trichosanthes kirilowii, and Triticum aestivum). These proteins found to be interacted with ribosome function in the infected cell and inhibited viral protein synthesis (Jassim and Naji, 2003). Thus, the first evidence that plants contain inhibitory substances came from virus-infected plants and subsequently the occurrence of virus inhibitory substances was noticed in a number of healthy plants, belonging to different families of Angiosperms, such as Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Nyctaginaceae, Phytolaccaceae, Solanaceae, and Verbenaceae (Cheesin et al., 1995).

Numbers of healthy plants, especially herbaceous species, have
been reported to contain virus inhibitory substances. **Duggar and Armstrong (1925)** reported for the first time in 1925 that the crude extract of pokeweed (*Phytolacca decandra* L.) markedly inhibited the activity of tobacco mosaic virus. **Melander (2004)** found that many plant species contain ribosome-inactivating proteins (RIPs), which have antiviral properties. A RIP from pokeweed (*Phytolacca americana*) was expressed in tobacco and potato, and was shown to confer broad-spectrum virus resistance both when inoculated mechanically and by aphids.

Pokeweed antiviral protein (PAP), a ribosome-inactivating protein (RIP) isolated from the leaves or seeds of *Phytolacca americana*. PAP displays broad-spectrum antiviral activity against plant viruses, inhibiting infection by seven different viruses, each representing a different plant virus group (*Lodge et al., 1993; Barbieri et al., 1997* and *Tumer et al., 1997*). PAP and its nontoxic mutants can directly depurinate brome mosaic virus (BMV) RNA *in vitro*, resulting in reduced viral protein translation (*Picard et al., 2005*).

**Barakat et al. (2005)** revealed that all tested RIPs showed potent antiviral activity against Tobacco Necrosis Virus (TNV) onto *Phaseolus vulgaris* plants, Tobacco Mosaic Virus (TMV) onto *Chenopodium amaranticolor* plants, and Bean Yellow Mosaic Virus (BYMV) onto its systemic host (*Vicia faba* plants).

Chemical analysis of clavillia (*Mirabilis jalapa*) was rich in many active compounds including triterpenes, proteins, flavonoids, alkaloids, and steroids. Purified an antiviral proteins from roots, shoots, leaves, fruits, and seeds of *Mirabilis jalapa* are employed for different affections. Thus, information about the reproductive pattern of this culture is important for implementing experimental procedures (*Leal et al., 2001*). MAPs in clavillia as being effective in protecting economically-important crops (such as tobacco, corn, and potatoes) from a large variety of plant
viruses (such as tobacco mosaic virus, spotted leaf virus and root rot virus) (Vivanco et al., 1999).

*Mirabilis jalapa* (Nyctaginaceae), containing a ribosome inactivating protein (RIP) called *Mirabilis* antiviral protein (MAP), against infection by potato virus X, potato virus Y, potato leaf roll virus, and potato spindle tuber viroid. Root extracts of *M. jalapa* sprayed on test plants 24 h before virus or viroid inoculation inhibited infection by almost 100%, as corroborated by infectivity assays and the nucleic acid spot hybridization test (Vivanco et al., 1999). They also, isolated *mirabilis* antiviral protein (MAP) from roots and leaves of *Mirabilis jalapa* L. which possess repellent properties against aphids and white flies. MAP showed antiviral activity against mechanically transmitted viruses but not against aphid transmitted viruses. MAP was highly effective in inhibiting TSWV at 60% saturation. A minimum concentration of 400µg/ml of MAP was sufficient to inhibit TSWV. (Devi et al., 2004).

β-farnesene volatiles emitted by the whole plant as well as by detached flowers of *Mirabilis jalapa*. Most remarkable were findings that assigned the use of β-farnesene as an alarm pheromone for aphids. By taking advantage of the aphid alarm signal, plants are able to repel herbivores as reported for the wild potato *Solanum berthaultii* (Effmert et al., 2005).

*Clerodendrum inerme* contain basic protein which resistant to proteases. Induces systemic resistance reversible by actinomycin D inhibits infectivity of many plant viruses (Verma et al., 1991).

Two systemic antiviral resistance-inducing proteins, namely CIP-29 and CIP-34, isolated from *Clerodendrum inerme* leaves, for ribosome-inactivating properties. CIP-29 has a polynucleotide: adenosine glycosidase (ribosome-inactivating protein), that inhibits protein synthesis both in cell-free systems and, at higher concentrations, in cells, and releases adenine from ribosomes, RNA, poly(A) and DNA. As compared with other known RIPS, CIP-29 deadenylates DNA at a high rate, and induces systemic antiviral resistance in susceptible plants (Olivieri et al., 1996).

The *Clerodendrum aculeatum*-systemic resistance inducing (CA-SRI) protein, a 34 kDa basic protein, plays a key role in inducing strong systemic resistance in susceptible plants against various plant viruses (Kumar et al., 1997).
Some plants synthesize enzymes that have antiviral activity and exhibit catalytic activity similar to that of the A chain of ricin. These plants include carnations (*Dianthus caryophyllus*) (*Houston et al.*, 1983).

Two proteins (dianthin 30 and dianthin 32) were isolated from the leaves of *Dianthus caryophyllus* (carnation). They act by damaging ribosomes in a less-than-equimolar ratio. Protein synthesis by intact cells is partially inhibited by dianthins at a concentration of 100µg/ml. Dianthins mixed with tobacco-mosaic virus strongly decrease the number of local lesions on leaves of *Nicotiana glutinosa*. They propose to name dianthin 30 and dianthin 32 on the basis of their respective molecular weights. Like the known 'A-chain-like' proteins, dianthins inhibit protein synthesis in a cell-free system by damaging ribosomes, but have little effect on whole cells. They also have strong inhibitory activity on the replication of tobacco-mosaic virus (*Stirpe et al.*, 1981).

A long-known virus inhibitor from carnation leaves has now been shown to be an inducer of systemic resistance to virus as well. Thus, it is possible that many of the other well-known virus inhibitors of plant origin may in fact be proven to be inducers of systemic resistance. While working on resistance induced by leaf extracts from carnation plants, observed that a very short period was required to the induced antiviral state in host tissue (*Ostermann et al.*, 1987).

Pyrethrin (*Pyrethrum*) is produced in the flowers of *Chrysanthemum cinerariaefolium* and is the forerunner of the synthetic pyrethroid insecticides. Pyrethrin is labelled against a large number of pests. An addendum to the label for one formulation of pyrethrin showed it to be moderately to highly effective (61-100% control) against the following pests of fruit: grape leafhopper, potato leafhopper, leaf curl plum aphid, blueberry flea beetle, blueberry thrips and blueberry sawfly. It is also effective against cranberry fruitworm. It is quickly broken down in the environment and may be used up to and including the day of harvest (*Kain and Kovach*, 1997).
3- MATERIALS AND METHODS

1- Survey for faba bean viruses:

Two restricted surveys for faba bean viruses was conducted during February and April 2004 in some districts at Qalyoubia Governorate (i.e., Banha, El-Qanater El-Khayria, Kafr Shoukr, Shebien El-Qanater and Toukh) to identify viruses infecting the faba bean (*Vicia faba*) crop. Faba bean plants showing symptoms suggestive of viral infection, such as stunting, vein clearing, leaf roll, mosaic, chlorosis, necrosis, yellowing and leaf distortion were visually inspected.

Symptomatic leaves [430 leaf samples, February 2004 (A) and 355 leaf samples, April 2004 (B)] naturally infected with many viral diseases were collected randomly from 5 different locations ([Table, i](#)). Disease incidence and severity was calculated separately for each survey as follows:

\[
\text{Disease incidence} = \frac{\text{Number of infected plant per location} \times 100}{\text{Total number of location}}
\]

\[
\text{Disease severity} = \frac{\text{Area of plant tissue affected by disease} \times 100}{\text{Total area}}
\]

**Table (i):** A total symptomatic collected leaf samples from faba bean fields in 5 different locations (Qalyoubia Governorate).

<table>
<thead>
<tr>
<th>Locations</th>
<th>Number of collected samples</th>
<th>February 2004</th>
<th>April 2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banha</td>
<td>70</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>El-Qanater El-Khayria</td>
<td>60</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Kafr Shoukr</td>
<td>80</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Shebien El-Qanater</td>
<td>100</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Toukh</td>
<td>120</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>430</strong></td>
<td><strong>355</strong></td>
<td></td>
</tr>
</tbody>
</table>

On the basis of symptoms, clearly viral symptomatic leaves were arranged in different 5 categories ([Plate, 1](#)) and tested at the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, for six faba viruses [*Faba bean necrotic yellows virus* (FBNYV); *Bean yellow mosaic virus*, (BYMV); *Broad bean stain virus* (BBSV); *Alfalfa mosaic virus* (AMV); *Broad Bean Wilt virus* (BBWV) and *Pea seed borne mosaic virus* (PSbMV)] using double antibody sandwich- enzyme-linked immunoassay (DAS-ELISA) according to [Makkouk et al. (2003)](#).
Plate (1): Symptomatic sample leaves showed naturally typical viral infection collected from different inspected faba fields during April 2004. (1: severe mosaic with blisters, downward leaf cup, 2: leaf narrow, mosaic with green vein banding, 3: vein clearing, bright yellowing, green vein banding, 4: severe malformation, mosaic and blisters and 5: severe mosaic with vein chlorosis).

2- Isolation and identification of the isolated virus.

The virus isolate used in this study was obtained from naturally faba bean symptomatic leaf samples showing stunting, vein clearing, leaf roll, mosaic, chlorosis, necrosis, bright yellowing and leaf distortion grown in different areas at Qalyoubia Governorate.

Infectious sap was prepared by grinding infected leaves with mortar and pestle [in the presence of 0.02 M phosphate buffer of pH 7.0, and to prevent oxidation of polyphenols, 1% sodium EDTA (ethylenediaminetetra-acetic acid)]. Extracted sap was strained through two layers of cheesecloth.

The obtained crude sap from each group was separately used for
mechanical inoculation of 600 mesh-dusted carborundum-leaves of *Chenopodium quinoa* Willd., *C. amaranticolor* Coste & Ryn. leaves as diagnostic host plants. Inoculation was carried out by rubbing of tested plants with the forefinger dipped in inoculum then rinsed with tap water. All inoculations were performed in an insect-proof greenhouse maintained at approximately 25°C and examined for external symptoms (within 20 days).

Chlorotic local lesions were observed on *C. quinoa* (15-20 days after inoculation). Single local lesions (showed the same color, the same pattern, the same size and on the same leaf) were cut out from inoculated leaves and macerated with few drops of buffer on a glass slide and inoculated onto the aforementioned diagnostic host as described by Basu and Giri (1992). Resultant extracts from the reformed local lesions on *C. quinoa* were used to inoculate tobacco (*Nicotiana clevelandii*) plants which served as the source of virus inoculum throughout this study.

3- Host range and symptomatology:

To study the host range of this virus isolate, twenty six plant species and cultivars belonging to five families were tested under Plant Pathology greenhouse conditions, Fac. Agric., Moshtohor, Banha University (Table, ii).
Table (ii): Plant species and cultivars used in studying host range of the isolated virus.

<table>
<thead>
<tr>
<th>Families</th>
<th>Host plant</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthaceae</td>
<td>Gomphrena globosa L.</td>
<td>Globe amaranth</td>
</tr>
<tr>
<td>Chenopodiaceae</td>
<td>Chenopodium quinoa Willd.</td>
<td>Quinoa</td>
</tr>
<tr>
<td></td>
<td>C.amaranticolor Coste &amp; Ryn.</td>
<td>Lamb’s-quarter</td>
</tr>
<tr>
<td></td>
<td>C. album L.</td>
<td>Lamb’s-quarter</td>
</tr>
<tr>
<td>Cucurbitaceae</td>
<td>Cucumis sativus L.</td>
<td>Cucumber</td>
</tr>
<tr>
<td></td>
<td>Cucurbita pepo L.</td>
<td>Squash</td>
</tr>
<tr>
<td>Leguminosae</td>
<td>Cicer arietinum</td>
<td>Chickpea</td>
</tr>
<tr>
<td></td>
<td>Phaseolus vulgaris L.</td>
<td>Kidney bean</td>
</tr>
<tr>
<td></td>
<td>Vigna unguiculata L.</td>
<td>Cowpea</td>
</tr>
<tr>
<td></td>
<td>Vicia faba L.</td>
<td>Broad bean</td>
</tr>
<tr>
<td></td>
<td>Lupinus albus</td>
<td>Lupine</td>
</tr>
<tr>
<td></td>
<td>Pisum sativum L.</td>
<td>Pea</td>
</tr>
<tr>
<td></td>
<td>Medicago sativa L.</td>
<td>Fenugreek</td>
</tr>
<tr>
<td></td>
<td>Lintus sativum L.</td>
<td>Lens</td>
</tr>
<tr>
<td></td>
<td>Trifolium hybridum</td>
<td>Clover</td>
</tr>
<tr>
<td>Solanaceae</td>
<td>Capsicum annuum L.</td>
<td>Pepper</td>
</tr>
<tr>
<td></td>
<td>Datura metel L.</td>
<td>Thorn apple</td>
</tr>
<tr>
<td></td>
<td>D. stramonium L.</td>
<td>Jimson weed</td>
</tr>
<tr>
<td></td>
<td>Lycopersicum esculentum</td>
<td>Tomato</td>
</tr>
<tr>
<td></td>
<td>Nicotiana clevelandii</td>
<td>Tobacco</td>
</tr>
<tr>
<td></td>
<td>N. tabacum L., cvs. Samsun</td>
<td>Tobacco</td>
</tr>
<tr>
<td></td>
<td>N. rustica L.</td>
<td>Tobacco</td>
</tr>
<tr>
<td></td>
<td>Petunia hybrida Vilm.</td>
<td>Petunia</td>
</tr>
<tr>
<td></td>
<td>Physalis floridana Ryd.</td>
<td>Ground-cherry</td>
</tr>
<tr>
<td></td>
<td>Solanum melongana L.</td>
<td>Egg-plant</td>
</tr>
<tr>
<td></td>
<td>Solanum nigrum L.</td>
<td>Nightshade</td>
</tr>
</tbody>
</table>

All test plants were grown from seed in a mixed soil (clay: peat : sand 1:1:1 v/v/v), fertilized weekly (Crystalon 20N: 20P : 20K) and regularly irrigated. The virus inoculum was prepared by grinding virus infected leaves in 0.01 M phosphate buffer, pH 7.0 containing 0.2% 2-mercaptoethanol at 10⁻¹ dilution. The inoculum was rubbed with muslin cloth pad to test plants dusted with 600-mesh carborundum. Inoculated and subsequently developed leaves were back inoculated to C. quinoa to confirm the virus infection under greenhouse conditions, development of symptoms for 4 weeks were observed for 4 weeks.
Four test plants sown in each clay pots (Ø 25 cm) were mechanically inoculated at the cotyledonary or four- to eight- leaf stage (according to the species) and were kept in insect-proof greenhouse. All plants not showing symptoms were tested 3 to 4 weeks after inoculation by back-inoculation to C. quinoa. Uninoculated plants were included as controls (Walkey, 1991).

Faba bean cultivars seeds were purchased from Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt and other seeds of the tested plants used were obtained kindly from Agronomy and Horticulture Departments, Faculty of Agriculture, Moshtohor, Banha Univ.

4. Mode of transmission:

A- Mechanical transmission:

The mechanical transmissible of the virus isolate was assayed by sap inoculation, using 0.1 M phosphate buffer, pH 7.0 as extraction buffer, 1% sodium EDTA (ethylenediaminetetra-acetic acid) to prevent oxidation of polyphenols and carborandum as an abrasive, on some host plants such as: Faba bean (Vicia faba), Chenopodium amaranticolor, C. quinoa, Nicotiana clevelendii, Pisum sativum and Phaseolus vulgaris.

Mechanical inoculation was performed by rubbing sap from leaves of infected source plant, with wet forefinger on the cotyledonary or true leaves of test plants (according to plant species). Immediately after inoculation the test plant leaves were washed with distilled water using an atomizer. Inoculated plants were kept in insect-proof greenhouse and visually inspected for typical viral symptoms.
B- Aphid transmission:

Pure identified colonies of both black bean aphid (*Aphis fabae* Scopoli) and green peach aphid (*Myzus persicae* Sulzer) were kindly obtained from Economic Entomology Branch, Plant Protection Department, Faculty of Agriculture, Moshtohor, Banha University.

These species were chosen as most dominant aphids in the faba bean field usually and related as the main faba-virus vectors.

Individual colony of each kept in the insect proof and reared on healthy cabbage seedlings (*Brassica oleracea* L. subsp. *oleracea*) until fourth instar nymph was appeared.

Separately homologous colony of apterus adults of both aphids were collected to evaluate as the isolated virus vectors.

Twenty-five of both aphids were starved for 2 hours (inside shaded Petri-dishes), allowed to acquisition feeding for 2 min on viral-infected leaves of faba bean plants, then transferred to 5 healthy faba bean seedlings (five aphids per seedling) for inoculation, feeding period of 24 hours.

For the control, the same procedure was used, but virus-free aphids where feeding for acquisition on healthy faba bean plants.

The inoculated seedlings were then sprayed with the insecticide malathion (0.1%). Symptoms and percentage of transmission were recorded 4 weeks after inoculation.

C- Seed transmission:

Seed transmissibility was evaluated through regular visually inspected of 50 seedlings resulted from faba bean seeds harvested from previous mechanically inoculated plants with the tested isolated virus for systemic viral symptoms.
For testing seed transmission of the isolated virus, seeds were germinating on moist paper towels and resulted seedlings grown in the pots in the greenhouse (McKirdy et al., 2000).

First four true leaves of immerged seedlings were grinding in the presence of 0.02 M phosphate buffer of pH 7.0, with mortar and pestle. Extracted sap was strained through two layers of cheesecloth.

Inoculation was carried out by rubbing of tested plants with the forefinger dipped in inoculum then rinsed with tap water. All inoculations were performed in an insect-proof greenhouse maintained at approximately 25°C and examined for external symptoms (within 20 days).

Seedlings were examined at regular intervals for one month to detect the development of any systemic or local symptoms.

5- Physical properties:

Crude sap from systemically infected leaves of tobacco (Nicotiana clevelandii), was used to study the thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) according to the method described by Fox (1993). C. quinoa was used as local lesions host plant.

a- Thermal Inactivation Point (TIP):

To determine the thermal inactivation point of the isolated virus, two ml of the infected sap pipette separately into each specimen tube. The tubes were heated for 10 minutes in a thermostatically controlled water-bath at the required temperature, i.e. 50, 55, 60, 65, 70, 75, and 80°C. The tubes were then immediately cooled by dipping in cold water. One tube of each infected sap was left without heating for comparison. Treated and untreated saps were used to inoculate five leaves of C.
quinoa, which were previously dusted with 600-mesh carborundum. The experiment was repeated three times and local lesion numbers were recorded after 21 days.

b- Dilution End Point (DEP):

Infectious sap of Nicotiana clevelandii leaves was diluted with distilled water. Several dilutions from $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$ and $10^{-7}$ were prepared. Each particular dilution was mechanically inoculated on five leaves of C. quinoa plants. The experiment was repeated three times and average numbers of local lesions were estimated.

c- Longevity in vitro (LIV):

To determine the in vitro stability of the isolated virus, infected sap extracted from Nicotiana clevelandii leaves was placed in sterilized small tubes (without any additives). The tubes were plugged and kept at room temperature ($25^\circ$C). Infectivity of virus isolate was evaluated up to 10 days through inoculation on C. quinoa. Numbers of local lesions were determined.

The all three experiments were repeated three times and average numbers of local lesions were estimated.

6- Cytopathological study:

a- Light microscopy

Stained epidermal strips (with the combination of Calcomine Orange and Luxol Brilliant Green dyes) obtained from faba bean leaves inoculated and non-inoculated with BYMV were examined by light microscopy to facilitate detection of inclusions. Cytoplasmic inclusions
photograph obtained after light microscope examination (1000 X magnification) (Edwardson and Christie, 1991).

b- Electron Microscopy:

Leaf-dip preparation of endemic isolates was used to determine virion morphology and size. Formvar carbon-coated 300-mesh grids were attached to sap drop squeezed from systemic infected faba bean leaf petiole, left for 5 min to dry, rinsed twice with distilled water. Grids negatively stained for 3 min with 2% aqueous uranyl acetate, pH 4.0, and air-dried and examined at a magnification of 60 to 80 KV on the Transmission Electron Microscopes JEOL (JEM 100 cxII & JEM 5A), Electron microscope Unit at Assiut University.

Particle size was determined by measuring the dimension of 70 virions on photographic plates and calculating the modal length and width according to method described by Damsteegt et al. (1999).

7- Virus Purification:

The isolated virus was purified partially by the following procedure. Systemically infected leaves of Nicotiana clevelendii L., harvested 25 days after inoculation, were homogenized with 0.1 M potassium phosphate buffer, pH 7.0, containing 0.3% 2-mercaptoethanol (1:1 w/v) in a Waring Blendor. The homogenized extract was strained through two layers of cheesecloth and clarified by adding 4% ethanol plus 4% CCl₄, followed by slow-speed centrifugation. The extract was stayed overnight, and the slow-speed centrifugation was repeated, if necessary. The virus was precipitated from the clarified juice by dissolving 0.5 M NaCl + 6% (w/v) of polyethylene glycol mol. wt. 6000, incubating the solution for 30 min and collecting the precipitated virus by low-speed centrifugation. The pellets were resuspended
in the original buffer (1/10 initial volume) and subjected to one or two cycles of differential centrifugation with suspension of the high-speed pellets in the same buffer. High-speed centrifugations were made for 45 min at 40000 rpm. Low-speed centrifugations were made in a Sorvall refrigerated centrifuge for 20 min at 7000 rpm in the SS-34 rotor.

Virus purification was performed at the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, according to method described by Gillaspie et al. (1998).

8- Serological Studies:

Antiserum preparation:

Specific antiserum against the isolated virus was produced by using the purified virus preparation. Two healthy white New Zealand rabbits were injected with purified virus emulsified 1 : 1 (v/v) in Freund’s complete adjuvant. Four injections (500 µg of the virus for first intramuscular injection, and 250 µg for three subsequent injections) were used at weekly intervals. Animal was bled two times after 21 and 27 days from last injection. After removal from the rabbit, the blood is allowed to clot overnight at (37°C) and the serum is carefully separated from the clot. The serum is then centrifuged at low speed 3,000 rpm for 5 min to remove any remaining corpuscles, and the resulting supernatant maintained. Stored specific antiserum against the isolated virus was subjected to serological tests. The binding of antibodies in the serum to the antigen was assayed with goat anti-rabbit immunoglobulin (IgG) conjugated to alkaline phosphatase to use for ELISA tests (Walkey, 1991).
**a- Gel double diffusion (SDS-immunodiffusion) test:**

In this test the antibody-antigen reaction is carried out in a gel agarose plates. The reactants are allowed to diffuse through the gel and combine.

Medium consists of 0.8-g agarose, 0.85 g NaCl and 0.5 g Sodium dodecyl sulphate (SDS) were dissolved in 100-ml phosphate buffer. Bring to the boil and allow cooling to approximately 50°C then 0.02-g sodium azide (NaN₃) was added. Medium maintains in water bath to prevent setting. Pipette about 15 ml of media onto new clean plastic Petri dishes (10 cm φ). Allow medium to solidify, standing dishes on a level surface.

The pattern in the medium plates consisted of 6 peripheral wells (5 mm φ each) around a central well (6 mm φ), with 5-mm distance between the closest edges of the center and peripheral wells. Both antibody (antiserum) and antigen (partial purified virus), were diluted with phosphate buffer at the same dilutions. Appropriate amounts of each were pipette into wells in the prescribed pattern, and arrangement was recorded.

Antisera against three *potyviruses* (*i.e.*, Bean yellow mosaic virus (BYMV), kindly obtained from (AGERI, Egypt); Pepper severe mosaic *potyvirus* (PSMV), Argentina (J.M. Feldman); Potato Y virus (PYV), Germany (Hans L. Weidemann). In addition to two *Tobamoviruses* antisera, Pepper mild mottle virus (PMMV) and Tobacco mosaic virus (TMV) previously obtained from Holland (E.Z. Maat) was used in the gel diffusion test. Serological studies used in this work were:

Plates were incubated in the humid chamber at room temperature for 1 week. Precipitin resulting bands (spurs) were photographed without staining and results were recorded (*Raj et al., 2002*).

Gelrite® [Merck & Co., Inc. (Rahway, NJ), Kelco Division, USA], is an agar substitute produced from a bacterial substrate producing a clear high strength gel at 0.3g/L was substituted with 0.8g/L agarose as solidification material. In addition, 0.02g/L sodium azide (NaN₃) was
replaced with 0.1% Kombucha (green fermented tea) as an antimicrobial which more safe and cheap.

b- Enzyme-Linked Immunosorbent Assay (ELISA):

Naturally and artificially viral infected faba bean samples directly detected with the double antibody Sandwich-enzyme Linked immunosorbent assay (DAS-ELISA) using antisera specific to 6 faba-related viruses (e.g., FBNYV, BYMV, BBSV, AMV, BBWV, PsbMV) as described by Clark and Adams (1977) and McLaughlin et al. (1984) as follows:

1- Coating microtiter (ELISA) plates:-
   a. Dilute 100 µl IgG in 100 ml coating buffer.
   b. Add 200 µl of this mixture to each well.
   c. Incubate for 3 hours at 37°C.
   d. Wash the plate 3 times with washing buffer (PBST).

2- Add 200 µl aliquots of the test sample (1g fresh infected tissue/10 ml of extraction buffer or sample buffer).

3- Incubate overnight at 4°C.

4- Wash the plate 3 times with washing buffer (PBST).

5- Dilute 100 µl conjugated IgG in 100 ml conjugated buffer and add 200 µl to each well.

6- Incubate at 37°C for 3 hours.

7- Wash the plate 3 times with washing buffer (PBST).

8- Prepare substrate solution immediately before use by adding p-nitrophenyl-phosphate at 0.75 mg/ml to the substrate buffer, add 200 µl to each well.

9- Incubate at room temperature for one hour to observe reaction (development of different intensities of yellow colour).

10- Stop reaction by adding 50 µl of 3 M NaOH to each well.

11- Assess results by:
   a. Visual observation
   b. Measurement of absorbance at 405 nm.
9- **Response of some faba bean cultivars to infection with the isolated virus under greenhouse conditions:**

A greenhouse-pot experiment was conducted to determine the response of some commercial faba bean cultivars to mechanical inoculation with the tested isolated virus. It was carried out under greenhouse conditions at Fac. Agric., at Moshtohor. Six faba bean cultivars (Sakha 1, Misr 1, Giza 2, Giza 3, Giza 716 and Giza 843) obtained from the Egyptian Agricultural Organization, Ministry of Agriculture, Cairo, Egypt were used. Twenty-five faba bean plants (each cultivar) were sown (5 plants/pot, 5 pots/cultivar) served as replicates for virus inoculation. The same numbers of faba bean plants from each cultivar were inoculated with double distilled water served as control to each treatment. All test plants were grown from seed in a mixed soil (clay: peat: sand 1:1:1 v/v/v), fertilized weekly (crystalon 20N: 20P: 20K) and regularly irrigated. Four true leaves stage faba bean seedlings were mechanically inoculated. The plants were observed and the systemically infected plants were counted until consistent numbers were reached. The percentages of infection of each cultivar per block were calculated according to the following equation:

\[
\text{Infection (\%)} = \frac{\text{No. of systemically infected plants}}{\text{Total No. of inoculated plants}} \times 100
\]

10- **Evaluate the induced systemic resistance activities of some medicinal plant extracts against faba bean viruses.**

- **Preparations:**

  Fresh plant parts of 6 medicinal plant [belonging 6 families] Table (iii) were collected from the botanical garden of the Faculty of
Agriculture, Moshtohor, Banha University. Fresh plant material was washed under running tap water.

Four healthy medicinal plants were chosen depending on previous information dealing with their systemic resistance inducers as producers for ribosomal inhibitor proteins (RIPs) such as: *Clerodendrum inerme* (Kumar et al., 1997), *Dianthus caryophyllus* (Stirpe et al., 1981), *Mirabilis jalapa* (Leal et al., 2001) and *Phytolacca americana* (Melander, 2004 and Barakat et al., 2005).

On the other hand, *Chrysanthemum cinerariaefolium* and *Schinus terebinthifolius* were tested as antiviral or insecticidal activities.

Stock aqueous crude extraction (botanical) for each individual tested plant (Table, iii) was made by blending 1 kg plant material in 1 liter distilled water. Then filtered through 8 layers of muslin cloth and centrifuged at 3000 rpm for 20 min. The supernatant was collected and stored in the refrigerator at -20°C until use.

From each stock extract, three dilutions were made (i.e., 2, 5, 20%) using distilled water.

The isolated virus inoculum was prepared by triturating 1 g of young viral symptomatic faba leaves cv. “Giza 2” in sterilized mortar by adding 20 ml (1:20 wt/vol) of sterile distilled water. The extract was filtered through muslin cloth then centrifuged for 20 min at 3000 rpm. The supernatant was used as inoculum.
Table (iii): Properties of selected medicinal plants used for antiviral activity against the isolated virus infection.

<table>
<thead>
<tr>
<th>Picture</th>
<th>Family: Nyctaginaceae</th>
<th>S. N.: Mirabilis jalapa L.</th>
<th>C. N.: Mirabilis</th>
<th>A. I.: Antiviral proteins (MAPs) and others</th>
<th>U. P.: Young shoots &amp; roots</th>
<th>Picture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phytolaccaceae</td>
<td>Phytolacca americana</td>
<td>Pokeweed</td>
<td>Pokeweed antiviral protein (PAP)</td>
<td>Leaves &amp; roots</td>
<td></td>
</tr>
</tbody>
</table>

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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Verbenaceae</td>
<td>Clerodendrum inerme (L.) Gaertn.</td>
<td>Seasid clerodendrum</td>
<td>Actinomycin D, Quinine, glycoside ester (Verbascoside)</td>
<td>Leaves</td>
<td></td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Asteraceae</td>
<td>Chrysanthemum cinerarifolium L.</td>
<td>Marguerite</td>
<td>Flavonoids, Pyrethrin</td>
<td>leaves</td>
<td></td>
</tr>
</tbody>
</table>

S.N.= Scientific Name, C.N.= Common Name, A.I.= Active ingredients, and U.P.= Used Parts.


**Greenhouse experiment:**

**Preliminary trial**

During August 2004, crude extracts of selected medicinal plants adjusted as 1:5 w/v and sprayed using Pressure Sprayer (2L) on ten *C. quinoa* leaves for each treatment without any dilutions 24 hours pre-inoculation with the isolated virus.

Leaves of *C. quinoa* dusted with Carborundum, 600-mesh, as an abrasive, mechanically inoculated with virus inoculum.

Total number of local lesions, from ten leaves for each treatment, was counted 14 days after virus inoculation. Efficiency of botanical sap in the inhibition of the isolated virus infection determined as local lesion formation on the indicator plant (*C. quinoa*).

\[
\text{Inhibition} \% = \left( \frac{A - B}{A} \right) \times 100
\]

where:

\[A = \text{Total number of local lesion on untreated plants with botanicals and inoculated with the isolated virus.}\]

\[B = \text{Total number of local lesion on treated plants with botanicals and inoculated with the isolated virus.}\]

**Avermently trial**

Plastic pots (Ø 25 cm) filled with suitable amount of sand-clay-loamy formalin sterilized soil mixture.

Ten seeds were sown per pot, and then thinned to 3 seedlings at 15 days after sowing. The sowing date was October 1 2004 and the experiment was conducted for about 45 days. Pots were irrigated with tap water whenever necessary but in equal amounts.
NPK fertilizers were applied at rates of 0.6 g of urea/pot, 0.75 g of Ca-super-phosphate/pot, and 0.25 g of K-sulphate/pot. Phosphorus was added during soil preparation (i.e. before sowing). Each of N and K were applied, in 2 equal doses, at thinning and 2 weeks after thinning.

Four-hundred twenty faba bean plants were divided into ten groups (15 plants for each in 5 pots), nine of them sprayed 24 hours pretreatment (virus-inoculation) with three concentrations of six tested medicinal plants. The last group (15 plants) served as control, pretreated with water and subsequently inoculated with the sap from virus-infected plants.

After one week from thinning and before 24 hours from virus inoculation, each treatment were separately sprayed, using Pressure Sprayer (2L), with the three previously concentrations from each botanical sap (i.e., 2, 5, 20%).

Primary four leaves of faba bean plants dusted with Carborundum, 600-mesh as an abrasive, mechanically inoculated with virus inoculum.

Treated plants were kept under insect proof house and observed for the appearance of local lesion formation.

Inhibitory effects of the tested medicinal plants were determined in both trials as described by Devi et al. (2004) using the following equation:

\[
\text{Inhibition} \% = \frac{A - B}{A} \times 100
\]

**where:**

- **A =** Total number of systemic infected plants untreated with botanicals and inoculated with the isolated virus.

- **B =** Total number of systemic infected plants treated plants with botanicals and inoculated with the isolated virus.
**Field experiment:**

Two field experiments were carried out in two consecutive seasons, with different sowing dates (first in October 15 2004 and second in November 16 2005) at Vegetable Farm, Faculty of Agriculture, Moshtohor, Banha University, on a homogeneous clay loamy soil. The cultivar Giza 2 was evaluated under six botanical spray regimes. These spray regimes were designed to provide an antiviral systemic resistance inducing activity.

According to **Abou El-Nour (2002)** the experimental design was randomized complete block with four replicates. The experimental plot size was 3.5 m long and 3 m wide. Each plot contained 5 ridges of 60 cm width. Seeds of faba bean cv. Giza 2 were secured from Seed Propagation Station, Ministry of Agriculture each year and sown at the rate of 75 kg/fed. (25 viable seeds/m²) on November 20 and 26 in 2004 and 2005 seasons, respectively. The crop was uniformly fertilized with 31 kg P₂O₅/fed in the form of single superphosphate (15.5% P₂O₅) during seed bed preparation and 20 kg N/fed in the form of ammonium nitrate (31%N) applied before the second irrigation. However, the soil potassium treatments were applied after 50 days from sowing. The supplemental foliar feeding with potassium was applied in two foliar sprays at rate of 2.5% potassium sulphate at 85 and 100 days from sowing.

The commonly known “Afear” method of sowing was used in which the soil was dried and the dry seeds drilled on the two sides of the ridge, then soil was irrigated. After emergence, seedlings were thinned to two plants per hill. Distance between hills was 20 cm.

Each replicate (4 plots) sprayed three times, 15, 30 and 45 days after sowing date, using Compression Sprayer (8L), with one of 20%...
botanical saps. Control plots sprayed, at the same three times with tap water.

Fourteen days after each spray, percentage of virus systemic infected plants to healthy one per replicates was recorded. Efficiency of the botanical treatments calculated as inhibitory percentage according to the method of Devi et al. (2004) using the following equation:

\[
\text{Inhibition} \% = \frac{A - B}{A} \times 100
\]

where:
- \( A \) = Total number of systemic infected plants (untreated with botanicals + virus inoculated).
- \( B \) = Total number systemic infected plants (treated with botanical sap + virus inoculated).

After 60 days from sowing, 5 young leaves from each plot were collected randomly from sprayed and non-sprayed faba bean plants with six botanical and screened at the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, for natural infection with any of six tested antisera of faba viruses, namely Faba bean necrotic yellows virus (FBNYV); Bean yellow mosaic virus (BYMV); Broad bean stain virus (BBSV); Alfalfa mosaic virus (AMV); Broad bean wilt virus (BBWV) and Pea seed borne mosaic virus (PSbMV) using double antibody sandwich- enzyme-linked immunoassay (DAS-ELISA).

At harvest, plot seed samples retained for seed size (hundred seed weight) measurement.

**Protein pattern profile:**

This trial was performed to demonstrate that the ribosomal inhibiting proteins were transferred from botanical extract via spray
applying to faba bean plants and induced systemic resistance against natural infection with any faba viruses.

**Plant materials:**

Five young leaves of faba bean sprayed with botanical extracts were collected from each plot randomly, washed carefully with distilled water, and then allow air dried. On the other hand, five leaves from each six botanical were prepared as above. Untreated faba bean leaves were also included as a control.

**Protein extraction and SDS-PAGE:**

- Ground tissue in a mortar with liquid nitrogen. Collect grounded material in a eppendorf tube (tube weight 1.0 g).
- Weight the material.
- Add 10% w/v trichloroacetic acid and 0.07% v/v 2-mercaptoethanol in COLD (-20°C) acetone (approx. 1ml for 0.3 g of tissue)
- Incubate for over night at -20°C.
- Centrifuge the precipitated proteins in microfuge for 25 min. at 10,000 rpm.
- Wash pellet with COLD acetone containing 0.07% v/v 2-mercaptoethanol (approx. 1 ml) to remove pigments and lipids until the pellet is colorless.
- Dry proteins under vacuum (5-10 min.)
- Resuspend proteins in the appropriate rehydration buffer
- Vortexing the extract proteins.
- Centrifuge and collect the supernatant containing predominantly soluble proteins.
**Electrophoresis:**

Fully denatured samples were those boiled for 3 min in Laemmli sample buffer containing 100 mM DTT as a reductant and 2% SDS. Twenty-five µg soluble proteins from leaves were separated by SDS-PAGE using the Laemmli (1970) system on a 12.5% running gel for 90 min at 100V. The gel was stained with Coomassie Brilliant Blue R-250 (EM Science, Gibbstown, NJ). Low-molecular mass protein markers (10 to 200 kDa, Bio-Rad) were run simultaneously for each electrophoresis gel.

**Coomassie Brilliant Blue/Page-Blue 83**

Make up stain: 0.2% CBB in 45:45:10 % methanol:water:acetic acid. Cover gel with staining solution, seal in plastic box and leave overnight on shaker (RT) or for 2 to 3 hours at 37°C also with agitation. Destain with 25% 65% 10% methanol water acetic acid mix, with agitation.

**4- EXPERIMENTAL RESULTS**

**1- Survey for faba bean viruses:**

Two restricted surveys for faba bean viruses were conducted during February and April 2004 in some districts at Qalyoubia Governorate (i.e., Banha, El-Qanater El-Khayria, Kafr Shoukr, Shebien El-Qanater and Toukh) to identify viruses affecting the faba bean (*Vicia faba*) crop. Faba bean plants showing symptoms suggestive of viral infection, such as stunting, vein clearing, leaf roll, mosaic, chlorosis, necrosis, yellowing and leaf distortion were visually inspected.

A total 430 viral symptomatic leaves of faba bean collected during the survey in the February of 2004 and 355 in the April of 2004 were
divided into 5 main categories, on the basis of the symptoms, and examined at the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, for six faba bean viruses [\textit{Faba bean necrotic yellows nanavirus} (FBNYV); \textit{Bean yellow mosaic potyvirus}, (BYMV); \textit{Broad bean stain comovirus} (BBSV); \textit{Alfalfa mosaic alfamovirus} (AMV); \textit{Broad bean wilt fabavirus} (BBWV) and \textit{Pea seed borne mosaic potyvirus} (PSbMV)] using double antibody sandwich- enzyme-linked immunoassay (DAS-ELISA). The ELISA reactions of faba bean collected samples from 5 different locations at Qalyoubia Governorate in both surveys [February (A) and April (B)] were recorded in Table (1). Frequencies of specific infection with the tested viruses were determined after disease incidence and disease severity and recorded in Tables (2 and 3) and Figs. (1 and 2).

Data in Table (1) show that all symptomatic leaf samples collected from 5 different locations was affected with many viruses either individual or in mixed infection as confirmed with ELISA test.

\textbf{Table (1):} ELISA reactions of both surveyed leaf samples, categorized on the basis of virus systemic infection in five groups (1 – 5) (see Plate, 1).

<table>
<thead>
<tr>
<th>Categories</th>
<th>Survey</th>
<th>\textbf{Antibodies (Ab\textsubscript{s})}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FBNYV</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>+</td>
</tr>
</tbody>
</table>
Disease incidence of virus infection in faba bean surveyed locations was recorded in Table (2) and Fig. (1). Data show that disease incidence in the first survey came in the descending order as follows: Toukh (55.55%), Kafr Shoukr (54.17%), Shebien El-Qanater (50.00%), Banha (47.62%) and El-Qanater El-Khayria (38.89%). Meanwhile, in the second survey were Shebien El-Qanater (94.12%), Banha (60.61%), Toukh (59.65%), Kafr Shoukr (52.38%) and El-Qanater El-Khayria (44.00%). There are variations in the sample size due to the faba bean cultivated area in each location. The disease incidence were increased in the second survey than the first one as a result of incurred virus symptoms, subsequently, a total leaf sample was decreased.

Table (2): The disease incidence of naturally viral infected faba bean plants in different locations (Qalyoubia Governorate).

<table>
<thead>
<tr>
<th>Locations</th>
<th>Disease incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>February 2004</td>
</tr>
<tr>
<td>1 Banha</td>
<td>47.62</td>
</tr>
<tr>
<td>2 El-Qanater El-Khayria</td>
<td>38.89</td>
</tr>
<tr>
<td>3 Kafr Shoukr</td>
<td>54.17</td>
</tr>
<tr>
<td>4 Shebien El-Qanater</td>
<td>50.00</td>
</tr>
<tr>
<td>5 Toukh</td>
<td>55.55</td>
</tr>
</tbody>
</table>
Disease severity of virus infection in faba bean surveyed locations was recorded in Table (3) and Fig. (2). Data show that disease severity was increased in the second survey than the first one also due to incurred specific virus symptoms. Toukh samples showed the highest disease severity in both surveys (23.26 and 23.94%), followed by Shebien El-Qanater (17.44 and 22.54%), Kafr Shoukr (15.12 and 15.49%), Banha (11.63 and 14.08%), then El-Qanater El-Khayria came at last (8.14 and 9.30%).
Table (3): The disease severity of naturally viral infected faba bean plants in different 5 locations (Qalyoubia Governorate).

<table>
<thead>
<tr>
<th>Locations</th>
<th>Disease severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>February 2004</td>
</tr>
<tr>
<td>1 Banha</td>
<td>11.63</td>
</tr>
<tr>
<td>2 El-Qanater El-Khayria</td>
<td>8.14</td>
</tr>
<tr>
<td>3 Kafr Shoukr</td>
<td>15.12</td>
</tr>
<tr>
<td>4 Shebien El-Qanater</td>
<td>17.44</td>
</tr>
<tr>
<td>5 Toukh</td>
<td>23.26</td>
</tr>
</tbody>
</table>

Fig. (2): Disease severity due to natural viruses affecting faba bean (*Vicia faba* L.) at 5 different locations in Qalyoubia Governorate during February and April 2004.
During the February of 2004, data show that Bean yellow mosaic potyvirus (BYMV) was the dominant one (67.00%) at all 5 surveyed locations, followed by FBNYV (21.00%), AMV (2.46%), PSbMV (1.50%), BBWV (2.14%) and BBSV (3.90%). Meanwhile, during April 2004, data showed the same trend where as Bean yellow mosaic potyvirus (BYMV) was the dominant one (67.65%) at all 5 surveyed locations, followed by FBNYV (24.81%), AMV (1.92%), PSbMV (0.68%), BBWV (1.61%) and BBSV (3.33%) (Table 4 and Fig., 3).

**Table (4):** Frequency percentage of 6 viruses infected faba bean in 5 different locations at Qalyoubia Governorate.

<table>
<thead>
<tr>
<th>Antisera tested against</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feb. 2004</td>
</tr>
<tr>
<td>1 Alfalfa mosaic alfamovirus (AMV)</td>
<td>2.46</td>
</tr>
<tr>
<td>2 Broad bean stain comovirus (BBSV)</td>
<td>3.90</td>
</tr>
<tr>
<td>3 Broad bean wilt fabavirus (BBWV)</td>
<td>2.14</td>
</tr>
<tr>
<td>4 Bean yellow mosaic potyvirus (BYMV)</td>
<td>67.00</td>
</tr>
<tr>
<td>5 Faba bean necrotic yellows nanavirus (FBNYV)</td>
<td>21.00</td>
</tr>
<tr>
<td>6 Pea seed borne mosaic potyvirus (PSbMV)</td>
<td>1.50</td>
</tr>
</tbody>
</table>
2- Isolation and identification.

On the basis of symptom, the crude sap (as source of inoculum) of the virus isolate was obtained from naturally infected broad bean (*Vicia faba* L.) leaves visually inspected from different locations at Qalyoubia Governorate. The infectious sap was mechanically inoculated on 600 mesh-dusted carborundum leaves of *Chenopodium amaranticolor* and *C. quinoa* as diagnostic host plants. Chlorotic local lesions were observed on *C. amaranticolor* and *C. quinoa* (20 and 9 days of inoculation, respectively). Single lesions were cut out and macerated on a glass-slide and inoculated onto the former diagnostic hosts. Extracts obtained from the reformed local lesions were inoculated on *Vicia faba* L., to confirm the pathogenicity of the isolated virus via Koch’s postulates and served as the source of virus inoculum throughout this study.

Symptoms induced by the virus isolate in experimentally inoculated
faba bean plants maintained in the greenhouse were similar to those expressed by filed plants (Plate, 2).

Plate (2): Typical bean yellow mosaic potyvirus symptoms (as vein clearing, vein banding, mild to severe mosaic, leaf deformation) on artificially inoculated leaves of faba bean (*Vicia faba* L.) Giza 2 cv.

**H:** healthy control  **D:** diseased artificially infected
3- Host range and symptomatology:

The host range of the isolated virus suggestive Bean yellow mosaic potyvirus (BYMV) which was restricted to Leguminous crops (Cicer arietinum, Lintus sativa, Lupinus albus, Medicago sativa, Phaseolus vulgaris, Pisum sativum, Trifolium subterraneum and Vicia faba) and some members of Amaranthaceae (Gomphrena globosa), Chenopodiaceae (Chenopodium album, C. amaranticolor and C. quinoa), and Solanaceae (Nicotiana clevelandii and Petunia hybrida).

Table (5) and Plate (3) show that most of tested leguminous hosts were reacted with infection systemically (vein clearing, mottle, mosaic, chlorosis and malformation etc...). In addition, Petunia hybrida reacted systemically with severe mosaic. On the other hand, Nicotiana clevelandii (propagative host) showed mild mosaic and was used as good sources for virus purification, and also for maintaining virus cultures (to avoid phenolic oxidation in faba bean).

Vicia faba was mechanically infected with the isolated virus and showed vein clearing, chlorosis followed by obvious green or yellow mosaic.

Chlorotic or necrotic local lesions were appeared on leaves of Chenopodium amaranticolor, C. album, C. quinoa and Gomphrena globosa mechanically inoculated.

On the contrary, symptomless hosts with negative back inoculation tests were: Cucumis sativus L. Cucurbita pepo L. (Cucurbitaceae), Vigna unguiculata L. (Leguminosae), Lycopersicum esculentum Mill, Capsicum annum L N. tabacum L., cvs. Samsun, Datura stramonium L., Datura metel, Solanum nigrum and Solanum melongana L. (Solanaceae).
Table (5): Host range of the isolated virus.

<table>
<thead>
<tr>
<th>Families</th>
<th>Host tested plant species</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amaranthaceae</strong></td>
<td><em>Gomphrena globosa</em> L.</td>
<td>NLL</td>
</tr>
<tr>
<td><strong>Chenopodiaceae</strong></td>
<td><em>C. album</em> L.</td>
<td>CLL</td>
</tr>
<tr>
<td></td>
<td><em>C. amaranticolor</em> Coste &amp; Ryn.</td>
<td>CLL</td>
</tr>
<tr>
<td></td>
<td><em>Chenopodium quinoa</em> Willd.</td>
<td>CLL</td>
</tr>
<tr>
<td><strong>Cucurbitaceae</strong></td>
<td><em>Cucumis sativus</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Cucurbita pepo</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Leguminosae</strong></td>
<td><em>Cicer arietinum</em></td>
<td>Mo</td>
</tr>
<tr>
<td></td>
<td><em>Lintus sativa</em> L.</td>
<td>Mo</td>
</tr>
<tr>
<td></td>
<td><em>Lupinus albus</em></td>
<td>Mo</td>
</tr>
<tr>
<td></td>
<td><em>Medicago sativa</em> L.</td>
<td>Mo</td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus vulgaris</em> L.</td>
<td>SM, Ch, Mf</td>
</tr>
<tr>
<td></td>
<td><em>Pisum sativum</em> L.</td>
<td>Ch, MM</td>
</tr>
<tr>
<td></td>
<td><em>Vicia fabae</em> L.</td>
<td>Ch, Mo</td>
</tr>
<tr>
<td></td>
<td><em>Vigna unguiculata</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Trifolium hybridum</em></td>
<td>Mo</td>
</tr>
<tr>
<td><strong>Solanaceae</strong></td>
<td><em>Capsicum annuum</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>D. stramonium</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Datura metel</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Lycopersicum esculentum</em> Mill.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>N. rustica</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>N. tabacum</em> L., cvs. Samsun.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Nicotiana clevelandii</em> Gray</td>
<td>Mo</td>
</tr>
<tr>
<td></td>
<td><em>Petunia hybrida</em> Vilm.</td>
<td>MM</td>
</tr>
<tr>
<td></td>
<td><em>Physalis floridana</em> Ryd.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Solanum melongana</em> L.</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td><em>Solanum nigrum</em> L.</td>
<td>NS</td>
</tr>
</tbody>
</table>

NLL = Necrotic local lesion, CLL = Chlorotic local lesions, Mo = Mosaic, MM = Mild mosaic, SM = Severe mosaic, Mf = Malformation, Ch = Chlorosis and NS = No symptoms.
Plate (3): Host range show positive reaction after mechanical inoculation with the isolated virus. (A) Local lesion hosts, 1 = Chenopodium album, 2 = C. amaranticolor, 3 = C. quinoa and (B) Systemic hosts, 1 = Nicotiana clevelandii, 2 = Petunia hybrida, 3 = Phaseolus vulgaris, 4 = Pisum sativum, 5 = Trifolium hybridum and 6 = Vicia faba.
4- Mode of transmission:

a- Mechanical transmission:

The isolated virus suggestive Bean yellow mosaic virus was mechanical transmissible readily (90 – 95%) with the infectious sap.

b- Insects transmission:

Both green peach aphid (*Myzus persicae* Sulz.) and faba aphid (*Aphis fabae*) were readily transmitted bean yellow mosaic potyvirus from infected faba bean (*Vicia faba* L.) source plants to healthy *C. quinoa* in a non-persistent manner with the average rate 85% and 73%, respectively.

c- Seed transmission:

Seeds were harvested from mechanically inoculated faba bean (*Vicia faba* L.) plants show obvious viral infections with the isolated virus were planted and the emerged seedlings showed no symptoms. To check for the presence of the isolated virus, inoculum were obtained from infected faba bean (*Vicia faba* L.) seedlings and inoculated to *C. quinoa* plants. No evidence that this virus was seed transmissible in the present work.

5- Physical properties:

The results of the virus stability in *in vitro* conditions shown in Table (6) were as follows:

a- Thermal Inactivation Point (TIP):

Infectious sap extracted from *Nicotiana clevelandii* as a source of the virus, was used to determine the thermal inactivation point of the present virus isolate. Inoculum was heated to 50, 55, 60, 65, 70, 75 and 80°C for 10 minutes. Treated and untreated sap was tested on leaves of
*C. quinoa* as an indicator plant.

The obtained results were cleared that, the virus isolate was lost its infectivity after heating for 10 min in crude sap at 65°C.

**b- Dilution End Point (DEP):**

Several dilutions up to $10^{-7}$ were prepared from infectious sap of *Nicotiana clevelandii* leaves. Each dilution was separately inoculated on leaves of *C. quinoa* as local lesion indicator plant.

The isolated virus still infectivity after its infectious sap was diluted to $10^{-4}$.

**c- Longevity in vitro (LIV):**

The effect of maintenance at room temperature (25°C) on the infectivity of the virus isolate in crude sap extracted from *Nicotiana clevelandii* was determined. Obtained data indicated that, the present virus isolate kept its infectivity for a period of 6 days on *C. quinoa* plants.
Table (6): Physical properties of the isolated virus of faba bean (*Bean yellow mosaic virus*, BYMV).

<table>
<thead>
<tr>
<th>Stability in vitro</th>
<th>Treatments and Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal inactivation point (TIP)</td>
<td>25°</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Dilution end point (DIP)</td>
<td>$10^0$</td>
</tr>
<tr>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Longevity in vitro (LIV)</td>
<td>0°</td>
</tr>
<tr>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

* Controls

+++ = High number of local lesions.

++ = Slightly number of local lesions.

+ = Low number of local lesions.

*(Chenopodium quinoa* was used as indicator plant).

6- Bean yellow mosaic Potyvirus particle properties:

**a- Light microscopy:**

Cytopathological examination of Bean yellow mosaic virus-infected faba bean cells showed presence of cytoplasmic vacuolated nuclear inclusion proteins of different size and shape were frequently found in epidermis cells (*Plate, 4*) as potyvirus cytoplasmic inclusions evidence.
Plate (4): Nuclear inclusion bodies in the cytoplasm of faba bean epidermal cells infected with bean yellow mosaic potyvirus stained with the combination of Orange and Brilliant Green dyes. [N = Nucleus and IN = Inclusions (X1000)].

b- Electron Microscopy:

Electron microscopic examinations of mechanically infected leaf sap (leaf-dip preparation of the isolated virus, negatively stained with uranyl acetate (2%) pH 4) showed the presence of filamentous flexuous virus particles (Plate, 5). The virions are counted (in number of micrographs) and the number extrapolated to estimate the modal length (Fig., 4).

Results showed the presence of filamentous virus particles with average dimension of approximately 750 x 13 nm.
Plate (5): Electron micrograph of negatively stained leaf-dip preparation of the isolated virus obtained from systemically infected *Nicotiana clevelandii* plants showed filamentous flexuous virus particles (Bar = 200 nm).
Fig. (4): Modal of particle length obtained from several electron micrographs of negatively stained leaf-dip preparation of the isolated virus obtained from systemically infected *Nicotiana clevelandii* plants.

7- Serological Studies:

a- Gel double diffusion (SDS-immunodiffusion) test:

In this test the antibody-antigen reaction is carried out in a gel agarose plates. The reactants are allowed to diffuse through the gel and combine.

Results illustrated in Plate (6) clearly show that, precipitin lines between antigen of the isolated virus (partially purified virus), and specific antiserum, produced against the isolated virus, were sharp and homologous without any cross in the ended, meaning that, there were strong serological relationship between both. The same properties between the isolated virus antigens and antibodies of BYMV, diffused
and weak were induced between isolated virus antigen and antibodies of both PVY and PSMV imported from Germany and Argentina, respectively. On the other hand, No reactions observed between the isolated virus antigen and antibodies of PMMV and TMV.

Obtained results indicated that, the isolated virus was related serologically to Potyviruses group. This may lead, to concede the isolated virus as a member of the plant Potyviruses.

**Plate (6A and B)** show comparison between the standard method for double diffusion (A) and modified one where 0.8-g/100ml agarose (as solidified material) was substituted with 0.3-g/100ml Gelrite which more clear and high strength gel (B). Also, 0.002g/100ml sodium azide (NaN₃) was replaced with 0.1% Kombucha (green fermented tea) as an antioxidant and antimicrobial more safe and cheap.
Plate (6): SDS-immunodouble diffusion test, using antisera of TMV and PMMV from tobamovirus, and PSMV, BYMV and PYV from potyvirus, respectively. Blank in both is empty.
These modifications make SDS-immunodouble diffusion test clearer and the results readily photographed and record. Avoid the toxicity of sodium azide with the same sterilization strength or more, beside useful of antioxidant activity of Kombucha.

**b- Enzyme-Linked Immunosorbent Assay (ELISA):**

The experimental results confirmed the presence of BYMV alone in the sample No. 3 and mixed with FBNYV, AMV, BBWV, PsbMV in other samples.

**8- Response of some faba bean cultivars to infection with the BYMV under greenhouse conditions:**

Data in Table (7) and Fig. (5) reveal that, among the 6 faba bean tested cultivars mechanically inoculated with BYMV, Giza 2 was highly susceptible one, followed by Shakha 1, Giza 843, Giza 716 and Giza 3. Meanwhile, Misr 1 cultivar was the least susceptible one. So, Giza 2 cultivar was used at all the followed studies in this work.

*Table (7):* Response of some faba bean cultivars to artificial infection with BYMV under greenhouse conditions.

<table>
<thead>
<tr>
<th>Faba bean cultivars</th>
<th>Infection percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sakha 1</td>
<td>48</td>
</tr>
<tr>
<td>2. Misr 1</td>
<td>32</td>
</tr>
<tr>
<td>3. Giza 2</td>
<td>92</td>
</tr>
<tr>
<td>4. Giza 3</td>
<td>36</td>
</tr>
<tr>
<td>5. Giza 716</td>
<td>40</td>
</tr>
<tr>
<td>6. Giza 843</td>
<td>44</td>
</tr>
</tbody>
</table>
Fig. (5): Response of some faba bean cultivars to artificial infection with BYMV virus under greenhouse conditions.

9- **Strategy for controlling faba viruses:**

Two field experiments were carried out in two consecutive seasons, with different sowing dates (first in October 15 2004 and second in November 16 2005).

On the apostil field experiment and regardless of results of spraying with the aforementioned six botanicals, current study observed that there was positive correlation between postponements of sowing date in the second season (November 16 2005) and enhancement of both virus inhibition rates and increasing of 100-seeds weight. Also, replacement of selected experimental area in the first season (2004) with a better on in
the second season (2005) show the obscure role regarding 100-seeds weight or systemic induced resistance against faba viruses.

The results of preliminary trial recorded in Table (8) and Fig. (6) show that root extracts of *Phytolacca americana* induced the highest systemic resistance against BYMV (99%) (as inhibitory percentage), followed by root extract of *Mirabilis jalapa* (98%), leaves extracts of *Clerodendrum inerme* and *Phytolacca americana*, and young shoots extract of *Mirabilis jalapa* (97%), leaves extract of *Dianthus caryophyllus* (91%), leaves extract of *Chrysanthemum cinerariifolium* (80%), then fruits and leaves extracts of *Schinus terebinthifolius* (76 and 72%, respectively).

Table (8): Antiviral inhibitory effect of some selected healthy medicinal plant extracts on BYMV (as local lesion reaction) on *C. quinoa* under greenhouse conditions.

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Used parts</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Chrysanthemum cinerariifolium</em></td>
<td>Leaves</td>
<td>80.00</td>
</tr>
<tr>
<td>2. <em>Clerodendrum inerme</em></td>
<td>Leaves</td>
<td>97.00</td>
</tr>
<tr>
<td>3. <em>Dianthus caryophyllus</em></td>
<td>Leaves</td>
<td>91.00</td>
</tr>
<tr>
<td>4. <em>Mirabilis jalapa</em></td>
<td>Young shoots</td>
<td>97.00</td>
</tr>
<tr>
<td>5. <em>Mirabilis jalapa</em></td>
<td>Roots</td>
<td>98.00</td>
</tr>
<tr>
<td>6. <em>Phytolacca americana</em></td>
<td>Leaves</td>
<td>97.00</td>
</tr>
<tr>
<td>7. <em>Phytolacca americana</em></td>
<td>Roots</td>
<td>99.00</td>
</tr>
<tr>
<td>8. <em>Schinus terebinthifolius</em></td>
<td>Fruits</td>
<td>76.00</td>
</tr>
<tr>
<td>9. <em>Schinus terebinthifolius</em></td>
<td>Leaves</td>
<td>72.00</td>
</tr>
</tbody>
</table>
Fig. (7): Antiviral inhibitory effect of some selected healthy medicinal plant extracts on BYMV (as local lesion reaction) on *C. quinoa* under greenhouse conditions.
Meanwhile, the results of avermently trial on faba bean recorded in Table (9) and Fig. (7) show that all three concentrations (i.e., 2, 5 and 20%) of all tested plant extracts, in general, gave encouraged results of virus-inhibition. The efficiency of inhibition was increased with increasing the concentration. The concentrations 20% of all extracts were the most effective one, where induced the highest systemic resistance against BYMV (as inhibitory percentage of systemic virus infection). Roots and leaves extract of both Mirabilis jalapa and Phytolacca americana gave the highest inhibition rate at all concentrations, followed by leaves extracts of Dianthus caryophyllus, Clerodendrum inerme. Least inhibitory rate was obtained using leaves extract of Chrysanthemum cinerariifolium and fruits and leaves extracts of Schinus terebinthifolius, respectively.
Table (9): The systemic inhibitory effect of some plant extracts (in three concentrations) on BYMV in faba bean plants under greenhouse conditions.

<table>
<thead>
<tr>
<th>Medicinal plant extracts</th>
<th>Used parts</th>
<th>Conc. (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysanthemum cinerariifolium</td>
<td>leaves</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>64</td>
</tr>
<tr>
<td>Clerodendrum inerme</td>
<td>leaves</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td>Dianthus caryophyllus</td>
<td>leaves</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td>Mirabilis jalapa</td>
<td>young shoots</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td>Mirabilis jalapa</td>
<td>roots</td>
<td>2</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td>Phytolacca americana</td>
<td>leaves</td>
<td>2</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td>Phytolacca americana</td>
<td>roots</td>
<td>2</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td>Schinus terebinthifolius</td>
<td>fruits</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>64</td>
</tr>
<tr>
<td>Schinus terebinthifolius</td>
<td>leaves</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>57</td>
</tr>
</tbody>
</table>
Fig. (7): The systemic inhibitory effect of some plant extracts (in three concentrations) on BYMV in faba bean plants under greenhouse conditions.
Applicable plan was achieved by repetition spraying with aqueous extracts of six healthy medicinal plants through field experiments.

Data recorded in Table (10) and Fig. (9) show that three sprays with 20% of all extracts fortnightly interval gave good results dealing inhibitory effect of natural virus infection. Leaves and roots extracts of *Phytolacca americana* came in the superior in the systemic induced resistance through three sprays, followed by roots and leaves extracts of *Mirabilis jalapa*. Leaves extract of both *Dianthus caryophyllus* and *Clerodendrum inerme* nearly equal in this concern. Least systemic resistance was induced by leaves extract of *Chrysanthemum cinerariifolium* and fruits and leaves of *Schinus terebinthifolius*, respectively.

Induced systemic resistance was achieved by using crude aqueous extracts of some medicinal plants suggestive contains endogenous proteins, previously identified as ribosome-inactivating proteins (RIPs), enzymes that act on ribosomes in a highly specific mechanism. Antiviral inhibitory activities were the means to record the induced systemic resistance of these medicinal extracts.

Generally, all spray treatments increased systemic induced resistance compared with the control in both seasons (2004 – 2005).
Table (10): The systemic inhibitory effect of some plant extracts (3 sprays applied) on naturally virus infection in faba bean crop under field conditions.

<table>
<thead>
<tr>
<th>Medicinal plant extracts</th>
<th>Treatments</th>
<th>Inhibitions (%)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2004</td>
<td>2005</td>
</tr>
<tr>
<td><em>Chrysanthemum cinerariifolium</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray*</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>66</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>71</td>
<td>70</td>
</tr>
<tr>
<td><em>Clerodendrum inerme</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>79</td>
<td>81</td>
</tr>
<tr>
<td>(leaves)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>85</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td><em>Dianthus caryophyllus</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>83</td>
<td>84</td>
</tr>
<tr>
<td>(leaves)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>86</td>
<td>87</td>
</tr>
<tr>
<td>(young shoots)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>(roots)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td><em>Phytolacca americana</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>87</td>
<td>89</td>
</tr>
<tr>
<td>(leaves)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td><em>Phytolacca americana</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td>(roots)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>61</td>
<td>69</td>
</tr>
<tr>
<td>(fruits)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>76</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>82</td>
<td>87</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; spray</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td>(leaves)</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; spray</td>
<td>60</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; spray</td>
<td>68</td>
<td>67</td>
</tr>
</tbody>
</table>

*1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> sprays applied after 15, 30 and 45 days from sowing date, respectively.
Fig. (8): The systemic inhibitory effect of some plant extracts (3 sprays applied) on naturally virus infection in faba bean crop under field conditions.
Data in Table (11) and Fig. (9) record positive correlation between successful of systemic induced resistance and faba bean productivity yield enhancement. Obvious increase in the weight of air-dried faba bean 100-seeds was recorded after three sprays of root and leaves extract of *Phytolacca americana* then leaves extract of *Mirabilis jalapa*. The 100-seeds weight then decreased after three sprays with leaves extracts of *Dianthus caryophyllus*, *Clerodendrum inerme*, *Chrysanthemum cinerariifolium* and fruit, leaves extracts of *Schinus terebinthifolius*, respectively. Generally, all spray treatments increased 100-seeds than the control (sprayed with tap water) in both seasons (2004 – 2005).

**Table (11):** Effects of spraying with some plant extracts on faba bean (cv. Giza 2) production (weight of dried 100-seeds in gram) under field conditions.

<table>
<thead>
<tr>
<th>Medicinal plants</th>
<th>Used parts</th>
<th>Weight of 100-seeds(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 <em>Chrysanthemum cinerariifolium</em></td>
<td>Leaves</td>
<td>76 78</td>
</tr>
<tr>
<td>2 <em>Clerodendrum inerme</em></td>
<td>Leaves</td>
<td>80 82</td>
</tr>
<tr>
<td>3 <em>Dianthus caryophyllus</em></td>
<td>Leaves</td>
<td>85 89</td>
</tr>
<tr>
<td>4 <em>Mirabilis jalapa</em></td>
<td>Young shoots</td>
<td>90 93</td>
</tr>
<tr>
<td>5 <em>Mirabilis jalapa</em></td>
<td>Roots</td>
<td>92 96</td>
</tr>
<tr>
<td>6 <em>Phytolacca americana</em></td>
<td>Leaves</td>
<td>93 95</td>
</tr>
<tr>
<td>7 <em>Phytolacca americana</em></td>
<td>Roots</td>
<td>94 97</td>
</tr>
<tr>
<td>8 <em>Schinus terebinthifolius</em></td>
<td>Fruits</td>
<td>71 73</td>
</tr>
<tr>
<td>9 <em>Schinus terebinthifolius</em></td>
<td>Leaves</td>
<td>76 79</td>
</tr>
<tr>
<td>10 Control (unsprayed)</td>
<td></td>
<td>59 70</td>
</tr>
</tbody>
</table>
Finally, after 60 days from sowing, 5 faba bean leaves (showing no virus symptoms) from each plot were randomly collected and tested at the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, for six faba viruses [Faba bean necrotic yellows virus (FBNYV); Bean yellow mosaic virus (BYMV); Broad bean stain virus (BBSV); Alfalfa mosaic virus (AMV); Broad bean wilt virus (BBWV) and Pea seed borne mosaic virus (PSbMV)] using double antibody sandwich- enzyme-linked immunoassay (DAS-ELISA).
Rarely or no positive reaction was recorded when faba bean leaf samples collected from plots sprayed with root and leaves extracts of *Phytolacca americana* and *Mirabilis jalapa* or leaves extracts of *Dianthus caryophyllus* and *Clerodendrum inerme* examined with DAS-ELISA against faba bean necrotic yellows virus (FBNYV); Bean yellow mosaic virus (BMYV); Broad bean stain virus (BBSV); Alfalfa mosaic virus (AMV); Broad bean wilt virus (BBWV) and Pea seed borne mosaic virus (PSbMV).

Moderately positive reaction was observed when faba bean leaf samples collected from plots sprayed with leaves extracts of *Chrysanthemum cinerariifolium* and fruit, leaves extracts of *Schinus terebinthifolius*, respectively, was tested using DAS-ELISA against the same previous faba viruses.

**Protein Pattern Profile:**

Electrophoretic analysis for protein pattern in both sprayed or non-sprayed faba bean leaves, in addition to leaf extract from six tested medicinal plants were clearly show in Plate (7). Extra bands between 24 to 32 kDa were released in the treated faba bean plants not found in the unsprayed one, but identical to those electrophoretic in leaves extracts of six botanicals.
**Plate (7):** The SDS-PAGE gel is of fully denatured samples run on a 12.5% polyacrylamide gel and stained for protein with Coomassie blue. Lane M contained molecular mass markers.

C: Control (unsprayed faba bean leaves)
1: Faba bean sprayed with leaf extracts of *Clerodendrum inerme*.
2: Faba bean sprayed with leaf extracts of *Dianthus caryophyllus*.
3: Faba bean sprayed with leaf extracts of *Mirabilis jalapa*.
4: Faba bean sprayed with leaf extracts of *Phytolacca americana*.
5: Faba bean sprayed with leaf extracts of *Chrysanthemum cinerariifolium*.
6: Faba bean sprayed with leaf extracts of *Schinus terebinthifolius*.

7: Leaf extract of *Clerodendrum inerme*.
8: Leaf extract of *Dianthus caryophyllus*.
9: Leaf extract of *Mirabilis jalapa*.
10: Leaf extract of *Phytolacca americana*.
11: Leaf extract of *Chrysanthemum cinerariifolium*.
12: Leaf extract of *Schinus terebinthifolius*.
5- DISCUSSION

1- Survey for faba bean viruses:

The results of a restricted survey for faba bean viruses which conducted in some districts at Qalyoubia Governorate (i.e., Banha, El-Qanater El-Khayria, Kafr Shoukr, Shebien El-Qanater and Toukh) during February and April of 2004 to identify viruses affecting the faba bean (Vicia faba) crop were recorded.

All symptomatic leaf samples collected from 5 different locations were affected with many viruses [Faba bean necrotic yellows nanavirus (FBNYV); Bean yellow mosaic potyvirus, (BYMV); Broad bean stain comovirus (BBSV); Alfalfa mosaic alfamovirus (AMV); Broad bean wilt fabavirus (BBWV) and Pea seed borne mosaic potyvirus (PSbMV)] either individual or in mixed infection as confirmed with ELISA test.

Frequencies of specific infection with the tested viruses, in addition to disease incidence and disease severity were also recorded.

These results are in agreement with those recorded by many investigators after surveying faba bean fields for virus infection and both disease incidence and severity. For example, in Egypt, Rizkalla (2002) surveyed a total 88 faba bean fields from Middle Egypt (Menia and Beni Suef), Delta Region (Qalyoubia, Menoufia, Sharkia, Gharbia, Dakahlia, Kafr-El Sheikh, Behairah and Noubaria) and Fayoum Governorate (Fayoum district, Tamia, Ebshowi and Senoris), a total of 1760 samples of faba bean were collected. The total samples were tested by ELISA in the virology Lab against the different antiserum and found 24.7% from the total samples of faba bean diseased by FBNYV and 41% diseased by BYMV and 3.5% diseased by BBWV. Generally, the major viruses problem on faba bean in Egypt was BYMV.
Also, serological tests showed that BYMV was identified in 89% of samples tested, was the most common virus. In most of the surveyed fields, BYMV symptoms were noted to occur at high levels (80–100% infection). BWYV, BLRV and BBSV were not detected in any of the tested samples. On the other hand, FBNYV, BYMV and PSbMV have previously been reported in faba bean (Makkouk et al., 2003).

The same trend was achieved with Makkouk et al. (1994) and Bekele et al. (2005).

2- **Isolation and identification.**

Pathogenicity tests revealed that mechanical inoculation with the crude sap extracted from naturally virus-infected faba bean leaves caused typical systemic symptoms on the same faba bean (Vicia faba L.) cultivar.

This result is in harmony with those obtained by Fortass et al. (1991), Fortass (1993) and Makkouk et al. (1994).

3- **Host range and symptomatology:**

The host range of the isolated virus suggestive that Bean yellow mosaic potyvirus (BYMV) was restricted to Leguminous crops (Cicer arietinum, Lintus sativa, Lupinus albus, Medicago sativa, Phaseolus vulgaris, Pism sativum, Trifolium subterraneum and Vicia fabae), some members of Amaranthaceae (Gomphrena globosa), Chenopodiaceae (Chenopodium album, C. amaranticolore and C. quinoa), and Solanaceae (Nicotiana clevelandii and Petunia hybrida).

*Vicia faba* mechanically infected with the isolated virus showed vein clearing, chlorosis followed by obvious green or yellow mosaic.

Many investigators depended on the host range among basic
procedures to identify the Bean yellow mosaic potyvirus (Russo and Rana, 1978; Borges, 1982; Makkouk et al., 1982; Bhardwaj et al., 1993; Fresno et al., 1997; Sasaya et al., 1997 and ICTVdB, 2006).

The host range of BYMV was restricted to leguminous crops except for *Nicotiana benthamiana*, *N. clevelandii*, *Chenopodium amaranticolor* and *C. quinoa* (Makkouk et al., 1982).

Over the obtained host range, some investigators recorded different other plants as a host to Bean yellow mosaic potyvirus with distinguish symptoms such as: chlorotic spots and light mottling on leaves of *Passiflora caerulea*, (Passifloraceae) (Parrella and Castellano, 2002), yellow and/or necrotic rings on leaves of *Aegopodium podagraria* (Bellardi and Bianchi, 2003), leaf mosaic on *Verbena x hybrida* (ornamental annual plant) (Guaragna et al., 2004), leaf chlorotic mosaic and flower colour breaking in gladiolus (*Gladiolus x hortulanus*) (Koenig, 1976; Orellana and Fan, 1978 and Arneodo et al., 2005). Hammond and Lawson (1988) isolated BYMV from infected Masdevallia (orchid). While, Skelton et al. (2006) isolated BYMV from *Dactylorhiza foliosa* (is a hardy orchid species) showed chlorotic mottle and streaking.

*Nicotiana clevelandii* L. was also recorded as a propagative host to maintain Bean yellow mosaic potyvirus by Koenig (1976), Lesemann and Koenig (1985) and ICTVdB (2006) this record was in agreement with the present study.

On the other hand, *Chenopodium album*, *C. quinoa*, *C. amaranticolor* and *Gomphrena globosa* were reacted with local lesions against mechanical inoculation with Bean yellow mosaic potyvirus (ICTVdB, 2006).

Most evaluated leguminous hosts mechanically inoculated with Bean
yellow mosaic potyvirus reacted with systematically differentiated infections (Lesemann and Koenig, 1985 and ICTVdB, 2006).

4- Mode of transmission:

a- Mechanical transmission:

The isolated virus suggestive Bean yellow mosaic virus was mechanical transmissible readily with the infectious sap.

This result was confirmed with the data obtained by Dafallah and Hussein (1994) who reported that Bean yellow mosaic potyvirus was the most common mechanically transmitted virus detected on faba beans. Moreover, Koenig (1976) observed, experimentally, that Bean yellow mosaic virus was easily transmitted from *Nicotiana clevelandii* to *Nicotiana clevelandii*, but only with difficulties from *N. clevelandii* to *Vicia faba*. From *V. faba* the virus was easily transmitted to *V. faba* and to *N. clevelandii*. A greatly increased infectivity for *V. faba* was retained after one passage on *N. clevelandii* following a passage on *V. faba*, it was lost, however, after several passages on *N. clevelandii*. Since the "adaptation" to *V. faba* was thus reversible and since single lesion isolates showed the same behavior, a selection of preexisting strains from the original inoculum cannot be the explanation for the observed phenomenon. The most likely explanation is that in *V. faba* variants are produced (induced or spontaneously) and that those variants which are especially well adapted to this host are propagated preferentially. After transfer to *N. clevelandii* other new variants may develop which gradually dilute out those with a high infectivity for *V. faba*.
**b- Insects transmission:**

Both green peach aphid (*Myzus persicae* Sulz.) and faba aphid (*Aphis fabae*) were readily transmitted Bean yellow mosaic potyvirus from infected broad bean (*Vicia faba* L.) source plants to healthy *C. quinoa* in a non-persistent manner with the average rate 85% and 73%, respectively.

**Makkouk et al. (1982)** recorded that, BYMV was transmitted by *Myzus persicae* and less effectively by *Aphis fabae*.

On the other hand, **Borges (1982)** noticed that a virus causing broad bean mosaic was transmitted by *Myzus persicae* and *Aphis fabae* to *Lathyrus ochrus*, lupin, pea, clover (*Trifolium pratense* and *T. subterraneum*), *Chenopodium amaranticolor*, *C. quinoa* and some bean (*Phaseolus vulgaris*) cultivars. **Eid (1983)** stated that Bean yellow mosaic virus (BYMV) transmitted by *Aphis craccivora* and *Myzus persicae* and cause severe damage to faba bean (*Vicia faba*) in Egypt.

**Nooh (1985)** reported that BYMV was transmitted by *Aphis gossypii* in a non persistent manner. **Vaid (1988)** reported that BYMV was also transmitted by aphid species *viz.*, *Myzus persicae*, *Brevicoryne brassicae* and *Aphis fabae*. Approximately 75% of aphid-vectored potyviruses are transmitted in a non-persistent (non-circulative) manner (Powell, 2005).

**c- Seed transmission:**

No evidence that bean yellow mosaic virus was seed transmissible in the present work.

This result was confirmed with those obtained by **Fidan and Yorganci (1990)** who tested seedlings of faba bean for seed transmission
and demonstrated not for alfalfa mosaic virus or Bean yellow mosaic potyvirus. Moreover, Bean yellow mosaic potyvirus is not known to be seed-transmitted in beans (Orellana and Fan, 1978). Also, not through the seeds of soybean (Nooh, 1985) or faba bean seeds (Vaid, 1988).

5- Physical properties:

- **Thermal Inactivation Point (TIP):**

  The obtained results were cleared that, the virus isolate was lost its infectivity after heating for 10 min in crude sap at 65°C.

- **Dilution End Point (DEP):**

  Results of trials showed that, the infectivity of the present virus isolate was preserved at dilution $10^{-4}$.

- **Longevity in vitro (LIV):**

  Data indicated that, the present virus isolate kept its infectivity for a period of 6 days in crude sap (without preservatives) of *C. quinoa* plants.

  There were similarity between the obtained results and those recorded by many investigators such as: TIP = 55 to 66°C, DEP = $10^{-4}$ to $10^{-5}$, LIV = up to 24 but not 36 hours at room temperature (Nooh, 1985); 66 to 68°C, $10^{-3}$ - $10^{-4}$ and 5-7 days (Borges, 1982); 60-65°C, $10^{-4}$-$10^{-6}$ and 3 days at room temperature and 5 days under refrigeration (Vaid, 1988); 60 and 65°C, $10^{-4}$ - $10^{-5}$, 3 and 5 days at room and refrigeration temperature (Bhardwaj et al., 1993) and 65°C, $10^{-3}$ - $10^{-5}$, and 2-7 days, respectively (ICTVdB, 2006).

  Whereas, obtained results were differed from those obtained by Verma et al. (1999) who recorded that BYMV withstood heating up to 55°C for 10 min and dilutions up to $10^{-3}$ and it had a longevity in crude sap at room temperature (25±5°C) of 30 hours and at 4°C for 56 hours.
6- Bean yellow mosaic Potyvirus Particle Properties:

A- Light microscopy:

Cytopathological examination of Bean yellow mosaic virus-infected faba bean cells showed presence of cytoplasmic vacuolated nuclear inclusion proteins of different size and shape were frequently found in epidermis cells.

This result is in harmony with those previously recorded by many investigators such as: Chang et al. (1988), Vaid (1988), Edwardson and Christie (1991), Staniulis and Zitikaite (1994), Hammond (1998), Bellardi and Bianchi (2003) and ICTVdB (2006).

7- Electron Microscopy:

Results showed the presence of filamentous virus particles with average dimension of approximately 750 x 13 nm.

Similar results were confirmed by Parrella and Castellano (2002) who reported that electron microscope observations of leaf dips from naturally infected plant showed a virus with filamentous particles ca. 750 nm in length. Bellardi and Bianchi (2003) stated that leaf sap (leaf-dip preparations, stained with uranyl acetate and phosphotungstic acid) showed the presence of filamentous virus particles ca. 760 nm in length.

On the other hand, Uyeda et al. (1982) recorded that the purified virus was a flexuous rod c. 760 nm long. Whereas, Vaid (1988) found that electron micrographs showed flexuous virus particles of 790x12 nm diameter.

The virus was purified using phosphate buffer and chilled chloroform and 2 cycles of differential centrifugation. Flexuous rod
particles approx. 750 x 11 nm were observed in the electron microscope. The virus was identified as a strain of BYMV \textit{(Verma et al., 1999)}. \textbf{Makkouk et al. (1982)} and \textbf{Staniulis and Zitikaite (1994)} agreed about the length of flexuous particles of Bean yellow mosaic potyvirus (750-800 nm).

Recently, \textbf{Skelton et al. (2006)} reported that subsequent examination of Bean yellow mosaic virus isolated from \textit{Dactylorhiza foliosa} (is a hardy orchid) by transmission electron microscopy revealed the presence of potyvirus-like particles, measuring ca. 750 nm in length.

\textbf{9- Serological Studies:}

\textbf{C- Gel double diffusion (SDS-immunodiffusion) test:}

The obtained results indicated that, the isolated virus was related serologically to Potyviruses group. This may lead, to concede the isolated virus as a member of plant Potyviruses.

This result is in the same trend revealed by \textbf{Makkouk et al. (1982)} who stated that Bean yellow mosaic potyvirus was reacted in SDS-immunodiffusion tests with BYMV antiserum. \textbf{Uyeda et al. (1982)} noticed, in agar gel double-diffusion tests, that 36 and 55 BYMV isolates were more closely related serologically to BYMV-N strain than to BYMV-CS strain; 132 was closer to 55 than to BYMV-CS. The disease that caused by BYMV-N strain including isolates 36 and 55. \textbf{Parrella and Castellano (2002)} reported that in the agar gel SDS-immunodiffusion tests, crude sap from naturally infected plant formed clear precipitin lines with an antiserum to BYMV.

\textbf{Moghal and Francki (1976)} studied antigenic relationships of six distinct potyviruses by immunodiffusion tests using highly purified
sonicated virus preparations and anti-intact virus sera devoid of detectable antibodies to host-plant antigens. Three variants of Bean yellow mosaic virus (BYMV) including BYMV sensu stricto and two variants of pea mosaic virus (PMV and SPMV) were shown to be antigenically very similar and also relatively closely related to lettuce mosaic virus (LMV). Distant antigenic relationships were detected between the BYMV variants and Bean common mosaic virus (BCMV); between BCMV and passionfruit woodiness virus (PWV); and between PWV and potato virus Y (PVY).

Regarding solidified media for immunodiffusion tests, gellan gum (Gelrite) is substantially equivalent to agar when used as a gelling agent in microbiological media and can also be used as a substitute for agarose for electrophoresis and isolation of DNA (Rath and Schmidt, 2001). GELRITE-solidified media may actually give higher viable cell recoveries than similar media solidified with agar. It is hoped that a few apparently manageable technical problems encountered during the course of this study, namely, hemolysis of blood at the elevated GELRITE setting temperature and slight difficulties in the streaking of certain media (blood and chocolate), will eventually be resolved (Shungu et al., 1983). In tests with this agar-like polysaccharide produced by Pseudomonas elodea (Gelrite), cucumber mosaic virus was detectable in Gelrite gels within half the time required for agar gels. Precipitin lines for tobacco mosaic and zucchini yellow mosaic viruses were also observed. The sensitivity of gel-diffusion tests performed in Gelrite appeared c. 100 times higher than in agar gels, indicating that the use of Gelrite makes these tests almost as sensitive as ELISA or IEM for the detection of
purified virus. Gelrite is readily available and is much cheaper than agar gel (Ohki and Inouye, 1987).

On the other hand, Kombucha (fermented tea) produced several metabolites including lactic, acetic, gluconic, glucoronic acids, as well as usnic acid which known as antimicrobial substances (Blanc, 1996). Fermented tea drink, Kombucha, can inhibit the growth of *Shigella sonnei*, *Escherichia coli*, *Salmonella enteritidis* and *Salmonella typhimurium* (Sreeramulu et al., 2001).

Jayabalan et al. (2007) reported that antioxidant and antimicrobial activities were achieved after fermenting sugared black tea, green tea or tea manufacture waste with tea fungus (Kombucha) for 12 to 15 days. The total phenol content increased up to 98% which implied that thearubigin might be subjected to biodegradation during fermentation, resulting in the release of smaller molecules with higher antioxidant activities.

**D- Enzyme-Linked Immunosorbent Assay (ELISA):**

The experimental results confirmed the presence of BYMV alone in the sample No. 3 and mixed with FBNYV, AMV, BBWV, PsbMV in other samples.

Many investigators found closely antigenically relationships between several Bean yellow mosaic potyvirus isolates and either its strains or other potyviridae including members as follows:

Demonstrated, using ELISA, that there was serologically related between antiserum of BYMV and antigen of the virus causing soybean mosaic, but not to SMV, PMV, CAMV, CMMV and SWBDMV (Nooh, 1985).

Bean yellow mosaic potyvirus was readily detected, using modified
ELISA, in sap of bioassay plants with the TuMV (Hammond and Lawson, 1988).

Gillaspie et al. (1998) observed strongly positive reaction using indirect-enzyme-linked immunosorbent assay with a general potyvirus monoclonal antibody and BYMV and white lupine mosaic virus (WLMV) polyclonal antisera.

Cheng and Jones (2000) found that all necrotic and non-necrotic isolates reacted with BYMV antiserum in ELISA but only two cross-reacted with antiserum to clover yellow vein virus (CYVV).

Bellardi and Bianchi (2003) identified the virus as an isolate of Bean yellow mosaic virus (BYMV) by serological tests including protein A sandwich enzyme-linked immunosorbent assay (PAS-ELISA).

Recently, Skelton et al. (2006) tested by ELISA for several viruses which are known to infect orchids including Tomato spotted wilt virus, Impatiens necrotic spot virus, Cymbidium mosaic virus, Odontoglossum ring spot virus and Bean yellow mosaic virus. Of these viruses, the sample tested positive only for Bean yellow mosaic virus (BYMV).

10- Strategy for controlling faba viruses:

Two field experiments were carried out in two consecutive seasons, with different sowing dates (first in October 1 2004 and second in November 16 2005).

Choosing suitable sowing date (early of second half of November), follow-up ideal agricultural practices, use resistant faba bean variety, and spray the crop fortnightly with some medicinal extracts were considered and permit the establishment of an integrated management option without the use of insecticides or other chemicals.
These suggesting was establishment with the current results and those obtained by ICARDA (2000) who reported that Middle Egypt is characterized by a mild winter where temperatures rarely fall below 5°C. Accordingly, aphids can actively move from such hosts and fly into the faba bean fields in October-December. They introduce the virus into faba bean plants when they start feeding on them. The virus is contained in the saliva of the aphid and transmitted while feeding takes place. In addition, the scientists evaluated different sowing dates. It was found that a delay (10-15 days) in sowing the crop until early November led to reduced virus spread. Infection was still present at low levels but the peak of aphid movement was taking place before the plants were fully developed as attractive hosts. Early October planting, by comparison, led to heavy infection levels three to four weeks after aphids flew into the newly-emerged crop. The collaborating scientists have shown it is possible to bring virus infection down from the 70-80% level to just 5-10% by integrating mid-November sowing with the early removal of any virus-infected plants, and the use of two systemic aphicide sprays in December and January. The success of this integrated virus disease management scheme in minimizing disease losses on experimental farms was such that the approach is now being taken to farmers’ fields.

**Ribosomal inhibiting proteins (RIPs):**

Electrophoretic analysis revealed that there were extra soluble protein bands found in the lanes of faba bean leaves sprayed with leaf extract of six botanicals and not found in unsprayed ones. On the other hand, the same extra bands were exactly found in the leaf extracts of tested medicinal plants.
The obtained results may be confirmed with those recorded by many researchers.

The use of antiviral principles, derived from higher plants, as biological control agents against viruses appears to be quite promising. Most of these substances have been found to be of proteinaceous nature and are known as antiviral proteins (AVPs). They can manifest their effects either by inactivating the viral pathogen or acting indirectly by inducing host resistance. The presence of such AVPs in extracts of several higher plants like *Phytolacca americana*, *Mirabilis jalapa*, *Dianthus caryophyllus*, *Clerodendrum* spp, *Bougainvillea* spp, *Chenopodium* spp and *Boerhaavia diffusa* has been reported. These have been shown to impart both non-systemic as well as systemic resistance. There is, as of yet, no clear understanding as to how these AVPs help in inducing resistance/protective mechanisms. Many of the AVPs from plants have also been shown to possess ribosome inactivating properties and thus known as ribosome inactivating proteins (RIPs). However, C-terminal deletion mutants of pokeweed antiviral protein (PAP) may inhibit viral infection but do not depurinate host ribosomes showing that RIP activity of PAP could be dissociated from its antiviral activity (Gholizadeh *et al.*, 2004).

Different RIPs have been reported from about 50 plant species covering 17 families. Some families include many RIP-producing species, particularly Cucurbitaceae, Euphorbiaceae, Poaceae, and families belonging to the superorder Caryophyllales. RIPs are divided into two categories depending upon the conformation of their subunits. Type I RIPs are single-chained proteins with a molecular mass of approximately 30 kD, whereas type II RIPs such as ricin and abrin possess two subunits,
a catalytic subunit (A chain) and a lectin subunit (B RIPs have shown broad spectrum antiviral activity against RNA, DNA, and plant and animal viruses. For instance, the RIP from *Mirabilis jalapa* has antiviral activity against the tobacco (*Nicotiana tabacum*) mosaic virus, potato (*Solanum tuberosum*) virus X, potato virus Y, and viroids such as the potato spindle tuber viroid. Also, some RIPs have specific DNA nuclease activity against supercoiled, covalently closed, circular plasmid DNA and single stranded phage DNA (*Sharma et al.*, 2004).


Two systemic antiviral resistance-inducing proteins, CIP-29 and CIP-34, isolated from *Clerodendrum inerme* leaves, for ribosome-inactivating properties. CIP-29 has a polynucleotide: adenosine glycosidase (ribosome-inactivating protein), that inhibits protein synthesis both in cell-free systems and, at higher concentrations, in cells, and releases adenine from ribosomes, RNA, poly(A) and DNA. As compared with other known RIPs, CIP-29 deadenylates DNA at a high rate, and induces systemic antiviral resistance in susceptible plants (*Olivieri et al.*, 1996).

The *Clerodendrum aculeatum*-systemic resistance inducing (CA-SRI) protein, a 34 kDa basic protein, plays a key role in inducing strong systemic resistance in susceptible plants against various plant viruses (*Kumar et al.*, 1997).

Two proteins (dianthin 30 and dianthin 32) were isolated from the leaves of *Dianthus caryophyllus* (carnation). They act by damaging
ribosomes in a less-than-equimolar ratio. Protein synthesis by intact cells is partially inhibited by dianthins at a concentration of 100µg/ml. Dianthins mixed with tobacco-mosaic virus strongly decrease the number of local lesions on leaves of *Nicotiana glutinosa*. They propose to name dianthin 30 and dianthin 32 on the basis of their respective molecular weights. Like the known 'A-chain-like' proteins, dianthins inhibit protein synthesis in a cell-free system by damaging ribosomes, but have little effect on whole cells. They also have strong inhibitory activity on the replication of tobacco-mosaic virus (*Stirpe et al.*, 1981).

A type 1 ribosome-inactivating proteins (RIPs) was isolated from leaves of *Dianthus caryophyllus* and examined their requirement for ATP and supernatant factors for full activity (*Carnicelli et al.*, 1997).

*Mirabilis jalapa* (Nyctaginaceae), containing a ribosome inactivating protein (RIP) called Mirabilis antiviral protein (MAP), against infection by potato virus X, potato virus Y, potato leaf roll virus, and potato spindle tuber viroid. Root extracts of *M. jalapa* sprayed on test plants 24 h before virus or viroid inoculation inhibited infection by almost 100%, as corroborated by infectivity assays and the nucleic acid spot hybridization test (*Vivanco et al.*, 1999). MAP was highly effective in inhibiting TSWV at 60% saturation. A minimum concentration of 400µg/ml of MAP was sufficient to inhibit TSWV (*Devi et al.*, 2004). In addition, Mirabilis antiviral protein (MAP) was isolated from roots and leaves of *Mirabilis jalapa* L. which possess repellent properties against aphids and white flies. MAP showed antiviral activity against mechanically transmitted viruses but not against aphid transmitted viruses (*Vivanco et al.*, 1999).
Pokeweed produces a suite of constitutive and induced RIPs in its leaves and seeds. For instance, PAP is a 29-kD constitutive RIP found in pokeweed leaves and localized in the cell wall matrix of leaf mesophyll cells. PAP II is a seasonal 30-kD RIP found in pokeweed leaves harvested in late summer, and PAP-S (29.8 kD) is expressed in seeds (Park et al., 2002).

Pokeweed antiviral protein (PAP) is a ribosome inactivating protein recognized primarily for its ability to depurinate the sarcin/ricin loop of the large rRNA. Studies have demonstrated that PAP also depurinates other RNA templates, such as Human immunodeficiency virus-1 RNA and Brome mosaic virus RNAs. However, the mechanism by which PAP accesses viral RNAs is not known. Considering that PAP was shown recently to bind the m7G of the cap structure, we speculated that PAP may interact with other factors involved in translation initiation (Wang and Hudak, 2006).

Barakat et al. (2005) reported that, many plant species are known to contain endogenous proteins, such proteins have been identified as ribosome-inactivating proteins (RIPs), enzymes that act on ribosomes in a highly specific way. Thereby, inhibiting protein synthesis. Six RIPs, including type I and 2 were isolated from leaves and/or seeds of certain plant species. Results revealed that all tested RIPs showed potent antiviral activity against Tobacco necrosis virus (TNV) onto Phaseolus vulgaris plants, Tobacco mosaic virus (TMV) onto Chenopodium amaranticolor plants, and Bean yellow mosaic virus (BYMV) onto its systemic host (Vicia faba plants).

Chemical analysis of clavillia (Mirabilis jalapa) was rich in many active compounds including triterpenes, proteins, flavonoids, alkaloids,
and steroids. Purified antiviral proteins from roots, shoots, leaves, fruits, and seeds of *Mirabilis jalapa* are employed for different affections. Thus, information about the reproductive pattern of this culture is important for implementing experimental procedures (*Leal et al., 2001*). MAPs in clavillia as being effective in protecting economically-important crops (such as tobacco, corn, and potatoes) from a large variety of plant viruses (such as tobacco mosaic virus, spotted leaf virus and root rot virus) (*Vivanco et al., 1999*).
6- SUMMARY AND CONCLUSION

This study was conducted at the Laboratory, Greenhouse, and Farm of Faculty of Agriculture, Moshtohor, Banha University and the Virology Laboratory of Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt, during two successive spring surveys (2003/2004 – 2004/2005).

The aims of this study are surveyed the viruses affecting faba bean crop at Qalyoubia Governorate, identified the collected infected samples via ELISA, determine the disease incidence and severity. On the basis of specific virus symptoms, estimated the viruses frequencies, subsequently, determine the dominant faba bean viruses at the surveyed fields. Completely, isolated and identified the dominant virus using symptomatology, host range, mode of transmissions, physical properties, serological diagnosis, particle properties (inclusion bodies, via light microscope and virus-particle dimension via transmission electron microscope). Study the protective means possibilities to reduce or eliminate the virus infection as early as.

The obtained results can be summarized as follows:

1- Through restricted two surveys for faba bean viruses conducted in some districts at Qalyoubia Governorate (i.e., Banha, El-Qanater El-Khayria, Kafr Shoukr, Shebien El-Qanater and Toukh) during February and April of 2004 to identify viruses affecting the faba bean (Vicia faba) crop. All faba bean plants showing symptoms suggestive of viral infection, such as stunting, vein clearing, leaf roll, mosaic, chlorosis, necrosis, yellowing and leaf distortion were visually inspected.

2- On the basis of the symptoms, a total virus infected leaf samples divided into 5 main categories, and examined against antisera for six
faba viruses [Faba bean necrotic yellows nanavirus (FBNYV); Bean yellow mosaic potyvirus (BYMV); Broad bean stain comovirus (BBSV); Alfalfa mosaic alfamovirus (AMV); Broad bean wilt fabavirus (BBWV) and Pea seed borne mosaic potyvirus (PSbMV)] using double antibody sandwich- enzyme-linked immunoassay (DAS-ELISA). The ELISA reactions proved that all viruses were found at all tested samples either alone or mixed.

3- Disease incidence in the first survey came in the descending order as follows: Toukh (55.55%), Kafr Shoukr (54.17%), Shebien El-Qanater (50.00%), Banha (47.62%) and El-Qanater El-Khayria (38.89%). Meanwhile, in the second survey were Shebien El-Qanater (62.75%), Banha (60.61%), Toukh (59.65%), Kafr Shoukr (52.38%) and El-Qanater El-Khayria (44.00%).

4- Disease severity was increased in the second survey than the first one also due to the incurred specific virus symptoms. Toukh samples showed the highest disease severity in both surveys (23.26 and 23.94%), followed by Shebien El-Qanater (17.44 and 22.54%), Kafr Shoukr (15.12 and 15.49%), Banha (11.63 and 14.08%), then El-Qanater El-Khayria came at last (8.14 and 9.30%).

5- ELISA tests showed that the frequencies of investigated viruses were recorded as follows: at the first survey, Bean yellow mosaic potyvirus (BYMV) was the dominant one (67.00%) at all 5 surveyed locations, followed by FBNYV (21.00%), BBSV (3.90%), AMV (2.46%), BBWV (2.14%) and PSbMV (1.50%). Meanwhile, during the second survey, Bean yellow mosaic potyvirus (BYMV) was the dominant one (67.65%) at all 5 surveyed locations, followed by FBNYV (24.81%), BBSV (3.33%), AMV (1.92%), BBWV (1.61%) and PSbMV (0.68%).
Host range of Bean yellow mosaic potyvirus (BYMV) was restricted. *Vicia faba* mechanically infected with the isolated virus showed vein clearing, chlorosis followed by obvious green or yellow mosaic. Chlorotic or necrotic local lesions were appeared on mechanically inoculated leaves of *Chenopodium amaranticolor, C. album, C. quinoa* and *Gomphrena globosa*. On the contrary, symptomless hosts with negative back inoculation tests were: *Cucumis sativus* L. *Cucurbita pepo* L. (Cucurbitaceae), *Vigna unguiculata* L. (Leguminosae), *Lycopersicum esculentum* Mill, *Capsicum annuum* L., *N. tabacum* L., cvs. Samsun, *Datura stramonium* L., *Datura metel*, *Solanum nigrum* and *Solanum melongana* L. (Solanaceae).

On the basis of symptoms, and host range (including differential and diagnostic hosts) isolation and primary identification (pathogenicity tests) revealed that faba bean plants of the same cultivar showed the typically systemic symptoms when inoculated with crude sap from naturally virus infected faba bean leaves.

Bean yellow mosaic potyvirus was mechanical transmissible readily with the infectious sap (90 – 95%). Both green peach aphid (*Myzus persicae* Sulz.) and faba aphid (*Aphis fabae*) were readily transmitted it from infected faba bean (*Vicia faba* L.) source plants to healthy *C. quinoa* in a non-persistent manner with the average rate 85% and 73%, respectively. No evidence that this virus was seed transmissible in the present work.

Infectious sap extracted from *Nicotiana clevelandii* as a source of the virus, was used to determine the physical properties of bean yellow mosaic potyvirus. Thermal inactivation point was 65°C. Dilution end
point was $10^{-4}$. The virus maintained its infectivity at room temperature (25°C) for 6 days on *C. quinoa* plants.

10- Cytopathological examination of Bean yellow mosaic potyvirus-infected faba bean epidermal cells showed presence of cytoplasmic vacuolated nuclear inclusion proteins of different size and shape. Its one of the evidence of the presence of potyvirus member.

11- Electron microscopic examinations of faba bean leaf-dip preparation showed the presence of filamentous flexuous virus particles with average dimension of approximately 750 x 13 nm.

12- Gel double diffusion (SDS-immunodiffusion) test revealed that there were serological relationships between the isolated virus and other potyvirus members (*e.g.* bean yellow mosaic virus, potato Y virus, pepper severe mosaic virus) BYMV, PYV, PSMV and not reacted with tobamoviruses (pepper mild mottle virus and tobacco mosaic virus) PMMV and TMV.

13- ELISA test confirmed the presence of BYMV alone or mixed in all the collected leaf samples or mixed with FBNYV, AMV, BBWV, PsbMV.

14- Response of six faba bean cultivars against mechanical inoculation with BYMV revealed that Giza 2 was highly susceptible, followed by Shakha 1, Giza 843, Giza 716 and Giza 3. Meanwhile, Misr 1 cultivar was the most resistant one.

15- Induced systemic resistance was demonstrated by estimate the inhibitory percentage on local lesion host. Using root extracts of *Phytolacca americana* induced the highest systemic resistance against the isolated virus (99%), followed by root extract of *Mirabilis jalapa* (98%), leaves extracts of *Clerodendrum inerme* and
Phytolacca americana, and young shoots extract of Mirabilis jalapa (97%), leaves extract of Dianthus caryophyllus (91%), leaves extract of Chrysanthemum cinerariifolium (80%), then fruits and leaves extracts of Schinus terebinthifolius (76 and 72%, respectively).

16- Enhancement in the faba bean productivity was recorded by estimating 100-seeds obtained from each plot of the field trials in the two seasons.

17- DAS-ELISA was used again at the final of each field experiments in two seasons to detect any faba virus infection after three sprays with 6 botanical saps. No or rarely positive reaction were achieved when leaves extracts of faba bean plots which sprayed with Roots and leaves of Phytolacca americana and Mirabilis jalapa or leaves extracts of Dianthus caryophyllus and Clerodendrum inerme were examined against 6 faba bean viruses antisera [faba bean necrotic yellows virus (FBNYV); Bean yellow mosaic virus (BYMV); Broad bean stain virus (BBSV); Alfalfa mosaic virus (AMV); Broad bean wilt virus (BBWV) and Pea seed borne mosaic virus (PSbMV). Meanwhile, moderately positive reaction against the same antisera using DAS-ELISA was observed when faba bean leaf samples collected from plots sprayed with leaves extracts of Chrysanthemum cinerariifolium and fruit, leaves extracts of Schinus terebinthifolius, respectively.

18- When protein pattern profile was carried out using electrophoresis. Results demonstrated that there were extra bands found in the faba bean leaves which sprayed with botanical extracts and not found in the unsprayed ones. Their harmony identical between extra bands with those found in the lanes of botanical proteins. The extra bands in the botanical act as antiviral proteins or enzymes.
CONCLUSION

The aims of this work were achieved by surveying the viruses affecting faba bean fields at different location distributed through Qalyoubia Governorate. On the basis of symptoms and ELISA test the wide spread viruses were detected. The dominant virus in the faba bean crop was isolated and identified as member of potyviruses (Bean yellow mosaic potyvirus) using symptomatology, host range, mode of transmission, physical properties, serological reactions, cytopathological examinations, virion particle shape and dimension. Response of some popular faba bean cultivars showed variation and Giza 2 was the highest susceptible for the isolated virus, whereas, Misr 1 was the highest resistant one. Good results dealing reduction of virus infection with faba viruses were obtained when aqueous extracts of some medicinal plants sprayed as systemic resistance inducers. Sowing in early second half of November enhanced the productivity of faba bean yield and help in the viral diseases reduction.
Recommendations

- Avoid the peaks of the famous insects which act as injury insect pests and plant viruses vectors.
- Cultivated the tolerant or resistant faba bean cultivars.
- Sowing faba bean crop in the early of the second half of November.
- Using the aqueous extracts of the above mentioned medicinal plants to their systemic induced resistance or anti-insect vectors activities as sprayed at gradual intervals.
- Eliminate the infected plants just when appeared.
- Replace the agarose and sodium azide in the double diffusion test with gelrite and Kombucha to obtain good clear results and avoid sodium azide toxicity and safe cost.
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