Inducing Systemic Resistance against Bean Yellow Mosaic Potyvirus Using Botanical Extracts

Mahdy¹ A.M.M.; R.N. Fawzy¹; M.A. Hafez¹; Hanan A.N. Mohamed² and Eman S.M. Shahwan¹

Mosaic with bright yellowing symptoms suggestive due to bean yellow mosaic potyvirus (BYMV) was the most frequently found and likely most damaging virus in faba bean crop. The virus was detected in different locations in Qalyoubia Governorate using DAS-ELISA. Enhancements were introduced to Ouchterlony double diffusion test. Response of some cultivated faba bean cultivars was studied under artificial inoculation by BYMV. Delaying the sowing date, and spraying faba bean seedlings with six aqueous botanical extracts under field conditions fortnightly interval, were achieved as a simple strategy for BYMV control. Ribosome-inactivating proteins (RIPs) were demonstrated by protein profile pattern of both faba bean and botanicals using SDS-PAGE. ELISA was used as a diagnostic tool at the beginning of the study and at the end to insure no faba virus infections after 3 botanical extract sprayers.

Key words: Faba bean, BYMV, induced systemic resistance, Botanical extracts

INTRODUCTION

Faba bean (Vicia faba L., Fabaceae) is considered as the most important nutritive popular food crop in Egypt and other world countries. 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 (producing 2,835,358 Ardab) in 2001 to 240,854 feddan producing 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). Bean yellow mosaic potyvirus (BYMV) is a common disease of legumes and many hosts, found worldwide (Bos, 1970). Moreover, BYMV infection reduced yield (kg/ha), and protein content but increased tannin content (mg/100 ml) (Babiker et al., 1995). In Egypt, Tolba (1980) reported that BYMV is the most widespread of all the viruses affecting broad bean crops. 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).


(Received December 2006)
(Accepted January 2007)
The present study aims to isolate Bean yellow mosaic potyvirus and to design an integrated program to eliminate or reduce faba virus- infections including natural alternatives as systemic resistance inducers.

MATERIALS AND METHODS

Source of the virus:

Faba bean young leaves was collected from different locations in Qalyoubia Governorate (during 2004 season) from plants showing stunting, vein clearing, mosaic, chlorosis, necrosis, bright yellowing and leaf distortion symptoms. Samples were tested for six faba viruses [Alfalfa mosaic virus (AMV); Broad bean stain virus (BBSV); Broad bean wilt virus (BBWV); Bean yellow mosaic virus, (BYMV); Faba bean necrotic yellows virus (FBNYV) and Pea seed borne mosaic virus (PsbMV)] using specific kits (produced by AGERI) for them by double antibody sandwich-enzyme-linked immunoassay (DAS-ELISA) at Agricultural Genetic Engineering Institute, AGERI, Agric. Res. Center, ARC., Giza, Egypt.

Isolation and propagation of BYMV:

Nicotiana clevelandii plants mechanically inoculated with sap from systemically infected faba bean plants in 0.01 mol L⁻¹ neutral phosphate buffers, which gave positive reaction with polyclonal antibody specific to BYMV and negative reaction with other tested viruses. Inoculated plants were kept for 4 weeks in an insect-free glasshouse at 18 – 24°C for recording symptom development.

Serological detection:

a- Ouchterlony (double diffusion) 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 according the method devised by Ouchterlony (1948).

Antisera specific to six faba viruses (AMV, BBSV, BBWV, BYMV, FBNYV and PsbMV) from AGERI were tested against infected samples using double diffusion test.

Gelrite® [Merck & Co., Inc. (Rahway, NJ), Kelco Division, USA], as solidification material (clear and high strength gel) at 0.3g/L was substituted with 0.8g/L agarose. In addition, 0.02g/L sodium azide (NaN₃) was replaced with 0.1% Kombucha (green fermented tea) as an antimicrobial and antioxidant, which more safe and
cheap. These considerable modified are enhancing this assay.

Plates were incubated in the humid chamber at room temperature overnight. Precipitin resulting bands were photographed without staining and results were recorded.

**b-DAS-ELISA:**

Naturally and artificially viral infected faba bean samples directly detected with the double antibody sandwich-enzyme linked immuno-sorbent assay (DAS-ELISA) using antisera specific to 6 faba-related viruses (e.g., AMV, BBSV, BBWV, BYMV, FBNYV and PsbMV) as described by Clark and Adams (1977).

**Control management:**

1- **Response of some faba bean cultivars to artificial infection with BYMV:**

Trial was carried under greenhouse conditions at Fac. Agric., at Moshtohor, Benha Univ. Six faba bean cultivars (Sakha 1, Misr 1, Giza 2, Giza 3, Giza 716 and Giza 843) all purchased from the Egyptian Agricultural Organization, Ministry of Agriculture, Giza, Egypt. Twenty-five faba bean plant (each cultivar) was sown (5 plants/pot, 5 pots/cultivar) served as replicates for virus inoculation. The same number of faba bean plants from each cultivar was inoculated with distilled water served as control. Seeds of all test plants were grown in a mixed soil (clay: peat : sand 1:1:1 v/v/v), fertilized weekly and regularly irrigated. Four true leaves stage faba bean seedlings were mechanically inoculated with BYMV isolate. The plants were observed and the systemically infected plants were counted until consistent numbers were reached (20-days post-inoculation). A scale of 1–5 categories was used to assess severity: 1=no symptom; 2=mild chlorotic patterns and slight distortion of leaves; 3=mosaic patterns on all leaves, leaf distortion; 4=mosaic patterns on all leaves, leaf distortion, and general reduction in leaf size; 5= severe mosaic on all leaves and stunting of whole plant. Disease severity (DS) percentage was calculated according to Wydra and Verdier (2002) using the following equation:

\[
DS (\%) = \frac{\sum n \times V}{5N} \times 100
\]

Where:

\( (n) = \) No. of infected leaves in each category.
\( (N)= \) Total No. of the leaves inspected.
\( (V)= \) Numerical value of the categories (1-5).

2- **Induced systemic resistance against BYMV:**

Fresh plant of 6 medicinal plant [belonging to 6 families] were collected from the botanical garden of the Faculty of Agriculture, Moshtohor, Benha University, except *Phytolacca americana* was obtained from the farm of Fac. of Pharmacy, Cairo University. Fresh

plant material was washed carefully under running tap water.  
*Chrysanthemum cinerariaefolium* (Asteraceae), *Clerodendrum inerme* (Verbenaceae), *Dianthus caryophyllus* (Caryophyllaceae), *Mirabilis jalapa* (Nyctaginaceae), *Phytolacca americana* (Phytolaccaceae) and *Schinus terebinthifolius* (Anacardiaceae) were chosen depending on previous information’s dealing their systemic resistance inducers as producers for ribosomal inhibitor proteins (RIPs).

Stock aqueous crude extraction for each individual tested plant was made by blended 1 kg plant material in 1 liter distilled water for 5 min. The mixture filtered through 8 layers of muslin cloth, afterwards the filtrate was centrifuged for 15 min. at 3000 rpm to separate the plant debris. Filtrates were sterilized using centre glass (G4), then stored in the refrigerator at -20°C until use.

From each stock extract, three dilutions were made (*i.e.*, 5, 10, 20%) using distilled water.

**Greenhouse experiment:**  
**Preliminary trial**  
During August 2004, crude botanical sap was sprayed using Pressure Sprayer (2L) on ten *C. quinoa* leaves for each treatment 24 hrs pre-inoculation with the virus isolate (each plant received about 20 ml solution).

Leaves of *C. quinoa* dusted with carborundum, 600-mesh, mechanically inoculated with virus inoculum.

Total number of local lesions, from ten leaves for each treatment, was counted 14 days post 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*).

**Avermently trial**  
Plastic pots (Ø 25 cm) filled with a suitable amount of formalin sterilized soil mixture.

Ten faba bean seeds were sown per pot, and then thinned to 3 seedlings 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, and fertilized when needed.

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 (5, 10 and 20%) 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.

Primary four leaves of faba bean plants dusted with carborundum 600-mesh, mechanically inoculated with virus inoculum.

Treated plants were kept under insect proof house and observed for the appearance of systemic symptoms formation.
Inhibitory effect of the tested botanical sap was determined in both trials as described by Devi et al. (2004) using the following equation:

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

where:

\[A = \text{Control}, \ B = \text{Treatment}\]

3- Sowing dates:
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) at Vegetable Farm, Faculty of Agriculture, Moshtohor, Benha 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.

Field experiment:
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). 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 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% crude 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).

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 extracts were screened for natural infection with any of six tested antisera of faba viruses aforementioned.

Protein Electrophoresis:
This method demonstrates that the ribosomal inhibiting proteins by SDS-PAGE were transferred from botanical extract, and induced systemic resistance against natural infection with faba viruses.
Extraction of botanical-related protein:
Protein extraction was conducted according to Bollag et al. (1996) using 1 g of young faba bean leaves collected after 4 sprays with botanical sap and subjected to natural virus-infection. Untreated leaves were used as control. As well as leaves of 6 botanicals.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

EXPERIMENTAL RESULTS

BYMV was recorded (Fig. 1), either alone or in mixed infection, the highest frequently percentage among 6 faba viruses found naturally infected faba bean samples. 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 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.

Artificially infected Vicia faba and Nicotiana clevelandii with the isolated virus showed systemic symptoms. Chlorotic local lesions were appeared on leaves of Chenopodium quinoa.

Fig. (1): Frequency percentage of 6 viruses infected faba bean plant collected from different locations at Qalyoubia Governorate.
1- Serological diagnosis:

a- Ouchterlony double diffusion test:

Strong serological relationship between BYMV antisrum and partially purified preparation from faba bean leaves was showed typical BYMV symptoms. Reaction was observed between partially purified virus and antisera of BBSV and BBWV, but no reaction was observed with AMV, FBNYV and PsbMV (Plate 1).

Reactions with the modified gel plates were clearer, sharpen and solidified than standard gel of Ouchterlony double diffusion test (Plate, 1).

b- DAS-ELISA:

This test used in this work at the beginning and the last experiments as diagnostic tools. In the early, DAS-ELISA confirmed the presence of BYMV alone in some infected samples and mixed with AMV, BBWV, FBNYV, PsbMV in the other samples. While, in the late demonstrated that inhibition percent of infection with any faba viruses in the treated faba plants with some botanical plant extracts.

Plate (1): Comparison between standard and modified Ochterlony double diffusion test. Using antisera of AMV, BBSV, BBWV, BYMV, FBNYV and PsbMV. Blank is empty.

2- Control management:

a- Response of some faba bean cultivars to BYMV:

Figure (2) illustrated that among the 6 faba bean tested cultivars mechanically inoculated with BYMV, under greenhouse conditions, Giza 2 was highly susceptible one (57%), followed by Sakha 1 (30%), Giza 843 (57.5%), Giza 716 (25%) and Giza 3 (22.5%). Meanwhile, Misr 1 cultivar was the more resistant one. So, Giza 2 cultivar was used at all the followed studies in this work.

![Figure 2: Response of six faba bean cultivars to artificial infection with BYMV virus under greenhouse conditions.](image)

Fig. (2): Response of six faba bean cultivars to artificial infection with BYMV virus under greenhouse conditions.

b- Sowing dates:

Field experiment for study the effect of some botanical extracts against faba bean viruses was repeated twice at the different sowing dates. The first was sown at October 1 2004 (as early sowing) and second in November 16 2005 (as late sowing). Data in Table (3) show that the second half of November was found to be suitable for least virus infection
in faba bean field than the first of October.

c- Induced systemic resistance against BYMV:

The results of preliminary trial recorded in Table (1) 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).

Meanwhile, the results of avermently trial on faba bean recorded in Table (2) show that all three concentrations (i.e., 5, 10 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 (1): 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>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chrysanthemum cinerariifolium</em></td>
<td>Leaves</td>
<td>80.00</td>
<td>Kain &amp; Kovach (1997)</td>
</tr>
<tr>
<td><em>Clerodendrum inerme</em></td>
<td>Leaves</td>
<td>97.00</td>
<td>Kumar et al. (1997)</td>
</tr>
<tr>
<td><em>Dianthus caryophyllus</em></td>
<td>Leaves</td>
<td>91.00</td>
<td>Stirpe et al. (1981)</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em></td>
<td>Young shoots</td>
<td>97.00</td>
<td>Effmert et al. (2005)</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em></td>
<td>Roots</td>
<td>98.00</td>
<td>Leal et al. (2001)</td>
</tr>
<tr>
<td><em>Phytolacca americana</em></td>
<td>Leaves</td>
<td>97.00</td>
<td>Melander (2004)</td>
</tr>
<tr>
<td><em>Phytolacca americana</em></td>
<td>Roots</td>
<td>99.00</td>
<td>Barakat et al. (2005)</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em></td>
<td>Fruits</td>
<td>76.00</td>
<td>Simons et al. (1963)</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em></td>
<td>Leaves</td>
<td>72.00</td>
<td></td>
</tr>
</tbody>
</table>

Inhibition (%) = Control ─ Treatment / Control x 100
Table (2): 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><em>Chrysanthemum cinerariifolium</em></td>
<td>leaves</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>64</td>
</tr>
<tr>
<td><em>Clerodendrum inerme</em></td>
<td>leaves</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td><em>Dianthus caryophyllus</em></td>
<td>leaves</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>71</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em></td>
<td>young shoots</td>
<td>5</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em></td>
<td>roots</td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td><em>Phytolacca americana</em></td>
<td>leaves</td>
<td>5</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td><em>Phytolacca americana</em></td>
<td>roots</td>
<td>5</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>99</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em></td>
<td>fruits</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>64</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em></td>
<td>leaves</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>57</td>
</tr>
</tbody>
</table>

Applicable plan was achieved by repetition spraying with aqueous extracts of six healthy medicinal plants through field experiments.

Data recorded in Table (3) show that three sprays with 20% of all extracts fortnightly interval gave good results dealing inhibitory effect of 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 botanical 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 (3):** 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><em>Chrysanthemum cinerariifolium</em> (leaves)</td>
<td>1st spray*</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>66</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>71</td>
<td>70</td>
</tr>
<tr>
<td><em>Clerodendrum inerme</em> (leaves)</td>
<td>1st spray</td>
<td>79</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>85</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td><em>Dianthus caryophyllus</em> (leaves)</td>
<td>1st spray</td>
<td>83</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em> (young shoots)</td>
<td>1st spray</td>
<td>86</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td><em>Mirabilis jalapa</em> (roots)</td>
<td>1st spray</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>89</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td><em>Phytolacca americana</em> (leaves)</td>
<td>1st spray</td>
<td>87</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td><em>Phytolacca americana</em> (roots)</td>
<td>1st spray</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>97</td>
<td>98</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em> (fruits)</td>
<td>1st spray</td>
<td>61</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>76</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>82</td>
<td>87</td>
</tr>
<tr>
<td><em>Schinus terebinthifolius</em> (leaves)</td>
<td>1st spray</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2nd spray</td>
<td>60</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>3rd spray</td>
<td>68</td>
<td>67</td>
</tr>
</tbody>
</table>

*1st, 2nd and 3rd sprays applied after 15, 30 and 45 days from sowing date, respectively.

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 botanical extracts are clearly shown (Plate, 2). Extra bands between 24 to 32 kDa were released in the treated faba bean plants and not found in the unsprayed one, but identical to those electrophoretic in leaves extracts of six botanicals.

Plate (2): 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.

Whereas:
C: Control (unsprayed faba bean leaves)
1-6: Lanes faba bean sprayed with leaf extracts of Clerodendrum inerme, Dianthus caryophyllus, Mirabilis jalapa, Phytolacca Americana, Chrysanthemum cinerariifolium and Schinus terebinthifolius, respectively.
7-12: Lanes of leaf extract of Clerodendrum inerme, Dianthus caryophyllus, Mirabilis jalapa, Phytolacca Americana, Chrysanthemum cinerariifolium and Schinus terebinthifolius, respectively.
DISCUSSION

BYMV was recorded, either alone or in mixed infection, the highest frequently percentage among 6 faba viruses found naturally infected faba bean samples. The same trend was observed in the results recorded by Makkouk et al. (1994); Rizkalla (2002); Makkouk et al. (2003) and Bekele et al. (2005).

Nicotiana clevelandii L. was 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 quinoa was reacted with local lesions against mechanical inoculation with Bean yellow mosaic potyvirus (ICTVdB, 2006).

Among the 6 faba bean tested cultivars mechanically inoculated with BYMV, under greenhouse conditions, Giza 2 was highly susceptible one (57%), followed by Sakha 1 (30%), Giza 843 (57.5%), Giza 716 (25%) and Giza 3 (22.5%). Meanwhile, Misr 1 cultivar was the more resistant one. So, Giza 2 cultivar was used at all the followed studies in this work. This result came in harmony with those recorded by Tolba (1980), in Egypt, who mention that of 90 lines and cultivars of Vicia faba exposed to infection with a locally occurring Egyptian strain of bean yellow mosaic virus, only five proved resistant.

The second half of November was found to be suitable for least virus infection in faba bean field than the first of October. 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 faba bean fields in October. They introduce the virus into faba bean plants when they start feeding on them. So, delay 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.

Generally, all spray treatments increased systemic induced resistance compared with the control in both seasons (2004 – 2005). 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).

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).

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).
Inducing Systemic Resistance against *Bean Yellow Mosaic*

*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).

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).

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).

Leaf sap of 30 plant species of 75 tested gave 95-100% reduction in tobacco mosaic virus (TMV) lesion production on *Nicotiana glutinosa*. Of these, 20 were succulents, mostly Crassulaceae. Non-succulent inhibitor sources not previously reported include *Eugenia paniculata*, *Lonicer (?)* caprifolium, *Schinus molle*, *Schinus terebinthifolius*, *Cyrtomium falcatum*, *Cedrus deodara*, *Juniperus communis*, and *Thuja orientalis* [*Platycladus orientalis*]. Many of the inhibitors withstood 80°C for 10 min. Attempts to establish the possible proteinaceous composition of the inhibitors in 5 succulents were only partially successful. Preparations from *Mesembryanthemum caprohetum* caused a 99% reduction at a dilution of 1: 1, 250. Sap from *Aloe* sp. and *Kleinia cylindrica* augmented lesion numbers by 100%. Several of the inhibitors were also active against potato virus Y in *Capsicum* var. California Wonder. It was concluded that the inhibitors are similar in effect and act on host plant susceptibility (Simons *et al.*, 1963).

REFERENCES

viral infection on yield, tannin and protein contents and in vitro protein digestibility of faba bean. Plant Foods for Human Nutrition, 47: 257-263.


Commonwealth Agricultural Bureaux, Slough.


Inducing Systemic Resistance against *Bean Yellow Mosaic*


**Ouchterlony, O. (1948):** *In vitro* method for testing toxin producing capacity of diphtheria.


عده مهدي محمد مهدي
محمود السيد حافظ سليم
إيمان شهوان محب الدين شهوان

قسم النباتات الزراعية (فرع أمراض النباتات) - كلية الزراعة - جامعة بنها - مصر
معهد الهندسة الوراثية الزراعية - مركز البحوث الزراعية - الجيزة - مصر

سجلت أعراض التبرقش والأصفرار التي تحدث نتيجة الإصابة بفيروس الموزايك الأصفر في عدة حقول منزرعة بالفول البلدي وكانت شديدة بدرجة أثرت سلباً على المحصول. وقد عزل من النباتات التي تحمل تلك الأعراض فيروس الموزايك الأصفر سواء منفرداً أو ضمن إصابة خليبية من عدة مناطق مختلفة بمحافظة الفيومية. وتم الكشف عن الفيروس بواسطة اختبار الإليزا. وعند إجراء اختبار الانتشار المزدوج خلال الجيل أدخلت عليه تعديلات حيث استبدل الأجور بالجبل رايت (كمادة تصلب) والشاي الأخضر المتخرم (كمبوش) بدلاً من الصوديوم أزيد (كمضاد للتلوث بالبيكوروات وأيضاً مضاد للأكسدة) وكانت النتائج أفضل من التركيبة التقليدية للاختبار وأكثر وضوحاً وأقل تكلفة وأكثر أماناً. للاعداد برنامج مقاومة متكامل تم اختيار حساسية ستة من أصناف وهم الفول البلدي المنزرعة للإصابة بفيروس الموزايك الأصفر. كذلك تضمن اختيار الموعد المناسب للزراعة لتجنب الإصابة وأخيراً تم رش نباتات الفول في الحقل بالمستخلص المائي لستة نباتات طبية لفترات احتوائها أربعة منها على بروتينات (إنزيمية) مثبتة للريبوسومات وتستحسن نباتات الفول على مقاومة الفيروسات النباتية. وقد تم الاستدلال على الانتشار الجهازي للفيروسات البروتينات في نباتات الفول ومنها ببعض إصابات الفيروسية بسبب انيفا مع انتشار الكهرباء للبروتينات. وقد استخدم اختبار الإليزا في بداية الدراسة للكشف عن نباتات التصويت في العينات المصابة ثم استخدم في نهاية التجارب للتأكد من خلو النباتات التي سبق رشها بالمستخلصات النباتية من الإصابة الفيروسية بنفس الفيروسات الستة.