

## Antiviral activity of latex from *Ficus nitida* against plant viruses

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### Abstract:

Latices tapped from *Ficus elastica*, *F. nitida* and *Euphorbia pulcherrima* plants were screened for antiviral activity against some plant viruses in different hosts. Three models of host-virus interaction were used. Two systemic infections, squash-zucchini yellow mosaic virus (ZYMV); broad bean-bean yellow mosaic virus (BYMV) and the third one bean-tobacco necrosis virus (TNV) as local infection. The greatest degree of antiviral activity against mechanical transmission of BYMV and ZYMV was observed with crud latex of *F. nitida*, compared with other latices. These results were obtained when the latex was mixed with the virus inoculum for 15 min or pretreatment for 48h, then the virus was challenged. Complete inhibition in local lesion numbers was achieved when serum latex of *F. nitida* (1 mg protein ml<sup>-1</sup>) was mixed with equal volume of purified TNV (1 µg ml<sup>-1</sup>) for 15 min. Latex infiltrated into the veins through the lower surfaces of broad bean leaves decreased BYMV transmission by *Aphis faba* (20% transmission compared with 65.0 % in water infiltrated). BYMV mixed with the least concentration of serum (0.5 mg protein ml<sup>-1</sup>) doesn't show any detectable virus through DAS-ELISA test. BYMV particles incubated with *F. nitida* serum latex 1.00 mg ml<sup>-1</sup> for 1 h showed aggregated, thickened, and malformed and lyses in some regions when examined by transmission electron microscopy.

### Key words:

*Ficus*, antiviral, BYMV, ZYMV, TNV, DAS-ELISA and serology ELISA and serology

### Introduction

Bean yellow mosaic and zucchini yellow mosaic viruses (BYMV and ZYMV) are well-established viruses with increasing economic importance as plant pathogens. This viruses has widest host range of any other plant viruses and they transmitted by aphids in non-persistent manner (El-Afifi and El-DougDoug, 1997 and Mahmoud *et al.*, 2004). These viruses affect the productivity of the crops grown around the world (Bailiss and Senananyake, 1984; Provvidenti *et al.*, 1985 and Montalbini *et al.*, 1991. The losses of market of broad bean and squash yields due to BYMV and ZYMV epidemics reduced the production by more than 50% in Egypt and England according to Bailiss and Senananyake,

1984 and Mahmoud *et al.*, 2004. No safe viricidal chemical that can eliminate viruses without adversely affecting their hosts has yet been found. This problem has become a serious challenge to plant pathologists, biochemists and molecular biologists to develop a long-term and a sustainable management strategy for plant viruses. After the first report of tobacco mosaic virus (TMV) inhibitor in the leaves of *Phytolacca* sp. by Allard (1914), many scientists have reported virus inhibitors in several plant species. The roll of a type 1 ribosome-inactivating protein (Type 1 RIP) purified from leaves of *Phytolacca heterotepala* was studied by Corrado, *et al.*, 2008. This protein inhibited protein translation in a cell-

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free assay and limited the local lesion formation from potato virus X infection in tobacco leaves. Several workers have reported antiviral activity of juices from different parts of higher plants including seeds of *Hordeum vulgare* L. and *Luffa cylindrical* L. (Barakat *et al.*, 2004); roots of *Cycas revolute* and *Mirabilis jalapa* L. (Rao *et al.*, 1984 and Kubo *et al.*, 1990); leaves of *Mirabilis jalapa* L., *Harpulia cupanioides* L., *Turnera ulmifolia* L. *Clerodendrum aculeatum* L. and *Qhytolacca americana* L. (Kujo *et al.*, 1990; Renuka Devi *et al.*, 2004; Figueira *et al.*, 1994; Verma *et al.*, 1996 and Irvin *et al.*, 1980). Canal systems of some plants containing various secretions, such as latex, resins, gums and mucilages are widespread in the plant kingdom (Radwan and Mahmoud, 2001). Although much information is available regarding virus inhibitors in plant species, a little attention was attributed to the plant bearing latex such as *Calotropis procera* against TMV (Paul Khurana and Singh, 1972) and *Euphorbia* genus against human viruses (Betancur-Galvis, *et al.*, 2002). Latex occurs in 12500 species belonging to 900 genera. It is a milky fluid composed of a liquid serum which holds, either in solution or suspension, and contain many of substances which are toxic to pathogens. Insects and pathogens that attack latex-producing plants are normally faced with a combination of physical and chemical defenses provided by latex (Lynn and Clevette-Radford 1987b). Enzymes carrying out varied functions are known to occur in various latex at high concentrations (Giordani *et al.*, 1992). The various exuded latex are known to contain glycosidases (Giordani and Lafon, 1993), proteases (Lynn and Clevette-Radford, 1986), acid phosphatase (Lynn and Clevette-Radford 1987a),

amylases (Freitas *et al.*, 2007), chitinases (Jekel *et al.*, 1991), trypsin inhibitors (Lin and Lu, 1994), and  $\beta$ -1,3-glucanase (Chye and Cheung, 1995). A large number of the plants, which are not affected by viral pathogens and insects, remain as an unexploited potential to be tapped, as sources of antiviral principles (Farrell *et al.*, 1991). Hence, the present work deals with screening and describes the inhibitory behaviors of latex to be explored for the management of plant viruses.

## Materials and methods

### Plants, viruses and virus inoculation

Seeds of squash (*Cucurbita pepo* cv. Eskandarani); Broad bean (*Vicia faba* cv. Giza 461) and bean (*Phaseolus vulgari* cv. Contender) were obtained from Horticulture Institute, Agriculture Research Centre (ARC), Dokki, Egypt. The seed surfaces were sterilized with sodium hypochlorite solution (5%) and then planted in pots containing 700 gm of soil. Pots were placed in a greenhouse of Botany Dept., Fac. Science, Assiut Univ., under a 16 h photoperiod at  $25 \pm 2^\circ\text{C}$ . *Chinopodium quinoa* seeds were obtained from Agric. Botany Dept., Fac. Agric., Sohag University and then planted as mentioned above.

Zucchini yellow mosaic virus (ZYMV), bean yellow mosaic virus (BYMV) and tobacco necrosis virus (TNV) were isolated in previous works in Virology Lab., Faculty of agriculture, Sohag University, Egypt. On the other hand, purified BYMV and TNV was obtained from the same source and stored at  $4^\circ\text{C}$ .

The virus isolates were maintained and propagated by serial inoculations on young actively grown squash (for ZYMV), broad bean (for BYMV) and bean (for TNV) plants in an insect-proof greenhouse according to the method cited by Anfoka and Buchenauer (1997). The inoculum of

each virus was prepared by grinding 1 gm young symptomatic leaves in 0.1 M phosphate buffer (pH 7) (1 ml per gm of leaf material) in a pestle and mortar. The pulp was passed through 2 layers of cheesecloth and centrifuged at 3000 rpm for 5 min. The clear supernatant thus obtained was diluted 1:5 for ZYMV or BYMV and 1:10 for TNV with buffer and used for inoculation purposes. Leaves of test plants were dusted with 600 meshes Carborandum and virus inoculum (200 µl / plant leaf) was applied with glass spatula on the upper leaf surface. All 4 fully expanded leaves in hypersensitive host (*C. quinoa* and *Ph vulgaris*) and 2 basal leaves in systemic hosts (Squash and broad bean) were inoculated. The inoculated leaves were washed with water and placed in the insect-proof greenhouse under controlled conditions.

#### **Latex collection and fractionation**

Latex of *F. elastica*, *F. nitida* and *Euphorbia pulcherrimia*, was obtained at morning of winter season from cultivated plants in Assiut Governorate then prepared according to method described by Freitas *et al.*, 2007. Superficial incisions on young stems of the trees provided milk like latex. The resulting latex drops were collected in ice-chilled plastic recipient into sterilized distilled water. Natural coagulation-like effect of the material was partially prevented by gentle agitation during collection. When a volume of latex equal to the starting volume of water was collected, the mixture was centrifuged for 20 min at 17,000 g (4 °C) in (SR4000 Prolabo, made in France) on the same day as collected. The latex separated into three layers; the top white zone containing natural rubber; a middle yellow clear aqueous centre layer called serum, and the sediment bottom layer containing a small quantity of

lute particles (lipids). The serum fraction was separated and preserved at 4 °C, and then tested for its antiviral activity.

#### **Screening test of crude latices against ZYMV and BYMV**

To detect the viral inhibitory activity in the different crude latex, three methods were used. (1) Latex were added to viral inocula in equal volumes and kept for 15 min, and then the mixtures was inoculated on leaves of squash or broad bean plants as mentioned above. The control consisted of each virus mixed with equal volume of distilled water instead of the latex. (2) Latex was applied on leaves of squash or broad bean plants 48 h before viral inoculation. (3) Latex was applied on leaves of squash or broad bean plants 48 h after viral inoculation. In the two later methods latex was diluted in equal volume of distilled water then rubbed on plant leaves with cotton pads soaked in it. Leaves of control plants were similarly rubbed with distilled water instead of the latex. Squash and broad bean plants were examined visually for external symptoms after 3 weeks from inoculation. In addition, biological and serological examination for confirmation the presence of ZYMV and BYMV was carried out by using *C. quinoa* and enzyme-linked immunosorbent assay (ELISA) according to Clark and Adams, 1977.

#### **Evaluation of serum latex from *F. nitida* against TNV and BYMV**

Effective latex from *F. nitida* was selected, whereas give highly activity which present in the serum only. So that the *F. nitida* serum was used in further studies as follow.

#### **1-Minimal inhibitory concentration (MIC) of serum latex on TNV**

Serum separated from latex of *F. nitida* was used and protein

concentrations (0.25, 0.50, 0.75 and 1.0 mg ml<sup>-1</sup>) were estimated according to Bradford (1976) with bovine serum albumin as standard. Each concentration was mixed with an equal volume of purified TNV (1.0 µg ml<sup>-1</sup>) for 15 min. The mixtures were rubbed on primary leaves of *Ph. Vulgaris*. On the other hand, suspension of 1.0 µg ml<sup>-1</sup> purified TNV was diluted with an equal volume of buffer and inoculated on another leaves as a control. In another experiment, serum (1.0 mg ml<sup>-1</sup>) was mixed with equal volumes of purified TNV at 0.5, 1, 5, 10, 20 and 30 µg ml<sup>-1</sup> for 15 min. The control ones were mixed with buffer and then the mixtures were inoculated on leaves of *Ph. vulgaris* as mentioned above. Six primary leaves were used for each treatment and the experiment was replicated twice.

## 2- Effect of time course on TNV

Leaves of *Ph. vulgaris* were mechanically inoculated with sap of TNV (0.5 µg ml<sup>-1</sup>). At 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 h before or after inoculation, *Ph. vulgaris* leaves were infiltrated with serum of *F. nitida*-latex (1.0 mg protein ml<sup>-1</sup>) or water using a syringe with a blunt-tipped needle (200 µl / leaf). The tip of the needle was placed against the leaf vein and the solutions were forced into the intercellular spaces of the lower surface of the whole leaf. The surfaces of the leaves were washed thoroughly with tap water. Treated leaves were mechanically inoculated with TNV (0.5 µg ml<sup>-1</sup>) after or before treatment at the same times mentioned above. Twelve primary leaves were used for each time as a replicates. The leaves were checked and numbers of local lesions were counted after 8 days from TNV inoculation then percentage of inhibition was calculated.

## 3- Effect of serum treatment on BYMV transmission by aphid

*Aphis fabae* (5 insects per plant) were used for BYMV transmission tests in broad bean plants. The aphids were maintained on virus-free Chinas cabbage plants and starved for 4 h before 8-10 min acquisition feed on broad bean infected with BYMV. After the acquisition feeding period, aphids were allowed to feed on small healthy seedlings of broad bean plants rubbed with cotton pads soaked in serum of *F. nitida* latex (1.0 mg ml<sup>-1</sup>) or plants treated with water alone as control. The aphids were caged for 24 h, and then killed. Alternatively, aphids were fed on BYMV-infected leaves at the leaves infiltrated (as mentioned above) with serum of *F. nitida*-latex (1.0 mg ml<sup>-1</sup>) or sterile distilled water for 4 h. The aphids were transferred and caged on small seedlings of broad bean healthy plants for 24 h, and then killed. In another experiment, after feeding on BYMV-infected leaves the aphids were transferred and caged on healthy plants of broad bean at leaves which had been infiltrated with serum solution (1.0 mg protein ml<sup>-1</sup>) or distilled water as control. The inoculated plants were maintained in the greenhouse then examined visually for symptoms and biologically by inoculation on *C. quinoa* as a local lesion host then confirmed serologically by DAS-ELISA test after 3 weeks.

## 4-Effect of serum latex from *F. nitida* on serological properties of BYMV

Serum separated from *F. nitida*-latex was used in concentrations of 0.25, 0.50, 0.75 and 1.0 mg protein ml<sup>-1</sup>. 100 µl of each concentration was added to 100 µl of purified BYMV (0.5 µg ml<sup>-1</sup>) for 15 min, then used as antigen and viral was detected by DAS-ELISA according to Clark and

**Adams, 1977.** Two-hundred  $\mu\text{l}$  of antigen per well (three wells as a replicates) was added to the ELISA plate previously coated with 200  $\mu\text{l}$  BYMV immunoglobulin (1/500 dilution) and incubated overnight at 4 °C. Sample buffer only or BYMV (0.5  $\mu\text{g ml}^{-1}$ , diluted with equal volume of sample buffer) were used instead of virus-serum mixtures as negative and positive controls, respectively. Alkaline phosphatase conjugated immunoglobulin was added at 1/2000 dilution and incubated for 3 h at 37 °C. Plates were washed four times between each step with phosphate buffered saline containing 0.05% Tween-20. Later, *p*-nitrophenyl phosphate was added at 1 mg  $\text{ml}^{-1}$  and incubated for 1 h at room temperature. The reaction was terminated with addition of 3 M NaOH. ELISA reactions were measured spectrophotometrically at 405 nm using ELISA-reader and positive reaction was confirmed by visual observations.

#### **5- Examination of BYMV particles treated with serum latex of *F. nitida* by transmission electron microscopy:**

To study the effect of serum of *F. nitida* latex on the constitutional structure, consequently the morphological shape of the viral particle, the purified BYMV preparation (1.0  $\mu\text{g ml}^{-1}$ ) mixed with equal volume of serum at concentration of 1.0 mg protein  $\text{ml}^{-1}$  and that control which mixed with sterilized distilled water were used. The mixtures were shaken gently, incubated for 30 min at room temperature (28  $\pm$ 2 °C, and then centrifuged at 95.000 g for 1 h at 4°C). The virus pellets were then subjected to three cycles of washing with buffer and ultracentrifugation to remove all traces of residual serum. Pellets were suspended in 200  $\mu\text{l}$  buffer, negatively stained with 2% (w/v) aqueous uranyl

acetate, and then examined with a zeiss10C transmission electron microscopy.

#### **Casein-embedded polyacrylamide gel electrophoresis (PAGE) for protease activity on serum of *F. nitida* latex**

Casein-PAGE (Cornish and Backhaus, 1990) was performed by including casein (0.5% w/v final concentration) to native polyacrylamide gel as described by Laemmli (1970). 100  $\mu\text{l}$  from serum of *F. nitida* latex sample was applied compared with 100  $\mu\text{l}$  of distilled water as a control and the electrophoresis was carried out at pH 8.8 and (100V, 20mA). Following electrophoresis the gel was washed three times in distilled water and then incubated at 37°C in 0.05 M sodium acetate buffer pH 5.5 for 30 min. After incubation the gel was stained overnight in 200 ml of Coomassie brilliant blue R-250 solution. The destaining solution was used for several times until background colour was removed. The presence of protease is detected as clear white band against a dark blue background due to the hydrolysis of casein.

#### **Calculation of infectivity inhibition**

The percentage of inhibition of local lesion formation or diseased plants by each treatment over the control was calculated based on the number of local lesions or diseased plants produced using the following formula:  $(1 - T / C) \times 100$  where, C = Number of local lesions or diseased plants in control; T = Number of local lesions or diseased plants treated with latex.

#### **Results and Discussions**

##### **Inhibition of ZYMV and BYMV on systemic hosts by crud-latex**

The results in Table (1) indicated that latex of *F. nitida* only showed significant inhibitory activity when mixed with virus inoculum or applied

48 h before virus challenge. On the other hand, no such inhibition was observed when *F. nitida* latex was applied 48 h after virus challenge in either squash or broad bean inoculated with ZYMV or BYMV, respectively. These results were confirmed by ELISA test reactions, which recorded the absorbance values as that of negative control. The others *E. pulcherrima* and *F. elastica* latices did not have any antiviral activity.

#### **Dose response of serum and TNV**

Serum of *F. nitida*-latex was found to inhibit the infection of TNV ( $1.0 \mu\text{g ml}^{-1}$ ) even at low concentrations when assayed on *Ph. vulgaris* as a local lesion host. Completely inhibition in local lesion numbers was performed by using  $1.0 \text{ mg protein ml}^{-1}$  of serum. But  $0.25 \text{ mg ml}^{-1}$  produced a 69.32% reduction in local lesion number (Table 2). Serum at concentration  $1.0 \text{ mg ml}^{-1}$  completely inhibited the infection of *Ph. vulgaris* by TNV at a range of concentrations between 0.5 and  $20 \mu\text{g ml}^{-1}$  only (Table, 3).

#### **Effect of serum time treatment on TNV infectivity**

Only 57.1% inhibition of TNV was obtained when serum of *F. nitida* latex ( $1.0 \text{ mg ml}^{-1}$ ) was infiltrated 3 h after viral inoculation. This percentage was dramatically reduced by time until 12 h (8.2%) after viral inoculation, and there was no inhibition when serum was applied 15 h after TNV inoculation (Figure, 1). Serum retained its inhibitory activity longer when injected into leaf intracellular spaces before virus inoculation. When infiltrated into the intracellular spaces 3 and 6 h before viral inoculation, TNV infection was approximately prevented (95.9 and 92.9 %, respectively), and after 36 h was still

inhibited by more than 30 % (Figure, 1).

#### **Relationship between aphid transmission of BYMV and serum treatment:**

The ability of aphids to transmit BYMV was affected (only 40.9% in latex-treated compared with 68.1% in non-treated) by serum of *F. nitida* latex when aphids were fed on BYMV infected leaves and subsequently on plants treated with latex (Table, 4, experiments 1). On the other hand, there are decrease in the ability of aphids to acquire BYMV from BYMV-infected leaves infiltrated with latex (36.7% transmission) compared with water (63.6% transmission) (Table, 4, experiments 2). However, when latex was infiltrated into healthy broad bean leaves onto which viruliferous aphids were transferred, there appeared to be highly decreased in the transmission of BYMV (20.0 % transmission on latex-treated, compared with 65.0% transmission in water treated) (Table, 4, experiment 3).

#### **Efficacy of serum from *F. nitida* latex on serological properties of BYM**

BYMV was assayed through DAS-ELISA in the mixtures of serum from *F. nitida* latex after 15 min as incubation period. Buffer was used to compare the efficacy of serum latex (Table, 5). The suspension of BYMV mixed with serum of *F. nitida* latex at concentrations of 0.50, 0.75 and  $1.0 \text{ mg ml}^{-1}$  did not show any significant variation in absorbance values when compared with sample buffer only (negative control). This result indicating the absence of any detectable virus. In lower concentration ( $0.25 \text{ mg protein ml}^{-1}$ ) of serum, the absorbance values as that of virus only (positive control).

**Table (1): Effect of crud latex on ability of squash and broad bean to infect by ZYMV and BYMV, respectively.**

treatments		Latex mixed with viral inocula			Latex was applied 48 h					
Host (virus)	Latex source	symptom	ELISA-value	result	Before viral inoculation			After viral inoculation		
					symptom	ELISA-value	result	Symptom	ELISA-value	result
<b>Squash</b> (ZYMV)	<i>Ficus nitida</i>	-	0.326	-	-	0.369	-	+	1.051	+
	<i>Euphorbia pulcherrima</i>	+	0.853	+	+	0.952	+	+	0.843	+
	<i>Ficus elastica</i>	+	0.744	+	+	0.843	+	+	1.077	+
	Control 1*	+	1.143	+	nd	nd	nd	nd	nd	nd
	Control 2**	nd	nd	nd	+	1.052	+	+	1.005	+
<b>Broad bean</b> (BYMV)	<i>Ficus nitida</i>	-	0.406	-	-	0.327	-	+	0.941	+
	<i>Euphorbia pulcherrima</i>	+	0.338	+	+	0.947	+	+	0.982	+
	<i>Ficus elastica</i>	+	0.692	+	+	0.885	+	+	1.158	+
	Control 1*	+	0.897	+	nd	nd	nd	nd	nd	nd
	Control 2**	nd	nd	nd	+	0.982	+	+	1.047	+

\*= Each virus mixed with equal volume of distilled water instead of the latex.

\*\*= Control plants were similarly rubbed with distilled water instead of the latex.

ELISA value at 405 nm, after incubation for 1 h at room temperature.

Positive control ELISA-values: 1.136 and 1.082 for ZYMV and BYMV, respectively from infected tissues.

Negative control ELISA-values: 0.281 and 0.279 for ZYMV and BYMV, respectively from healthy tissues.

Note: the ELISA value that equal two folds of healthy tissue was considered as a positive (+) results

**Table (2): Inhibition of TNV at concentration 1.0 µg ml<sup>-1</sup> infection by serum of *F. nitida* latex**

Serum concentration (mg ml <sup>-1</sup> )	Number of local lesions*		Inhibition (%)
	Serum (+)	Serum (-)	
0.25	27a**	88a	69.32
0.50	15b	92a	83.7
0.75	3c	97a	96.91
1.00	0d	86a	100

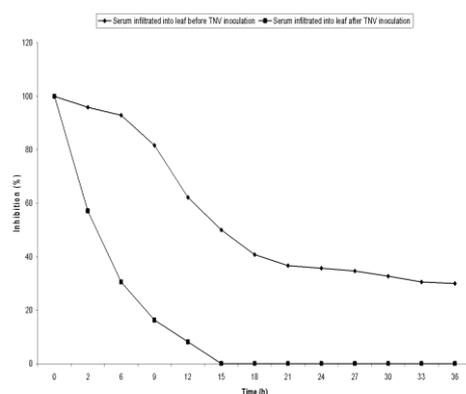
\*1.0 µg ml<sup>-1</sup> purified TNV was diluted with an equal volume of serum (+) or buffer (-), and then inoculated on *Ph. vulgaris* primary leaves. Six primary leaves were used for each treatment and the experiment was replicated twice. \*\* The mean values with different letters in the same column are significant different.

**Table (3): Inhibition of TNV infection inoculated at different concentrations by serum from *F. nitida* latex at concentration 1 mg ml<sup>-1</sup>.**

TNV concentration ( $\mu\text{g ml}^{-1}$ )	Number of local lesions*	
	Serum (+)	Serum (-)
0.5	0a**	58a
1	0a	102b
5	0a	143b
10	0a	195c
20	0a	241c
30	12b	386d

\*No of local lesions induced on *Ph vulgaris* primary leaves by using TNV concentrations mixed with equal volume of serum latex (+) or buffer (-). Six primary leaves were used for each treatment and the experiment was replicated twice.

\*\* The mean values with different letters in the same column are significant different.

**Figure (1): Effect of serum from *F. nitida* latex (1 mg ml<sup>-1</sup>) infiltrated into leaves before or after inoculation with TNV.****Table (4): Effect of serum from *F. nitida* latex on transmission of bean yellow mosaic virus to broad bean by *Aphis fabae***

Treatments		Transmission			
		Serum (-)		Serum (+)	
Plant tested	Experiment*	No of infected / inoculated plants	%	No of infected / inoculated plants	%
broad bean	1	15 / 22	68.1	9 / 22	40.9
	2	14 / 22	63.6	8 / 22	36.7
	3	13 / 20	65.0	4 / 20	20.0

\*1: Aphid (five/plant) fed on BYMV-infected plants, then transferred and caged on healthy plants treated with serum (+) or water (-). 2: aphids fed on BYMV-infected leaves infiltrated with sterile distilled water (-) or serum (+) and then transferred and caged on healthy plants. 3: aphid fed on BYMV-infected plants, then transferred and caged to healthy plants at positions infiltrated with sterile distilled water (-) or serum (+).

**Table (5): Effect of serum from *F. nitida* latex on BYMV detection in purified preparation as determined by DAS-ELISA.**

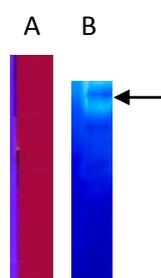
Serum concentration (mg ml <sup>-1</sup> )	O.D. value at 405 nm	Viral presence
0.25	0.217a***	+
0.50	0.069b	-
0.75	0.072b	-
1.00	0.075b	-
Negative control*	0.062b	-
Positive control**	0.238a	+

\*sample buffer only was used instead of serum-virus mixtures. \*\*BYMV (0.5 µg ml<sup>-1</sup>) was used instead of serum-virus mixtures. \*\*\* The mean values with different letters in the same column are significant different.

### Effect of serum of *F. nitida* latex on BYMV particles:

Electron micrographs of purified BYMV preparations indicated the presence of thick, quasi-malformed, aggregates and lysis of rod virus particles (Fig. 2B), when the purified preparations (1.0 µg ml<sup>-1</sup>) mixed with an equal volume of serum (1 mg ml<sup>-1</sup>) from *F. nitida*. The control which treated only with sterilized distilled water, normal of filamentous particles

with model size of 750 x 17 nm were observed (Fig. 2A) when negatively stained with 2% aqueous uranyle acetate and examined by transmission electron microscopy. This result was accompanied with protease activity in serum. Casein-embedded PAGE used specifically to detect proteases also shows activity band (Fig. 3B). Bands visualized indicated presence more than isoform.



**Figure (3): Casein-embedded PAGE stained with Commasie brilliant blue for detecting protease activity in latex serum of *F. nitida*. (A) Control lane applied with water, (B) Lane applied with latex.**

Due to rapid spread of plant viruses, the development of effective materials against plant infection is dependent upon the identification of novel agents. There is elucidation of the role of latex in plants. Most of the work done on those exudates is based on the assumption that they are involved in defense mechanisms of plants against pests. It's well known that latices commonly contain several enzymatic and inhibitory activities of potential action against insects and pathogens (Lynn and Clevette-Radford, 1987b; Paul Khurana and Singh, 1972 and Sritanyarat *et al.*, 2006). The authors suggested this study because plants secretion latex remains

unexploited as a source of antiviral principles. The present study definitely shows that the *F. nitida* plant contains a potent inhibitor of viral infection. Genus *Ficus* contains more than 180 named species making them one of the largest genres in the Moraceae family. Serum of *F. nitida* is a basic protein (Caffini *et al.*, 1988), sharing several properties with that of *Phytolacca americana*. The latices of plant species screened in the present study were not used previously against plant viruses. Paliwal (1961); Paul-Khurana and Singh (1972) reported complete inhibition of sunhemp mosaic virus and TMV by papaya and *Calotropis procera* latices. Our study

indicated that the almost 100% inhibition was achieved by the preinoculation treatment to the leaf surface. It's considered that *F. nitida* latex is not the inhibitor of viral synthesis but that of viral infection because no appreciable inhibition was observed when latex was applied after virus inoculation. A noteworthy feature of latex is that its inhibitory activity is exhibited systemically. However, it seems unlikely that the proteinaceous inhibitor is capable to penetrate into leaf cells and translocation to long distance in sufficient amount to block virus infection directly. A direct action of serum from *F. nitida* on virus in a mixture could not be separated from the virus particles by ultracentrifugation. However, electronic micrographs indicated that latex components adhered to particles. The results suggested that, latex inhibitor conjugates with virus for its action and also, may be to non-specifically bind to proteins and may have interfered with virus binding to its receptors inside the plant cell. Because many of the antiviral proteins are also ribosome-inactivating proteins (RIPs) (Corrado *et al.*, 2008), a further study is needed to determine whether the serum from *F. nitida* latex inhibits virus infection by inactivating ribosome in the host.

When the mixture of TNV and latex protein was inoculated on the leaves of *Ph. vulgaris*, TNV inhibition occurred, even if the TNV concentration was higher. Thus the antiviral activity of *F. nitida*-latex is depended on the concentration of latex and not on the concentration of virus. Serum of *F. nitida* latex as antiviral proteins could completely inhibited TNV infection at 1.0 mg ml<sup>-1</sup>. In another study, Kumon *et al.*, 1990 reported that only 0.01 mg ml<sup>-1</sup> of pokeweed antiviral proteins is needed for inhibition of the TMV infection. Defense-related proteins reported to be present in latex include chitinase,  $\beta$ -1,3-glucanase, heveamines, hevein, glucosidase,  $\beta$ -galactosidase,  $\beta$ -N-acetylglucosaminidase, polyphenol oxidase, and a protease inhibitor (D'Auzac and Jacob, 1989; Kim *et al.*, 2003). The free radical scavenging property may be one of the mechanisms by which this latex is effective as antiviral agent. Most of the tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants (Larson, 1988). Details on the latex structure and compositions, including rubber

biosynthesis and defense-related proteins have been extensively reviewed (Wititsuwannakul and Wititsuwannakul, 2001). This study supports concept that, plant latices may be important in the potential discovery of natural antiviral product, which benefit in plant viral control.

## References

- Allard, H. A. 1914. The mosaic disease of tobacco. *U. S. Dep. Agric. Bull* (40): 1.
- Anroka, G. & Buchenauer H. 1997. Systemic acquired resistance in tomato against *Phytophthora infestans* by pre-inoculation with tobacco necrosis virus. *Physiol. Pl. Pathol.* (50): 85-101.
- Bailiss, K. W. & Senananyake S. 1984. Virus infection and reproductive losses in faba bean (*Vicia faba* L.). *Plant Pathology* (33): 185-192.
- Barakat, A.; Bahieldin A.; Gebreel H. M. & Rashad Z. A. 2004. Chromatographic fraction of antiviral principles depurinating plant ribosomes from certain seeds and leaf extracts. *Egyptian J. Virol.* (1): 383-391.
- Betancur-Galvis, L. A.; Morales G. E.; Forero J. E. & Roldan J. 2002. Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the *Euphorbia* genus. *Mem. Inst. Oswaldo Cruz, Rio de Janeiro* 97(4): 541-546.
- Boller, T. 1986. Roles of proteolytic enzymes in interaction of plants with other organisms. In: *Dalling, M.J. (Ed.), Plant Proteolytic Enzymes*, vol. 1. pp. 67-96. CRC Press, Boca Raton, FL.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Annal. Biochem.* (72): 248-254.
- Caffini, N.O.; Lopez, L.M.I.; Natalucci, C.L. & Priolo, N.S. 1988. Proteases of higher plants. General features, physiological roles and applications. *Acta. Farm. Bonaerense* (7): 195-213.

- Chye, M. L. and Cheung K. Y. 1995.  $\beta$ -1,3-glucanase in highly-expressed in laticifers of *Hevea brasiliensis*. *Plant Mol. Bio.* (39): 397-402.
- Clark, M.F. & Adams A.N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. of General virology* (34):475-483.
- Cornish, K. & Backhaus R.A. 1990. Rubber transferase activity in rubber particles of gryllus. *Phytochemistry* (29): 3809–3813.
- Corrado, G.; Scarpetta M.; Aljoto D.; Di Maro A.; Polito L.; Parente A. & Roa R. 2008. Inducible antiviral activity and rapid production of the ribosome-inactivating protein 1 from *Phytolacca heterotepala* in tobacco. *Plant Science* (174): 467-474.
- D'Auzac, J. & Jacob J.L. 1989. The composition of latex from *Hevea brasiliensis* as a laticiferous cytoplasm. In: d' Auzac, J., Jacob, J.L., Chrestin, H. (Eds.), *Physiology of Rubber Tree Latex*. CRC Press, Florida, pp. 59–98.
- El-Afifi, (Sohier) I. & El-DougDoug Kh. A. 1997. Identification of some mechanically transmitted viruses affecting faba bean plants in Egypt. *Annals Agric. Sci., Ain Shams Univ., Cairo* 42(1): 29-49.
- Farrell, B. D.; Dussourd D. E. & Mitter C. 1991. Escalation of plant defense: Do latex and resin canals spur plant diversification? *Amer. Natural.* (138): 881-900.
- Figueira, A. R.; Sodeck L. & Costa A. S. 1994. Partial purification and some properties of a virus inhibitor from *Turnera ulmifolia*. *Fitopatol. Bras.* 19(3): 365-369.
- Freitas, C. D.; Oliveira J. S.; Maria R. A.; Macedo N. M. R.; Sales M. P.; Villas-Boas L. A. & Ramos M. V. 2007. Enzymatic activities and protein profile of latex from *Calotropis procera*. *Plant Physiology and Biochemistry* (45): 781-789.
- Giordani, R.; Benyahia S.; Teissere M. & Noat G. 1992. Purification and properties of N-acetyl- $\beta$ -D-glucosaminidase from *Hevea Brasiliensis* latex. *Plant Sci.* (84): 25-34.
- Giordani, R. & Lafon L. 1993. Action of *Carica papaya* latex on cell wall glycosidases from *Lactuca sativa*. *Phytochemistry* (34): 1473-1475.
- Irvin, J. D.; Kelly T. & Robertus J. D. 1980. Purification and properties of a second antiviral protein from *Phytolacca americana* which inactivates eukaryotic ribosomes. *Arch. Biochem. Biophys.* (200): 418-425.
- Jekel, P. A.; Hartmann B. H. & Beintema J. J. 1991. The primary structure of hevamine, an enzyme with lysozyme chitinase from *Hevea brasiliensis* latex. *Eur. J. Biochem.* (200): 123-130.
- Kim, J.S.; Kim Y.O.; Ryu H.J.; Kwak Y.S.; Lee J.Y. & Kang H. 2003. Isolation of stress-related genes of rubber particles and latex in fig tree (*Ficus carica*) and their expressions by abiotic stress or plant hormone treatments. *Plant Cell Physiol.* (44): 412–414.
- Kubo, S.; Ikeda T.; Imaizumi S.; Takanami Y. & Mikami Y. 1990. A potent plant virus inhibitor found in *Mirabilis jalapa* L. *Ann. Phytopath. Soc. Japan* (65): 481-487.
- Kumon, K.; Sasaki J.; Sejima H.; Takeuchi Y. & Hagashi Y. 1990. Interactions between tobacco mosaic virus, pokeweed antiviral proteins and tobacco cell wall. *Phytopathology* (80): 636-641.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* (227): 680-685.
- Larson, R. A. 1988. The antioxidants of higher plants. *Ohytochemistry* 27(4): 969-978.
- Lin, Y. H. & Lu C. L. 1994. Latex trypsin inhibitors of sweet potato (*Ipomoea*

- batatas* Lam.). *Bot. Bull. Acad. Sin.* (35): 153-159.
- Lynn, K. R. & Clevette-Radford N. A. 1987a. Acid phosphatases from latices of Euphorbiaceae. *Phytochemistry* (26): 655-657.
- Lynn, K. R. & Clevette-Radford N. A. 1987b. Biochemical properties of latices from the Euphorbiaceae. *Phytochemistry* (26): 939-944.
- Lynn, K. R. & Clevette-Radford N. A. 1986. Isolation and characterization of proteases from *Euphorbia lacta* and *E. lactea cristata*. *Phytochemistry* (25): 807-810.
- Mahmoud, S. Y. M.; Soweha H. E. & Abdel-Ghaffar M. H. 2004. Control of Zucchini yellow mosaic potyvirus by antiphytoviral glycoproteins. *Proc. 3<sup>rd</sup> Int. Conf. Biol. Sci. Fac. Sci., Tanta Univ.*, 28-29 April 2004 Vol (3): 241-256.
- Montalbini, P.; Bellezla G.; Marte M. & Polverari A. 1991. Severe attacks of bean yellow mosaic virus in *Vicia faba* in Umbria (Central Italy). *Informatore Fitopatologico* (2): 45-48.
- Paliwal, Y. C. 1961. Inhibition of sunnhemp mosaic virus by plant extracts and ultra violet and gamma radiations. *M. Sc. Thesis, P. G. School, I.A.R.I, New Delhi*.
- Paul Khurana, S. M. & Singh S. 1972. Studies on *Calotropis procera* latex as inhibitor of tobacco mosaic virus. *Phytopath. Z.* (73): 341-346.
- Provvidenti, R.; Manger H. M. & Paulus A. O. 1985. Epidemics of Zucchini yellow mosaic virus and other cucurbit viruses in Egypt in the spring of 1983. *Cucurbit Genet. Coop.* (7): 78-79.
- Radwan, H. M. & Mahmoud S. Y. M. 2001. The protein and mucilage constituents of both *Mesembryanthemum forsskalei* (Hochst) and *Halocnemum strobilaceum* (Pall) and their antiphytoviral activity. *Egyptian J. Appl. Sci.* 15(5): 39-50.
- Rao, G. P.; Baghel A. K. S.; Singh R. K. & Chatterji K. S. 1984. Antiviral activity of caralloid root of *Cycas revoluta* extract against some viruses of tomato plants. *Experientia* (40): 1257-1258.
- Renuka Devi, P.; Doraiswamy S.; Nakkeeran S.; Rabindran R.; Ganapathy T.; Ramiah M. & Mathiyazhagan S. 2004. Antiviral action of *Harpulia cupanioides* and *Mirabilis jalapa* against tomato spotted wilt virus (TSWV) infecting tomato. *Archives of Phytopath. Pl. Prot.* (37): 245-259.
- Sritanyarat, W.; Pearce G.; Siems W.F.; Ryan C. A.; Wititsuwannakul R. & Wititsuwannakul D. 2006. Isolation and characterization of iso inhibitors of the potato protease inhibitor I family from the latex of the rubber trees, *Hevea brasiliensis*. *Phytochemistry* (67): 1644-1650.
- Verma, H. N.; Varsha S. & Kumar D. 1996. Induction of systemic resistance in plants against viruses by a basic protein from *Clerodendrum aculeatum* leaves. *Phytopathology* (86): 485-492.
- Wititsuwannakul, D. & Wititsuwannakul, R. 2001. Biochemistry of natural rubber and structure of latex. In: Koyama, T., Steinbuechel, A. (Eds.), *Biopolymers, Polyisoprenoids*, vol. 2. Wiley-VCH, Germany, pp. 151-201.