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STUDIES ON SOME VIRUSES INFECTING CUCURBITS

BY

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INTRODUCTION

The cucurbits (family *Cucurbitaceae*) form a diverse group consists of about 90 genera and 750 species, grown around the world under many different conditions for many different purposes. The cultivated species are important crops in the tropics, subtropics and milder portion of the temperate zones.

In Egypt annual cultivated areas (Feddan) with watermelon, squash, cucumber, cantaloupe, sweet melon, snake cucumber, melon (shahd), melon(quoz) and pumpkin were 164526, 91054, 84261, 71362, 22560,10460, 2148, 867 and 242, respectively. Such areas produced 2025185, 698606, 802644, 745953, 211257, 104637, 23917, 7803 and 1672 ton in case of watermelon, squash, cucumber, cantaloupe, sweet melon, snake cucumber, melon (shahd), melon (quoz) and pumpkin, respectively. (A. R. E. Ministry of Agriculture and Land Reclamation. Economic Affairs Sector. Vol. 2. 2006).

There are over 200 known cucurbit diseases of diverse etiologies. Virus diseases are a major problem in cucurbit planting areas all over the world. They are difficult to control and result in tremendous crop losses. At least 45 viruses have been reported to infect cucurbit plants all over the world (Lovisolo, 1980; El-Saleh, 1994; Brunt *et al.*, 1996 and Fegla *et al.*, 2008). Out of these viruses 24 were recorded in Arab regions (Zouba *et al.*, 1997; Hag-Kasem *et al.*, 2005; El-Chaabi *et al.*, 2006 and Fegla *et al.*, 2008). Among of these viruses the most common are *Cucumber green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV), *Cucurbit aphid borne yellow virus* (CABYV), *Papaya ring spot virus* (PRSV), *Squash mosaic virus* (SqMV),*Tobacco ring spot virus* (TRSV), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV).

Little work has been done on virus diseases affecting cucurbits in northern Egypt; therefore the aim of the present investigation is directed mainly to study the following:

1- Distribution and prevalence of cucurbit viruses in different locations of northern Egypt.
2- Characterization of the most prevalent viruses (WMV and ZYMV) using host range and symptomology, stability in sap, mode of transmission, U.V. absorption spectra of purified viruses, molecular weight of coat protein subunit, reverse transcription-polymerase chain reaction (RT- PCR), multiplex- RT- PCR and electron microscopy.
3- Preparation of antisera against WMV and ZYMV
4- Some control measures such as distance among hills, barrier crops, soil mulches, insecticide and oil sprays and their effect on productivity.

MATERIALS AND METHODS

1. Survey studies of some viruses affecting cucurbits

A survey studies were conducted in different locations of three governorates namely Alexandria, El-Beheira and Kafr El-Sheikh from February through October during the growing seasons 2004, 2005 and 2006.

Surveys were performed to study the prevalence and distribution of viruses affecting cucurbits growing in open fields as well as in the plastic houses.

Periodical inspection was done every one month. Plants were randomly evaluated based on symptoms suspected to be caused by virus infection such as chlorosis, vein clearing, mottle, mosaic, yellowing, blister, stunting and leaf malformation and fruit distortion.

Samples of plants exhibiting virus like symptoms were collected and the symptoms of plants were recorded. Each plant sample was kept separately in a plastic bag.

Estimation of diseases incidence was based on external symptoms and serological diagnosis.

1.1. Visual survey

Incidence of viral disease symptoms was estimated visually by counting the number of plants showing symptoms out of 100 consecutive plants in each patch selected, at random, from each inspected field and plastic house.

1.2. Serological diagnosis

Serological diagnosis was carried out using indirect ELISA.

1.2.1 Source of antisera

Antisera of *Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus* (CGMMV), *Squash mosaic virus* (SqMV), *Papaya ring spot virus* (PRSV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) *and Tobacco ring spot virus* (TRSV) were used.

The antiserum to CMV was locally prepared in laboratory of Plant Virology, Faculty of Agriculture, Alexandria University (Younes, 1995), ZYMV antiserum was supplied by Agricultural Genetic Engineering Research Institute (AGERI), ARC, Egypt, CGMMV antiserum was kindly supplied by Dr. M. EL-Mazaty, Faculty of Agriculture, El-Mansoura University, TRSV antiserum was supplied by antiserum-Bank, Institute of Seed Pathology for Developing Countries, Denmark while SqMV, PRSV and WMV antisera were provided by Agdia, the catalog numbers were CAB 26400, CAB 53500 and CAB 5400, respectively.

1.2.2. Indirect ELISA

The indirect ELISA was carried out as described by Younes (1995) and Fegla *et al*. (1997). Disposable polystyrene flat bottom micro ELISA plates were used. The plant

samples were ground in coating buffer (0.5 M carbonate, pH 9.6) (Appendix 1) 1:10 (w/v) using a mortar and pestle, then strained through double layered cheesecloth. Wells were coated with antigens by adding 100 µl of each sample to the bottom of the well and incubated for 3 hours at 37°C or overnight at 4°C. The plates were rinsed three times by flooding wells with phosphate buffer saline + Tween 20 (PBST) (Appendix 1), 5 minutes each. To reduce non specific reactions, antisera requiring cross adsorption were diluted 1:500 with filtered extract from healthy tissues 1:20 (w/v) in serum buffer [PBS-Tween 20 containing 2% soluble ployvinylpyrolidone ((PVP) and 0.2% bovine serum albumin (BSA)] (Appendix 1), and incubated for 45 min. at 37ºC.

The precipitate, which had formed, was removed by centrifugation for 10 min. at 500 rpm, 100 µl aliquots from the diluted antisera were added to each well, after which the plates were incubated at 37°C for 2 hours or at 4°C overnight , then washed as before.

Goat anti-rabbit gamma globulin conjugated to alkaline phosphatase (whole molecule, enzymatic activity 475 units /ml) obtained from Sigma Chem. Co. St Louis, Mo (Production # A 8025) was diluted 1:1000 in serum buffer and 100 µl were added to each well , followed by one hour incubation at 37°C , then washed as before.

One hundred µl of the enzyme substrate, 0.5 mg/ml para-nitrophenylphosphate (Sigma#104) in 10% diethanolamine buffer, pH 9.8 (Appendix 1) were added to each well and incubated at room temperature (25°C) for about 30 minutes. The enzyme activity was stopped by adding 50µl of 3M NaOH.

The ELISA values, measured by multiskan MS ELISA reader, were expressed as absorbance at 405 nm. Absorbance values of at least double that of healthy control, were considered positive.

In each set of test, wells lacking antigen (coating buffer only) were included as a blanks.

2. Characterization of the most prevalent viruses

The most prevalent viruses were *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus* (WMV). WMV and ZYMV which used in this study were isolated from squash plants. The isolated viruses were separately maintained on squash plants cv. Eskandrani that served as a source for subsequent studies. Except otherwise stated, virus inocula were prepared by grinding infected leaf tissues 1:10 (w/v) with a mortar and pestle in 0.1M phosphate buffer, pH 7.0. Leaves of plants to be inoculated were first dusted with carborandum (600 mesh) and then inoculated with freshly prepared inoculum using forefinger. Inoculated plants were rinsed with tap water shortly after inoculation.

Characterization of the isolated viruses was based mainly on host range and symptomology, stability in sap, mode of transmission, U.V. absorption spectra of purified viruses, molecular weight of coat protein subunit, reverse transcription-polymerase chain reaction (RT-PCR) and electron microscopy.