PREPARATION OF ANTISERA FOR SOME PLANT VIRUSES

By

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B.Sc. (Agric. Microbiology), Fac. Agric.,
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ABSTRACT

Rehab Ali mohamed Dawoud, Preparation of antisera for some plant viruses. Unpublished M.Sc. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 1999.

Preparation of purified virus suspension was carried out using polyethylene glycol (PEG) and ultracentrifugation for tomato yellow leaf curl virus (TYLCV) and pepper mottle virus (PeMoV). Purified virus suspensions was determined biologically (infectivity assay) spectrophotometrically and electron microscopic. Production of specific antisera for TYLCV and PeMoV was performed using rabbits immunization with total and half antigen amounts (5.64, 2.82 mg of TYLCV, 7.68 and 3.84 mg of PeMoV). The titer of the prepared antisera were determined using tube precipitin test. The suitability, efficiency and sensitivity of different serological tests; indirect- ELISA, dot- ELISA, rocket immuno-electrophoresis, tube pre-cipitin, Ouchterlony double diffusion and serological blocking of virus transmission by insect; were studied for assay, detection and diagnosis of the two viruses. Indirect-ELISA procedures were found to be much better suited for diagnostic work for the two viruses because of its high sensitivity. The concentration of TYLCV and PeMoV purified suspension was determined using rocket immunoelectrophoresis. The present results clearly indicated that Ouchterlony were coast effective but less sensitive compared to the other methods. Tube precipitin test proved to be efficient for the determination of antiserum titer and virus antigen end-point. Serological blocking of virus transmission by insect was in agreement with relationships shown in serological tests based on agar - double - diffusion.

Key Words: TYLCV, PeMoV, Serology, ELISA, Electron microscopy

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INTRODUCTION

It can be said that, the losses in the yield of some economic crops are attributed to virus infection. Tomato yellow leaf curl geminivirus (TYLCV) and pepper mottle potyvirus (PeMoV) are of great importance since they cause great losses in tomato and pepper crops.

One of the main economic advantage to be gained from research on plant viruses is linked to the improving yield of the agricultural crops by an early diagnosis of the infection. Biological methods used for plant virus diagnosis take more time, space consuming and the results obtained are relatively inaccurate in many cases.

Although this approach remains valuable today, it is considered to be less sensitive than serological methods **(Ramsdell *et al*., 1979).** Plant virologists have found serological techniques to be extremely useful for the routine diagnosis of virus diseases. In addition, serological tests provide a rapid and convenient results for virus assay, detection and diagnosis. The main advantages of the serological tests are the specificity of the reaction that allow virus to be measured even in the presence of host materials or their impurities and the results obtained in few hours or overnight compared with days for infectivity assay. Enzyme-linked immunosorbent assay (ELISA) has gained a considerable recognition and considered as a sensitive diagnostic procedure for plant viruses, most notably viruses.

Since the work of **Clark and Adams (1977),** ELISA technique have been developed with many variants to increase the sensitivity of virus diagnosis. The main purpose of this work was (1). The preparation of purified viruses (TYLCV and PeMoV) for production of sensitive for the application of serological diagnostic methods. (2) Study the effect of decreasing the total amount of antigen on the titer of antiserum produced in order to determine the lowest amount of antigen required for

immunization that give good titer of antiserum, and comparison the sensitivity and efficiency of six different serological tests (Indirect enzyme-linked immunosorbant assay, dot-ELISA, rocket immunoelectrophoresis, ouchterlony double diffusion, tube precipitin and serological blocking of virus transmission by insect-vectors) for the detection and diagnosis of the two viruses in the infected sap and purified suspension

REVIEW OF LITERATURE

1. Virus propagation and indicator hosts :

The choice of host plant for propagating a virus may be of critical importance for it's successful purification, in which the virus reaches a high concentration **(Van Regenmortel 1982).** In addition, the suitable species or varities of host plant that give clear characteristic and consistent symptoms for virus or virus studied under greenhouse conditions provide one of the most basic tools for routine diagnosis **(Matthews, 1991).**

1.a. Tomato yellow leaf curl geminivirus (TYLCV) :

Russo *et al* 1980; Osaki and Inouye 1981; Mazyad *et al* 1982 (a); Zaher 1985 and Youssef 1998 found that tomato plants were very susceptible to virus infection in which the infected tomato plants showed clearing of the veins, stunting and marked reduction in the leaf size accompanied by shortening of the internodes. **Allam *et al*, 1994 and Youssef 1998** isolated TYLCV from naturally infected tomato and it was maintained in tomato plants cv. Castle rock. They reported that, infected tomato plant cv. Castle rock can be used as a propagative host for virus purification.

Cohen and Nitzany 1966; Zaher 1973; Nakhla 1977; Abdel Salam 1991b; Allam *et al*, 1994, Aref and El-Dougdoug 1996 and Youssef 1998, indicated that *Datura stramonium* was stated to be susceptible to TYLCV and exhibited clear symptoms of the disease. **Nakhla 1977** found that *N. glutinosa* L. and *N. sylvesteris* developed systemic symptoms vein clearing, curling and chlorosis.

In addition **Youssef 1998** reported that *Nicotiana glutinosa* and *Nicotiana benthamiana* developed leaf curling vien clearing yellowing, and deformation.

1.b. Pepper mottle poty virus (PeMoV) :