APPROVAL SHEET

SEROLOGICAL STUDIES FOR DIAGNOSIS OF SOME PLANT VIRUSES

BY

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B. Sc. Microbiology 1984
M. Sc. Microbiology 1991

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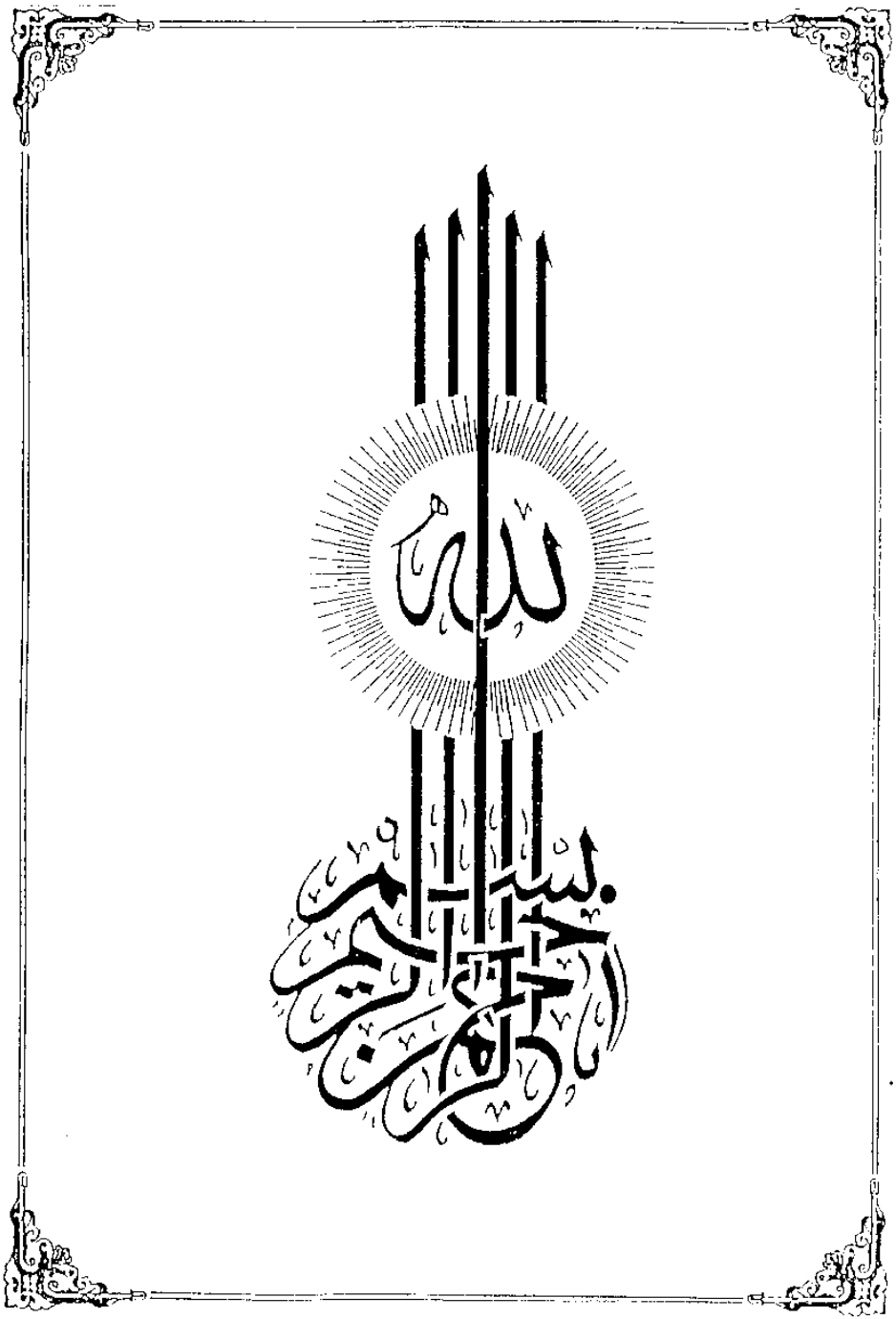

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Abstract

Preparation of virus purified suspension was carried out using Polyethylene glycol (PEG) for tobacco mosaic virus (TMV), ammonium sulphate and ultra centrifugation for tobacco necrosis virus (TNV) and PEG and ultracentrifugation for potato virus X (PVX). Purified virus suspensions obtained indicated a good virus concentrations as determined biologically (infectivity assay) and spectrophotometrically. Electron microscopic examination of the purified suspensions was carried out using negative staining technique with uranyl acetate. Production of specific antisera for TMV, TNV and PVX was preformed using rabbits immunization with total amounts injected; 12.87 mg of TMV, 7.874 mg of TNV and 8.922 mg of PVX. The titer of the prepared antisera was determined using tube precipitin test . The lowest amount of purified virus suspension needed for the production of virus specific antiserum with adequate titer was determined. The suitability , efficiency and sensitivity of different eight serological tests (Indirect-ELISA, indirect-DAS-ELISA, dot-ELISA, rocket immunoelectrophoresis, tube precipitin, Ouchterlony double diffusion, single radial immunodiffusion and immunoelectron microscopy) for assay, detection and diagnosis of the three viruses were

determined. Indirect-ELISA procedures were found to be much better suited for diagnostic work because of its high sensitivity. Immunoelectron microscopy was rapid and very sensitive for virus detection and diagnosis involving two properties (virus morphology and serological specificity). The concentration of TMV, TNV and PVX infected sap was determined using single radial immunodiffusion and rocket immunoelectrophoresis. The present results clearly indicated that both Ouchterlony and radial immunodiffusion were cost-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.

Key words: TMV, TNV, PVX, Serology, ELISA, electron microscopy.

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