affinity correction factors[15]. The new staining procedure affords a convenient, simple and stable staining method for widespread use in electrophoresis.

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STUDIES ON TOBACCO RING SPOT VIRUS FROM BRINJAL (*SOLANUM MELONGENA* L) WITH PARTICULAR REFERENCE TO PURIFICATION AND ASSESSMENT OF LOSSES

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ABSTRACT

Virus causing ring spot symptoms on brinjal is studied and identified to be due to Tobacco ring spot virus (TRSV). The virus is spherical with 26 mμ in diameter and is related to TRSV in other physical properties also. The loss in yield due to this virus infection is 55·2% to 70·3%.

RING spot symptoms of brinjal were found to be of common occurrence in the fields around Tirupati (Andhra Pradesh) and Bangalore (Karnataka). The disease incidence ranged from 60% to 80%. The characteristic symptoms of the disease were chlorotic concentric rings as well as mosaic mottling of leaves (Fig. 1). The infected plants produced less fruits which were small, disfigured and with concentric rings. Sastry and Nayudu[6] reported that the virus producing concentric rings or brinjal induced only necrotic local lesions without becoming systemic on forty-five plant species belonging to the families like Solanaceae, Chenopodiaceae, Cucurbitaceae and Leguminosae. The virus had thermal inactivation point between 65–70° C, the dilution end point between 1/1000 to 1/3000 and longevity *in vitro* for 7 days (28–32° C). Further investigations pertained to the purification of the virus and the losses in yield caused by this virus are presented in this paper.

FIG. 1. A diseased brinjal plant showing chlorotic rings.

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Virus source and infectivity.—The virus was maintained in an insect proof glass house on 30-day old brinjal plants var. 'Pusa Purple Long'. Cowpea [(*Vigna sinensis* (L.) Savi. Ex. Hassk.] was found to be a good local lesion host for bioassay of the virus and was used in the present studies[6]. The infectivity of the virus was very less whenever the inoculum was prepared in distilled water. The maximum infectivity was obtained by macerating the diseased tissues in phosphate buffer, pH 7 (0·01 M).

Purification.—Systemically infected brinjal leaves were harvested and used for purification. For initial clarification of the extract, the sap was filtered and centrifuged at 10,000 rpm. By passing the light green coloured supernatant, through the agar granules column or charcoal-celite cake, the filtrate was colourless, but most of the infectivity was lost. Attempts, therefore, were made to isolate the virus by following different purification methods and the procedure given below yielded higher infectivity. The infected leaves were macerated in phosphate buffer pH 7 (0·01 M) containing 0·02 M mercaptoethanol, at the rate of 2 ml/gram infected material. The sap was filtered and centrifuged at 10,000 rpm for 30 minutes. The supernatant obtained was centrifuged at 40,000 rpm for 90 minutes in Beckman L3–50 ultra centrifuge. The pellets were resuspended in 0·01 M phosphate buffer pH 7 and this suspension had higher infectivity when tested. Another cycle of low speed and high speed centrifugation was given to remove green pigment if present. It was further purified by following rate zonal density-gradient centrifugation Linear sucrose gradient columns were prepared by following the method of Brakke[1], by layering 7, 7, 7 and 4 ml of 0·01 M phosphate buffer pH 7-containing 40%, 30%, 20% and 10% sucrose solution respectively (in a tube of 1 × 3 in.). A sample of two ml of partially purified virus suspension was floated on the linear sucrose gradient columns and centrifuged at 23,000 rpm for 2 hours in a spinco SW 25·1 rotor. After centrifugation, the tubes were examined in a dark room by projecting a narrow beam of light down into the tube from the top and two light absorbing zones were noticed. The bottom zone was seen at 18–20 mm and the upper zone at 15–16 mm from the miniscus. One milli liter (ml) fraction from the top was collected by using syringe and 0·5 ml was diluted to 2 ml with phosphate buffer pH 7 (0·01 M) and infectivity was tested by inoculating to five cowpea plants. The remaining 0·5 ml was used for taking absorption spectrum at 240, 260 and 280 mμ in spectrophotometer and the results are given in Fig. 3B. The bottom zone exhibited typical nucleoprotein absorption spectrum having a peak of ultra-violet absorption at 260 mμ, with a minimum at 240 mμ and the infectivity was associated to this zone only. While the top zone had maximum absorption at 280 mμ and minimum at 260 mμ, giving the indication that it is of protein nature as it did not have the infectivity (Fig. 3A). The purified preparation when centrifuged at 23, 150 rpm in model E-Analytical ultracentrifuge, exhibited two schlieren optic peaks and the 'S' value of these two peaks was 75 and 117 (Fig. 2). The final purified preparation showed typical nucleic acid absorption spectrum and the 260/280 and 260/240 ratios were 1·8 and 1·4 respectively. The virus preparation stained with 2% PTA was examined under Hitachi electron microscope, and spherical particles of 26 mμ diameter were seen (Fig. 4). In serological tests, the virus under study reacted positively with the Tobacco ring spot virus antiserum.

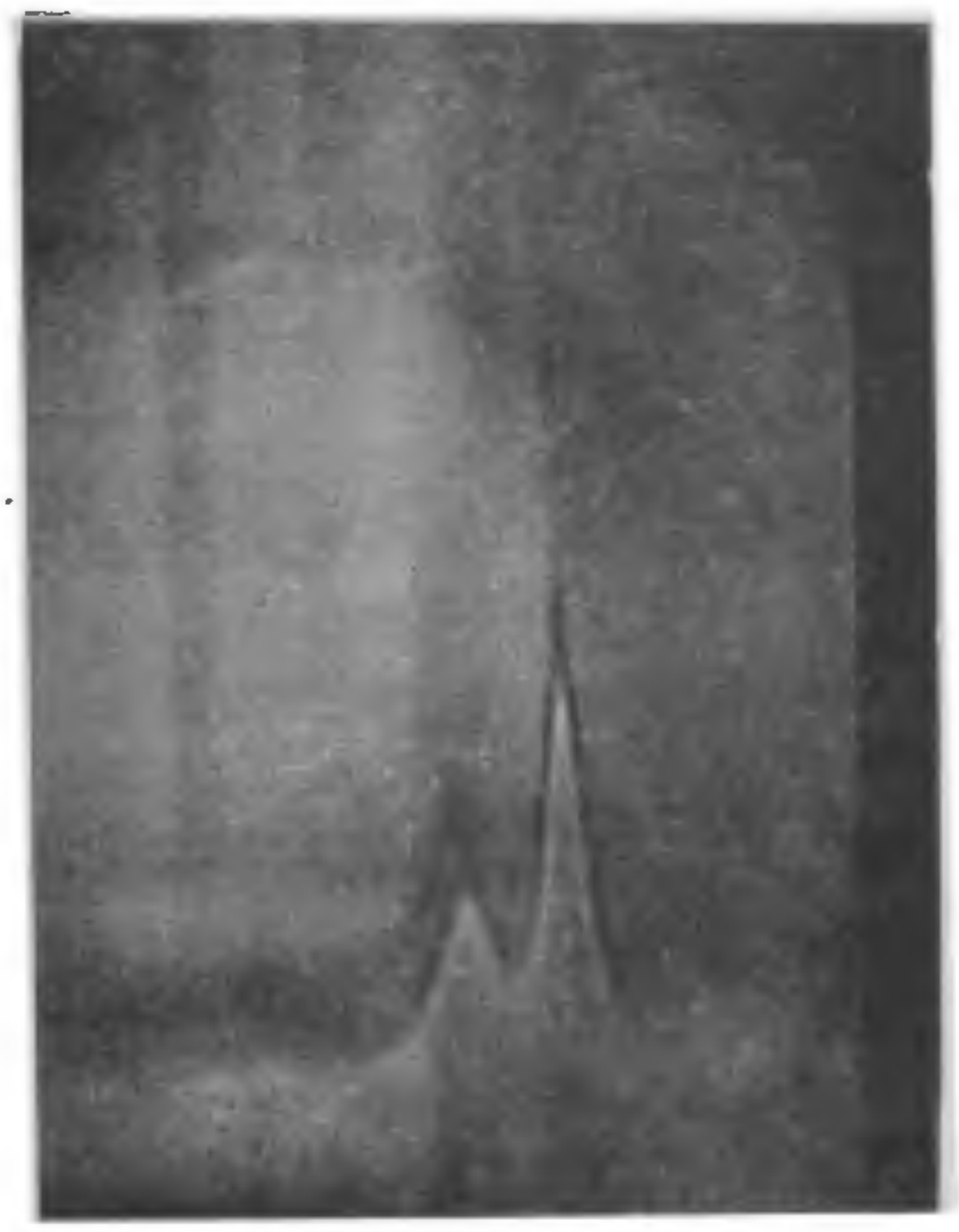

FIG. 2. Sedimentation pattern of purified virus, photographed through schlieren optics during analytical centrifugation run at 23, 150 rpm.

Assessment of losses.—Two independent field experiments were conducted to study the extent of losses caused by the virus under study at the experimental station of Indian Institute of Horticultural Research, Bangalore. The plants were inoculated when 30 day old and the yield data indicated that the average yield in the control plots was 144·26 and 169·73 Q/ha, as compared to 50·33 and 64·60 Q/ha from the infected plots. Approximately 55·2 to 70·3% loss in yield was noticed with this virus.

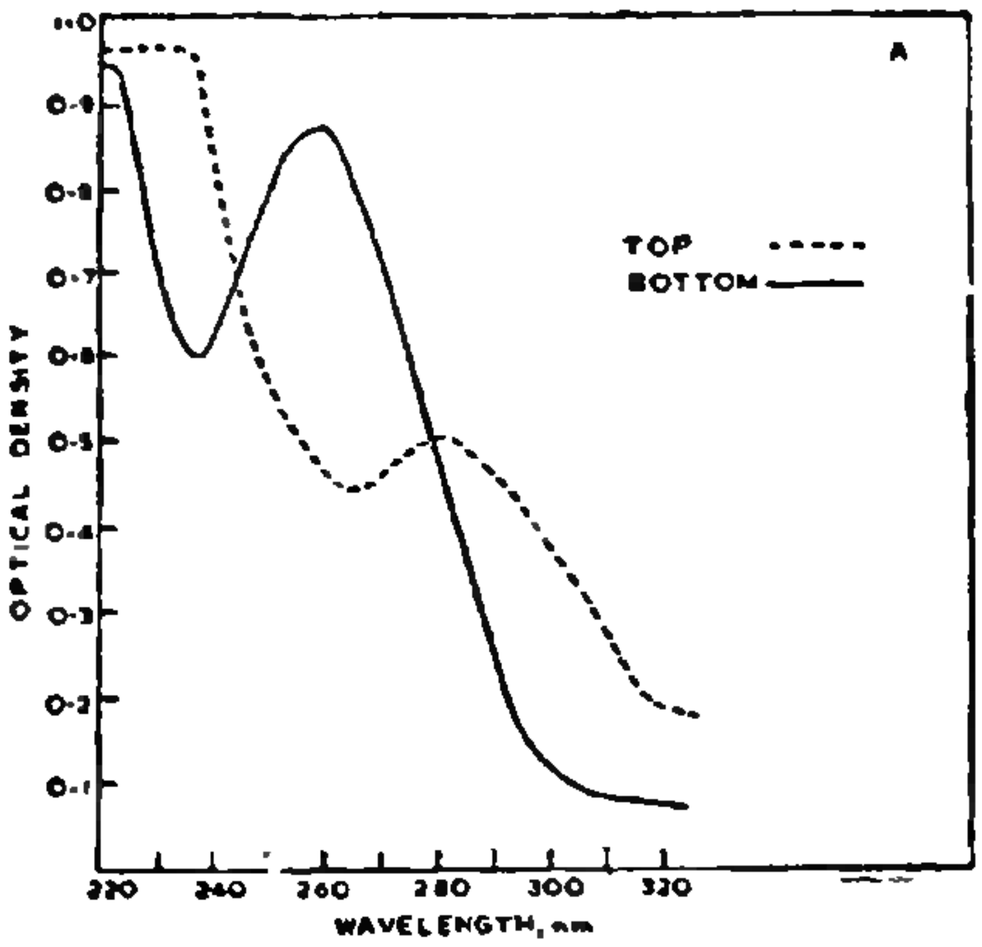

FIG. 3. A: Ultraviolet-absorbance spectrum of the purified virus preparation.

B: Ultraviolet-absorbance profile of different fractions at 260 mμ wavelength after rate zonal density gradient centrifugation (O———O); The infectivity in different fractions was examined on the primary leaves of cowpea plants (O - - - O).

DISCUSSION

Earlier, different viruses like tobacco mosaic virus[6], potato virus y[5], cucumber mosaic virus[2,7,8], tobacco etch virus[10], brinjal mild mottle virus[3], a graft transmissible mosaic disease[4] and some unidentified viruses[8] were reported from India, infecting brinjal under natural conditions. From Trinidad, Valleau[9] reported that the cause of eggplant yellows was tobacco ring spot virus and no further information is available on this virus. With the present studies, based on particle morphology, 'S' values and serology, it is concluded that the virus producing ring spot symptoms on brinjal under natural conditions in Andhra Pradesh and Karnataka, is a strain of tobacco ring spot virus and is reported for the first time from India.

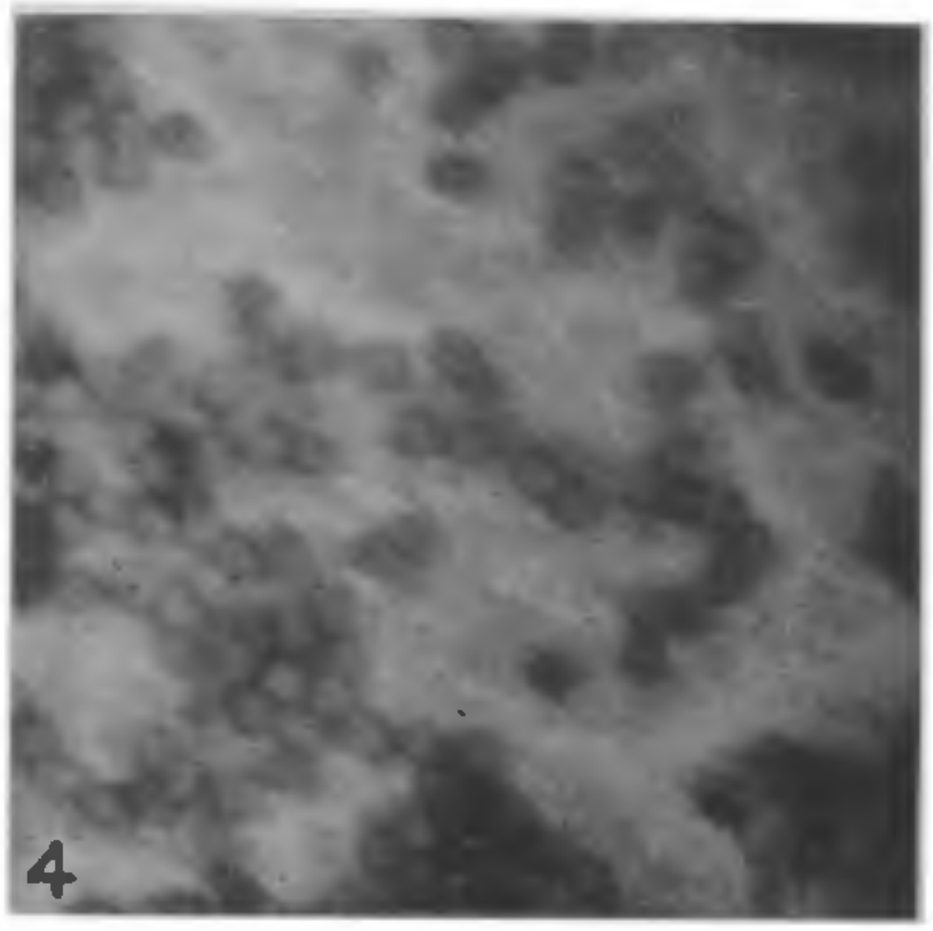

FIG. 4. Electron micrograph of a purified preparation stained with phosphotungstic acid (× 126,000).

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