obtained because of balance among growth regulators. It is well established that auxin–cytokinin balance facilitates organogenesis and is necessary for the regeneration of plants in a culture[6–9]. In this plant, of the various cytokinins used kinetin produced the maximum shoot buds, whereas among auxins NAA was the best for root formation.

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ASSOCIATION OF GEMINIVIRUS-LIKE PARTICLES WITH YELLOW MOSAIC DISEASE OF *DOLICHOS LABLAB* L.

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YELLOW mosaic symptoms were observed on the leaves of *Dolichos lablab* L. in Lucknow. Similar symptoms have been reported from Pune[1], and whitefly (*Bemisia tabaci* Genn.) has been established as a vector of the disease agent[2]. We report here the transmission of the disease agent and type of particles associated with the disease. For whitefly transmission, the method detailed earlier[3] was used for acquisition access feeding (AAF) and inoculation access feeding (IAF). The whiteflies were allowed AAF of 24 h followed by an IAF of 96 h. Tests were carried out by using *D. lablab* both as donor and recipient host. Twenty to twenty-five whiteflies were used per test plant. Insects were killed by spraying an insecticide after IAF and plants were transferred to an insect-proof glasshouse for 5 to 7 weeks. Back indexing was done 6–8 weeks after inoculation in the manner described earlier. *D. lablab* plants showing typical yellow mosaic symptoms were harvested 4 weeks after inoculation with whiteflies and homogenized with 0.1 M phosphate buffer, pH 8.0, containing 1% 2-mercaptoethanol and 0.01 M sodium ethylenediaminetetraacetate (1:3). The extract was squeezed through cheesecloth, clarified at 8000 rpm for 20 min, and 4% polyethylene glycol (PEG) and 1.5% sodium chloride were added. The mixture was incubated at 4°C for 3 h and centrifuged at 10,000 rpm for 20 min. The pellets were resuspended in 0.01 M phosphate buffer, pH 7.5. The suspension was clarified at 8000 rpm for 10 min, stirred with 2.5% Triton X-100 for 1 h at 4°C, and centrifuged for 10 min at 10,000 rpm. The supernatant thus obtained was loaded on 10 ml of 20% sucrose, prepared in phosphate buffer, and centrifuged at 35,000 rpm for 2 h in a Beckman Ti 45 rotor. Pellets were resuspended in 5 ml PB, centrifuged at low speed (10,000 rpm) for 10 min and finally at high speed (40,000 rpm) for 2 h in a Beckman SW 50.1 rotor. The pellet was resuspended in 2 ml of buffer and subjected to a low-speed centrifugation for 10 min at 8,000 rpm. Linear sucrose gradients (10–40%) were prepared in 0.1 M phosphate buffer, pH 7.0, containing 0.001 M EDTA. The partially purified preparation (0.5 ml) was layered on the gradients and centrifuged at 40,000 rpm for 2.5 h in a Beckman SW 50.1 rotor. Two distinct light-scattering bands observed were collected and concentrated by pelleting at 40,000 rpm for 2 h. The samples thus obtained were stained with 2% uranyl acetate (pH 4.0) and examined under a Philips 410 transmission electron microscope. Particles were measured with an X7 Bausch and Lamb magnifier directly from negatives.

During purification of the particles two light-scattering bands were observed after 10–40% sucrose density gradient centrifugation. The UV absorption spectrum of the lower band was typical of nucleoproteins with maximum absorbance at

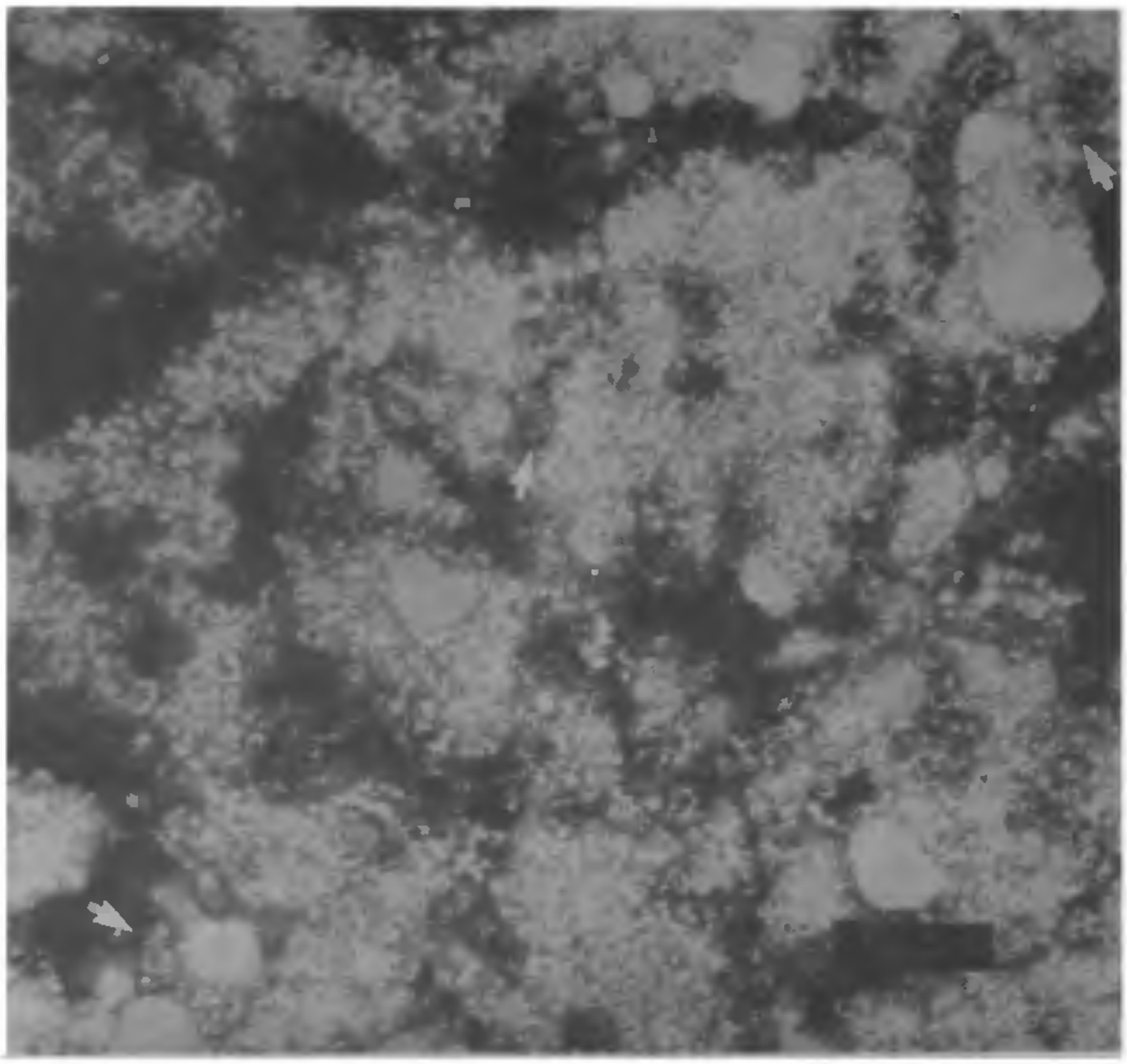

Figure 1. Electron micrograph of geminivirus-like particles stained with 2% uranyl acetate. Arrows indicate dimers. Bar represents 100 nm.

258 nm and minimum at 242 nm. The A_{260}/A_{280} ratio was 1.46 (uncorrected for light scattering), similar to that of several geminiviruses[4]. Electron microscopy of the sample obtained in the upper band revealed the presence of 12-nm particles, whereas 20 × 40 nm germinate particles were found in the lower band (figure 1). However, the intact geminate particles were only a few in number (1 or 2 particles per field) but most of the particles appeared to be degraded. The smaller number of geminate particles may be due to their susceptibility to the 2% uranyl acetate used for staining, as geminiviruses have been reported to be very susceptible to negative stains[5]. Particles of 12 nm appear to be phytoferritins.

Whiteflies transmitted the disease but at a very low transmission rate (2/50). These findings confirm the earlier report[2], where only 3% transmission of the disease was achieved by *B. tabaci*.

The yellow mosaic disease agent exhibits the characteristics shared by some suspected or proven geminivirus group members, including whitefly transmissibility and particle morphology[6,7]. The geminiviruses may be responsible for the yellow mosaic in *D. lablab*. However, for unequivocal proof of their involvement in the disease, an extensive investigation is needed.

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MICROBIAL CONVERSION OF MURRAYANINE TO MUKOEIC ACID

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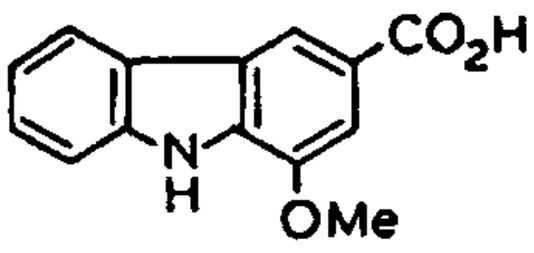
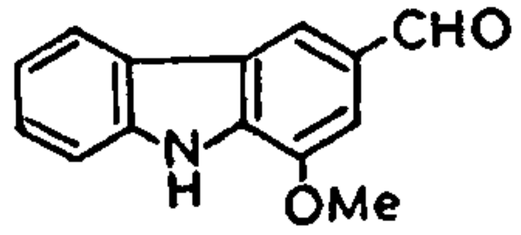
MUKOEIC acid[1], a carbazole alkaloid isolated from *Murraya koenigii*, has been assigned structure (I) based on physical evidences and also based on its formation from murrayanine (II)[2,3]. The biological oxidation of murrayanine to mukoeic acid was suggested based on the occurrence of both of them in the same plant[4]. The present study was undertaken to investigate microbial means of conversion of (II) into (I).

Initially, we searched for an appropriate organism capable of oxidizing an aromatic aldehyde to its corresponding acid, since the natural product II contains an aromatic aldehyde group. As a model system, we investigated the possibility of microbial oxidation of salicylaldehyde (III), a commercially available compound. For this purpose, strains of *Pseudomonas aeruginosa*, *Escherichia coli* B and *Bacillus firmis* were grown in medium (pH 6.8) containing KH_2PO_4 1 g, K_2HPO_4 2 g, $CaCl_2$ 0.02 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KNO_3 1 g, NaCl 0.2 g, yeast extract 0.3 g, and glucose 10 g per litre. Salicylaldehyde was added to a concentration of 250 μg/ml after 24 h of growth. After incubation for another 48 h the culture filtrates were extracted with ethyl acetate. On removal of solvent, only the *Pseudomonas* culture filtrate yielded a white solid. The solid was crystallized from aqueous alcohol. The crystalline compound (yield 60%), m.p. 159°C, was identified as salicylic acid (IV) from its physical and chemical properties and by direct comparison with a pure sample of salicylic acid.

In a similar study *P. aeruginosa* was also found to be quite efficient in converting compound II to I. Maximum conversion could be achieved 24 h after addition of the substrate to a final concentration of 150 μg/ml to the culture. The culture filtrate was extracted with ethyl acetate. On removal of the solvent a semi-solid mass was obtained. It was then chromatographed over silica gel using a series of solvents of increasing polarity, and three compounds were isolated. One was identified as the unconverted compound (II). The second compound (yield 50%), m.p. 241°C, has been assigned molecular formula $C_{14}H_{11}NO_3$ (M^+ 241). The UV spectrum (λ_{max} 236, 270 and 320 nm log ε 4.50, 4.58, 3.92) and IR spectrum (ν_{max} 3431, 1690, 1635, 1615, 1610 cm^{-1}) of the compound were strikingly similar to those of mukoeic acid (I), and identity was finally confirmed by direct comparison with natural mukoeic acid (m.m.p., UV, IR). The other compound, m.p. 228°C, molecular formula $C_{13}H_9NO_3$ (M^+ 227), gave violet coloration with $FeCl_3$, indicating the presence of phenolic hydroxyl group. The UV spectrum (λ_{max} 240, 266, 286, 315 and 323 nm, log ε 4.59, 4.64, 3.8, 3.78, and 3.18) bears a strong similarity to that of 1-hydroxycarbazole with carboxyl substitution at the 3 position[5]. The IR spectrum indicated it was an aromatic substance with an NH (3430 cm^{-1}), one hydroxyl (3200 cm^{-1}) and one carboxylic acid group (1690 cm^{-1}). From all these data the compound has been assigned the structure V.



(I)



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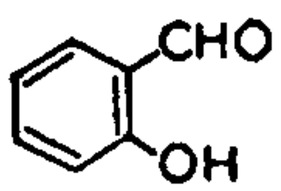

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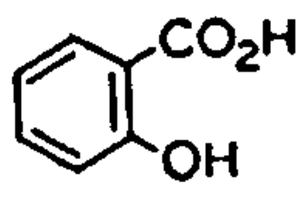
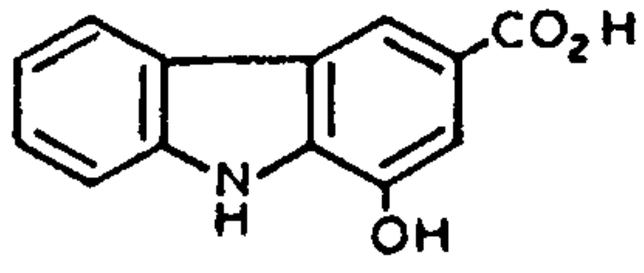


(IV)



(V)