Marigold Mottle Virus in Aligarh, India

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ABSTRACT

Naqvi, Q. A., Hadi, S., and Mahmood, K. 1981. Marigold mottle virus in Aligarh, India. Plant Disease 65:271-275.

Marigold mottle virus, a mechanically transmissible virus with rod-shaped particles about 675 nm long, was isolated from diseased marigolds in Aligarh, India. Symptoms included mottling and green mosaic in the leaves, stunting of plant growth, discoloration of *Nicotiana sanderae* flowers, and suppression of spines of *Datura metel* fruit. The virus was maintained on *N. glutinosa* and readily transmitted through sap and also by *Myzus persicae*. Ultraviolet absorption was maximum at 260 nm and minimum at 234 nm; A_{260}/A_{280} ratio was 1:28. The virus sedimented as a single band in sucrose density gradient columns and had one centrifugal component with a sedimentation coefficient of 152 S. Marigold mottle virus may be a member of the potyvirus group and serologically related to Datura mosaic and tobacco etch viruses.

A mosaic disease of marigold (*Tagetes erecta* L.), similar to that in Gorakhpur, Haldwani and Nainital (4), and Delhi (8), is prevalent in Aligarh. Affected plants show severe mosaic or mottling (Fig. 1A), are stunted, and produce small distorted flowers; many of the plants die.

The causal virus differed markedly from the virus previously reported to cause marigold mosaic (3,4,8). This article reports the host range, transmission, purification, and some properties of this marigold mottle virus (MMV).

MATERIALS AND METHODS

The virus was isolated from a marigold plant with characteristic symptoms and was propagated in *Nicotiana glutinosa* L. or *Datura metel* L. by periodic sap inoculation. All plants were grown in a glasshouse in pots containing an autoclaved mixture of soil, sand, and compost (2:1:1). Inoculation was done manually by abrasion with 600-mesh Carborundum. Inoculum was prepared by grinding infected leaves in a mixture of 0.1 M PO_4 buffer, pH 7.0, and 0.02 M Na_2SO_3 (v/v). In host range studies, at least three plants of a species were inoculated, and a similar number served as uninoculated controls. Symptomless inoculated plants were back indexed on *Tetragonia expansa* Murr. or *Chenopodium amaranticolor* Coste & Reyn. Virus for property studies was obtained from the sap extracted from *N. glutinosa* plants inoculated 10–12 days earlier; bioassays were done with *T. expansa*, a local lesion host for MMV.

Aphis gossypii Glov., *Brevicoryne brassicae* L., and *Myzus persicae* Sulz. were reared on Chinese cabbage (*Brassica pekinensis* (Lour) Rupr.), tobacco (*Nicotiana rustica* L.), and brinjal (*Solanum melongena* L.), respectively. Nonviruliferous aphids were starved for 4

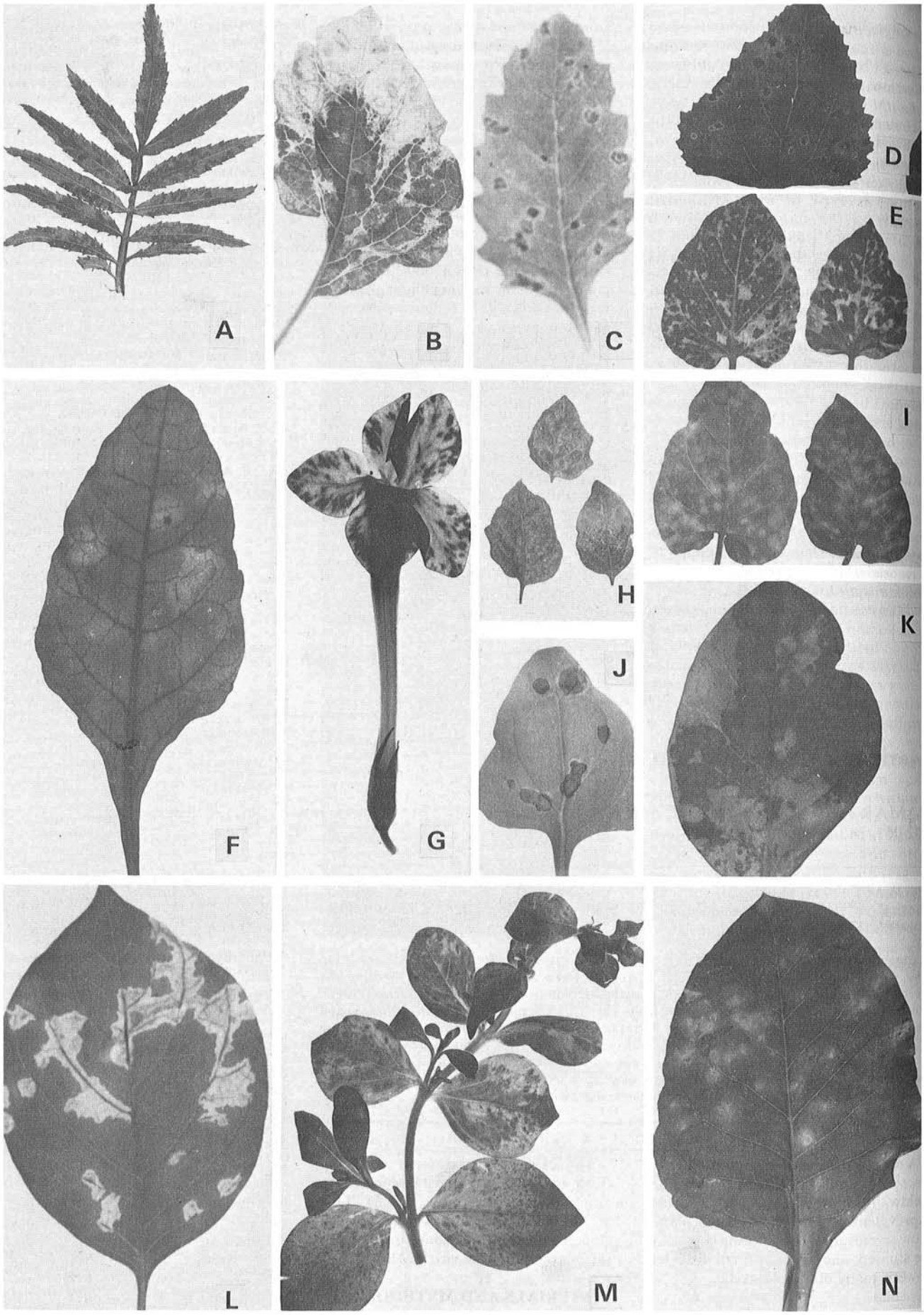

Fig. 1. Symptoms of marigold mottle virus infection on the leaves of **(A)** *Tagetes erecta*, **(B)** *Datura metel*, **(C)** *Chenopodium ambrosioides*, **(D)** *C. amaranticolor*, **(E)** *Nicotiana glutinosa*, and **(F)** *Beta vulgaris saccharifera;* on the flower of **(G)** *N. sandereae;* and the leaves of **(H)** *Solanum nigrum*, **(I)** *N. glutinosa*, **(J)** *Tetragonia expansa*, **(K)** *N. tabacum* 'Harrison's Special,' **(L)** *Achyranthes aspera*, **(M)** *Petunia hybrida*, and **(N)** *N. tabacum* 'Samsun NN.'

hr in the dark, then allowed acquistion feeding on diseased *N. glutinosa* leaves for 15 sec to 10 min, and transferred in groups of one to five to young, healthy *N. glutinosa* plants.

Purification and electron microscopy. Infected *N. glutinosa* tissue was homogenized in 0.1 M PO_4 buffer (1 g of tissue, 2 ml of buffer), pH 7.0, containing 2-mercaptoethanol made up to 0.02 M, and the homogenate was clarified by addition of chloroform (20–25%, v/v).

After low-speed centrifugation, the supernatant was centrifuged at 30,000 rpm in a Beckman Type 30 rotor for 3 hr. Pellets were suspended in 0.01 M PO_4 buffer, pH 7.0, and after two low-speed centrifugations, the supernatant was centrifuged at 40,000 rpm in a Beckman Type 50 rotor for 2 hr. Final pellets were resuspended in the same buffer and clarified by low-speed centrifugation.

Sucrose gradients (2) were prepared by layering 7, 7, 7, and 4 ml of 0.01 M PO_4 buffer, pH 7.0, containing 400, 300, 200, and 100 mg of sucrose per milliliter, respectively, in a 2.5 × 7.5 cm tube. Two milliliters of partially purified virus suspension was floated on the gradient, and the tube was centrifuged for 3 hr in a Beckman Type SW25.1 rotor. Material from the virus zone was removed with a needle bent twice at right angles and attached to a hypodermic syringe.

Sedimentation studies were performed in a Spinco model E analytical ultracentrifuge with Schlieren optics. Exposures were taken at 4-min intervals at a rotor speed of 31,410 rpm at 20 C. Sedimentation coefficients were determined by the graphic method (5).

Ultraviolet absorption spectra of purified preparations of the virus were determined with a Beckman DU2 spectrophotometer.

Samples from the virus-containing zones of density gradients were diluted, stained with 2% phosphotungstate, and examined with a Siemens-Elmiskope 1 electron microscope. Particles in a negative were measured with an eyepiece micrometer and low-power microscope, using a diffraction grating replica (22,835/cm) as a calibration standard.

Serology. An antiserum against MMV was prepared by immunizing a rabbit with purified preparations of the virus. Two 2-ml intravenous injections were given 1 wk apart, followed by intramuscular injections of 1 ml of the virus emulsified 1:1 with Freund's incomplete adjuvant, at weekly intervals for 4 wk. The titer of the antiserum was determined by the microprecipitin method (1). The relationship of MMV to some other viruses with elongated particles was determined by immunodiffusion tests in 0.8% agar containing 0.1% sodium azide and 0.5% sodium dodecyl sulphate.

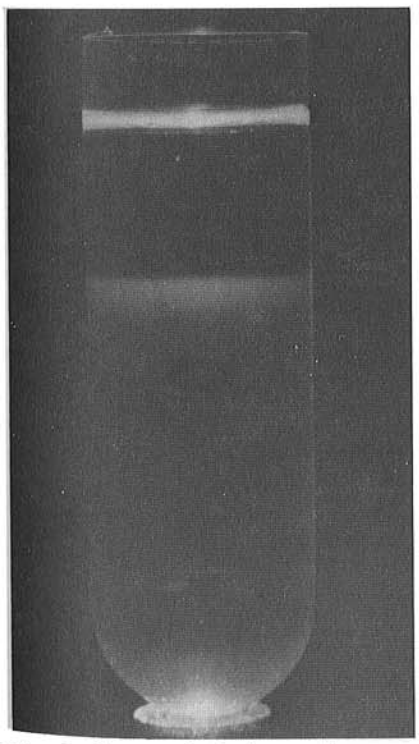

Fig. 2. Rate-zonal density gradient tube showing zone containing marigold mottle virus.

RESULTS

Host range and symptomatology. Of 79 plant species, 29 were susceptible to MMV (Table 1); 21 species were infected systemically and usually showed vein clearing followed by mosaic symptoms (Fig. 1 A–N.). Local lesions formed on *Abelmoschus esculentus, Achyranthes aspera, C. amaranticolor, C. ambrosioides,* amd *T. expansa.* The following species failed to develop symptoms after inoculation and the virus was not

Table 1. Host range of marigold mottle virus isolated from *Tagetes erecta* L.

Family / Species	Symptoms[a] Local	Symptoms[a] Systemic	Back inoculation[b]
Amaranthaceae			
Achyranthes aspera L.	CLL	···	–
Amaranthus			
caudatus L.	CLL	VC, MOS, S	+
hypochondriacus L.	CLL	VC, MOS, N, LD	+
Aizoaceae			
Mesembryanthemum criniflorum L.	CLL	VY, MOS, N, S	+
Tetragonia expansa Murr.	LL	···	–
Caryophyllaceae			
Stellaria media Cyr.	···	VY, MOS, DC, LD	+
Chenopodiaceae			
Beta vulgaris saccharifera L.			
'Katari 6'	NLL	···	–
Chenopodium			
amaranticolor Coste & Reyn.	LL	···	–
ambrosioides L.	LL	···	–
Compositae			
Calendula officinalis L.	CR	VC, MMOS, S	+
Centaurea			
imperialis Hort.	···	MOS, DC, LD	+
moschata L.	LL	MMOS, LD, DF, S	+
Tagetes			
erecta L.	···	VC, MMOS, D, S	+
minuta L.	···	VC, MMOS, DF, S	+
patula L.	···	VC, MOS, S	+
Leguminosae			
Cassia tora Roxb.	NLL	···	–
Dolichos lablab L.	···	VC, MMOS, LD	+
Malvaceae			
Abelmoschus esculentus Moench.	NLL	···	–
Scrophulariaceae			
Antirrhinum majus L.	···	VC, MOS, LD	+
Solanaceae			
Capsicum annuum L.	···	VC, MOS, S, TN	+
Datura metel L.	NLL	VC, MOS, BMR, MF, SS	+
Lycopersicon lycopersicum			
(L.) Karsten.	···	VC, MOS, S	+
Nicotiana			
glutinosa L.	CLL	VC, MOS, D, S, TN	+
tabacum			
'Harrison's Special'	···	VC, MOS, DC, S	+
'Samsun NN'	···	VC, MOS, S	+
'Xanthi-nc'	···	VC, MMOS, N, S	+
Petunia hybrida Vilm.	···	MOS, LD, S	+
Solanum nigrum L.	···	MMOS, LD, S	+

[a] BMR = bare midrib, CLL = chlorotic local lesion, D = distortion, DC = downward curling, DF = decoloration of flower, LL = local lesion, LD = leaf deformation, MOS = mosaic, MMOS = mosaic mottle, MF = malformation, N = necrosis, CR = chlorotic ring, S = stunting, SS = suppression of spines on fruit, TN = top necrosis, VC = vein clearing, VY = vein yellowing.
[b] + = positive, – = negative results.

recovered on back inoculation to local lesion hosts: *Ageratum conyzoides* L., *Amaranthus blitum* L., *A. caudatus* L., *A. cruentus* L., *A. gracilis* Desf., *A. leucocarpus* S. Wat., *A. spinosus* L., *A. tricolor* L., *Ammi majus* L., *Aster indicus* L., *Benincasa hispida* Cogn., *Beta vulgaris saccharifera* L. 'Brasov,' 'Bushmona,' and 'Stupnism.,' *Boerhaavia diffuss* L., *Brassica oleracea* L., *Cajanus cajan* Druce., *Catharanthus roseus* (L) G. Don., *Celosia cristata* L., *Chenopodium album* L., *C. murale* L., *Cineraria chinensis* Sprang., *Coccinia cordifolia* Cogn., *C. indica* Wight & Arn., *Coleus blumei* Benth., *Cosmos bipinnatus* Cav., *Cucumis sativus* L., *Cucurbita pepo* L., *C. moschata* Dusch., *Cyamopsis tetragonoloba* L., *Croton bonplandianum* Baill., *Dahlia pinnata* Cav., *Dianthus barbatus* L., *Daucus carota* L., *Eclipta alba* Hassk., *Gomphrena globosa* L., *G. celesioides* Mart., *Impatiens balsamina* L., *Lagenaria leucantha* (Dusch.) Rusby, *Launea aspleniifolia* Hook., *Peristrophe bicalyculata* Nees., *Phlox drummondii* Hook., *Phyllanthus fraternus* Web., *Physalis peruviana* L., *Pisum sativum* L., *Raphanus sativus* L., *Sesbania aculeata* (Willd.) Poir., *Sida rhombifolia* L., *Solanum melongena* L., *Sonchus asper* Wulf., *S. oleraceus* L., *Spinacia oleracea* L., *Trapaeolum majus* L., *Vigna sinensis* L., and *Zinnia elegans* Jacq.

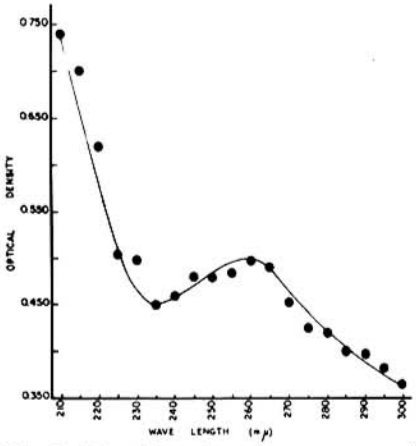

Fig. 3. Ultraviolet absorption spectrum of purified marigold mottle virus.

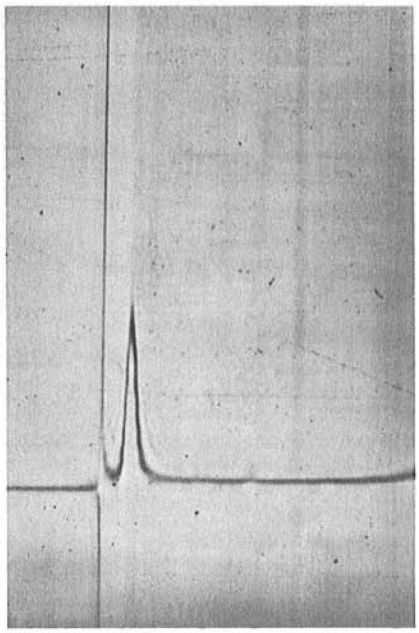

Fig. 4. Schlieren pattern of purified marigold mottle virus at 31,420 rpm at 20 C in 0.1 M PO_4 buffer, pH 7.0. Photographed when the rotor attained maximum speed after 16 min.

Transmission. MMV was easily sap transmissible, with 70–80% transmission when sap from infected marigold or *N. glutinosa* was used. Of the aphid species, only *M. persicae* transmitted the virus from infected to healthy *N. glutinosa* plants. In one trial, 6 of 10 plants became infected by allowing one aphid a 10-min inoculation access after a 20-sec acquisition feeding. Attempts to transmit the virus by *Cuscuta campestris* and *C. reflexa* were unsuccessful.

Virus properties. In *N. glutinosa* sap, the virus retained infectivity as long as 136 hr at room temperature (25 ± 5 C). The dilution end point was between 10^{-1} and 10^{-2}, and the thermal inactivation point was between 45 and 50 C.

In purification of the virus, rate-zonal density gradient centrifugation removed the last traces of normal proteins. After 3-hr centrifugation, a light scattering band was present 27–30 mm below the meniscus of the gradient (Fig. 2). Material removed from this band consistently was infectious. The ultraviolet spectrum of purified preparations was typical of nucleoproteins, with a maximum at 260 nm and minimum at 234 nm (Fig. 3). The A_{260}/A_{280} ratio was 1:28. Analytical centrifugation of purified preparations revealed a single Schlieren peak (Fig. 4) with a sedimentation coefficient of 152 S. Purified preparations contained long flexuous particles (Fig. 5) ranging from 650 to 700 nm, with a mean length of 675 nm (50 particles).

Serology. Antiserum against the virus had a 1:512 titer and formed a single band with the homologous antigen in double diffusion tests (Fig. 6). Immunodiffusion tests with other viruses having similar morphology indicated that MMV was serologically related to Datura mosaic and tobacco etch viruses but not to brinjal mild mosaic and Melilotus mosaic viruses.

DISCUSSION

MMV has a fairly wide host range, infecting 26 species among nine families of angiosperms. Mosaic and stunting were typical, although *Capsicum annuum* invariably showed top necrosis, which is diagnostically important. *Abelmoschus esculentus* and *Achyranthes aspera*, previously not reported as hosts for viruses infecting marigold, were found to be suitable local lesion hosts.

MMV differs from other viruses reported to cause mosaic disease in marigold. The virus causing a severe mosaic of *Tagetes patula* (3) was sap transmissible to *Datura stramonium*, whereas MMV failed to infect this host. It also failed to infect *Cucumis sativus*, *Cucurbita pepo*, *Physalis peruviana*, *Vigna sinensis*, and *Zinnia elegans*, which were reported as systemic hosts for a mosaic-causing virus of marigold (4). MMV also differs from marigold mosaic virus (8) in its physical properties, host reaction, mode of transmission, and particle morphology. The virus described by Sang and Verma (8) has isometric particles, 42–45 nm in diameter, with a few 30 nm in diameter.

MMV particles are similar to those of

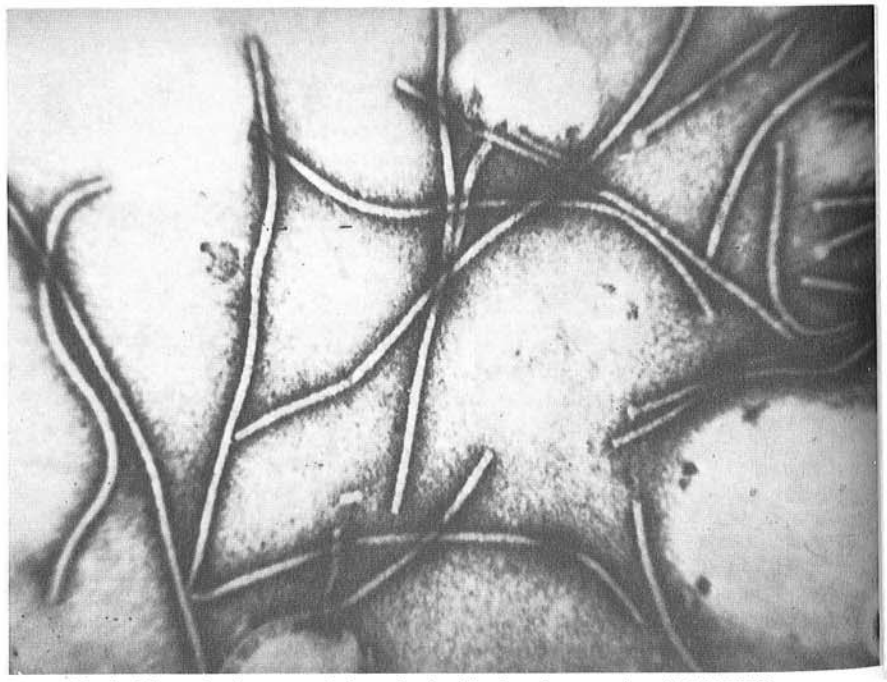

Fig. 5. Marigold mottle virus negatively stained with phosphotungstic acid (×80,000).

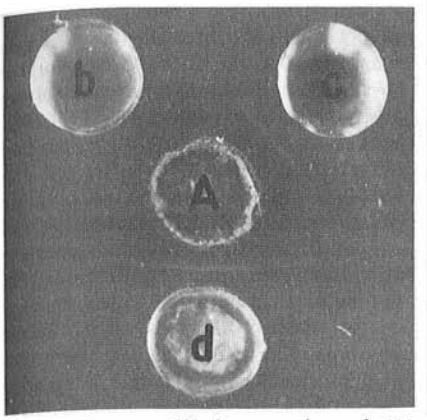

Fig. 6. Immunodiffusion reaction of **(A)** marigold mottle virus (MMV) antiserum with **(B)** sap from healthy *N. glutinosa* plant, **(C)** sap from MMV-infected *N. glutinosa* plant, and **(D)** purified MMV preparation.

brinjal mild mosaic and Melilotus mosaic viruses (6,7). Brinjal mild mosaic virus is restricted to Solanaceae and is not transmitted by aphids. Melilotus virus differs from MMV in host range and some biophysical properties. Serologic tests indicated that MMV was related to tobacco etch and Dtura mosaic viruses but not to brinjal mild mosaic virus and Melilotus virus. The length of MMV particles is not typical of potyviruses, but transmission properties and serologic relationships suggest that MMV may be a member of the potyvirus group.

ACKNOWLEDGMENTS

We thank D. Z. Maat of Het Institute Voor Plantenzicken Kunding, Wageningen, for providing antisera of viruses and also M. Atreyi for help in analytical ultracentrifugation.

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