area[1,11–12] suggests that pelitic sediments metamorphosed to mica schists were manganiferous in places to give rise to gondites and/or pink colouration to some muscovite schist. It is significant that brownish pink, pink or reddish pink piedmontite is present in gondites and only in those mica schists which are rose coloured. But since piedmontite occurs as a minor accessory in these rose mica schists, its contribution to the rose colour of the schist is trivial. Nevertheless its presence in rose mica schists of the present area is symptomatic for their manganiferous nature. The fairly high MnO content of the rose muscovites compared to the colourless muscovite and their distinct pleochroism in shades of rose colours suggest that the rose colour is primarily due to high manganese content in rose muscovites. Thus, the role of manganese appears to be the most important factor for the rose colour of these muscovites.

1. Chakraborty, A., Unpublished M.Sc. thesis, Calcutta University, 1985.
2. Heinrich, E. Wm. and Levinson, A. A., *Am. Mineral.*, 1953, **38**, 25.
3. Richardson, S. M., *Am. Mineral.*, 1975, **60**, 73.
4. Annersten, H. and Hålenius, U., *Am. Mineral.*, 1976, **61**, 1045.
5. Richardson, S. M., *Am. Mineral.*, 1976, **61**, 1051.
6. Richardson, S. M. and Richardson, Jr., J. W., *Am. Mineral.*, 1982, **67**, 69.
7. Finch, J., Gainsford, A. R. and Tennant, W. C., *Am. Mineral.*, 1982, **67**, 59.
8. Deer, W. A., Howie, R. A. and Zussman, J., *Rock-forming Minerals 3*, Longmans, London, 1962.
9. Bilgrami, S. A., *Mineral. Mag.*, 1956, **31**, 236.
10. Roy, S., *Syngenetic Manganese Formations of India*, Jadavpur University, 1966, p. 219.
11. Mukherjee, S. and Bandyopadhyay, B. K., *J. Geol. Soc. India*, 1975, **16**, 485.
12. Gangopadhyay, S., Unpublished M.Sc. thesis, Calcutta University, 1985.

ACKNOWLEDGEMENTS. We thank Mr Subrata Sinha, Dr N. D. Mitra and Dr B. Gangopadhyay (Geological Survey of India, Calcutta), Prof. P. K. Gangopadhyay (Geology Department, Presidency College) and Arijit Roy (Durgapur Govt. College) for help.

26 March 1990; revised 30 June 1990

Purification and some properties of a virus causing chlorotic mottle disease in carnations (*Dianthus caryophyllus* L.)

S. K. Raj, M. Aslam and B. P. Singh
Plant Virus Laboratory, National Botanical Research Institute, Lucknow 226 001, India

A virus causing vein chlorosis, leaf mottling, chlorotic spots, growth stunting and flower damage in carnations (*Dianthus caryophyllus* L.) was transmitted by *Myzus persicae* in a non-persistent manner. In *D. barbatus* sap, the virus had thermal inactivation point 55–60°C, dilution end point 10^{-2}–10^{-3} and longevity *in vitro* 4–5 days at room temperature. The virus was purified by PEG precipitation and sucrose density gradient centrifugation. Virus particles were flexuous rods of 718 × 12 nm.

Dianthus caryophyllus L. is an important economic ornamental plant for cut flowers in floriculture trade. Viruses adversely affect the flowers deteriorating their market value. Carnation latent virus[1], carnation vein mottle virus[2], carnation yellow fleck virus[3], carnation necrotic fleck virus[4] and carnation streak virus[5] occur naturally in carnations. During the past few years, a disease characterized by vein chlorosis, leaf mottling and chlorotic spots on the leaf (Figure 1) followed by stunting and flower damage (Figure 2) was observed on carnations. This note reports the transmission, host range, purification and electron microscopic studies of the virus-isolate causing chlorotic mottle disease of carnations studied for the first time in India.

Inocula were prepared by grinding infected leaf tissues in 0.02 M potassium phosphate buffer, pH 7.5. Carborundum (600 mesh) was added as an abrasive and test plants were inoculated by rubbing with forefinger dipped in inoculum. Five to ten plants of different species of Caryophyllaceae, Amaranthaceae, Leguminosae,

Figure 1. Vein chlorosis, mottle and spots on infected leaves of *Dianthus caryophyllus* L.

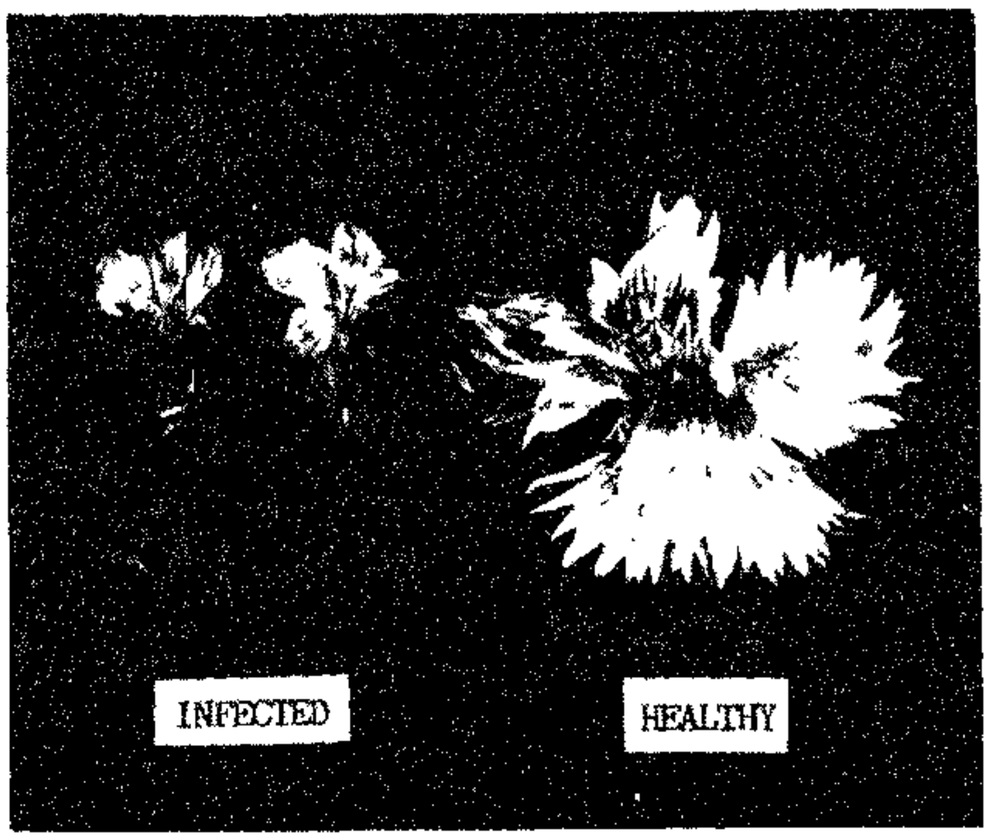

Figure 2. Stunting and flower damage in infected *D. caryophyllus* L.

Solanaceae, Chenopodiaceae and Cucurbitaceae were inoculated. Back inoculations to *C. amaranticolor* were carried out 3–5 weeks after inoculation to check the infectivity. *Myzus persicae* and *Aphis gossypii* were tested for aphid transmission. After a starvation period (1–2 h) the aphids were allowed to probe on infected carnation plants (1–2 min) and were then placed on healthy carnations (*D. caryophyllus*) and *D. barbatus* for 24 h. Aphids were sacrificed by spraying with 0.02% Rogor. Stock culture of the virus was maintained on *D. barbatus* (Sweet William) plants by sap inoculation. Biophysical properties of virus were determined in infected *D. barbatus* sap following the procedure described by Noordam[6].

A modified procedure[3] was adopted for purification of virus from infected *D. caryophyllus* leaves using 0.05 M sodium phosphate buffer pH 7.5. Partial purification of virus was done by polyethylene glycol precipitation (4% PEG + 1.17% NaCl) followed by differential speed centrifugation. The virus was further purified by loading the partially purified preparation onto linear gradient of sucrose (10–40% w/v). A light scattering band observed after centrifugation at 85,000 g for 2.5 h in Beckman SW 20 rotor was collected, diluted and concentrated at 85000 g for 90 min. The final preparation obtained was considered to be a purified virus.

The UV absorption spectrum of the purified virus preparation was recorded in Pye-Unicam spectrophotometer. For electron microscopy, virus preparations were stained with 2% uranyl acetate (pH 4.2) and examined under Philips CM 10 transmission electron microscope.

The virus could be transmitted by *Myzus persicae* in a non-persistent manner from carnation to Sweet William (*D. barbatus*); 8/30 plants produced systemic symptoms after 3–4 wk of aphid transmission. The virus was transmitted mechanically from carnations to a few plant species, producing chlorotic local lesions on *Vaccaria pyramidata, Chenopodium amaranticolor, Dolichos lablab* and *Ageratum conyzoides* without systemic spread. Chlorotic rings, vein chlorosis on *D. caryophyllus* and mosaic mottling and stunting in *D. barbatus* were observed after 3–4 wk of virus inoculation. No symptoms developed on *Nicotiana tabacum* cv. Samsun NN, *N. glutinosa, N. rustica, Chenopodium album, C. murale, C. quinoa, Lycopersicon esculentum, Solanum melongena, Datura stramonium, Cucumis sativus* and *Vicia faba.*

In Sweet William sap, the virus had a thermal inactivation point between 55 and 60°, a dilution end point between 10^{-2} and 10^{-3} and longevity *in vitro* between 4 and 5 days. The virus particles were flexuous rods of varying lengths (Figure 3). End to end aggregation of particles was also observed. The normal dimensions of the virus particles calculated on the basis of 100 particles was 718 × 12 nm (mean maximum), a characteristic of potyvirus group[7]. UV absorption spectrum was typical of nucleoproteins. Based on A_{260}/A_{280} ratio (1.17), the content of nucleic acid in the virus was estimated as being about 5%[8]. In SDS-immunodiffusion tests[9], the virus did not react with antiserum to potato virus Y^N, clover yellow vein and bean yellow mosaic viruses.

The virus causing chlorotic mottle disease in carnations resembles the members of polyvirus group in particle morphology[7], biophysical properties and transmission by aphids in non-persistent manner. The virus isolate was precipitated by 4% PEG + 1.17% NaCl, without losing its infectivity as with many polyviruses[10]. It resembles carnation vein mottle virus, a polyvirus[2], in

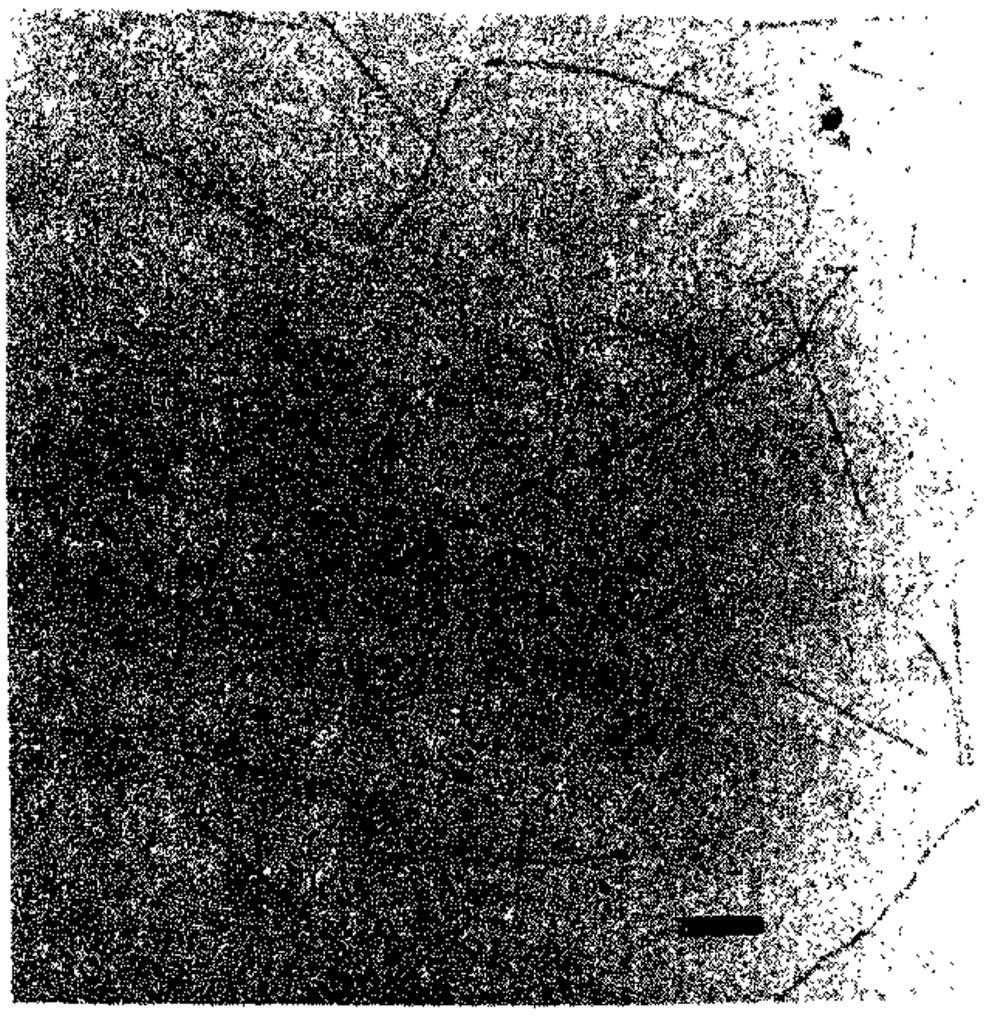

Figure 3. Electron micrograph of purified virus preparation. Bar = 200 nm.

particle morphology and physical properties but serological relationship of present isolate with other polyviruses including carnation vein mottle virus has to be confirmed. To our knowledge, it is the first report of a virus causing a disease in carnations, in India.

1. Wetter, C., CMI/AAB, 1971, 61.
2. Hollings, M. and Stone, O. M., CMI/AAB, 1971, 78, 1.
3. Smookler, M. and Loebenstein, G., *Phytopathology*, 1974, **64**, 979.
4. Inouye, T., CMI/AAB, 1974, 136.
5. Poupet, A., Cardin, A., Marais, A. and Caldihac, B., *Annales de Phyto-pathogie*, 1975, 7, 277.
6. Noordam, D., *Identification of plant viruses, Methods and Experiments*, Oxford & IBH Publishing Co., New Delhi, 1973, 175.
7. Hamilton, R. I., Edwardson, J. R., Francki, R. I. B., Hsu, H. T., Hall, R., Koenig, R. and Milne, R. G., *J. Gen. Virol.*, 1981, **54**, 223.
8. Gibbs, A. J. and Harrison, B. D., *Plant Virology, The principles*, Arnold, London, 1976, 292.
9. Purcifull, D. E. and Batchelor, D. L., *Fla. Agric. Exp. Tech. Bull.*, 1977, **788**, 39.
10. Rajyalakshmi, R., Nayudu, M. V. and Sreenivasulu, P., *Curr. Sci.*, 1984, **53**, 332.

ACKNOWLEDGEMENTS. The authors are thankful to Dr. P. V. Sane, Director, NBRI, Lucknow for providing facilities, Mr. V. K. Lall, scientist for electron microscopy and Dr. K. M. Srivastava, Scientist for his critical reading of the manuscript.

2 September 1989; revised 14 June 1990

Fungus and insect spoiled maize for ethanol production by *Saccharomyces cerevisiae* var *ellipsoideus* and *Zymomonas mobilis*

R. C. Kanta and Y. F. Neelgund*
Department of Post Graduate Studies in Microbiology, Gulbarga University, Gulbarga 585 106, India

The alcohol yield (w/v) from maize of mould-infected, insect-infested and mould-insect affected grains using *Saccharomyces cerevisiae* var *ellipsoideus* were 7.67, 7.32 and 6.85; and by *Zymomonas mobilis* were 7.26, 6.92 and 6.49 respectively. The fermentation efficiencies (%) by *S. cerevisiae* var *ellipsoideus* were 97.58, 97.08 and 97.49; and by *Z. mobilis* were 92.36, 92.44 and 92.31 respectively. The present observations using spoiled maize grains are promising.

OUR studies using maize (*Zea mays*) Ganga-5 (hybrid variety) as substrate, fermented by *Saccharomyces cerevisiae* var *ellipsoideus* and *Zymomonas mobilis* revealed that maize could be used as a potential biomass for ethanol production[1,2]. Often ill storage and improper harvesting processes lead to fungal and insect infestations of maize and so disqualify this grain for human consumption. Such spoiled grains are used as animal feed or manure. Very scanty literature is available on the use of spoiled grains for alcohol production. Calleja *et al*[3], suggested that spoiled grains and roots could well serve as raw materials for production of ethanol. A few studies carried out on the fate of mycotoxin-contaminated grains, used as substrate for the fermentative alcohol, have shown no toxin in the distilled alcohol[4–8]. Therefore, an attempt is made here to use spoiled maize grains for production of an industrially valuable product such as ethanol.

Samples of spoiled maize grains of Ganga-5 (hybrid variety), were randomly collected from different warehouses, storehouses and grain shops in clean polythene bags. These samples were analysed to find out causative agent/s for spoilage. The causes were found to be infection by mould, primarily being *Aspergillus niger* and infestation by Coleopteran storage pest, *Sitophilus oryzae* (Linne).

Such grains were assorted into three sets, comprising grains of fungus-infected (Set I), grains of insect-infested (Set II) and third set as grains spoiled by both the agents (Set III). Another set (Set IV) using unaffected, healthy maize grains was also processed similarly as control. These samples were further cleaned and washed separately to free them from contaminants. Each set of grains were pulverised (30 mesh) separately to prepare 20% solids' slurry.

The slurry was prepared out of 30 mesh flour, on dry weight basis by using clean tap water. Liquefaction of the slurry was carried out by enzyme process by adjusting pH of the slurry to 6.0 using 2.5 N HCl. Bacterial alpha-amylase was added at the concentration of 3 g/kg of flour. An amount of 0.022% $CaCl_2$ and 0.014% NaCl were added to the slurry for the satisfactory activity of the enzyme as described by Srikanta *et al*[9]. Then the whole slurry was cooked in a water bath at 80–85°C for 30 min.

The slurry thus hydrolysed was subjected to saccharification by lowering the temperature of the slurry to 60°C under running tap water. The pH of the slurry was adjusted to 4.5 by using 2.5 N HCl. The slurry was then treated with fungal amyloglucosidase (glucozyme) enzyme at the concentration of 5 g/kg of flour and was maintained at 60°C for 48 h in a thermostatically controlled device, as suggested by Srikanta *et al*[9].

The reducing sugars of the saccharified mash were determined by Shaffer-Hartmans' micromethod[10], which were considered as initial sugars for fermentation. Fermentation was carried out using *S. cerevisiae* var *ellipsoideus* and *Z. mobilis*. The ethanol content was determined by A.O.A.C. method[11].

The initial and final reducing sugar, ethanol yield

*For correspondence.