Discussion
Coccinia grandis (L.) Voigt, a wild plant occurring in Uttar Pradesh specially in Aligarh was found to be infected with a virus disease showing characteristic symptoms of mosaic and mottling followed by leaf distortion. At advanced stage of infection, the plants showed reduction in leaf size with retarded vegetative growth of the plants. The disease was investigated and the identity of its causal agent was established. During the survey, it was found that the disease incidence was lower at the seedling stage (3.20%). However, as the plants grow older, the disease spread and its maximum incidence was 33.00% during September 1998.

The disease was found to be transmitted by usual mechanical sap inoculation and by three species of aphids viz. Aphis gossypii Glov., A. crassivora Koch. and Myzus persicae Sulz. but was not transmitted by whitefly (Bemisia tabaci Genn.), dodder (Cuscuta reflexa Roxb.), soil and seeds. However, the disease was transmitted by grafting.

Experimental host range studies revealed that the virus under study has a restricted host range. It infects some 24 species belonging to 10 families. Most of the hosts were from Chenopodiaceae, Cucurbitaceae and Solanaceae. The plants belonging to Chenopodiaceae and Cucurbitaceae generally showed necrotic and chlorotic local lesions, while those of Solanaceae showed varied type of mosaic on the
leaves and stunting of the plants. *Nicotiana glutinosa* L. was used as propagation host. Different *Chenopodium* spp. were found to react with localized lesions to this virus, but *C. amaranticolor* Coste & Reyn was used as local lesion host for quantitative assay as it gave maximum and countable local lesions.

Virus in crude sap lost its infectivity after heating at 60°C for 10 min. but remained infective at 55°C, and lost infectivity at a dilution of $10^{-5}$. It remained infective for 6 days when stored at room temperature (20 ± 5°C) and 10 days at 4°C. No infectivity was recorded beyond these periods.

Phosphate buffer (0.1 M, pH 7.0) was found to be the most suitable extraction medium for retaining virus infectivity. Other buffers tried such as acetate, borate, citrate and tris HCl were found to inhibit the activity of the virus. Addition of sodium sulphite also inhibited the virus infectivity. Virus attained maximum concentration in *N. glutinosa* L. plants after 14 days of inoculation. All parts (root, stem, leaf and flower) of this propagation host contained virus but the maximum concentration of the virus was present in leaves.

Virus was purified by a procedure involving extraction from *N. glutinosa* L. plant leaf tissues (infected with the
virus) in 0.1 M phosphate buffer, pH 7.0. Chilled butanol (30%) was used to clarify the sap. The virus was precipitated by using PEG (6%) having molecular weight 6,000 and 0.1% NaCl followed by two cycles of differential centrifugation.

Further purification was achieved by rate zonal density gradient centrifugation. Partially purified virus preparation when centrifuged on linear sucrose columns for 2 h at 23,000 rpm formed a light scattering band. The material forming the light scattering band was found to be infective containing virus particles.

The purified preparation gave a spectrum typical of nucleoproteins. The nucleic acid isolated by phenol-chloroform method was found to be RNA by Orcinol test. The nucleic acid (RNA) percentage was found to be 6.02 as A\textsubscript{260}/A\textsubscript{280} ratio was 1.1838. The extinction coefficient, buoyant density and partial specific volume were 2.7651, 1.3024 g/cm\textsuperscript{3} and 0.7678 respectively. SDS-PAGE electrophoresis of viral capsid protein showed only one type of protein subunit having a molecular weight of c. 36,000 daltons.

Electron microscopic studies of clarified virus preparation revealed the presence of flexuous filamentous particles measuring c. 760 × 12 nm. Ultrastructural studies of infected leaf tissues of \textit{N. glutinosa} L. plant revealed
three types of cytoplasmic inclusion bodies viz. long lamellar aggregates, scrolls and pin wheels in the cells.

Antiserum raised against the virus showed a titre of 1:2048 in tube precipitin test. In agar-gel double diffusion test, the virus showed serological relationship with Zucchini Yellow Mosaic Virus (ZYMV) antiserum. In immuno-specific electron microscopy, the virus showed close relationship with ZYMV as moderate decoration were obtained with the antiserum of this virus.

Only few viruses are reported to cause mosaic and mosaic mottling diseases on Coccinia grandis L. Voigt including watermelon mosaic virus strain-2 (Bhargava et al., 1975) and a serologically related strain of watermelon mosaic virus-2 designated as Trichosanthes Virus (TV) (Purcifull et al., 1989). It is difficult to compare the virus isolate under investigation with the viruses reported earlier, as very meagre information is available on these viruses (except few). Physico-chemical properties of the virus, particle morphology and serological studies give more reliable information regarding the relationship of a virus with other viruses.

Comparison made by present isolate with the virus described by Auger et al. (1974) i.e. WMV-2 strain differs on
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particle morphology especially in their lengths though both are flexuous rods.

*Coccinia grandis* was shown to be a ready source of WMV-2 strain by Bhargava et al. (1975) and *Aphis gossypii* was an important vector in the spread of the virus. The virus under study was also transmitted through the vector i.e; *A. gossypii*. But host range and transmission are the least criteria for a meaningful comparison. Therefore, no comparison of this strain could be made with the present isolate due to paucity of information.

This Aligarh isolate induced the pin wheels and scrolls in the host similarly as induced by strain 2 of WMV studied by Martelli and Russo (1976). However, no comparison be made due to lack of particle morphology of the virus described by these authors.

The virus strain detected by Arteaga et al. (1976) measured c. 740-760 nm. It was transmitted by mechanical sap inoculation and by *Aphis gossypii* and *Myzus persicae* in a non-persistent manner. This strain differs from the virus under study as the present isolate measures c. 760 nm exactly though the mode of transmission is same i.e. non-persistent. Other properties such as serological relationship and electron microscopy of the virus described by Arteaga et
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al., could not be compared with the present isolate due to non availability of the information.

The present virus is similar in symptom expression to the strain of WMV studied by Chen et al., (1982) which causes mosaic, mottling, stunting and distortion of the host plant but is distinct from the virus under study in having a different particle size measuring c. 770 x 16 nm whereas the present virus measures c. 760 x 12 nm.

The studies carried out by Dikova et al., (1983) on cucumber show the occurrence of WMV. They identified the strain on the basis of host range, properties in sap, serological tests, ultrastructural studies and Electron microscopy. The present isolate is similar in bio-physical properties as both have a DEP of 10^{-4}, TIP of both the strains lies between 55- 60°C and LIV is also approximately similar. The typical potvirus inclusions were seen associated with both the strains but both are distinct in having different particle sizes. The strain studied by Dikova et al. measures c. 705.4 nm.

The Aligarh isolate under study differs from the strain of WMV described by Almeida and Borges (1983) in having different bio-physical properties. The antiserum obtained by them had a high titre (1:16000) than that obtained from the present isolate (1:2048).
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Zucchini yellow mosaic virus described by Purcifull \textit{et al.} (1984) was transmitted mechanically and by \textit{Myzus persicae} in a non-persistent manner. They were flexuous filamentous particles measuring c. 760 nm and induced striated inclusions in the cytoplasm of host cells. They resemble the present virus isolate in some aspects. Both the viruses have almost the same host range and are similar in symptom expression. In immuno-specific electron microscopy, both the viruses show close relationship with zucchini yellow mosaic virus (ZYMV) antiserum as comparatively maximum trapping (apart from homologous antiserum) was obtained with antiserum to ZYMV. But exact comparison of the present virus with this strain was not possible because information about the RNA\%, buoyant density and other physico-chemical properties of the strains described by Purcifull \textit{et al.} are not available.

A strain of watermelon mosaic virus described by Tripathi and Joshi (1985) is similar in host range and symptom expression to the virus under investigation but is distinct from it in having a high TIP of 65°C and LIV of 26-27 days at 32-34°C and 42 days at 17-19°C.

The strain of watermelon mosaic virus described by Meer \textit{et al.} (1987) is similar in symptom expression and host range to the virus under investigation. Both have the same
molecular weight of 36,000 daltons but the strain described by Meer et al. differs from the present isolate in having a different particle morphology ranging in between 706-770 nm.

There is much affinity between the present isolate and the virus strain WMV-2 designated as Trichosanthes Virus (TV) by Purcifull et al. (1989) in host reaction, mode of transmission etc. but their comparison remain incomplete in the absence of information regarding particle morphology and physico-chemical properties of the virus, as these were not reported by the above authors.

On the basis of the properties of the virus (under investigation), filamentous particles measuring c. 760 x 12 nm, a buoyant density of 1.3024g/cm³, presence of RNA (6.02%) and protein subunits of molecular weight 36,000 daltons, induction of cytoplasmic inclusions (pin wheels, lamellar aggregates and scrolls), transmission by mechanical sap inoculation and by aphids (Aphis gossypii, A. crassivora and Myzus persicae) in a non- persistent manner, restricted host range and serological relationship with Potato Virus Y (specifically in ISEM) and Zucchini Yellow Mosaic Virus (ZYMV), it is concluded that the virus under investigation is a member of potyvirus group and appears to be a strain of Watermelon Mosaic Virus-2 (WMV-2) naturally infecting Coccinia grandis (L.) Voigt.

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