DISCUSSION

During survey of turnip fields conducted in 1983-84, a mosaic disease of turnip was found to be prevalent in and around Aligarh district. The disease was investigated and the identity of its causal virus established. Survey records showed that the disease incidence was lower at seedling stage. However, as the plants grow older the disease spreads throughout the field, the disease incidence being the maximum (80%) during January and February 1984, when the plants were fully grown.

The infected plants showed characteristic symptoms which included mosaic, mottling and curling of leaves followed by severe stunting of plants. Infected plants produced only few flowers and distorted roots. The disease was transmitted by mechanical inoculation but not by aphids, soil, seed and dodder (Cuscuta reflexa). Experimental host range studies revealed that the virus under study has a wide host range. Out of one hundred and six species of plants belonging to 27 families, sixty three species belonging to 20 families were found susceptible, while forty three species belonging to 7 families were not infected when inoculated with the present virus. Most of the hosts were in the family Solanaceae, Cruciferae and Asteraceae (Compositae). Nicotiana glutinosa L. was used as propagation host. Chenopodium amaranticolor, C. album, C. murale and C. quinoa were found to be good local lesion hosts of the virus, the first being most suitable for the quantitative assay.
Virus in crude sap lost its infectivity after heating at 65°C and at a dilution of $10^{-5}$, it remained infective for 72 h when stored at room temperature (20±5°C) and 144 h at 4°C and no infectivity was noted beyond these periods.

Of the several buffers tried 0.1M phosphate buffer, pH 7.5 was found to provide the most suitable environment to maintain the infectivity. Various organic solvents such as butanol, carbon tetrachloride and chloroform either alone or in combination were used for the clarification of the infected crude sap and out of them, a combination of butanol-chloroform (1:1) was found suitable and others had an adverse effect on the infectivity of the virus.

The virus isolate attained highest concentration in Nicotiana glutinosa plants after 13 days of inoculation and this species proved to be a suitable propagation host. All parts of the plant contained virus but the maximum concentration was attained in leaves.

Among the procedures used for purification of the virus under study, the procedure involving extraction from systemically infected N. glutinosa leaf tissues in 0.1M phosphate buffer (7.5 pH) containing 0.01M EDTA and 0.1% sodium sulphite, clarification with 20% chilled butanol and chloroform (1:1), treatment with 1% Triton X-100 followed by two cycles of differential centrifugation proved most successful. Preparations at different stages of the procedure were infective. Further purification was
achieved by rate zonal density gradient centrifugation. Partially purified preparation when centrifuged on linear sucrose columns for 90 min at 40,000 rpm and in CsCl column for 12 h at 40,000 rpm formed a light scattering band. Material forming the light scattering band was found to be infectious.

Sedimentation studies revealed the presence of one component and the sedimentation constant was 118S.

Purified preparation stained with 2% uranyl acetate contained flexuous rod shaped particles 580 nm in length and 13 nm in width with a buoyant density of 1.32 g/cm³ in cesium chloride (CsCl). Preparations showed a UV-spectrum characteristic of nucleoprotein with 260/280 ratio of 1.25 indicating approximately 5-6% nucleic acid in virion. Viral capsid contained only one type of protein subunit having a molecular weight of ca. 25,000±500 daltons with helical arrangements.

Ultrastructural studies of infected leaf of *Nicotiana glutinosa* fixed with 3% glutaraldehyde showed three types of cytoplasmic inclusion bodies viz., laminate, banded and bundle type in the mesophyll cells.

Antiserum against the virus under study raised in rabbit showed a titre of 1:512 in tube precipitin test. The antigen in the crude sap of the infected leaves reacted up to the dilution of 1:256. In agar-gel double diffusion test only one line of precipitate was formed. In gel diffusion test the virus
showed no serological reaction with antisera to the following
flexuous viruses: potato virus Y (PVY), potato virus S (PVS),
potato aucuba mosaic virus (PoAMV), cymbidium mosaic virus
(CybMV), white clover mosaic virus (WCIMV), narcissus mosaic
virus (NaMV) and papaya mosaic virus (PaMV) but it strongly
reacted with potato virus X (PVX) antiserum. In immuno-specific
electron microscopy the virus particles were trapped with PVX
antiserum on grids. Virus particles were also decorated with
antibodies when incubated with PVX antiserum.

Hoggan and Johnson (1935) described the host range and
biophysical properties of a virus isolated from turnip. The pro-
properties are not similar to the virus under study, except necrotic
lesions on tobacco. Present virus differs from turnip mosaic
virus of Chamberlain (1937) in symptoms and in being not aphid
transmissible. Tompkins (1938) described a mosaic disease of
turnip from USA which in host range and biophysical properties
appears to be similar to the present virus, but differ in aphid
transmission. Turnip mosaic of Ling and Yang (1940) from China
also resembles with the virus studied herein in host range, TIP
and DEP but differs in aphid transmission and symptoms on turnip.
A virus disease of certain crucifers including turnip was obser-
ved by Dale (1948) which is different in host range, symptoms
(except necrotic lesions on tobacco) when compared with the virus
under study.
Tochihara (1960) described a virus from cabbage as cauliflower mosaic virus which is different from the virus under study in transmission, biophysical properties and virus particles but shows some similarity in host range. The present isolate markedly differs in biophysical properties from turnip mosaic virus described by Chenulu (1961) and is also different from a virus isolated from radish (Kou, 1961) as it could not be transmitted by any species of aphids, but has much resemblance in biophysical properties and almost similar host range except *N. rustica* which is a non-host for the present one.

The Japanese strain of turnip mosaic virus described by Yoshii *et al.* (1963) from Japanese radish could not be compared with the present isolate as it totally differs in biophysical properties and also in particle size.

Turnip mosaic virus described under different names from various hosts is aphid transmissible (Kvicala, 1974; Lee and Paik, 1977; Fujisawa and Iizuka, 1985) but the present virus was not transmitted by aphids.

Capsid protein heterogeneity is of common occurrence in purified viruses of potato virus Y group (Hiebert and Mc Donalds, 1976). Michelin-Lausarot and Papa (1975) found that the protein obtained by degradation of particles of turnip mosaic virus (*A₁TuMV*), a member of PVY group, was heterogeneous and polyacrylamide gel electrophoresis experiment showed 3 components I, II and III (estimated mol. wt. 38,000; 30,000 & 27,500, respectively)
while the virus under study showed only one type of protein estimated as 25,000±500 d, thus it differs from $A_1$TuMV.

HS and K30 isolates of turnip mosaic virus isolated from turnip and cabbage (Juretic et al., 1976) and an Iris isolate of TuMV (Inouye and Mitsuhata, 1978) have different biophysical properties from the virus discussed herein. All these isolates were transmitted by *Myzus persicae* in a non-persistent manner which never transmitted this isolate. Particle size of these isolates (730-750 nm) is also different from the particle of the present isolate (580 nm). HS and K30 isolates produced oval or irregular X-bodies, often containing crystalline needles while Iris strain produced pinwheels, circular and short curved laminate aggregate bundles in infected tissues whereas the present virus produced banded and laminate inclusions in infected tissues of *N. glutinosa*.

The inclusion bodies reported in the case of cabbage black ring spot virus (Rubio-Huertos, 1956) and in radish mosaic virus (Edwardson and Purcifull, 1970) were found to be dissimilar with the inclusion bodies found in the present isolate which suggests that the virus under study is different from radish mosaic and cabbage black ring spot viruses.

Cyttoplasmic inclusions, which have been observed in most known groups of plant viruses, differ considerably in size, location, composition and ultrastructural organization and have diverse origin and functions. Some viruses are reported to induce
inclusions in the infected host cells and the inclusions are quite characteristic of the virus group (Martelli and Russo, 1976a). Turnip mosaic virus, a member of potyvirus group is the most widely occurring virus on turnip. Members of PVY group produce peculiar type of inclusion bodies in the infected cells. Potyviruses induce the formation of conical or cylindrical cytoplasmic inclusions, the structure of which has been elucidated from ultrastructural studies of infected plants (Edwardson, 1966a) and freeze etch electron microscopy (McDonald and Hiebert, 1974b). Recently, it has been further elucidated by computer-assisted analytical geometry (Mernaugh, Gardner and Yocom, 1980). Cytoplasmic inclusions seen in transverse section are described as 'pinwheels' and in longitudinal section as 'bundles'. Potyviruses have been sub-grouped according to the predominant type of cytoplasmic inclusions they induce (Edwardson, 1974a). Such characteristic type of inclusion bodies induced by potyviruses were not observed in cells of tissue of N. glutinosa infected with the virus under study.

The 'fibrous masses' composed of bundles of filamentous particles have been seen in many hosts infected with potyviruses (Purcifull et al., 1966; Kozar and Sheludko, 1969; Zettler et al., 1968; Shalla and Shepard, 1972; Doraiswamy and Lesemann, 1974). Conspicuous virus aggregates, having a periodicity arising from the order by alignment of particles in stacked layers, form 'banded' inclusion like those of members of potex virus group (Amelunxen, 1956, 1958). Successive layers are not continuous with one another being usually separated by thin cytoplasmic strands containing
ribosomes, which are likely to be trapped within the inclusion in the course of its formation (Purcifull et al., 1966; Esau, 1968). Different authors (Kozar and Sheludko, 1969; Stols et al., 1970; Shalla and Shepard, 1972; Doraiswamy and Lesemann, 1974; Pennazio and Appiano, 1975) reported laminate inclusions of multilayered bundles of proteinaceous sheet which have been found consistently associated with the infection of potato virus X. PVX induced laminate inclusions consist of sheets mostly free of virus but heavily studded on both sides with beads. These sheets and beads are composed of proteins which were not related antigenically to PVX (Shalla and Shepard, 1972). The same inclusion bodies (laminate and banded) characteristics of the member of the PVX were observed in the ultrastructural studies of leaf tissues of N. glutinosa infected with this virus which suggest that the present virus is of the same group.

Thus, it is evident on the basis of differential hosts, biophysical properties, mode of transmission and positive serological reaction with PYX antiserum that the virus isolate under study belongs to PVX group. Further, the properties of purified virus such as particle morphology, buoyant density, mol. wt. of protein, helical arrangement of protein sub-unit and cytoplasmic banded and laminar inclusions supports the above view. This has been again confirmed by using different serodiagnostic techniques such as trapping and decoration of virus particles with potato virus X (PVX) antiserum and double diffusion test. Thus the properties of the virus under study strongly support the view
that it is a new strain of potato virus X (PVX) and belongs to the potex group. It is hitherto unrecorded from turnip and may be called as Aligarh strain of potato virus X causing mosaic of turnip followed by leaf curl.