

ISOLATION AND IDENTIFICATION OF VIRUSES OF PUMPKIN (Cucurbita moschata Poir)

THESIS SUBMITTED TO
BIDHAN CHANDRA KRISHI VISWA VIDYALAYA
WEST BENGAL FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN AGRICULTURE (PLANT PATHOLOGY)

BY
SUBRATA KUMAR GHOSH

DEPARTMENT OF PLANT PATHOLOGY
FACULTY OF AGRICULTURE
BIDHAN CHANDRA KRISHI VISWA VIDYALAYA
1976

Bidhan Chandra Krishi Viswa Vidyalaya

P. O. KALYANI, Dist. NADIA,
WEST BENGAL
Telephone :

No.....

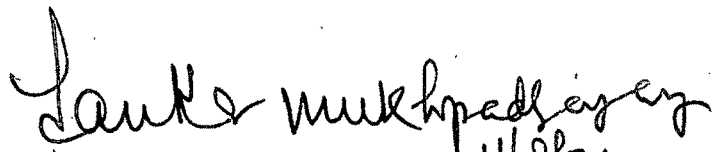
Dated197

Dr. S. Mukhopadhyay, M.Sc.(Cal.), Ph.D.(Missouri),
Reader, Department of Plant Pathology,
Faculty of Agriculture,
Bidhan Chandra Krishi Viswa Vidyalaya.

This is to certify that the thesis entitled
" ISOLATION AND IDENTIFICATION OF VIRUSES OF PUMPKIN
(Cucurbita moschata Poir)" submitted in fulfilment of
the requirement for the Degree of Doctor of Philosophy
in Agriculture (Plant Pathology) of Bidhan Chandra Krishi
Viswa Vidyalaya, is a record of bonafide research carried
out by Sri Subrata Kumar Ghosh, under my guidance and
supervision and no part of the thesis has been submitted
for any other degree or diploma.

Sri Ghosh worked in the Department of Plant
Pathology, Faculty of Agriculture for three years as
Research Fellow under the project " Studies on Cucurbit
Viruses in West Bengal". During his stay in the Department
and association with me as a student he earned distinction
for his amicable manners and good behaviour.

Assistance and help received during the course
of investigation and sources of literature have been fully
acknowledged.


(S. Mukhopadhyay) 11/8/76

A C K N O W L E D G E M E N T

I express my deep sense of gratitude and indebtedness to Dr. S. Mukhopadhyay, B.Sc.(Cal.), Ph.D.(Missouri), Reader in Plant Pathology, Faculty of Agriculture, Bidhan Chandra Krishi Viswa Vidyalaya for suggesting the problem, inspiration and guidance throughout the course of investigation and pains taking interest in the preparation of the manuscript.

My sincere thanks are due to Dr. S.B. Chattopadhyay, B.Sc.(Cal.), Ph.D. (Lond.), D.I.C.(Lond.), Vice-Chancellor, Bidhan Chandra Krishi Viswa Vidyalaya and Dr. A.K. Sinha, B.Sc.(Cal.), Ph.D.(Lond.), D.I.C.(Lond.), Head, Department of Plant Pathology for providing facilities and valuable suggestions during the course of present investigation.

My grateful thanks are due to Dr. P. Sathukhan, Bio-physics Laboratory, Saha Institute of Nuclear Physics, Calcutta for providing laboratory facilities and continuous help during electromicroscopic studies with the virus isolates.

Grateful thanks are due to Dr. P.R. Sengupta, Reader, Dr. A.K.M. Kaiser, Dr. Md. Mahasin of the Department of Plant Pathology and Dr. H.H. Choudhuri, Veterinary Surgeon, Bidhan Chandra Krishi Viswa Vidyalaya for their help during serological studies.

Thanks are due to Dr. H.B. Webb, U.S.D.A. and Dr. S.P. Kapoor, Plant Virus Research Institute, Poona, India for providing seeds of some indicator plants for testing the virus isolates. Thanks are also due to Dr. A.L. Steere, U.S.D.A. and Dr. S.P. Raychaudhuri, formerly Head, Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi, India for their valuable suggestions on different techniques for isolation and identification of cucurbit viruses during their visit to this laboratory.

Grateful thanks are due to Indian Council of Agricultural Research, New Delhi, India for providing Research Fellowship under the project "Studies on Cucurbit Viruses in West Bengal", during the tenure of which this work was carried out.

Assistance and help received from Dr. A.K. Mukhopadhyay, Reader, Department of Agricultural Chemistry and Soil Science, Dr. T.K. Gupta, Reader, Department of Statistics and Dr. H. Roy, Deputy Librarian, Bidhan Chandra Krishi Viswa Vidyalaya are also fully acknowledged.

Finally, I am grateful to all of my colleagues for their co-operation and help throughout the course of investigation.

Kalyani


(Subrata Kumar Ghosh)

Dated 11th Aug. 1976.

C O N T E N T S

	<u>Page</u>
I. INTRODUCTION ...	1
II. REVIEW OF LITERATURE ...	3
III. MATERIALS AND METHODS ...	31
A. Incidence of virus diseases ...	31
B. Sap transmissibility of Type II symptom complex. ...	31
C. Separation and Identification of isolates of Type II symptom complex. ...	32
a) Determination of Physical properties of the isolates. ...	33
1. Determination of Thermal inactivation point of the isolates. ...	33
2. Determination of Dilution End Point of the isolates. ...	34
3. Determination of Ageing <u>in vitro</u> of the isolates. ...	34
b) Host range of the Isolates. ...	35
c) Insect Transmissibility of the Isolates. ...	39
d) Electronmicroscopy of the Isolates. ...	44
e) Serological relationship of the Isolates. ...	46
D. Biochemical changes in the leaves of <u>Cucurbita Moschata</u> Poir plants inoculated with different virus isolates at different stages of pathogenesis. ...	50

	<u>Page</u>
a) Determination of chlorophyll content (a,b and total) ...	50
b) Determination of buffer soluble protein content. ...	51
c) Determination of total (TCA precipitable) sap protein content. ...	53
d) Determination of reducing sugar and starch content. ...	53
e) Determination of ribonucleic acid (RNA) content. ...	56
IV. RESULTS ...	59
A. Incidence of virus diseases ...	59
B. Sap transmissibility of Type II symptom complex. ...	64
C. Identification of isolates of Type II symptom complex. ...	70
a) Determination of Physical properties of the isolates. ...	70
1. Determination of Thermal inactivation point of the isolates. ...	70
2. Determination of Dilution End Point of the isolates. ...	71
3. Determination of Ageing <u>in vitro</u> of the isolates. ...	72
b) Host range of the Isolates. ...	80
c) Insect Transmissibility of the Isolates. ...	135
d) Electronmicroscopy of the Isolates. ...	139
e) Serological relationship of the Isolates. ...	141

D. Biochemical changes in the leaves of <u>Cucurbita moschata</u> plants inoculated with different virus isolates at different stages of pathogenesis.	...	146
a) Determination of chlorophyll content (a,b and total).	...	146
b) Determination of buffer soluble protein content.	...	155
c) Determination of total (TCA precipitable) sap protein content.	...	158
d) Determination of reducing sugar and starch content.	...	161
e) Determination of ribonucleic acid (RNA) content.	...	167
V. DISCUSSION	...	170
VI. SUMMARY	...	208
VII. BIBLIOGRAPHY	...	(i) - (xvii)

LIST OF TABLES

<u>Table Number</u>	<u>S u b j e c t</u>	<u>Page</u>
1	Name of the plants used in the study of host range of the isolates obtained from Type II symptom complex of pumpkin (<u>C. moschata</u> Poir)	36-38
2	Relative incidence of virus diseases of pumpkin (<u>Cucurbita moschata</u> Poir) in different districts of West Bengal during February-March 1973.	60
3	Symptoms of virus diseases of pumpkin (<u>Cucurbita moschata</u> Poir) found in the fields of different districts of West Bengal during February-March 1973	61-62
4	Relative incidence of different types of field symptoms of virus diseases of pumpkin (<u>Cucurbita moschata</u> Poir) in different districts of West Bengal during February-March 1973	63
5	Sap transmissibility of Type II symptom complex of pumpkin (<u>Cucurbita moschata</u> Poir).	65
6	Types of symptoms obtained from Type II symptom complex of <u>C. moschata</u> Poir on sap transmission	66-69
7	Infectivity of the standard inocula of different isolates obtained from Type II symptom complex after treating them at different temperatures for 10 minutes.	75
8	Infectivity of the standard inocula of different isolates obtained from Type II symptoms complex at different dilution	76
9	Determination of ageing in vitro at room temperature 24-32°C) of different isolates of Type II symptom complex.	77

<u>Table Number</u>	<u>S u b j e c t s</u>	<u>Page</u>
9a	Infectivity of the standard inocula of the isolate A, when stored for different hours	78
10	Comparative physical properties of different isolates of Type II symptom complex.	79
11	Host range of the isolates obtained from <u>Cucurbita moschata</u> Poir.	82-83
12	Transmissibility of the isolates by <u>Aphis gossypii</u> Glover, <u>Myzus persicae</u> Sulz. and <u>Bemisia tabaci</u> Conn.	136
13	Serial transmission of the isolates at every 24 hours by the viruliferous <u>Aphis gossypii</u> Glover, <u>Myzus persicae</u> Sulz. and <u>Bemisia tabaci</u> Conn. on <u>C. moschata</u> Poir plants.	138
14	Size of the virus particles of the nine different isolates.	139
15	Antigenic relationship between the virus isolates	144
16	Chlorophyll a content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	148
17	Chlorophyll b content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with nine pumpkin virus isolates.	151
18	Total chlorophyll content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	154
19	Buffer soluble protein content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	157

(iii)

<u>Table Number</u>	<u>S u b j e c t</u>	<u>Page</u>
20	Total (TCA precipitable) sap protein content of the leaves of pumpkin (<u>C.moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	160
21	Reducing sugar content of the leaves of pumpkin (<u>C.moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	163
22	Starch content of the leaves of pumpkin (<u>C.moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	166
23	Ribonucleic acid (RNA) content of the leaves of pumpkin (<u>C. moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	169

...

LIST OF PLATES

<u>Plate Number</u>	<u>S u b j e c t</u>	<u>Page</u>
PLATE I	Different types of leaf symptoms observed in virus infected pumpkin plants in the fields.	60-61
PLATE II	Different types of leaf symptoms observed in virus infected pumpkin plants in the fields	60-61
PLATE III	Different types of leaf symptoms observed in virus infected pumpkin plants in the fields.	61-62
PLATE IV	Leaf symptoms of different isolates from type II symptom complex.	65-66
PLATE V	Leaf symptoms of different isolates from type II symptom complex	66-67
PLATE VI	Leaf symptoms of different isolates from type II symptom complex	67-68
PLATE VII	Leaf symptoms of different isolates from type II symptom complex	68-69
PLATE VIII	Enation symptoms due to isolates A ₂ and A ₃ on the undersurface of pumpkin leave	68-69
PLATE IX	Symptoms of different isolates on <u>Lagenaria vulgaris</u>	80-81
PLATE X	Symptoms of different isolates on <u>Lagenaria vulgaris</u>	85-86
PLATE XI	Symptoms of different isolates on <u>Trichosanthes anguina</u>	93-94
PLATE XII	Symptoms of different isolates on <u>Trichosanthes anguina</u> .	93-94
PLATE XIII	Symptoms of different isolates on <u>Luffa acutangula</u>	101-102
PLATE XIV	Symptoms of different isolates on <u>Citrullus lanatus</u> cv. Charleston Gray.	107-108

<u>Plate Number</u>	<u>S u b j e c t</u>	<u>Page</u>
PLATE XV	Symptoms of different isolates on <u>Cucurbita pepo</u> cv. Early Prolific Straightneck	110-111
PLATE XVI	Symptoms of different isolates on <u>Cucurbita pepo</u> cv. Early Prolific Straightneck	111-112
PLATE XVII	Symptoms of different isolates on <u>Cucurbita pepo</u> cv. Early Prolific Straightneck	113-114
PLATE XVIII	Symptoms of different isolates on <u>Cucurbita pepo</u> cv. Early Prolific Straightneck	115-116
PLATE XIX	Symptoms of different isolates on <u>Cucumis melo</u> (Cantaloupe Selection B 63-3)	117-118
PLATE XX	Symptoms of isolate A ₈ on <u>Cucumis sativus</u> cv. Long Green	124-125
PLATE XXI	Symptoms of isolate A ₂ and A ₅ on <u>Petunia hybrida</u> .	129-130
PLATE XXII	Electronmicrographs of the particles for different isolates	139-140
PLATE XXIII	Electronmicrographs of the particles for different isolates	139-40
PLATE XXIV	Electronmicrographs of the particles for different isolates	139-40
PLATE XXV	Antigenic relationship of different isolates	143-144
PLATE XXVI	Antigenic relationship of different isolates	143-144

LIST OF TEXT FIGURES

<u>Text Figure Number</u>	<u>S u b j e c t</u>	<u>Page</u>
1-2	Antigenic relationship of different isolates	141-142
3-4	Antigenic relationship of different isolates	142-143
5	Antigenic relationship of different isolates	143-144
6	Chlorophyll a content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates	146-147
7	Chlorophyll b content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus plants	149-150
8	Total chlorophyll content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates	152-153
9	Buffer soluble protein content of the leaves of pumpkin (<u>C. moschata</u> Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	155-156
10	Total (SDS precipitable) sap protein content of the leaves of pumpkin (<u>C. moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	158-159
11	Reducing sugar content of the leaves of pumpkin (<u>C. moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	161-162

(II)

<u>Text Figure Number</u>	<u>S u b j e c t</u>	<u>Page</u>
12	Starch content of the leaves of pumpkin (<u>C. moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates	164-165
13	Ribonucleic acid (RNA) content of the leaves of pumpkin (<u>C. moschata</u> Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates.	167-168

I. INTRODUCTION

Pumpkin (Cucurbita moschata Poir) is an important cucurbitaceous crop of West Bengal. This crop is grown in most of the districts of this State either as summer or as rainy season crop. In the districts like Hooghly and Bardwan, this crop is usually cultivated as companion to potato or as an additional crop in between potato and rice or as a second crop to rice. As such, a large portion of the total cultivable land in West Bengal is utilized for growing this crop. The common varieties, that are grown in this area are usually belonged to two broad categories namely summer pumpkins and rainy pumpkins. The yield potentials of these varieties are not very high and the average yield has been estimated to be 7.27 to 10.00 metric tons/acre (Hand Book of Agriculture, Indian Council of Agricultural Research, 1967).

Pumpkins are grown in West Bengal, primarily as a vegetable crop. Because of its low cost of production, this vegetable is sold in the market at a comparatively cheaper rate. As a vegetable, it is a good source of carbohydrate. Besides, it is also a good source of many vitamins and minerals. Ripe pumpkin fruits are also industrially important. It is used in preparing sweets and jams. It can also be candied or fermented to give a beverage.

Inspite of the wide use of pumpkin as vegetables and as raw materials for several industrial products, the management of this crop in the fields still remains to be very much neglected. Very limited work has so far been done on the diseases and pests of

this crop. As for diseases, Saha (1968) and Das (1971) found the large scale incidence of virus diseases in the field. It is, however, known that there occur at least 17 types of cucurbit viruses (Martyn, 1968) and most of them can infect pumpkins on inoculation. But no informations are, however, available on the viruses that usually infect pumpkins grown in the fields of West Bengal. Identification of the local pumpkin viruses was felt essential in view of the fact that such a study is a pre-requisite for any kind of varietal improvement programme, so that the improved varieties may be tested against the specific cucurbit viruses. Moreover, epidemiological studies with the cucurbit viruses known to occur in this State, may also be made for recommending feasible control practices. From this point of view, the present study on the isolation and identification of the common pumpkin viruses in West Bengal has been undertaken.

II. REVIEW OF LITERATURE

Cucurbita moschata Poir belongs to the family Cucurbitaceae.

Most of the species of this family harbour a very specialized group of plant viruses which usually do not infect plants belonging to other families. There, however, occurs a few cucurbit viruses which can also infect plants belonging to a wide number of families. Martyn (1968) reported that at least seventeen cucurbit viruses may occur in nature which include Arabis mosaic (Cucumber stunt mottle), Bottlegourd mosaic, Cucumber green mottle mosaic, Cucumber necrosis, Cucumber mosaic, Cucurbit latent, Cucurbit mosaic, Melon mosaic, Muskmelon necrotic spot, Muskmelon vein necrosis, Pumpkin mosaic, Pumpkin yellow vein mosaic, Squash mosaic, Tobacco mosaic, Tobacco ringspot (Cantaloupe mosaic), Cucumber ringspot and Watermelon mosaic virus. Of these seventeen viruses, only five viruses namely Cucumber mosaic virus, Squash mosaic virus, Cucumber green mottle mosaic virus, Watermelon mosaic virus and Pumpkin mosaic virus have been studied in details by several workers while informations available on other viruses are very much limited.

Cucumber Mosaic Virus (CMV)

Physical properties

The first virus that has been isolated from Cucurbits is CMV or Cucumber virus 1 (Doolittle, 1920). The thermal inactivation point (TIP), dilution end point (DEP) and ageing in vitro at room temperature for this virus have been reported to be 60°C, 5×10^{-3} and about 6 days respectively (Lindberg et al., 1956; Walker, 1969).

Transmissibility

This virus was found to be transmitted by sap (Elmer, 1922), Aphis gossypii Glover (Doollittle, 1924), Myzus persicae Sulz. , M. pseudosolanii Theob., M. circumflexus Buckt. and Macrosiphum euphorbiae (Hoggan, 1929). Coudriet (1962) observed the transmissibility of this virus by rusty plum aphid (Hysteroneura setariae). Two additional insect vectors namely Phorodon humuli (Schrank) and P. cannabis (Pass.) for this virus have also been reported by Schmidt and Karl (1968). According to Doollittle and Walker (1925) the virus could be transmitted through the seed of wild cucumber (Micrampolis lobata) but not through the seed of the cultivated cucumber. On the otherhand, Bowley (1923) considered that the virus was seed borne in the cultivated cucumber. Mahoney (1935) offered evidence that the virus was carried in the seeds of muskmelon (Cucumis melo). Vasudeva and Ravgi (1945) suspected the infection of watermelon seeds with this virus. Similarly, Mukhopadhyay and Saha (1968) also suspected that the virus could be transmitted through the seed of Cucurbita moschata.

Host range and local lesion hosts

Elmer (1922) and Doollittle and Walker (1923) observed that this virus had a wide host range and it also could infect plants belonging to families other than Cucurbitaceae. Doollittle (1921) observed that CMV produced different symptoms in different hosts. Considering this variation CMV was erroneously reported as Southern Celery mosaic or Celery virus 1 (Wellman, 1934), Lily mosaic virus (Price, 1937), Infectious Chlorosis of Banana

(Magee, 1940). A mosaic disease of Celery which was probably due to this virus has been reported in Italy by Gigante (1936) and he stated that the insect vector in this case has been found to be an aphid, Cavariella pastinacae.

Sill and Walker (1952) reported that Cowpea could be used as local lesion host for assaying CMV. Uschdrawait (1955) reported the use of Chenopodium quinoa as indicator plant for this virus. Later on, C. amaranticolor was also reported to be a local lesion host for this virus (Wilno et al., 1969). Hollings (1959) reported two more plants namely Gomphrena globosa and Spinacea oleracea which could act as indicator plants for this virus.

Particle length

Sill et al. (1952) found that the shape of the particles of CMV were spherical having the diameter of 35 m/ μ approximately but Scott (1963) and Corola et al. (1965) reported that the particles were isodiametric and measured 28 to 30 m/ μ in diameter. Murant (1965) reported that the particles were actually polyhedral rather than spherical and about 30 m/ μ in diameter.

Strains

Bewley (1923) reported two types of CMV in Great Britain, one resembling Aucuba type, characterized by localized yellow patches and the other marked by mottling and blistering of the leaves and stunting of the plants. Price (1934) tabulated the reactions of this virus on certain differential plants and described

six cucumber virus strains. A mosaic disease of vegetable marrow was described by Ogilvie and Mulligan (1931) in England which showed symptoms quite different from that described by Doolittle in America (1920). There seemed little doubt that the same virus or a strain of it affected vegetable marrow both in America and Europe but there is still uncertainty whether the virus causing mosaic of vegetable marrow is CMV or a strain of the type virus. Bhargava (1951) reported "Spinach strain" of CMV. The occurrence of this strain later was confirmed by Paccioli (1972). Simmons (1958) reported that Southern Cucumber mosaic virus and Pepper Calico virus were the strains of CMV. Reddy and Mariani (1963) also reported one strain of it from vegetable marrow. Grellana and Quacquarelli (1968) reported "Sunflower strain" of CMV and designated as CMV-SF. Goel and Varma (1973) reported a new strain of CMV and designated it as "Luffa strain" and a new insect vector, Brevicoryne brassicae of this virus. Similarly Seth and Raychoudhuri (1973) also reported another new strain of CMV. Recently Waterworth and Povish (1975) also reported one more strain of this virus from Ixora plants and designated it as CMV-1x.

Relationship with other viruses

Mink (1969) reported a direct serological relationship between CMV and PSV-W (Peanut stunt virus - Western).

Squash Mosaic Virus (SMV)

Physical properties

The occurrence of this virus was first suspected by Kendrick (1934). But the virus was actually identified by Freitag

(1941a, 1941b; 1956) and thus his work has been accepted as the first report on the incidence of this virus disease. The TIP, DEP and ageing in vitro at room temperature for this virus have been found to be 75°C, 10^{-6} and 6 weeks and 6 months at 0°F (Freitag, 1941a). These preliminary observations were later confirmed by Freitag (1956), Lindberg et al., (1956) and EL-Hadidi et al. (1966). According to their observations the TIP and DEP of this virus lied between 70 to 75°C and 10^{-4} to 10^{-5} respectively.

Transmissibility

It was found to be transmitted mechanically and also through seed (Kendrick, 1934; Middleton, 1944; Grogan et al., 1959; Milne et al., 1969). Transmission of SMV by the beetles was first demonstrated by Freitag (1941a; 1941b; 1952; 1956). He observed that Western striped cucumber beetle (Diabrotica trivittata Fabr. = Acalymma trivittata (Mann.)) and Western twelve spotted cucumber beetle (D. soror = D. undecimpunctata (Barber)) were the vectors for the virus. Sitterly (1960) and Lastra (1968) respectively recorded that D. balteata Lec. and A. thiemel thiemel could also act as vectors.

Host range and local lesion hosts

While describing the host range of SMV, Middleton (1944) recorded that the disease could be produced by inoculation in 15 species of plants distributed in 11 genera within 4 families. He did not however, mention the names of the families, genera and species concerned. Freitag (1956) demonstrated the ability of

this virus to infect many members of the family Cucurbitaceae such as Citrullus vulgaris, Cucurbita maxima, C. pepo and Cucumis sativus but not the members of other families tested such as Nicotiana tabacum, H. rustica, Datura stramonium, Vigna sinensis and Zinnia elegans. McLean and Meyer (1961) confirmed Freitag's observation but they did not find any infection in C. vulgaris. Cohen and Nitzany (1963) in Israel observed the systemic symptoms of this virus in Cucurbita moschata and Echallium olaterium.

Cohen and Nitzany (1963) observed sporadic local lesions on C. amaranthicolor when inoculated with SMV during winter season. Lastra et al. (1975) reported that Cucumis metuliferous could be good local lesion host of this virus.

Particle length

Takahashi and Rawlins (1947) found that the shape of the particles of SMV was polyhedral with average diameter of 30 m/μ. Subsequently, many workers made electromicroscopic studies with this virus but the average diameter recorded by them varied to some extent. Rice et al. (1955) found the average diameter of the freeze-dried particles to be 30 m/μ. Nelson and Milbrath (1965) recorded it as 28 m/μ. Lindberg et al. (1956) found it to be 36.7 m/μ. EL-Hadidi et al. (1966) observed that the average diameter of this virus varied between 35 to 37 m/μ. Lastra and Munz (1969) confirmed the observations of Nelson and Milbrath (1965).

Strains

Occurrence of different strains of SMV was first pointed

out by Nelson et al. (1965). They have found that an isolate from Arizona differed from the type collection in its host reaction. It produced mild mosaic on squash but marked stunting and leaf distortion on watermelon. According to them, this isolate infected only the members of Cucurbitaceae and was not transmitted by Acalymma trivittata. The physical properties of this isolate was also found to differ from the type virus. When serological tests were made, the Arizona isolate was found to be related with the type culture but not with any other virus. From these observation Nelson and his workers designated this isolate as Watermelon Stunt virus (WSV) and recorded it as a strain of SMV. Later, Knutson and Nelson (1968) designated this isolate as SMV-W. Knutson and Nelson (1968) and Demski (1969) also worked on many other isolates and recorded at least 6 strains including SMV-V. They designated the strains as SMV-C, SMV-P, SMV-M, SMV-W (10-28-4), SMV-W (4-25-26) and SMV-Y. According to their observations SMV-C and SMV-P caused severe systemic mottle and distortion in C. pepo and very mild mosaic in C. melo. None of them could infect C. vulgaris. SMV-M also could not infect C. vulgaris but it produced mild mosaic both in C. pepo and C. melo. SMV-W (10-28-4) and SMV-W (4-25-26) caused chlorotic rings in C. pepo at the beginning of infection which later transformed into mild mosaic. The mild mosaic caused by the later strain only was found to persist. Besides that, these two strains also differed in their reaction to watermelon. SMV-W (10-28-4) caused severe stunt, necrosis and chlorotic lesions but SMV-W (4-25-26) caused only mild mosaic. The strain SMV-Y was isolated by Demski. This strain was found to induce chlorotic

rings in C. pepo on the first 2-3 leaves with mild mottle on the last formed leaves. It was also found to cause local lesions on Black diamond watermelon. All these strains, however, could not be serologically differentiated. From this stand point, only two strains could be categorized, one which could infect watermelon and the other which could not.

Relationship with other viruses

No direct serological relationship of SMV with any other viruses has been reported but two serotypes of SMV were obtained by using serological technique by Knutson and Nelson (1968). Nelson and Knutson (1973) also observed the variability of SMV biotypes on serological comparison.

Watermelon Mosaic Virus (WMV)

Physical properties

Occurrence of WMV was first suspected by Walker (1933). He also described the symptoms of the disease produced by it. The TTP, DEP and ageing in vitro at room temperature for this virus were 55° to 60°C, 1: 10,000 to 1: 30,000 and 9 to 10 days respectively (Anderson, 1954a).

Transmissibility

It was found to be transmitted by sap (Anderson, 1954a; Toba, 1962), Aphis gossypii (Walker, 1933; Van Regenmortel et al., 1962) and Myzus persicae (Anderson, 1954a; Toba, 1962). Couderiet (1962) reported lettuce seed stem aphid (Macrosiphum barri) and rusty plum aphid (Hysteroneura setariae) as vectors of WMV.

Host range and local lesion hosts

Anderson (1954b) reported the infectivity of WMV to 12 cucurbitaceous crops. Lindberg et al. (1956) identified WMV from plants belonging to other families. Infectivity of vegetable marrow by this virus has been found by Bhargava and Joshi (1960) and Anderson (1961). Komuro (1962) reported the infectivity of one isolate of WMV to Okra, pea and broad bean. But Toba (1962) and Nagarajan and Ramakrishnan (1971a) reported that the host range of WMV was restricted to Cucurbitaceae. Toba (1962) further reported that Momordica balsamina might be the commonest wild reservoir of this virus in Hawaii. Foster and Dennis (1963) reported symptomless transmission of a local strain of WMV in some cantaloupe breeding lines. Inouye (1964) reported an isolate of WMV that could infect Cucurbitaceae, broad bean, sweet pea, beans, crimson clover, Astragalus sinicus, Crotalaria spectabilis and sesame. Variation in host range of WMV has been reported by Schmelzer (1965). Bhargava and Tewari (1970) reported Trichosanthes dioica as another host of WMV. Molnar and Schmelzer (1964) observed a wide host range for a strain of WMV. According to them 71 plant species in 17 plant families proved susceptible; of these, 43 species became systemically infected and 28 responded with local lesions only.

Schmelzer (1965) found Lavatera trimestris as a good indicator plant for WMV. C. album and C. strictum have also been found to react with this virus by producing local lesions (Denski, 1968).

Particle length

Van Regenmortel (1960) found that a typical WMV isolate of Southern California was a flexuous rod having a modal length of 700 to 800 x 13 m/μ. Van Regenmortel ^{et al.} (1962) further studied different isolates of WMV and reported that the virus particles were flexuous rods of 725 x 15 m/μ in size. Inouye (1964), however, reported the length of WMV particles as 700 to 800 m/μ. Schnelzer (1966) observed that the particles of both Cuban and European isolate of WMV were filiform with normal length of 750 m/μ. Raychoudhuri and Varma (1975) reported the virions of vegetable marrow isolate of WMV as flexuous filamentous rods measuring 720 to 760 x 19 m/μ. Purcifull et al. (1969) reported that a preparation from Lupin infected with WMV showed filaments of 765 m/μ in length.

Strains

Anderson (1954b) reported two strains of WMV, one designated as type strain (Marmor citrulli) and the other yellow strain (M.C. flavidanum or yellow WMV) which were differentiated on their stability in physical properties and difference in host reaction. These strains of WMV could be distinguished on the basis of their reactions on squash and citron plants. From virus samples isolated in Europe, Cuba and South Africa, Molnar and Schnelzer (1964) differentiated three strains designated "General Watermelon Mosaic Virus", the only one found in Europe; "Specific Watermelon Mosaic Virus", newly reported from Cuba and "South African Watermelon Mosaic Virus". Raychoudhuri and Varma (1975) reported two strains of WMV, one causing mosaic of pumpkin and the

other mosaic of vegetable marrow. Pumpkin isolate was found to be restricted to Cucurbitaceae whereas vegetable marrow isolate had wider host range. They further reported that none of the viruses was seed transmitted but both isolates were transmitted by Aphis craccivora, A. gossypii, A. nerii, M. persicae and Rhopalosiphum maidis.

Considering the reports of different workers from different places it was observed that WMV consisted of many unrelated viruses and thereby all the isolates of WMV have been grouped into two groups namely WMV -I and WMV-II.

Webb (1963) first reported that WMV was consisted of two or more unrelated viruses. Webb and Scott (1964) divided 10 isolates of WMV into 2 groups, namely WMV-I (Freitag type) and WMV- II on the basis of host reaction, cross protection and serology. According to Webb (1965) the reaction on Luffa acutangula could differentiate these two viruses.

WMV- I

Physical properties

The T₁₀, D₅₀ and ageing in vitro at room temperature for this virus were 55°C, 10⁻⁴ and 50 days respectively (Webb and Scott, 1965). Webb and Scott (1965) and Webb et al. (1965) further reported the variability in physical properties of WMV-I.

Transmissibility

It could be transmitted by sap, A. gossypii and Myadaphis pseudobrassicae (Adlerz, 1974). Webb and Scott (1965) found it to be transmissible by M. persicae.

Host range and local lesion hosts

Webb and Scott (1965) observed that this virus had a limited host range and it was restricted to Cucurbitaceae family. Later on, this was confirmed by Quiet et al. (1971).

Webb and Scott (1965) reported muskmelon P.I. 180280 Selection B.633-3 as a local lesion host for WMV-I. The unsuitability of this plant for WMV-I detection has however been pointed out by Wilne et al. (1969).

Particle size

According to Schmelzer (1966) the length of this virus was 750 m/ μ and he described the particles as filiform. According to Webb (1971) the particles were long and flexuous measured between 700 and 740 m/ μ .

Strains

Shanmugasundaram et al. (1969) reported a strain of this virus and designated it as "Kauai strain of WMV-I". Bhargava et al. (1975) reported a strain of WMV, presumably a strain of WMV-I, from India. This was found to be transmitted by A. gossypii and M. persicae in a non-persistent manner. In addition to its restricted host range to Cucurbitaceae they found that it could infect Vigna unguiculata var. Black turtle. The particle size of this virus has been found to be 730 to 750 m/ μ in length and 15 m/ μ in width.

WMV- IIPhysical properties

Webb and Scott (1965) reported that TTP, DDP and ageing in vitro at room temperature of this virus were 55°C, 10^{-4} and 50 days respectively. Wide variations of this virus in physical properties was also observed by Webb and Scott (1965) and Webb et al. (1965). Inouye (1964) reported one isolate of WMV, presumably WMV-II which was found to have TTP, DDP and ageing in vitro as 55° to 60°C, 10^{-3} to 5×10^{-3} and 8 to 16 days at 20°C respectively.

Transmissibility

This virus was found to be transmitted by sap and M. persicae (Augar et al., 1974).

Host range and local lesion hosts

Webb and Scott (1965) reported that in addition to the members of family Cucurbitaceae, this virus could also infect the members of Leguminosae. Later on, this was confirmed by many workers (Milne et al., 1969; Milne and Grogan, 1969 and Lastra, 1968).

Nelson and Tuttle (1969) reported that WMV-II caused local lesion on C. amaranticolor. Local lesion on muskmelon P.I. 180280 Selection B 633-3 by this virus was also reported by Webb (1965).

Particle length

Inouye (1964) reported the length of one isolate

of this virus to be 700 to 800 m μ . Webb (1971) found that the shape of the particles of WMV-II was rod having the length measured between 700 to 740 m μ . He also observed the variations in length of this virus. Augar et al. (1974) reported that all WMV-II isolates were 747 m μ long flexuous rods. Raychoudhuri and Varma (1975) reported that WMV from vegetable marrow, an isolate of WMV-II were flexuous filamentous measuring 720 to 760 $\times$ 19 m μ .

Strains

Close association of WMV-II and Cucurbit Latent virus, described by Webb and Bohn (1961), was observed by Milbrath and Nelson (1967). Weideman and Mostafawy (1972) reported a strain of WMV-II which was transmitted by A. roseumii, H. euphorbiae, A. nasturtii and H. persicariae. Fischer and Lockhart (1974) reported a strain of WMV-II and they found Malva parviflora as a natural source of inoculum.

Considering the wide variation and erroneous naming of this virus from different places, Schnelzer (1967) reported that for WMV-II name General Watermelon Mosaic Virus (GWMV) be used because of general distribution, for WMV-I, the name Specific Watermelon Mosaic Virus (SWMV) should be used and WMV investigated by Van Regenmortel (1960) should be named as South African Watermelon Mosaic Virus (SAWMV).

Relationship with other viruses

There has been some doubt as to the exact identity of this virus (WMV). Some workers have regarded it as a strain of Melon Mosaic Virus. Van Regenmortel et al. (1962) showed their

isolate to be distantly related serologically to Potato Virus Y and Bean Yellow Mosaic Virus (Martyn, 1968). Webb and Scott (1964) on the basis of serology reported that WMV-I and WMV-II were two distinct viruses and not related. But Milne (1968) and Milne and Grogan (1969) disclaimed it and reported that WMV-I and WMV-II were the strains of WMV and not unrelated. They further reported that an isolate of Papaya Ringspot Virus was found to be serologically related to WMV.

Cucumber Green Mottle Mosaic Virus (CGMMV)

Physical properties

Bowley (1926) observed a mosaic disease of cucurbit and described it as Cucumber Mild or Ordinary Mosaic virus disease. Later on, it was studied by Ainsworth (1935a) and named it as Cucumber Green Mottle Mosaic Virus and he designated the type strain as CV 3. Knight (1955) later on confirmed the identity of this virus and concluded that this could not be a strain of Tobacco Mosaic Virus. The T₁₀₀ and ageing in vitro of this virus were 80° to 90°C and one year or longer (Ainsworth, 1935b). Inouye et al. (1967) reported that it could withstand dilution upto 10^{-6} and ageing for 8 months at 20°C.

Transmissibility

It has been found to be transmitted by sap, probably through seed but not by A. gossypii and cucurbit leaf beetle (Inouye et al. ,1967).

Host range and local lesion hosts

Price (1940) observed that this virus had a limited host range and it was found to be more or less restricted to Cucurbitaceae. Ainsworth (1935b) reported that although this virus was restricted to the members of Cucurbitaceae family but it was not transmissible to G. pepo and Bryonia dioica. Inouye et al. (1967) reported that an isolate of this virus was not confined to only members of Cucurbitaceae but it could infect solanaceous plants also such as N. tabacum var. Samsun and N. tabacum var. White Burley, Potunia hybrida and D. stramonium. Moreover, it could infect G. pepo.

Ainsworth (1935b) and Kado and Knight (1970) reported the failure of this virus to produce local lesion either on D. stramonium or G. amaranticolor. But Inouye et al. (1967) reported that their CGMYV was able to produce local lesions on D. stramonium and P. hybrida. They further reported to observe local latent infection on N. tabacum var. Samsun and N. tabacum var. White Burley. They also confirmed the inability of CGMYV to produce local lesion on G. amaranticolor. Break et al. (1962) also reported the infectivity of CGMYV to Samsun and Xanthi-ec tobacco. Systemic infection of G. murale was also observed by them.

Particle length

Knight and Oster (1947) reported that the shape of the particles of this virus was rod having the dimension of $297 \pm 8.6 \text{ m}/\mu \times 13 \pm 0.6 \text{ m}/\mu$. But Inouye et al. (1967) reported that the particles of this virus were RV-like rods having dimension of $300 \text{ m}/\mu \times 18 \text{ m}/\mu$.

Strains

Cucumber aucuba mosaic virus which was described by Bowley (1923) has been found to be the first strain reported of this virus and Ainsworth (1935b) designated it as Cucumber Yellow Mosaic Virus. This strain seemed to bear the same relationship to CGMMV. Porter (1931) reported a virus in the name of CV 2 which had got some similarities with this virus. Ainsworth (1935a) reported CV 4 as another virus which later on was found to be a strain of CGMMV (Franklin, 1956; Johnson, 1951; Knight, 1955; Rochow, 1956; Holmes and Franklin, 1958) having high thermal death point (inactivated at 90°C for 10 minutes) and ageing in vitro (9 months or longer). Capeor and Varma (1948) reported a strain of this virus from Lagenaria vulgaris and named it as CV 2B. According to them the TIP, DEP and ageing in vitro for this virus were 98°C, 1:10,000 and 308 days respectively. They further reported its symptomless transmission through vegetable marrow and Momordica charantia. Another strain of this virus in the name of CV 2C was reported from L. leucantha by Vasudeva et al. (1949). According to them the TIP, DEP and ageing in vitro for this virus were 86 to 88°C, 1:1000 (standard extract) and 1:10,000 (pure sap) and 90 days respectively. This strain was differentiated by them on the basis of symptom differences and symptomless transmission through D. stramonium, L. acutangula, C. vulgaris and M. charantia. Vasudeva and Mariani (1952) reported the inability of CV 2C to infect the members of Solanaceae symptomlessly. Anand (1960) performed electromicroscopic studies with CV 2C and claimed the virus to be rod having an average modal length of

800 m/ μ and width of 25 m/ μ . Seed transmissibility of CV 2C through L. siceraria has been observed by Lal and Chouhan (1961). Mehta and Lal (1962) made electromicroscopic studies with CV 2C and reported that 72.9 per cent of the particles of it had a length of 100 to 500 m/ μ (minimum 20 m/ μ , maximum 1290 m/ μ though few were below 50 m/ μ or above 600 m/ μ). But Shankar et al. (1971) performed purification and electromicroscopic studies which CV 2C and reported the virus was rigid rod with a medial length of 280 m/ μ and width 17 m/ μ . Infectivity of CV 2C to D. stramonium and transmission by red pumpkin beetle (Aulacophora spp.) was also observed (Anonymous, 1954c). Anderson (1954b) regarded Bottlegourd mosaic virus as a strain of CGMMV. One more strain of CGMMV was also reported in the name of CV 2A (Valentin, 1958; Van Koot and Van Dorst, 1958; Van Koot and Van Dorst, 1959). But Break et al. (1962) reported CV 4 as CGMMV and CV 3 as a strain of it. They reported that CV 4 was able to produce local lesion on Samsun and Xanthi-ne tobacco but not on N. glutinosa. Production of local lesion on C. glaucum by CV 4 and CV 3 (Strain of it) was observed whereas CV 4 caused systemic infection on C. murale only. Komuro et al. (1971) and Nozu et al. (1971) reported one watermelon strain of CGMMV. Nozu et al. (1971) reported that the virus isolated from cucumber was able to produce local lesion on D. stramonium but not on C. amaranticolor whereas that obtained from watermelon was able to produce local lesion on C. amaranticolor. On the other hand, the type strain (CV 3) and cucumber aucuba mosaic strain (CV 4) was able to produce no local lesion on either D. stramonium or C. amaranticolor (Ainsworth,

1935b; Kado and Knight, 1970). From these reactions on these plants, these Japanese strains have been distinguished as the cucumber strain and the watermelon strain.

Relationship with other viruses

Knight (1955) reported a distant serological relationship of CGMMV with TMV. But Inouye et al. (1967) reported that one isolate of CGMMV has got weak serological relationship with ORSV (Odontoglossum ringspot virus) but not with TMV.

Pumpkin Mosaic Virus (PMV)

Physical properties

The TTP of this virus was found to be 50°C and this virus was found to be inactivated in vitro by one hour in 90 per cent ethanol at 35°C (Anonymous, 1954b). Shankar et al. (1972) studied this virus in details and reported the TTP, D₅₀ and ageing in vitro at room temperature of this virus were 56°C, 1: 500 and 8 hours respectively.

Transmissibility

The virus was found to be transmitted by sap and A. gossypii (Anonymous, 1954b). The transmissibility of this virus by H. persicae and Sitobion rosaeformis was also observed by Shankar et al. (1972). This virus was not found to be transmitted through seed (Shankar et al., 1972).

Host range and local lesion hosts

Shankar et al. (1972) found that this virus had a limited host range and it was found to be restricted to Cucurbitaceae

family only.

No local lesion host of this virus has yet been reported.

Particle length

Shankar et al. (1972) observed that the shape of the particles of PMV was flexuous rod having the dimension of 840 x 15 m/ μ .

Strains

No strain of this virus has yet been reported.

Relationship with other viruses

Shankar et al. (1972) reported that PMV resembled CV 3 (CGMMV) reported on vegetable marrow and bottlegourd from India and had close affinities with WMV reported from other countries and it did not react with antiserum of CV 1 and CV 2.

Other Viruses

Bottlegourd mosaic virus (BGMV)

Occurrence of a mosaic disease of bottlegourd was first described by Vasudeva and Lal (1943). The T₁₀, D₅₀ and ageing in vitro at room temperature for this virus were 60°C, 1:500 and 6 hours respectively (Vasudeva and Lal, 1943). This virus has found to be transmitted by sap only (Vasudeva and Lal, 1943). The insect transmissibility of this virus has not yet been reported. Vasudeva and Lal (1943) observed that this virus had a limited host range and it was found to be restricted to Cucurbitaceae family. Not much work has been done on this virus. However, Janardhan et al. (1969) reported a strain of BGMV. Cohen and

Nitzany (1960) reported one BGMV from Israel which was also restricted to Cucurbitaceae family and was transmissible by sap and Bemisia tabaci only. To avoid confusion the name Cucumber Vein Yellowing Virus (CVYV) was reported instead of BGMV (Harpas and Cohen, 1965). Raychoudhuri and Varma (1975) reported serological relationship of BGMV to Muskmelon Mosaic Virus.

Yellow vein mosaic of pumpkin (YVMP)

Occurrence of yellow vein mosaic of pumpkin was reported in 1954 (Anonymous, 1954a). It was found to be transmissible by B. tabaci only and not by sap (Anonymous, 1954a). Varma (1955) confirmed the identity of this virus and described the symptoms produced by it and reported that it did not go to Malvaceae.

Cucurbit latent virus (CLV)

Latent infection of viruses on cucurbitaceous crops was observed by Webb and Bohn (1961). They isolated and named it as Cucurbit Latent Virus (CLV). This was later on confirmed by Webb (1964) and Nelson et al. (1966). The TIP, DEP and ageing in vitro at 22°C for this virus were 50°C, 1:100 and 3 days (Webb and Bohn, 1961). This virus was found to be transmitted by sap and M. persicae (Webb and Bohn, 1961). Webb and Bohn (1961) reported that the virus had wide host range. In addition to the members of Cucurbitaceae, it could infect garden pea var. Laxton Progress, pinto bean (Phaseolus vulgaris), spinach var. Dixie Market but the virus failed to infect tobacco plants. This virus was found to produce local lesion on C. amaranticolor (Webb and

Bohn, 1961; Nelson et al., 1966). But Milne et al. (1969) failed to obtain any local lesion on C. amaranticolor with this virus. Close association of this virus with WMV II has been reported by Milbrath and Nelson (1967). But Milne et al. (1969) on the other hand has created a doubt about the existence of this virus. No further work has been done for its clear identification.

Bittergourd mosaic virus

Nagarajan and Ramakrishnan (1971b) reported a new virus, Bittergourd Mosaic Virus which was found to be transmitted by M. persicae, A. gossypii, A. nerii and B. brassicae and not by sap or seed. They further reported that host range of this virus has been found to be restricted to Cucurbitaceae family. No further work has been done on it.

Kakri mosaic virus (KMV) and Tori mosaic virus (ToMV)

Raychoudhuri and Varma (1975) reported two more additional viruses, KMV and ToMV infecting Cucurbitaceous crops. They have reported that KMV was found to be restricted to Cucurbitaceae whereas ToMV was restricted to Cucurbitaceae and Chenopodiaceae. None of these viruses was found to be transmitted by insect vector. No further work has been done on these two viruses.

Incidence of Different Cucurbit Viruses in the Fields

Although a large number of Cucurbit viruses have been reported and described from time to time but very limited informations are available on their relative incidence in crops grown in particular localities. However some workers (Anderson, 1951a, 1951b; Grogan et al., 1959; McLean and Meyer, 1961; Webb, 1961; Cohen and Nitzany, 1963; Nelson et al., 1966; Salama and Sill, 1968; Lastra, 1968; Shanmugasundaram et al., 1969; Provvidenti and Schroeder, 1970 and Quiot et al., 1971) attempted to study the prevalence of different types of cucurbit viruses under field condition. In a survey of cucurbit viruses in Central Florida, Anderson (1952) observed that cucumber mosaic and watermelon mosaic virus were more abundant in the fields. Anderson (1951b) further reported the appearance of Southern squash mosaic virus on cucurbits and also observed that CMV did not predominate over Southern squash mosaic virus or the reverse in either hosts taken. Grogan et al. (1959) reported that the most prevalent viruses infecting cucurbits in the desert valleys of Southern California during 1956-1958 were strains of WMV, second in prevalence was strains of CMV. According to them occurrence of SMV was rare in that location. McLean and Meyer (1961) surveyed Lower Rio Grande Valley of Texas during 1959-1960 for recording the relative incidence of different virus diseases. For this purpose they collected 140 diseased samples and identified the viruses involved. According to them out of 140 samples, 124 samples contained tobacco ringspot virus, 18 contained WMV, 10 contained SMV and only 2 contained CMV. Symptoms of combined

effect of TRSV and CMV were observed in some watermelon samples. Webb (1961) surveyed the incidence of virus diseases in Northwest melon producing areas. According to him TRSV was the most prevalent in cucurbits from S. Texas and often found to be mixed with CMV which was most prevalent in California and Arizona, while CMV and SMV were rare in these areas. Ringspot was not detected in squash from one centre in California or in cantaloupes from Mesa, Arizona. The major Texas viruses seemed to differ from those of the other 2 States. Cohen and Hitzany (1963) while surveying reported 5 viruses on cucurbits in Israel. They found the incidence of BGMV, CMV, WMV, SMV and a new virus which they named as squirting cucumber mosaic virus. Joshi (1962) in a study in U.P. (India) observed a strain of WMV infecting vegetable marrow, Cyclanthera pedata, squash and cucumber. Toba (1962) in a survey at Hawaii reported the prevalence of WMV. Schmelzer (1965) reported the incidence of WMV and CMV singly or in combination of both in the fields of Germany. Schmelzer (1966) reported the occurrence of Specific WMV in Cuba. Nelson et al. (1966) while surveying the fields in the vicinity of Culiacan and Los Mochis, State of Sinaloa, Mexico, reported the presence of WMV I and WMV II, CMV, GYMV, SMV and TRSV. They however recorded the consistent occurrence of only WMV I and CMV. Salama and Sill (1968) while surveying the fields in Kansas reported the incidence of SMV and CMV. According to them SMV accounted to 45 per cent of the isolates from squash, cucumber, muskmelon, pumpkin and gourd, whereas CMV accounted to 40 per cent isolates from cucumber, squash and muskmelon. They also observed mixed infection of SMV

and CMV in squash and muskmelon. 12 per cent of the isolates from watermelon and muskmelon were identified by them as TRSV and they identified one isolate from squash as TMV. Lastra (1968) while surveying the fields in Venezuela reported the occurrence of WMV I, WMV II (the most prevalent) , CMV and SMV (the least) in Venezuela. Milne et al. (1969) surveyed the fields in California where cucurbit crops were grown during 1966-1968 for recording the relative incidence of virus diseases and reported that SMV was common in one area of the State whereas WMV was found only in N. California. According to them 67 samples from Sinaloa, Mexico contained 62 per cent WMV II and 21 per cent WMV I. The occurrence of both CMV and SMV was also observed by them in the samples from Mexico but they failed to observe the presence of CMV in any of the samples. Shanmugasundaram et al. (1969) reported the prevalence of WMV I, WMV II, Kauai strain of WMV I and CMV in Hawaii. Mixed infection of WMV I and WMV II in the fields of Central New York was reported by Provvidenti and Schroeder (1970). Guillet et al. (1971) while surveying the fields in French West Indies reported the incidence of WMV I. Raychaudhuri and Varma (1975) while surveying cucurbitaceous crops in Delhi (India) reported 6 viruses. They were identified as two strains of bottlegourd mosaic virus, one causing mosaic disease of bottlegourd (BMV) and mottling of watermelon (WMV) and the other mild mosaic disease of muskmelon (MMV); two strains of watermelon mosaic virus, one causing mosaic of pumpkin (PMV) and the other mosaic disease of vegetable marrow (VMV); kakri mosaic virus (KMV) and Tori mosaic virus (TMV).

Alterations in Chlorophyll, Buffersoluble Protein, Total (TCA precipitable) Sap Protein, Reducing Sugar, Starch and Ribonucleic acid Contents Due to Virus Infection

Virus infection usually causes reduction in chlorophyll content (Diener, 1963; Goodman et al., 1967; Matthews, 1970). Elmer (1925) and Peterson (1931) reported the reduction of chlorophyll content in mosaic infected leaves. Reduction of the sugar content from 7.2 to 6.7 per cent was observed by Verplancke (1929) in case of beet root infected by virus diseases. Dunlap (1930) reported that mosaic diseases were accompanied by an increase of total nitrogen and a decrease in total carbohydrate content. According to him the carbohydrate to nitrogen ratio was decreased in mosaic affected leaves. Ludtke (1930) reported the accumulation of starch in mosaic infected tobacco plants. Malhotra (1931) reported while working on mosaic disease of potato that percentage of non-reducing sugar and starch was higher in healthy leaves, whereas nitrogen was relatively more abundant in diseased individuals. Cordingley et al. (1934) observed reduction of carbohydrate content which was found to be more in mosaic affected tobacco. They also observed reduction of chlorophyll and marked increase of total nitrogen content when tobacco plants infected with a mosaic virus. Watson and Watson (1951) reported that older leaves of infected plants had a higher content of reducing sugar and sucrose and usually of starch in dark. They also observed that the carbohydrate content of sugar beet leaves was little damaged by infection with beet mosaic virus. Basler and Commoner (1956) found that in TMV infected tobacco leaves, the buffer

soluble nucleic acid content increased to a large extent immediately after infection and it gradually decreased after the development of symptom. Yoshu (1958) observed that the nucleic acid content in rice leaves was greatly increased due to infection with rice dwarf virus. Honeycutt and Hillikan (1964) observed reduction in RNA content in cherry leaves due to virus infection.

Mariharasubramanian (1964) on the other hand, observed the increase of RNA content in Dolichos lablab infected with Dolichos enation mosaic virus. Subsequently, many workers have studied the nucleic acid content in virus infected leaves and obtained controversial results (Matthews, 1970). Weilkie et al. (1967) reported that due to CMV infection on resistant and susceptible strains of cowpea, most of the amino acids especially asparagin was increased.

Jeyarajan (Jayarajan) and Ramakrishnan (1968) reported the decrease of reducing sugar, sucrose and starch content in the leaves of Chilli plants infected by potato virus Y. Mandahar and Singh(1971) observed the destruction of chlorophyll a and b in Abelmoschus esculentus due to infection of yellow vein mosaic virus, in Luffa aegyptica due to infection of cucurbit mosaic virus and in Cucurbita moschata due to infection of pumpkin mosaic virus.

Destruction of chlorophyll a and b in Abelmoschus esculentus due to infection of yellow vein mosaic virus was later on confirmed by Ramiah et al. (1972). Mandahar and Garg (1972) further reported that in Luffa aegyptica due to CMV infection, total chlorophyll content was decreased by 46 per cent, starch was increased by 12 per cent, reducing sugar was increased by 44 per cent and total sugar was increased by 16 per cent in the infected tissue.

Choudhury and Mukhopadhyay (1974) reported that reducing sugar content decreased and starch content increased due to Rice Tungro Virus (RTV) infection in Padma, Jaya and IR-8 varieties of rice. Anonymous(1966), Raychoudhuri et al. (1969), Anonymous(1970) and Choudhury (1973) reported the reduction in chlorophyll and increase of RNA in the RTV infected rice leaves. Choudhury (1973) further reported the increase of buffer soluble protein content of RTV infected rice leaves. Awasthi and Singh (1974) reported the differential increase of RNA content in Chilli plants due to infection of CMV. Ziemiński and Wood (1975) reported that soluble protein content was quantitatively changed when cucumber cotyledons were inoculated with different strains of CMV. Quaseem et al. (1975) found a positive correlation between bean mosaic infection in beans and soluble and insoluble proteins and RNA content of the leaves. They, however, observed an inverse relationship between mosaic infection and the soluble and insoluble carbohydrate contents. But no informations are available on chlorophyll, buffer soluble protein, total (TCA precipitable) sap protein, reducing sugar, starch and RNA content of mosaic infected pumpkin (Cucurbita moschata Poir) plants at different stages of pathogenesis.

III. MATERIALS AND METHODS

A. INCIDENCE OF VIRUS DISEASES

The incidence of virus diseases of pumpkin (Cucurbita moschata Poir) has been studied by surveying the fields where this crop is grown, particularly in the districts of Burdwan, Hooghly, Malda, Nadia and 24 Parganas during the months of February-March 1973. In each district one location where this cucurbit is grown in large scale was considered and in each location, three pumpkin fields of uniform size were selected at random. In each field, 100 plants were taken for observation. The percentage of diseased plants was determined by counting the number of them in the fields. The symptoms of these diseased plants were further critically studied to tabulate the types of symptoms that occurred in the fields. To observe the relative incidence of different types of symptoms in different locations, samples were collected from the fields previously selected in the districts of Burdwan, Hooghly and Nadia only and the percentages of samples showed different symptoms were recorded.

B. SAP TRANSMISSIBILITY OF TYPE II SYMPTOM COMPLEX

A large number of young leaves showing type II symptoms (complex) in the fields were brought to the laboratory from time to time and transmission tests were performed following the usual sap transmission technique.

To perform the sap transmission technique, young leaf samples were taken and thoroughly washed with tap water followed by glass distilled water. After washing, the samples were kept in freeze for one hour. Frozen samples were then cut into small pieces and grinded in a glass mortar and pestle. While grinding 0.1 M citrate-phosphate buffer (pH 8.0) containing 10 micromole (mM) diethyl-dithiocarbamate (DIECA) at the ratio of 1 gram/milli litre weight/volume (gm/ml) was added as extraction medium. The sap thus obtained was clarified by straining through cheese cloth (four fold). The clarified sap was used as inoculum.

Series of healthy pumpkin (Cucurbita moschata Poir) seedlings were raised in sterilized soil under insect-proof condition and inoculated with the standard inoculum at the first leaf stage. Before inoculation, carborundum powder (400 mesh) was dusted on the leaf, to be inoculated. Immediately after inoculation, the leaf was washed with glass distilled water to remove excess inoculum from its surface. The inoculated plants were kept under insect proof condition for 20 days to observe the development of the symptom.

C. SEPARATION AND IDENTIFICATION OF VIRUSES OF TYPE II SYMPTOM COMPLEX

Separation of viruses as pure isolates

To separate the viruses involved in the type II symptom complex as pure isolates, the plants showing different types of symptoms during the transmission studies were separately taken. Standard inocula were prepared from each type of symptom and inoculated to the healthy test seedlings (C. moschata Poir)

following the usual procedure. The inoculated plants were kept under insect proof conditions for 20 days for the development of the symptom true to the types observed during the transmission studies of the type II symptom complex. The pure isolates thus obtained were maintained in C. moschata Poir plants. These plants were considered as the sources of the isolates for all the future experiments.

Identification of the isolates

a) Determination of the physical properties of the pure isolates

To determine the physical properties of the isolates (1) thermal inactivation point (2) dilution end point and (3) ageing in vitro of the standard inocula obtained from the leaves of C. moschata Poir plants inoculated with the nine different isolates under identical conditions, were performed.

1) Determination of thermal inactivation point

Standard inocula obtained from young plants (C. moschata Poir) inoculated with each isolate, were divided into samples of 5 ml each in pyrex test tubes of uniform size and capacity. These samples were exposed to varied range of temperatures, viz. 40°C, 45°C, 50°C, 55°C, 60°C, 65°C and 70°C for 10 minutes in a temperature controlled water bath (Ultra Thermostat Type HBB, VSB MLW PRÜFGERÄTE-WERK MEDINGEN SITZ FRISTAL) in such a way that the portion of the tube containing extract (standard inocula) was completely immersed in water and the tubes were not touching the walls of the water bath. Immediately after exposure for 10 minutes,

tubes were taken out and cooled by placing them in ice-cold water. Young and healthy leaves (first leaf) of C. moschata Poir previously dusted with carborundum powder (400 mesh) were inoculated with each sample, exposed to different temperatures. Suitable controls were also kept by inoculating the test plants with standard inoculum without any exposure to temperature. All the inoculated plants were kept under insect proof condition and observed for 20 days for symptom expression.

2) Determination of dilution end point

Standard inocula from young plants (C. moschata Poir) previously inoculated with different isolates were separately prepared. Serial dilutions viz. 10^{-1} , $10^{-1.5}$, 10^{-2} , $10^{-2.5}$, 10^{-3} , $10^{-3.5}$ and 10^{-4} with the standard inocula of each isolate were made with sterilized glass distilled water. Young leaves (first leaf) of healthy C. moschata Poir plant previously dusted with carborundum powder (400 mesh) were inoculated with the inocula of different dilutions. All the inoculated plants were kept under insect proof condition and observed for 20 days for symptom expression.

3) Determination of ageing in vitro

Standard inocula were obtained from young plants (C. moschata Poir) inoculated with each isolate. 5 ml of each inoculum was taken in test tubes and stored under ordinary laboratory condition (temperature ranged from 24° to 32°C). Samples were taken out from each inoculum at different lengths of time namely after 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days and 10 days

respectively. Since the ageing in vitro of one isolate (Isolate A₁) was found to be less than 12 hours, the freshly prepared inoculum of that isolate was again kept for 2 hours, 4 hours, 6 hours, 8 hours, 10 hours and 12 hours at room temperature for determining its ageing in vitro. Young leaves (first leaf) of healthy C. moschata Poir previously dusted with carborundum powder (400 mesh) were inoculated with the inocula, taken out at different time intervals. All the inoculated plants were kept under insect proof condition and observed for 20 days for symptom expression.

b) Host range of the isolates

To study the host range of isolates, species of plants belonging to different families were selected. The name of the plants used in this study are presented in the Table 1.

The selected plants were grown in sterilised soil under insect proof condition. The plants were tested for the presence of any virus in them and only virus free plants were considered for the present experiment. All these virus free plants (10-15 days old) were inoculated separately with the nine isolates under all possible identical conditions.

The inoculated plants were kept under insect proof condition for 30 days and they were observed once in a week for symptom development.

After the period of observation, leaves were collected from all the inoculated plants and standard inocula were obtained from them following the usual procedure. These inocula were then back inoculated to healthy C. moschata Poir plants (10 days old).

Table 1. Name of the plants used in the study of host range of the isolates obtained from Type II symptom complex of pumpkin (C. moschata Poir)

Sl. No.	Name of the plant		Name of the family	Sources from which the seeds were obtained.
	Common name	Scientific name		
1	Pumpkin	<u>Cucurbita moschata</u> Poir	Cucurbitaceae	Sutton & Sons (India) Pvt. Ltd.
2	Bottlegourd	<u>Lagenaria vulgaris</u> Ser.	"	"
3	Bittergourd	<u>Momordica charantia</u> L.	"	"
4	Ashgourd	<u>Benincasa cerifera</u> Savi.	"	"
5	Snakegourd	<u>Trichosanthes anguina</u> L.	"	"
6	Dhurdul	<u>Luffa cylindrica</u> Roem.	"	"
7	Ribgourd	<u>Luffa acutangula</u> Roxb.	"	Dr. R. B. Webb, U.S. Deptt. of Agriculture.
8	Tinda	<u>Citrullus vulgaris</u> Schrad var. <u>fistulosus</u>	"	Sutton & Sons (India) Pvt. Ltd.
9	Watermelon	<u>Citrullus lanatus</u> cv. Charleston Gray	"	Dr. R. B. Webb, U.S. Deptt. of Agriculture.
10	Squash	<u>Cucurbita pepo</u> L. cv. Early Prolific Straightneck	"	"
11	Cantaloupe	<u>Cucumis melo</u> L. Selection B 63-3	"	"
12	Cucumber	<u>Cucumis sativus</u> L. cv. Local White	"	Dr. S. P. Kapoor, Plant Virus Res Station, Agri. College Estate, Poona, India.
13	Cucumber	<u>Cucumis sativus</u> L. cv. Long Green	"	"

Sl. No.	Name of the plant		Name of the family	Sources from which the seeds were obtained
	Common name	Scientific name		
14	Chilli	<u>Capsicum annum</u> L. var. <u>Suryamukhi</u>	Solanaceae	Sutton & Sons (India) Pvt. Ltd.
15	Thornapple	<u>Datura stramonium</u> L.	"	Dr. S. P. Kapoor, Plant Virus Res. Station, Agri. College Estate, Poona, India.
16	Tomato	<u>Lycopersicon</u> <u>esculentum</u> Mill. var. <u>Marglobe</u> Supreme	"	Sutton & Sons (India) Pvt. Ltd.
17	Potato	<u>Solanum tuberosum</u> L. var. <u>Kumri Chandramukhi</u>	"	All India Potato Improvement Project D.C.E.V.V., Kalyani Centre
18	Brinjal	<u>Solanum melongena</u> L. var. <u>Muktakoshi</u>	"	Sutton & Sons (India) Pvt. Ltd.
19	Tobacco	<u>Nicotiana tabacum</u> L.	"	Central Tobacco Res. Institute, Rajamundri, A.P.
20	Tobacco	<u>N. tabacum</u> L. cv. <u>Virginia Gold</u>	"	"
21	Tobacco	<u>N. tabacum</u> L. cv. <u>Kanthi</u>	"	Dr. S. P. Kapoor, Plant Virus Res. Station, Agri. College Estate, Poona, India.
22	Tobacco	<u>N. glutinosa</u> L.	"	"
23	Tobacco	<u>N. sylvestris</u> L.	"	"
24	Tobacco	<u>N. rustica</u> L.	"	Central Tobacco Res. Institute, Rajamundri, A.P.
25	Petunia	<u>Petunia hybrida</u> Vilm.	"	Sutton & Sons (India) Pvt. Ltd.
26	Broad bean	<u>Vicia faba</u> L.	Leguminosae	"
27	French bean	<u>Phaseolus vulgaris</u> L.	"	"

Sl. No.	Name of the plant		Name of the family	Sources from which the seeds were obtained
	Common name	Scientific name		
28	Pea	<u>Pisum sativum</u> L.	Leguminosae	Sutton & Sons (India) Pvt. Ltd.
29	Mustard	<u>Brassica campestris</u> L. var. Sarson	Cruciferae	"
30	Spinach	<u>Spinacea oleracea</u> L.	"	"
31	Papaya	<u>Carica papaya</u> L. var. Washington Special	Caricaceae	"
32	-	<u>Chenopodium amaranticolor</u> Coste et Reyn	Chenopodiaceae	I.A.R.I., New Delhi., India
33	-	<u>C. quinoa</u> Willd.	"	Dr. S.P. Kapoor, Plant Virus Res. Station, Agri. College Estate, Poona, India.
34	Sugarbeet	<u>Beta vulgaris</u> L.	"	Sutton & Sons (India) Pvt. Ltd.
35	Periwinkle	<u>Vinca rosea</u> L.	Apocyanaceae	"
36	Gomphrena	<u>Gomphrena globosa</u> L.	Amaranthaceae	I.A.R.I., New Delhi, India.
37	Zinnia	<u>Zinnia elegans</u> Jasq.	Compositae	Sutton & Sons (India) Pvt. Ltd.

These back inoculated plants were then kept under insect proof condition for 30 days to observe the symptom development in them.

c) Insect transmissibility of the isolates

To study the transmissibility of the isolates obtained from type II symptoms complex of C. roschata by insect vectors, melon aphid (Aphis gossypii Glover), green peach aphid (Myzus persicae Sulz.) and white flies (Bemisia tabaci Conn.) were selected.

1. Collection and rearing

i) Aphis gossypii and Myzus persicae

Apterae aphids were collected from cauliflower plants (Brassica oleracea var. capitata) in the month of January, 1975. To collect the aphids, a sheet of paper was held beneath the infested leaves of the plant and the leaves were vibrated in such a way that the aphids got detached from the leaf surface and fell on the paper sheet. Pieces of leaves heavily infested with the aphids were also detached from the plant. The aphids thus obtained were kept in a receptacle having adequate facility for aeration. Healthy cauliflower plants of 15 days old were raised before hand under insect proof condition in pots containing sterilized soil. The aphids collected in the receptacle were then released on the leaves of those plants. For this purpose, aphids were carefully picked up from the receptacle with a small Camel's hair brush slightly moistened with water and carefully placed on a piece of paper. Then the pieces with aphids were also placed on the plants, so that they could easily crawl to the leaves

of the plants. The plants were then kept within a insect proof chamber and the aphids were allowed to multiply for several generations on these plants. Fresh aphids were collected from these plants in the same way as before and placed on another set of cauliflower plants of 15 days old, kept in rearing chamber and allowed to multiply there. Aphids from this second chamber were considered for transmission studies.

In case of Myzus persicae the apterae aphids were collected from Chilli plants (Capsicum annum) in the month of October, 1975 in the same way as in case of Aphis gossypii. Rearing of M. persicae was also done in Chilli plants in the same way as above.

ii) Bemisia tabaci

White flies were collected from Petunia hybrida plants in the month of June, 1975. The adult white flies were collected with the help of a suitable aspirator in the early morning. The white flies thus obtained were kept in a receptacle having adequate facility for aeration. Healthy Petunia plants of 15 days old were raised before hand under insect proof condition in pots containing sterilized soil. The white flies collected in the receptacle were then released on healthy Petunia plants of 15 days with the help of aspirator. The plants were then kept within an insect proof chamber and the white flies were allowed to multiply for several generations on these plants. Fresh white flies were collected from these plants in the same way as before and transferred to another set of Petunia plants of 15 days, kept in a rearing chamber and allowed to multiply there. In the same way as before one more transfer of fresh white flies was done

on another set of Petunia plants of 15 days, kept in a rearing chamber. Fresh white flies from the third rearing chamber were considered for transmission studies.

2. Acquisition of the isolates

1) Aphis gossypii and Myzus persicae

Fresh aphids were collected from the rearing chamber in a tube with the help of a small Camel's hair brush slightly moistened with water. The aphids were kept on fasting for 1 hour without providing any food for them in the tube. Healthy C. moschata plants of 10 days old, raised under insect proof condition, were separately inoculated with the nine isolates and kept in insect proof chambers. 20 days after inoculation these plants were taken as sources for the isolates to the aphids for their acquisition feeding. After 1 hour of fasting the aphids were picked up and released on the source plants for acquisition feeding following methods described earlier. The aphids were kept on the inoculated plants for 24 hours to provide sufficient time period for the acquisition of respective viruses.

ii) Bemisia tabaci

Fresh white flies were collected from the rearing chamber in a receptacle with the help of aspirator. The white flies were kept on fasting for 1 hour without providing any food in the receptacle for them. Healthy C. moschata plants of 10 days old, raised under insect proof condition, were separately inoculated with the nine isolates and kept in insect proof chambers. 20 days after inoculation these plants were taken as sources for the isolates to the white flies for their acquisition

feeding. After 1 hour of fasting the white flies were picked up and released on the source plants for acquisition feeding following methods described by Nene (1972). The white flies were kept on the inoculated plants for 24 hours to provide sufficient period for acquiring the respective viruses.

3. Inoculation of the isolates to the test plants by the viruliferous insects

1) Aphis gossypii and Myzus persicae

Healthy C. moschata seedlings (10 days old) were raised in sterilized soil under insect proof condition. Aphids after acquisition feeding on the inoculated plants were transferred to the test plants at the rate of 15 aphids/plant following the usual technique and the plants were kept covered with glass chimneys. After 24 hours of inoculation feeding, the glass chimneys were removed and Sumithion (0.03 per cent) was sprayed on the plants to kill the aphids. The plants thus inoculated were kept under observation in insect proof condition for 30 days.

11) Bemisia tabaci

Healthy C. moschata seedlings (10 days old) were raised in sterilized soil under insect proof condition. White flies after acquisition feeding on the inoculated plants were transferred to the test plants at the rate of 15 white flies/plant following the usual technique. The inoculation feeding was done within transmission cages according to the method described by Nene (1972). After 24 hours of inoculation feeding

the transmission cages were removed and Sumithion (0.03 per cent) was sprayed on the plants to kill the white flies. The plants thus inoculated were kept under observation in insect proof condition for 30 days.

4. Persistence of the isolates in *Aphis gossypii*,
Myzus persicae and *Bemisia tabaci*

To study the persistence of isolates, serial transmissions of them to the healthy *C. moschata* plants by the viruliferous insects were performed.

1) *Aphis gossypii* and *Myzus persicae*

Healthy *C. moschata* plants of 10 days old were raised in sterilized soil under insect proof condition. Aphids after acquisition feeding on the inoculated plants were transferred to the test plants at the rate of 15 aphids/plant following the usual technique and the plants were kept covered with the help of glass chimneys. Requisite number of healthy *C. moschata* plants of 10 days old were raised before hand. After 24 hours of inoculation feeding successive transfer of aphids to 10 days old healthy *C. moschata* plants were done at an interval of 24 hours for 5 days (120 hours) following usual techniques. In case of each set of experiment the plants were kept covered with glass chimneys. After the completion of experiment the plants were sprayed with Sumithion (0.03 per cent) to kill the aphids. The plants after such treatment were kept under observation in insect proof condition for 30 days for the development of the symptom.

ii) Denisia tabaci

Healthy C. moschata plants of 10 days old were raised in sterilized soil under insect proof condition. White flies after acquisition feeding on the inoculated plants were transferred to the test plants at the rate of 15 white flies/plant with the help of aspirator and inoculation feeding was done in transmission cage following the procedure described before. Requisite number of healthy C. moschata plants of 10 days old were raised before hand. After 24 hours of inoculation feeding successive transfer of white flies to 10 days old healthy C. moschata plants were done at an interval of 24 hours for 5 days (120 hours) following usual technique. In case of each set of experiments, after completion, the plants were sprayed with Sumithion (0.03 per cent) to kill the white flies. The plants after such treatments were kept under observation in insect proof condition for 30 days for the development of the symptom.

d) Electronmicroscopy of the isolates*

Healthy squash plants (Cucurbita pepo cv. Early Prolific Straightneck) were raised in pots containing sterilized soil under insect proof condition. These plants at the age of 10 days were separately inoculated with the isolates following the usual sap inoculation technique. The inoculated plants were kept under insect proof condition upto 20 days till

* The electronmicroscopy of the isolates was done at the Biophysics Laboratory, Saha Institute of Nuclear Physics, 37, Belgachia Road, Calcutta-37.

the characteristic symptoms developed. Young leaves showing distinct symptoms were collected from these plants and the sap from these leaves were electronmicroscopically observed following the dip method (Brandes, 1957; 1964) using negative staining with 2 per cent potassium phosphotungstate (PTA) having pH 6.5 (Hitchborn and Mills, 1965).

To perform the method, Copper grids (400 mesh of 3 millimeter in diameter) were taken as mounting media. These grids were then coated with thin films of carbon. Each carbon coated grid was then picked up with a fine pointed forcep, placed horizontally and kept in that position with the help of a clamp. A drop of 2 per cent PTA at pH 6.5 was put on each grid.

Leaf samples after being collected from the inoculated plants, were kept in refrigerator for one hour. Then small pieces were cut out from each sample with the help of a sharp razor blade and the cut edge was very quickly dipped for 5 seconds in the stain on the grid, so that the sap with the virus might be dropped into the stain. The stain was then dried and each grid was put on a piece of filter paper in a Petridish. While opening the forcep from the grid, the grid was slightly pressed against the filter paper with the opposite side of a bent needle so that the grid might not stick to the forcep. Each grid was then observed under a microscope with the low power objective to check the continuity of the film on it. In case of any break in the film, the grid was to be discarded. In this way, 18 grids were prepared with two replicates for each sample obtained from squash plants inoculated with nine different isolates.

Each grid was then examined under an electromicroscope (Siemens Elmiskop-I at 60 Kilo Volt and at an electronic magnification of 8000X).

After examining the locations in each grid where virus particles were present in the electron microscope, suitable photographs were taken. In this way, a number photographs of the virus particles of each isolate were taken. The measurement of the size of the particles was made by measuring the length and breadth of the photographic image of each particle and then dividing the data by the electronic and photographic magnification of it. The photographic magnification was determined with the help of a crossed rule grating having known length. Photograph of the grating was taken under the same electronic magnification and the magnification of the grating obtained was considered as the photographic magnification.

e) Serological relationship of the isolates

To determine the serological relationship of the isolates, studies were undertaken for antigenic comparison between them by performing " Agar double diffusion " technique (Grewle, 1961). Antigens were obtained from the inoculated plants. Antisera were obtained from rabbits after injecting them with the respective antigens. Antigens-Antisera reactions were studied in agar plates, and the relationship between the isolates were recorded by observing the appearance of characteristic precipitation bands in specific areas as a result of antigens-antisera reactions.

1) Preparation of viral antigens

Healthy C. moschata plants of 10 days old were raised in sterilized soil under insect proof condition. These plants were separately inoculated with nine different isolates following usual technique and the inoculated plants were kept under insect proof condition for 20 days for the development of symptoms. The plants showing typical symptoms for the respective isolates were used as sources for the preparation of viral antigen of each isolate.

Antigen of each isolate was extracted from young infected plants by grinding the leaf tissue (4 gms) with a fixed volume (4 ml) of phosphate buffered saline (0.01M, $\text{Na}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$ and 0.85 per cent NaCl) at pH 7.1 in a mortar by adding a little sterile neutral sand at 4°C. The homogenate was then subjected to centrifugation at 15000 x g for 20 minutes at 4°C using International IEC Refrigerated Centrifuge Model PR-2. The clear supernatant layer was decanted and used as viral antigen for immunization of rabbits. Freshly prepared antigen of each isolate was used for immunizing rabbits.

2) Preparation of antisera

To prepare the antisera with respect to different antigens healthy Belgian white Rabbits (2-2.5 Kilo gram body weight) were obtained. The antigens were intravenously injected into the rabbits through their marginal ear veins. Each rabbit was injected nine times with increasing concentrations of each antigen at four days interval. Before each injection, the

respective antigen was emulsified with equal volume of 0.05 per cent Sodium alginate as an adjuvant (Slavin, 1950). The first injection was made with 0.1 ml antigen or in other words with 0.2 ml antigen-adjuvant mixture. The second and third injection was made with 0.2 ml antigen or in other words with 0.4 ml antigen-adjuvant mixture. In the fourth injection, the concentration of the antigen was raised to 0.4 ml (0.8 ml antigen-adjuvant mixture) and in the fifth injection, the concentration of the antigen was raised to 0.5 ml (1.0 ml antigen-adjuvant mixture). In the subsequent injections, however, this concentration of the antigen was used. After the ninth injection, the rabbits were kept for 10 days and then they were bled by cardiac puncture.

The blood samples were aseptically collected in 15 ml centrifuge tubes and allowed to coagulate by keeping the tubes at room temperature (25°C) for 1 hour. The coagulated blood clots were then loosened with the help of a sterilized glass rod and the samples were then kept overnight at 4°C. The liquid portion of the samples were then decanted and centrifuged at 10,000 x g for 30 minutes at 4°C. The clear straw coloured supernatant thus obtained was considered as antisera. The antisera were then dispensed into sterilized serum bottles and preserved in deep freeze for further use.

3) Agar double diffusion technique

Agar double diffusion technique was performed according the method of Crowle (1961). Good quality laboratory grade agar was washed overnight in running tap water

followed by washing in absolute alcohol. The agar was then thoroughly washed in tap water followed by repeated washing in glass distilled water. The washed agar thus obtained was then dried in a hot air oven at 68°C for 24 hours and was used in the experiment. The agar plates (previously sterilized) for the test were made up of 2 per cent agar in 0.01M phosphate buffer at pH 7.1. Then with the help of a sterilized cork borer five wells (one well in the centre and other four wells were around it) were made within each agar plate. Each well was 3 mm deep and 6 mm wide and the distance between the wells was 10 mm. 0.4 ml of undiluted antisera were taken at the central well of each agar plate. Out of the four surrounding wells, three were filled up with different undiluted antigens (0.4 ml for each well) and the fourth one was filled with 0.4 ml of phosphate buffered saline as control. In this way, antigens obtained from three isolates were accommodated in one petridish and so for nine isolates, three Petridishes were used. The experiment was performed in triplicates.

The plates were incubated at 4°C and examined periodically for the appearance of characteristic precipitation bands upto 14 days. The plates were examined within a special dark chambered box against reflected light as suggested by Crowle (1961). The precipitation bands that appeared as a result of the antigens-antisera reactions were suitably drawn. Suitable photographs were also taken wherever possible. The precipitation bands were numbered according to their mode of appearance and their relative distance from the respective reactant wells.

D. BIOCHEMICAL CHANGES IN THE LEAVES OF CUCURBITA MOSCHATA POIR PLANTS INOCULATED WITH DIFFERENT VIRUS ISOLATES AT DIFFERENT STAGES OF PATHOGENESIS

Healthy C. moschata seedlings were raised in sufficient number under insect proof condition. The seedlings were separately inoculated at the age of 10 days with nine different isolates, maintained in C. moschata plants following usual sap inoculation technique. The plants thus inoculated were kept under insect proof condition for the development of symptoms.

Leaf samples (apical three leaves) were collected from the inoculated plants and the healthy plants as well at three different stages of pathogenesis. The first collection was made at the time of symptom expression or in other words 10 days after inoculation (Stage I). The second collection was made at 1 month after symptom expression that means 40 days after inoculation (Stage II) and third collection was made at 2 months after symptoms expressions or in other words 70 days after inoculation (Stage III).

The samples, in triplicates, obtained after such collection were analysed for the (a) chlorophyll (a, b and total) (b) buffer soluble protein (c) total (TCA precipitable) sap protein (d) reducing sugar and starch and (e) ribonucleic acid (RNA) content in them.

a) Chlorophyll (a, b and total)

To estimate the chlorophyll content (a, b and total) of the samples of all the collections, the samples were first thoroughly washed with glass distilled water. The samples air dried and the method described by Mackinney (1941) and Yoshida et al. (1971)

was utilised for determination of chlorophyll contents. 2 grams of each sample were cut into small pieces. The tissues were then grinded and homogenized in a glass mortar and pestle adding requisite quantity of 80 per cent acetone (Analar Grade). The homogenization was continued till tissues became completely chlorophyll free. The supernatant was then decanted through filter paper (Whatman No. 42). The extraction process was repeated thrice following the similar procedure. Total volume of the supernatant was made upto 10 ml in a volumetric flask with 80 per cent acetone. Then 5 ml of this solution was transferred into a 50 ml volumetric flask and the volume was made up with 80 per cent acetone. These samples were utilized for estimating chlorophyll content by spectrophotometric technique. 3 ml of the prepared solution was taken in each cuvette and the absorbancy of this solution was determined at two different wave lengths namely 663 m μ (for chlorophyll a) and 645 m μ (for chlorophyll b) in a Beckman Model DU Spectrophotometer. The absorbancy recorded in this way was converted to obtain the quantity of chlorophyll a and b present in the test solution by following the method of yoshida et al. (1971). To estimate the total chlorophyll content in the test sample, the quantity of chlorophyll a and b were added together according to the method described by Yoshida et al. (1971).

b) Buffer soluble protein

To estimate the buffer soluble protein content of the samples of all the collections, the samples were first thoroughly washed with glass distilled water, then the samples were air dried and kept in a refrigerator for half an hour.

The extraction of buffer soluble protein from the chilled samples was done following the method of Gornal et al. (1949). 10 grams of chilled leaf tissues were homogenized in a pre-cooled mortar and pestle with M/15 phosphate buffer (pH 6.0, pre-cooled). The homogenised tissues were kept in a refrigerator for 30 minutes and then centrifuged at 3000 x g for 15 minutes. The supernatant obtained was taken in a centrifuge tube and equal volume of 40 per cent trichloroacetic acid was added to it. The mixture was then kept at 0°C for overnight, and centrifuged at 4000 x g. The precipitate obtained was washed with ether (Analar Grade) for several times to remove the traces of trichloroacetic acid and finally dissolved in 3 ml phosphate buffer (pH 6.0).

The estimation of the protein present in the solution was done according to the method of Gornal et al. (1949). To 3 ml of solution 6 ml of " Biuret reagent " * was added and kept for 30 minutes till the characteristic pink colour developed.

The absorbancy of the coloured samples at 540 m μ was then determined in a Beckman Model DU Spectrophotometer. The protein contents of the samples were calculated by comparing the absorbancy

* The "Biuret reagent" was prepared by dissolving 110 gms of A.R. quality of sodium hydroxide in 100 ml of distilled water in a 500 ml conical flask. The solution was left for a couple of days to settle down the sodium carbonate (Na_2CO_3), if any, present in the solution. 92 ml of this carbonate free sodium hydroxide solution was taken in a 500 ml volumetric flask and 208 ml of distilled water was added. 100 ml of 1 per cent solution of crystalline copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was added to the sodium hydroxide (NaOH) solution and the solution was stirred for thorough mixing.

of the sample solution with the standard curve prepared with "Bovine albumin" (B.D.H., England) at different dilutions.

c) Total (TCA precipitable) sap protein

To estimate the total TCA precipitable protein in the sap of the samples of all the collections, the samples were thoroughly washed with glass distilled water and then air dried. 10 gms of leaf tissues were homogenised with slight distilled water and the sap obtained was strained through muslin cloth (four fold). To each sap equal volume of 20 per cent trichloroacetic acid (TCA-E. Merck) was mixed and allowed to stand overnight at 4°C. The mixture was centrifuged at 17,500 x g for 10 minutes at 0°C. The precipitate thus obtained was separated out and suspended in 5 ml of ice-cold 95 per cent ethanol containing 0.1N potassium acetate and 5 ml of isopropyl ether. This suspension was centrifuged again and again till the lipid and colour pigments were completely removed and the final precipitate obtained was then mixed with 1 ml of 1 N NaOH and agitated until TCA agitated protein was completely dissolved. The solution was then diluted 100 times with glass distilled water.

The protein content in the sample were determined according to the method of Lowry et al. (1951). 1 ml aliquot of the diluted protein solution was transferred into a test tube

containing 5 ml of reagent C*. After 10 minutes 0.5 ml of reagent D* was added and mixed thoroughly at once and kept for 30 minutes. The absorbancy of this mixture at 750 m μ was recorded in a Beckman Model DU Spectrophotometer.

To estimate the protein content in the samples, a standard curve was prepared with known concentrations of "Bovine albumin" (B.D.H., England) and the absorbancy of the samples were compared with the standard curve.

d) Reducing sugar and starch

To estimate the reducing sugar and starch content of the samples of all the collections, the samples were thoroughly washed with glass distilled water and air dried. Then the samples were dried in an oven at 80°C for 48 hours. The dried leaves were cut into small pieces and then powdered in a grinder (Arthur H. Thomas Co., U.S.A.) using 80 mesh sieve. The reducing sugar and starch content of the samples were then determined following the procedure of Yoshida *et al.* (1971). 100 mg of dried samples was taken into a 15 ml centrifuge tube, 10 ml of 80 per cent ethanol was added. The mixture was kept in a water bath at

Reagent A- Dissolved 20 gms of Na_2CO_3 , 4 gms of NaOH and 200 mg of KNa-tartrate in distilled water and diluted to 1000 ml.

B- Dissolved 5 gms of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in distilled water and diluted in 1000 ml.

*C -Mixed 50 volume of reagent A with 1 volume of reagent B.

*D -Diluted Folin-Ciocalteu reagent (B.D.H., England) with an equal volume of distilled water.

80°C to 85°C for 30 minutes and then centrifuged at 3000 x g for 15 minutes. The supernatant was decanted into a 50 ml beaker and evaporated on a water bath at 80°C to 85°C to reduce the volume to about 3 ml. Then the volume was made upto 25 ml with distilled water and used for estimating reducing sugar.

The residue was dried in an oven at 80°C. The dried material was taken in a centrifuge tube and 2 ml of distilled water was added. The tubes were put in boiling water bath for 15 minutes with occasional stirring. The tubes were then cooled and 2 ml of 9.2 N perchloric acid was added to it with constant stirring. The mixture was allowed to stand for 15 minutes with occasional stirring. The volume of the suspension was made upto 10 ml with distilled water and centrifuged at 1200 x g for 15 minutes and supernatant was collected. To the centrifuge tube 2 ml of 4.6 N perchloric acid was added, the volume was made upto 10 ml with distilled water and centrifuged again at 1200 x g for 10 minutes. The volume of the supernatant was made upto 50 ml with distilled water and this solution was utilized for estimating starch.

5 ml of these sugar and starch were taken individually in the volumetric flasks. The volume were made upto 100 ml with distilled water. 2 ml of each of these solutions were taken in a Pyrex test tube and placed in an ice-bath. To each tube, 4 ml of "Anthrone" reagent (prepared by dissolving 200 mg anthrone in 100 ml of concentrated sulphuric acid, Analar Grade) was slowly added allowing the reagent to run down the side of the tube. The mixture was stirred slowly with a glass rod. After stirring the tubes were put into a boiling water bath for exactly 7.5 minutes and then cooled immediately. In case of starch, only 0.6 ml of 0.46 N

perchloric acid was added in the tubes. The absorbancy of this solution at 630 m μ was measured using a Beckman Model DU Spectrophotometer. A standard curve was prepared with different known concentrations of pure glucose. The reducing sugar contents in the samples were determined by comparing the data obtained from the samples with those obtained from the standard curve. In case of starch also a standard curve was prepared with different known concentrations of pure glucose after treating with perchloric acid as suggested by Yoshida et al. (1971). The starch contents in the samples were determined by comparing the data obtained from the samples with those obtained from the standard curve.

e) Ribonucleic acid (RNA)

To estimate the RNA content of the samples of all the collections, the samples were thoroughly washed with glass distilled water and air dried. 1 gm of fresh leaf was taken and it was thoroughly crushed with neutral glass powder in a glass mortar and pestle. The homogenized material thus obtained was utilized for estimating RNA content of the samples following the procedure described by Kirby (1956).

The homogenized material was transferred in stoppered conical flask. 3 ml of 0.1M phosphate buffer (pH 7.0), 3 ml of glass distilled water and 3 ml of 90 per cent phenol were added to it and shaken by flask shaker (CAT. No. GL 83, Toshniwal Co., India) for 6 hours. Then the mixture was centrifuged at 3000 x g for 10 minutes. The upper aqueous layer was taken out and the rest was centrifuged twice at 3000 x g for 10 minutes adding small amount of 0.1M phosphate buffer. The upper aqueous layer

obtained was mixed with that obtained earlier. To this 4 volumes of cold 95 per cent ethanol was added and left for overnight in cold (4°C) for complete precipitation. The mixture was then centrifuged at $4500 \times g$ for 15 minutes and the precipitate was washed 3-4 times with absolute ethanol.

The precipitate obtained in this way was dissolved in small quantity of 0.1M phosphate buffer (2 ml). Equal volume of chloroform:octanol (4:1) mixture was added to it and shaken vigorously for 30 minutes. The mixture was then centrifuged at $20,000 \times g$ for 15 minutes. The precipitate obtained was treated with 4 volumes of cold 95 per cent ethanol and kept overnight in cold at 4°C . The mixture was then centrifuged at $3000 \times g$ for 20 minutes. The precipitate obtained was treated again with chloroform:octanol (4:1) mixture following the same procedure. The precipitate finally obtained was dissolved in 5 ml of 0.1M phosphate buffer (pH 7.0).

1 ml of the above solution was taken in a Pyrex test tube and diluted with 1 ml of 0.1M phosphate buffer (pH 7.0) and 2 ml of "Hejbaum's reagent", prepared by dissolving 100 mg Orcinol in 10 ml of concentrated hydrochloric acid (Analar Grade) containing 0.1 per cent ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), was added to this solution. The mixture was thoroughly shaken and placed on a boiling water bath for 10 minutes with occasional stirring till blue green colour developed. A blank was also made by taking 2 ml of buffer and 2 ml of reagents and similarly heated for 10 minutes. The optical density of the mixture was measured at $660 \text{ m}\mu$ in a Beckman Model DU Spectrophotometer using the blank as control.

standard curve was prepared with different known concentrations of "Yeast RNA " (B.D.H., England). The RNA contents in the samples were determined by comparing the data obtained from the samples with those obtained from the standard curve.

IV. R E S U L T

A. INCIDENCE OF VIRUS DISEASES

Data obtained on the relative incidence of virus diseases of pumpkin (C.moschata Poir) in different districts of West Bengal in the month of February-March, 1973 have been presented in the Table-2. It was observed that 60.00 ± 4.58 to 89.33 ± 3.47 per cent plants were infected by virus diseases under conditions prevailed during the period of observation.

In the critical study with the diseased plants, it was observed that the types of symptoms differed. The types of symptoms observed in different fields have been presented in Table-3. It was observed that at least six different types of symptoms appeared in the fields (Plate I - III, Figures 1 - 6). Results obtained on the relative incidence of different types of field symptoms during the period of observation have been presented in Table-4. It is apparent from the Table-4 that the type II symptom complex predominated in the fields during the period of observations.

Table 2. Relative incidence of virus diseases of pumpkin (Cucurbita moschata Poir)
in different districts of West Bengal during February - March 1973

Sl.No.	Name of the district	Actual location	Percentage of diseased plants			Average ($\bar{x} \pm S.E. (\bar{x})$)
			Field I	Field II	Field III	
1	Burdwan	Memari	61.0	69.0	67.0	65.66 $\pm$ 2.40
2	Hooghly	Sarakeswar	72.0	68.0	71.0	70.33 $\pm$ 1.2
3	Malda	Malda	57.0	54.0	69.0	60.00 $\pm$ 4.58
4	Nadia	Harinagata	90.0	95.0	83.0	89.33 $\pm$ 3.47
5	24 Parganas	Baruipur	75.0	80.0	78.0	77.66 $\pm$ 1.41

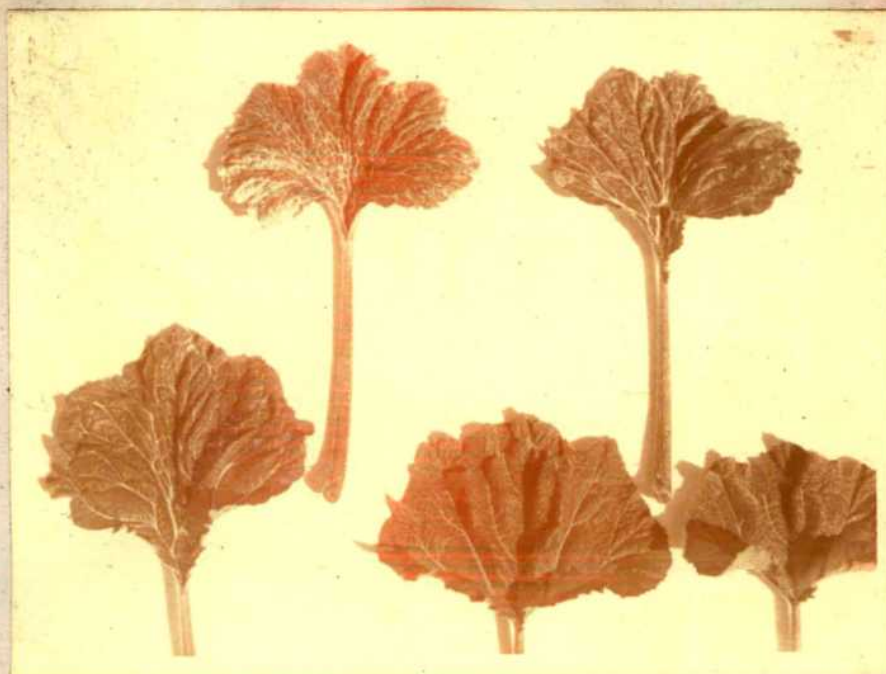


Figure 1. Pumpkin plants showing Type I symptoms

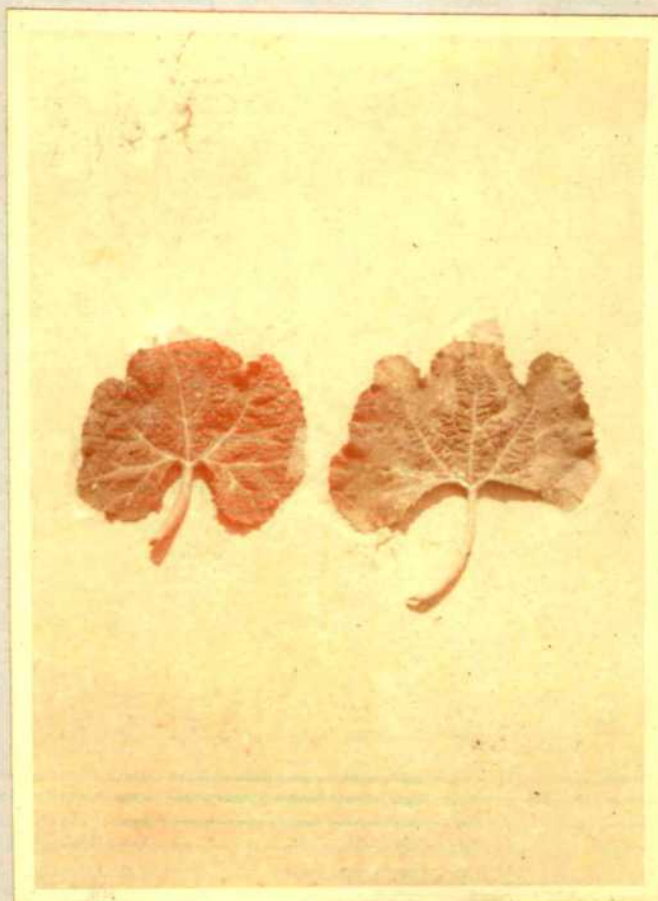


Figure 2. Pumpkin plants showing Type II symptoms

PLATE - I Different types of Leaf symptoms observed in virus infected pumpkin plants in the fields.

Figure 3. Pumpkin plants
showing Type III
symptoms



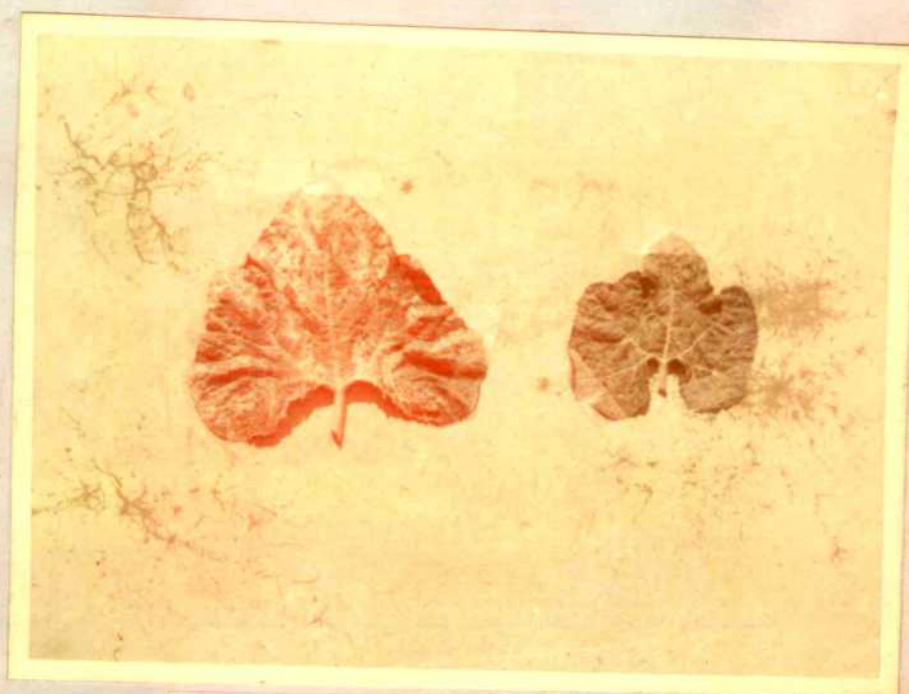
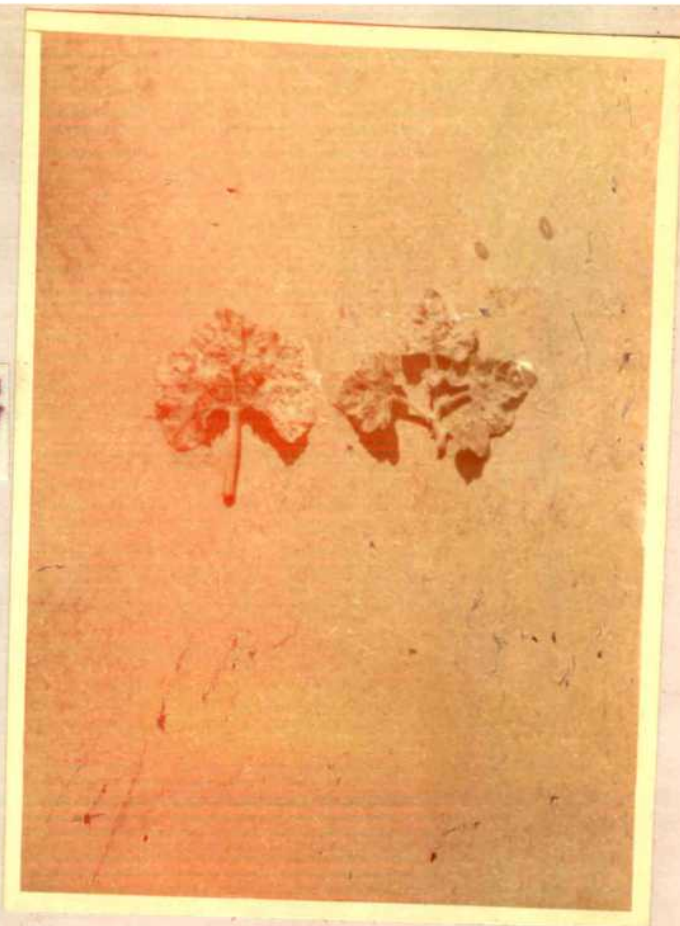
Figure 4. Pumpkin plants
showing Type IV
symptoms



Table 3. Symptoms of virus diseases of pumpkin (*Cucurbita moschata* Poir) found in the fields of different districts of West Bengal during February - March 1973

Sl.No.	Types of symptom	Characteristic alterations in the young leaves
1	I	The lamina appeared to be reduced in size. The upper surface of the lamina showed yellow mottle with scattered dark green blisters. Green bands were found around all the veins. The veins tended to coalesce near the base of the lamina. The surface of the lamina eventually became wrinkled and puckered, the margins of it became irregular and the shape became distorted (Plate I, Figure 1).
2	II	The lamina appeared to be reduced in size. The upper surface of the lamina showed mottling. Yellowing appeared near the margins which were found to extend gradually into the entire lamina. Green banding along the veins which were more pronounced than that in type I. Margins became irregular and slightly serrated. The shape of the lamina became distorted (Plate I, Figure 2).
3	III	The upper surface of the lamina showed yellowish chlorosis which started at the margin and gradually extended into the entire lamina. Dark green blisters were found around the veins particularly near the base of the lamina. The surface of the lamina became wrinkled. The margins became serrated and the shape was found to be distorted (Plate II, Figure 3).

**Figure 5. Pumpkin plants
showing Type V
symptoms**



**Figure 6. Pumpkin plants
showing Type VI
symptoms**

**PLATE -III Different types of leaf symptoms observed in
virus infected pumpkin plants in the fields.**

Table 3(Contd.)

Sl.No.	Types of symptom	Characteristic alterations in the young leaves
4	IV	The lamina appeared to be reduced in size. The upper surface of the lamina was mottled and rugose. The lamina appeared to be puckered and leathery. Scattered dark green blisters were found on the surface of the lamina. The veins became bright yellow in colour. The margins remained more or less entire but the shape was slightly distorted (Plate II, Figure 4).
5	V	The upper surface of the lamina showed intense yellow mosaic. In advanced stage the chlorotic tissue became bleached, veins became yellow and the surface became wrinkled. Margins appeared to be serrated but the serrations were very small in size (Plate III, Figure 5).
6	VI	The lamina appeared to be reduced in size. The upper surface of the lamina showed mosaic. The chlorotic tissues appeared to be light yellow to yellow in colour. These tissues gradually became necrotic (Plate III, Figure 6).

Table 4. Relative incidence of different types of field symptoms of virus diseases of pumpkin (*Cucurbita moschata* Poir) in different districts of West Bengal during February - March 1973

Sl.No.	Name of the district	Percentage of incidence of different types					
		Type I $\bar{X} \pm S.E.(\bar{X})$	Type II $\bar{X} \pm S.E.(\bar{X})$	Type III $\bar{X} \pm S.E.(\bar{X})$	Type IV $\bar{X} \pm S.E.(\bar{X})$	Type V $\bar{X} \pm S.E.(\bar{X})$	Type VI $\bar{X} \pm S.E.(\bar{X})$
1	Burdwan	21.66 $\pm$ 0.33	36.66 $\pm$ 1.20	8.66 $\pm$ 0.87	14.66 $\pm$ 0.33	2.00 $\pm$ 0.57	16.33 $\pm$ 0.87
2	Hooghly	13.66 $\pm$ 0.87	43.66 $\pm$ 1.45	7.00 $\pm$ 1.00	12.00 $\pm$ 1.15	7.00 $\pm$ 0.57	16.66 $\pm$ 0.87
3	Nadia	13.66 $\pm$ 1.20	51.00 $\pm$ 1.52	8.00 $\pm$ 1.15	9.33 $\pm$ 1.20	5.00 $\pm$ 1.15	13.00 $\pm$ 1.52

B. SAP TRANSMISSIBILITY OF TYPE II SYMPTOM COMPLEX

Data obtained on the transmissibility tests with type II symptom complex have been presented in the Table -5. It appears from the table that 31 to 80 per cent transmission of the symptoms was obtained by sap transmission technique. It is further apparent that the symptoms developed in all the inoculated plants were not identical to each other and at least nine different types of symptoms were observed in the inoculated plants which have been presented in the Table- 6 and Plate IV - VII, Figure 6 - 14.

Nine types of symptoms however, were not obtained from a single collection. When the type II symptom complexes were collected from time to time in batches and the sap extracted from them were inoculated to test plants, these nine types of symptoms were appeared from the different batches of samples.

Table 5. Sap transmissibility of type II symptom complex of pumpkin (Cucurbita
moschata Poir)

Sl.No.	Number of batches (leaf samples collected from time to time)	Number of plants inoculated	Percentage of plants showed symptoms	Remarks
1	1	25	80	All inoculated plants did not show identical symptoms.
2	2	25	62	All inoculated plants did not show identical symptoms.
3	3	25	31	All inoculated plants did not show identical symptoms.
4	4	25	55	All inoculated plants did not show identical symptoms.
5	5	25	42	All inoculated plants did not show identical symptoms.

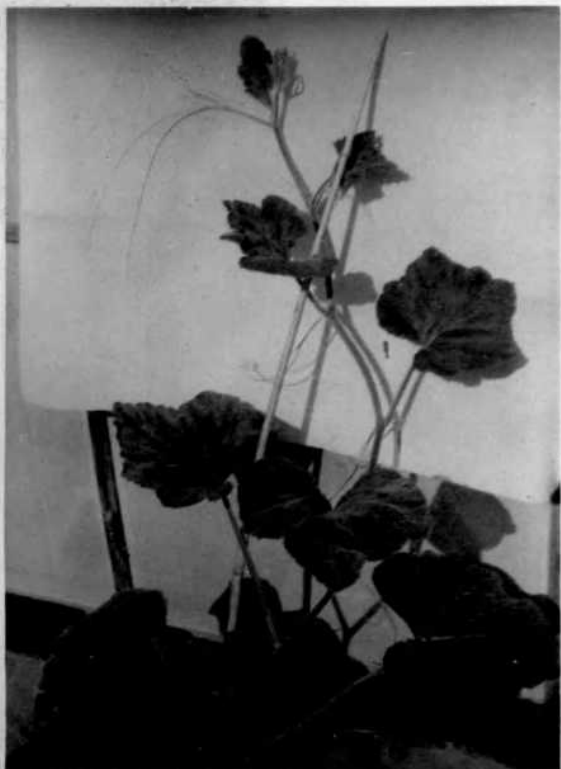


Figure 7. Pumpkin plants showing symptoms of isolate A₁



Figure 8. Pumpkin plants showing symptoms of isolate A₂

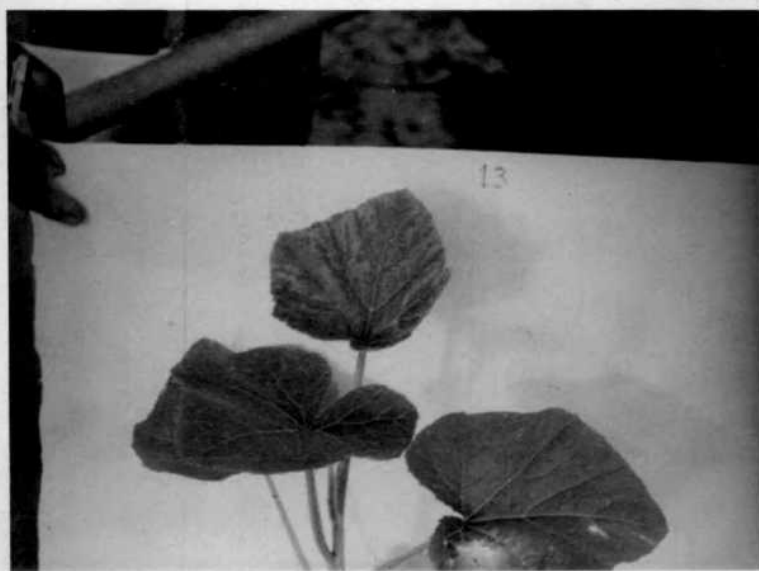


Figure 9. Pumpkin plants showing symptoms of isolate A₃

Table 6. Types of symptoms obtained from type II symptom complex of
C. roschata Poir on sap transmission

Sl. No.	Types of symptom obtained	Description of symptoms produced
1	A ₁	Irregular chlorotic spots appeared at the margins of the lamina which gradually coalesced and became yellow. The surface of the lamina showed mottling with prominent green blisters and green vein banding. There was no vein yellowing and apparently there was no rugosity and puckering of the leaf (Plate IV, Figure 7).
2	A ₂	Irregular chlorotic spots appeared at the margins of the lamina which gradually coalesced and became yellow. The surface of the lamina showed mottling with prominent green vein banding and blisters. In advanced stage of symptom development the green vein banding gradually became less prominent and veins became yellow and more prominent. The surface of the lamina did not show any rugosity but puckering was observed at the advanced stage of symptom development. When transmission was done in winter months, in addition to the above mentioned symptoms organoid enations were observed at the under surface of the lamina. The enations were cup shaped to boat shaped in appearance (Plate IV, Figure 8 and Plate VIII, Figure 16).

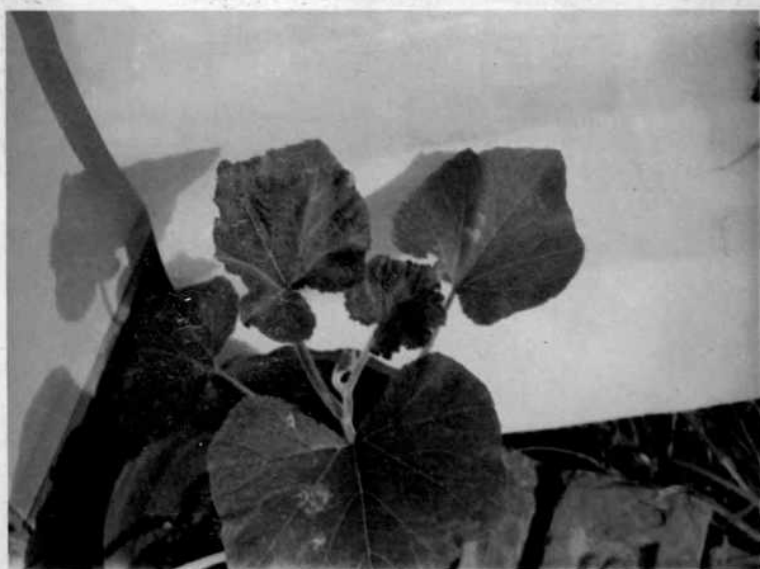


Figure 10. Pumpkin plants showing
symptoms of isolate A₄



Figure 11. Pumpkin plants showing
symptoms of isolate A₅

Table 6 (Contd.)

Sl.No.	Types of symptom obtained	Description of symptoms produced
3	A ₃	Irregular chlorotic spots appeared at the margins of the lamina which gradually coalesced and became yellow. The surface of the lamina showed mottling with prominent green vein banding and mild green blisters. The surface of the lamina did not show any rugosity or vein yellowing but at the advanced stage of disease development slight puckering of the lamina was observed (Plate IV, Figure 9).
4	A ₄	Irregular chlorotic spots appeared at the margins of the lamina which gradually coalesced and became yellow. The surface of the lamina showed mottling with prominent green blisters and ill defined green vein banding. The surface of the lamina was rugose but there was no vein yellowing. No puckering of lamina was also observed (Plate V, Figure 10).
5	A ₅	Irregular chlorotic spots appeared at the margins of the lamina which gradually coalesced and became yellow. The surface of the lamina showed mottling with mild green blisters and green vein banding. There was no vein yellowing and apparently there was no rugosity and puckering of the lamina (Plate V, Figure 11).

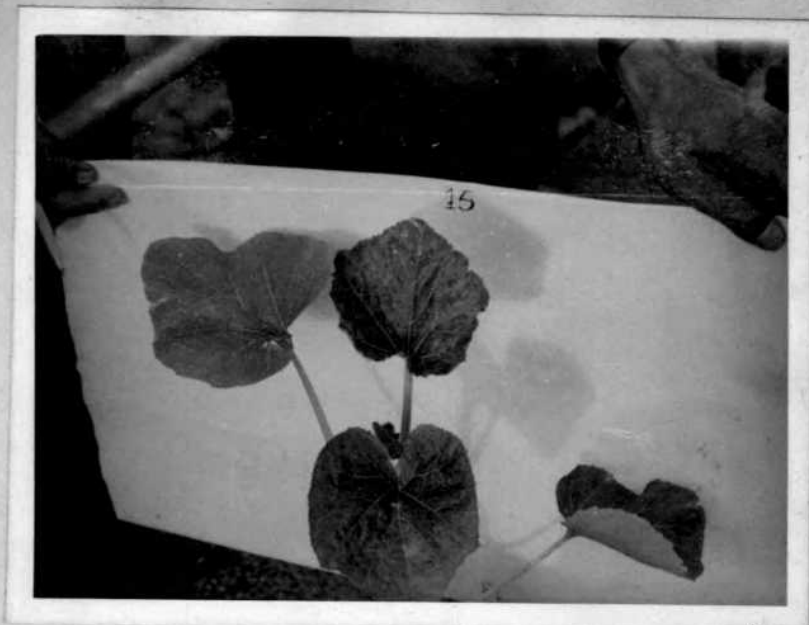


Figure 12. Pumpkin plants showing
symptoms of isolate A₆



Figure 13. Pumpkin plants showing
symptoms of isolate A₇

Table 6 (Contd.)

Sl.No.	Types of symptom obtained	Description of symptoms produced
6	A ₆	Irregular chlorotic spots appeared on the entire lamina. The spots gradually coalesced and the lamina gradually appeared to be yellow in colour. The surface of the lamina showed mottling with mild green blisters and ill defined green vein banding. There was no vein yellowing or rugosity on the surface of the lamina but puckering of the lamina was observed in the advanced stage of the symptom expression (Plate VI, Figure 12).
7	A ₇	Irregular chlorotic spots appeared on the entire lamina. The spots gradually coalesced and the lamina gradually appeared to be greenish yellow in colour. The surface of the lamina showed mottling with mild green blisters and green vein banding. There was no vein yellowing, no rugosity and no puckering of the lamina (Plate VI, Figure 13).
8	A ₈	Irregular chlorotic spots appeared at the margins of the lamina which gradually coalesced and became yellow. The surface of the lamina showed mottling with irregularly distributed chlorotic spots. There was no green vein banding or green blisters on the surface of the lamina. There was no vein yellowing, no rugosity and no puckering of the lamina. When transmission was done in winter months, in addition to the above mentioned symptoms organoid enations were observed at the under surface of the lamina. The enations were cup shaped to boat shaped in appearance (Plate VII, Figure and Plate VIII, Figure 17).



Figure 14. Pumpkin plants showing
symptoms of isolate A₈



Figure 15. Pumpkin plants showing
symptoms of isolate A₉

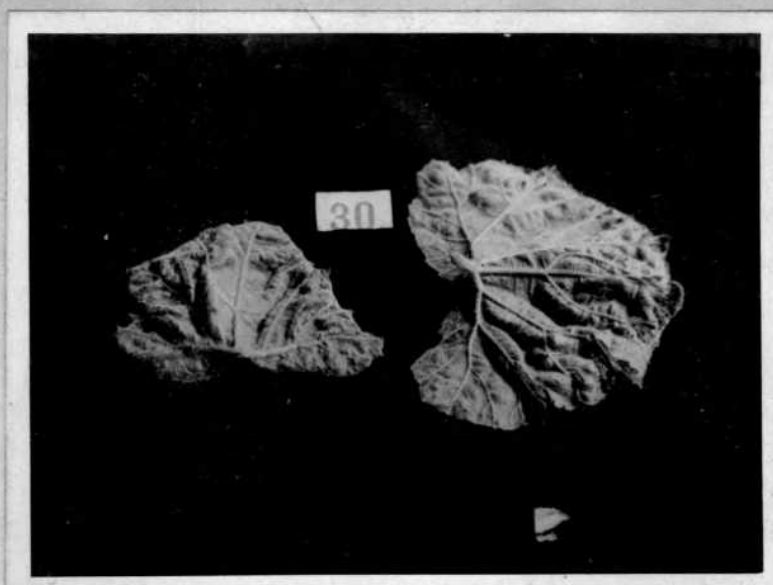


Figure 16. Enation symptom on pumpkin leaves due to infection of isolate A_2

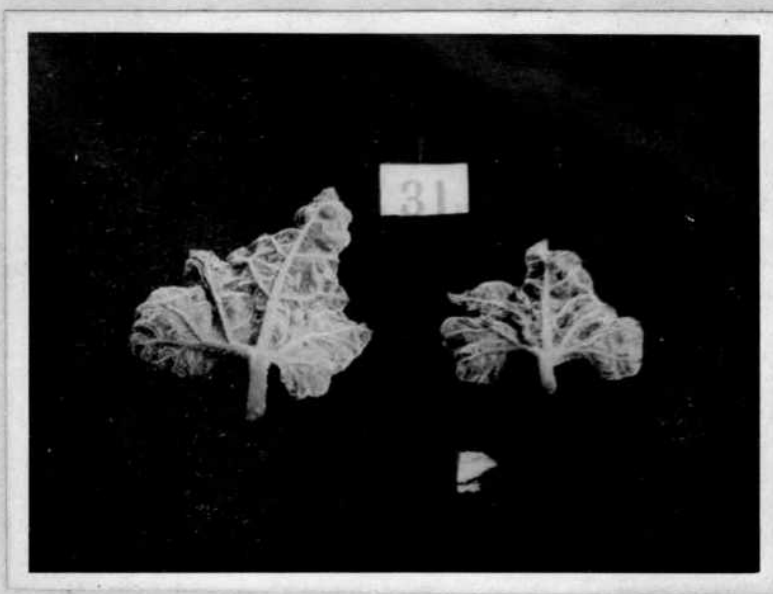


Figure 17. Enation symptom on pumpkin leaves due to infection of isolate A_8

Table 6 (Contd.)

Sl.No.	Types of symptom obtained	Description of symptoms produced
9	A ₉	Irregular chlorotic spots appeared on the entire lamina. The spots gradually coalesced and the lamina gradually appeared to be pale yellow in colour. The surface of the lamina showed mottling with mild green blisters but no green vein banding was observed. There was no vein yellowing, no rugosity and no puckering of the lamina. (Plate VII, Figure 15).

C. IDENTIFICATION OF THE ISOLATES

a) Determination of the physical properties of the pure isolates

1. Determination of thermal inactivation point

From the Table-7, it is evident that treating the standard inocula of the isolates $A_1 - A_9$ at different temperatures namely 40°C , 45°C , 50°C , 55°C , 60°C , 65°C and 70°C for 10 minutes and utilizing these treated inocula for inoculating healthy C. moschata plants to determine the thermal inactivation of the isolates under consideration, the standard inocula of none of the isolates treated at temperature above 50°C , produced any symptom in the inoculated plants. The standard inocula of A_1 and A_5 , produced symptoms, even when treated at 50°C . The percentages of infection with the inocula of A_1 treated at 40° , 45° and 50°C were 52, 39 and 8. The percentages of infection with the inocula of A_5 treated at 40° , 45° and 50°C were 33, 11 and 4 respectively. The standard inocula of A_2 , A_4 , A_6 , A_7 and A_8 produced symptoms, when treated at temperatures upto 45°C . The percentages of infection with the inocula of these isolates treated at 40° were 34, 26, 43, 40 and 9. The percentages of infection with the inocula of these isolates (A_2 , A_4 , A_6 , A_7 and A_8), treated at 45°C were 3, 7, 10, 2 and 2 respectively. The standard inocula of the isolates A_3 and A_9 produced symptoms only upto treatment at 40°C . The percentages of infection with the inocula of these isolates treated at 40°C were 12 and 8.

The results indicated that the standard inocula of the isolates A_1 and A_5 became inactivated when treated at temperature between 50° - 55° C. The standard inocula of the isolates A_2 , A_4 , A_6 , A_7 and A_8 became inactivated when treated at temperature between 45° - 50° C. The standard inocula of the isolates A_3 and A_9 became inactivated when treated at temperature between 40° - 45° C.

2. Determination of dilution end point

From the table 8, it is evident that the infectivity of the standard inocula obtained from the different isolates were different at different dilutions. The standard inocula of the isolate A_2 could produce symptoms even when diluted upto 10^{-3} . The percentages of infection with the standard inocula of this isolate at the dilutions of $10^{-1.0}$, $10^{-1.5}$, $10^{-2.0}$, $10^{-2.5}$ and $10^{-3.0}$ were 51, 33, 27, 12 and 9 respectively. The standard inocula of the isolate A_7 could produce symptoms when diluted upto $10^{-2.5}$. The percentages of infection with the standard inocula of this isolate at dilutions of $10^{-1.0}$, $10^{-1.5}$, $10^{-2.0}$ and $10^{-2.5}$ were 59, 36, 10 and 2 respectively. The standard inocula of the isolates A_3 , A_4 and A_6 could produce symptoms when diluted upto $10^{-2.0}$. The percentages of infection with the standard inocula of the isolate A_3 at dilutions $10^{-1.0}$, $10^{-1.5}$ and $10^{-2.0}$ were 59, 25 and 5 respectively. The percentages of infection with the standard inocula of the isolate A_4 at the above dilutions were 39, 12 and 4 respectively. The percentages of infection with the standard inocula of the isolate A_6 were 52, 27 and 6 respectively. The standard inocula of the isolates A_5 and A_9 could produce symptoms when diluted upto $10^{-1.5}$. The respective percentages of infection with the standard

inocula of A_5 at the dilutions of $10^{-1.0}$ and $10^{-1.5}$, were 42 and 17 respectively. The respective percentages of infection with the standard inocula of A_9 at the above dilutions, were 28 and 7 respectively. The standard inocula of the isolates A_1 and A_8 , could not produce any symptom even at the dilution of $10^{-1.0}$.

The results showed that the standard inocula of the isolate A_2 lost its infectivity at dilution between $10^{-3.0}$ to $10^{-3.5}$. The standard inocula of the isolate A_7 lost its infectivity at the dilution between $10^{-2.5}$ to $10^{-3.0}$. The standard inocula of the isolates A_3 , A_4 and A_6 , lost their infectivity at the dilution between $10^{-2.0}$ to $10^{-2.5}$. The standard inocula of the isolates A_5 and A_9 lost their infectivity at the dilution between $10^{-1.5}$ to $10^{-2.0}$. The standard inocula of the isolates A_1 and A_8 did not show infectivity at the dilution of $10^{-1.0}$.

3. Determination of ageing in vitro

From the Table 9, it is evident that the infectivity of the standard inocula obtained from different isolates differed when they were stored for different length of time under ordinary laboratory conditions. The standard inocula of the isolate A_7 could produce symptom even after being stored for 7 days. The percentages of infection with this standard inocula stored for 12 hours, 24 hours, 36 hours, 48 hours and 60 hours, were 52, 31, 22, 12 and 4 respectively. The same inocula when stored for 72 hours, 84 hours, 4 days, 5 days, 6 days and 7 days, the percentage of infection was 1 in each case. The standard inocula of the isolate A_6 could produce symptoms when stored upto

84 hours. The percentages of infection with the standard inocula of this isolate stored for 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours and 84 hours were 43, 27, 11, 7, 3, 2 and 2 respectively. The standard inocula of the isolate A_3 could produce symptom when stored upto 60 hours. The percentages of infection with the standard inocula of this isolate stored upto 12 hours, 24 hours, 36 hours, 48 hours and 60 hours were 52, 30, 20, 12 and 7 respectively. The standard inocula of the isolates A_2 , A_5 and A_9 could produce symptoms when stored upto 48 hours. The percentages of infection with the standard inocula of the isolate A_2 stored for 12 hours, 24 hours, 36 hours, and 48 hours were 45, 27, 15 and 2 respectively. The percentages of infection with the standard inocula of the isolate A_5 stored at 12 hours, 24 hours, 36 hours and 48 hours were 26, 20, 5 and 2 respectively. The percentages of infection with the standard inocula of the isolate A_9 stored for 12 hours, 24 hours, 36 hours and 48 hours were 39, 13, 9 and 1 respectively. The standard inocula of the isolates A_4 and A_8 could produce symptoms when stored upto 12 hours and the percentages of infection under this condition were 37 and 14 respectively. The standard inocula of the isolate A_1 could not produce any symptom when stored up to 12 hours.

The results indicated that the standard inoculum of the isolate A_7 lost its infectivity when stored for more than 7 days. The standard inoculum of the isolate A_6 lost its infectivity when stored for more than 84 hours. The standard inoculum of the isolate A_3 lost its infectivity when stored for more than 60 hours. The standard inocula of the isolates A_2 , A_5 and A_9 lost their

infectivity when stored for more than 48 hours. The standard inocula of A_1 and A_0 lost their infectivity when stored for more than 12 hours. The standard inoculum of the isolate A_1 on the otherhand lost its infectivity when stored for more than 6 hours (Table 9a).

Table 7. Infectivity of the standard inocula of different isolates obtained from type II symptom complex after treating them at different temperatures for 10 minutes

	Sl. No.	Isolates	Control (untreated) Percentage of infec- tion	Temperature (°C)						Percentage of infec- tion
				40	45	50	55	60	65	70
				Per- centage of infec- tion	Per- centage of infec- tion	Per- centage of infec- tion	Per- centage of infec- tion	Per- centage of infec- tion	Per- centage of infec- tion	Per- centage of infec- tion
1	A ₁	64	52	37	8	-	-	-	-	-
2	A ₂	59	34	3	-	-	-	-	-	-
3	A ₃	66	12	-	-	-	-	-	-	-
4	A ₄	50	26	7	-	-	-	-	-	-
5	A ₅	51	33	11	4	-	-	-	-	-
6	A ₆	61	43	10	-	-	-	-	-	-
7	A ₇	75	40	2	-	-	-	-	-	-
8	A ₈	28	9	2	-	-	-	-	-	-
9	A ₉	60	8	-	-	-	-	-	-	-

Table 3. Infectivity of the standard inocula of different isolates obtained from type II symptom complex at different dilutions

Sl.No.	Isolation	Control (undiluted) percentage of infec- tion	Dilutions						
			10 ^{-1.0} Percent- age of infection	10 ^{-1.5} Percent- age of infection	10 ^{-2.0} Percent- age of infection	10 ^{-2.5} Percent- age of infection	10 ^{-3.0} Percent- age of infection	10 ^{-3.5} Percent- age of infection	10 ^{-4.0} Percent- age of infection
1	A ₁	68	-	-	-	-	-	-	-
2	A ₂	62	51	33	27	12	9	-	-
3	A ₃	71	50	25	5	-	-	-	-
4	A ₄	54	39	12	4	-	-	-	-
5	A ₅	57	42	17	-	-	-	-	-
6	A ₆	65	52	27	6	-	-	-	-
7	A ₇	81	59	35	10	2	-	-	-
8	A ₈	29	-	-	-	-	-	-	-
9	A ₉	53	28	7	-	-	-	-	-

Table 9. Determination of ageing in vitro at room temperature (24-32°C) of different isolates of type II symptom complex

Sl. No.	Period of storage	I	A ₁	A ₂	A ₃	A ₄	A ₅	A ₆	A ₇	A ₈	A ₉
		Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion	Per- cent- age of infect- ion
1	12 hrs	-	45	52	57	26	43	52	39	14	39
2	24 hrs	-	27	30	-	20	27	31	13	-	13
3	36 hrs	-	15	20	-	5	11	22	9	-	9
4	48 hrs	-	2	12	-	2	7	12	1	-	1
5	60 hrs	-	-	7	-	-	3	4	-	-	-
6	72 hrs	-	-	-	-	-	2	1	-	-	-
7	84 hrs	-	-	-	-	-	2	1	-	-	-
8	4 days	-	-	-	-	-	-	1	-	-	-
9	5 days	-	-	-	-	-	-	1	-	-	-
10	6 days	-	-	-	-	-	-	1	-	-	-
11	7 days	-	-	-	-	-	-	1	-	-	-
12	8 days	-	-	-	-	-	-	-	-	-	-
13	9 days	-	-	-	-	-	-	-	-	-	-
14	10 days	-	-	-	-	-	-	-	-	-	-
15	Control	60	55	68	52	51	61	79	28	54	54

Table 9a. Infectivity of the standard inocula of the isolate A₁
when stored for different hours

Period of storage (hours)

2 4 6 8 10 12

Percentage of infection

48

22

5

Table 10. Comparative physical properties of different isolates of type II symptom complex

Sl.No.	Isolates	Dilution end point (DEP)	Thermal inactivation point (TIP)	Ageing in vitro at room temperature (24-32°C)
1	A ₁	$\angle 10^{-1.0}$	50 - 55°C	6 hours
2	A ₂	$10^{-3.0} - 10^{-3.5}$	45 - 50°C	48 hours
3	A ₃	$10^{-2.0} - 10^{-2.5}$	40 - 45°C	60 hours
4	A ₄	$10^{-2.0} - 10^{-2.5}$	45 - 50°C	12 hours
5	A ₅	$10^{-1.5} - 10^{-2.0}$	50 - 55°C	48 hours
6	A ₆	$10^{-2.0} - 10^{-2.5}$	45 - 50°C	84 hours
7	A ₇	$10^{-2.5} - 10^{-3.0}$	45 - 50°C	7 days
8	A ₈	$\angle 10^{-1.0}$	45 - 50°C	12 hours
9	A ₉	$10^{-1.5} - 10^{-2.0}$	40 - 45°C	48 hours

b) Host range of the isolates

The symptoms produced by the nine isolates in the plants belonging to Cucurbitaceae, Solanaceae, Leguminosae, Cruciferae, Caricaceae, Chenopodiaceae, Apocyanaceae, Amaranthaceae and Compositae families have been recorded and the data have been presented in Table-11 according to the observations made with respect to the families. The observation made on the back inoculation of the isolates from the different plants to the C. moschata Poir have also been recorded along with the above observations.

Family 1. Cucurbitaceae

Host 1. Cucurbita moschata Poir

Isolates A₁ - A₉

The symptoms produced by all the nine isolates in C. moschata Poir have been presented in the Table-6. When the isolates were back inoculated from C. moschata Poir to C. moschata Poir plants, similar symptoms were also observed.

Host 2. Lagenaria vulgaris Ser.

Isolate A₁

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic areas on the entire lamina. Within 20 days after inoculation the chlorotic areas gradually increased in size. The lamina became

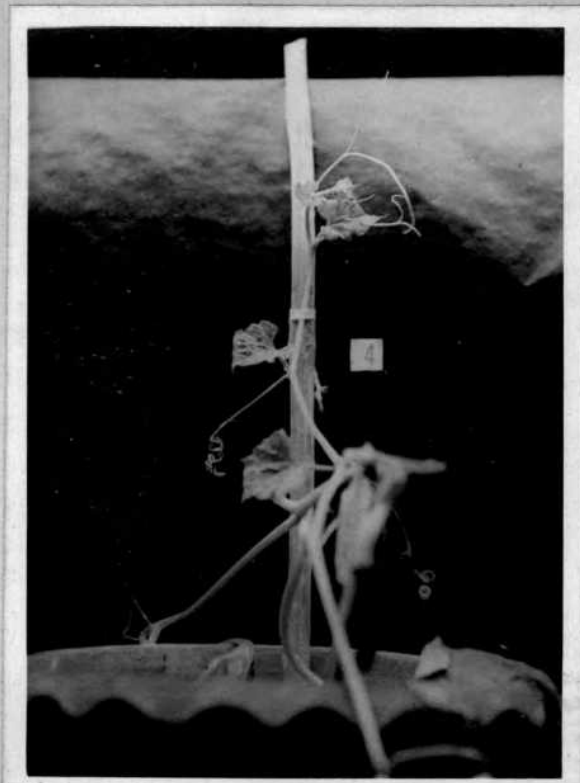


Figure 18. Symptoms of isolate
A₁ on L. vulgaris



Figure 19. Symptoms of isolate
A₆ on L. vulgaris

Symptoms of different isolates on Lagenaria
vulgaris

netttled and greyish yellow in colour. Within 30 days after inoculation numerous dark green blisters with slight vein banding appeared on the lamina and it gradually became wrinkled. As the infection advanced the size of the lamina was reduced. The margins became wavy and the shape became distorted with the downward curving of the margin (Plate IX, Figure 18).

When the isolate was back inoculated from L. vulgaris to C. moschata Poir Plants, symptoms similar to isolate A₁ on C. moschata Poir (Table 6) were observed.

Isolate A₂

First visible symptoms appeared after 10 days of inoculation. The symptoms produced by the isolate A₂ were found to be similar to that found with the isolate A₁ (Plate IX, Figure 18). The results with the back inoculation to C. moschata Poir plants appeared to be similar to the isolate A₂ on C. moschata Poir (Table 6).

TABLE II. HOST RANGE OF DIFFERENT ISOLATES OBTAINED FROM CUCURBITA MOSCHATA POIR

SL. No.	NAME OF THE PLANTS	NAME OF THE FAMILY	ISOLATES								
			A ₁	A ₂	A ₃	A ₄	A ₅	A ₆	A ₇	A ₈	A ₉
1	CUCURBITA MOSCHATA POIR	CUCURBITACEAE	+	+	+	+	+	+	+	+	+
2	LAGENARIA VULGARIS SER.	"	+	+	+	S.C.	S.C.	+	S.C.	+	+
3	MOMORDICA CHARANTIA L.	"	+	-	+	+	+	-	+	+	+
4	BENINCASA CERIFERA SAVI	"	+	-	+	-	-	-	-	S.C.	-
5	TRICHOSANTHES ANGUINA L.	"	+	+	+	+	+	+	+	+	+
6	LUFFA CYLINDRICA ROEM.	"	+	S.C.	-	+	-	+	-	-	+
7	LUFFA ACUTANGULA ROXB.	"	-	S.C.	+	-	+	+	+	-	+
8	CITRULLUS VULGARIS SCHRAD VAR. FISTULOSUS	"	+	S.C.	+	S.C.	S.C.	-	-	-	S.C.
9	CITRULLUS LANATUS CV. CHARLESTON GRAY	"	+	+	+	S.C.	+	S.C.	+	+	-
10	CUCURBITA PEPO L. CV. EARLY PROLIFIC STRAIGHTNECK	"	+	+	+	+	+	+	+	+	+
11	CUCUMIS MELO L. SELECTION B 63-3	"	-	-	-	+	+	+	-	-	-
12	CUCUMIS SATIVUS L. CV. LOCAL WHITE	"	+	+	-	+	+	+	-	+	+
13	CUCUMIS SATIVUS L. CV. LONG GREEN	"	+	+	-	+	+	+	-	+	+
14	CAPSIUM ANNUUM L. VAR. SURYAMUKHI	SOLANACEAE	-	-	-	-	-	-	-	-	-
15	DATURA STRAMONIUM L.	"	-	-	-	-	-	-	-	-	-
16	LYCOPERSICON ESCULENTUM MILL. VAR. MARGLOBE SUPREME	"	-	-	-	-	-	-	-	-	-
17	SOLANUM TUBEROSUM L. VAR. CHANDRAMUKHI	"	-	-	-	-	-	-	-	-	-
18	SOLANUM MELONGENA VAR. MUKTAKESI	"	-	+	+	-	+	-	-	-	-

TABLE 11. (CONTD.)

SL. No.	NAME OF THE PLANTS	NAME OF THE FAMILY	ISOLATES								
			A ₁	A ₂	A ₃	A ₄	A ₅	A ₆	A ₇	A ₈	A ₉
19	NICOTIANA TABACUM L.	SOLANACEAE	-	-	-	-	-	-	-	-	-
20	NICOTIANA TABACUM L. CV. VIRGIANA GOLD	"	-	-	-	-	-	-	-	-	-
21	NICOTIANA TABACUM L. CV. XANTHI	"	-	-	-	-	-	-	-	-	-
22	NICOTIANA GLUTINOSA L.	"	-	-	-	-	-	-	-	-	-
23	NICOTIANA SYLVESTRIS L.	"	-	-	-	-	-	-	-	-	-
24	NICOTIANA RUSTICA L.	"	-	-	-	-	-	-	-	-	-
25	PETUNIA HYBRIDA VILM.	"	-	-	-	-	-	-	-	-	-
26	VICIA FABA L.	LEGUMINOSAE	-	+	+	+	+	-	-	-	-
27	PHASEOLUS VULGARIS L.	"	-	-	-	-	-	-	-	-	-
28	PISUM SATIVUM L.	"	-	-	-	-	-	-	-	-	-
29	BRASSICA CAMPESTRIS L. VAR. SARSON	"	-	-	S.C.	-	-	-	-	-	-
30	SPINACEA OLERACEA L.	CRUCIFERAE	-	-	S.C.	-	-	-	-	-	-
31	CARICA PAPAYA L. VAR WASHINGTON SPECIAL	CARICACEAE	-	-	-	-	-	-	-	-	-
32	CHENOPODIUM AMARANTICOLOR COSTEET REYN	CHENOPODIACEAE	-	-	-	-	-	-	-	-	-
33	CHENOPODIUM QUINOA WILLD.	"	-	-	-	-	-	-	-	-	-
34	BETA VULGARIS L.	"	-	-	S.C.	-	-	-	-	-	-
35	VINCA ROSEA L.	APOCYNACEAE	-	-	-	-	-	-	-	-	-
36	GOMPHRENA GLOBOSA L.	AMARANTHACEAE	-	-	-	-	-	-	-	-	-
37	ZINNIA ELEGANS JASQ.	COMPOSITAE	-	-	-	-	-	-	-	-	-

+ SYMPTOMS APPEARED - NO SYMPTOMS APPEARED S.C. SYMPTOMLESS CARRIER

Isolate A₃

First visible symptoms appeared after 10 days of inoculation. The symptoms produced by the isolate A₃ were found to be similar to that found with the isolate A₁ (Plate IX, Figure 18). The results with the back inoculation to C. moschata Poir plants appeared to be similar to the isolate A₃ on C. moschata Poir (Table 6).

Isolate A₄

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the lamina. But when the isolate was back inoculated from the inoculated L. vulgaris to C. moschata plants, typical symptoms of isolate A₄ on C. moschata (Table 6) were observed on C. moschata.

Isolate A₅

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the lamina. But when the isolate was back inoculated from the inoculated L. vulgaris to C. moschata plants, typical symptoms of isolate A₅ on C. moschata (Table 6) were observed on C. moschata.

Isolate A₆

First visible symptoms appeared after 10 days

of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation the chlorotic spots gradually coalesced and the lamina appeared to be pale yellow in colour. The surface of the lamina showed mottling with irregularly scattered mild blisters. Within 30 days after inoculation slight rugosity and ill defined vein banding appeared on the surface of the lamina. At the advanced stage of infection the lamina appeared to be bleached and it became reduced in size. The shape became distorted with downward curving of the lamina (Plate IX, Figure 19).

When the isolate was back inoculated from L. vulgaris to C. moschata plants, symptoms similar to isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₇

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the lamina. But when the isolate was back inoculated from the inoculated L. vulgaris to C. moschata plants, typical symptoms of isolate A₇ on C. moschata (Table 6) were observed on C. moschata.

Isolate A₈

First visible symptoms appeared after 10 days of inoculation. The lamina became mottled and extremely reduced. It became pale greenish



Figure 20. Symptoms of
isolate A₈ on
L. vulgaris

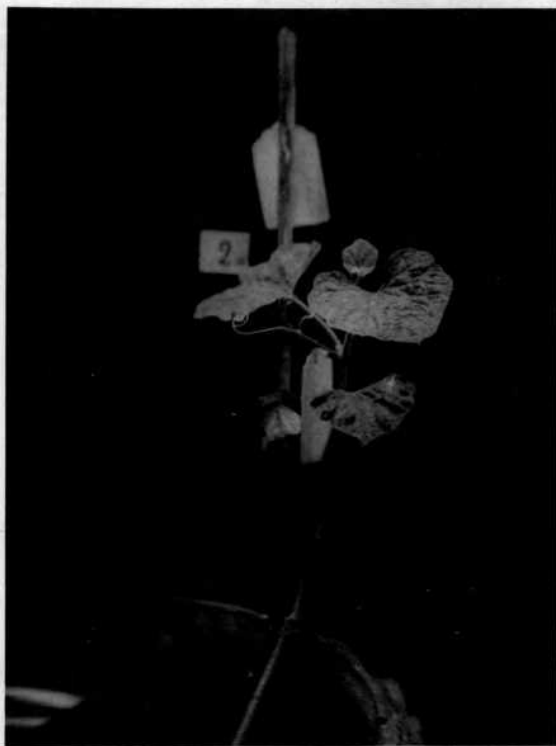


Figure 21. Symptoms of
isolate A₉ on
L. vulgaris

yellow in colour. Within 20 days of inoculation numerous green blisters appeared on the surface of the lamina. Within 30 days of inoculation green vein banding on the surface of the lamina appeared. The growth of the plant became severely stunted at the advanced stage of infection (Plate X, Figure 20).

When the isolate was back inoculated from L. vulgaris to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.

Isolate A₉

First visible symptoms appeared after 13 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days of inoculation the chlorotic spots gradually increased in size. The surface of the lamina became mottled. Within 30 days after inoculation, the entire lamina became chlorotic with green vein banding and irregular green blisters. At the advanced stage of infection, the growth of the plant was found to be stunted (Plate X, Figure 21).

When the isolate was back inoculated from L. vulgaris to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

Host 3. Momordica charantia L.

Isolate A₁

First visible symptoms appeared after 8 days of inoculation in the form of slight mottling with irregularly distributed small yellowish chlorotic spots on the surface of the lamina. Within 20 days after inoculation, slight blistering on the surface of the lamina was observed. Within 30 days after inoculation, the lamina appeared to be pale green in colour.

When the isolate was back inoculated from M. charantia to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

Even keeping the plants for 20 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated M. charantia to C. moschata plants, no symptoms of isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

First visible symptoms appeared after 10 days of inoculation in the form of irregular small chlorotic spots on the surface of the lamina. Within 20 days after inoculation, the chlorotic

spots gradually coalesced and the lamina appeared as pale green in colour. Within 30 days after inoculation, mottling with slight green blisters were observed on the surface of the lamina. At the advanced stage of infection, the size of the lamina was reduced. Growth of the plant was also found to be slightly stunted.

When the isolate was back inoculated from M. charantia to C. moschata plants, symptoms similar to isolate A₃ on C. moschata (Table 6) were observed.

Isolate A₄

First visible symptoms appeared after 8 days of inoculation in the form of irregular small chlorotic spots on the surface of the lamina and the lamina showed mottle to mosaic. Within 20 days after inoculation, slight vein yellowing on the surface of the lamina was observed. No additional symptoms on the surface of the lamina were observed within 30 days of inoculation.

When the isolate was back inoculated from M. charantia to C. moschata plants, symptoms similar to isolate A₄ on C. moschata (Table 6) were observed.

Isolate A₅

First visible symptoms appeared after 10 days of inoculation in the form of slight mottling with irregular chlorotic spots on the surface of

the lamina. Within 20 days of inoculation, the chlorotic spots gradually coalesced and the lamina appeared as pale yellow in colour. No additional symptoms on the surface of the lamina were observed within 50 days of inoculation.

When the isolate was back inoculated from infected M. charantia to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated M. charantia to C. moschata plants, no symptoms of isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₇

First visible symptoms were appeared after 10 days of inoculation on the surface of the lamina. The symptoms produced by the isolate A₇ were found to be similar to that found with the isolate A₁. The results with the back inoculation to C. moschata plants, appeared to be similar to the isolate A₇ on C. moschata (Table 6).

Isolate A₈

First visible symptoms appeared after 10 days of inoculation in the form of slight mottling and irregularly distributed yellow chlorotic spots on the surface of the lamina and the lamina appeared as pale yellow in colour. Within 20 days after inoculation, green blisters appeared on the surface of the lamina and it became puckered. No additional symptoms on the surface of the lamina were observed within 30 days after inoculation.

When the isolate was back inoculated from the infected M. charantia to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.

Isolate A₉

First visible symptoms appeared after 9 days of inoculation. The symptoms produced by the isolate A₉ were found to be similar to that found with the isolate A₅. The results with the back inoculation from infected M. charantia to C. moschata plants, appeared to be similar to that with the isolate A₉ on C. moschata (Table 6).

Host 4. Benincasa cerifera

Isolate A₁

First visible symptoms appeared after 13 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days of inoculation, the surface of the lamina became mottled. At the advanced stage of infection, the lamina became reduced in size and puckered. Slight rugosity was also observed on the surface of the lamina.

When the isolate was back inoculated from infected B. cerifera to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on surface of the lamina. When the isolate was back inoculated from inoculated B. cerifera to C. moschata plants, no symptoms of isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

First visible symptoms appeared after 10 days of inoculation in the form of mottling of lamina. Within 20 days after inoculation, irregular

chlorotic spots appeared on the entire surface of the lamina. The lamina became pale greenish in colour. The size of the lamina was found to be reduced.

When the isolate was back inoculated from infected B. cerifera to C. moschata plants, symptoms similar to that with the isolate A_3 on C. moschata (Table 6) were observed.

Isolate A_4

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated B. cerifera to C. moschata plants, no symptoms of isolate A_4 on C. moschata (Table 6) were observed.

Similar results were obtained with the isolate A_5 , A_6 , A_7 and A_9 .

Isolate A_8

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated B. cerifera to C. moschata plants, typical symptoms of isolate A_8 on C. moschata (Table 6) were observed on C. moschata.

Host 5. Trichosanthes anguina L.

Isolate A₁

First visible symptoms appeared 12 days after inoculation in the form of irregular small chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed mottling and ill defined green vein banding. At the advanced stage of infection, the lamina appeared to be pale yellowish green in colour (Plate XI, Figure 22:10)

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

First visible symptoms appeared 10 days after inoculation in the form of irregular chlorotic spots on the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced in a linear way. The lamina appeared to be yellowish green in colour. The surface of the lamina showed slight mottling. At the advanced stage of infection, the margins of the lamina appeared to be slightly bleached (Plate XII, Figure 24:14).

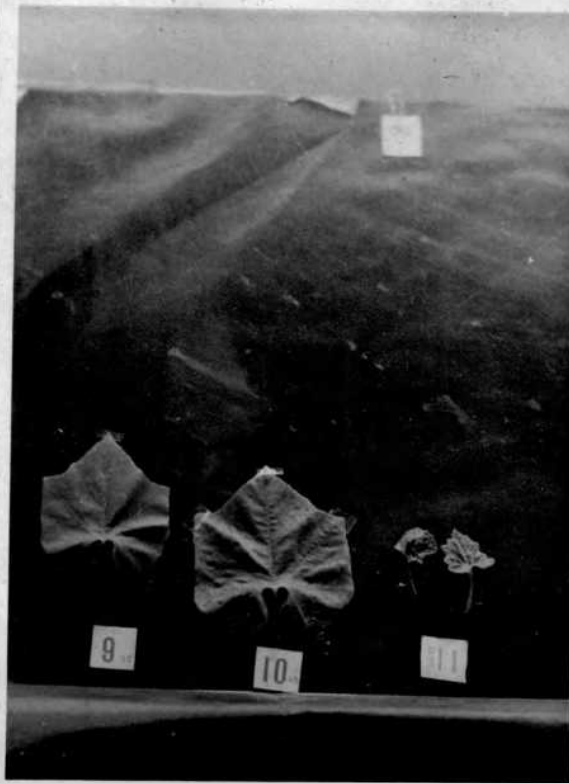


Figure 22. Symptoms of isolate A_1, A_4
on T.anguina
9=healthy, 10= A_1 , 11= A_4



Figure 23. Symptoms of
isolate A_5 on
T.anguina

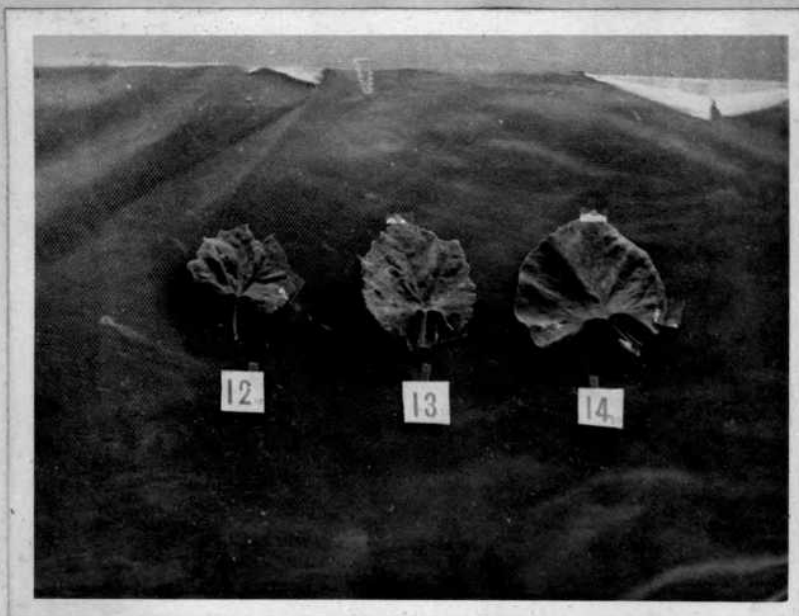


Figure 24. Symptoms of isolate
A₆, A₃, and A₂ on
T. anguina
12= A₆, 13=A₃, 14=A₂



Figure 25. Symptoms of isolate
A₉, A₇, A₈ on T. anguina
15=A₉, 16=A₇, 17=A₈

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed mottling with irregularly distributed green blisters. The shape of the lamina was slightly distorted and the size of it was slightly reduced (Plate XII, Figure 24:13).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₃ on C. moschata (Table 6) were observed.

Isolate A₄

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the chlorotic spots enlarged and gradually coalesced. The surface of the lamina showed intense mottling. At the advanced stage of infection, the size of the lamina was

extremely reduced and the shape of it was slightly distorted and it appeared to be bleached (Plate XI, Figure 22:11).

When the isolate was back inoculated from infected E. anguina to C. moschata plants, symptoms similar to isolate A₄ on C. moschata (Table 6) were observed.

Isolate A₅

First visible symptoms appeared after 9 days of inoculation in the form of numerous irregular yellow chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed mottling and green blisters were found around the veins (Plate XI, Figure 23).

When the isolate was back inoculated from infected E. anguina to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots on the surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed prominent mottling with irregular dark green

blisters. At the advanced stage of infection, the size of the lamina was found to be reduced and curved downwards(Plate XII, Figure 24:12).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₇

First visible symptoms appeared after 11 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina and the lamina appeared to be pale yellowish in colour. Within 20 days after inoculation, the surface of the lamina was slightly mottled and dark green. Blisters were found to be irregularly distributed on it. The size of the lamina was slightly reduced (Plate XII, Figure 25:16).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₇ on C. moschata (Table 6) were observed.

Isolate A₈

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the surface of the lamina appeared

to be slightly mottled but it did not show any dark green blisters (Plate XII, Figure 25:17).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.

Isolate A₉

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina appeared pale yellowish in colour. The surface of the lamina showed slight mottling and irregularly distributed dark green blisters. The surface of the lamina also became slightly wrinkled (Plate XII, Figure 25:15).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

extremely reduced and the shape of it was slightly distorted and it appeared to be bleached (Plate XI, Figure 22:11).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₄ on C. moschata (Table 6) were observed.

Isolate A₅

First visible symptoms appeared after 9 days of inoculation in the form of numerous irregular yellow chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed mottling and green blisters were found around the veins (Plate XI, Figure 23).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots on the surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed prominent mottling with irregular dark green

blisters. At the advanced stage of infection, the size of the lamina was found to be reduced and curved downwards(Plate XII, Figure 24:12).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₇

First visible symptoms appeared after 11 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina and the lamina appeared to be pale yellowish in colour. Within 20 days after inoculation, the surface of the lamina was slightly mottled and dark green. blisters were found to be irregularly distributed on it. The size of the lamina was slightly reduced (Plate XII, Figure 25:16).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₇ on C. moschata (Table 6) were observed.

Isolate A₈

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the surface of the lamina appeared

to be slightly mottled but it did not show any dark green blisters (Plate XII, Figure 25:17).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.

Isolate A₉

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina appeared pale yellowish in colour. The surface of the lamina showed slight mottling and irregularly distributed dark green blisters. The surface of the lamina also became slightly wrinkled (Plate XII, Figure 25:15).

When the isolate was back inoculated from infected T. anguina to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

Host 6. Luffa cylindrica Roem.

Isolate A₁

First visible symptoms appeared after 6 days of inoculation in the form of minute chlorotic spots at the margin of the lamina and the lamina appeared to be pale greenish yellow in colour. Within 20 days of inoculation, the surface of the lamina was slightly mottled. The spots remained to be minute and pale yellow in colour.

When the isolate was back inoculated from infected L. cylindrica to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated L. cylindrica to C. moschata plants, symptoms similar to isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated L. cylindrica to C. moschata plants, no symptoms of isolate A₃

on C. moschata (Table 6) were observed.

Similar results were obtained with the isolates A₅, A₇ and A₈.

Isolate A₄

First visible symptoms appeared after 6 days of inoculation. The symptoms produced by the isolate A₄ were found to be similar to that found with the isolate A₁. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₄ on C. moschata (Table 6).

Isolate A₆

First visible symptoms appeared after 7 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually spread on the entire surface of the lamina. The surface of the lamina showed slight mottling. The spots remained to be minute and pale yellow in colour.

When the isolate was back inoculated from the infected L. cylindrica to C. moschata plants, symptoms similar to that with the isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₉

First visible symptoms appeared after 7 days of inoculation in the form of irregular isolated chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed slight mottling. At the advanced stage of infection, slight vein clearing was observed. The size of the lamina was also found to be slightly reduced with downward curving of the margin.

When the isolate was back inoculated from the infected L. cylindrica to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

Host 7. Luffa acutangula Roxb.Isolate A₁

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated L. acutangula to C. moschata plants, no symptoms of isolate A₁ on C. moschata (Table 6) were observed.

Similar results were obtained with the isolates A₄ and A₃.

Isolate A₂

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated L. acutangula to C. moschata plants, symptoms similar to that with the isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

First visible symptoms appeared after 6 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the surface of the lamina appeared to be slightly mottled. Within 30 days after inoculation, the leaves became extremely reduced, bleached and puckered. At the advanced stage of infection, the plants became severely stunted (Plate XIII, Figure 26).

When the isolate was back inoculated from the infected L. acutangula to C. moschata plants, symptoms similar to isolate A₃ on C. moschata (Table 6) were observed.

Figure 26. Symptoms
of isolate
A₃ on
L. acutangula



Figure 27. Symptoms of isolate A₅ on
L. acutangula



Figure 28. Symptoms of isolate A₇
on L. acutangula

Isolate A₅

First visible symptoms appeared after 7 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the spots gradually became increased in number and appeared to be distorted on the entire lamina. Within 30 days after inoculation, the spots remained minute and gradually became slightly necrotic. The surface of the lamina showed slight mottling (Plate XIII, Figure 27).

When the isolate was back inoculated from infected L. acutangula to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

First visible symptoms appeared after 6 days of inoculation. The symptoms produced by the isolate A₆ were found to be similar to that found with the isolate A₅. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₆ on C. moschata (Table 6).

Isolate A₇

First visible symptoms appeared after 7 days of inoculation in the form of numerous irregularly distributed chlorotic spots on the

entire surface of the lamina and the lamina appeared as slightly pale yellow in colour. Within 20 days after inoculation, the surface of the lamina showed slight mottling. Within 30 days after inoculation, the chlorotic spots were minute, gradually became bleached to slightly necrotic (Plate XIII; Figure 28).

When the isolate was back inoculated from infected L. acutangula to C. moschata plants, symptoms similar to isolate A₇ on C. moschata (Table 6) were observed.

Isolate A₉

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated L. acutangula to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

Host 8. Citrullus vulgaris Schrad var. fistulosus

Isolate A₁

First visible symptoms appeared after 6 days of inoculation in the form of irregular chlorotic spots appeared at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina became

pale yellow in colour. The surface of the lamina showed prominent blistering and slight vein yellowing. At the advanced stage of infection, the entire lamina became intense yellow with a limited green tissue distributed at the margin or around the veins. The growth of the plant was found to be stunted.

When the isolate was back inoculated from infected C. vulgaris var. fistulosus to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated C. vulgaris var. fistulosus to C. moschata plants, symptoms similar to isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

First visible symptoms appeared after 8 days of inoculation. The symptoms produced by the isolate A₃ were found to be similar to that found with the isolate A₁. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₃ on C. moschata (Table 6).

Isolate A₄

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated C. vulgaris var. fistulosus to C. moschata plants, typical symptoms of isolate A₄ on C. moschata (Table 6) were observed on C. moschata.

Isolate A₅

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated C. vulgaris var. fistulosus to C. moschata plants, typical symptoms of isolate A₅ on C. moschata (Table 6) were observed on C. moschata.

Isolate A₆

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated C. vulgaris var. fistulosus to C. moschata plants, no symptoms of isolate A₆ on C. moschata (Table 6) were observed.

Similar observation was made with isolate A₇ and isolate A₉.

Isolate A₈

First visible symptoms appeared after 8 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared as pale yellow in colour. Within 20 days after inoculation, the surface of the lamina showed mottling with mild blisters but no vein yellowing was observed. At the advanced stage of infection, the entire lamina became intensely yellow and the size of the lamina became reduced. The growth of the plant was found to be stunted.

When the isolate was back inoculated from infected C. vulgaris var. fistulosus to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.

Host 9. Citrullus lanatus cv. Charleston GrayIsolate A₁

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the surface of the lamina. Within 20 days after inoculation, the lamina showed mottling and appeared to be pale yellowish green in colour. No additional symptoms on the surface of the lamina were observed within 30 days after inoculation.

When the isolate was back inoculated from infected C. lanatus cv. Charleston Gray to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the spots spread over the entire surface of the lamina and the lamina appeared yellowish in colour. The surface of the lamina showed mottling and ill defined green bands appeared around the veins. Within 30 days after inoculation, the green bands gradually disappeared. The chlorotic spots did not completely coalesce and were found to remain more or less around the veins and in the interveinal tissues. No marked reduction in the size of the lamina was found and the growth of the plant was not found to be stunted (Plate XIV, Figure 29).

When the isolate was back inoculated from infected C. lanatus cv. Charleston Gray to C. moschata plants, symptoms similar to isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

First visible symptoms appeared after 12



Figure 29. Symptoms of
isolate A₂
on C. lanatus



Figure 30. Symptoms of
isolate A₅
on C. lanatus

days of inoculation. The symptoms produced by the isolate A₃ were found to be similar to that found with the isolate A₁. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₃ on C. moschata (Table 6)

Isolate A₄

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated C. lanatus cv. Charleston Gray to C. moschata plants, typical symptoms of isolate A₄ on C. moschata (Table 6) were observed.

Isolate A₅

First visible symptoms appeared after 9 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina appeared to be chlorotic. The surface of the lamina showed mottling and puckering. Within 30 days after inoculation, the size of the lamina was reduced. The shape of it appeared to be filiform and the growth of the plant was severely stunted (Plate XIV, Figure 30).

When the isolate was back inoculated from infected C. lanatus cv. Charleston Gray to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

Even keeping the plants for 30 days after inoculations, no visible symptoms were appeared on the surface of the lamina. But when the isolate was back inoculated from inoculated C. lanatus cv. Charleston Gray to C. moschata plants, typical symptoms of isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₇

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared to be pale yellow in colour. Within 20 days after inoculation, the surface of the lamina showed slight mottling but no puckering was observed. Within 30 days after inoculation the lamina was found to be slightly distorted.

Isolate A₈

First visible symptoms appeared after 13 days of inoculation. The symptoms produced by

the isolate A_8 were found to be similar to that found with the isolate A_7 . The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A_8 on C. moschata (Table 6).

Isolate A_9

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated C. lanatus cv. Charleston Gray to C. moschata plants, no symptoms of isolate A_9 on C. moschata (Table 6) were observed.

Host 10. Cucurbita pepo L. cv. Early Prolific Straightneck

Isolate A_4

First visible symptoms appeared after 7 days of incubation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and spread on the entire surface of the lamina. The surface of the lamina showed vein yellowing and mottling with scattered dark green blisters. Within 30 days after inoculation, ill defined vein banding and puckering of the lamina were observed. The size of the leaf became reduced. The furrows of the lamina became slightly



Figure 31. Healthy plant
of C. pepo



Figure 32. Symptoms of
isolate A,
on C. pepo

deeper (Plate XV, Figure 32).

When the isolate was back inoculated from C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A_1 on C. moschata (Table 6) were observed.

Isolate A_2

First visible symptoms appeared after 7 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared yellowish in colour. Within 20 days after inoculation, the surface of the lamina showed dark green blisters and slight vein banding. Slight puckering of the lamina was also observed. Within 30 days after inoculation veins became yellow and the green bands around the veins gradually disappeared. The margins of the lamina became yellow and the shape of it became distorted (Plate XVI, Figure 33).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A_2 on C. moschata (Table 6) were observed.

Isolate A_3

First visible symptoms appeared after 6 days of inoculation in the form of mottling of the



Figure 33. Symptoms of isolate A₂ on C. pepo



Figure 34. Symptoms of isolate A₃ on C. pepo



Figure 35. Symptoms of isolate A₄ on C. pepo

lamina and dark green bands appeared around the veins. Within 20 days after inoculation, irregular chlorotic spots appeared on the surface of the lamina which gradually coalesced. Within 30 days after inoculation, the furrows of the lamina became deeper and the lamina became puckered. The size of the lamina was reduced and the shape of it was distorted (Plate XVI, Figure 34).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A₃ on C. moschata (Table 6) were observed.

Isolate A₄

First visible symptoms appeared after 7 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually advanced towards the base of the lamina. The lamina appeared greenish yellow in colour. The surface of the lamina showed mottling. Within 30 days after inoculation, slight puckering of the lamina and vein yellowing on the surface of the lamina were observed. There was no marked distortion of the shape of the lamina. The growth of the plant was, however, stunted (Plate XVI, Figure 35).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A_4 on C. moschata (Table 6) were observed.

Isolate A_5

First visible symptoms appeared after 7 days of inoculation in the form of irregular isolated chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina appeared chlorotic yellow. The surface of the lamina showed slight blistering. Within 30 days after inoculation, the leaves became extremely reduced in size, lamina became puckered and the furrows of the lamina became very deep so that the lamina appeared filiform. The growth of the plant was also stunted (Plate XVII, Figure 37).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A_5 on C. moschata (Table 6) were observed.

Isolate A_6

First visible symptoms appeared after 7 days of inoculation in the form of irregular chlorotic spots appeared on the entire surface



Figure 36. Symptoms of isolate A₆ on C. pepo



Figure 37. Symptoms of isolate A₅ on C. pepo



Figure 38. Symptoms of isolate A₇ on C. pepo

of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina appeared chlorotic yellow. The surface of the lamina showed mottling and the veins became yellow. Within 30 days after inoculation, the surface of the lamina became crinkled and puckered. The size of the lamina was reduced. The furrows of the lamina became slightly deeper and the shape of the lamina appeared to be slightly distorted (Plate XVII, Figure 36).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₇

First visible symptoms appeared after 6 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots appeared on the entire surface of the lamina and the lamina appeared yellowish in colour. The surface of the lamina showed mottling, mild green vein banding and slight blistering. Within 30 days after inoculation, the green bands around the veins gradually disappeared and the veins became yellow. The size of the lamina became reduced

The furrows of the lamina became deeper so that the lamina appeared to be filiform. The lamina became slightly puckered and the shape of it appeared to be distorted(Plate XVII, Figure 38).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A₇ on C. moschata (Table 6) were observed.

Isolate A₈

First visible symptoms appeared after 8 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina and the lamina appeared pale greenish yellow in colour. Within 20 days after inoculation, the surface of the lamina showed slight mottling and vein yellowing. Within 30 days after inoculation, slight puckering of the lamina was observed. The furrows of the lamina appeared to be slightly deeper. The size of the lamina was slightly reduced and the shape of it was slightly distorted (Plate XVIII, Figure 39).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.



Figure 39. Symptoms of isolate A₈
on C. pepo



Figure 40. Symptoms of isolate A₉
on C. pepo

Isolate A₉

First visible symptom appeared after 7 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina and the lamina appeared pale green in colour. Within 20 days after inoculation, the chlorotic spots spread towards the base of the lamina. The surface of the lamina showed prominent dark green vein banding and the lamina became slightly mottled. The size of the lamina reduced. Within 30 days after inoculation, size and shape of the lamina was found to be distorted. Slight puckering on young apical lamina was observed. Infected lamina did not show any blistering and vein yellowing. The growth of the plant was found to be stunted (Plate XVIII, Figure 40).

When the isolate was back inoculated from infected C. pepo cv. Early Prolific Straightneck to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

Host 11. Cucumis melo L. Selection B 63-3Isolate A₁

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was

back inoculated from inoculated C. melo Selection B 63-3 to C. moschata plants, no symptoms of isolate A₁ on C. moschata (Table 6) were observed.

Similar results were also obtained with isolate A₂, A₃, A₇, A₈ and A₉.

Isolate A₄

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared pale greenish in colour. Within 20 days after inoculation, the surface of the lamina showed slight vein yellowing and dark green blisters (Plate XIX, Figure 41).

When the isolate was back inoculated from infected C. melo Selection B 63-3 to C. moschata plants symptoms similar to isolate A₄ on C. moschata (Table 6) were observed.

Isolate A₅

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the chlorotic spots gradually coalesced and the lamina appeared yellowish green in colour. The surface of the

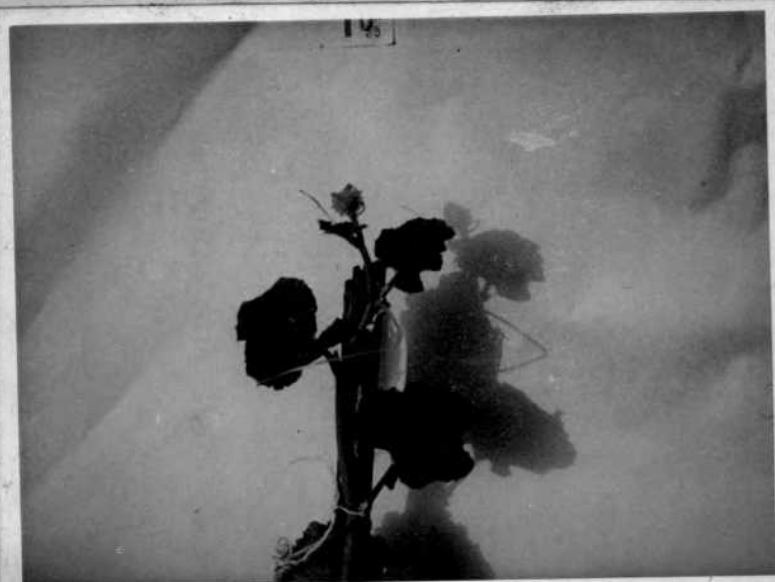


Figure 41. Symptoms of isolate A₄
on C. melo



Figure 42. Symptoms of isolate A₅
on C. melo

lamina showed mosaic mottling and vein yellowing. At the advanced stage of infection, deep green blisters appeared on the surface of the lamina and the size of the lamina was found to be slightly reduced (Plate XIX, Figure 42).

When the isolate was back inoculated from infected C. melo Selection B 63-3 to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

First visible symptoms appeared after 10 days of inoculation. The symptoms produced by the isolate A₆ were found to be similar to that found with the isolate A₄. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₆ on C. moschata (Table 6)

Host 12. Cucumis sativus L. cv. Local White

Isolate A₁

First visible symptoms appeared after 9 days of inoculation in the form of mild chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina showed mottling and slight vein clearing. Within 30 days after inoculation, dark green blisters were appeared on the surface of the lamina.

When the isolate was back inoculated from infected C. sativus cv. Local White to C. moschata plants, symptoms similar to isolate A₁ on C. moschata (Table 6) were observed.

Isolate A₂

First visible symptoms appeared after 10 days inoculation. The symptoms produced by the isolate A₂ were found to be similar to that found with the isolate A₁. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₂ on C. moschata (Table 6).

Isolate A₃

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated C. sativus cv. Local White to C. moschata plants, no symptoms of isolate A₃ on C. moschata (Table 6) were observed.

Similar results were also obtained with isolate A₇.

Isolate A₄

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina

showed mottling and prominent vein clearing. Within 30 days after inoculation, occasional vein banding at the marginal portion of the lamina was observed. The size of the lamina was found to be reduced.

When the isolate was back inoculated from infected C. sativus cv. Local White to C. moschata plants, symptoms similar to isolate A₄ on C. moschata (Table 6) were observed.

Isolate A₅

First visible symptoms appeared after 12 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared to be yellow in colour. Within 20 days after inoculation, the surface of the lamina showed mottling and vein clearing. Within 30 days after inoculation, less pronounced green bands appeared around the veins. The size of the lamina was also reduced.

When the isolate was back inoculated from infected C. sativus cv. Local White to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

First visible symptoms appeared after 7 days of inoculation in the form of irregularly

distributed chlorotic spots on the entire surface of the lamina and the lamina appeared to be yellow in colour. Within 20 days after inoculation, the lamina showed mild mottling with slight vein clearing. No additional symptoms were observed on the lamina at 30 days after inoculation.

When the isolate was back inoculated from infected C. sativus cv. Local White to C. moschata plants, symptoms similar to isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₈

First visible symptoms appeared after 9 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared to be yellow in colour. Within 20 days after inoculation, the surface of the lamina showed mottling and prominent vein clearing. Irregularly distributed raised dark green areas were observed on the surface of the lamina. Within 30 days after inoculation, green vein banding was also occasionally observed on the lamina. At the advanced stage of infection, downward curving of the lamina was observed and the size of the lamina was found to be reduced (Plate XX, Figure 43).

When the isolate was back inoculated from infected C. sativus cv. Local White to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) was observed.

Isolate A₉

First visible symptoms appeared after 10 days of inoculation. The symptoms produced by the isolate A₉ were found to be similar to that found with the isolate A₈. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₉ on C. moschata (Table 6).

Host 13. Cucumis sativus L. Cv. Long Green

Isolate A₁

First visible symptoms appeared after 10 days of inoculation. The symptoms produced by the isolate A₁ were found to be similar to that found with the isolate A₁ on C. sativus cv. Local White. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₁ on C. moschata (Table 6).

Isolate A₂

First visible symptoms appeared after 10 days of inoculation in the form of irregular

chlorotic spots on the entire surface of the lamina and the lamina appeared to be yellow in colour. Within 20 days after inoculation, the surface of the lamina showed mottling and vein clearing. Within 30 days after inoculation, the chlorotic areas became more enlarged and the size of the lamina became reduced.

When the isolate was back inoculated from infected C. sativus cv. Long Green to C. moschata plants, symptoms similar to isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated C. sativus cv. Long Green to C. moschata plants, no symptoms of isolate A₃ on C. moschata (Table 6) were observed.

Similar results were also obtained with the isolate A₇.

Isolate A₄

First visible symptoms appeared after 12 days of inoculation. The symptoms produced by the isolate A₄ were found to be similar to that found with the isolate A₄ on C. sativus cv. Local

White. The results with the back inoculation to C. moschata plants appeared to be similar to the isolate A₄ on C. moschata (Table 6).

Isolate A₅

First visible symptoms appeared after 10 days of inoculation in the form of small chlorotic spots on the entire surface of the lamina and the lamina showed mosaic to mottle in appearance. Within 20 days after inoculation, the surface of the lamina showed slight vein clearing. Within 30 days after inoculation, the size of the lamina was found to be reduced and the growth of the plant was found to be stunted.

When the isolate was back inoculated from infected C. sativus cv. Long Green to C. moschata plants, symptoms similar to isolate A₅ on C. moschata (Table 6) were observed.

Isolate A₆

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots on the entire surface of the lamina and the lamina appeared yellowish in colour. Within 20 days after inoculation, the surface of the lamina showed mottling and slight green vein banding. No additional symptoms on the lamina



Figure 43. Symptoms of isolate A₃
on C. sativus

were observed at 30 days after inoculation.

When the isolate was back inoculated from infected C. sativus cv. Long Green to C. moschata plants, symptoms similar to isolate A₆ on C. moschata (Table 6) were observed.

Isolate A₈

First visible symptoms appeared after 11 days of inoculation. The symptoms produced by the isolate A₈ were found to be similar to that found with the isolate A₈ on C. sativus cv. Local White. The results with the back inoculation from inoculated C. sativus cv. Long Green to C. moschata plants, symptoms similar to isolate A₈ on C. moschata (Table 6) were observed.

Isolate A₉

First visible symptoms appeared after 10 days of inoculation in the form of irregular chlorotic spots at the margin of the lamina. Within 20 days after inoculation, the chlorotic spots gradually extended to the entire surface of the lamina. The surface of the lamina showed mottling and slight vein banding. Within 30 days after inoculation, a few dark green blisters were also found on the surface of the lamina. The size of the lamina was found to be reduced.

When the isolate was back inoculated from infected C. sativus cv. Long Green to C. moschata plants, symptoms similar to isolate A₉ on C. moschata (Table 6) were observed.

Host 14. Capsicum annuum L. var. Suryamukhi

No visible symptoms appeared within 30 days after inoculation with any of the isolates. On back inoculation of C. annuum var. Suryamukhi to Suryamukhi to C. moschata also did not produce any symptom on C. moschata.

Host 15. Datura stramonium L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. On back inoculation of D. stramonium to C. moschata also did not produce any symptom on C. moschata.

Host 16. Lycopersicon esculentum Mill. var. Marglobe Supreme

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of L. esculentum var. Marglobe Supreme to C. moschata also did not produce any symptom on C. moschata.

Host 17. Solanum tuberosum L. var. Kufri Chandramukhi

No visible symptoms appeared within 30 days after inoculation with any of the isolates.

Back inoculation of S. tuberosum var. Kufri Chandramukhi to C. moschata also did not produce any symptom on C. moschata.

Host 18. Solanum melongena L. var. Mukta Keshi

Isolate A₁

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated S. melongena var. Mukta Keshi to C. moschata plants, no symptoms of isolate A₁ on C. moschata (Table 6) were observed.

Similar results were also obtained with isolate A₄, A₆, A₇, A₈ and A₉.

Isolate A₂

First visible symptoms appeared after 20 days of inoculation in the form of very mild mottle to mosaic symptoms on the surface of the lamina which could be clearly seen when the inoculated leaves were examined by placing them against light. At the advanced stage of infection, the size of the lamina was found to be slightly reduced.

When the isolate was back inoculated from infected S. melongena var. Mukta Keshi to C. moschata plants, symptoms similar to isolate A₂ on C. moschata (Table 6) were observed.

Isolate A₃ and Isolate A₅

First visible symptoms appeared after 15 days of inoculation. The symptoms produced by the isolate A₃ and Isolate A₅ were found to be similar to that found with the isolate A₂. The results with the back inoculation to C. moschata plants appeared to similar to the isolate A₃ on C. moschata (Table 6) and A₅ on C. moschata (Table 6).

Host 19. Nicotiana tabacum L.

No visible symptoms appeared within 30 days after inoculation with any of the isolate. Back inoculation of N. tabacum to C. moschata also did not produce any symptom on C. moschata.

Host 20. Nicotiana tabacum L. cv. Virginia Gold

No visible symptoms appeared within 30 days after inoculation with any of the isolate. Back inoculation of N. tabacum cv. Virginia Gold to C. moschata also did not produce any symptom on C. moschata.

Host 21. Nicotiana tabacum L. Cv. Xanthi

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of N. tabacum cv. Xanthi to C. moschata also did not produce any symptom on C. moschata.

Host 22. Nicotiana glutinosa L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of N. glutinosa to C. moschata also did not produce any symptom on C. moschata.

Host 23. Nicotiana sylvestris L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of N. sylvestris to C. moschata also did not produce any symptom on C. moschata.

Host 24. Nicotiana rustica L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of N. rustica to C. moschata also did not produce any symptom on C. moschata.

Host 25. Petunia hybrida Vilm.

Isolate A₁

Even keeping the plants for 30 days after inoculation, no visible symptoms were appeared on the surface of the lamina. When the isolate was back inoculated from inoculated P. hybrida to C. moschata plants, no symptoms of isolate A₁ on C. moschata (Table 6) were observed.



Figure 44. Symptoms of
isolate A₂
on P.hybrida



Figure45. Symptoms of
isolate A₅ on
P.hybrida

Similar results were also obtained with the isolate A_4 , A_6 , A_7 , A_8 and A_9 .

Isolate A_2

First visible symptoms appeared after 10 days of inoculation in the form of small yellow spots irregularly distributed on the entire surface of the lamina. Within 20 days after inoculation, the surface of the lamina appeared to be mottled with slight interveinal chlorosis. The margin of the lamina showed upward curving (Plate XXI, Figure 44).

When the isolate was back inoculated from infected *P. hybrida* to *C. moschata* plants, symptoms similar to isolate A_1 on *C. moschata* (Table 6) were observed.

Isolate A_3

First visible symptoms appeared after 9 days of inoculation. The symptoms produced by the isolate A_3 were found to be similar to that found with the isolate A_2 . The results with the back inoculation to *C. moschata* plants appeared to be similar to the isolate A_3 on *C. moschata* (Table 6).

Isolate A_5

First visible symptoms appeared after 10 days

of inoculation in the form of irregular yellow spots on the lamina. Within 20 days after inoculation the surface of the lamina showed mottling and interveinal chlorosis. The margins of the lamina showed upward curving. The size of the leaf (including lamina and the petiole) was found to be reduced. The length of the internodes was also reduced. As a result, clustering of the leaves took place which gave a rosette like appearance for the plant (Plate XXI, Figure 45.).

When the isolate was back inoculated from infected P. hybrida to C. moschata plants, symptoms similar to isolate A₄ on C. moschata (Table 6) were observed.

Host 26. Vicia faba L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of V. faba to C. moschata also did not produce any symptom on C. moschata.

Host 27. Phaseolus vulgaris L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of P. vulgaris to C. moschata also did not produce any symptom on C. moschata.

Host 28. Pisum sativum L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of P. sativum to C. moschata also also did not produce any symptom on C. moschata.

Host 29. Brassica campestris L. var. Sarson.

No visible symptoms developed within 30 days after inoculation with any of the isolates. On back inoculation from B. campestris var. Sarson to C. moschata however, isolate A₃ produced typical symptoms on C. moschata.

Host 30. Spinacea oleracea L.

No visible symptoms developed within 30 days after inoculation with any of the isolates. But on back inoculation from S. oleracea to C. Moschata only isolate A₃ produced typical symptoms on C. moschata.

Host 31. Carica papaya L. var. Washington

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of C. papaya var. Washington to C. moschata also did not produce any symptom on C. moschata.

Host 32. Chenopodium amaranticolor Coste et Reyn

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of C. amaranticolor to C. moschata also did not produce any symptom on C. moschata.

Host 33. Chenopodium quinoa Willd.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of C. quinoa to C. moschata also did not produce any symptom on C. moschata.

Host 34. Beta vulgaris L.

No visible symptoms developed within 30 days after inoculation with any of the isolates. But on back inoculation from B. vulgaris to C. moschata only isolate A₃ produced typical symptoms on C. moschata.

Host 35. Vinca rosea L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of V. rosea to C. moschata also did not produce any symptom on C. moschata.

Host 36. Gomphrena globosa L.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of G. globosa to C. moschata

also did not produce any symptom on C. moschata.

Host 37. Zinnia elegans Jacq.

No visible symptoms appeared within 30 days after inoculation with any of the isolates. Back inoculation of Z. elegans to C. moschata also did not produce any symptom on C. moschata.

c) Insect transmissibility of the isolates

Data obtained on the transmissibility of the isolates for the type II symptom complex by A. gossypii, M. persicae and B. tabaci have been presented in the Table-12.

Data obtained on the persistence of isolates in A. gossypii, M. persicae and B. tabaci have been presented in the Table - 13.

It is evident from the Table-12 that A. gossypii transmitted the isolates A₅, A₇, A₈ and A₉. The percentages of transmission of these isolates were found to be 37, 43, 29 and 22 respectively. Time required for the appearance of symptoms was found to be 5-7 days. These isolates however could not be transmitted by M. persicae and B. tabaci. Isolates A₄ and A₆ were found to be transmissible only by M. persicae and not by A. gossypii and B. tabaci. The percentage of transmission of these isolates were found to be 40 for both the case. Time required for the appearance of symptom was found to be 8 days. The isolate A₃ alone was found to be transmissible by B. tabaci. The percentage of transmission was only 32. Time required for the appearance of symptom was found to be 10 days. This isolate was however, could not be transmitted either by A. gossypii or M. persicae.

Table 12. Transmissibility of the isolates by Aphis gossypii Glover, Myzus persicae Suls. and Bemisia tabaci Genn.

Sl No.	Isolates	<u>Aphis gossypii</u>			<u>Myzus persicae</u>			<u>Bemisia tabaci</u>		
		No. of days required for the appearance of symptom	Percentage of infection	No. of days required for the appearance of symptom	Percentage of infection	No. of days required for the appearance of symptom	Percentage of infection	No. of days required for the appearance of symptom	Percentage of infection	No. of days required for the appearance of symptom
1	A ₁	-	-	-	-	-	-	-	-	-
2	A ₂	-	-	-	-	-	-	-	-	-
3	A ₃	-	-	-	-	-	-	10	32	-
4	A ₄	-	-	-	8	40	-	-	-	-
5	A ₅	5	37	-	-	-	-	-	-	-
6	A ₆	-	-	8	40	-	-	-	-	-
7	A ₇	7	43	-	-	-	-	-	-	-
8	A ₈	6	29	-	-	-	-	-	-	-
9	A ₉	6	22	-	-	-	-	-	-	-

In the experiment for the serial transmission of the respective isolates by A. gossypii, M. persicae and B. tabaci (Table-13), it was found that the percentages of transmission with respect to time varied with the isolates. Isolates A₅, A₇, A₈ and A₉ were A. gossypii transmissible. Once virulified with A₇ and A₉, the aphids could transmit the respective viruses upto 96 hours, but the aphids virulified with A₅ and A₈ could transmit the respective viruses upto 72 hours and 24 hours only. The isolates A₄ and A₆ were M. persicae transmissible. Once virulified, the aphids could transmit both the viruses upto 96 hours. The isolate A₃ was B. tabaci transmissible. Once virulified, this white fly could transmit this isolate upto 48 hours only.

Table 13. Serial transmission of the isolates at every 24 hours by the viruliferous
Aphis gossypii Glover, Myzus persicae Sulz. and Tomisia tabaci Germ. on
C. moschata Poir plants

Sl.No.	Isolates	<u>Aphis gossypii</u> Glover					<u>Myzus persicae</u> Sulz.					<u>Tomisia tabaci</u> Germ.				
		Percentage of infection					Percentage of infection					Percentage of infection				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
1	A ₁	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	A ₂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	A ₃	-	-	-	-	-	-	-	-	-	-	32	15	-	-	-
4	A ₄	-	-	-	-	-	40	29	25	9	-	-	-	-	-	-
5	A ₅	37	22	5	-	-	-	-	-	-	-	-	-	-	-	-
6	A ₆	-	-	-	-	-	40	23	18	10	-	-	-	-	-	-
7	A ₇	43	38	25	10	-	-	-	-	-	-	-	-	-	-	-
8	A ₈	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	A ₉	22	18	11	5	-	-	-	-	-	-	-	-	-	-	-

d) Electronmicroscopy of the isolates

Data obtained on the determination of the length and breadth of the virus particles of the isolates have been presented in the Table-14.

Table 14. Size of the virus particles of the nine different isolates

Sl.No.	Isolates	Average length(m/ μ) (Average of 10 observations)	Average Breadth (m/ μ) (Average of 10 observations)
1	A ₁	712.5	12.5
2	A ₂	587.5	14.0
3	A ₃	725.0	12.5
4	A ₄	525.0	12.5
5	A ₅	743.6	15.0
6	A ₆	675.0	12.5
7	A ₇	825.0	12.5
8	A ₈	874.9	20.8
9	A ₉	785.0	12.5

It is evident from the Table 14 and Plate XXII - XXIV, Figure 46-54 that the shape of all the virus particles appeared to be flexuous rods, but the size of them varied considerably. The length of the particles of the isolates varied from 525.0 m/ μ to 874.9 m/ μ and breadth varied from 12.5 m/ μ to 20.8 m/ μ . Lengths of virus particles in case of isolate A₄ and A₂ were found to be 525.0 m/ μ and 587.5 m/ μ respectively. The breadth of the particles of these two isolates was found to be 12.5 m/ μ and 14.0 m/ μ .

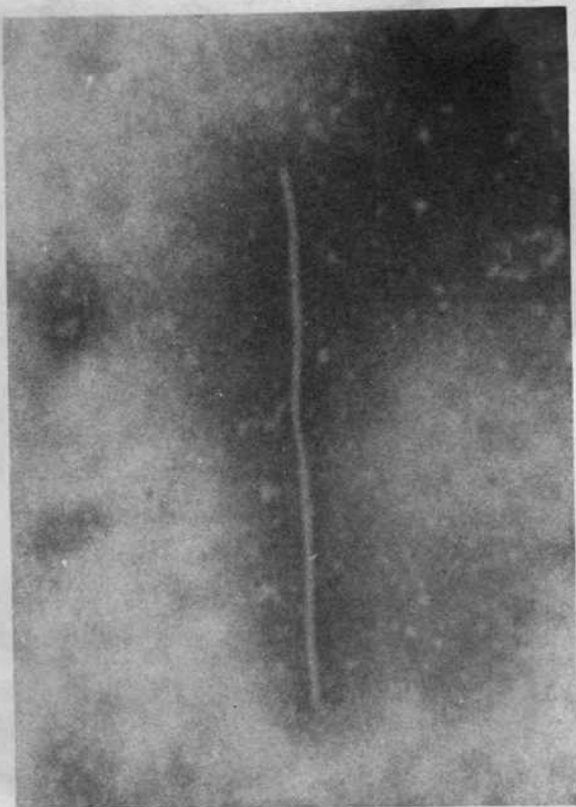


Figure 46. Virus particle of isolate A₁



Figure 47. Virus particle of isolate A₂

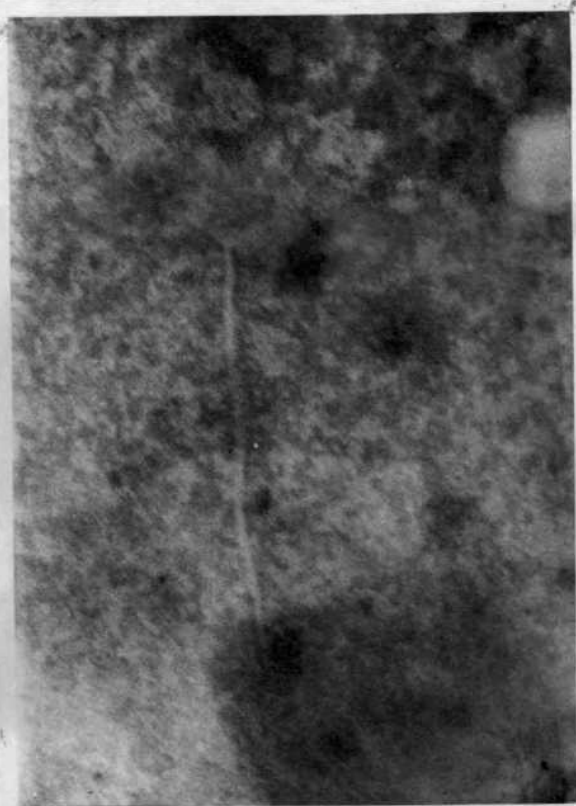


Figure 48. Virus particle of isolate A₃

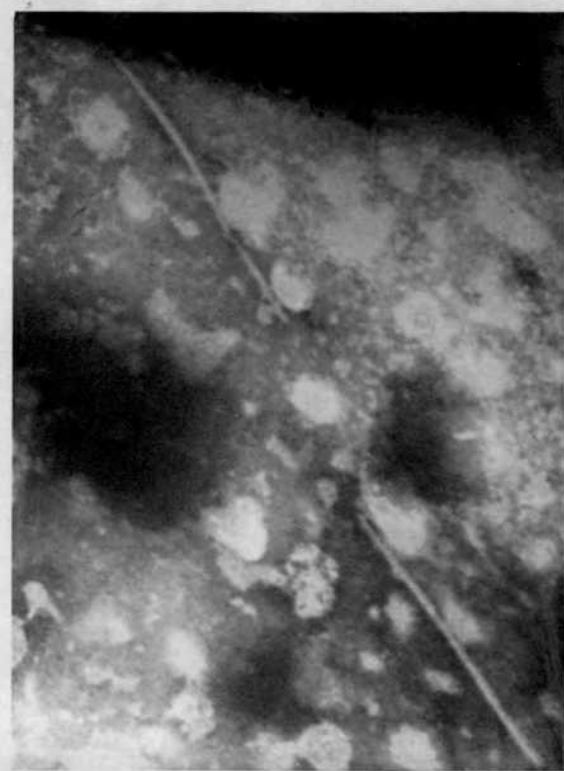


Figure 49. Virus particles of isolate A₄

PLATE -XXII Electronmicrographs of the particles from different isolates.

from different isolates.



Figure 54. Virus particles of isolate A₈

PLATE -XXIV Electronmicrographs of the particles
from different isolates.

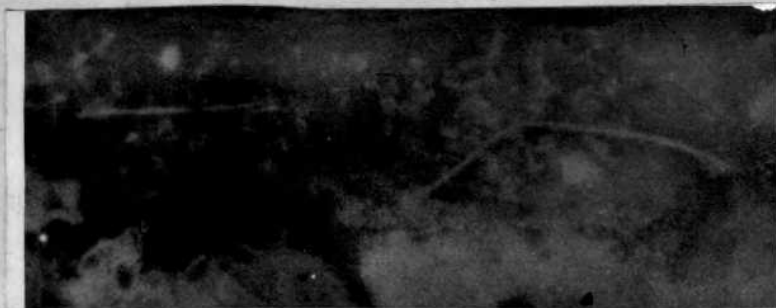


Figure 50. Virus particles
of isolate A₅

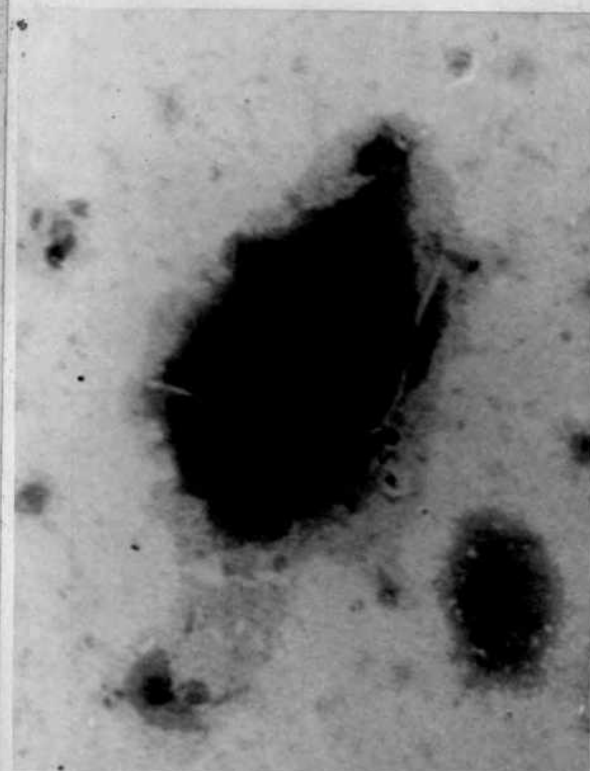


Figure 51. Virus particle
of isolate A₆



Figure 52. Virus particle
of isolate A₇



Figure 53. Virus particle
of isolate A₉

respectively. In case of isolate A_6 the length of the particle was found to be 675.0 m/ μ and breadth was found to 12.5 m/ μ . The length of the particles in case of isolate A_1 , A_3 , A_5 and A_9 varied from 712.5 m/ μ to 785.0 m/ μ . The respective length of the particles of these isolates was found to be 712.5 m/ μ , 725.0 m/ μ , 743.6 m/ μ and 785.0 m/ μ . The breadth of the particles in case of these isolates varied from 12.5 m/ μ to 15.0 m/ μ . In case of isolate A_1 , A_3 and A_9 the breadth of the particles was found to be 12.5 m/ μ whereas in case of isolate A_5 , the breadth was found to be 15.0 m/ μ . The length of the particles in case of isolate A_7 and A_8 was found to be 825.0 m/ μ and 874.9 m/ μ respectively and the breadth was found to be 12.5 m/ μ and 20.8 m/ μ respectively.

e) Serological relationship of the isolates

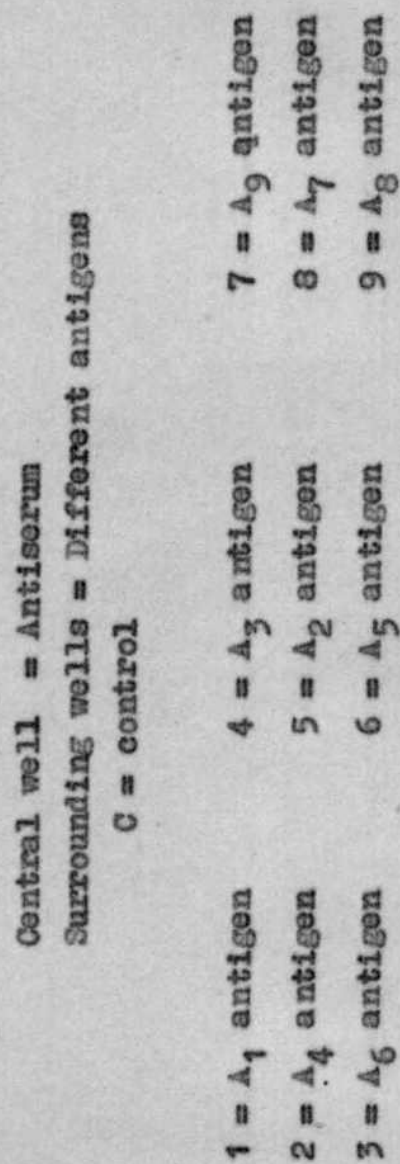
Data obtained on serological studies have been presented in the Table-15, Plate XXV- XXVI and Text figure 1-5.

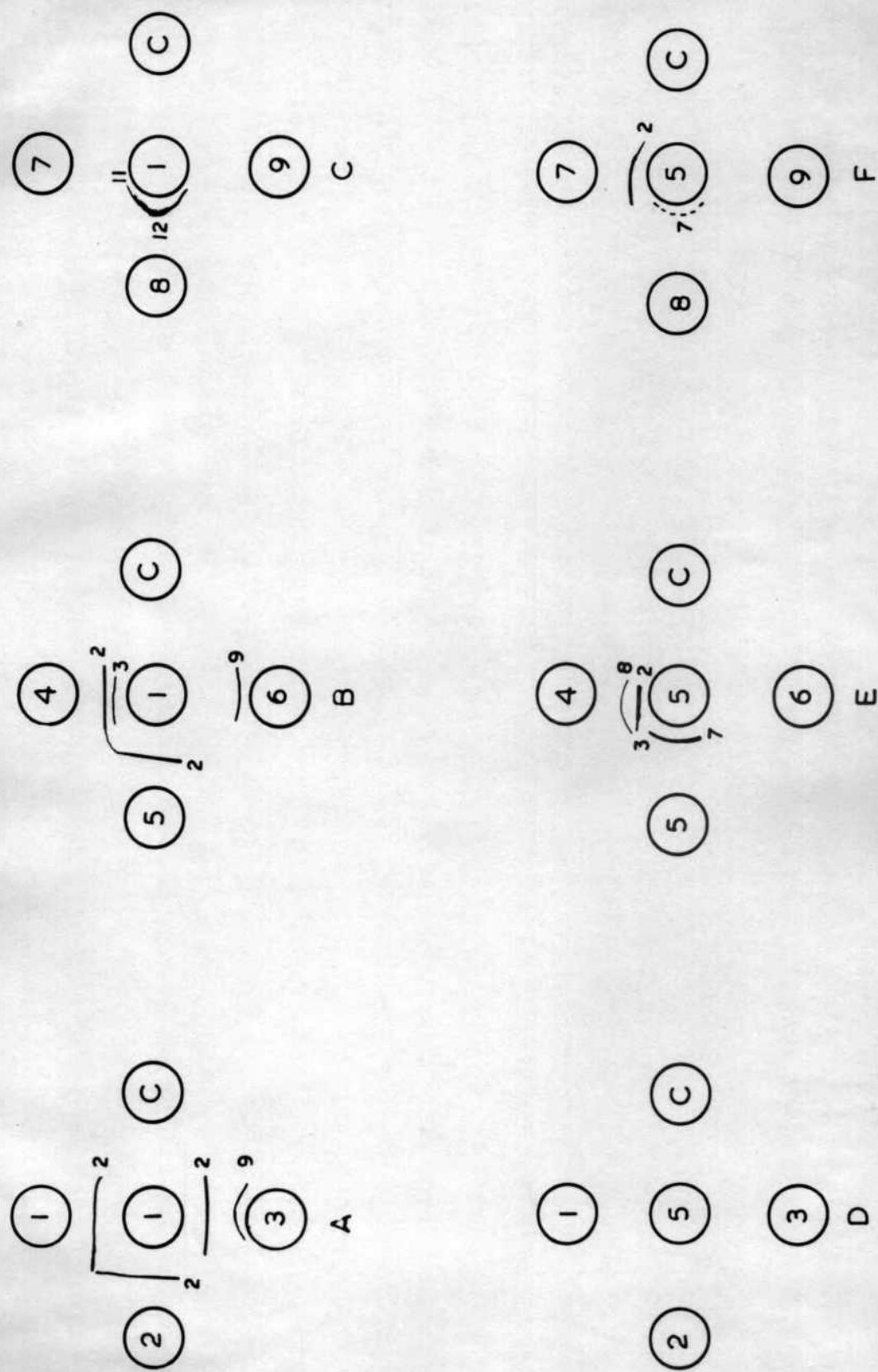
When the antigens of isolate A_2 , A_3 and A_5 were exposed to the antiserum of isolate A_1 (a_1) three characteristic precipitation bands (band 2, band 3 and band 9) appeared. But exposing the antigens of isolate A_1 , A_4 and A_6 to the same antiserum only two precipitation bands (Band 2 and Band 9) were obtained. Similar results were also obtained with the antigens of the isolate A_7 , A_8 and A_9 reacting with a_1 . But in this case precipitation band number 11 and 12 were observed (Text figure 1 (A,B,C) and Figure 59 (Plate XXV) and 66 (Plate XXVI) .

When the antigens of isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_2 (a_2) no precipitation bands were appeared. But exposing the antigens of the isolate A_2 , A_3 and A_5 to the same antiserum only four precipitation bands (band 2, band 3, band 7 and band 8) were obtained. Similar results were also obtained with the antigens of the isolate A_7 , A_8 and A_9 reacting with a_2 . But in this case precipitation band number 2 and 7 were observed (Text figure 1 (D,E and F).

When the antigens of isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_3 (a_3) two characteristic precipitation bands (band 2 and band 12) appeared. But exposing the antigens of isolate A_2 , A_3 and A_5 to the same antiserum three precipitation bands (band 2, band 5 and band 9) were obtained. With the antigens of the isolate A_7 , A_8 and A_9 reacting with the

Text Figure 1. Antigenic relationship of different isolates
(A diagrammatic representation)

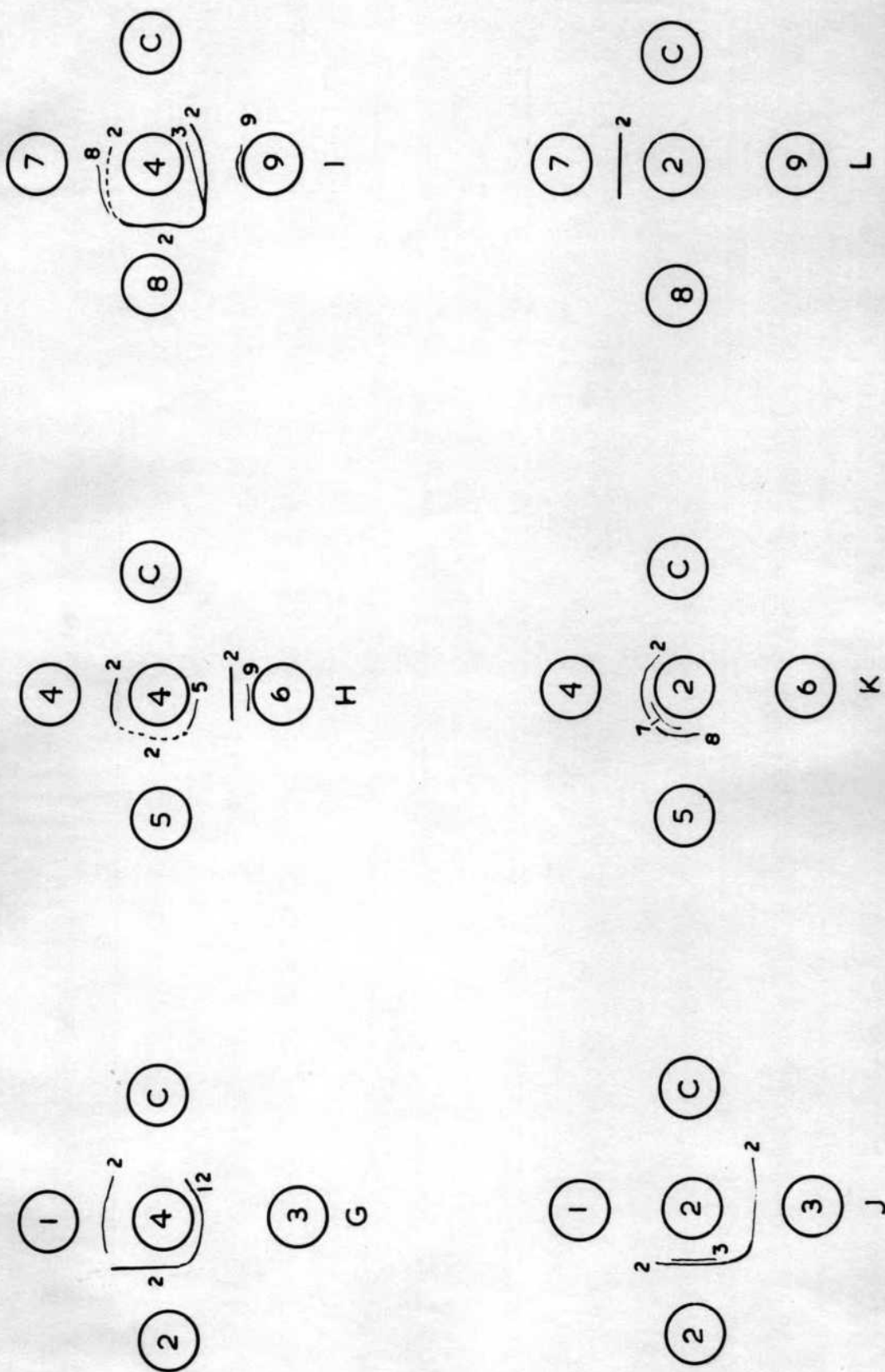




TEXT FIGURE 1. ANTIGENIC RELATIONSHIP OF DIFFERENT ISOLATES.

Text Figure 2. Antigenic relationship of different isolates
(A diagrammatic representation)

Central well		= Antiserum
Surrounding wells		= Different antigens
Control C = Control		
1 = A ₁ antigen	4 = A ₃ antigen	7 = A ₉ antigen
2 = A ₄ antigen	5 = A ₂ antigen	8 = A ₇ antigen
3 = A ₆ antigen	6 = A ₅ antigen	9 = A ₈ antigen



TEXT FIGURE 2. ANTIGENIC RELATIONSHIP OF DIFFERENT ISOLATES.

same antiserum four precipitation bands (band 2, band 3, band 8 and band 9) were observed (Text figure 2(G,H and I) and Figure 60 (Plate XXV), 62 and 64 (Plate XXVI).

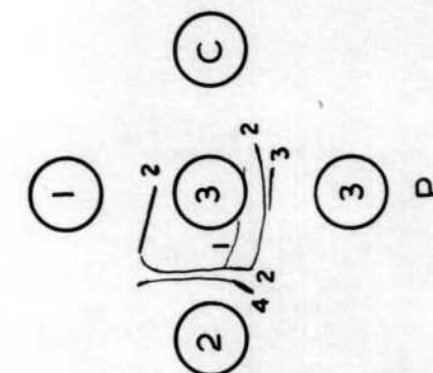
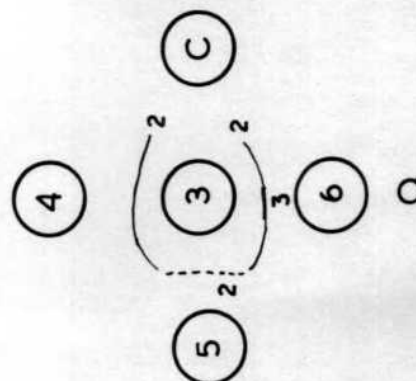
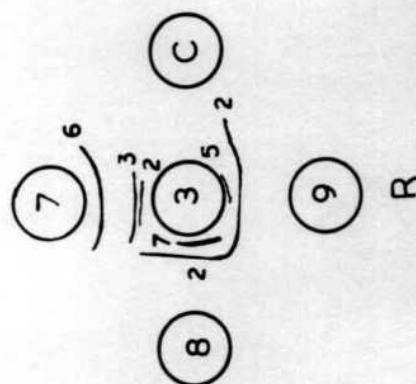
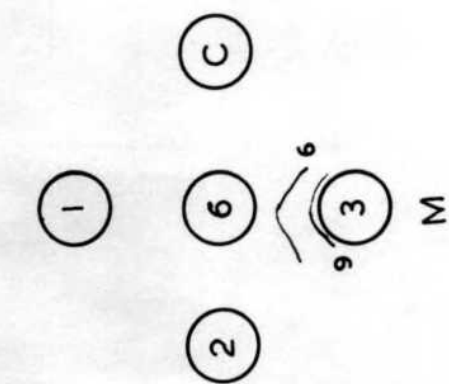
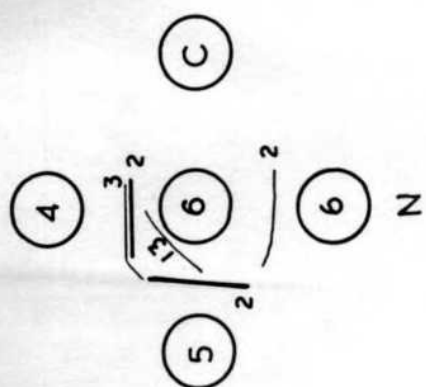
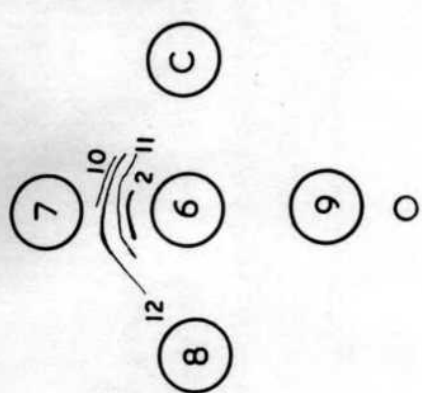
When the antigens of isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_4 (a_4) two precipitation bands (band 2 and band 3) appeared. But exposing the antigens of isolate A_2 , A_3 and A_5 to the same antiserum three characteristic precipitation bands (band 2, band 7 and band 8) were obtained. Reacting with the same antiserum the antigens of the isolate A_7 , A_8 and A_9 produced only one precipitation band (band 2) (Text Figure 2 (J, K and L) and Figure 65 (Plate XXVI).

When the antigens of the isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_5 (a_5) two characteristic precipitation bands (band 6 and band 9) were observed. But exposing the antigens of isolate A_2 , A_3 and A_5 to the same antiserum three characteristic precipitation bands (band 2, band 3 and band 13) appeared. Reacting with the same antiserum, the antigens of the isolate A_7 , A_8 and A_9 produced four precipitation bands (band 2, band 10, band 11 and band 12) (Text Figure 3 (M,N and O) and Figure 57 and 58 (Plate XXV).

When the antigens of the isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_6 (a_6) four precipitation bands (band 1, band 2, band 3 and band 4) appeared. But exposing the antigens of isolate A_2 , A_3 and A_5 to the same antiserum only two precipitation bands (band 2 and band 3) were observed. Reacting with the same antiserum, the antigens of the

Text Figure 3. Antigenic relationship of different isolates
(A diagrammatic representation)

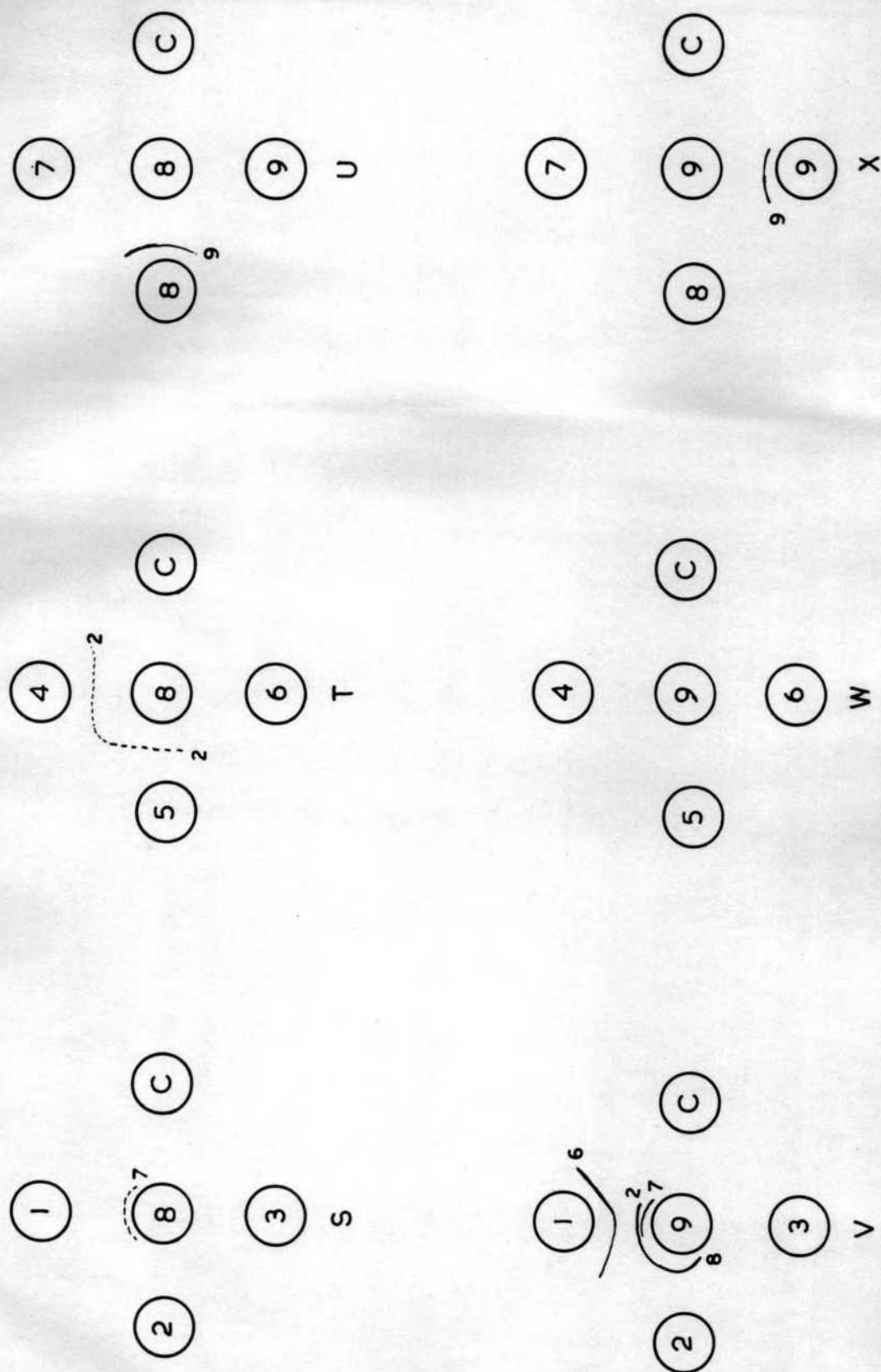
Central well = Antiserum	
Surrounding wells = Different antigens	
C = Control	
1 = A ₁ antigen	7 = A ₉ antigen
2 = A ₄ antigen	8 = A ₇ antigen
3 = A ₆ antigen	9 = A ₈ antigen
	4 = A ₃ antigen
	5 = A ₂ antigen
	6 = A ₅ antigen



TEXT FIGURE 3. ANTIGENIC RELATIONSHIP OF DIFFERENT ISOLATES.

Text Figure 4. Antigenic relationship of different isolates
(A diagrammatic representation)

	Central well	= Antiserum	
	Surrounding wells	= Different antigens	
	C = Control		
1 = A ₁ antigen	4 = A ₃ antigen	7 = A ₉ antigen	
2 = A ₄ antigen	5 = A ₂ antigen	8 = A ₇ antigen	
3 = A ₆ antigen	6 = A ₅ antigen	9 = A ₈ antigen	



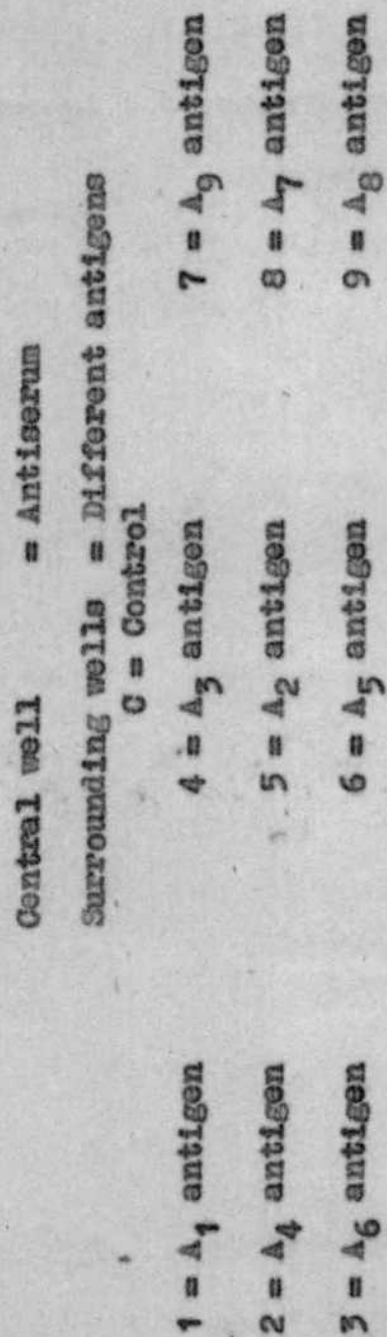
isolate A_7 , A_8 and A_9 produced five precipitation bands (band 2, band 3, band 5, band 6 and band 7) (Text Figure 3 (P, Q and R) and Figure 61 (Plate XXVI).

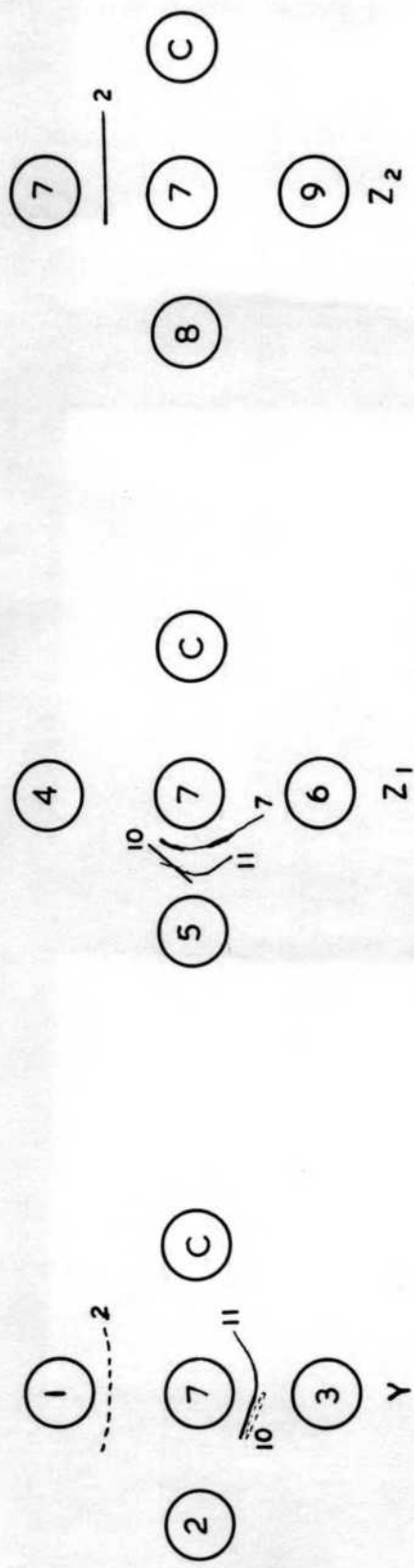
When the antigens of the isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_7 (a_7) only one precipitation band (band 7) appeared. Similarly exposing the antigens of isolate A_2 , A_3 and A_5 to the same antiserum only one precipitation band appeared. But in this case precipitation band number 2 was observed. Reacting with the same antiserum, the antigens of the isolate A_7 , A_8 and A_9 also produced one precipitation band (band 9) (Text Figure 4 (S, T and U).

When the antigens of the isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_8 (a_8) four characteristic precipitation bands (band 2, band 6, band 7 and band 8) appeared. But exposing the antigens of the isolate A_2 , A_3 and A_5 to the same antiserum no precipitation bands were observed. Reacting with the same antiserum, the antigens of the isolate A_7 , A_8 and A_9 produced only one precipitation band (band 9) (Text Figure 4 (V, W and X).

When the antigens of the isolate A_1 , A_4 and A_6 were exposed to the antiserum of the isolate A_9 (a_9) three characteristic precipitation bands (band 2, band 10 and band 11) were observed. Exposing the antigens of the isolate A_2 , A_3 and A_5 to the same antiserum also, three precipitation bands appeared.

Text Figure 5. Antigenic relationship of different isolates
(A diagrammatic representation)





TEXT FIGURE 5. ANTIGENIC RELATIONSHIP OF DIFFERENT ISOLATES.

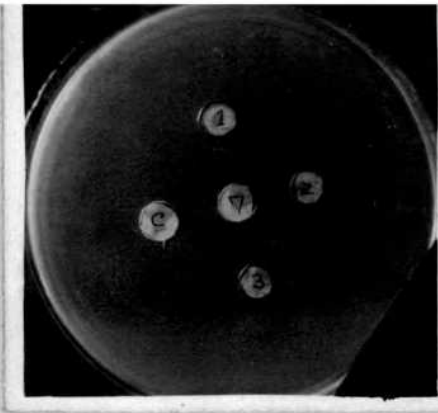


Figure 55

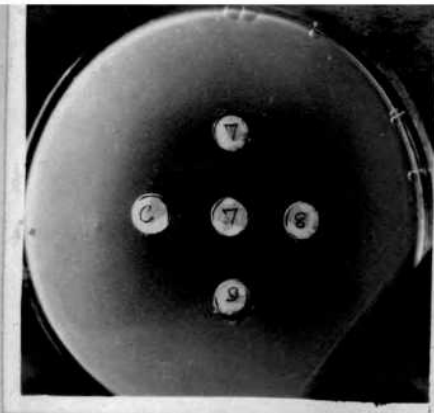


Figure 56

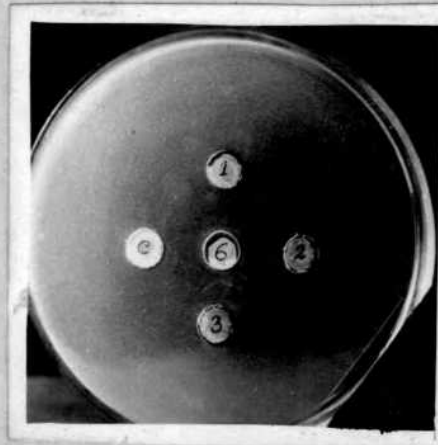


Figure 57

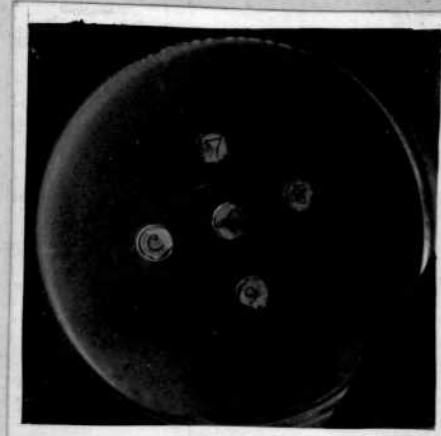


Figure 58



Figure 59

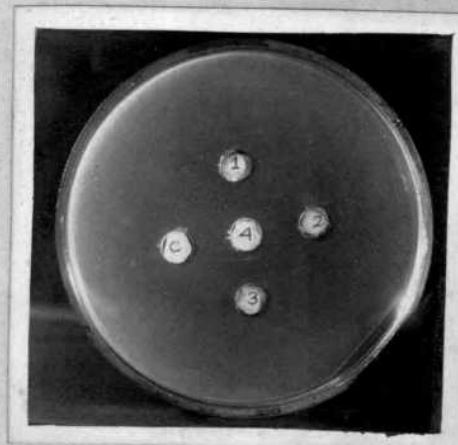


Figure 60

PLATE-XXV Antigenic relationship of different isolates.

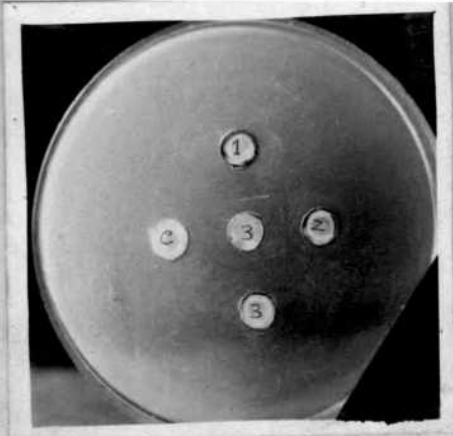


Figure 61

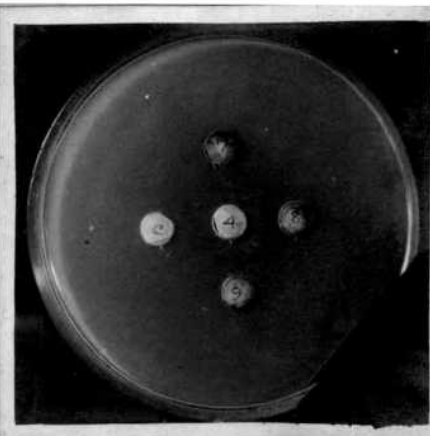


Figure 62

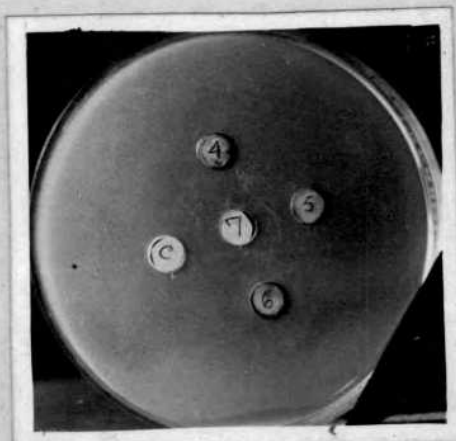


Figure 63

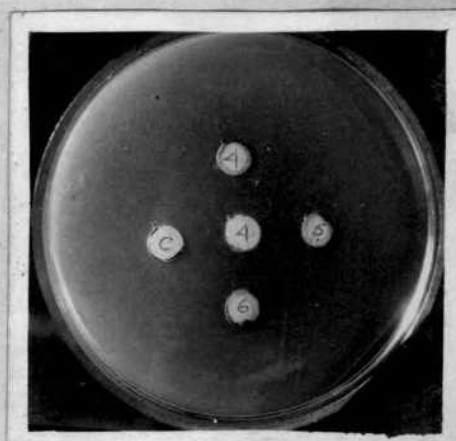


Figure 64

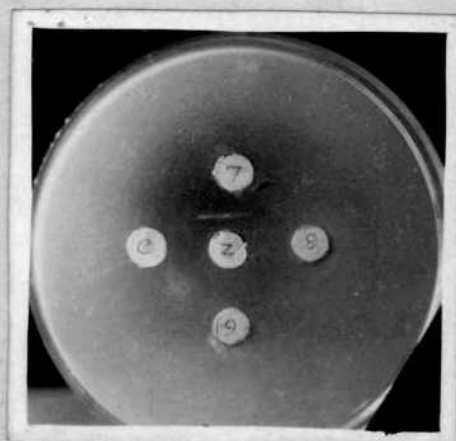


Figure 65

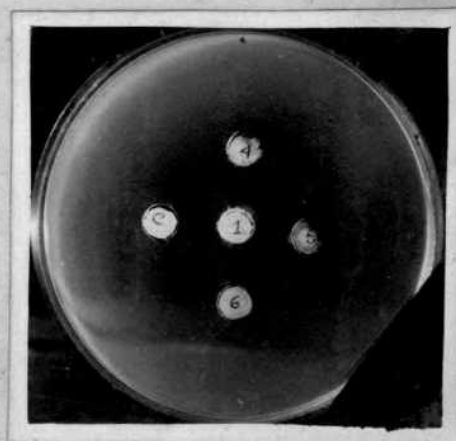


Figure 66

But in this case band number 7, 10 and 11 were observed. Reacting with the same antiserum, the antigens of the isolate A₇, A₈ and A₉ produced only one precipitation band (band 2) (Text Figure 5 (Y, Z₁ and Z₂) and Figure 55, 56 (Plate XXV) and 63 (Plate XXVI).

Table 15. Antigenic relationship between the virus isolates

Sl. No.	Anti-sera	A n t i g e n								
		A ₁	A ₂	A ₃	A ₄	A ₅	A ₆	A ₇	A ₈	A ₉
		Number of precipitation bands*								
1	a ₁	2	2	2,3	2	9	2,9	11,12	x	x
2	a ₂	x	7	2,3,8	x	x	x	7	x	2
3	a ₃	2	2	2	2	2,5,9	12	2	2,3,9	2,8
4	a ₄	x	7,8	2	2,3	x	2	x	x	2
5	a ₅	x	2,13	2,3	x	2	6,9	x	x	2,10,11 12
6	a ₆	2	2	2	2,4	2,5	1,2,3	2,7	2,5	2,3,6
7	a ₇	7	2	2	x	x	x	9	x	x
8	a ₈	2,7,6	x	x	8	x	x	x	9	x
9	a ₉	2	7,10,11	x	x	x	10,11	x	x	2

* The designation of the precipitation bands has been made according to Noorden (1973).

x = No precipitation bands appeared.

From the Table 15 it is evident that when antiserum of one isolate was exposed to antigen of other isolates, different types of

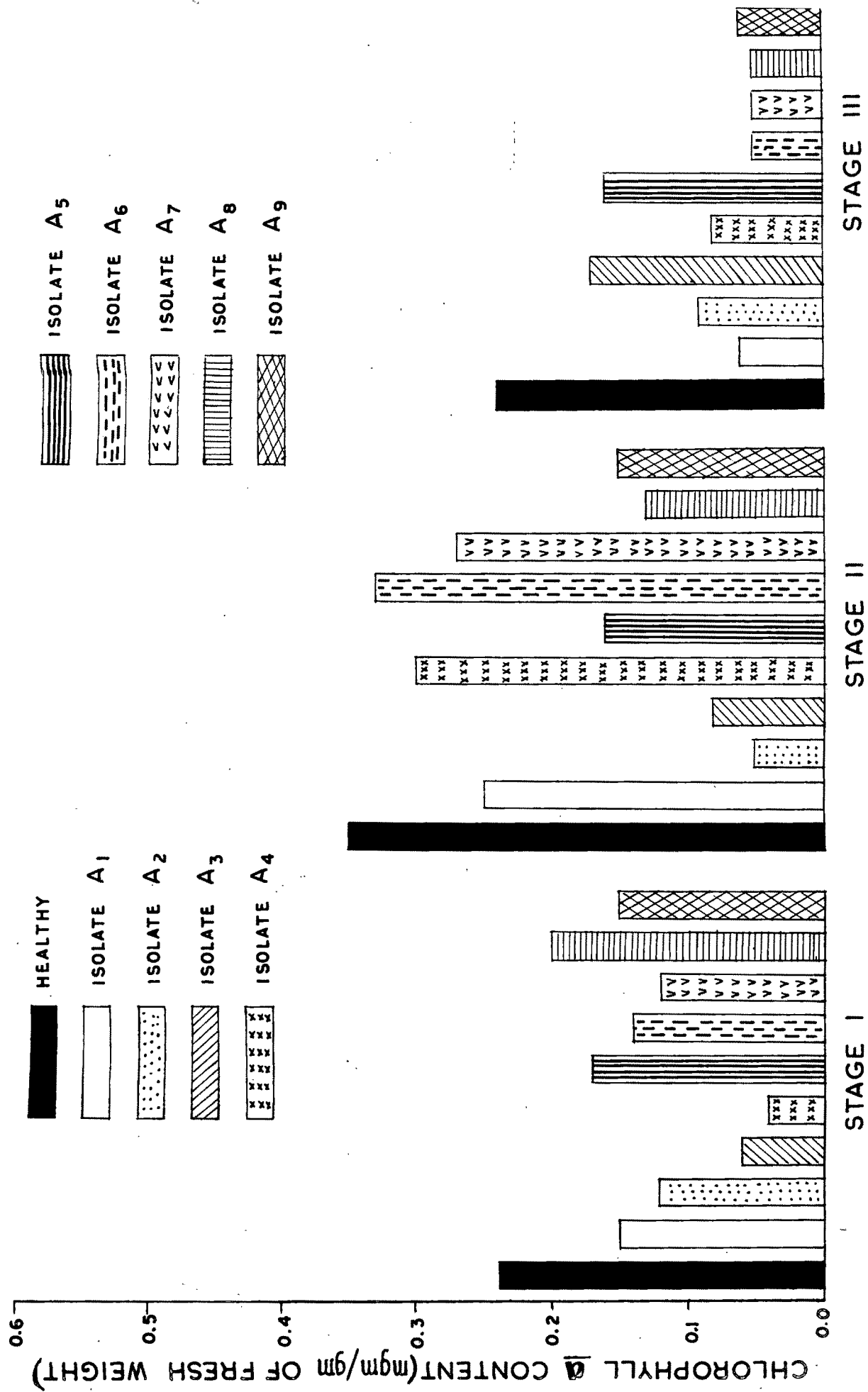
precipitation lines, as a result of antigens-antisera reactions, were observed. In all, 13 types of precipitation lines were observed in these isolates. When the antiserum a_1 was exposed to the antigens of nine isolates ($A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8$, and A_9), it produced five precipitation lines namely band number 2, 3, 9, 11 and 12. When the antiserum a_2 was exposed to the antigens of nine isolates, it produced six precipitation lines namely band number 2, 3, 5, 8, 9 and 12. When the antiserum a_3 was exposed to the antigens of nine isolates, it produced eight precipitation bands namely band number 2, 3, 6, 8, 9, 10, 11 and 12. Reacting with the antigens of nine isolates the antiserum a_4 was found to produce four precipitation lines in common with antiserum a_4 and they were namely band number 2, 3, 7 and 8. When the antiserum a_5 was exposed to the antigens of nine isolates, it produced seven precipitation bands namely band number 1, 2, 3, 4, 5, 6 and 7. When the antiserum a_6 was exposed to the antigens of nine isolates, it produced only two precipitation bands namely band number 2 and 7. When the antiserum a_7 was exposed to the antigens of nine isolates, it produced five precipitation bands namely band number 2, 6, 7, 8 and 9. When the antiserum a_8 was exposed to the antigens of nine isolates, it produced four precipitation bands namely band number 2, 7, 10 and 11.

D. BIOCHEMICAL CHANGES IN THE LEAVES OF CUCURBITA MOSCHATA POIR
PLANTS INOCULATED WITH DIFFERENT VIRUS ISOLATES AT DIFFERENT
STAGES OF PATHOGENESIS

Data obtained on (a) chlorophyll (a,b and total)
 (b) buffer soluble protein (c) total (TCA precipitable) sap
 protein (d) reducing sugar and starch and (e) ribonucleic acid
 (RNA) content have been presented in Tables 16 - 23 and Text
 Figures 6 - 13.

(a) Chlorophyll (a, b and total)

It is evident from the Table-16 that due to inoculation of all the isolates to healthy pumpkin plants, reduction of chlorophyll a content was observed. At State I minimum reduction was observed in case of isolate A₈ where chlorophyll a content was found to be 0.20mgm as compared to healthy where chlorophyll a was found to be 0.24 mgm. Moderate reduction of chlorophyll a content was observed in case of isolate A₁, A₂, A₅, A₆, A₇ and A₉ where chlorophyll a content were found to be 0.15 mgm, 0.12 mgm, 0.17 mgm, 0.14 mgm, 0.12 mgm and 0.15 mgm respectively. Maximum reduction was observed in case of isolate A₃ and A₄ where chlorophyll a content was found to be 0.06 mgm and 0.04 mgm respectively. At Stage II minimum reduction was observed in case of isolate A₆ where chlorophyll a content was found to be 0.33 mgm. In case of isolate A₁, A₄ and A₇ also, reduction of chlorophyll a was found to be very close to the minimum and their respective contents were found to be 0.25 mgm, 0.30 mgm and 0.27 mgm. But this reduction was more



TEXT FIGURE 6. CHLOROPHYLL a CONTENT OF THE LEAVES OF PUMPKIN (C. MOSCHATA POIR) PLANTS AT THF DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATE

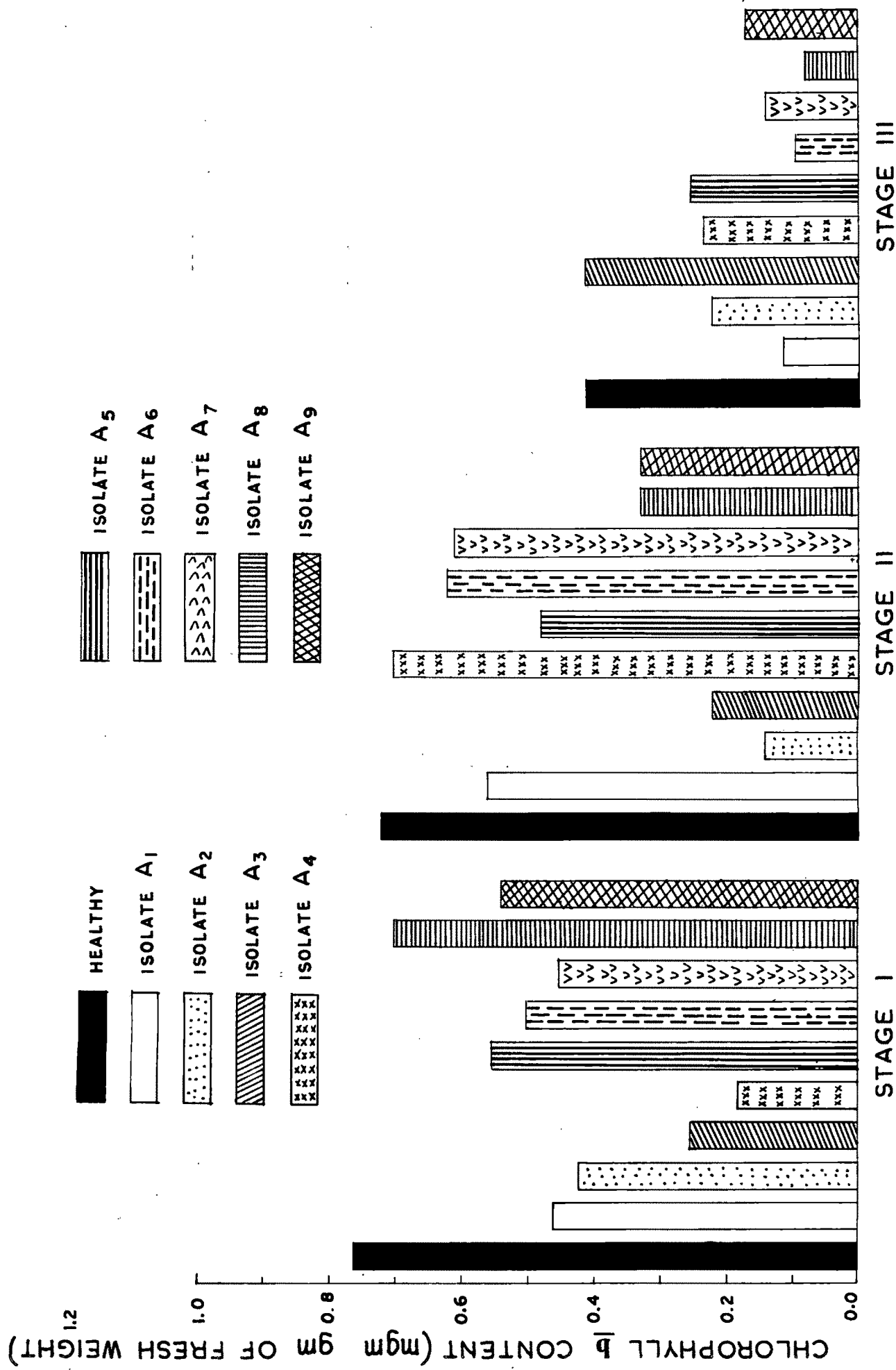
as compared to isolate A₆. Moderate reduction was observed in case of isolates A₅, A₈ and A₉ whose chlorophyll a contents were found to be 0.16 mgm, 0.13 mgm and 0.15 mgm respectively. Maximum reduction was observed in case of isolate A₂ and A₃ where chlorophyll a contents were found to be 0.05 mgm and 0.08 mgm respectively. At Stage III the reduction of chlorophyll a was found to be too high in case of all isolates except A₃ and A₅ where moderate reductions were observed and the chlorophyll a contents in these two cases were found to be 0.17 mgm and 0.16 mgm respectively. In case of isolate A₁, A₂, A₄, A₆, A₇, A₈ and A₉ the chlorophyll a contents were found to be 0.06 mgm, 0.09 mgm, 0.08 mgm, 0.05 mgm, 0.05 mgm, 0.05 mgm and 0.06 mgm respectively. In case of healthy leaves the chlorophyll a content was found to be 0.24 mgm (Text Figure 6).

When stage mean was considered, only Stage III significantly reduced chlorophyll a content. When isolate mean was considered all the isolates significantly reduced chlorophyll a content. At Stage I, all the isolates except isolate A₉ significantly reduced chlorophyll a content. At Stage II all isolates except isolate A₄ and A₆ reduced it significantly. At Stage III all the isolates were found to reduce chlorophyll a content significantly.

Table 16. Chlorophyll a content of the leaves of pumpkin (C. moschata Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates

Sl. No.	Isolates	Chlorophyll a content (mgm/gm of fresh weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	0.15	0.25	0.06	0.15		
2	A ₂	0.12	0.05	0.09	0.08		
3	A ₃	0.06	0.08	0.17	0.10		
4	A ₄	0.04	0.30	0.08	0.14		
5	A ₅	0.17	0.16	0.16	0.16	*	0.02
6	A ₆	0.14	0.33	0.05	0.17		
7	A ₇	0.12	0.27	0.05	0.14		
8	A ₈	0.20	0.13	0.05	0.12		
9	A ₉	0.15	0.15	0.06	0.12		
10	Healthy	0.24	0.35	0.24	0.27		
Average		0.13	0.20	0.10			
'F' test		*					
C.D. at 5%		0.01					
Stage x Isolate		'F' test = *					
		C.D. at 5% = 0.05					

It is evident from the Table-17 that due to inoculation of all the isolates to healthy pumpkin plants, reduction of chlorophyll b content was observed. At state I minimum reduction was observed in case of isolate A₈, where chlorophyll b content was found to be 0.70 mgm as compared to healthy where chlorophyll b was found to be 0.76 mgm. Moderate reduction of chlorophyll b content was observed in case of isolates A₁, A₂, A₅, A₆, A₇ and A₉ where chlorophyll b contents were found to be 0.46, 0.42, 0.55, 0.50, 0.45 and 0.54 mgm respectively. Maximum reduction was observed in case of isolate A₃ and A₄ where chlorophyll b contents were found to be 0.25 and 0.18 mgm respectively. At Stage II minimum reduction was observed in case of isolate A₄ where chlorophyll b content was found to be 0.70 mgm as compared to healthy where chlorophyll b content was found to be 0.72 mgm. Moderate reduction of chlorophyll b content was observed in case of isolate A₁, A₅, A₆ and A₇ where chlorophyll b content was found to be 0.56, 0.48, 0.62 and 0.61 mgm respectively. Isolates A₈ and A₉ reduced chlorophyll b content too much and in both the cases the content was found to be 0.33 mgm. Maximum reduction was observed in case of A₂ and A₃ where chlorophyll b contents were found to be 0.14 and 0.22 mgm respectively. At Stage III all the isolates were found to reduce the content too much. The chlorophyll b contents with the isolates A₁, A₂, A₃, A₄, A₅, A₇ and A₉ were found to be 0.11, 0.22, 0.41, 0.23, 0.25, 0.14 and 0.17 mgm respectively. Maximum reduction of the chlorophyll b content was observed with the isolate A₆ and A₈. The chlorophyll b contents with them were found to be 0.09 and 0.08 mgm respectively.



TEXT FIGURE 7. CHLOROPHYLL b CONTENT OF THE LEAVES OF PUMPKIN (C. MOSCHATA POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES.

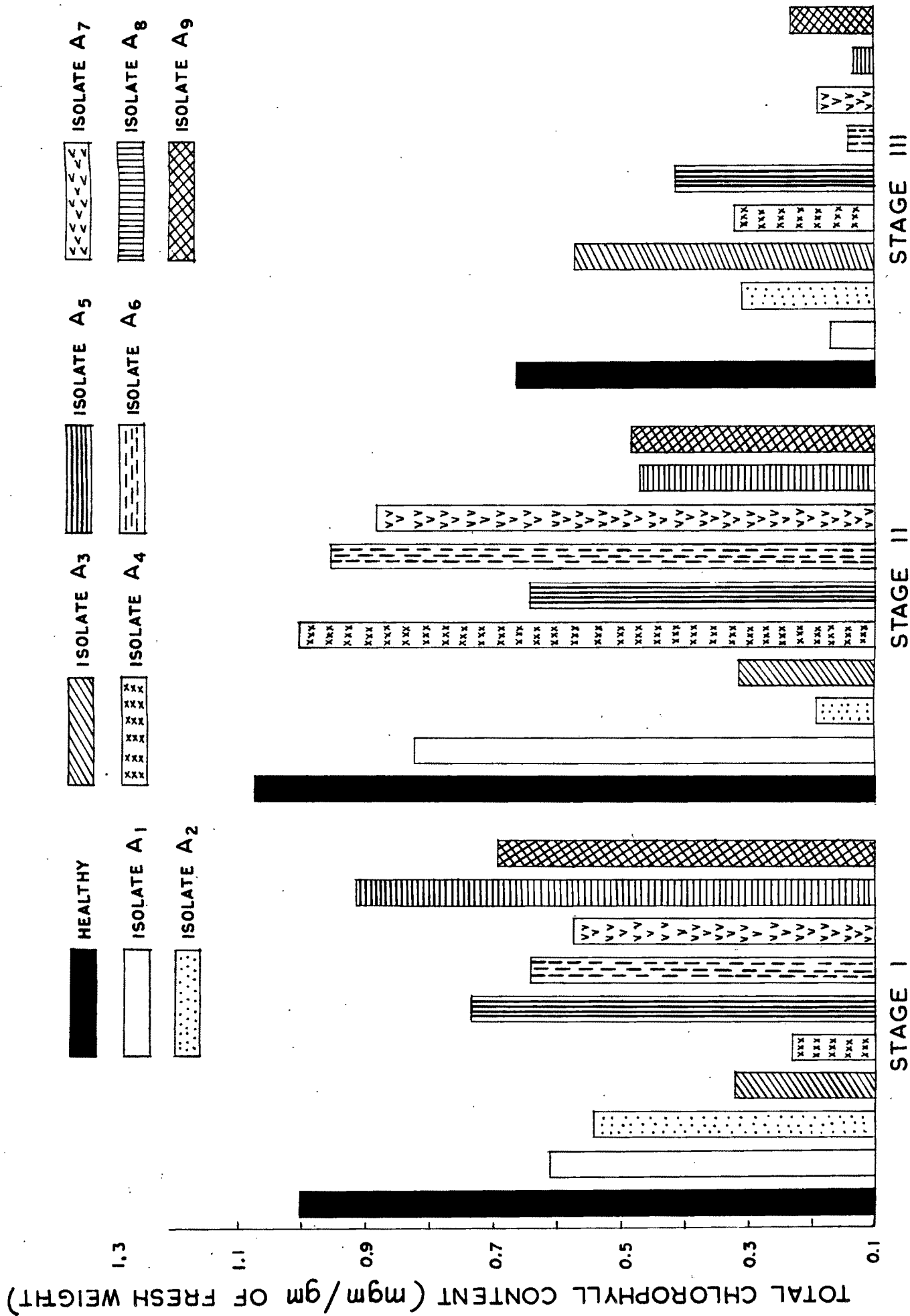
When the chlorophyll b content of healthy leaves at different stages were compared, it was also found to decrease with age (Text Figure 7).

When stage mean was considered, Stage III significantly reduced chlorophyll b content. When isolate mean was considered all the isolates significantly reduced chlorophyll b content. At Stage I, all isolates except A₈ significantly reduced chlorophyll b. At Stage II, isolate A₂, A₃, A₅, A₈ and A₉ reduced it significantly. At Stage III significant reduction of chlorophyll b content was observed in case of isolates A₁, A₆, A₇, A₈ and A₉.

Table 17. Chlorophyll b content of the leaves of pumpkin
(C. moschata Poir) plants at three different stages
of pathogenesis after being inoculated with the nine
pumpkin virus isolates

Sl. No.	Isolate	Chlorophyll <u>b</u> content ($\mu\text{gm/gm}$ of fresh weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	0.46	0.56	0.11	0.38		
2	A ₂	0.42	0.14	0.22	0.26		
3	A ₃	0.25	0.22	0.41	0.29		
4	A ₄	0.18	0.70	0.23	0.37		
5	A ₅	0.55	0.48	0.25	0.43	*	0.11
6	A ₆	0.50	0.62	0.09	0.40		
7	A ₇	0.45	0.61	0.14	0.40		
8	A ₈	0.70	0.33	0.08	0.37		
9	A ₉	0.54	0.33	0.17	0.35		
10	Healthy	0.76	0.72	0.41	0.63		
Average		0.48	0.47	0.21			
'F' test		*					
C.D. at 5%		0.06					
Stage x Isolate		'F' test = *					
		C.D. at 5% = 0.20					

It is evident from the Table-18 that due to inoculation of all the isolates to healthy pumpkin plants, reduction of total chlorophyll content was observed. At Stage I minimum reduction was observed in case of isolate A_8 where total chlorophyll content was found to be 0.91 mgm as compared to healthy where total chlorophyll was found to be 1.00 mgm. Moderate reduction of total chlorophyll content was observed in case of isolates A_5 , A_9 , A_6 , A_1 , A_7 and A_2 where total chlorophyll contents were found to be 0.73, 0.69, 0.64, 0.61, 0.57 and 0.54 mgm respectively. Maximum reduction was observed in case of isolates A_3 and A_4 where total chlorophyll contents were found to be 0.32 and 0.23 mgm respectively. At Stage II minimum reduction of total chlorophyll content was observed in case of isolate A_4 , A_6 , A_7 and A_1 where the respective chlorophyll contents were found to be 1.00, 0.95, 0.88 and 0.82 mgm. Moderate reduction of total chlorophyll was observed in case of isolate A_5 , A_9 , A_8 and A_3 where the contents were found to be 0.64, 0.48, 0.47 and 0.31 mgm respectively. Maximum reduction was observed in case of isolate A_2 where the chlorophyll content was found to be 0.19 mgm. In case of healthy, the total chlorophyll content was found to be 1.07 mgm. At Stage III in case of healthy leaves the total chlorophyll content was found to be 0.66 mgm. Minimum reduction of total chlorophyll content was observed in case of isolate A_3 and A_5 where the chlorophyll contents were found to be 0.57 and 0.41 mgm respectively. Moderate reduction was observed in case of isolate A_2 , A_4 and A_9 where the chlorophyll contents were found to be 0.31, 0.32 and 0.23 mgm respectively. Maximum reduction was observed in case of isolate A_1 , A_6 and A_8



TEXT FIGURE 8. TOTAL CHLOROPHYLL CONTENT OF THE LEAVES OF PUMPKIN (C. MOSCHATA POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES.

where the chlorophyll contents were found to be 0.17, 0.14 and 0.13 μgm respectively(Text Figure 8).

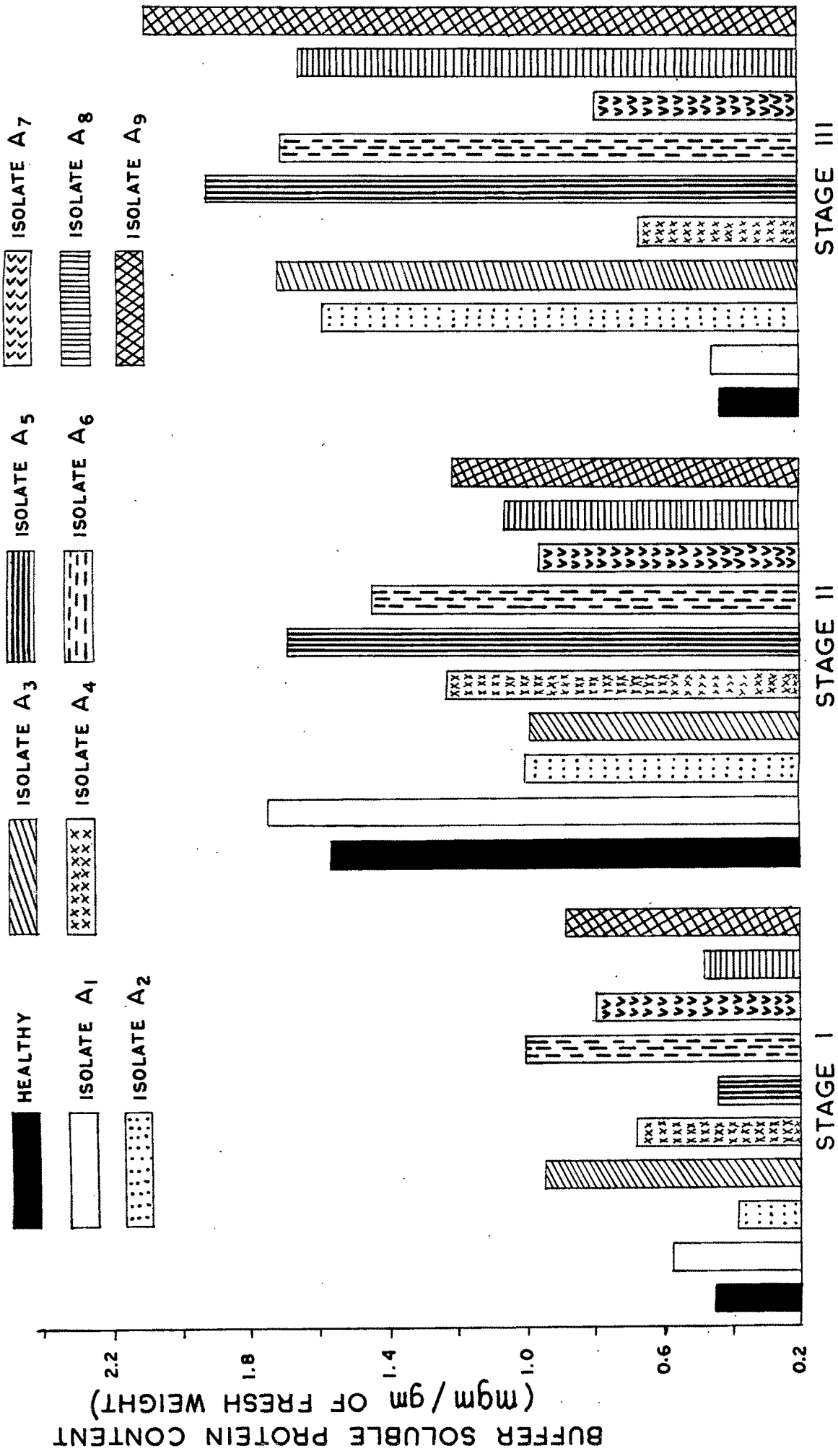
When stage mean was considered, Stage III was found to significantly reduce total chlorophyll content. When isolate mean was considered, all the isolates were found to decrease total chlorophyll content. In all the three stages all isolates were found to decrease total chlorophyll content significantly when compared with healthy of each stage.

Table 13. Total chlorophyll content of the leaves of pumpkin (*C. moschata* Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates

Sl. No.	Isolate	Total chlorophyll content ($\mu\text{gm/gm}$ of fresh weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	0.61	0.82	0.17	0.53		
2	A ₂	0.54	0.19	0.31	0.35		
3	A ₃	0.32	0.31	0.57	0.40		
4	A ₄	0.23	1.00	0.32	0.52		
5	A ₅	0.73	0.64	0.41	0.59	*	0.01
6	A ₆	0.64	0.95	0.14	0.58		
7	A ₇	0.57	0.88	0.19	0.55		
8	A ₈	0.91	0.47	0.13	0.50		
9	A ₉	0.69	0.48	0.23	0.47		
10	Healthy	1.00	1.07	0.66	0.91		
Average		0.62	0.63	0.31			
'F' test		*					
C.D. at 5%		0.006					
Stage x Isolate		'F' test		= *			
		C.D. at 5%		= 0.02			

b) Buffer soluble protein

It is evident from the Table-19 that all the isolates except isolate A_2 and A_5 increased the buffer soluble protein when compared with healthy at Stage I. Healthy leaves were found to have 0.45 mgm of buffer soluble protein. Maximum increase of buffer soluble protein content was observed in case of isolate A_6 where it was found to be 1.00 mgm. Moderate increase was observed in case of isolate A_3 , A_9 , A_7 , A_4 and A_1 where the buffer soluble protein contents were found to be 0.94, 0.88, 0.79, 0.67 and 0.57 mgm respectively. Isolate A_8 increased the buffer soluble protein very little and it was found to be 0.48 mgm. Isolate A_2 and A_5 were found to decrease it and the buffer soluble protein contents with these were found to be 0.38 and 0.44 mgm respectively. At Stage II in case of healthy the buffer soluble protein content was found to be 1.56 mgm. In this stage the buffer soluble protein content was found to be decreased in case of all isolates. Maximum decrease of the content was observed in case of isolate A_7 and A_3 where the contents of buffer soluble protein were found to be 0.96 and 0.99 mgm respectively. Moderate decrease was observed in case of isolate A_2 , A_4 , A_6 , A_8 and A_9 where the contents of buffer soluble protein were found to be 1.00, 1.22, 1.44, 1.05 and 1.21 mgm respectively. Minimum decrease was found in case of isolate A_5 where the content was found to be 1.69 mgm only. Isolate A_1 was found to increase it and the content was found to be 1.75 mgm. At Stage III the healthy leaves were found to have



TEXT FIGURE 9. BUFFER SOLUBLE PROTEIN CONTENT OF THE LEAVES OF PUMPKIN (C. MOSCHATA POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES.

buffer soluble protein content as 0.43 mgm. In this Stage all the isolates were found to increase the buffer soluble protein content. Maximum increase was observed in case of isolate A₉ where the buffer soluble protein content was found to be 2.10 mgm. Moderate increase was observed in case of isolate A₂, A₃, A₅, A₆ and A₈ where the contents were found to be 1.59, 1.72, 1.92, 1.71 and 1.66 mgm respectively. Minimum increase was observed in case of isolate A₁, A₄ and A₇ where the contents were found to be 0.45, 0.66 and 0.79 mgm respectively (Text Figure 9).

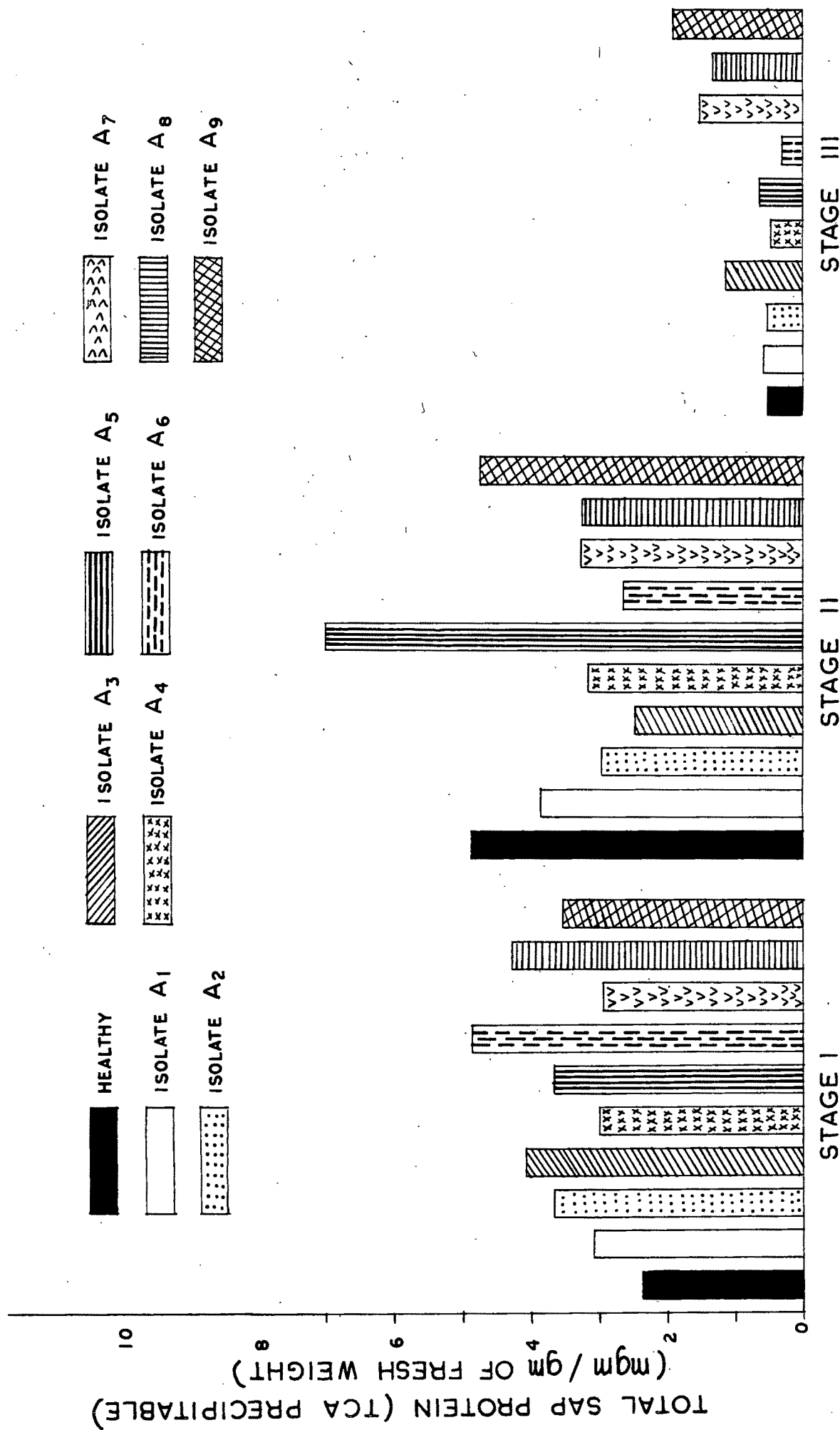
When stage mean was considered, Stage II was found to increase buffer soluble protein content significantly. When isolate mean was considered all isolates were found to increase it significantly. At Stage I, all isolates except A₂ and A₅ significantly increased buffer soluble protein content in comparison to that of the healthy. At Stage II, all isolates except A₁ and A₅ significantly reduced it. But at Stage III, all the isolates were found to increase buffer soluble protein content with respect to that of healthy.

Table 19. Buffer soluble protein content of the leaves of pumpkin (*C. moschata* Poir) plants at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates

Sl. No.	Isolate	Buffer soluble protein content			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	0.57	1.75	0.45	0.92		
2	A ₂	0.38	1.00	1.59	0.99		
3	A ₃	0.94	0.99	1.72	1.21		
4	A ₄	0.67	1.22	0.66	0.85		
5	A ₅	0.44	1.69	1.92	1.35	*	0.01
6	A ₆	1.00	1.44	1.71	1.38		
7	A ₇	0.79	0.96	0.79	0.84		
8	A ₈	0.48	1.05	1.66	1.06		
9	A ₉	0.38	1.21	2.10	1.39		
10	Healthy	0.45	1.56	0.43	0.81		
Average		0.66	1.28	1.30			
'F' test		*					
C.D. at 5%		0.007					
Stage x Isolate		'F' test = *					
		C.D. at 5% = 0.02					

c) Total (TCA precipitable) sap protein

It is evident from the Table-20 that at Stage I all the isolates were found to increase total (TCA precipitable) sap protein content. Maximum increase was observed in case of isolate A₆ where the total protein content was found to be 4.83 mgm. Moderate increase was observed in case of isolate A₂, A₃, A₅, A₇, A₈ and A₉ where the protein contents were found to be 3.74, 4.06, 3.77, 2.90, 4.36 and 3.53 mgm respectively. Minimum increase was observed in case of isolate A₁ and A₄ where the contents were found to be 3.03 and 3.00 mgm respectively. In case of healthy the total (TCA precipitable) sap protein content was found to be 2.37 mgm. At stage II all the isolates except A₅ decreased total (TCA precipitable) sap protein content. Minimum decrease was observed in case of isolate A₉ where the content was found to be 4.77 mgm. Moderate decrease was observed in case of isolate A₁, A₄, A₇ and A₈ where the contents were found to be 3.87, 3.18, 3.23 and 3.23 mgm respectively. Maximum decrease was observed in case of A₂, A₃ and A₆ where the respective contents were found to be 2.97, 2.46 and 2.63 mgm. Only isolate A₅ was found to increase the total (TCA precipitable) sap protein content and the protein content in this case was found to be 7.00 mgm as compared to healthy where the content was found to be 4.68 mgm. At Stage III in case of healthy the total (TCA precipitable) sap protein content was found to be 0.43 mgm. In this stage all the isolates except isolate A₆ were found to increase the total (TCA precipitable) sap protein content. Maximum increase



TEXT FIGURE 10. TOTAL (TCA PRECIPITABLE) SAP PROTEIN CONTENT OF THE LEAVES OF PUMPKIN (C. MOSCHATA POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES.

was observed in case of isolate A_9 where the content was found to be 1.83 mgm. Moderate increase was observed in case of isolate A_3 , A_7 and A_8 where the contents were found to be 1.15, 1.53 and 1.35 mgm respectively. Minimum increase was observed in case of isolate A_1 , A_2 , A_4 and A_5 where the contents were found to be 0.59, 0.55, 0.48 and 0.61 mgm respectively. Only in case of isolate A_6 the total (TCA precipitable) sap protein content was found to decrease and the protein content in this case was found to be 0.36 mgm (Expt Figure 10).

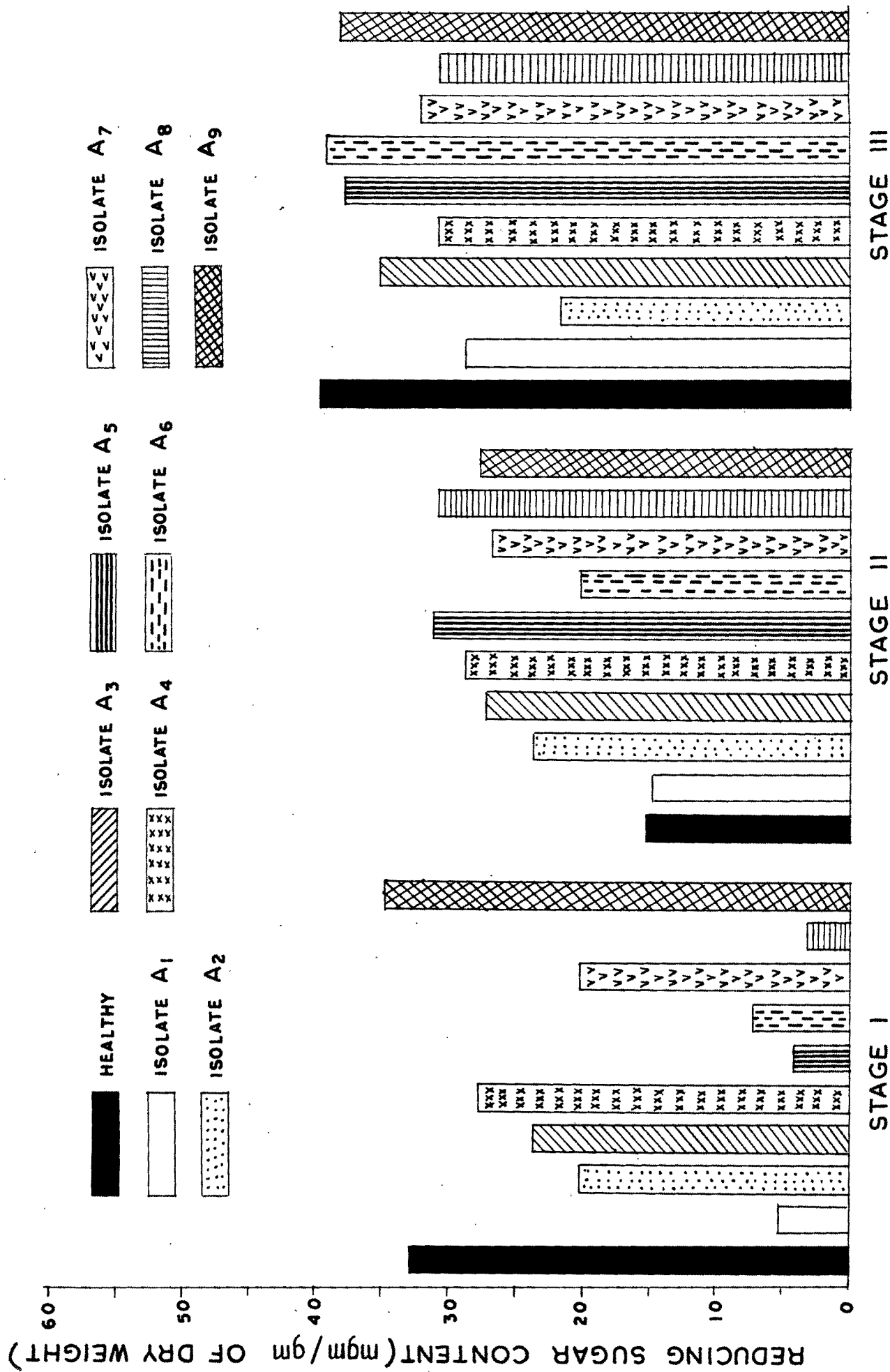
When stage mean was considered Stage II was found to increase total (TCA precipitable) sap protein content significantly. When isolate mean was considered, isolate A_5 , A_6 , A_8 and A_9 were found to increase total (TCA precipitable) sap protein content significantly whereas isolates A_1 , A_2 , A_3 , A_4 , and A_7 significantly reduced it when compared with the healthy. At Stage I and III, all the isolates significantly increased total (TCA precipitable) sap protein content. At Stage II isolate A_5 significantly increased whereas all other isolates significantly reduced total (TCA precipitable) sap protein content.

Table 20. Total (TCA precipitable) sap protein content of the leaves of pumpkin (*C. moschata* Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates

Sl. No.	Isolate	Total sap protein(TCA precipitable) content (ngn/gm of fresh weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	3.03	3.87	0.58	2.49		
2	A ₂	3.74	2.97	0.55	2.42		
3	A ₃	4.06	2.46	1.15	2.55		
4	A ₄	3.00	3.18	0.48	2.22		
5	A ₅	3.77	7.00	0.61	3.79	*	0.02
6	A ₆	4.83	2.63	0.36	2.60		
7	A ₇	2.90	3.23	1.53	2.55		
8	A ₈	4.36	3.23	1.35	2.98		
9	A ₉	3.53	4.77	1.83	3.37		
10	Healthy	2.37	4.88	0.43	2.56		
Average		3.55	3.82	0.88			
'F' Test		*					
C.D. at 5%		0.01					
Stage x Isolate		'F' test = *					
		C.D. at 5% = 0.04					

d) Reducing sugar and starch

It is evident from the Table-21 that at Stage I the reducing sugar content in healthy leaves was found to be 32.50 mgm. In case of all isolates except A₉ the reducing sugar content was found to be decreased. Minimum decrease of reducing sugar content was observed in case of A₄ where the content was found to be 27.50 mgm. Moderate decrease was observed in case of A₂, A₃ and A₇ where the contents were found to be 20.00, 23.50, and 20.00 mgm respectively. Maximum decrease was observed in case of A₁, A₅ and A₈ where the respective sugar contents were found to be 5.00, 4.00 and 3.00 mgm. In case isolate A₉, the sugar content was found to be 34.50 mgm. At Stage II the reducing sugar content in case of healthy was found to be 15.00 mgm. In this stage the reducing sugar content was found to increase with all the isolates except isolate A₁. Maximum increase was observed in case of isolate A₅ and A₈ where the contents were found to be 32.00 and 30.50 mgm respectively. Moderate increase was observed in case of isolate A₂, A₃, A₄, A₇ and A₉ where the contents were found to be 25.50, 27.00, 28.50, 26.50 and 27.50 mgm respectively. Minimum increase was observed in case of isolate A₆ where the content was found to be 20.00 mgm. In case of isolate A₁ the reducing sugar was found to decrease and the content was found to be 14.50 mgm. At Stage III the reducing sugar content of healthy leaves was found to be 39.50 mgm. In this stage the reducing sugar content in case of all isolates were found to decrease. Minimum decrease was observed in case of isolate A₆, A₉ and A₅ where the contents



TEXT FIGURE 11. REDUCING SUGAR CONTENT OF THE LEAVES OF PUMPKIN (C. MOSCHATA POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES.

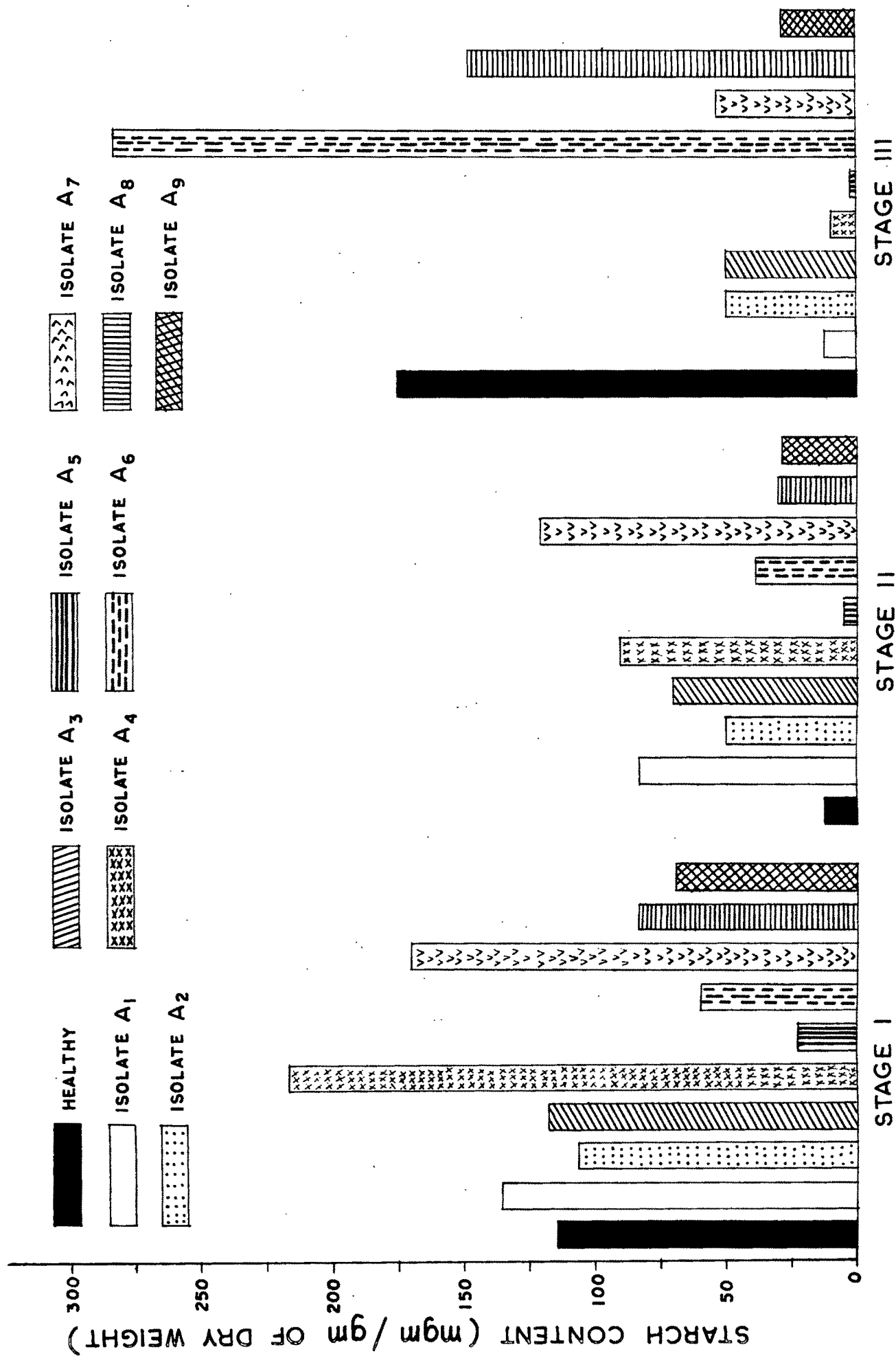
were found to be 39.00, 38.00 and 37.50 mgm respectively. Moderate decrease was observed in case of isolate A₁, A₃, A₄ and A₈ where the contents were found to be 28.50, 35.00, 30.50, 32.00 and 30.50 mgm respectively. Maximum decrease was observed in case of isolate A₂ where the contents were found to be 21.50 mgm (Text Figure 11).

When stage mean was considered Stage I significantly decreased the reducing sugar content whereas significant increase of this compound was observed with Stage III. When isolate mean was considered, significant decrease of the reducing sugar was observed when compared with healthy. Some isolates were found to increase it but that increase was found to be insignificant. At Stage I significant reduction of reducing sugar content by isolates A₁, A₂, A₅, A₆, A₇ and A₈ was observed. At Stage II isolates A₄, A₅, A₈ and A₉ were found to increase it significantly. At Stage III no significant effect was observed but in case of isolate A₂ significant reduction of reducing sugar content was observed.

Table 21. Reducing sugar content of the leaves of pumpkin
(*C. moschata* Poir) at three different stages of
pathogenesis after being inoculated with the nine
pumpkin virus isolates

Sl. No.	Isolate	Reducing sugar content (mgm/gm of dry weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	5.00	14.50	28.50	16.00		
2	A ₂	20.00	23.50	21.50	21.67		
3	A ₃	23.50	27.00	35.00	28.50		
4	A ₄	27.50	28.50	30.50	28.83		
5	A ₅	4.00	32.00	37.50	24.50	*	6.99
6	A ₆	7.00	20.00	39.00	22.00		
7	A ₇	20.00	26.50	32.00	26.17		
8	A ₈	3.00	30.50	30.50	21.33		
9	A ₉	34.50	27.50	38.00	33.33		
10	Healthy	32.50	15.00	39.50	29.00		
Average		17.70	24.50	33.20			
'F' test		*					
C.D. at 5%		3.83					
Stage x Isolate		'F' test = Not significant					

It is evident from the Table-22 that at Stage I in case of healthy leaves starch content was found to be 115.00 mgm. Isolate A₁, A₃, A₄ and A₇ was found to increase starch content whereas A₂, A₅, A₆, A₈ and A₉ decreased it. Maximum increase of starch content was observed in A₄ where the content was found to be 217.00 mgm. In case of A₁, A₃ and A₇ the content was found to be 136.00, 118.00 and 170.00 mgm respectively. Maximum decrease of starch content was observed in A₅ where the content was found to be 23.00 mgm. In case of isolate A₂, A₈ and A₉, the starch content was found to be 107.50, 60.00, 83.00 and 69.00 mgm. At Stage II in healthy leaves the starch content was found to be 12.00 mgm. In this stage all the isolates except A₅ increased starch content. Maximum increase was observed in case of A₇ where the content was found to be 121.00 mgm. Moderate increase was observed in case of A₁, A₂, A₃ and A₄ where the contents were found to be 83.00, 50.00, 70.00 and 92.50 mgm respectively. Minimum increase was observed in case of isolate A₆, A₈ and A₉ where the contents were found to be 38.50, 30.00 and 29.00 mgm respectively. Isolate A₅ was found to decrease the starch content and the content was found to be only 5.00 mgm. At Stage III the starch content of healthy leaves was found to be 175.00 mgm. At this stage all the isolates except isolate A₆ were found to decrease the starch content. Minimum decrease was found in case of isolate A₈ where the content was found to be 148.00 mgm. Moderate decrease was observed in case of isolate A₁, A₂, A₃, A₇ and A₉ where the content was found to be 12.00, 50.00, 50.00, 53.00 and 28.00 mgm respectively. Maximum decrease



TEXT FIGURE 12. STARCH CONTENT OF THE LEAVES OF PUMPKIN (*C. MOSCHATA* POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES.

was observed in case of isolates A_4 and A_5 where the respective starch contents were found to be 9.00 and 1.00 mgm. In case of isolate A_6 , the starch content was found to increase maximum and the content was found to be 282.50 mgm (Text Figure 12).

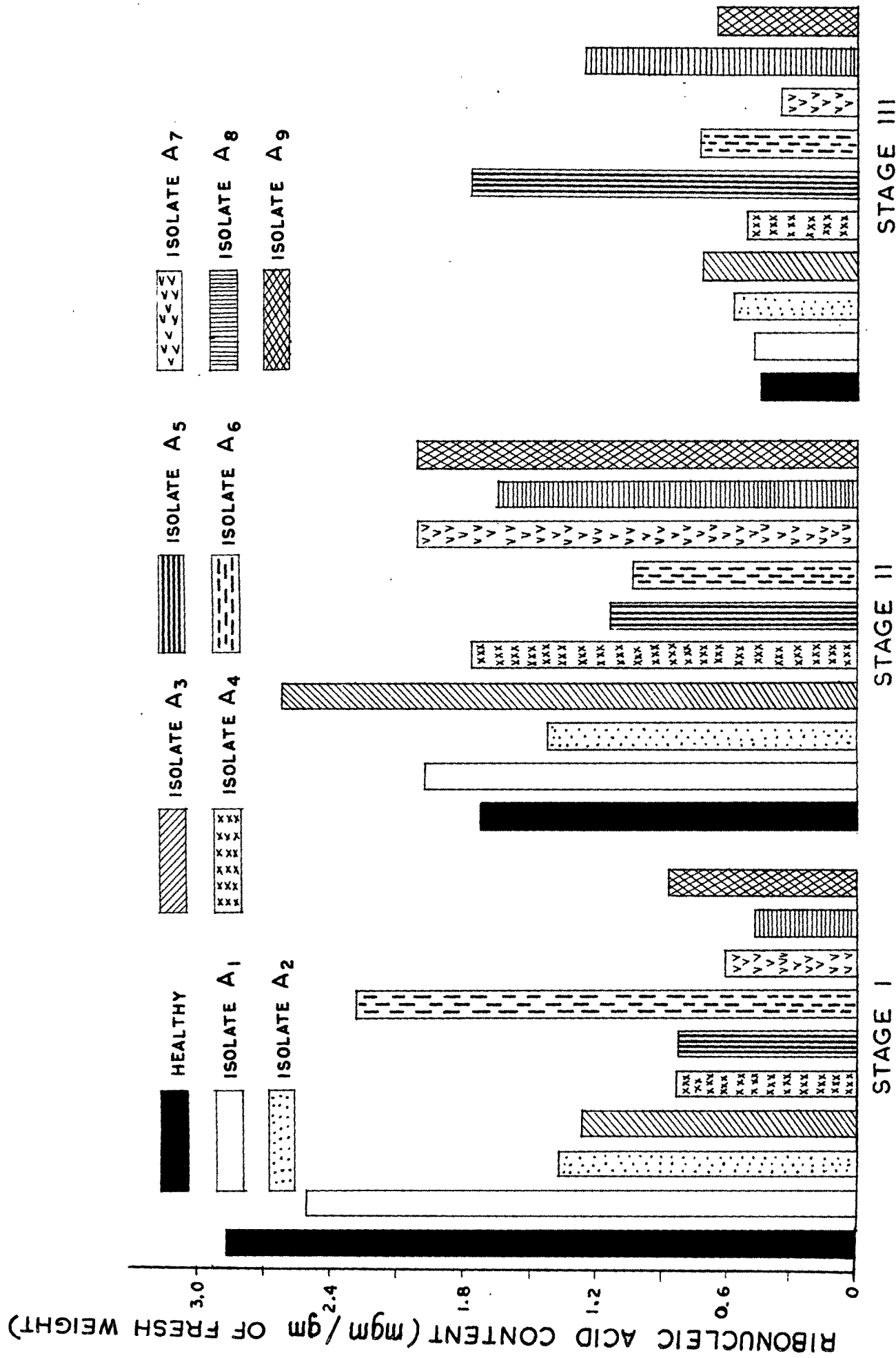
When stage mean was considered, Stage I significantly increased starch content whereas Stage II significantly decreased it. When isolate mean was considered some isolates (A_1 , A_2 , A_3 , A_5 , A_8 and A_9) were found to decrease and some isolates (A_4 , A_6 and A_7) were found to increase it significantly. At Stage I, A_1 , A_4 and A_7 significantly increased and A_2 , A_5 , A_6 , A_8 and A_9 reduced significantly. At stage II, all the isolates except isolate A_5 increased starch content significantly. At Stage III, all isolates reduced it significantly except isolates A_6 where significant increase was observed.

Table 22. Starch content of the leaves of pumpkin (*C. moschata* Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates

Sl. No.	Isolate	Starch content (mgm/gm of dry weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	136.00	83.00	12.00	77.00		
2	A ₂	107.50	50.00	50.00	69.17		
3	A ₃	118.00	70.00	50.00	79.33		
4	A ₄	217.00	92.50	9.00	106.17		
5	A ₅	23.00	5.00	1.00	9.67	*	2.14
6	A ₆	60.00	33.50	282.50	27.00		
7	A ₇	170.00	121.00	53.00	114.67		
8	A ₈	83.00	30.00	148.00	87.00		
9	A ₉	69.00	29.00	28.00	42.00		
10	Healthy	115.00	12.00	175.00	100.67		
Average		109.85	53.10	60.85			
'F' test		*					
C. D. at 5%		1.16					
Stage x Isolate		'F' test		= *			
		C.D. at 5%		= 3.72			

e) Ribonucleic acid (RNA)

It is evident from the Table-23 that at Stage I the RNA content in healthy leaves was found to be 2.85 mgm. In this stage all the isolates were found to decrease RNA content. Minimum decrease was observed in case of isolate A₁ and A₆ where the contents were found to be 2.50 and 2.28 mgm respectively. Moderate decrease was observed in case of isolate A₂, A₃, A₄, A₅ and A₉ where the contents were found to be 1.36, 1.25, 0.81, 0.60 and 0.87 mgm respectively. Maximum reduction was observed in case of isolate A₇ and A₈ where the contents were found to be 0.60 and 0.45 mgm respectively. At Stage II, the RNA content of healthy leaves was found to be 1.70 mgm. At this stage isolates A₁, A₃, A₄, A₇ and A₉ increased RNA content. Maximum increase was observed in case of A₃ where the content was found to be 2.60 mgm. In case of isolate A₁, A₄, A₇ and A₉ moderate increase was observed and the contents were found to be 1.97, 1.75, 2.00 and 1.99 mgm respectively. Isolates A₂, A₅, A₆ and A₈ were found to decrease it. Minimum decrease was observed in case of A₈ where the content was found to be 1.63 mgm. Minimum decrease was observed in case of A₂, A₅ and A₆ where the contents were found to be 1.40, 1.13 and 1.02 mgm respectively. At Stage III, RNA content in healthy leaves was found to be 0.43 mgm. At this stage, all the isolates except A₇ increased RNA content. Maximum increase was observed in case of A₅ and A₈ where the respective RNA contents found to be 1.77 and 1.25 mgm. Moderate increase was observed in case of



TEXT FIGURE 13. RIBONUCLEIC ACID CONTENT OF THE LEAVES OF PUMPKIN (*C. MOSCHATA* POIR) PLANTS AT THREE DIFFERENT STAGES OF PATHOGENESIS AFTER BEING INOCULATED WITH THE NINE PUMPKIN VIRUS ISOLATES

A₂, A₃, A₄, A₆ and A₉ where the RNA contents were found to be 0.57, 0.71, 0.50, 0.72 and 0.65 mgm respectively. Minimum increase was observed in case of isolate A₁ where the content was found to be 0.47 mgm. Isolate A₇ was found to decrease RNA content and the content was found to be 0.35 mgm (Text Figure 13).

When stage mean was considered Stage II was found to increase RNA content significantly. When isolate mean was considered significant reduction of RNA content due to virus infection was observed. At Stage I, all the isolates were found to reduce RNA content significantly. At Stage II isolates A₁, A₃, A₄, A₇ and A₉ were found to increase it significantly. At Stage III, all isolates except isolate A₇ were found to increase RNA content significantly.

Table 23. Ribonucleic acid (RNA) content of the leaves of pumpkin (*C. moschata* Poir) at three different stages of pathogenesis after being inoculated with the nine pumpkin virus isolates

Sl.No.	Isolate	Ribonucleic acid content (mgm/gm of fresh weight)			Average	'F' test	C.D. at 5%
		Stage I	Stage II	Stage III			
1	A ₁	2.50	1.97	0.47	1.64		
2	A ₂	1.36	1.40	0.57	1.11		
3	A ₃	1.25	2.60	0.71	1.52		
4	A ₄	0.81	1.75	0.50	1.02		
5	A ₅	0.80	1.13	1.77	1.23	*	0.02
6	A ₆	2.28	1.02	0.72	1.34		
7	A ₇	0.60	2.00	0.35	0.98		
8	A ₈	0.45	1.63	1.25	1.11		
9	A ₉	0.07	1.99	0.65	1.17		
10	Healthy	2.85	1.70	0.43	1.66		
Average		1.37	1.71	0.74			
'F' test		*					
C.D. at 5%		0.01					
Stage & Isolate		'F' test = *					
		C.D. at 5% = 0.04					

V. D I S C U S S I O N

Cucurbita moschata Poir, popularly known as pumpkin, is widely grown in West Bengal particularly in the districts of Burdwan, Hooghly, 24 Parganas and Nadia as one of the major rabi vegetables. The crop is usually sown in the month of January-February and harvested in the month of May-June. Every year during the middle of their growth period, symptoms of virus diseases appear in the leaves in many fields. When the fields were surveyed during February-March 1973 for the incidence of virus diseases, symptoms were observed in 60.00 ± 4.58 - 89.33 ± 3.47 per cent plants in the fields of all the districts under consideration. The leaf symptoms found in the fields were not identical to each other and at least six types of symptoms (type I-VI) were observed. The incidence of type I, II, III, IV, V and VI symptoms were 13.66 ± 0.37 - 21.66 ± 0.33 , 36.66 ± 1.20 - 51.00 ± 1.52 , 7.00 ± 1.00 - 8.66 ± 0.87 , 9.33 ± 1.20 - 14.66 ± 0.33 , 2.00 ± 0.57 - 7.00 ± 0.57 and 13.00 ± 1.52 - 16.66 ± 0.87 per cent respectively. It is apparent from these observations that the incidence of type II symptoms was much more common in the fields. As such, the isolation and identification studies on the viruses involved in the type II symptoms were undertaken. When the leaves showing type II symptoms were collected from the fields from time to time and sap transmission studies were performed, 31-80 per cent transmission was obtained, but symptoms produced in the inoculated plants were again found to differ. Thus the type II symptoms

in the field was considered as complex and attempt was made through sap transmission technique to separate the types associated with this complex. Samples showing type II symptom complex were brought in batches from the fields. On sap transmission, each batch of sample produced two or more types of symptoms in the inoculated plants. When inoculum was obtained from these plants and again transferred to the test plants, symptoms true to the type was observed. In this way from series of samples, nine different types of symptoms were identified ($A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8$ and A_9). $A_1, A_2, A_3, A_4, A_5, A_6$ and A_7 showed prominent vein banding which was not apparent in A_8 and A_9 . In A_1, A_2 and A_3 , vein banding was much more prominent than in A_4, A_5, A_6 and A_7 . Prominent green blisters were apparent in A_1 and A_2 but not in A_3 . In between A_1 and A_2 , vein yellowing was found in A_2 and not in A_1 . Amongst A_4, A_5, A_6 and A_7 , yellow chlorosis was found to start at the margin of the lamina in A_4 and A_5 whereas in A_6 and A_7 yellow chlorosis started irregularly upon the entire lamina. Prominent green blisters were found in A_4 but not in A_5 . In between A_6 and A_7 , puckering of the lamina was observed in A_6 but not in A_7 . In between A_8 and A_9 , yellow chlorosis was found to start from the margin of the lamina in A_8 whereas in A_9 yellow chlorosis started irregularly upon the entire lamina. The viruses from the nine types of symptoms obtained from the type II symptom complex of the field could be isolated and maintained in C. moschata plants in pure forms.

When the physical properties of the isolates were compared, the thermal inactivation points (TIP) of all isolates

were found to be in between 40° to 55°C . The TIP of A_3 and A_9 was $40-45^{\circ}\text{C}$, the TIP of A_2 , A_4 , A_6 , A_7 and A_8 was $45-50^{\circ}\text{C}$ whereas the TIP of A_1 and A_5 were found to be $50-55^{\circ}\text{C}$. Similarly the dilution end point (DEP) of all the isolates were found to be in between $\angle 10^{-1} - 10^{-3.5}$. The DEP of A_1 and A_8 was found to be less than 10^{-1} . The DEP of A_5 and A_9 was found to be $10^{-1.5}-10^{-2}$. The DEP of A_3 , A_4 and A_6 was $10^{-2} - 10^{-2.5}$, the DEP of A_7 was $10^{-2.5} - 10^{-3}$ and the DEP of A_2 was $10^{-3} - 10^{-3.5}$. The ageing in vitro of all the isolates at room temperature ($24-32^{\circ}\text{C}$) were found to be in between 6 hours to 168 hours. The ageing in vitro of A_1 was 6 hours. The ageing in vitro of A_4 and A_8 was 12 hours. The ageing in vitro of A_2 , A_5 and A_9 were 48 hours and the ageing in vitro of A_3 , A_6 and A_7 were 60 hours, 84 hours and 168 hours (7 days) respectively (Table -10).

In the host range studies, it was observed that all the isolates except A_2 , A_3 and A_5 could be transmitted only to the most of the cucurbitaceous hosts under consideration and not to the plants belonging to other families. 13 plants of Cucurbitaceae, 12 plants of Solanaceae, 3 plants of Leguminosae, 2 plants of Cruciferae, 1 plant of Caricaceae, Apocynaceae, Amaranthaceae, Compositae and 3 plants of Chenopodiaceae were tested. The isolate A_1 was found to be transmissible to all the cucurbits tested except L. acutangula and C. melo (Cantaloupe Selection B 63-3). It was not transmissible to S. melongena var. Mantakochi, B. hybrida, B. campestris var. Sarson, S. oleracea or B. vulgaris. The isolate A_2 was found to be transmissible to all the cucurbits except M. charantia, B. cerifera and C. melo (Cantaloupe Selection B 63-3). It however could not produce any symptom in L. cylindrica,

L. acutangula and C. vulgaris var. fistulosus. These plants were found to be symptomless carrier of the isolate. As regards to the non-cucurbitaceous hosts, it could infect and produce symptoms in S. melongena var. Muktakeshi and P. hybrida. But it did not infect B. campestris var Saroon; S. oleracea and B. vulgaris. The isolate A_3 was transmissible to all the cucurbits except L. cylindrica, C. melo (Cantaloupe Selection B 63-3), C. sativus var. Local White and C. sativus var. Long Green. It produced distinct symptoms in all the cucurbits in which it was transmissible. As regards to the non-cucurbitaceous hosts, it could infect and produce symptoms in S. melongena var. Muktakeshi and P. hybrida. Mustard, Spinach and Beet were found to be symptomless carrier of this isolate. The isolate A_4 was found to be transmissible to all the cucurbits except B. corifera and L. acutangula. It, however, could not produce any symptom in L. vulgaris, C. vulgaris var. fistulosus and C. lanatus. As such, these plants appeared to be symptomless carriers of this isolate. This isolate was not found to be transmissible to any of the non-cucurbitaceous hosts tested. The isolate A_5 was found to be transmissible to all the cucurbits except B. corifera and L. cylindrica. It however, could not produce any symptoms in L. vulgaris and C. vulgaris var. fistulosus and presumably these two plants were symptomless carriers for this isolate. Amongst the non-cucurbitaceous hosts, this isolate could infect and produce symptoms only in S. melongena var. Muktakeshi and P. hybrida. The isolate A_6 could not infect M. charantia, B. corifera and C. vulgaris var. fistulosus. It could infect but could not produce any symptom only in C. lanatus. This isolate however, was

not transmissible to any non-cucurbitaceous hosts. The isolate A_7 could not infect B. cerifera, L. cylindrica, C. mole (Cantaloupe Selection B 63-3), C. sativus var. Local White and C. sativus var. Long Green. L. vulgaris has been found to be the only symptomless carrier for this isolate. This isolate, however did not infect any non-cucurbitaceous hosts. The isolate A_8 could not infect L. cylindrica, L. acutangula and C. mole (Cantaloupe Selection B 63-3). B. cerifera has been found to be the only symptomless carrier for this isolate. This isolate also did not infect any non-cucurbitaceous hosts. The isolate A_9 could not infect B. cerifera, C. vulgaris var. fistulosus, C. lanatus and C. mole (Cantaloupe Selection B 63-3). L. acutangula has been found to be the only symptomless carrier for this isolate. It did not infect any non-cucurbitaceous hosts.

From the host range studies, it is apparent that the transmissibility of the isolates A_1 , A_4 , A_6 , A_7 , A_8 and A_9 was strictly restricted to the cucurbitaceous hosts, whereas the isolates A_2 , A_3 and A_5 could be transmitted to a few non-cucurbitaceous hosts. These three isolates could infect and produce symptoms in S. melongena var. Muktakochi and E. hybrida. The isolate A_3 on the otherhand could infect B. campestris var. Sarson, C. colocasia and B. vulgaris but could not produce any symptom in them. Amongst the isolates, restricted to the cucurbitaceous hosts, A_9 could not infect Citrullus lanatus. A_5 and A_7 could not infect cucumber long green and Cucumber local white. A_3 and A_6 could not infect M. charantia. A_1 , A_4 , A_8 could not infect L. acutangula. A_6 , A_7 and A_9 could not infect C. vulgaris var. fistulosus. A_3 , A_5 , A_7

and A₈ could not infect L. cylindrica. A₁, A₂, A₃, A₇, A₈ and A₉ could not infect C. melo (Cantaloupe Selection B 63-3) and none of the isolate except A₁, A₃ and A₈ could infect B. corifera (Table 11).

In the transmission studies of the isolates with A. gossypii Clover, Myzus persicae Sulz. and B. tabaci Conn. it was observed that A₁ and A₂ were not transmissible by them. A₃ was transmissible by B. tabaci and not by A. gossypii or M. persicae. A₄ and A₆ were transmissible by only M. persicae. A₅, A₇, A₈ and A₉ were transmissible by only A. gossypii. In case of A. gossypii transmissible isolates, the efficiency of transmission, was, however, found to differ. The percentages of transmission of the isolates A₅, A₇, A₈ and A₉ with this aphid were 37, 43, 29 and 22 respectively. Moreover, the period of retention of these isolates in this aphid after the virulification was also found to differ. An A. gossypii once virulified could retain the isolates A₅, A₇, A₈ and A₉ upto a period of 72 hours, 96 hours, 24 hours and 96 hours respectively. In case of the M. persicae transmissible isolates A₄ and A₆, the efficiency of transmission was found to be the same. With both the isolates 40 per cent transmission was obtained. An aphid M. persicae, virulified with either A₄ or A₆, was found to retain the isolate for the same period or in other words for a period of 96 hours only. The percentage of transmission of A₃ with B. tabaci was found to be only 32 and the retention period of this isolate in the white fly once virulified was found to be 48 hours.

In the electronmicroscopic studies, the particles of all the isolates were found to be flexible rods of varied length. The length of the particles of A_2 and A_4 were in between 500 m/ μ - 600 m/ μ . The length of the particle A_2 was 587.5 m/ μ and that of A_4 was 525.0 m/ μ . The length of the particles of A_6 was 675.0 m/ μ . The length of the particles of A_1 , A_3 , A_5 and A_9 were in between 700 m/ μ - 800 m/ μ . The respective lengths were 712.5 m/ μ , 725 m/ μ , 743 m/ μ and 785.0 m/ μ . The length of the particles of A_7 and A_8 were in between 800 m/ μ - 900 m/ μ and the respective lengths were 825.0 m/ μ and 874.9 m/ μ .

When serological studies were made with these isolates, it was observed that all the isolates were related to each other (Table 15). Exposing the antisera of all the isolates to the antigens of all the isolates, at least one common band (band 2) was found to each combination. Amongst these isolates again, when antiserum a_1 was exposed to the antigens $A_2, A_3, A_4, A_5, A_6, A_7, A_8$ and A_9 , one band was observed with A_4 (band 2) and also with A_5 (band 9), two bands were produced with A_3 (bands 2 and 3), A_6 (bands 2 and 9) and A_7 (bands 11 and 12) and no band was observed with A_8 and A_9 . When antisera a_4 and a_5 were exposed to the antigen A_1 no band was observed. When the antisera a_3, a_6 and a_7 were exposed to the A_1 antigen, one band was found in all the cases, with a_3 and a_6 band 2 was observed but with a_7 a different band (band 7) was found. Exposing the antiserum a_8 to the A_1 antigen, three bands (band 2, band 6 and band 7) were found and when the a_2 antiserum was exposed to A_1 antigen no band was formed. Thus it may be said that exposing the A_1 antigen to

different antisera and exposing the a_1 antiserum to different antigens, common bands were observed only with A_3 and A_6 . Thus A_1 may be more closely related to A_3 and A_6 .

When antiserum a_2 was exposed to the antigens of all the isolates, no band was observed with the antigens of A_1 , A_4 , A_5 , A_6 and A_9 . With the antigen of A_3 only one band was observed and with the antigen of A_7 , three bands (band 2, band 3 and band 8) were observed. When the antisera a_9 and a_3 were exposed to the antigen A_2 , the same band 2 was observed with a_3 and three different bands (band 7, band 10 and band 11) were observed with a_9 . When the antisera a_1 , a_4 , a_5 , a_6 , a_7 and a_8 were exposed to the antigen A_2 , one band (band 2) was observed respectively with a_1 , a_6 and a_7 and two bands were observed with a_4 (band 7 and band 8) and a_5 (band 2 and band 13). No band was, however, observed with a_8 . From these observations, it is apparent that when the A_3 antigen was exposed to a_2 antiserum bands 2, 3 and 8 appeared. When antiserum a_3 was exposed to A_2 antigen, band 2 also appeared. Thus the isolate A_3 seems to be more close to A_2 than any other isolate.

When antiserum a_3 was exposed to the antigens of A_1 , A_2 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 , only one common band (band 2) was found with A_1 , A_2 , A_4 and A_7 , one different band (band 12) was found with A_6 , two bands (band 2 and band 8) were found with A_9 and three bands were formed with A_5 and A_8 . The bands with A_5 were 2, 5 and 9 and those with A_8 were 2, 3 and 9. When the antisera a_1 , a_2 , a_4 , a_5 , a_6 , a_7 , a_8 and a_9 were exposed to the antigen A_3 , one

common band (band 2) was formed with a_4 , a_6 and a_7 . Two bands (band 2 and band 3) were found with a_1 and a_5 , three bands were found with a_2 (band 2, band 3 and band 8) and no bands were found with a_8 and a_9 . It is apparent that when a_3 antiserum was exposed to the different antigens and A_3 antigen was exposed to different antisera, common bands were observed only with A_1 , A_2 , A_4 , A_5 , A_6 and A_7 . Thus A_3 may be closely related to all these isolates.

When the antiserum a_4 was exposed to the antigens of A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 , only one band was found with A_3 , A_6 and A_9 . A_1 , A_5 , A_7 and A_8 did not produce any band. A_2 produced two different bands (band 7 and band 8). When antisera a_1 , a_2 , a_3 , a_4 , a_5 , a_6 , a_7 , a_8 and a_9 were exposed to the antigen A_4 two bands (band 2 and band 4) were found with a_6 and one band (band 8) was found with a_8 , with a_2 , a_5 , a_7 and a_9 no band was observed. With a_1 and a_3 one common band (band 2) was observed. It is apparent that when a_4 antiserum was exposed to the different antigens and A_4 antigen was exposed to different antisera, common bands were found only with A_3 and A_6 . Thus A_4 may be more closely related to A_3 and A_6 .

When the antiserum a_5 was exposed to the antigens A_1 , A_2 , A_3 , A_4 , A_5 , A_6 and A_7 , A_8 and A_9 , two bands were formed with A_2 (band 2 and band 13), A_3 (band 2 and band 3) and A_6 (band 6 and band 9) and four bands (band 2, band 10, band 11 and band 13) were formed with A_9 , A_4 , A_7 and A_8 did not produce any band. When the antisera a_1 , a_2 , a_3 , a_4 , a_5 , a_6 , a_7 , a_8 and a_9 were exposed to antigen

A_5 , two different bands (band 2 and band 3) were formed with a_6 . a_2 , a_4 , a_7 , a_8 and a_9 did not produce any band but a_1 produced a different band (band 9). It is apparent that when a_5 antiserum was exposed to the different antigens and A_5 antigen was exposed to different antisera, common bands were formed only with A_3 . Thus A_5 may be closely related only to A_3 .

When the antiserum a_6 was exposed to antigens A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 , two bands were formed with A_4 (band 2), A_5 (band 2 and band 3), A_7 (band 2 and band 7) and A_8 (band 2 and band 5) and three bands were formed with A_9 (band 2, band 3 and band 6). With A_1 , A_2 and A_3 , only one band was formed (band 2). When the antisera a_1 , a_2 , a_3 , a_4 , a_5 , a_6 , a_7 , a_8 and a_9 were exposed to the antigen A_6 , no bands were formed with a_2 , a_7 and a_8 . Two different bands were produced respectively by a_1 (band 2 and band 9), a_5 (band 6 and band 9) and a_9 (band 10 and band 11). It is apparent that when a_6 antiserum was exposed to the different antigens and A_6 antigen was exposed to different antisera, common bands were found only with A_1 and A_4 . Thus A_6 may be closely related to these two isolates.

When the antiserum a_7 was exposed to the antigens A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 , no band was observed with A_4 , A_5 , A_6 , A_8 and A_9 . One band was respectively found with A_1 (band 7), A_2 (band 2), A_3 (band 2). Similarly exposing the antisera a_1 , a_2 , a_3 , a_4 , a_5 , a_6 , a_8 and a_9 to the Antigen A_7 , no band was also observed with a_4 , a_5 , a_8 and a_9 . One band was respectively observed with a_2 (band 7) and a_3 (band 2) and two

bands were observed with a_1 (band 11 and band 12). When antiserum a_6 was exposed to the antigen a_7 , two bands (band 2 and band 7) were also observed. It is apparent that when antiserum a_7 was exposed to different antigens and antigen A_7 was exposed to different antisera, common bands were found with only A_3 . Thus these two isolates may be very closely related.

When the antiserum a_8 was exposed to the antigens, $A_1, A_2, A_3, A_4, A_5, A_6, A_7$ and A_9 , no band was formed with A_2, A_3, A_5, A_6, A_7 and A_9 . One band was formed with A_4 (band 8) and three bands were formed with A_1 (band 2, band 6 and band 7). When the antisera $a_1, a_2, a_3, a_4, a_5, a_6, a_7$ and a_9 were exposed to the antigen A_8 , no band was formed with a_1, a_2, a_4, a_5, a_7 and a_9 . Three bands (band 2, band 3 and band 9) were formed with a_3 and two bands were formed with a_6 (band 2 and band 5). Thus it is apparent that A_8 may not be closely related to any of the isolates.

When the antiserum a_9 was exposed to the antigens $A_1, A_2, A_3, A_4, A_5, A_6, A_7$ and A_8 , no band was formed with A_3, A_4, A_5, A_7 and A_8 . Three bands (band 7, band 10, band 11) were formed with A_2 , two bands (band 10 and band 11) were formed with A_6 and one band was observed with A_1 (band 2). When the antisera $a_1, a_2, a_3, a_4, a_5, a_6, a_7$ and a_8 were exposed to the antigen A_9 , no band was observed with a_1, a_7 and a_8 . Two bands were observed with a_3 (band 2 and band 8), three bands were observed with a_6 (band 2, band 3 and band 6) and four bands were observed with a_5 (band 2, band 10, band 11 and band 12). It is apparent that when the antiserum a_9 was exposed to the different antigens and the antigen A_9 was exposed to different antisera, no precipitation band was observed. Thus it may be said that the isolate A_9 is not

closely related to any other isolate (Table 15).

From the serological studies, it appears that the isolate A₁ was closely related to the isolate A₃ and A₆. Isolate A₂ was closely related to the isolate A₃. Isolate A₃ was closely related to A₁, A₂, A₃, A₄, A₅, A₆ and A₇. Isolate A₄ was closely related to A₃ and A₆. Isolate A₅ was closely related to A₁ and A₄. Isolate A₇ was closely related to A₃. But the isolates A₈ and A₉ were not related to any isolate. Thus the isolates may form three groups, based on their serological relationship, Group 1 included isolate A₁, A₂, A₃, A₄, A₅, A₆ and A₇. Group 2 included isolate A₈ and Group 3 included isolate A₉.

When the transmission characteristics of the isolates of the Group 1 were compared, it was found that the isolates of this group can be further sub-divided on the basis of insect transmissibility (Table 12). The subgroup 1 included the isolate A₁ and A₂ which were found to be only sap transmissible. The subgroup 2 included A₃ which was found to be transmissible by sap and white flies. The subgroup 3 included A₄ and A₆ which were found to be transmissible by sap and by H. persicae. The subgroup 4 included A₅ and A₇ which were found to be transmissible by sap and by A. gossypii.

The isolates A₁ and A₂ on the other hand, were found to widely differ in their physical properties but the physical properties of A₄ and A₆ were found to be more or less identical to each other except the ageing in vitro. The isolates A₅ and A₇ again were found to differ in their physical properties. Thus it

can be said that all the isolates, obtained from the fields were distinct and different from each other and one isolate in no way appeared to be completely identical to any other isolate. But according to their similarities and dissimilarities, particularly with respect to their serological properties, transmissibility and to some extent the host range, the isolates may be arranged in six groups namely A_1 and A_2 ; A_3 ; A_4 and A_6 ; A_5 and A_7 ; A_8 and A_9 (Table 15, 12 and 11).

When the properties of the isolates were compared with the properties of the known cucurbit virus, isolate A_1 was found to resemble Bottlegourd mosaic virus of Vasudeva and Lal (1943). Isolate A_6 was found to be very close to the Cucurbit latent virus of Webb (1963). Isolate A_4 also appeared to share many common properties with this virus. Isolate A_3 was found to be close to Vein yellowing virus of Harpaz and Cohen (1965). Isolate A_5 was found to be close to the Cucurbit mosaic virus of Hariharasubramanian and Badani (1964) and isolate A_7 was found to be very close to the Watermelon mosaic virus 1 of Webb and Scott (1965). Considering the similarities and dissimilarities, the isolate A_2 was found to be a distantly related new strain of Bottlegourd mosaic virus of Vasudeva and Lal (1943). The isolates A_8 and A_9 however, did not resemble any known Cucurbit virus, thus these two isolates may be considered as new viruses.

Isolate A₁ resembled Bottlegourd mosaic virus (Vasudeva and Lal, 1943) in many respects. Both of them has the same range of TTP and ageing in vitro. Both of them could infect C. sativus var. Local white and C. sativus var. Long green, C. pepo cv. Early Prolific Straightneck, L. vulgaris and M. charantia. Both of them are sap transmissible and could not infect chilli or tobacco. The dilution end point of these two viruses, however, have been found to differ but this may be due to the fact that the sap utilized for the test were collected from different hosts and under different environmental conditions. Although much work has been done in India, on two other bottlegourd mosaic viruses reported by Kapoor and Varma (1948) and Vasudeva et al. (1949), no further informations, however, are available on the bottlegourd mosaic virus reported by Vasudeva and Lal (1943). As such comparison could not be made on the insect transmissibility and particle size of these two viruses. But considering their similarities, the isolate A₁ may be regarded as a strain of the Bottlegourd mosaic virus of Vasudeva and Lal (1943). It has been mentioned before that the isolate A₂ was close to the isolate A₁ with respect to serological properties and transmissibility. Both of them were found to be only sap transmissible. Both of them were able to infect L. vulgaris, T. anguina, L. cylindrica C. vulgaris var. fistulosus, C. lanatus, C. pepo cv. Early Prolific Straightneck and C. sativus cv. Long green and Local White. In case of A₂ L. cylindrica was found to be symptomless carrier. Both of them were unable to infect C. melo (Cantaloupe Selection B 63-3), B. campestris var. Carson, S. oleracea and B. vulgaris.

In addition to these cucurbitaceous hosts, isolate A_2 was also able to infect S. melongena var. Muktakeshi, and P. hybrida plants which isolate A_1 was not able to infect. Besides these similarities, isolate A_2 was also found to differ from A_1 in its ability to induce enations in the inoculated leaves of C. moschata under favourable condition. Both of them have got their thermal inactivation point close to each other but their dilution end point and ageing in vitro were found to differ greatly. The dilution end point of A_1 was found to be 10^{-1} whereas of isolate A_2 was found to be $10^{-3} - 10^{-3.5}$. The ageing in vitro of isolate A_1 was found to be 6 hours whereas that of isolate A_2 was found to be 48 hours. The length of the particle of isolate A_1 was found to be 712.5 m/ μ and that of the particle of isolate A_2 was found to be 587.5 m/ μ . Thus considering the similarities and dissimilarities of this isolate with the isolate A_1 , the isolate A_2 may be considered as a strain of the Bottlegourd mosaic virus, but very distantly related to it.

Isolate A_3 was found to infect most of the plants reported to be susceptible to Bottlegourd mosaic virus except C. sativus var. Local White and Long Green. It also did not infect C. annuum var. Suryamukhi or Nicotiana spp. The thermal inactivation point and dilution end point of this isolate were also found to be very close to that of vein yellowing virus (Harpaz and Cohen, 1965), but the ageing in vitro of both the viruses were found to differ. Complete similarity of transmission of both these viruses by B. tabaci has also been existed. Since no information on the particle size of the vein yellowing virus,

this property could not be compared. From this comparative study it may be said that the isolate A_3 has some similarities with the vein yellowing virus (Harpaz and Cohen, 1965) and it may be distantly related to it.

The properties of the isolate A_6 resembled the properties of the cucurbit latent virus (Webb, 1963) in many respects. The thermal inactivation point and the dilution end point of both of them were more or less similar. The ageing in vitro of the isolate A_6 , however, was found to be slightly more than that of other virus but this variation may be due to the variation of the experimental conditions. The isolate A_6 produced similar symptoms in C. sativus var. Local White, C. sativus var. Long Green, C. pepo cv. Early Prolific Straightneck and C. melo (Cantaloupe Selection B 63-3) but it did not produce any symptom in C. amarananticolour. According to Milne et al. (1969) however, some isolates of the Cucurbit latent virus did not infect C. amarananticolour. As regards to the transmissibility, both the viruses are sap transmissible and also transmissible by N. persicae. Since no informations are available on the particle size of the Cucurbit latent virus, the observed length of the particle of A_6 could not be compared with that of the latent virus. But considering the similarity of these two viruses with respect to physical properties, host range and transmissibility, the isolate A_6 may be regarded as a strain of Cucurbit latent virus. The isolate A_4 has been found to be very close to the isolate A_6 . It is similar to A_6 in all respects except ageing in vitro and its reaction to L. vulgaris, N. charantia and

L. acutangula. The ageing in vitro of A_4 was 12 hours whereas that of A_6 was 24 hours. L. vulgaris appeared to be symptomless carrier of A_4 but A_6 produced distinct symptoms in this plant. A_4 produced symptoms in M. charantia but A_6 did not infect this plant. In L. acutangula symptoms were produced by A_6 and not by A_4 . As regards to their particle size, slight differences in length was observed. The length of the particle of A_4 was 525.0 m/ μ and that of the particle of A_6 was 675.0 m/ μ . Thus the isolate A_4 may be considered a different strain of cucurbit latent virus.

The isolate A_5 was found to possess many properties similar to the Cucurbit mosaic virus (Hariharasubramanian and Badani, 1964). Both of them had the same thermal inactivation point and more or less similar host range. Both of them produced symptoms in the common cucurbitaceous plants and on C. pepo cv. Early Prolific Straightneck they produced almost identical symptoms. Both of them were sap and aphid transmissible. A_5 has been found to be transmitted by M. persicae and the cucurbit mosaic virus was found to be transmitted by A. laburnii and no report is available on the transmissibility of cucurbit mosaic virus by M. persicae. The two viruses, however, differed in their dilution end point, ageing in vitro and their reaction to L. vulgaris. The differences in dilution end point and ageing in vitro may be due to the variation in the experimental conditions, particularly in the differences in the source plants of the virus. In case of cucurbit mosaic virus, C. pepo was used whereas in case of A_5 C. moschata

was used. Regarding the reaction of these two viruses to L. vulgaris, this plant was found to be symptomless carrier for A₅ whereas cucurbit mosaic virus produced definite symptoms in it. As regards to the particle size, it could not be compared as no information is available on the length of the particle of cucurbit mosaic virus. Thus from these wide similarities, the isolate A₅ may be regarded as identical to or a strain of Cucurbit mosaic virus.

The isolate A₇ resembled the watermelon mosaic virus 1 (Webb and Scott, 1965) in many respects. The dilution end point and ageing in vitro of both the viruses were more or less the same. The thermal inactivation point of them was very close to each other. The host range of both of them was restricted to Cucurbitaceae and both of them were sap and aphid transmissible. Both of them produced identical symptoms in C. pepo cv. Early Prolific Straightneck, C. lanatus (Charleston Gray Watermelon) and L. acutangula. But isolate A₇ did not infect C. melo (Cantaloupe Selection 3 63-3). Milne et al. (1969), however, pointed out the unsuitability of this plant for testing watermelon mosaic virus 1 (WMV-1). The shape of the particles of both the viruses was flexuous rod but they to some extent differed in length. The length of the particle of WMV-1 is 746.0 m μ whereas that of the isolate A₇ is 625.0 m μ . Thus the isolate A₇ may be regarded to be a virus very close to WMV-1 or in other words it may be considered as a strain of WMV-1.

The remaining two isolates namely A₈ and A₉ did not resemble any known Cucurbit virus. As regards A₈, it was somewhat

found to be a little bit close to A_1 in host range. Both of them produced infection in L. vulgaris, L. charantia, T. anguina, C. vulgaris var. fistulosus, C. lanatus, C. pepo cv. Early Prolific Straightneck and C. sativus var. Local white and Long Green. The host range of them was restricted only to the plants belonging to the family Cucurbitaceae. But the physical properties excepting dilution end point largely differed. The thermal inactivation point of A_0 was found to be 45-50°C whereas that of A_1 was found to be 50-55°C. The ageing in vitro of A_0 was found to be 12 hours whereas that of A_1 was found to be 6 hours. As for the particle length, wide differences were observed. The particle length of the isolate A_0 was found to be 874.9 m μ and that of A_1 was found to be 712.5 m μ . Besides these, the characteristic differences between these two isolates were, however, found in insect transmissibility and production of symptoms in C. moschata. A_0 was aphid transmissible whereas A_1 was only sap transmissible. A_0 produced enations in C. moschata under favourable conditions but A_1 did not produce any enation in C. moschata under any condition. The isolate A_0 could not be compared with any other isolates because of its wide differential reactions on cucurbitaceous hosts, insect transmissibility, dilution end point and particle characteristics. So, this isolate may be regarded as a new virus and may be designated as pumpkin enation mosaic virus.

The isolate A_0 also did not resemble any known cucurbit virus. The isolate A_0 was found to be both sap and A. gossypii transmissible. The isolate A_0 was also found to

vary widely from all other isolates. Slight similarity of this isolate in host reaction was found to be observed with cucurbit mosaic virus (Hariharasubramanian and Padani, 1964) but the physical properties of isolate A_9 and cucurbit mosaic virus were found to differ greatly. The dilution end point, thermal inactivation point and ageing in vitro of A_9 were found to be $10^{-1.5} - 10^{-2}$, $40-45^{\circ}\text{C}$ and 48 hours respectively whereas that of cucurbit mosaic virus were $1:5000$, 55°C and 72 hours respectively. So, the isolate A_9 could not be compared with cucurbit mosaic virus. The host range of this isolate was found to be restricted to only the members of family Cucurbitaceae. It was found to infect and produce symptoms on L. vulgaris, L. charentia, T. anguinum, L. cylindrica, C. pepo cv. Early Prolific Straightneck and C. sativus cv. Local white and C. sativus cv. Long green. L. acutangula was found to be symptomless carrier of this virus. D. cerifera, C. vulgaris var. fistulosus, C. lanatus and C. melo (Cantaloupe Selection B 63-3) were not found to be infected by this isolate. The characteristic differences of this isolate with all other isolates are the production of symptoms on C. moschata, the particle characteristics, insect transmissibility, dilution end point and the nature of reactions on different host species. The length of the particle of A_9 was found to be 785.0 m μ . So, this isolate may be regarded as a new virus and may be designated as Pumpkin mild mosaic virus.

From the studies it is apparent that type II symptoms of C. moschata Poir plants, predominated in the fields, is a complex of eight different viruses. These eight viruses were

found to differ from one another only in their specialized properties. The viruses involved in this complex were found to be as Strains of Bottle-gourd mosaic virus (isolate A₁ and A₂), vein yellowing virus (isolate A₃), Strains of Cucurbit latent virus (isolate A₄ and A₆), Cucurbit virus (isolate A₅), Watermelon mosaic virus-1 (isolate A₇) and two new viruses as Pumpkin enation mosaic virus (isolate A₈) and Pumpkin mild mosaic virus (isolate A₉).

Considering the fact that the different isolates obtained from the pumpkins produced different symptoms in the leaves of Cucurbita moschata plants on inoculation, attempts have been made to study the alterations of some important biochemical components such as (a) chlorophyll (a,b and total) (b) buffer soluble protein (c) Total (TCA precipitable) sap protein (d) reducing sugar and starch and (e) ribonucleic acid content in the leaves of the inoculated plants at different stages of pathogenesis namely stage I (10 days after inoculation), stage II (40 days after inoculation) and stage III (70 days after inoculation).

In case of chlorophyll a content, it was observed that all the isolates after being inoculated to healthy pumpkin plants, significantly reduced this component. When the first stage of pathogenesis was considered, it was observed that maximum reduction of chlorophyll a content took place with isolates A₃ and A₄, where the percentages of reduction were found to be 75.0 and 63.3. Minimum reduction of chlorophyll a content was observed with the

isolate A_8 where the percentage of reduction was found to be 16.6. In case of the other isolates namely A_1 , A_2 , A_5 , A_6 , A_7 and A_9 , the respective percentages of reduction were found to be 37.5, 50.0, 29.1, 41.6, 50.0 and 37.4.

When the second stage of pathogenesis was considered, it was observed that the chlorophyll a contents of healthy leaves were found to increase. The percentage of increase in this case was found to be 45.8. Increase in chlorophyll a content was also observed at this stage with the isolates A_1 , A_3 , A_4 , A_6 and A_7 . The percentages of increase of the chlorophyll a content with respect to these isolates were 66.6, 33.3, 650.0, 135.7 and 125.0 respectively. But the chlorophyll a contents of the leaves with respect to isolates A_2 , A_5 and A_8 were found to further decrease at this stage of pathogenesis from that observed at the first stage. The respective percentages of decrease were 58.3, 5.8 and 35.0. With the isolate A_9 , however the chlorophyll a content remained more or less same at both the stages.

When the third stage of pathogenesis was considered, it was observed that the chlorophyll a content of healthy leaves was found to decrease. The percentage of decrease was found to be 31.4. At this stage of pathogenesis reduction of chlorophyll a content was found with all the isolates except A_2 and A_3 . The percentages of reduction with respect to the second stage for the isolates A_1 , A_4 , A_6 , A_7 , A_8 and A_9 were 76.0, 73.3, 84.8, 81.4, 61.5 and 60.0 respectively. With the isolates A_2 and A_3 , the chlorophyll a contents, however, were found to increase. The percentages of increase at this stage with respect

to the second stage were 80.0 and 112.5 respectively. With the isolate A₅ on the other hand, the chlorophyll a content did not alter from that found at the second stage.

Thus it is apparent that the isolates altered the chlorophyll a content in the inoculated leaves, but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the chlorophyll a content was found to decrease with all the isolates but with the advance of infection, however, the level of alterations differed. After 40 days of inoculation, the chlorophyll a content was found to increase to a large extent from that found after 10 days of inoculation with A₄, A₆ and A₇, high increase was found with A₁, moderate increase was found with A₃. On the other hand, high decrease was found with A₂, moderate decrease was found with A₈, very slight decrease was found with A₅ and no change was found with A₉. With the further advance of infection, or in other words, 70 days after inoculation, the chlorophyll a content was found to decrease to a large extent from that found after 40 days of inoculation in A₁, A₄, A₆, A₇, A₈ and A₉. In A₃ it was found to increase in large extent but moderate increase was found with A₂, whereas with A₅ the chlorophyll a content remained more or less the same.

In case of chlorophyll b content also it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly reduced this component. When the first stage of pathogenesis was considered, it was observed that as

in case of chlorophyll a, maximum reduction of chlorophyll b content was observed with isolates A_4 and A_3 , where the percentages of reduction were found to be 76.3 and 67.1. Minimum reduction of chlorophyll b content was observed with the isolate A_8 , where the percentage of reduction was found to be 7.8. In case of the other isolates namely A_1 , A_2 , A_5 , A_6 , A_7 and A_9 , the respective percentages of reduction were found to be 39.4, 44.7, 27.6, 34.2, 40.7 and 28.9.

When the second stage of pathogenesis was considered, it was observed that in contrast to chlorophyll a, the chlorophyll b content of healthy leaves were found to decrease. The percentage of decrease in this case was found to be 5.2. But this component was found to increase at this stage with the isolates A_1 , A_4 , A_6 and A_7 . The percentages of increase of the chlorophyll b content with respect to these isolates were 21.7, 288.8, 24.0 and 35.5 respectively. But the chlorophyll b contents of the leaves with respect to isolates A_2 , A_3 , A_5 , A_8 and A_9 were found to decrease at this stage of pathogenesis from that found at the first stage. The respective percentages of decrease were 66.6, 12.0, 12.7, 52.8 and 38.8.

When the third stage of pathogenesis was considered, it was observed that the chlorophyll b content of healthy leaves was found to decrease further. The percentage of decrease was found to be 43.0. At this stage of pathogenesis reduction of chlorophyll b content was found with all the isolates except A_2 and A_3 . The percentages of reduction with respect to the second

stage for the isolates A_1 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 were 20.3, 67.1, 47.9, 65.4, 77.0, 75.7 and 48.4 respectively.

With the isolates A_2 and A_3 , the chlorophyll b contents, however, were found to increase. The percentages of increase at this stage with respect to the second stage were 57.1 and 86.3 respectively.

Thus, it is apparent that the isolates altered the chlorophyll b content in the inoculated leaves, but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the chlorophyll b content was found to decrease with all the isolates but with the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the chlorophyll b content was found to increase to a large extent from that found after 10 days of inoculation with A_4 and moderate increase was observed with A_1 , A_6 and A_7 . On the other hand, high decrease was found with A_2 , moderate decrease was found with A_8 and A_9 and very slight decrease was found with A_3 and A_5 . With the further advance of infection, or in other words 70 days after inoculation, the chlorophyll b content was found to decrease to a large extent from that found after 40 days of inoculation in A_1 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 . But with A_3 it was found to increase in large extent and with A_2 the chlorophyll b content was found to be moderately increased.

In case of total chlorophyll content also, it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly reduced this component. When the

First stage of pathogenesis was considered maximum reduction of total chlorophyll content was observed with isolates A₃ and A₄, where the percentages of reduction were found to be 68.0 and 77.0. Minimum reduction of total chlorophyll content was observed with isolate A₈ where the percentage of reduction was found to be 9.0. In case of the other isolates namely A₁, A₂, A₅, A₆, A₇ and A₉, the respective percentages of reduction were found to be 39.0, 46.0, 27.0, 36.0, 43.0 and 31.0.

When the second stage of pathogenesis was considered, the total chlorophyll content of healthy leaves were found to increase. The percentage of increase in this case was found to be 7.0. Increase of total chlorophyll content was also observed at this stage with the isolates A₁, A₄, A₆ and A₇. The percentages of increase of the total chlorophyll content with respect to these isolates were 34.4, 334.7, 48.4 and 54.3 respectively. But the total chlorophyll contents of the leaves with respect to isolates A₂, A₃, A₅, A₈ and A₉ were found to further decrease at this stage of pathogenesis from that found at the first stage. The respective percentages of decrease were 64.8, 3.1, 12.5, 48.5 and 30.4.

When the third stage of pathogenesis was considered, it was observed that the total chlorophyll content of healthy leaves was found to decrease. The percentage of decrease was found to be 38.3. At this stage of pathogenesis, reduction of total chlorophyll content was found with all isolates except A₂ and A₃. The percentages of reduction with respect to second

stage for the isolates A_1 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 were 79.2, 60.0, 35.9, 85.2, 70.4, 72.3 and 52.0 respectively. With the isolates A_2 and A_3 the total chlorophyll contents, however, were found to increase. The percentages of increase at this stage with respect to second stage were 65.1 and 93.8 respectively.

Thus it is apparent that the isolates altered the total chlorophyll content in the inoculated leaves, but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the total chlorophyll content was found to decrease with all the isolates but with the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the total chlorophyll content was found to increase to a large extent from that found after 10 days of inoculation with A_4 , high increase was found with A_6 and A_7 and moderate increase was found with A_1 . On the other hand, high decrease was found with A_2 , moderate decrease was found with A_5 , A_8 and A_9 and very slight decrease was found with A_3 . With the further advance of infection, or in other words 70 days after inoculation, the total chlorophyll content was found to decrease to a large extent from that found after 40 days of inoculation with A_1 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 . But with A_3 it was found to increase in large extent and with A_2 the total chlorophyll content was found to be moderately increased.

In case of buffer soluble protein content, it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly altered this component. When the first stage of pathogenesis was considered, it was observed that maximum increases of buffer soluble protein content was observed with isolate A₃ and A₆, where the percentages of increase were found to be 110.0 and 123.3 respectively. Minimum increase of buffer soluble protein content was found with the isolate A₃ where the increase was found to be 8.0. In case of other isolates namely A₁, A₄, A₇ and A₉ the respective percentages of increases were found to be 20.0, 50.0, 76.6 and 96.6. Isolates A₂ and A₅ were found to reduce this component and the percentages of reduction in these cases were found to be 15.3 and 2.0 respectively.

When the second stage of pathogenesis was considered, it was observed that the buffer soluble protein content of healthy leaves were found to increase. The percentage of increase in this case was found to be 246.6. Increase was also observed at this stage with the isolates A₁, A₂, A₃, A₄, A₅, A₆, A₇, A₈ and A₉. The percentages of increase of the buffer soluble protein content with respect to these isolates were 20.70, 163.1, 5.3, 82.0, 234.0, 44.0, 21.5, 118.7 and 37.5 respectively.

When the third stage of pathogenesis was considered, it was observed that the buffer soluble protein content of healthy leaves was found to decrease. The percentage of decrease was found to be 72.4. At this stage of pathogenesis, further

increase of buffer soluble protein content was found with the isolates A_2 , A_3 , A_5 , A_6 , A_8 and A_9 . The percentage of increase with respect to second stage for these isolates were found to be 59.0, 73.7, 13.6, 18.7, 58.0 and 73.5 respectively. With the isolates A_1 , A_3 and A_7 , the buffer soluble protein contents, however, were found to be reduced. The percentages of reduction in these case at this stage with respect to second stage were found to be 74.2, 45.9 and 17.7 respectively.

Thus it is apparent that the isolates altered the buffer soluble protein content in the inoculated leaves but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the buffer soluble protein content was found to be increased with all the isolates except A_2 and A_5 . With the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the buffer soluble protein content was found to increase to a large extent from that found after 10 days of inoculation with A_5 and A_1 , high increase was found with A_2 and A_8 , moderate increase was observed with A_4 , A_6 , A_7 and A_9 and very slight increase was observed with A_3 . With the further advance of infection, or in otherwords 70 days after inoculation, the buffer soluble protein content was found to be increased to a large extent from that found after 40 days of inoculation with A_3 and A_9 , moderate increase was observed with A_2 and A_8 , and slight increase was observed with A_5 and A_6 . But with A_1

it was found to reduce it in large extent. With A_4 it was moderately reduced and with A_7 only slight reduction of it was observed.

In case of total (TCA precipitable) sap protein content, it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly altered this component. When the first stage of pathogenesis was considered, it was observed that maximum increase of total (TCA precipitable) sap protein was observed with A_6 , where the percentage of increase was found to be 103.7. Minimum increase of total (TCA precipitable) sap protein was found with the isolates A_7 , A_4 and A_1 where the percentages of increase were found to be 22.3, 26.5 and 27.5 respectively. In case of other isolates namely A_2 , A_3 , A_5 , A_8 and A_9 , the respective percentages of increase were found to be 57.8, 71.3, 59.07, 83.9 and 48.9 respectively.

When the second stage of pathogenesis was considered, it was observed that the total (TCA precipitable) sap protein contents of healthy leaves were found to increase. The percentage of increase in this case was found to be 105.9. Increase was also observed at this stage with the isolates A_1 , A_4 , A_5 , A_7 and A_9 . The percentages of increase of the total (TCA precipitable) sap protein content with respect to these isolates were 27.7, 6.0, 85.6, 11.3 and 35.1 respectively. But the total (TCA precipitable) sap protein content of the leaves with respect

to isolates A₂, A₃, A₆ and A₈ were found to decrease further at this stage of pathogenesis from that found at the first stage. The respective percentages of decrease were 20.5, 39.4, 45.5 and 25.9.

When the third stage of pathogenesis was considered, it was observed that the total (TCA precipitable) sap protein contents of healthy leaves were found to decrease. The percentage of decrease was found to be 91.1. At this stage of pathogenesis, reduction of total (TCA precipitable) sap protein content was found with all isolates. The percentages of reduction at this stage with respect to second stage for the isolates A₁, A₂, A₃, A₄, A₅, A₆, A₇, A₈ and A₉ were found to be 85.0, 81.4, 53.2, 84.9, 91.2, 86.3, 52.6, 58.2 and 61.6 respectively.

Thus it is apparent that the isolates altered the total (TCA precipitable) sap protein content in the inoculated leaves but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the total (TCA precipitable) sap protein content was found to increase with all the isolates but with the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the total (TCA precipitable) sap protein content was found to increase to a large extent from that found after 10 days of inoculation with A₆, moderate increase was observed with A₁, A₇ and A₉ and very slight increase was observed with A₄. On the otherhand, high decrease was found with A₆ and moderate decrease was found with A₃ and A₈. With the further advance of infection,

or in otherwords 70 days after inoculation, the total (TCA precipitable) sap protein content was found to decrease to a large extent from that found after 40 days inoculation with A_1 , A_2 , A_4 , A_5 , A_6 and A_9 and moderately with A_3 , A_7 and A_8 .

In case of reducing sugars, it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly reduced this component. With the first stage of pathogenesis was considered, it was observed that maximum reduction of reducing sugar content was observed with A_1 , A_5 and A_9 , where the percentages of reduction were found to be 84.6, 87.6 and 90.7. Minimum reduction of reducing sugar content was observed with A_4 , where the percentage of reduction was found to be 15.3. In case of other isolates namely A_2 , A_3 , A_6 and A_7 the respective percentages of reduction were found to be 38.4, 27.6, 78.4 and 38.4.

When the second stage of pathogenesis was considered, it was observed that the reducing sugar contents of healthy leaves were found to decrease. The percentage of decrease in this case was found to be 53.8. Decrease was also observed at this stage with the isolate A_9 . The percentage of decrease of the reducing sugar content in this case was found to be 20.2. But the reducing sugar contents of the leaves with respect to isolate A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 and A_8 were found to increase from that observed at the first stage. The respective percentages of reduction of reducing sugar content in these cases were found to be 190.0, 17.5, 14.8, 3.6, 700.0, 185.7, 32.5 and 916.6 respectively.

When the third stage of pathogenesis was considered, it was observed that the reducing sugar content of healthy leaves was found to increase again. The percentage of increase was found to be 163.3. At this stage of pathogenesis, increase of reducing sugar content was observed with all isolates except A_2 . The percentages of reduction with respect to the second stage for the isolates A_1 , A_3 , A_4 , A_5 , A_6 , A_7 and A_9 were found to be 96.5, 29.6, 7.3, 17.1, 95.0, 2.7 and 38.1 respectively. With the isolate A_2 the reducing sugar content, however, was found to decrease the percentage of decrease at this stage with respect to second stage for this isolate was found to be 8.5. With the isolate A_8 on the otherhand, the reducing sugar content did not alter from that found at the second stage.

Thus it is apparent that the isolates altered the reducing sugar content in the inoculated leaves but the pattern of alternation differed with the isolates. Within 10 days after inoculation, the reducing sugar content was found to decrease with all the isolates except A_9 but with the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the reducing sugar content was found to increase to a large extent from that found after 10 days of inoculation with A_5 and A_8 , high increase was found with A_1 and A_6 , moderate increase was found with A_2 , A_3 and A_7 and very slight increase was observed with A_4 . On the otherhand, moderate decrease was found with A_9 . With the further advance of infection or in otherwords 70 days after inoculation, the reducing sugar content was found to increase to a large extent

from that found after 40 days of inoculation in A₁ and A₆ and moderate increase was observed with A₃, A₄, A₅ and A₉ and very slight increase was observed with A₇. But with A₂ slight decrease of reducing sugar content at this stage was found. With the isolate A₈ on the otherhand, the reducing sugar content remained more or less the same.

In case of starch, it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly altered this component. When the first stage of pathogenesis was considered, it was observed that maximum reduction of starch content was observed with isolate A₅, where the percentage of reduction was found to be 80.0. Minimum reduction of starch content was observed with A₂, where the percentage of reduction was found to be 6.5. In case of other isolates namely A₆, A₈ and A₉ the percentages of reduction were found to be 47.8, 27.8 and 40.0 respectively. But the isolates A₁, A₃, A₄ and A₇ were found to increase it. Maximum increase was observed with A₄, where the percentage of increase was found to be 88.8. Minimum increase was found with A₃, where the percentage of increase was found to be 2.6. In case of other isolates namely A₁ and A₇, the respective percentages of increase were found to be 18.2 and 47.8.

When the second stage of pathogenesis was considered, it was observed that the starch content of healthy leaves was found to decrease. The percentage of decrease in this case was found to be 89.5. Decrease of starch content was also observed with all isolates. The respective percentages of decrease for

the isolates A₁, A₂, A₃, A₄, A₅, A₆, A₇, A₈ and A₉ were 38.9, 53.4, 40.6, 576.0, 78.2, 35.8, 28.8, 63.8 and 57.9 respectively.

When the third stage of percentages was considered, it was observed that the starch content of healthy leaves was found to increase again. The percentage of increase was found to be 1358.3. At this stage of pathogenesis, further reduction of starch content was found with all the isolates except A₆ and A₈. The percentages of reduction with respect to second stage for the isolates A₁, A₃, A₄, A₅, A₇ and A₉ were 85.8, 28.5, 90.2, 80.0, 56.1 and 3.4 respectively. With the isolates A₆ and A₈, the starch contents, however, were found to increase. The percentages of increase at this stage with respect to second stage for these isolates were 633.7 and 393.3 respectively. With the isolate A₂ on the otherhand, the starch content did not alter from that found at the second stage.

Thus it is apparent that the isolates altered the starch content in the inoculated leaves, but the pattern of alteration differed with the isolates. Within 10 days after inoculation the starch content was found to decrease with isolates A₂, A₅, A₆, A₈ and A₉ and with respect to isolates A₁, A₃, A₄ and A₇ the starch content was found to be increased. But with the advance of infection, however, the level of alterations differed with the isolates. After 40 days inoculation, the starch content was found to decrease to a large extent from that found after 10 days of inoculation with A₄, high decrease was found with A₅ and A₈, moderate decrease was found with A₁, A₂, A₃, A₆, A₇ and A₉. With

the further advance of infection, or in otherwords 70 days after inoculation, the starch content was found to decrease to a large extent from that found after 40 days of inoculation with A_4 , high decrease was found with A_1 and A_5 , moderate decrease was observed with A_3 and A_7 and very slight decrease was observed with A_9 . But with A_6 it was found to increase in large extent and high increase was also observed with A_8 but with A_2 the starch content remained more or less the same.

In case of ribonucleic acid (RNA) it was observed that all the isolates, after being inoculated to healthy pumpkin plants, significantly reduced this component. When the first stage of pathogenesis was considered, it was observed that, maximum reduction of RNA content was observed with isolates A_8 , where the percentage of reduction was found to be 84.2. Minimum reduction of RNA content was observed with A_1 , where the percentage of reduction was found to be 12.2. In case of other isolates namely A_2 , A_3 , A_4 , A_5 , A_6 , A_7 and A_9 , the respective percentages of reduction were 52.2, 56.1, 71.8, 20.0, 78.9 and 69.4.

When the second stage of pathogenesis was considered, it was observed that the RNA contents of healthy leaves were found to decrease. The percentage of decrease in this case was found to be 405.5. Decrease was also observed at this stage with the isolates A_1 and A_6 . The percentages of decrease of the RNA content with respect to these isolates were 21.2 and 55.2. But the RNA contents of the leaves with respect to isolates A_2 , A_3 , A_4 , A_5 , A_7 , A_8 and A_9 were found to further increase at this stage of pathogenesis from that found at the first stage. The

respective percentages of decrease were 2.9, 108.0, 116.0, 41.2, 233.3, 262.2 and 28.7.

When the third stage of pathogenesis was considered, it was observed that the RNA content of healthy leaves was found to decrease further. The percentage of decrease was found to be 74.7. At this stage of pathogenesis, reduction of RNA content was found with all isolates except A₅. The percentages of reduction with respect to second stage for the isolates A₁, A₂, A₃, A₄, A₆, A₇, A₈ and A₉ were 76.1, 59.2, 72.6, 71.4, 29.4, 82.5, 23.3 and 67.3 respectively. With the isolate A₅, the RNA content, however, was found to increase. The percentage of increase at this stage with respect to second stage for this isolate was found to be 56.6.

Thus it is apparent that the isolates altered the RNA content in the inoculated leaves but the pattern of alteration differed with the isolates. Within 10 days after inoculation, the RNA content was found to decrease with all the isolates but with the advance of infection, however, the level of alterations differed with the isolates. After 40 days of inoculation, the RNA content was found to increase to a large extent from that found after 10 days of inoculation with A₇ and A₈, high increase was found with A₃, moderate increase was found with A₅ and A₉ and very slight increase was found with A₂. On the otherhand, moderate decrease was observed with A₁ and A₆. With the further advance of infection, or in other words 70 days after inoculation,

the RNA content was found to decrease to a large extent from that found after 40 days of inoculation with A₇ and moderately with A₁, A₂, A₃, A₄, A₆, A₈ and A₉. But with A₅ it was found to increase the RNA content moderately.

Abstract

VI. SUMMARY

1. The fields particularly in the districts of Burdwan, Hooghly, 24 Parganas, Malda and Nadia were surveyed during February-March 1973 for recording the incidence of virus diseases. Symptoms of virus diseases were observed in $60.00 \pm 4.58 - 89.33 \pm 3.47$ per cent plants in the fields of all districts under consideration. Six different types (I-VI) of symptoms were found to occur in the fields. The incidence of Type I, II, III, IV, V and VI symptoms were $13.66 \pm 0.87 - 21.66 \pm 0.33$, $36.66 \pm 1.20 - 51.00 \pm 1.52$, $7.00 \pm 1.00 - 8.66 \pm 0.87$, $9.33 \pm 1.20 - 14.66 \pm 0.33$, $2.00 \pm 0.57 - 7.00 \pm 0.57$ and $15.00 \pm 1.52 - 16.66 \pm 0.87$ per cent respectively. The type II symptoms were found to predominate in all the fields taken under consideration and the percentages of infection of type II symptoms in the districts of Burdwan, Hooghly and Nadia were 36.66 ± 1.20 , 43.66 ± 1.45 and 51.00 ± 1.52 .
2. When the leaves showing type II symptoms were collected from the fields from time to time and sap transmission studies were performed 31-80 per cent transmission was obtained. The symptoms produced in the inoculated plants were again found to differ. Thus the type II symptoms in the field was considered as complex.
3. On serial transmission tests from different batches of collections, nine different types of symptoms were identified. These nine types of symptoms was maintained in young Cucurbita moschata Poir plants in pure forms under insect proof condition

and the viruses involved therein were designated as A₁, A₂, A₃, A₄, A₅, A₆, A₇, A₈ and A₉.

4. The thermal inactivation points (TIP) of all the isolates were found to be in between 40-55°C. The TIP of A₃ and A₉ was 40-45°C, the TIP of A₂, A₄, A₆, A₇ and A₈ was 45-50°C whereas the TIP of A₁ and A₅ was found to be 50-55°C.

5. The dilution end points (DEP) of all the isolates were found to be in between $\angle 10^{-1} - 10^{-3.5}$. The DEP of A₁ and A₈ was found to be less than 10^{-1} . The DEP of A₅ and A₉ was found to be $10^{-1.5} - 10^{-2}$. The DEP of A₃, A₄ and A₆ was found to be $10^{-2} - 10^{-2.5}$, the DEP of A₇ was found to be $10^{-2.5} - 10^{-3}$ and the DEP of A₂ was found to be $10^{-3} - 10^{-3.5}$.

6. The ageing in vitro of all the isolates at room temperature (24-32°C) were found to be in between 6 hours to 168 hours. The ageing in vitro of A₁ was 6 hours, of A₄ and A₈ was 12 hours, of A₂, A₅ and A₉ was 48 hours and of A₃, A₆ and A₇ was 60 hours, 84 hours and 168 hours (7 days) respectively.

7. As regards the host range 37 plants belonging to Cucurbitaceae, Solanaceae, Leguminosae, Cruciferae, Caricaceae, Chenopodiaceae, Apocynaceae, Amaranthaceae and Compositae families were tested. It was observed that all the isolates except A₂, A₃ and A₅ could infect only the plants belonging to the cucurbitaceae family. The isolates A₂ and A₅ were found to infect S. melongena and P. hybrida plants besides the cucurbitaceous hosts.

Isolate A₃ was found to infect S. melongena, P. hybrida, B. campestris var. Sarson, S. oleracea and B. vulgaris plants besides the cucurbitaceous hosts. But in case of B. campestris var. Sarson, S. oleracea and B. vulgaris this virus was present symptomlessly. As regards cucurbitaceous hosts in case of A₂ Luffa acutangula, Luffa cylindrica and Citrullus vulgaris var. fistulosus, in case of A₄ Lagenaria vulgaris Citrullus vulgaris var. fistulosus and Citrullus lanatus, in case of A₅ Lagenaria vulgaris and Citrullus vulgaris var. fistulosus, in case of A₆ Citrullus lanatus, in case of A₇ Lagenaria vulgaris in case of A₈ Benincasa cerifera and in case of A₉ Luffa acutangula only were found to be symptomless carriers of the respective viruses.

8. In the transmission studies of the isolates with Aphis gossypii Glover, Myzus persicae Sulz. and Benicisia tabaci Gemm. it was observed that A₁ and A₂ were not transmissible by them. A₃ was transmissible by B. tabaci and not by A. gossypii or M. persicae. A₄ and A₆ were transmissible by only M. persicae. A₅, A₇, A₈ and A₉ were transmissible by only A. gossypii. In case of A. gossypii transmissible isolates namely A₅, A₇, A₈ and A₉, the percentages of transmission were 37, 43, 29 and 22 respectively. In case of M. persicae transmissible isolates namely A₄ and A₆ the percentage of infection for both the cases were same (40%). The percentage of transmission of A₃ by B. tabaci was 32. An A. gossypii once virulified could retain the isolates A₅, A₇, A₈ and A₉ upto a period of 72 hours, 96 hours, 24 hours and 96 hours respectively. An aphid M. persicae virulified with either

A_4 or A_6 was found to retain the isolate for the same period or in other words for a period of 96 hours only. The retention period of isolate A_3 in B. tabaci once virulified was found to be 48 hours.

9. In the electronmicroscopic studies, the particles of all the isolates were found to be flexible rods of varied length. The length of the particles of A_2 and A_4 were in between 500 m/ μ - 600 m/ μ . The length of the particle of A_2 was 587.5 m/ μ and that of A_4 was 525.0 m/ μ . The length of the particle of A_6 was 675.0 m/ μ . The length of the particles of A_1 , A_3 , A_5 and A_9 were in between 700 m/ μ - 800 m/ μ . The respective lengths of them were 712.5 m/ μ , 725.0 m/ μ , 743.6 m/ μ and 785.0 m/ μ . The length of the particle of A_7 and A_8 were in between 800 m/ μ - 900 m/ μ and the respective lengths were 825.0 m/ μ and 874.9 m/ μ .

10. [From the serological studies, it appeared that the isolate A_1 was closely related to the isolate A_3 and A_8 . Isolate A_2 was closely related to the isolate A_3 . Isolate A_3 was closely related to A_1 , A_2 , A_4 , A_5 , A_6 and A_7 . Isolate A_4 was closely related to A_3 and A_6 . Isolate A_5 was closely related to A_1 and A_4 . Isolate A_7 was closely to A_3 .] But the isolate A_8 and A_9 were not related to any other isolate and also unrelated to each other. Thus the isolates may form three groups, based on their serological relationship, Group 1 included isolate A_1 , A_2 , A_3 , A_4 , A_5 , A_6 and A_7 . Group 2 included isolate A_8 and Group 3 included isolate A_9 .

11. When the properties of the isolates were compared with those of the known cucurbit viruses, it appears that the isolate A_1

resembled Bottlegourd mosaic virus reported by Vasudeva and Lal (1943). Since isolate A_2 was close to A_1 in many respects, A_1 and A_2 may be considered as two different strains of bottlegourd mosaic virus. Isolate A_3 was found to be close to Vein yellowing virus reported by Heryns and Cohen (1965). Isolate A_4 and A_6 were found to be very close to Cucurbit latent virus reported by Lebb (1963). Isolate A_5 was found to be close to Cucurbit Mosaic virus reported by Hariharasubramanian and Radani (1964). Isolate A_7 was found to be very closer to Internelson Mosaic virus 1 reported by Lebb and Scott (1965). The isolate A_8 and A_9 did not resemble any known cucurbit virus and thus these two isolates may be considered as new viruses and may be designated as Pumpkin Anation Mosaic virus and Pumpkin Mild Mosaic virus respectively.

12. In the biochemical studies, it was observed that the inoculation of all the isolates altered (a) chlorophyll (a,b and total), (b) buffer soluble protein (c) total (PCA precipitable) sap protein (d) reducing sugar and starch and (e) ribonucleic acid contents in the leaves of C. moschata plants at different stages of pathogenesis.

a) The chlorophyll a content was reduced with respect to all isolates within 10 days after inoculation. At 40 days after inoculations this component was increased from that found at 10 days of inoculation with respect to A_1 , A_3 , A_4 , A_6 and A_7 . But it was found to be reduced with A_2 , A_5 and A_8 . No change of this component was however observed with A_9 . At 70 days after inoculation the chlorophyll a content was reduced with A_1 , A_4 , A_6 ,

A₇, A₈ and A₉ whereas with A₂ and A₃ it was found to increase from that found at 40 days after inoculation. At the stage of pathogenesis, no change of chlorophyll a content was observed with A₅.

Chlorophyll b content was reduced with respect to all isolates at 10 days after inoculation. A₁, A₄, A₆ and A₇ increased this component from that found at 10 days after inoculation and A₂, A₃, A₅, A₈ and A₉ decreased it as compared to 10 days after inoculation. At 70 days after inoculation A₁, A₄, A₅, A₆, A₇, A₈ and A₉ decreased this component from that found at 40 days after inoculation. But A₂ and A₃ increased it from that at 40 days after inoculation.

Total chlorophyll content was also decreased with all the isolates at 10 days after inoculation. At 40 days after inoculation A₁, A₄, A₆ and A₇ increased it over that found at 10 days after inoculation. But A₂, A₃, A₅, A₈ and A₉ decreased it from that found at 10 days after inoculation. At 70 days after inoculation A₁, A₄, A₅, A₆, A₇, A₈ and A₉ decreased it from that found at 40 days after inoculation. But A₂ and A₃ increased it from that observed at 40 days after inoculation.

b) In case of buffer soluble protein, A₁, A₃, A₄, A₆, A₇, A₈ and A₉ increased it at 10 days after inoculation whereas A₂ and A₅ decreased it. At 40 days after inoculation this component was increased with all the isolates. At 70 days after inoculation A₂, A₃, A₅, A₆, A₈ and A₉ increased it from that observed at 40 days after inoculation but A₁, A₄ and A₇ reduced it from that observed at 40 days after inoculation.

c) In case of total (TCA precipitable) cap protein, all the isolates increased it at 10 days after inoculation. At 40 days after inoculation A_1 , A_4 , A_7 and A_9 increased it from that observed at 10 days after inoculation. But A_3 , A_6 and A_8 decreased it from that observed at 10 days after inoculation. At 70 days after inoculation all isolates increased it from that observed at 40 days after inoculation.

d) In case of reducing sugar at 10 days after inoculation all the isolates except A_9 decreased it. At 40 days after inoculation reducing sugar increased with A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 and A_8 but A_9 decreased it from that observed at 10 days after inoculation. At 70 days after inoculation A_1 , A_3 , A_4 , A_5 , A_6 , A_7 and A_9 increased it whereas A_2 decreased it from that observed at 40 days after inoculation. At this stage of pathogenesis no change of reducing sugar content was observed with A_8 .

In case of starch at 10 days after inoculation A_2 , A_5 , A_6 , A_8 and A_9 decreased it whereas A_1 , A_3 , A_4 and A_7 increased it. At 40 days after inoculation starch content was decreased with all the isolates. At 70 days after inoculation A_1 , A_3 , A_4 , A_5 , A_7 and A_9 decreased it from that found at 40 days after inoculation whereas A_6 and A_8 increased this component but with A_2 no change of this component was observed with the respect to 40 days after inoculation.

c) In case of RHA of 10 days after inoculation all the isolates decreased it. At 40 days after inoculation A_2 , A_3 , A_4 , A_5 , A_7 , A_8 and A_9 increased it and A_1 and A_6 decreased it from that observed at 10 days after inoculation. At 70 days after inoculation A_1 , A_2 , A_3 , A_4 , A_5 , A_6 , A_7 , A_8 and A_9 decreased it whereas A_5 increased this component from that observed at 40 days after inoculation.

VII. B I B L I O G R A P H Y

- Adlerz, W.C. 1974. Wind effects on spread of Watermelon mosaic virus 1 from local virus sources to watermelon. J. Econ. Ent. 67 : 361-364.
- *Adsuar, J. 1964. A mosaic disease of watermelon at Laja Valley. J. Agric. Univ., P.Rico. 48: 352.
- *Ainsworth, G.C. 1935a. Virus diseases of cucumber. J. Minist. Agric. xiii, 4: 338-344 (R.P.P. 14: 1935).
- Ainsworth, G.C. 1935b. Mosaic diseases of the cucumbers. Ann. appl. Biol. 22: 55-67.
- Anderson, C.W. 1951a. Some preliminary observations on cucurbit viruses in Florida. Pl. Dis. Repr. 35: 233-234.
- Anderson, C.W. 1951b. Further observations of some cucurbit viruses from Central Florida. Pl. Dis. Repr. 35: 396-398.
- Anderson, C.W. 1952. The distribution of cucurbit viruses in Central Florida. Pl. Dis. Repr. 36: 377-379.
- Anderson, C.W. 1954a. Two muskmelon mosaic virus strains from Central Florida. Phytopathology 44 : 371-374.
- Anderson, C.W. 1954b. Two watermelon mosaic strains from Central Florida. Phytopathology 44: 198-202.
- Anderson, C.W. 1961. Annual Rep. Agric. Exp. Sta., Florida, June 30, 1960, pp. 364.
- Anonymous 1954a. Rep. (1952-53) Indian Council of Agric. Res., New Delhi, India, 2-45.
- Anonymous 1954b. Rep. (1952-53) Indian Council of Agric. Res., New Delhi, India, 79-84.
- Anonymous 1954c. Rep. (1952-53) Indian Council of Agric. Res., New Delhi, India, 75-87.

- Anonymous 1966. International Rice Research Institute, Annual Rep. (1966), Los Banos, Philippines.
- Anonymous, 1970. All India Coordinated Rice Improvement Project, Annual Rep. (1970), Hyderabad, India.
- Auger, J. G., Escaffi, O. and Hone, P.S. 1974. Occurrence of watermelon mosaic virus 2 on Cucurbits in Chile. Pl. Dis. Repr. 58 : 599-602.
- Awasthi, D.N. and Singh, B.P. 1974. Influence of Cucumber mosaic virus on alcohol soluble amino acids and RNA content of infected and non-infected plants of tolerant and susceptible Chilli (Capsicum sp.) varieties. Indian Phytopath. 27: 224-228.
- Basler, E. and Commoner, B. 1956. The effect of Tobacco mosaic virus biosynthesis on the nucleic acid content of Tobacco leaf. Virology 2: 13-28.
- *Bewley, W.F. 1923. 8th Annual Rep. (1922), Cheshunt Exp. Sta., Hertfordshire, 34-45.
- *Bewley, W.F. 1926. 11th Annual Rep. (1925), Cheshunt Exp. Sta., Hertfordshire, 86-89.
- Bhargava, K. 1951. Some properties of four strains of Cucumber mosaic virus. Annl. appl. Biol. 38: 377-388.
- Bhargava, K.S. and Joshi, R.D. 1960. Detection of watermelon mosaic virus in Uttar Pradesh. Curr. Sci. 29: 443-444.
- Bhargava, K.S. and Tewari, J.P. 1970. Trichosanthes dioica, an additional natural host of watermelon mosaic virus. Pl. Dis. Repr. 54: 727-728.
- Bhargava, B., Bhargava, K.S. and Joshi, R.D. 1975. Perpetuation of Watermelon mosaic virus in Eastern Uttar Pradesh, India. Pl. Dis. Repr. 59: 634-636.
- *Brandes, J. 1957. Eine elektronenmikroskopische Schnellmethode zum Nachweis faden- und stabchenformiger Viren, insbesondere in Kartoffel dunkelkeimen. Nachr. bl. dt. PflSchutzd. Braunschweig, 9: 151-152. (Eng. Summ.).

- * Brandes, J. 1964. Identifizierung von gestreckten pflanzen pathogenen Viren auf morphologischer Grundlage. Mitt. biol. Bundesanst. Ld-u., Forstw., 110: 1-130 (Eng. Summ.).
- Break, J., Ulrychova, Lise H. and Cech, H. 1962. Infection of Tobacco and some Chenopodium species by the CV 4 (and 3) and by its nucleic acid. Virology 16 : 105-114.
- Capoor, S.P. and Varma, P.M. 1948. A mosaic disease of Jageria vulgaris Sor. in the Bombay Province. Curr. Sci. 17 : 274-275.
- Choudhury, A.K. 1973. "Studies on Tungro virus disease of Rice in W. Bengal", Ph.D. Thesis submitted at the University of Kalyani, W.Bengal, India, 1973.
- Choudhury, A.K. and Mukhopadhyay, S. 1974. Effect of Rice Tungro Virus (RTV) infection on starch, reducing sugar and phosphorus contents of leaves of different varieties of Rice. Curr. Sci. 43: 280-281.
- * Cohen, S. and Nitzany, P.E. 1960. A White fly transmitted virus of Cucurbits in Israel. Phytopath. medit. 1: 44-46.
- Cohen, S. and Nitzany, P.E. 1963. Identity of viruses affecting cucurbits in Israel. Phytopathology 53: 193
- Cordingley, H., Grainger, J., Pearsall, W.H. and Wright, A. 1934. The effect of mosaic disease upon certain metabolic products in the Tobacco plant. Ann. appl. Biol. 21: 78-89.
- Coudriet, D.L. 1962. Efficiency of various insects as vectors of cucumber mosaic and watermelon mosaic viruses in Cantaloupes. J. econ. Ent. 55: 519-520.
- Crowle, A.J. 1961. Immunodiffusion. Academic Press, New York, pp. 353.
- Das, H.N. 1971. "Studies on some Virus Diseases of Cucurbita moschata", M.Sc.(Ag.) Thesis submitted at the University of Kalyani, W.Bengal, India, 1971.

- Demski, J.W. 1968. Local lesion reactions of Chenopodium species to watermelon mosaic virus 2. Phytopathology 58: 1196-1197.
- Demski, J.W. 1969. Local reactions and cross protection for strains of squash mosaic virus. Phytopathology 59: 251-252.
- Diener, T.O. 1963. Physiology of virus infected plant. Ann. Rev. Phytopath. 1: 197-218.
- Dunlap, A.A. 1930. The total nitrogen and carbohydrates, and the relative rates of respiration, in virus infected plants. Amer. J. Bot. 17: 348-357.
- *Doolittle, S.P. 1920. The mosaic disease of cucurbits. U.S. Dept. Agric. Bull. No. 87.
- Doolittle, S.P. 1921. The relation of wild host plants to the over-wintering of Cucurbit Mosaic. Phytopathology (Abstr.) 11: 47.
- *Doolittle, S.P. 1924. Control of cucurbit mosaic in the green house. U. S. Dept. Agric. Circ. 321: 5.
- * Doolittle, S.P. and Walker, M.N. 1923. Cross-inoculation studies with Cucurbit mosaic. Science, N.S. lvii: 477 (R.P.P. 2, 1923).
- Doolittle, S.P. and Walker, M.N. 1925. Further studies on the overwintering and dissemination of cucurbit mosaic. J. agric. Res. 31: 1-58.
- *Elmer, O.H. 1922. Mosaic cross inoculation and insect transmission studies. Science, N.S., lvi : 370-372 (R.P.P. 2, 1923).
- *Elmer, O.H. 1925. Transmissibility and pathological effects of the mosaic disease. Iowa agric. Exp. Sta. Bull. 82: 39-91.

- El-Madidi, A.P., Salama, E.A. and Sill (Jr.), U.H. 1966. Comparative studies of host range, biological and biochemical properties of pumpkin mosaic virus and squash mosaic virus. Phytopathology 56: 877.
- *Faccioli, G. 1972. Un ceppo di virus del mosaico cetriolo isolato da spinacio in Emilia. Phytopath. medit. 11: 67-70 (Eng. Summ.).
- Fischer, H.U. and Lockhart, B.E.L. 1974. Serious losses in cucurbits caused by Watermelon mosaic virus in Morocco. Pl. Dis. Reprtr. 58: 143-146.
- Foster, R.E. and Dennis, H.E. 1963. Resistance in Muskmelon to a strain of Watermelon mosaic virus. Phytopathology 53: 600-602.
- Franklin, R.E. 1956. X-ray diffraction studies of Cucumber virus 4 and three strains of Tobacco mosaic virus. Biochem. Biophys. Acta 19 : 203-211.
- Freitag, J.H. 1941a. Insect transmission, host range and properties of squash mosaic virus. Phytopathology (Abstr.) 31: 8.
- Freitag, J.H. 1941b. A comparison of the transmission of four Cucurbit viruses by cucumber beetles and by aphids. Phytopathology (Abstr.) 31 : 8.
- Freitag, J.H. 1952. Seven virus diseases of cucurbits in California. Phytopathology (Abstr.) 42: 8.
- Freitag, J.H. 1956. Beetle transmission, host range and properties of squash mosaic virus. Phytopathology 46: 73-81.
- *Gerola, F.M., Bassi, M. and Belli, G. 1965. II. Nicotiana glutinosa systemically infected with cucumber mosaic virus, strain Y. Caryologia 18: 567-597.
- *Gigante, R. 1936. Una nuova virosi della rosa in Italia. Boll. Staz. Pat. veg. Roma, (n.s.), 16: 76-94.

- Goel, R.H. and Varma, J.P. 1975. Mosaic disease of ridgegourd (*Melothra acutangula* Roxb.) in Haryana. Haryana Agric. Univ. J. Res. 3: 135-144.
- Goodman, W.R., Kiraly, S. and Kaitlin, M. 1967. The biochemistry and physiology of infectious plant diseases. Van Nostrand Company, Inc., U.S.A.
- Gornall, A.G., Bardawill, C.D. and David, H.H. 1949. Determination of serum protein by means of the Biuret Reaction. J. Biol. Chem. 177: 751.
- Grogan, A.G., Hall, D.H. and Nimble, R.A. 1959. Cucurbit mosaic viruses in California? Phytopathology 49: 366-376.
- Hariharasubramanian, V. 1964. Influence of *Dolichos* emment mosaic virus on the nucleic acid levels of *Dolichos lablab* L. Ind. J. Exp. Biol. 2: 163-164.
- Hariharasubramanian, V. and Badami, R.S. 1964. A virus disease of Cucurbits from India. Phytopath. 54: 274-279.
- Harpas, I. and Cohen, S. 1965. Semipersistent relationship between cucumber vein yellowing virus (CVYV) and its vector, the Tobacco white fly (*Trialeurodes vaporariorum* Comnadius). Phytopath. 55: 240-248.
- Hitchborn, J.H. and Mills, G.J. 1965. The use of negative staining in the electron microscopic examination of plant viruses in crude extracts. Virology 27: 528-540.
- Hoggan, I.A. 1929. The peach aphid (*Myzus persicae* Sulz.) as an agent in virus transmission. Phytopathology 19: 109-123.
- Hollings, H. 1939. Host range studies with fifty-two plant viruses. Ann. appl. Biol. 47: 93-108.
- Holmes, K.G. and Franklin, R.S. 1958. The radial density distribution in some strains of CMV. Virology 6: 328-336.
- Honeycutt, R.P. and Hillman, B.P. 1964. Changes in nucleic acid content of *Lycopersicon* leaves as a result of virus infection. Phytopathology 54: 1109-1111.

- Inouye, T. 1964. A virus disease of Pea caused by Watermelon mosaic virus. Ber. Ohara Inst. Landw. Biol. 12: 133-143.
- Inouye, T., Inouye, N., Asotani, M. and Mitsuhashi, K. 1967. Studies on the cucumber green mottle mosaic virus in Japan. Ber. Ohara Inst. Landw. Biol. 14: 49-69.
- Janardhan, A., Varaguntala, R.C. and Govindu, H.C. 1969. Studies on a mosaic disease of Bottlegourd. Mysore J. agric. Sci. 3: 444-448.
- Jeyarajan (Jayarajan), R. and Ramakrishnan, K. 1968. Studies on the physiology of chilli plants (Capsicum annuum L.) affected by Potato virus Y. I. Effect on metabolism of carbohydrates, organic acids, phenolics and respiration. Phytopath. 58: 142-152.
- Johnson, J. 1951. Virus particles in various plant species and tissues. Phytopathology 41: 78-93.
- Joshi, R.D. 1962. A study of virus diseases of economic plants of U.P.(India). Agra. Univ. J. Res. 11: 103-104.
- Kado, C.I. and Knight, C.A. 1970. Host specificity of plant viruses. I. Cucumber virus 4. Virology 40: 997-1007.
- Kendrick, J.B. 1934. Cucurbit mosaic transmitted by muskmelon seed. Phytopathology 24: 820-823.
- Kirby, E.S. 1956. A new method of isolation of RNA from mammalian tissues. Biochem. J. 64: 405-408.
- Knhutsen, H.K. and Nelson, H.R. 1968. Identification of two serotypes in squash mosaic virus strains. Phytopathology 58: 345-347.
- Knight, C.A. 1955. Are cucumber viruses 3 and 4 strains of Tobacco mosaic virus? A review of the problem. Virology 1: 261-267.

- *Knight, G.A. and Oster, G. 1947. The size of the particles of some strains of Tobacco mosaic virus as shown by the electron microscope. Arch. Biochem., N.Y., 15: 289-294.
- Komuro, Y. 1962. Virus diseases of cucumber and watermelon caused by Melon mosaic virus. Ann. Phytopath. Soc. Japan. 27: 31-36 (Eng. Summ.).
- Komuro, Y., Tochiwara, H., Fukatsu, R., Nagai, Y. and Yoneyama, S. 1971. Cucumber green mottle mosaic virus (watermelon strain) in watermelon and its bearing on deterioration of watermelon fruit known as "Konnyaku" disease. Ann. Phytopath. Soc. Japan. 37: 34-42 (Eng. Summ.).
- Lal, S.B. and Chauhan, L.S. 1961. Distribution of CV 20 in mosaic infected bottlegourd (Lagenaria siceraria Standl.) plants. Curr. Sci. 30: 273.
- Lastra, R. 1968. Occurrence of Cucurbit viruses in Venezuela. Pl. Dis. Reptr. 52 : 171-174.
- Lastra, R. and Munz, K. 1969. Purification and electron microscopy of squash mosaic virus. Phytopathology 59: 1429-1435.
- Lastra, J.R., Acosta, J.M. and Uzcategui, R. de. 1975. Cucumis metuliferus as a local lesion host for Squash mosaic virus. Pl. Dis. Reptr. 59: 693-696.
- Lindberg, G.D., Hall, D.H. and Walker, J.C. 1956. A study of melon and squash mosaic viruses. Phytopathology 46: 489-495.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin-Phenol reagent. J. Biol. Chem. 193: 265-275.
- Lutke, H. 1930. Contribution to the knowledge of metabolism of mosaic diseased and healthy tobacco plants. Phytopath. 2. 2: 341-359.
- MacKinney, G. 1941. Absorption of light by chlorophyll solutions. J. Biol. Chem. 140: 315-322.

(1x)

- *Magee, C.J.P. 1940. Transmission of infectious chlorosis or heart rot of the banana and its relationship to cucumber mosaic. J. Austr. Inst. Agric. Sci. 6: 44-47.
- *Mahoney, C.H. 1935. Seed transmission of mosaic in inbred lines of muskmelon. Proc. Amer. Soc. Horti. Sci. 1934, 32: 477-480.
- Malhotra, R.C. 1931. Biochemical investigation of mosaic in Solanum tuberosum. J. Biochem. 13: 473-487.
- Mandahar, C.L. and Singh, J.S. 1971. Destruction of chlorophyll a and b in virus infected leaves. Sci. Cult. 37: 485-487.
- Mandahar, C.L. and Garg, I.D. 1972. Effect of Cucumber mosaic virus on chlorophyll content, photosynthesis, respiration and carbohydrates of infected Luffa aegyptica Mill. Phytopath. Z. 75: 181-186.
- Martyn, A.B. 1968. Plant virus names, Commonwealth Mycological Institute, Kew, Surrey, England.
- Matthews, R.B.F. 1949. Criteria of relationship between plant virus strains. Nature 163: 175.
- Matthews, R.B.F. 1970. Plant virology, Academic Press, Inc., New York.
- McLean, D.M. and Meyer, H.M. 1961. A survey of Cucurbit viruses in the lower Rio Grande Valley of Texas: Preliminary report. Fl. Dis. Reprtr. 45: 137-139.
- Mehta, S.C. and Lal, R. 1962. Note on the variation in the length of the Cucumis virus. Curr. Sci. 31: 98-99.
- Middleton, J.E. 1944. Seed transmission of Squash mosaic virus. Phytopathology 34 : 405-410.
- Milbrath, G.M. and Nelson, M.R. 1967. The close association of Watermelon mosaic virus 2 and Cucurbit latent virus. Ann. Meeting of the Pacific Div. Amer. Phytopath. Soc., Univ. Calif., June 21-23, 1966. Phytopathology (Abstr.) 57: 100.
- *Milne, K.S. 1968. Identification of Cucurbit viruses in California. Ph.D. Thesis, Univ. Calif. pp. 68.

(x)

- Milne, K.S. and Grogan, R.G. 1969. Characterization of Watermelon mosaic virus strains by Serology and other properties. Phytopathology 59: 803-818.
- Milne, K.S., Grogan, R.G. and Kimble, R.A. 1969. Identification of viruses affecting Cucurbits in California. Phytopathology, 59: 819-828.
- Mink, G. I. 1969. Serological relationships among Cucumber mosaic virus, tomato aspermy type viruses and Peanut stunt virus. Phytopathology 59: 1889-1893.
- *Molnar, A. and Schmelzer, K. 1964. Beitrage zur Kenntnis des Wassermelonemosaik virus. Phytopath. 2. 51 : 361-384.
- Mukhopadhyay, S. and Saha, K. 1968. Transmission of Cucumis virus (Cucumber mosaic virus) through seeds of Cucurbita maxima L. Sci. Cult. 34 : 436-437.
- Murant, A.F. 1965. The morphology of Cucumber mosaic virus. Virology 26: 538-544.
- Nagarajan, K. and Ramakrishnan, K. 1971a. Studies on Cucurbit viruses in Madras State. III. Watermelon mosaic virus on Snake gourd (Trichosanthes anguina L.). J. Indian Bot. Soc. 50A : 25-30.
- Nagarajan, K. and Ramakrishnan, K. 1971b. Studies on Cucurbit viruses in Madras State. I. A new virus disease on bittergourd (Momordica charantia L.). Proc. Indian Aca. Sci., B 73 : 30-35.
- Nelson, M. R. and Milbrath, G.M. 1965. Heterogeneity of particle size in preparations of Squash mosaic virus. Phytopathology 55: 1069.
- Nelson, M.R. and Tuttle, D.H. 1969. The epidemiology of Cucumber mosaic and Watermelon mosaic 2 of Cantaloupes in an Arid Climate. Phytopathology 59: 849-856.

- Nelson, H.R. and Knutson, H.K. 1973. Squash mosaic virus variability: review and serological comparison of six biotypes. Phytopathology 63: 920-926.
- Nelson, H.R., Matejka, J.C. and McDonald, H.H. 1965. Systemic infection of Watermelon by a strain of Squash mosaic virus. Phytopathology, 55: 1362-1364.
- Nelson, H.R., Laborde, J.A. and McDonald, H.H. 1966. Cucurbit virus diseases on the West Coast of Mexico. Pl. Dis. Rept. 50: 947-950.
- Nene, Y.L. 1972. A survey of the viral diseases of Pulse crops in Uttar Pradesh, Final Tech. Rep., C.B.P. Univ. Agri. and Tech., India, Res. Bull. No. 4, pp. 191.
- Noordam, D. 1973. Identification of plant viruses- Methods and experiments, Centre for Agricultural Publishing and Documentation (Pudoc), Wageningen, pp. 207.
- Nozu, Y., Tsuchihara, H., Komuro, Y. and Okada, Y. 1971. Chemical and immunological characterization of Cucumber green mottle mosaic virus (Watermelon strain) protein. Virology, 45: 577-585.
- *Ogilvie, L. and Mulligan, B.O. 1931. Diseases of vegetable marrow. Rep. Agric. Hortic. Res. Sta. Bristol, 1930, 144-145.
- Orellana, R.C. and Quacquarelli, A. 1968. Sunflower Mosaic caused by a strain of Cucumber Mosaic Virus. Phytopathology 58: 1439-1440.
- Peterson, P.D. 1931. Plastid pigment and chlorophyllase contents of Tobacco plants as influenced by three types of mosaic. Phytopathology (Abstr.) 21: 119.
- Porter, R.H. 1931. The reaction of Cucumbers to types of mosaic. Iowa State Coll. J. Sci. 6: 95-120.
- Price, W.C. 1934. Isolation and study of some yellow strains of Cucumber mosaic. Phytopathology 24: 743-761.
- Price, W.C. 1935. Classification of Southern Celery mosaic virus. Phytopathology 25: 947-954.

- Price, W.C. 1937. Classification of Lily mosaic virus. Phytopathology 27: 561-569.
- Price, W.C. 1940. Comparative host ranges of six plant viruses. Amer. J. Botany 27 : 530-541.
- Provvidenti, A. and Schroeder, W.T. 1970. Epiphytotic of Watermelon mosaic among Cucurbitaceae in Central New York in 1969. Pl. Dis. Reprtr. 54: 744-748.
- Quaseem, S.M., Ahmed, A., Khalique, A. and Jamal, A. 1975. Studies on Some Biochemical Changes in Foliar Tissues Induced by Mosaic virus. Proc. 62nd Indian Sci. Cong. Part III (Abstr.): 76
- *Quiot, J.B., Kaan, F. and Beranis, M. 1971. Identification of a Watermelon mosaic strain in the French West Indies. Annls. Phytopath. 3: 125-130.
- Ramiah, M. , Vidhyasekaran, P. and Kandasamy, T.K. 1972. Changes in photosynthetic pigments of bhendi infected by Yellow vein mosaic disease. Madras Agric. J. 59: 402-404.
- Raychoudhuri, M. and Varma, A. 1975. Virus diseases of Cucurbits in New Delhi. Proc. 62nd Indian Sci. Congr. Part III (Abstr.): 76.
- Raychoudhuri, S.P., Mishra, M.D. and Ghosh, A. 1969. Occurrence of paddy virus and virus like symptom in India. In: "The virus disease of rice plant", Proc. Symp. Indian Agricultural Research Institute , April 1967. Baltimore Maryland, John Hopkins Press.
- Reddy, K.R.C. and Mariani, T.K. 1963. Studies on mosaic diseases of vegetable marrow (Cucurbita pepo L.). Indian Phytopath. 16: 260-267.
- Rice, G.V., Lindberg, G.D., Raesberg, P., Walker, J.C. and Stahmann, H.A. 1955. The three components of squash mosaic virus. Phytopathology 45 : 145-148.
- Rochow, W.F. 1956. Interference with Tobacco mosaic infection by Cucumber viruses 3 and 4. Phytopathology 46: 133-137.

- Saha, K.S. 1968. "Studies on the Mosaic Diseases of Cucurbita maxima L.", M.Sc.Ag. Thesis submitted at the University of Kalyani, W. Bengal, India, 1968.
- Salama, B.A. and Sill (Jr.), W.H. 1968. Distribution of Cucurbit viruses in Kansas. Pl. Dis. Rept., 52: 11-14.
- *Schmelzer, K. 1965. Das Wassermelonemosaik-virus tritt auch in Deutschland auf. Z. Nachr. Pflanzensch. 3: 69-71 (Eng. Summ.).
- *Schmelzer, K. 1966. Nachweis des spezifischen Wassermelonemosaik-virus in Kuba. Naturwissenschaften, 53: 619 (Eng. Summ.).
- Schmelzer, K. 1967. Some more recent knowledge on Cucurbit viruses. "Plant Virology", Proc. 6th Confer. Czech. Plant Virologists, Olomouc 1967, 206-240.
- *Schmidt, H.S. and Karl, E. 1968. Untersuchungen über eine Scheckung an Japanischen Hopfen (Humulus scandens (Lour.) Merr.). Phytopath. Z., 62: 272-278 (Eng. Summ.).
- Scott, H. A. 1963. Purification of Cucumber mosaic virus. Virology 20: 103-106.
- Seth, H.L. and Raychoudhuri, S.P. 1973. Further studies on a new mosaic disease of brinjal (Solanum melongena L.). Proc. Indian Nat. Sci. Acad. B 39: 122-128.
- Shankar, G., Nariani, T. and Nam Prakash, 1969. Purification and Serology of Bottlegourd (Lagomaria siceraria -Standl.) mosaic virus. Indian J. Microbiol. 11: 43-52.
- Shankar, G., Nariani, T. and Nam Prakash 1972. Studies on pumpkin mosaic virus (PMV) with particular reference to purification, electromicroscopy and serology. Indian J. Microbiol. 12: 154-165.
- Shannugasundaram, S., Ishii, M., Gilbert, J.C. and Nagai, H. 1969. Cucurbit virus studies in Hawaii. Pl. Dis. Rept. 53: 70-74.

- Sill, W.H. and Walker, J.C. 1952. Cow Pea as an assay host for Cucumber virus 1. Phytopathology 42: 328-330.
- Sill, W.H., Burger, W.C., Stahmann, H.A. and Walker, J.C. 1952. Electron microscopy of Cucumber virus 1. Phytopathology 42 : 420-422.
- Simmons, J.N. 1958. Titres of three non-persistent aphid-borne viruses affecting pepper in South Florida. Phytopathology 48: 265.
- Sitterly, W.R. 1960. A new insect vector of squash mosaic virus. Pl. Dis. Reprtr. 44: 134-135.
- Slavin, D. 1950. Production of antisera in rabbits using calcium alginate as an antigen. Nature 165 : 4186.
- Takahasi, W.N. and Rawlins, T.M. 1947. An electron microscope study of Squash mosaic virus. Amer. J. Bot. 34: 271-272.
- Toba, H.H. 1962. Studies on the host range of Watermelon mosaic virus in Hawaii. Pl. Dis. Reprtr. 46: 409-410.
- *Uschdraweit, H.A. 1955. The green mottle mosaic of cucumber. Chenopodium quinoa as test plant for cucumber mosaic. NachrBl. dtsh. Pflsch Dienst (Braunsch), Stuttgart 7: 150-151; 9: 151-152.
- *Valentin, H. 1958. Das Gurkengelbmosaik (Cucumis virus 2A, Smith). NachrBl. Dtsch. PflschDienst. 10: 93-94.
- *Van Koot, Y. and Van Dorst, H.J.M. 1958. Die invloed van enkele virussen op de bloemvorming b i j Komkommer. Tijdschr. Plziekt 65 (Van Slogteren Number): 432-439 (Eng. Summ.).
- *Van Koot, Y. and Van Dorst, H.J.M. 1959. Virussiekten van de Komkommer in Nederland. Tijdschr. Plziekt 65: 257-271(Eng. Summ.).
- Van Regenmortel, M.H.V. 1960. Zone electrophoresis and electronmicroscopy of a Watermelon mosaic virus from South Africa. Virology 12: 127-130.
- Van Regenmortel, M.H.V., Brandes, J. and Bercks, R. 1962. Investigations on the properties of Watermelon mosaic virus. Phytopath. Z. 45: 205-216.

- Varma, P.M. 1955. Ability of the Whitefly to carry more than one virus simultaneously. Curr. Sci. 24: 317-318.
- Vasudeva, R.S. and Lal, T.B. 1943. A mosaic disease of Bottle Gourd Indian J. Agric. Sci. 13: 182-191.
- Vasudeva, R.S. and Pavgi, H.S. 1945. Seed transmission of Melon mosaic virus. Curr. Sci. 10: 271-272.
- Vasudeva, R.S. and Mariani, T.K. 1952. Host range of bottlegourd mosaic virus and its inactivation by plant extracts. Phytopathology 42: 149-152.
- Vasudeva, R.S., Raychoudhuri, S.P. and Singh, J. 1949. A new strain of Cucumis virus 2. Indian Phytopath. 2 : 180-185.
- *Verplancke, G. 1929. Beet root diseases caused by filterable viruses. La Sucrerie Belge, xlix, 7 : 121-127 (R.P.P. 9, 1930).
- Walker, J.C. 1969. Plant Pathology. McGraw Hill Book Company, 3rd edition.
- Walker, M.N. 1933. Occurrence of watermelon mosaic. Phytopathology 23: 741-744.
- Waterworth, H.E. and Povish, H.R. 1975. A virus related to Cucumber mosaic virus isolated from imported Ixora plants. Phytopathology 65 : 728-729.
- Watson, M. (arion), A. and Watson, D.J. 1951. The effect of infection with Beet yellows and Beet mosaic viruses on the carbohydrate content of sugar Beet leaves and on translocation. Ann. appl. Biol. 38: 276-288.
- Webb, R.E. 1961. Distribution of some Cucurbit viruses in some South West Melon producing areas. Pl. Dis. Repr. 45: 851-853.
- Webb, R.E. 1963. Local lesion hosts for some isolates of Watermelon mosaic virus. Pl. Dis. Repr. 47: 1036-1038.

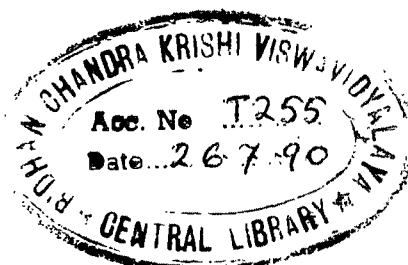
- Webb, R.E. 1964. Distribution of the Cucurbit latent virus (CLV) in some cucurbit growing areas. Pl. Dis. Repr. 48 : 321-323.
- Webb, R.E. 1965. Luffa acutangula for separation and maintenance of watermelon mosaic virus 1 free of watermelon mosaic 2. Phytopathology 55: 1379-1380.
- Webb, R.E. 1971. Watermelon mosaic viruses 1 and 2 in Squash on the Atlantic Seaboard. Pl. Dis. Repr. 55: 132-135.
- Webb, R.E. and Dohn, G.W. 1961. A virus latent in some cucurbits. Pl. Dis. Repr. 45: 677-679.
- Webb, R.E. and Scott, H.A. 1964. Relation of 10 isolates of Watermelon mosaic virus (WMV). 21st Ann. Meeting of the Potomac Div. Amer. Phytopath. Soc., Beltsville, Md. March 5-6, 1963. Phytopathology(Abstr.) 54: 749.
- Webb, R.E. and Dohn, G.W. 1965. Isolation and Identification of Watermelon mosaic virus 1 and 2. Phytopathology 55: 895-900.
- Webb, R.E., Dohn, G.W. and Scott, H.A. Watermelon mosaic viruses 1 and 2 in Southern and Western Cucurbit producing areas. Pl. Dis. Repr. 49: 532-535.
- *Weidemann, H.L. and Mostafawy, M. 1972. Watermelon mosaic virus type 2 in Iran and its transmissibility by different aphids species. Iranian J. Pl. Patho. 9 : 46-51.
- Weillie, G.V., Tang, S.F. and Miller, G.W. 1967. Metabolic changes induced by cucumber mosaic virus in resistant and susceptible strains of Cow Pea. Phytopathology 57: 472-475.
- Wellman, F.L. 1934. Identification of Celery Virus 1, the cause of Southern Celery mosaic. Phytopathology 24: 695-725.
- Yoshida, S., Farns, D.A. and Cook, J.H. 1971. Laboratory Manual for Physiological studies of rice. Int. Rice Res. Inst., Los Banos, Philippines.

(xvii)

Yosha, H. 1958. Plant virus transmission by insects. IV. Metabolic Changes in rice affected with the dwarf disease virus. Chem. Abstr. 53 : 959.

Zieniecki, A. and Wood, R.H. 1975. Changes in Soluble protein constitutions of cucumber cotyledons following infection with two strains of cucumber mosaic virus. Physiol. Pl. Patho. 7 : 79-89.

* Original not seen



633.629 8
6H0