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RESEARCH GUIDE
DR. D.M. SAWANT SIR**

**STUDIES ON SAP TRANSMISSIBLE VIRAL DISEASES
OF GROUNDNUT (*Arachis hypogaea* L.)**

A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

in partial fulfilment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

PLANT PATHOLOGY

by

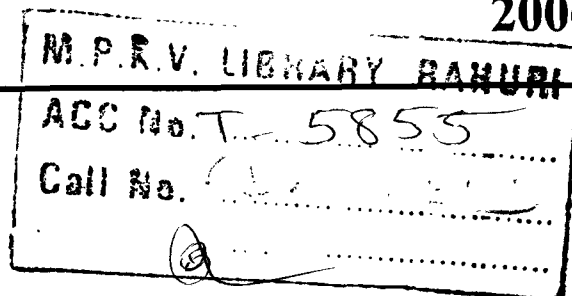
Krishna Sarjerao Badak

(Reg.No. 02031)

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL
MICROBIOLOGY

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
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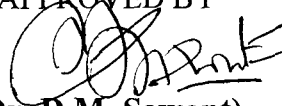
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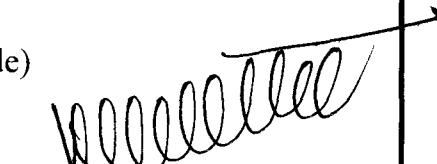
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MAHARASHTRA, INDIA

2006

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Associate Dean,
College of Agriculture,
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Maharashtra State (INDIA)


CERTIFICATE

This is to certify that the thesis entitled, "**STUDIES ON SAP TRANSMISSIBLE VIRAL DISEASES OF GROUNDNUT (*Arachis hypogaea* L.)**", submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, M.S. for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **PLANT PATHOLOGY**, embodies the results of a *bona fide* research carried out by **MR. KRISHNA SARJERAO BADA**K, under my guidance and supervision and that no part of the thesis has been submitted for any other Degree or Diploma.

The assistance and help received during the course of this investigation have been acknowledged.

Place : MPKV, Rahuri

Dated : 13 /02/2006


(D.M. Sawant)

Research Guide

Dr. D.V. Kasar

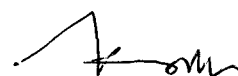
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Place : MPKV, Rahuri

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(D.V. Kasar)

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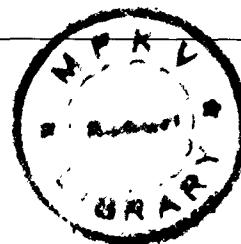
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LIST OF ABBREVIATIONS

$^{\circ}\text{C}$:	Degree Celcius
C.C.	:	Central Campus
cv.	:	Cultivar
DAC-ELISA	:	Direct Antigen Coating - Enzyme Linked Immunsorbent Assay
dia.	:	Diameter
E.M.	:	Electron microscopy
<i>et al.</i> ,	:	And others (et alli)
g	:	Gram
ha	:	Hectare
hrs.	:	Hours
i.e.	:	That is
I.S.E.M.	:	Immunsorbent electron microscopy
kg	:	Kilogram
KJ	:	Kilo joule
M	:	Molarity
mg	:	Milligram
min.	:	Minute (s)
ml	:	Mililitre
mm	:	Milimeter
N	:	Normality
ng	:	Nanogram
nm	:	Nanometer
q	:	Quintal
Rs.	:	Rupees
var.	:	Variety
<i>viz.</i> ,	:	Videlicet (Namely)
%	:	Per cent
μl	:	Microlitre
/	:	Per

ABSTRACT

**STUDIES ON SAP TRANSMISSIBLE VIRAL DISEASES OF
GROUNDNUT (*Arachis hypogaea* L.)**

By

Krishna Sarjerao Badak

A candidate for the degree

of

DOCTOR OF PHILOSOPHY

Mahatma Phule Krishi Vidyapeeth,

Rahuri - 413 722

Research Guide	:	Dr. D.M. Sawant
Department	:	Plant Pathology and Agricultural Microbiology

The virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11) showing different symptoms of stripes and green blotches collected from Groundnut Improvement Project, C.C., M.P.K.V., Rahuri and Oilseed Research Station, Jalgaon were studied in detail to identify the causal virus. The symptomatology on groundnut revealed production of vein clearing, dark green stripes, continuous banding along the lateral veins of young leaves and the appearance of chlorotic rings surrounding the blotching of young leaflets.

Host range of virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11) was limited to family leguminosae, solanaceae and chenopodiaceae. No any

kind of symptoms were observed on inoculated plants belonging to the different plant species of cucurbitaceae, compositae, malvaceae, amaranthaceae and pedalaceae families.

The virus was easily mechanically transmissible and could be recovered at the greatest extent from leaves of diseased plant. The virus was not seed transmitted. Among the aphid species tested namely *M. persicae* and *A. craccivora*; the *M. persicae* proved to be most efficient vector for transmission. The virus isolates were not transmitted by *A. gossypii*, *R. maidis* and *Bemisia tabaci*.

Studies on physical properties of virus isolates of groundnut revealed that the virus isolates SB-XI, Kopergaon-1, Kopergaon-3 and Karad 4-11 had longevity *in vitro* (LIV) of 72-84 hrs, dilution end point (DEP) of 10^{-4} – 10^{-5} and thermal inactivation point (TIP) of 65-70 °C and virus isolate JL-24 had LIV of 64-72 hrs, DEP of 10^{-3} – 10^{-4} and TIP of 60-65°C.

The particle morphology of virus isolates showed that the virus particles were found to be flexuous rods. The virus particles of all the isolates of groundnut were found to be in the range of 722-765 nm (743 ± 21 nm) in length and 13-17 nm (15 ± 2 nm) in breadth.

In immunosorbent electron microscopy, the production of specific antibody halo around the virus particles clearly indicated one group of one strain (PStV) of potyvirus

Abstract contd.....**K.S. Badak**

group. In DAC-ELISA, maximum O.D. values of 1.23 and 0.984 was noticed in Karad 4-11 and SB-XI virus isolates respectively and minimum O.D. values of 0.691, 0.765 and 0.806 was noticed in JL-24, Kopeegaon-1, Kopeegaon-3 virus isolates, respectively. The virus isolates Karad 4-11 and SB-XI showed maximum infection as well as virus titre.

The symptomatology, host range, transmission by various methods, physical properties, electron microscopy, serology indicated virus isolates under study belonged to potyviridae family and classified them into peanut stripe virus (PStV).

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Chapter Opener Page



INTRODUCTION



1. INTRODUCTION

Groundnut or Peanut (*Arachis hypogaea* L.) is one of the principal economic crops of the world, ranking 13th among the food crops. The genus *Arachis* belongs to the family Leguminosae which is distributed in many diverse agro environments i.e. from South of Amazon to 34°S latitude and from the eastern East Coast to the eastern slopes of the Andes (Gregory *et al.*, 1980). *Arachis hypogaea* L., the cultivated groundnut, is generally referred to as 'Peanut' in North America whereas those first recorded from other parts of the world generally incorporated the term 'groundnut' to designate the host *Arachis hypogaea* L. is also known as Monkeynut and Goobernut.

Groundnut is now grown throughout the tropical and warm temperate regions of the world. Although groundnut is predominantly a crop of the tropics, the approximate limit of present commercial production lies between latitudes 40°N and 40°S. Thus, it is grown on an area of 20 million hectares in about 103 countries. Groundnut is a crop of global economic significance not only for its widespread production but also for even wider areas of processing and consumption. Major products and by products are all sought after material on world markets with good history of relatively stable price level and considerable potential for value added processing (Purseglove, 1968; Weiss, 1983 and Nigam, 1991).

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Groundnut is important in global agricultural economy. Oilseed map of world shows that groundnut is the king of oilseeds, which plays an important role in human life. It has been aptly described as nature's master piece of food values containing 36-54 per cent oil with 21.36 per cent proteins and has a high energy value of 2,363 KJ/100 g. Seeds are used for oil extraction, as food and as an ingredient in confectionery products. After oil extraction, the residual cake is processed largely for use as animal feed and to some extent it is also used for human consumption. The haulms are valued as fodder.

Groundnut oil is rich in unsaturated fatty acids (80 %), oleic and linoleic acids accounting for 38 to 58 per cent and 16 to 38 per cent each, respectively. Among the saturated fatty acids, palmitic acid is the major one with the proportion of about 10 to 16 per cent, higher iodine value (82-106) and refractive index values (1.4697 to 1.4719 ND²⁰) indicating its susceptibility to oxidation. Raw groundnut oil has very good stability. It can be stored at room temperature for 18 months without any deterioration. Continuous deep fat frying for 10 hours also did not greatly alter its quality. The stability of oil may be due to the presence of tocopherols (Vitamin-E) which act as auto oxidative stabilizers. Oil is used in deep frying. It brings out the flavour of herbs and spices without contributing its own flavour (Nagaraj, 1995).

The total carbohydrate content of the kernel and meal is 10-20 and 38 per cent, respectively. The reducing sugars are low (1.2 to 1.8 %). Sucrose is the most important sugar which

ranges between 2.86 to 6.35 per cent. Glucose, fructose and galactose are the other minor sugars present. Oligosaccharides stachyase and raffinase are also present. It is a rich source of minerals like phosphorus, calcium, riboflavin, magnesium, potassium, zinc, copper, iron and manganese (Nagaraj, 1995).

Groundnut is a soil enriching food legume. Biological nitrogen fixation is not usually a limiting factor to the groundnut production in the locations with a history of groundnut cultivation (Nigam *et al.*, 1990).

It is reported that the groundnut plant has a universal ability to utilize soil nutrients that are relatively unavailable to other crops and is very effective in extracting nutrients from sandy soils of low nutrient supply (Arnon, 1972).

About two thirds of the crop produced in the world is crushed to extract oil and one third is used to make other edible products. India accounts for 40 per cent of the world area and 30 per cent of world output of groundnut.

Groundnut is cultivated in India both as a kharif and rabi crop but the output is higher in kharif. In the rabi season groundnut is cultivated only in the south i.e. Andhra Pradesh, Karnataka and Tamil Nadu which produce about 15 lakh tonnes groundnut.

Though the share of groundnut in the total oilseeds production in India has been falling since the 1950s when it was 70 per cent to the present level of 33 per cent, groundnut is still a major oilseed crop in India. Its production decides not only the

price of groundnut oil in any year but also the prices of most other oils.

Considering the oilseeds scenario of world during 2002, the crop was grown on 24,672 thousand hectares with annual production of 34,075 thousand metric tonnes and average yield was 1381 kg/ha (Anonymous, 2002a).

In India, the crop was grown on 6800 thousand hectares with annual production of 5400 thousand metric tonnes in the year 2002 and average yield was 794 kg/ha (Anonymous, 2002a).

In Maharashtra, the crop was grown on 429 thousand hectares with annual production of 492 thousand tonnes in the year 2001-02 and average yield was 11.5 q/ha (Anonymous, 2002b).

The exports of groundnut extractions were higher at 68,450 tonnes, valued at Rs. 43.31 crores in 2001-02 against 53,550 tonnes (April-March) valued at Rs. 29.03 crores in 2000-01. Earlier the exports of groundnut extractions had fallen in 1999-2000 to 25,000 tonnes valued at Rs. 10 crores from 1 lakh tonnes valued at Rs. 50 crores in 1998-99 (Singhal, 2003).

At present the US, Argentina and China are world's largest suppliers of edible peanuts. India and South Africa are only minor players in the world peanut export market.

Over 12 per cent of the total groundnut produced in the country is utilized for seed purpose, 6 per cent for domestic use, 81 per cent for oil extraction and about 1 per cent for export. Oilseeds play an important role in Indian economy

sharing 5 per cent of gross national product providing 13 per cent job to rural and industrial labour. The production of edible oil was 83 per cent of its requirement. According to the National Economic Survey, Indian adult could consume 7.2 kg/year per capita i.e. 20 g oil per day against 55 g per day per capita in India and 100 g per day/capita in western countries. This is just a starvation for fats (Anonymous, 1989 and Joshi, 1993).

Groundnut needs good sunshine and high temperature to produce more pods (Cox, 1979 and Ong, 1986) leading to higher productivity. Summer is, therefore, the ideal season for the cultivation of groundnut wherever irrigation facilities are available and soil is suitable.

The groundnut soils in the Maharashtra are light to medium black. About 60 per cent of the total groundnut area is planted to the Spanish-bunch varieties. The most important districts growing Spanish-bunch groundnut are Jalgaon, Dhulia, parts of Nashik and the northern parts of Ahmednagar. It is also grown in the eastern parts of Marathwada region in the districts of Aurangabad and Jalna.

About 30 per cent ground area is under Virginia-runner type varieties. The districts where Virginia-runner varieties are under cultivation are Solapur, Sangli, Eastern Satara, Pune, Kolhapur, Osmanabad and Beed. About 5 per cent of the groundnut area is under Virginia-bunch varieties. These varieties are raised in small pockets in the Virginia-runner belt, depending upon the soil type. Virginia-bunch types are grown in

light to medium soils while the runner types are cultivated in heavier soils.

The released groundnut varieties in Maharashtra are AK-12-24, SB-XI, TG-1 (Vikram), Phule Pragati (JL-24), TG-17, UF-70-130, Kopergaon-1, Kopergaon-3 and Karad-4-11 which are cultivated in large areas.

During recent years, the average per ha yield of groundnut in India still remained low. In addition to many other reasons, diseases and pests which attack crop are important factors responsible for low production.

The principle diseases of groundnut are tikka or leaf spot, caused by *Cercosporidium personatum*, *C. arachidicola*, rust caused by *Puccinia arachidis* (Triharso, 1972), collar rot caused by *Aspergillus niger*, *A. pulvarulentus*, wilt caused by *Sclerotium rolfsii*, stem rot caused by *Macrophomina phaseolina* and root rot by *Pellicularia filamentosa*. About 15 diseases of groundnut caused by fungi, bacteria and viruses have so far been reported from different parts of our country.

The sap transmissible viral diseases infecting groundnut are peanut mottle, peanut clump, peanut stripe, groundnut rosette, tomato spotted wilt, peanut stunt, cowpea mild mottle and cucumber mosaic (Reddy, 1991).

In Rahuri region the groundnut crop is found severely infected with viruses showing symptoms of vein clearing, stripes and green blotches on leaves. These viral diseases are considered most destructive since they can be spread within the crop during

cultural operations and also by insect vectors thereby causing maximum reduction in crop yield.

Since these viral diseases from Rahuri region are not studied in detail and considering the importance of viral diseases occurring on groundnut, the systematic studies were therefore undertaken with the following objectives

- i. To collect the samples of disease from field infected groundnut crop.
- ii. To transfer the viruses by mechanical sap inoculation to glasshouse grown groundnut seedlings so as to carry out detailed systematic study.
- iii. Symptomatology
- iv. Host range
- v. Transmission by various methods
 - a. Mechanical transmission
 - b. Seed transmission
 - c. Insect transmission
- vi. Physical properties
 - a. Thermal inactivation point (TIP)
 - b. Dilution end point (DEP)
 - c. Longevity *in vitro* (LIV)
- vii. Particle morphology
- viii. Serology

The material used and methods followed as well as results obtained are presented in succeeding chapters.

Chapter Opener Page



REVIEW OF LITERATURE



2. REVIEW OF LITERATURE

2.1 Occurrence

Zimmerman (1907) reported the first virus disease of groundnut from East Africa as 'Krasuel Krankheit' and also reported for the first time the occurrence of groundnut rosette disease from Tanganyika (now called Tanzania) and has since been reported in several other countries in Sub-Saharan Africa.

Sundararaman (1926) reported groundnut rosette disease for the first time in India as clump disease.

Sundararaman (1926, 1932) was the first to report a virus disease of groundnut at Tamilnadu Agricultural Research Station, Chennai with mosaic symptoms.

Sundararaman (1927) reported a clump disease of groundnut from India in 1927. Later it was reported from Bambey in Senegal (Bouhot, 1967) and Saria in Burkino Faso (Germani and Dhery, 1973).

Storey and Bottomley (1928), Hayes (1932) and Storey and Ryland (1957) reported the occurrence of groundnut rosette virus (GRV) in Africa, South of Sahara, including Madagascar causing severe crop damage.

Fulton (1950) reported cucumber mosaic virus (CMV) in Arkansas in which he described six isolates of CMV from Spinach.

Kuhn (1965) reported peanut mottle virus (PMV) for the first time from the U.S.A. and has since been positively identified in South America (Herold and Munz, 1969), Japan

(Inouye, 1969), Australia (Behncken, 1970), Africa (Bock, 1973), West Malaysia (Geh and Ting, 1973), Philippines (Benigno and Favali-Hedayat, 1977), India (Reddy *et al.*, 1978) and Sudan (Ahmed and Idris, 1981).

Miller and Troutman (1966) reported undescribed peanut disease in the United States, designated as stunt in Virginia on August 3, 1964 on a farm near Manry and caused an estimated 10 per cent loss of marketable nuts. The disease was observed on September 17, 1964 on a farm near Littleton in Sussex County, where it caused an estimated 50 per cent loss.

Echandi and Hebert (1970) reported that stunt had appeared the previous summer in epiphytotic form in home gardens and commercial pole and snapbean plantations in North Carolina in 1970. Beczner and Devergne (1979) for the first time reported natural occurrence of a PSV strain in Hungary.

Later the peanut stunt virus disease was reported from Japan (Tsuchizaki, 1973), the U.S.S.R. (Kracv *et al.*, 1977), France (Douine and Devergne, 1978), Morocco (Fisher and Lockhart, 1978), Spain (Diaz-Ruiz *et al.*, 1979) and Poland (Twardowicz-Jakusz and Pospieszny, 1983).

Brunt and Kenten (1973) and Brunt and Phillips (1981) reported cowpea mild mottle virus (CMMV), which has been known to occur on cowpea and tomato in Africa. While Brunt and Phillips (1977) reported CMMV on soybean in Thailand.

Germani and Dhery (1973) observed peanut clump virus during the summer of 1969 in peanut at the agricultural

station of Saria in Uppervolta, where about 6 out of 10 ha were affected. Thouvenel *et al.* (1974) reported peanut clump, a soil borne virus disease on peanut in West Africa.

Halliwell and Philley (1974) first reported tomato spotted wilt virus (TSWV) on peanut in Texas in 1972. Reddy *et al.* (1968) first reported bud necrosis disease (BND) from India. It was subsequently shown that BND was caused by a virus which resembled tomato spotted wilt virus (Ghanekar *et al.*, 1979; Reddy *et al.*, 1991). Later the TSWV was reported from Brazil (Costa, 1941), South Africa (Dyer, 1949), Punjab in India since 2000 (Cheema *et al.*, 2003) and Alabama (Hagan *et al.*, 1990).

Griffin *et al.* (1982) found peanut stunt virus (PSV) in beans in a breeding nursery in Fayetteville, AR and in white clover surrounding the bean and cowpea breeding nurseries. PSV was not recovered from white clover or other perennial plants from three other locations in Arkansas. Ahmed and Mills (1985) for the first time reported PSV in the Sudan.

Iizuka *et al.* (1982) recorded CMMV on peanut in India. Mali and Nirmal (1987) observed CMMV every year since 1980 on summer groundnut crops at Parbhani and the command areas in the Marathwada region of Maharashtra.

Reddy *et al.* (1983) observed Indian peanut clump in Punjab state of India in 1977.

Demski *et al.* (1984) for the first time reported occurrence of peanut stripe potyvirus (PStV) on peanut in Georgia in the United States during 1982.

In India, PStV was first observed in 1987 simultaneously in experimental plots in Andhra Pradesh, Gujarat and Karnataka (Prasad Rao *et al.*, 1988). Since then, the virus was reported from five major peanut growing states of the country : Andhra Pradesh, Gujarat, Karnataka, Maharashtra and Tamil Nadu (Varma *et al.*, 1994).

Later the peanut stripe disease was reported from Thailand, China, Philippines, Malaysia, Indonesia (Reddy *et al.*, 1985; Wongkaew, 1987; Xu *et al.*, 1983), Florida, North Carolina, Texas, Virginia and Oklahoma (Demski *et al.*, 1985, Demski *et al.*, 1984) and South Korea (Choi, 2001).

Xu and Barnett (1984) reported a virus isolated from an infected peanut seedling grown from seed collected in Jing Country, Liaoning Province, China and was identified as a strain of cucumber mosaic virus, designated CMV-CA.

2.2 Symptomatology

Kuhn *et al.* (1972) reported symptoms on soybean caused by peanut mottle virus. Small chlorotic areas appeared on the first two trifoliates in 6-9 days. Although the areas enlarged, they were not continuous and striking dark green islands occurred on the young leaves. Chlorotic patches and line patterns appeared on the third and fourth trifoliolate leaves. Paguio and Kuhn (1971) reported that strains M₁ and M₂ induced mild mottle in peanut whereas N₁S and CLP caused necrosis, severe mosaic and chlorotic line pattern, respectively.

Sun and Hebert (1972) observed typical symptoms of PMV-S in peanut leaves consisting of pale yellow or greenish-

yellow mosaic patterns with variable green patches. In plants inoculated at the two-leaf stage, the youngest leaves developed an intensive chlorosis, sometimes necrosis, mosaic pattern and remained small and severely distorted. Inoculations at later growth stages initially caused faint yellow spots.

Waterworth *et al.* (1972) reported that symptoms in field plants of peanut were noticed in August and were characterized by bright yellow chlorosis, usually along the veins of leaflets. In some instances, leaflets were entirely chlorotic, distorted or showed epinasty. Affected plants were not stunted or malformed.

Thouvenel *et al.* (1976) reported symptoms of IPCV on *Arachis hypogaea* cv. TE₃. Mottle and chlorotic ringspots appeared on new tip leaves, 7-10 days after inoculation. These symptoms rapidly faded, then disappeared when the leaves turned dark green. The plant then stopped growing and found stunted.

Ghanekar *et al.* (1979) observed chlorotic ringspots or chlorotic leaf specking, terminal bud necrosis, axillary shoot proliferation and severe stunting of groundnut and were shown to be caused by tomato spotted wilt virus (TSWV).

Iwaki *et al.* (1982) observed that symptoms of CMMV on soybean varied with the cultivar. Cultivars SJ4, Shiotsurunoko and Okuharawase showed slight veinclearing and leaf malformation, either downward curling or upward cupping. Cultivar Toyosuzu showed distinct mosaic, vein necrosis and top necrosis. Muniyappa and Reddy (1983) also

noticed symptoms of CMMV on soybean plants consisted of veinclearing and veinal necrosis of leaves followed by downward curling of leaves by 10 days after inoculation. Two weeks after inoculation, newly emerged leaves showed mosaic and puckering.

Thouvenel *et al.* (1982) reported symptoms of cowpea mild mottle virus (CMMV). Naturally infected soybeans were stunted and showed a light green mosaic. On inoculated soybean seedlings, veinclearing evolving into yellow mosaic with occasional crinkling developed on youngest leaves 9-12 days after inoculation. Inoculated plants were reduced in size compared with healthy plants.

Xu *et al.* (1983) studied the symptomatology of peanut mottle virus and observed the top leaflets of infected plants showing distinct chlorotic spots or ring spots. After several days, the chlorotic spots and ring spots disappeared and the leaves showed typical mottling with dark green islands on a light green background. Diseased plants were not noticeably stunted.

Reddy *et al.* (1983) observed typical symptoms of Indian peanut clump virus (IPCV) on newly emerged 2-3 week old seedlings. Plants were stunted and the new quadrifoliates showed mosaic mottling with chlorotic rings. Subsequently, the infected leaves turned dark green and showed faint mottling when viewed against light.

Demski *et al.* (1984) reported characteristic striping, discontinuous vein banding along the lateral veins and oakleaf mosaic in groundnut.

Xu and Barnett (1984) reported symptoms of the cucumber mosaic virus (CMV) which were characterized by chlorotic spots on young emerging leaves, chlorosis of young expanded leaves that were smaller and rolled, mosaic or mottling of some leaves and moderate stunting of the plant.

Demski and Lovell (1985) described the peanut stripe virus as two symptom variants, stripe and blotch, caused by serologically indistinguishable potyviruses. Peanut plants infected with the stripe variant typically exhibited dark green stripes along the lateral leaf veins. As stripe infected plants matured and the disease progressed, an oak leaf pattern developed on the leaves and became the predominant symptom expression. Plants infected with blotch variant developed dark green circular areas on the leaves.

Reddy *et al.* (1985a) reported the GRV isolates from Malawi, such as GRV (MC) and GRV (MM), which showed symptoms in *Nicotiana benthamiana* similar to those reported for isolates from Nigeria except that the initial host reaction was usually slightly more severe, with veinal chlorosis or necrosis in the first one or two systemically infected leaves instead of a mild mottle. However, some GRV isolates from Malawi occasionally induced small yellow flecks on systemically infected leaves of *N. benthamiana*.

Zeyong *et al.* (1986) reported severe symptoms including leaf malformation, ring spots and necrotic lesions on blackeye cowpea by isolates in the first group *viz.*, PSV-W, PSV-1, PSV-3, PSV-5 and PSV-E whereas isolates of the third group

viz., PSV-60-4, PSV-65-24, PSV-80-11, PSV-100-26, and PSV-101-16 caused a mild mosaic on these plants. In the first group, PSV-W caused malformation and systemic necrotic spots on Perfected Wales pea and malformation of Pinto bean. In the second group, isolates PSV-76-69, PSV-2 and PSV-V caused only a faint mosaic on Pintobean, whereas PSV-V and PSV-76-69 caused dwarfing.

Mali and Nirmal (1987) reported a disease of groundnut in Maharashtra, associated with vein clearing and downward rolling of leaves followed by necrosis and severe stunting and was shown to be caused by cowpea mild mottle virus (CMMV).

Demski *et al.* (1988), Reddy *et al.* (1988 b), Wongkaew and Dollet (1989) reported symptomatology of PStV and observed discontinuous chlorotic stripes along the lateral veins of young quadrifoliate leaves. Fukumoto *et al.* (1986) reported chlorotic rings surrounding the blotching of young leaflets from Thailand and Indonesia. Zeyong *et al.* (1983) reported isolate in China inducing mild mottle symptoms in peanut.

Warwick and Demski (1988) observed symptoms like stripe, blotch and mid mottle on peanut. Symptoms on soybeans ranged from systemic necrosis, mosaic and mid mottle to no reaction.

Nasir Saleh *et al.* (1989) reported peanut stripe virus in Indonesia which was characterized by the presence of green blotches. These blotches were either randomly distributed over

the entire leaflets or concentrated along the veins; sometimes they were surrounded by light green rings.

Prasada Rao *et al.* (1989) reported that during surveys in 1987 rainy season on a multilocational groundnut varietal trial of the All India Co-ordinated Oilseeds Research Project (AICORPO), leaflets of several genotypes showing discontinuous stripes, green blotches and oakleaf mosaic were observed.

Chang *et al.* (1990) reported that the virus (Ts) related to peanut stripe virus (PStV) causing severe mosaic, necrotic and stunt symptoms on peanut, distinct from those caused by peanut mottle virus was detected in Southern Taiwan. In 1982, other isolates of this virus (Tc), which induced stripe or vein banding but no necrosis symptoms were also detected.

Murant and Kumar (1990) described the symptomatology of groundnut rosette virus. In chlorotic rosette, the leaves showed a bright yellow chlorosis which affected the whole leaf or only parts of the leaf, in the latter event causing a mosaic. The symptoms appeared over almost the entire plant or only in parts of the plant, affecting perhaps some shoots but not others, or the distal portions of shoots but not the proximal portions. In green rosette, the leaves were darker green than normal, or showed a light green dark green mosaic and are much reduced in size. In both forms there was stunting of the plants and this was especially severe in green rosette.

Prasada Rao and Chakrabarty (1990) reported that the during 1988, when groundnut seeds imported from Myanmar were tested by ELISA. In the growing-out tests, the PStV infected

plant initially showed discontinuous stripes along the lateral veins of the young leaves that were later replaced by green blotches.

Sreenivasulu and Demski (1991) reported chlorotic spots and concentric rings on peanut leaflets inoculated with TSWV-T two weeks after inoculation. Fully expanded leaflets below the terminal bud later became flaccid, with necrosis of the petiole and terminal bud. This led to cessation of apical growth and proliferation of axillary shoots bearing mottled and distorted leaflets.

Naidu *et al.* (1999) reported two predominant symptom types of groundnut rosette disease viz., “chlorotic” and “green” rosette. Chlorotic rosette was ubiquitous in sub-saharan Africa, while the distribution of green rosette was unknown. The expression in young plants of either form of the disease affected the entire plant and caused severe stunting due to shortened internodes and reduced leaf size, leading to a bushy appearance. In contrast, plants infected late in their growth showed symptoms only in some branches or parts of branches.

Jain *et al.* (2000) observed similar type of symptoms of PStV those observed by Prasad Rao *et al.* (1988, 1989).

Choi *et al.* (2001) reported that severe mosaic symptoms resembling those reported for a blotch isolate of peanut stripe virus were observed in the year 1998 in Suwon, South Korea on several peanut cultivars.

2.3 Host range

Mink *et al.* (1969) reported the behaviour of PSV-W on various plant species. The plant species developed systemic symptoms and virus was recovered from uninoculated young growth : *Arachis hypogaea*, *Capsicum frutescens*, *Crotalaria spectabilis*, *Cucumis sativus* cv. 'National Pickling' , *Datura stramonium*, *Glycine max*, *Lycopersicon esculentum*, *Medicago sativa*, *Nicotiana tabacum*, *Petunia hybrida*, *Phaseolus acutifolius*, *P. vulgaris*, *Pisum sativum*, *Vigna sinensis* and *Zinnia elegans*.

Chlorotic lesions developed on *C. quinoa*, *C. amaranticolor* and *Cyamopsis tetragonoloba* and virus was recoverable only from inoculated leaves of *C. quinoa*, *C. amaranticolor* and *Cyamopsis tetragonoloba*.

Echandi and Hebert (1970) tested several specimens of *Plantago major*, *Oxalis striata*, *Rumex obtusifolius* and *Gleditsia triacanthos* from around infected fields, for the presence of the PSV, but no PSV was detected in any of these plants.

Beczner and Devergne (1979) reported that the peanut stunt virus (PSV) produced very pronounced systemic infection on *Gomphrena globosa*, *Nicotiana megalosiphon*, *Phaseolus vulgaris*, *Pisum sativum* and *Vigna sinensis*.

Ghanekar *et al.* (1979) studied the tomato spotted wilt virus (TSWV) which induced the chlorotic as well as necrotic local lesions in *Beta vulgaris*, *Cajanus cajan*, *Chenopodium amaranticolor*, *C. quinoa*, *Crotalaria juncea*, *Cucumis sativus* cv. National Pickling, *Gomphrena globosa*, *Nicotiana rustica*, *N. tabacum* cv. 'Xanthi-nc', *Petunia hybrida* cv. Coral Satin and

chlorotic or necrotic spots followed by systemic infection in *Canavalia ensiformis*, *Datura stramonium*, *Dolichos uniflorus*, *Glycine max* cv. Bragg, *Lycopersicon esculentum* cvs. Pusa Ruby and Perfection, *Nicotiana clevelandii*, *N. glutinosa* a hybrid between *N. glutinosa* x *N. clevelandii*, *Phaseolus vulgaris* cvs. Bountiful and Topcrop, *P. lunatus* cv. Henderson Bush Lima, *Physalis floridana*, *Pisum sativum* cv. Bonneville, *Vigna radiata* cv. Hy-45, *V. mungo* cv. UPU-1, *V. unguiculata* cvs. C-152 and California Black Eye, *Vinca rosea* and *Zinnia elegans*.

Ahmed and Idris (1981) reported 20 plants species in seven families. *P. vulgaris* cvs, 'Topcrop' and 'Prince' reacted to sap inoculation of PMV with faint chlorotic lesions and severe systemic mottle. *A. hypogaea* cvs. 'Ashford', 'Barberton', 'Libian', MH-383 and 'Nigerian', *Cassia occidentalis*, *Glycine max* cv. 'William', *Medicago sativa*; and *Vigna sinensis* reacted with severe systemic symptoms without local lesions. Plant species that did not react to inoculation included *Abelmoschus esculentus*, *Beta vulgaris*, *Brassica oleracea*, *Capsicum annum*, *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativa*, *Datura metel*, *D. stramonium*, *Daucus carota*, *Gossypium barbadense*, *N. glutinosa*, *N. tabacum* and *Vicia faba*. Virus was not recovered from these plants in back inoculations to Topcrop beans.

Thouvenel and Fauquet (1981) found previously (Thouvenel, 1976) that PCV could not be sap transmitted from *C. amaranticolor* to groundnut, probably because of the presence of inhibitors in *C. amaranticolor* sap. Later it was found that

groundnut could be infected by inoculation with virus purified from *C. amaranticolor* (Dollet *et al.*, 1976).

Thouvenel *et al.* (1982) reported that the CMMV was readily transmitted by mechanical inoculation as follows :
 Amaranthaceae : *Gomphrena globosa* (mottle); Chenopodiaceae : *Beta vulgaris*, *Chenopodium amaranticolor*, *C. quinoa* and *C. foetidum* (chlorotic local lesions); Leguminoceae *Arachis hypogaea* (mild crinkling), *Cajanus cajan* (chlorosis), *Canavalia ensiformis* (mosaic and crinkling), *Crotalaria usaramoensis* (Chlorosis), *Glycine max* (mosaic), *Phaseolus lathyroides* (mosaic), *P. lunatus* (chlorosis), *P. mungo* (chlorosis), *P. vulgaris* (chlorotic spots), *Pisum sativum* (chlorosis), *Psophocarpus tetragonolobus* (mottle), *Vigna cylindrica* (mosaic), *V. sinensis* (mosaic), *V. unguiculata* (mosaic) and *Voandzeia subterranea* (light mosaic); Pedaliaceae : *Sesamum indicum* (chlorotic spots); Scrophulariaceae : *Penstemon hirsutus* (chlorosis); Solanaceae : *Browallia demissa* (chlorosis), *B. speciosa* (chlorosis), *Nicotiana clevelandii* (chlorosis), *N. megalosiphon* (mottle) and *Solanum carolinense* (chlorosis).

Demski *et al.* (1984) reported nine species of the Chenopodiaceae, Leguminoceae and Solanaceae as a host of peanut stripe virus.

Xu and Barnett (1984) reported CMV-CA (China Arachis Strain) infected 31 of 36 plant species in six families. Plants systemically infected showed mosaic or mottle symptoms (except those with other symptom types in parentheses)
 Amaranthaceae : *Gomphrena globosa*, *Vinca rosea* and *Zinnia*

elegans, Chenopodiaceae : *Beta vulgaris* and *Spinacia oleracea* (stunt); Cucurbitaceae : *Cucumis sativus* cvs. 'Chicago Pickling' (latent); Gramineae : *Zea mays* 'Golden Cross Bantam' (stunt and wilt); Leguminoceae : *A. hypogaea*, *Canavalia ensiformis* (stunt), *Cassia occidentalis*, *Dolichos lablab*, *Phaseolus lunatus* cv. 'Henderson Bush', *P. vulgaris* cvs. 'Bountiful', 'Pinto' and 'Topcrop', *Pisum sativum* cvs. 'Dwarf Grey Sugar' and 'Perfected Wales' (systemic necrosis and wilt), *Trifolium incarnatum*, *Vicia faba* (streak) and *Vigna unguiculata* subsp. *unguiculata*; Solanaceae : *Lycopersicon esculentum* (latent), *N. benthamiana*, *N. clevelandii*, *N. glutinosa*, *N. megalosiphon*, *N. occidentalis*, *N. tabacum* cv. 'Burley 21' and *Solanum melongena*. Plants infected only on inoculated leaves showed local lesions Chenopodiaceae : *Chenopodium album*, *C. amaranticolor*, *C. quinoa*; Leguminoceae : *Phaseolus aureus*; Solanaceae : *Datura stramonium*. Plants not infected were Gramineae : *Triticum aestivum*, Leguminoceae : *Glycine max* cvs. 'Bragg', 'Davis' and 'Jackson', *Trifolium hybridum*, *T. pratense* and *T. repens*.

Ahmed and Mills (1985) reported peanut stunt virus which produced chlorotic or necrotic local lesions and systemic symptoms on *A. hypogaea*, *Capsicum annum*, *Chenopodium amaranticolor*, *C. quinoa*, *Datura stramonium*, *Dolichos lablab*, *N. bigelovii*, *N. debneyi*, *N. glutinosa*, *N. rustica*, *N. tabacum* cvs. 'Harrow velvet', 'White burley' and Xanthi; *N. virginiae*, *Petunia hybrida*, *Phaseolus vulgaris*; *Vicia faba* and *Vigna sinensis*. Local lesions with symptomless systemic infection occurred in

Gomphrena globosa, and local lesions without systemic infection occurred in *Cucumis sativus* cv. 'Telegraph Improved'.

Demski and Lovell (1985) observed that some cultivars of soybeans, cowpeas, lupines and forage legumes such as arrowleaf, crimson and subterranean clovers were susceptible to PStV. Sesamum and beggarweed growing next to infected peanuts were found to be infected.

Reddy *et al.* (1985) reported GRV (C) which infected nine out of 32 species in three out of nine families. It caused local lesions without systemic infection in *C. amaranticolor*, *C. murale* and *C. quinoa* and systemic symptoms in *Glycine max*, *N. benthamiana*, *N. clevelandii* and *Phaseolus vulgaris* as well as in groundnut.

Mali and Nirmal (1987) reported that CMMV was readily sap transmitted to ten species in the Leguminosae, Chenopodiaceae and Solanaceae families. *Beta vulgaris*, *C. amaranticolor*, *Cyamopsis tetragonoloba* produced local lesions without systemic invasion. *C. quinoa* produced chlorotic lesions on inoculated and newly produced leaves. *Cajanus cajan* produced local lesions followed by transient vein clearing on the trifoliate leaves. The virus produced necrotic lesions and veinal necrosis on inoculated leaves followed by systemic severe mosaic on *G. max* cvs. Bragg, Monetta, MACS-75, T-49, PK-395, Kalitur and only systemic necrosis on the cultivars N-19, JS-75-280, JS-76-190, JS-75-185.

Nolt *et al.* (1988) reported that at least 10 of the 15 inoculated host range plants including *A. hypogaea*, *Canavalia*

ensiformis, *C. amaranticolor*, *C. murale*, *C. quinoa*, *Nicotiana clevelandii* x *glutinosa* hybrid, *P. mungo*, *P. vulgaris* cvs. 'Local' and 'Top crop', *Vicia faba* and *V. unguiculata* subsp. *unguiculata* cvs. 'C-152 and Early Ramshorn' were infected by at least one of the five IPCV isolates tested.

Nicotiana bigelovei and *N. rustica* were not infected by any of the isolates tested. All five isolates produced identical symptoms on *A. hypogaea*, *P. mungo* and *V. faba*. Five host range plants were selected on which the five IPCV isolates produced a differential symptom response. The B-IPCV and C-IPCV isolates produced similar symptoms on all host plants; whereas the L-IPCV, H-IPCV and T-IPCV isolates each produced a unique set of symptoms. *C. ensiformis* and *N. clevelandii* x *glutinosa* hybrid were the most useful hosts for separating isolates on the basis of symptom expression.

Nasir Saleh *et al.* (1989) reported the host range of PStV which caused chlorotic local lesion on *C. amaranticolor*, *C. quinoa*, *Glycine max* 'Wilis', *Phaseolus vulgaris* 'Bataaf' and *P. vulgaris* 'Kintoki'. Neither of the isolates was recovered from inoculated leaves of *Cucumis sativus*, *Gomphrena globosa*, *N. glutinosa*, *N. tabacum* 'Samsun NN', *P. vulgaris* 'Topcrop', *Pisum sativum* 'Koroza', *P. sativum* 'Juweel', *Vicia faba* 'Kompakta', *Vigna radiata* 'Merak' and *Vigna unguiculata* 'Blackeye'.

Prasad Rao *et al.* (1989) reported PStV which infected 14 plant species in 4 families. It caused chlorotic local lesions in *C. amaranticolor*, *C. quinoa*, *C. murale*, *P. vulgaris* cv. Bataaf and *Cassia occidentalis* while it produced mosaic mottle systemic

symptoms in *Cyamopsis tetragonoloba* cv. Pusa Navbahar, *Glyciune radiata* cv. PDU-5, *V. unguiculata* cv. C-152, *Sesamum indicum* and *N. benthamiana*.

Chang *et al.* (1990) reported the host range of PStV in Taiwan which included the forty-four species in eight plant families which were inoculated with either Ts or Tc1. Chlorotic lesions appeared on systemically infected leaves of four cultivars of *A. hypogaea* (Tainung No. 4, Tainung No. 5, Tainan Selection No. 9 and Tainan No. 11). In addition to *A. hypogaea*, *N. benthamiana*, *V. unguiculata* subsp. *unguiculata* 'California Blackeye', *V. angularis*, *V. mungo*, *V. umbellata*, *Glycine max* and *V. unguiculata* subsp. *sesquipedalis* became infected by Ts and developed foliar systemic mottle symptoms but were not infected by Tc1.

The following species developed local lesions on inoculated leaves and the viruses were not recovered from uninoculated symptomless ones : *V. radiata* and *C. amaranticolor*. On *C. quinoa* willd, Ts induced both local lesions and systemic mosaic symptoms, whereas Tc1 induced only local lesions.

Reddy (1991) observed all PStV isolates induced local lesions in *Chenopodium amaranticolor* and *C. quinoa* leaves. Unlike peanut mottle virus (PMV), PStV did not infect *Phaseolus vulgaris* (cv. Topcrop).

Kumar *et al.* (1991) reported the host range of groundnut rosette isolates GRV (MC) and YB both caused tiny necrotic local lesions in *C. amaranticolor* and gave symptomless

infection, in inoculated leaves only of *Nicotiana debneyi* and *N. tabacum* cv. Samsun NN. Neither of the isolates was recovered from inoculated or uninoculated leaves of *Capsicum annuum* cv. Bell Boy, *Datura stramonium*, *Lycopersicon esculentum* cv. Kondine Red, *N. glutinosa* or *N. tabacum* cv. White Burley.

Li *et al.* (1991) reported that peanut mottle virus was manually transmitted to *N. benthamiana* and to seven of the eight species of leguminosae included in host range studies. PM₀V-VS did not infect broad bean (*Vicia faba* L.) or any of the following species in other families : *Capsicum annuum* *Cucumis sativus*, *Cucurbita pepo*, *Datura stramonium*, *Gomphrena globosa*, *Gossypium hirsutum*, *Lycopersicon esculentum*, *N. glutinosa*, *N. rustica*, *N. tabacum*, *Zinnia elegans*. PM₀V-VS induced systemic mosaic symptoms in bambarra groundnut (TVSU-334), lima bean and *Macroptilium lathyroides*.

Prasada Rao *et al.* (1997) reported the peanut stripe virus which produced chlorotic local lesions on *Chenopodium amaranticolor*, systemic symptoms on *Glycine max* cv. Bragg, *Vigna unguiculata* cv. C-152, *Arachis hypogaea* cv. TMV-2 and JL-24.

2.4 Transmission

2.4.1 Mechanical transmission

Storey and Bottomley (1928) had proved that rosette disease was not readily transferred by the direct inoculation of juice. Forty-five plants inoculated by the injection of the juice of diseased plants into the region of the growing point failed to develop rosette. Similar results were obtained with 24 plants



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inoculated through wounds on the leaves and with a like number inoculated by insertion of small portions of diseased tissue into the stem.

Hull and Adams (1968) reported that the buffer containing magnesium or sodium bentonite (25 mg/ml), 1 per cent K_2HPO_4 , 0.01 M DIECA at pH 7.3 favoured higher rate of transmission.

Echandi and Hebert (1971) reported fifteen peanut stunt virus isolates which were collected from commercial pole and snapbean plantations in western North Carolina and sap transmitted to Ramshorn blackeye peas [*Vigna sinensis*], *Nicotiana glutinosa*, *N. tabacum* and return inoculations were made to beans. One isolate was used throughout this work and was maintained by mechanical transfers to young Ramshorn blackeye peas.

Reddy *et al.* (1978) reported that plants showing mottling and interveinal depression were collected from the crop raised by farmers in Samrala district of the Punjab State. Extracts from leaves were mechanically inoculated on peanut and the disease was subsequently maintained in a screen-house in '*Arachis hypogaea*' TMV-2 and *Glycine max* 'Bragg' by mechanical sap inoculation. Germani *et al.* (1975) reported that the peanut clump virus was easily transmitted by mechanical inoculation.

Demski and Lovell (1985) observed that single lesions from *Chenopodium* plants used to mechanically inoculate individual peanut plants. The majority of these plants developed

banding and dark stripes along the lateral leaf veins that in many cases resembled stripes, so the virus was named peanut stripe virus (PStV). A few peanut plants inoculated from single lesions from *Chenopodium* developed dark green circular areas that were not associated with the veins and these isolates were called peanut blotch.

Reddy *et al.* (1985) reported that the extracts made from each of the 22 groundnut plants imported from Nigeria with chlorotic rosette disease. The extracts in 0.02 M phosphate buffer, pH 8.0, containing 1 litre Mg-bentonite were each rubbed on to carborundum dusted leaves of 10 groundnut and two *N. clevelandii* plants. Two of the extracts failed to induce symptoms in any of the test plants. Three of the extracts induced a reaction characteristics of cowpea mild mottle virus in the groundnut seedlings.

Prasada Rao *et al.* (1989) reported that PStV was found infected in nature as well as under artificial sap inoculation. Mechanical inoculations were done as per procedure described by Ghanekar *et al.* (1979). In host range studies, at least 5 plants of each species were sap inoculated and maintained in a screen house.

Rehcigl *et al.* (1989) mechanically transmitted peanut stripe virus and maintained in the greenhouse in peanut cultivar Florigiant or NC-7. Plants were inoculated 14-16 days after seedling by rubbing neutral phosphate buffer, extracted sap or torn, infected leaf edges onto carborundum (600 mesh) dusted leaflets of the first two quadrifoliate leaves.

Chang *et al.* (1990) maintained T_s and T_c isolates either on peanut or on blackeye cowpea [*Vigna unguiculata* (L.) Walp. Subsp. *unguiculata* cv. 'California Blackeye' by manual inoculations.

Reddy (1991) reported that two potyviruses *viz.*, peanut mottle virus (PMV) and peanut stripe virus (PStV), two cucumoviruses *viz.*, peanut stunt virus (PSV) and cucumber mosaic virus (CMV), peanut clump furovirus, cowpea mild mottle carlavirus, tomato spotted wilt tospovirus, groundnut rosette virus and its satellite RNA were transmitted by mechanical inoculations.

Mandal *et al.* (2001) studied on evaluation of peanut germplasm for tomato spotted wilt virus (TSWV) resistance which has been slowed by the difficulty in achieving a high rate of mechanical transmission of the virus to peanut. In this study, improvements were made and a highly efficient mechanical transmission protocol was developed. Several factors that affect the transmission efficiency were identified. Use of two antioxidants (sodium sulfite and mercaptoethanol) and two abrasives (Celite and Carborundum) and application of the inoculum by rubbing with a cotton swab dipped in the inoculum as well as pricking with an inoculation needle resulted in a significantly higher transmission rate.

2.4.2 Seed transmission

Storey and Bottomley (1928) reported during the 1923-24 season, 330 seedlings were raised from seeds of selected rosetted plants which was sown at monthly intervals from

September to February in the open at Pretoria and 40 in the greenhouse; all seedlings remained free from rosette. In 1926, 90 seeds from diseased plants were sown in a gauze-protected greenhouse at Durban and produced 86 healthy plants, only four seeds failing to germinate. There was therefore no evidence to suggest that possible virus bearing seeds might undergo delayed germination and so escape notice.

Troutman *et al.* (1967) and Kuhn (1969) reported that peanut stunt virus was seed transmitted but with a low frequency. Large seeds, especially from late-infected plants, yield only 0.01 per cent or fewer infected plants, whereas small seeds, selected from relatively less severely stunted plants, may yield up to 0.20 per cent infected plants.

Herold and Munz (1969) observed that peanut mottle virus was seed borne to the extent of 2 per cent. Adams and Kuhn (1977) reported that the four isolates of the peanut mottle virus differed in the frequency of seed transmission in Starr peanut, the per cent transmission ranging from 0 to 8.5 per cent. The embryo was infected by the virus, when groundnut plants were maintained at 21 or 35°C during flowering and pegging, the percent transmission through seeds was reduced to one third. Reddy *et al.* (1986) reported that seed transmission of peanut mottle virus did not occur in certain groundnut genotypes like EC 76446 (292), NcAc 17133 (RF) and Ah 7171 which were used in breeding for resistance to this disease.

Brunt and Kenten (1973) observed that cowpea mild mottle virus (CMMV) was seed borne in *Vigna unguiculata*,

Glycine max and *Phaseolus vulgaris* but not in *Nicotiana clevelandii*.

Ghanekar *et al.* (1979) reported that of nearly 6000 seeds collected from the infected groundnut plants, 1800 seeds (30 %) produced normal plants, 540 seeds (9 %) produced malformed plants and the remainder failed to germinate. None of the plants developed disease symptoms and assays on cowpea from the malformed stunted plants gave negative results. The results indicate that the virus was not seedborne.

Demski and Lovell (1985) observed that PSTV was seed transmitted upto 37 per cent in groundnut that were artificially infected. PSTV infected naturally soybean in which also it was seed borne.

Demski and Warwick (1986) noticed that the PSTV was consistently detected by ELISA in the cotyledonary and embryonic tissues of infected seed but not in the testa.

Culver and Sherwood (1988) detected PSTV antigen in peanut cotyledonary tissue by MAB (Monoclonal antibodies) indirect ELISA format. All five peanut cultivars *viz.*, Spanco, Pronto, Tamnut 74, Argentine and Florunner were shown to transmit PSTV by seed at rates ranging from 0.4 to 5.0 per cent.

Warwick and Demski (1988) found that none of the three virus isolates of peanut stripe virus were transmitted in the 15,000 soybean seeds from infected plants harvested from all susceptible cultivars. Neither infective virus nor serologically detectable PSTV was recovered from cotyledons or embryo of mature seeds.

Prasada Rao *et al.* (1989) reported that the seed transmission of PStV in five groundnut cultivars *viz.*, Robut 33-1, ICGS-11, J-11, TMV-2 and JL-24 varied from 12 to 28.88 per cent.

Chang (1990) observed that the out of 400 seeds harvested from Ts infected peanut plants, 12.5 per cent were infected. The study involved four replicates consisting of 100 seeds each; the number of infected seedlings from each replicate was five, nine, twenty five and eleven. The average germination rate of seeds from infected plants was 81.53 per cent compared to 93.3 per cent for seeds from non-infected plants.

Parasada Rao and Chakrabarty (1990) reported that the during 1988, when groundnut seeds imported from Myanmar were tested by ELISA, one seed out of 410 was found to contain peanut stripe virus (PStV).

Reddy (1991) reported that peanut clump virus (PCV) was seed transmitted upto 11 per cent in groundnut. PCV is also transmitted in seed of pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*) and foxtail millet (*Setaria italica*). Thus, long distance dispersal through seed of cereal crops was possible

Zeyong *et al.* (1991) tested peanut seeds positive for PStV by DAC-ELISA to determine the location of the virus in infected seed and results showed that the PStV was consistently detected by ELISA-DAC in the cotyledonary and embryonic tissues of 65 infected seeds but not in the testae. In this group of tests, ELISA values (P/N) of cotyledonary and embryonic

tissue averaged three and 2.8 fold higher, respectively than healthy controls. The average of ELISA values (P/N) of testae was less than one. In another test with a group of 39 mature or immature infected seeds collected before harvest, PStV was detected by DAC-ELISA in both cotyledonary and embryonic tissues of five seeds, 32 seeds and in the testa of two seeds.

Jain *et al.* (2000) noticed that the seedlings of peanut raised out of seeds collected from plants infected with peanut stripe virus were symptomless.

Choi *et al.* (2001) reported PStV in South Korea which was seed transmitted to varying degrees depending on the cultivar and a maximum seed transmission of 15.7 per cent was observed in cv. Aul.

2.4.3 Insect transmission

Watson and Okusanya (1967) reported that *A. craccivora* from Nigeria could transmit four strains of rosette virus, while the race of *A. craccivora* from Kenya transmitted only two strains from East Africa.

Troutman *et al.* (1967) observed that the peanut stunt virus was transmitted by aphid vectors *M. persicae*, *A. craccivora* and parasitic dodder *Cuscuta sp.* The virus has been shown to be stylet-borne in nature.

Herold and Munz (1969) reported that *A. craccivora* and *M. persicae* transmitted the peanut mottle virus in a stylet-borne manner with equal efficiency.

Fischer and Lockhart (1978) reported that PSV was transmitted by non-persistent aphid transmission using *M.*

persicae, four of eight assay plants of Bountiful bean and two of four Early Ramshorn cowpeas developed typical symptoms of systemic infection.

Ghanekar *et al.* (1979) found that the TSWV was transmitted by the thrips *Frankliniella schultzei* consistently and to a lesser extent by *Scirtothrips dorsalis*. Adults of *S. dorsalis* collected from plants with bud necrosis successfully transmitted TSWV to 14 of 65 plants. Similarly, thrips that acquired virus from infected leaves and were then allowed inoculation feeds of 12 to 15 days transmitted the disease to 31 out of 164 groundnut plants. None of the 160 plants exposed to thrips from healthy colonies were infected.

Amin *et al.* (1981) reported that tomato spotted wilt virus (TSWV) was transmitted by thrips *F. schultzei* which was shown to be the principal vector in the Indian subcontinent.

Ahmed and Idris (1981) noticed that the PMV was transmitted to groundnut seedlings in a non-persistent manner by starved *A. craccivora* (10 aphids per plant) when allowed acquisition feedings of 45 min. The aphids transmitted the virus to 38 to 80 test plants.

Thouvenel *et al.* (1982) observed that the CMMV was not transmitted by *A. craccivora* (0/100) nor by *A. spiraecola* (0/100) from soybean to soybean.

Muniyappa and Reddy (1983) reported that the individual *Bemisia tabaci* adults acquired cowpea mild mottle virus (CMMV) in 10 minutes and transmitted it within 5 minutes to soybeans. Starvation before acquisition had no effect upon

transmission, but starvation after acquisition decreased transmission frequency. Irrespective of the length of acquisition, ability to transmit CMMV was retained in the whitefly for only four successive inoculation access periods of 5 minutes each, but adults that lost the ability to transmit the virus could reacquire and transmit the virus.

Kuhn and Demski (1984) reported that peanut mottle virus which was transmitted in the non-persistent manner by several aphid species including *Aphis craccivora*, *A. gossypii*, *M. persicae*, *Hyperomyzus lactucae*, *Rhopalosiphum padi* and *R. maidis*.

Demski *et al.* (1984) reported that after a 1 min acquisition access period, *A. craccivora* transmitted PStV to 11 of 14 groundnut plants.

Xu and Barnett (1984) noticed that the cucumber mosaic virus was transmitted to five of 10 *Nicotiana clevelandii* plants by *Macrosiphum euphorbiae*.

Reddy *et al.* (1986) and Nolt *et al.* (1988) reported that the phycomycete fungus *Polymyxa graminis* was considered to be vector of the peanut clump virus.

Sreenivasulu and Demski (1988) reported that the *Aphis craccivora* and *Myzus persicae* transmitted PStV from peanut to peanut. *M. persicae* was a more efficient vector than *A. craccivora*. From singly infected plants, *M. persicae* and *A. craccivora* transmitted PStV at 29 and 17 per cent, respectively.

Prasada Rao *et al.* (1988) studied on *A. craccivora* colonies maintained on *Vigna unguiculata*, were fasted for 6 hr

and then allowed an acquisition access of 1 minute followed by 18 h. inoculation access period in PStV.

Warwick and Demski (1988) reported that the PStV was transmitted efficiently (16%) from peanut to soybean by the aphid *Myzus persicae* but inefficiently (1 %) by *Aphis craccivora*.

Prasada Rao *et al.* (1989) observed *A. craccivora* transmitted the PStV to 7 of 10 groundnut plants.

Chang *et al.* (1990) used twenty peanut seedlings to test the aphid (*M. persicae*) transmissibility of Ts and Tc1, 18 and 15 seedlings, respectively, became infected.

Chang (1990) conducted aphid transmission trials with *M. persicae* which were starved for 3-4 hours before access feeding of approximately 60 seconds on Ts-infected cowpea or Tc-infected peanut leaves. Groups of five aphids were transferred to each of 25 test plants. After a testing feeding period of 4-5 hours, the aphids were removed and the plants were placed in a screenhouse for observation.

2.5 Physical properties

Kuhn (1965) studied the *in vitro* stabilities of the peanut mottle virus in crude sap and found that the virus remained infective for 24 hours but not for 48 hours at 25°C, at a dilution of 10⁻³ but not at 10⁻⁴ and after 10 minute heating at 60°C but not at 65°C.

Okusanya and Watson (1966) reported the physical properties of groundnut rosette as DEP between 1:10 and 1:100, TIP at 50°C and LIV for one week at 18°C and four weeks at 20°C.

. Herold and Munz (1969) studied physical properties of PMV as the virus remained active in undiluted sap from pea leaves for 24 hr but not 48 hr at 4°C, after 10 min. at 62°C, but not at 65°C and in a dilution with distilled water of 10⁻³ but not 10⁻⁴.

Echandi and Hebert (1971) reported that the PSV was inactivated between 55 to 60°C for 10 min. and lost its infectivity between 1 : 1000 and 1 : 10,000 dilution. Unbuffered crude sap remained infective after 24 but not 48 hr. at room temperature 25°C.

Sun and Hebert (1972) reported physical properties of PMV-S (severe strain) as DEP 10⁻³-10⁻⁴, TIP 60-65°C and LIV 12-14 hr.

Brunt and Kenten (1973) determined that the physical properties of CMMV using the sap from systemically infected *Glycine max* was infective to *C. quinoa* after dilution to 10⁻³ but not 10⁻⁴, after 10 min. at 65°C but not 70°C and after at least 8 days at 20°C. Lyophilized sap remained infective for at least 4 years.

Thouvenel *et al.* (1976) studied the physical properties of peanut clump virus as DEP 10⁻⁴-10⁻⁵, TIP 60-64°C and LIV 15 months at 20°C, 12 months in frozen sap and 50 days by alternate freezing and thawing.

Fischer and Lockhart (1978) studied physical properties of Moroccan peanut stunt virus (PSV) isolate and found that the thermal inactivation point occurred at 50-52°C. In 10 fold dilution steps, virus infectivity was lost between 10⁻⁴

and 10^{-6} and the infectivity was retained for 24-48 hr at room temperature 24°C .

Reddy *et al.* (1978) studied physical properties of PMV in India as DEP 10^{-3} - 10^{-4} , TIP 55 - 60°C and LIV 48-72 hr.

Beczner and Devergne (1979) studied the physical properties of PSV-Tp (Peanut stunt virus-*Trifolium pratense*) and found that sap extracted from *Nicotiana megalosiphon* and assayed on *N. megalosiphon* remained infectious when heated to 75°C for 10 min. However, heating to 80°C resulted in no infection. In 10 fold dilution steps, virus infectivity was lost between 10^{-4} to 10^{-5} . Sap containing PSV-Tp usually remained infective at laboratory temperature (about 22°C) for only 10-12 days.

Ghanekar *et al.* (1979) reported the physical properties of TSWV as DEP $10^{-2.5}$ - $10^{-3.0}$, TIP 45 - 50°C and LIV 4 hr but not for 5 hr at room temperature (30°C).

Ahmed and Idris (1981) reported physical properties of PMV as DEP 10^{-3} - 10^{-4} , TIP 55 - 60°C and LIV 1-2 days

Thouvenel *et al.* (1982) reported the physical properties of CMMV as DEP was between 10^{-4} - 10^{-5} , TIP was 65 and 70°C and infectivity was retained for 2 days at 25°C , 28 days at 4°C and more than 2 months at -20°C .

Reddy *et al.* (1983) studied the physical properties of IPCV (Indian peanut clump virus) as DEP was between 10^{-3} - 10^{-4} , TIP between 60 - 65°C and LIV 20 days at room temperature (25 - 30°C).

Xu and Barnett (1984) studied the properties of CMV-CA (China Arachis Strain) and found that CMV-CA infectivity was lost at dilutions between 10^{-2} and 10^{-3} , by heating for 10 min. in between 55 and 60°C and by storage at 28°C between 6 and 7 days.

Demski *et al.* (1984) determined physical properties of stripe virus of peanut using the extracted sap of systemically infected peanut and reported that DEP was between 10^{-3} and 10^{-4} , TIP was between 60°C and 65°C and the virus remained infective in crude sap for 3 days at 20°C.

Ahmed and Mills (1985) reported physical properties of PSV as DEP 10^{-2} and 10^{-3} , TIP 50°C and LIV 1-2 days.

Reddy *et al.* (1985) reported an isolate of GRV (C) from Nigeria remained infective in *N. clevelandii* leaf extracts for 1 day at room temperature and for 15 days at 4 °C, but lost infectivity after 1 day at -20 °C or after dilution to 10^{-4} .

Mali and Nirmal (1987) studied the physical properties of CMMV and found that the TIP was between 75°C and 80°C, DEP was between 10^{-3} and 10^{-4} and the virus remained infective at room temperature for 9 days.

Prasada Rao (1989) studied physical properties of peanut stripe virus and found that the thermal inactivation was between 60 and 65°C, the infectivity dilution end point was between 10^{-3} and 10^{-4} and the infectivity was retained for 2 days at room temperature (18 to 30°C).

2.6 Particle morphology

Williams and Wycoff (1944) reported the early studies on virus particles which were carried out by using metal shadowing techniques. These procedures required relatively pure virus preparations.

Brenner and Horne (1959) studied on electron microscopy of plant viruses occurred with the development of negative contrast staining. This technique is now universally used and procedures have been developed that allow simple and rapid examination of virus infected leaf material without the necessity of using purified virus preparations.

Brandes (1960) for the first time described the quick leaf dip procedure, in which a drop of infected leaf sap, obtained by squeezing sap from the freshly cut surface of leaf, which is examined under electron microscope for identification of viruses.

Hitchborn and Hills (1965) modified quick-leaf-dip method by epidermal strip method, in which a strip of epidermal tissue, peeled from the under surface of an infected leaf placed on to a drop of negative stain.

Troutman (1966) reported that the purified peanut stunt virus preparations showed spherical particles. Mink *et al.* (1969) noticed that the western strain of PSV was measured 26-30 nm in diameter.

Herold and Munz (1969) studied electron microscopy of peanut mottle virus and found the thread-like virus particles with a mean of 812 x 15 nm are present in dip preparations of systemically infected groundnut plants. According to Schmidt

and Schmeezer (1966) the virus particles measure 763 x 15 nm. Paguio and Kuhn (1973a) reported the purified virus particles of a mild mottle strain having a particle length of 725 nm.

Thouvenel *et al.* (1976) reported that the peanut clump virus particles which was rod shaped and of two predominant lengths of 190 and 245 nm.

Ghanekar (1980) studied electron microscopy of tomato spotted wilt virus (TSWV) and found that in thin sections of groundnut leaves infected with the disease, membrane bound particles 70-90 nm in diameter associated with endoplasmic reticulum are present.

Demski *et al.* (1984) observed cytoplasmic inclusions in thin sections of infected groundnut tissue. Virus particles in purified preparations stained with ammonium molybdate, pH 6.5, had a mean diameter of 13 ± 1 nm and a mean length 752 nm (mean of 330 particles).

Reddy *et al.* (1985) reported that plants infected with groundnut rosette assistor virus (GRAV) contained isometric particles 25 nm in diameter which were detectable by immunosorbent electron microscopy on grids coated with antisera to several luteoviruses, especially with antisera to bean leaf roll, potato leaf roll and beet western yellow viruses. Rajeshwari *et al.* (1987) observed similar type of particle morphology as mentioned earlier.

Culver *et al.* (1987) studied electron microscopy which was done by leaf dip assay. Two microlitres of a saline solution was placed on formvar-coated electron microscope grids. A leaf

from a test plant was cut perpendicular to the midvein and the cut edge was allowed to make contact with the saline on the grid for 2 min. The grid was negatively stained with 1 per cent uranyl acetate for 2 min. and wicked dry with filter paper. Ten to 15 grid openings in each grid were examined to determine the presence or absence of PStV. In electron microscopy positive samples had rod-shaped particles of the same length and diameter as particles found in the PStV-infected cultivar Argentine control. Negative samples showed no such particles.

Prasada Rao and Chakrabarty (1988) reported dip preparations of peanut leaf samples stained with 1 per cent uranyl acetate showed flexuous, rod shaped particles.

Rajeshwari and Murant (1988) reported the purified preparations from GRV which contained isometric particles typical of a luteovirus, 25 nm diameter in uranyl acetate negative stain of 28 nm.

Prasada Rao *et al.* (1989) carried out electron microscopic demonstration of PStV and particles measured 750 nm length in groundnut and clusterbean.

Chang (1990) studied particle morphology of PStV and reported that particles were flexuous, rod shaped in negatively stained peanut extracts infected with Ts and Tc. Numerous flexuous rods were also observed in purified preparations of Ts. A total of 121 Ts particles and 107 Tc particles were chosen for examination. The mean lengths were 760 and 747 nm respectively; while Choi (2001) reported filamentous rod shaped virus having modal length of 720 nm of PStV in South Korea.

Amin (1992) reported PStV particles were flexuous filaments 752 nm long and 13 ± 1 nm diameter.

Narayanasamy (1993) reported the virus particles of groundnut rosette disease which were isometric with a diameter of 25-28 nm in the purified preparation.

2.7 Serology

Oudin (1952) and Mancini *et al.* (1965) used one type of immunodiffusion test i.e. radial diffusion for identification of viruses.

Van Slogtesen (1955) used slide agglutination or chloroplast agglutination test which has been particularly useful in the past for the rapid detection of virus infected potatoes in the field.

Ackers and Steere (1967) reported immunodiffusion test, in which antibody-antigen reaction was carried out in a gel instead of liquid.

Derrick (1973) was the first to use electron microscope serology when he 'trapped' viruses on to antibody-coated grids (ACG).

Noordam (1973) reported the micro-precipitin tests which were very economical in the use of antiserum, antigen and are quite sensitive.

Milne and Luisoni (1975) used two other electron microscope serology techniques for identification of plant viruses. The first involved the mixing of virus particles and antisera prior to their being placed on a grid for electron microscope examination. In the second technique, the antibodies

are added to a grid that already has virus particles attached, so that the particles became coated or decorated with the antibodies. Besides its use for virus identification, the 'decoration' procedure has also been used to demonstrate degrees of relationship between viruses (Walkey and Webb, 1984).

Thouvenel *et al.* (1976) reported the peanut clump virus which was not serologically related to tobacco rattle, pea early browning or soil borne wheat mosaic viruses or to a virus associated with a rhizomania-like disease of beet.

Voller *et al.* (1976) and Voller and Bidwell (1977) developed the ELISA procedure for the first time.

Clark and Adams (1977) reported that the Double Antibody Sandwich-Enzyme Linked Immunosorbent Assay (DAS-ELISA) method was commonly used.

Casper *et al.* (1983) found GRAV in ELISA reacted only with best Western yellow viruses antiserum and in subsequent work other antisera to other luteoviruses failed to detect GRAV reliably.

Koenig and Paul (1983) reported the numerous variations of ELISA used for plant virus identification.

Clark and Bar-Joseph (1984) reported the Direct Antigen Coated-Enzyme Linked Immunosorbent Assay (DAC-ELISA) method.

Demski *et al.* (1984) reported in dir-ELISA, PSTV cross reacted strongly with blackeye cowpea mosaic virus (BICMV), clover yellow vein virus (CYVV) and SMV antisera. With the

exception of a weak reaction with pepper veinal mottle virus (PVMV) antiserum, none of the other potyvirus antisera tested reacted with PStV. In indirect ELISA using F (ab')₂ fragments, PStV reacted strongly with antiserum to BICMV, CYVV to a lesser extent with PVMV and SMV. Nasir Saleh (1989) reported that the virus isolated from groundnut plants in Indonesia reacted positively with antisera to SMV, BICMV, CYVV and PStV. It has been demonstrated that BICMV, CYVV and SMV did not infect groundnut.

Milne and Lesemann (1984) reported the immunosorbent electron microscopy method (ISEM) for identification of plant viruses.

Reddy *et al.* (1986) reported the clump virus in India was serologically unrelated to the West African clump virus.

Culver and Sherwood (1988) compared indirect and double-antibody sandwich enzyme linked immunosorbent assay (ELISA) formats using polyclonal antibodies alone, monoclonal antibodies (MAB) alone or the two in combination, for the detection of peanut stripe virus (PStV) in peanut seed. The MAB indirect ELISA format was shown to be the most sensitive, detecting 2.5 ng/ml of virus and detecting one PStV-infected seed part diluted in 32 healthy seed parts.

Lynch *et al.* (1988) reported ELISA analyses for PStV infection in peanut leaves in 1985 showed that plants were not infected before inoculation. Plants in all control plots (Caged and uncaged) remained free of PStV infection throughout the growing season. PStV was detected 20 days after inoculation in plants

inoculated at emergence. Likewise, later inoculations with PStV for the other inoculation treatments resulted in significantly higher ELISA values than for the caged and uncaged control by 20 days after inoculation.

Rajeshwari and Murant (1988) reported the double antibody sandwich – ELISA (DAS-ELISA), in which Ig from GRAV antiserum was used as both plate-coating detecting antibody, positive reactions were obtained with GRAV (C), GRAV (G), GRAV (MC) and GRAV (MM) in groundnut leaf extracts.

Prasada Rao *et al.* (1989) showed that in DAC-ELISA, the virus strongly reacted with antisera to PStV and black eye cowpea mosaic (BICMV) and did not react with peanut green mosaic virus (PGMV) antisera.

Chang (1990) used direct and indirect enzyme-linked immunosorbent assays (ELISA) to study relationships between Ts, PStV and other potyviruses and results indicated that the Ts and Tc reacted closely to each other and to blackeye cowpea mosaic virus, but not to six other potyviruses tested. In contrast to the result obtained in the SDS immunodiffusion test, neither isolate of bean common mosaic virus reacted against antiserum of non-degraded Ts. In indirect ELISA, antiserum to non-degraded Ts reacted weakly with soybean mosaic virus. The absorbance values of homologous and heterologous antigens were similar in indirect ELISA, whereas in direct ELISA the absorbance values of homologous antigens were always higher than those of heterologous antigens.

Jain *et al.* (2000) studied on serological relationship of PStV with other potyviruses which was determined by Direct antigen coating ELISA and ISEM tests and result showed that the PStV isolate not only reacted with homologous antiserum but also to cowpea aphid borne mosaic (CABMV) and soybean mosaic (SbMV) antisera. Antisera to bean common mosaic (BCMV), bean yellow mosaic (BYMV), eggplant mottle (EMoV), potato virus Y (PVY), sugarcane mosaic (SCMV), sweet potato feathery mottle virus (SPFMV) and watermelon mosaic (WMV-1), however, failed to react with PStV in DAC-ELISA.

2.8 Identification of sap transmissible viral diseases of groundnut

Zimmerman (1907) reported the first virus disease of groundnut from East Africa as 'Krausel Krankheit', and also reported for the first time the occurrence of groundnut rosette disease from Tanganyika (now called Tanzania) and has since been reported in several other countries in Sub-Saharan Africa.

Sundararaman (1926, 1932) was the first to report a virus disease of groundnut at Tamilnadu Agricultural Research Station, Chennai with mosaic symptoms.

Sundararaman (1927) reported a clump disease of groundnut from India in 1927.

Fulton (1950) reported cucumber mosaic virus (CMV) in Arkansas in which he described six isolates of CMV from Spinach.

Kuhn (1965) reported Peanut Mottle Virus (PMV) for the first time from the U.S.A. and India (Reddy *et al.*, 1978).

Echandi and Hebert (1970) reported that stunt had appeared the previous summer in epiphytotic form in home gardens and commercial pole and snapbean plantations in North Carolina in 1970.

Brunt and Kenten (1973) and Brunt and Phillips (1981) reported cowpea mild mottle virus (CMMV), which has been known to occur on cowpea and tomato in Africa.

Germani and Dhery (1973) observed peanut clump virus during the summer of 1969 in peanut at the agricultural station of Saria in Uppervolta, where about 6 out of 10 ha were affected.

Halliwell and Philley (1974) first reported tomato spotted wilt virus (TSWV) on peanut in Texas in 1972. Reddy *et al.* (1968) first reported bud necrosis disease (BND) from India.

Griffin *et al.* (1982) found Peanut Stunt Virus (PSV) in beans in a breeding nursery in Fayetteville, AR and in white clover surrounding the bean and cowpea breeding nurseries. PSV was not recovered from white clover or other perennial plants from three other locations in Arkansas.

Iizuka *et al.* (1982) recorded CMMV on peanut in India.

Demski *et al.* (1984) detected for the first time peanut stripe virus (PStV), a potyvirus in the United States in Georgia in 1982. It had apparently been introduced through germplasm lines from the People's Republic of China (Demski and Lovell, 1985).

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MATERIAL AND METHODS



3. MATERIALS AND METHODS

The details of materials used and methods adopted for this study were as follows.

3.1 Materials

3.1.1 Diseased plant samples from groundnut

The groundnut plant samples showing characteristics symptoms of stripe and blotch were collected for the extraction of isolates of stripe and blotch virus from different varieties of groundnut and maintained on glasshouse grown groundnut seedling var. SB-XI for further use.

The diseased samples of groundnut were collected from Groundnut Improvement Project, Mahatma Phule Krishi Vidyapeeth, C.C., Rahuri and Oilseed Research Station, Jalgaon.

3.1.2 Seeds

Genetically pure seeds of groundnut were obtained from Groundnut Improvement Project, M.P.K.V., C.C., Rahuri. The other plant species required for the host range studies were procured from other sources and also from Department of Plant Pathology and Agril. Microbiology, M.P.K.V., Rahuri.

3.1.3 Miscellaneous

All the required glasswares were of corning brand, while water bath, mortars and pestles and earthen pots available at Department of Plant Pathology and Agricultural Microbiology were used.

3.2 Methods

The present investigations were carried out in insect free well managed glasshouse. Various methods followed for carrying out present investigation were described under appropriate headings as below.

3.2.1 Raising of seedlings

The experimental test plants viz., groundnut, cowpea, soybean, bean and hosts of different plant species belonging to various families were raised from seeds in earthen pots of suitable size and filled with sterilized mixture of sand, medium black soil and well decomposed farm yard manure. Seed were treated with 0.3 per cent captan (N-trichloromethyl thio-4-cyclohexene-1,2 dicarboximide) to prevent them from rotting.

The glasshouse was sprayed with 0.05 per cent monocrotophos (Dimethyl-cis-1-methyl-2-methyl-carbamoylvinyl phosphate) at an interval of 12 to 15 days to keep it free from the insects.

3.2.2 Maintenance of virus isolates of groundnut

Virus isolates from different varieties of groundnut were collected for isolation of viruses from Groundnut Improvement Project, C.C., M.P.K.V., Rahuri and Oilseed Research Station, Jalgaon as shown in Table - 1.

Table 1. Locationwise diseased samples of groundnut collected

Sr. No.	Varieties	Location
1.	SB-XI	Groundnut Improvement Project, M.P.K.V., C.C., Rahuri
2.	Phule Pragati (JL-24)	Groundnut Improvement Project, M.P.K.V., C.C., Rahuri
3.	Kopergaon-1	Oilseed Research Station, Jalgaon
4.	Kopergaon-3	Oilseed Research Station, Jalgaon
5.	Karad-4-11	Oilseed Research Station, Jalgaon

3.2.3 Symptomatology

The symptoms of groundnut viruses were observed periodically on infected plants under field condition as well as an artificial inoculated experimental plants based on symptoms described by earlier workers (Fukumoto *et al.*, 1986; Zeyong *et al.*, 1983 and Demski *et al.*, 1984) which included the appearance of chlorotic rings surrounding the blotching of young leaflets, mild mottle, dark green stripes and continuous banding along the lateral veins of young leaves .

3.2.4 Host range

For host range studies, the hosts of different plant species belonging to various families viz., Chenopodiaceae, Leguminosae, Cucurbitaceae, Malvaceae, Amaranthaceae, Pedalaceae, Compositae and Solanaceae were mechanically sap inoculated. Twenty plants of different plant species were

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inoculated at 3-5 leaf growth stage with sap extracted from different varieties of groundnut by conventional leaf rub method. Inoculated plants were kept in insect proof glasshouse and observations on symptom expressions were carried out at periodical intervals upto one month after inoculation. The plants, which did not show any symptoms, were back indexed on test plants of *Chenopodium amaranticolor*.

3.2.5 Determination of virus concentration

Samples of roots, stems and leaves from virus infected groundnut plants var. SB-XI were separately collected for determination of virus concentration. The crude sap was separately extracted from roots, stems and leaves and inoculated to twenty seedlings of *C. amaranticolor*. Inoculated plants were labelled and maintained in an insect free glasshouse. Observations were recorded for number of local lesions appeared.

3.2.6 Transmission

3.2.6.1 Mechanical inoculation

a. Preparation of inoculum

Inocula prepared from diseased plants of different varieties of groundnut by routine crude sap extraction methods were used in transmitting the viruses. Young leaf tissues showing typical dark green stripes and blotching of young leaflet symptoms were selected for sap extraction.

The leaves of virus isolates of groundnut were used for inoculation to the test plant of most susceptible groundnut variety for their maintenance. The leaves of virus isolates of groundnut were gently washed in tap water immediately wiped

off excess water with the help of blotting paper and weighted on the chemical weighing balance. Then these leaves were macerated in sterilized and chilled mortar and pestle by adding equal quantity of phosphate buffer. The crude sap was then obtained by squeezing the pulp through a double layered muslin cloth.

The inocula of all the isolates were kept at 8°C and in some cases the inocula were inoculated immediately.

b. Inoculation procedure

Before inoculation, the carborundum powder or celite, an abrasive was dusted on the leaves to be inoculated. The leaves were then inoculated by the inoculum following gentle rubbing method of Ghanekar *et al.* (1979) with the help of cotton swab. Immediately after inoculation, the leaves were slightly washed in the stream of tap water to remove excess inoculum. The seedlings were labelled properly and kept in the insect proof glasshouse for recording observations. The inoculated plants were observed periodically and observations were recorded as and when the symptoms appeared.

3.2.6.2 Seed transmission

The seeds were collected from virus infected groundnut plants. Five hundred seeds of different varieties of groundnut were sown in earthen pots and kept in an insect free glasshouse. Observations were recorded for plants showing virus symptoms.

The level of seed transmission were calculated by counting diseased or infected plants from total populations.

3.2.6.3 Transmission by insects

i. Collection and identification of insects

For insect transmission studies the colonies of ten non-viruliferous aphids namely *Aphis craccivora* on young healthy cowpea, *Myzus persicae* on pepper, *Rhopalosiphum maidis* on young healthy sorghum, *Aphis gossypii* on cotton and a whitefly (*Bemisia tabaci*) on tobacco were raised in an insectary in small musclin covered cages. These insects were identified from Department of Agricultural Entomology, M.P.K.V., Rahuri.

Wingless adult females were employed in all insect transmission experiments. Young 10-15 days old plants of groundnut were used as a test plant. During fasting periods, insects were placed in the test tubes after collecting from their colonies by means of a camel hair brush and the open ends of the test tubes were covered with musclin cloth. For acquisition feeding, insects were liberated separately on detached, diseased groundnut leaves kept in petridishes.

After a requisite acquisition feeding, the insects were liberated for transmission feeding on healthy groundnut test plants which were covered with celluloid cages, the open ends of which were pressed into the soil. The virus transmission by aphids (*Aphis craccivora* and *Myzus persicae*) were carried out as per the methods described by Sreenivasulu and Demski (1988). After a requisite transmission feeding, plants were sprayed with malathion to kill the insects and kept in glasshouse under observations for 25 days.

Detailed studies were carried out to ascertain the virus vector relationship, the various procedures adopted are given here under.

ii. Aphid transmission efficiency of virus isolates of groundnut

The preliminary tests were carried out with two different aphid species namely *Aphis craccivora* and *Myzus persicae* with a view to ascertain the efficiency of these insects for transmission of virus isolates of groundnut. The following treatments were given and 20 seedlings were tested.

Pre acquisition fasting	:	2 hrs
Acquisition feeding	:	10 min
Number of insects per test plant	:	10
Total virus isolates	:	5

The individual aphids from colonies were picked up carefully using camel hair brush for testing the efficiency.

The leaves of viral infected plants with different virus isolates kept in separate petriplates and starved aphids (*Aphis craccivora* and *Myzus persicae*) were transferred on it for ten min. acquisition feeding period. Ten aphids of two species were transferred individually to each test seedlings of groundnut var. SB-XI for transmission feeding of 15 min.

After transmission feeding, the aphids were killed by spraying malathion and plants were maintained in an insect free glasshouse for recording the observations. This experiment was conducted twice for accurate results and the observations were recorded for transmission percentage by aphid species.

iii. Number of aphids required to transmit virus isolates of groundnut by *A. craccivora* and *M. persicae*

Varying number viz., 1, 5, 10, 15, 20, 25, 30 of viruliferous aphids of *A. craccivora* and *M. persicae* were transferred to individual test seedlings of groundnut and allowed transmission feeding for 15 min. As stated before aphids were allowed pre-acquisition fasting for 2 hrs and acquisition feeding for 10 min. on various virus isolates of groundnut and 20 seedlings were exposed to each group.

iv. Effect of acquisition threshold on transmission of virus isolates of groundnut by *A. craccivora* and *M. persicae*

Aphids viz., *A. craccivora* and *M. persicae* were starved for 2 hrs before access feeding for varying periods viz., 1, 5, 10, 15, 30, 60 min. A minimum number of 10 aphids were liberated on each test plant and allowed to feed on it for 15 min. For each test 20 seedlings of groundnut (var. SB-XI) were exposed to the aphids.

v. Effect of inoculation threshold on transmission of virus isolates of groundnut by *A. craccivora* and *M. persicae*

After pre-acquisition fasting for 2 hrs, the aphids viz., *A. craccivora* and *M. persicae* were allowed for 10 min. acquisition feeding and then liberated on each test seedlings in groups of 10 aphids (*A. craccivora* and *M. persicae*) for infection feeding for different periods viz., 1, 5, 10, 15, 30, 60, 120, 180, 360 min. For each test, 20 groundnut seedlings were exposed to the aphids.

vi. Effect of pre and post acquisition starvation on transmission efficiency of virus isolates of groundnut by *A. craccivora* and *M. persicae*

Two separate tests were carried out to ascertain the effect of pre and post-acquisition starvation periods on transmission efficiency of the vector. Aphids viz., (*A. craccivora* and *M. persicae*) were starved for varying periods viz., 0, 1/2, 2, 3, 4, 5, 10, 15, 20 hrs and then allowed access feeding for 10 min. These were then released in groups of 10 aphids per test plant and allowed to feed on them for 15 min.

For post-acquisition starvation, fasting for 2 hrs and acquisition feeding for 10 min. were given. These aphids viz., *M. persicae* and *A. craccivora* were starved for different periods viz., 0, 1/2, 2, 3, 4, 5, 10, 15, 20 hrs before transferring them in groups of ten aphids per test seedlings for 15 min. For each test, 20 groundnut seedlings (var. SB-XI) were exposed to the aphids.

vii. Persistence of virus in *A. craccivora* and *M. persicae* test with virus isolates of groundnut.

Aphids viz., *A. craccivora* and *M. persicae* were starved for 2 hrs for various virus isolates of groundnut and allowed acquisition feeding for 10 min. Thereafter specific group of aphids of *A. craccivora* and *M. persicae* were transferred to series of 10 test seedlings at an intervals of 10 min. each.

3.2.7 Physical properties

The studies on physical properties viz., (i) Thermal inactivation point (TIP), (ii) Dilution end point (DEP) and (iii)

Longevity *in vitro* (LIV) of virus isolates of groundnut were carried out by using standard procedures (Noordam, 1973).

3.2.7.1 Thermal inactivation point

The virus infected leaves of various virus isolates of groundnut were taken.

Four ml sap of all these five isolates filled in ten thin test tubes separately and labelled with respective range of temperature. Each test tubes were individually exposed to temperature starting from 40, 45, 50, 55, 60, 65, 70, 75, 95 °C for 10 min. in hot water bath. Immediately after heating period, the test tubes were cooled in the ice cold water. The sap filled in the tenth test tube were kept untreated to serve as control.

The treated sap from the test tubes along with control were mechanically inoculated on the five leaves of *C. amaranticolor*. The inoculations were started from highest temperature i.e. 95°C to lowest temperature i.e. untreated to avoid contamination. All inoculated plants of virus isolates were maintained in an insect free glasshouse. Observations were recorded for number of local lesions appeared.

3.2.7.2 Dilution end point (DEP)

The crude sap of virus isolates of groundnut were extracted as per method described earlier. A series of dilutions viz., 1:10, 1:100, 1:1,000, 1:10,000, 1:1,00,000, 1:10,00,000, 1:1,00,00,000, 1:10,00,00,000, 1:1,00,00,00,000 were made from crude sap in distilled water.

From the crude sap, 1 ml sap was pipetted out and mixed in next test tube containing 9 ml distilled water. This

dilution was made 1:10 (10^{-1}). From this dilution, 1 ml of crude sap was pipetted out and mixed with the next test tube containing 9 ml of distilled water. This dilution was 1:100 (10^{-2}). In this way, other dilutions upto 10^{-9} were prepared. This was done for all virus isolates of groundnut.

For each dilution, separate pipette was used to avoid contaminations and to have accurate results.

The inoculation was started from highest dilution to lowest dilutions and lastly undiluted sap (control) to avoid contamination. Inoculation was done on five leaves of *C. amaranticolor*. The all inoculated plants of virus isolates of groundnut were maintained in an insect free glasshouse. Observations were recorded for number of local lesions appeared.

3.2.7.3 Longevity *in vitro*

To study the longevity *in vitro* of virus isolates of groundnut, crude sap of above virus isolates were extracted as described earlier.

A quantity of 4 ml sap were filled in test tubes of virus isolates of groundnut. The test tubes were plugged for various isolates and stored at room temperature (22-35°C) for further studies.

Immediately after extraction of the sap, inoculations were done mechanically on five leaves of *C. amaranticolor* that was served as control, whereas sap from other test tubes stored at room temperature were inoculated at an specific interval, i.e., 4, 8, 12, 16, 20, 24, 36, 48, 64, 72, 84 and 96 hrs on five leaves

of *C. amaranticolor* for each inocula. This was done for all virus isolates of groundnut.

For each inoculation, the sap from virus isolates of groundnut were used from separate test tubes filled at the same time.

The inoculated plants were labelled properly and maintained in an insect free glasshouse. Observations were recorded for number of local lesions appeared.

3.2.8 Electron microscopy

The leaf dip procedure was used for detecting virus particles associated with viral diseases of groundnut. Leaf dip preparations stained with uranyl acetate were examined under electron microscope (JEOL 100 C x II) at Advanced Centre for Plant Virology, Indian Agricultural Research Institute, New Delhi.

3.2.8.1 Preparation of grid carbon coating

Copper grids (3 mm diameter, 400 mesh) were cleaned with acetic acid and loaded on a filter paper placed at the bottom of a clean petridish with sterile distilled water. A clean slide was then coated with a thin film of carbon in a vacuum coating unit (BIORAD, E 640, Evaporation PSU) and gently brought in contact with water in the petridish containing the grids.

The carbon film was gently allowed to float off on the water. The filter paper was gently lifted along with the grids and lifted off with the floating carbon film. In the process, the grids were coated with the carbon film. The carbon coated grids were

air dried in the dark at room temperature for 12-24 hrs or in an oven at 37°C for 30 min.

3.2.8.2 Selection of tissue

Virus particles occur in varying concentrations in different parts of the plant. Usually young leaves contain high concentrations of virus particles. Diseased leaf bits were cut with the cork borer from infected young leaves of groundnut. The cut leaf bits contained veins as well as interveinal portions.

3.2.8.3 Preparation of virus extract from leaf tissues

Selected leafbits were macerated on a clean glass slide with a flat ended glass rods in 2-3 drops of phosphate buffer (0.07 M, pH 6.5) and allowed with finely homogenized material to settle for a few seconds. The clear supernatant was then taken with the help of fine glass capillary and used for mounting the grids.

3.2.8.4 Mounting

Freshly carbon coated grids were mounted with drops of homogenized leaf extract. After one minute, the extract from the grid surface was sucked off by touching filter paper on one side of the grids. The grids were then washed with 5-6 drops of distilled water before staining with 2-3 drops of uranyl acetate (aqueous 2 %, pH 4.2) which was used as the negative stain. Excess stain was removed and blotted dry by touching the edge of the grid with a strip of filter paper. The grid was dried for 1-2 min. and examined under E.M.

3.2.8.5 Grid examination for measurement of particles

Negatively stained grids were loaded on the grid holder, which was introduced into the EM and scanned. Electron micrographs were taken on cut film. Virus particles for their morphology and size were studied from negatives with good contrast. The photographs of virus particles were taken in transmission electron microscope with a magnification of $1 \times 1,20,000$ and $1 \times 1,35,000$. The electron micrographs were further magnified ten times and projected on a white paper, with the help of an enlarger on which the dimensions of the particles were measured. The particle size was calculated in 'nm' by using a formula.

$$\text{Size of virus particles (nm)} = \frac{\text{Length and breadth of particles in nm}}{\text{Magnification}} \times 10^6$$

3.2.9 Immunosorbent Electron Microscopy (ISEM)

Immunosorbent electron microscopy is an important diagnostic method of plant pathogens. In this procedure an EM support film is first coated with antibodies which can trap the virus particles from a solution containing them.

ISEM is used for main purposes, one is to trap comparatively large number of particles on the grid, with less amount of host material. The second purpose of ISEM is to detect individual viruses from a mixture of two or more viruses (by decoration). This test was also carried out at Advanced Centre for Plant Virology, I.A.R.I., New Delhi.

3.2.9.1 Decoration

First four step of the leaf dip preparation were repeated except that the washing at fourth step will be in PO_4 buffer. Again the grids were put over specific antibody (1'50 to 1'100) for 30 min. at room temperature. The grids were washed with double distilled water and stained with 2 per cent uranyl acetate and mounted as described earlier under leaf dip preparation.

Observations in respect of production of specific antibody halo around the virus particles was recorded.

3.2.9.2 Trapping

The carbon coated grids were flooded as described earlier over a drop of 10 μ l of antiserum diluted 1 : 1000 in phosphate buffer. The grids were left for half an hour at 37°C. The grids were washed for 10-15 min. in phosphate buffer. The grids were drained off briefly and then placed them over a drop of 10 μ l of extract containing virus and left them for 45 min. at room temperature.

The grids were removed and washed with approximately 20 drops of double distilled water and the grids are stained with 2-4 drops of 2% freshly, prepared uranyl acetate solution. The grids were drained off to dry and examined in electron microscope at 20,000 magnification. The number of particles were counted from ten different areas of the grid and compared with a grids prepared by leaf-dip preparation.

3.2.10 Direct Antigen Coating : Enzyme-Linked Immunosorbent Assay (DAC-ELISA)

The enzyme linked immunosorbent assay (ELISA), first introduced into plant virology about 23 years ago, is one of the most widely used serological tests for plant viruses detection, identification, quantification and comparison owing to its simplicity, sensitivity (detection limits of 1-10 ng/ml) and adaptability. The amount of virus present is proportional to the amount of enzyme labelled specific antibody forms the basis of the test.

3.2.10.1 Requirements

The polystyrene microtitre plates, antisera of peanut stripe virus and enzyme labelled antirabbit immunoglobulins available at Advanced Centre for Plant Virology, IARI, New Delhi was used for these studies. The virus infected samples showing most diagnostic symptoms of different virus isolates were maintained in insect proof glasshouse of Department of Plant Pathology and Agricultural Microbiology, MPKV., Rahuri carried to IARI, New Delhi under frozen condition was used for preparation of fine sap.

3.2.10.2 Solution

Following different buffers, solutions and antibody diluted buffer were prepared using different ingredients as given below

1. Stock buffer (Phosphate buffer saline 1 × PBS, pH 7.4)

NaCl	:	8.0 g
Na ₂ HPO ₄ ·2H ₂ O	:	1.44 g or

Na₂HPO₄·12H₂O : 2.90 g or
Na₂HPO₄ : 1.50 g
KH₂PO₄ : 0.20 g
KCl : 0.20 g
Distilled water : To make 1 litre

2. Wash buffers (PBS – Tween, PBS-T)

Add 0.5 ml Tween – 20 to 1 litre PBS.

3. Coating buffer (Carbonate buffer pH 9.6)

Na₂CO₃ : 1.59 g
NaHCO₃ : 2.93 g
Distilled H₂O : to make 1 litre

4. Enzyme conjugate diluent/buffer (PBS-T Polyvinyl-Pyrrolidone Ovalbumin, PBS – TPO)

Add 20 g Polyvinyl Pyrrolidone (PVP, MW 44,000) and 2.0 of egg ovalbumin to 1 litre PBS-T.

5. Antibody diluent/buffer

Same as PBS – TPO

6. Substrate buffer (diethanolamine buffer, pH 9.8)

Diethanolamine : 97 ml
Distilled water : 800 ml

Adjust the pH to 9.8 with 1 N HCl about 67 ml and make up the volume to 1 litre with distilled water.

7. Blocking solution

Add 5.0 g Bovine serum albumin (BSA/spray dried milk SDM) to 1 litre PBS-T.

3.2.10.3 Protocol

Two hundred fifty milligram of virus infected samples of virus isolates were weighed and made it in the form of fine powder with the help of liquid nitrogen. Finally a fine sap was prepared after addition of 5 ml coating buffer. 195 microlitre of sap of virus isolates was added to each well and the ELISA plate was incubated at 37 °C for 1 hr. After incubation, three washing was done for 3 min. each with PBS-T solution. 195 microlitre blocking solution was added to each well and then incubated at 37 °C for 1 hr. After incubation, three washings were done for 3 min. each with PBS-T solution. 195 microlitre crude antiserum diluted in PBS-TPO solution was added to each well and incubated at 37 °C for 2 hrs. After incubation, three washings were done for 3 min. each with PBS-T solution.

One hundred ninety five microlitre of enzyme labelled antirabbit IgG/ Secondary antibody diluted in PBS-TPO was added to each well and was incubated at overnight for 40°C. Later the plates were washed as described earlier. 195 microlitre freshly prepared substrate was added to each well and was incubated at room temperature for 1 hr. The ELISA plate was put on ELISA reader. The presence or absence of yellow colour to each well was recorded.

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EXPERIMENTAL RESULTS



4. EXPERIMENTAL RESULTS

The systematic studies on symptomatology, host range, physical properties, transmission, particle morphology and serological relationship were undertaken of isolates of groundnut collected from virus infected groundnut fields with a view to identify the viruses present in this region. The results obtained are presented here under.

4.1 Collection of virus isolates of groundnut

The groundnut samples showing different symptoms of stripe and green blotch were collected from Groundnut Improvement Project, C.C., Rahuri and Oilseed Research Station, Jalgaon. Incidence of the virus was found to be 60-90 per cent in the fields visited to collect the samples. Total 15 samples were collected in various periods and brought to the laboratory for isolation purpose.

4.1.1 Isolation and maintenance of virus isolates of groundnut

Virus isolates of groundnut were collected from Rahuri and Jalgaon regions and these isolates were maintained on glasshouse grown groundnut seedlings (var. SB-XI) by conventional leaf rub method, by using carborundum 800 mesh as an abrasive. The symptoms produced by these isolates are presented in Table 2.

Table 2. Symptoms produced by virus isolates of groundnut on test plant var. SB-XI

Sr. No.	Virus isolates	Location	Incubation period (days)	*Symptom patterns
1.	SB-XI	Rahuri	12-13	VC, MVC, SVC, ST, GB
2.	JL-24	Rahuri	15-16	VC, MVC, ST, GB
3.	Kopergaon-1	Jalgaon	14-15	VC, MVC, SVC, ST, GB
4.	Kopergaon-3	Jalgaon	14-15	VC, MVC, SVC, ST, GB
5.	Karad-4-11	Jalgaon	12	VC, ST, GB

*GB : Green blotches

ST : Striping

VC : Vein clearing

MVC : Mild vein clearing

SVC : Severe vein clearing

Incubation period was 15-16 days in Phule Pragati (JL-24) virus isolate, collected from Rahuri areas. While two virus isolates showed 14-15 days of incubation period (Kopergaon-1 and Kopergaon-3 from Jalgaon region). Minimum incubation period of 12 and 12-13 days recorded by Karad 4-11 and SB-XI virus isolates respectively indicating thereby highly susceptible nature of variety.

The appearance of symptoms on SB-XI after sap inoculation of virus isolates indicated that out of five groundnut isolates inoculated, JL-24 (collected from Rahuri) showed chlorotic spotting and vein clearing after 7 days of inoculation and discontinuous stripes along lateral veins of young leaflets appeared 15-16 days ^{after} inoculation.

Virus isolates Kopergaon-1, Kopergaon-3 and Karad 4-11 (collected from Jalgaon) expressed chlorotic spotting, vein clearing followed by discontinuous stripes along lateral veins of young leaflets and older leaflets showed green blotches and occasionally an oakleaf pattern was noticed.

4.2 Symptomatology

In present studies symptoms of groundnut virus isolates were studied in detail in naturally infected as well as artificially sap inoculated groundnut plants.

4.2.1 Symptoms of disease in naturally infected plants

Under field condition green blotches were exhibited on leaves. The youngest leaves showed vein clearing (VC). Along lateral veins of young leaflets, discontinuous stripes were appeared. The older leaflets showed green blotches (Plate - 1). The infected plants were found stunted (Plate - 2).

4.2.2 Symptoms of disease in artificially inoculated plants

Virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad-4-11) were collected and symptoms on the leaves were critically observed. The crude sap of each isolates were separately inoculated on test plant groundnut variety SB-XI. The symptoms exhibited by test plant were periodically observed upto two months as described here under.

4.2.2.1 Isolate SB-XI

The virus isolate SB-XI on groundnut leaves showed dark green stripes and green blotches along the lateral leaf veins (Plate 3). On inoculation to SB-XI, groundnut leaves showed



Plate – 1 Groundnut plantation showing vein clearing and green blotches on leaves under field condition



Plate – 2 Groundnut leaves showing stunt symptoms under field condition



Plate – 3 Groundnut isolate SB-XI showing stripes, dark green blotches along the lateral leaf veins under field condition



Plate – 4 Virus isolate of groundnut SB-XI showing the symptoms of vein clearings, stripes and dark green blotches on test plant (var. SB-XI) after 12-13 days of inoculation under glasshouse condition

chlorotic spotting and vein clearing 7 days of inoculation and discontinuous stripes and green blotches along lateral veins of young leaflets appeared in about 12-13 days of inoculation (Plate 4)

4.2.2.2 Isolate JL-24

The virus isolate JL-24 on groundnut leaves showed vein clearing. Discontinuous stripes were appeared along lateral veins of young leaflets and the green blotches were either randomly distributed over the entire leaflets or concentrated along the veins, sometimes they were surrounded by light green rings. The inoculated plants remained extremely stunted (Plate 5). On inoculation to SB-XI, the initial symptoms of vein clearing was noticed 10-11 days of inoculation, later appearance of stripes was observed followed by dark green blotches randomly distributed over the entire leaflet 15-16 days of inoculation (Plate 6).

4.2.2.3 Isolate Kopergaon-1

The virus isolate Kopergaon-1 on groundnut leaves showed stripes, which was characterized by dark green stripes, discontinuous banding along lateral veins of young leaves (Plate 7). On inoculation to SB-XI, the initial symptoms of mild vein clearing was noticed 11 days of inoculation, later these cleared veins coalesced each other giving appearance of stripes followed by green blotches 14-15 days of inoculation. The inoculated plants were slightly stunted (Plate 8).

4.2.2.4 Isolate Kopergaon-3

The virus isolate Kopergaon-3 on groundnut leaves showed discontinuous stripes and vein banding along the lateral veins. Older leaves developed mosaic in the form of green islands



Plate – 5 Groundnut isolate JL-24 showing the symptoms of vein clearings and dark green blotches under field condition



Plate- 6 Virus isolate of groundnut JL-24 showing the symptoms of vein clearings followed by stripes and the green blotches randomly distributed over the entire leaflets on test plant (var. SB-XI) after 15-16 days of inoculation under glasshouse condition



Plate – 7 Virus isolate of groundnut Kopergaon – 1 showing green blotches along the lateral veins of young leaves under field condition



Plate – 8 Virus isolate of groundnut Kopergaon – 1 showing the symptoms of vein clearings, stripes and green blotches on test plant (var. SB-XI) after 14-15 days of inoculation under glasshouse condition

(blotches). Early infected plants were stunted and symptoms persisted on older leaves (Plate – 9). On inoculation to SB-XI, the initial symptoms of vein clearing was noticed 11 days of inoculation, later appearance of stripes was observed followed by dark green blotches 14-15 days of inoculation (Plate – 10).

4.2.2.5 Isolate Karad-4-11

The virus isolate Karad-4-11 on groundnut leaves showed dark green stripes along the lateral leaf veins. The plants infected with blotch developed dark green circular areas on the leaves (Plate – 11). On inoculation to SB-XI, the initial symptoms of vein clearing was noticed 7 days of inoculation, later these cleared veins coalesced each other giving appearance of stripes followed by green blotches 12 days of inoculation. The inoculated plants were stunted (Plate – 12).

4.3 Host range studies

4.3.1 Isolate SB-XI

The results presented in Table 3 showed that virus isolate SB-XI could infect groundnut, soybean, clusterbean, cowpea, mungbean while bean, horsebean and peas did not show any symptoms nor the virus was recovered from them on back indexing onto SB-XI.

In groundnut (vars. SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad-4-11) this virus isolate exhibited vein clearing, later these cleared vein coalesced each other giving appearance of stripes followed by green blotches. The cluster bean, cowpea and mungbean showed mosaic mottle symptoms. Maximum infection of 90 per cent was noticed in groundnut cv.



Plate – 9 Virus isolate of groundnut Kopergaon – 3 showing discontinuous stripes symptoms under field condition



Plate – 10 Virus isolate of groundnut Kopergaon – 3 showing discontinuous stripes and dark green blotch symptoms on test plant (var. SB-XI) after 14-15 days of inoculation under glasshouse condition



Plate – 11 Virus isolate of groundnut Karad 4-11 showing the symptoms of green blotches which developed dark green circular areas on the leaves under field condition



Plate – 12 Virus isolate of groundnut Karad 4-11 showing vein clearings and stripes followed by irregular green blotches on test plant (var. SB-XI) after 12 days of inoculation under glasshouse condition

Table 3. Host range of SB-XI virus isolate

Sr. No.	Family	Symptom pattern*	Percentage of infection
	Leguminosae		
1.	Groundnut (<i>Arachis hypogaea</i> L.)		
a.	SB-XI	VC, MVC, SVC, ST, GB	85
b.	JL-24	VC, MVC, ST, GB	65
c.	Kopergaon-1	VC, MVC, SVC, ST, GB	80
d.	Kopergaon-3	VC, MVC, SVC, ST, GB	80
e.	Karad 4-11	VC, ST, GB	90
2.	Soybean (<i>Glycine max</i> (L.) Merr.)	VC, MM	85
3.	Cowpea (<i>Vigna unguiculata</i>)	MM	85
4.	Clusterbean/Guar (<i>Cyamopsis tetragonoloba</i>)	MM	75
5.	Mungbean (<i>Vigna radiata</i>)	MM	55
6.	Bean (<i>Phaseolus vulgaris</i>)	-	-
7.	Pea (<i>Pisum sativum</i>)	-	-
8.	Horsebean (<i>Vicia faba</i>)	-	-

*GB : Green blotches

ST : Striping

SVC : Severe vein clearing

MVC : Mild vein clearing

MM : Mosaic mottle

VC : Vein clearing

Karad 4-11, while 85 per cent infection was recorded in groundnut cv. SB-XI. The soybean and cowpea showed 85 per cent infection, while groundnut cvs. Kopergaon-1 and Kopergaon-3 showed 80 per cent infection. The cluster bean and mungbean showed varying percentage of infection.

4.3.2 Isolate JL-24

The results presented in Table 4 revealed that only groundnut, soybean and cowpea were the host of virus isolate JL-24 whereas clusterbean, mungbean, bean, pea and horsebean did not show any symptom nor the virus was recovered from them on back indexing onto SB-XI.

On groundnut, this virus isolate expressed primary symptoms of vein clearing, discontinuous stripes were appeared along lateral veins of young leaflets and the green blotches were either randomly distributed over the entire leaflets. The soybean showed vein clearing and mosaic mottle symptoms while cowpea showed the mosaic mottle symptoms.

The virus isolate JL-24 showed different percentage of infection. Maximum infection of 85 per cent was noticed in groundnut cv. Karad 4-11, while 80 per cent infection was observed in groundnut cv. SB-XI. The groundnut cvs. Kopergaon-1, Kopergaon-3 and soybean recorded 70 per cent of virus infection. The cowpea showed 75 per cent infection.

4.3.3 Isolates Kopergaon-1 and Kopergaon-3

Results presented in Table 5 indicated that both the virus isolates showed similar host range. These isolates could infect groundnut, soybean, clusterbean, cowpea and mungbean.

Table 4. Host range of JL-24 virus isolate

Sr. No.	Family	Symptom pattern*	Percentage of infection
	Leguminoceae		
1.	Groundnut (<i>Arachis hypogaea</i> L.)		
a.	SB-XI	VC, MVC, SVC, ST, GB	80
b.	JL-24	VC, MVC, ST, GB	55
c.	Kopergaon-1	VC, MVC, SVC, ST, GB	70
d.	Kopergaon-3	VC, MVC, SVC, ST, GB	70
e.	Karad 4-11	VC, ST, GB	85
2.	Soybean (<i>Glycine max</i> (L.) Merr.)	VC, MM	70
3.	Cowpea (<i>Vigna unguiculata</i>)	MM	75
4.	Clusterbean/Guar (<i>Cyamopsis tetragonoloba</i>)	-	-
5.	Mungbean (<i>Vigna radiata</i>)	-	-
6.	Bean (<i>Phaseolus vulgaris</i>)	-	-
7.	Pea (<i>Pisum sativum</i>)	-	-
8.	Horsebean (<i>Vicia faba</i>)	-	-

*GB : Green blotches

ST : Striping

SVC : Severe vein clearing

MVC : Mild vein clearing

MM : Mosaic mottle

VC : Vein clearing

Table 5. Host range of Kopergaon-1 and Kopergaon-3 virus isolates

Sr. No.	Family	Percentage of infection (Kopergaon-1)	Symptom pattern*	Percentage of infection (Kopergaon-3)	Symptom pattern*
	Leguminosae				
1.	Groundnut (<i>Arachis hypogaea</i> L.)				
a.	SB-XI	80	VC, MVC, SVC, ST, GB	85	VC, MVC, SVC, ST, GB
b.	JL-24	65	VC, MVC, ST, GB	65	VC, MVC, ST, GB
c.	Kopergaon-1	75	VC, MVC, SVC, ST	70	VC, MVC, SVC, ST
d.	Kopergaon-3	80	VC, MVC, SVC, ST	80	VC, MVC, SVC, ST
c.	Karad 4-11	85	VC, ST, GB	85	VC, ST, GB
2.	Soybean (<i>Glycine max</i> (L.) Merr.)	75	VC, MM	75	VC, MM
3.	Cowpea (<i>Vigna unguiculata</i>)	80	VC, MM	75	MM
4.	Clusterbean/Guar (<i>Cyamopsis tetragonoloba</i>)	75	VC, MM	65	VC, MM
5.	Mungbean (<i>Vigna radiata</i>)	60	VC, MM	70	VC, MM
6.	Bean (<i>Phaseolus vulgaris</i>)	-	-	-	-
7.	Pea (<i>Pisum sativum</i>)	-	-	-	-
8.	Horsebean (<i>Vicia faba</i>)	-	-	-	-

*GB : Green blotches

ST : Striping

SVC : Severe vein clearing

MVC : Mild vein clearing

MM : Mosaic mottle

VC : Vein clearing

Other hosts namely bean, horsebean and peas did not show any symptoms nor the virus isolates were recovered from them on back indexing onto SB-XI.

In groundnut (cv. SB-XI) virus isolates Kopergaon-1 and Kopergaon-3 expressed earliest symptoms of vein clearing within 11 days of inoculation. These vein cleared areas coalesced each other giving appearance of stripes followed by green blotches 14-15 days of inoculation.

In soybean, cowpea, clusterbean and mungbean, the virus isolates Kopergaon-1 and Kopergaon-3 showed primary symptoms of vein clearings within 6-7 days of inoculation. Two to three vein cleared areas were formed mosaic mottle symptoms.

The virus isolate Kopergaon-3 could infect groundnut var. SB-XI and Karad 4-11 at the maximum percentage of 85 per cent while three other varieties of groundnut (Kopergaon - 1, Kopergaon-3, Karad 4-11), soybean, cowpea, clusterbean, mungbean showed varying percentage of infection.

4.3.4 Isolate Karad 4-11

The results presented in Table 6 indicated that virus isolate Karad 4-11 showed early symptoms on groundnut (SB-XI) of vein clearing within 7 days of inoculation. This isolate also showed dark green stripes along the lateral leaf veins. The plants infected with blotch developed dark green circular areas on the leaves.

The virus isolate Karad 4-11 in soybean and cowpea showed vein clearing and mosaic mottle symptoms.

Table 6. Host range of Karad 4-11 virus isolate

Sr. No.	Family	Symptom pattern*	Percentage of infection
	Leguminoceae		
1.	Groundnut (<i>Arachis hypogaea</i> L.)		
a.	SB-XI	VC, MVC, SVC, ST, GB	80
b.	JL-24	VC, MVC, ST, GB	60
c.	Kopergaon-1	VC, MVC, SVC, ST, GB	75
d.	Kopergaon-3	VC, MVC, SVC, ST, GB	80
e.	Karad 4-11	VC, ST, GB	85
2.	Soybean (<i>Glycine max</i> (L.) Merr.)	VC, MM	60
3.	Cowpea (<i>Vigna unguiculata</i>)	VC, MM	70
4.	Clusterbean/Guar (<i>Cyamopsis tetragonoloba</i>)	-	-
5.	Mungbean (<i>Vigna radiata</i>)	-	-
6.	Bean (<i>Phaseolus vulgaris</i>)	-	-
7.	Pea (<i>Pisum sativum</i>)	-	-
8.	Horsebean (<i>Vicia faba</i>)	-	-

*GB : Green blotches

ST : Striping

SVC : Severe vein clearing

MVC : Mild vein clearing

MM : Mosaic mottle

VC : Vein clearing

Table 7. Host range of virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11)

Sr. No.	Family	Reactions*
1.	Chenopodiaceae	
a.	<i>Chenopodium amaranticolor</i>	CLL, NLL
b.	<i>C. murale</i>	-
c.	<i>C. quinoa</i>	CLL, NLL
2.	Solanaceae	
a.	<i>Nicotiana tabacum</i> L.	-
b.	<i>N. glutinosa</i>	-
c.	<i>N. benthamiana</i>	CLL, NLL
d.	<i>Capsicum annum</i> L. (Chilli)	-
e.	<i>Lycopersicon esculentum</i> (Tomato)	-
f.	<i>Datura stramonium</i> (Datura)	-
3.	Cucurbitaceae	
a.	<i>Luffa cylindrica</i> Roem. (Sponge gourd)	-
b.	<i>Cucumis sativus</i> L. (Cucumber)	-
c.	<i>Lagenaria siceraria</i> (Molina) Standl. (Bottle gourd)	-
d.	<i>Momordica charantia</i> L. (Bitter gourd)	-
4.	Malvaceae	
a.	<i>Abelmoschus esculentus</i> (Okra)	-
5.	Amaranthaceae	
a.	<i>Gomphrena globosa</i> L. (Bachelor's button)	-
6.	Pedalaceae	
a.	<i>Sesamum indicum</i> L. (Sesamum)	-
7.	Compositae	
a.	<i>Helianthus annuus</i> (Sunflower)	-
b.	<i>Carthamus tinctorious</i> (Safflower)	-

*CLL = Chlorotic local lesions NLL – Necrotic Local lesions

The virus isolate Karad 4-11 showed different percentage of infection in host plants. Maximum infection was noticed in groundnut variety Karad 4-11, while 80 per cent infection was observed in groundnut cv. Kopergaon-3 and SB-XI. The groundnut cv. Kopergaon-1 recorded 75 per cent of virus infection. The groundnut cv. Karad 4-11 and cowpea infected 85 and 70 per cent respectively, while the groundnut cv. JL-24 and soybean infected 60 per cent each.

The results of different plant species belonging to seven families, mechanically sap inoculated with various virus isolates of groundnut presented in Table 7 indicated that all the plants species in chenopodiaceae family except *Chenopodium murale* showed chlorotic local lesions (CLL) in the range of 4-7 days and necrotic local lesions (NLL) 7-10 days after inoculation (Plate-13 to Plate-18). While the *Nicotiana benthamiana* in solanaceae family showed CLL, NLL after 5-6 and 8-9 days of inoculation respectively (Plate – 19).

The remaining plant species in families viz., Solanaceae, Cucurbitaceae, Malvaceae, Pedalaceae, Compositae and Amaranthaceae neither showed any symptom reaction nor the virus was recovered from them by back indexing on *C. amaranticolor*.

4.4 Transmission of virus isolates of groundnut

4.4.1 Transmission by mechanical sap inoculation

Infectivity of inocula prepared from extracts of different parts of diseased groundnut plants were carried out to recover the virus from different parts of diseased plants.

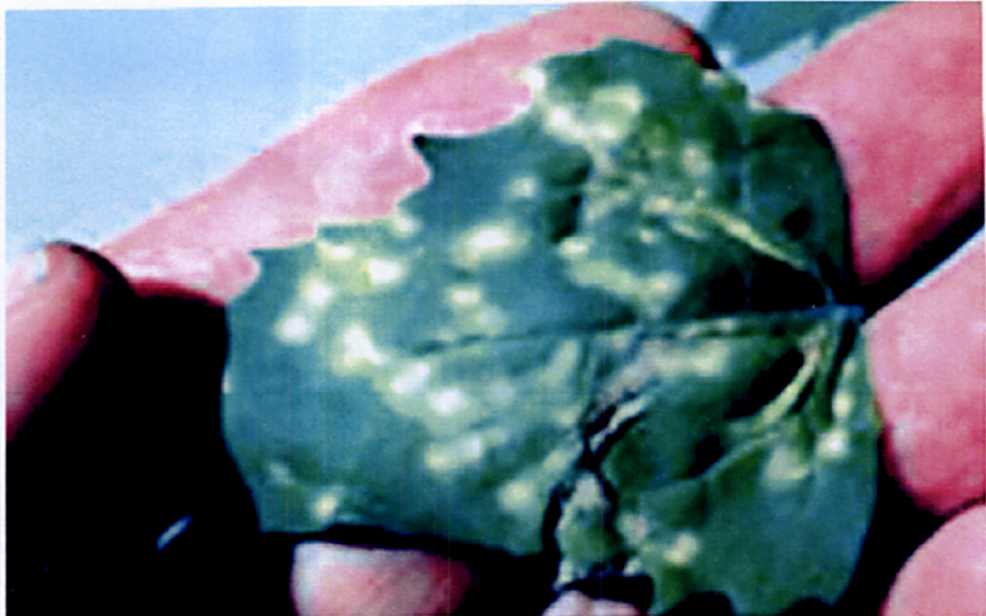


Plate -13 Virus isolate of groundnut SB-XI showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on *Chenopodium amaranticolor* appeared after 4-6 and 7-8 days of inoculation respectively



Plate -14 Virus isolate of groundnut JL-24 showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on *Chenopodium amaranticolor* appeared after 6-7 and 9-10 days of inoculation respectively



Plate -15 Virus isolate of groundnut Kopergaon - 1 showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on *Chenopodium amaranticolor* appeared after 5-7 and 8-9 days of inoculation respectively



Plate -16 Virus isolate of groundnut Kopergaon - 3 showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on *Chenopodium amaranticolor* appeared after 5-6 and 8-9 days of inoculation, respectively

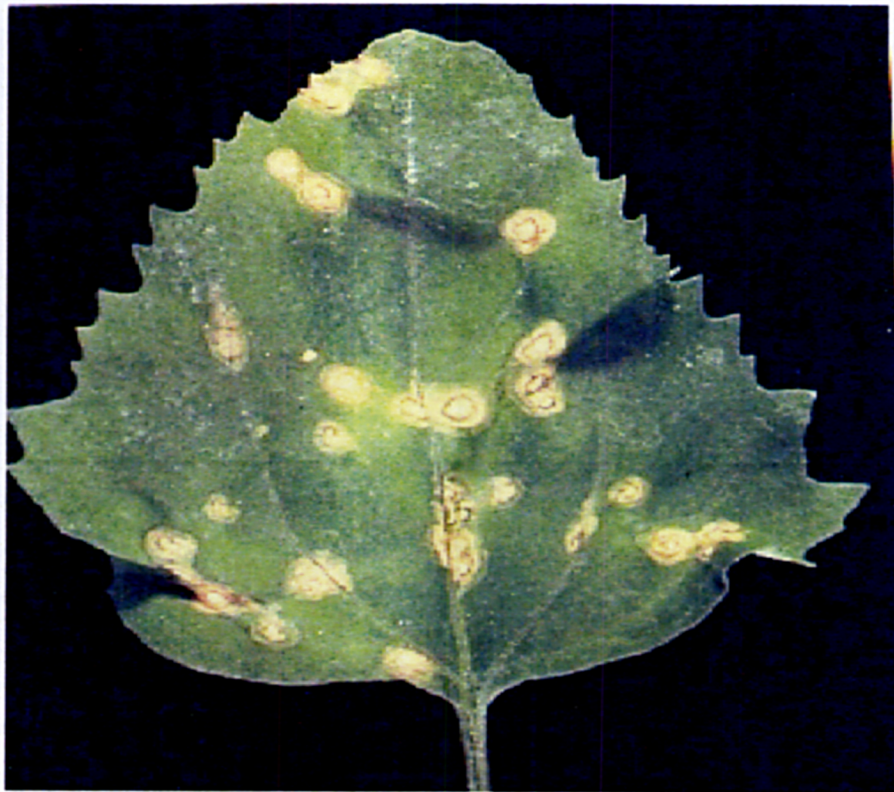


Plate -17 Virus isolate of groundnut Karad 4 -11 showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on *Chenopodium amaranticolor* appeared after 4-5 and 7-8 days of inoculation, respectively

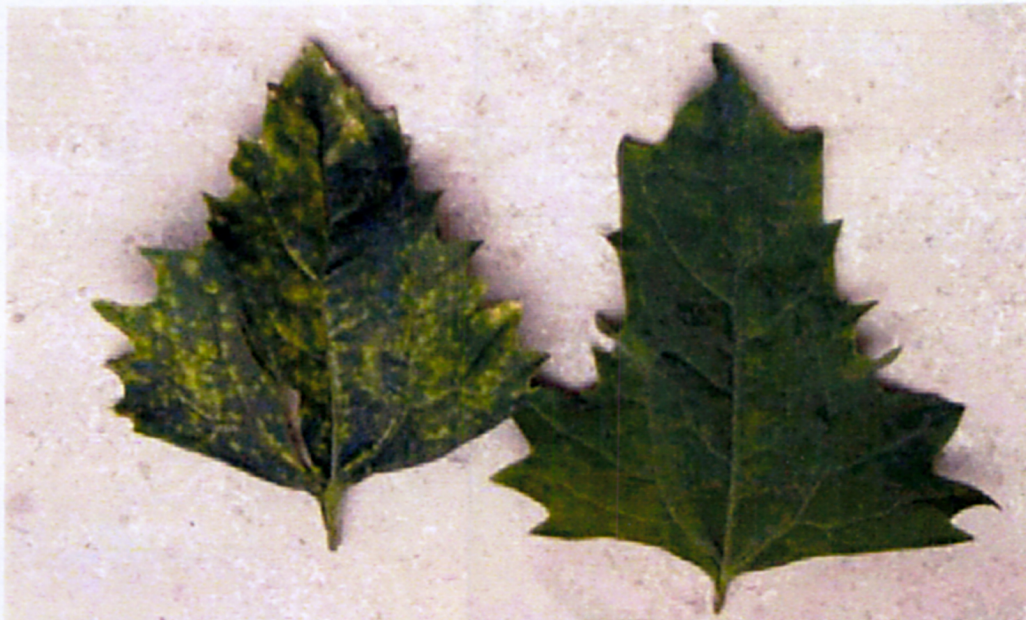


Plate -18 Virus isolates of groundnut viz., SB-XI, JL-24, Kopergaon - 1 , Kopergaon - 3 and Karad 4 -11 showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on *Chenopodium quinoa* appeared after 4-5 and 7-8 days of inoculation respectively

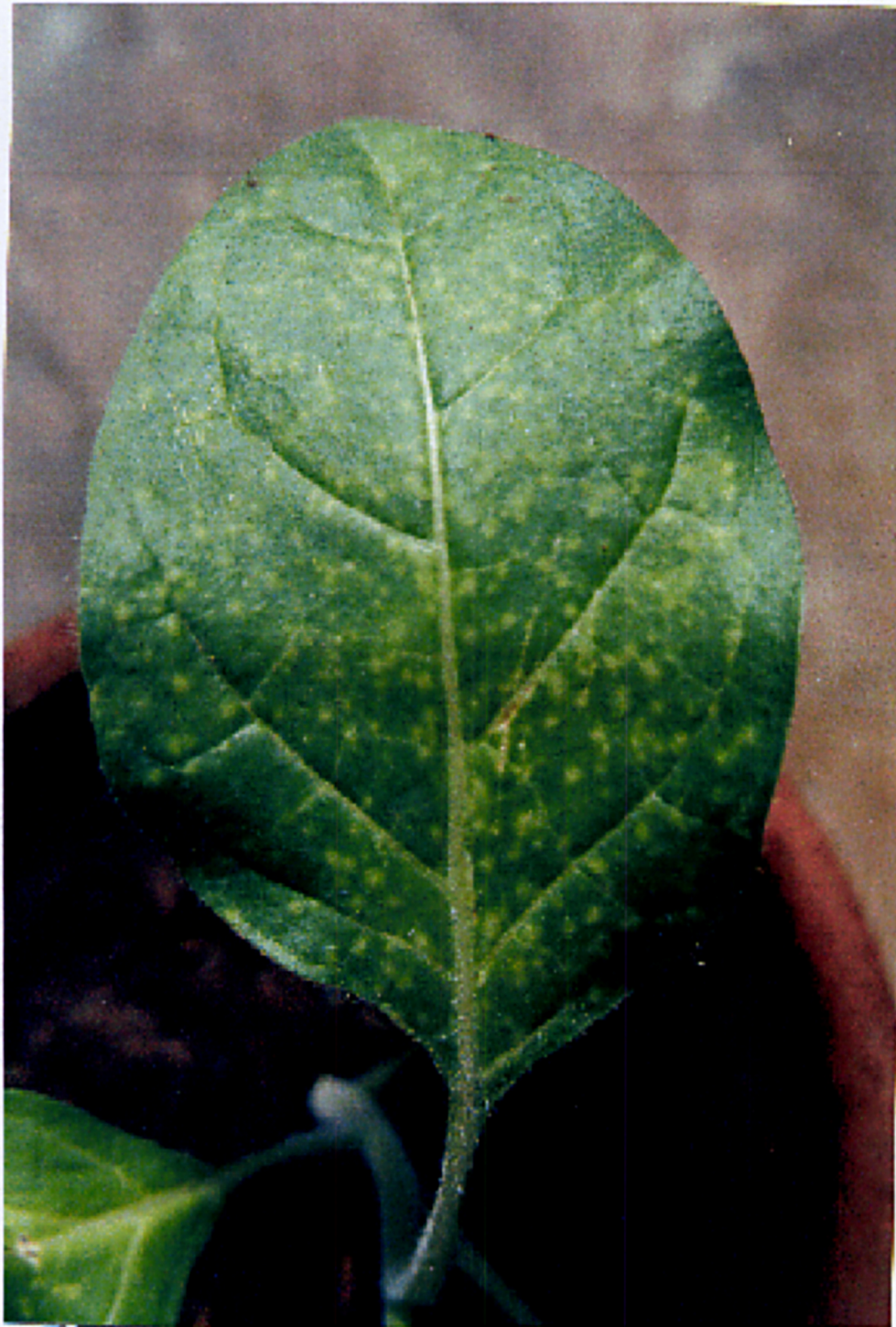


Plate -19 Virus isolates of groundnut viz., SB-XI, JL-24, Kopergaon - 1 , Kopergaon - 3 and Karad 4 -11 showing chlorotic local lesions (CLL) and necrotic local lesions (NLL) on leaves of *Nicotiana benthamiana* appeared after 5-6 and 8-9 days of inoculation, respectively

The data given in Table 8 showed that the extracts prepared from the leaves of groundnut proved to be most infectious containing the highest concentrations of the virus as compared to the extracts obtained from other plants parts. Subsequently inoculum was prepared only from the leaves of groundnut plants.

Table 8. Determination of virus titre in leaf, stem and root tissues of infected SB-XI groundnut plants by mechanical sap inoculation on leaves of *Chenopodium amaranticolor*

Sr. No.	Extract from	Number of Local Lesions on <i>C. amaranticolor</i> *
1.	Infected	
a.	Leaves	48.8
b.	Stems	22.0
c.	Roots	00
2.	Healthy	
a.	Leaves	00
b.	Stems	00
c.	Roots	00

* Average of five leaves

4.4.2 Transmission through seeds

In order to determine the possibility of transmission of virus through seeds, five hundred seeds of five varieties of groundnut viz., SB-XI, JL-24, Koperagaon-1, Koperagaon-3 and Karad 4-11 were collected from infected plants of groundnut.

The germination of seeds was varied and 433, 482, 450, 475, 365 seeds were germinated respectively but none of the

seedlings showed the symptoms and no virus was recovered in composite sample used for indexing on local lesion host. This indicated that virus isolates were not transmitted through seeds collected from diseased plants (Table 9).

Table 9. Seed transmission of virus isolates of groundnut

Sr. No.	Virus isolates	Number of seeds sown	Number of seed germinated	Percentage seed germinated	Number of plants showing symptoms	Percentage seed transmission
1.	SB-XI	500	433	86.60	0	0
2.	JL-24	500	482	96.40	0	0
3.	Kopergaon-1	500	450	90.00	0	0
4.	Kopergaon-3	500	475	95.00	0	0
5.	Karad-4-11	500	365	73.00	0	0

4.4.3 Transmission through insects

In present studies tests on transmission of virus isolate SB-XI of groundnut were carried out using four different species of aphids and whitefly and the virus vector relationship was determined. The results were as under.

i. Comparative efficiency of different insects to transmit virus isolate SB-XI

The results given in Table 10 showed that *Aphis craccivora* and *Myzus persicae* transmitted virus isolate SB-XI while it was not transmitted by *Rhopalosiphum maidis*, *Aphis gossypii* and whitefly (*Bemisia tabaci*). Out of four aphid species and whitefly tested, *Myzus persicae* proved to be the most efficient vector which transmitted virus isolate SB-XI to the greater extent (75 %).

Table 10. The efficiency of different insects to transmit SB-XI virus isolate

Sr. No.	Insect species	Number of plants infected/ exposed	Percentage of infection
1.	<i>Myzus persicae</i>	15/20	75
2.	<i>Aphis craccivora</i>	14/20	70
3.	<i>Aphis gossypii</i>	0/20	00
4.	<i>Rhopalosiphum maidis</i>	0/20	00
5.	<i>Bemisia tabaci</i>	0/20	00

ii. Aphid transmission efficiency of virus isolates of groundnut

The results on studies of aphid transmission efficiency of virus isolates of groundnut to cv. SB-XI are presented in Table 11 and indicated that two species of aphid vectors viz., *Myzus persicae* and *Aphis craccivora* transmitted the virus isolates of groundnut to groundnut particularly on test plant (cv. SB-XI) whereas highest transmission rate of SB-XI virus isolate by *Myzus persicae* was 75 per cent in cv. SB-XI and Karad 4-11 while by *Aphis craccivora* was 70 per cent in cv. SB-XI and Karad 4-11. The transmission of virus by *Myzus persicae* was 65 per cent in Kopergaon-1 isolate and 70, 60 per cent in Kopergaon-3 and JL-24 isolates, respectively. The transmission of virus by *Aphis craccivora* was 55 per cent in JL-24 while 60, 65 per cent in Kopergaon-1 and Kopergaon-3 isolates, respectively.

Table 11. Efficiency of insects (Aphids) to transmit virus isolates of groundnut to groundnut (cv. SB-XI)

Sr. No.	Virus isolates	<i>Myzus persicae</i>	Per cent transmission	<i>Aphis craccivora</i>	Per cent transmission
1.	SB-XI	15/20	75	14/20	70
2.	JL-24	12/20	60	11/20	55
3.	Kopergaon-1	13/20	65	12/20	60
4.	Kopergaon-3	14/20	70	13/20	65
5.	Karad-4-11	15/20	75	14/20	70

However, they differed in their efficiency to transmit the virus from peanut to soybean plant (Table 12). *Myzus persicae* was found to be comparatively efficient vector since 20 per cent transmission was obtained in virus isolate SB-XI. While the 15 per cent transmission was obtained in groundnut isolates viz., JL-24, Kopregaoon-1, Kopergaon-3 and 10 per cent in Karad-4-11 isolate. The transmission percentage by *A. craccivora* was 5 per cent in all virus isolates of groundnut.

Table 12. Efficiency of insects (Aphids) to transmit virus isolates of groundnut to soybean

Sr. No.	Virus isolates	<i>Myzus persicae</i>	Per cent transmission	<i>Aphis craccivora</i>	Per cent transmission
1.	SB-XI	4/20	20	1/20	5
2.	JL-24	3/20	15	1/20	5
3.	Kopergaon-1	3/20	15	1/20	5
4.	Kopergaon-3	3/20	15	1/20	5
5.	Karad-4-11	2/20	10	1/20	5

iii. Number of aphids required to transmit virus isolates of groundnut by *M. persicae* and *A. craccivora*

The results given in Table 13 a and 13 b indicated that even a single viruliferous adult female of *M. persicae* and *A. craccivora* was able to acquire and transmit the virus of various isolates of groundnut. Percentage of transmission was increased with the increase in number of aphids per test plant. There was decrease in transmission percentage when 15, 20, 25 and 30 aphids were released on each test plant, the reason for this decrease in transmission percentage was not understood.

iv. Effect of acquisition threshold on transmission of virus isolates of groundnut by *M. persicae* and *A. craccivora*

The results given in Table 14a and 14b showed that *M. persicae* and *A. craccivora* could acquire the virus of various isolates of groundnut after access feeding for one min.

The maximum transmission was obtained after an access feeding for 10 min in various virus isolates of groundnut. Access feeding beyond 10 min reduced transmission efficiency of the aphid.

v. Effect of inoculation threshold on transmission of virus isolates of groundnut by *M. persicae* and *A. craccivora*

The results given in Table 15a and 15b indicated that *M. persicae* and *A. craccivora* could transmit the virus of various isolates of groundnut in a minimum feeding period of 1 min but maximum transmission was obtained after infection feeding for 15 min. in various virus isolates of groundnut.

Table 13a. Per cent virus transmission of various isolates of groundnut produced by varying number of *M. persicae*

Number of aphids per test plant (<i>M. persicae</i>)	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
1	25	15	20	20	30
5	35	25	30	30	40
10	70	60	65	70	75
15	65	55	60	60	70
20	60	50	50	50	60
25	50	45	50	40	55
30	40	35	35	40	45

Table 13b. Per cent virus transmission of various isolates of groundnut produced by varying number of *A. craccivora*

Number of aphids per test plant (<i>A. craccivora</i>)	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
1	20	10	20	20	20
5	30	20	25	25	30
10	65	60	60	65	70
15	60	50	55	55	60
20	50	45	45	45	55
25	45	40	40	40	50
30	40	30	30	30	40

Table 14a. Effect of access feeding period on transmission of virus isolates of groundnut by *M. persicae*

Access feeding period (min) <i>M. persicae</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
0.5	0	0	0	0	0
1	35	30	30	30	35
5	55	50	50	50	55
10	75	65	70	70	75
15	60	55	55	60	65
30	50	40	45	45	50
60	15	10	15	15	20

Table 14b. Effect of access feeding period on transmission of virus isolates of groundnut by *A. craccivora*

Access feeding period (min) <i>A. craccivora</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
0.5	0	0	0	0	0
1	20	10	15	15	25
5	55	45	50	50	55
10	70	60	65	70	70
15	50	40	45	45	50
30	20	15	25	25	30
60	15	10	10	15	20

Table 15a. Effect of various infection feeding period on transmission of virus isolates of groundnut by *M. persicae*

Infection feeding period (min) <i>M. persicae</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
1	15	5	10	10	15
5	15	10	20	25	30
10	25	20	30	35	40
15	70	65	70	70	75
30	50	45	50	55	50
60	45	40	45	45	40
120	35	30	40	40	35
180	20	10	15	15	25
360	0	0	0	0	0

Table 15b. Effect of various infection feeding period on transmission of virus isolates of groundnut by *A. craccivora*

Infection feeding period (min) <i>A. craccivora</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
1	15	5	10	10	10
5	10	10	15	15	20
10	20	20	25	30	30
15	70	60	60	65	75
30	50	45	45	50	55
60	45	35	40	40	40
120	30	30	35	40	30
180	15	5	15	10	20
360	0	0	0	0	0

vi. Effect of pre and post acquisition starvation on transmission efficiency of virus isolates of groundnut by *M. persicae* and *A. craccivora*

The results given in Table 16a and 16b showed that when *M. persicae* and *A. craccivora* were not starved, transmitted the virus to a very low percentage of plants with virus isolates of groundnut but maximum transmission was obtained when they were starved for 2 hrs before access feeding.

On the contrary, when the aphids were not starved after access feeding and before inoculation feeding they transmitted the virus to the maximum percentage, whereas the results presented in Table 16c and 16d indicated that post acquisition fasting brought about loss in transmission ability of aphids. The longer the fasting period, the greater was the loss in infectivity.

vii. Persistence of virus in *M. persicae* and *A. craccivora* tested with virus isolates of groundnut.

The results given in Table 17a and b revealed that the *M. persicae* and *A. craccivora* failed to infect a test plant of virus isolates of groundnut after a period of 60 min in a serial successive feeding showing thereby that *M. persicae* and *A. craccivora* did not retain the virus beyond 60 min and there was no latent period. These results clearly indicated that both the aphid vectors transmitted the virus isolates in non-persistent manner.

Table 16a. Effect of pre-acquisition starvation on the efficiency of vector to transmit virus isolates of groundnut by *M. persicae*

Pre-acquisition starvation (hr) <i>M. persicae</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
0	10	5	10	10	10
½	30	15	20	25	30
2	70	65	70	70	75
3	60	65	65	60	60
4	45	55	50	55	50
5	45	55	50	55	50
10	40	35	45	50	45
15	20	25	30	25	20
20	00	00	00	00	00

Table 16b. Effect of pre-acquisition starvation on the efficiency of vector to transmit virus isolates of groundnut by *A. craccivora*

Pre-acquisition starvation (hr) <i>A. craccivora</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
0	10	5	5	10	10
½	25	10	15	20	30
2	70	60	65	65	75
3	65	60	60	60	60
4	50	55	45	55	45
5	40	35	40	45	45
10	30	30	25	25	30
15	10	20	15	20	10
20	00	00	00	00	00

Table 16c. Effect of post acquisition starvation on the efficiency of vector to transmit virus isolates of groundnut by *M. persicae*

Post-acquisition starvation (hr) <i>M. persicae</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
0	70	60	65	65	75
½	40	30	30	35	45
2	15	5	10	10	20
3	00	00	00	00	00
4	00	00	00	00	00
5	00	00	00	00	00
10	00	00	00	00	00
15	00	00	00	00	00
20	00	00	00	00	00

Table 16d. Effect of post acquisition starvation on the efficiency of vector to transmit virus isolates of groundnut by *A. craccivora*

Post-acquisition starvation (hr) <i>A. craccivora</i>	Virus isolates of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
0	65	55	55	60	70
½	30	20	25	25	35
2	10	5	5	10	10
3	00	00	00	00	00
4	00	00	00	00	00
5	00	00	00	00	00
10	00	00	00	00	00
15	00	00	00	00	00
20	00	00	00	00	00

Table 17a. Infection obtained in serial transfer at intervals of 10 min. in *M. persicae* of virus isolates of groundnut

Virus isolates	Aphid number	Test plant infected by <i>M. persicae</i> (min.)										Total number of plants infected	
		1	2	3	4	5	6	7	8	9	10		
SB-XI	1	+	+	-	-	-	-	-	-	-	-	-	2
	2	+	-	-	+	-	-	-	-	-	-	-	2
	3	-	+	-	+	+	-	-	-	-	-	-	3
	5	+	-	+	+	-	-	-	-	-	-	-	3
	7	-	+	+	-	+	+	-	-	-	-	-	4
	10	+	+	-	+	+	+	-	-	-	-	-	5
JL-24	1	+	-	+	-	-	-	-	-	-	-	-	2
	2	+	+	-	-	-	-	-	-	-	-	-	2
	3	+	+	-	+	-	-	-	-	-	-	-	3
	5	+	-	+	+	-	-	-	-	-	-	-	3
	7	+	+	-	-	+	-	-	-	-	-	-	3
	10	+	+	+	-	-	+	-	-	-	-	-	4
Kopergaon-1	1	+	-	+	-	-	-	-	-	-	-	-	2
	2	+	+	-	-	-	-	-	-	-	-	-	2
	3	+	-	-	+	-	-	-	-	-	-	-	2
	5	-	+	+	-	+	-	-	-	-	-	-	3
	7	+	-	+	+	+	-	-	-	-	-	-	4
	10	+	+	-	+	+	+	-	-	-	-	-	5
Kopergaon-3	1	-	+	-	+	-	-	-	-	-	-	-	2
	2	-	+	+	-	-	-	-	-	-	-	-	2
	3	+	-	+	+	-	-	-	-	-	-	-	3
	5	+	-	-	+	+	-	-	-	-	-	-	3
	7	+	+	-	+	+	+	-	-	-	-	-	5
	10	+	-	+	+	+	+	-	-	-	-	-	5
Karad 4-11	1	+	+	-	-	-	-	-	-	-	-	-	2
	2	+	-	-	+	-	-	-	-	-	-	-	2
	3	+	+	+	-	-	-	-	-	-	-	-	3
	5	+	+	-	+	+	-	-	-	-	-	-	4
	7	+	-	+	-	+	+	-	-	-	-	-	4
	10	+	-	+	+	+	+	-	-	-	-	-	5

Table 17b. Infection obtained in serial transfer at intervals of 10 min. in *A. craccivora* of virus isolates of groundnut

Virus isolates	Aphid number	Test plant infected by <i>A. craccivora</i> (min.)										Total number of plants infected
		1	2	3	4	5	6	7	8	9	10	
SB-XI	1	+	+	-	-	-	-	-	-	-	-	2
	2	-	+	+	-	-	-	-	-	-	-	2
	3	+	-	+	-	-	-	-	-	-	-	2
	5	-	+	+	+	+	-	-	-	-	-	4
	7	+	-	+	-	+	+	-	-	-	-	4
	10	+	+	-	+	+	+	-	-	-	-	5
JL-24	1	-	+	-	+	-	-	-	-	-	-	2
	2	+	+	-	-	-	-	-	-	-	-	2
	3	+	-	+	-	-	-	-	-	-	-	2
	5	+	+	-	-	-	-	-	-	-	-	2
	7	+	-	-	+	+	-	-	-	-	-	3
	10	+	-	-	+	-	+	-	-	-	-	3
Kopergaon-1	1	+	+	-	-	-	-	-	-	-	-	2
	2	+	-	+	-	-	-	-	-	-	-	2
	3	+	+	-	-	-	-	-	-	-	-	2
	5	+	-	+	+	-	-	-	-	-	-	3
	7	+	+	+	+	-	-	-	-	-	-	4
	10	+	-	+	+	-	+	-	-	-	-	4
Kopergaon-3	1	+	+	-	-	-	-	-	-	-	-	2
	2	-	+	+	-	-	-	-	-	-	-	2
	3	+	-	-	+	-	-	-	-	-	-	2
	5	+	+	-	-	+	-	-	-	-	-	3
	7	+	+	+	-	-	+	-	-	-	-	4
	10	+	-	-	+	+	+	-	-	-	-	4
Karad 4-11	1	-	+	+	-	-	-	-	-	-	-	2
	2	+	-	+	-	-	-	-	-	-	-	2
	3	+	+	-	+	-	-	-	-	-	-	3
	5	+	-	+	+	+	-	-	-	-	-	4
	7	+	+	-	+	+	+	-	-	-	-	5
	10	+	+	-	+	+	+	-	-	-	-	5

4.5 Physical properties of virus isolates of groundnut.

The experiments were carried out to determine the thermal inactivation point, dilution end point, longevity *in vitro* of virus isolates of groundnut.

4.5.1 Thermal inactivation point

The results presented in Table 18 indicated that the virus isolates SB-XI, Kopergaon-1, Kopergaon-3 and Karad 4-11 remained infective after heating for 65°C for 10 min but not at 70°C and JL-24 isolate remained infective at 60°C but lost the infectivity at 65°C.

Table 18. Thermal inactivation point of virus isolates of groundnut

Treatment (°C)	Number of local lesions on <i>C. amaranticolor</i> *				
	SB-XI isolate	JL-24 isolate	Kopergaon-1 isolate	Kopergaon-3 isolate	Karad 4-11 isolate
Control	49.4	44.2	46.8	47.2	49.6
40	48.6	43.6	45.4	46.6	48.8
45	45.4	40.4	41.2	42.4	45.8
50	37.2	30.2	33.4	35.2	37.6
55	27.8	21.6	22.6	25.4	28.0
60	16.8	11.4	13.8	14.2	17.2
65	2.6	0	2.2	2.4	2.8
70	0	0	0	0	0
75	0	0	0	0	0
95	0	0	0	0	0

*Average of 5 leaves

4.5.2 Dilution end point

The results presented in Table 19 revealed that dilution end point were in between 10^{-4} to 10^{-5} for SB-XI, Kopergaon-1, Kopergaon-3 and Karad 4-11 and 10^{-3} to 10^{-4} for virus isolate JL-24.

Table 19. Dilution end point of virus isolates of groundnut

Treatment	Number of local lesions on <i>C. amaranticolor</i> *				
	SB-XI isolate	JL-24 isolate	Kopergaon-1 isolate	Kopergaon-3 isolate	Karad 4-11 isolate
Control (Undiluted)	49.4	44.2	46.8	47.2	49.6
10^{-1}	42.2	40.0	41.2	41.8	43.0
10^{-2}	37.4	31.4	33.2	34.4	38.2
10^{-3}	10.2	7.4	8.0	8.8	10.6
10^{-4}	2.6	0	1.8	2.2	2.8
10^{-5}	0	0	0	0	0
10^{-6}	0	0	0	0	0
10^{-7}	0	0	0	0	0

*Average of 5 leaves

4.5.3 Longevity *in vitro*

The results of storing the five virus isolates of groundnut at room temperature presented in Table 20 showed that SB-XI, Kopergaon-1, Kopergaon-3 and Karad-4-11 remained infective for 72 hrs but not at 84 hrs. While the virus isolate JL-24 remained infective for 64 hrs but lost the infectivity at 72 hrs.

Table 20. Longevity *in vitro* of virus isolates of groundnut

Treatment (hrs)	Number of local lesions on <i>C. amaranticolor</i> *				
	SB-XI isolate	JL-24 isolate	Kopergaon-1 isolate	Kopergaon-3 isolate	Karad 4-11 isolate
Control	49.4	44.2	46.8	47.2	49.6
4	48.6	43.4	45.6	46.6	48.8
8	44.4	40.2	42.4	43.2	44.8
12	39.2	34.2	38.2	38.6	39.6
16	36.2	30.4	34.8	35.2	37.4
20	30.8	26.6	28.2	29.4	31.6
24	25.8	21.8	23.4	24.6	26.2
36	21.6	17.6	19.6	20.8	22.2
48	16.8	12.2	14.4	15.2	17.0
64	11.6	8.4	9.6	10.4	11.8
72	2.4	0	2.0	2.2	2.6
84	0	0	0	0	0
96	0	0	0	0	0

* Average of 5 leaves

4.6 Particle morphology

Glasshouse infected samples of various virus isolates of groundnut were examined under electron microscopy (JEOL 100 C x II) by leaf dip method at the Advanced Centre for Plant Virology, I.A.R.I., New Delhi. In leaf dip preparations only few particles were visible having flexuous rods of all five virus isolates. The virus particles were found to be of two types based on length and breadth (Table 21). The virus particles of five isolates viz., SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad-4-11 were found in the range of 722-765 nm (743 ± 21

nm) in length (Plate 20) and 13-17 nm (15 ± 2 nm) in breadth. This clearly indicated that all the virus isolates under study belongs to potyvirus.

Table 21. Particle morphology of virus isolates of groundnut

Sr. No.	Virus isolates	Particle morphology*	
		Length (nm)	Breadth (nm)
1.	SB-XI	740-790	16-22
2.	JL-24	700-740	10-14
3.	Kopergaon-1	710-750	12-15
4.	Kopergaon-3	700-745	11-15
5.	Karad 4-11	760-800	14-18

* Average of 100 virus particles

4.7 Immunosorbent Electron Microscopy (ISEM)

This test was done at Advanced Centre for Plant Virology, I.A.R.I., New Delhi for trapping comparatively large number of particles on the grid with less amount of host material and also detecting individual viruses from a mixture of two or more viruses (by decoration) using antiserum of peanut stripe virus (PStV). In leaf dip preparation only few particles were visible as against several particles in trapping (Plate 21). From the particle morphological studies of leaf dip preparations of all virus isolates undertaken earlier, it was clearly evident that virus isolates were noticed based on length and breadth of virus particles.

A representative composite sample from these isolates were used for immuno-sorbent electron microscopy.

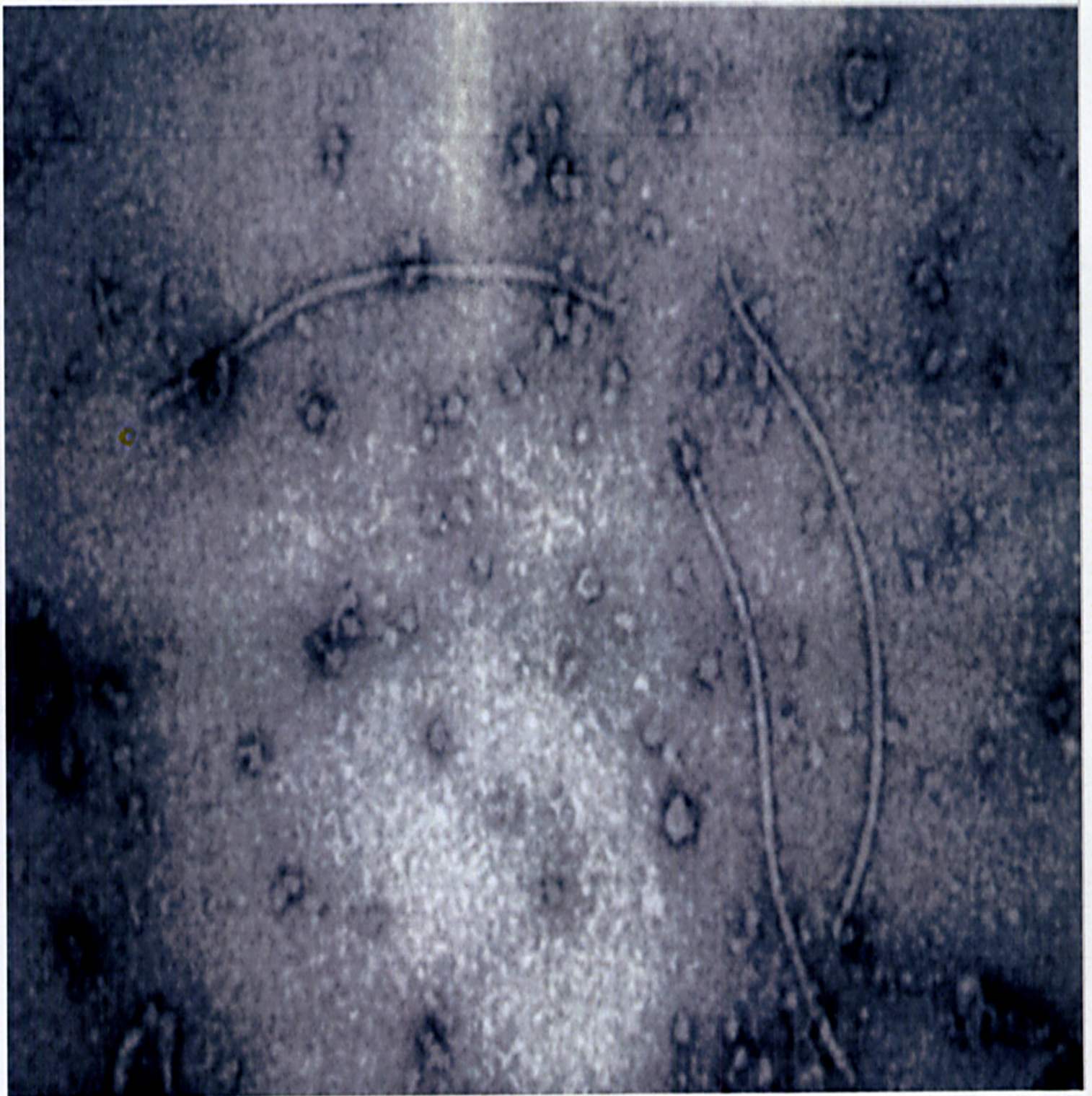


Plate - 20 Electron micrograph showing flexuous rod shaped particles of virus measuring $722 - 765 \text{ nm}$ ($743 \pm 21 \text{ nm}$) in length and $13-17 \text{ nm}$ ($15 \pm 2 \text{ nm}$) in breadth

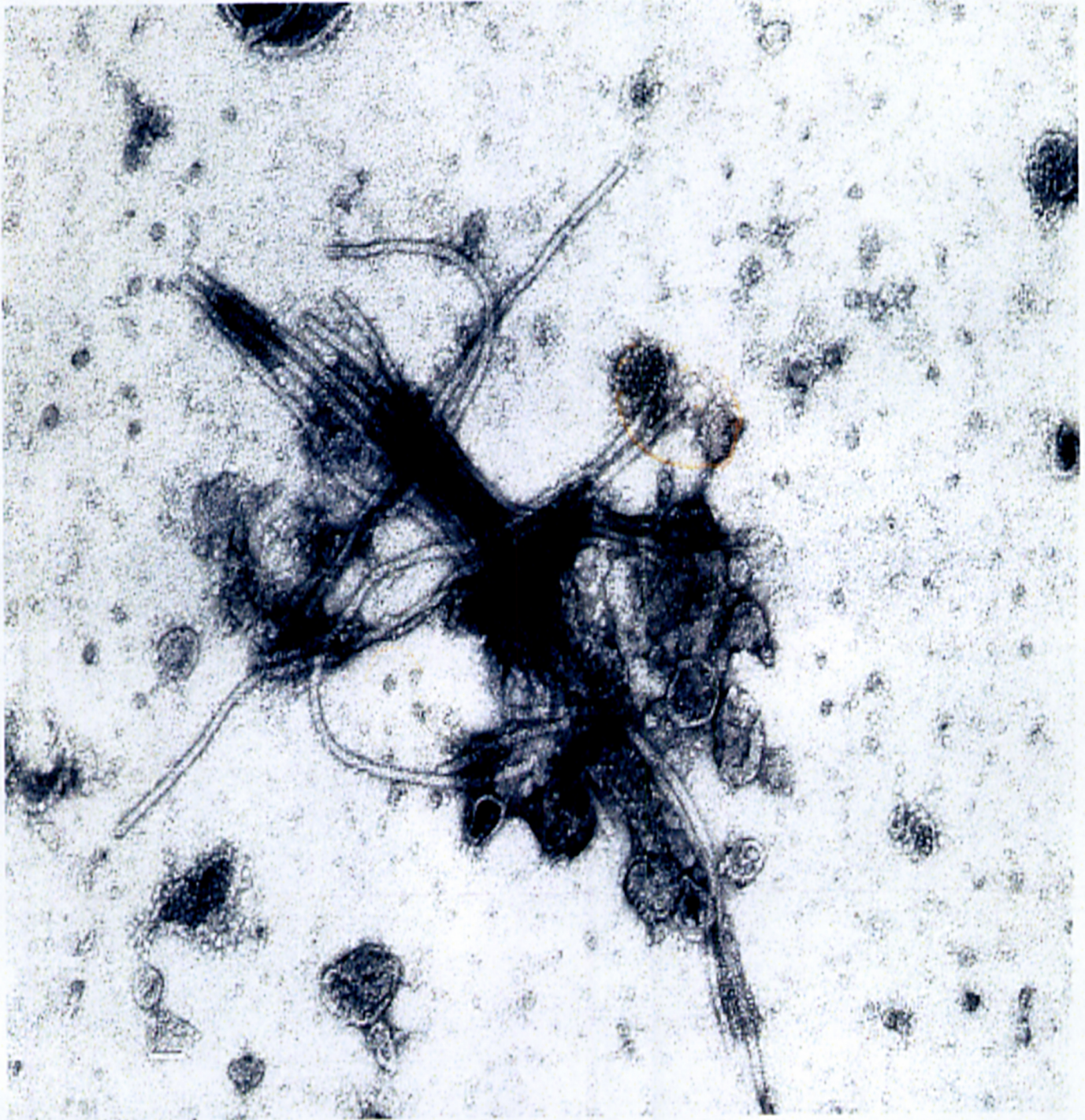


Plate - 21 Electron micrograph showing trapping of flexuous rod shaped particles of various virus isolates of groundnut by peanut stripe virus (PStV) antiserum (80,000 x)

The production of specific antibody halo around virus particles (Plate 22) clearly indicated that virus particles of various isolates were strains of potyvirus groups.

4.8 Direct Antigen Coating : Enzyme - Linked Immunosorbent Assay (DAC-ELISA)

The O.D. values at 405 nm of virus isolates of groundnut were tested by DAC-ELISA which are tabulated in Table 22.

Table 22. Detection of O.D. value from virus isolates of groundnut by DAC-ELISA

Sr. No.	Virus isolates	O.D. value at 405 nm
1.	SB-XI	0.984
2.	JL-24	0.691
3.	Kopergaon-1	0.765
4.	Kopergaon-3	0.806
5.	Karad 4-11	1.23

These results indicated that the maximum O.D. values of 1.23 and 0.984 was shown by two virus isolates namely Karad 4-11 and SB-XI, respectively which clearly pointed out the maximum infection in these virus isolates. Minimum O.D. values of 0.691, 0.765 and 0.806 i.e. low infection percentage was noticed in three isolates i.e. JL-24, Kopergaon-1 and Kopergaon-3, respectively.



Plate - 22 Electron micrograph showing flexuous rod shaped particles decorated by peanut stripe virus (PStV) antiserum (80,000 x)

Chapter Opener Page



DISCUSSION



5. DISCUSSION

The systematic studies on sap transmissible viral diseases of groundnut were carried out in respect of symptomatology, host range, various modes of transmission, physical properties, particle morphology, immunosorbent electron microscopy means trapping and decoration methods, DAC-ELISA techniques to establish the identity of the viruses under investigation.

5.1 Symptomatology and host range

5.1.1 Symptomatology

In present studies various isolates of groundnut virus (SB-XI, JL-24, Kopergaon-1, Kopergaon-3, Karad-411) showed the symptoms on test plant (var. SB-XI) *viz.*, vein clearings, chlorotic spotting, discontinuous stripes along lateral veins of young leaflets, the green blotches were either randomly distributed over the entire leaflets or concentrated along the veins; sometimes they were surrounded by light green rings. The inoculated plants remained extremely stunted. Similar symptoms were observed by several workers (Prasada Rao *et al.*, 1980; Zeyong *et al.*, 1983; Fukumoto *et al.*, 1986; Demski *et al.*, 1988; Reddy *et al.*, 1988b, Wongkaew and Dollet, 1989 and Jain *et al.*, 2000) as shown in Table 23.

Table 23. Cross inoculation of virus isolates on different varieties of groundnut

Virus isolates	Symptom pattern exhibited by cultivars of groundnut				
	SB-XI	JL-24	Kopergaon-1	Kopergaon-3	Karad 4-11
SB-XI	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB
JL-24	VC, MVC, ST, GB	VC, ST, GB	VC, MVC, ST, GB	VC, ST, GB	VC, MVC, ST, GB
Kopergaon-1	VC, MVC, ST, GB	VC, ST, GB	VC, MVC, ST, GB	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB
Kopergaon-3	VC, MVC, SVC, ST, GB	VC, MVC, ST, GB	VC, MVC, ST, GB	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB
Karad 4-11	VC, MVC, SVC, ST, GB	VC, MVC, ST, GB	VC, MVC, ST, GB	VC, MVC, SVC, ST, GB	VC, MVC, SVC, ST, GB

GB : Green blotches
 ST : Striping
 VC : Vein clearing

MVC : Mild vein clearing
 SVC : Severe vein clearing

5.1.2 Host range

Demski *et al.* (1984) observed nine species of the Chenopodiaceae, Leguminosae and Solanaceae as hosts of peanut stripe virus.

Reddy *et al.* (1991) reported that all PStV isolates induced local lesions on *C. amaranticolor* and *C. quinoa* leaves. Unlike peanut mottle virus, PStV did not infect *Phaseolus vulgaris* (cv. Topcrop).

The virus isolates of groundnut under investigation had limited host range distributed only in Leguminosae (groundnut, soybean, cowpea, clusterbean and mungbean), Chenopodiaceae (*Chenopodium amaranticolor* and *C. quinoa*) and

Solanaceae (*Nicotiana benthamiana*). The virus isolates of groundnut produced chlorotic local lesions and necrotic local lesions on leaves of *C. amaranticolor*, *C. quinoa* and *N. benthamiana* while the host range in the Leguminosae family showed mosaic mottle symptoms except groundnut. These results were in agreement with other workers (Demski *et al.*, 1984; Demski and Lovell, 1985; Reddy *et al.*, 1991, Li *et al.*, 1991 and Prasada Rao *et al.*, 1997).

Ahmed and Idris (1981) reported plant species that did not infect to PMV inoculation which included *Abelmoschus esculentus*, *Capsicum annuum*, *Cucumis sativus*, *Datura stramonium*, *Nicotiana tabacum*, *N. glutinosa*, *C. amaranticolor*, *C. quinoa*, *Brassica oleracea*. Virus was not recovered from these plants in back inoculations to Topcrop beans.

Li *et al.* (1991) reported that peanut mottle virus was manually transmitted to *N. benthamiana*. PMoV-VS did not infect *Capsicum annuum*, *Cucumis sativus*, *Gomphrena globosa*, *Lycopersicon esculentum*, *N. glutinosa*, *N. tabacum* and *Zinnia elegans*.

The various host plants from other families *viz.*, Chenopodiaceae (*C. murale*), Solanaceae (*N. tabacum*, *N. glutinosa*, *Capsicum annum*, *Lycopersicon esculentum* and *Datura stramonium*), Cucurbitaceae (*Luffa cylindrica*, *Cucumis sativus*, *Lagenaria siceraria* and *Momordica charantia*), Malvaceae (*Abelmoschus esculentus*), Amaranthaceae (*Gomphrena globosa*), Pedalaceae (*Sesamum indicum*) and Compositae (*Helianthus annus* and *Carthamus tinctorious*) were mechanically inoculated

with virus isolates of groundnut. None of the host plant from these families showed symptom reaction to any of the virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad-4-11). The virus was also not recovered from inoculated plants of these hosts. Similar results were observed by Ahmed and Idris (1981) and Li *et al.* (1991).

5.2 Transmission

5.2.1 Transmission by mechanical sap inoculation

Germani *et al.* (1975) reported the peanut clump virus which was easily transmitted by mechanical sap inoculation.

Rehcigl *et al.* (1989) transmitted PStV by mechanical sap inoculation method by rubbing neutral phosphate buffer, extracted sap, infected leaf edges onto carborundum dusted leaflets of the first two quadrifoliate leaves.

Investigations on the transmission of the virus from different plant parts indicated that highest concentration of the virus was in the leaves of infected groundnut plant than other parts of the same plant. These results are in agreement with those reported by several other workers (Germani *et al.*, 1975; Demski and Lovell, 1985; Rehcigl *et al.*, 1989; Prasada Rao *et al.*, 1989; Chang *et al.*, 1990 and Mandal *et al.*, 2001) as indicated in Table 24.

Reddy *et al.* (1991) reported that eight peanut viruses viz., peanut mottle potyvirus, peanut stripe potyvirus, peanut stunt cucumovirus, cucumber mosaic cucumovirus, peanut clump furovirus, cowpea mild mottle carlavirus, tomato spotted

Table 24. Sap transmissible viral diseases infecting groundnut

Authority and Year	Country	Name of virus	Physical properties			Transmission			Particle morphology	Serological relationship
			DEP	TIP	LIV	Sap	Vector	Seed		
Kuhn (1965)	USA	PMV	10 ⁻³ -10 ⁻⁴	60-65°C	1-2 days	+	Aphids	+	Flexuous 700 x 12 nm	East African PMV isolate
Herold and Munz (1969)	South America									
Inouye (1969)	Japan									
Behncken (1970)	Australia									
Bock (1973)	East Africa									
Geh and Ting (1973)	West Malaysia									
Behncken and McCarthy (1973)	Queensland									
Benigno and Favli-Hedayat (1977)	Philippines									
Reddy <i>et al.</i> (1978)	India									
Ahmed and Idris (1981)	Sudan	TSWV								
Costa (1950)	Brazil		10 ^{-2.5} - 10 ^{-3.0}	45-50°C	4 hrs	+	Thrips	-	Spherical membrane bound 70- 90 nm dia.	
Dyer (1949)	South Africa									
Helms <i>et al.</i> (1961)	Australia									

Table 24 contd.....

Authority and Year	Country	Name of virus	Physical properties			Transmission			Particle morphology	Serological relationship
			DEP	TIP	LIV	Sap	Vector	Seed		
Reddy <i>et al.</i> (1968) Halliwell and Philley (1974) Hagan <i>et al.</i> (1990) Cheema <i>et al.</i> (2003)	India United States Alabama India(Punjab)									
Miller and Troutman (1966) Echandi and Hebert (1970) Tsuchizaki (1973) Kracv <i>et al.</i> (1977) Fischer and Lockhart (1978) Douine and Devergne (1978) Beczner and Devergne (1979) Diaz-Ruiz <i>et al.</i> (1979)	United States North Carolina Japan USSR Morocco France Hungary Spain	PSV	10 ⁻² -10 ⁻³	50°C	1-2 days	+	Aphids	+	Spherical 30 nm dia.	PSV, PSV-E, V, W

Table 24 contd.....

Authority and Year	Country	Name of virus	Physical properties			Transmission			Particle morphology	Serological relationship
			DEP	TIP	LIV	Sap	Vector	Seed		
Brunt and Kenten (1973)	Africa	CMMV	10 ⁻³ -10 ⁻⁴	75-80°C	9 days	+	Whitefly	-	Flexuous rods 15 nm width 610 ± 10 nm length	CMMV (do not react with PMV, PCV, TSWV antisera)
Brunt and Phillips (1977)	Thailand									
Iizuka <i>et al</i> (1982)	India									
Mali and Nirmal (1987)	India (M. S.)									
Fulton (1950)	Arkansas	CMV	10 ⁻² -10 ⁻³	55-60°C	6-7 days	+	Aphids	+	Spherical 28.7 nm dia.	CMV-CA antisera (China arachis)
Xu and Barnett (1984)	China									
Zimmerman (1907)	Tanzania	GRV and its satellite RNA	10 ⁻¹ -10 ⁻²	50°C	One week (at 18°C) four week at 20°C	+	Aphids	-	Isometric 25-28 nm dia.	-
Bisht <i>et al.</i> (1963)	India									
Kousalya <i>et al.</i> (1967)	India (Tamilnadu)									
Singh and Gupta (1968)	India (Rajasthan)									

Table 24 contd.....

Authority and Year	Country	Name of virus	Physical properties			Transmission			Particle morphology	Serological relationship
			DEP	TIP	LIV	Sap	Vector	Seed		
Badak and Sawant (2005)	India (M.S.)	PStV	10 ⁻³ -10 ⁻⁵	60-70 °C	64-84 hrs.	+	Aphids	+	Flexuous rod shaped 722-765 nm length, 12-17 nm breadth	Related with peanut stripe virus (PStV)

CMMV = Cowpea mild mottle virus
 DEP = Dilution end point
 IPCV = Indian peanut clump virus
 PCV = Peanut clump virus
 PStV = Peanut stripe virus
 TIP = Thermal inactivation point

CMV = Cucumber mosaic virus
 GRV = Groundnut rosette virus
 LIV = Longevity *in vitro*
 PMV = Peanut mottle virus
 PSV = Peanut stunt virus
 TSWV = Tomato spotted wilt virus

wilt tospovirus and groundnut rosette virus and its satellite RNA were transmitted by mechanical sap inoculation.

Hull and Adams (1968) reported that the buffer containing magnesium or sodium bentonite (25 mg/ml), 1 per cent K_2HPO_4 , 0.01 M DIECA at pH 7.3 favoured higher rate of TSWV transmission. Mandal *et al.* (2001) observed the use of two antioxidants (sodium sulfite and mercaptoethanol) and two abrasives (Celite and Carborundum) and application of the inoculum by rubbing with a cotton swab dipped in the inoculum as well as pricking with an inoculation needle resulted in a significantly higher transmission rate.

In present studies, addition of abressive and buffers was found to transmit the virus isolates more effectively and efficiently. These results are in agreement with Hull and Adams (1968) and Mandal *et al.* (2001).

5.2.2 Transmission through seeds

Zeyong *et al.* (1991) showed that the PStV was consistently detected by DAC-ELISA in the cotyledonary and embryonic tissues of infected seeds but not in the testae. While Warwick and Demski (1988) found that none of the three virus isolates of PStV were transmitted in the 15,000 soybean seeds from infected plants harvested from all susceptible cultivars. Neither infective virus nor serologically detectable PStV was recovered from cotyledons or embryo of mature seeds.

Ghanekar *et al.* (1979) reported that out of nearly 6000 seeds collected from the infected tomato spotted wilt virus plants, 1800 seeds (30 %) produced normal plants, 540 seeds (9

%) produced malformed plants and the others failed to germinate. None of the plants developed disease symptoms and assays on cowpea from the malformed stunted plants gave negative results. The results indicated that the virus was not seed borne.

Results of seed transmission in present studies indicated that the five virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad-4-11) were not seed borne in nature. These results were in agreement with other workers (Ghanekar *et al.*, 1979; Reddy *et al.*, 1986; Warwick and Demski, 1988; Jain *et al.*, 2000) for seed transmission of peanut stripe virus, peanut mottle virus and tomato spotted wilt virus as indicated in Table 24.

5.2.3 Insect transmission

Sreenivasulu and Demski (1988) observed *Aphis craccivora* and *Myzus persicae* as vectors of peanut stripe virus (PStV). Kuhn and Demski (1984) observed *A. craccivora*, *A. gossypii*, *M. persicae*, *Hyperomyzus lactucae*, *Rhopalosiphum padi* and *R. maidis* as vectors of PMV.

In present studies, the most abundant species namely *R. maidis*, *M. persicae*, *A. craccivora*, *A. gossypii* and whitefly (*Bemisia tabaci*) were used for virus transmission and only two species of aphids viz., *M. persicae* and *A. craccivora* transmitted all the virus isolates but not by *A. gossypii*, *R. maidis* and *Bemisia tabaci*. These results were in agreement with earlier workers (Watson and Okusanya, 1967; Troutman *et al.*, 1967; Fischer and Lockhart, 1978; Ahmed and Idris, 1981; Muniyappa

and Reddy, 1983; Sreenivasulu and Demski, 1988; Prasada Rao *et al.*, 1988; Chang, 1990) as shown in Table 24. However, the aphid species *Aphis craccivora* transmitted the various viruses viz., peanut stripe virus, groundnut rosette virus, peanut mottle virus, peanut stunt virus and *M. persicae* transmitted peanut stunt virus, peanut stripe virus, peanut mottle virus. While cowpea mild mottle virus was transmitted by *Bemisia tabaci*, peanut mottle virus by *A. gossypii* and *R. maidis* (Reddy *et al.*, 1991) which were different from the isolates of present virus.

Sreenivasalu and Demski (1988) observed that *Myzus persicae* was more efficient in transmitting PStV (29 %) and PMV (14 %) than *A. craccivora* (17 and 4 %, respectively).

In present studies, aphid species *M. persicae* transmitted all the five virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11) to the extent of 75, 60, 65, 70, 75 per cent, respectively. While *A. craccivora* transmitted all the virus isolates to the extent of 70, 55, 60, 65, 70 per cent, respectively. The results showed that the *M. persicae* was more efficient in transmitting all virus isolates of groundnut than *A. craccivora*. Similar results were recorded by Sreenivasulu and Demski (1988).

Demski *et al.* (1984) conducted aphid transmission trials by *Aphis craccivora*. They used 10 aphids for acquisition access and results showed that the 11 of 14 groundnut plants was infected by PStV. Chang (1990) used group of five aphids for transmission of PStV.

In present studies, the transmission of various isolates of groundnut virus by *M. persicae* and *A. craccivora* was increased with increased with increase in number of aphids per test plant. Maximum transmission was obtained with 10 adult aphids per test plant. Similar results on aphid transmission was recorded by Demski *et al.* (1984).

Chang (1990) conducted aphid transmission trials with *M. persicae* which were starved for 3-4 hrs before access feeding of approximately 60 sec on Ts infected cowpea or Tc infected peanut leaves. Groups of five aphids were transferred to each of 25 test plants. After a test feeding period of 4-5 hrs the aphids were removed and the results showed that twenty peanut seedlings were used to test the aphid transmissibility of Ts and Tc; 18 and 15 seedlings, respectively became infected.

In present studies, maximum access feeding for 10 min and transmission feeding for 15 min was found necessary to produce maximum transmission to an extent of 75 per cent by *M. persicae* and 70 per cent by *A. craccivora*. These results were in agreement with other workers (Fischer and Lockhart, 1978; Ahmed and Idris, 1981; Prasada Rao *et al.*, 1988; Sreenivasulu and Demski, 1988 and Chang, 1990).

Muniyappa and Reddy (1983) observed that the individual *Bemisia tabaci* adults acquired CMMV in 10 min and transmitted it within 5 min to soybeans. Starvation before acquisition had no effect upon transmission, but starvation after acquisition decreased transmission frequency.

In present studies, the pre-acquisition starvation was found to increase percentage of infection. Maximum infection was obtained with two hours of starvation before access feeding. Aphids not starved after access feeding and before inoculation feeding were found to transmit the virus to the maximum percentage. Post acquisition fasting, on the other hand, decreased transmission efficiency. These results were in confirmation with other workers (Troutman *et al.*, 1967; Fischer and Lockhart, 1978; Ahmed and Idris, 1981; Muniyappa and Reddy, 1983; Kuhn and Demski, 1984; Prasada Rao *et al.*, 1988; Sreenivasalu and Demski, 1988 and Chang, 1990).

Muniyappa and Reddy (1983) observed that the irrespective of the length of acquisition, ability to transmit CMMV the whitefly retained for only four successive inoculation access periods of 5 min each, but adults lost the ability to transmit the virus which required reacquisition to transmit the virus.

Sreenivasulu and Demski (1988) reported that the in sequential feeding trials (aphid fed first on a PMV – infected leaf and then on a PStV–infected leaf and vice versa), PStV was transmitted to healthy peanut plants at a higher percentage than PMV, regardless of the sequence of feeding. Transmission of peanut stripe virus was enhanced to 35 per cent with *A. craccivora* and 45 per cent with *M. persicae* when the aphid vectors fed on PMV-infected plants before feeding on PStV source plants. Simultaneous transmission of both viruses by a single aphid from doubly infected plants or sequential feeding trials did

not occur with *A. craccivora* and at 3 per cent or less for *M. persicae*.

In present studies, the viruliferous aphids *viz.*, *M. persicae* and *A. craccivora* failed to infect test plants of various virus isolates of groundnut after a period of 60 min in a serial successive feeding showing thereby that *M. persicae* and *A. craccivora* did not retain the virus beyond 60 min. These results clearly indicated that both the aphid vectors transmitted the virus in a non-persistent manner (Table 24).

Non-persistent transmission was reported for various viruses *viz.*, peanut mottle virus (PMV), peanut stripe virus (PStV), peanut stunt virus (PSV), cowpea mild mottle virus (CMMV) and cucumber mosaic virus (CMV) by Reddy *et al.* (1991). While the groundnut rosette virus (GRV) and tomato spotted wilt virus (TSWV) was transmitted in a persistent or circulative manner by aphid and thrips, respectively.

5.3 Physical properties

Thouvenel *et al.* (1976) studied the physical properties of peanut clump virus as DEP 10^{-4} - 10^{-5} , TIP 60-64°C and LIV 15 months at 20°C, 12 months in frozen sap and 50 days by alternate freezing and thawing.

Demski *et al.* (1984) reported physical properties of PStV as DEP between 10^{-3} and 10^{-4} , TIP between 60°C and 65°C and LIV for 3 days at 20°C.

Present studies on physical properties of five virus isolates revealed that isolate number SB-XI, Kopergaon-1, Kopergaon-3 and Karad 4-11 had TIP of 65-70°C, LIV of 72-84

hrs and DEP of 10^{-4} - 10^{-5} . While isolate JL-24 had LIV of 64-72 hrs, DEP of 10^{-3} - 10^{-4} and the TIP in between 60-65°C.

It is clearly evident that physical properties of five virus isolates under study were identical with those of PStV reported by earlier workers (Demski *et al.*, 1984 and Prasada Rao, 1989) shown in Table 24.

5.4 Particle morphology

Early studies on size of virus particles were carried out using metal shadowing techniques (Williams and Wycoff, 1944) required relatively pure virus preparations.

Brenner and Horne (1959) studied electron microscopy of plant viruses occurred with the development of negative contrast staining. This technique is now universally used and procedures have been developed that allow simple and rapid examination of virus infected leaf material, without the necessity of using purified virus preparations.

Brandes (1960) for the first time described the quick leaf dip procedure of electron microscopy for identification of plant viruses.

Hitchborn and Hills (1965) modified quick-leaf dip procedure by epidermal strip method.

Culver *et al.* (1987) studied electron microscopy of PStV by leaf dip assay.

The present studies on particle morphology of five virus isolates of groundnut *viz.*, SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11 were found in the range of 722-765

nm (743 ± 21 nm) in length and 13-17 nm (15 ± 2 nm) in breadth.

The virus particles of these isolates were similar in morphology with those reported by earlier workers (Schmidt and Schmeezer, 1966; Paguio and Kuhn, 1973a; Demski *et al.*, 1984; Prasada Rao, 1989; Chang, 1990; Amin, 1992 and Choi, 2001) as indicated in Table 24.

5.5 Serology

Serological tests may be decisive in the final identification of an unknown virus and important for studying the relationships between related virus isolates and strains.

A various serological tests used for virus identification which included Precipitin tests, Immunodiffusion tests, Agglutination tests, Electron microscope serology and Enzyme linked immunosorbent Assay (ELISA).

Noordam (1973) reported the micro-precipitin tests which were very economical in the use of antiserum, antigen and are quite sensitive.

Ackers and Steere (1967) reported immunodiffusion test in which antibody antigen reaction was carried out in a gel instead of liquid.

Oudin (1952) and Mancini *et al.* (1965) used one type of immunodiffusion test i.e. radial diffusion for identification of viruses.

Van Slogteren (1955) used slide agglutination or chloroplast agglutination test which has been particularly useful

in the past for the rapid detection of virus infected potatoes in the field.

Derrick (1973) was the first to use electron microscope serology when he trapped viruses on to antibody-coated grids (ACG).

Milne and Luisoni (1975) used two other electron microscope serology techniques for identification of plant viruses. The first involved the mixing of virus particles and antisera prior to their being placed on a grid for Electron microscopy (EM) examination. In the second technique, the antibodies were added to a grid that already had virus particles attached, so that the particles became coated or decorated with the antibodies. Besides its use for virus identification, the decoration procedure had also been used to demonstrate degrees of relationship between viruses (Walkey and Webb, 1984).

Voller *et al.* (1976) and Voller and Bidwell (1977) developed the ELISA procedure for the first time. Koenig and Paul (1983) reported the numerous variations of ELISA used for plant virus identification.

Clark and Adams (1977) reported the Double Antibody Sandwich ELISA (DAS-ELISA) method which is commonly used.

Clark and Bar-Joseph (1984) reported the Direct Antigen-Coated Enzyme Linked Immunosorbent Assay (DAC-ELISA) method. Milne and Lesemann (1984) reported the Immunosorbent Electron Microscopy Method (ISEM) for identification of plant viruses.

In the present investigation, virus particles on the grids were trapped by decoration using antiserum of peanut stripe virus (PStV). In leaf dip preparation only few particles were visible as against several particles in trapping. The production of specific antibody halo around the virus particles was clearly visible. These results are in agreement with earlier workers (Culver and Sherwood, 1988; Demski *et al.*, 1984; Nasir Saleh, 1989; Prasada Rao *et al.*, 1989; Chang, 1990 and Jain *et al.*, 2000) as indicated in Table 24.

In present studies, a composite samples of five virus isolates of groundnut (SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11) were used for serological relationship by trapping and decoration using antiserum of PStV. The production of specific antibody halo around the virus particles was clearly visible.

The enzyme linked immuno-sorbent assay (ELISA) an extremely sensitive technique was used in present investigation. DAC-ELISA technique was employed for detection of higher concentration of the virus. The results of DAC-ELISA indicated that the maximum O.D. values of 1.23 and 0.984 was shown by two isolates *viz.*, Karad 4-11 and SB-XI which clearly suggested that the maximum infection as well as virus titre in these virus isolates. Further it was also evident that minimum O.D.values of 0.691 to 0.806 i.e. low infection percentage was noticed in three isolates *viz.*, JL-24, Kopergaon-1 and Kopergaon-3.

The results were in agreement with other workers (Demski *et al.*, 1984; Culver and Sherwood, 1988; Nasir Saleh,

1989; Prasada Rao *et al.*, 1989; Chang, 1990 and Jain *et al.*, 2000) as shown in Table 24.

5.6 Identification of the virus isolates

The five isolates of virus disease of groundnut were studied after artificial inoculation on their respective hosts showed greater similarities in symptomatology. These results were in agreement with other workers (Prasad Rao *et al.*, 1980; Zeyong *et al.*, 1983; Fukumoto *et al.*, 1986; Demski *et al.*, 1988; Reddy *et al.*, 1988b; Wongkaew and Dollet, 1989 and Jain *et al.*, 2000).

The host range of all the five isolates of virus disease of groundnut had limited to family leguminosae, chenopodiaceae and solanaceae. Based on host range, these isolates were grouped in three categories *viz.*, Severemost (SB-XI, Karad 4-11), Moderate (Kopergaon-1, Kopergaon-3) and Mild (JL-24). The host plants from other families such as Cucurbitaceae, Malvaceae, Amaranthaceae, Pedalaceae and Compositae did not show any symptom reaction inoculated with different virus isolates of groundnut.

The results of transmission of virus isolates of groundnut by mechanical sap inoculation and by aphids particularly *M. persicae* and *A. craccivora* indicated that the virus was transmitted by sap and also by aphids in a non-persistent manner. Maximum transmission of virus was received with *M. persicae*. These results were in agreement with other workers (Demski and Lovell, 1985; Prasada Rao *et al.*, 1989; Rechcigl *et*

al., 1989; Chang *et al.*, 1990; Mandal *et al.*, 2001; Sreenivasulu and Demski, 1988 and Demski, 1984).

Based on symptomatology, host range, transmission by various methods, physical properties, electron microscopy and serology (Table 23, 24) the present virus isolates belongs to potyvirus group and also strain of peanut stripe virus (PStV).

One of the five virus isolates studied, two isolate *viz.*, SB-XI and Karad 4-11 were found to show severest symptoms in a short period and two isolates namely Kopergaon-1 and Kopergaon-3 were found to show moderate symptoms while the fifth isolate JL-24 showed mild symptoms.

Based on the symptom expression in different hosts and their transmission by aphid vectors, the five isolates of the virus under study were grouped under severe most (SB-XI, Karad 4-11), moderate (Kopergaon-1 and Kopergaon-3) and mild (JL-24).

The further studies related to their detailed genomic studies are to be completed to prove their differences.

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SUMMARY



6. SUMMARY

Groundnut crop at Mahatma Phule Krishi Vidyapeeth, C.C., Rahuri and Oilseed Research Station, Jalgaon was seriously affected by viral diseases. The viral diseases produced symptoms like vein clearings, chlorotic spotting, discontinuous stripes along lateral veins of young leaflets, the green blotches were either randomly distributed over the entire leaflets or concentrated along the veins; sometimes they were surrounded by light green rings. The inoculated plants remained extremely stunted.

Host range of various isolates of groundnut (SB-XI, JL-24, Kopregaoon-1, Kopergaoon-3 and Karad 4-11) was limited to family Leguminosae, Chenopodiaceae and Solanaceae. Based on symptom pattern expressed on host range, these isolates were grouped into 3 categories *viz.*, Severest (SB-XI and Karad-4-11), Moderate (Kopergaoon-1 and Kopergaoon-3) and Mild (JL-24) which showed symptom reaction of CLL and NLL on *C. amaranticolor* and *N. benthamiana*. While the host plants from other families such as Cucurbitaceae, Malvaceae, Amaranthaceae, Pedalaceae and Compositae did not show any symptom reaction of inoculated different virus isolates of groundnut. The studies regarding mechanical sap inoculation from different plant parts of diseased groundnut plants revealed maximum recovery of virus from leaves.

Transmission studies indicated that all the virus isolates are easily sap transmissible but not seed transmitted.

Among the aphid species and whitefly species tested *M. persicae* and *A. craccivora* were found vectors of all the isolates of virus under study. However, *M. persicae* proved to be most efficient vector for transmission of various isolates of virus and all the isolates^{were transmitted} in a non-persistent manner.

The studies on physical properties of virus isolates of groundnut revealed that isolate number SB-XI, Kopergaon-1, Kopergaon-3 and Karad-4-11 had TIP of 65-70°C, LIV of 72-84 hrs and DEP of 10^{-4} – 10^{-5} . While isolate JL-24 had LIV of 64-72 hrs, DEP of 10^{-3} – 10^{-4} and the TIP between 60-65°C.

The electron microscopy showed that the virus particles of five isolates *viz.*, SB-XI, JL-24, Kopergaon-1, Kopergaon-3 and Karad 4-11 were found in the range of 722-765 nm (743 ± 21 nm) in length and 13-17 nm (15 ± 2 nm) in breadth.

Based on particle morphological studies, one group of virus isolates were noticed based on length and breadth of virus particles. A representative composite sample from this group were used for immunosorbent electron microscopy using antiserum of peanut stripe virus (PStV). The production of specific antibody halo around the virus particles clearly indicated that virus particles belongs to potyvirus group.

In present studies, DAC-ELISA (Direct antigen coating – Enzyme linked immunosorbent assay) technique was employed for detection of higher concentration of the virus. The maximum O.D. values of 1.23 of 0.984 was shown by two virus isolates *viz.*, Karad-4-11 and SB-XI which clearly pointed out the maximum

infection as well as virus titre in the isolates. Minimum O.D. values of 0.691 to 0.806 i.e. low infection percentage was noticed in three isolates i.e. JL-24, Kopergaon-1 and Kopergaon-3.

The symptomatology, host range, transmission by various methods, physical properties, electron microscopy, serology indicated that virus isolates under study belonged to strains of potyvirus group and classified as peanut stripe virus (PStV).

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7. LITERATURE CITED

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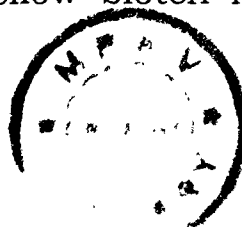
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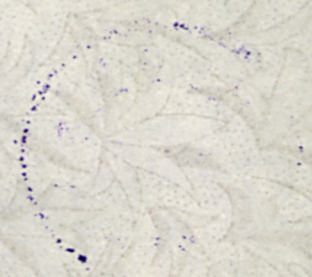
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Chapter Opener Page



VITA



8. VITA

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of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

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