STUDIES ON BHENDI YELLOW VEIN MOSAIC VIRUS WITH SPECIAL REFERENCE TO PURIFICATION AND ELECTRON MICROSCOPY

MANJULA RAO

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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STUDIES ON BHENDI YELLOW VEIN MOSAIC VIRUS WITH SPECIAL REFERENCE TO PURIFICATION AND ELECTRON MICROSCOPY

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MANJULA RAO

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C E R T I F I C A T E

This is to certify that the thesis entitled "STUDIES ON BHENDI YELLOW VEIN MOSAIC VIRUS WITH PARTICULAR REFERENCE TO PURIFICATION AND ELECTRON MICROSCOPY" submitted by MANJULA RAO, for the degree of MASTER OF SCIENCE (AGRICULTURE) in Plant Pathology, of the University of Agricultural Sciences, Bangalore, is a record of research work done by her during ther period of her study in the University under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Dr. V.Muniyappa
Major Adviser

APPROVED BY:

Chairman: _____ 11/11/86

(V. Muniyappa)

Members :

1. _____

(Dr. H.S.Savithri)

2. _____

(Dr. R. Ram Mohan)

3. _____

(Mr. S. Vishwanath)

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BANGALORE
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(MANJULA RAO)

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INTRODUCTION

I INTRODUCTION

Bhendi, or lady's finger, also called okra [*Abelmoschus esculentus* (L.) Moench] is one of the important vegetable crops grown throughout the tropical and sub-tropical parts of the world mainly for its tender pods which are rich in calcium and Vitamin C and to a little extent for seed production. Bhendi has occupied an area of 9013 ha in Karnataka, of which Bangalore district alone accounted for 385 ha and statewide production being 67597 tonnes.

Bhendi is infected with a number of diseases causing substantial losses in yields. Of all the diseases reported, bhendi yellow vein mosaic virus (BYVMV) is one of the most severe diseases which takes a heavy toll of the crop in India (Capoor, 1953; Muniyappa, 1980; Singh, 1980). Sinha and Chakrabarthi (1978) observed 86.00 per cent seed loss when bhendi plants were infected 33 days after sowing. In Karnataka, the disease is more serious when sowing is carried out from January to May (Singh, 1980).

Bhendi yellow vein mosaic is characterised by vein clearing, yellowing of veins, reduction in leaf size and fruit yield. The disease is transmitted by the whitefly *Bemisia tabaci*. Though the disease has been known since a long time, the available information on the pathogen-vector relationship, sources of infection, epidemiology, host preference to the whitefly is inadequate. The causal agent of the disease is not known. Therefore, attempts were made to study the disease with the following objectives:

1. Survey for the incidence of BYVMV disease.

2. Sources of infection and host range.

3. Transmission of the disease by agents other than whiteflies.

4. Preference of host plants to the whitefly Bemisia tabaci.

5. Isolation and characterisation of the virus, and

6. Relationships of BYVMV to other whitefly transmitted diseases.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Occurrence and losses

Bhendi yellow vein mosaic (BYVM) was first reported by Kulkarni (1924) and later studied by Uppal et al. (1940) and Capoor and Varma (1950). Study of the effect of BYVM infection on the growth and yield of bhendi was conducted by Sastry and Singh (1973b), who showed that the growth of the plants was very much stunted when plants were infected in the early stages of the crop. The estimated loss in yield amounted to 93.8 per cent (35 day old crop) and 83.63 per cent (35 days later). Chelliah and Murugesan (1976b) reported on the estimation of loss due to BYVM disease. The number of fruits harvested from plants expressing symptoms of BYVM 30, 45 and 60 days after sowing was reduced by 76, 54.9 and 47.8 per cent respectively. On other plants that showed symptoms of BYVM 30, 45 and 60 days after sowing, the fruit number harvested was reduced to 4, 7 and 8 respectively compared to 16 in the healthy plant.

Bhendi grown in Bihar suffers extensively from BYVM and is particularly severe in vegetable belts where the crop is almost continuously cultivated throughout the year. The percentage of infection has been found to vary from 50-90 per cent (Jha and Mishra, 1955).

Bhendi plants were infected by BYVM at various stages of plant growth (Mandahar and Singh, 1972; Sastry and Singh, 1974). The disease had an adverse effect on plant height, number of

branches, number and size of fruits and seed yield. The highest loss of yield (86.23%), occurred in plants showing symptoms on the 33rd day after germination, and the least (32.85%) on the 75th day (Sinha and Chakrabarthi, 1978).

Symptomatology

The earliest symptom of BYVMV infected plants is vein clearing (Kulkarni, 1924; Uppal et al., 1940; Fernando and Udurevan, 1942; Raychaudhuri and Nariani, 1977; Nariani and Seth, 1958; Capoor and Varma, 1950). Vein clearing starts on the small veins and then extends to the larger ones (Uppal et al., 1940). Sometimes the yellow network of veins is followed by the thickening of veins and veinlets (Nariani and Seth, 1958; Raychaudhuri and Nariani, 1977).

Vein clearing is soon followed by veinal chlorosis (Uppal et al., 1940; Fernando and Udurevan, 1942; Raychaudhuri and Nariani, 1977; Nariani and Seth, 1958; Capoor and Varma, 1950). In severe cases, chlorosis will be followed by complete yellowing of leaves (Kulkarni, 1924; Uppal et al., 1940; Raychaudhuri and Nariani, 1977; Nariani and Seth, 1958).

Fernando and Udurevan (1942) reported that yellow vein banding may be followed by interveinal clearing and minute enations on the axial side of leaves. Finally the fruits that arise from such diseased plants are malformed and bleached (Kulkarni, 1924; Fernando and Udurevan, 1942; Raychaudhuri and Nariani, 1977; Nariani and Seth, 1958).

Transmission

Seed transmission : Transmission through seeds was not observed in bhendi seeds (Capoor and Varma, 1949; Uppal et al., 1940; Costa, 1969).

Mechanical transmission : The virus was not transmitted by sap (Capoor and Varma, 1949; Costa, 1969; Uppal et al., 1940; Raychaudhuri and Mariani, 1977).

Graft transmission : Successful graft transmission of BYVM was reported by Capoor and Varma (1949) and Uppal et al. (1940).

Dodder transmission : No reports were found, so far, on dodder transmission of BYVM.

Insect transmission : BYVMV was transmitted exclusively by whitefly Bemisia tabaci (Uppal et al., 1940; Raychaudhuri and Mariani, 1977; Varma, 1952).

Acquisition access period : Acquisition access period was reported as 4-6 hrs (Raychaudhuri and Mariani, 1977), 12-24 hrs (Varma, 1952). Preliminary fasting markedly increased the efficacy of whiteflies as vectors (Varma, 1952).

Inoculation access period : A feeding period of 30 minutes was sufficient to transmit the virus, but a five minute probe was not sufficient (Varma, 1955a). Varma (1955b) reports that the whitefly B.tabaci is capable of harbouring different viruses simultaneously and can readily cause infection in healthy host plant susceptible to the respective

viruses on the same day and can continue to do so for several days without having reaccess to the source of infection.

Epidemiology

Fortnightly sowing of bhendi and the record of incidence of BYVM and the vector B.tabaci were carried out for a period of 2 years (Chelliah et al., 1975). Multiple regression analysis lead to the conclusion that in the 30 day old crop, increase of whitefly population brought about 18.5 per cent of the disease, while 1 per cent decrease in relative humidity increased the disease incidence by 1.2 per cent. In the 45 day old crop, minimum temperature alone exerted positive influence on the disease incidence. During the 45-60 days of crop age, increase in the maximum temperature by 5°C, resulted in 6.3 per cent increase of the disease. Significant increase of the disease incidence was observed in the bhendi crop sown in March-May (Chelliah and Murugesan, 1976a).

Control

Cultural : Capoor and Varma (1949) suggested the following control measures (i) eradication of Hibiscus tetraphyllus, the wild host of the virus, (ii) observing a closed season of atleast 2 months during summer between 2 successive crops (iii) reguing of diseased bhendi plants at the earliest stage of their infection and (iv) keeping the fields free from weeds, which may help in the multiplication of the whitefly.

Chemical control : Sastry and Singh (1973b) conducted a field trial of insecticides for the control of the whitefly (B.tabaci) in relation to the incidence of BYVM. Results revealed that spraying in the initial stages of the crop just after germination is the most important. If the crop was not sprayed within 20 days after germination, the incidence of BYVM would be as high as 100 per cent resulting in low yields, even if crop was sprayed regularly after 20 days after germination. Four to six applications of systemic insecticides such as metasystox, rogor and dimecron as foliar sprays and one to two applications of the granular thimet and dysystox to the soil, not only reduced the whitefly population, but also the incidence of BYVM to a greater extent, when compared to the yield and profit of those plots where no insecticide has been applied.

Further trials were taken up by Sastry and Singh (1973a) on the restriction of BYVM spread through the control of the vector B.tabaci. Virus spread was restricted by 4 sprays, each of Parathion (0.02%), oxydemeton methyl (0.02%) or dimethoate (15 kg/ac) at sowing (Sastry and Singh, 1973b). A higher incidence of BYVM occurred when sprays were delayed.

According to Palaniswamy et al. (1973), BYVM can be effectively minimised by the furrow application of aldicard and carbofuran granules at 1.0 kg a.i./ha at the time of sowing. These treatments have accounted for 13.8 per cent and 76.2 per cent reduction in the disease incidence over the control, respectively. Phorate, fensulphothion, and mephospholan have

also reduced the incidence by more than 50 per cent over the control.

Chakrabarthi and Mukhopadhyay (1977) evaluated 6 insecticides, viz., methyl demeton, phosphomidon, endosulfan, carbofuran, and fenitrothion on 'Pusa Sawani' variety of bhendi. The insecticides significantly reduced the number of diseased plants and the whiteflies, irrespective of the dates of sowing. The total yields of treated plants was also significantly higher than that of the control.

Chelliah (1976) reported that by the application of aldicarb granules (1.0 kg a.i./ha) a week after sowing and spraying with endosulfan (0.07%) at 30 days after sowing and endosulfan (0.07%) at 50 days were effective in reducing incidence of BYVM.

Utthasami et al. (1977) reported that application of aldicarb granules followed by endosulfan resulted in the reduction of BYVM incidence and enhanced fruit yield.

Basha and Balasubramanyam (1982) observed that the application of aldicarb (0.04 at 0.5 kg a.i./ha) on the 15th day of sowing followed by spraying with endosulfan (0.03 kg a.i./ha) twice on the 45th day and 60th day of sowing or spraying monocrotophos 0.04 per cent or endosulfan 0.05 per cent four times at fortnightly intervals commencing from 15 days after sowing were found to be effective in reducing the yellow vein mosaic disease of bhendi.

Control through resistance : In 1962, Singh et al. conducted experiments on breeding for field resistance to BYVM disease. Inheritance studies in the crosses between IC 1542 as the resistant parent and 'Pusa Makhmali' S 91 and S 72 as the susceptible parents, suggest that 2 loci are involved, the presence of dominant alleles at both loci being necessary for causing susceptibility for the disease. The field resistance variety IC 1542 could then be assigned the genotype YV_1, YV_1, YV_2, YV_2 and the susceptible parents Yv_1, Yv_1, Yv_2 and Yv_2. The field resistance is shown by variety IC 1542, and Pusa Sawani is a symptomless carrier.

Sandhu et al. (1974) studied the sources of resistance to jassid and whitefly in bhendi germplasm. Of ninetyfour lines of bhendi germplasm tested, three lines, viz., IC 1542, selection 1-1, selection 2-2, exhibited resistance to BYVM transmitted by B.tabaci. A.manihot, a wild species from Ghana, was almost immune to BYVM disease and also resistant to jassid.

Arumugam and Muthukrishnan (1978) found free amino acids in the resistance mechanism of BYVM disease. Leaf analysis of parents and F_1 differing in reaction to BYVM virus showed that glycine, histidine and some unidentified amino acids were present in substantial amounts only in the resistant plants and asparagine only in the susceptible ones.

Singh and Thakur (1979) studied the nature of resistance to BYVM in A.manihot manihot. The following hybrids were used in the experiment.

1. A.esculentus (L.) Moench cv. Pusa Sawani and Pusa Makhmali.

2. A.manihot (L.) medicus sub sp. manihot.

3. F_1 (A.esculentus and Pusa Sawani x A.manihot sub sp. manihot.

Results indicated that the F_1 and species A.manihot acted as symptomless carriers.

In crosses between 2 susceptible varieties of bhendi and 2 resistant forms of A.manihot, resistant to BYVM was not associated with any independent economic character, indicating the scope for effective selection (Arumugam and Muthukrishnan, 1979).

On crossing the wild species A.manihot and A.manihot sub sp. manihot, both resistant to BYVMV, with the susceptible A.esculentus cv Pusa Sawani; the hybrids were resistant and partially fertile. Resistance was shown to be controlled by a single dominant gene (Jambhale and Nerkar, 1981).

Sharma and Sharma (1981) found a high degree of resistance to the symptomless carrier type in bhendi (A.manihot sub sp. manihot). Resistance was found to be dominant over susceptibility and has been incorporated into the susceptible cultivars of bhendi. The resistance appears to be influenced by temperature. The new lines evolved in addition to resistance are also superior in horticultural traits as compared with the commercially cultivated variety 'Pusa Sawani'.

Varma and Mukherjee (1955) tested 43 genotypes for BYVMV and some showed resistance to the virus. Three types of symptoms, viz., yellow vein, yellow lamina and green mosaic, were observed on the genotypes. Of 37 varieties tested for BYVMV resistance, none were found to be resistant. However, 3 lines, viz., F_3, L_1 bulk and AB7 were found less susceptible recording less than 10 per cent infection (Gunathilagraj et al., 1977).

Rhizosphere microflora : A higher population of Rotylenchus reniformis (Reniform nematode) was observed in the rhizosphere of yellow mosaic infected bhendi plants (Shivakumar and Merenazamuddin, 1973).

Singh and Tiwari (1979) observed that the rhizosphere of healthy bhendi plants contained higher fungal populations at pre-flowering and post-flowering stages than plants infected with BYVMV. The differences in the rhizosphere of healthy and diseased plants during flowering seemed to be due to a change in the C/N ratio and in amino acids due virus infection.

Physiological changes due to BYVMV infection

Photosynthesis: Mandahar and Singh (1971) observed that both the chlorophylls a and b were destroyed by BYVMV, cucurbit mosaic virus and pumpkin mosaic virus. Ramiah et al. (1972) reported that the chlorophyll content (a, b and total) of the leaves decreased markedly due to BYVMV

infection. The reduction in the chlorophyll content was interpreted to be due to the enhancement of chlorophyllase enzymatic activity. Xanthophyll and carotene content was also reduced due to infection.

Enzymatic activity : Ramiah et al. (1973a) reported that the catalase and peroxidase activity increased in the leaves of BYVMV infected plants. Polyphenol oxidase activity markedly decreased. The ascorbic acid oxidase activity decreased initially, but increased in severely infected leaves.

Proteins : Singh and Srivastava (1974) reported that the fruits of BYVMV infected plants showed a significantly higher amount of protein than the healthy plants. However, the total amino acid content decreased with the age of infection. Jamal et al. (1975) reported that total proteins increased in the BYVMV infected cultivar Pusa Sawani.

Nucleic acids : Jamal et al. (1975) reported that the RNA content rose in BYVMV infected bhendi plants, while there was a fall in the DNA content.

Phenols and flavenoids : Arumugam and Muthukrishnan (1977) tested 2 bhendi cultivars susceptible to BYVMV, and 2 A.manihot cultivars (one immune to BYVMV and the other, a symptom less carrier), for their phenolic and flavenoid content. They reported that the phenolic and flavenoid contents were high in resistant parents and very low in the susceptible ones.

Minerals : Ramiah et al. (1973b) reported that in the leaves of BYVMV infected plants, phosphorus, potassium, magnesium, sodium, iron and sulphur content accumulated whereas the calcium content decreased.

Sugars and carbohydrates : BYVMV inoculated plants showed a lower content of total sugar. However, crude fibre and carbohydrate was recorded as higher than the healthy plants (Potty and Wilson, 1973). Singh and Srivastava (1974) reported that fruits from infected plants of bhendi contained significantly lower amounts of total reducing sugars and starch compared to those from healthy plants. Jamal et al. (1975). reported that tissues of BYVMV infected cultivar Pusa Sawani showed a reduction in total carbohydrates although the insoluble fraction was slightly increased.

Permeability alterations and ultrastructural changes : Kothari et al. (1981) worked on the permeability alterations and ultrastructural changes in yellow vein mosaic of bhendi. Electron micrographs of a symptomatic leaves (initial stage of pathogenesis) showed the disorganisation of cell structure including the loosening of microfibrillar tubules, of the cell wall, detachment of the plasma membrane from the cell wall, formation of vesicles near the plasma membrane, disorganisation of grana and the loosening of thylakoids, and the appearance of plastoglobuli in the cytoplasm. The mitochondria were unaffected. At the vein clearing stage (later stage of pathogenesis), the cytoplasm was highly

vacuolated and necrose, thylakoids were associated with multivesiculate bodies, formation of peramural bodies occurred with the degradation of osmeophilli globules. Mitochondria showed no changes in membranes, however, the cristae were swollen.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Survey

A survey was carried out in some of the bhendi growing areas of Bangalore, Kolar and Tumkur districts of Karnataka, to record the incidence of yellow vein mosaic disease, and where the bhendi crop is less than 0.5 acre the total number of plants and the infected plants were recorded. Where the plot size was one acre or more than one acre, twenty rows of 10 metre length were selected randomly and the total number of plants and infected plants were recorded to calculate the percentage of infection.

Raising healthy bhendi seedlings

Bhendi (local variety 'white velvet' Halebende) seeds were sown in earthen pots of size 9 x 6 inches or polythene bags of size 9 x 4 inches filled with farm yard manure. When the seedlings were 7-15 days old, they were used for various experiments in the laboratory.

BYVMV culture

The culture of the disease was obtained from the farmers fields near Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore and graft inoculated to healthy bhendi plants by using viruliferous whiteflies (*Bemisia tabaci*) in an insect proof glass house. The local bhendi variety white velvet (Halebende) was used in all the transmission experiments (unless otherwise stated).

Vector cultures

An aspirator comprising of a glass tube (30 cm length and 0.5 cm diameter) and a rubber tube of 40 cm length was used for the collection of whiteflies. The whiteflies were collected by turning the leaves slightly upwards, and they were sucked into the glass tube. The whiteflies (Fig. 1) were released on cotton plants (Gossypium hirsutum var. Lakshmi) kept in insect rearing cages and subsequently maintained on cotton by frequently introducing the young cotton seedlings into the rearing cage (Fig. 2).

Whitefly rearing cage

A wooden frame measuring 45 x 45 x 30 cm was constructed. Muslin cloth was fixed on three sides and on the top, with fevicol. The front side was covered with a glass door which could be easily moved on the grooves made in the wooden frame. This frame was fitted on a wooden rectangular base (45.5 x 45.5 x 10 cm). Potted cotton plants were kept inside the cage and the whiteflies were released.

Transmission

Whitefly transmission

Whiteflies were collected from rearing cages and released into polyvinyl chloride (PVC) tubes in which a BYVMV infected branch was inserted previously and allowed to feed for 24 hrs

Fig. 1. Whitefly Bemisia tabaci was maintained on cotton (Gossypium hirsutum cv. Varalakshmi) for experimental use.

Fig. 2. Rectangular wooden cage used for rearing the whitefly B.tabaci.

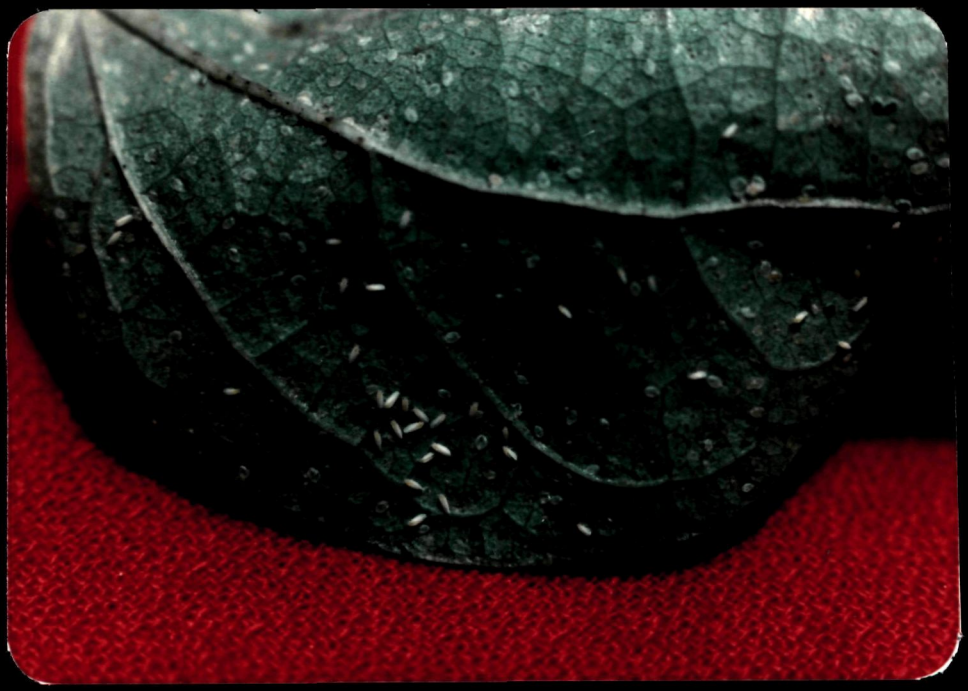


Fig. 1

Fig. 2

(acquisition access period) (Fig. 3). The viruliferous whiteflies were released onto healthy bhendi seedlings by using small plastic tubes and were allowed to feed for 24 hours (inoculation access period). After inoculation, the plants were removed, and sprayed with 0.1 per cent malathion or 0.1 per cent dimethoate to kill all the whiteflies. The inoculated plants were kept in the glass house for symptom production.

Graft transmission

Side grafting method was employed. The diseased scions from BYVMV infected plants were made into a 'V' shaped structure. The scions were inserted into slanting cuts made on the stock plants of bhendi.

The grafted portion was tied with a polythene strip and the scion was covered with a polythene bag. The inoculated plants were kept in a cool place in the glass house for symptom production.

Mechanical transmission

The BYVMV infected leaves were harvested 15 days after inoculation and macerated in a pestle and mortar by adding 2 ml/g of 0.1 M phosphate buffer, pH 7.0, containing 1 per cent 2-mercaptoethanol. The resulting pulp was strained through a muslin cloth. Carborundum was spread on the upper surface of healthy bhendi leaves and inoculation was made by

Fig. 3. Round polyvinyl chloride (PVC) cage used in various transmission experiments. Whitefly B.tabaci was released on BYVMV infected bhendi plants for acquisition access.

Fig. 3

rubbing the surface of leaves with cotton dipped in the extract (inoculum). Excess inoculum was washed with water using a wash-bottle and the inoculated plants were kept in an insect-proof glass house for symptom production. Different host plants were inoculated.

Host range

Host range studies were conducted by raising seedlings of various plant species through seeds and inoculating them with BYVMV through the agent B.tabaci. Whiteflies were given a 24 hr acquisition access and 48 hr inoculation access periods. The inoculated seedlings were kept in a glass house for symptom production. The plant species that did not produce any symptoms were back indexed on healthy bhendi seedlings to know if there were any symptomless carriers.

Epidemiology

Fortnightly sowings were done starting from January, 1985 to December, 1985 at the Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore. The local variety 'white velvet' (Halebende) was used. Sowing was carried out at a spacing of 60 x 30 cm in an area of 10 x 15 feet and replicated thrice. The incidence of the disease was recorded at 4, 8, 12 and 14 weeks after sowing.

Whitefly population in relation to sowing dates

Fortnightly sowings were done as described under disease incidence in relation to sowing dates. The number of adults B.tabaci were recorded at 4, 8, 12 and 14 weeks after sowing by direct counting on the undersurface of the leaves and in a yellow water pan (diameter 15 cm), from January, 1985 to December, 1985. In direct counting, 40 leaves were examined for adults in each replication. Yellow water pans were kept in the centre of each plot from 8 a.m. to 5 p.m. (Fig. 4). The number of whiteflies fallen into the water pan were counted at 5 p.m.

Transmission of different whitefly transmitted viruses to bhendi

Whiteflies (B.tabaci) were given a 24 hr acquisition access period on Tomato leaf curl virus, Horsegram yellow mosaic virus, Malvastrum yellow vein mosaic virus, and Croton yellow vein mosaic virus infected plants separately, and they were released on healthy bhendi seedlings for 48 hrs. The inoculated plants were kept in the glass house for symptom production.

Biology of B.tabaci on bhendi

Twenty pairs of whiteflies (twenty males and twenty females) were released into a small plastic cage. A single leaf was inserted into the cage and the hole was plugged with

ig. 4. Yellow plastic pan with water kept in the centre of a bhendi plot for monitoring whitefly population.

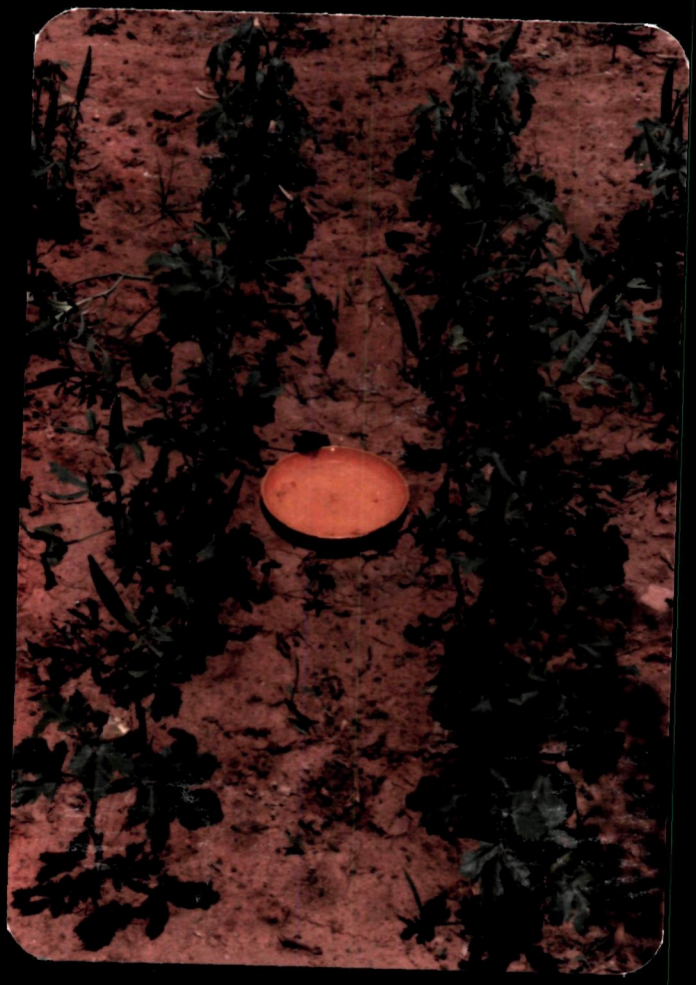


Fig. 4

cotton. Whiteflies were allowed to oviposition on the undersurface of leaves for 72 hr on 10 leaves and the adults were removed. The eggs laid were counted and were examined daily for hatching. The development of 1st, 2nd and 3rd instars of B.tabaci and pupae were daily examined under a stereobinocular microscope. The number of adults emerged and the adult longevity were also recorded. The variation in the fecundity and duration of different stages of B.tabaci were recorded.

Relative attractiveness of different host plants to the whitefly B.tabaci

One month old seedlings of different host plants were kept in a small glass chamber (10 ft x 10 ft x 15 ft). About 1000 whiteflies were released into the chamber. The whiteflies were allowed to settle on the plants. Twenty four, 48 and 72 hours after release, the whiteflies were counted on different host plants separately.

Purification of BYVMV

Purification methods described for Bean golden mosaic virus (Goodman et al., 1977) and horsegram yellow mosaic virus (Muniyappa et al., 1986, in press) were employed (with slight modifications) for the purification of virus from yellow mosaic infected bhendi plants. Bhendi seedlings were inoculated soon after germination with viruliferous whiteflies. First or second leaves showing typical yellow vein mosaic

symptoms were harvested 10-15 days after inoculation. The infected leaves were homogenized in a blender with 0.1 M potassium phosphate buffer, pH 7.5 containing 1 per cent, 2-mercaptoethanol, 10 mM sodium ethylene diamine tetra acetate (EDTA) and 1 mM cysteine hydrochloride. Three-four ml of buffer was added per gram of leaf tissue. The extract was strained through a cheese cloth and clarified at 10,000 rpm for 20 minutes. To the supernatent, polyethylene glycol (PEG 6%) and sodium chloride (0.2 M) were added and the mixture was kept at 4°C for 3 hr and then centrifuged at 10,000 rpm for 20 minutes. The pellets were dissolved in 0.02 M phosphate buffer, pH 7.5 (0.5 ml/gm of tissue) and clarified at 10,000 rpm for 10 min. Triton X-100 (0.5%) was added and the mixture gently stirred. Twentyfive ml of the extract were layered on a 13 ml column of 10 per cent sucrose prepared in 0.02 M phosphate buffer, pH 7.5 and centrifuged at 25,000 rpm for 3 hr in a Beckman SW 28 rotor, and the pellets were resuspended in phosphate buffer. The suspension was subjected to density gradient centrifugation. The gradient columns were prepared by layering 6, 9, 9 and 9 ml of 100, 200, 300 and 400 gm/L of sucrose in phosphate buffer (0.02 M) and then stored overnight at 4°C before use. Five ml of virus preparation was layered on each gradient column and centrifuged at 25,000 rpm for 3 hr in an SW 28 rotor. The fractions were collected and screened in a UV spectrophotometer. The fractions which showed maximum absorbance at 260 mm were pooled and centrifuged at 36,000 rpm for 3 hr. The pellets were dissolved in 0.5-1 ml of phosphate buffer.

The purification method used for okra mosaic virus (Givord and Hirth, 1973)was also attempted, for purification of BYVMV. Two hundred grams of frozen diseased leaves were homogenised in a waring blender with 1.5 volume of 0.01 M sodium phosphate buffer, pH 7, containing sodium bisulfite, 0.5 per cent, 1 per cent bentonite and 10 mg magnesium chloride. After being filtered through a cheese cloth, the filterate was centrifuged for 20 min at 12,000 g. The supernatant was then emulsified for 5 min. with 0.5 volume of n-butanol and incubated for 1 hr at 4°C and then centrifuged for 5 min. at 1500 g. The aqueous phase was centrifuged for 10 min at 8000 g. The supernatant collected was centrifuged for 120 min at 100,000 g. The pellet obtained was resuspended overnight in 0.1 M sodium phosphate buffer (pH 7.3) containing 0.01 M EDTA. The suspension was further centrifuged for 20 minutes at 12,000 g. The supernatant was then centrifuged for 120 min. at 100,000 g. The pellet obtained was resuspended overnight in 0.01 M phosphate buffer pH 7, using 1/10th of the initial volume. The supernatant was centrifuged for 20 min at 12,000 g. The virus sample was negatively stained with 2 per cent uranyl acetate, pH 3.7 and examined in a Philips 301 C electron microscope. Particle diameter measurements were made on micrographs.

Ultramicrotomy

Small pieces of infected tissue (2 mm x 5 mm) mainly the main and secondary veins were fixed in 1 per cent glutaraldehyde in phosphate buffer (PB, 0.1M) pH 7.2 for 3-4 hr

at 4°C. After fixation, the tissues were washed 4 times in PB for 10 min each. The tissue was then transferred to 2 per cent osmium tetroxide in 0.1 M PB. After post fixation the material was washed 4 times in the buffer for 10 min each.

The specimen was dehydrated in 25, 50, 75 per cent acetone for 15 min each at 4°C and twice in 100 per cent acetone at room temperature.

The material was infiltrated in 2:1 ratio followed by 1:2 acetone : resin mixture (without an accelerator) for 30 min each and then transferred to the pure resin mixture and kept overnight.

The material was embedded in spurrmedium in a rubber mould and polymerised at 70°C for 8 hr.

Composition of spurrmixture (Spurr, 1968)

ERL 4206	(Vinyl cyclohexene dioxide)	10 gm
DER 736	(diglycidyl ether of polpropylene glycol)	6.0 gm
NSA	(nonenyl Succinic anhydride)	26.0 gm
SI	(dimethyl amino ethanol) or DMAE	0.4 gm

After proper polymerisation, the blocks were trimmed to a pyramid shape to expose the embedded tissue for sectioning. About 600-700 A thickness sections were taken in Ultracut microtome using sharp glass knives. The sections were

collected on copper grids. The thin sections collected on grids were stained with 2 per cent uranyl acetate and sodium citrate. The samples were examined in JEOL 100 CX electron microscope.

Serological relationships

Double diffusion gel technique

Serological relationships of purified BYVMV with other plant viruses like Belladonna mottled virus (BDMV), cucumber mosaic virus (CMV) and okra mosaic virus (OMV) was tested by the agar gel double diffusion technique using 1 per cent agar in 0.1 M phosphate buffer. The purified virus was placed in the central well and antibodies to CMV, BDMV and DMV were placed in the outer wells. The samples were allowed to diffuse overnight and examined for precipitin lines the next day.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the experiments on the studies of bhendi yellow vein mosaic virus disease are presented in the following pages.

1. Survey for the incidence of the disease

A survey was undertaken to assess the incidence of bhendi yellow vein mosaic virus disease in some of the bhendi growing areas of Bangalore, Kolar and Tumkur districts. Incidence of the disease was recorded and data were presented in Table 1. The data indicated that the percentage of BYVMV varied with the season.

The incidence of disease varied from 1.8 per cent to 80 per cent depending on the season in which the crop was grown. Incidence was high in April-May and low in August-October sown crops. Some of the plots surveyed were very severely infected (Fig. 5, 6).

Mechanical transmission

Mechanical transmission was attempted to find out whether the BYVMV is transmissible and to know if there are any local lesion hosts. Different host species were inoculated with BYVMV as described under materials and methods. None of the inoculated plants showed any symptoms (Table 2).

5. Bhendi var. Pusa Sawani was infected with BYVMV under field conditions. Young stage of the crop is being infected.

6. Bhendi var. Pusa Sawani was infected with BYVMV under field conditions. Infected plants produced a few pods.

Fig. 5

Fig. 6

Graft transmission

BYVMV was transmitted by grafting from infected bhendi to healthy bhendi. Eighty seven to 100 per cent transmission was observed. Inoculated plants took 15-20 days to show the symptoms (Table 3).

Whitefly transmission

Whitefly Bemisia tabaci was tested for transmission of BYVMV as described under materials and methods. B.tabaci transmitted BYVMV to bhendi and the per cent transmission was found upto 100 per cent. Symptoms appeared 10-15 days after inoculation through B.tabaci (Table 4; Figs. 7, 8).

Membrane feeding

Whiteflies fed through parafilm membrane on the infected extract and partially purified virus sample were inoculated on healthy bhendi plants for 48 hrs. None of the inoculated plants showed symptoms (Table 5).

Host range

In order to determine the host range of BYVMV different host species were inoculated with B.tabaci, as described under materials and methods. The inoculated plants were kept under observation for 2-3 months in a glass house. The results of the host range are presented in Table 6. Of 31 plant species inoculated, Euphorbia sp. and Althaea rosea (Fig. 9) were

Fig. 7. Bhendi infected with BYVMV showing yellow vein mosaic and slight reduction in leaf size.

Fig. 8. Bhendi leaves showing bright yellow vein mosaic symptoms after inoculation with BYVMV.

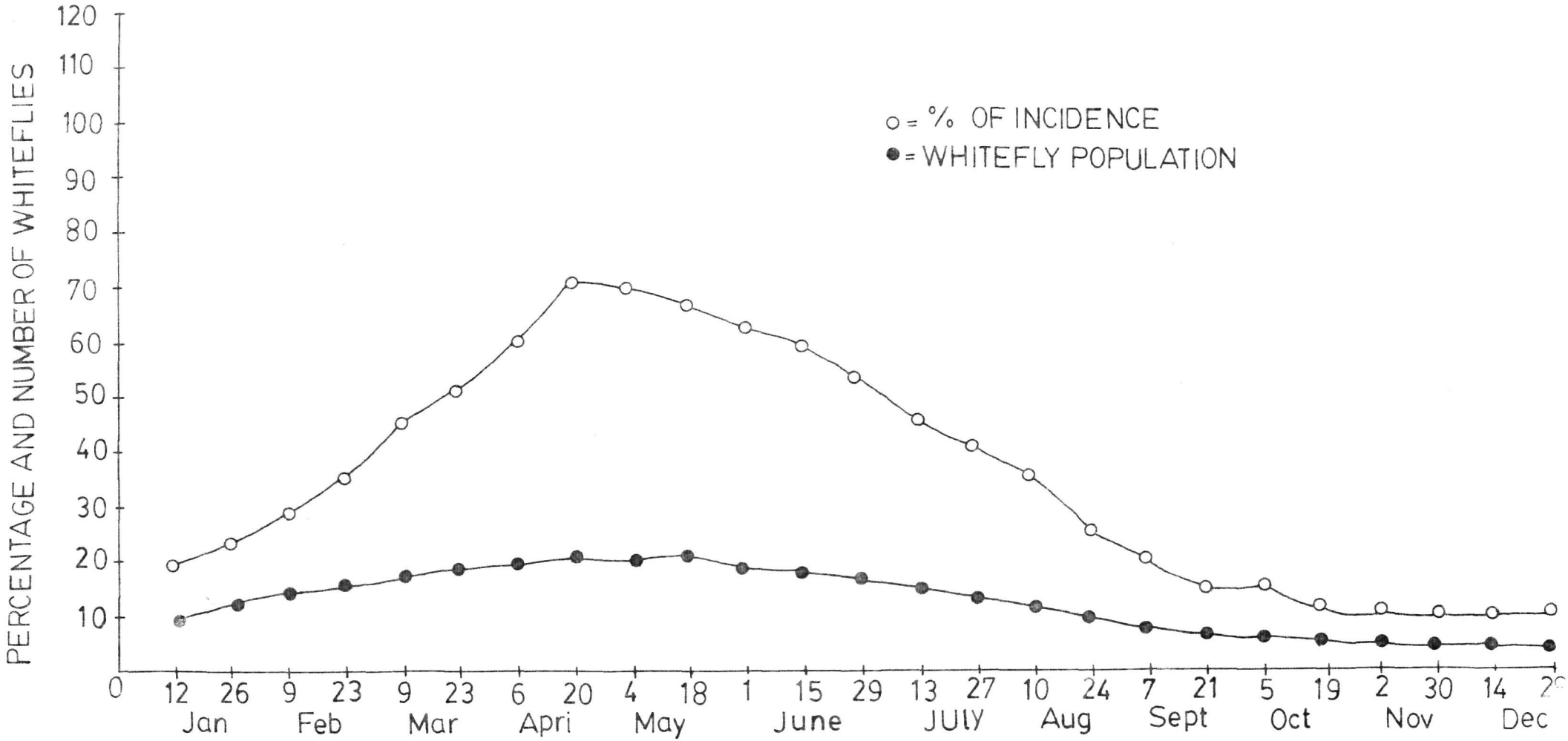
Fig. 9. *Althaea rosea* showing symptoms of leaf curling and crinkling after inoculation with BYVMV.

Fig. 9

suspected to be infected with BYVMV. Crinkling, curling and vein thickening were the symptoms seen on these plants, which are different from the typical BYVMV symptoms.

Disease incidence and vector population in relation to sowing dates

The data on the effect of fortnightly sowing on bhendi yellow vein mosaic incidence are presented in Table 7. The incidence varied from less than one per cent to 60 per cent depending on the season. Crops sown from January to May showed high incidence (21-60%). Crops sown from late June to November showed low incidence, while the crops sown in early June and December showed moderate incidence (21-25%). The spread of the disease was slow upto 4 weeks and Moderate from 4th to 8th week. The incidence rapidly increased from 8th to 14th week. In March sown crops (23-3-85) the incidence upto 4 weeks, the incidence from 4 to 8th week, 8-12th week and 12-14th week was 3.9, 20.10, 47.6 and 60.1 per cent respectively. The whitefly population was more in crops sown from January to early June and low from late June to early December. Whitefly population started increasing from late December and January reaching peaks in February, March, April and May, and then slowly decreased upto June. From July to November, the decrease in whitefly population was very rapid. Whitefly population was correlated with the disease incidence (Table 7; Fig. 10).



FIG·10, INCIDENCE OF BYVMV IN BHENDI IN RELATION TO DATE OF SOWING AND WHITEFLY POPULATION

Weather data was collected from the meteorological observatory located in the Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore, where all the experiments were conducted during 1985. The data on maximum and minimum temperature, relative humidity and rainfall are presented in Table 8. High vector population was directly correlated with the high temperature in summer. The low whitefly population in late kharif and rabi was correlated with high rainfall, low temperature and high humidity.

Transmission of different whitefly transmitted viruses to bhendi, and to their respective host plants

The results reported in Table 9 indicates that the bhendi was not infected with tomato leaf curl, horsegram yellow mosaic, Malvastrum yellow vein mosaic, Croton yellow vein mosaic and cassava mosaic viruses. Tomato leaf curl, limabean yellow mosaic, Malvastrum yellow vein mosaic and Croton yellow vein mosaic were transmitted by B.tabaci to tomato, limabean, Malvastrum and Croton respectively (Tables 10, 11, 12). Though the symptoms on Malvastrum and Croton were similar to symptoms of bhendi yellow vein mosaic virus, they were not transmitted to bhendi (Tables 10, 11, 12).

Biology of B.tabaci on bhendi

Twenty pairs of adult whiteflies were released on bhendi and cotton plants and allowed to oviposit for 72 hr as described under materials and methods. Fecundity, duration of each stage and survival rate were recorded, and results are presented in Tables 13 and 14.

The duration of eggs, 1st, 2nd, 3rd instar nymphs, pupae and adults as well as egg to adult was found to be 4-6, 3-5, 2-5, 2-4, 3-6, 8-21 and 21-49 days respectively on bhendi. The total duration of egg to adult in cotton was 20-41 days (Table 14). Nymphal instars and adult duration was more on bhendi compared to cotton. The number of eggs laid was high on cotton and less in bhendi. When 20 pairs of whiteflies were released per leaf for 72 hr on bhendi and cotton, the whiteflies laid 321 eggs on cotton and 101 eggs on bhendi. Cotton is therefore highly preferred for egg laying. In bhendi, out of 101 eggs, 85 eggs hatched and became 1st instar nymphs. Finally, 42 adults were obtained. There was mortality at each stage. Mortality of each stage was higher in bhendi compared to cotton (Table 14).

Relative attractiveness of different host plants to whitefly B.tabaci

One-and-a-half month old seedlings of bhendi, A.conyzoides, C.sparsiflorus, cotton, horsegram, Malvastrum and tomato were kept in a small glass house room (10 ft x 10 ft x 15 ft). About 1000 whiteflies were released into the room. Twentyfour, 48 and 72 hrs after release, the whiteflies were counted on the hosts separately. The data are presented in Table 15. The results revealed that cotton (185), horsegram (158), Ageratum (128) and tomato (132) attracted more adult whiteflies, while

Malvastrum (65), Croton (56) and bhendi (54) attracted less whiteflies. Bhendi attracted the least number of whiteflies. Results indicate that bhendi is not a preferred host in the presence of several other host plants.

In addition to keeping several plants together, cotton and bhendi, horsegram and bhendi, tomato and bhendi, Ageratum and bhendi, Croton and bhendi and Malvastrum and bhendi were seperately kept in glass chambers and about 200 whiteflies were released and counted. The numbers of whiteflies on bhendi and other host plants were recorded after 24, 48 and 72 hrs. The results are presented in Table 16, 17, 18, 19, 20 and 21. The results revealed that in all the experiments bhendi could not attract many adult whiteflies. Whether the bhendi plant is kept along with many host plants or individual host plants, it attracted less whiteflies. When all the host plants are removed, the whiteflies settled on bhendi (Table 22).

Relative attractiveness of infected and healthy bhendi to B.tabaci

When whiteflies were released on healthy and infected plants of bhendi, the yellow vein mosaic infected plants attracted more whiteflies (95 after 72 hr) compared to healthy plants (36 after 72 hr) (Table 23).

Purification

Partially purified preparations were made from bhendi plants 15 days after inoculation with BYVMV (Fig. 11). Bhendi

Fig. 11. Inoculation of bhendi seedlings with BIVMV for purification of virus. Leaves were harvested 15 days after inoculation.

Fig.11

leaves contain abundant quantity of mucilage which makes extraction more difficult. Concentration of virus was measured by spectrophotometric observation of virus zones following sucrose density gradient centrifugation (Fig. 12).

The virus concentration seems to be very low (1-2 mg/ml/300 g of virus sample). The fractions that showed a peak absorbance at 260 nm were pooled and pelleted. Treatment of extracts with 0.5 per cent Triton X-100 before layering on sucrose cushion resulted in a virus preparation with less host material.

Electron microscopy

Electron microscopy of purified virus preparations showed spherical virus particles 20-25 nm size (Fig. 13,14). Uranyl acetate was used for staining the particles. However, ultra-thin sections observed under the electron microscope did not show any virus particles.

Serological relationships

In the agar gel double diffusion method partial purified BYVMV failed to react with okra mosaic virus (obtained from Dr. L. Givord), Belladonna mottle virus and cucumber mosaic virus antisera (obtained from Dr. H.S. Savithri).

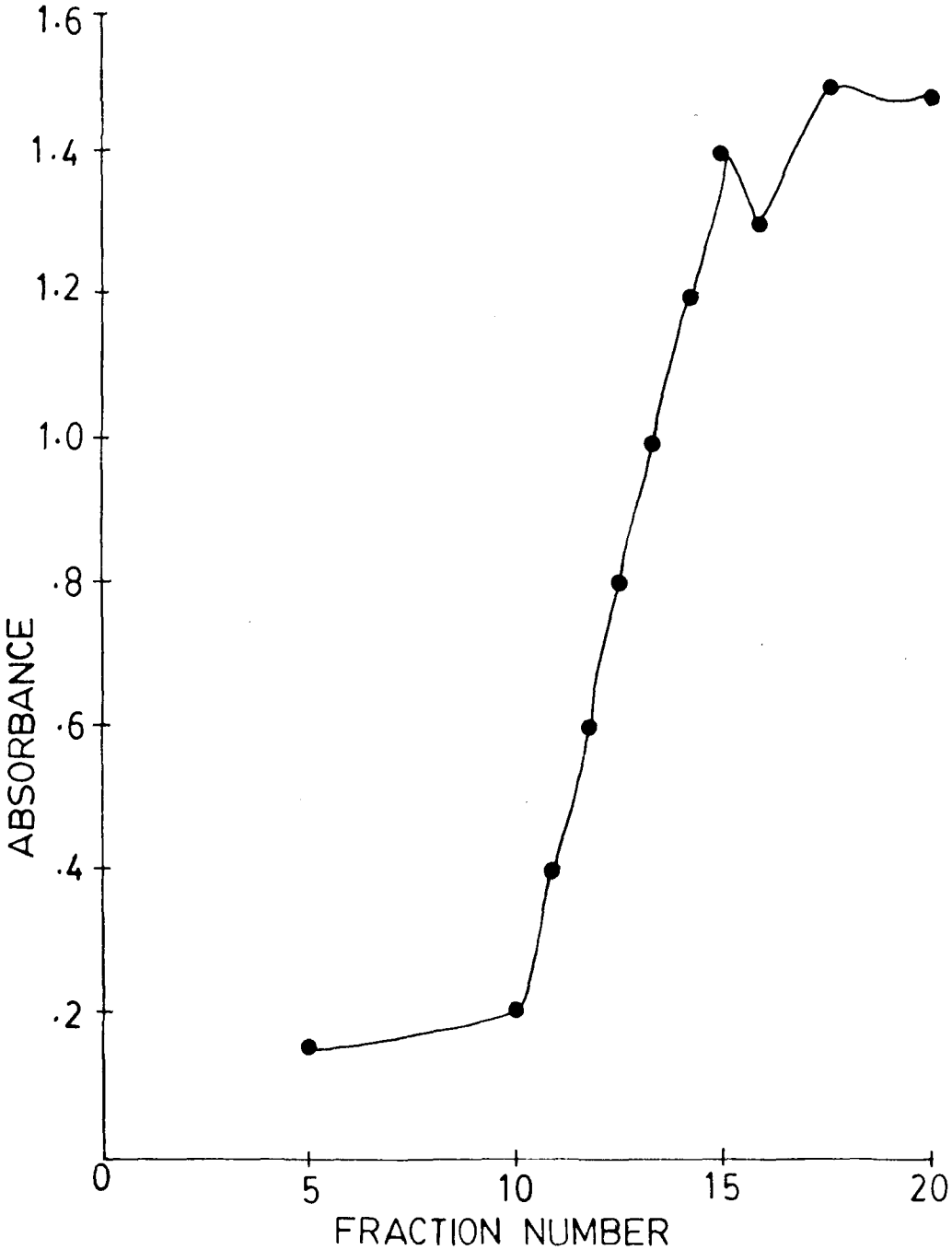


FIG.12, SPECTROPHOTOMETRIC OBSERVATION OF VIRUS ZONES FOLLOWING SUCROSE DENSITY GRADIENT CENTRIFUGATION

Fig. 13. Electron micrograph of BYVMV particles measuring 20-25nm under EM.

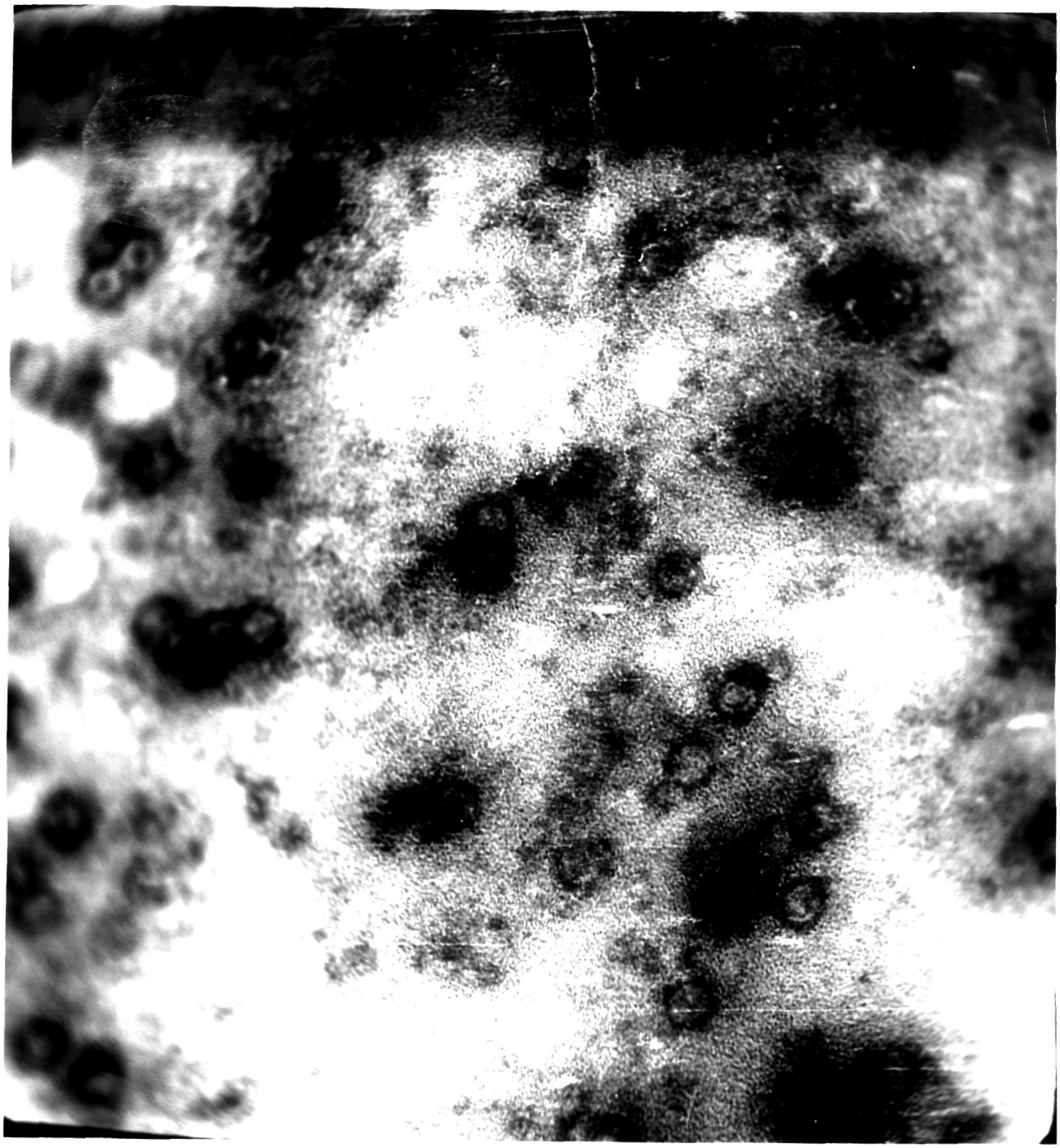


Fig.13

Fig. 14. Electron micrograph of BYVMV particles measuring 20-25mm under EM.

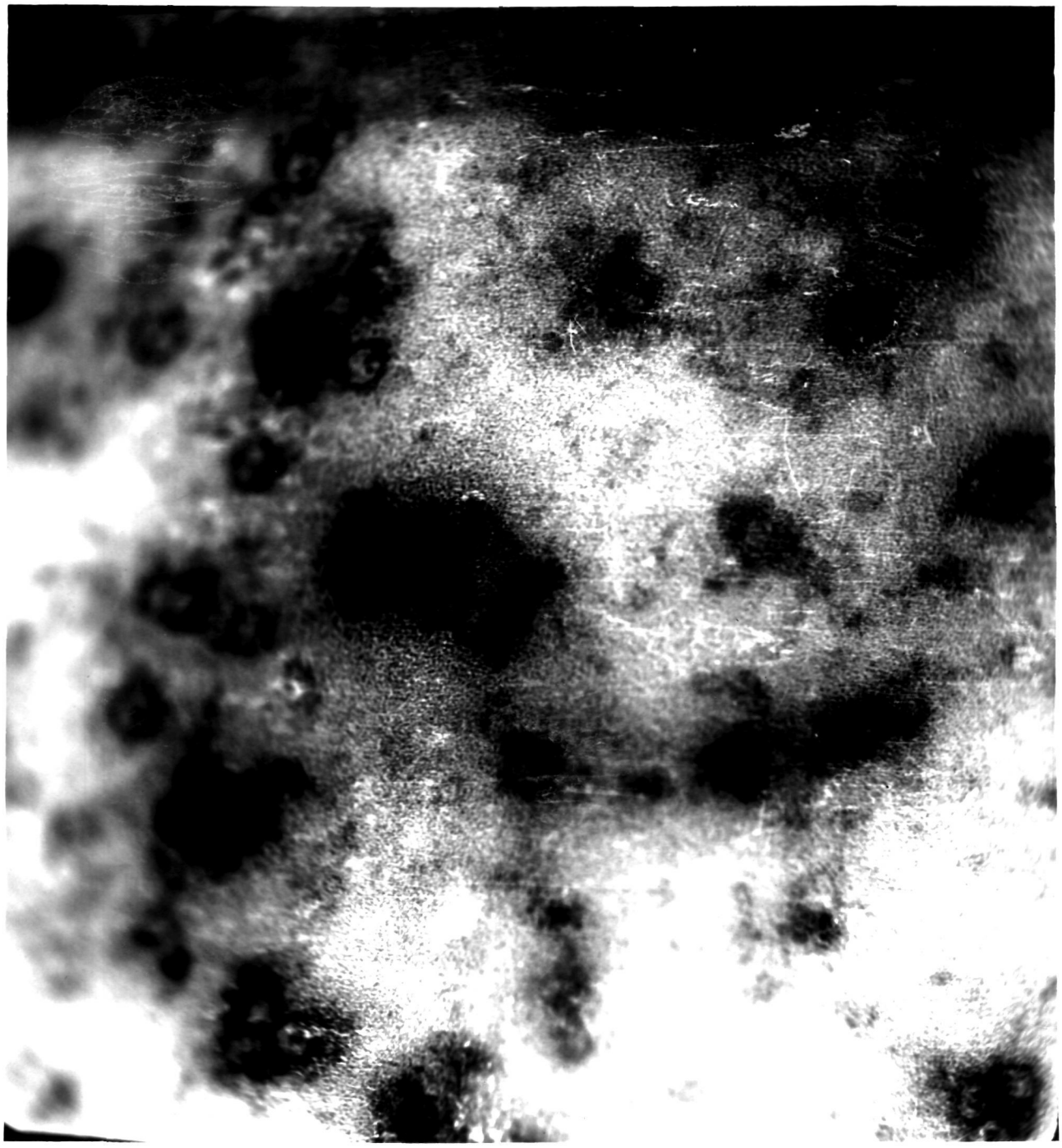


Fig.14

Table 1. Incidence of BYVMV in different parts of Karnataka

District	Taluk	Variety	Date of sowing	Month of recording	Area cultivated (acres)	Per cent incidence
1	2	3	4	5	6	7
Bangalore	Doddaballapura	Pusa Sawani	22-4-85	June	0.50	65.00
	Doddaballapura	Local	7-5-85	July	1.00	35.50
	Hosakote	Pusa Sawani	Aug. 85	Nove. 85	0.50	4.50
	Nelamangala	Pusa Sawani	1-6-85	August	0.50	12.00[a]
	Nelamangala	Pusa Sawani	15-5-85	July	0.50	21.00[a]
	Nelamangala	Pusa Sawani	15-5-85	July	0.75	11.50[a]
	Kengeri	Pusa Sawani	7-5-85	July	1.00	80.00
	Kengeri	Pusa Sawani	15-5-85	July	1.00	70.00
	Devanahalli	Pusa Sawani	10-5-85	July	1.00	60.50
	Devanahalli	Pusa Sawani	15-5-85	July	0.50	40.50
	Magadi	Pusa Sawani	10-5-85	July	0.75	25.00
	Magadi	Pusa Sawani	5-5-85	July	0.50	20.50
Tumkur	Tumkur	Pusa Sawani	25-5-85	August	0.50	70.00

Table contd.. 1).

1	2	3	4	5	6	7
Kolar	Gouribidanur	Pusa Sawani	14-5-85	July	0.50	21.80
	Gouribidanur	Pusa Sawani	10-5-85	July	0.50	60.00
	Gouribidanur	Pusa Sawani	11-5-85	July	0.75	50.00
	Gouribidanur	Pusa Sawani	13-5-85	August	0.50	20.50[a]
	Kolar town	Pusa Sawani	21-5-85	August	0.50	45.40[a]
	Kolar	Halebende	Oct.85	Dec. 85	1.00	2.50
	Kolar	Local variety	Sept.85	Nov. 85	0.50	1.00
	Srinivaspure	Local variety	Oct. 85	Dec.85	0.50	3.00
	Malur	Halebende	Aug. 85	Nov.85	0.50	3.50

[a]Sprayed twice with rogor or metecid one month after sowing.

Table 2. Sap inoculation of BYVMV to different host plants

Host inoculated [a]	No. infected / No. inoculated	Per cent infection
Abelmoschus esculentus	0/25	0
Chenopodium amaranticolor	0/25	0
Croton sparsiflorus	0/25	0
Datura stramonium	0/60	0
Euphorbia sp	0/20	0
Malvastrum coromandelianum	0/60	0
Nicotiana glutinosa	0/20	0
Phaseolus vulgaris cv. top crop	0/40	0

[a] 20-25 whiteflies (which were previously given 24 hr acquisition access period) were released per plant and allowed to feed for 24 hr.

Table 3. Graft transmission of BYVMV to bhendi.

Experiment	Number infected / Number inoculated[a]	Per cent transmission	Incubation period in days
1	35/40	87.50	15-20
2	20/20	100.00	15-20

[a]Only the graft established plants were infected.

Table 4. Whitefly transmission of BYVMV[a]

Number infected / Number inoculated[b]	Per cent transmission	Incubation period in days
50/50	100	10-15
80/80	100	10-17

[a]10 viruliferous whiteflies were transferred to each seedling.

[b]Whiteflies were given 24 hr acquisition and 24 hr inoculation access periods.

Table 5. Feeding whiteflies on the extract from the BIVMV infected leaves through a membrane and inoculating healthy bhendi plants.[a]

Experiment	Number infected / Number inoculated[b]	Per cent transmission	Incubation period in days
1	0/20	0	-
2	0/25	0	-

[a] 10 whiteflies were inoculated to each seedling.

Table 6. Host range of BYVMV

Host inoculated[a]	Family	No.infected / No.inoculated	per cent transmission	Incubation period in days
Abelmoschus esculentus	Malvaceae	25/25	100	10-15
Abelmoschus moschatus	Malvaceae	0/15	0	-
Acalypha indica	Malvaceae	0/20	0	-
Althaea rosea[b]	Malvaceae	1/10	10	20-35
Arachis hypogaea	Leguminosae	0/20	0	-
Ageratum conyzoides	Asteraceae	0/25	0	-
Cajanus cajan	Leguminosae	0/20	0	-
Capsicum annuum	Solanaceae	0/20	0	-
Carica papaya	Caricaceae	0/20	0	-
Catharanthus roseus	Apocynaceae	0/10	0	-
Croton sparsiflorus	Euphorbiaceae	0/20	0	-
Cucumis sativus	Cucurbitaceae	0/25	0	-
Euphorbia sp[b]	Euphorbiaceae	4/30	16	20-25
Gossypium hirsutum	Malvaceae	0/20	0	-
Glycine max	Leguminosae	0/30	0	-
Hibiscus rosa-sinensis	Malvaceae	0/20	0	-
Hibiscus sabdariffa	Malvaceae	0/20	0	-
Lablab purpureus	Leguminosae	0/20	0	-
Lycopersicon esculentum	Solanaceae	0/20	0	-
Macrotyloma triquetridatum	Malvaceae	0/20	0	-

Table 6 contd..)

Host inoculated[a]	Family	No.infected / No.inoculated	Per cent trans-mission	Incuba-tion period in days
Macrotyloma uniflorum	Leguminosae	0/20	0	-
Nicotiana glutinosa	Solanaceae	0/10	0	-
N.tabacum	Solanaceae	0/40	0	-
Phaseolus lunatus	Leguminosae	0/20	0	-
Sesamum indicum	Pedaliaceae	0/20	0	-
Phaseolus vulgaris	Leguminosae	0/20	0	-
Psophocarpus tetragonolobus	Leguminosae	0/20	0	-
Sida cordifolia	Malvaceae	0/20	0	-
Sonchus oleraceus		0/10	0	-
Vigna unguiculata	Leguminosae	0/20	0	-

[a]10-15 viruliferous whiteflies were released onto each plant.

[b]Crinkling of leaves.

Table 7. Incidence of BYVMV and whitefly population in relation to date of sowing

Date of sowing	Per cent incidence and whitefly population recorded weeks after sowing							
	4		8		12		14	
	BYVM	WF[a]	BYVM	WF[a]	BYVM	WF[a]	BYVM	WF[a]
12-01-85	1.50	2.45	10.00	7.00	30.00	8.50	35.50	4.00
26-01-85	2.10	3.50	14.00	6.00	31.00	9.00	37.50	4.50
9-02-85	2.80	3.00	20.50	6.50	48.00	8.75	56.00	6.00
23-02-85	3.10	3.50	19.50	6.25	47.50	7.50	54.30	4.50
9-03-85	4.00	3.25	21.50	7.25	46.00	8.00	55.00	4.00
23-03-85	3.90	4.50	20.10	6.00	47.60	7.50	60.10	5.00
6-04-85	4.50	4.00	18.50	7.25	43.00	8.00	58.00	4.00
20-04-85	4.80	5.00	17.50	5.50	41.10	7.50	27.00	3.50
4-05-85	3.70	4.50	15.00	5.50	33.00	6.50	36.00	3.00
18-5-85	3.10	3.50	14.50	5.75	27.50	6.50	29.50	2.75
1-06-85	2.90	2.50	10.70	4.50	19.50	5.50	21.30	1.75
15-06-85	1.90	2.00	8.10	3.00	19.40	4.50	22.00	2.00
29-06-85	1.50	2.00	7.60	2.00	17.00	3.50	19.50	2.25

Table 7 contd..)

Date of sowing	Per cent incidence and whitefly population recorded weeks after sowing							
	4		8		12		14	
	BYVM	WF[a]	BYVM	WF[a]	BYVM	WF[a]	BYVM	WF[a]
13-07-85	1.00	1.50	6.10	2.00	14.10	3.00	15.70	2.50
27-07-85	0.90	1.75	5.90	1.75	12.50	2.50	13.50	2.50
10-08-85	1.30	1.00	4.50	2.50	9.10	3.75	10.50	2.00
24-08-85	1.10	1.00	3.50	2.75	8.50	2.50	10.20	1.50
7-09-85	0.00	1.00	2.25	3.00	5.50	2.75	7.00	1.50
21-09-85	0.00	1.00	2.00	3.00	4.50	3.00	6.50	1.75
5-10-85	0.00	0.00	1.80	2.75	3.50	4.00	4.50	2.50
19-10-85	0.00	0.50	1.50	3.00	2.70	4.50	3.50	2.50
2-11-85	0.00	0.25	0.25	4.50	1.00	6.00	1.75	3.00
30-11-85	0.00	1.00	1.00	5.50	3.50	7.00	4.10	4.00
14-12-85	0.50	1.25	4.25	6.50	9.00	8.00	18.00	4.50
28-12-85	0.50	1.00	5.90	7.00	13.50	8.75	25.10	5.00

[a]Each figure is average of 40 leaves.

Table 8. Weather data in relation to whitefly population and incidence of BYVMV during 1985

Months	Temperature (°C)		Relative humidity(%)		Rain-fall (mm)
	Maximum	Minimum	720 hr	1420 hr	
January	28.60	14.00	86.00	40.00	-
February	30.60	15.70	77.03	32.64	30.60
March	33.40	17.20	74.19	30.00	0.40
April	34.20	21.90	77.00	34.00	-
May	37.30	21.50	74.00	38.00	-
June	29.40	20.00	87.00	54.00	-
July	28.40	19.40	92.00	60.00	-
August	28.76	19.60	90.00	57.20	-
September	28.99	19.66	91.00	59.00	-
October	27.82	17.98	90.00	54.00	-
November	26.16	16.15	90.20	50.40	-
December	27.18	16.44	89.23	51.23	-

Table 9. Transmission of whitefly transmitted viruses to bhendi

Name of virus	Host inoculated	No.of plants infected / No.of plants inoculated	Per cent transmission	Incubation period in days
Tomato leaf curl	Bhendi	0/20	0	-
Limabean yellow mosaic	Bhendi	0/20	0	-
Cassava mosaic	Bhendi	0/20	0	-
Malvastrum yellow vein mosaic	Bhendi	0/50	0	-
Croton yellow vein mosaic	Bhendi	0/50	0	-

Table 10. Transmission of Croton aparsiflorus yellow vein mosaic to bhendi and other host plants

Host inoculated[a]	Family	No. infected / No. inoculated	Per cent transmission	Incubation period in days
Abelmoschus esculentus	Malvaceae	0/30	0	-
Capsicum annuum	Solanaceae	0/25	0	-
Cassava manihot esculenta	Euphorbiaceae	0/20	0	-
Croton aparsiflorus[b]	Euphorbiaceae	20/20	100	7-10
Euphorbia sp	Euphorbiaceae	0/10	0	-
Lablab purpureus	Leguminosae	0/20	0	-
Macrotyloma uniflorus	Leguminosae	0/20	0	-
Phaseolus lunatus	Leguminosae	0/20	0	-
P. vulgaris	Leguminosae	0/20	0	-

[a] 10-15 viruliferous whiteflies were released on each plant.

[b] Yellow vein.

Table 11. Transmission of Malvastrum yellow vein mosaic to bhendi and other host plants

Host inoculated[a]	Family	No. infected / No. inoculated	Per cent transmission	Incubation period in days
Abelmoschus esculentus	Malvaceae	0/20	0	-
Arachis hypogaea	Leguminosae	0/10	0	-
Ageratum conyzoides	Asteraceae	0/20	0	-
Capsicum annuum	Solanaceae	0/20	0	-
Carica papaya	Caricaceae	0/10	0	-
Croton sparsiflorum	Euphorbiaceae	0/20	0	-
Lablab purpureus	Leguminosae	0/20	0	-
Lycopersicon esculentum	Solanaceae	0/20	0	-
Malvastrum coromandelianum	Malvaceae	25/25	100	7-10
Macrotyloma uniflorum	Leguminosae	0/20	0	-
Nicotiana glutinosa	Solanaceae	0/10	0	-
N. tabacum	Solanaceae	0/20	0	-
Sesamum indicum	Pedaliaceae	0/20	0	-

[a] 10-15 viruliferous whiteflies were released onto each seedling.

Table 12. Transmission of different whitefly transmitted viruses to their respective host plants.

Name of the virus	Host inoculated	No. of plants infected / No. of plants inoculated[a]	Per cent transmission	Incubation period in days
Bhendi yellow vein mosaic virus	Bhendi	10/10	100	10-15
Tomato leaf curl virus	Tomato	10/10	100	10-12
Horsegram yellow mosaic virus	Horsegram	10/10	100	7-10
Cassava mosaic virus	Cassava	0/10	0	-
Malvastrum yellow vein mosaic virus	*Malvastrum coromandelianum*	10/10	100	10-15
Croton yellow vein mosaic virus	*Croton sparsiflorus*	10/10	100	10-15

[a] 10-15 viruliferous whiteflies were released onto healthy seedlings

Table 13. Duration of egg, different instars and adults of *B. tabaci* on bhendi and cotton

Period	Crops[a]	Eggs	Duration (days)[b] Nymphal instars			Pupae	Adult	Egg to adult
			1st	2nd	3rd			
March 1985	Bhendi	4-6	3-5	2-5	2-6	3-6	8-21	21-49
April 1985	Cotton	3-4	3-5	2-4	2-4	3-5	7-19	20-41

[a]20 pairs of whiteflies were released per leaf for 48 hr.

[b]Average of 10 leaves.

Table 14. Fecundity and development of different stages of *B.tabaci* on bhendi and cotton

Period	Crop[a]	Eggs	Numbers[b] Nymphal instars			Pupae	Adults	Duration Egg to adult in days
			1st	2nd	3rd			
March 1985	Bhendi	101	85	70	53	50	42	21-49
April 1985	Cotton	321	290	265	243	230	205	19-41

[a]20 pairs of whiteflies released per leaf for 72 hr.

[b]Average of 10 leaves.

Table 15. Relative attractiveness of different host plants to whitefly *B.tabaci*[a]

Host[b]	Number of whiteflies recorded hour after release		
	24	48	72
Bhendi	54	59	45
Ageratum conyzoides	128	120	110
Croton sparsiflorus	56	51	47
Cotton	185	190	158
Horsegram	158	109	83
Malvastrum	65	61	53
Tomato	132	125	95

[a]Average of 2 experiments

[b]Approximately 1000 whiteflies were released into glass chamber.

Table 16. Relative attractiveness of cotton and bhendi to whitefly *B. tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
Cotton	184	142	123
Bhendi	15	22	19

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released into glass chamber.

Table 17. Relative attractiveness of horsegram and bhendi to whitefly *B.tabaci*[a]

Crop[b]	Number of whiteflies recorded hourse after release		
	24	48	72
Horsegram	155	123	78
Bhendi	28	31	15

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released into glass chamber.

Table 18. Relative attractiveness of tomato and bhendi to whitefly *B.tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
Tomato	127	136	119
Bhendi	48	26	24

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released into glass chamber.

Table 19. Relative attractiveness of *Ageratum conyzoides* and bhendi to whitefly *B. tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
Ageratum conyzoides	92	77	65
Bhendi	28	17	10

[a]Average of 2 experiments.

[b]Approximately 200 whiteflies were released into glass chamber.

Table 20. Relative attractiveness of *Croton sparsiflorus* and bhendi to whitefly *B. tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
Croton sparsiflorus	99	73	90
Bhendi	35	39	24

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released into the glass chamber.

Table 21. Relative attractiveness of *Malvastrum coromandelianum* and bhendi to whitefly *B. tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
Malvastrum coromandelianum	48	35	26
Bhendi	18	8	10

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released into the glass chamber.

Table 22. Relative attractiveness of bhendi to whitefly *B.tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
Bhendi	179	160	151

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released into the glass chamber.

Table 23. Relative attractiveness of infected and healthy bhendi to *B. tabaci*[a]

Crop[b]	Number of whiteflies recorded hours after release		
	24	48	72
BYVMV infected bhendi	125	120	95
Healthy bhendi	55	45	36

[a]Average of 2 experiments

[b]Approximately 200 whiteflies were released.

DISCUSSION

V. DISCUSSION

Bhendi yellow vein mosaic virus (BYVMV) is one of the most economically important diseases of bhendi in India. The disease was first reported in Maharastra (Kulkarni,1924). Though the disease is present in India since a very long time, no systematic efforts have been made to characterise the virus and to know the sources of infection. Many aspects of the disease are not well understood.

The present investigations therefore were undertaken on sources of infection, epidemiological aspects of the disease, purification of the virus and electron microscopy.

The survey conducted to assess the incidence of BYVMV in some bhendi growing areas of Bangalore, Kolar and Tumkur districts revealed that the percentage of incidence varied from season to season. The disease was found in all the bhendi fields surveyed. During the kharif season, the incidence varied from less than 1 to 15 per cent. The incidence was higher and increased markedly in summer months and the incidence ranged from 11 to 80 per cent (Table 1). Higher incidence during summer could be attributed to higher whitefly population (Table 7).

BYVMV infected plants exhibited yellow vein mosaic symptoms, vein clearing, slight curling of infected leaves and slight reduction in the leaf size.

Early infected plants were severely stunted. Infected plants produced few fruits of a small size. These symptoms were similar to those described by Fernando and Udurawan (1942) and Kulkarni (1924) and Uppal et al. (1940).

BIVMV was not transmissible by sap inoculation (Table 2). Earlier studies also proved that BIVMV was not sap transmissible (Raychaudhuri and Nariani, 1977). However, some of the whitefly transmitted diseases are transmitted by mechanical means. They are mosaic of Euphorbia prunifolia (Costa and Carvalho, 1960), cassava mosaic (Marchoux et al., 1960), bean golden mosaic virus (Meiners et al.,1975) and mungbean yellow mosaic disease (Honda et al., 1983). Failure to transmit mechanically in the present investigation may be due to various factors like the buffer combination, physiology of the test plant, virus concentration in the leaf samples and failure to reach the phloem tissue. It was observed that BIVMV was transmitted only by the whitefly Bemisia tabaci. Symptoms appeared within 10-15 days after inoculation by B.tabaci. Similar observations were also reported by various workers (Singh, 1980; Muniyappa and Veeresh, 1984; Varma,1952; Summanwar, 1980).

In order to know the sources of infection to bhendi, several host plants (Table 6) were inoculated with BIVMV by B.tabaci. BIVMV was not transmitted to tomato, Ageratum, Malvastrum, Cassava, Croton and horsegram. It is still not known as to how the bhendi crop gets infected in nature.

Experiments were carried out to know whether the yellow vein mosaic diseases present on Croton and Malvastrum were similar to the yellow vein mosaic of bhendi. Cross inoculations from Croton and Malvastrum to bhendi and from bhendi to Croton and Malvastrum did not result in any transmission (Tables 10, 11). Summanwar (1980) reported that Althaea roses was infected with BYVMV.

The season had a profound effect on the incidence of disease. The incidence was low when sowings were done from late June to early December and the incidence was high from late December to early June sown crops. Higher the population of the vector, higher was the incidence of the disease. BYVMV incidence varied with different seasons (Table 7). The whitefly population gradually increased from late December and reached a peak in February and March. The population then decreased gradually, reaching the lowest numbers from August to November.

High temperature, lower or no rainfall, and low humidity contributed to the increase in the vector population from January to May. Low whitefly population during the months of July to November was related to high rainfall, low temperature and high humidity. Similar results of seasonal variations of the whitefly population have been reported by Chelliah et al. (1975), Singh et al. (1977) and Singh (1980).

Preference of whiteflies to different host plants showed that the maximum number of eggs, nymphs, pupae and and adults were found on cotton, followed by horsegram. Ageratum, tomato, Malvastrum, Croton and bhendi in a decreasing order respectively.

Biology of B.tabaci on bhendi and cotton showed that the duration of eggs, 1st, 2nd, 3rd instar nymphs, pupae, and adults were found in 4-6, 3-5, 2-5, 2-4, 3-4, 8-21 and 21-49 days respectively on bhendi. Nymphal instars and adult duration was more on bhendi compared to that on cotton. Egg laying was better on cotton than on bhendi. Though the preference of whiteflies to bhendi is less, minimum number of whiteflies present in the field were enough to cause and spread the disease. Biology of B.tabaci was studied on different host plants by several workers (Pruthi and Samuel, 1942; Hussain and Trehan, 1933; Johnson et al., 1982; Murugesan and Chelliah, 1978; Saikia, 1985; Annapurna, 1985).

Under laboratory conditions, cotton, horsegram, Ageratum, and tomato attracted more whiteflies and Malvastrum, Croton and bhendi attracted a low numbers. In addition, more number of whiteflies were attracted to yellow mosaic infected plants. Vetten and Allen (1983) observed that B.tabaci preferred lima-bean golden mosaic susceptible P.lunatus, and more pupae of B.tabaci occurred in cowpea susceptible to cowpea golden mosaic. Oviposition of B.tabaci was higher on yellow mosaic

infected mungbean plants than on healthy plants (Murugesan and Chelliah, 1978). Annapurna (1985) also observed more oviposition on yellow mosaic infected legume plants than on healthy plants.

Attempts to purify BYVMV from bhendi was successful and spherical particles 20-25 nm diameter were observed in the electron microscope. Several methods were employed for the initial purification. Because of mucilage in the tissue, the extraction was more difficult. It is assumed that some virus is trapped in the mucilage. Efforts to break mucilage in the initial stages might help in the better recovery of virus particles. Several workers have attempted to purify the virus from BYVMV infected plants but have failed to do so. Many of the whitefly transmitted yellow mosaic diseases have been shown to be caused by geminate viruses (Goodman). It is not clear from the electro microscopy whether BYVMV also is a geminate virus. Preliminary experiments on serological identity of the virus have suggested that BYVMV is neither a Tymovirus nor a cucumo virus. However the lack of cross reactivity with antiserum for EDMV and CMV could also be due to the low concentration BYVMV used. Further experiments using more concentrated BYVMV and antisera to other geminate viruses would be necessary for complete identification.

The purification of BYVMV would help in producing antiserum which is useful in detecting the BYVMV antigen in host and vector tissues. It would also be useful in

epidemiology and testing germplasm for the presence of BYVMV infection.

Conclusions

BYVMV is one of the main constraints in the cultivation of bhendi in India. The spread of the disease is related to season and abundance of vector population. The disease is severe in summer and early kharif and low during the late kharif and rabi seasons in Karnataka.

Integrated control program has to be evolved for effective management of the disease. In addition, resistant sources for the disease have to be identified and incorporated in the breeding for disease resistance.

How a bhendi crop gets infected in the field is still not known. There may be host plants infected with BYVMV which may serve as sources of infection to the bhendi crop. This is very vital in understanding the epidemiology and control of the disease.

In antiserum is produced; for the BYVMV several host plants artificially inoculated and also plants from fields could be tested for the presence of BYVMV infection. Even symptomless carriers could be identified.

We have been successful in the partial purification of BYVMV and have identified virus particles in the electron micrographs for the first time. This would help in further characterisation the virus.

SUMMARY

VI. SUMMARY

The results of the studies of bhendi yellow vein mosaic virus are summarised hereunder:

1. Typical yellow vein mosaic symptoms were observed in bhendi plants infected with BYVMV. Furthermore, vein clearing, thickening of veins and veinlets and dwarfed fruits were also observed in infected plants.

2. A survey of some of the bhendi growing areas of Bangalore, Tumkur and Kolar districts revealed that the percentage of disease incidence varied from season to season. Higher incidence of the disease was observed in the summer and low in kharif seasons.

3. BYVMV was not transmissible through sap inoculation, but was transmitted by grafting and whitefly B.tabaci.

4. Of 31 plant species inoculated Euphorbia sp and Althaea rosea showed crinkling and curling and vein thickening.

5. Date of sowing on the incidence of the disease as well as whitefly population showed that the season had a profound effect on the incidence of the disease as well as whitefly population. Crops sown from January to May showed high incidence (21-60%) while crops sown from June to December showed low incidence. The incidence of disease was directly correlated to vector population.

6. Bhendi was not infected with tomato leaf curl, horsegram yellow mosaic, Malvastrum yellow vein mosaic, Croton yellow vein mosaic, cassava mosaic viruses.

7. The duration of the eggs, 1st, 2nd, 3rd instar nymphs, pupae, and adults were found to be 4-6, 3-5, 2-5, 3-4, 8-21 and 21-49 days respectively on bhendi. The total duration of egg to adult in cotton eggs laid was high on cotton and less in bhendi. Mortality of each stage was higher in bhendi compared to cotton.

8. Preference of whiteflies to different host plants showed that the maximum number of adults were attracted to cotton, followed by horsegram, Ageratum, tomato, Malvastrum, Croton and bhendi in a decreasing order, respectively.

9. Attempts to purify the BYVMV from bhendi was partially successful and spherical particles of 20-25 nm in diameter were observed in an electron microscope.

10. In limited double diffusion tests, BYVMV did not react with antiserum of okra mosaic virus. Belladona mottle virus and a cucumber mosaic virus.

REFERENCES

VI. REFERENCES

Annapurna, S., 1985. Epidemiology of horsegram yellow mosaic virus in legume crops. M.Sc.(Agri.) Thesis (Unpublished), University of Agricultural Sciences, Bangalore, 95 pp.

Arumugam, R. and Muthukrishnan, C.R., 1977. Phenolics and flavenoids composition of bhendi yellow vein mosaic virus affected leaves of bhendi. Madras Agri. J., 64 : 839-840.

Arumugam, R. and Muthukrishnan, C.R., 1978. Free amino acids in the resistance mechanism of the yellow vein mosaic disease of bhendi (Abelmoschus esculentus (L.) Moench). Madras Agri. J., 65 : 208-210.

Arumugam, R. and Muthukrishnan, C.R., 1979. Association of resistance to yellow vein mosaic with economic characters of okra. Indian J. Agri. Sci., 49 : 605-608.

Basha, A.A. and Balasubramanyam, M., 1982. Control of yellow vein mosaic disease of bhendi through the chemical control of the vector Bemesia tabaci. In All India Symposium on Vector and Vector-borne diseases. (Trivandrum), (Feb. 26-28, 1982), p. 54.

Capoor, S.P., 1953. Bhendi mosaic and its control in Poona. Ind. Fmg., 2 : 14-16.

Chakraberti, R. and Mukhopadhyay, S., 1977. Effect of some pesticides on the yellow vein mosaic disease of bhendi. Pesticides, 11 : 19-20.

Chellish, S. and Murugesan, S., 1976a. Seasonal incidence of bhendi yellow vein mosaic. Annamalai Univ. Agri. Res., 6 : 167-168.

Chelliah, S. and Murugesan, S., 1976b. Estimation of loss due to yellow vein mosaic disease in bhendi. Annamalai Univ. Agri. Res., 6 : 169-170.

Chelliah, S., Sellammal, M., Murugesan, S., 1975. Influence of weather factors on the incidence of yellow vein mosaic disease of bhendi. Madras Agri. J., 62 : 412-419.

Costa, A.S., 1969. Whiteflies as virus vectors. In Viruses, Vectors and Vegetation. Ed. by K. Maramorosch. Interscience Pub., N.Y. pp. 95-119.

Costa, A.S. and Carvalho, A.M.B., 1960. Comparative studies between Abutilon and Euphorbia mosaic viruses. Phytopathology, 38 : 129-152.

Fernando, H.F. and Uduraven, S.B., 1942. Nature of the mosaic disease of bandeka, Hibiscus esculentus. Trop. Agric., 98 : 16-24.

Gunathilagraj, K., Padmanabhan, V. and Kumaraswami, T., 1977. Susceptibility of bhendi varieties to yellow vein mosaic virus. Madras Agri. J., 64 : 486-487.

Honda, Y., Iwaki, M., Saito, Y., Thongmeearkom, P., Kittisak, K. and Deema, N., 1983. Mechanical transmission, purification and some properties of whitefly borne mungbean yellow mosaic virus in Thailand. Plant Disease, 67 : 801-804.

Igwegbe, E.C.K., 1983. New Strain of okra mosaic virus in Nigeria. Plant Disease, 67 : 320-322.

Jamal, A., Quasseem, S.M., Wasiuddin, A.A., and Khalique, A., 1975. Biochemical aberration of yellow vein mosaic virus in Abelmoschus esculentus (L.) Moench. var. Pusa Sawani, Geobios, 2 : 90-91.

Jambhale, N.D. and Narkar, Y.S., 1981. Inheritance of resistance to okra yellow vein mosaic disease in interspecific crosses of Abelmoschus. Theoretical and Applied Genetics, 60 : 313-316.

Jha, A. and Mishra, J.N., 1955. Yellow vein mosaic of bhendi in Bihar. In. Proc. Bihar Acad. of Agri. Sci., 4 : 129-130.

Kothari, I.L., Dube, H.C. and Doshi, S.K., 1981. Studies on the permeability alterations and ultrastructural changes in yellow vein mosaic of bhendi (Abelmoschus esculentus). In. 3rd International Symp. Pl. Path. (New Delhi), (Dec. 14-18), p. 122.

Kulkarni, G.S., 1924. Mosaic and other related diseases of crops in the Bombay presidency. Poona Agri. Cult. Magazine, XVI : 6-12.

Mandahar, C.L. and Singh, J.S., 1971. Destruction of chlorophylls a and b in virus infected leaves. Sci. Cult., 37 : 485-487.

Mandahar, C.L. and Singh, J.S., 1972. Effect of bhendi yellow vein mosaic virus on its host. Acta.Phytopath. Acad. Scien. Hung., 7 : 187-191.

Marchoux, G., Leclant, F. and Mathai, P.J., 1960. Maladies de type Jannisse et maladies voi sines affectant principalementios solanecee et transmises per de insects. Ann. Phytopathol., 2 : 735-773.

Meiners, J.P., Lawson, H.H., Smith, E.F. and Diaz, A.J., 1975. Mechanical transmission of whitefly (Beniaia tabaci) borne disease agents of beans in El Salvadore. In : 'Tropical Diseases of legumes'. Eds. J. Bird and K. Maramorosch. pp. 61-69. Acad. Press, New York.

Muniyappa, V. and Veeresh, G.K., 1984. Plant virus diseases transmitted by whiteflies in Karnataka. Proc. Ind. Acad. Sci., 93 : 397-406.

Mariani, T.K. and Seth, M.L., 1958. Reaction of Abelmoschus and Hibiscus species to yellow vein mosaic virus. Indian Phytopath., 11 : 137-143.

Oblisami, G., Kandasami, D. and Bhaskaran, R., 1974. Phyllosphere microflora of bhendi plants as influenced by yellow mosaic virus infection. Zeitschrift Fur Pflanzenkrankheiten und. Pflanzenschutz. 81 : 522-524.

Palaniswamy, P., Thirumurthy, S., and Subramanian, T.R., 1973. Effect of systemic granular insecticides on the incidence of yellow mosaic disease of okra (Abelmoschus esculentus L.). S. Ind. Hort., 21 : 104-106.

Potty, V.P. and Wilson, K.I., 1973. Studies on the physiology of virus disease of bhendi plants. Agri. Res. J., Kerala, 11 : 65-68.

Potty, V.P. and Wilson, K.I., 1974. Effect of the yellow vein mosaic virus infection on the phyllosphere microflora of bhendi. Agri. Res. J., Kerala, 12 : 168-171.

Ramiah, M., Vidhyasekaran, P. and Kandaswamy, T.K., 1972. Changes in the photosynthetic pigments of bhendi infected by yellow vein mosaic diseases. Madras Agri.J., 59 : 402-404.

Ramiah, M., Vidhyasekaran, P. and Kandaswamy, T.K., 1973a. Changes in the activities of oxidative enzymes in yellow mosaic disease of bhendi leaves. Madras Agri.J., 60 : 609-610.

Ramiah, M., Vidhyasekaran, P., Kandaswamy, T.K. and Govindaswamy, E.V., 1973b. The effect of yellow vein mosaic virus on the mineral content of bhendi leaves. Madras Agri. J., 60 : 609-610.

Raychaudhuri, S.P. and Nariani, T.K., 1977. Viruses and mycoplasma diseases of plants in India. Oxford and IBH Publishing Co., New Delhi, pp. 160-167.

Saikia, A.K., 1985. Epidemiology and control of tomato leaf and virus. Ph.D. thesis (Unpublished), University of Agricultural Sciences, Bangalore, 229 pp.

Sandhu, G.S., Sharman, B.R., Balkarn Singh and Bhalla, J.S., 1974. Sources of resistance to jassid and whitefly in okra germplasm. Crop Improvement, 1 : 77-81.

Sastry, K.S.M. and Singh, S.J., 1973a. Restriction of yellow vein mosaic spread in okra through the control of vector Bemisia tabaci. Indian J. Mycol. Pt. Path., 3 : 76-80.

Sastry, K.S.M. and Singh, S.J., 1973b. Field evaluation of insecticides for the control of whitefly (B.tabaci) in relation to the incidence of yellow vein mosaic of okra (Abelmoschus esculentus). Indian Phytopath.,26:129-138.

Sastry, K.S.M. and Singh, S.J., 1974. Effect of yellow vein mosaic virus infection on the growth and yield of the okra crop. Indian Phytopath., 27 : 294-297.

Sharma, B.R. and Sharma, D.P., 1981. Resistance to yellow vein mosaic virus in okra. In. 3rd Int. Symp. Pt.Path., (New Delhi) (14-18 Dec.), p.159.

Shivakumar, C.V. and Mereneganuddin, M., 1973. Effect of yellow vein mosaic disease of okra on the reniform nematode (Rotylenchus reniformis). S.Ind. Hort., 21 : 68-69.

Singh, A.B., Joshi, B.S., Khanna, P. and Gupta, P.S., 1962. Breeding for field resistance to yellow vein mosaic of bhendi. Ind. J.Genet. Pt. Breeding, 22 : 137-144.

Singh, M. and Thakur, M.R., 1979. Nature of resistance to yellow vein mosaic in A.manihot sub sp. manihot. Curr. Sci., 48 : 164-165.

Singh, R. and Srivastava, R.P., 1974. Physiological changes in bhendi (Abelmoschus esculentus) fruit affected by yellow vein mosaic virus. Curr. Sci., 43 : 89-91.

Singh, S.J. and Tewari, R.P., 1979. Rhizosphere microflora of healthy and yellow vein mosaic virus infected okra (A.esculentus) plants. Acta. Micro. Pol., 4 : 311-314.

Sinha, S.N., and Chakrabarthi, A.K., 1978. Effect of yellow vein mosaic virus infection on okra seed production. Seed Res., New Delhi, 6 : 67-70.

Summanwar, A.S., 1980. Whiteflies as vectors of plant pathogens. In. Group Discussion on Plant Virology. Lucknow, Nat. Bot. Res. Inst., p. 179.

Utthasami, S., Chelliah, S. and Balasubramanian, M., 1977. Insecticidal control of pests and the yellow vein mosaic of bhendi. Sci. and Cult., 43 : 510-512.

Uppal, B.N., Varma, P.M. and Capoor, S.P., 1940. Yellow vein mosaic of bhendi. Curr. Sci., 9 : 227-228.

Varma, P.M., 1952. Studies on the relationship of the bhendi yellow vein mosaic virus and vector, the white-fly (Bemisia tabaci). Indian J. Agr. Sci., 22 : 75-91.

Varma, P.M., 1955a. Persistance of yellow vein mosaic virus of Abelmoschus esculentus (L.) Moench in its vector Bemisia tabaci (Gen.). Indian J. Agri. Sci., 25:293-302.

Varma, P.M., 1963. Transmission of plant viruses by whiteflies. Bull. Nat. Inst. Sci., India, 24 : 11-23.

Varma, P.M. and Mukherjee, S.K., 1955. Studies on the varietal classification of virus resistance in lady's finger (Abelmoschus esculentus). Proc. 42nd Ind. Sci. Congr. (Baroda), p. 371-372.

VII. REFERENCES

Capoor, S.P. and Varma, P.M., 1950, Yellow vein mosaic of H.esculentus L. Ind. Jour. Agri. Sci., 20 : 217-230.

Chellian, S., 1976, Seasonal incidence of Bhendi Yellow vein mosaic disease. Annamalai Univ. Agri. Res. A., 6 : 167-168.

Goodman, R.M., Bird, J. and Thongmeearkom, P., 1977, An unusual virus like particle associated with golden yellow mosaic of beans. Phytopath., 67 : 37-42.

Givord, L. and Hirth, L., 1973, Identification, purification and some properties of mosaic virus of Okra (H.esculentus). Ann. Appl. Biol., 74 : 359-370.

Hussain, M.A. and Trehan, K.N., 1933, Observations on the life history, bionomics and control of the whitefly on cotton (B.gossypiperda M & L). Ind. J. Agri. Sci., 3 : 701-753.

Johnson, M.W., Toscano, N.C., Reynolds, H.T., Sylvester, E.S., Kido, K. and Natwick, E.T., 1982, Whiteflies cause problems for S.california growers. Calif. Agri., 36(9/10) : 24-26.

Muniyappa, V., 1980, Whiteflies. In. Vectors of plant pathogens. ed. by K.F. Harris and K. Maramorosch. pp. 39-85. Acad. Press, New York.

Muniyappa, V., Rajeshwari, R., Bharathan, N., Reddy, D.V.R. and Nolt, B.L., 1986, Isolation characterisation of gemini virus causing yellow vein mosaic disease of Horsegram (Macrotyloma uniflorum) in India. Phytopath. Z., (in Press).

Pruthi, H.S. and Samuel, C.K., 1942, Entomological investigations on the leaf curl disease of tobacco in Northern India. 5. Biology and population of the whitefly vector B.tabaci (Genn). in relation to the incidence of the disease. Ind. J. Agri. Sci., 12 : 35-37.

Ramaiah, M., Vidyasekharan, P., Kandaswamy, T.K., 1972, Changes in the photosynthetic pigments of bhendi infected by yellow vein mosaic disease. Madras Agric. J., 59 : 402-404.

Singh, S.J., 1980, Studies on epidemiology of yellow vein mosaic virus of okra. Ind. J. Mycol. and Pt. Patn., 10 : 35.

Singh, S.J., Sastry, K.S. and Sastry, K.S.M., 1977, Yellow vein mosaic of bhendi (okra) and methods of control. Ind. Hort., 22 : 14-15.

Varma, P.M., 1955b, Ability of the whitefly to carry more than one virus simultaneously. Curr. Sci., 24 : 317-318.

Vetten, H.J. and Allen, D.J., 1983, Effects of environment and host on vector biology and incidence of two whitefly spread disease of legumes in Nigeria. Ann. Appl. Biol., 102 : 219-227.