STUDIES ON BUD NECROSIS VIRUS DISEASE OF WATERMELON (*Citrullus lanatus* Thunb.)

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DEPARTMENT OF PLANT PATHOLOGY
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STUDIES ON BUD NECROSIS VIRUS DISEASE OF WATERMELON (*Citrullus lanatus* Thunb.)

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CERTIFICATE

This is to certify that the thesis entitled " **STUDIES ON BUD NECROSIS DISEASE OF WATERMELON (*Citrullus lanatus* Thunb.)**" Submitted by **Mr. KRUPA SHANKAR. M.R.** 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 him during the period of his study in this 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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INTRODUCTION

I INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum and Nakai] is an annual summer crop native to tropical Africa. It is commonly called 'Tarbooz' in most of the Indian languages. It is a major crop of various river beds in Uttar Pradesh, Pujab, Haryana, Rajasthan, Bihar, Gujrat, Maharastra, Andhra Pradesh and Karnataka. In India, this crop occupies an area of 16,194 ha with the production of 2,05,884 tonnes/yr (Gill and Tomer,1993). It can be grown up to an elevation of 1524 m above mean sea level.

Watermelon is a common man's fruit relished by both rich and poor alike. The fruit is also consumed by many after adding a pinch of salt and black pepper. The juice is delicious and nourishing and exerts a cooling effect in hot summer months. The fruit contains 95% water, 0.2% protein, 0.3% minerals and 3.3% carbohydrates per 100 gram of fresh weight (Aykroyd, 1963). It is also a rich source of iron. The seed kernels are used in various sweets and other delicacies. The unripe fruits are also cooked as vegetable in some parts of India.

The cultivars like Sugarbaby, Ashahi Yamato, Arka Manik and Arka Jyothi and some of the other varieties and hybrids developed by private companies are being cultivated extensively in different watermelon growing areas of India.

Among the diseases affecting watermelon, those caused by viruses are difficult to control and can be very destructive. Their incidence and severity may vary, depending on the complex relationships of pathogens, hosts, vectors, the environment and the localities in which they occur. It is important to identify the causal agents of virus diseases and when possible, adopt strategies to minimise their impact on the yield and quality of watermelon.

Watermelon cultivation is affected by several viral diseases caused by cucumber green mottle mosaic tobamo virus (Komuro *et al.*, 1971; Vani and Verma 1993), Watermelon mosaic II poty virus (Komuro, 1962; Bhargava *et al.*,

1975) cucumber mosaic cucumovirus (Komuro *et al* 1971), Papaya ringspot poty virus W strain and tospoviruses (Iwaki *et al.*, 1984; Yeh *et al.*, 1992; Singh and Krishna Reddy 1996). Among these viral diseases, bud necrosis disease caused by a virus belonging to tospovirus group of the family Bunyaviridae has become increasingly important as it causes heavy yield losses ranging from 20-100% (Krishna Reddy and Singh, 1993) depending upon the time of sowing and variety/hybrid.

In Karnataka, watermelon cultivation around Bangalore has got a severe set back due to the attack of bud necrosis virus disease (Singh and Krishna Reddy, 1995). It has attained a major status as a disease in the past 3-4 years causing enormous losses in yield and quality of watermelons.

Information is scanty on the incidence, intensity and yield losses caused by this virus in different watermelon growing areas of the country and there is a need for detailed study of this disease in respect of seasonal variation, transmission, host range, serology, histopathology and electron microscopy. Further, sufficient studies are needed with respect to the characterisation of the causal agent and management aspects of this disease. In order to devise suitable management practices of the disease, preliminary studies were undertaken with the following objectives.

1. Survey of different watermelon growing areas for the disease incidence, intensity and thrips population
2. Seasonal variation of disease incidence
3. Identification of the thrips vector
4. Transmission studies
5. Host range
6. Serological detection of the virus
7. Histopathology
8. Electron microscopy

The results obtained have been presented in this thesis.

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

'Bud necrosis' a viral disease of watermelon was observed for the first time during 1991-92 in Bangalore in the watermelon fields at Indian Institute of Horticultural Research, Hesarghatta lake, Bangalore, and was reported to be caused by a tospovirus (Singh and Krishna Reddy, 1995). Because of the characteristic necrosis of the terminal buds and petioles in the initial stages and die back symptoms in the later stages of affected watermelon plant, it was named as 'bud necrosis' disease (Krishna Reddy and Singh, 1993).

Infection of watermelon plants by a tospovirus was for the first time observed in the silver mottle diseased watermelon plants in Japan by Iwaki *et al.* 1984. Yeh *et al.* (1992) reported a similar virus with different kind of disease symptoms in Taiwan.

The occurrence of tospoviruses in India on various crops like peanut (Ghanekar *et al.*, 1979), tomato (Prasada Rao *et al.*,1980), urdbean and mungbean (Amin *et al.*, 1985; Krishna Reddy and Varma, 1990), peas (Prasada Rao *et al.*, 1984), cowpea, chilli, brinjal and clusterbean (Prasada Rao *et al.*, 1987;Krishna Reddy and Varma, 1990) have been reported so far.

Economic importance

The incidence of watermelon bud necrosis was reported to be 39-100% with an estimated yield loss of 60-100% ((Krishna Reddy and Singh, 1993).

Surveys in the watermelon growing areas of Karnataka state, South India revealed that the incidence of the disease varied from 20-100% depending upon the time of infection and varieties/hybrids grown (Singh and Krishna Reddy, 1995).

Symptomatology

Under field condition of watermelon fields of Changhua county in Central Taiwan (during the growing season of 1988), watermelon plants exhibited mosaic mottling, crinkling, yellow spotting and narrowing of leaf laminae. Plants were severely stunted and had short internodes accompanied with upright growth of younger branches, tip necrosis and die-back. Fruit set was reduced and the fruit that developed remained small, malformed and showed necrotic spots (Yeh *et al.*, 1992).

In July 1991, approximately 3% of melon (*Cucumis melo* L) plants in a field at CNPH/EMBRAPA in Brazil showed chlorosis of new growth, concentric ring spots on leaves and fruit, fruit malformation, and overall stunting (Boiteux *et al.*, 1994) .

Symptoms of watermelon bud necrosis disease occurring in India included necrosis of the buds and petioles, necrotic spots on leaves and necrotic streaks on vines. The vines eventually wilt and dry, and fruits from infected plants of a few varieties exhibited broken concentric rings with corky texture on the surface (Singh and Krishna Reddy 1996).

Causal Virus

Tospovirus is a genus of family Bunyaviridae. Its members infect a wide range of plant species, including field crops, vegetables, fruits, ornamental crops, weed hosts etc. and cause devastating diseases throughout the world in both greenhouse and field crops. Tomato spotted wilt virus (TSWV) is a type member of the *Tospovirus* genus. It has got unusual physical properties, chemical composition, vector relationships and a very wide host range (Black *et al.*, 1963; Best and Katekar 1964; Best and Palk 1964; Best 1968; Mohammed *et al.*, 1973; Francki and Hatta, 1981).

The causal virus of the silver mottle disease that occurred in watermelon in Okinawa, Japan, was identified as tospovirus on the basis of particle morphology, host reaction, instability in crude sap, effects of sodium sulphite and L- cysteine on stability and thrips transmissibility. This is the first report of a tospovirus naturally occurring in cucurbits (Iwaki *et al.*, 1984).

The causal agent of an unusual disease affecting watermelon in Taiwan was identified as tomato spotted wilt like virus (TSWV-W) in the tospovirus group on the basis of particle morphology, host reaction, serology and transmission by thrips (Yeh *et al.*, 1992). Later TSWV-W, naturally infecting melon was reported from Brazil (Boiteux *et al.*, 1994; Pozzer *et al.*, 1994).

The bud necrosis disease of watermelon affecting the watermelon cultivation in India was shown to be caused by the watermelon strain of tomato spotted wilt virus (TSWV-W) (Krishna Reddy and Singh, 1993). Later, Singh and Krishna Reddy (1996) identified the causal agent of the disease by host range, thrips transmission, virus particle morphology and serology.

The watermelon tospovirus (TSWV-W) was reported as a distinct isolate (Yeh and Chang, 1995) and different from the TSWV-Lettuce isolate (TSWV-L) and Impatiens necrotic spot virus (INSV) (Low and Moyer, 1990, Adam *et al.* 1993, Krishnareddy and Singh, 1993).

Transmission

a. Sap

Better transmission of tospovirus was achieved by keeping the plants in darkness for 24 hours prior to inoculation (Halliwell and Philley, 1974). For successful transmission of TSWV, young infected leaves should be used as source of inoculum and the inoculum should be prepared in chilled extract using cold mortar and pestle (Ghanekar *et al.*, 1979).

Chohan (1967) had succeeded in transmitting Indian peanut bud necrosis virus by sap inoculation. The virus was not infective in sap normally, but infectivity was retained by extracting the sap in a potassium phosphate buffer (pH 7.0). Addition of 0.02 M 2-mercapto ethonal as an anti oxidant in the phosphate buffer ensured successful transmission of the virus through sap. Use of sodium sulphite (0.05%) was reported to give inconsistent results of transmission (Ghanekar and Nene, 1979).

Iwaki *et al.* (1984) had succeeded in transmitting watermelon silver mottle virus (WSMV) by sap inoculation. Naturally infected watermelon leaves were macerated in a mortar with 0.05 M sodium and potassium phosphate buffer, pH 7.0, containing 0.5% sodium sulphite and 1 mM L-cysteine and rubbed on to 600-mesh carborundum - dusted leaves of test plants grown in a glass house.

Tomato spotted wilt like virus infecting watermelon in Taiwan was also found to be sap transmissible by Yeh, *et al.* (1992). They used phosphate buffer containing sodium sulphite, pH 7.0, for the extraction of the virus in sap.

b. Thrips Vector

The tospoviruses are transmitted by thrips which are minute insects of the order Thysanoptera and family Thripidae (Sakimura,1962, 1969 ; Amin *et,al.*, 1981 ; German *et al.*, 1992 ; Iwaki, *et al.*, 1984; Kobatake, *et al.*, 1984; Reddy and Wightman 1988; Wijkamp, *et al.*, 1995; Mound, 1996; Ullman, 1996). Although thrips are minute, their tendency to occur in large populations across broad host, geographic and climatic ranges make them significant as direct crop pests and virus vectors. These biological entities lie at the foundation of the tospovirus pathosystem ; thrips vectors, tospoviruses (species, serogroups and serotypes), and the plant species, serving as hosts for both the viruses and the vector species. Epidemics can occur only when these biological entities coincide in an appropriate environment (Ullman, 1996).

More than 5,000 species of thrips are currently recognised world wide (Mound,1996), only seven species are competent to transmit tospoviruses .

Confirmed thrips vectors of tospoviruses

Vector Species		**Tospoviruses**	**References**
Latin name	Common name		
Frankliniella occidentalis Pergande	Western flower thrips	TSWV, INSV	Paliwal (1976) Cho *et al.*, (1988) Mau *et al.* (1990)
Frankliniella fusca Hinds	Tobacco thrips	TSWV	Sakimura (1963)
F. intosa Trybom	Tobacco thrips	TSWV	Mound (1995)
F. schultzei Trybom	common blossom or cotton bud thrips	TSWV, BNV	Amin *et al.* (1981) Sakimura (1969)
Thrips tabaci Lindeman	Onion thrips	TSWV	Cho *et al.* (1989)
T. setosus Moulton	no common name	TSWV	Sakimura, (1962)
T. palmi Karny	Melon thrips	TSWV-W, BNV	Yeh *et al.* (1992)
Scirtothrips dorsalis Hood	chilli thrips	BNV	Amin *et al.* (1981)

The claims of *Scirtothrips dorsalis* and other thrips species needs to be confirmed (Amin, *et.al.*, 1981; Kormelink, 1994; Zitter, *et.al.*, 1989; Ullman *et.al.*, 1996).

Transmission is the result of the different processes that start with the ingestion of the virus on infected plants and end with the successful transmission

of virus to a healthy plant. These events coincide with host finding and feeding activities of the thrips. The ratio at which thrips become viruliferous and transmit can be quantified in terms of the efficiency with which the virus can be acquired and subsequently transmitted and the length of the latent period. Its dynamics are the results of complex interactions between plants, vectors and viruses. (Peters, *et al.*, 1996)

Only larval thrips can acquire tospovirus while both larval and adult thrips transmit the virus in a persistent manner. Widely varying acquisition and inoculation thresholds and latent periods have been reported (Sakimura, 1962), although thrips-virus interactions underlying this variation is not clearly known. Recent evidence suggests that viral replication may be an important determinant of thrips infectivity *(Ullman et. al.*, 1992).

Two types of thrips. *Frankliniella schultzei* Trybom and *Scirtothrips dorsalis* Hood, have been reported to transmit TSWV in India (Ghanekar *et al.*, 1979 and Amin *et al.*, 1981). Later, Ranga Rao and Vijaya Lakshmi (1993) showed that *F. schultzei* was a poor vector of PBNV. The same species also spread the silver mottle disease of watermelon in Okinawa (Honda *et al.*, 1989).

In India, Singh and Krishna Reddy (1995) reported *Thrips flavus* Schrank (Thysanoptera : Thripidae) as a new vector of a tospovirus infecting watermelon plants. About 10-15 nymphs of *Thrips flavus* require 3-4 days acquisition access period and about 15-20 days of inoculation feeding period to transmit the virus successfully (Singh and Krishna Reddy, 1995).

Host range

TSWV has an extremely wide host range exceeding 200 species of plants covering 34 families of which 5 are monocotyledons. Almost half of the known hosts belong to Solanaceae (Halliwell and Philley, 1974). Ghanekar *et al.*, (1979) reported that the virus could induce chlorotic and /or necrotic local lesions in

some hosts and on others it could produce chlorotic or necrotic spots followed by systemic infection.

WSMV (from Taiwan) and TSWV-NY (tomato isolate of New York) caused similar symptoms on test plants in Amaranthaceae, chenopodiaceae, and solanaceae (Yeh *et al.*, 1992). With the exception of WSMV from Japan (Iwaki *et al.*, 1984) and watermelon isolate of TSWV from Taiwan (Yeh *et al.*, 1992) the most tospoviruses induce only local infection on cucurbitaceae (Ie, 1970; Reddy and Wightman, 1988). Singh and Krishna Reddy (1996) showed that the watermelon tospovirus isolate from India systemically infected watermelon, muskmelon and other cucurbits.

Hosts indicating only necrotic local lesions include *Chenopodium amaranticolor, Petunia hybrida, Solanum melongena, Lycopersicon esculentum cv, Arka Sourabh,* and hosts exhibiting only chlorotic local lesions include *Vigna unguiculata* cv, C-152, *Vigna mungo* and *Sesamum indicum.* Hosts showing chlorotic or necrotic spots followed by systemic infection include *Citrullus lanatus* cv, Arka Manik, *Citrullus lanatus* cv Madhu, *Cucumis melo, Cucumis sativus, Cucurbita maxima, Cucurbita moschata, Cucurbita pepo, Lagenaria siceraria, Trichosanthes anguina, Arachis hypogaea* cv-JL-24, *Cassia tora, Lablab purpureus, Glycine max* cv. Bragg, *Phaseolus lanatus, Phaseolus vulgaris* cv Arka komal, *Capsicum annum* cv Yellow Wax, *Datura stromonium, Lycopersicon esculentum* cv Pusa Ruby, *L. esculentum* cv Arka Sourabh, *Nicotiana glutinosa, Nicotiana rustica, Nicotiana tobacum* cv *Samsun, Physalis floridana, Gomphrena globosa, Emilia sonchifolia, Zinnia elegans* (Yeh *et al.*, 1992, Singh and Krishna Reddy 1996).

According to latest review on host range of TSWV, it can infect more than 500 species of plants in more than 50 families which include important ornamental, fruit and vegetable crops. More than 100 species have been reported in Solanaceae and compositae alone (Peters *et al.*, 1996; Stobbs *et al.*, 1992). Many of these plants are hosts for both virus and thrips and they serve as reservoirs of infection that contribute to epidemics in crops plants (Cho *et al.*, 1986).

Only a few attempts have been made to use serology for the detection and identification of TSWV owing to difficulties in getting purified virus suitable for use as an immunogen. Reports of the serological studies with TSWV have been enlisted in the following table.

References	Purpose of Studies	Titre of antiserum
Best and Harihara Subramanian (1967)	Differentiation of virus strains	1/256 to 1/512 by tube precipitin tests.
Feldman and Bonninsegna (1968)	Virus detection	1/10 by gel diffusion tests.
Tsakirdis and Gooding (1972)	Virus detection	1/8 by gel diffusion tests.
Joubert *et al.* (1974)	Virus detection	1/32 by gel diffusion tests.
Paliwal (1974, 1976)	Virus detection	1/30 by ring tests
Tas *et al.* (1977)	Antigenic analysis of virus	1/32 to 1/28 by gel diffusion tests.
Gonsalves & Trujilla (1986)	Virus detection	up to 1µg/1ml by ELISA.
Sreenivasulu *et al.* (1991)	Virus detection	up to 1:5000 by ELISA.
Hsu and Lawson (1991)	Detection of the virus	1mg/ml dilution of biotinylated monoclonal antibodies for DBIA
Singh and Krishna Reddy (1996)	Serological relationship of TSWV-W isolates	1:1000

During the first 50 years since the discovery of tospoviruses, TSWV was considered to be monotypic in the group and isolates of different crops were thought to be strains of the virus (Ie, 1970; Matthew's, 1982). In 1992, the placement and unique status of TSWV was significantly challenged (Law and

Moyer, 1990; Law *et al.*, 1992). Data on the molecular biology of the virus revealed a taxonomic relationship with Bunyaviridae, a recognised group of animal viruses. As a result, the tospovirus genus was established within the family Bunyaviridae, and the TSWV is the type member of this new genus (Francki *et al.*, 1991). In addition, research on serologically distinct isolates, formerly designated as strains TSWV-L or Lettuce strain and TSWV-I or impatiens strain revealed differences sufficiently distinct to warrant establishment of at least two separate viruses, now called TSWV (formerly TSWV-L) and impatiens necrotic spot virus (INSV, formerly TSWV-I) within the *Tospovirus* genus (Law *et al.*, 1992).

After the discovery of INSV, two additional isolates with a great overlap in host range with TSWV were recognised. Tomato chlorotic spot virus (TCSV) and groundnut ring spot virus (GRSV) were recognised only after serological and genome-sequence analysis of the nucleocapsid (N) protein. TCSV was reported form Sotuh America and GRSV only from South Africa (Peters *et al.*, 1996).

A fifth virus, Watermelon silver mottle virus (WSMV), was been described in Japan and Taiwan (Yeh and Chang 1995). The virus from Taiwan that reacts with GRSV antiserum has an N-protein gene that is identical in base sequence to WSMV (Heinze *et al.*, 1995). This virus and WSMV forms with GBNV, a fourth serogroup.

Yeh and Chang (1995) reported that the low degree of nucleotide sequence identity and amino acids similarity of the N-gene with those of the TSWV and INSV, coupled with negative hybridisation relationships, indicated that watermelon tospovirus isolate should be named as watermelon silver mottle virus (WSMV).

Serological tests by Enzyme Linked Immuno Sorbent assay (ELISA) and western blotting with polyclonal and monoclonal antibodies demonstrated that the virus infecting watermelon in Taiwan was serologically related to the

Tospovirus that caused silver mottle symptoms in Okinawa (Japan) but not to tomato, lettuce and amaryllis isolates of TSWV from other countries (Yeh *et al.*, 1992;Adam *et. al.*, 1993).

Watermelon bud necrosis virus in India was also found similar to watermelon tospovirus from Japan and Taiwan and closely related to Indian peanut bud necrosis virus (PBNV), but is distinct from, or not related to, the lettuce strain of TSWV and INSV (Singh and Krishna Reddy, 1996).

Serological and physiochemical properties of nucleocapsid of tospovirus isolated from watermelon (TSWV-W) were compared with three isolates of TSWV from Dahlia (TSWV-D), pepper (TSWV-P) and tomato (TSWV-N) in Japan. Nucleocapsid of TSWV-W had three major RNA species (RNA1, RNA2,and RNA3) and one protein. TSWV-W contained larger RNA3 (Mr.1.2 x 10^6) and larger protein (32K) than those of the other 3 isolates, although nucleocapsids of TSWV-N, TSWV-P and TSWV-D contained 3 major RNA with Mrs of 2.7 x 10^6 (RNA1), 2.6 X 10^6 (RNA2) and 1x10^6 (RNA3) and one kind of protein (30k). Serological relationships among the nucleocapsids of TSWV-W and TSWV-N revealed that nucleocapsids of TSWV-W and TSWV-N reacted clearly with their homlogous antisera but rarely or only weak with heterologous antisera. Nucleoapsids of TSWV-P and TSWV-D were serologically closely related to that of TSWV-N but not TSWV-W (Hanada *et.al.*, 1993) .

Several tospoviruses have been reported so far as serologically distinct from TSWV and INSV. Among these, established species (Peters *et al.*, 1996) are Tomato spotted wilt virus (TSWV), Groundnut ring spot virus (GRSV), Tomato chlorotic spot virus (TCSV), Impatiens necrotic spot virus (INSV), watermelon silver mottle virus (WSMV),Peanut bud necrosis virus (PBNV), Melon spotted wilt virus (MSWV), Peanut chlorotic fern leaf virus (PCFV), peanut yellow spot virus (PYSV). The following table enlists tospoviruses with serologically distinct nucleocapsid proteins reported so far :-

Sero group		Name of the virus/disease Distinct Viruses	N- Protein sequence	Economic hosts	Reference
I		Tomato spotted wilt virus (TSWV)	100%	Many Di and Monocots	Francki *et al.* (1991)
II	1	Tomato chlorotic spot virus	76%	-	-
	2	Ground nut ring spot virus	78%	-	-
III		Impatiens necrotic spotvirus (INSV)	55%	Floral crops, Mint	Law *et al.* (1991)
IV	1	Watermelon silver mottle virus (WSMV)	29%	Watermelon	Yeh *et al.* (1995)
	2	Peanut bud necrosis virus	29%	Peanut	Satyanarayana *et.al.* (1996b)
	3	Melon spotted wilt virus (MSWV)	35%	Melons	-
	4	Watermelon bud necrosis virus (WBNV)	-	Muskmelon Watermelon	Singh and Krishna Reddy (1996), Jain *et al.* (1997)
V		Peanut yellow spot virus (PYSV)	-	Peanut	Satyanarayana *et.al.* (1996a)

HISTOPATHOLOGY

Necrosis and growth abnormalities were two principal types of pathological changes induced by viruses in the tissues mainly affected by them (Mandahar, 1987) All histological changes result from alterations in cells, which in turn, alter the tissues to which they belong (Sutic and Sinclair, 1992).

Informations regarding histological changes due to tospoviruses are very much scanty. Therefore histopathological changes in leaves caused by viruses in general are briefly reviewed here.

Several viruses which are obligate parasites in epidermal cells induces hypertropy and and hyperplasia in infected as well as other tissue cells. As a consequence of such changes, small galls were formed, for example, in virus infected plants of *Narcissus species* (Buttler and Jones, 1949). Collapsed upper epidermal layer cells were observed in the localised areas of cucumber mosaic virus infected leaves of *Cucurbita pepo*, showing mild mosaic symptoms(Bansal,*et.al.*,1992).

The mesophyll of susceptible hosts can be infected by all or almost all viruses, except some histotrophic viruses like citrus tristeza virus (Price, 1966). Hypoplasma of mesophyll cells results from inadequate or reduced activity of meristem cells of leaf primordia. For example, broad bean leaf mesophyll infected with pea enation mosaic virus showed only a single type of tissue consisting primarily of irregular cells (Ulrich and Quantz ,1964). Due to such changes, the diameter of infected leaf blades are reduced by a third to half (Schmidt, 1980).

Disorders in cell differentiation and hypotrophy frequently occurred in leaf parts with chlorotic mosaic, which can be caused by several viruses. According to Sutic (1980), underdeveloped or immature palisade cells are particularly susceptible to hypotrophy, so that those leaf parts with such cells are thinner than normal and have a rough surface. Mathews (1970) also stated that yellow zones on leaves infected with many of the mosaic viruses are thinner than green one's ; that mesophyll cells are less differentiated with a smaller number of chloroplasts, and that only a few or no intercellular spaces are formed. Schmidt (1980) observed that cells in the yellow zones of sugarbeet leaves infected with sugar beet mosaic virues are closely arranged and that these leaf parts are thinner than green ones due to the absence of palisade and spongy tissue differentiation. Bawden (1956) also showed that palisade cells in leaves infected with a mosaic virus are shorter than normal with smaller intercellular spaces, and that chlorotic leaf parts usually are thinner than the green ones.

Abnormal shapes of palisade cells with a fewer number of chloroplasts and spongy parenchyma with lesser air space were observed in CMV infected leaves of *Cucurbita pepo* (Bansal *et.al.*, 1992)

Electron Microscopy

Detection of Tospovirus virus particles and their identification by electron microscopy can usually be achieved by examination of leaf dip preparation or of thin sections of infected cells (Milne, 1970). Fixation is found to be essential to avoid flattening and distortion of particles before staining (Francki and Halta, 1981). Ammonium molybdate has found to be a good negative stain. The isolated particles lack uniformity of size and shape and hence are not readily distinguished from some normal cell materials. The problems associated with negative staining of TSWV are due to the presence of viral envelope. Preparation of thin sections from infected tissues of the plant, though time consuming and requires skill, still is recomended as a very reliable method of identifying TSWV. The particles in the sections are relatively uniform and cannot be mistaken for those of any other virus. In addition, presence of viroplasms in many of the cells, is characteristic of TSWV infection (Francki and Hatta, 1981) .

TSWV particles can be detected in negatively stained dip preparations and also by electron microscopy of ultra-thin sections of TSWV-W infected watermelon plants (Milne 1970; Iwaki *et al.*, 1984; Urban *et al.*, 1991; Kitazima *et al.*, 1992; Yeh *et al.*, 1992;Singh and Krishna Reddy, 1995).

Morphology of the tospoviruses are typical of the members of family Bunyaviridae and the particles are 80-110 nm in diameter, spherical and membrane bound (German *et al.*, 1992).

Iwaki *et al.* (1984) observed roughly spherical TSWV particles with 80-100 nm diameter in the dip preparations and particles of 80-90 nm diameter in the ultra-thin sections of the watermelon silver mottle disease infected watermelon plants. Large quasi- spherical particles of similar dimension were also observed in TSWV infected watermelon plants of Taiwan (Yeh *et al.*, 1992) and bud necrosis infected watermelon plants of India (Singh and Krishna Reddy, 1996).

MATERIAL AND METHODS

III MATERIALS AND METHODS

The present investigations on bud necrosis virus disease of watermelon (*Citrullus lanatus* Thunb) were carried out during the years 1996-97. Culture of the disease was obtained from the fields of watermelon crop grown in the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. The culture was further maintained by frequently inoculating on to the healthy watermelon plants var. Arka Manik with the sap extracted from diseased watermelon leaves by mechanical inoculation, in insect proof cages in the glass house. The watermelon cultivar used for all the experimental purposes was Arka Manik. The assay host used in all the experiments was *Vigna unguiculata* (L) Walp cv C-152.

3.1 SURVEY FOR THE INCIDENCE AND SEVERITY OF THE DISEASE

Surveys were conducted to study the percentage incidence of bud necrosis virus disease of watermelon in parts of Karnataka. Watermelon fields in the four districts of Karnataka namely, Bangalore, Kolar, Mandya and Chitradurga were surveyed from December 1996 to April 1997. The incidence of bud necrosis virus disease was recorded after 30 and 60 days of planting. All the plants in the selected plot area of the fields were first counted and then the number of plants showing bud necrosis symptoms were recorded separately to calculate the percentage of infection.

The disease intensity in each plot was graded as mild, moderate and severe. Mild symptoms included mottling, crinkling, yellowing, brittleness of the twig and small brown to black spots on the younger leaves. Conspicuous black spots on the leaves, reduced internodal length, necrotic black streaks on the stem and drying of the tendrils and fruits showing chlorotic and corky ring spots were graded as moderate disease symptoms. Typical die-back symptoms, malformed fruits and necrosis of the stem and tendrils were considered to be the symptoms of severely affected plants.

The methodology of survey employed was random sampling method (Kulkarni, 1996). In each of the four districts surveyed, two to three taluks were randomly selected and in each taluk two to three villages with intensive watermelon cultivation were surveyed.

At village level, a watermelon plot was randomly selected for recording observations. In each field 5 lines were randomly selected and the total number of plants and the number of plants showing bud necrosis symptoms were counted and percentage disease incidence was calculated.

3.2. SEASONAL VARIATION OF DISEASE INCIDENCE AND VECTOR POPULATION

Seasonal variation of disease incidence was observed in the monthly sown crops starting from September 1996 (Rabi season) to August 1997 (Kharif season) in the watermelon fields of IIHR, Bangalore. The per cent disease incidence 60 DAS (days after sowing) and time taken for symptom expression were recorded at regular intervals.

The disease incidence was calculated by using the total number of plants showing bud necrosis symptoms and the total plant population.

In the watermelon plot, 20 plants were selected randomly and one of the buds of each selected plants was tapped on the black paper, in order to count the number of thrips per bud. Thrips were counted while aspirating them in to the aspirator. The average thrips count per bud in the plot in three seasons (rabi 1996, summer and kharif 1997) was noted and calculated. Thrips count below 10 per bud was graded as low, between 10-25 as medium and above 25 as high.

3.3 TRANSMISSION STUDIES

3.3.1 Mechanical inoculation

3.3.1.1 Preparation of standard inoculum

Young infected leaves showing black spots, crinkling, reduced leaf size and brittleness symptoms were collected, washed thoroughly in running tap water to remove dirt, and blotted dry. The inoculum was prepared by grinding young infected leaves in chilled 0.1 M phosphate buffer, pH 7.0, containing 0.02 M 2-mercapto-ethanol. The buffer was added at the rate of one ml per gram of infected leaf tissue while macerating. The resulting pulp after maceration was squeezed between two folds of sterile absorbent cotton. This extract was used as "Standard inoculum" for further studies.

3.3.1.2 Method of inoculation

Celite (600 mesh size) was added to the standard inoculum at the rate of 0.025 g/ ml of inoculum. The plants to be inoculated were kept in darkness for 24 hr, prior to inoculation. Such plants were inoculated by gently smearing/rubbing unidirectionally on the upper surface of the young leaves of watermelon and on fully expanded young primary leaves or trifoliate leaves in case of cowpea and other legumes. The inoculated leaves were washed a few minutes after inoculation to remove the excess of inoculum on the leaves with a jet of sterile water from a squeeze bottle. The inoculated plants were kept under observation in the insect proof cages in the glass house for expression of symptoms up to 40 days.

3.3.1.3 Assay host

Several plant species produced necrotic or chlorotic lesions or both on mechanically inoculated leaves. As cowpea (*V unguiculata* (L) Walp) cv. C-152 consistently produced more local lesions within 4-5 days after inoculation, it

was selected as an assay host. Fully expanded primary leaves were chosen for mechanical inoculation.

3.3.2 Insect transmission

Thrips transmission assay were conducted as described by Amin *et al.* (1981). The acquisition access, latent, and inoculation access periods were 24h, 5-6 days, and 5 days respectively. Plants raised in an 80 mesh cage were used in all transmission experiments.

Adult thrips collected from naturally infested watermelon plants were transferred to 10 healthy watermelon plants at four leaf stage and similarly on to the tomato and tobacco plants. Each test plant received 10 adult thrips, and after three days of inoculation access feeding period, thrips were killed by spraying 0.05% monocrotophos. The test plants were kept in glass house for further observation.

3.4 IDENTIFICATION OF VECTOR THRIPS

The adult thrips were collected from the watermelon fields of IIHR, Bangalore and removed with a fine brush into collecting vials. The vials contained AGA, a mixture of 10 parts of 60 per cent ethyl alcohol, one part of glycerine and one part of acetic acid, which helped in prolonged safe storage of adult thrips.

A set of thrips thus collected were sent for identification to Mr.Laurence A. Mound, CSIRO Division of Entomology, Canberra 2601, ACT, Australia.

3.5 HOST RANGE

The host range studies were conducted by raising seedlings of various plant species through seed. Plant species belonging to families Amaranthaceae,

Asteraceae, Chenopodiaceae, Cucurbitaceae, Fabaceae were used in the host range studies. Species of *Nicotiana* and *Chenopodium* were inoculated at 6-8 leaf stage, whereas cucurbits and legumes were inoculated at primary leaf stage and others at 2-4 leaf stage. Six plants of each were mechanically inoculated and observed for 30-40 days in a greenhouse at 20-30°C. All inoculated plants were checked for virus by assays on cowpea and by ELISA.

3.6 SEROLOGICAL STUDIES

The antibodies used for serological studies were obtained from following sources:

1. Watermelon silver mottle virus antiserum (WSMV) - Provided by Y.Honda, Japan.
2. Peanut bud necrosis virus (PBNV)-Provided by D.V.R. Reddy, India.

3.6.1. Enzyme Linked Immuno Sorbent Assay (ELISA)

Direct antigen coating (DAC) indirect ELISA was performed as per the procedure of Hobbs *et al.* (1987) and included the following steps. The various buffers used in ELISA were prepared as follows:

A. Coating Buffer (carbonate buffer) :

Sodium carbonate ($Na_2 Co_3$)	- 1.59 g
Sodium hydrogen carbonate ($NaHCo_3$)	- 2.93 g
Distilled water	- 1.00 L
pH	- 9.60

Then diethyl dithiocarbomate (Sodium salt) (DIECA) was added to this at 0.001 M concentration (1.71 g/litre)

B. Phosphate buffer (PBS) :

Disodium hydrogen phosphate (Na_2HPO_4)	- 2.38 g

Potassium dihydrogen phosphate (KH_2PO_4)	- 0.40 g
Potassium chloride (KCl)	- 0.40 g
Sodium chloride (NaCl)	- 16.00 g
Distilled water	- 2.00 L
pH	- 7.40

C. Wash buffer (PBST) :

PBS	- 1.0 L
Tween-20	- 0.5 ml

D. Antibody buffer :

PBS - tween	- 100.0 ml
Polyvinyl pyrrolidone (PVP) (40,000 MW)	- 2.0 g
Ovalbumin (Crystallised)	- 0.2 g

E. Substrate buffer :

Diethanol amine	- 97.0 ml
Distilled water	- 800.0 ml

pH was adjusted to 9.8 using 1 N HCl and the volume was made up to one litre. This was preserved in a brown bottle at room temperature. Substrate used was P-nitrophenyl phosphate (PNPP). When dissolved in substrate buffer it gave colourless solution.

PNPP was dissolved in diethanol amine buffer just before use at the rate of 1.0 mg/ml and the substrate mixture was stored in dark by covering the substrate container with aluminium foil.

Procedure of DAC-ELISA

1. Crude plant extracts were prepared in coating buffer using dilution of 1:10 (1 g of leaf sample/10 ml buffer). These were dispensed into each well of

microtitre plate at the rate of 200 µl per well using a micropipette and the plates were incubated at 37°C for 2-2.5 h.

2. The contents of the plates were poured off and rinsed in PBS-tween. This was followed by washing the plates in three changes of PBS-tween allowing 3 min for each wash.
3. Optimum dilutions of 1:1000 of crude antisera were added to each well. The plates were incubated at 37 °C for 2-2.5 h and were washed in PBS-tween as in Step- 2.
4. Alkaline phosphatase (ALP) labelled antirabbit IgG was diluted in antibody buffer to a concentration of 1:8000 dilution. The plates were incubated at 37°C for 2 h and were washed in PBS-tween as in Step 2.
5. Substrate was then dispensed to each well of microtitre plate @ 200 µl/well and incubated at room temperature for 20 minutes. Light orange to yellow colour development indicated a weak to strong positive reaction and the results were quantitatively recorded on a EL 310 C ELISA reader at 405 nm.

3.6.2. Dot Blot Immuno Binding Assay (DIBA)

Procedure :

All steps were performed at room temperature. For the detection of virus by DIBA method, Nitrocellulose sheet (15 x 9.2 cms, 0.45 µm) was cut to an appropriate size, marked with a grid of 1 x 1 cm squares with a soft pencil and immersed in TBS buffer for 15 min. Then the membrane was dried on a filter paper for 5 min.

1. The antigens about (1-5µl) were dotted on to each square and allowed to dry for 5 min.
2. The membrane was kept in blocking buffer for 30 min, blotted and dried briefly with filter paper and transferred to distilled water.
3. The membrane after washing in TBS buffer was transferred to a second glass petridish containing virus specific antibody and incubated for 1h.
4. The membrane was removed from the first antibody solution dipped in distilled water, and washed twice by agitation for 10 min in wash buffer.

5. The membrane again was dipped in distilled water and transferred to a third glass petridish containing antirabbit IgG of enzyme conjugates and incubated for 1 h in conjugate buffer.
6. The membrane was removed from second antibody solution, dipped in distilled water and then washed for 10 min in wash buffer.
7. Then the membrane was transferred to substrate buffer and incubated for 10-30 min.

Reagents and solutions

Antigen buffer (TBS buffer)

0.2 M Tris-HCl pH 7.5

0.5 M NaCl

0.02% sodium azide

Wash buffer (TBST buffer)

TBS buffer

0.05% Tween 20

0.02% Sodium azide

Blocking buffer

TBS Buffer

0.05% Tween 20

2% polyvinyl pyrrolidone (PVP, mol. wt 40,000)

2% Bovine serum albumin (fraction V)

0.02% Sodium azide

Antibody buffer

TBS buffer

0.05% Tween-20

2% PVP

0.2% BSA

0.02% Sodium azide

Conjugate buffer (AP buffer)

0.1 M Tris-HCl pH 9.5

0.1M NaCl

0.005M $MgCl_2$

0.02% Sodium azide

Substrate buffer : AP buffer

5 mg/30 ml BCIP, 10 mg/30 ml NBT

3.7 HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIES

Histopathological and histochemical studies were made using the healthy and diseased leaf bits.

Fixation and dehydration

The leaf bits (10 x 3 mm) were killed and fixed in Carnoy's B fixative (6 parts ethyl alcohol + 3 parts of chloroform + 1 part of 80 percent alcohol) for 15 minutes and subjected to dehydration using series of 70, 80, 90 percent alcohol and absolute alcohol (2 changes). Then the leaf bits, were passed through alcohol butanol mixtures (3:1, 1:1, 1:3) and pure butanol twice.

Infiltration

The materials were transferred from the medium of pure butanol to small vials and chips of paraffin were added successively until the medium reached a saturation point at the room temperature and later under the table lamp (40 watts). Finally, the materials were given 5-6 changes with molten pure paraffin in an oven at 60°C thus replacing the last traces of butanol with paraffin.

Embedding

Molten paraffin was poured into suitable sized paper boats. Lower layer of wax was allowed to cool and solidify. Then the molten wax with the leaf bits were poured into the paper boat and leaf bits were arranged properly before the wax gets solidified. After the surface of the wax was cooled and solidified, the boat was plunged into cold water. Later, when the entire block was cooled it was removed from water.

Sectioning and affixing sections to slides

The paraffin bits of suitable size containing the material were cut from the block and fixed to the surface of a wooden peg, which was fixed to the block holder of the microtome. The microtome was set to 8 µm thickness and the sections were taken. Gelatin (0.2%) with a 100 mg of potassium dichromate was used as an adhesive for affixing the sections to slides. Adhesive was taken on the slide and paraffin sections were arranged on the surface of the adhesive and warmed for proper stretching at 45°C. Then the slides were dried for 24 hours in a dust free chamber.

Before staining, the slides with sections were kept in xylol for 5 minutes to deparaffinize and later passed through butanol, alcohol successively for 5 minutes in each grade. Celloidin coating was given to prevent floating of sections. After staining, the sections were dehydrated using alcohol butanol series, cleared in three changes of xylol and mounted in DPX.

Staining

Insoluble polysaccharides (periodic acid and Schiffs method) (Jenson, 1962)

After hydration the slides were placed in 0.5 per cent periodic acid for 15 minutes and were stained in Schiff's reagent for 20 minutes and rinsed in water for 10 minutes. Sites of polysaccharides appeared magenta.

DNA and RNA (Toluidine blue method) (Feder and O'Brien, 1968)

After hydration the sections were kept in 0.1 per cent toluidine blue stain for 10 minutes and washed in running water. DNA and RNA containing sites stained green and blue respectively.

The dimensions of the cells and layers of the leaf tissues were measured with the help of oculometer and a stage micrometer. The value of the oculometer scale divisions changes with the change in the eye piece and objectives. Each division in the scale of stage micrometer is equal to 10μm and is fixed.

While measuring dimensions of the microscopic objects the stage micrometer was removed and the slide with the specimen was focussed. With the help of standardised (calibrated) oculometer, the size of the microscopic objects was measured and the readings were converted into absolute values. The values of each oculometer division was calibrated or standardised as 15.3μm and 3.85μm in 10 x and 40 x magnifications respectively in this experiment. These values were multiplied with the ocular readings to the absolute dimension of the object.

3.8 ELECTRON MICROSCOPY

Leaf-dip preparations were prepared by grinding small pieces of diseased leaves previously fixed in 4% gluteraldehyde for 1hr. The crude sap was placed on formvar-coated copper grids for 30S, rinsed three times with sterile distilled water, stained with 2% uranyl acetate (each for 10s) and examined under a Jeol 100s transmission electron microscope.

For ultrathin sectioning, 1 cm pieces of infected leaves, petioles, and young vines were cut and fixed for 3h in 3% gluteraldehyde in 0.01 M

cacodylate buffer (pH 7.2). The samples were washed 3 times in 0.01 M phosphate buffer (pH 7.2) and post fixed with 2% osmium tetroxide, for 2 h at 4°C. After dehydration with ethanol, the samples were embedded in 2:1 ratio Spur medium and kept for polymerisation at 70°C for 8 hr. Ultra thin sections were cut with a glass knife mounted on a LICA ultra-cut microtome. Sections were stained with 2% uranyl acetate followed by lead citrate and rinsed with distilled water, placed on copper grids, and examined under transmission election microscope.

The photographs of the virus particles were taken and the size of particles was calculated by the following formula

$$\text{Actual size of the particle on the negative} = \frac{\text{Measured size in nm}}{\text{Magnification}} \times 10{,}00{,}000$$

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

4.1 SYMPTOMATOLOGY

Watermelon plants infected with 'Bud necrosis' disease showed symptoms on all plant parts above the ground.

Foliar symptoms included mild mottling, crinkling, yellowing and dark brown or black coloured necrotic spots, rugosity of young leaves and narrowing of leaf lamina (Plate 1A and 1B). Initial disease symptoms on leaves were black spots spread over the entire leaf lamina and reduced leaf size. Necrosis of the mid rib of leaves was one of the major symptoms of the disease (Plate 2A). Mid veins and all the lateral veins turn black, become thick and at times distorted.

Severe disease symptoms were observed on the young tender branches (Plate 2B). Affected plants were severely stunted, had shortened internodes and became very brittle (Plate 2B and 3A). Upright growth of younger branches, unopening of flower buds, bud necrosis, and die-back were the major symptoms of this disease (Plate 3A and 3B).

Another conspicuous symptom was the presence of longitudinal brown necrotic streaks on vines (Plate 3B) tendrils, petioles, and fruit stalks. As the disease progresses, the stem splits and started drying from the tip.

Fruit set and yield were drastically reduced. Fruits of a few varieties showed necrotic or chlorotic ring spots with corky texture (Plate 4A and 4B). Some fruits showed chlorotic mottle symptoms and uneven surface. Six of the fruit was drastically reduced and were malformed. Fruit quality was also affected to the extent that they were not marketable.

Plate 1 A. Photograph showing necrosis of the leaf lamina of watermelon bud necrosis virus (WBNV) infected leaf.

1B. Photograph showing black spots, mottling or yellowing on the leaf margin of watermelon infected with WBNV.

2 A. Photograph showing necrosis of the veins of WBNV infected leaf.

2B Photograph showing typical bud necrosis symptoms on the WBNV infected twigs.

Plate 3A. Photograph showing up-right, brittle WBNV infected watermelon twigs.

3B. Photograph showing WBNV infected twigs with black streaks on the stem.

3C. Photograph showing watermelon twig infected with both WBNV and WMV (watermelon mosaic virus).

Plate 4 A. Photograph showing chlorotic rings and mottling symptoms on the watermelon fruit infected with WBNV.

4B. Photograph showing necrotic ring spots with corky textures on the WBNV infected watermelon fruit.

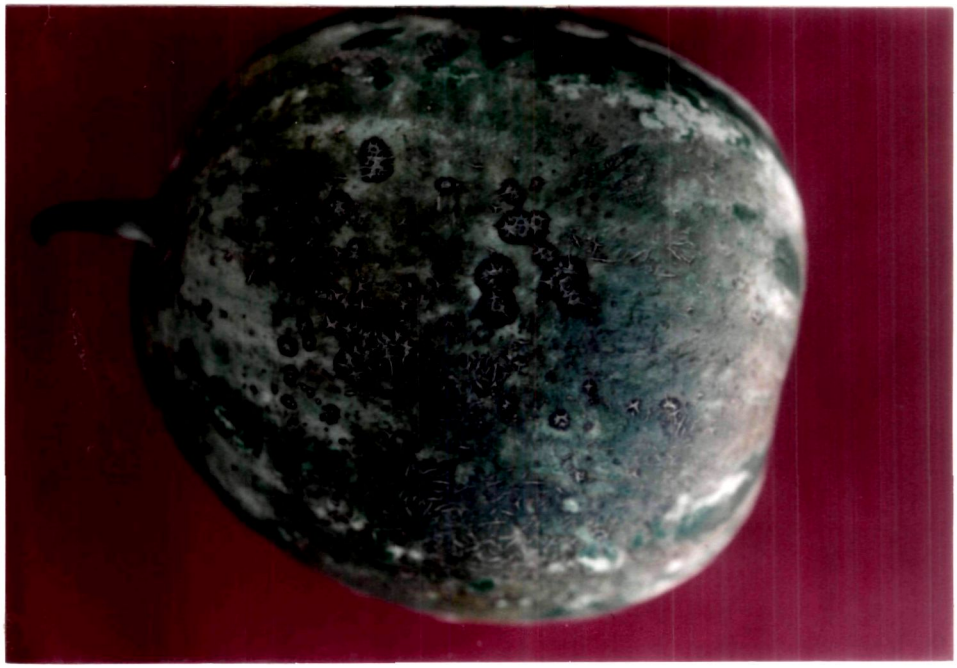


Plate 5. Photograph showing moderate field infection of watermelon plants with WBNV.

Most of the plants infected with bud necrosis virus were invariably infected with watermelon mosaic virus-2 (WMV-2) (Plate 3C) In case of mixed infections the symptoms became very severe.

4.2 SURVEY FOR THE INCIDENCE AND SEVERITY OF BUD NECROSIS DISEASE

Surveys were undertaken to assess the percentage incidence of bud necrosis disease of watermelon as described under 'Material and Methods'. The observations were recorded two times after 30 days of sowing and the data are presented in the Table 1.

The over all incidence of bud necrosis virus disease (BND) in the four districts of Karnataka namely Bangalore, Kolar, Chitradurga and Mandya Districts ranged between 0.7-10% upto 30-45 days after sowing (DAS) and 10-100% up to 60-100 days after sowing.

In three taluks of Bangalore district surveyed during the months of December 1996 and January 1997, the overall mean incidence of BND was found to be 1.2% between 30-45 days after sowing (DAS) and 61.9% between 65-105 days after sowing. The highest overall mean incidence of 95.5% was found in Hessaraghatta and Nelamangala villages of Bangalore rural district after 60DAP, compared to Doddaballapur taluk (69.4%) and Devanahalli taluk (45.4%).

The overall mean incidence of BND in 3 taluks of Kolar District surveyed during the months of January to April 1997 was found to be 0.6% between 30-40 DAS and 57.9% between 60-100 DAS. The highest overall mean incidence of 75% was found in Chintamani taluk when compared to Chikkaballapur taluk (66.98%) and Kolar taluk (23.3%). The least incidence of 20% at 70-75DAS was found in Kamadhenahalli of Kolar Taluk and highest incidence was found in Talagavara village of Chintamani taluk (100% at 75 DAS) and Marenahalli of Cikkaballapur taluk (100% at 100DAS).

Table 1 : Incidence of bud necrosis disease in some parts of Karnataka.

Sl. No.	Place	Variety	Area in ha	Month of survey	Days after sowing	% incidence	Intensity*
1	Bangalore						
A	Bangalore Rural						
	Hessaraghatta	Arka Manik	0.6	Dec-Jan1996-97	30	0.0	-
					90	97.6	Severe
	Nagamangala	Arka Manik	0.5	"	40	3.5	-
					90	93.4	Severe
B	Devanahalli Tq						
	Aradipura	Arka Manik	1.6	"	30	0.0	-
					70	50.0	Moderate
	Chemachanahalli	Arka Manik	0.6		30	0.0	-
					65	27.3	Moderate
	Naganayakanahalli	Arka Manik	0.8	"	65	6.9	-
					105	64.7	Severe
		Madhu	0.6		45	1.2	-
					95	39.7	Moderate
C.	Doddaballapura						
	Doddarajaghatta	Arka Manik	0.7	"	35	0.0	-
					85	48.9	Moderate
	Hunasanahalli	Arka Manik	1.2	"	35	0.0	-
					85	78.4	Severe
		Madhu	0.6	"	35	0.0	-
					85	57.4	Moderate
2	Kolar district						
A	Chikkaballapur Tq						
	Korenahalli	Arka Manik	1.0	Jan-Feb	30	0.0	
					70	19.8	Mild

(Table No. 1 Continued)

Sl. No.	Place	Variety	Area in ha	Month of survey	Days after sowing	% incidence	Intensity
		Madhu	0.6		35	0.9	-
					80	59.5	Moderate
	Marenahalli	Arka Manik	0.5		40	0.7	-
					75	73.3	Severe
		Madhu	0.3		45	2.8	-
					100	100	Severe
	Vaneganahalli	Arka Manik	0.4		40	1.9	-
					80	82.3	Severe
B	Chintamani Tq						
	Mohammadpur	MHW-6	1.2		30	1	-
					90	75	Severe
	Talagavara	Namdhari	0.8		30	1	-
					75	100	Severe
		Arka Manik	1.6		25	0.0	-
					75	75	Severe
	H-Cross	Namdhari	1.0		30	0.0	-
					70	50	Moderate
C	Kolar Tq						
	Kamadenahalli	Namdhari	0.8		30	0	-
					75	20	Mild
		Namdhari	0.8		30	0	-
					70	20	Mild
	Poovandahalli	Arka Manik	1.2		40	0	Mild
	Odagur	Arka Manik	1.0		30	0	Mild

(Table no. 1 Continued)

Sl. No.	Place	Variety	Area in ha	Month of survey	Days after sowing	% incidence	
	Kyalanur	Mahyco	0.8		30	0	-
		hybrid			85	30	Mild
3	Chitradurga Dist						
A	Hiriyur Tq	Namdhari	0.5	Jan	40	0	-
					80	10	Mild
		Namdhari	0.5		40	0	-
					75	5	Mild
	Adivala	Namdhari	1.0		45	0	-
					80	10	Mild
	Metikurke	Mahyco	0.5		45	0	-
		hybrid			75	5	Mild
4	Mandya Dist						
	Nagamangala Tq	Arka Manik	0.5	October	90	10	Mild
		Namdhari	1.0		100	10	Mild
	Mandya Tq	Namdhari	1.0		85	0	Mild
		Namdhari	1.5		95	0	Mild

* Arbitrary categorization

The incidence of BND during the Month of January 1997 in Hiriyur taluk of Chitradurga District was found to range from 5-20 per cent between 60-80DAS.

Disease incidence ranging from 0-10% was found in Nagamangala and Mandya taluks of Mandya District during the months of September and October 1996, at 75-90 DAS.

4.3 SEASONAL VARIATION OF DISEASE INCIDENCE

Seasonal variation of disease incidence was observed in the monthly sown crop starting from Rabi season of 1996 to Kharif season of 1997. The percent disease incidence after 60 DAS and the time taken for symptom expression were recorded in a regular interval (Table 2).

The Kharif season crop sown during the months of May- August 1997 showed the least overall mean incidence of 32.5%. The time taken for symptom expression was found to be more in kharif sown crops (50-60DAS) compared to the crops sown in during summer and Rabi seasons.

The highest overall mean disease incidence of 87.5% was found in crops sown during the months of January, February, March and April. Early disease symptom expression (30-40 DAS) was also observed during this period when compared to other months.

The Rabi season crop sown during the months of September-December showed moderately high overall mean disease incidence of 62.25% . The days taken for symptom expression was ranged from 40-50DAS.

The crops sown during the months of June and July showed mild symptoms of the disease. Moderate disease symptoms were observed in the crops sown during May, September, October and November months. The crops sown

Table 2 : Seasonal variation of disease Incidence

	Season	Month of sowing	% incidence (two months after sowing)	Days taken for symptom expression after sowing	Intensity
1	Rabi	September 1996	50	45	Moderate
2	Rabi	October 1996	70	50	Moderate
3	Rabi	November 1996	50	40	Moderate
4	Rabi	December 1996	80	40	Severe
5	Summer	January1997	100	40	Severe
6	Summer	February1997	100	40	Severe
7	Summer	March 1997	100	35	Severe
8	Summer	April1997	50	35	Severe
9	Kharif	May1997	45	60	Moderate
10	Kharif	June1997	10	50	Mild
11	Kharif	July1997	25	50	Mild
12	Kharif	August 1997	50	50	Moderate

Index : Thrips populations
< 10 per bud - Low
10-25 per bud - Medium
> 25 per bud - High

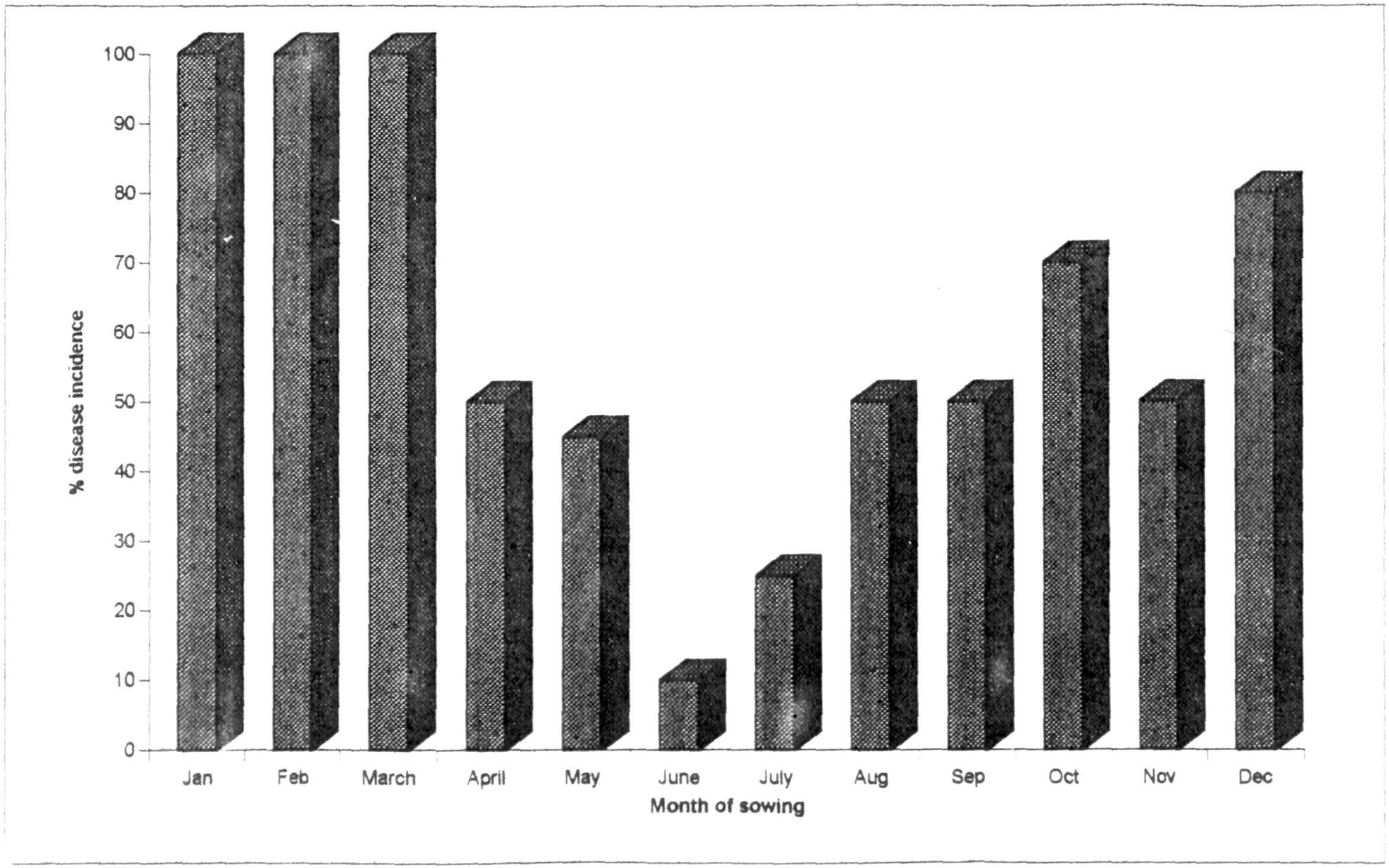


Fig 1. Seasonal variation of disease incidence

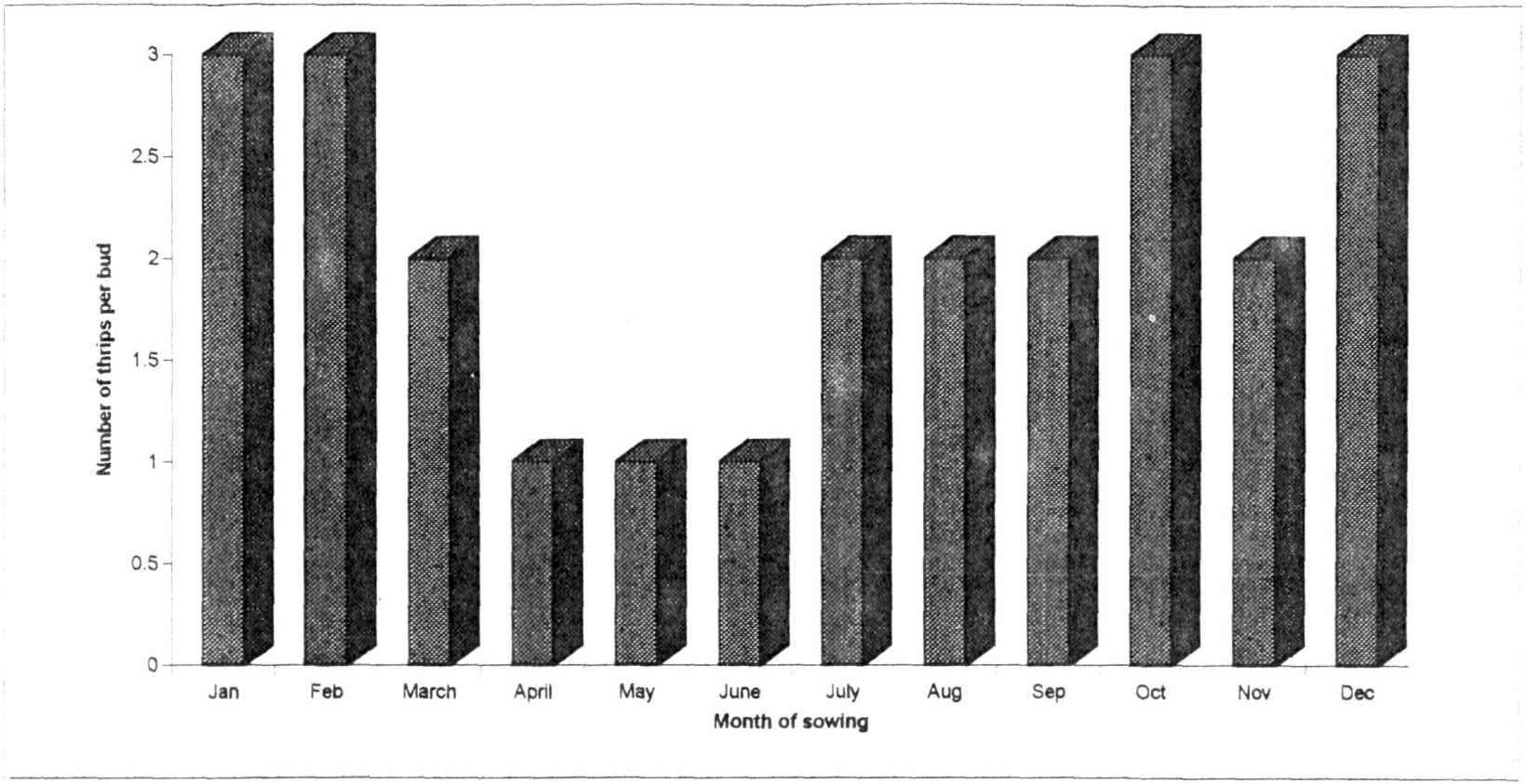


Fig 2. Seasonal variation of thrips population

Index
Number of thrips per bud
1=low (<10 per bud)
2=Medium(10-25 per bud)
3=High (>25 per bud)

during the months of December, January, February , March and April months showed severe disease incidence.

Thrips population was found to be medium to high in Rabi and summer crops and low in Kharif season crop.

4.3 TRANSMISSION

4.3.1 Mechanical transmission

The crude extracts from watermelon bud necrosis virus (WBNV) infected leaves were inoculated on to the young glass house grown seedlings of three local lesion host species viz., *Vigna unguiculata* cv. C-152, *Nicotiana rustica* and *Chenopodium amaranticolor* by sap inoculation following the procedure outlined under "Material and Methods". The experiment was repeated thrice and the results obtained were noted (Table-3).

As can be seen from Table-3, the crude viral extract produced local lesions on cowpea within five days after inoculation (Plate 6A). The percentage transmission varied from 20-80% depending upon the plant species and the inoculated plants developed symptoms in 10-15 days. *Chenopodium amaranticolor* (Plate 6B) took 8-10 days, and *Vigna unguiculata* Walp. cv. C-152 took 4-5 days for expression of local lesion symptoms. *Nicotiana rustica* took 10-15 days for symptom expression (Plate 7a).

4.3.2. INSECT TRANSMISSION

Thrips palmi, a species of thrips was tried for the transmission of WBNV as per the procedure described in 'Material and Methods'. The data presented in the Table 4 revealed that *Thrips palmi* could transmit the virus from watermelon to watermelon. Adult thrips collected from naturally infected watermelon showed 30% transmission and the plant took 30-40 days for symptom expression. Virulent adults also transmitted the virus to tobacco and tomato with 30% and

Table-3 Sap transmission of Watermelon bud necrosis virus on 3 local lesion hosts

	Host species inoculated	Experiment	No of plants Infected/ Inoculated	Per cent transmission of WBNV	No. of days taken for expression of symptoms
1	*Vigna unguiculata* (L) Walp cv C-152	I	1/5	20	4-5
		II	2/5	40	4-5
		III	1/5	20	4-5
2	*Chenopodium amaranticolor*	I	4/5	80	8-10
		II	4/5	80	8-10
		III	4/5	80	8-10
3	*Nicotina rustica*	I	3/5	60	10-15
		II	2/5	40	10-15
		III	2/5	40	10-15

Plate 6 A. Photograph showing chlorotic local lesions on cowpea leaf after mechanical inoculation

6B. Photograph showing chlorotic local lesions on *Chenopodium amaranticolor* leaves after mechanical inoculation.

Plate 7 A. Photograph showing chlorotic local lesions on *Nicotiana rustica* leaf after mechanical inoculation

7B. Photograph showing mild chlorotic local lesions on *Nicotiana glutinosa* leaves after mechanical inoculation.

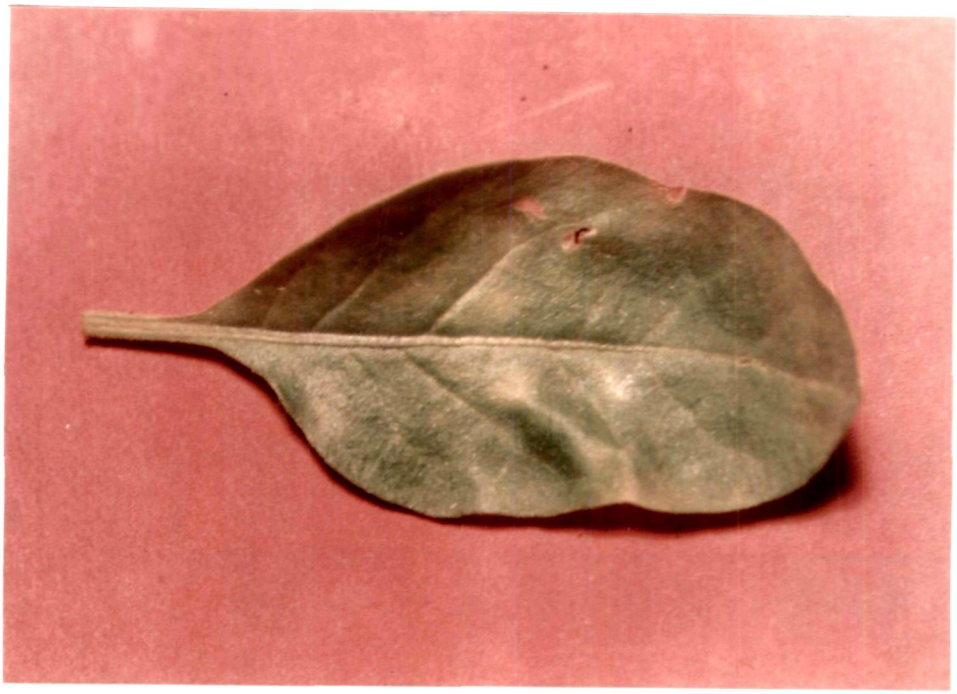


Table. 4 : Thrips transmission of water melon bud necrosis virus

Thrips species tested	Name of the Plant	No. of plants inoculated	No. of plants infected	% Transmission	Days required for symptom expression (after sowing)
Thrips palmi	Watermelon	10	3	30	30-40
Thrips palmi	Tobacco	10	3	30	15-25
Thrips palmi	Tomato	10	2	20	15-25

Plate 8. Photograph of adult thrips of *Thrips palmi* Karny .

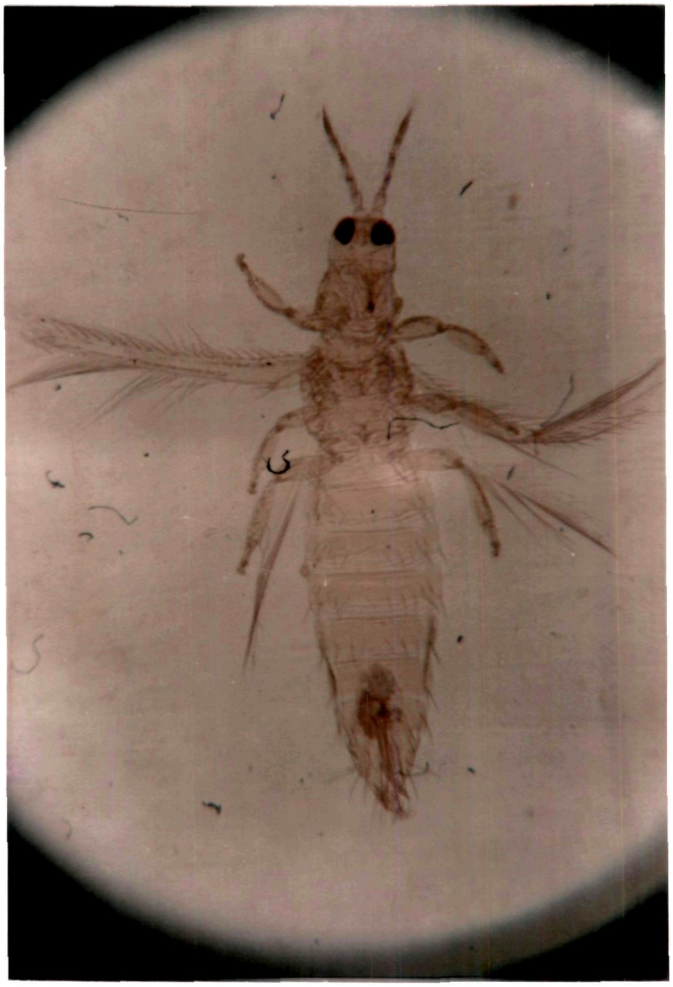
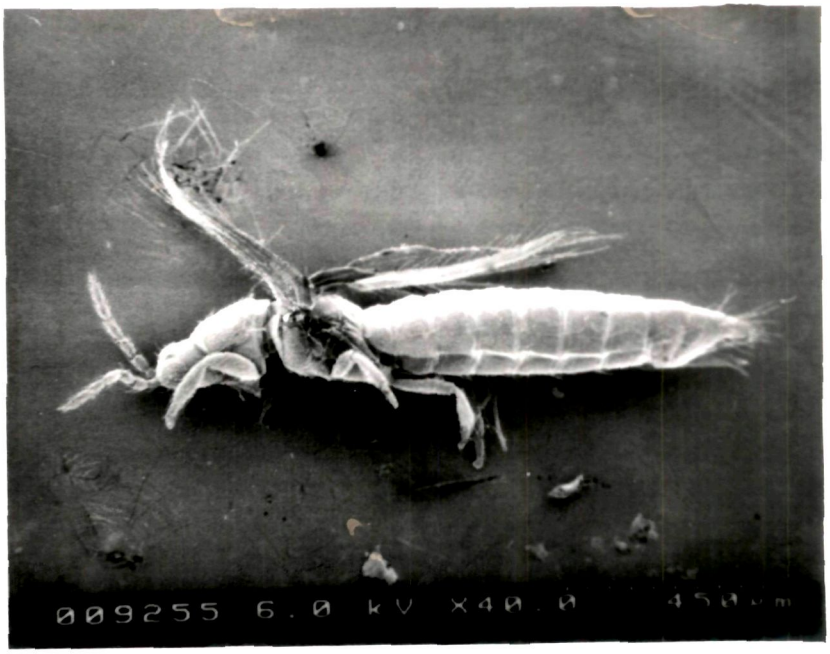
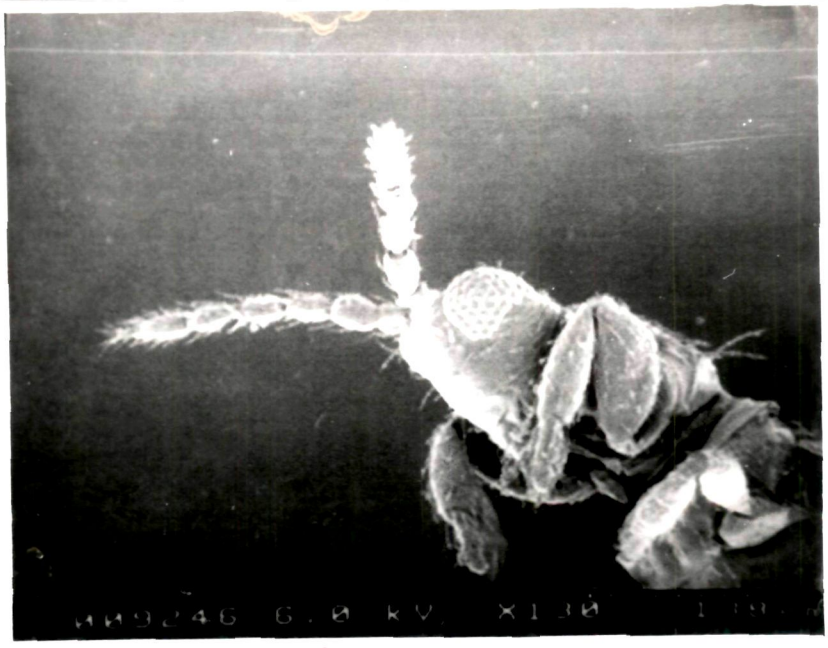


Plate 9 A. Scanning electron micrograph of full view of *Thrips palmi* Karny.

9B. Scanning electron micrograph of close view of *Thrips palmi* Karny.


009255 6.0 kV X40.0


009246 6.0 kV X130

20% transmission respectively. In the above case the inoculated plants took 15-25 days for symptom expression.

The main symptoms included yellow mottling, distortion and necrotic spots on leaves similar to those induced by mechanical transmission by the virus. The presence of the virus in test plants was confirmed by inoculation to cowpea and also by ELISA test.

4.4. Identification of thrips vector.

The insect vector of WBNV collected from Kolar and Bangalore, was identified as *Thrips palmi* Karny by Mr. Laurence A. Mound, CSIRO Division of Entomology, Canberra, Australia (Plate 8).

Light microscopic observations of mounted specimens of thrips revealed yellow coloured body, unshaded forewing, red coloured ocellar pigment and abdominal pleurotergites without setae.

The results of identification from Dr. A. Jagadish, Associate Professor, Department of Entomology, UAS, Bangalore, also confirmed the presence of *Thrips palmi* on water melon.

Scanning electron micro graphs (SEM) of the thrips vector (*Thrips palmi*) have also been taken (provided by Mr. W.B. Hunter) (Plate 9) .

4.5 HOST RANGE

The plants belonging to different families when observed after sap inoculation, showed different types of symptoms. *Gomphrena globosa* showed necrotic local lesions on the leaves. Chlorotic local lesions turning to necrotic lesions were observed in *Chenopodium amaranticolor* (Plate 6B) and *Chenopodium quinoa*. Watermelon plants (*Citrullus lantus* cv.Arka Manik and cv. Madhu)

Table 5 : Host-range of Watermelon bud necrosis virus

Family	Test plant	Symptoms on inoculated plants
Amaranthaceae	*Gomphrena globosa*	Necrotic local lesions on leaves
Chenopodiaceae	*Chenopodium amaranticolor*	Necrotic local lesions on leaves
	Chenopodium quinoa	Necrotic local lesions on leaves
Cucurbitaceae	*Citrullus lanatus cv.* Arka Manik.	Chlorotic local lesion and systemic infection
	Citrullus lanatus, cv. Madhu	Chlorotic local lesion and systemic infection
	Cucumis sativus	Chlorotic local lesion
	Cucurbita pepo	Chlorotic local lesion
Fabaceae	*Arachis hypogaea* cv. JL-24	Crinkling of leaves and chlorotic local lesions
	Glycine max cv. Bragg	Chlorotic local lesion on leaves
	Vigna mungo	Chlorotic local lesion on leaves
	Vigna unguiculata cv. C-152	Chlorotic local lesion on leaves
Solanaceae	*Capsicum annum* cv. Yellow Wax	Chlorotic local lesion on leaves
	Datura stromonium	Necrotic local lesion
	Lycopersicon esculentum cv. Pusa Ruby	Necrotic local lesion
	L esculentum cv. Arka Sourabh	Necrotic local lesion
	Nicotiana rustica	Chlorotic and necrotic local lesions on leaves
	Nicotiana benthamiana	Crinkling, vein clearing and systemic infection
	Nicotiana glutinosa	Chlorotic local lesions
	Physalis floridana	Necrotic local lesion.

showed chloratic local lesions followed by necrotic local lesions and systemic infection. Other cucurbitacious plants like *Cucumis sativus* and *Cucumis melo* showed mild chlorotic spots on the leaves. Similar spots were observed in *Glycine max* cv. Bragg, *Vigna mungo* and *Capsicum anum* cv Yellow Wax plants. Chlorotic followed by necrotic local lesions were observed in *Datura stromonium*, *Lycopersicon esculentum* cv PusaRuby, *Lycopersicon esculetum* cv Arka Sourabh and *Physalis floridana*. *Arachis hypogaea* cv JL-24 produced chlorotic local lesions and crinkling of the leaves. *Vigna unguiculata* cv C-152 (Plate 6A) and *Nicotiana rustica* produced conspicuous chlorotic followed by necrotic local lesions on the leaves. *Nicotiana benthamiana* showed systemic symptoms like paleness and dwarfing of the leaves of the plant. *Nicotiana glutinosa* showed mild chlorotic local lesions on the leaves (Plate 7B).

The details of the host range have been mentioned in Table-5.

4.6 SEROLOGICAL DETECTION

Serological detection of WBNV was done by using indirect ELISA and DIBA as described under "Material and Methods".

4.6.1 Detection by ELISA

WBNV was detected by using WSMV antiserum in all the diseased samples collected from the different watermelon growing areas. Details of the ELISA tests have been presented in the Table 6. Fruit samples collected from Talagavara and Hessaraghatta showing initial green rings and corky rings and mottling symptoms showed highest virus concentration (1.023-1.449 OD values) followed by young crinkled leaf samples collected from H-cross showing 1.076 OD values, brittle stem with black streak samples collected from Devanahalli showing 0.911 OD value and samples with die-back symptom collected from Kamadhenahalli showing 1.013 OD value with considerable virus concentrations when compared with healthy control sample showing 0.463 OD value after 1

hour of substrate addition. But the samples with fully blackened twigs collected from Kyalanur showed less virus concentration (0.941)

Virus concentrations in different parts of the diseased plants (Table 7) was detected by using PBNV antiserum. Highest virus concentration showing highest OD values were recorded in fruits showing ring symptoms followed by stems, which were brittle with black stripes, buds with initial necrosis symptoms, tender leaves with black spots and older leaves with necrosis of the midrib. All tested plant parts showed considerably high OD values when compared to healthy, both under 1: 10 and 1: 100 antigen dilutions.

Detection of the virus by using different dilutions of antisera and different dilutions of the crude virus extract was conducted. The results indicated that high OD values were obtained at lower dilution of antigen in virus infected samples. The reaction of the virus to different antibody dilutions showed that the virus could be effectively detected even at 1:20000 dilution. The OD values of buffer control and healthy control were very low indicating no nonspecific reaction to the host proteins (Table 8).

4.6.2 Detection by DIBA

Dot immuno-binding assay(DIBA) was conducted to detect the virus in different dilutions of the crude plant extract (Plate 10). Positive reactions were indicated by the development of purple colour in the blotted area on nitrocellulose membrane. Virus was detected up to 1 : 1000 antigen dilution. The lower dilution of antigen gave strong colour reaction indicating high virus titer. Dot blots from healthy plant extract did not show any colouration in the blotted area in all the dilutions tested.

Plate 10 . Photograph of membranes showing Dot Immuno-Binding Assay (DIBA) results of WBNV at different antigen dilution.

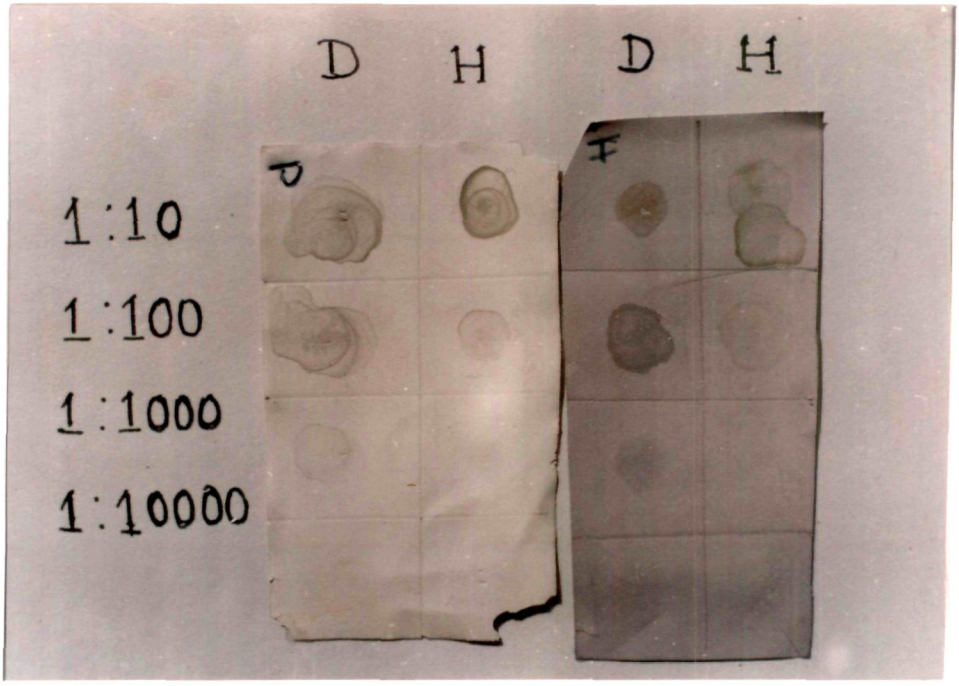

D
H
D
H
1:10
1:100
1:1000
1:10000

Table 6 : ELISA based detection of WBNV in the diseased samples of watermelon collected from different watermelon growing regions of Karnataka, showing different types of symptoms.

Sl. No.	Symptoms	Source	Variety	Absorbance values at 405nm		Reaction
				After 20 min	After 1 hr	
1.	Young twigs with initial die back symptom	Kamadhenahalli (Kolar taluk)	Arka Manik	0.198	1.013	++
2.	Fully blackened twigs	Kyalanur (Kolar taluk)	Sugar baby	0.326	0.941	+
3.	*Young leaves with black spots*	*Chintamani*	Arka Manik	*0.240*	1.527	++
4.	Young crinkled leaves	H-cross (Chintamani TQ)	Mahyco hybrid	0.231	1.076	+
		Aradipura (Devanahalli TQ)		0.117	0.709	-
5.	Initial green rings on fruits	Talagavara (Chintamani TQ)	Namdhari	0.283	1.383	++
6.	Corky rings on fruits	Hessaraghatta (Bangalore North)	Arka Manik	0.551	1.449	++
7.	Mottling on fruits	Hessaraghatta (Bangalore North)	Sugar Baby	0.331	1.023	+
8.	Leaf mosaic symptom & curling	Chikkaballapura	Arka Manik	0.102	1.089	+
9.	Brittle stem with black streaks	Devanahalli	Arka Manik	0.306	0.9115	++
10.	Healthy watermelon leaves			0.075	0.463	
11.	Buffer control			0.052	0.3015	

Antiserum used = WSMV in 1: 10,000 dilution
Antigen dilution = 1: 10
- Indicates OD values < 0.463 (Healthy control)
+ Indicates OD values 0.463 to 0.826 (Double of healthy control)
++ Indicates OD values between 0.826 to 1.527

Table 7 : Detection of the virus concentration in different parts of the plant by DAC ELISA

Sl. No	Plant part	Symptoms	Absorbance values at 405nm					
			After 5 min		After 30 min		After 1 hour	
			1:10	1:100	1:10	1:100	1:10	1:100
1.	Leaves	Healthy	0.45	-	0.093	-	0.126	-
2.	Tender leaves	Pale, curled with black spots	0.166	0.103	0.805	0.422	1.334	0.719
3.	Older leaves	necrosis of mid rib	0.120	0.085	0.755	0.402	1.249	0.648
4.	Buds	Brittle and showing initial symptoms of necrosis	0.378	0.292	1.988	1.649	2.352	2.095
5.	Tendrils	Initial necrosis	0.154	0.101	0.876	0.728	1.431	1.229
6.	Stems	Brittle with black stripes	0.565	0.519	2.232	2.160	2.210	2.095
7.	Midrib	Necrosis	0.263	0.216	1.465	1.117	2.143	1.878
8.	Fruit rind	Showing ring spots	0.654	0.525	2.312	2.341	2.247	2.224
9.	Buffer control		0.0045	-	0.025	-	0.025	-

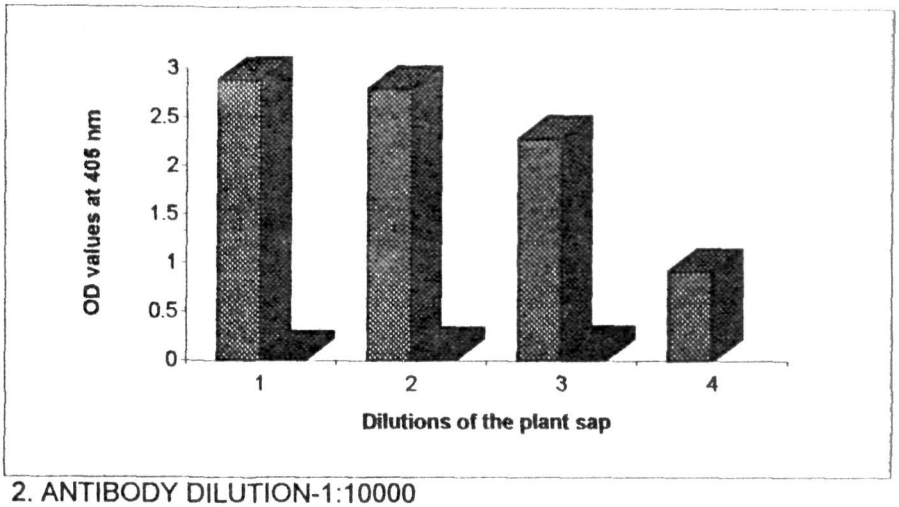
Antiserum Used - PBNV
Antiserum dilution - 1: 10,000

Table-8 : Detection of WBNV at different combinations of antigen and antibody dilutions

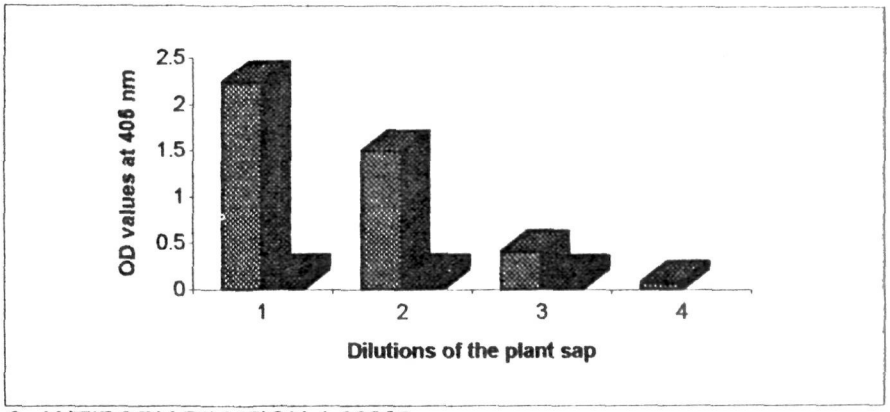
Test sample	Antigen Dilution	Antibody dilution		
		1 : 5000	1 : 10,000	1 : 20,000
Infected	10^{-1}	2.877	2.2334	1.632
	10^{-2}	2.791	1.4925	0.7115
	10^{-3}	2.288	0.4100	0.215
	10^{-4}	0.9095	0.079	0.140
Healthy	10^{-1}	0.0625	0.1545	0.167
	10^{-2}	0.101	01545	0.167
	10^{-3}	0.114	0.1460	0.157
Buffer	-	0.005	0.05	0.115

Antiserum used - PBNV

1. ANTIBODY DILUTION-1:5000



2. ANTIBODY DILUTION-1:10000



3. ANTIBODY DILUTION-1:20000

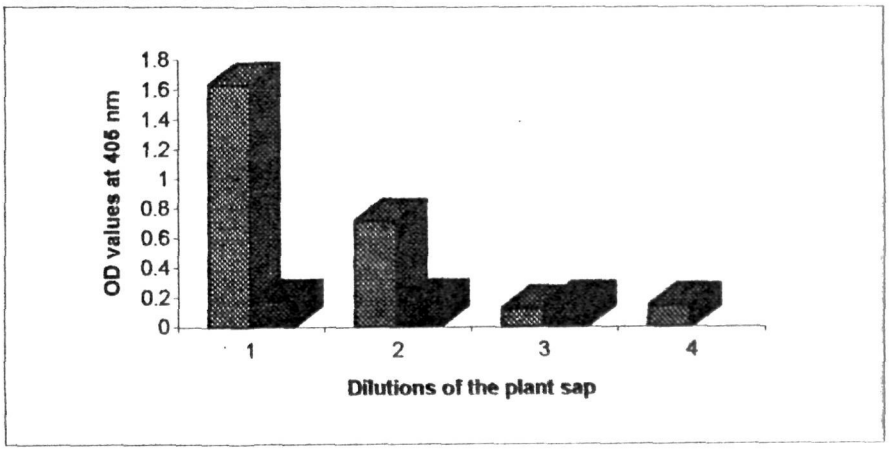


Fig.3 Graph showing ELISA results of WBNV at different antigen and antibody dilutions

Index 1
Dilutions of the plant sap or anitgen dilution
1=1:10
2=1:100
3=1:1000
4=1:10000

Index 2
Ist bar in each dilutions-Infected sap values
IInd bar in each dilutions-Healthy sap values

4.7 HISTOPATHOLOGY

HEALTHY LEAF

The cross section of healthy leaf revealed that both the epidermal layers were intact with numerous hairy outgrowths. The upper epidermal layer was thickly coated with cuticle. The palisade cells were compact and densely packed with parietally located chloroplasts. The spongy parenchymatous cells were isodiametric to oval in shape and loosely arranged. Vascular bundles along with ground tissue were quite intact and no disorders either in cell structure or in cellular contents were observed (Plates 11A, 11B and 12A).

INFECTED LEAF

The changes in infected watermelon leaves showing black spots and curling were observed to be due to changes in the structure of cells and tissues. Different histological changes recorded are given in Table 9.

a. Hyperplasmic changes

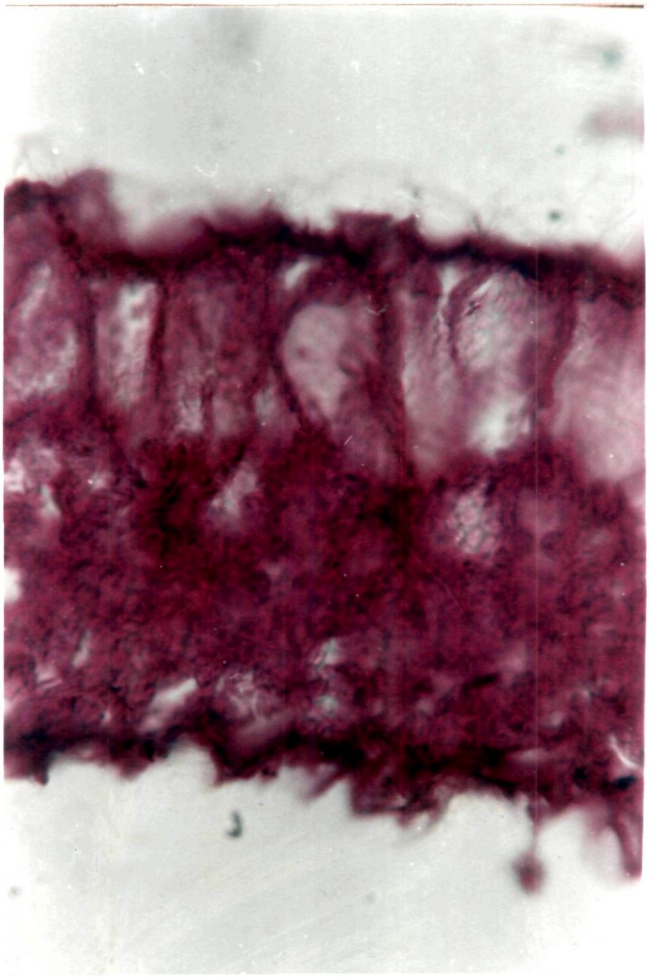
The cross section of infected leaves showing curling symptoms showed increased number of cells per unit area. The section of advanced stage of infection showed tissues with densely packed cells lacking any air space (Plates 11B, 12A and 12B).

b. Histotrophic and Histomorphological changes :

Histotrophic changes like changes in the thickness of the infected leaf, epidermis, spongy tissue and palisade layer were observed. Infected leaves showed 48.57% of reduction in their thickness due to the reduction in the thickness of epidermis (65.19%), palisade layer (60%) and spongy tissue layers (29%).

Plate 11 A. Transverse section of healthy watermelon leaf stained with per-iodic acid Schiffs reagent (PAS) X 250.
A = Upper epidermis
B = Palisade layer
C = Spongy layer
D = Lower epidermis

11B. Transverse section of WBNV infected watermelon leaf stained with PAS X 250.



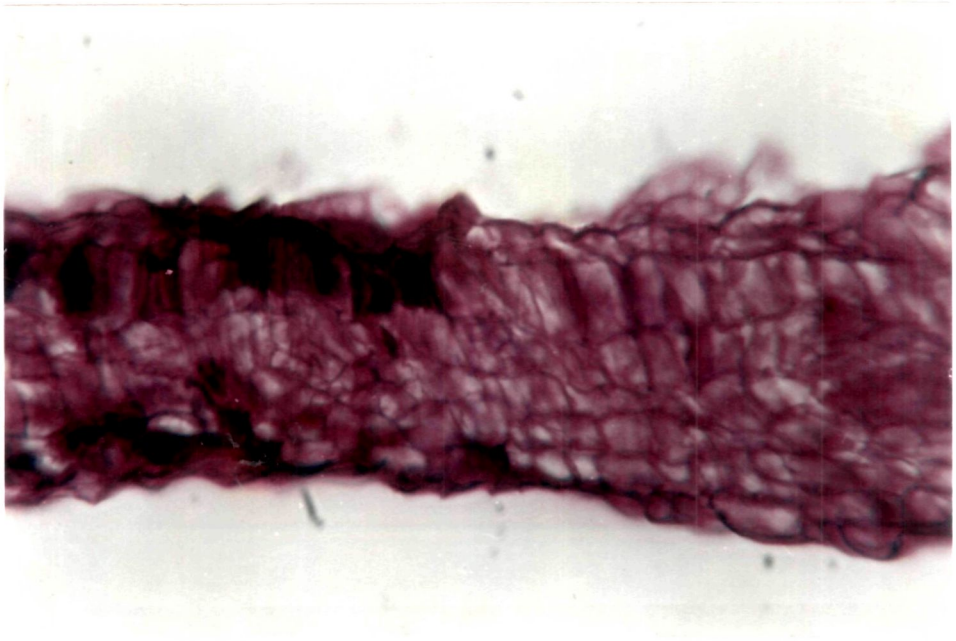
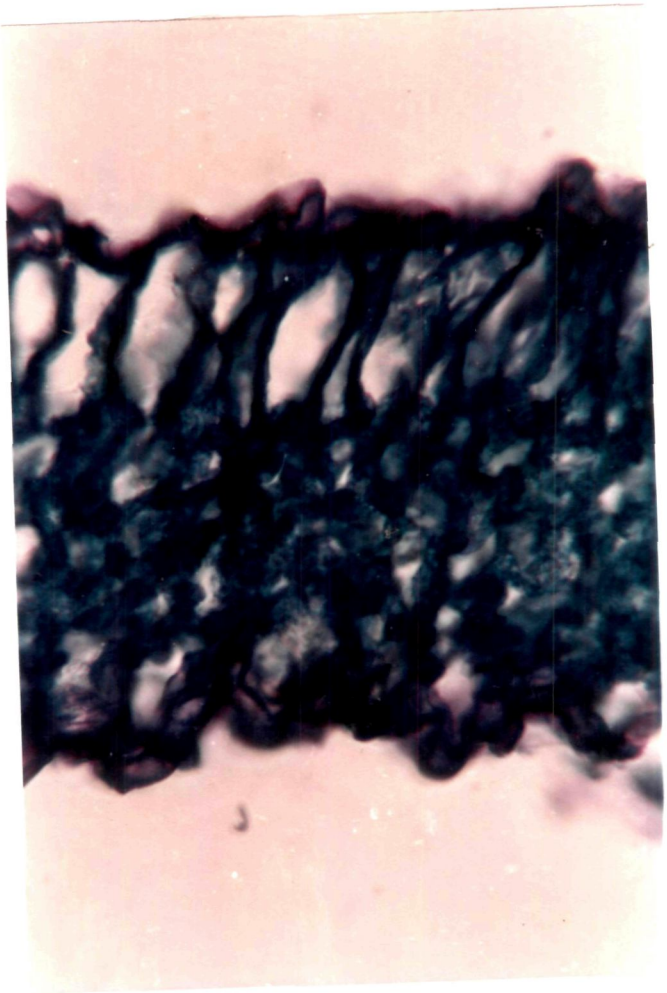


Plate 12 A. Transverse section of healthy watermelon leaf stained with Toluidine Blue (TB) X 250.
A = Upper epidermis
B = Palisade layer
C = Spongy layer
D = Lower epidermis

12B. Transverse section of WBNV infected watermelon leaf stained with TB X 250.



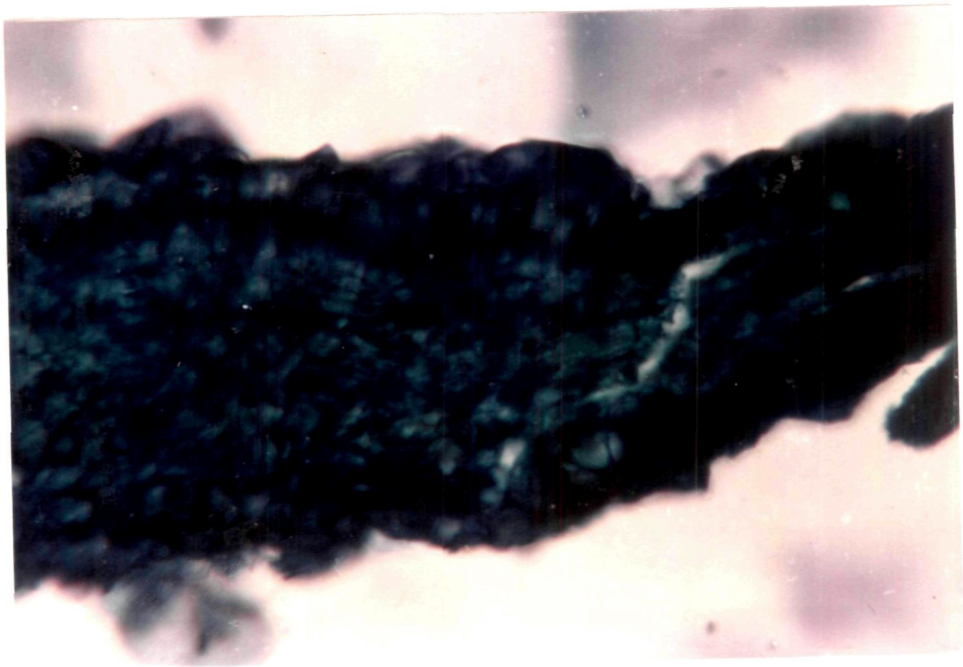


Plate 13 . Transverse section of infected watermelon leaf showing necrotic spotted area and stained with TB.

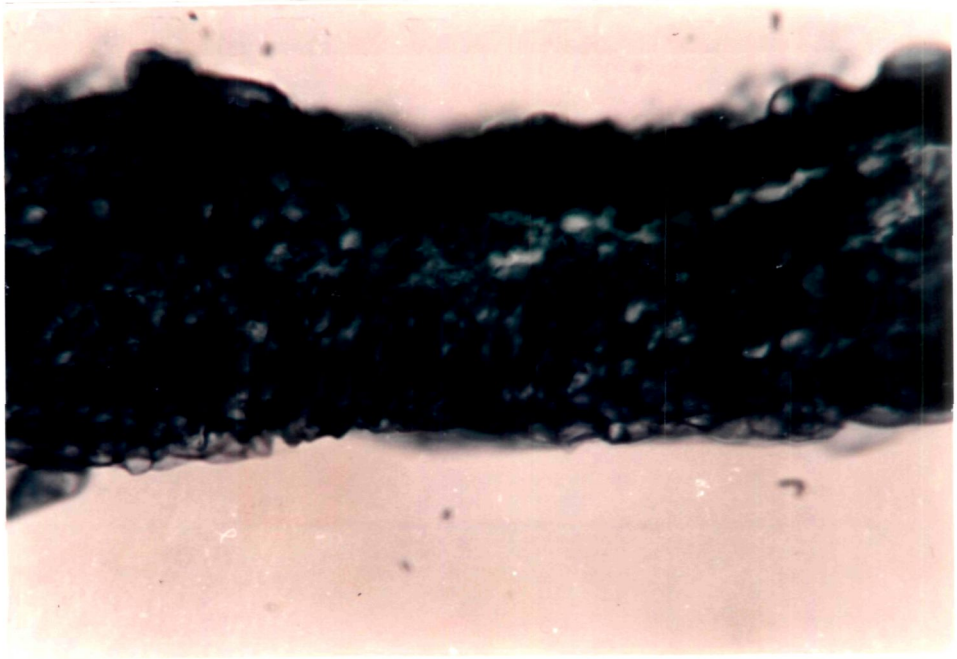


Table 9.Histological changes in watermelon leaves due to infection by watermelon bud necrosis virus

Sl. No.	Tissue	Healthy (in µm)	Infected µm)	% Reduction
1.	Palisade parenchyma cells			
s	-Length	38.5	15.4	60
	-breadth	23.10	6.7	70.99
	- size(area)	889.35	103.18	88
2.	Thickness of epidermis	19.25	6.7	65.19
3.	Thickness of palisade layer	38.50	15.4	60
4.	Thickness of spongy tissue	57.65	40.5	29
5.	Thickness of leaf	134.75	69.3	48.57

Histotrophic changes, as a result of cytotrophic changes, were characterised by changes in cell size and volume. The palisade parenchyma cells of infected leaves showed considerable reduction in their size/area (88%), due to the reduction in their length (60%) and breadth (70.99%).

Histomorphological changes like changes in cell forms were observed in the infected tissues. The cross section of the infected leaf showing black spots exhibited that the upper epidermal layer cells were collapsed in the necrotic areas (Plate 13). Palisade cells situated just below the collapsed region were seen in various abnormal shapes (Plate 12B and 13). In case of severe necrosis the palisade cells were also seen collapsed in the black spots region. The spongy parenchymatous cells also depicted varied shapes and lesser air spaces as compared to healthy ones.

C. Histochromatic changes

Characteristic changes with brownish to black pigments were observed in the infected young leaves showing black spots. The pigments were first seen in the palisade layer followed by epidermis(Plates 11B, 12A and 12B). The epidermis had collapsed in the pigmented region. Small patches of pigments in the tissues later coalised to give a black necrotic appearance on the plant parts. These were prominently seen in the leaf midrib, tendrils and tender stem parts giving a black streak like appearance.

4.8 ELECTRON MICROSCOPY

Leaf dip preparations from infected watermelon plants and tobacco revealed the presence of spherical, enveloped virus particles when viewed under Transmission Electron Microscope (TEM). The diameters of the particles in negative stain were greater than in ultra-thin sections and more variable, presumably because of flattening. Similar particles were not observed in healthy plant preparations.

Plate 14. Electron micrograph of spherical particles of WBNV present in the periphery of the watermelon leaf cell.
ER = Endoplasmic reticulum
CW = Cell wall
V = Virus

Plate 15 . Electron micrograph of ultra-thin section of watermelon leaf showing densely packed WBNV particles in the cell.
Vp = Viroplasm
CW = Cell wall

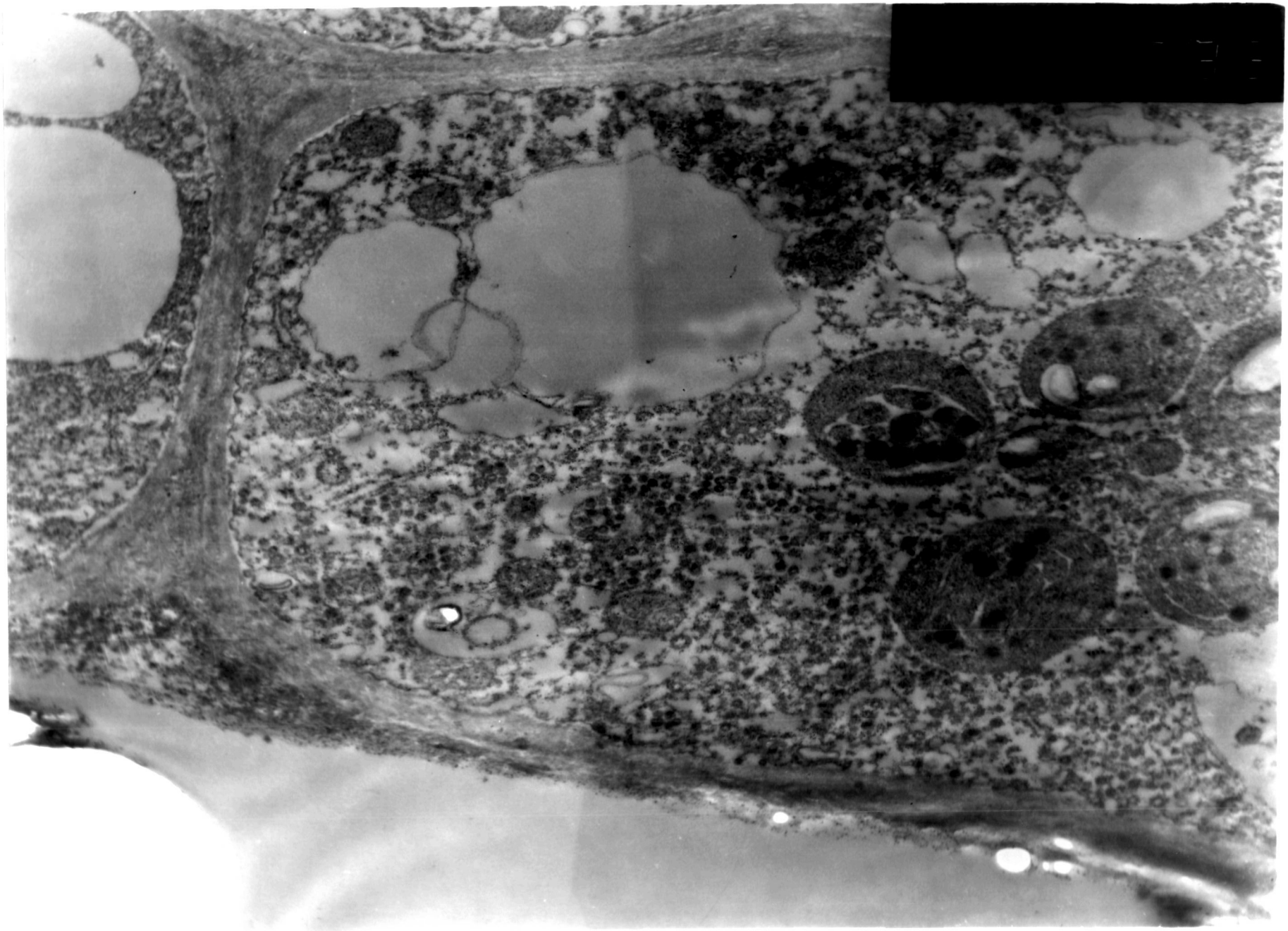
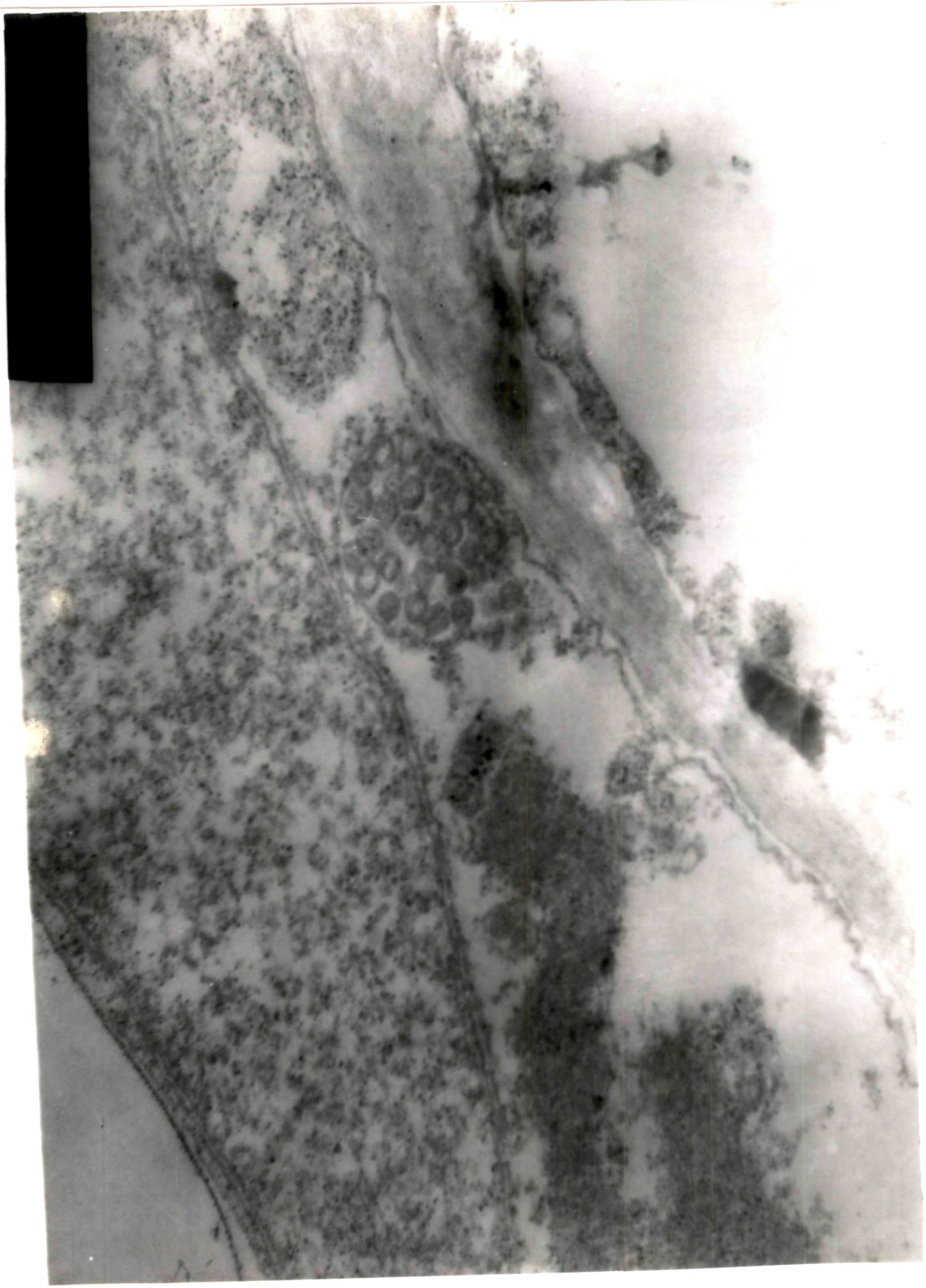


Plate 16 . Electron micrograph of ultra-thin section of watermelon leaf showing isometric particles embedded in a sac.
ER = Endoplasmic reticulum
CW = Cell wall
C = Cisternae



Large quasi-spherical particles with membranes, 80-90nm in diameter (Plate 14) were observed in ultra-thin sections of leaves of diseased watermelon plants collected from field and mechanically inoculated in the green house. These particles appeared more regular in shape and were grouped in cisternae of the enodplasmic reticulum in the cytoplasm (Plate 15).

Viroplasm and densely stained amorphous masses were observed in the cytoplasm and were associated with the endoplasmic reticulum that sometimes contained spherical particles (Plate 16). These particles were observed in cells of leaf epidermis and mesophyll as well as in seive tubes and phloem parenchyma of leaf veins.

DISCUSSION

V DISCUSSION

The occurrence of bud necrosis disease on watermelon (*Citrullus lanatus*) was for the first time observed from India by Singh and Krishna Reddy (1995). It was shown that the bud necrosis disease (BND) was caused by a virus which resembled tomato spotted wilt virus (TSWV) (Yeh *et al.*, 1992; Krishna Reddy and Singh, 1993; Singh and Krishna Reddy , 1996). Since 1991, BND has been observed in the watermelon growing areas of Bangalore in Karanataka and is on the increase year after year. The results of the present studies carried out on the bud necrosis virus disease of watermelon in some parts of Karnataka with respect to Symptomatology, survey, transmission, seasonal variation, identification of vector thrips, host range, serological studies, histopathology and electron microscopy are discussed in detail here under.

Symptomatological observation in India showed that symptoms like stunting of the plant with short internodes, upright growth of younger branches, tip necrosis and dying back were similar to the symptoms observed in Taiwan (Yeh *et al.*, 1992), but sympotms like necrotic spots on leaves and necrotic streaks on vines were found to be prominent in the Indian field conditions. This may be due to the warm tropical climate of India.

TSWV strains reported till 1984 produced only chlorotic local lesions on inoculated leaves of cucurbitacious plants but did not invade them systemically. But our observations showed the systemic infection of the watermelon plants by a tospovirus not only in the field condition but also on the mechanically inoculated plants in the glass house condition. This is in conformity with the results obtained in Japan, Taiwan and India (Iwaki *et al.*, 1984; Yeh *et al.*, 1992 ; Singh and Krishna Reddy 1996) . ELISA results of our present investigation confirmed the presence of watermelon bud necrosis virus in buds, leaves, tendrils, stem and fruit rind of the plant (Table 7).

The studies on survey for incidence of bud necrosis virus disease in Karnataka indicated that its incidence ranged from 10 to 100 percent in various parts of the state. The present investigation revealed that the incidence of BND was comparatively higher in Kolar district (75%) indicating that BND preferred dry climatic conditions for its proper expression of symptoms and for thrips multiplication and spread. The studies on seasonal variation of disease incidence has shown that summer crop sown during the months of January, February and March had shown severe disease incidence (100%), when compared to crops sown during Rabi and Kharif season. Reddy *et al.* (1991) also opined that Peanut bud necrosis tospovirus symptom was common on crops grown in dry seasons in India and probably associated with high temperature.

Studies on mechanical transmission of bud necrosis virus infected samples revealed that the virus produced chlorotic or necrotic lesions on cowpea, *Chenopodium amaranticolor* and *Nicotiana rustica* indicating that the virus can be successfully mechanically transmitted. They produced local lesions within 4 to 15 days of inoculation. From this study it is clear that cowpea (*Vigna unguiculata* (L) Walp. cv.C-152) was a good assay host for watermelon bud necrosis virus since it produced local lesions with-in 4 to 15 days of inoculation. These findings are in accordance with the reports of Iwaki *et. al.* (1984), Yeh *et al.* (1992), Krishna Reddy and Singh, (1993) and Singh and Krishna Reddy (1996).

Our results demonstrate that *Thrips palmi* Karny, can successfully transmit the watermelon bud necrosis virus. Previously, the same species was reported to be the vector of a tospovirus causing Silver mottle disease of watermelon in Japan (Iwaki *et al.*, 1984) and in Taiwan (Yeh *et al.*, 1992). Recently *Thrips palmi* Karny has been found to be a potential vector of peanut bud necrosis virus in India (Vijayalakshmi *et al.*, 1991).

Earlier, Singh and Krishna Reddy (1995), reported that bud necrosis disease of watermelon was transmitted by thrips which was identified as *Thrips flavus* Shrank. Lot of similarities were assumed to be existing between *Thrips*

flavus Shrank and *Thrips plami* Karny. Later detailed studies on the vector and identification by L.A. Mound indicated that *Thrips palmi* Karny is the potential vector for watermelon bud necrosis disease (unpublished).

In the light of above understanding, a study on identification of thrips species present in Karnataka which could transmit water melon bud necrosis virus was under taken.

Based on the thrips specimen collected from Bangalore and following the identification key proposed by Reddy *et al.* (1991), Mound (1996),it was observed that thrips species commonly prevailing on bud necrosis infected plants of watermelon in Karnataka had yellow coloured body, with unshaded forewing, red colored ocellar pigment and the abdominal pleurotergites without setae. This evidence confirmed that thrips species present in the areas studied were none other than *Thrips palmi* Karny. A set of thrips collected from Bangalore areas were identified by Dr. Laurence A. Mound, CSIRO, Division of Entomology, Australia, and also by Dr. A. Jagadish, Associate Professor, Department of Entomology, UAS, Bangalore as *Thrips palmi* only.

Studies on the host range of bud necrosis virus of watermelon revealed that the virus could infect 18 host plant species belonging to 13 genera in five families by mechanical inoculations. Some hosts produced chlorotic local lesions and some produced necrotic local lesions on the host. The host range of water melon isolates of tospoviruses were similar to those of other tospovirus isolates, with the exception of systemic invasion of cucurbitaceae (Iwaki *et al.*, 1984; Yeh *et al.*, 1992; Singh and Krishna Reddy, 1996). It can infect field crops like *Arachis hypogaea, Vigna mungo* and *Vigna unguiculata.* These crops are grown in Kharif and Rabi seasons in some parts of Karnataka and are invariably infested by thrips vector.

The virus could also infect vegetable crops like *Lycopersicon esculentum* Mill, *Cucumis sativus, Cucumis melo* and ornamental and weed hosts like

Gomphrena globosa and etc. The vegetable host species and ornamental plants are grown round the year nearby watermelon fields, and these could harbour vectors carrying the virus. The weed plants also harbour the virus as well as vectors. All these plant species could serve as a good reservoirs of virus inoculum as well as vector mediators for the onset of epiphytotics of bud necrosis virus disease on watermelon crop year after year.

WSMV was found to infect 23 plant species representing six families (Iwaki *et al.*, 1984). Yeh *et al.* (1992) also identified twelve plant species representing five families as the hosts of TSWV infecting watermelon in Taiwan. Singh and Krishnareddy (1996) listed 34 plant species belonging to 23 genera in 7 families to be infected by WBNV. A recent report also enlists more than 500 species of plants in more than 50 families to be infected by TSWV (Ullman *et al.*, 1992). Owing to its wide host range, survival of the virus on various alternative hosts through vectors seems to be probable reason for its appearance in high proportions on watermelon crop in recent years.

Watermelon bud necrosis virus was detected serologically both by using WSMV antiserum obtained from Japan and peanut bud necrosis virus (PBNV) antiserum obtained from ICRISAT, India.

ELISA allows a quantitative estimate of tospovirus antigens in infected plants with high sensitivity and specificity (Gonsalves and Trujillo, 1986 ; Hobbs *et al.*, 1987). In our experiment we employed indirect ELISA method for the detection of the virus.

Reddy *et al.* (1992) have discussed in length about serological relationship among the tospoviruses and indicated that member of this genus could be distinguished based on the serological differences in the N protein.

Earlier, serological tests by ELISA and Western blotting using polyclonal and monoclonal antibodies demonstrated that the watermelon virus was

serologically related to the tospovirus causing silver mottle on watermelon in Okinawa (Japan) (Iwaki *et al.*, 1984), TSWV-W isolate of Taiwan (Yeh *et al.* 1992) and peanut isolate causing bud necrosis in India (Adam *et al.*, 1993 ; Reddy *et al.*, 1992) but distinct from TSWV and INSV (Adam *et al.*, 1993; Yeh *et al.*, 1992). Further, TSWV-W nucleocapsid polyclonal antibodies revealed that TSWV isolates from dahlia, pepper and tomato are serologically distinct (Hanada *et al.*, 1993). Yeh and Chang (1995) reported that the low degree of nucleotide sequence identity and amino acid similarity of the N-gene with those of the TSWV and INSV, coupled with negative hybridization relationships, indicated that the watermelon strain of TSWV should be named as watermelon silver mottle virus (WSMV).

The nucleocapsid protein gene of the tospovirus infecting watermelon in India has been cloned and sequenced. The NP gene of the watermelon tospovirus shared 81 per cent and 79 per cent identity with PBNV and WSMV. In contrast 44 to 46 per cent sequence identity with NP gene of other tospoviruses belonging to sero groups I, II, III and V (Jain *et al.* 1997).

Considering the distinct differences in host range and the NP gene sequence, the watermelon tospovirus from India, designated as watermelon bud necrosis virus (WBNV) constitutes a new virus species in sero group IV (Singh and Krishna Reddy, 1996; Jain *et al.*, 1997).

The WBNV in the present study is different from most other tospoviruses in host reaction, vector relationships and serology. Although the present isolate is serologically related to Indian PBNV, host range is very much different from it with respect to host range and N gene sequence homology (Jain *et al.*, 1997).

Light microscopic observations of the cross section of the infected leaf showed the reduction in the dimension of leaf cells resulting in the reduction in thickness of infected leaf. Palisade cells showed drastic reduction in their size. According to Sutic (1980) underdeveloped or immature palisade cells are

particularly susceptible to hypotrophy, so that those leaf parts with such cells are thinner than normal and have a rough surface. Our observations also showed the reduction in the thickness of epidermis and spongy tissue. Epidermis was collapsed in the necrotic spotted areas. Similar observations were made in case of *Cucurbita pepo* leaves infected with cucumber mosaic virus (Bansal *et al.*, 1992). The mesophyll of susceptible host can be infected by all or almost all viruses, except some histotrophic viruses (Price, 1966). Hypoplasma of mesophyll cells results from inadequate or reduced activity of meristem cells of leaf primordia (Ulrich and Quartz, 1964). Reduction in the leaf thickness due to viral infection has been reported for several plant diseases (Mathews 1970; Sutic 1980; Schmidt, 1980). Lack of air spaces in spongy tissues of the infected tissue was in conformity with some of the viral diseases (Bawden 1956; Schmidt, 1980; and Bansal *et al.*, 1992).

Detection of Tospovirus virus particles and their identification by electron microscopy can usually be achieved by examination of leaf dip preparation or of thin sections of infected cells (Milne, 1970). Fixation is found to be essential to avoid flattening and distortion of particles before staining (Francki and Halta, 1981). Ammonium molybdate has found to be a good negative stain. The isolated particles lack uniformity of size and shape and hence are not readily distinguished from some normal cell materials. The problems associated with negative staining of TSWV are due to the presence of viral envelope. Preparation of thin sections from infected tissues of the plant, though time consuming and requires skill, still is recomended as a very reliable method of identifying TSWV. The particles in the sections are relatively uniform and cannot be mistaken for those of any other virus. In addition, presence of viroplasms in many of the cells, is characteristic of TSWV infection (Francki and Hatta, 1981) .

Electron microscopic studies have showed that for the detection of TSWV particles in negatively stained dip preparations, samples must be fixed (Franki and Hatta, 1981; Milne, 1970). Fixation prevents much of the destortion of virus particles, and consequently the envelope around the core particle can be

observed (Plate 16). Detection of WBNV particles in negatively stained dip preparations and ultra thin sections of infected plants showed that tospovirus particles were always present in the cisternae of the endoplasmic reticulum. These results concur with those reported for other tospoviruses (Milne, 1970; Iwaki *et al.*, 1984; Urban *et al.*, 1991; Kitazima *et al.*, 1992; Yeh *et al.*, 1992 and Singh and Krishnareddy 1996).

SUMMARY

VI SUMMARY

The present investigations on bud necrosis virus disease on watermelon in Karnataka include survey and seasonal variation of disease incidence, identification of vector thrips, transmission and electron microscopic studies of the diseased tissue. The experiments were conducted at Indian Institute of Horticulture Research, Bangalore during the years 1996 and 1997. Histopathalogical studies of the diseased tissue was conducted at the Department of Botany, University of Agricultural Sciences, Bangalore. The results of the studies are summarised here under.

1. The over all incidence of bud necrosis virus disease in four districts of Karnataka namely Bangalore, Kolar, Chitradurga and Mandya ranged between 0.7-10% up to 30-45 days after sowing and 10-100% up to 60-100 days after sowing.
2. Studies on seasonal variation of disease incidence showed that the incidence was found to be the highest in summer season crop (87.5%) followed by Rabi season crop (61.25%) and very less incidence in Kharif season crop. The disease intensity was also found to be in the same order.
3. Watermelon bud necrosis virus could be successfully transmitted by mechanical inoculation using 0.02M 2-mercapto ethanol in 0.1 M potassium phosphate buffer (pH 7.0). Cow pea (*Vigna unguiculata* L. Walp C-152) was found to be a good assay host.
4. Watermelon bud necrosis virus was found to be potentially transmitted by thrips vector. From the present studies on the identification of vector species it was confirmed that the thrips species which transmits bud necrosis virus on watermelon in Karnataka is *Thrips palmi* Karny.
5. Watermelon bud necrosis virus (WBNV) could infect 18 plant species belonging to 13 genera in 5 families. The host range of WBNV include *Gomphrena globosa* belonging to Amaranthaceae, *Chenopodium amaranticolor* and *Chenopodium quinoa* belonging to chenopodiaceae ; *Citrullus lanatus* cv. Arka manik, *Citrullus lanatus* cv. Madhu, *Cucumis sativus* and *Cucumis melo*,

belonging to cucurbitaceae; *Arachis hypogaea* cv. J.L-24, *Glycine max* cv. Bragg, *Vigna mungo* and *Vigna unguiculata* cv. C-152 belonging to Fabaceae; and *Capsicum annum* cv. Yellow Wax, *Datura stromonium, Lycopersicon esculentum* cv. Pusa Ruby, *Lycopersicon esculentum* cv. Arka Sourabh, *Nicotiana rustica, Nicotiana benthamiana* and *Physalis floridana* belonging to solanaceae .

6. Watermelon bud necrosis virus was detected serologically by using DAC ELISA in all the diseased samples collected from different watermelon growing areas of Karnataka. Systemic infection of the infected plant was confirmed when the virus was detected in all the parts of the plant. The reaction of the virus to different antibody dilutions showed that the virus could be effectively detected up to 1: 20, 000 dilution.

   The virus was detected up to 1:1000 antigen dilution using PBNV antiserum by Dot Immuno Binding Assay(DIBA). The WBNV showed strong serological relationship to Peanut bud necrosis virus antiserum (PBNV) but not to TSWV and INSV.

(7) Histopathological changes of diseased leaves observed under light microscope were reduction in the dimension of, palisade parenchyma cells, epidermis, spongy tissue leading to the over all reduction in the thickness of the leaf. Characteristic brown to blackish pigments were seen in the palisade layer of the infected leaves.

(8) Transmission electron microscopic studies of the ultra thin sections of the diseased leaf tissue showed the presence of large quasi-spherical viral particles with membranes, 80-90nm in diameter grouped in the cisternae of the endoplasmic reticulum in the cytoplasm. Dip preparations from infected watermelon and tobacco leaves also revealed the presence of spherical enveloped watermelon bud necrosis virus particles.

FUTURE LINE OF WORK

1. More precise epidemiological studies have to be undertaken in order to avoid further spread of the disease.

2. ELISA based survey of all the watermelon growing areas of Karnataka has to be undertaken to know the incidence of the disease.
3. Detailed study on acquisition and transmission of WBNV by thrips has to be done.
4. Purification of the WBNV will be more useful to study the physical and chemical properties of the virus.
5. Polyclonal and monoclonal antibodies have to be produced to precisely determine the viral identity and this helps to know whether the virus belong to already reported sero groups of tospoviruses.
6. Available germplasm has to be screened to locate source of resistance.
7. There is an urgent need to develop suitable management practices to reduce the losses caused by watermelon bud necrosis virus disease.

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*** Originals not seen**