Results

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Part I-Isolation and Identification of the virus:

- **Virus isolation:**

A virus was found naturally infecting *Lactuca sativa* in a field near Benha, Kalubia, Egypt induced severe mosaic mottling, vein banding, blisters, crinkle, rugosity and epinosty of the leaves (fig. 1).

Many trials were made to isolate the virus on lettuce plants but unfortionatly not succeeded because the virus was thought to be seed transmitted. For this reason *Hibiscus schizopetalus* was selected as a suitable local lesion host for virus isolation.

The inoculated leaves of *Hibiscus schizopetalus* showed chlorotic local lesions which developed clear limited fine round and colored within 5 days during summer season (30-40 °C) fig.(4) but in winter season (10-20 °C) no symptoms appeared on inoculated leaves.

Fig. (1) Naturally infected leaves of *Lactuca sativa* showing severe mosaic symptoms, vein banding, blisters, crinkle, rugosity and epinosty.

- **Virus Identification:**

To identify this virus many experiments were done and we have the following results:

I-1. Symptomology:-

The inoculated lettuce plants under greenhouse conditions showed severe mosaic, vein banding ,blisters, crinkle, rugosity, epinosty and dwarfing of the infected plants.

I-2.Host Range: -

72 different host plant species belong to 29 families were mechanically inoculated with the sap expressed from the naturally infected *Lactuca sativa* plants.

The host plants reacted in two ways:

a- Local necrotic lesions,

b- Systemic symptoms,

The results are summarized in table (1)

- Table (1). Symptoms produced by virus under test on various host species (arranged alphabetically)

Plant species	Family	Symptoms
Acalypha wilkesiana Müll. Arg .	Euphorbiaceae	Necrotic local lesions appeared after 6 days.
Brassica rapae L. var. rapifera koch.	Cruciferae	A large number of necrotic local lesions appeared after 3 days.
Chenopodium album L.(white goose foot)	Chenopodiaceae	Chlorotic local lesions appeared after 6-7 days. Fig. (2).
Chenopodium quinoa willd.	Chenopodiaceae	Local lesions appeared after 7 days as faint yellow rings. Followed by mosaic mottling appeared on the new leaves after 2 weeks.
Colocacia antiquorum schott.	Araceae	Necrotic local lesions appeared after 4-5 days.
Corchorus olitorius L.	Tiliaceae	Necrotic local lesions appeared after 6 days.
Eruca sativa Miller. (Rocket)	Cruciferae	A large number of necrotic local lesions appeared after 5 days. Fig. (3).
Gazania splendens. Hort. Ex E. G.Henders. and A. Henders. (Treasure flower).	Compositae	Necrotic local lesions appeared after 7 days.
Helianthus annuus L.(sun-flower)	Compositae	Necrotic local lesions appeared after 5 days.

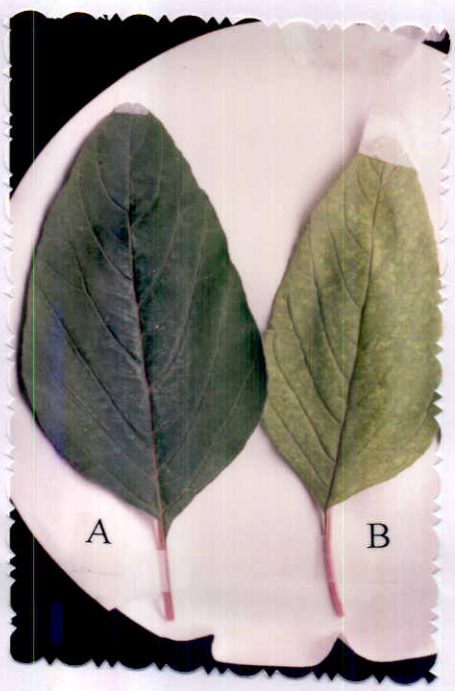

Fig.(2) *Chenopodium album.*(A) healthy leaf , (B) infected leaf showing chlorotic lesions.

Fig.(3) Infected leaves of *Eruca sativa* showing necrotic local lesions.

(arrow)

continuous

Hibiscus schizopetalus (M. T. Mast.) Hook. F.	Malvaceae	A large number of necrotic local lesions appeared after 5 days Fig.(4)
Lupinus termis Forssk (Egyptian lupin).	Leguminosae	Necrotic local lesions appeared after 6 days.
Lycopersicon esculentum Mill. (Tomato)	Solanaceae	Systemic chlorotic spots appeared after 2 weeks. The newly formed leaves appeared yellowed and distorted. Fig. (5).
Malva parviflora L. (small flowered mallow)	Malvaceae	Necrotic local lesions appeared after 5 days.
Pelargonium zonale Ait.	Geraniaceae	Necrotic local lesions appeared after 6 days. Fig. (6).
Pisum sativum L.(Garden pea)	Leguminosae	Vein clearing and mosaic, followed by malformation of leaves appeared after 12 days.
Raphanus sativus L. var. aegyptiaca Sick.(Radish)	Cruciferae	A large number of necrotic local lesions appeared after 5 days. Fig (7).
Solanum melongena L. var. atroviolacea Sick.	Solanaceae	Necrotic local lesions appeared after 6 days.
Solanum tuberosum L. (potato)	Solanaceae	Necrotic local lesions appeared after 5 days.
Sonchus oleraceus L. (sow-thistle)	Compositae	Necrotic local lesions appeared after 7 days.

Fig. (4) Infected leaf of *Hibiscus schizopelalus* showing large number of necrotic local lesions.

Fig. (5) Infected leaves of *Lycopersicon esculantum* showing chlorotic (A) spots followed by yellowing and malformation (B).

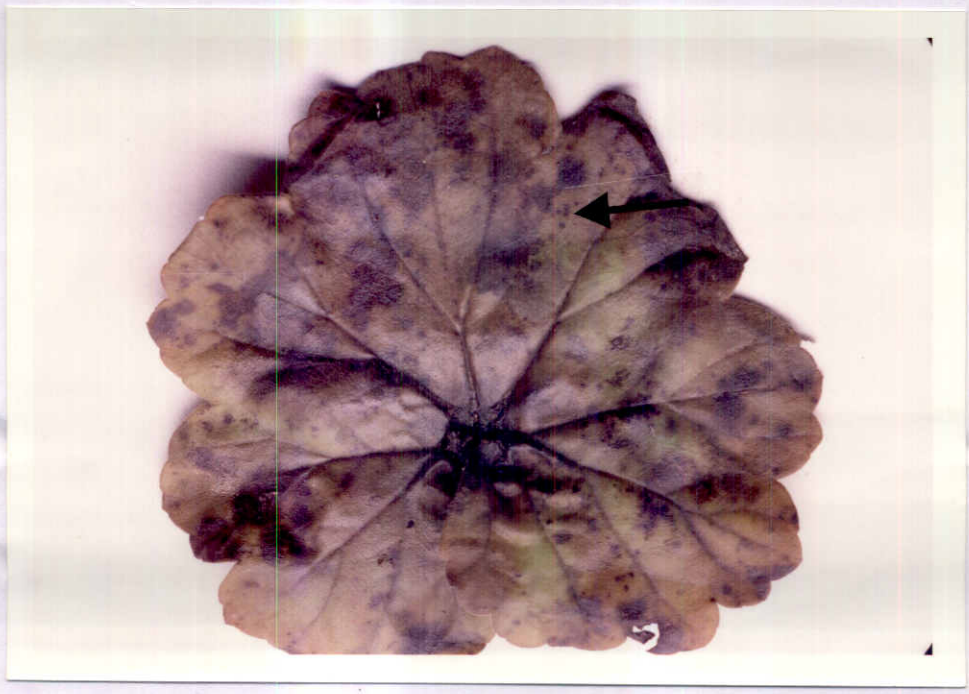

Fig. (6) Infected leaf of *Pelargonium zonale* showing necrotic local lesions.(arrow).

Fig.(7) Infected leaf of *Raphanus sativus* showing necrotic local lesions.

continuous

Spinacia oleracea L. (Garden-spinach)	Chenopodiaceae	Fine necrotic lesions appeared on the inoculated leaves after 6 days. Fig. (8).
Trifolium alexandrinum L. (Egyptian clover)	Leguminosae	Necrotic local lesions appeared after 7 days.
Zinnia elegans Jacq.	compositae	Necrotic ring spots appeared within 6 days in the form of brown concentric rings. The lesions were gradually fuse and form dead areas causing the leaves to wither. Fig.(9).

Fig. (9) Infected leaf of *Zinnia elegans* showing necrotic ring spots in the form of brown rings.

The following list of plants did not react to inoculation when back inoculated by the infected sap extracted from these plants and mechanical inoculation on indicator plant (*Hibiscus schizopetalus*):

Family: Acanthaceae (*Acanthus mollis* L. Artist's A and *Adhatoda vasica* Nees. (Malabar nut tree).

Family: Agavaceae (*Cordyline terminalis* (L.) kunth).

Family: Anacardiaceae(*Schinus terebinthifolius* Raddi.(Brazilian pepper tree).

Family: Apocynaceae (*Nerium oleander* L. (Oleander, south sea rose), *Thevitea neriifolia* A.Juss. ex Steud.(Luky nut) *and Vinca rosea* L.)

Family: Cannaceae (*Canna indica* L. (Indian shot).

Family: Caprifoliaceae (*Lonicera japonica* Thunb).

Family: Cruciferae (*Brassica oleracea* L. var. capitata L.(cabbage)

Family: Convolvulaceae (*Convolvulus arvensis* L. (lesser lindweed).

Family: Cucurbitaceae (*Citrullus vulgaris* schrad., *Cucurbita pepo* L and *Cucumis melo* L. (Melon).

Family: Euphorbiaceae (*Euphorbia peplus* L., *Phyllanthus nivosus* W.G.Sm.(Snowbush,foliage flower) and *Ricinus communis* L. (Castor oil plant).

Family: Gramineae (*Triticum vulgare* Vill. (Wheat) and *Zea mays L.*

Family: Labiatae (*Mentha piperita* L. (pepper ment) and *Ocimum basilicum* L. (Basil).

Family: Leguminosae (*Phaseolus vulgaris* L. (Kidney bean) , *Vicia faba* L. cv. "Detae" Sick. (Broad bean), *Vigna sesquipedalis* (L.) Wight, *V. sinensis* L. Saviex Hcessk. (Cow pea) and *V. ungicutat* (L.) Walp.).

Family: Liliaceae (*Allium kurrat* Regel).

Family: Malvaceae (*Abutilon barwinii* Mill, *Hibiscus esculentus* L.(Ochra) and *H. rosa-sinensis* L.(Chinese Hibiscus)

Family: Moraceae (*Ficus benghalensis* L. (Banyan tree), *F. carica* (L.), *F. nitida* Thunb.(Indian laurel, Malay Bayan) and *F. religiosa* L.(Bos-tree, peepul).

Family: Myrtaceae (*Psidium guajava* L.)

Family: Oleaceae (*Jasminium grandiflorum* L., *J. sambac* (L.) Ait. (Sambac) and *Olea europaea* L. (Olive tree).

Family: Plantaginaceae (*Plantago major* L. (Broad-Leaved plantain).

Family: Ruttaceae (*Citrus sinensis* L. Osbeck.(Sweet orange).

Family: Solanaceae (*Capsicum frutescens* L.(Red pepper), *Datura metel* L. (Garden datura) *Nicotiana glauca* R.C. Graph.(Tree tobacco) and *Petunia hybrida* (Garden petunia)

Family: Tropaeolaceae (*Tropaeolum majus* L. (Indian cress)

Family: Verbenaceae (*Duranta plumeri* Jacq.(Golden-dewdrop) and *Lantana camara L.* (yellow Sage).

Family: Vitaceae (*Vitis vinifera* L. (Wine G.).

I-3.Transmission of virus :-

3.1.Seed transmission:

Infected seeds were collected from infected lettuce plants grown in open field and healthy seed from the agricultural research center-vegetable research department-Giza (Khass Rokeen) and 100 seeds were cultivated in sterilized pots (15 cm Ø) in a green house (25 ±5 °C).About 20% of the grown lettuce seedlings showed different mosaic symptoms. This indicates that the virus was seed transmissible.

3.2.Mechanical inoculation:

The infected sap of the virus was mechanically inoculated on many hosts. *Hibiscus schizopetalus* leaves were preferably chosen as a test host since the virus under test develops clear necrotic local lesions on the inoculated leaves within 1-2days during summer (30-40° C) (fig.4).

But in winter (16-20° C) no symptoms appeared on inoculated leaves.

I-4.Electron Microscopy:

Examination of negatively stained partially purified preparation of the virus under test with 2% phosphotungestic acid revealed flexuous rod-shaped virus particles with mean length 740 X13 nm (fig.11).

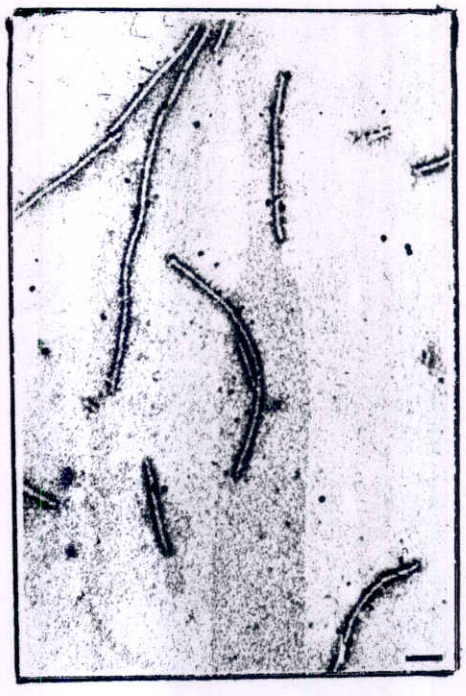

Fig.(11) Electron micrograph of partially purified virus , contrasted with 2% phosphotungstic acid. X 40,000

I-5.Physical properties of virus: -

5.1 Thermal inactivation point (TIP): -

The results are represented in table (2) and fig. (12), in which the percentage of leaves showing infection, is plotted against temperature.

The table and figure showed that the thermal inactivation point of the virus under test was 70° C for 10 min.

- Table (2) Thermal inactivation point of virus under test,

Temp. (°C)	No. of leaves showing symptoms	% of leaves showing symptoms
Room temp.	19	95
50°C	17	85
60°C	15	75
62°C	7	35
65°C	1	5
70°C	0	0

(No. of inoculated leaves were 20)

5.2 Dilution end-point (DIP): -

The results are represented in table (3) and fig. (13). In which the percentage of leaves showing infection is plotted against dilution. The figure showed that the dilution end point of the virus under test was completely inactivated when diluted to 10^{-7}.

Since the percentage of leaves showing symptoms at dilution 10^{-6} were 20% and that at 10^{-7} were 0%, The dilutions in between were tested. The dilution end point was found to be $1{:}4\times10^{-6}$ (table 4 and fig. 14).

- Table (4) Dilution End-point of virus.

Dilution	No. of leaves showing symptoms	% of leaves showing symptoms
Undiluted	19	95
10^{-1}	16	80
10^{-2}	15	75
10^{-3}	14	70
10^{-4}	12	60
10^{-5}	10	50
10^{-6}	4	20
10^{-7}	0	0

(No. of inoculated leaves were 20)

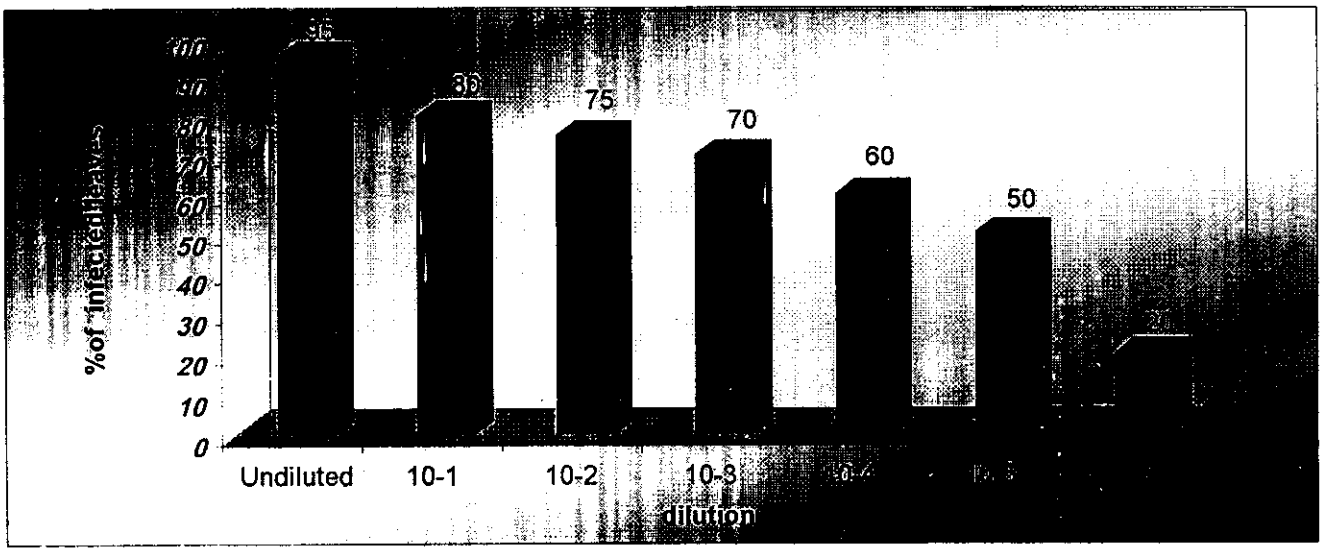

Fig. (13) Dilution end point.

- Table (4) Dilutions in between (10^{-6} and 10^{-7})

Dilution	No. of leaves showing symptoms	% of leaves showing symptoms
10^{-6}	4	20
$1:2\times10^{-6}$	2	10
$1:4\times10^{-6}$	1	5
$1:8\times10^{-6}$	0	0
10^{-7}	0	0

(No. of inoculated leaves were 20)

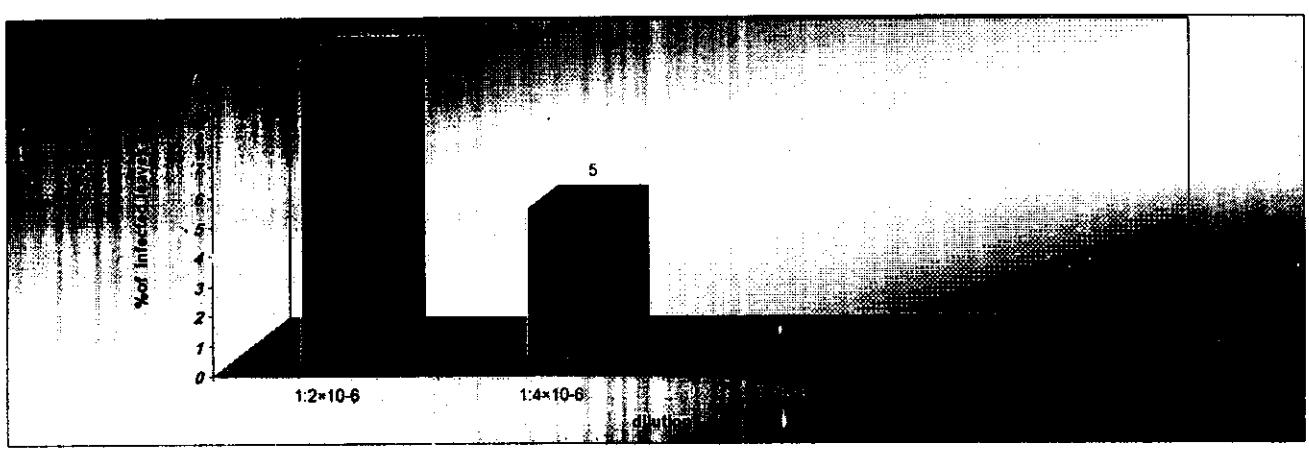

Fig. (14) Dilutions in between.

5.3 Longevity *in vitro* (LIV): -

The results represented graphically in table (5) and fig. (15), In which the percentage of leaves showing infection is plotted against period of storage in months. The table showed that the virus isolate longevity under (16-30 °C) was 210 days.

- Table (5) Longevity in vitro.

Storage period (days)	No. of leaves showing symptoms	% of leaves showing symptoms
10	16	80
40	14	70
70	12	60
90	10	50
110	4	20
210	0	0

(No. of inoculated leaves were 20)

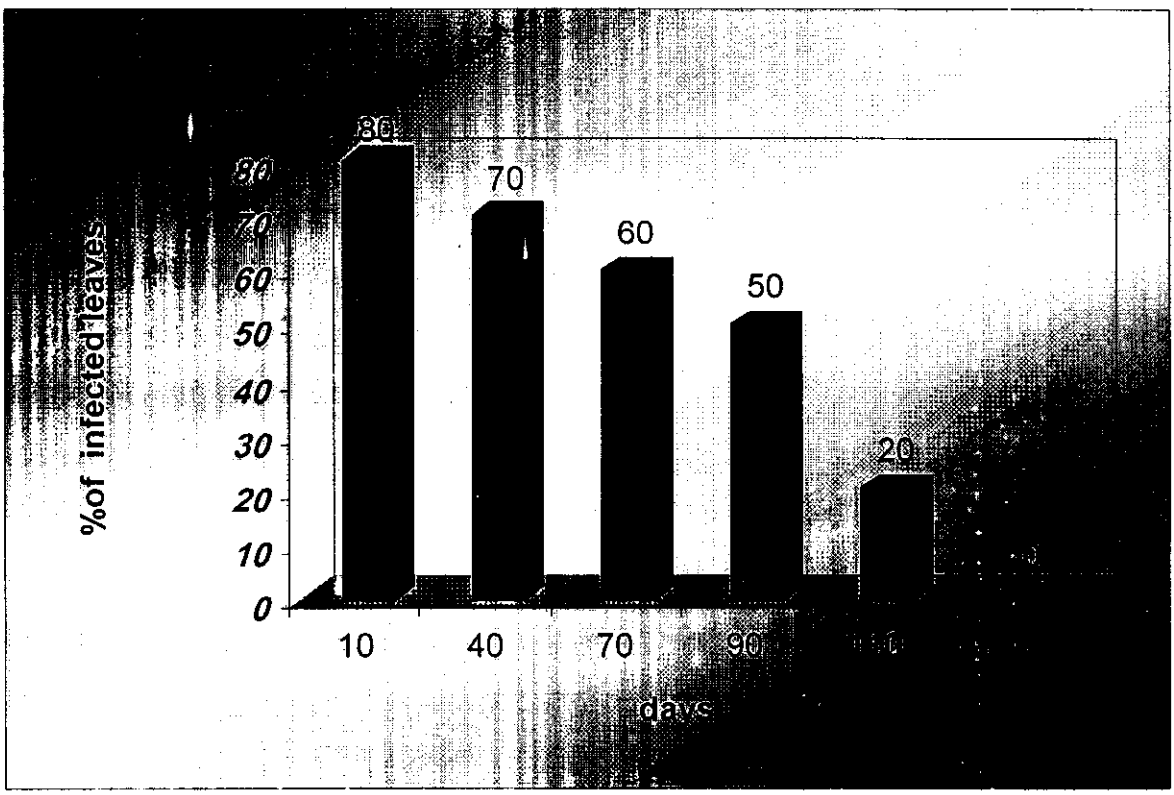

Fig. (15) Longevity in vitro

Part II. Histopathology of virus-infected leaves showing necrotic local lesions:

Healthy leaf

Examination of healthy leaf sections of *Hibiscus schizopetalus* plants, revealed that: the lamina is flat with the midrib or lateral veins bulging from upper and lower sides (fig. 16). The epidermal cells were mostly barrel shaped, contained stomata specially in the lower epidermis and covered with thin cuticle. The cells of the mesophyll are differentiated into loosely packed elongated palisade cells, and spongy tissue with wide intercellular spaces. Both palisade and spongy tissue contain chloroplasts. Few palisade cells and much spongy cells contain druses calcium oxalate crystals (figs. 17 and 18).

Few large spaces are found in between the palisade cells, it may be empty cystolith (fig. 19). The xylem vessels are arranged in rows, with the protoxylem directed upwards and the metaxylem downwards. The phloem appears in the form of small patches at the lower side of the xylem. Phloem cells and the surrounding parenchyma cells show great number of druses calcium oxalate crystals (figs. 20 and 21).

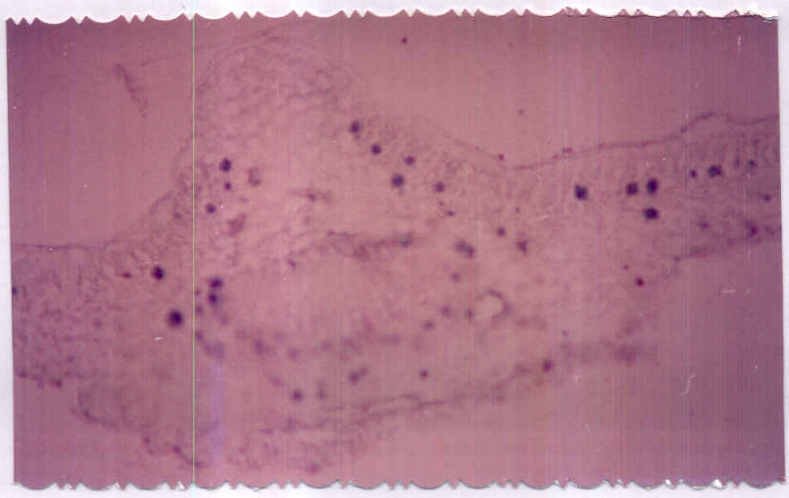

Fig. (16)Transection of healthy leaf showing flat lamina (Magn. X 63)

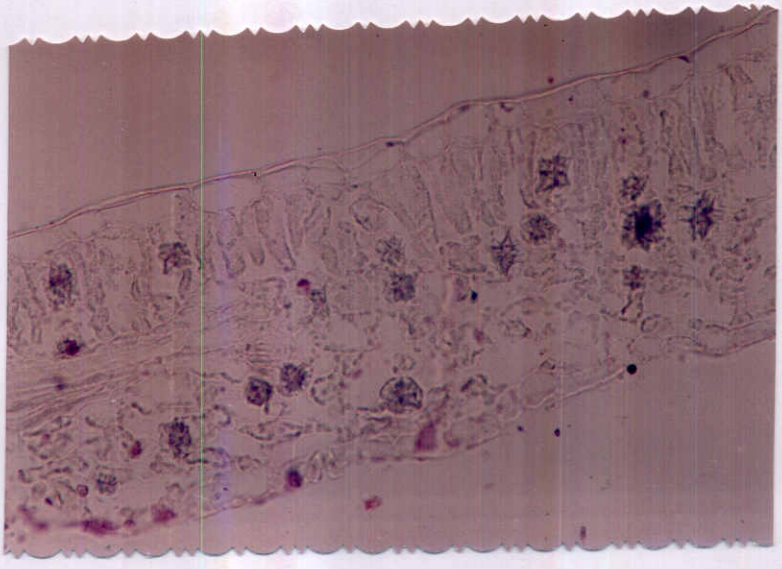

Fig. (17) magnified part of the blade showing the presence of druses crystals in the mesophyll tissue (Magn. X160)

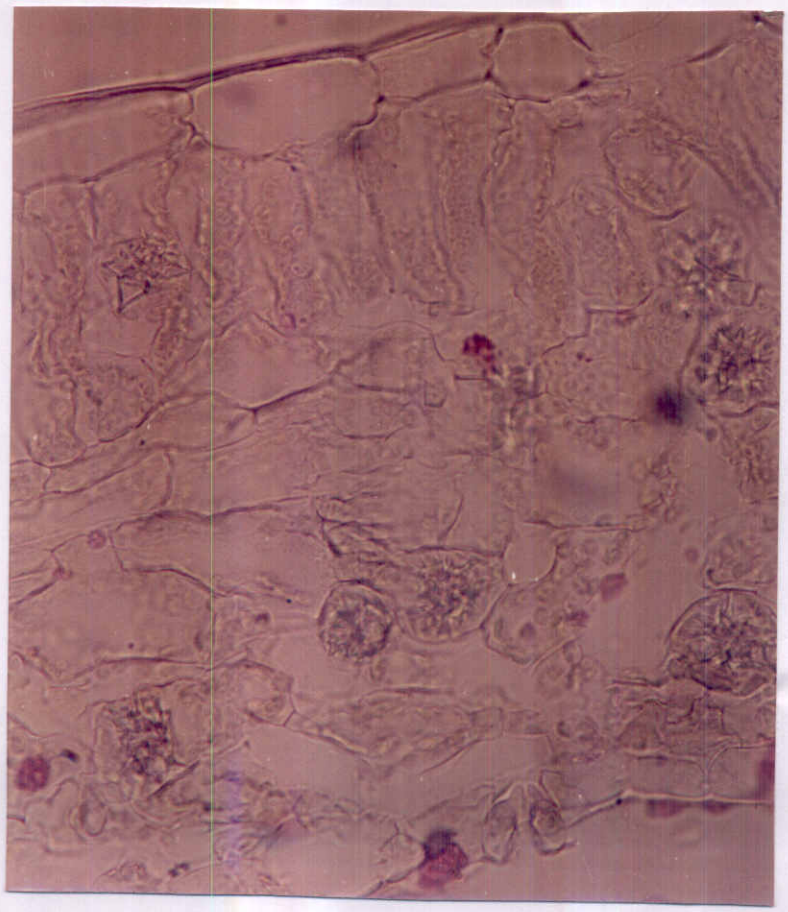

Fig. (18) Magnified part of the blade showing that the mesophyll cells are differentiated into loosely packed elongated palisade cells and spongy tissue with wide intercellular spaces.(Magn. X 400).

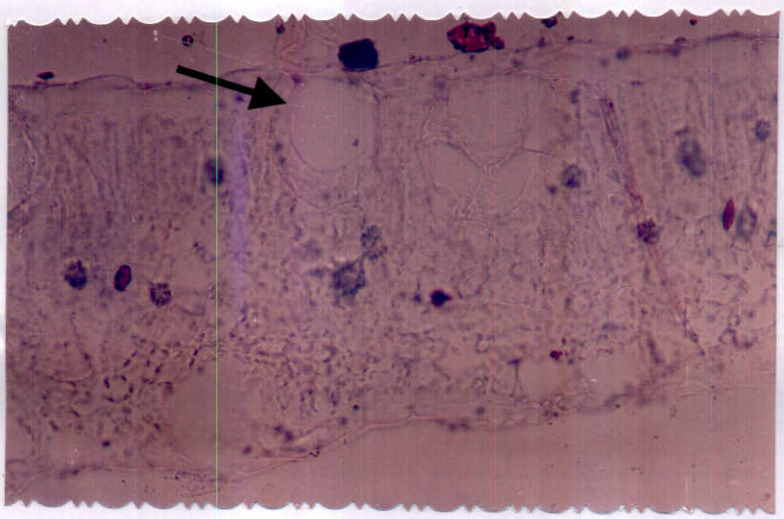

Fig. (19) Magnified part of the blade showing the presence of few large spaces in between the palisade cells (arrow). (Magn. X 160).

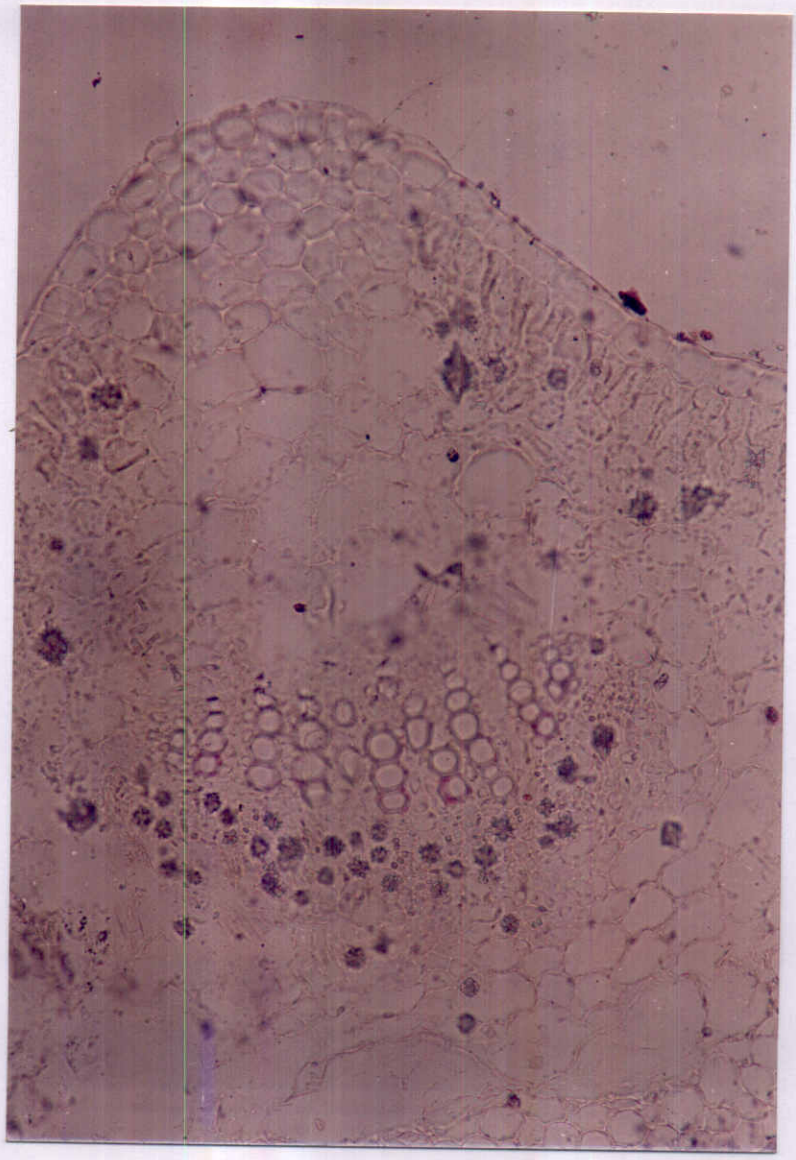

Fig. (20) Transection of healthy midrib showing the presence of great number of druses calcium oxalate crystals in the phloem and the surrounding parenchyma cells. (Magn. X 160).

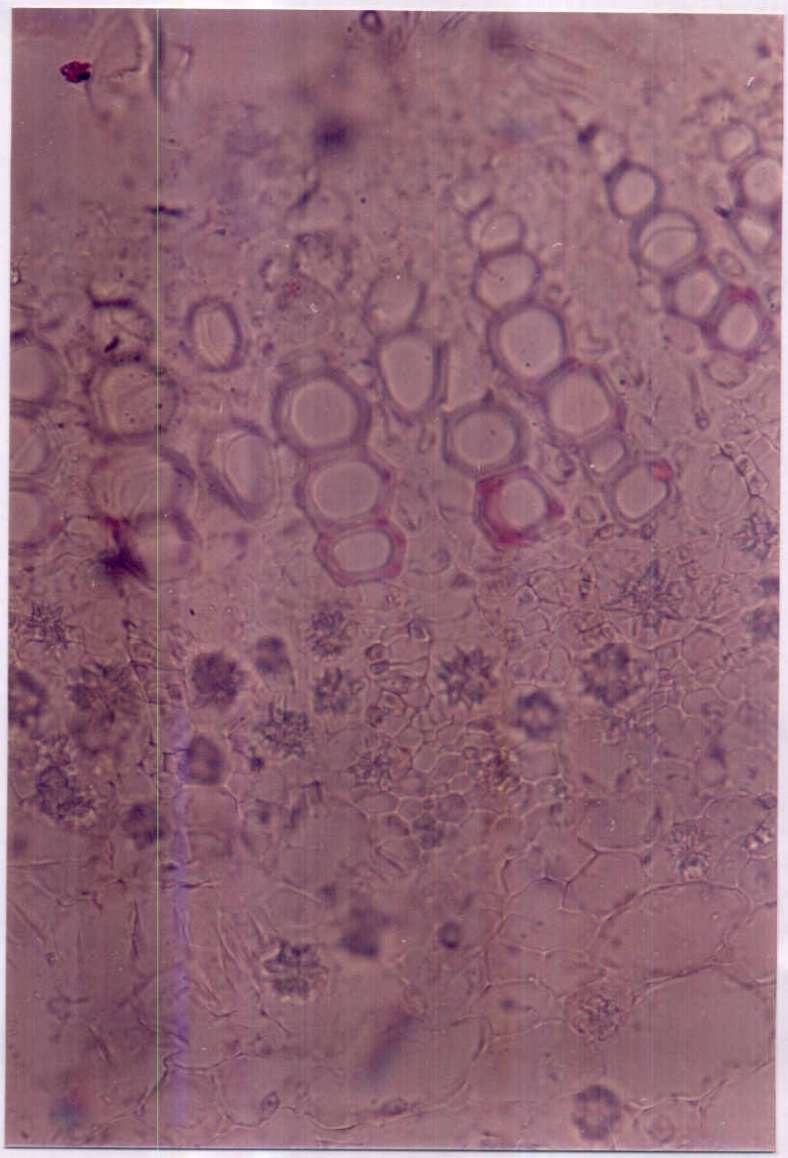

Fig. (21) Magnified part of the above section. (Magn. X 400).

Diseased leaf

Cross sections in diseased leaf showed that the blade is not clearly flat. The thickness of the blade is unequal on both sides of the midrib. The upper and lower edge of the midrib showed clear collenchyma cells (fig. 22,23). The epidermal cells appear somewhat isodiametric, with few distorted stomata and covered with thick cuticle. The mesophyll cells appear compact without differentiation into palisade and spongy tissues. The chloropalsts are few. The number of druses calcium oxalate crystals is clearly few (figs. 24, 25, 26 and 27).

Necrotic spots appear on both upper and lower epidermis densely stained with safranin and the cells around the necrotic spots are irregular in shape (figs. 28, 29, 30, 31 and 32).

Cross sections in the midrib and lateral veins of the diseased leaves showed that the infection of *Hibiscus schizopetalus* with LMV resulted in reduction of number and size of xylem, phloem tissues when being compared with healthy leaves. The number of druses calcium oxalate crystals clearly reduced. The parenchyma cells surrounding the vascular tissue appear wavy walled (figs.31 and 32).

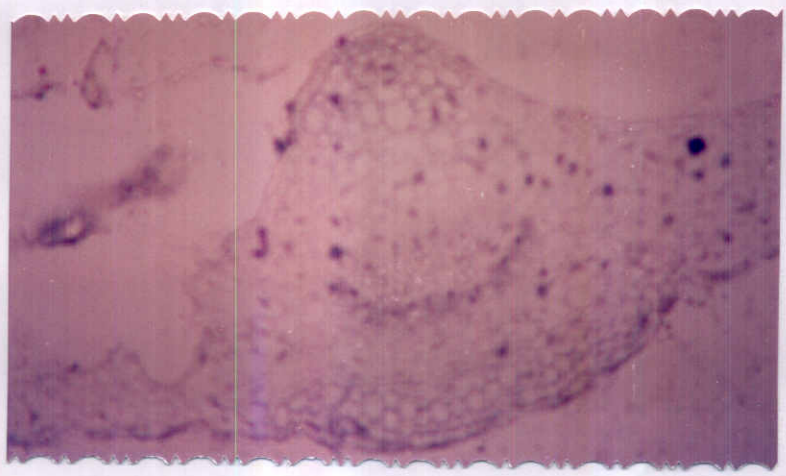

Fig. (22) Transection of infected leaf of showing that the blade is not clearly flat. (Magn. X 63).

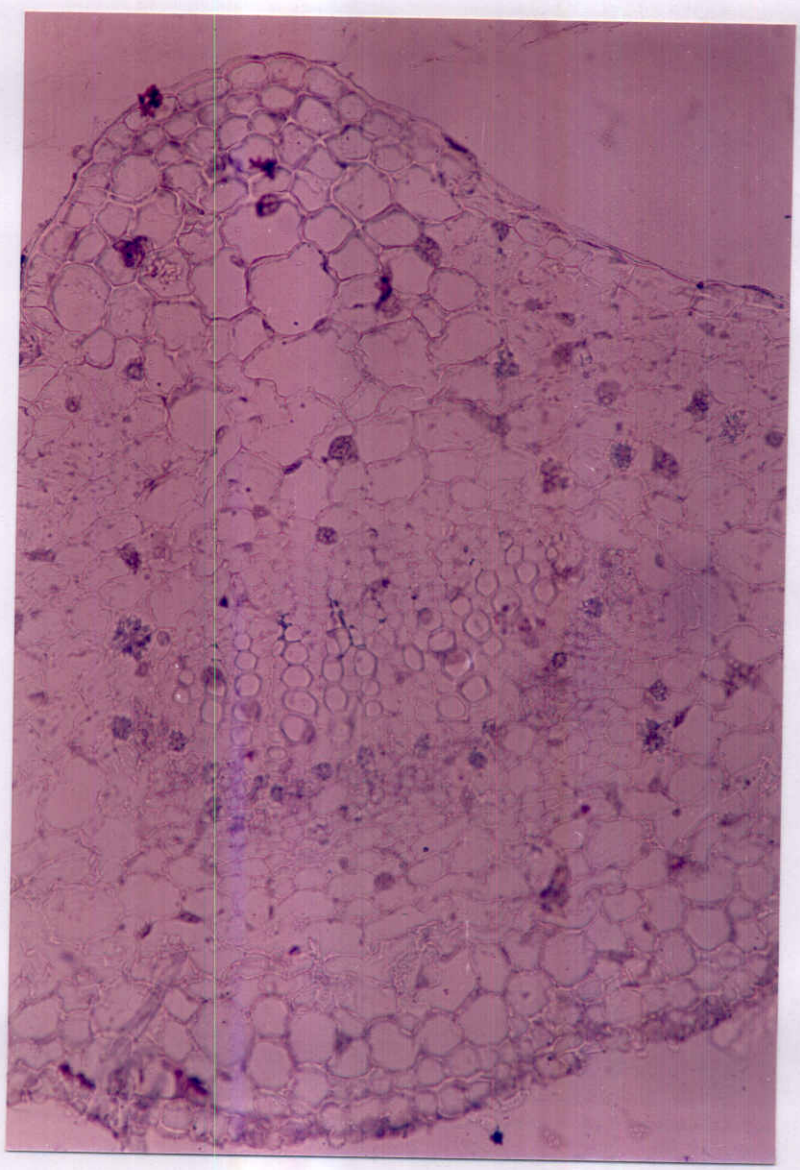

Fig. (23) Transection of infected leaf showing clear collenchyma cells on the upper and lower edges of the midrib (Magn. X 160).

Fig. (24) Transection of infected blade showing compact mesophyll cells without differentiation into palisade and spongy tissues. (Magn. X 63).

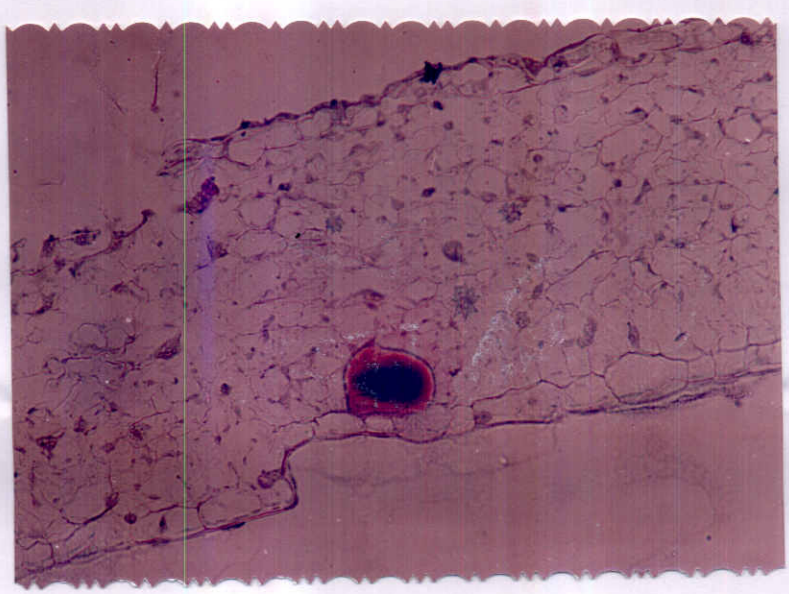

Fig. (25) Magnified part of the above section showing distorted stomata and thick cuticle. (Magn. X 160).

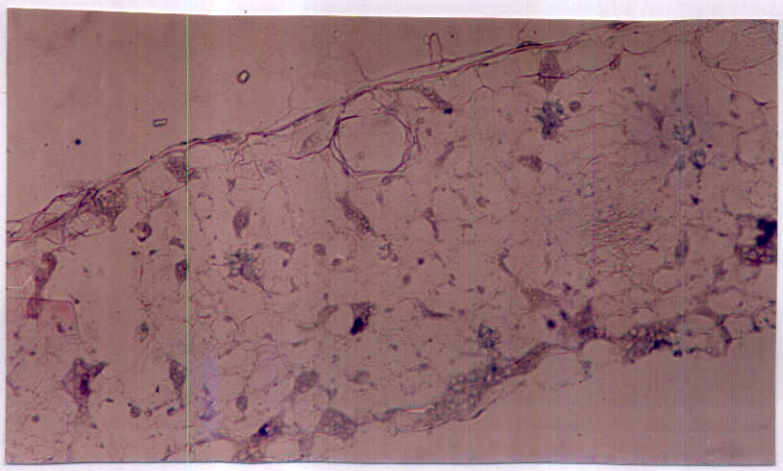

Fig. (26) Transection of infected blade showing compact mesophyll cells and few chloroplasts. (Magn. X 160)

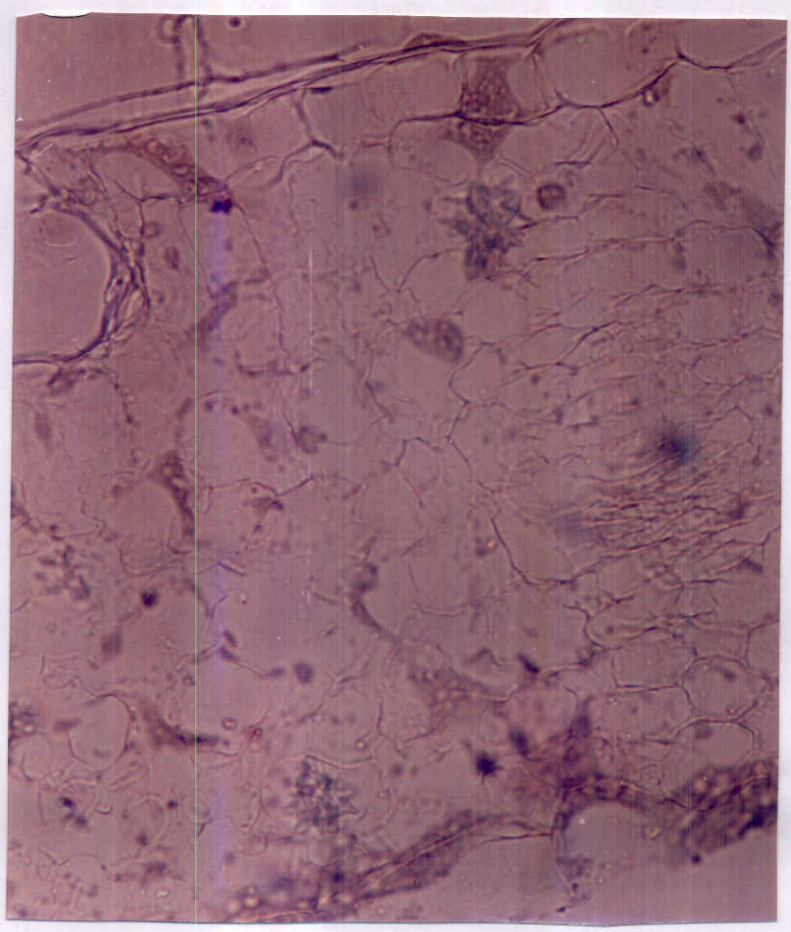

Fig. (27) Magnified part of the above section (Magn. X 400).

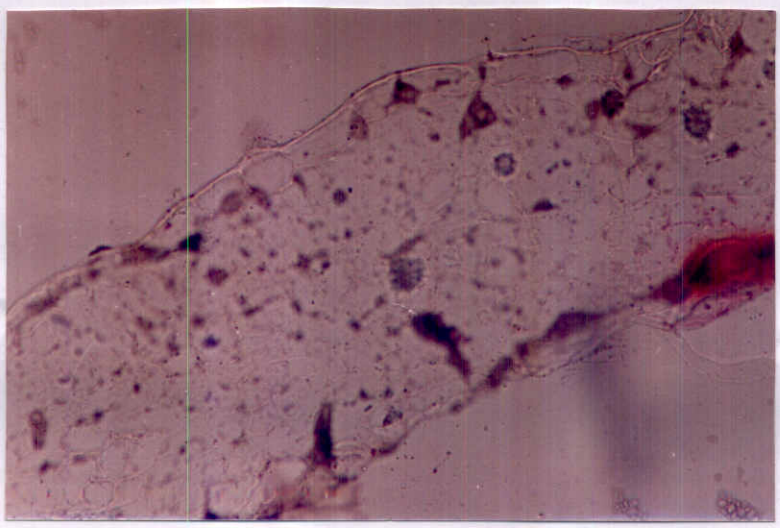

Fig. (28) Transection of infected blade showing necrotic spot on lower epidermis densely stained with safranin.(Magn. X 160).

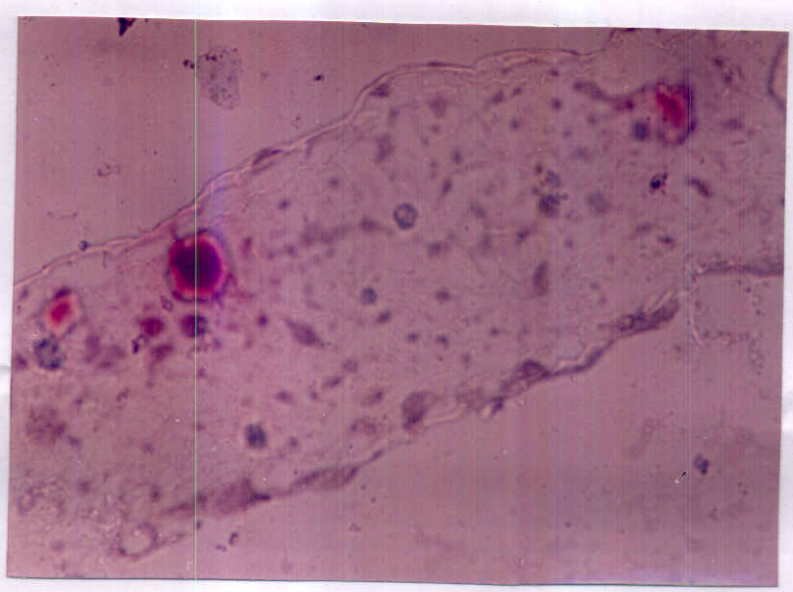

Fig. (29) Other part of the infected blade showing necrotic spot on the upper epidermis densely stained with safranin. (Magn. X 160).

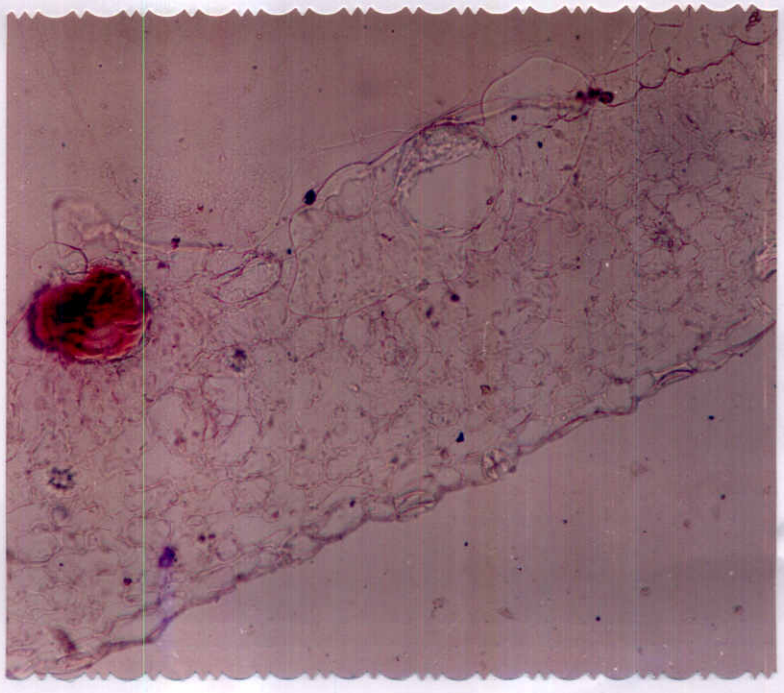

Fig. (30) Other part of the infected blade showing necrotic spot on the upper epidermis densely stained with safranin. (Magn. X 160).

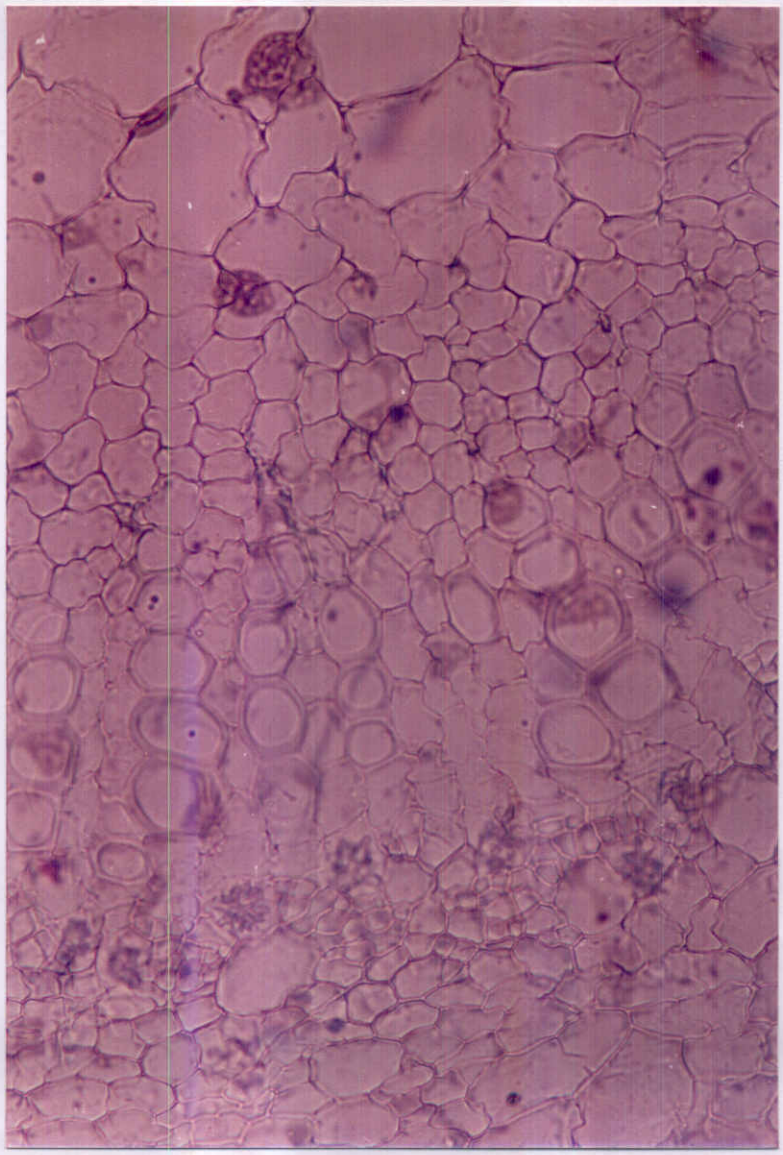

Fig. (31) Transection of the infected midrib showing normal xylem and phloem cells. The number of druses calcium oxalate crystals are reduced. (Magn. X 400).

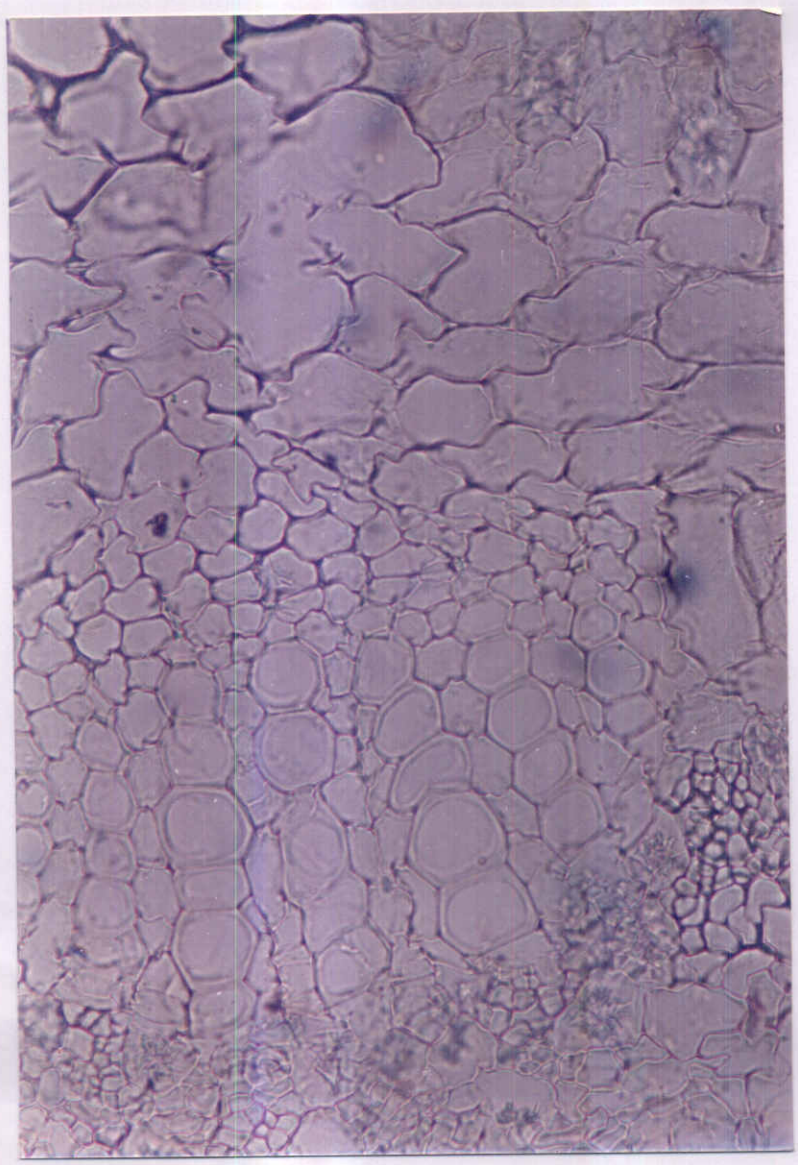

Fig. (32) Transection of the infected midrib showing wavy walled parenchyma cells surround the vascular tissue. (Magn. X 400).