SYMPTOMATOLOGY AND ETIOLOGY OF LITTLE LEAF DISEASE OF PEPPER

(*Piper nigrum* L.)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

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Department of Plant Pathology

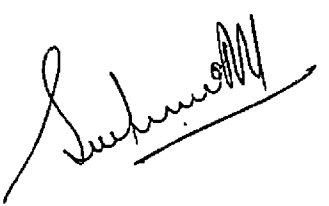
COLLEGE OF HORTICULTURE

VELLANIKKARA - THRISSUR

1995

DECLARATION

I hereby declare that this thesis entitled **Symptomatology and Etiology of Little Leaf Disease of Pepper (*Piper nigrum* L.)** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.



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CERTIFICATE

Certified that the thesis entitled **Symptomatology and Etiology of Little Leaf Disease of Pepper (*Piper nigrum* L.)** is a record of the research work done independently by **Miss P. K. Sreekumari** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship or other similar titles to her.

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Dedicated To All My Well wishers

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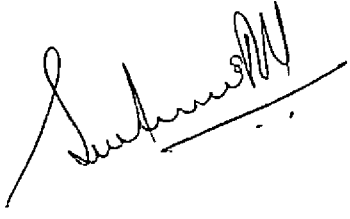

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INTRODUCTION

Black pepper (*Piper nigrum* L.) the 'King of Spices' is a major export earning spice of India. In India, the cultivation of pepper is mostly confined to southern states and Kerala accounts for an area of 1,83,480 ha producing 49,670 t annually (Anonymous, 1995).

In Kerala, it is mainly grown in Western Ghats and to a lesser extent in homesteads. Idukki and Wayanad districts account for 39,163 ha (21.34 %) and 32,613 ha (17.77 %) in area and 14,195 t (28.57%) and 10,242 t (20.62%) in production respectively (Anonymous, 1995).

According to a survey conducted by 'the Hindu' on Indian Agriculture it is pointed out that export of black pepper from India have shown a decrease from 29,985 t which account for Rs.102.40 crores in 1990 - '91 to 20,565 t (Rs. 74.21 crores) in 1991-'92 (Nandakumar, 1992) and to 25,480 t in 1992- '93 (Rs.83.17 crores) (Peter, 1994).

The various diseases affecting pepper in Kerala is a constraint in the cultivation of black pepper which ultimately resulted in dwindling the quantum of export of black pepper.

The foot rot (quick wilt) caused by the fungus *Phytophthora capsici* is the predominant one among the diseases which takes a heavy toll, often resulting in 100 per cent loss to the farmers.

Among other diseases, little leaf is of recent occurrence. The disease is commonly known by the name 'kathi' meaning knife because of the characteristic symptoms exhibited on leaves.

Little leaf disease was observed for the first time in 1975 in Pulppally of Wayanad district and Neriyamangalam of Ernakulam district.

A detailed survey conducted by the Spices Board in 1993 revealed that the total number of plants affected by the disease in Wayanad alone is 13,815. Pulppally panchayat accounts for 11,015 plants and in Mullankolly panchayat there are 2,800 diseased vines. The disease was victorious in conquering newer areas. But due to the adoption of cutting and removal strategy of affected plants by the Spices Board, the number of affected plants in Pulppally was reduced to 3,109 but that of Mullankolly is 75,000 in 1994 - '95.

Since the disease is of recent occurrence in Kerala and informations on symptomatology, epidemiology, etiology and

control measures are scanty, the present study has been undertaken with the following objectives.

1. To study the detailed symptomatology of the disease

2. To prove the etiology

3. To find the role of micro nutrients in the disease production.

4. To reveal the mode of transmission, and

5. To estimate the loss in yield due to the disease.

REVIEW OF LITERATURE

2.1. Distribution of the disease

Little leaf disease of black pepper was reported for the first time from Sri Lanka by Randombage and Bandara (1984) based on a survey conducted by them during 1981. However, the disease was observed in Neriyamangalam of Ernakulam district and Pulppally of Wayanad district in Kerala from 1975, eventhough it was reported only during 1988 (Sarma *et al.* 1988).

Sitepu and Kasim (1991) also reported the occurrence of a similar disease on black pepper from Indonesia during 1984 and it was named as "stunted growth "of pepper.

2.2. Varietal difference in disease expression

Randombage and Bandara (1984) observed variation in disease symptom expression among varieties Kuching and Panniyur 1 of pepper with little leaf disease.

2.3. Symptomatology of the disease

Information on symptoms on roots of pepper infected by little leaf disease was not available.

Jackson (1945) reported that in little leaf disease of southern pines, excessive exfoliation of bark, producing brown patches, dieback of the fine roots and large pitchy,

canker like lesions were present. Internodes of twigs were shortened which gave the crown a sparse tufted appearance. Marked slowing down of diameter and also reduced needle length and varying degrees of yellowing were recorded.

Anjaneyulu and Ramakrishnan (1972) found that the roots of little leaved eggplant were severely stunted and they were reduced to about 50 per cent in length and about 75 per cent by weight. Branching of roots were very poor and hence they weighed less. The internodes of the stem were shortened and large number of axillary buds were stimulated to grow into short branches with small leaves which gave the plant a bunchy appearance. They have observed in 1968 that affected eggplant had very short leaves. In cotton small leaf disease, root development was adversely affected and the taproot was malformed and it ended abruptly, giving rise to a number of secondary roots. The diseased plant could easily be pulled out of the ground with the taproot intact (Raychaudhuri and Nariani 1972)

The symptom appearance of little leaf and witches' broom on stems of different crops are closely resembling each other.

Sitepu and Kasim (1991) reported that stunted growth in black pepper was characterized by abnormal and malformed shoots with reduced internodal length. Initial symptoms

of the disease were the occurrence of abnormal leaves and shoots. The affected plants showed malformation of young leaves, shoots and berries. Later, mosaic symptom, reduction in size, form and quality of leaves, berries and internodes were seen. This was followed by shedding of leaves and berries.

Randombage and Bandara (1984) found that there were more nodes in a standard length (50cm) of the lateral branches on little leaf infected pepper vines. The internodes on lateral branches were shorter in diseased plants. On variety Kuching, proliferation of branches was a more predominent feature. Irregular yellow spots on both leaf surfaces were noticed which increased in size and lead to severe infection, leaves eventually turned yellow. Leaves on infected vines often showed bright conspicuous yellow mottling or mosaic patterns. The diseased leaves were constantly puckered and crinkled and the tissues were stiff and brittle.. The leaves were smaller compared to healthy. They observed marked reduction in the leaf area of affceted Panniyur-1 variety of pepper plants. Both the length of the flower spike and the number of flowers per spike were reduced significantly. Some spikes had no flowers, and enlargement and greening of the floral bracts were observed in some cases. There was an increase in aborted berries and berries which did mature were smaller

as indicated by lower 100 berry weight. Premature drop of berries were also observed.

Similar observations on little leaf affected pepper plants were recorded by Sarma *et al.* (1988). The leaves of affected pepper branches were very small, narrow, thick and leathery.

Multiple, spindly, wire-like shoots were observed by Plakidas (1949) in the case of witches' broom of Arizona ash. Yellow leaves, about 1/4 to 1/3 of natural size were produced in diseased plants.

2.4. Seasonal occurrence of the disease

On perusal of the literature, no study was seen conducted on the seasonal occurrence of little leaf of black pepper. However, the study conducted on little leaf disease of eucalypts (*Eucalyptus tereticornis*) by Ghosh *et al.* (1985) revealed that symptoms were influenced by climatic conditions. They found that the sprouts produced during summer had normal sized leaves and those produced during monsoon were invariably with typical little leaf symptoms.

2.5. Relationship between age of the plant and disease

No study was seen conducted on the relationship between age of the plant and disease in the case of little leaf of pepper.

Doi *et al.* (1967) reported that witches' broom affected potato and *Poulownia* exhibited shortened internodes. Subnormal plant growth and filamentous stems in witches' broom of potato was also observed. Simple leaves reduced in size were found in both the diseases.

Proliferation, dwarfing and reduction in size of leaves were reported in little leaf affected legume (Bowyer, 1969).

Several of ash trees attacked by witches' broom had abnormal basal sprouts, exhibiting a compact upright growth. Damage due to stunted growth was permanent and irrecoverable. Small, simple rather than large compound leaves, bud break from axillary buds that are normally dormant and yellowish than normal dark green (Schall and Agrios, 1973)

Witches' broom appearance due to shortening of internodes was also observed in rose rosette by Doudrick and Millikan (1983).

Mc Coy *et al.* (1983) reported that witches' broom of pigeon pea was characterized by proliferation of shoots from axillary buds with shortening of internodes. Witches' broom occurred as smaller dense tufts of proliferated shoots with greatly reduced leaves developing from nodes of affected branches. Stunting of leaves were also noticed.

2.6. Etiology of the disease

2.6.1. Detection of Zinc deficiencey

Deficiency of Zinc was reported to induce little leaf symptoms in crops like pepper, tea, clove, cocoa etc.

According to Nybe (1986), the first visible symptom of Zn deficiency in pepper was manifested as interveinal chlorosis of younger leaves leaving a prominent network of dark green veins. The size of the new leaves produced was very much reduced. The terminal growth was retarded and a number of lateral branches with shortened internodes and small leaves were produced from the terminal portion of the vine which resulted in bunching or rosetting. Abscission of leaf was seldom noticed. The number of leaves in Zn deficient plants were more than in healthy ones.

2.6.2. Detection of mycoplasma like organisms in the phloem tissues.

Seliskar *et al.* (1973) reported that in the phloem region of black locust plant affected with witches' broom, mycoplasmalike bodies were observed by Dienes' stain method. Similar results were recorded in little leaf of eucalypts (Ghosh *et al.*, 1985) and in witches' broom of *Syringa* (Hibben *et al.*, 1986).

2.7. Transmission studies

2.7.1. Sap transmission

Sap transmission was not found successful in diseases similar to little leaf of pepper. Failure of sap transmission was reported in witches' broom of ash tree (*Fraxiues americana*) by Hibben and Wolanski (1971), in witches' broom of bleeding heart (*Dicentra spectabilis*) by Hiruki and Shukla (1973), in rose rosette by Doudrick and Millikan (1983), and in apple little leaf by Ahlawat and Chenulu (1986).

2.7.2. Vegetative propagation

2.7.2.1. Transmission through cuttings.

Sarma *et al.* (1988) reported that the little leaf of pepper could be transmitted through cuttings of diseased plants.

Plakidas (1949) obtained successful transmitted of witches' broom disease of Arizona ash through cuttings, while Mc Coy *et al.* (1983) failed to transmit witches' broom through stem cuttings in pigeon pea.

2.7.2.2. Transmission through grafting

Graft transmission of little leaf of brinjal was reported by Thomas and Krishnaswami (1939). Anjaneyulu and

Ramakrishnan (1968 and 1969) found that wedge grafting from infected tomato to eggplant expressed symptoms of little leaf in eggplant after 15-20 days.

Stem, branch and root parts were successfully grafted on seedlings, saplings and adult short leaf pine (*Pinus echinata*) by bark patch and approach grafting methods and transmitted the little leaf disease by Jackson and Bratislav (1949).

In the case of witches' broom of Arizona ash, Plakidas (1949) found that the disease causing agent was graft transmissible by inarching.

Graft transmission of little leaf of *Eucalyptus citriodora* was reported by Sastry *et al.* (1971). But Ghosh *et al.* (1985) failed to get similar results.

Transmission of witches' broom disease of rose (Crowe 1983), rose resette (Doudrick and Millikan, 1983), little leaf of apple (Ahlawat and Chenulu, 1986) and witches' broom of lilac (Hibben *et al.*, 1986) was obtained through grafting.

2.7.3. Dodder transmission

Transmission of little leaf of legume to *Nicotiana glutinosa* L. was sucessfully done by Maramorosch *et al.* (1970) with Australian dodder (*Cuscuta australis* E. Br.)

The infectious agent of peach rosette was transmitted from peach to *Vinca rosea* by *Cuscuta campestris* but not by *C. subinclusa* (Kirkpatrick *et al.* 1975).

Transmission of little leaf of *Eucalyptus tereticornis* and *E. grandis* in Kerala through *C. chinensis* and *C. reflexa* was not successful (Ghosh *et al.*, 1985). After an initial establishment for two weeks on diseased and healthly twigs, the dodder dried off.

2.7.4. Insect transmission

Several little leaf disease of plants were reported to be transmitted through vectors - mainly leafhoppers.

Little leaf of brinjal was found transmitted by *Hishimonus* (*Eutettix*) *phycitis* Distant (*Cestius phycitis*) and by *Amrasca biguttula biguttula* Ishida by Thomas and Krishnaswami (1939).

Transmission of legume little leaf by *Orosius argentatus* (Evans) was recorded by Hutton and Grylls (1956). *Desmodium intortum* (Mill) Urb. was reported as the insect vector of the same disease by Bowyer *et al.* (1969).

Kar and Panda (1990) reported that the periwinkle little leaf could be transmitted by *Hishimonus phycitis*.

2.8. Tetracycline administration and remission of symptoms

Temporary remission of symptoms of yellow leaf of Bermuda grass (*Cynodon dactylon* L. Pers.) was obtained with oxytetracycline treatment when cuttings soaked for 120 min. in 200ml solution (Bar Joseph *et al.*, (1975).

Partial remission (37.57%) of peach rosette symptoms occurred when treated with 500 mg tetracycline - hydrochloride and lesser remission (9.1%) with chlortetracycline - hydrochloride and oxytetracycline dihydrate was obtained when the tetrtacyclines were injected into the bark. However, it was not successful when the solution of the chemicals were sprayed (Kirkpatrick *et al.*, 1975).

There was a spontaneous remission of phyllody symptoms of bottle gourd with three foliar sprayings of oxytetracycline - hydrochloride (OTC) at 500ppm at weekly interval (Sastry and Singh, 1970).

Remission of symptoms after prolonged infusion with oxytetraycline was recored in pear decline by Mc Intyre *et al.* (1979).

Successful remission of symptoms was reported in mulberry dwarf (Brown *et al.*, 1979), white leaf of Bermuda grass (Muniyappa *et al.*, 1979), pear decline (Lacy and Mc Intyre, 1981) root (wilt) of coconut (Anonymous, 1983 and

Pillai *et al.*, 1991), turnip phyllody (Gangopadhyay, 1984), little leaf of eucalypts (Ghosh *et al.*, 1985), sandal spike (Ali *et al.* (1987), rice yellow dwarf (Reddy and Jayarajan, 1991) and X-disease of peach(Cooley *et al.*, 1992) with tetracycline treatments.

2.9. Assessment of loss in yield

There is no report on the assessment of loss in yield due to little leaf disease of pepper.

Joshi and Bose (1983) reported 99 per cent of yield reduction due to little leaf in eggplant which was mainly due to reduction in number and size of fruits.

According to Singh (1990), yield loss due to witches' broom of potato was 100 per cent.

Materials and Methods

MATERIALS AND METHODS

3.1 Location of the experiment

The study on little leaf disease of pepper was conducted during 1992-'94 at the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur. Since the disease was more prevalent in Pulppally panchayat of Wayanad district, the field studies were conducted at Pulppally.

The site selected for the study was the pepper garden of Sri. K. P. Abraham, Kanhirakkattu house in Chettappalam of Pulppally panchayat in Padichira Village of Sulthan Bathery Taluk in Wayanad district. The map of the Panchayat is given as Appendix I.

The altitude of the area is 950 m above mean sea level. The average annual rainfall is 2000 mm. The rainfall data for the period 1988-1993 is given in Appendix II. The soil type is forest soil with a pH of 5-6.

The farmer has 2.65 ha of land in which pepper is grown along with cocount, banana and ginger. Pepper is trailed on live standards of *Erythrina indica*

Pepper varieties Karimunda, Arakkulam munda, Aimpiriyan, Vellanamban and Panniyur 1 were cultivated in the experimental plot.

3.2. Varietal difference in disease expression

The differences in the disease symptoms observed in the five varieties commonly cultivated in Wayanad namely Karimunda, Arakkulam munda, Aimpiriyan, Vellanamban and Panniyur 1 were recorded. Ten plants each from the five different varieties grown in the plantation was used for the study. All the plants were five year old. Incidence of the disease in the selected plants were recorded at monthly intervals for a period of two years. The intensity of the disease shown by these varieties were calculated using the formula.

$$\text{Disease intensity} = \frac{\Sigma \text{ Total numerical observations}}{\text{Total no. of plants observed x Maximum disease category}} \times 100$$

3.3. Symptomatology of the disease

The detailed symptomatology of the disease was studied and observations were recorded at monthly interval. The morphological characters of roots, stem, leaves, spikes and berries were studied for this purpose. The symptoms observed on all the five varieties (Karimunda, Arakkulam munda, Aimpiriyan, Vellanamban and Panniyur-1) were recorded.

Based on the observations of typical disease symptoms on above ground parts, five disease categories (grades) were recognized, as given below in Table 3.1.

Table 3.1. Disease scoring chart for little leaf disease of pepper

Disease category	Intensity of symptom development	Disease score
I	Healthy plants with no trace of disease symptoms on any of the above ground parts.	0
II	Apparently healthy vine; upto 25 per cent of the canopy showing diseased leaves and the remaining were normal stem, branches leaves, spikes and berries.	1
III	26-50 per cent of canopy bearing diseased stem, branches, leaves,spikes and berries	2
IV	51-75 per cent of the canopy showing disease symptoms on stem, branches,leaves spikes and berries.	3
V	75-100 per cent of the vine showing typical disease symtoms on the stem, branches, leaves, spikes and berries	4

Plates 1 to 5 show the disease score.

Detailed observations on the changes in the morphological characters of roots, stem, leaves, spikes and yield of berries were recorded from ten plants each of karimunda variety of uniform age selected in the five disease categories during 1993 and 1994. The plants in the category I (healthy) served as control.

Plate 1. Disease category I (Healthy plants)

Plate 2. Disease category II

Plate 3. Disease category III

Plate 4. Disease category IV

Plate 5. Disease category V

The mainroot length was measured by removing the soil from the base of the plants, while the diameter of the mainroot was measured at the collar region.

Height of the plant was measured from the tip to the collar region at soil level. For measuring the length of internode, the distance between third and fourth node from top was selected. Diameter at the centre portion of the internodal region was measured.

The changes in the foliage symptoms in the diseased plants were studied in detail. Length and breadth of the leaf produced at the third node from top was measured. Breadth of the leaf was measured at three points of the same leaf (at 1/3, 1/2 and 3/4th of the length of the leaf)

For measuring length of spike and average number of berries per spike, ten spikes each were selected at random from ten plants of the five disease categories and mean values were calculated.

3.4. Seasonal occurrence of the disease

Seasonal variations in the appearance of disease symptoms were recorded for two consecutive seasons in 1993 and 1994.

3.5. Relationship between age of the plant and disease

To study the influence of age of the plant on disease development, ten healthy plants of age group 2-5 years were observed in the plantation over a period of two years. Rooted cuttings of healthy plants grown in polythene bags were kept in the plantation for a period of two years and observations were recorded at monthly intervals.

3.6. Etiology of the disease

3.6.1. Detection of Zinc deficiency

Zinc sulphate ($ZnSO_4$) at a concentration of 5,000, 10,000 and 15,000 ppm were applied to the diseased plants by soil drenching, wick feeding, dipping and spraying. Different concentrations of $ZnSO_4$ solution were drenched @ 2 litres/plant at the base of the diseased plants.

Cotton thread of length 15 cm and 0.1 cm thick dipped in hot water for 30 minutes was used for wick feeding. Sterile coconut shell containing 200 ml of the required concentration was kept at 30 cm above ground level near the stem of the plant. Three small vertical slits having enough space to insert the wick were made on the main stem of the plant 2 cm apart at 20 cm above ground level and one end each of the cotton wick was inserted in it. The other end

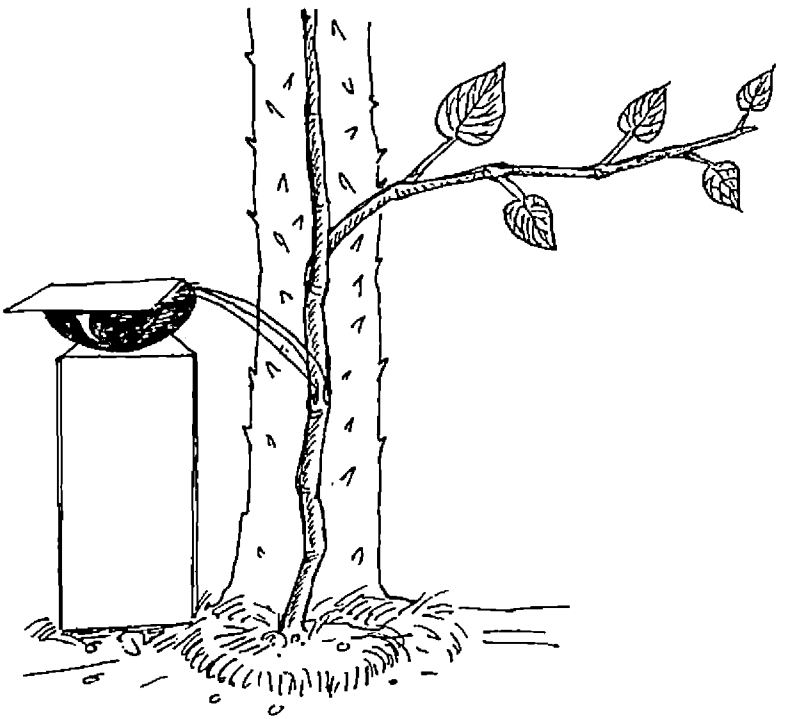


Fig. 1. Method of wick feeding

of the wick was dipped in the solution taken in the cocount shell. The shell was covered for preventing evaporation of the solution (Fig. 1). After complete absorption of the first 200 ml of the solution, another 200 ml was given and this process continued until one litre of the solution was absorbed. It took five days to get complete absorption of the solution.

The cuttings collected from diseased plants were dipped in $ZnSO_4$ solution for varying periods ranging from one hour to 72 hours before planting. However, for large scale field trials, the cuttings were dipped in $ZnSO_4$ solutions for 24, 48 and 72 hours before planting.

Zinc sulphate solution of different concentrations were sprayed on the diseased plants using a knapsack sprayer so as to get a uniform coverage on the foliage.

All the treatments were given in the early morning during March 1993.

Observation on the symptom development were recorded at regular intervals.

3.6.2. Detection of mycoplasma like organisms (MLO) in the phloem tissues

Dienes' staining technique (Dienes *et al.*, 1948) was used for detection of MLO in the phloem tissues of

diseased and healthy plant parts (Appendix III). Freehand sections of fresh tissues of diseased and healthy plants were stained with Dienes' stain solution. For staining, third node of young stem, petiole of third leaf from the top and fibrous roots were kept in 0.2 per cent solution of filtered Dienes' stain for 10 minutes. The sections were then washed in distilled water, mounted in glycerine and viewed under microscope for the indication of the presence of MLO in the phloem tissues. Photomicrographs of the stained sections of plant parts were taken.

3.7. Transmission studies

3.7.1. Sap transmission

Ten grams of young newly opened leaves showing typical disease symptoms were crushed in a sterile mortar and pestle using 10 ml distilled water. The sap was then sieved through a sterile absorbant cotton pad. A small quantity of 600 mesh carborundum (Silicon carbide) was also added to this. The sap was then swabbed with sterile cotton gently on second and third leaves from top of the plant. Leaves were then washed with distilled water. The plants were then kept under shade for observation.

3.7.2. Vegetative propagation

3.7.2.1. Transmission through cuttings

Ten numbers of three noded cuttings of runners of the plants in the five disease categories were planted in

polythene bags containing potting mixture. Each treatment was replicated ten times. Observation of the symptom development on the newly produced leaves was recorded.

3.7.2.2. Graft transmission

Scions collected from healthy and diseased plants of all categories were wedge grafted on healthy and diseased one year old rootstock of all categories. Healthy rootstocks were supplied by the Regional Agricultural Research Station, Ambalavayal, Wayanad distirct. The grafts were then kept under shade. The experiment was repeated 10 times and the observations on disease symptoms on newly produced leaves were made.

3.7.3. Dodder transmission

The dodder, used for the transmission studies *(Cuscuta campestris)* was collected from Ambalavayal and cuttings of size 10-25 cm were trailed on healthy one year old potted plants. In order to prevent drying up of cuttings, the lower end of cuttings was dipped in water, taken in bottles. After proper establishment, they were then trailed on established diseased cuttings grown in polythene covers. The dodder was again trailed on healthy plants. The experiment was repeated 10 times and symptom production if any on newly produced leaves were recorded.

3.7.4. Insect transmission

A detailed survey of insects prevalent on the diseased plants was conducted during 1993-94. Insects were trapped, using sweeping nets both during day and night, at weekly intervals. Surface feeders were not used for the transmission studies. Sucking insects with comparatively higher population were identified and used for transmission studies. Adults of these insects were collected using an aspirator. The insects were subjected to pre-acquisition fasting for periods ranging from one to 48 hours. However, for transmission studies, only insects subjected to 24 and 48 hours of pre-acquisition fasting were used. After pre-acquisition fasting the insects were released on diseased plants kept in insect proof cage of 45 cm length and 15 cm diameter made out of 200 gauge transparent polythene sheet, for acquisiton feeding. After varying periods of acquisition feeding, ranging from 1-24 hours and from 1-10 days, ten insects each were released on healthy potted plants kept in insect proof cage. Based on the preliminary observation the acquisition feeding period for transmission studies were fixed as 1-5 days.

3.8. Tetracycline administration and remission of symptoms

Terramycin tablets manufactured by M/s Pfizer India Limited, Thane, Bombay were used for oxytetracycline -

hydrochloride (OTC) treatment. OTC of varying concentrations of 250, 500, 750, 1000, 1500 and 2000 ppm were prepared in sterile distilled water and were applied as soil drenching, wick feeding, dipping and spraying. The quantity of OTC solution and method of application were similar to that used for $ZnSO_4$ application.

3.9. Assessment of loss in yield

From each disease category, ten plants belonging to the same age group were selected at random and yield data of these plants for two consecutive seasons (1993 and 1994) were collected.

Total fresh weight, 100 berry weight (fresh and dry, after four days of sundrying) and the percentage weight loss by drying were recorded.

Results and Discussion

RESULTS AND DISCUSSION

4.1 Location of the experiment

The study on symptomatology and etiology of little leaf disease of pepper (*Piper nigrum* L.) was confined to Wayanad district of Kerala state. In Wayanad, the disease was found mainly in Pulppally and Mullankolly panchayats. In Pulppally, the disease was noticed in Adikkolly, Amarakkuni, Eriappally, Shed, Cheeyambam, Chettappalam and Bhagda and in Mullankolly it was observerd in Kappiset, Vandikkadavu, Majjanda, Alathoor, Perikkalloor and Antony Kavala.

Sarma *et al.* (1988) and Sarma (1992) reported the occurrence of little leaf disease of black pepper in Pulppally of Wayanad and Neriyamangalam of Ernakulam districts. During the present investigation, stray occurrence of the disease was noticed in a few pepper gardens in Changanassery, Kottayam and Thrissur areas also.

The location selected for the present experiment was situated in Pulppally panchayat. Pepper with an area of 3,610 ha is the main crop of this panchayat. Pepper is cultivated either as a pure crop or as mixed crop with coconut, banana and ginger. The harvesting season of pepper usually starts from January.

The plot selected for the experiment was owned by Sri. K. P. Abraham, Kanhirakkattu House, Chettappalam in Pulppally panchayat (Appendix I). The pepper is trailed on live standards of *Erythrina indica*. Crops like banana, ginger and coconut are grown as intercrops.

4.2. Varietal difference in disease expression

Difference in the intensity of disease shown by the varieties commonly grown in Wayanad is given in the Table 4.1. The disease had shown an increase in intensity during the period under study in all the varieties.

Among the varieties Karimunda was the most susceptible with 79.81 per cent intensity in 1993 and 91.81 per cent in 1994. All the other varieties were also found infected by the disease but with lesser intensity than in Karimunda. Panniyur-1 was observed to have very low incidence of disease (4.23% in 1993 and 6.29% in 1994).

Thus, the study revealed that the varietal characters have a direct role on the tolerance/susceptibility to the little leaf disease of pepper.

Table 4.1. Varietal difference in the expression of little leaf disease of pepper

Varieties	Per cent disease intensity *	
	1993	1994
Karimunda	79.81	91.81
Arakkulam munda	49.34	58.72
Aimpiriyan	42.87	54.33
Vellanamban	45.13	53.10
Panniyur-1	4.23	6.29

* Mean of ten observations

4.3. Symptomatology of the disease.

4.3.1. Roots

Changes in the morphological characters of the roots in the five disease categories of plants were recorded and presented in Table 4.2.

In the category I, the mean length of the mainroot of Karimunda variety was found to be 62.83 cm in 1993 and 64.93 cm in 1994 (Plate 6). This category included plants which were healthy. So the increase in root length was normal. The length of mainroot showed a reduction with an increase in the disease intensity. Thus the mean mainroot length was

Table 4.2. Changes in the morphological characters of roots in the five disease categories of little leaf affected pepper plants *

Characters	Year	Category I	Category II	Category III	Category IV	Category V
Length of mainroot (cm)	1993	62.83	58.91	44.65	38.71	31.96
	1994	64.93	60.07	40.36	36.63	27.96
Diameter of mainroot (cm)	1993	4.32	4.12	3.52	3.99	2.94
	1994	5.22	4.79	3.39	3.73	3.02
Number of fibrous roots	1993	14.60	13.20	8.40	7.90	6.80
	1994	16.20	15.10	7.00	8.90	5.30
Average length of fibrous roots (cm)	1993	11.80	8.14	9.53	9.03	6.31
	1994	13.50	9.62	8.95	8.29	6.64

* Mean of ten observations

Plate 6. Root characters

Plate 7. Root characters

of disease category I

of disease category V

58.91 cm, 44.65 cm 38.71 cm and 31.96 cm in disease categories II, III, IV and V respectively during 1993 and 60.07 cm, 40.63 cm ,36.63 cm and 27.96 cm in 1994. Nearly 50 per cent reduction in the length of mainroot was noticed in category 5 compared to category I (Plate 7). In the category I and II, there was an increase in the mainroot length during 1994 compared to 1993. However, in category II to V , a marked reduction of the mainroot length was observed in 1994 compared to 1993. This clearly indicates that over a period of time in diseased plants mainroot length gets reduced and which inturn may affect the stand and yield of crop. The reduction in mainroot length may be due to the death of roots.

Reduction in length of mainroot of little leaf of southern pines (Jackson, 1945), little leaf of eggplant (Anjaneyulu and Ramakrishnan, 1972) and small leaf diseases of cotton plants (Raychaudhuri and Nariani, 1972) were reported. However, there was no report of reduction in mainroot length in the case of little leaf of pepper.

The diameter of mainroot in diseased plants were less than that observed in the healthy. The reduction in the diameter was more pronounced in disease category III to V. In general, diameter of a healthy mainroot increased as the

plant grows old. This is clearly evident in the category I where the diameter was 4.32 cm during 1993 and 5.22 cm in the next year. However in diseased plants the diameter did not show an increase (2.94 cm and 3.02 cm in 1993 and 1994 in category V) during the period of study.

Number and length of fibrous roots is an indication of the health of a plant. In the healthy five year old Karimunda plants there were 14.60 fibrous roots in 1993 while it was 16.20 in 1994. With an increase in disease severity there was a corresponding decrease in the number of fibrous roots. The total number of fibrous roots in category V during 1993 was only 6.80 and 5.30 in 1994. The mean root length of fibrous root increased as the plants grow old. In healthy roots of category I, the average root length was 11.80 cm and 13.50 cm during 1993 and 1994 respectively. A gradual reduction in the root length was oberved with an increase in disease intensity as seen in category II and V the root length were 8.14 cm and 6.31 cm in 1993 and 9.62 cm and 6.64 cm in 1994. The uptake and translocations of nutrients gets reduced with a decrease in fibrous root length and number. The stunted growth of diseased plants thus may be due to poor uptake of nutrients.

Brown patches were observed in root system of little leaf diseased pepper plants. Similar symptoms were also observed by Jackson (1945) in little leaf of southern pines.

4.3.2. Stem

The changes in the stem characters like height, total number of branches, number of diseased branches, number of healthy branches, average length of internodes and the average diameter of internodes due to the disease were recorded during 1993 and 1994 and the results were presented in Table 4.3.

Average height of 10 plants was 4.14 m and 4.31 m in 1993 and 1994 respectively in the disease category I. When intensity of the disease increased from category II to V, height of the plants showed reduction from 3.78 m and 4.00 m in category II, 3.34 m and 3.89 m in category III, 3.32 m and 3.46 m in category IV and 2.52 m and 2.45 m in category V during the periods 1993 and 1994 respectively (Table 4.3). This clearly indicate that as the disease intensity increased there was a corresponding reduction in the height of the plants.

Disease intensity had no direct influence on the total number of branches produced by the plants. The number of diseased branches increased with an increase in disease intensity. In disease category I, none of the branches were diseased where as in category V, 76.4 and 74.4 branches were diseased during 1993 and 1994 respectively showing an increase of about 75 per cent.

Table 4.3. Changes in the morphological characters of stem in the five disease categories of little leaf affected pepper plants *

Characters	Year	Category I	Category II	Category III	Category IV	Category V
Height of the plant (m)	1993	4.14	3.78	3.34	3.32	2.52
	1994	4.31	4.00	3.89	3.46	2.45
Total number of branches	1993	109.30	70.50	58.60	98.20	78.40
	1994	113.80	74.70	63.80	94.70	75.80
Number of diseased branches	1993	-	16.60	28.50	76.80	76.40
	1994	-	19.90	33.40	70.00	74.40
Number of healthy branches	1993	109.30	53.90	30.10	21.40	2.00
	1994	113.80	54.80	30.40	28.10	1.40
Average length of internodes (cm)	1993	13.80	11.96	8.68	7.20	4.52
	1994	14.71	12.58	7.62	7.06	4.25
Average diameter of inter nodes (cm)	1993	0.87	0.63	0.50	0.59	0.50
	1994	0.99	0.83	0.46	0.50	0.43

* Mean of ten observations

Plate 8. Comparison of stem characters of disease category I and V

Plate 9. Stem characters of disease category V

The mean length of internodes in the healthy plants during 1993 and 1994 was 13.80 cm and 14.71 cm respectively (Table 4.3 and Plates 8 and 9). With an increase in the intensity of the disease, there was a corresponding reduction in the internodal length and in the disease category V, the internodal length was 4.52 cm and 4.25 cm during 1993 and 1994, showing about 60 per cent reduction (Plate 9) over the healthy plants.

The mean diameter of the internodes in the healthy plants was 0.87 cm during 1993 and 0.99 cm during 1994. A reduction in the diameter of the internodes in the diseased plants was noticed. In the disease category V, 42.5 per cent and 56.5 per cent reduction in the diameter over the healthy was observed during 1993 and 1994 respectively.

The characteristic reduction in internodal length and diameter contributed to the reduction in height of the plant which in turn gave a rosette or bush like appearance to the diseased plants.

The reduction in internode length due to little leaf disease of black pepper was reported by Sitepu and Kasim (1991), Randombage and Bandara (1984) and Sarma *et al.* (1988). However, there were no report about the reduction in the diameter of the vine due to the disease.

4.3.3. Leaves

Reduction in length and breadth of leaves gave the name little leaf to this disease.

A gradual reduction in the leaf length with an increase in the disease was observed in the disease category V where the leaf length was only 4.74 cm and 3.63 cm in 1993 and 1994 which was 53.7 and 64.3 per cent less than in healthy plants while it was 41.8 and 46.5 per cent compared to category II (Plate 10) (Table 4.4).

The mean breadth of leaves also reduced as the disease progressed. Healthy plants had a mean leaf breadth of 5.69 cm to 5.64 cm in 1993 and 1994 respectively, while it was 1.87 cm and 1.20 cm in category V during the same period. In certain plants in the disease category V breadth of leaves was as low as 0.5 cm.

Sarma *et al.* (1988) observed that the leaves of little leaf affected branches were very small and narrow.

Leaves of the diseased plants were distorted. Sometimes the lamina on one side of the midrib alone was found enlarged giving the shape of a knife and hence the vernacular name 'kathi', meaning knife to this abnormality. The downward curling of the leaves was also noticed in some

Table 4.4. Changes in the morphological characters of leaves in the five disease categories of little leaf affected pepper plants *

Characters	Year	Category I	Category II	Category III	Category IV	Category V
Length (cm)	1993	10.24	8.14	6.45	5.33	4.74
	1994	10.17	8.35	6.92	4.64	3.63
Average breadth (cm)	1993	5.69	4.49	3.61	2.85	1.87
	1994	5.64	4.59	3.75	3.25	1.20

* Mean of ten observations

Plate 10. Comparison of leaf characters of disease category I and V

cases (Plate 10). The veinlets of some of the diseased leaves were prominent resembling a net. This was more prevalent in larger leaves while, in leaves of very small size the veinlets were not evident.

The diseased leaves showed a chlorotic appearance (Plate 10). A pale green colour and drying up of the spathe -like covering of the leaf at its bud stage were the initial indication of the disease. The diseased leaves were brittle, leathery and hard.

Randombage and Bandara (1984) have also observed chlorotic patches, puckering, crinkling, stiffening, brittleness and small size of leaves in little leaf affected pepper vines. The present observation revealed that irrespective of the position, disease symptoms can be noticed on newly produced leaves (Plates 2 to 5).

4.3.4 Spikes

The size and shape of flowers produced in plants affected by little leaf disease were normal. However, the length of spike and the number of flowers per spike were considerably reduced and the spikes showed an abnormal curving.

The mean number of spikes per plant in 1993 for the disease category I was 2101.80 and in 1994 it was 1684.40

Table 4.5. Changes in the morphological characters of spikes in the five disease categories of little leaf affected pepper plants *

Characters	Year	Category I	Category II	Category III	Category IV	Category V
Total number of spikes	1993	2101.80	837.40	669.70	1185.40	582.00
	1994	1684.40	662.80	464.60	729.30	398.80
Average length of spike (cm)	1993	11.48	9.97	9.48	7.49	3.72
	1994	12.21	9.38	9.12	7.55	3.95
Average number of berries per spike	1993	53.38	36.21	24.21	19.46	11.84
	1994	43.52	33.96	23.08	17.15	9.23

* Mean of ten observations

Plate 11. Spike production in response to character of leaf

Plate 12. Comparison of spike characters of disease category I and V

(Table 4.5). For the disease categories II, III, IV and V, the values for 1993 were 837.40 669.70, 1185.40 and 582.00 and that for 1994 were 662.80, 464.60, 729.30 and 398.80 respectively (Table 4.5)

The reduction in number of spikes in the diseased plants may be due to the reduction in leaf area, which resulted in the reduced capability of photosynthesis. It was observed that irrespective of the intensity of the disease in a plant, a spike produced opposite to a healthy leaf was invariably healthy and those produced opposite to a diseased leaf was diseased (Plate 11). The number of spike in the diseased plants reduced considerably with an increase in disease intensity.

Mean length of spikes was found to be reduced with an increase in intensity of the disease (Table 4.55 and Plate 12). Compared to a mean length of 11.48 cm and 12.21 cm in 1993 and 1994, in the healthy plants, the spike length was only 3.72 cm and 3.95 cm in category V during 1993 and 1994 respectively. The variation in length of spikes was found to be erratic in the two years.

Average number of berries per spike for the healthy plants was 53.38 in 1993 and 43.52 in 1994. When the plants showed 25 per cent disease (category II) the average number of berries per spike were 36.21 and 33.96 during 1993 and

1994. The results clearly indicated a reduction in the number of berries per spike with an increase in intensity of the disease (Plates 8 and 12). Thus the disease intensity has a direct effect on the number of berries per spike.

The reduction in the number of berries per spike was due to poor setting of flowers and premature dropping of berries.

The reduction in number and length of spikes and the presence of disfigured spikes were reported earlier in little leaf disease of pepper by Randombage and Bandara (1984). Increased number of aborted flowers and berry drop were also reported by Sarma *et al.* (1988).

4.4. Seasonal occurrence of the disease

Sarma *et al.* (Personal communication) first noticed little leaf disease of pepper in Pulppally in the pepper garden of Sri. K.P. Abraham after the onset of south west monsoon in 1988. (Personal communication)

In the present study, typical disease symptoms were observed soon after the showers from May to August and October to November on the newly opened leaves. The seasonal occurrence of the disease is presented in the Table 4.6.

Table 4.6. Occurrence and intensity of little leaf disease of pepper in Pulppally of Wayanad district

Month	Normal growth pattern during the period	Occurrence of the disease in plants	Per cent disease intensity
April	Growth commenced	No disease	0.0
May	Summer showers obtained and new flushes along with new inflorescences appeared.	Some of the new leaves and inflorescences were diseased	9.7
June	South West monsoon Development of new flushes and inflorescences.	Gradual increase in the development of diseased flushes and spikes	12.2
July			20.6
August			31.4
September	Berry formation commenced.	Disease observed in some of the new flushes formed	39.8
October	North East monsoon New Flushes produced	Considerable increase in the intensity of the disease	45.3
November	- do -	- especially on the newly formed leaves	51.6
December	Berry maturing stage	Slow progress in disease as no new flushes are produced	56.4
January	Fly picking of selected mature berries	Berries of diseased plants (Category IV and V) matured early. Already infested plants show increased severity.	58.4
February	Second round harvesting	Moderately infested plants harvested (category II and III)	62.5
March	Harvesting completed	Healthy plants (category I) were harvested	65.0

During late summer and months in which reproductive structures arise, no new flushes appeared and as a result there was no marked increase in the intensity of the disease.

Symptoms were expressed in the inflorescence as reduction in its length, production of aborted flowers, dropping of berries and curved spike. New flushes appeared during summer, in irrigated gardens showed disease symptoms.

4.5. Relationship between age of the plant and disease

The disease was observed on plants of all age groups as it attacked both rooted cuttings and yielding vines. The results of the present investigation clearly showed that that incidence of the disease is more as the vines grew old (Table 4.7).

Out of the ten plants observed for disease incidence in 1993 in each group only two plants infected in one year old group whereas, four, five, six and seven plants were infected in the age group of two, three, four and five years respectively. The corresponding figure were three, four, six, six and eight in 1994.

Table 4.7. Influence of age of pepper plant on the occurrence of little leaf disease

Age of the plant	Year	Plants Number 1	2	3	4	5	6	7	8	9	10	Total
Rooted cuttings (one year old)	1993	-	-	+	-	-	-	-	-	+	-	2
	1994	+	-	-	+	-	-	+	-	-	-	3
2 years (one year after planting the rooted cuttings	1993	+	-	-	+	-	+	-	-	-	+	4
	1994	-	+	-	-	+	-	+	-	+	-	4
3 Years	1993	-	+	-	-	+	-	+	-	+	+	5
	1994	+	-	-	+	+	+	-	+	-	+	6
4 Years	1993	+	-	+	+	-	-	+	-	+	+	6
	1994	-	+	-	+	+	+	-	+	-	+	6
5 Year onwards	1993	+	-	+	+	-	-	+	+	+	+	7
	1994	-	+	+	+	+	+	-	+	+	+	8

+ Presence of disease

- Absence of disease

4.6. Etiology of the disease

4.6.1. Detection of Zinc deficiency

Zinc deficiency exhibited symptoms similar to little leaf in many plants. In order to find out the effect of Zn on symptom remission, Zn in the form of zinc sulphate at 5,000 ppm, 10,000 ppm and 15,000 ppm was administered by foliar spray , soil drenching, dipping and by wick feeding. In none of the treatments, remission of the disease symptom was noticed. The results thus indicated that the disease is not due to Zn deficiency.

4.6.2. Detection of mycoplasma like organisms (MLO) in the phloem tissues.

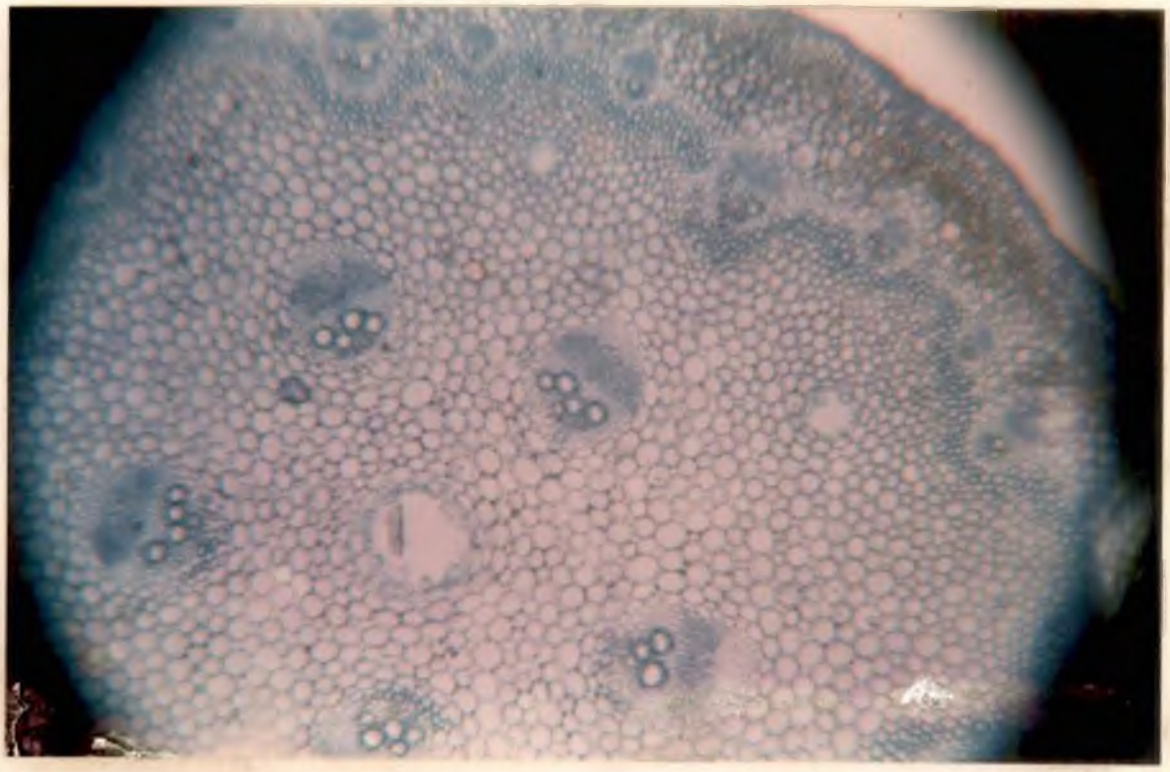
Dienes' staining technique was employed to find out the presence of MLO in the diseased plants. The third node from the top was used for the staining purpose. The cross section of the diseased stem after staining gave blue colour in the phloem region indicating the involvement of MLO (Plates 13 and 14). Phloem of the healthy plants were not stained blue (Plate 15 and 16). The petioles of the diseased leaf when stained with Dienes' stain also showed the involvement of MLO in the phloem region. The feeder roots collected from diseased plants also stained positive.

Plate 13. Photomicrograph of petiole of third leaf from the top of disease category V showing the blue colour after staining with Dienes' stain

10 × 5

Plate 14. Photomicrograph of the vascular bundle (enlarged) of petiole of third leaf from the top of disease category V showing the blue colour after staining with Dienes' stain

10 × 45



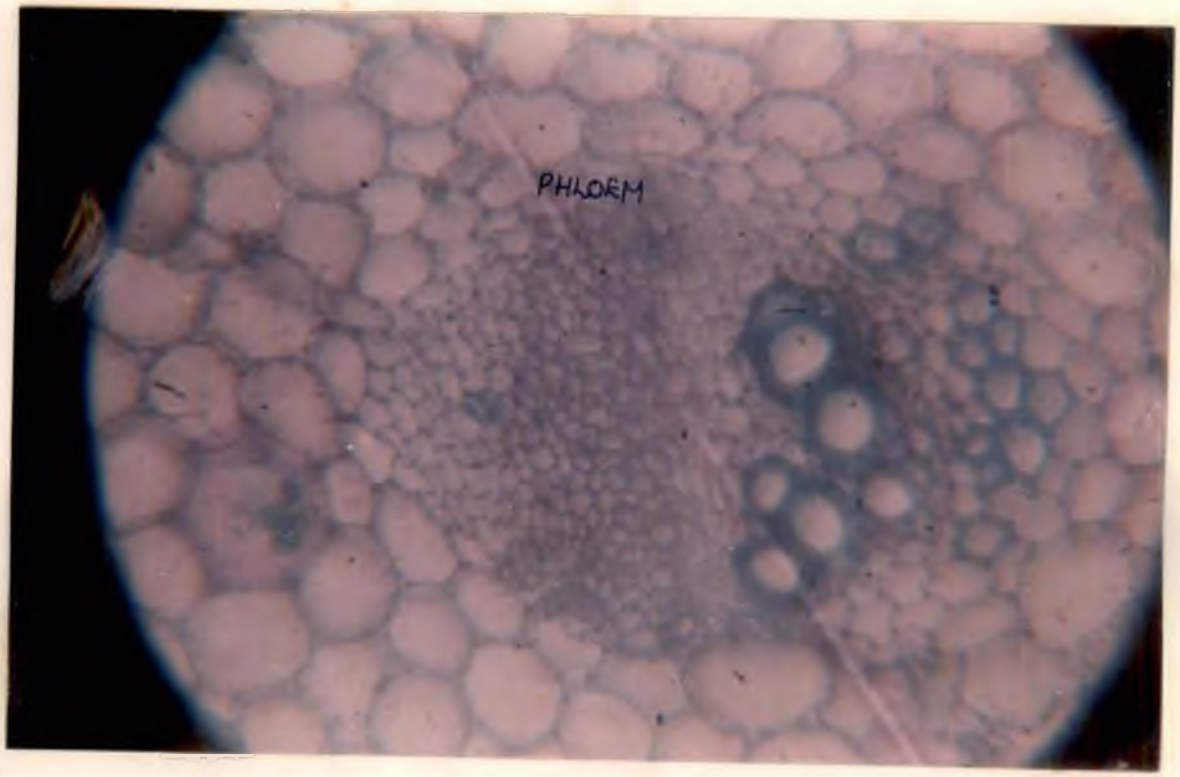
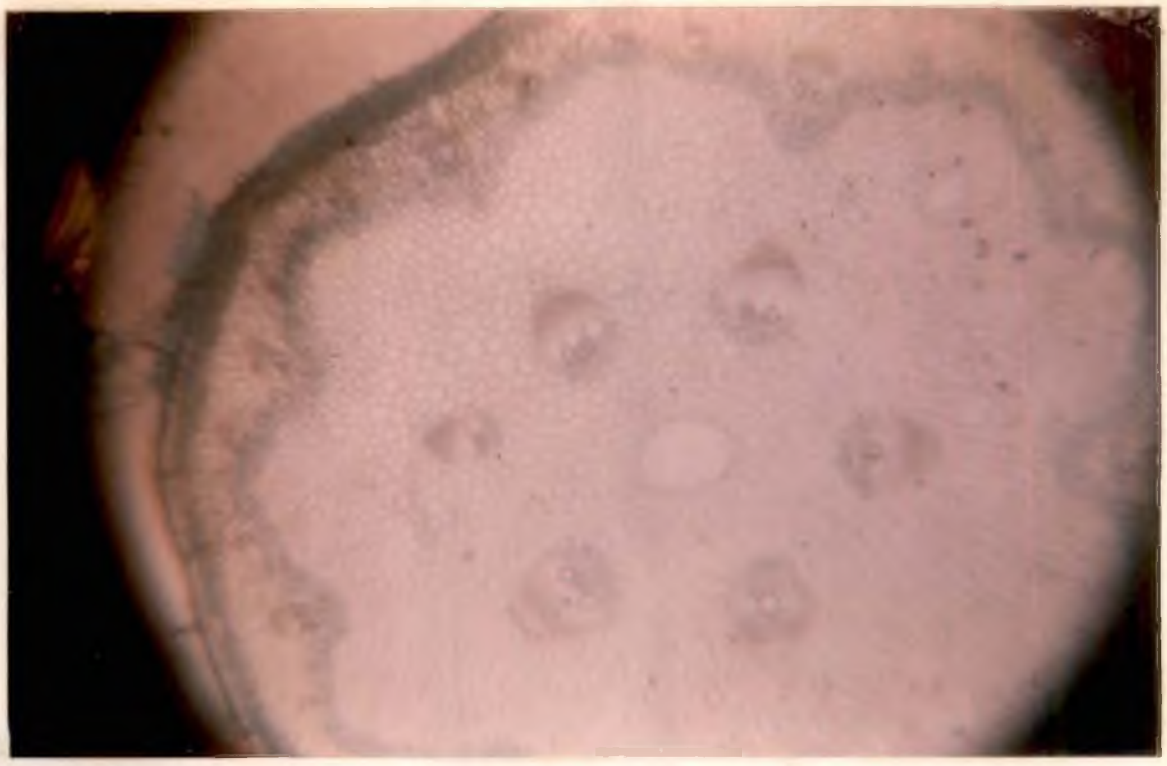

PHLOEM

Plate 15. Photomicrograph of petiole of third leaf from the top of disease category I showing the absence of blue colour after staining with Dienes' stain

10 X 5

Plate 16. Photomicrograph of the vascular bundle (enlarged) of petiole of third leaf from the top of disease category I showing the absence of blue colour after staining with Dienes' stain

10 X 45



Dienes' stain is specific for detecting MLO from the tissues. This staining technique was used to confirm the presence of MLO in eucalypts (Ghosh *et al.*, 1985) and in Syringa (Hibben *et al.*, 1986).

4.7. Transmission studies

4.7.1. Sap transmission

In spite of the repeated and constant efforts made for transmitting the disease through sap, the disease could not be transmitted. There is no report of sap transmission of little leaf disease of pepper. A number of workers have tried sap transmission of little leaf disease of other crops and found that the disease was not sap transmissible as in the case of witches' broom of ash tree (*Fraxinus americana*) by Hibben and Wolanski (1971), in witches' broom of bleeding heart (*Dicentra spectabilis*) by Hiruki and Shukla (1973), in apple little leaf by Ahlawat and Chenulu (1986). The results clearly indicated that the causal agent of the disease is not sap transmissible.

4.7.2. Vegetative propagation

4.7.2.1. Transmission through cuttings

Out of 10 cuttings each planted for rooting, only eight of them were rooted in healthy plants (disease category I)

after 18 days (Table 4.8). However, in the case of disease categories II, III, IV and V, the cuttings rooted only after 25,32,45 and 56 days of planting and the number of cuttings rooted were 7,6,6 and 3 respectively. In these categories the number of days taken for symptom production were 28,35,45 and 59 respectively. On an average it took 41 days for disease symptoms to appear once the cuttings were planted. The experiment was repeated 10 times and the data presented in Table 4.8 are mean of these experiments.

Table 4.8. Transmission of little leaf disease of pepper through cuttings

Disease category	No. of cuttings planted for rooting	No. of cuttings rooted	No. of cuttings produced symptoms	No. of days taken for	
				Rooting	Symptom production
I	10	8	Nil	18	Nil
II	10	7	7	25	28
III	10	6	6	32	35
IV	10	6	6	42	45
V	10	3	3	56	59

The experiments have proved beyond doubt that the disease could be transmitted through cuttings. Further, as the disease intensity increased cuttings took more number of days to establish. The transmission of little leaf disease of pepper through cuttings was already reported by Sarma *et al.* (1988).

4.7.2.2. Transmission through grafting

Even with repeated effort for transmitting the disease through grafting with diseased scion from all disease categories and healthy root stock, disease could not be transmitted. It took 8,12,14, 16 and 18 days to get the graft union (Table 4.9). Even when a diseased scion was grafted on a healthy rootstock the leaves produced after successful graft union were invariably healthy (Plate 17).

However, when healthy scion was grafted on diseased root stock, disease symptoms were observed after successful graft union. Usually the disease symptoms were noticed after 21, 29, 37 and 45 days in disease categories II, III, IV and V. On an average it took 21 days to get the successful graft union and 33 days to get the transmission (Table 4.9). This clearly indicated that the disease could be successfully transmitted through grafting .

There were no previous reports on graft transmission of little leaf disease of pepper. But several of the little leaf disease of crops were graft transmissible. Swingle (1938) reported that when healthy scion was intoduced to diseased root stocks as a patch graft, the union produced necrosis disease in elm. Leaf casting yellows disease of

Table 4.9. Transmission of little leaf disease of pepper through grafting

Disease category	No. of plants grafted with the type of scion & root stock		No. of sucessful grafts	No. of grafts produced symptoms	No. of days taken for	
					Graft union	Symptom production
I	A	10	6	6	15	-
	B	10	5	-	8	-
II	A	10	7	7	21	21
	B	10	8	-	12	-
III	A	10	5	5	23	29
	B	10	6	-	14	-
IV	A	10	4	4	24	37
	B	10	6	-	16	-
V	A	10	7	7	25	45
	B	10	8	-	18	-
Mean	A	10	5.8	5.8	21	33
	B	10	6.6	-	13	-

A - Healthy scion and diseased root stock

B - Scion from diseased plant and healthy root stock

Plate 17. Resultant plant of grafting healthy rootstock with diseased scion.

peach was reproducible after eight months when healthy top was grafted on diseased stock and not vise versa in peach to sweet cherry *(Prunus avium)* (Thomas *et al.*, 1940).

In the present study instead of a downward transport of the disease causing agent, an evidence for upward movement was found. This aspect of grafting needs further detailed study.

Mean number of days taken for graft union in the case of grafting with healthy scion and diseased root stock (A) was 21 and that for diseaseed scion and healthy root stock (B) was 13 (Table 4.9). On an average the number of successful grafts in the former case (A) was 58 per cent and that in latter (B) 66 per cent.

4.7.3. Dodder transmission

In each disease category 10 plants were used for transmission of disease through dodder *(Cuscuta campestris)*. The bits of dodder taken from disease free location in Ambalavayal were used for the transmission studies. The bits were trailed around healthy, one year old Karimunda rooted cuttings. It took, on an average, 33 days for the dodder to get established in Karimunda (Table 4.10).

Table 4.10. Transmission of little leaf disease of pepper through dodder

No. of plants observed	No. of days taken for establishment of dodder in			No. of days taken for transmission of disease
	Healthy plants	Diseased plants of category	New healthy plants	
10	18	I	Nil	Nil	Nil
10	26	II	Nil	Nil	Nil
10	28	III	Nil	Nil	Nil
10	45	IV	Nil	Nil	Nil
10	52	V	Nil	Nil	Nil

When the dodder first trailed on the healthy rooted cuttings was then trailed on diseased rooted cuttings of diferent disease categories, it dried up within 10 days, without any growth in the diseased plants. In all the trials the same results were obtained. Hence from the diseased plants to the healthy plants trailing could not be done.

This clearly indicated that the diseased pepper plants were not a preferred host of dodder. Since pepper is a cash crop, no neglected garden could be observed with dodder trailed naturally on them. Thus there is little chance of dodder (*Cuscuta campestris*) causing the transmission of the disease.

No previous report on dodder transmission was reported in the case of little leaf disease of black pepper.

4.7.4. Insect transmission

By sweeping with a net on the plants and sucking with an aspirator, six different types of insects namely *Austroagallia* sp., *Mandera beta* Owarokowska, *Liothrips karnyi*, tortoise beetles, different species of wasps and *Spodoptera litura* were obtained.

Table 4.11. Insect population on different disease categories of pepper plants affected by little leaf disease in the diseased plot at Pulppally during different periods.

Sl. No.	Name of the insects obtained	Disease category	Average number of insects obtained through 10 sweeps											
			4 am	5 am	6 am	7 am	8 am to 3 pm	4 pm	5 pm	6 pm	7 pm	8 pm	9 pm	Mean
1.	Austroagalliasp.	I	33	53	47	45	Nil	7	8	27	42	59	64	38.5
		II	26	28	34	26	Nil	18	13	20	31	35	42	27.3
		III	17	21	28	25	Nil	12	15	18	24	29	33	22.2
		IV	12	15	13	9	Nil	8	9	13	15	15	19	12.8
		V	7	8	5	3	Nil	3	5	9	12	15	19	8.6
2.	Mandera beta	I	9	12	18	3	Nil	Nil	7	12	23	28	30	14.3
		II	3	8	6	-	Nil	-	7	9	11	15	21	8.0
		III	4	6	7	-	Nil	-	-	-	8	11	15	5.1
		IV	2	2	3	-	Nil	-	-	-	3	4	8	2.2
		V	-	-	1	-	Nil	-	-	-	1	2	2	0.6
3.	Liothrips karnyi	I	2	3	4	1	Nil	1	2	3	4	4	5	2.9
		II	-	1	-	2	Nil	1	1	-	-	-	-	0.5
		III	-	2	-	-	Nil	3	-	-	-	-	-	0.5
		IV	-	-	-	-	Nil	-	-	-	2	-	-	0.2
		V	-	-	1	-	Nil	-	-	-	1	-	-	0.2

... continued

Table 4.11. continued

Sl. No.	Name of the insects obtained	Disease category	Average number of insects obtained through 10 sweeps											
			4 am	5 am	6 am	7 am	8 am to 3 pm	4 pm	5 pm	6 pm	7 pm	8 pm	9 pm	Mean
		I	-	-	1	1	Nil	-	1	2	-	-	-	0.5
		II	-	-	-	-	Nil	-	1	-	-	-	-	0.1
4.	Tortoise beetles	III	-	-	1	-	Nil	-	-	-	-	-	-	0.1
		IV	-	-	-	1	Nil	-	1	-	-	-	-	0.2
		V	-	-	-	1	Nil	-	-	-	-	-	-	0.1
		I	-	-	1	-	Nil	-	1	-	-	-	-	0.2
		II	-	-	-	-	Nil	-	-	-	-	-	-	0.0
5.	Wasps	III	-	-	-	-	Nil	-	1	-	-	-	-	0.1
		IV	-	-	-	-	Nil	-	-	-	-	-	-	-
		V	-	-	-	-	Nil	-	-	-	-	-	-	0.1
		I	-	-	1	-	Nil	1	-	1	-	-	-	0.3
		II	-	-	1	-	Nil	-	-	-	-	-	-	0.1
6.	Spodoptera litura	III	-	-	-	-	Nil	-	-	1	-	-	-	0.1
		IV	-	-	-	-	Nil	-	-	-	-	-	-	0.0
		V	-	-	-	-	Nil	1	-	-	-	-	-	0.1

In ten sweeps conducted at various parts of the plot, ie. in areas with diseased plants of all categories at different time of the day, those insects which were constatly observed were used for studies.

Maximum population of insects were obtained during 5 am and 6 am and after 7 pm (Table 4.11). During the peak hours of their occurrence, they were found under the leaves of the plants. More number of insects were found on the healthy plants (disease category I) compared to diseased ones.

Since *Austroagallia* sp. was constantly found associated with pepper it was selected for studying transmission of the disease.

Adult insects of *Austroagallia* sp. failed to survive fasting of more than two days. Hence they were subjected to pre-acquisition fasting for one and two days, and then released to the plants of different disease categories. Sixty eight and twenty eight per cent of insects survived after one and two days of fasting respectively. For the acquistion feeding the insects were released on plants of different disease categories for a period of 1 to 5 days. The insects failed to survive on diseased plants for more than 5 days. The insects after acquisition feeding were released on healthy plants kept in insect proof cages. These plants were kept under observation. Inspite of

Table 4.12. Insect transmission of little leaf disease of pepper through Austroagallia sp.

Disease category	Number of plants observed	Number of insects put for pre-acquisition fasting		Number insects survived after the fasting	Number of insects released for acquisition feeding of					Number of insects survived after the acquisition feeding of					Number of days the insects survived after acquisition feeding of					Number of days taken for transmission of disease after acquisition feeding of				
					1 day	2 days	3 days	4 days	5 days	1 day	2 days	3 days	4 days	5 days	1 day	2 days	3 days	4 days	5 days	1 day	2 days	3 days	4 days	5 days
1	10	1day	10	8	10	10	10	10	10	6	4	3	2	1	5	5	3	2	1	-	-	-	-	-
	10	2days	10	4	10	10	10	10	10	4	2	2	1	1	4	3	3	2	1	-	-	-	-	-
2	10	1day	10	7	10	10	10	10	10	5	3	2	1	1	4	4	3	2	1	-	-	-	-	-
	10	2days	10	3	10	10	10	10	10	3	2	2	1	0	3	2	1	1	0	-	-	-	-	-
3	10	1day	10	6	10	10	10	10	10	4	2	2	1	0	4	2	1	1	0	-	-	-	-	-
	10	2days	10	3	10	10	10	10	10	2	1	1	0	0	3	2	1	0	0	-	-	-	-	-
4	10	1day	10	7	10	10	10	10	10	4	3	1	1	1	3	2	2	1	1	-	-	-	-	-
	10	2days	10	2	10	10	10	10	10	2	2	1	0	0	2	1	1	0	0	-	-	-	-	-
5	10	1day	10	6	10	10	10	10	10	3	2	0	0	0	2	1	0	0	0	-	-	-	-	-
	10	2days	10	2	10	10	10	10	10	3	1	0	0	0	2	1	0	0	0	-	-	-	-	-
Mean	10	1day	10	6.8	10	10	10	10	10	4.4	2.8	1.6	1.0	0.6	3.6	2.8	1.8	1.2	0.6	-	-	-	-	-
	10	2days	10	2.8	10	10	10	10	10	2.8	1.6	1.2	0.4	0.2	2.8	1.8	1.2	0.6	0.2	-	-	-	-	-

repeated efforts, the disease could not be transmitted to healthy plants using *Austroagallia* sp. (Table 4.12).

Mandera beta was another insect found associated with pepper plants. This insect was also suspected to be a pest of *Erythrina indica*, the common live standard of pepper. Artificial inoculation using *Mandera beta* on the same lines as that of *Austroagallia* was also unsuccessful. These two insects were not reported from Kerala on any of the crops.

Transmission of the disease using *Liothrips karnyi* was also not successful.

There is no report of the insect transmission of little leaf disease of black pepper.

4.8. Tetracycline administration and remission of symptoms

When oxytetracycline hydrochloride (OTC) at 500 ppm was applied through wick, remission of symptoms was noticed after 28 days. As the concentration of OTC increased from 500 ppm to 2000 ppm, there was reduction in the time taken for the remission of symptoms. At 2000 ppm concentration, new healthy leaves were produced 61 days after spraying, 42 days after drenching, 50 days after dipping for 24 hours, 38 days after dipping for 72 hours and 22 days after wick feeding (Table 4.13).

Table 4.13. Tetracycline administration and symptom remission in little leaf affected pepper plants *

OTC concentration (ppm)	Number of plants treated	Number of days taken for symptom remission(R1) and symptom re-appearance (R2) through											
						Dipping and planting after						Wick feeding	
		Spraying		Drenching		24 hours		48 hours		72 hours			
		R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
250	10	-	-	-	-	-	-	-	-	-	-	-	-
500	10	73	93	70	118	73	179	65	187	53	189	28	Not re-appeared
750	10	65	108	60	128	68	185	57	191	45	193	25	-do-
1000	10	64	113	45	131	62	189	53	195	42	199	24	-do-
1500	10	62	115	43	135	55	193	48	198	40	201	22	-do-
2000	10	61	118	42	138	50	199	45	201	38	205	22	-do-

* Mean of ten observations

Plate 18. Resultant plant of wick feeding with 500 ppm oxytetracycline hydrochloride.

The remission of symptoms in OTC sprayed; drenched or dipped plants was observed for a period of 3-9 months. At 500 ppm concentration, the re- appearance of the symptoms on sprayed plants was observed three months after the remission of symptom was noticed. Thus the efficiency of this treatment lasted only for 93 days. However, the efficiency of 500 ppm application of OTC was the best in cuttings dipped for 24 hours where the plants remain healthy for a period of 7-8 months. As the concentration of the OTC increased, the plants remained healthy for a longer period of time. Thus the plants dipped for 24 hours in OTC solution at 2000 ppm remained healthy for a period of 8 months. However, at concentration above 750 ppm, the plants showed mild scorching symptoms. The time taken for the re-appearence of the symptoms in 750 ppm was four months in spraying, four months in drenching and 6 - 7 months in dipping which was on par with that of plants treated with 500 ppm.

Among the various methods of application of OTC, wick feeding was found to be the best. At 500 ppm and above the remission of symptoms was noticed within 22-28 days after treatment. None of these plants reverted back to a diseased condition till the end of the experiment (two years) (Plate 18). Thus this treatment has been found to be effective in saving the plants from the disease. However, this treatment require further detailed study.

The better results obtained in wick feeding may be due to the fact that higher quantity of OTC was directly absorbed into the system of the plant, while the absorption might have been less in plants sprayed or dipped with OTC. In cuttings dipped in OTC a tendency of the solute to settle at the bottom of the container might have blocked the absorption of the chemical. The quantity of OTC required for drenching and spraying was higher than that required in dipping and wick feeding . Lot of chemicals get lost by evaporation and seepage when the chemicals were applied by spraying and drenching.

The inhibitory effect of OTC on MLO in eucalypts (Ghosh *et al.*, 1985) and sandal spike (Ali *et al.*, 1987) have already been reported. From the study it is clear that OTC at 500 ppm applied through the wick is the best method for remission of the symptoms followed by dipping of cuttings for 24 hours in the same solution.

The presence of blue colour of the pholem after Dienes' staining in diseased plants and remission of the symptoms by the use of OTC clearly indicate that the causative organism of the little leaf disease of black pepper is MLO. This is the first report of the relationship of MLO with little leaf disease of black pepper.

4.9. Assessment of loss in yield

During 1993, the average yield recorded per plant in the category I was 7.42 kg while it was 5.54 kg in 1994. This yield reduction may be attributed to the climatic condition. As the incidence of the disease increased there was a corresponding reduction in the yield. Thus, in the disease category V, the difference between 1993 and 1994 yields showed a drastic difference. This clearly indicated that the disease can bring down the yield of the plant considerably.

The fresh weight of 100 berries in the disease category I during 1993 was 10.43 g, while it was 9.74 g for the disease category 2 and 8.29 g in disease category 5. During 1994, the corresponding figures were 9.21 g, 10.24 g and 8.21 g. This indicated that apart from reducing number of berries per spike (Table 4.14) disease could also reduce the yield by reducing the weight of the berries.

The dry weight of 100 berries after four days of sundrying decreased from 65.47 per cent (category I) to 57.01 per cent (disease category IV). Thus there is reduction in the dry weight of diseased plants.

Table 4.14. Yield loss due to little leaf disease of pepper in the five disease categories *

Characters	Year	Category I	Category II	Category III	Category IV	Category V
Total yield (fresh) kg	1993	7.42	2.54	1.61	1.66	0.86
	1994	5.54	1.76	1.06	1.36	0.42
100 berries weight (fresh) (g)	1993	10.43	9.74	9.50	9.15	8.29
	1994	9.21	10.24	10.35	8.96	8.21
100 berries weight (dry) (g)	1993	4.22	3.87	3.77	3.77	3.05
	1994	3.37	3.55	3.81	3.83	2.94
Percentage loss in weight due to drying	1993	65.47	66.33	66.54	58.69	63.11
	1994	63.37	65.33	62.90	57.01	64.02

* Mean of ten observations

Summary

SUMMARY

1. The study 'symptomatology and etiology of little leaf disease of pepper (*Piper nigrum* L.)' was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during 1992-'94. The field trials were conducted at Pulppally in Wayanad district as the disease was seen more in that area.

2. A disease scoring chart (0 to 4) was perfected based on the external symptoms of the disease.

3. The diseased plants had shorter mainroot and lesser feeder roots. The roots of the diseased plants turned brown.

4. A reduction in the internodal length was observed in diseased vines which give a bushy appearance to the plant.

5. The leaves on the diseased plants were distorted, downwardly curved, chlorotic, brittle with wire-net type veinlets.

6. The shape and size of flowers in diseased plants were normal in appearance. But the number of flowers per spike decreased and the aborted flowers increased.

7. Only few berries reached proper maturity.

8. Little leaf disease was seen on plants of all age groups. However, the disease increased with an increase in age.

9. Karimunda was highly susceptible to the disease while Arakukkulam munda, Aimpiriyan and Vellanamban showed lesser degrees of infection. Panniyur 1 was comparatively tolerant to this disease.

10. Zinc application did not inhibit the symptoms.

11. The phloem portion of the roots, nodes and petioles of the diseased plants stained blue with 0.2% Dienes' stain indicating the presence of mycoplasma like organisms. Phloem portion of the healthy plant did not take up the stain.

12. The disease could not be transmitted by sap or dodder.

13. It could be transmitted by grafting and by cutting. Grafting was successful only when healthy scion was wedge grafted using diseased root stock. But when diseased scion was grafted on healthy root stock the transmission was not observed.

14. Insects *Austroagallia* sp. and *Mandera beta* Owarokowska were found constantly associated with diseased plants.

Transmission studies using these insects and thrips (*Liothrips karnyi*) was not successful.

15. Remission of the symptoms of the disease was observed when oxytetracycline hydrochloride (OTC) was used at concentrations more than 500 ppm. OTC at 250 ppm was not effective. Application of OTC by drenching, spraying, dipping and wick feeding was effective. However, wick feeding was found to be the best. Symptom remission in trials where OTC was applied by spraying, drenching and dipping lasted for less than 6 months. But plants treated with OTC through wick application, the re-appearance of symptoms was not noticed even after two years. OTC was phytotoxic when applied at a concentration of more than 750 ppm.

16. Yield reduction was noticed in diseased plants.

17. The results of the symptomatology and etiological studies indicate that the causative agent of little leaf disease of pepper is a mycoplasma like organism.

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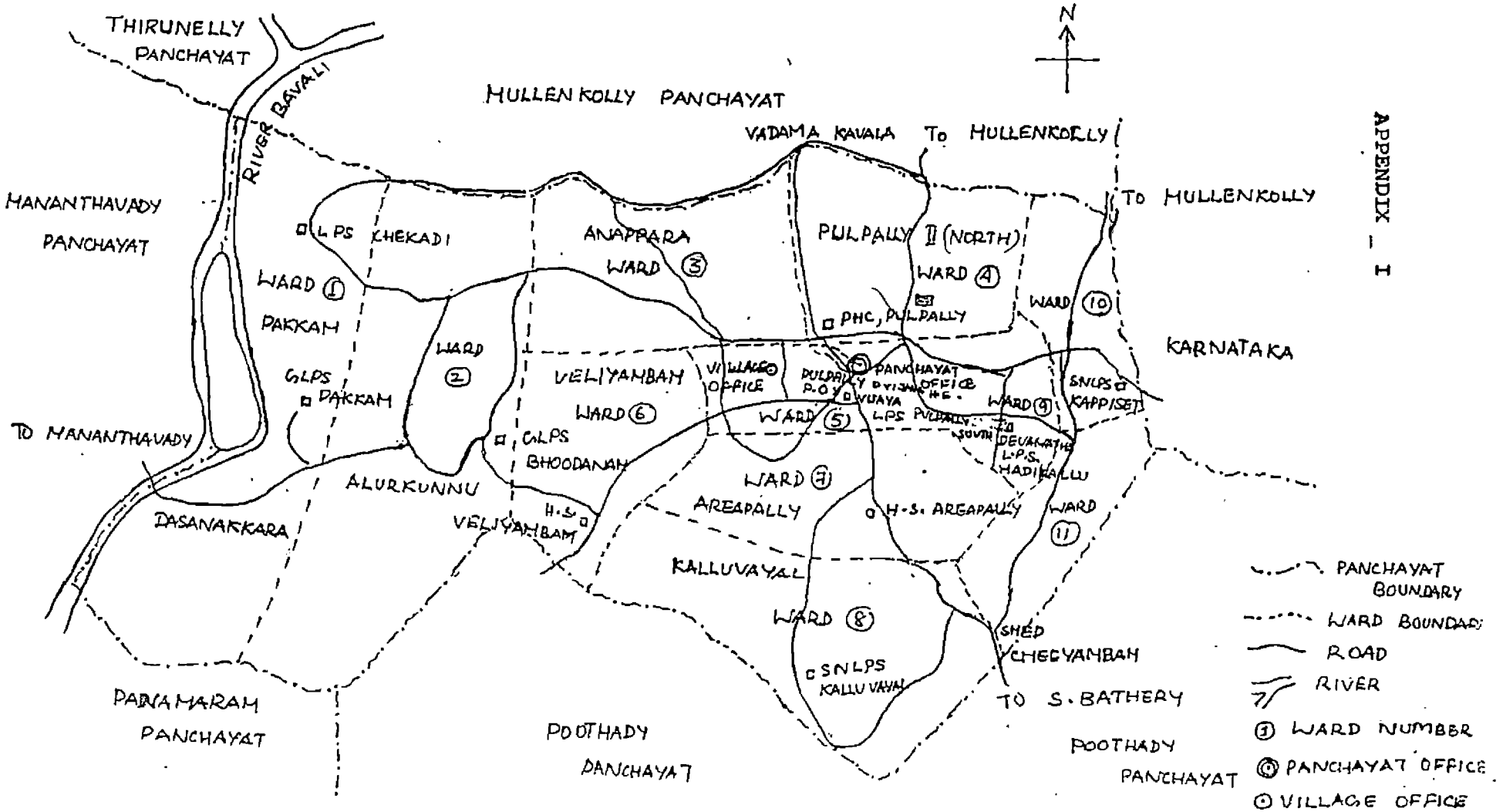
* Orginals not seen

Appendices

MAP OF PULPALLY PANCHAYAT



APPENDIX - II

APPENDIX II

Distribution of rainfall in Pulpally panchayat over five years from 1988-1993 as recorded by Pambra coffee plantations

Month	1988	1989	1990	1991	1992	1993
January	Nil	Nil	5.0	Nil	Nil	Nil
February	86.4	Nil	Nil	0.5	Nil	3.5
March	48.8	45.9	57.7	30.2	Nil	72.2
April	257.2	84.0	122.9	114.0	107.4	213.6
May	94.7	124.6	122.5	248.7	169.7	122.3
June	170.2	317.0	179.8	375.4	622.6	171.2
July	409.2	480.0	264.7	505.5	492.7	395.0
August	196.9	180.4	314.2	217.4	249.5	209.0
September	201.9	110.7	38.8	141.5	169.5	55.8
October	39.3	131.6	307.9	105.1	157.7	247.4
November	10.2	61.2	32.5	27.3	153.6	32.0
December	25.1	35.3	Nil	Nil	Nil	65.2
Total for the year	1569.9	1570.7	1446.0	1765.6	2122.4	1588.0

APPENDIX III

Composition of Dienes' stain

Methylene blue	- 2.5 g
Azure II	- 1.25 g
Maltose	- 10 g
Sodium carbonate	- 0.25 g
Distilled water	- 100 ml

SYMPTOMATOLOGY AND ETIOLOGY OF LITTLE LEAF DISEASE OF PEPPER

(*Piper nigrum* L.)

By

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ABSTRACT OF A THESIS

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ABSTRACT

Symptomatology and etiology of little leaf disease of pepper (*Piper nigrum* L.) was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur and at Pulppally in Wayanad district during 1992-'94.

Based on the external symptoms of the disease a disease scoring chart (0 to 4) was perfected.

The diseased plants had shorter mainroot and less number of feeder roots. Brown discolouration was noticed on diseased roots. Internodal length was reduced which give a bushy appearance to the plant. Leaves were distorted, downwardly curved, chlorotic, brittle with wire-net type veinlets. Eventhough the size and shape were normal, the number of flowers per spike decreased and the aborted flowers increased. Proper maturity was obtained by very few berries.

Disease was observed to attack all age group of plants, but the intensity increased with age.

Variety Karimunda was highly susceptible and Punniyur-1 was observed to be tolerant though the disease attacked Arakkulam munda, Aimpiriyan and Vellanamban with lesser degree than Karimunda.

Application of Zinc did not inhibit the symptoms.

When 0.2% Dienes' stain was used for the phloem portion of the roots, nodes and petioles of the diseased and healthy plants, only diseased plant parts stained blue indicating the presence of mycoplasma like organisms in them.

Disease could not be transmitted by sap or dodder.

It could be transmitted by cuttings and by wedge grafting using diseased root stock and healthy scion not vice versa.

Transmission studies with insects *Austroagallia sp.* *Mandera beta* Owarokowska and *Liothrips karnyi* were not successful.

Applications of oxytetracycline hydrochloride (OTC) by drenching, spraying, dipping and wick feeding were effective at concentrations more than 500 ppm for remission of disease symptoms. OTC at 250 ppm was not effective. Above 750 ppm, the treatment caused phytotoxicity. Among methods, wicks feeding was found to be the best, which prevented re-appearance of symptom even after two years where as in all the other methods, the symptom remission lasted for less than 6 months.

Yield was observed to be reduced with an increase in disease intensity.

The study revealed the symptomatology of little leaf disease of black pepper and resulted in the identification of etiology as mycoplasma like organism.