CHAPTER III

MATERIALS & METHODS
1. Brown spot disease Survey and assessment of disease severity levels under different agro-ecological situations of KLS region

A field survey for the incidence of brown spot disease was carried out in Flue Cured Virginia (FCV) tobacco growing areas in Southern Transition Zone (STZ) of Karnataka during 1996-97 crop season. The survey was conducted in all the FCV tobacco growing agro-ecological situations (AES) of STZ (Lokamanya et al., 1994). At each location, disease incidence and severity levels were assessed on randomly selected 100 plants of 90 to 100 days old crop at three or more fields, based on visual symptoms. Brown spot disease severity was scored on a new scale, developed based on the FAO methods of plant disease assessment and standardised for the purpose (Chiarappa, 1970 and Anon., 1997). The new rating scale of disease severity comprises 0 to 7 grades, wherein 0 = no symptoms, 1 = less than 1% leaf area spotted, 2 = 1-5% leaf area spotted, 3 = 6-10% leaf area spotted, 4 = 11-20% leaf area spotted, 5 = 21-50% leaf area spotted, 6 = 51-80% leaf area spotted, 7 = above 80% leaf area spotted (Fig. 4). These ratings were assigned to each leaf in each randomly selected plant. The per cent leaves affected (PLA) and per cent disease index (PDI) were calculated. PDI was calculated using the formula of Wheeler (1969) as given below.

\[
PDI = \frac{\text{Sum of numerical grade values}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum grade value}}
\]
Fig. 4. Grades (1 to 7) of brown spot disease severity rating scale
2. Epidemiological studies

(a) Study of brown spot disease progress curve and factors influencing the disease spread

A replicated field trial was conducted for three seasons from 1996-97 through 1998-99 at Sollepura farm of CTRI Research Station, Hunsur, which has been identified as a ‘hot-spot’ area for brown spot disease, with the aim of identifying critical meteorological factors influencing brown spot disease spread, besides establishing relationship between date of planting, age of the crop and disease severity.

Geographically, the farm is located at 12° 17' 55" North latitude, 70° 17' 35" East longitude and at an altitude of 827 MSL (Krishnamurthy et al., 1993). The local conditions are of general representative type of Karnataka light soil (KLS) region in transitional zone of western ghats with its tropical monsoon climate of lower diurnal temperatures, higher relative humidities, frequent rains and an average annual rainfall of 650 to 1000 mm of which 80% is received during May to October period, coinciding with FCV tobacco crop season (Shenoi, 1998). The planting period extends from early May to mid July. Depending on planting period, Gopalachari (1984) classified the KLS crop as ‘early’ (crop planted in the month of May), ‘middle’ (crop planted up to 20th June) and ‘late’ (crop planted beyond June 20th).

The soil of experimental plot was sandy-clay loam in texture with acidic to neutral pH, normal in soluble salt content, containing very low chlorides. The status of the soil with respect to organic matter, nitrogen and phosphorus was low to medium while potassium status was high (Krishnamurthy et al., 1993).

The trial in each season was conducted in a randomised block design with two replications. The treatments consisted of three varieties and seven dates of planting commencing from 10th May at 10 days interval. The varieties recommended by CTRI Research Station, Hunsur were used in the study. Details of the trial are as follows:
Planting dates (seven)

1. May 10  Early planting
2. May 20
3. May 30

4. June 10  Middle planting
5. June 20

6. June 30  Late planting
7. July 10

Variedes (Three)
1. FCV Special
2. Bhavya
3. Swarna

Replications:  Two

Seasons:  Three (1996-97, 97-98 & 98-99)

Plot size:  6 x 16.5m. (6 x 30 = 180 plants)

About 60 days old healthy seedlings raised in a nursery adopting recommended package of practices were transplanted to field according to the schedule (Fig. 5). Seedlings were transplanted 55 cm. apart on to ridges, spaced at 100 cm. Thus, each block contained 180 plants at a spacing of 100 x 55 cm. Fertilizers NPK @ 50 : 80 : 80 kg/ha. were applied according to normal schedule. All the package of practices recommended by CTRI Research Station, Hunsur (Anon, 1996) were strictly followed for growing field crop and natural epiphytotic disease development was permitted.

To ascertain role of planting date and crop age in brown spot disease manifestation, observations on brown spot incidence and severity were recorded at weekly intervals on 10 randomly selected plants in each plot, commencing from 60 days after transplantation (DAT). Disease severity was recorded on a 0 to 7 standard scale and PDI was calculated as explained earlier. The data was subjected to statistical analysis. The rate of spread of the disease was also calculated from PDI recorded at weekly intervals, from 60 DAT to 95 DAT. Overall rate of spread was also calculated for this
Fig. 5. Experimental plot showing crop at different stages of growth in different planting dates

Fig. 6. Meteorological observatory in the crop canopy
period in each DOP. The rate of disease spread was calculated as apparent infection rate 
\( r \) per unit per day using the formula of VanderPlank (1963). The formula is:

\[
2.3 \frac{X_2 (1 - X_1)}{t_2 - t_1} = \log_{10} \left( \frac{X_1 (1 - X_2)}{t_2 - t_1} \right)
\]

Where, \( r \) = apparent infection rate,

\( X_1 = \) PDI at \( t_1 \),

\( X_2 = \) PDI at \( t_2 \) and

\( t_2 - t_1 = \) Time interval between two observations.

Based on mean values of PDI, brown spot disease progress curves (DPCs) were 
developed for each variety at each date of planting for all the three seasons as well as for 
mean of three seasons.

For studying the role of weather factors in brown spot disease spread, a 
temporary meteorological observatory was established in the centre of experimental plot 
(Fig. 6). Day-to-day changes in important weather parameters were recorded by 
equipping the observatory with thermohygrograph and maximum and minimum 
thermometers housed in a Stevenson’s screen. Sunshine recorder, rain gauge and dew 
gauge were also established in the centre. Daily recording of weather factors in crop 
canopy was done till final harvest of July planted crop. The factors recorded include 
maximum and minimum temperature, relative humidity during night and day hours, 
sunshine hours per day, rainfall and dew fall.

Brown spot disease initiation and its progress was recorded at each harvest of the 
crop in regular intervals. Leaves were harvested at their maturity, generally at six days 
interval starting from 60 DAT. At each harvest, the disease was scored in 500 randomly 
selected leaves from every plot as per the disease severity rating scale and PDI values 
were calculated. Mean values of meteorological parameters during ten days period prior 
to each harvest were calculated. Mean values were also calculated for PDI and 
meteorological factors for entire period of harvest in each DOP. Based on this, linear
correlation and regression coefficients between the weather parameters and disease severity (PDI) were computed (Snedecor and Cochran, 1967).

To develop a quantitative relationship between disease progress and weather variables, multiple regression analysis (MRA) was done with PDI as dependent variable (Y) and meteorological factors such as mean maximum temperature (X1), mean minimum temperature (X2), per cent night relative humidity (X3), per cent day relative humidity (X4), total number of wet nights (i.e., nights with precipitation either in the form of rain or dew) (X5) and mean sunshine hours per day (X6) as independent variables. MRA was performed for each variety at every planting date. The predicted equation for PDI i.e., \( Y = a + b_1 (X_1) + b_2 (X_2) + \ldots + b_6 (X_6) \) was derived by MRA, where \( Y \) denotes the predicted brown spot PDI on tobacco; \( a \) denotes the intercept and \( b_1 \) to \( b_6 \) denotes partial regression coefficients for \( X_1 \) to \( X_6 \) weather factors, respectively. Significance of coefficient of multiple determination (\( R^2 \)) and partial regression coefficient (b) values were followed at 5 per cent level of probability (Snedecor and Cochran, 1967).

(b) Critical study of the period between the inoculation of leaves and disease manifestation

Progress in spot development was studied under glass house conditions which were modified to suit the requirements for disease development. Potted plants were inoculated at 80 DAT with \( A. alternata \) by spraying conidial suspension. Suspension was prepared in sterile water by grinding dried, infected leaves of the previous crop, stored in polythene bags (Lucas, 1975). Adequate humidity and moisture were given during night hours with the help of humidifiers. Air conditioners were used to maintain favourable temperature regime. During day hours, the potted plants were exposed to direct sunshine.

With the aim of identifying acute stage during spot development in terms of production of conidia, a replicated experiment was conducted using the infected plants from glass house. A total of six stages were visually identified in the development of brown spot, based on size and appearance of the spot, commencing from water soaked lesions of pin head size and ending with 'Shot-holes'. At each of these six stages of development a known area of spot was incubated under darkness at 90% RH and less than 20°C temperature for a period of 48 hours for the purpose of maximum sporulation.
The entire set up for each stage was replicated six times. After maximum sporulation, conidial suspension was prepared for each stage in 10 ml sterile water. Number of conidia from each suspension were counted using a Haemocytometer. The procedure followed for counting and calculating conidial production at each stage of spot development is as follows -

Depth of Haemocytometer = 100 µ or 0.01 cm.

Area used for counting = 50 mm or 0.5 cm²

.: Total volume of suspension used = Area x depth

= 0.5 cm² x 0.01 cm

= 0.005 cm³

= 0.005 ml.

Hence, of the 10 ml suspension prepared for each sample, only 0.005 ml is utilised for counting the conidia under the Haemocytometer.

For 0.005 ml suspension number of conidia counted = X

.: For 10 ml suspension number of conidia counted = \( \frac{10 \times X}{0.005} \)

Area of infection spot = \( \gamma \) mm²

Hence for \( \gamma \) mm² area of spot the conidia produced = \( \frac{10 \times X}{0.005} \)

.: For one mm² area of infection spot conidia produced = \( \frac{10X \times 1}{0.005 \times \gamma \text{mm}^2} \)

Thus, the conidia produced from each stage of the developing spot was presented as mean of six replications per mm² of infected area and the data was subjected for statistical analysis.
Role of abiotic factors other than meteorological factors, particularly, nitrogen (N), phosphorus (P) and potassium (K) on brown spot disease manifestation was studied in three separate trials.

**Nitrogen trial:**

To study the effect of nitrogen (N), a trial was conducted in a randomised block design. The treatment combinations of three levels of N (20+10, 30+10 and 40+10 kg/ha) and three levels of FYM (0, 5 and 10 t/ha.) were replicated thrice. The recommended dosage of 40 + 10 kg N with 10 tonnes of FYM/ha served as check. FYM was given as pre-planting application and N was given in two doses with basal dressing at 10 DAT through diammonium phosphate (DAP) as per treatment and top dressing at a uniform rate of 10 kg through calcium ammonium nitrate (CAN) on 30\textsuperscript{th} DAT. The recommended dosage of P and K (80 kg each) were supplied to all the treatments.

**Potassium trial:**

To study the effect of potassium (K), a trial was conducted in a randomised block design having three replications and five treatments. Levels of K (0+0, 10+10, 20+20, 40+40 and 80+0 kg/ha) served as treatments. The recommended dosage of 80 kg K in a single dose as basal fertilizer was considered as control. The basal dose of potassium on 10 DAT and the top dressing given at 30 DAT were in the form of Sulphate of Potash (SOP). N and P were applied as per the standard recommendation.

**Phosphorous trial:**

A trial was conducted to study the effect of phosphorous (P) on brown spot disease severity. The treatments consisting of five levels of P (0, 20, 40, 60 and 80 kg/ha) were replicated thrice in a randomised block design. Entire quantity of P was applied as basal dressing at 10 DAT in the form of either DAP or single super phosphate (SSP) as per the treatments. The recommended dosage of 80 kg phosphorous was considered as check. N and K were given according to standard dosage.
The above three trials were conducted at Sollepura farm of CTRI Research Station, Hunsur, which has been identified as a 'hot-spot' for brown spot disease. Susceptible variety, Bhavya, transplanted late in the season (June 30) was used for all the three studies to ensure high disease pressure. All the recommended package of practices were strictly followed. Leaves were harvested as soon as they ripened, generally at six days interval, starting from 60 DAT. At each harvest, leaves were assessed for disease parameters such as PDI, per cent leaves having more than 10% area damaged and per cent leaves having more than 50% area damaged. The data were subjected to standard statistical analysis for drawing valid inferences.

(d) Influence of biotic factors on disease perpetuation

A study was conducted to understand overseasoning and survival of brown spot pathogen and also to identify sources of primary inoculum. The study included documentation of weed host range for *Alternaria alternata*, and viability of the pathogen on host crop residues.

(i) Weed host range studies:

Weeds in the vicinity of tobacco fields which might play the role of collateral hosts were identified initially. As a first phase of investigation, these weeds with symptoms of spots from field location were selected, the pathogen involved was isolated and maintained as pure cultures as per standard procedure (Dhingra and Sinclair, 1986). Isolations of suspected *Alternaria* cultures obtained from these weeds were identified as *Alternaria alternata* at IARI, New Delhi. Further confirmation of weed hosts was made in glass house studies by applying Koch's postulates to establish their role as collateral hosts for *Alternaria alternata*.

(ii) Survival of *Alternaria alternata* on host crop residues:

Survival of pathogen on infected crop residues such as leaf trash and stubble was studied. Bulk samples of naturally infected leaves and infected stalks were collected at the end of crop season and were air-dried before placing them in punctured polythene bags, which were tied with thread and kept separately under three different conditions viz., (1) in the laboratory, (2) on the ground in the field and (3) buried in field soil at a depth of
about 15 cm. Air-dried, uninfected healthy leaves and healthy stubble kept in the punctured polythene bags and stored in laboratory conditions served as control. Each treatment was replicated four times. Regular isolations were carried out at bimonthly (once in two months) intervals to determine the longevity of the pathogen by putting 100 bits on PDA slants after surface sterilising the samples with 0.1% mercuric chloride solution and incubating for four days. The isolations obtained were tested for the presence of Alternaria alternata. Besides, an attempt to isolate the pathogen from infected, cured tobacco was also made. Quantification of conidia produced in infected crop residues during the period of overseasoning of fungus was done during the study. From the samples kept for survival studies, 100 infected bits, with mean spotted area of 10 to 15 mm for leaf samples and 3 to 6 mm for stubble, were incubated at bimonthly intervals to estimate the conidial production by infected crop residues kept under different conditions. Infected bits were surface sterilised with 0.1% mercuric chloride and incubated at 90% RH, less than 20° C temperature and under darkness for a period of 48 hours to maximise sporulation. After maximum sporulation, conidial suspension was prepared for each sample in 10 ml sterile water. The conidia from each suspension were counted using haemocytometer as described earlier and presented as conidia produced per sq. mm. of infected area.

(e) Interaction with root-knot nematode

Two pot culture experiments under glass house conditions, and replicated field trials were conducted to study the interaction if any, between two major pathogens causing root knot and brown spot diseases in tobacco crop.

**Pot experiment-1:**

In the first pot experiment, eight week old, healthy seedlings of var. FCV Special raised in fumigated bed were used. Fumigation was done to prepared beds with methyl bromide @ 18 ml/sq. m for 24 hours and sowing was taken up after 24-48 hours of aeration (Anon, 1997). The seedlings were transplanted singly in surface sterilized earthen pots filled with sterilized soil. The treatments consisting of four levels of nematode (second stage juveniles- J2) inoculum load (0, 500, 2000 and 5000 J2 of
Meloidogyne spp. per plant) were replicated five times. The treatment with no nematode inoculum was considered as control. At the time of transplanting, along with transplanting water, seedlings were inoculated at root zone with juveniles of mixed population of *M. incognita*, *M. javanica*, and *M. arenaria* according to the treatment schedule.

**Pot experiment-2:**

In another pot experiment, seedlings of variety, FCV Special, raised in root-knot nematode sick nursery site were used. Eight week old transplantable seedlings were pulled out and categorised based on their root-knot index (RKI) as less infected (RKI =1), moderately infected (RKI =2 and 3) and highly infected seedlings (RKI = 4). Very highly infected seedlings (RKI = 5) were rejected, since they do not establish in the field. Healthy seedlings, free from root-knot infection, obtained from fumigated bed served as control. Five replications were maintained for each treatment. The seedlings were transplanted singly in surface sterilized earthen pots filled with sterilized soil. Variety, FCV Special, was selected due to its high susceptibility to both root-knot and brown spot pathogens.

**Pot experiment-3:**

A similar trial, like pot experiment-2, was conducted with another variety, Beinhart 1000-1, which is susceptible to root-knot but resistant to brown spot to make an indepth study on possible interaction between two diseases and possible breakdown of disease resistance.

In all the above experiments, potted plants kept in the glass house were inoculated twice at 65 DAT and 75 DAT with *A. alternata* by spraying conidial suspension. Suspension was prepared in sterile water by grinding dried, infected leaves of the previous crop stored in polythene bags (Lucas, 1975). The conditions suitable for brown spot were created in the glass house as described previously.
Field studies:

Further studies on interaction of brown spot and root-knot pathogens were conducted under field conditions. A trial with five treatments and four replications in nematode infested soil using variety FCV Special, was conducted in a field endemic to brown spot disease. Gall free, healthy seedlings planted in nematode free plots (the plots were treated with phenamiphos, a nematicide) were considered as check plots. Healthy, light, medium and severe galled seedlings planted in nematode sick soil formed other treatments. A similar trial was also conducted with brown spot resistant variety, Beinhart 1000-1, to study the possible break down of brown spot resistance.

For all the above experiments, conducted both under glass house and field conditions, the recommended package of practices were strictly followed. Brown spot disease severity was recorded on harvested leaves using the standardised scale. Leaves were harvested at their maturity, generally at six days interval, starting from 60 DAT. At each harvest, leaves were assessed for brown spot disease parameters such as PDI, per cent leaves having more than 10 per cent area damaged and per cent leaves having more than 50 per cent area damaged. Mean values were calculated at the end of the experiment. Separate mean values of brown spot disease parameters under epiphytotic conditions were calculated for the field trial with variety, FCV Special. Root-knot index (RKI) was recorded after final harvest on 0 to 5 scale of Taylor and Sasser (1978) where, 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100 and 5 = more than 100 galls. Besides this, for the experiment conducted under field conditions with variety FCV Special, yield parameters were recorded and the loss in yield and quality due to the diseases was assessed based on % decrease in yield of total green leaf, total cured tobacco and bright grade tobacco in nematode infested plots over healthy, nematode-free check plots.
3. Crop loss assessment - Quantification of yield and quality loss due to brown spot disease

A study was conducted to assess loss in yield and quality of tobacco due to brown spot disease and also the possible alterations in important chemical and technological parameters in cured tobacco. Besides this, an attempt was also made to quantify effect of brown spot on market value of the produce.

Brown spot susceptible variety, Bhavya, which is also the ruling variety in KLS region is used for the studies. Harvested leaves were classified as per the 0 to 7 disease rating scale described earlier and loss in yield was assessed at each disease severity level. PDI was also calculated in each category as described earlier and a simple linear regression analysis was done to find out the correlation between the disease severity levels and yield and quality of FCV tobacco using the formula, \( Y = a + bX \), where, \( Y \) = estimated loss in yield or quality; \( a \) = regression line intercept; \( b \) = regression coefficient and \( X \) = PDI.

Yield parameters were studied as described by Main and Chaplin (1972) and Monga (1988). One thousand harvested leaves (replicated four times) of ‘L’ position, from each category of disease rating scale were collected from a field at Sollepura farm of the CTRI Research Station, Hunsur during 1997 crop season and the weights of Green leaf (GL), cured leaf (CL), Bright grades (BG), medium grades (MG) and low grades (LG) were recorded. Market value for each category was calculated with the prevailing KLS market rates of Rs.60 per kg for BG, Rs.40 per kg for MG and Rs.20 per kg for LG tobaccos. Important chemical and technological parameters such as Nicotine (Nic), Reducing Sugars (RS), Equilibrium moisture content (EMC) and Filling value (FV) were analysed at chemistry division of CTRI, Rajahmundry, for samples in each category following standard procedures (A.O.A.C., 1990; Harvey et al., 1969; Artho et al., 1963 and Chakraborty et al., 1965). An attempt was also made to quantify effect of brown spot on seed production by comparing the seed yields of healthy and disease affected crops.
4. Disease management studies

(a) In vitro evaluation of potentially effective botanical species and fungicides for the suppression of growth and sporulation of *A. alternata*

(i) Evaluation of Botanicals species:

Crude leaf extracts of commonly occurring 45 botanical species were evaluated in vitro for studying their antifungal properties, if any, against *A. alternata*. 25% leaf extract of each plant species was prepared as per the method described by Gupta et al. (1996). Effect of each plant extract on radial growth of the test pathogen was assessed by 'poisoned food technique' as described by Grover and Moore (1962). Potato dextrose agar (PDA) medium amended with fresh leaf extract of each botanical filtered through Whatman no.1 filter paper at dilution of 1:1 (extract: medium) was used in 10 replications. Medium with sterile distilled water served as a control. Mycelial disc of 4 mm diameter of actively growing single spore culture of *A. alternata* was used for inoculation. The inoculated culture plates were incubated at 28 ± 2°C. Mean mycelial growth of the pathogen was recorded on seventh day. The % increase or decrease of mycelial growth over control was calculated using the formulae, I= C-T/C x 100 and J= T-C/C x100, respectively, where I = % decrease; J= % increase; C = mean mycelial growth in control plate and T= mean mycelial growth in culture plate amended with leaf extract. The data was subjected to statistical analysis and the fungitoxicity of leaf extracts were compared at 5% level of significance.

The botanicals which were found promising against brown spot pathogen were further evaluated at a lower dilution of 1:10 (extract: medium) in the similar way as described above.

(ii) Evaluation of fungicides:

With the aim of identifying promising chemical fungicides against brown spot disease in tobacco fields, initially, a total of 28 fungicides were evaluated in vitro by poisoned food technique (Shervelle, 1979) at three concentrations of 1000, 500 and 100 ppm. Required concentration of each fungicide based on commercial formulations was prepared in sterilised distilled water and added to an autoclaved PDA to obtain desired dilutions. For each concentration, separate control was maintained by adding required
amount of sterile distilled water to the medium. The culture plates with PDA media, mixed with known concentration of test fungicide was inoculated with 5 mm culture bit from seven days old actively growing single spore culture of *A. alternata*. Five replications were maintained. The culture plates were incubated at 28 ± 2 °C temperature and diameter of mycelial colony was recorded at 7th day after inoculation. Extent of inhibition of mycelial growth by each fungicide was calculated by estimating the per cent reduction in mean growth over that of pathogen in control, which served as the criterion for identifying the potentiality of a fungicide at a particular dosage. The data was subjected for statistical analysis.

The fungicides were also tested for their efficacy in inhibiting the conidial germination at three concentrations, 1000, 500 and 100 ppm, using test tube dilution method (Grover, 1983). Paired drops of spore suspension in fungicide solutions were kept on glass slides in humid chamber and incubated at 28 ± 2 °C for 24 hours. Five replications were maintained for each treatment along with suitable controls. The extent of conidial germination was measured after 24 hours by counting 100 spores in each treatment.

The fungicides found promising in the above tests were further evaluated under field conditions.

(b) Evaluation of chemical fungicides and determining effective spray schedules for disease management

The promising fungicides from *in vitro* studies were further evaluated for their efficacy in controlling brown spot disease under field conditions in a 'hot-spot' area. Three trials were conducted at Sollepura farm of CTRI Research Station, Hunsur, to identify most effective chemicals and their spray schedules. Under Chemical control trial-I, fungicides selected from *in vitro* studies were evaluated and promising ones are identified. In trial-II, promising fungicides of Trial-I were further evaluated for their efficacy under two different leaf harvesting schedules i.e., timely and delayed. Based on the results of first two trials, a third trial (Chemical control trial-III) was taken up to identify the effective spray schedule for the promising fungicides.
Chemical control trial I - Field evaluation of fungicides:

This trial was conducted in a randomised block design with susceptible variety, Bhavya, for two seasons at Sollepura farm to identify effective chemicals for the control of brown spot disease under field conditions. 13 treatments were replicated thrice and each plot contained four rows with 12 plants in each row. Two side rows and the end plants of two central rows were considered as guards. Two central rows except guards received spraying in each plot. The treatments consisted of 12 fungicides viz., Baycor 25 EC (bitertanol), Bayleton 25 WP (triadimefon), Beam 75 WP (thiobendazole), Cuman-L 27 SC (ziram), Foltaf 80 DS (captafol), Indofil M.45 75 WP (mancozeab), Punch 40 EC (flusilazole), Raxil 25 WP (tebuconazole), Ridomil MZ 72 WP (metalaxyl + mancozeb), Score 25 EC (difenoconazole), Thiram 75 WP (thiram) and Tilt 25 EC (propiconazole) and an unsprayed control.

Each fungicide was sprayed six times, at six days interval, with first spray commencing at 60 DAT. Subsequent sprays were given soon after each harvest. At each harvest, leaves from two central rows except guard plants were assessed for disease. Efficacy of fungicides was judged based on the extent of reduction in PDI and leaves with severe damage as compared to the untreated check. Yield parameters like green leaf, cured leaf and bright grade were recorded. Loss in yield and quality due to the disease in untreated control plots was also assessed by comparing with effective chemical control treatments.

Chemical control Trial II - Effect of harvesting period and fungicides:

Of the 12 fungicides tested in Trial-I, four promising ones viz., Foltaf, Indofil M 45, Score and Tilt were selected for assessing their performance in two different leaf harvest situations i.e., under timely harvest, a recommended practice, and under delayed harvest condition, a commonly observed trend in the farmers' fields. Though harvesting commenced at 60 DAT in both the situations, under timely harvest, leaves were harvested at right maturity level, mostly at 6 days interval whereas under delayed harvest, the reaping of leaves was delayed by two to three days resulting in an interval of eight to nine days between two harvests.
The trial was conducted for three seasons with variety, Bhavya, in a randomised block design. A total of 10 treatments i.e., four chemicals + one unsprayed control, with one set under timely harvest and another set under delayed harvest conditions, were replicated three times. Each plot contained 48 plants, 12 each in four rows. Two side rows and the end plants of two central rows were considered as guards as in Trial I. Fungicides were sprayed five times at seven days interval, commencing the first spray at 60 DAT. Two central rows except guards received spraying in each plot. At each harvest, leaves from these plants were assessed for disease parameters. Yield and quality parameters were also recorded as mentioned above and compared with that of control. Rate of infection (r) was calculated for each treatment using Vanderplank’s formula (1963) as described earlier. Economic efficacy of the fungicides under two harvest conditions was calculated by comparing the fungicidal treatments with respective controls.

Chemical control trial III - Spray schedules:

Based on the results of first two trials on chemical control of brown spot disease, a third trial was attempted to identify effective spray schedule for the promising fungicides to control brown spot incidence and to study their influence on yield and quality of FCV tobacco.

Two systemic fungicides, Score and Tilt, and two protectant fungicides, Foltiaf and Indofil M 45, which were found promising, were tested along with control (unsprayed). The trial was done with FCV tobacco variety, Bhavya, at Sollepura farm for two seasons. Each fungicide was tested under four spray schedules designated as A, B, C & D, where A - six sprays were given soon after each harvest, B - five sprays at weekly intervals, C - four sprays at 10 days intervals, D - five sprays, three at 60, 70 and 78 DAT and the remaining two soon after subsequent harvests at six days interval. In all the four schedules, first fungicidal spray was given at 60 DAT. So, a total of 17 treatments i.e., 16 fungicidal treatments (four fungicides each under four different spray schedules) and a control were accommodated in a randomised block design. Each plot contained 48 plants, 12 each in four rows. Two side rows and the end plants of two central rows were considered as guards as in the previous experiments. Two central rows except guards
received spraying in each plot according to corresponding spray schedule. Leaves were harvested simultaneously from all the treatments under different schedules as soon as they ripened generally at 6 days interval starting from 60 DAT. At each harvest leaves were assessed for disease parameters. Yield and quality parameters were also recorded and compared with that of control like previous trials. Economic efficacy of fungicides under different spray schedules was also calculated.

(c) Evaluation of tobacco germplasm for identification of resistant donors and studies on disease inheritance

(i) Germplasm evaluation

A total of 375 lines of *Nicotiana tabacum* were screened against brown spot pathogen in a ‘hot-spot’ at Sollepura farm of CTRI Research Station, Hunsur. 20 plants of each ‘accession’ were planted in two rows and were flanked from all sides by Bhavya (susceptible check). Brown spot disease observations were made on five randomly selected plants. All the recommended package of practices were followed strictly except that, the planting was delayed and the plants were not topped or suckered and the leaves were not harvested to ensure high disease pressure. Natural infection by *A. alternata* was sufficient enough to allow a visual rating of brown spot intensity. Each entry was rated on a standardised scale of zero (no symptoms) to seven (leaves having more than 80 per cent area spotted) as described earlier. PDI was calculated for each entry and compared with that of Bhavya. The entries were classified according to their reaction to the brown spot disease as compared to susceptible check, Bhavya. The lines which recorded more than 75% decrease in PDI over Bhavya were classified as resistant, lines which recorded 50 to 75% decrease in PDI as moderately resistant, lines which recorded 25 to 50% decrease in PDI moderately susceptible and lines which recorded less than 25% decrease in PDI as susceptible.
(ii) Brown spot inheritance studies

An attempt has been made to study genetic architecture involved in the inheritance of brown spot resistance in tobacco. Beinhart 1000-1, a cigar filler tobacco selection from the Dominican Republic, which is confirmed to be having brown spot resistance during the investigation, was crossed with Bhavya, a popular FCV tobacco variety grown in Karnataka, which is highly susceptible to brown spot disease. Crosses were made over a period of two years from 1996-97 to 1997-98, both during season (Kharif) and off-season to obtain reciprocal F1 and F2 crosses as well as four back crosses. These populations were studied during 1998-99 Kharif season along with the parents for their reaction to brown spot disease in a randomised block design with three replications under epiphytotic conditions in field at Sollepura farm, a ‘hot-spot’ for brown spot disease. Every block contained two rows of 50 plants of each test material and the experimental block was surrounded from all sides by susceptible check, Bhavya, to ensure evenness of disease spread. All recommended package of practices were followed (Shenoi, 1998), except that, the plants were not topped or suckered and the leaves were not harvested to ensure high disease pressure.

Natural infection by A. alternata was sufficient to allow a visual rating of brown spot severity. The disease severity was recorded when it reached epiphytotic levels in the susceptible check line, Bhavya, at around 80 DAT. A standard 0 to 7 scale described earlier was used for scoring disease severity. These ratings were assigned to each leaf in each plant. After inspecting all the leaves of a plant, the PDI was calculated using the formula of Wheeler (1969) as described earlier.

All the plants of each entry in each replication were categorised as resistant (PDI less than 15) or susceptible (PDI above 15). The data were subjected to X² test to determine the goodness of fit of the observed ratios.