STUDIES ON THE BIOLOGY OF BROWN BLIGHT DISEASE OF TEA, *CAMELLIA SINENSIS* (L.) O. KUNTZE WITH SPECIAL REFERENCE TO THE FACTORS AFFECTING ITS INCIDENCE

THESIS SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN SCIENCE (BOTANY)
OF THE
UNIVERSITY OF NORTH BENGAL

Library

Shraboni Datta, M.Sc.

DEPARTMENT OF BOTANY
UNIVERSITY OF NORTH BENGAL
2000

ST - VERI

Ref
581.2326
D 2325

STOCK TAKING - 2011

140420
16 MAR 2011

UNIVERSITY OF NORTH BENGAL

Department of Botany

Dr. Usha Chakraborty
M.Sc. (Gold), Ph.D., F.P.S.I.
Reader

P.O. N.B.U. - 734430, Siliguri, India
✆ (0353) 450411(O) ; 450347 (R)
Fax : (0353) 450546 ; 450593

April 5, 2000

This is to certify that Ms Shraboni Datta has carried out her research work under my supervision. Her thesis entitled "Studies on the biology of brown blight disease of tea, *Camellia sinensis* (L.)O.Kuntze with special reference to the factors affecting its incidence", is based on her original work and is being submitted for the award of Doctor of Philosophy (Science) degree in Botany in accordance with the rules and regulations of the University of North Bengal.

Usha Chakraborty

(Usha Chakraborty)

Usha Chakraborty
Reader
Department of Botany
University of North Bengal

Acknowledgement

First and foremost I would like to express my enormous gratitude to my supervisor, Dr.(Mrs) Usha Chakraborty, Department of Botany, North Bengal University, for her guidance, kind and valuable suggestions and discussion throughout my work.

I would also like to acknowledge my great thanks to Prof. B.N. Chakraborty, Department of Botany, North Bengal University for his valuable guidance all the time.

I thankfully acknowledge the help of the Head of Department of Botany, North Bengal University and other teachers of this Department.

I also want to thank my friends Dr. A.Saha, Dr.G.Das, Dr. S. Das, Dr. P. Basu, Dr.R. Das, Dr B.Sarkar, Ms. S.Dutta, Ms D.Sarker, Mr D. Deb, Ms. J. Das for their encouragement. I also record my thanks to Mr.Anil Sarki, Fieldmean for his help at various times.

My special thanks also goes to Mr. Talukdar and Mr. S.K. Pathak of Tea Research Association, Bengdubi.

My sincere thanks goes to Mr. A.N. Banerjee, and Mr. D. Pradhan of Hansqua Tea Garden, R. D. Tea Limited, for their help and co-operation to my research work in the field.

My special thanks goes to Prof. Urmila Chakraborty, North Bengal University and Mr. Amitava Roy for their invaluable contribution and moral support.

I would like to thank Mr. K. Deb and Mr. B. Biswas for providing of computers and composing the matters of this thesis.

My special gratitude goes to my husband Subir Datta, who shared every moment of my research work, and my daughters Sujata and Subhadra who co-operated with me at all times.

Finally I would like to express my gratitude to my parents and parents in-law for their great moral support and blessings.

Shraboni Datta

Contents

Introduction

Tea, *Camellia sinensis* (L) O Kuntze is one of the most important plantation crops of India and contributes significantly and substantially to the national economy. Its cultivation is spread over more than 3,96,000 hectares of land, divided into two distinct regions - the North Indian tea belt located between 22 - 27^0 N and the South Indian tea belt, located at 7^{0}N. North-East India produces 75% of the total Indian tea in three different landscapes (Jain, 1991) - the hilly terrain of Darjeeling upto an elevation of 2000 m, yielding the world's finest quality teas; the extensive riverine flat plains at the base of Himalayan ranges - the Terai and Dooars; and the Brahmaputra valley of Assam located at 100 m above sea level which is the largest flat plains of the world and which accounts for more than half of Indian production. With great emphasis being given to increase productivity in tea, more and more non-conventional areas are being brought under tea cultivation, coupled with replanting old sites with vigorous and highly productive tea (Banerjee, 1993).

The young leaves and unopened buds of the tea plant are plucked for beverage production. Foliar diseases of tea cause enormous losses as they reduce the quality and quantity of tea productin. Among the many foliar fungal diseases of tea, brown blight, caused by *Glomerella cingulata* (Stoneman) Spauld & Schrenk is common in all tea growing areas. The fungus generally gains entrance through a wound or into tissues that in some way have been weakened (Baxter, 1974; Bertus, 1974; Dickens and Cook, 1989). The disease patches usually start on the margin of the leaves and spread inwards. When two or more patches occur side by side, the whole leaf may be affected. The edges of the patches are sharply defined and mostly marked with a delicate concentric zonation. The colour of the upper surface is yellowish to chocolate browh at first, gradually changing to grey from centre outwards. Minute black, scattered dots which are the fructifications, appear on both sides of the diseased patch. The affected portion of the leaf finally turns over and shrivels up.

Environment plays an important role in regulating a plant's growth and development. Plant responses to the environment are normally expressed by changes in total dry matter production and harvestable yield (Ng'etich, 1997). Climatic changes also affect plant disease production, but research on impacts of climate change on plant disease has been limited. Most of the research work has concentrated on the effects of a single atmospheric constituent or metereological variable on the host, pathogen, or the interaction of the two under controlled conditions. Climate changes could alter stages and rates of

development of the pathogen, modify host resistance, and result in changes in the physiology of host pathogen interactions (Coakley *et al;* 1999).

Tea, being a perennial, is subjected to a large number of environmental stresses throughout the course of its life, the most important being the climatic variables. The main climatic variables influencing the yield of tea are temperature, the saturation vapour pressure deficit of the air and, through their influence on plant and soil water deficits, rainfall, evotranspiration (Ng'etich, 1997). Along with the influence of climatic variables on the growth and development of tea, it is also expected that they will also influence the development of various diseases. Since no previous work on the role of environmental factors on brown blight disease development of tea has been reported, the present work has been undertaken with the following major objectives :

(i) to determine pathogenicity of *Glomerella cingulata* on different tea varieties; to evaluate the effect of various factors on disease development following artificial inoculation;

(ii) to record the occurrence of brown blight disease of different tea varieties under natural conditions;

(iii) to correlate disease occurrence with various climatic variables- temperature, rainfall, humidity, hours of sunshine, etc;

(iv) to determine the effect of different factors on mycelial growth and spore germination of *G. cingulata;*

(v) to determine changes in phenolic contents of tea leaves of different tea leaves following inoculation with *G. cingulata;*

(vi) to determine the effect of different factors on production of phenols;

(vii) to quantify diffusible phenolics from susceptible and resistant varieties;

(viii) to determine the changes in chlorophyll contents of tea leaves as affected by age of leaves, varietal differences, infection;

(ix) to determine the epicticular wax contents of resistant and susceptible varieties; and

(xi) to study anatomical characteristics of leaves of different tea varieties.

The materials used and methods adopted to achieve the above objectives have been outlined in the following pages along with the results achieved. In the beginning, a review of literature along lines related to the work has also been presented.

Literature Review

The establishment of disease in any plant is influenced by a large number of factors and is the final outcome of a series of complex interactions. The factors generally involved are host factors, pathogen factors as well as environmental factors and the interaction among these. Considering the importance of environmental factors on disease development, which again is also dependent on the response of the host and pathogen, there have been a large number of studies in different plants. Previous attempts have been made to understand the role of environmental factors on pathogen development in the host leading to production of disease.

In the following pages, a short, selective review pertaining to the present study has been presented. The review has been divided into three parts : (a) Influence of environmental factors on disease development; (b) Facrors affecting fungal growth *in vitro;* (c) Biochemical changes in plants associated with infection.

2.1 Influence of environmental factors on disease development

Prakash and Jhooty (1987), reported that conidia of powdery mildew of ber initiated germination and appressoria formation after 2 and 4 hours, respectively at 20 ± 2^0C in moist saturated atmosphere. Sporulation started 96 hours after incubation on susceptible ber leaves. Cardinal temperatures for powdery mildew development were below 10, 20 and 30^{0}C. Relative humidity of 32% and above favoured development of powdery mildew. Temperature had negative significant correlation, whereas relative humidity had positive non significant correlation with disease.

Ragazzi *et al.* (1987) carried out a series of observations on olive tree orchards (cv Moraiolo) in the countryside around Florence. The trees were infected by *Verticillium dahliae.* The object of this research was to contribute to the knowledge of *V. dahliae* epidemiology in Tuscany. The authors found that it was possible to isolate the fungus all over the year, but this possibility decreased from summer to winter. They observed no correlation with climatic data. A hypothesis to verify was that the mycelium of *V. dahliae* can be active for a part of the year. For the alternation of period in which it was more or less easy to isolate the fungus, it could be a situation in which the fungus can be successfully fought.

The incubation period of *Puccinia arachidis* in cultivars SB-XI of groundnut under field conditions was gradually influenced by temperature and relative humidity (R.H). It was prolonged as the mean or maximum temperature increased with which the relative humidity was negatively correlated. Multiple regression analysis of different environmental combinations including rainfall, evaporation rates explained more than 96% of the observed variation in incubation period (Mayee and Kokate, 1987). Leaf rust was reported commonly to occur during 86 and 87 but was most severe on winter wheat in the orange free state. Seven races were characterized during both years. Pathotypes 35AI 34 (avirulence / virulence formula) Lr 39, 3bg, 3ka, 10, 11, 16, 20, 24, 26, 30/ 1, 2a, 2b, 2c, 14a, 15, 17 and 35A 140 (Lr 3a, 3bg, 3ka, 29, 2b, 2c, 10, 14, 15, 17, 24, 26) were identified for the first time -35A 13u in 1986 and 35 AI140 in 1987. In south Africa, virulence to Lr 26 has not previously been recorded. Pathotype 35A 132 (virulent to Lri, 2a, 2b, 2c, 10, 14a, 15, 17, 24) was isolated most frequently during both years - 44.1% in 1986 and 34.2% in 1987; Pretorius and Leroux, 1988). Biggs and Northover (1988) reported that nonwounded peach and sweet cherry [*P. avium*] fruits were inoculated with a conidial suspension of *Monilinia fructicola* at temperature of 15-30^{0}C at 2.5^0 intervals, and wetness duration of 3-15 hr (peach) or 6-18 hr (cherry) at 3hr. intervals. Both peach and cherry had an increased incidence of fruit infection with increased wetness duration over the range of temperature tested. Optimum observed temperature for cherry fruit infection was 20-22.5^{0}C with greater than 80% infection of 15 hrs of wetness. After 18 hrs of wetness infection was greater than 80% at all temperature, except 30^{0}C. Optimum observed temperature for infection of peach fruit was 22.5-25^{0}C. Greater than 70% infection occurred after 12 hr, at all remperatures except 27.5^{0}C and 30^{0}C. Nontransformed polynomial and logistic equations for peach and cherry respectively were chosen as the best regression models to describe the incidence of fruit infection as function of temperature and wetness duration. Coefficients of determination for 2 sweet cherry and 2 peach experiment were .89 and .90 and 0.71 and 0.84 respectively.

The effect of temperature on colonization of maize (*Zea mays*) and the subsequent invasion of kernels by *A. flavus* was studied in controlled environments. At the post-inoculation temperature regimes of 34^{0}C / 30^{0}C (34^{0}Cx9hr, 30^{0}C x 15hr) and 26/22^{0}C, as many as 28 and 2.4% of the kernels respectively, were infected. Infected kernels were present in all areas of the ear and neither temperature nor time of incubation, affected the location of

infected kernels on the ear. At 34/30^{0}C the fungus entered the ear tip in one day and was present in the base by 4 days. Internal infection of the ear did not occur until 8 days after inoculation and the percentage of infection of kernels increased greatly between 28-32 days, when kernels moisture was < 32% (Payne *et al.* 1988). Epidemiological study of 2 fungus diseases *Puccinia digitariae* Pole Evans (a rust) on *Digitaria criantha* and *Phyllactiara paspalicola* on *D. maximum* jacq, ws undertaken by Rey and Garnett (1988). Both incidence (% leaves infected purtuft) and severity (% leaf area infected) were recorded throughout the growing season in 1978/79 and 1979 / 80. Disease progress curve revealed growth on both rust tar spot through out the season with the maximum incidence (I) of rust reaching 59% in both season. Severity of incidence (S.I.) of rust attained a maximum of 0.24 in March 1980 and 0.14 in December 1978. The highest tarspot occurred in February 1979. The relationship between (I) and (S.I.) were also determined. The rust on *Digitaria criantha* appeared to increase relatively more by (I) than (S.I) in 1978/79 and early month of 1979/80 and later stage of disease development in 1980. Tarspot increased more by I relative to S.I. in 1978/79 but conversely in 1979/80. Comparison between disease progress curves of the 2 disease and between and seasons have also been presented. The influence of selected macro cimatic parameters on disease development is discussed.

Chee (1988), reported that *Corynespora cassiicola* infected leaves of *Hevea* rubber and caused leaf fall. Leaves were most susceptible to infection for upto 4 weeks. *Hevea* clones differed in their degree of susceptibility, but immunity was common. Infection of leaf in the laboratory was correlated with field susceptibility. 137 clones were screened for resistance in both laboratory and field test. Conidia from RRIC 103-the most susceptible clone were particularly aggressive. None of the several hosts tested was infected by the isolates from *Hevea. C. cassiicola* released its spores from 08:00 and attained a peak around noon. It gradually fell to a very low level in the evening and remained low throughout the early hours of the morning. There was no clear cut seasonal pattern of spore rate in relation to rainfall. The results showed that in the region of Nevesinje, Gacko, Duvno and kupres had possible favourable conditions for very intensive development. In vegetation period the temperature were 12^{0}C-16^{0}C and rainfall was intensive with development of *Puccinia graminis tritici* at temperature of 16^{0}C and development of *Erysiphe graminis tritici* on lower temperature. It proved to be a great flexible development on a spring wheat of *Erysiphe graminis tritici* (Boskovic 1988).

Infected plant debris of soyabean stem have been reported to have very important role in epidemiology of *Diaporthe phaseodorum* var. *caulivora.* The fungus formed in mass perithecia with asci and ascospores on infected plants debris of soyabean stem. During rainfall the vegetation determined the time of perithecia occurring and the dynamics of ascospores discharge. The formation of perithecia and the liberation of ascospores occurred in temperatural intervals from 10^0C-27^0C with optimum between 20^0C-25^0C. The ascospores germinated at temperatures from 10^0C-32^0C with optimum at 22.5^0C. The fungus overwintered in soyabean debris on soil surface and formed perithecia in these plants debris in spring most frequently at the end of May or in the first half of June. The beginning of ascospore discharged occurred 5-10 days after appearance of perithecial neck on the stem of soyabean. The ascospores were discharged successively during all vegetation periods usually after abundant rainfalls. The ascospores were oozed through opening of the neck where they formed mucous. The ascospores were deposited on plants by rain-drops and wind. The infection of soyabean plants took place directly through leaf blade, petioles or stem wounds (Vidic and Jasnic, 1988).

An integrated pest management experiment for the control of avocado root rot caused by *Phytophthora cinnamomi* was established in a commercial orchard at Allixco puebla (Mexico) in 1982. Avocado trees were severely pruned. The flooding irrigation system was modified to watering individual tree basins. Chemical fertilisers were applied and aerial disease were controlled. Experimental trees were tested with fresh bovine manure, alfafa hay. Metalaxyl and their combinations. Incidence in time and space of *Phytophthora cinnamomi* in the root system of avacado tree was evaluated in 1984-85. Dynamics of fungus incidence and root growth followed a modified logistic model. Maximum rate of infection occurred at the beginning of August and coincided with rainfall. Bovine manure had the lowest infection rate in time and depth. Highest incidence of root pathogen occurred in the control tree whose management was not modified (Mora *et al,* 1988) .

Chaudhury and Dhar (1988) reported that blast disease caused by *Pyricularia oryzae* is a serious problem of rice in Arunachal pradesh. Disease on foliage throughout appeared by end of April only, June was found to be the epiphytic period and there after foliage infection declined by its self. Flowering stage was found to influence the declining phenomenon. Bhargava and Khare (1988) correlated temperature and relative humidity index for the first time with disease build up. Initiation of foliage infection was recorded at 66 while. THI of 74

and above was responsible for epiphytic. Sowing chickpea on different dates did not significantly influence intensity of *Alternaria* blight. The pattern of disease development was similar in different planting dates. The disease was first seen at flowering stage when temperature were between 25-27^{0}C and the relative humidity around 80%. Particularly the periodic rain with consequent high relative humidity, favoured disease development. Maximum numbers of spores were trapped in field during the night. Environmental temperature influenced expression of red rot symptoms and its overall development in sugarcane. Under low temperature conditions, the susceptible cultivars behaved as intermediate and intermediate one as resistant. The age of sugarcane plant and high sugar content appeared to have no effect on red rot development in the internodal tissue. Under warm conditions, favourable to disease, the pathogen caused disease within 15 days of inoculation (Hiremath *et al.* 1988).

Mends and Filho (1989) reported monocylic parameters of been rust (*Uromyces phaseoli* var *typica*) was quantified in growth chamber on two been cultivars for three temperature (17,21, 25^{0}C), 2 types of leaves (uni and trifoliate leaves) and nine leaf wetness period (0,4,7,10,13,16,19,22 and 25 hrs). The expression of disease was greatly influence by post inoculation temperature. The incubation and latent periods were shorter at 21^{0}C for both cultivars and leaf types for both cultivars, trifoliate leaves were more succeptible than unifoliate leaves. A wetness period of at least 4 hours was required for disease to occur. The maximum disease efficiency for both cultivars, occurred with 22 hr. of leaf wetness at 17^{0}C. The disease efficiencies for temperature of 17-29^{0}C and leaf wetness period of 0-25 hrs were adequately described by a response surface model. Because of the great influence of temperature and leaf wetness on infection, bean rust is unlikely to occur at high temperature (>25^{0}C) and short leaf wetness period (<7 hours). Rolando *et al.*(1989) also reported that the level of infection by *Puccinia psidi* varied with temperature, leaf wetness period and photoperiod. The highest disease index occurred at 20^{0}C and 25^{0}C after 24 hours of free water on the leaf surface. At 20^{0}C, maximum infection was estimated at 31 h of leaf wetness; infection and uredospores germination (2% water agar) were inversely correlated with the length of light exposure during the nucleation phase. Periods of light exposure greater than shows in the initial stage of infection incubation uredospores germination and infection by *Puccinia psidi*. Uredospore and teliospore production at 20 and 25^{0}C was significantly greater than at 30^{0}C; at 15^{0}C no sporulation occurred. The optimum temperature for infection was estimated at approximately 23^{0}C.

Klavis (1989) compared 2 years results of plot experiments and gave a first plot idea of the mildew (*Erysiphe graminis* sp. *hordei*) infection behaviour of spring barley varieties with different overcome or infact resistance source in manufactured variety mixtures. The obvious relationship between infection of the mixture and its components turned out to be quite simple and constant during the limited test period. Components with overcome resistance seemed to influence only in a quantitative way with their specific susceptibility established in pure stands the infestation in mixture the overall level of components, retaining gradual varietal difference in susceptibility. The relationship between disease severity and the time of inoculum application was best described by cauchy distribution models. These models predict exponentially increasing disease incidence in plant exposed to inoculum from emergence to a maximum for those exposed to inoculum at 22 and 24 days (V_3 stage). Post planting plants exposed from the V_3 stage through the V_3 V_{10} stage developed progressively less disease. Even a highly susceptible cultivar would not develop disease when exposed after the vegetative stage (Smith and Backman, 1989). Jang *et al.* (1989) observed that the results of spur ear blaze was more sensitively affected than fungi by increasing nitrogen concentration in stand culture. The optimum temperature for proliferation of *Alternaria* leaf spot was about 30^{0}C and the infection was more expanded to lower leaves as humidity increased. As calcium concentration increased, disease occurrence showed a decreasing trend in spur earl blaze, while potassium and magnesium affected positively in fungi variety. The severity of the south American leaf blight (*Microcyclus ulecii*)was quantified under field conditions during 1 year in Ponte nova-MG. A hygrothermograph in the experimental area was placed in order to register the relative humidity of the air and the air temperature. Also an aspergigraph was set to monitor free water on the leaf surface and a pluviometer to monitor rainfall. Disease severity was not significantly correlated with the total rainfall nor with the average or maximum temperature of the period but, it was positively correlated with free water on the leaf surface, relative humidity $\geq$90% and minimum average temperature. A negative correlation was found with periods of temperature $\leq$ 20^{0}C (Gasparotto *et al,* 1989).

Temperature and duration of wetness period during the inoculation period influenced the development of shot hole disease on almond leaves caused by *Wilsonomyces carpophilus (Stigmina carpophila).* In control environment studies, a 14 hr wetness period resulted in 0.1 and 45.0 lesion per leaf after 10

days at 8°C and 22°C, respectively. Extended wetness periods during the infection period increased the number of lesion per leaf regardless of the temperature, Number of lesion increased as temperature was increased from 8° to 22°C for wetness period greater than 12hr. At 8°C, 4.7 and 45.6 lesion per leaf developed after wetness period of 30 and 48hr. respectively, and at 15°C, 110.0 lesion developed at 15°C. After a wetness period of 28hr. temperature after the infection period influenced symptoms expression, the rate of lesion development, and lesion abscission, but not the number of lesion formed. Lesion abscission, or shot hole, was significantly higher at 22°C (70.0% of lesion showing abscission) than at 8°C (0.3%) and 15°C (3.9%). Additional wetness period after an initial infection period resulted in a greater number of lesion and a higher disease index after 8 days at 8,15 and 22°C (Shaw *et al.* 1990). Infection of wheat seedlings by *Puccinia striiformis,* f. sp. *tritici* was investigated under both laboratory (constant temperature) and field conditions using 15 hours period of 100% relative humidity (Park, 1990). In laboratory studies infection decreased from 100% at 15.4°C to 0.8% at 20.5°C, and it was estimated that no infection would occur at or above 20.8±0.2°C. In contrast, high levels of infection occurred under field conditions even when temperature fluctuated within the range 19-30°C. An experiment was carried out under controlled conditions by using growth chambers at different temperatures .Fourty days old soybean plants (Parana Cultivar) were inoculated by using a uredospore suspension of *Phakospora pachyrhizi*; after inoculation plants were kept in the wet chamber at 12, 16, 20, 24 and 28°C for different period of leaf surface wetness (0,3,6,12 and 24 hours). After that leaves were artifically dried and returned to the same temperature conditions without any leaves wetness period. Average number of lesion / cm^2 and latent period of infection were determined. A multiple regression equation was estimated for the effect of the binomial temperature (T) length of leaf surface wetness period (DMF) on the number of lesion / cm^2 of leaf area. The estimate equation was $Y= -115.17 + 12.549\ T-0.307T^2 + 1{\cdot}49\ DMF - 0{\cdot}045\ DMF^2$ reported (Valefx *et al,* 1990).

Isotopes of *Cladosporium allii* and *Cladosporium allii cepae* grew at 20°C and 15-20°C respectively on 2·5% malt extract agar and both species tolerated wide pH range with maximum sporulation of *Cladosporium allii cepae* occurred between pH 5-8·5. *Cladosporium allii* did not sporulate in the light in culture and grew more rapidly in darkness. At least 8 hours darkness was necessary for optimum conditions of both fungi on lesion of leck but

conidiophores were produced in light and dark conditions after 2-12 hours incubation at >90% relative humidity. Radiation of $2W/m^2$ and $3W/m^2$ (the approximate equivalent to strong moon light) prevented conidiation of *C. allii* and *C. allii cepae* respectively. Relative humidity of 95-100% increased sporulation of both fungi and leaf lesion. Laboratory and field experiments to measure the effect of temperature, light and relative humidity on sporulation showed that maximum sporulation of both pathogens ond leaf lesion occurred in the late autumn (Nov-Dec) and secondary peak in the early spring (March-April). The pattern of spore release for both fungi was similar with maximum and minimum concentration of conidia recorded during late morning or early afternoon (Jordan *et al.* 1990).

The development of sheath blight caused by *Rhizoctonia solani* Kuhn under different culture conditions was studied. Plants appeared to be most susceptible at panicle initiation booting stage with more severity in symptoms and extent of damage during the Aus (March-July) season. The rate of lesion length development was high during the 3days immediately after initiation of infection and it decreased with time after infection. Varieties differed in lesion length, developing higher in susceptible than in moderately resistant cultivars. (Shahjahan *et al.* 1990). Quantification of disease progress and the effect of rainfall and temperature variable on *Phytophthora* blight of pepper [*Capsicum annum*] caused by *Phytophthora capsici* was carried out by Bowers *et al.* (1990). From point source of inoculum (diseased plants) the incidence of disease was observed to speread outwards over time to the central, primary foci. Disease progress was observed to be influenced by rainfall and movement of water soil and plastic mulch. Path coefficient analysis was conducted to determine which rainfall variables and relatively large, direct or indirect effects on the incidence and the rate of disease progress without the confounding influence of multicolinearity. The cumulative amount of rainfall had the largest absolute direct effect on one trial. The cumulative number of days with rainfall, the cumulative daily average temperature and chronological time had far lesser effects indicating their lack of influence on disease progress. After inoculation of winter wheat (v. longbow) a single time, lesion *Mycosphaerella graminicola* were produced over long intervals starting 15-35 days after inoculation dependent on temperature. There were no evidence that a single infection gave rise to more than one lesion. After the initial infection period at 100% relative humidity keeping leaves wet for 10 hours / day did not shorten latent period on seedling. Experiments in controlled enviroment chambers

demonstrated a minimum latent period at approximately 17°C. Variation in the latent period of individual lesion was also minimum at this temperature the latent period varied according to the cultivars tested.cv. longbow having the shortest cv. Avalon having almost the longest field observation broadly confirmed the results of experiments in constant environment (Shaw, 1990). Singh and Pande (1991) investigated the exact correlation between weather conditions and the incidence of *Pyricularia* spore in the air and an incidence of leaf spot disease of bajra *(Pennisetum typhoides)* using conventional spore trap. The maximum concentration of *Pyricularia* spore in the air and the incidence of disease at boot leaf stage of crop coincided with seed forming stage of the crop. They suggested that this clearly indicated an impact of environment parameters on the incidence of leaf spot disease as compared to the crop growth stage. Kapoor *et al.* (1991) reported that ascospores of *Sclerotina sclerotiorum* are disseminated by air currents and initiate infection of cabbage seed plants. The leaf scar left behind on the stump region owing to the detachment of senile leaves and blossom are preferential sites of infection by ascospores however, maximum infection occurred during flowering stage. The infection restricted to the above ground parts and on soil level.

The influence of cultural factors on mildew *(Erysiphe graminis)* development was analysed and quantified on the basis of data collected by state run plant protection institution. Mildew development was promoted by water saturated soils and sheltered locations preceding crops oats rape, potato legumes (only influence on the early infestation) ,vegetables (only influence on early infestation), plant emergence not later them 30 September and nitrogen fertilization exceeding 140kg / ha (exception loess soil). Mildews development is inhibited by alluvial soil preceding crops-winter barly, spring barley, legume (only influence on late infestation), plant emergence later than 1st October, nitrogen fertilization less than 80kg / ha (on less soil more than 140kg/ ha) plant density less than 400 ear bearing culms/ m^2 (Eberhard, 1991). Formation of primary, secondary, tertiary hypae, mycelial growth and production of conidiophores and conidia of *Uncinula nector* were very rapid at 25°C followed by 20°C and 30°C. There was no powdery mildew development at 35°C and 40°C. Some conidia successfully established host parasite relationship even at a low percent relative humidity, but the extremely low r.h. adversely affected the growth of the pathogen on the host, higher humidity levels but below saturated atmosphere seemed to favour the development of primary, secondary and tertiary hyphae of the pathogen (Munshi and Singh, 1999).

Chakraborty *et al.* (1995) studied the factors associated with conidial germination and appressoria formation of *Glomerella cingulata* causing the brown blight disease of tea (*Camellia sinensis)*. Maximum spore germination and appressoria formation were evident at 24 hours incubation period. At a concentration of conidia - $1x10^6$ / ml, 10 days old culture, *G. cingulata* exhibited maximum germination and appressoria formation. A maximum production of lesion was also evident on detached tea leaves at this spore concentration and in diffuse light. Diffusates of a phenolic mature collected from tea varieties, susceptible and resistant to *G. cingulata* inhibited spore germination and appressoria formation. Diffusates from resistant varieties were more fungi toxic than those from susceptible varieties. Some phenolics known to be present in tea leaves, when tested *in vitro,* exhibited varying degrees of fungitoxicity. Pyrogallol totally inhibited spore germination, while pyrocatechol and phloroglucinol completely inhibited appressoria formation.

Detailed studies were undertaken an various epidemiological factors affecting white rust disease development of mustard by *Albugo candida* (Kumar *et al.* 1995). Older leaves were more susceptible than the younger leaves. Older leaves showed symptoms 4 days after inoculation with disease intensity of 11.2% as compared with younger leaves which showed symptoms 6 days after inoculation with disease intensity of 5.7%. On lower surface the symptoms appeared 3 days after inoculation while on upper surface it appeared 5 days after. Panwar *et al.* (1995), reported that under Haryana conditions ergot appeared during 2nd week of September, when mean temperature was 27.9°C, r.h. about 89%. As the mean temperature increased (above 30°C) ergot did not progress inspite of high r.h.. When mean temperature was about 25°C and r. h. about 75% it progressed significantly and high disease intensity was recorded. Mayee (1995) reported that infection rate of ground nut rust reaches 0·20 to 0·35 unit per day during August to October which is governed by temperature of 22°C to 25°C, infrequent rain not exceeding 100 mm in a week and r.h. above 80%. (Sidhartha *et al.* 1995) reported that the epidemiological studies taken upon karnal bunt (*Neovossia indica*) of wheat revealed that the density and the viability of teliospores were reduced with the increase of soil depth. Effect of snowing and thawing also showed that chilling reduced viability of teliospores and prolonged the germination period. Therefore the chance of disease occurrence where snowing occurs are very rare. The diurnal periodicity in release of secondary sporidia was observed which showed that maximum sporidia were released between morning hours 2·0 - 6·0 AM under high r.h.

and leaf wetness. The sporidial release was negatively correlated with solar radiation and wind speed. The sporidial count was more at lower heights during last week of February 1st week of March, which coincides with the anthesis period of crop. Low temperature (15^{0}C) before inoculation predisposed the host to infection, whereas an optimum post inoculation temperature (18^{0}C) was favourable for spread of disease. Moses (1995) observed that bararas and gola the cultivated varieties of ber (*Zizyphus mauritiana* L.) were affected by mouldy leaf spot (*Psariopsis india* var. *zizyphi*) and powdery mildew (*Oidium* sp.). Mouldy leaf spot appeared during Oct-Nov when the temperature were moderate with high r. h., rainfall and cloudiness. The disease had inverse correlation with temperature and positively correlated with r.h..The incidence of powdery mildew was correlated with moderate temperature and r.h. .The disease continued to appear in spite of regular pruning because of its survival on the collateral hosts. Figueroa *et al.* (1995) reported that the effects of temperature on the development of light leaf spot (*Pyrenopeziza brassicae)* on winter oil seed rape were investigated in controlled environment experiments. The proportion of conidia which germinated on leaves, the growth rate of germ tubes, the severity of light leaf spot and the production of conidia increased with increasing temperature from spot. Lesion decreased when temperature increased from 5-15^{0}C. The time to 50% germination of conidia and the inocubation and latent periods of light leaf spot lesion decreased when temperature increased from 5-15^{0}C. At 20^{0}C, however, light leaf spot severity and production of conidia were less and the incubation and latent periods were longer than at 15^{0}C. There were difference between *Pyrenopeziza brassicae* isolates and oilseed rape cultivars in the severity of light leaf spot, the production of conidia and the length of the incubation period but not in the length of the latent period. The responses to temperature for lesion severity and incubation and latent periods appeared to be approximately linear over the temperature range 5-15^{0}C and could be quantified using linear regression analysis. Web blight of moth bean (*Vigna acontifolius)* caused by *Thanatephorus cucumeris* (Fr) donk occurred through soil, seed and naturally infected collateral hosts. Secondary spread of disease was due to basidiospores and contact between diseased and healthy plant part. Plants got infected at any stage of growth but 30-60 days plants were highly susceptible. 25-28^{0}C temperature and 85% or above r.h. were favourable for disease development and basidiospore production (Dwivedi *et al.* 1995). The progressive development of powdery mildew (*Erysiphe polygoni* DC) on 33 mungbean (*Vigna radiata* L. wiltczet) and 18 urd bean (*V. mungo* L. Hepper) varieties was most rapid during winter

1991-92 and 92-93 when the average maximum temperature varied from 27·4 - 34·3°C, r.h. from 67·9% during morning and 12-38% during noon and wind velocity from 2·3-4·1 km/hr. . There was positive correlation between mildew severity and temperature and wind velocity in most of the-varieties. However the correlation with r.h. was negative and significant except few varieties. The pooled infection rate / unit / day on mung bean CV. N. 89047 and urdbean CV, AKU4 was less then 0·1 with disease score of 9 and 7 respectively as against highest infection rates of 0.323 (mungbean) and 0.317 (urdbean) on susceptible varieties (Thakur and Agrawal, 1995). The influence of weather factor on development of leaf spot of sunflower caused by *Alternaria alternata* was studied under field conditions during kharif 1990 and 1999. The most important weather factors favouring disease development were the temperature and relative humidity ranging from 27-29°C and 78-80% respectively, whereas rainfall did not affect the disease development because it was erratic and abnormal during both the years. The disease intensity was the highest in the last week of August in both years there after there was a gradual decline in disease severely (Kumar and Singh, 1996).

Botrytis allii colonies incubated at low temperature have been reported to produce larger conidia that germinate faster and give rise to long germtubes than those grown at room temperature. A comparative study was made on effect of conidia produced at 20°C and at 0 and 2°C on their pathogencity to artificially inoculated white onion bulbs and effect of conidial concentration ($5x10^3$ and $5x10^4$ conidia / ml) on disease incidence, lesion area, incubation and latent period during storage temperature and periods tested. Conidia produced at -2°C caused a higher disease incidence and larger areas of rot than those produced at higher temperatures, when the conidial producing duration of incubation on the bulb inoculated with $5x10^4$ conidia / ml was more (Bertolini and Tion, 1997). The pathogenic variability of the barley scald fungus *Rhynchosporium secalis* in Central Norway was examined by Salamati and Tronsmo (1997). The climate in this region was reported to be usually cold and wet during the growing season of spring barley and leaf blotch is prevalent causing significant yield losses. Forty two isolates of the fungus from naturally infected spring barley in 4 countries, were differentiated into 32 pathotypes by the standard differential set for *R. secalis*. All pathotypes were complex and virulent for 9 to 22 differentials. The cultivars *osiris* was resistant to all isolates tested. The cultivars (·1·8162, Hudson, Atlas·46 and C·1·3515 were resistant to the majority of the isolate. Several differentials

with various resistance genes were susceptible to up to 100% of the isolate. Isolates were derived from local cultivers with no known resistance genes suggesting that *Rhynchosporium secalis* in Central Norway are characterlized by the high degree of seemingly unnecessary pathogenicity.

The growth and sclerotial formation of *Sclerotinia* was found to be within the temperature range of 10-25^{0}C, optimum being at 20-25^{0}C. However a temperature range of 15-20^{0}C was found to be the most favourable for number and dry weight of sclerotia whereas size of sclerotia was maximum at 5^{0}C and progressively decreased with the increase in temperature. One or two months duration of preconditioning showed increased myceliogenic and carpogenic germination. Ascospores started germinating in 4 hours within a temperature range of 5-25^{0}C optimum being at 20^{0}C. Longer the incubation period better was the spore germination even at sub optimum temperature of 12^{0}C. Sclerotia of *S. sclerotiorum* were found to survive more than one year in soil, sclerotial germination and decomposition showed decreasing trend with increasing soil depth under wet humid conditions, whereas both were reverse in snow bound areas (Sharma and Kapoor, 1997).

Khare *et al.* (1997) reported that powdery mildew severity was positively correlated with maximum temperature in all the varieties while only in some varieties with minimum temperature. Non significant correlation between disease severity and r.h. was found in all varieties whereas some varieties showed positive and significant correlation with sunshine. The powdery mildew incidence reduced grain yield of a susceptible variety PS-16 significantly, compared with other varieties. This is because PS-16 got infected at the vegetative stage while other varieties showed symptoms at much later stage. Because of late infection the grain yield of the remaining varieties was not affected.

The incidence and progress of cucumber mosaic cucumo virus (CMV) in commercial brinjal, chilli and tomato crops around Tirupati, chittor district, Andrapradesh during 1992-94 kharif and rabi season ranged from 9-21% in the young crops (10-15 days after transplanting) and progressed upto 74-86% as the crop aged. There was no significant difference in the incidence and progress of CMV in the 3 crops between kharif and rabi season (Kiranmal *et al.* 1998).

Models of a banana bunchy top virus disease epidemic were developed to incorporate the 2 key features of an epidemic in a plantation in the Philippines-

140420 16 MAR 01

an exponential increase in disease over 10 years and a declining gradient of incidence from the outside edge of the plantation to the centre. A non spatial model consisted of 3 different equations to describe the numbers of latently infected and of infections plants in the plantation and the size of inoculum source out side the plantation. In a spatial model the outside portion of the plantation was divided into 8 block running parallel and to the out side edge. The dispersal gradient of the inoculum was assumed to be negative exponential. Analysis of the 2 models showed that for disease incidence to increase exponentially over time, the rate of disease progress could be dependent either on internal spread and growing rate (proportion of diseased plants removed and replaced per unit time) or on the rate of increase of external inoculum pressure (Smith *et al.* 1998).

Didymella rabici grew saprophytically on pieces of artificially and naturally infected chickpea stem debris under artificial incubation conditions and formed pseudothecia and pycnidia. The extent of growth was not significantly affected by temperature of incubation within the range 5-25^{0}C but was significantly reduced as relative humidity decreased from 100% to 86% when no growth occurred. Pseudothecia matured at 10^{0}C and constant 100% relative humidity or at 5 and 10^{0}C and alternating 100% / 34% relative humidity under these conditions. Pseudothecial maturations, assessed by a pseudothecia maturity index increased over time according to the logistic model. For temperature higher then 10^{0}C or relative humidity lower than 100% pseudothecia either did not form ascospores or ascospores did not mature and their content degenerated (Cortes *et al.* 1998). Gupta and Singh (1999) compared relationship of environmental factors with downy mildew development in pearl millet. Under dried climate % disease incidence (PDI) at 25 days after sowing (DAS) has positive correlation with relative humidity and albedo (%) and negative correlation with global radiation. At 70 DAS, PDI showed a negative correlation with global radiation, incoming short wave solar radiation net radiation, soil temperature and sunshine but positive with relative humidity. Maximum disease incidence was observed in the crop grown below Netlon shading net as it provided conducive environment for pathogen development on account of drastic reduction in global radiations (86.9%), incoming short wave solar, radiation (88.7d.), net radiation (77·7%), temperature of surface (12·7^{0}C) and subsoil (8·8^{0}C). As compared to open field relative humidity was also maximum (79·4%).

Mittal (1999) reported that, anthracnose caused by *Colletotrichum truncatum*

(Schwein) Andrus and Moore was the main disease observed during the study of leaf spot caused by *Cercospora canescens* Ellis and Martin and *C. cruenta* sace were also observed in all the tested years, but developed and spread late in the season. Roger *et al.* (1999 a) reported that the effects of temperature (5-30^{0}C) and the duration of moisture on the development of ascochyta blight (*Mycospha crella pinodes)* on pea seedling, grown under controlled conditions, were investigated. The optimum temperature for monocyclic processes was 20^{0}C. At the temperature pynidiospores germinated after 2 hours, appressoria formed after 6 hours and the germtube penetrated the leaf cuticle after 8 hours. Disease symptoms were evident after 1day of incubation and the 1st pycnidia formed after 3 days. Longer wetting periods were required for disease development and pycnidial formation at non optimum temperature. Disease severity and the number of pycnidia formed on leaves increased with temperature from 5to 20^{0}C then decreased between 20 and 30^{0}C, Roger *et al.* (1999 a) further reported that the effect of interrupted wet period on pycnidiospores of *Mycosphaerella pinodes* was studied by assessing spore viability, infection and disease development on pea seedlings. Pycnidiospores survived dry periods of upto 21 days after inoculation. Re wetting restored the infection capacity of the pycnidiospores resulting in high levels of disease. The effect of dry and wet cycles depended on when the dry period occurred during the infection process. No disease symptoms appeared when dry periods occurred during germination; a low level of disease occurred after rewetting in high relative humidity if the interruption of the wet period was long. (Roger *et al.* 1999 b).

Lukose *et al.* (1999) studied the effect of rainfall on development of downy mildew disease on different pearl millet lines. The percentage of lines with downy mildew disease on the 30th day of the crop increased with an increase in rainfall. The lines which recorded an increased percentage of disease during the period from 30 to 60 days after sowing were rainfall significantly less in the years of maximum and minimum rainfall. Lines with reduced percentage of disease and the lines in which the disease incidence remained constant were higher in the years of drought and higher rainfall. Suggesting that such conditions considerably reduced the secondary spread of disease. Significantly less percentage of lines with green ear in the year of higher rainfall established that the development of system infection was suppressed under such condition. Studies were made on the effect of the soil moisture, temperature, relatively humidity (r.h). and age of inoculum and host plant on charcoal rot development

in cowpea caused by *macrophomina phaseolina,*(Sandhu and Singh, 1999). They obtained that unfavourable soil moisture, low (40%) as well as high (100%), for sowing of cowpea caused maximum pre-emergence rot, but post emergence mortality of seedling was maximum only under moisture stress contition (40% soil moisture). The optimum temperature 35°C for growth of the pathogen also favoured the maximum disease development under artificial inoculatin. There was no positive correlation between soil moisture and r.h as regards their influence on seedling mortality. It was regards their influence on seedling mortality. It was found that younger the pathogenic culture, more aggressive it was in infecting the seedlings, while the susceptibility of the crop increased with the increasing plant age.

2.2. Factors affecting fungal growth *in vitro*

Singh *et al.*(1987) investigated the effects of temperature, r.h. and light on sporangial production of the Pearl millet downy mildew pathogen.The process of sporulation was completed in about 6 hours and high r.h. (95-100%) was essential only during the last 3 hours. Maximum sporulation occurred when infected leaves were incubated for 6-12 hours at 30°C prior to exposure to high r.h.

Rao *et al.* (1987) observed the effect of temperature on conidial germination and germtube growth of 6 *Peronosclerospora sorghi* isolates collected from maize in Arsikere' Bylakuppe. Dharwan Hyderabad ICRISAT kadur and Mysore. The isolates varied in their temperature requirements for both conidial germination and germtube growth. An isolate from Mysore showed increased conidial germination than isolates from other localities at all temperature. The varied temperature requirement of different isolates of the pathogen indicated the adaptation of the isolates to different environmental conditions. Penaranda *et al.* (1988) studied the effect of temperature upon the mycelial growth sporulation and the infection of *Phytopthora nicotianae var. parasitica* in tomato fruits. Between 16 and 36°C the growth reached the greatest values, laying the optimum near 28°C. The best sporulation was measured near 28°C and no infection was observed at 14,15 and 16°C, in the small plants inoculated. The fruits infection occurred between 20 and 32°C, lying the optimum near 28°C.

It has been reported by Dillard (1988) that the germination of conidia of *Colletotrichum coccoides* was greatest at 22°C after 24 hours. Conidia did not germinate at 7°C after 24 hours and <70% of the conidia germinated at 31°C.

Germination of conidia and growth from sclerotia were optimum at pH6. Water agar was osmotically adjusted using either Kcl, Nacl, $Cacl_2$ or sucrose, and maximum germination of conidia and growth from sclerotia occurred at the highest osmotic potentials (-510-isobars). Little or no germination of conidia occurred at -45 bars except when $Cacl_2$ was used to adjust the osmotic potential of the medium.

Rewal and Grewal (1989) recorded that minimum, optimum and maximum temperature for germination of conidia of all 3 strains of *Botrytis cinerea* were found to be 5, 20 and 30°C. Mycelial strain B_4 showed more then 50% germination at 10-30°C, sclerotia strain B_5 at 15-25°C and sporulating strain B_1 at 20-25°C. Maximum conidial germination of strain B_1 was recorded in continuous light, strain B_4 in alternating 12 hours of light / darkness and of strain B_5 in complete darkness. Strain B_1 needed 93-100% strain B_4 81-100% and strain B_5 75-100% r.h. for their conidial germination. The inhibitory effect of catechins on the spore germination of *Cochliobolus miyabenous* was studied for (-) - epicatechin (EC), (-) - epigallocatechin (EGC), (-) epicatechin-3 gallate (ECg) and (-)- epigallocatechin - 3- gallate (EGC3) by Mukai *et al* (1989). *C. miyabeanus* was inoculated on the leaves after spraying of EGCS solution (1000 ppm) on the leaves of rice plants grown in a greenhouse. Protective effects was measured by the number of disease spots on rice leaves. Activity of the synergistic effects of caffeine on spore germination was investigated using mixture having equivalent amount of caffeine of each EC.EGC. ECg and EGCg.

Piza and Ribeiro (1989) evaluated the effects of 5 temperature, 5 kinds of water and 7 incubation periods on germination of uredospores of *Puccinia psidii*. The data indicated that temperature within 15 and 18°C, dew chamber for atleast 6 hours and distilled water with sterilization were the best conditions for uredospores germination. Studies on *Septoria tritici* revealed that the sporulation in submurged culture and the use of molasses corn culture media in submurged culture made it possible to increase conidial yield in the medium by 7 times compared with potato sucrose medium. *S. tritici* inoculum obtained on submurged molasses corn culture medium was used for preparing infectious material (Davydov *et al.* 1989). Themts and Ogawa (1989) compared survival of mycelia and sporangiospores of *Mucor piriformis* (California isolate) [CA] and chile isolate [CH] at temperatures of 35-60°C. The thermal death point of the mycelia and sporangiaspores were 46° and 55°C respectively, for isolate (CA and 43°C and 52°C, respectively for isolate CH. After 2 days incubation

at 25°C, both isolates exhibited yeast like growth on agar medium; sporangiospore was erratic at 27°C and, when subsequently kept at 25°C, germtubes were abnormally swollen and produced no viable colonies. Reduction in viability was greater in wet than in dry sporangispores.

Nawal and Ho (1990) reported that mycelial growth of *Ganoderma boninense* (from *Elaeis guineensis*) from floating inoculum disks inoculum in flask swirled daily was significantly more abundant than that from submerged inoculum disk in static flask. The optimum temperatures for mycelial growth in both solid and liquid media showed an initial lag phase of little growth (1-3 days), then a phase of rapid growth (6-8 days) and finally a phase of autolysis and decline in dry weight. It has been reported that during growth of *Pythium sp.* the pH of the medium changed correspondingly with the changes in the growth pattern. As mycelial growth increased, the pH of medium decreased and when mycilial growth decreased during prolonged incubation pH of the medium increased, optimum pH for mycelial growth was between 3·7-5·8. Inqune and Takio (1990) reported that - MES [2-(N morpholiono) ethanesulfonic acid] had a superior buffering capacity for two species of *Pythium* although the phosphate buffer had relatively high capacity. Optimum pH range for mycelial growth by MES (50 mM) was considered at about 4·5 or a slightly higher value for *Pythium paddicum* and about 5·5 for *P. iwaymi*. High humidity and temperature at 20-25°C were most conducive to white mould of pea (*Pisum sativum L.*) caused by *Sclerotinia sclerotiorum* (Ub), which causes 10-70% losses in potential seed yields. Senescent flowers and other dead ends of the plants parts were the convenient invading foci for the pathogen to start the initial infection. Out of fungicides tried, bavistin followed by benlate and captan provided the best control by reducing the disease incidence and increasing the seed yields (Sharma and Munshi, 1990). The growth and sporulation of *Alternaria brassiceae* was found to be influenced by temperature, r.h., pH as well as exposure to light and darkness. The growth and the sporulation occurred at 5-30°C being greatest at 23°C, Alternate light and darkness was better for the r.h. levels tried; gradual increase in r.h. correspondingly, enhanced the mycelial growth and sporulation being the optimum at 95-100% and the maximum at 100%. Mycelial growth occurred at all the pH levels tried, the higher being at 6.5. An increase or decrease from this level (pH 6.5) gradually suppressed the growth and sporulation was noticed at all level except 2.9 and 9.2 (Nisar *et al.* 1990). Hywel and Gillespie (1990) studied the growth of *Metarhizium anisopliae* and *Beauveria brassiana* at 20-

30°C. The lag phase for *M. anisopliae* strains was generally less than for *B. brassiana* at given temperature and dependent, on temperature. However *B. brassiana* strain 206-85 had a very short lag phase that was independent of temperature. The germination rate was species dependent with *M. anisopliae* strains having faster rate than *B. brassiana.* The combination of germination rate and lag phase resulted in *M. anisopliae* strains having higher (germination levels (95% 10-14h) compared with the best *B. brassiana* strain (206-85-95% germinated in 14-15h). The results demonstrated intraspecific difference in the germination response which the authors feel should be considered when selecting isolates for development as myco insecticides. Spore germinatin of *Colletotrichum graminicola,* the causal agent of anthracnose of sorghum was obtained only after 2 hours of incubation; maximum spore germination was seen in host seed extract followed by glucose and sucrose solution. The germinated spore produced hyaline germtube with spherical or pyriform appressoria. Cultural studies on different solid media showed that the PDA and host seed extract agar were good for growth and sporulation. In liquid media there was increase in growth of the fungus up to eight days of incoulation and there was decrease in growth from nine day onwards. Temperature requirement of fungus in culture showed 30°C was good for both growth and sporulation and 35°C was good for growth of fungus. The fungus exposed to alternate cycle of light and darkness produced maximum growth and sporulation when compared to continuous light and continuous darkness, (Safeeula and Ranganathan, 1990). Growth, sporulation and production of oospore of *Peronospora parasitica* were found to be dependent on environmental factors such as temperature, light, pH of medium and age of culture. Optimum temperature of 23°C was required and chilling had no marked effects on oospore germination. At pH 7.5, 42%. germination was recorded while at pH 4.5 only 1% of oospore germinated. Germination increased with increase in their age (Jang and Safeeula, 1990).

Studies on factors affecting growth of *Rhizoctonia bataticola,* causing root rot of Okra showed that group G_1 produced maximum growth at temperature of 20° to 30°C. Maximum growth of group G_2 was recorded at 24 to 35°C whereas group G_3 showed maximum growth at 35°C. The pathogen grew over a wide range of pH. Growth was less at pH4 and 5 in all the 3 groups. G_1 produced significantly higher growth at pH 6 and 7, whereas the growth of G_2 and G_3 was maximum at pH9. A tendency of the pathogen to bring pH to neutrality was observed in all the 3 groups (Brar and Bedi, 1991). Ando (1991)

reported that the tea brown blight fungus *Glomerella cingulata,* on culture medium formed conidia. i-e acervulus. Free conidia were derived directly from the tips of hyphae and not form acervuli. The conidia were morphologically similar to those derived from acervuli on the host, the germination pattern and subsequent formation of appressoria were similar to those of the conidia derived from acervuli. The acervuli free conidia were formed abundantly in shaking culture with potato sucrose medium, which was suitable for the mass production of the conidia of *G. cingulata.* Verma (1991) isolated 4 isolates I_4 I_2 and I_3 of *Corticium salmonicolour* berk the incident of pink canker disease from diseased twigs of apple, lime and pear respectively obtained from different parts of the country. The isolates were studied for their comparative ability to utilize various carbon, nitrogen and vitamin source and pathogenicity of apple. The isolates were found to show a significant variability in their physiological behaviour. Okhovat and Hedjaroude (1991), reported the effects of environmental factors on sporulation of *Pyricularia oryzae.* Studies showed that the number of trapped spores increased in June and reached their maximum in July. During these times the plants were at tillering stage and susceptible to the diseas. Correlation between the stage of plant (x) and leaf lesion (Y) during 1979-80 was Y= 1227.34-10.948x, r=0.73.

Murray and Campbell (1991) reported that the sporulation by *Cephalosporium gramineum* on mineral salt agar (MSA) containing phosphate or citrate phosphate buffer was greatest at 20 and least at 5^{0}C, which corresponded to the temperature for greatest and least hyphal growth respectively on this medium. Sporulation on oat kernels artifically colonized by *C. gramineum* or on naturally colonized wheat straw on or buried 2cm below the soil surface was two fold to three fold greater in soil pH 4.5-5.5 that at 6.5-7.5. In contrast to effects of temperature *in vitro*, sporulation by *C. gramineum* on oat kernels on soil was 28 fold to 50 fold greater at 5 than at 15^{0}C sporulation on oat kernals or straw on soil increased from two fold to 10^3 fold as soil metric potential decreased from - 0.001 to -0.07 times. Greater sporulation of *C. gramineum* at low soil pH may partially explain why *Cephalosporium* stripe is more severe in acid soils (pH 4.5-5.5) than in soil of higher pH. However the influence of soil matric potential on sporulation observed in this study was not consistent with high soil moisture contents.

Ahuja (1991) reported that incidence of jowar rust infection was observed at growth stage of jowar even after harvest on ratoon fillers. Infection resulted in heavy losses due to its distorting effects on leaves and general weaking of the

plants. Air born urediniospore were found to be mainly responsible for the development of epiphytotics causing immense loss in grain and fodder.

Trichoderma viride and *Aspergillus niger* were identified as potential anlagonist of *Fusarium solani* (Mart. sacc.) causing antagonist muskmelon wilt by Asalmon *et al.* (1991). *In vitro* studies on temperature and pH requirements of these three individual organisms and combined effect of temperature and pH on their antagonism were carried out. Optimum temperature and pH for growth of the individual organism were found as 30°C and 7·4 for *F. solani,* 35°C and 4·2 for *A. niger* and 25°C and 5 for *T. viride.* Antagonism, when measured by inhibition zone technique revealed that there was no inhibition in acidic range of 4·2 to 5 pH and 30°C with both the antagonistic organisms.

Sharma and Gupta (1991), reported that conidia of *Podosphaera leucotricha,* incitant of apple powdery mildew germinated on glass slide after 6h.at temperature ranging from 10-30°C with maximum germination at 20°C. The optimum temperature for germtube growth was 25°C followed by 20°C. Conidial germination initiated at 96% r.h. with maximum obtained at 100% r.h. The germination of conidia however, in vitro was erratic and poor. No germination was observed in free film of water.

Mishra and Gupta (1994) observed that maximum radial growth, sporulation and spore germination of *Colletotrichum dematium* occurred at 27°C as compared to 20, 25, 30°C. But the same counts were highest of all at 100% r.h. at room temperature (28±2°C) as compared to incubation at other temperature done and at room temperature with 80, 85, 90% r.h.. Maximum increase in lesion size was obtained with continuous light at room temperature as compared to alternate light and dark and total dark followed by room temperature with 100% r.h. and 25°C temperature alone. Increased spore of *C. dematium* was trapped at drizzling rains, 28.11°C temperature and 81.78% r.h.. Spores trapped at 1 PM were found in decreasing trends in later hours of the day. Sahran and Meel (1994), reported that *Alternaria* blight of sunflower caused by *A. helianthi* (Hansf) Tub & Nishihara is an important disease during kharif season under Haryana conditions causing considerable yield losses. In the spore germination study, 25°C temperature and 90% r.h. was found best. Leaf exudates from resistant cvs adversely affected conidial germination. 100 ppm glutamic acid concentrateion was best for maximum spore germination but cystein and glycine adversely affected spore germination. Injury was essential for maximum infection and disease development. Older plants (60 days) at anthesis stage

were most susceptible. Three hours of leaf wetness and 25°C temperature was most congenial for maximum disease development. Rapid progression of *Alternaria* blight was recorded during the period when temperature was between 24.3 to 32.2°C with r.h. of more than 85%. Prashar and Sood (1994) reported that soyabean seedlings inoculated with spore suspension ($6.25x10^6$ spore / ml) were incubated at 20±1, 24±1, 28±1, 32±1 and 34±1°C for 12, 24, 30 and 48 hours and high humidity was maintained by covering the seedlings with glass chimnies lined with moist blotting paper. The inoculated seedlings were observed for the appearance of disease for 20 days. The brown spot development occurred between a temperature range of 20°C to 32°C with a minimum duration of 24h of leaf wetness. The number of spots per leaf increased with the increase in duration of leaf wetness. The maximum number of spots were formed at 24°C with all the duration of leaf wetness. Brown spot did not develop with 12h leaf wetness and also at 34°C.

In vitro spore germination studies using aqueous and alcohol leaf leachates showed that the % of spore germination in cv. CLI-317 was low than in cv. Armoor. This indicated the presence of alcohol soluble fungitoxic subtance in the leachates of vacuum concentrated and TLC separated leachates that developed black spot with a RF value of 0.4. These may be terpenes that proved highly fungitoxic (Reddy *et al.* 1995). Dartar (1995) reported that 6 fungi viz. *Alternaria alternata, Aspergillus niger, Fusarium moniliforme, Fusarium solani, Drechslera australiensis, Colletotrichum capsici* were associated with chilli fruit rot of and were pathogenic when artifically inoculated. Of these fungi *A. niger, D. australiensis* and *F. solani* causing fruit rot of chilli constituted new host records from India. Fruit rot development by all the fungus pathogens was completely arrested when inoculated chillies were kept at 0 and 5°C while disease development was slow at 10-15°C; temperature between 20° and 30°C were found conducive for fruit rot development in chillies.

Blight caused by *Alternaria dianthi* Stev and Hall has been reported to be a major disease affecting carnation plants in Himachal Predesh on the basis of 3 year experiments (1988-91) on the progress and management of the disease under field conditions. The data on disease severity were recorded at 15 days intervals following 0-5 scale based on area under disease and meteorological data were collected from observatory. The relationship between disease severity and various environmental factors have been estimated six fungicides were evaluated at 10 days intervals as foliar spray under field conditions. The 1st symptoms of the disease appeared in the month of December / January whereas

maximum disease development occurred in July-October (temperature 29.7-15·4^{0}C). The disease severity had a highest correlation with minimum temperature (r=0.8128) followed by rainfall revealed that only the minimum and maximum temperature have significant impact on the disease (Madhu and Malhotra, 1995). Boyle *et al.* (1995) studied the influence of r.h. during conidiogenesis and germination of *Blumeria graminis* f.sp. *tritici,* the powdery mildew of wheat agent. Conidiogenesis at high r.h. resulted in a higher conidial water content than in conidia formed under drier conditions. In general a r.h. of 97% was the optimum for maintenance of conidial turgor. Above that level, the number of burst conidia increased in comparison to conidia formed under drier conditions. Optimum germtube growth and appressorium development were obtained at 97% r.h.. Conidia exposed to higher humidities during conidiogenesis had a somewhat higher optimum. No appressoria were formed at lower humidities.

Xu (1996) reported that the magnitude of nonlinear effects on fungal development is shown to depend on the types of non linear models and, on the extent of temperature fluctuation. A method has been described which has been used in other disciplines to fit non linear models directly to varying temperature. Hypothetical data were generated to demonstrate the usefulness of this method with the underlying rate equations being non linear models derived from average temperature under estimate the rates of intermediate temperature the greater the temperature fluctuation the greater this underestimation.

Balmurali Krishan and Jeyarajan (1997), on the basis of their 3 year studies on powdery mildew reported that conidial production, germination and infection of grapes by *Uncinula necator* are greatly influenced by weather factors . Data on maximum and minimum temperature, r.h., rainfall and number of rainydays were correlated with disease incidence. The multiple regression analysis was used to correlated the mean maximum temperature, mean minimum temperature mean relative humidity. Total rainfall and rainy days for the period of one week prior to disease observation with mean PDI for that period of observation taken at 15 days intervals.

Optimum temperature for the germination and germ tube elongation of *Colletotrichum truncatum* was 20^{0}C and that of soyabean pod infection was 25^{0}C. 3 hours light followed by 9 hours dark was found best for spore germination and germ tube elongation. 12h light followed by 12 hours dark

was most suitable for pod infection. However, continuous light took significantly more time for pod infection and development of acervuli; very high r.h. (100%) was required for pod infection and development of acervuli. In silde germination tests however, none of the spores germinated even at 100% r.h. (Kaushal *et al.* 1998). A combination of 18^0C temperature and more than 80 percent relatively humidity was found to be the best to get maximum conidial germination of *Peronospora trigonella* (Prakash and Saharan, 1999). Similarly, exposure of conidia to light had inhibitory effect while it was stimulatory in darkness, Glucose as a nutrition gave better condial germination than sucrose cystine and glutamic acid. Conidia collected after 12h of formation gave maximum germination. Younger and older than 12h of age conidia showed drastic reduction in germination. Washing of conidia at 5000 rpm showed highest germination of 80.26 percent. Conidial germination was better in distilled water than on host leaf and agar medium.

2.3. Biochemical changes in plants associated with infection

Bakshi and Chauhan (1986) reported that increase in phenolic contents of leaf exudates of sheshum (*Dalbergia sissoo*) was found after infection with *Phyllactinia dalbergiae,* but phenolic content decreased as the disease progressed. Healthy leaf exudates showed a gradual decline in phenolic contents as the leaves matured. Changes in phenolic concentration of sheath blight infected rice tissue were studied by Rao *et al.* (1986). A general decrease in the concentration of soluble phenols and an increase in that of bound phenols were observed in diseased tissue of both susceptible (TKM9) and moderately resistant (IR20) varieties. Thin layer chromatographic analysis of the bound phenolic fractions revealed the presence of 2 monohydric phenols, viz. P-coumaric and ferulic acids and 4 other unidentified compounds. P-coumaric and ferulic acids present in bound form were quantified and their possible role in the host defence machanism has been discussed by the authors. Bold seeded chickpea cultivars have been reported to suffer more due to *Alternaria* blight. Resistant cultivars contained more phenols, orthodihydric phenols, total aminoacid and sugar in leaf tissue as compared to susceptible ones. Similar trends was in N, P, K, Zn, Fe and Mn. Stomal morphology of resistant and susceptible cultivars did not differ (Bhargava and Khare, 1988).

There was a marked decrease in the total chlorophyll, carotenoids, sugar,

phenols and ascorbic acid in betelvine varieties, Karcyele and Ampadi infected with *Colletotrichum gloeosporioides* in necrotic area than that of holo and preholo region. However no carotenoid content was observed in the necrotic areas of both the varieties (Naik *et al.* 1988).

Vascular wilt caused by *Fusarium oxysporium* f.sp. *lentes* is a serious disease of lentil. Various factors related to environment and host are responsible for the disease incidence. The disease is severe in sandy loam soil with 7·5 to 8·0 pH, temperature 20 to 30°C. Mortality of lentil due to pathogen was maximum at 25% soil moisture which decreased with increase in moisture level. Morphological and anatomical characters as well as biochemical constituents of lentil root were found responsible for susceptibility and resistance in the host and influenced the disease incidence. Lentil varieties having shorter root and less secondary root exhibited low incidence of the disease. Compact cork cambium and narrow metaxylem were observed in these varieties. Higher amount of amino acid, sugar, phenols, orthodihydric phenols, phosphorus and potassium and low permeability and nitrogen in root of lentil were responsible for resistance against wilt. (Sexena and Khare, 1988). Leaves of lucerne varieties resistant and susceptible to downy mildew did not exhibit significant difference in their total phenols and total soluble sugar; however, resistant varieties had high concentration of orthophenols nitrogen, potassium, Zn, Fe and low concentration of reducing sugar and structural carbohydrates than susceptible ones. The amount of total soluble sugar, non reducing sugar and zn increased reducing sugar, N, P, Mn and Fe contents decreased after infection in susceptible varieties (Luthra *et al.* 1988). Susceptible sugarcane varieties infected with smut showed an increase in total phenols, reducing sugar and total free aminoacids with a decrease in orthodihydric and total sugar. The ascorbic acid content showed no marked variation between healthy an smutted plants. Smut infected plants had less nitrogen, phosphorus Mg, high K content compared to healthy plants. There was no marked difference in calcium content of healthy and smutted plants. (Padmanabhan *et al.* 1988). Kaur and Dhillon (1988) reported that the size, frequency and index of stomata were significantly higher in the susceptible varieties of groundnut whereas the resitant varieties had higher palisade index values. P_1 25a 747, the most resistant variety had in addition highest frequency of trichomes and calcium oxalate. These characteristics seem to provide greater degree of defence against penetration and invasion of *Cercosporidium personatum* in the resistant varieties.

The role of the abaxil leaf surface waxes of *Lolium* spp in resistance to powdery

mildew pathogen *Erysiphe graminis* was determined by Carver *et al.* (1990). Field and glass house observation of *Lolium* spp. grasses indicated that the lower abxial leaf surface was rarely infected even when the upper abxial leaf surface was densely colonised. Experiments showed that conidia of 2 strains of *Erysiphe graminis* from *Lolium* and one from *Avena* germinated equally well on both surface of *Lolium* and *Avena* leaves, but that the subsequent growth and development of germlings was impaired on the lower surface. They suggested that the sheet waxes prevent the pathogen gaining access to rupture of cuticular membrane which trigger normal germling development. Kumar *et al.* (1990) studied the biochemical changes in pearl millet shoot infected with downy mildew pathogen *Sclerospora graminicola* (Sacc). Total leaf chlorophyll, total phenols and total free amino acid contents in the shoots and the specific activity of the enzyme nitrate reductase in both shoots and roots of the host were analysed. The estimation revealed that the total leaf chlorophyll, total phenol and total free aminoacid contents were found low whereas the nitrate reductase activity was found to be high in both the diseased shoot and roots of pearl millet (*Pennisetum americanum L.* leck). Nine polyhydroxy phenolic compounds, one tea extract and a conventional fungicide Benlate (Benomyl) were screened in vitro for toxicity against *Hypoxylon serpens*. All polyhydroxy phenolic compounds except (+) - catechin significantly inhibited growth of the fungus. The naphthalene based pehnolics were most toxic and completely inhibited fungal growth throughout the experimental period, irrespective of the concentration used. The tap root bark extract with unknown composition inhibited fungal growth more than the control and (+)- catechin (Onsando and Owuor, 1990).

Total phenols have been implicated in the resistance and susceptibility of plants. Application of systemic fungicides on foliage results in quantitative changes in phenol content of the plant. Looking to the phytotoxic effects, 4 fungicides namely mancozeb, zine, triforine and carbendazim were applied at their recommendation rates during rabi 1986. The results indicated that the phenol content in leaves were significantly increased by carbendazim 4.5mg/gm. and triforine 4.3 mg / gm as compared to dithiocarbamates. (Kotasthane *et al.* 1991). The levels of total phenol and activities of total oxidizing enzymes such as catalase and peroxidase in the levels of resistant cultiars (Co Vu-2, V-269) and susceptible cultivars (CSSR-IRC-48) of cowpea was determined at different intensities of powdery mildews development. Inherently, the resistant cultivars had higher phenol content compared to susceptible cultivars. (Rao and Kumar, 1991).

Verma *et al.* (1991) evaluated the blochemical changes of fruits infected with three *Aspergillus* species. Healthy and apparently uninjured ripen bael fruits of uniform size and weight were analysed for N, protein, total free amino acid, total sugar, reducing sugar, non reducing sugar total phenol and ash contents. Total phenol was found more in infected tissue than in healthy tissue. The accmulation of total phenol was higher in fruits infected with *Aspergillus luchuensis* followed by those infected with *Aspergillus niger* and *A. fumigatus.* Total sugar content was greatly reduced in infected tissue by all the 3 species of *Aspergillus.* Chaudhuri and Nair (1991) reported that changes in protein and polyphenols were observed in plants and in calli when they were infected by rhizobia. Protein content increased with age and was greater in the infected than in the uninfected plants / callus. Polyphenol content was greater in the younger than the mature plants / callus and in the infected rather in the uninfected plants / callus. Infestation of Chinia and Malbhog varieties of banana by six dominate fungi viz. *Botryodiplodia theobromae, Fusarium oxysporum, Helminthosporium spiciferrum, Curvularia lunata, Aspergillus flavus* and *Trichothecium roseum* exhibited a gradual fall in all the forms of proteins under pathogensis. The maximum reduction of proteins was observed due to *B. theobromae* at the end of the incubation period in both the varieties of banana. Storage of healthy chinia and Malbhog varieties of banana showed an initial increase upto 2nd day which subsequently decreased with the increased in the incubation period. However, infestation of two varieties of banana fruits by all the six isolates viz. *B. theobromae, F. oxysporum, A. spiciferum, C. lunata, A. flavus, T. roseum* induced a gradual decline in the amount of total phenol. Maximum reduction in both the fruits was recorded due to *B. theobromae* on 10th day of incubation. (Prasad and Kumari, 1994).

Sharma and Kaul (1995) reported that nine apple cultivars were analysed for phenolics and related enzymatic activity in healthy and scab inoculated leaves throughout the pathogenesis. There were no significant difference in the total phenol contents of the younger healthy leaves of test cultivars. However an upsurge in total and orthodihydirc phenols was noticed after inoculation, the increase was however more conspicuous in the resistant combinations. Level of phloridzin and phloretin were also assayed; whereas, phloridzin showed a direct relationship, phloretin did not. Also higher enzymatic activity of β-glucosidase, polyphenol oxidase and peroxidase was associated with the resistant cultivars which increased in the leaves when challenged by scab pathogen, *Venturia inaequalis.*

Various phenols were examined in root exudates from 10 day old seedling of chickpea varieties grown in sand culture and in root, stem and leaf tissue of different stage grown in wilt sickplot. Effect of these phenols on spore germination and mycelial growth of fungus was also examined. Hydroquinone and chlorogenic acid were higher in root exudates. Salicylic acid was higher in leaf tissue of resistant varieties at pre infection stage. Also at disease initiation stage, chlorogenic acid and p-coumaric acids were higher in root and leaf tissue and hydroquinone and umbelliferone were higher in stem tissue of resistant varieity. Most of the phenols inhibited spore germination and mycelial growth of the fungus under *in vitro* condition salicylic acid was most effective (Mandavia *et al.* 1995). Choudhury (1995) studied the biochemical changes associated with induction of resistance to rust infection in ground nut caused by *Puccinia arachidis* speg. Results revealed that all the chemicals provided significant protection. Best results were achieved with IAA. When infected plants were analysed for biochemical changes the treated plants always recorded higher levels of phenolics, proteins and oxidase activity as compared to untreated plants. Gupta *et al.* (1995) reported changes in the level of total phenol and specific activities of polyphenol oxidase, peroxidase and catalase in healthy and *Alternaria* leaf blight susceptible leaves of *Brassica* species. *B.* species which are tolerant (*B. carinata, B. napus*) and susceptible (*B. juncea, B. campestris)* to *Alternaria brassicola* and *Alternaria brassicae* were used in the study. Healthy and infected leaf samples were collected at 20 days intervals after 40 days of sowing for various biochemical analysis. Result indicated an initial increase in the level of total phenol followed by continuous decrease with the age of plants in all *B.* species.Tolerant species of *Brassica* registered considerable higher amount of total phenol compared to susceptible ones at all stage of plant development.

Changes in phenolic compounds, carbohydrates and mineral elements in resistant (M-147, G-201) and susceptible (NC-13 GK-19) cultivars of ground nut were studied. Total phenols and orthodihydric phenols were high, whereas total reducing and non reducing sugar were low in resistant-cultivars in comparison to susceptible Ones. Amount of phenolic compounds increased whereas carbohydrates decreased after infection in all the cultivars. Ten aminoacids were detected in resistant cultivars as compared to nine in susceptible cultivars. Nitrogen, Mn, and Fe contents were low whereas and Fe, P, K, Cu, Zn were high in resistant as compared to susceptible. N, P, Cu and Mn decreased and K, Zn, Fe increased in all the cultivars after infection (Sindhan and Parashar, 1996).

Healthy plants of chickpea genotype ICC 1096, resistant to *Botrytis cinerea* had significantly less amount of total soluble sugar and free aminoacid but higher amount of total phenol than the susceptible genotype BGM 408 irrespective of plant part tested. The amount of sulphur containing aminoacids, methionine and cystine was almost double in the resistance genotype compared to susceptible one. Shoot tips had higher amount of sugar and free aminoacid but lower phenol content compared to middle and lower leeaves irrespective of genotypes. Sugar and phenol decreased after inoculation by *B. cinerea.* The decrease in phenol was more in susceptible genotypes BGM 408 but sugar content declined markedly in resistant genotype ICC 1069. Free pool of amionoacid increased by 280% in BGM 408 after inoculation as compared to only 22% in ICC 1069. All the aminoacids except aspartic acid decreased after inoculation. (Mitter *et al.* 1997).

The development and senescence of pigeonpea cultivars H-77-216 were studied at vegetative and flowering stage at weekly intervals upto 35 days from leaf emergence. Leaf development leading to senescence followed significant decrease in fresh weight to dry weight ratio. Chlorophyll content total soluble carbohydrate, starch, proteins and leaf carotenoids were comparatively stable while total leaf N, activities of cellulase and amylase and protease increased significantly at flowering stage (Singh *et al.* 1998a).

Singh *et al.* (1998b) reported that the studies on the effects of CMV on chlorophyll content and mineral elements were carried out in two resistant and two susceptible chilli varieties. Resistant genotypes in general recorded higher content of chlorophyll, phosphorus and magnesium and lower of Zn and manganese than susceptible genotypes. Virus infection showed significant reduction as compared to the resistant ones. The level of Mg was found to be relatively higher in resistant varieties but it showed very little increase after infection.

In sunflower leaves higher amount of chlorophyll, amino acid ortho phenol were observed in cultivars tolerant to *Puccinia calcitrapae* var. *centaureae* whereas in susceptible cultivars total soluble and reducing sugar were more. Total chlorophyll, chlorophyll a and b, total aminoacid and soluble sugar declined while the total and orthodihydirc phenol content increased after rust infections. (Singh *et al.* 1998c).

Materials and Methods

3.1 Plant Material

3.1.1. Selection

Nine different varieties originally obtained from Tea Research Association, Tocklai, Jorhat, Assam and being maintained in the Tea Germ plasm Bank, Department of Botany, University of North Bengal were selected for experimental purpose in this study. The selected varieties were -TV-30, 29, 26, 25, 23, 22,18,9 and Teenali 17. These were selected based on the growing stability as observed under field conditions over the years by Bezbaruah and Singh (1988).

For field studies a Tea Estate in the Terai region i.e., Hansqua Tea Estate was selected (Plate I). For field survey, out of the nine varieties selected for experimental purposes, six varieties available in the Tea Estate were selected. These were TV 26, 25, 23, 22, 9 and Teenali 17. The selected tea bushes ranged in age from 15 - 20 years and were well maintained in different plots (Plates - II and III).

3.1.2. Propagation

Tea plants were propagated in the experimental garden by cuttings. For cuttings, sandy soil (sand and soil 3:1) was used and p^H was adjusted to 4.8 - 5.0 by treating with 2% Aluminium Sulphate solution. Before planting, the soil was heated 60 - 80^{0}C on a metal sheet with fire below to kill eel warms if any present in the soil.

Polythene sleves (6") were filled up with the soil and arranged in rows in a bed and watered thoroughly. All cuttings were allowed for rooting after dipping them in rooting hormone. These cuttings were kept under shade until new leaves appeared (Plate IV).

3.1.3. Plantation

One year old well grown seedlings (Plate V) were used for planting in the field. Before planting, simazine @ 75 gm./ 20 liter water and glyophosate @ 1:200 were used in the experimental plots for suppression of weeds (Borpujari and Banerjee, 1994). Pits (1½' x 1½' x 1½') were dug at intervals of 2' between plants and 3.5' between rows. Planting mixture was prepared in the ratio 4.5 kg. well rotten dry cattle manure, 30 gm. rock phosphate, 30 gm. superphosphate and 2.5 gm. phorate [O, O' - diethyl - (ethylthio methyl) phosphoro - dithioate]. At the bottom of each pit rock phosphate was placed following which half portion was covered with cattle manure - soil mixture. Phorate was mixed with a portion of excavated soil and was applied approximately 5 cm below ground level.

Plate I

Plate II (figs. A & B) - Bushes of different tea varieties

Plate III (figs. A & B) - Bushes of different tea varieties

Plate IV (figs. A-C) – Propagation of tea varieties by cuttings in polythene sleeves under shade in the nursery

Plate V (figs. A-C) - One -year-old tea seedlings of different varieties in the nursery

Following soil conditioning, plants were planted in the prepared soil. Pits were filled with conditioned soil.

3.1.4. Maintenance

Manuring was done in young plants using mixture as follows - Ammonium sulphate - 8 parts by weight, Ammonium - phosphate sulphate (16:20) - 35 parts by weight; potassium sulphate - 15 parts by weight Magnesium sulphate-15 parts by weight and zinc sulphate - 3 parts by weight. The total mixture was dissolved @ 30 gm. in one liter of water and applied @ 50 ml/ plant as suggested by Ranganathan and Natesan (1987).

Mature plants were maintained by applying a soluble mixture of N,P,K. consisting of 10 kg. Urea-46% N, 20 kg. Ammonium Phosphate - 11% P_2O_5, 8 kg.Muriate of potash-60% K_2O in the soil. Greenol (Triacontanol) was sprayed at regular intervals for good growth of bushes.

3.2. Fungal Culture

3.2.1. Source

A virulent strain of *Glomerella cingulata* (Stoneman) Spauld and Schrenk was originally isolated from naturally infected leaves of tea growing in the experimental garden. This strain was identified at Common wealth Mycological Institute, Kew, Surrey, U.K. (IMI N. 356860) and was used in all studies after completion of Koch's postulate.

3.2.2. Koch's Postulate

Young tea leaves were collected from Phytopathological experimental garden and inoculated with conidial suspension of the isolate *Glomerella cingulata* following detached leaf inoculation technique. After 72 hours of inoculation the infected tea leaves were washed thoroughly and small pieces were cut from the infected areas. These were taken in small vials, disinfected with 0.1% $HgCl_2$ for 3 to 5 minutes followed by washing with sterile distiled water several time. Pieces were then transfered aseptically into Richard's Medium Agar Slants. These isolates were examined after 12 days of incubation at 28 $\pm$ 2°C and the identity of the organism was confirmed by comparing with the stock culture.

3.2.3. Stock Culture Maintenance

The fungus thus obtianed was subcultured on RMA (Richard's medium agar) slants. After 2 weeks the culture was stored under 3 different conditions (5°C,

20°C and at ambient temperature (30 ± 2°C). Apart from weekly transfers for experiemental work, the culture of *Glomerella cingulata* was also examinedat regular intervals to test its pathogenicity.

3.2.4. Assessment of Mycelial Growth

3.2.4.1. Solid media

To assess mycelial growth of *G. cingulata* in solid media, the fungus was first grown in Petridishes, each containing 20 ml. of RMA and incubated for 7 days at 30°C. Agar block (4mm diameter) containing the growing mycelia was cut with a sterile cork borer from the advancing zone of mycelial mat and transferred to each petridish containing 20 ml of sterilzed soild media. All petridishes were then incubated at 30°C for the desired period. Diameter of mycelial growth and the nature of the growth, sporulation etc. were noted at definite intervals.

3.2.4.2. Liquid Media

To assess mycelial growth of *G. cingulata* in liquid media, the fungus was first allowed to grow in petridishes containing 20 ml of RMA and incubated at 30°C for 6 days. From the advancing zone mycelial block (4 mm dia.) was cut with a sterilized cork borer and transferred to each Ehrlenmeyer flask (250 ml) containing 50 ml of sterilized medium and incubated for desired period at 30°C. Finally the mycelia was strained through muslin cloth, dried at 60°C for 96 hours, cooled in a desiccator and weighed.

3.3. Composition of Media

A number of media - both solid and liquid, were used in the course of experiments in this study. The composition of different media are as follows:

Richard's medium (RMA)

KNO_3	10 gm.
K_2HPO_4	5.0 g
$MgSO_4.7H_2O$	2.5 g
KCl	0.5 g
$FeSO_4.7H_20$	0.01 g
Sucrose	30.0 g
Agar	20.0 g
Distilled water	1 L.

Potato Dextrose Agar (PDA)

Potato (Peeled and sliced)	400.0 g
Dextrose	20.0 g
Agar	20.0 g.
Distilled water	1 L

Carrot Juice Agar (CJA)

Grated Carrot	20.0 g
Agar	20.0 g
Distilled water	1 L

(Grated carrot boiled, strained through muslin bag, volume made upto 1L, agar added , boiled till dissolved).

Green Tea Agar (GTA)

Green Tea Leaves	100.0 g

(Crushed with distilled water, strained and final volume made upto 1 L.).

Agar	20.0 g

Boiled Tea Agar (BTA)

Tea leaves	100.0 g

(cut, boiled and final volume made upto 1L.)

Agar	20.0 g

Nutrient Agar (NA)

Peptone	5.0 g
Beef extract	3.0 g
Nacl	3.0 g
Agar	20.0 g
Distilled water	1 L.

Czapek - Dox Agar (CDA)

$NaNO_3$	2 g
K_2HPO_4	1 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	0.01 g
Sucrose	30.00
Agar	20.00
Water	1 L

Potato Sucrose Agar (PSA)

Potato (Peeled and sliced)	400.0 g
Sucrose	20.0 g
Agar	20.0 g
Distilled water	1 L

Elliott's Agar

KH_2PO_4	1.36 g
$MgSO_4.7H_2O$	0.50 g
Na_2CO_3	1.06 g
Dextrose	5.00 g
Asparagine	1.00 g
Agar	15.00 g
Water	1 L

Yeast Extract - Dextrose Agar

Yest extract	7.5 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1 L

For liquid media, compositions were the same as respective solid media, without addition of agar.

Experimental

3.4. Inoculation Techniques

3.4.1. Detached leaf

A detached leaf inoculation technique as described by Dickens and cook (1989) was followed for artificial inoculation of leaves. Fresh, Young, fully expanded tea leaves were detached from plants and placed in plastic trays lines with moist blotting paper. Wounds were made on the adaxial surface of each leaf and inoculated either with spore suspension or mycelial block of the fungus. Mycelial block inoculation was done by placing plug (2mm dia) of mycelia bearing conidia from 10 day old culture aseptically on the inoculation site. Sterile Richard's medium blocks or sterile distilled water were used as control.

For inoculation with spore suspension, 20 µl droplets of conidial suspension (1.6×10^6 conidia/ml) of the fungus (prepared from 10 day old culture) were placed (2-4 drops/leaf) on the adaxial surface of each leaf with a hypodermic syringe after making appropriate needle scratches. In control sets, drops of sterile distilled water were placed on the leaves.

Each tray was covered with a glass lid and sealed with petroleum jelly in order to minimize the drying of drops during inoculation.

3.4.2. Cut Shoot

Cut shoot inoculation technique was followed as described by Yanase and Takeda (1989). Twigs (with 3 - 4 leaves) of tea plant grown in the experimental garden were cut carefully and immediately introduced into glass vials containing sterile tap water and taken to the laboratory. Leaves were inoculated by making 2 mm light scratch with a sharp sterilized needle on the adaxial surface of the leaf and placing mycelial plugs on the scratches, Mycelial plug inoculated cut shoots were placed into the holes of styrofoam board which was floated on modified Hoagland and Knop's solution and kept in a glass chamber (45 cm x 30 cm) for one week with aeration.

3.5 Assessment of Disease Intensity

3.5.1 Artificial inoculation

3.5.1.1 Detached leaf

Disease intensity was assessed on the basis of number of inoculum drops that resulted in lesion production out of the total number of inoculum drops. Percent drops or mycelial plugs that resulted in lesion production was calculated after

48,72 and 96 h of inoculation as discribed by Chakraborty and Saha (1994). Diameter of lesions were also noted. Observations were based on 50 inoculated leaves for each treatment and average of 3 separate experiments.

3.5.1.2. Cut shoot

The actual number of lesions that developed on the artificially inoculated shoots were counted after 48,72 and 96h. Diameters of individual lesions were measured. They were graded into four groups and a value was assigned to each group. Very small restricted lesion, 1-2 mm dia. = 0.1; 2-4mm dia with sharply defined margin = 0.25; lesion with slow spread beyond 4 mm = 0.5 and spreading lesion variable in size with diffused margin = 1.0 Number of lesion in each group was multiplied by the value assigned to it and the sum total of such values were noted and disease index was computed as the mean of observation of 50 cut shoot per treatment.

3.5.2. Natural infection

Occurrence of brown blight disease in the field was assessed, and disease incidence was calculated as the percentage of the infected leaves per bush. Bushes were selected at random and for each replicate a minimum of 25 bushes were surveyed. In each case the symptom of the disease was carefully noted and differentiated from other foliar diseases.

3.6. Obtaining meteriological data

Monthly record of meteriological data for a period of three years (1996, 1997, 1998) was obtained from the Gangaram meteriological station, Tea research association Terai branch, Bengdubi, West Bengal. Meteriological data included maximum and minimum temperature, percentage relative humidity (morning and afternoon), hours of sunshine and monthly rainfall. Hours of observation were 0635 and 1335 ISD.

3.7. Collection of diffusible compound from tea leaves

Diffusible compounds from tea leaves were collected following drop diffusate technique of Muller (1958) with modifications. Leaves were collected from tea plants, washed in sterile distilled water and blotted dry with blotting paper. Fifty leaves were placed on moist blotting paper in each plastic tray (30cm x 30cm). Twenty µl droplets (2 - 4 per leaf) of sterile distilled water or conidial suspension of *G. cingalata* (1.2 x 10^6 conidia/ml) prepared from 10 day old cultures with sterile distilled water were placed on the adaxial surface of each

leaf. Each tray was covered with a glass lid and sealed with a smear of white petroleum jelly to maintain necessary moisture. Drops were collected from leaf surfaces, combined and centrifuged; this supernatant was treated as the diffusate. Similarly, the water drops were also collected from the leaf surface combined centrifuged and the supernatant obtained was the exudate, Finally, the diffusates and exudates were passed through sintered glass filter and then used for experimental purpose.

3.7.1. Spore Germination Bioassay

For spore germination bioassay, method of Trivedi and Sinha (1976) was followed with modification. A clean grease free slide was taken and a drop 10 µl of the fungal spore suspension was placed on it and incubated in humid petridishes for the desired period. Finally 1 drop of lactophenol cotten blue was added to each drop to fix the germinated spore. Slide was observed under the microscope and percentage germination, appresoria formation and germtube length were determined.

3.8. Extraction of phenols from tea leaves

Phenols were extracted from tea leaves following the method of Mahadevan and Sridhar (1982) with modification.Leaves were cut into small pieces and put directly into boiling 80% ethanol for 5 minutes, cooled and crushed in a mortar with pestle, passed through 2 layers of cheese cloth and filtered through filter paper. The final volume was made upto 5 ml with 80% ethanol. This filtrate was used for estimation of both total and orthodihydroxy phenols.

3.9. Estimation of total and orthodihydroxy phenol contents

3.9.1. Total phenol

The total phenol was estimated by Folin Ciocalteau's method as described by Bray and Thorpe (1954). One ml alcohol extract was taken in a 25 ml test tube; 1 ml of Folin Ciocalteau's reagent was added to it, followed by 2 ml of 20% Na_2Co_3 solution. The tube was shaken and heated on boiling water bath for 1min. The tube was cooled under tap water and volume raised to 25ml. A blank was prepared with all reagents except the sample, to adjust the zero reading. Quantity of total phenol was estimated with caffeic acid standard in a Systronics Photoelectric Colorimeter Model 101 at 515 nm wavelength.

3.9.2. Orthodihydroxyphenol

Quantitative estimation of orthodihydroxy phenols was done by Arnow's method

(1933). Initially 1 ml of the alcohol extract was taken in a tube and the following reagents were added - 2 ml of 0.05 (N) HCl, 1 ml of Arnow's reagent ($NaNo_2$ - 10 g; Na_2MoO_4 - 10 g; distilled water 100 ml) and 2 ml of 1N NaOH. The volume was raised to 25 ml and absorbance was noted using a Photoelectric Colorimeter Model 101 at - 515 nm wavelength. A blank containing all the reagents except Arnow's was used to adjust the absorbance to zero. Quantity of phenol was estimated following the standard curve of caffeic acid.

3.10. Extraction of chlorophyll from tea leaves

Chlorophylls were extracted from tea leaves in 80% acetone as described by Harborne(1976).Leaves were crushed in 80% acetone in a mortar with pestle.This slurry was filtered through filter paper and a final volume made up to known amount using 80% acetone,the whole procedure was carried out in dim light.

3.11. Estimation of chlorophyll content

Chlorophyll estimation wasdone by directly noting absorbance value at 663 and 645 nm as dscribed by Harborne (1976). Quantification of Total chlorophyll, chlorophyll a and chlorophyll b was done from the following formula.

Toral chlorophyll = 20.2 A 645 + 8.02 663 µg/ml.

Chlorophyll a = 12.7 A 663 - 2.69 A 645 µg/ml

Chlorophyll b = 22.9 A 645 - 4.68 A 663 µg/ml

3.12. Extraction of epicuticular wax from tea leaf surfaces

Tea leaves were collected cut into discs and the area of both the surfaces determined. Discs were immersed in chloroform for 15 seconds. The extract was filtered and evaporated on boiling water bath as suggested by Ebercon et al (1977).

3.13. Estimation of epicuticular wax content

Estimation of epicuticular wax content of tea leaves was done colorimetrically as described by Ebercon et al, (1977). Reagent was prepared by mixing 40 ml deionized water with 20 gm powdered potassium dichromate. The resulting slurry was mixed vigoursly with one litre sulphuric acid and heated (below boiling) until a clear solution was obtained. 5 ml of this reagent was added to each wax

sample (after evaporation of chloroform extract) and placed on boiling water bath for 30 minutes. After cooling, 12 ml of deionized water was added and incubated for 5 to 10 minutes until colour developed. Following this, optical density of the sample was taken 590 nm in a Systronics Photoelectric Colorimeter Model 101.

Standard curve was prepared with Polyethylene glycol - 3000 (Carbo wax 3000). Waxes were dissolved in chlorofrom and 15 ml aliquots containing range of concentration (1 - 5 mg) were prepared. These aliquots were carried through the above analytical procedure.

3.14. Sample preparation for anatomical studies

For anatomical studies, transverse section of leaves were prepared and stained either with lactophenol-cotton blue (infected leaves) and mounted in glycerine or stained with saffranin light green and permanent preparation were made. The sections were the observed under Leica Microscope and specific anatomical features noted.

4.1. Pathogencity test of *G. cingulata* on different tea varieties

Resistance of nine varieties of tea (TV -30, 29, 26, 25, 23, 22, 18, 9 and Teenali 17) released by Tocklai Experimental Station, Jorhat, were tested against *G. cingulata* by detached leaves and cutshoot inoculation techniques. Details of inoculation techniques and disease assessment methods have been described in material and methods. Results are given below :

4.1.1. Detached leaves

Leaves of the nine selected varieties were artificially inoculated as described previously. Disease assessment was done after 48, 72 and 96 hours of inoculation. It was observed that in all varieties lesions appeared only after 48h of inoculation (Plate VI) Lesions were observed to coalesce after five days. Results showed that among all tested varieties TV-18 was most susceptible followed by TV-25, while TV-26 was the most resistant followed by TV-29 (Table 1).

Table 1 : Pathogenicity test of *G. cingulata* on different tea varieties (detached leaf inoculation).

Varieties	% lesion production after *		
	48h	72h	96h
TV - 30	41.8 ± ·434	52.77 ± ·447	56·11 ± ·200
TV - 29	41·6 ± ·577	50·50 ± ·894	55·84 ± ·583
TV - 26	36·11 ± ·577	41·60 ± 1·58	47·2 ± ·99
TV - 25	50·0 ± 1·90	59·20 ± ·216	65·01 ± ·623
TV - 23	45·0 ± ·497	58·00 ± ·470	70.0 ± ·577
TV - 22	47.2 ± ·996	52.7 ± ·333	63.88 ± 1·90
Teenali-17	43.00 ± ·516	51.38 ± ·447	60·0 ± 1·90
TV - 18	52·77 ± ·447	65·55 ± 1·86	80·55 ± 1·21
TV-9	40.60 ± ·428	50·95 ± ·683	56·66 ± ·921
Mean	**44.2**	**53.62**	**61.69**
(CD) (5%)	**8.90**	**4.92**	**6.97**

* Average of 3 experimental sets and 50 leaves inoculated per set; ± = standard error.

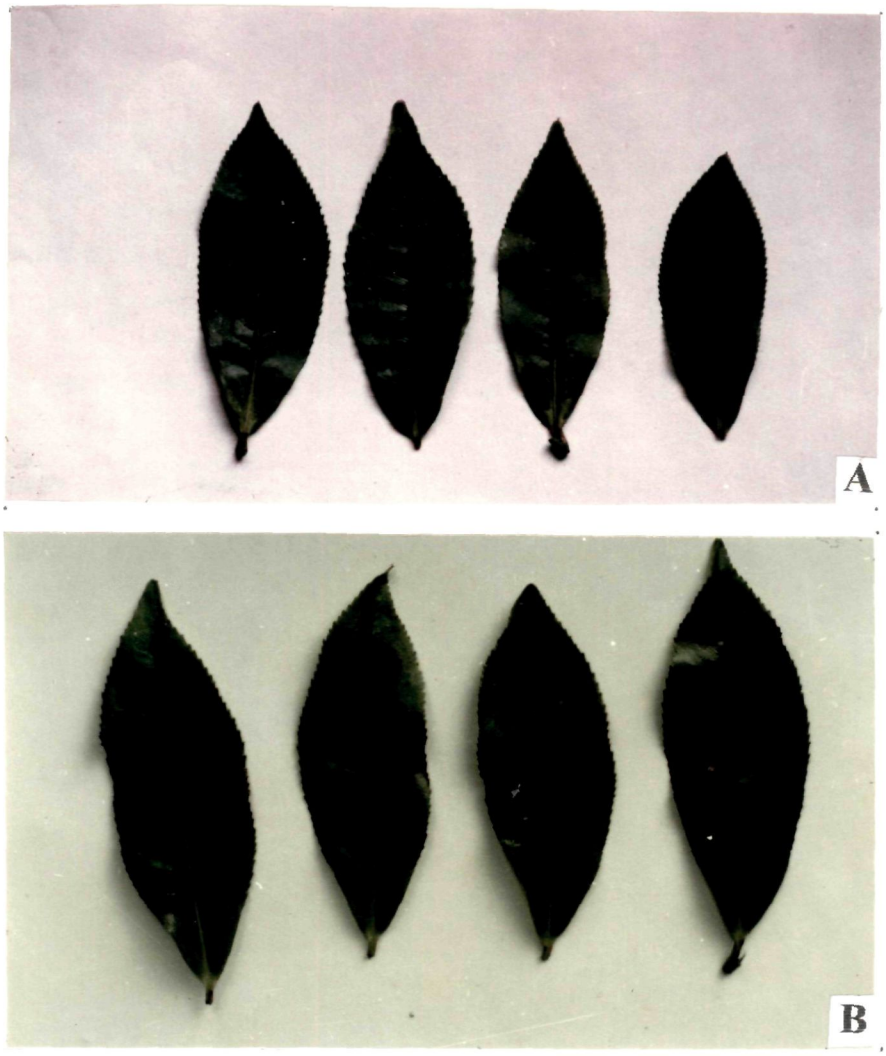


Plate VI (Figs. A & B) – Detached leaves of TV-18; A –Healthy ; B - Artificially inoculated with *Glomerella cingulata*

4.1.2. Cutsoot

Pathogenicity of *G. cingulata* on the selected varieties were also tested by cutshoot inoculation technique as described under materials and methods Disease was assessed on the basis of mean number of lesions per shoot from which mean disease index per shoot was calculated. Results (Table 2) showed that in this case also TV-18 was most susceptible and TV-26 was most resistant.

Table 2 : Varietal resistance test of different tea varieties against *G. cingulata* (cul-shoot inoculation).

Varieties	48h		72h		96h	
	Mean No of* lesion/shoot	Mean disease index/shoot	Mean No of* lesion/shoot	Mean disease index/shoot	Mean No of * lesion/shoot	Mean disease index/shoot
TV - 30	3.00	0.75	5.00	1.42	7.00	2.63
TV - 29	3.00	0.73	**4.00**	1.02	**4.00**	1.52
TV - 26	2.00	0.62	**3.00**	0.92	**3.00**	1.04
TV - 25	6.00	2.28	**7.00**	2.49	10.00	4.7
TV - 23	6.00	**2.14**	**7.00**	2.33	9.00	4.2
TV - 22	5.00	**1.09**	**7.00**	2.42	**8.00**	3.9
TV - 18	8.00	3.5	10.00	4.6	12.00	5.9
Tenali 17	6.00	2.24	**7.00**	2.52	**8.00**	3.8
TV - 9	4.00	0.81	**6.00**	2.08	**7.00**	2.42

* Average of 50 shoots / variety

Results of both detached leaf and cutshoot inoculation techniques confirm that TV-18 and TV-26 are most susceptible and resistant, respectively to *G. cingulata* (Fig. 1). The other varieties exhibited varying degrees of susceptibility and resistance.

4.2. Factors affecting brown blight disease development following artificial inoculation

Disease development in any plant is expected to be influenced by different factors - some of which are environmental and others related to the pathogen. A series of experiments were performed in order to determine the effect of a few factors i.e. age of culture, concentration of inoculum, light period and seasonal difference on lesion production by *G. cingulata* on six selected tea varieties i.e. TV-18, 23 and 25 (susceptible) and TV-26, 29 and 9 (moderately resistant).

Pathogenicity test of *Glomerella cingulata* on different tea varieties

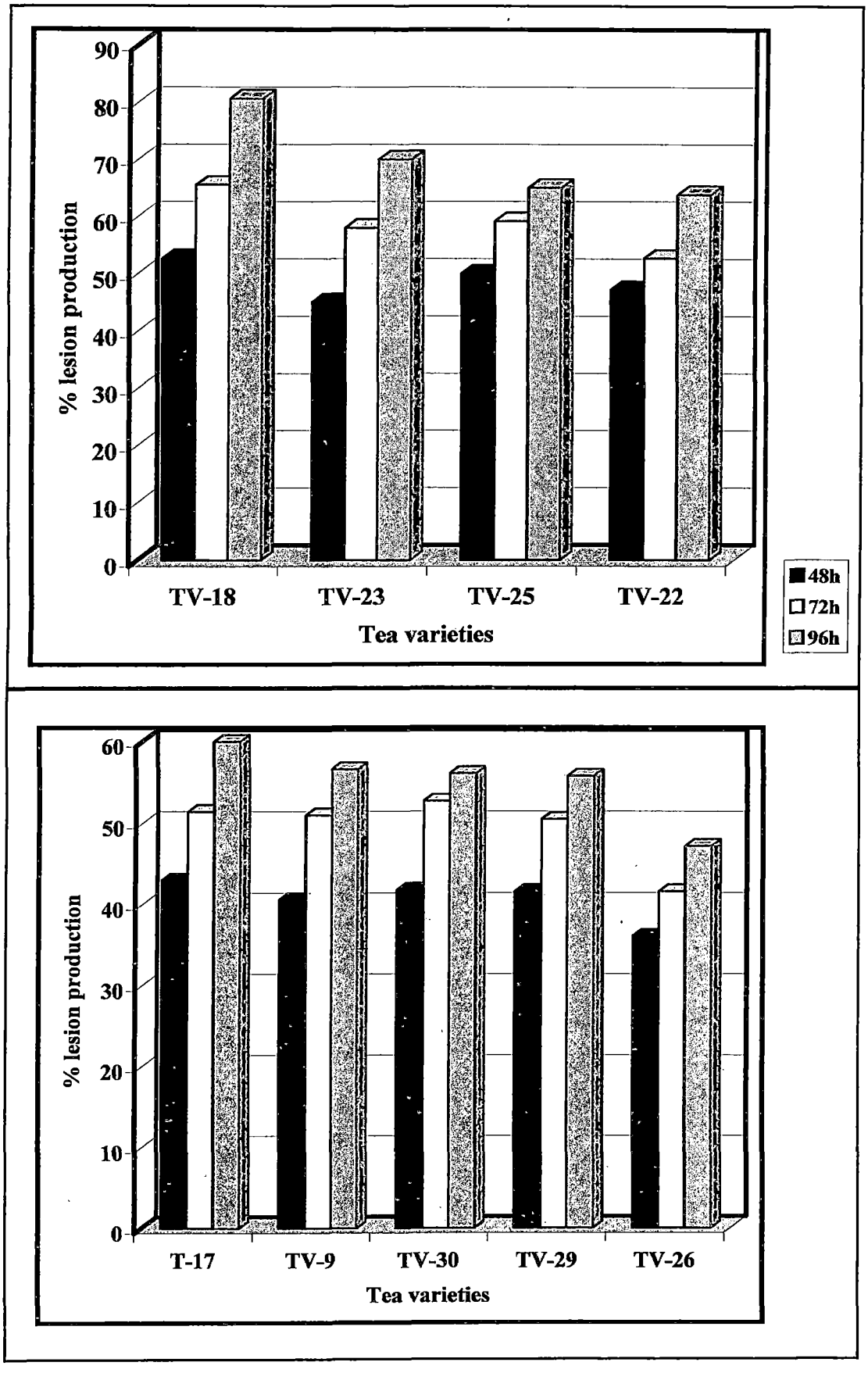


Fig.1

4.2.1. Age of culture

Detached leaves were inoculated with spore suspension obtained from culture of 5, 10, 15, 20, 25 and 30 days; lesion development was determined after 48, 72 and 96h. of inoculation. Results (Tables 3-5 and Fig. 2) revealed that spores from 10 day old culture had maximum infective capacity. Lesion, however, could be observed in all cases. After 10 days the infective capacity of the spores showed gradual decline. In TV-18, 10 day old culture produced approximately 68% lesion which declined to 47% in 30 days old culture (96h of inoculation).

4.2.2. Spore concentration

Spore concentrations ranging from $1.6x10^3$ to $1.6x10^6$/ml were used for inoculation of detached leaves. Optimum spore concentration for disease production was $1.6x10^5$ spore/ml. At this concentration maximum lesion production was obtained in all varieties (Table 6, Fig. 3).

4.2.3. Light

In order to determine the effect of light on lesion production, inoculated leaves were kept under three light conditions i.e one set was kept under total light, second set under diffused light and the third set was kept under total dark condition for upto 96h. Percentage, lesion production was determined after 48, 72 and 96 of inoculation. Results revealed that in all tested varieties maximum lesion production was obtained under diffused light and minimum under total dark (Table 7, Fig. 4, Plate VII).

4.2.4. Different seasons

Disease development following artificial inoculation was also recorded during four seasons i.e. summer, rainy, autumn and winter (April, July, October and January, respectively) No lesion production was observed during winter, while maximum disease development occurred during rainy season. Lesion production in summer and autumn were more or less similar (Table 8; Fig.5).

4.3. Brown blight disease occurrence under natural conditions

Plots were selected in a particular experimental area containing different varieties of tea bushes ranging in age from 15-20 years, Disease incidence was calculated as percentage of infected leaves / bush. For survey of natural incidence, six varieties from the original nine, which are commonly planted in

Table 3 : Effect of age of culture on brown blight disease development in different tea varieties (48h after inoculation).

Varieties	% lesion production*					
	Age of Culture					
	5d	10d	15d	20d	25d	30d
TV - 29	22.2 ± 1·00	30.66 ± 1.09	29·13 ± ·584	28·95 ± 1.02	27·00 ± ·912	20·55 ± 1·21
TV - 26	19·3 ± ·323	27·77 ± ·632	26·2 ± ·489	25·8 ± ·804	24·52 ± ·622	18·34 ± ·564
TV - 25	33·33 ± ·670	38·88 ± ·439	43·50 ± ·540	42·00 ± 1·19	41·65 ± 1·47	35·63 ± 1·20
TV - 23	36·33 ± ·933	41·50 ± ·291	41·66 ± ·729	40·70 ± ·750	40.52 ± ·655	34·86 ± 1.39
TV - 18	44·4 ± ·313	52·75 ± ·933	50·0 ± ·912	49·50 ± 1.82	47·56 ± ·676	40·21 ± ·906
TV - 9	27·77 ± ·686	34·10 ± 1.14	33.5 ± ·695	32·35 ± ·569	30·15 ± ·150	28·20 ± 1.82

* Average of 3 experimental sets
Average of 50 leaves inoculation per set
±-Standard error.

Table 4 : Effect of age of culture on brown blight disease development in different tea varieties (72h after inoculation).

Varieties	% lesion production*					
	Age of Culture					
	5d	10d	15d	20d	25d	30d
TV-29	30.55± ·288	34.66 ± ·116	33.33 ± ·323	32·65 ± ·645	28·21 ± 2·04	22·19 ± ·577
TV- 26	25·0 ± ·577	33·33 ± ·516	31.92 ± 1·76	30·50 ± ·645	27·25 ± ·924	20·18 ± ·555
TV- 25	38·8 ± 1·39	50·00 ± 1·18	48·6 ± ·697	47·80 ± ·804	46·16 ± ·656	36·13 ± ·533
TV- 23	41·66 ± ·577	47·22 ± ·996	45·83 ± 1.21	44·85 ± ·912	44·33 ± 4.51	36·73 ± 1·31
TV- 18	50.5 ± ·894	58·33 ± 1·26	55·55 ± 3·17	53·01 ± 1·47	52·53 ± 1·05	42·59 ± 1·67
TV- 9	33·33 ± ·816	38·88 ± ·933	36·11 ± ·366	35·01 ± ·408	34·89 ± ·852	30·14 ± ·875

* Average of 3 experimental sets
Average of 50 leaves inoculation per set
± = Standard error.

Table 5 : Effect of age of culture on brown blight disease development in different tea varieties (96h after inoculation).

Varieties	% lesion production *					
	Age of Culture					
	5d	10d	15d	20d	25d	30d
TV-29	38.88± 1.37	45·83 ± ·865	44·44 ± ·216	44·00 ± ·912	40·25 ± ·520	25·58 ± 1.29
TV-26	30·55 ± ·880	38·88 ± ·933	43·05 ± ·966	42·50 ± 1.04	35·27 ± ·530	23·59 ± 1·67
TV-25	44·44 ± ·632	55·55 ± ·880	54·20 ± ·454	53·48 ± 1·21	53·32 ± ·554	38·85 ± ·830
TV-23	47·22 ± ·163	52·77 ± ·333	51·38 ± ·383	51·00 ± 1·47	50·63 ± ·713	38·96 ± ·890
TV-18	58·33 ± ·334	68·44 ± ·248	66·66 ± 1·63	60·89 ± ·852	56·56 ± 1·06	47·11 ± 1·47
TV-9	47·33 ± ·437	52·70 ± ·477	50·0 ± ·912	50·00 ± ·912	46·23 ± 1·66	34·19 ± 1·01

* Average of 3 experimental sets and 50 leaves inoculated per set
± = Standard error.

Table 5A : Analysis of variance of disease intensity in defferent tea varieties with *G. cingulata* culture of different ages (Data presented in Table 5).

Source of variation	d.f	S.S.	M.S.	F
Between age of culture	5	1317·04	263·41	55·69
Between varieties	5	2114·34	422·87	89·41
Residual	25	118·21	4·73	—

Both 'F' values highly significant of 1% level.

Effect of age of culture on brown blight development in different tea varieties

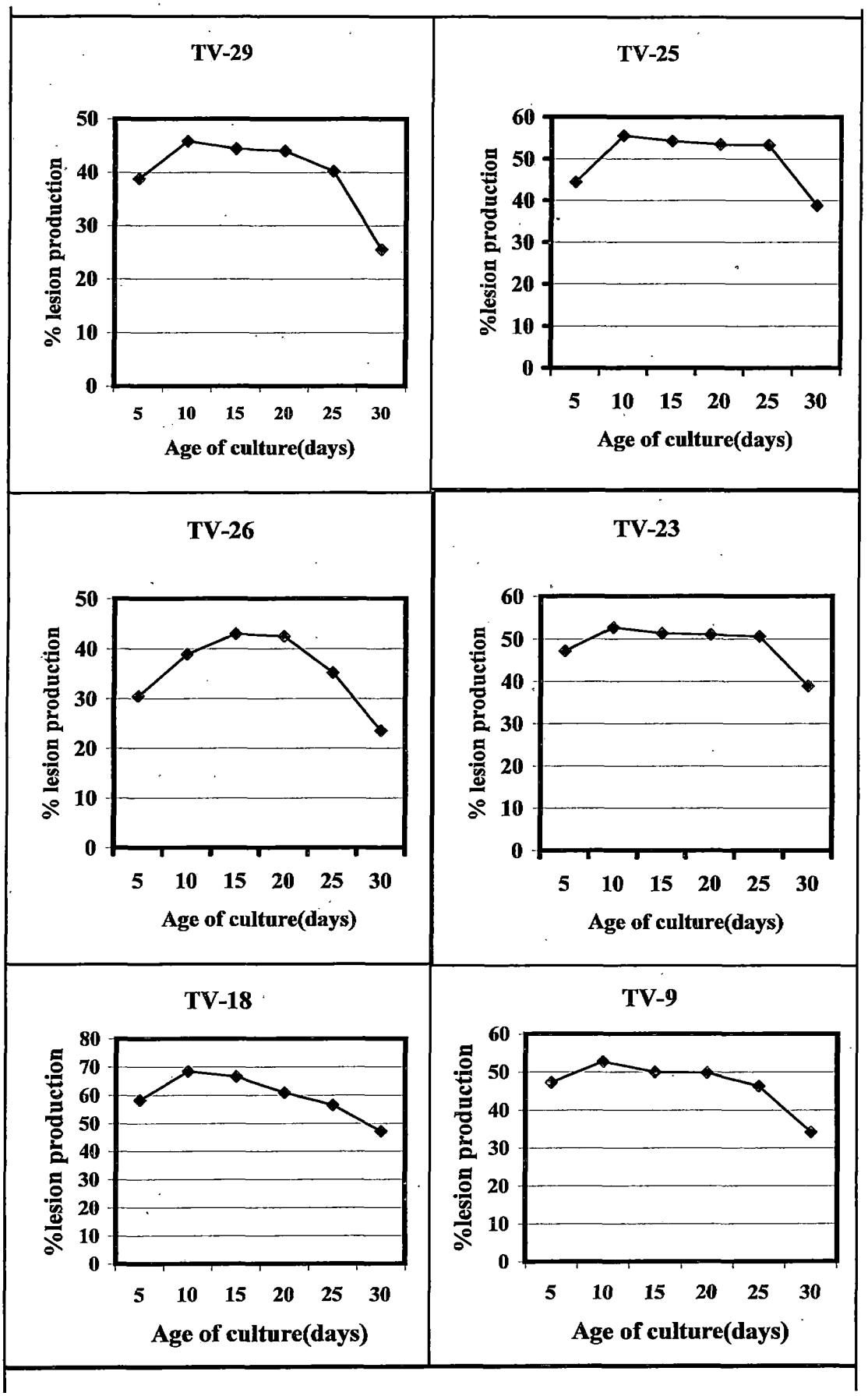


Fig.2

Table 6 : Effect of spore concentration on lesion production on different tea varieties by *G. cingulata.*

Spore concentration (conidia / ml)	Hours after inoculation	% lesion Production *					
		TV-29	TV-26	TV-25	TV-23	TV-18	TV-9
$1{\cdot}6 \times 10^6$	48	36·1 ± ·519	33·3 ± 1·01	46·8 ± ·40	46·2 ± ·40	48·0 ± 1·25	38·3 ± ·76
	72	52·7 ± ·460	44·4 ± 1·84	66·6 ± 1·67	58·3 ± ·53	68·0 ± 1·47	54·0 ± ·96
	96	56·2 ± 1·8	45·8 + ·223	68·2 ± 1·72	65·8 ± 1·47	78·6 ± 1·84	58·6 ± 1·8
$1{\cdot}6 \times 10^5$	48	46·1 ± ·40	41·6 ± 1·23	56·6 ± ·24	50·0 ± ·47	58·0 ± ·96	48·2 ± ·95
	72	55·5 ± ·76	52·7 ± 1·7	75·5 ± 1·67	76·7 ± ·40	81·0 ± 1·82	67·7 ± ·24
	96	68·34 ± 1·25	65·2 ± 1·6	86·3 ± ·76	88·2 ± 1·84	92·2 ± 1·86	72·85 ± 1·84
$1{\cdot}6 \times 10^4$	48	25·0 ± 1·19	17·1 ± ·94	35·7 ± ·64	32·7 ± ·48	41·6 ± 1·79	27·7 ± ·88
	72	32·0 ± ·16	27·7 ± 2·4	55·0 ± ·22	54·4 ± ·40	69·4 ± 1·72	50·0 ± 1·47
	96	39·5 ± 1·23	32·8 ± 1·8	66·3 ± ·22	65·0 ± 1·25	72·2 ± ·29	56·5 ± 1·84
$1{\cdot}6 \times 10^3$	48	7·5 ± ·12	5·6 ± ·21	12·5 ± ·20	13·8 ± ·33	16·6 ± 1·95	9·5 ± ·78
	72	10·11 ± ·47	8·3 ± ·47	29·4 ± ·24	36·1 ± ·51	38·3 ± ·67	17·11 ± ·12
	96	15·23 ± 1·19	12·2 ± 1·67	34·6 ± ·40	40·0 ± 1·87	45·38 ± 1·25	20·6 ± ·96

* Average of 3 experimental sets and average of 50 leaves inoculated per set.
± = Standard error.

Table 6A : Analysis of variance of disease in different tea varieties spore concentrations. (data of 72h after inoculation)

Source of variation	d.f.	S.S.	M.S.	F
Between spore concentration	5	6616.09	1323.38	66.14
Between varieties	3	2875.06	958.35	47.89
Residual	15	300.02	20.01	

Both 'F' values highly significant at 1% level.

Effect of spore concentration on brown blight development in different tea varieties

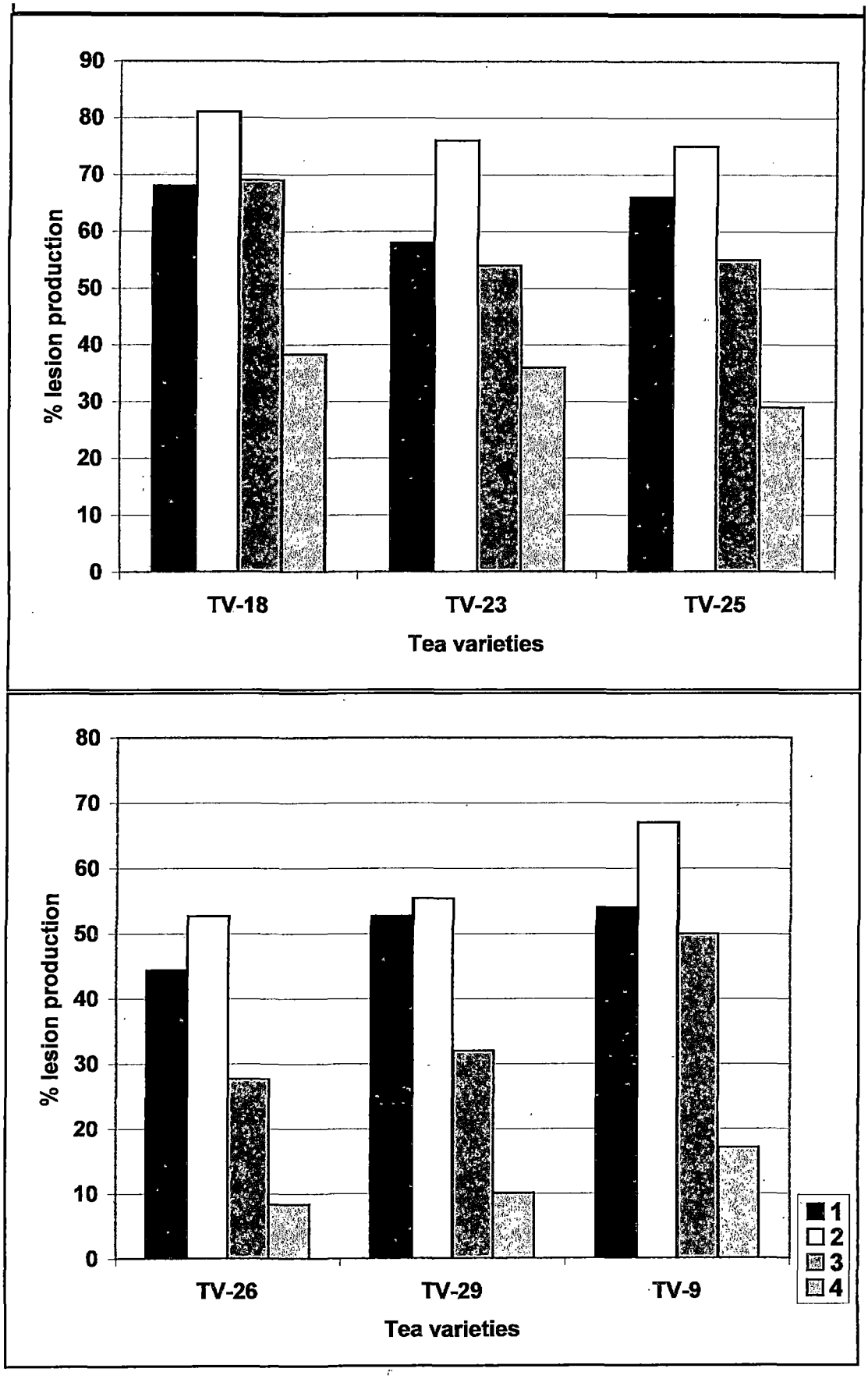


Fig.3

Table 7 : Effect of light on brown blight disease development in different tea varieties.

Varieties	% lesion production *								
	Total light			Diffused light			Total dark		
	48h	72h	96h	48h	72h	98h	48h	72h	96h
TV-18	65.25 ± .333	78.25 ± .333	86.3 ± 1.47	680 ± 1.72	86.46 ± .293	90.68 ± 1.31	68.0 ± .333	70 ± .858	72.8 ± 1.02
TV-9	41.6 ± .966	54.60 ± .943	62.72 ± 1.29	50 ± .966	61.00 ± 1.82	68.23 ± 1.08	41.6 ± .966	46.36 ± 1.31	52.77 ± .645
TV-23	55.7 ± .709	64.8 ± .675	72.56 ± 1.24	54.1 ± 2.04	68.9 ± .659	79.86 ± 1.47	52.14 ± 1.19	64.8 ± 1.35	70.86 ± 1.47
TV-25	55.14 ± .177	62.8 ± .720	78.26 ± .333	59.2 ± .888	66.8 ± 1.08	80.42 ± 1.82	54.2 ± .489	60.7 ± 1.19	68.26 ± 1.31
TV-26	21.00 ± .966	21.571 ± 1.82	44.63 ± 1.47	25.42± .966	38.00 ± 1.29	52.62 ± 1.31	14.23 ± 1.02	15.33 ± .333	25.86 ± 1.82
TV-29	31.50 ± 1.50	43.75 ± .853	50.00 ± 1.47	52.63 ± 1.29	55.6 ± .294	63.00 ± 1.08	18.75 ± 1.31	21.87 ± .858	28.52 ± 2.04

* Average of 3 experimental sets and average of 50 leaves inoculated per set;
± = Standard error.

Table 7A : Analysis of Variance of disease in different tea varieties under different light conditions data of (96h after inoculation)

Source of variation	d.f.	S.S.	M.S.	F
Between light sources	5	1141.93	228.39	8.85
Between varieties	2	4271.86	2135.93	82.79
Residual	10	258.03	25.80	

Both 'F' values significant at 1% level.

Effect of light periods on brown blight development in different tea varieties

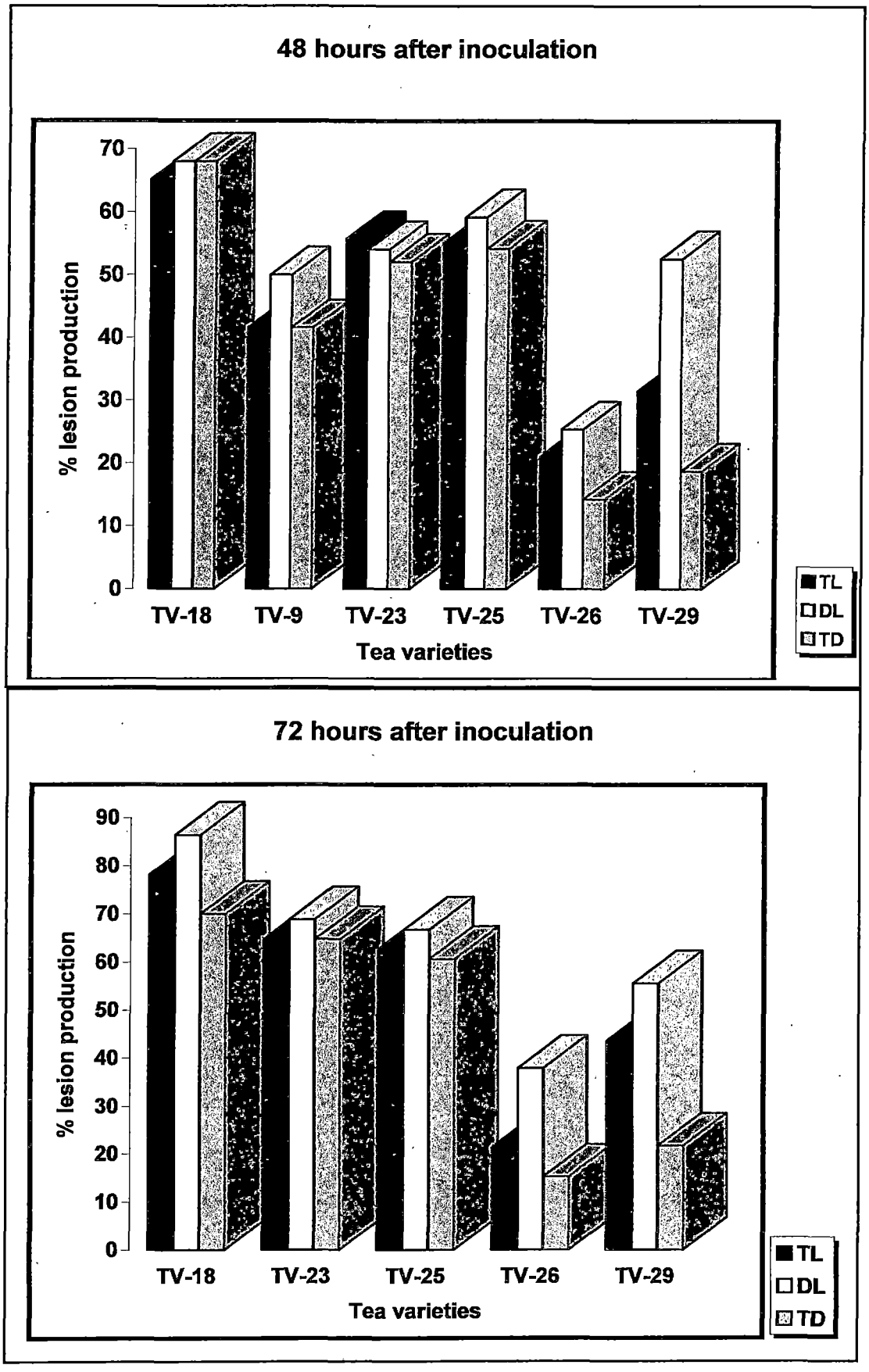


Fig.4

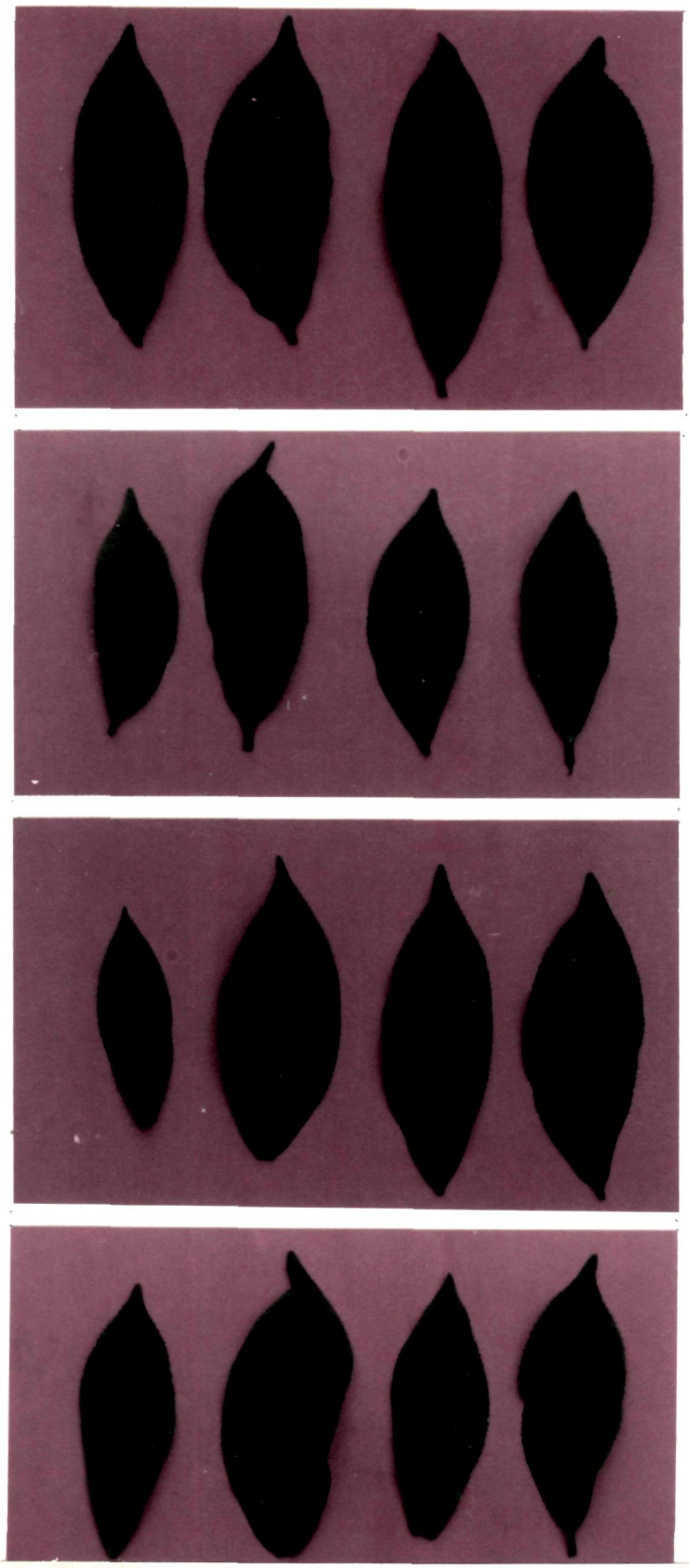


Plate VII (Fig.A-D)-Detached leaves of TV-18; A - Healthy ; B-D-Artificially inoculated with *G. cingulata* and kept under different light conditions; B-Diffused light; C-Total light ; D-Total Dark

Table 8 : Disease intensity in artificially inoculated tea leaves during different seasons

Varieties	% lesion production *								
	Summer			Rainy			Autum		
	48h	72h	96h	48h	72h	96h	48h	72h	96h
TV-29	27.77 ± .632	33.3 ± .516	41.60 ± .632	41.6 ± .577	50.50 ± .894	55.8 ± 1.90	23.25 ± .210	25.62 ± 1.02	27.84 ± .583
TV-26	25.0 ± .730	30.5 ± .774	38.8 ± .933	36.11± .577	41.6 ± 1.58	47.2 ± .99	20.35 ± .447	23.68 ± .210	24.98 ± .583
TV-25	38·8 ± .577	44.4 ± 1·39	55·5 ± 1·43	50·0 ± 1·90	.59·20 ± ·216	65.0 ± .623	37.32 ± 427	38.96 ± .670	40.24 ± .912
TV-23	30·5 ± ·774	44·4 ± .632	55·5 ± ·447	45·0 ± ·497	58·00 ± ·470	70.0 ± .577	36.45 ± .509	37.23 ± .427	39.48 ± .707
TV-18	52·77 ± ·933	58·33 ± 1·19	69·4 ± ·707	52·77± ·447	65·55 ± 1·86	80·55 ± 1·21	40.36 ± .774	36.00 ± .142	38.50 ± .583
TV- 9	33·33 ± ·365	38·8 ± 1·39	47·2 ± ·933	40·60 ± .428	50·95 ± ·683	56·66 ± ·921	30.56 ± .707	33.32 ± .928	35.46 ± .991

* Average of 3 experimental sets and average of 50 leaves inoculated per set, Summer - April,; Rainy - July; Autumn - October.
± = Standard error.

Table 8A : Analysis of Variance of disease in different tea varieties under different seasons (data of 96h of inoculation).

Source of variation	d.f.	S.S.	M.S.	F
Between light sources	5	24043	480.86	25·05
Between varieties	2	1351·6	675·8	35·17
Residual	10	191·93	19·19	

Both 'F' values highly significant at 1% level.

Effect of different seasons on brown blight development in different tea varieties

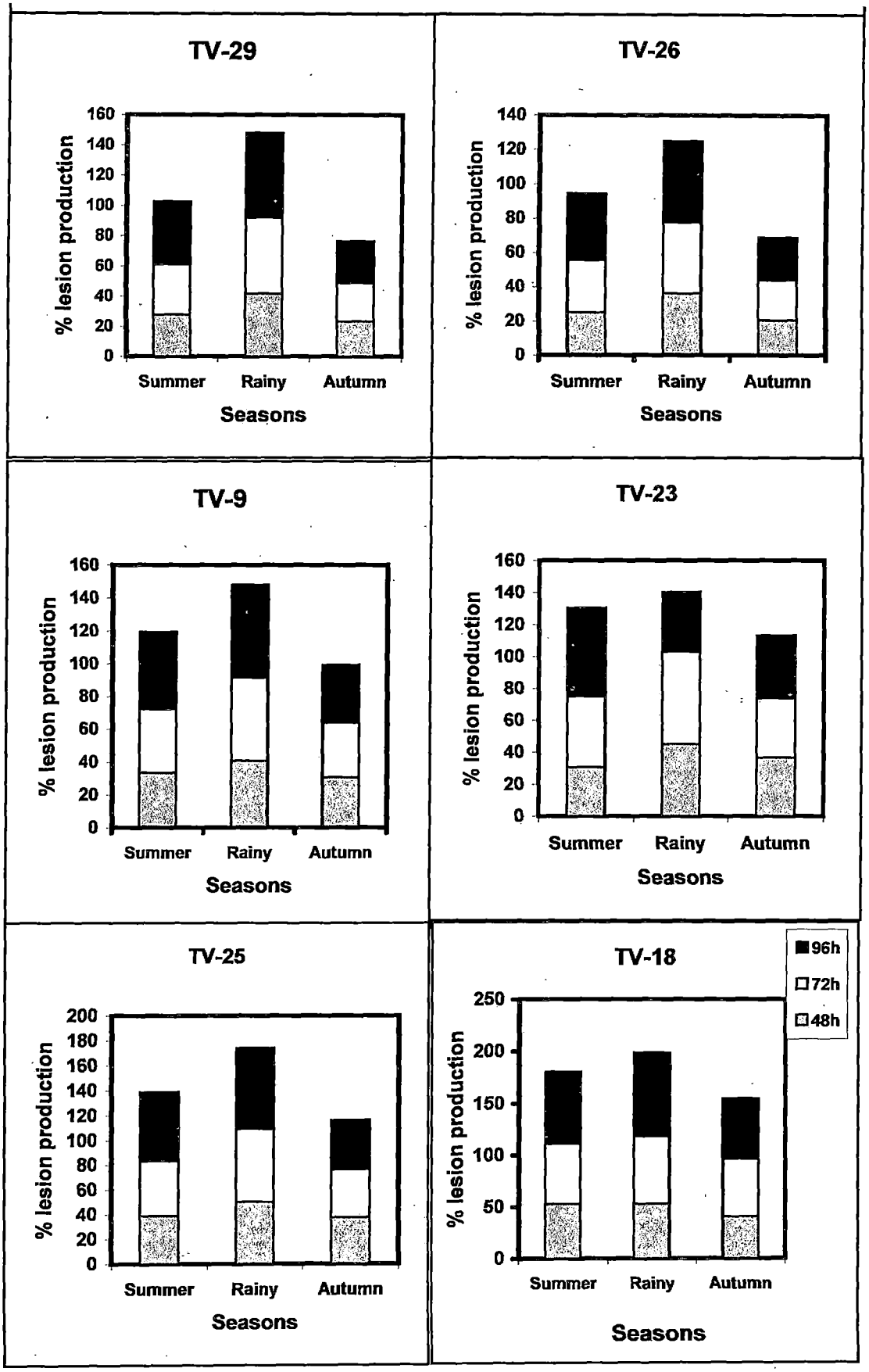


Fig.5

the Tea estates were selected, i.e. TV-26, 25, 23, 22, 9 and Teenali-17. In each case a minimum of 25 bushes were surveyed. The survey was under taken on a monthly basis during 3 successive years i.e. 1996, 1997 and 1998. Results are presented in Table 9-12 and Fig.6.

Symptoms of brown blight disease were common on the lower leaves (PlateVIII). in all three years tested. Disease occurrence was negligible during the winter months of December, January & February.

4.4. Meteorological data of three years

Monthly meteorological data were collected for 1996, 1997 and 1998 which included maximum and minimum temperature, percentage of relative humidity in the morning and afternoon, hours of sunshine and monthly rainfall. Maximum temperature ranged from approximately 22°C to 33°C while minimum ranged from 9°C to 25°C (Table 13, Fig. 7).

Relative humidity ranged from 87% to 95% in the morning and from 42% to 75% in the afternoon (Table 14; Fig.8). The mean hour of sunshine ranged from approximately 4.5 in January to 9 in May (Table 15; Fig. 9A).Monthly rainfall showed maximum rain during the rainy season, i.e. June-September. A mean monthly rainfall of 1040 mm was obtained in July (Table 16; Fig. 9B).

4.5. Correlation of environmental factors with disease occurrence

In order to determine whether disease development in nature is correlated with different environmental factors, Karl Pearson's correlation coefficient was calculated in respect of disease occurrence and the various factors. Mean data of the three years was used in all cases. When mean disease incidence of all varieties for three years was correlated with the different factors, positive significant correlation was obtained with minimum temperature, relative humidity and rainfall while negative correlation with hour of sunshine was seen. A correlation co efficient of 0.78 was obtained with disease and minimum temperative whereas maximum temperature was not correlated to disease occurrence ($r = 0.06$). Disease was more significantly correlated to relative humidity in the afternoon (RH2) with an 'r' value of 0.82 while the 'r' value for R.H.1 (morning humidity) and disease also showed correlation ($r = 0.70$) at 5% level. Disease occurrence was also significantly positively correlated with rainfall ($r=0.84$) and negatively correlated with hours of sunshine ($r=0.73$). Results of the correlation coefficient showing 2- tailed significance is presented in Table 17. When disease occurrence in different varieties was correlated

Table 9 : Occurrence of brown blight disease during 1996 in different tea varieties.

	% Disease incidence*					
Month	**TV-26**	**TV-9**	**TV-22**	**TV-23**	**TV-25**	**Tecnali 17**
March	3.55 ±1·08	6.36 ±·534	6·40 ±2.21	8·54 ±1·40	7·75 ±1·73	6·42 ±·912
April	3·6 ±1·11	6·38 ±2·62	6.44 ±1·47	8·59 ±1·15	7·80 ±1·69	6·53 +1·89
May	3·66 ±1·60	6·40 ±1·47	6·46 ±2·04	8·62 ±2·10	7·90 ±1·29	6·53 ±1·69
June	3·70 ±2·44	6·41 ±1·86	7·49 ±1·84	8·64 ±1·20	7·91 ±1·36	6·59 ±1·77
July	4·73 ±1·85	7·44 ±1·46	7·51 ±1·69	9·66 ±1·05	7·95 ±·907	7·62 ±1·84
August	4·76 ±2·01	7·46 ±1·85	7·54 ±1·82	9·70 ±1·17	7·97 ±1·60	7·67 ±1·10
Sept.	4·78 ±·966	7·48 ±2·44	7·56 ±1·29	9·73 ±·912	7·49 ±1·73	7·69 ±2·10
October	3·44 ±·966	6·31 ±1·68	6·26 ±1·29	7·43 ±1·69	6·65 ±1·55	6·19 ±1·50
Nov.	3·33 ±1·47	6·27 ±1·99	6·16 ±1·82	7·26 ±1·68	6·19 ±·743	5·95 ±1·29
Mean	3·94	6·72	6·86	8·68	7·56	6·80
CD(5%)	·156	·136	·-83	·415	·262	·265

* Average of 25 bushes

± = Standard error.

Table 10 : Occurence of brown blight disease during 1997 in different tea varieties.

	% Disease incidence*					
Month	**TV-26**	**TV-9**	**TV-22**	**TV-23**	**TV-25**	**Tecnali 17**
March	3·72 ± 1·88	7·15 ± ·743	7·50 ± 1·17	9·60 ± 1.36	8·23 ± 1·60	7·25 ± 1·10
April	3·98 ± ·907	7·20 ± 1·08	7·55 ± 1·18	9·70 ± 1·36	8·75 ± 1·68	7·53 ± 1·77
May	4·20 ± ·600	7·33 ± 1·11	7·65 ± 1·84	9·98 ± 1·81	9·00 ± 1·29	7·88 ± 1·97
June	4·35 ± 2·01	7·50 ± 1·60	7·80 ± 1·16	10·23 ± ·912	9·15 ± 1·55	8·17 ± 1·56
July	4·50 ± 1·17	8·00 ± 1·47	8·10 ± 2·62	10·35 ± 1·44	9·50 ± 1·55	8·43 ± 1·07
August	4·68 ± 1·84	8·23 ± 2·04	8·55 ± 2·92	10·66 ± 1·83	10·25 ± 1·10	8·73 ± 2·22
Sept.	4.70 ± 2·44	8·50 ± 1·47	8·79 ± 1·92	10·75 ± 1·79	10·83 ± 1·99	9·02 ± ·912
October	4·00 ± ·966	7·52 ± 1·82	8·05 ± 1·29	9·50 ± 1·93	9·62 ± 1·99	9·00 ± 1·29
Nov.	3·90 ± 1·47	7·00 ± 1·68	7·40 ± 1·01	9·43 ± 1·93	9·12 ± 1·47	8·33 ± 1·05
Mean	4·22	7·60	7·89	10·02	9·38	8·26
CD(5%)	··345	·189	·170	·163	·338	·244

* Average of 25 bushes

± = Standard error.

Table 11 : Occurence of brown blight disease during 1998 in different tea varieties.

	% Disease incidence*					
Month	**TV-26**	**TV-9**	**TV-22**	**TV-23**	**TV-25**	**Tecnali 17**
March	3·60 ± ·966	7·06 ± ·967	7·39 ± 1·41	9·42 ± 1·47	7·75 ± 1·33	7·42 ± 2·05
April	3·69 ± 1·39	7·10 ± ·534	7·50 ± 1·60	9·52 ± 1·20	7·86 ± 1·74	7·53 ± 1·05
May	3·81 ± 1·72	7·20 ± 1·24	7·70 ± 2·21	9·58 ± 1·40	7·95 ± 2·01	7·60 ± ·724
June	3·86 ± 2·04	7·33 ± 2·22	7·79 ± 1·71	10.00 ± 1·47	8·50 ± 1·47	7·75 ± 2·47
July	4·90 ± 1·29	7·83 ± 1·94	8·70 ± 1·86	10·50 ± 2·04	9·60 ± 2·10	8·00 ± 1·73
August	4·66 ± 1·76	7·00 ± 1·82	8·98 ± 1·46	11·00 ± 1·82	10·00 ± ·912	8·10 ± ·912
Sept.	4·33 ± ·614	7·00 ± 1·82	9·52 ± 1·76	11·25 ± 1.05	10·50 ± 1·29	8·30 ± 1·15
October	3.40 ± ·812	6·01 ± 1·39	8·00 ± 1·47	9·20 ± 1·47	9·40 ± 1·50	8·13 ± 1·89
Nov.	3·56 ± 1·78	6·00 ± 1·39	7·60 ± ·702	9·00 ± 1·68	9·00 ± 1·29	7·40 ± 1.69
Mean	3·97	6·94	8·13	9·94	8·95	7·80
CD (5%)	·186	·279	·251	·279	·334	·092

* Average of 25 bushes

± = Standard error.

Table 12 : Mean occurence of brown blight disease during three years.

	% Disease incidence (Mean)*					
Month	**TV-26**	**TV-9**	**TV-22**	**TV-23**	**TV-25**	**Tecnali 17**
March	3·62 ± 1·30	6·85 ± ·747	7·09 ± 1·59	9·18 ± 1·41	7·92 ± 1·55	7·03 ± 1·35
April	3·75 ± 1·13	6·89 ± 1·41	7·16 ± 1·41	9·27 ± 1·23	8·13 ± 1·70	7·19± 1·57
May	3·89 ± 1·30	6·97 ± 1·27	7·27 ± 2·03	9·39 ± 1·77	8·28 ± 1·53	7·35 ± 1·29
June	3·97 ± 2·16	7·08 ± 2·89	7·69 ± 1·57	9·62 ± 1.19	8·52 ± 1·44	7·50 ± 1·93
July	4·71 ± 1·43	7·75 ± 1·62	8·10 ± 2·05	10·17 ± 1·51	9·01 ± 1·51	8·01 ± 1.54
August	4·70 ± 1·87	7·56 ± 1·90	8·35 ± 2·06	10·45 ± 1·60	9·40 ± 1·20	8·16 ± 1·41
Sept.	4·60 ± 1·34	7·66 ± 1·91	8·62 ± 1·65	10.56 ± ·900	9·50 ± 1.65	8.33 ± 1.38
October	3·61 ± ·914	6·61 ± 1·63	7·43 ± 1.35	8·71 ± 1.69	8·55 ± 1.68	7·77 ± 1.56
Nov.	3.59 ± 1.57	6·42 ± 1.67	7·057 ± 1·18	8·56 ± 1·68	8·10 ± 116	7·22 ± 1·34
Mean	4·04	7·08	7·64	9·54	8·60	7·61
CD(5%)	·067	·078	·082	·116	·092	·062

* Average of 25 bushes
± = Standard error.

Table 12A : Analysis of variance of natural occurrence of brown blight disease during different months of the year (data presented in Table 12).

Source of variation	d.f.	S.S.	M.S.	F
Between varieties	8	157.25	19.66	393.2
Between months	5	13.14	2.63	52.6
Residual	40	1.82	0.05	

Both 'F' values significant 1% level.

Monthly incidence of brown blight disease on different tea varieties

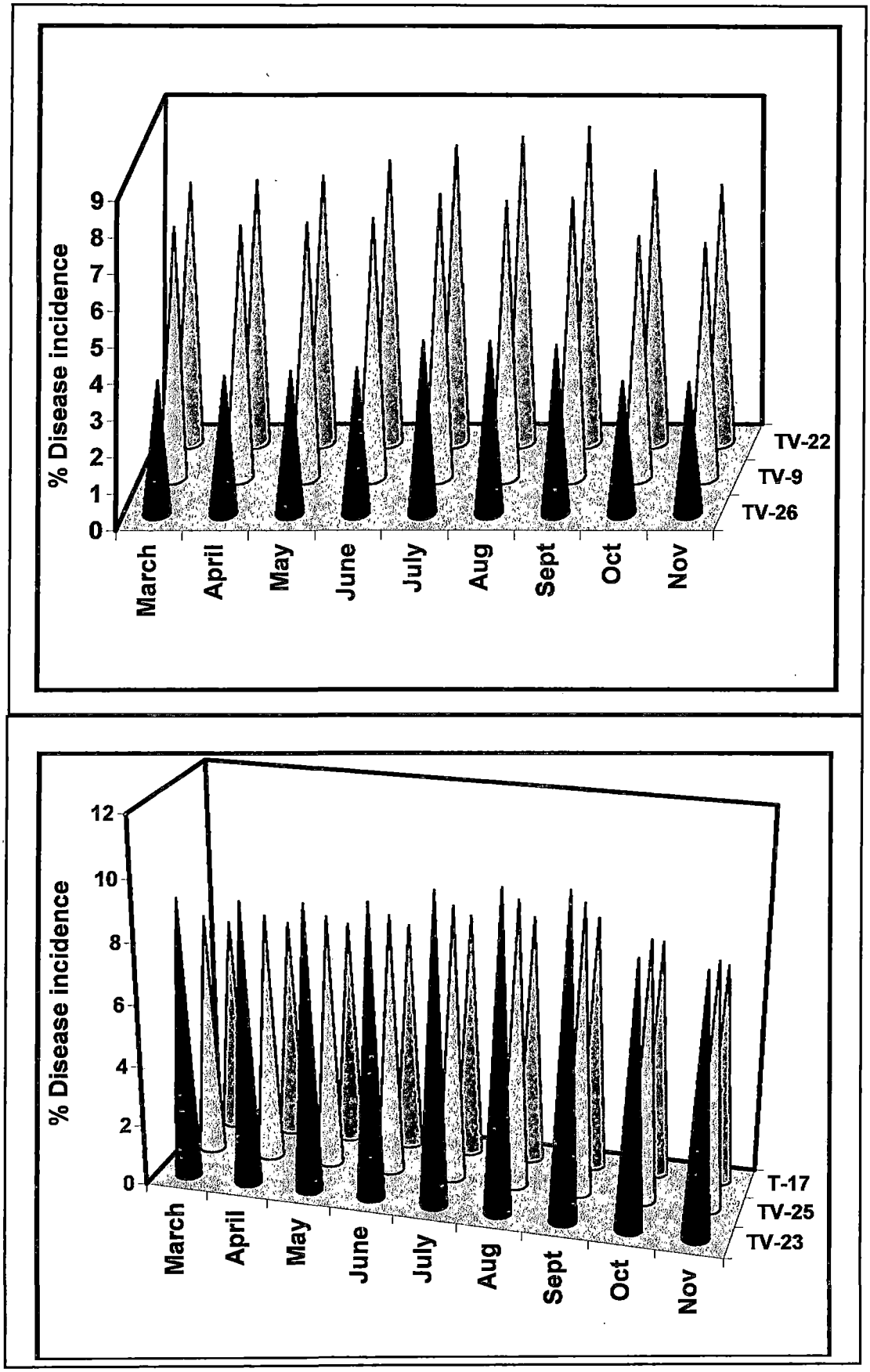


Fig.6

Plate VIII (figs.A & B) – *Portion of healthy (A) and naturally brown blight infected (B) bush of TV-23*

Table 13 : Maximum and minimum temperature during three years (1996-1998).

	Maximum temperature (°C)				Maximum temperature (°C)			
Month	**1996**	**1997**	**1998**	**Mean**	**1996**	**1997**	**1998**	**Mean**
January	23.5	22.7	21.1	22·4 ± ·705	10·0	9·0	8·4	9·1 ± ·549
February	27.0	23.3	25.1	25·1 ± 1·06	13·2	9·3	11·9	11·4 ± 1·14
March	32·0	30·8	30·9	31·2 ± ·385	17·8	16·3	16·7	16·9 ± ·449
April	34·1	33·2	32·6	33.3 ± ·435	22·3	21·3	21·7	21·8 ± ·241
May	33·5	33·6	34·4	33·8 ± ·285	22·8	23·8	24·6	23·7 ± ·521
June	33·8	33·4	32·6	33·2 ± ·355	24·5	25·1	24·7	24·7 ± ·182
July	32·5	31·4	29·4	31·1 ± ·907	25·3	24·9	24·5	24·9 ± ·230
August	33·1	34·1	31·4	32·8 ± ·789	24·9	25·4	24·5	24·9 ± ·261
September	32·9	32·5	32·9	32·7 ± ·141	24·5	24·2	24·8	24·5 ± ·173
October	33·4	32·7	33·0	33·0 ± ·204	20·8	20·5	23·0	21·4 ± ·788
November	31·6	29·8	32·1	31.1 ± ·472	16·4	17·3	19·1	17·6 ± ·793
December	27·3	26·7	28·1	27·3 ± ·408	11·1	11·5	12·5	11·7 ± ·416

± = Standard Error.

Monthly maximum amd minimum temperatures for 1996-1998

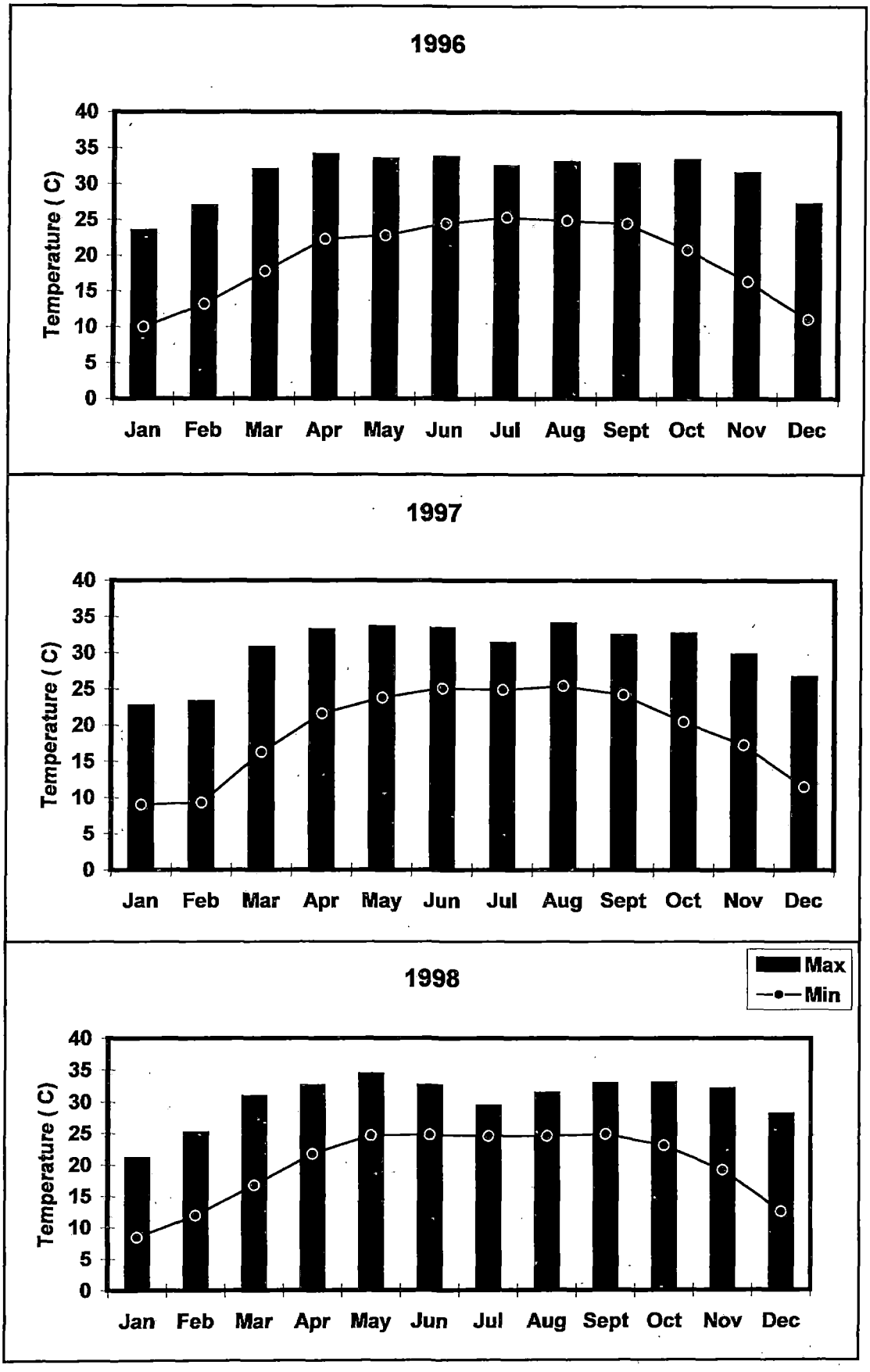


Fig.7

Table 14 : Relative humidity during three years (1996-1998).

	% Relative humidity - 1				% Relative humidity - 2			
Month	**1996**	**1997**	**1998**	**Mean**	**1996**	**1997**	**1998**	**Mean**
January	91	94	95	93·3 ± 1·20	53	42	53	49·3 ±3·66
February	89	94	93	92 ± 1·52	48	59	49	52·0 ±3·51
March	90	89	88	89 ± ·577	54	48	44	48·6 ±2·45
April	88	90	92	90 ± 1·15	55	55	59	56·3 ± 1·33
May	85	88	90	87·6 ± 1·45	62	58	67	62·3 ± 2·60
June	91	92	93	92 ± ·577	63	65	71	66·3 ± 2·40
July	93	93	95	93·6 ± ·668	65	75	86	75·3 ± 6·06
August	94	92	95	93·6 ± ·883	69	62	78	69·66 ± 4·62
September	95	95	94	94·6 ± ·336	63	69	64	65·3 ± 1·85
October	92	94	95	93·6 ± ·883	64	63	59	62·0 ± 1·52
November	94	95	85	91·3 ± 3·17	53	53	42	49·3 ± 3·66
December	96	94	95	95 ± ·577	40	46	40	42·0 ± 200

R.h. 1 = Morning; R.h. 2 = Afternoon.
± = Standard error.

Monthly relative humidities for 1996-1998

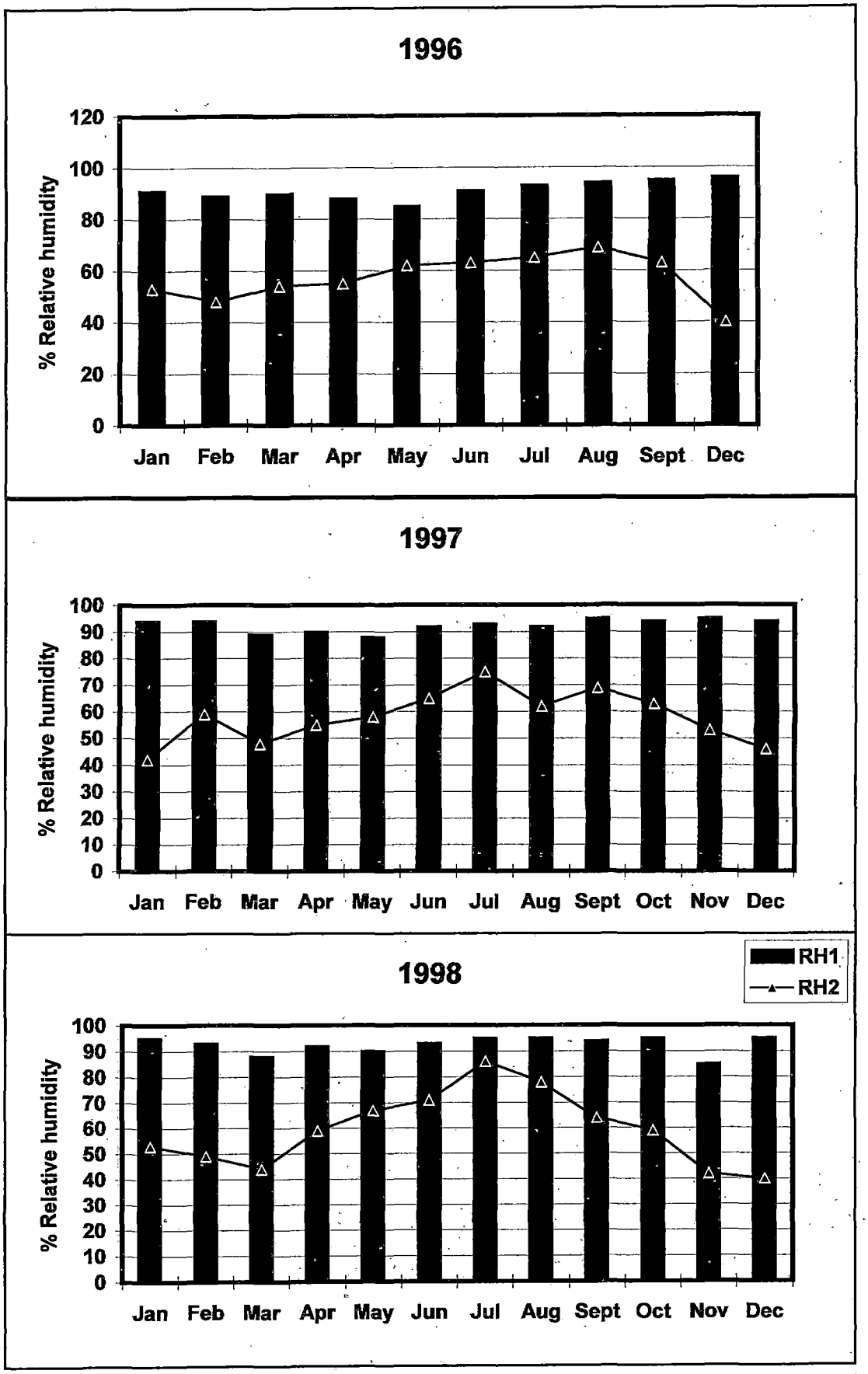


Fig.8

Table 15 : Average hours of Sunshine during 1996-1998.

Month	1996	1997	1998	Mean
January	6·2	4·8	2·4	4·46 ± 1·10
February	7·9	4·3	5·5	5·9 ± 1·05
March	8·4	8·6	6·9	7·9 ± ·538
April	8·9	6·4	7·2	7·5 ± ·737
May	8·0	8·8	9·5	8·7 ± ·435
June	7·8	8·1	7·0	7·63 ± 1·63
July	5·9	7·2	1·7	4·93 ± 1·650
August	7·5	7·4	4·9	6·6 ± ·850
September	6·9	5·7	6·9	6·5 ± ·400
October	8·3	8·1	7·1	7·83 ± ·220
November	7·5	7·9	7·6	7·6 ± ·129
December	7·3	5·9	7·3	6·83 ± ·476

± = Standard error.

Mean monthly rainfall for 1996-1998

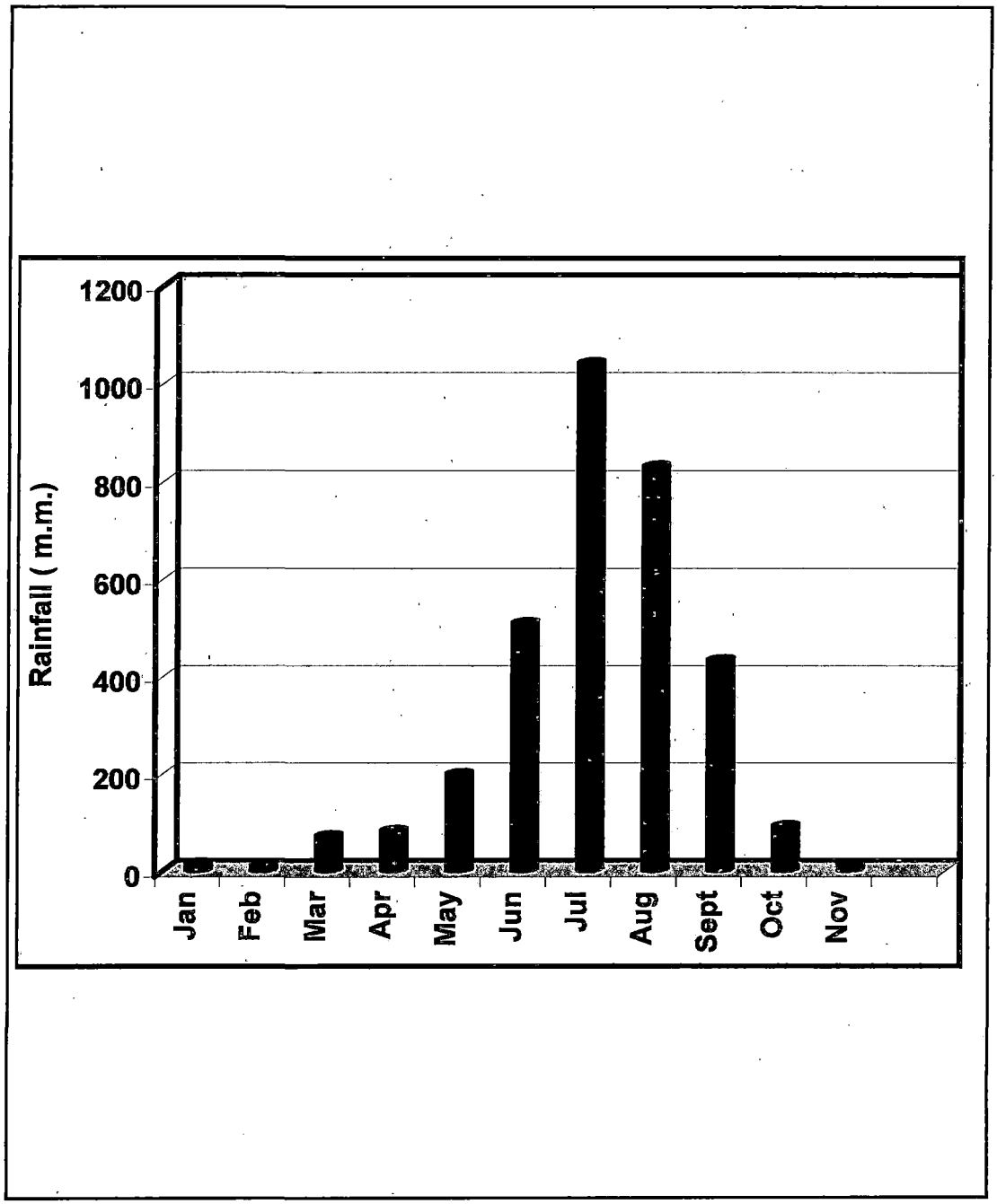


Fig.9A

Table 16 : Average monthly rainfall during 1996-1998.

	Rainfall (mm)			
Month	**1996**	**1997**	**1998**	**Mean**
January	36·3	8·3	00·0	14·0 ± 8·08
February	5·5	5·9	15·1	8·8 ± 3·13
March	Trace	55·1	156·9	70·66 ± 29·15
April	13·8	55·4	174·3	81·16 ± 48·08
May	323·1	71·9	197·4	197·46 ± 48·515
June	260·3	481·4	782·4	508·03 ± 151·30
July	1069·9	765·3	1284·2	1039·8 ± 150·54
August	758·8	439·5	1289·1	829·13 ± 247·76
September	225·7	622·3	447·8	431·93 ± 114·76
October	123·1	4·8	144·4	90·7 ± 43·42
November	00·0	Trace	28·8	9·6 ±
December	00·0	76·2	00·0	25·4

± = Standard error.

Average hours of sunshine during 1996-1998

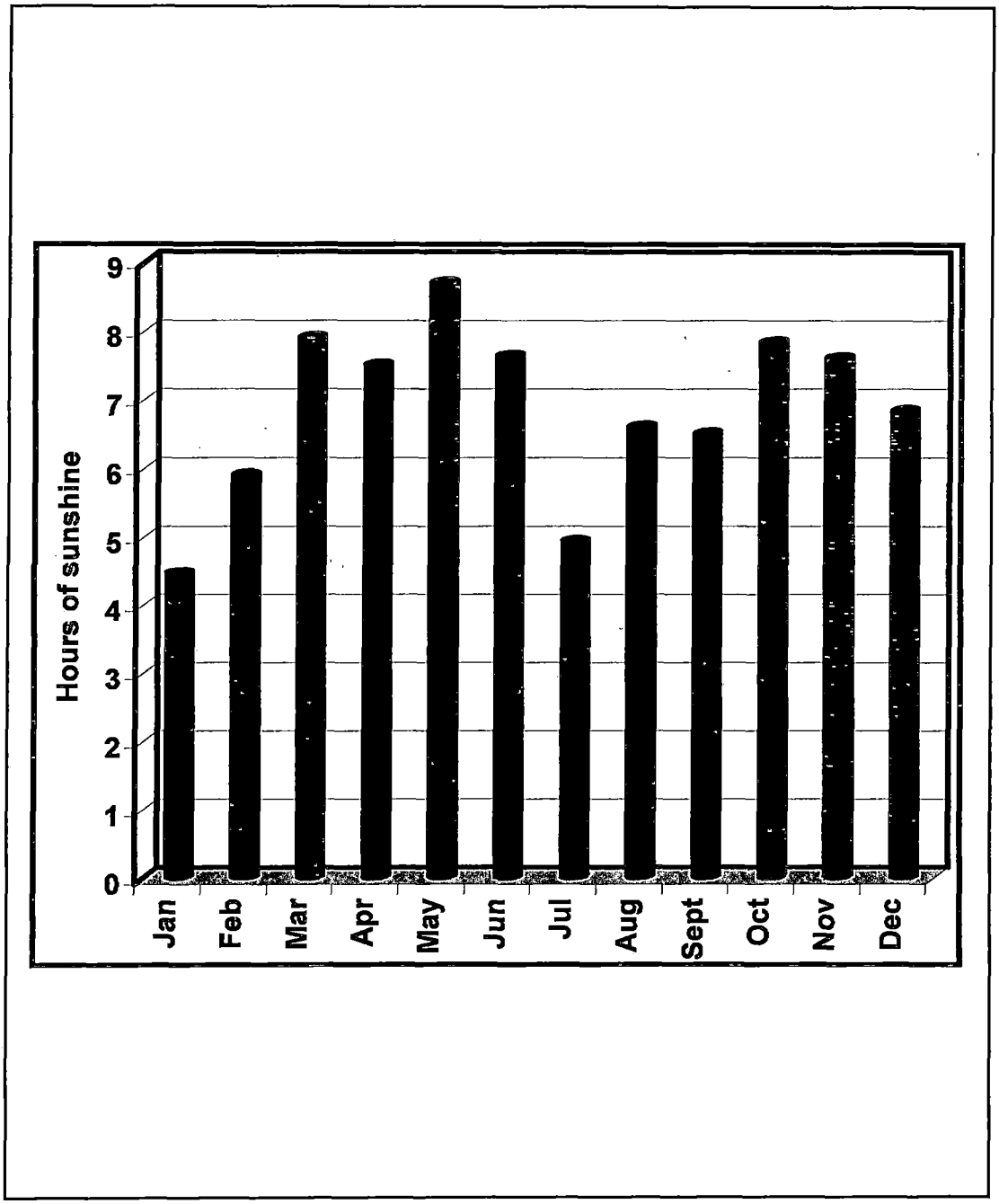


Fig.9B

Table 17 : Correlation matrix showing correlation between brown blight disease and environmental factors during 3 years (1996-1998). (2-tailed significance).

	Diasese[b]	Max. Temp.	Min Temp.	R.H.I[a]	R.H.2[a]	Rainfall	House of sunshine
Disease[b]	1·00	·056	·776*	·697**	·816*	·838*	-·7348**
Max Temp.	·056	1·00	·526	-·147	·244	-·127	-·459
Min. Temp.	·776*	·526	1·00	·445	·927*	·742**	-·447**
R.H.I.	·697**	-·147	·445	1·00	·583	·559	-·705**
R.H.2	·816*	·224	·927*	·583	1·00	·886*	-·651**
Rainfall	·838*	-·127	·742**	·559	·886*	1·00	-·821*
House of sunshine	-·734**	·459	-·447	-·705**	-·651**	-·821**	1·00

'r' values are pearson's correlation co-efficient.

* significant at P = 0·01, ** significant at P=0·05, Rest insignificant.

[a] R.H.1 = % Relative humidity (morning) 2

R.H.2 = % Relative humidty (afternoon)

[b] Disease-computed as % incidence of mean of all varieties.

separately with the environmental factors the trend in correlation was more or less similar as in the mean of all varieties. All the varieties (TV-9, 22, 23, 25, 26 and Teenali 17) separately showed significant correlation in disease occurrence with minimum temperature, r.h.2, rainfall (positive) and hours of sunshine (negative). However significant correlation of disease occurrence with r.h.1 was obtained only in TV-22 (r=.77), 25 (r=.80) and T 17 (r=.85). In the other 3 varieties correlation was not significant. In none of the varieties was disease occurrence correlated to maximum temperature (Table18) Results are also presented graphically in Figs. 10, 11, 12 & 13.

Table 18 : Correlation of brown blight disease occurrence in different tea varieties with environmental factors.

	'r' values[a]					
Varieties	**Max. Temp.**	**Min. Temp.**	**R.H.I.**	**R.H.2**	**Rainfall**	**House of sunshine**
TV-9	·004	·762*	·510	·804*	·875*	-·768*
TV-22	·076	·749**	·774*	·777*	·775*	-·701**
TV-23	·110	·758*	·488	·736*	·804*	-·656**
TV-25	·115	·747**	·803*	·782*	·748*	-·675**
TV-26	·035	·762*	·615	·824*	·906*	-·804*
T-17	·097	·722**	·850*	·792*	·753*	-·677**

[a] Pearson's correlation coefficient.
* Significant at P=0·01, ** Significant at P=0·05, rest insignificant.
Data for correlation are mean of three years (1996-1998).

4.6. Factors affecting mycelial growth *in vitro*

Results of previous experiments indicated that disease development both in the field and under laboratory condition is affected by various factors. In order to determine the effect of different factors on mycelial growth of *G. cingulata in vitro,* the following experiments were under taken. Effects of different media, incubation period, pH of medium, different carbon and nitrogen sources on the mycelial growth of *G. cingulata* were studied.

4.6.1. Different media

Mycelial growth of *G. cingulata* was studied on a number of solid and liquid media, in order to determine the nature of growth, type of mycelia, sporulation etc.

4.6.1.1. Solid media

Nine different media (potato dextrose agar, potato sucrose agar, boiled tea agar, crushed tea agar, czapex dox agar, yeast agar, Richard's agar, Elliot's agar and carrot juice agar) were used to study the growth and sporulation of *G. cingulata.* Observation was taken after 4,6, 8, and 10 days of incubation. Of all the media, maximum growth and sporulation was observed in Richard's agar medium (Table 19). Mycelial growth was white fluffy with profuse orange acervuli (Plate-IX). Czapex dox agar was also conducive to good growth and

Correlation of average temperatures and hours of sunshine with occurrence of brown blight disease in tea (TV-26)

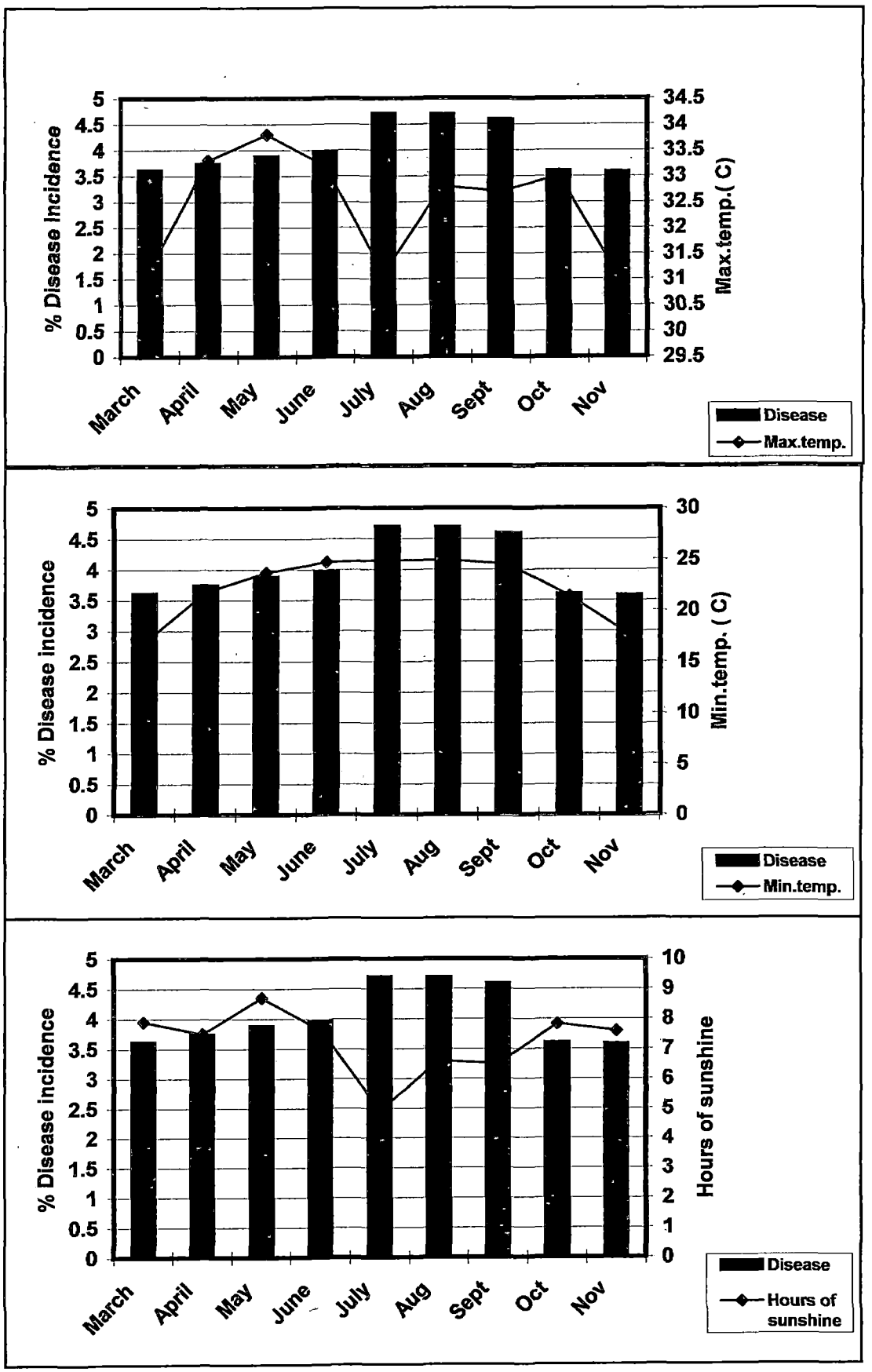


Fig.10

Correlation of average relative humidities and rainfall with occurrence of brown blight disease in tea (TV-26)

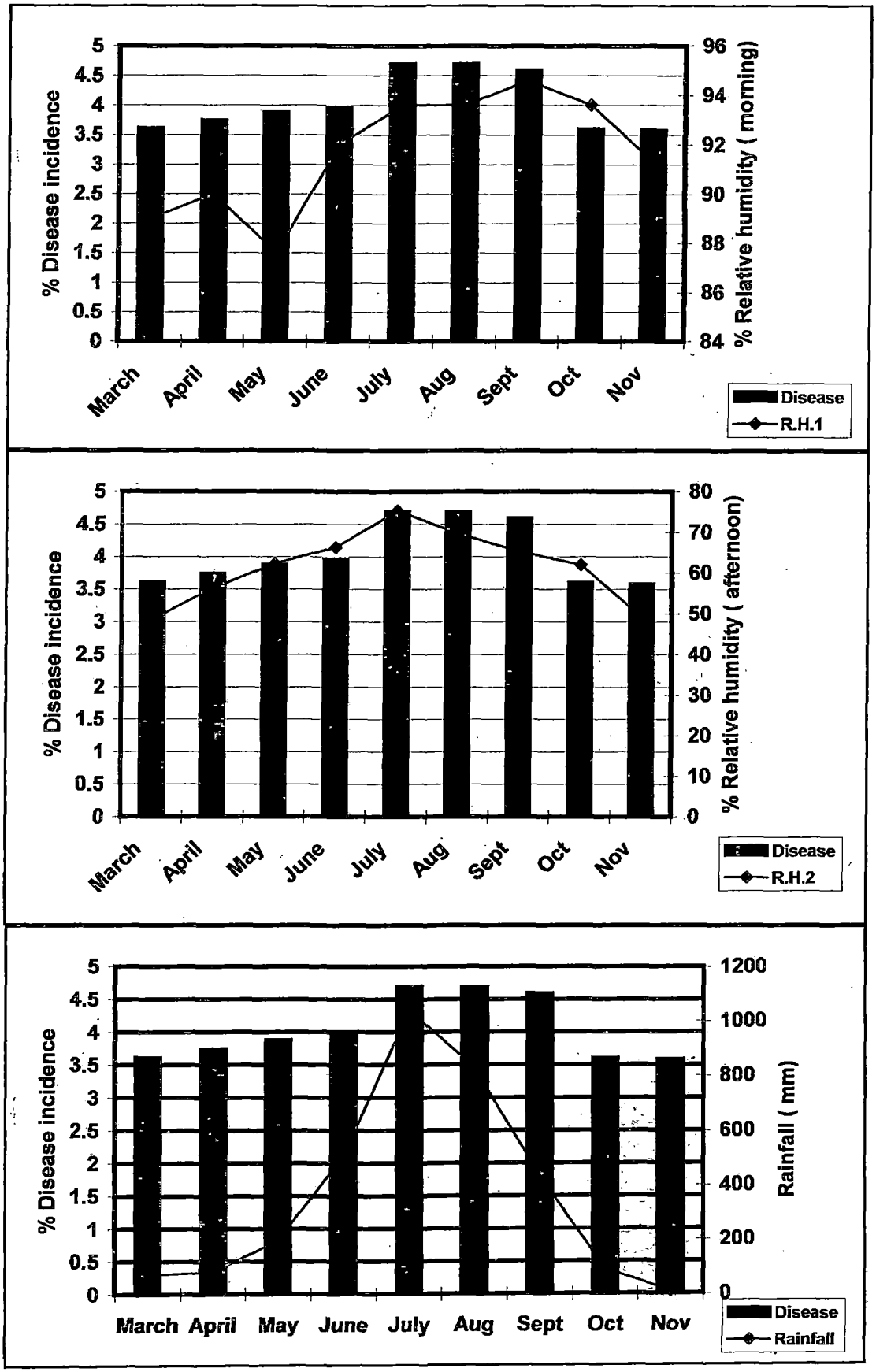


Fig.11

Correlation of average temperatures and hours of sunshine with occurrence of brown blight disease in tea (TV-23)

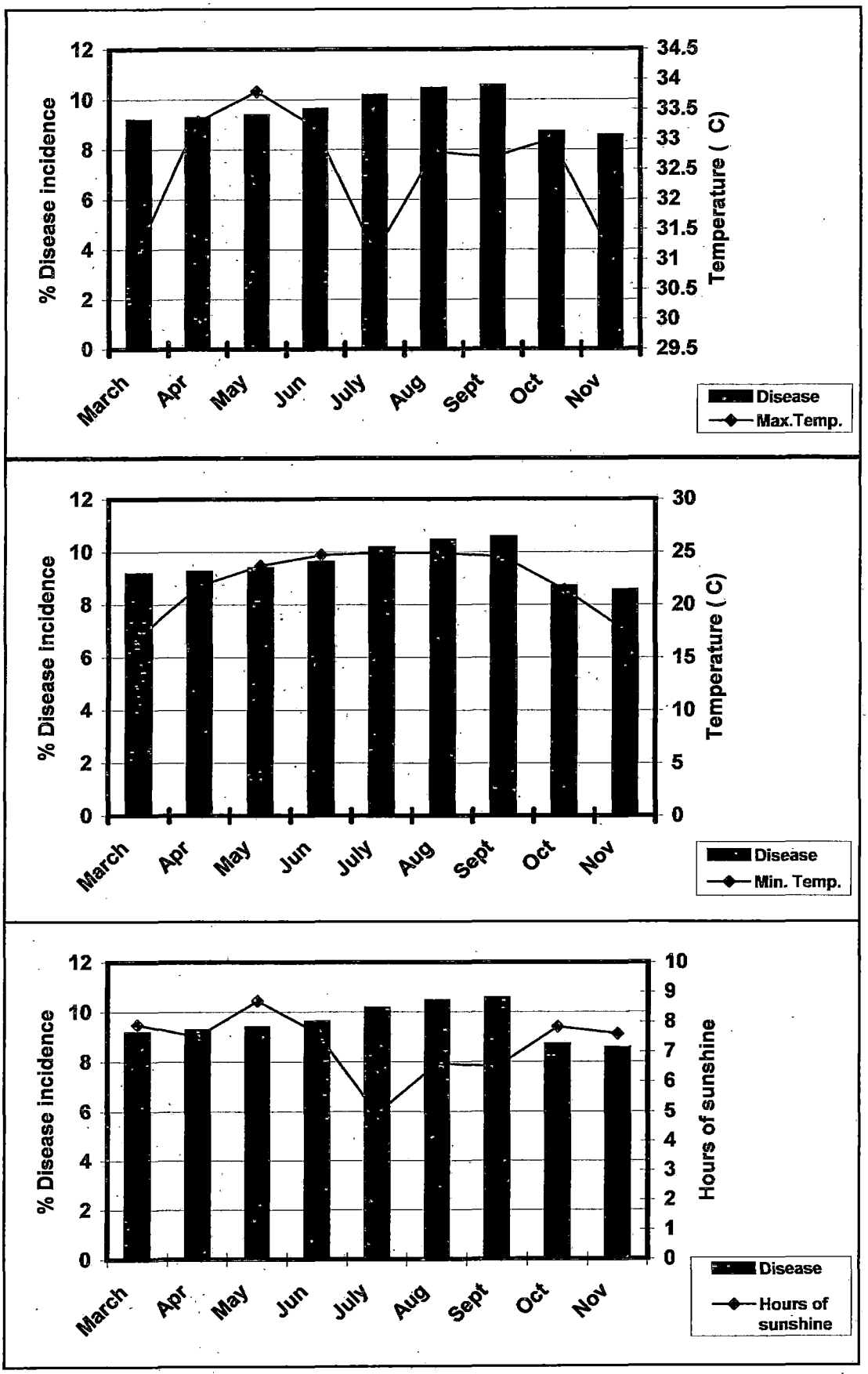


Fig.12

Correlation of relative humidities and rainfall with occurrence of brown blight disease in tea (TV-23)

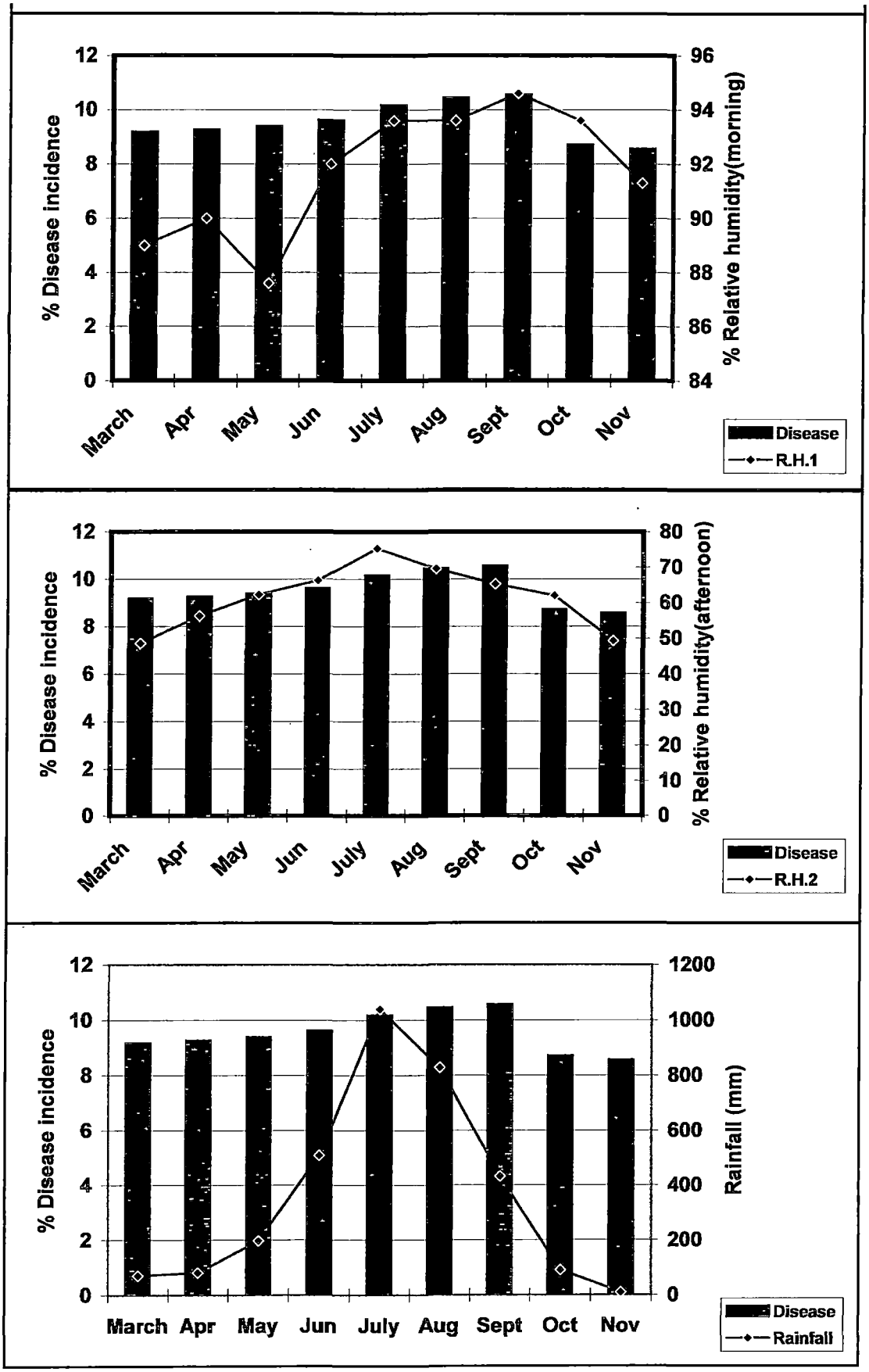


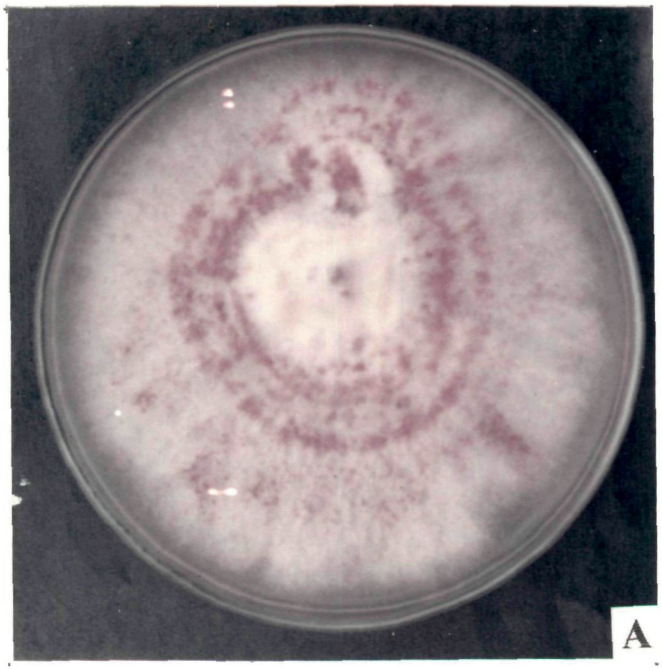
Fig.13

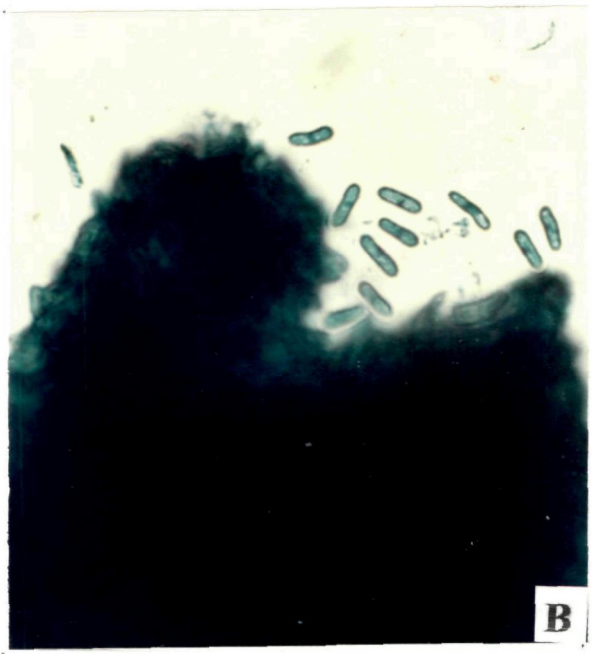
Table 19 : Effect of different solid media on mycelial growth.

Media	Average diameter (c.m)*				Nature of mycelia[a]	Sporulation[a]
	4d	6d	8d	10d		
Richard's Agar	5·16 ± ·306	7·1 ± ·306	8·00 ± 1·00	9·00 ± 1·00	Fluffy, white	Excellent, orange acervuli
Potato dextrose Agar	3·1 ± ·294	4·15 ± ·663	5·8 ± ·577	6·6 ± ·336	Fluffy, grayish white	Blackish acervuli
Potato sucrose Agar	4·3 ± 1·00	6·3 ± 1·00	7·8 ± ·716	7·00 ± ·577	Fluffy, grayish white	Blackish acervuli
Czapek dox Agar	4·71 ± ·334	5·9 ± ·493	7·0 ± ·330	8·6 ± 1·33	White fluffy	Good sporulation orange acervuli
Carrot juice Agar	3·4 ± 1·00	3·8 ± ·754	4·63 ± ·549	4·9 ± ·550	Submerged	No sporulation
Crushed tea Agar	3·4 ± 1·00	3·9 ± ·330	5·53 ± ·522	5·6 ± ·141	Submerged	No sporulaiton
Boiled tea Agar	2·4 ± 1·00	3·2 ± ·200	5·20 ± 200	5·3 ± ·200	Submerged	No sporulation
Yeast extract dextrose Agar	4·4 ± 1·00	5·6 ± ·141	7·30 ± ·200	7·8 ± ·366	Slightly fluffy	Little sporlation
Elliott's Agar	2·9 ± ·330	3·6 ± ·115	4·83 ± ·366	5·2 ± 20	Submerged	No sporulation

*Average of 3 replicates; a = observation of 10 days' growth

± = standard error.





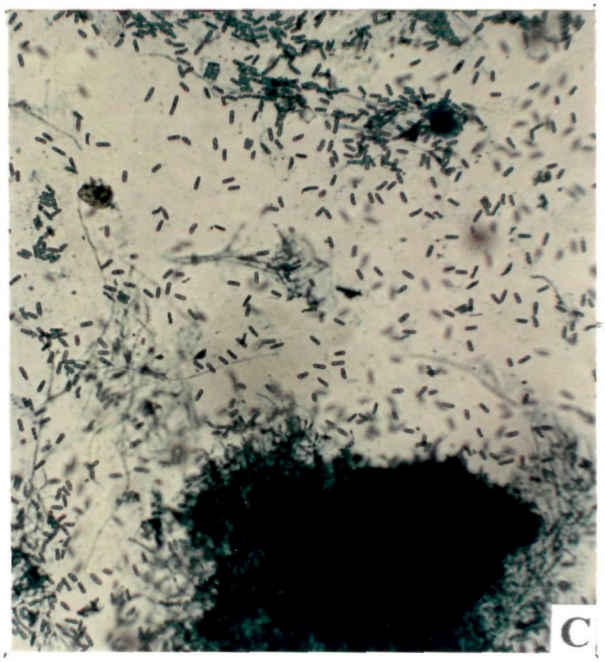


Plate IX (figs. A-C) : A- *G. cingulata* growing on Richard's Agar medium, showing orange acervuli; B & C- Acervulus and conidia of *G.cingulata* , under high power (45x) and low power (10x) magnification

sporulation. In both of these media within 10 days of growth the whole petridish (9cm dia) was covered. On both potato dextrose agar and potato sucrose agar mycelia were grayish white with fluffy growth; and black acervuli acuvuli were noticed. In the other tested media mycelial growth was submerged with little or no sporulation (Plate X).

4.6.1.2. Liquid media

Six different liquid media (Richard's broth, nutrient broth, potato dextrose broth, (czapek dox broth, yeast extract broth, potato sucrose broth were also tested for their effect on mycelial growth of *G. cingulata.* Dry weights were taken after 3,6,9,12 and 15 days of incubation. Among the tested media maximum growth (620mg) was observed in Richard's medium and least in yeast extract dextrose broth (285mg). All the others were more or less similar (Table 20).

Table 20 : Effect of different liquid media on growth of *G. cingulata.*

	Average dry wt of mycelia (mg)*				
Media	**3d**	**6d**	**9d**	**12d**	**15d**
Potato dextrose borth	121·0 ± 1·35	280 ± 1·15	423·3 ± 2·35	489 ± 1·67	399 ± ·577
Nutrient broth	50·0 ± 1·25	90 ± 1·67	110 ± 1·15	490 ± 1·77	470 ± 1·77
Richard's broth	150·0 ± 2·44	269 ± 1·67	487 ± 100	626 ± 2·00	375 ± ·570
Czapek dox broth	48·0 ± 5·7	262 ± ·816	390 ± 2·51	450 ± 1·73	345 ± 1·67
Yeast dextrose broth	60·0 ± ·577	260 ± 1.22	357 ± ·577	285 + 1·10	268 ± 1·14
Potato sucrose broth	80·2 ± 2·44	95·6 ± 1·77	140·8 ± 1·15	400 ± 1·25	420 ± 1·35

* Average of 3 replicates.
±=standard error.

4.6.2. Incubation period

G. cingulata was grown in Richards medium for a period of 30 days and mycelial growth was recorded after 5, 10, 15, 20, 30 days of incubation. Growth increased from 52mg at 5 days to 576 mg at 10 days of growth, after which steady decline in growth was observed. After 30 days growth of mycelial dry weight had declined to 246mg (Table 21). Good sporulation was observed from 10-20 days of growth. In all the other incubation periods sporulation was meager.

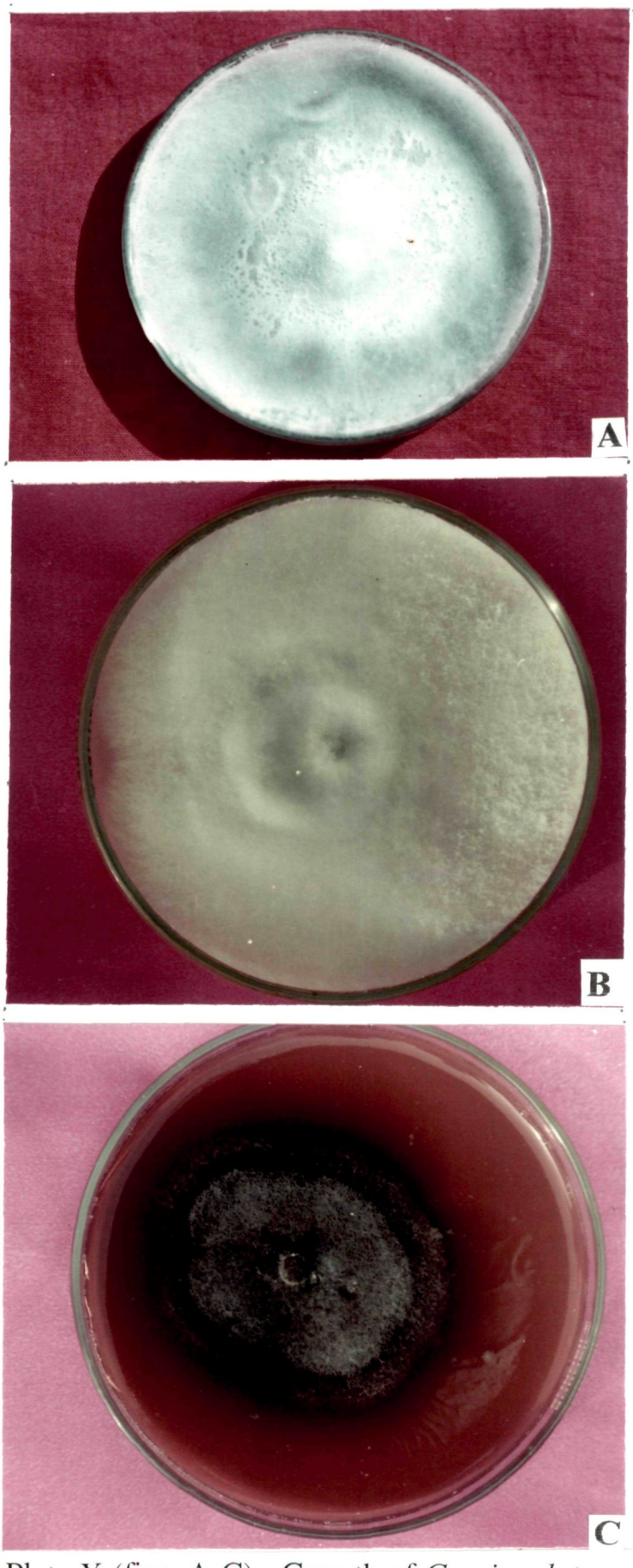


Plate X (figs. A-C) : Growth of *G. cingulata* in different solid media. A-Potato sucrose agar; B- Potato dextrose agar; C- Boiled tea agar

Table 21 : Effect of incubation period on mycelial growth of *G. cingulata*

Incubation period	Mean mycelial dry weight (mg)*
5	52·0 ± 1·15
10	572·0 ± 4·24
15	376·6 ± 2·88
20	300·0 ± 5·77
25	273·0 ± 2·30
30	246·0 ± 4·61

* Average of 3 replicates

± = Standard error.

4.6.3. pH

Growth of microorganisms is greatly influenced by the pH of the medium in which they grow. In order to study the effect of pH on growth, buffer systems have to be used to stabilize the pH which will otherwise vary Buffer solution with pH values ranging from 4-9 were prepared by mixing KH_2PO_4 and K_2HPO_4 each at a concentration of M/30. The medium (double strength RM) and the buffer was sterilized separately by autoclaving for 15 minutes at 15 lbs p.s.i pressure Equal parts of the buffer solution and the medium were mixed before use. Each flask containing 50 ml of medium with desired pH was then inoculated with mycelial block of *G. cingulata* and incubated for 10 days at 30°C. Results (Table 22) revealed that *G. cingulata* grew to certain extent in all tested pH (4,5,6,7,8 and 9), showing maximum growth (460mg) at pH 7. Minimum growth was recorded at pH 4 and 9. Sporulation was good in pH ranges from 5-7 above and below which little or no sporulation was observed.

Table 22 : Effect of different pH on mycelial growth of *G. cingulata.*

PH	
5	52·0 ± 1·15
4	295·0 ± 2·88
5	350· ± 1·15
6	415·0 ± 1.73
7	4650·0 ± 5·77
8	342·0 ± 2·30
9	310·0 ± 3·46

* Average of 3 repticates

± = Standard error.

4.6.4. Carbon source

Carbohydrates are major nutrients in any medium and the ability of microorganisms to grow in different media depends on their capacity to utilize the available nutrients. All carbohydrates are not utilized by the fungus at the same rate and hence growth rate varies with different carbon source. In the present study seven different carbon sources i.e. mannitol, sucrose, fructose, maltose, starch, dextrose, and lactose were tested for their effect on the growth of *G. cingulata.* Basal medium used was Richard's medium and sucrose of RM was replaced by equivalent amount of each carbohydrate. For control basal medium without any carbohydrate was used, Mycelia were harvested after 3,6,9, 12 & 15 days of incubation. Results (Table 23) revealed that maltose supported maximum growth followed by dextrose and sucrose. Good sporulation was also observed in these cases. Minimum growth was recorded when mannitol and lactose was used as the carbon sources. There was little growth in absence of any carbohydrates.

Table 23 : Effect of different carbon sources on mycelial growth of *G. cingulata.*

	Average dry wt. of mycelia (mg)*				
Media	**3d**	**6d**	**9d**	**12d**	**15d**
Mannitol	27·33 ± 120	94·3 ± 1·47	140·0 ± 1·20	138·2 ± ·883	130 ± ·336
Fructose	36·30 ± ·334	160·2 ± 1·00	310·6 ± ·84	302·2 ± ·88	300·0 ± 1·42
Maltose	38·5 ± 1·67	300·2 ± 1·20	328·2 ± ·40	420·0 ± 1·52	400·0 ± 1·42
Starch	28·3 ± 3·26	189·2 ± ·661	208·6 ± 3·36	214·6 ± 1·66	210 ±1·57
Dextrose	44·6 ± 3·36	143·3 ± 3·2	390·6 ± 1·67	395·2 ± 2·32	300·5 ± 1·42
Sucrose	38·2 ± 1·00	283·5 ± 1·67	380·0 ± 1·67	384·6 ± 1·67	292 ± 1·87
Lactose	24·0 ± 1·42	105·0 ± 1·47	144·0 ± 1·20	140·3 ± 1·42	93·0 ± 336
Basal medium (without -C)	10·5 ± 2·90	18·0 ± 1·00	21·3 ± 1·66	22·0 ± 2·2	20·5 ± 3·2

* Average of 3 replicates

±Standard error

Basal medium - Richard's Medium.

4.6.5. Nitrogen sources

Nitrogen is undoubdtedly the most important single nutrient necessary for the growth of any organism. The availability of nitrogen depends to a great degree on the form in which it is supplied. Hence, the most suitable nitrogen source for any particular microrganism can be only determined by testing a number of sources including both inorganic and organic. The effect of inorganic nitrogen sources (calcium nitrate, sodium nitrate, ammonium nitrate and potassium nitrate) as well as complex organic sources (peptone, urea, yeast extract and beef extract) on the mycelial growth of *G. cingulata* was tested. A basal medium (RM) without any nitrogen source was considered as control. Data recorded after 3,6,9,12 and 15 days of growth showed potassium nitrate to be optimum for growth of *G. cingulata,* followed by sodium nitrate, among all tested sources both inorganic and organic (Table 24). Among the organic sources, beef extract supported maximum growth.

Table 24 : Effect of different nitrogen sources on mycelial growth of *G. cingulata.*

Nitrogen Source	**Average dry wt. of mycelia (mg)***				
Inorganic	**3d**	**6d**	**9d**	**12d**	**15 d**
KNO_3	25·90 ± ·507	320·6 ± 1·67	460·2 ± 1·67	448 ± 4.68	440·2 ± 1·82
NH_4NO_3	22·30 ± 1·20	285·2 ± 1·67	339·6 ± 1·82	343·6 ± 1·87	320·2 ± 1·67
$Ca(NO_3)_2$	22·0 ± 1·67	238·2 ± 2·50	432·5 ± 1·50	430·2 ± 1·67	390·6 ± 1·87
$NaNO_3$)	22·0 ± 1·67	315·3 ± 1·67	434·6 ± 2·51	431·2 ± 3·53	398·2 ± 1·20
Organic					
Peptone	11·9 ± 1·67	195·0 ± 3·05	265·0 ± ·714	285·0 ± 1·67	200·0 ± 2·88
Urea	9·0 ± 1·67	143·7 ± ·336	210·0 ± 1·57	230·0 ± 1·00	190.0 ± 1·35
Yeast extract1	7·0 ± ·99	180·2 ± 1·67	203·7 ± 1·40	215·0 ± 4·58	175·0 ± 6·24
Beefextract	13·9 ± ·651	195·0 ± 1·67	296·3 ± 1·21	300·2 ± 1·21	290·1 ± 1·67
Control[a]	7·0 ± 1·67	10·1 ± ·617	11·9 ± ·667	12·3 ± ·617	9·5 ± ·313

* Average of 3 replicates; Basal medium - RM

a Control - without N;

± = Standard error.

Result of the above experiments clearly indicates that *G. cingulata* grows best in Richard's medium, after 10-12 days of incubation, at a pH of 6.5-7.0 and with maltose as a carbon source and potassium nitrate as nitrogen source. (Fig. 14).

4.7. Factors affecting spore germination and appressoria formation

The first step in the establishment of disease of any pathogen on the leaf surface is spore germination and appressoria formation. These processes are influenced by a large number of factors — both biotic and abiotic. Thus the factors affecting spore germination and its subsequent growth are of vital importance in deciding the fate of the pathogen in the initial stages. *G. cingulata* produces conidia in acervuli forming orange or black masses depending on the medium. Conidia appearing scantily on solitary phialides but normally in orange sporodochia; a basal stromatic cushion is covered with dense layer of cylindrical, slightly tapering phialides upto 20 µm long. Sometimes these are interspersed with dark brown, tapering, blunt, septate setae. Conidia cylindrical with a rounded apex and slightly truncated base, hyaline filled with granular cytoplasm, 12-18 x 3-5 µm; forming orange - red slimy masses, germinating by irregularly rounded brown appressoria (Plates IX & XI). In view of the importance of spore germination and appresoria formation in the establishment of the disease, effect of several factors including incubation period, pH, temperature, light, age of culture and concentration of spores have been studied in this investigation.

4.7.1. Incubation period

Spores of *G. cingulata* were suspended in sterile distilled water and allowed to germinate for different time periods ranging from 4-24h. At each desired period the spores were fixed, stained and observed under the microscope and percentage germination, appressoria formation and germtube length were determined. Results (Table 25) revealed that no spore germination was detected after 4h of incubation while, at 8h approximately 30% spores had germinated. Spore germination and appresoria formation progressively increased from 8h. onwards till 24h. Germtube length was also maximum at 24h of incubation.

Effect of cultural factors on mycelial growth of *Glomerella cingulata*

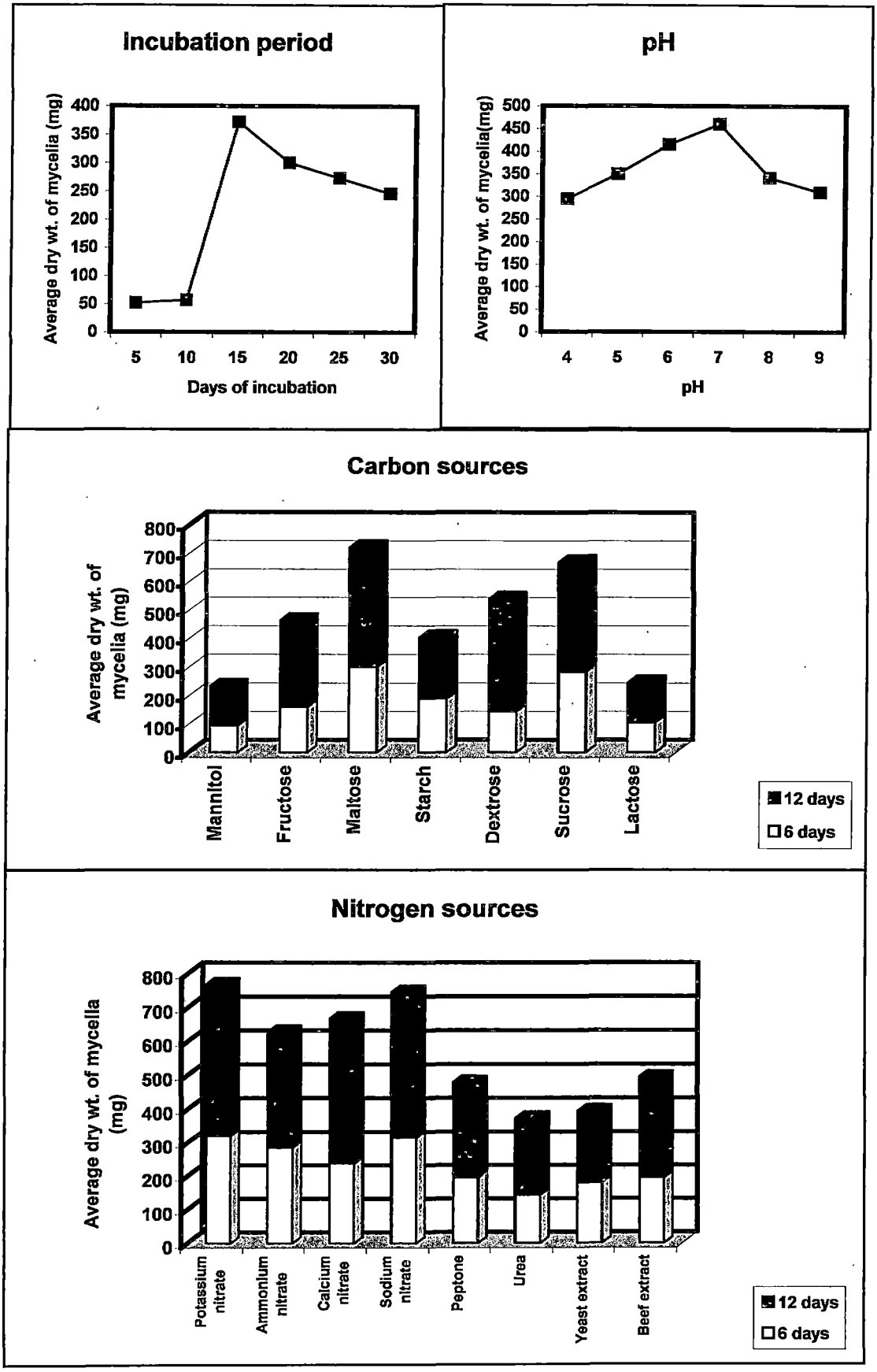


Fig.14

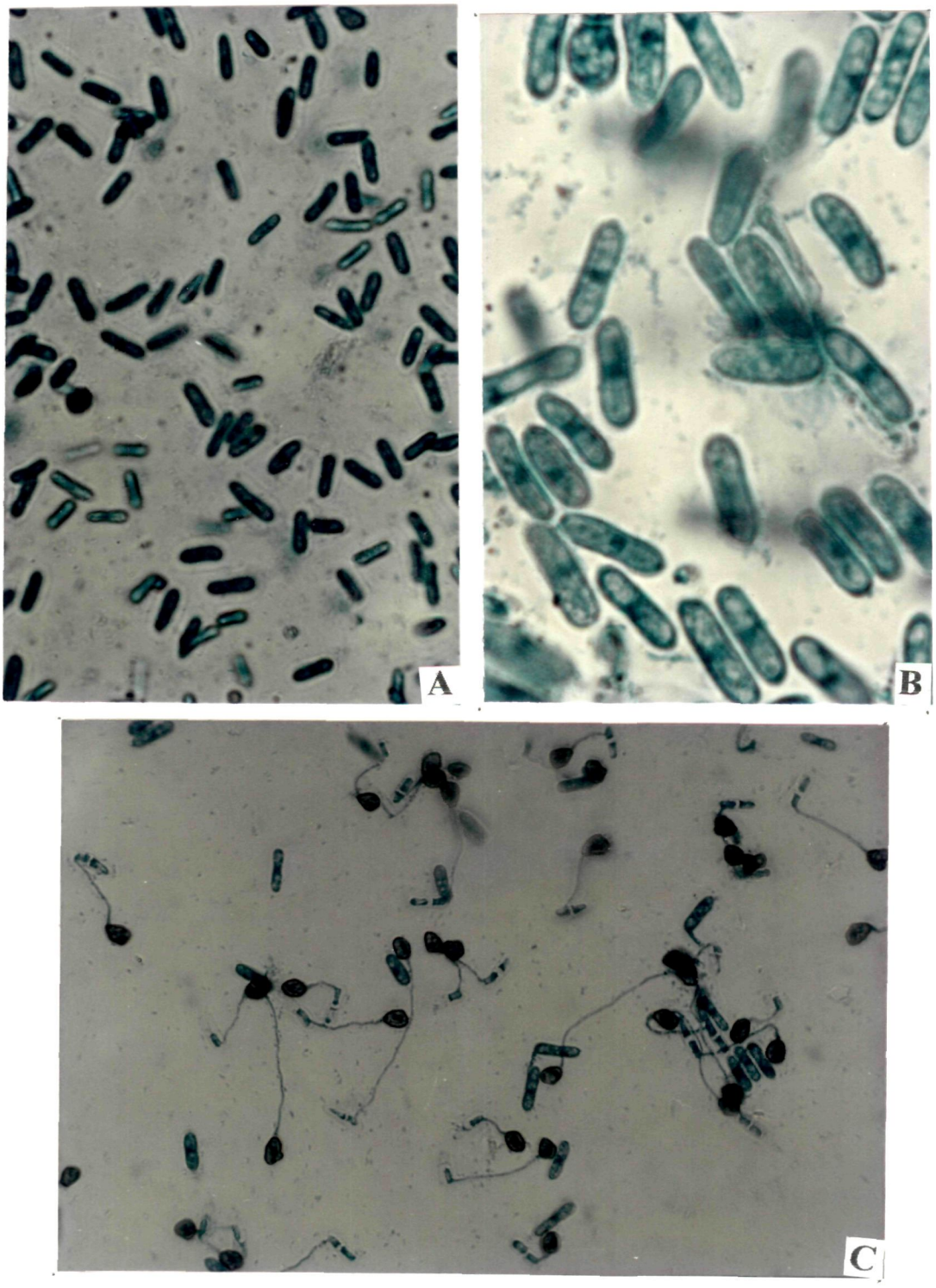


Plate XI(figs. A-C) : Conidia of *G.cingulata* under 45x(A) and 100x (B) magnifications; C – germinated conidia with acervuli

Table 25 : Effect of incubation period on spore germination of *G. cingulata.*

Incubation Period(hour)	% spore germination[a]	% appressoria formation[b]	Germ tube* length (µm)[b]
4	—	—	—
8	29·29 ± 2·49	13·8 ± 2·13	80·9 ± 4·3
12	36·0 ± 3·0	23·6 ± 3·6	184· ± 3·8
16	46·99 ± 3·05	40·2 ± 2·60	319 ± 5·62
20	77·71 ± 1·45	46·6 ± 2·56	425 ± 6·08
24	87·46 ± 1·58	68·5 ± 2·49	539·6 ± 9·59

[a] Average of 200 spores
[b] Average of 20 germlings
± = Standard error.

4.7.2. pH

Effect of different pH (4-8) on spore germination and appresoria formation were determined using .01M sodium phosphate buffer. Results (Table 26) revealed that pH 5.5. was optimum for both spore germination and appressoria formation. Spore germination and appressoria formation at pH 4 was also quite high (67% and 60% respectively) while at pH 8 there was a significant reduction in spore germination and appressoria formation.

Table 26 : Effect of different pH on spore germination of *G. cingulata.*

Different pH	% spore germination[a]	% appressoria[b] formation[b]	Germ tube length[b]
4·0	66·8 ± 3·6	60·5 ± 3·2	400·5 ± 3·08
5·0	70·8 ± 2·60	62·6 ± 3·1	425·6 ± 6·08
5·5	85·20 ± 2·49	80·6 ± 2·4	540·0 ± 9·54
6·0	55·75 ± 2·56	30·8 ± 2·8	180·6 ± 3·40
7·0	15·60 ± 1·45	13·7 ± 3·1	350·4 ± 4·62
8·0	10·2 ± 1·58	3·0 ± 2·6	150·3 ± 5·63

[a] Average of 200 spore
[b] Average of 20 germlings
± = Standerd error.

4.7.3. Temperature

A range of temperature from 10-40°C wee tested for their effect on spore germination and appressoria formation of *G. cingulata* Results (Table 27) revealed that the spore germination and appressoria formation were maximum at 25°C. At 10°C a germination percentage of apporimately 60% was evident while at 40°C about 45% spores had germinated.

Table 27 : Effect of different temperatures on spore germination of *G. cingulata*

Different temp.	% spore germination[a]	% appressoria formation[b]	Germ tube length (µm)[b]
10	60·2 ± 2·6	7·7 ± 2·9	91·3 ± 1·4
15	62·3 ± 3·1	21·6 ± 3·7	164·6 ± 2.8
20	65·0 ± 2·2	16·9 ± 2·9	289·3 ± 1·9
25	83·0 ± 2·9	80·9 ± 3·3	542·1 ± 3·3
35	42·7 ± 2·3	21·4 ± 3·1	438·6 ± 4·6
40	45·0 ± 3·8	20·5 ± 3·6	213·1 ± 2·9

[a] Average of 200 spores
[b] Average of 20 germilings
± = Standard error.

4.7.4. Light period

The effect of light on spore germination was studied using three types of light sources i.e. complete light, diffused light and total darkness. It was observed that maximum spore germination and appressoria formation occurred under diffused light conditions whereas total darkness was not conducive either to spore germination or appressoria formation (Table 28).

Table 28 : Effect of light on spore germination of *G. cingulata.*

Light period	% spore germination	% appressoria[a] formation[a]	Germ tube length[b] (µm)
Dark	16·31 ± 2·2	5·8 ± 3·1	357·61 ± 4·41
Diffused	69·90 ± 2·9	50·8 ± 3·8	528·76 ± 13·59
Total light	33·41 ± 3·1	20·6 ± 2·6	397·41 ± 5·26

[a] Average of 200 spores; [b] Average of 20 germlings
± = Standard error.

4.7.5. Age of culture

Spores from 5-30 days old culture of *G. cingulata* germinated, with the optimum age being 10 days (Table 29). 5 days old culture showed a germination of about 70% and 30 days old culture of 78%.

Table 29 : Effect of age of culture on spore germination of *G. cingulata.*

Ags	% spore germination[a]	% appressoria formation[a]	Germ tube length[b] (µm)
5	70·7 ± 3·6	64·2 ± 3·2	348·62 ± 4·3
10	93·0 ± 3·2	85·3 ± 3·1	524·40 ± 3·39
15	88·0 ± 2·49	56·0 ± 2·8	530·22 ± 3·78
20	84·0 ± 3·0	50·0 ± 2·4	368·3 ± 4·86
25	82·0 ± 1·58	43·5 ± 2·6	330·05 ± ·826
30	77·6 ± 1·45	40·0 ± 2·56	302·42 ± 2·21

[a] Average of 200 spores; [b] Average of 20 germlings
± = Standard error.

4.7.6. Concentration of spores

Spore germination and appressoria formation differed with the concentration of spores. The optimum concentration was at $1.6x10^5$ spores / ml above and below which spore germination reduced (Table 30).

Table 30 : Effect of different spore concentration on spore germination of *G. cingulata.*

Spore concentration (conidia/ml)	% spore germination	% appressoria formation[a]	Germ tube length[b] (µm)[a]
$1·6 \times 10^6$	64·4 ± 3·3	58·6 ± 2·5	240·58 ± 1·29
$1·6 \times 10^5$	80·0 ± 3·7	76·8 ± 2·9	521·16 ± 5·39
$1·6 \times 10^4$	65·3 ± 3·1	60·2 ± 2·5	541·37 ± 3·38
$1·6 \times 10^3$	52·5 ± 2·7	40·2 ± 2·2	517 ± 4·28

[a] Average of 200 spores; [b] Average of 20 germlings
± = Standard error.

All the tested factors influenced spore germination, appressoria formation and germtube length. Optimum conditions were — incubation period of 24h, pH 5.5., 25°C, diffused light and 10 days old culture at a concentration of $1.6x10^5$ spores / ml (Fig. 15).

4.8. Studies on factors affecting phenolic contents of tea leaves

Phenols are known to be major constituents of several plant species and in tea, polyphenols are the most important of all constituents. Phenolic compounds, like other biochemical constituents are known to be influenced by several factors. In the present investigation, therefore effect of various factors on phenolic contents of tea leaves have been determined. These include varietal difference, seasonal differences, difference in age of leaves as well as inoculation with *G. cingulata.*

4.8.1. Varietal difference

In order to determine the level of phenolics in different varieties nine varieties selected previously were used. Both total and orthodihydroxy phenol contents were determined following the method described previously. Among the nine varieties, total phenol content varied from 28 mg / g tissues to 46mg/g tissue. Most of the varieties showed a phenol content of about 45 to 46 mg/gm tissue (Table 31). Orthodihydroxy phenol content varied from ca.8-20mg / g tissue.

Table 31 : Total and orthodihyroxy phenol contents in different tea varieties.

Varieties	Phenolic content (mg/gm tissue)	
	Total phenol	Orthodihyroxy phenol
TV - 30	46·2	10·00
TV - 29	47·5	7·75
TV - 26	46·2	19·50
TV - 25	28·7	8·25
TV - 23	41·2	9·25
TV - 22	35·5	12·87
TV - 18	43·7	10·87
Tenali - 17	46·0	14·37
TV - 9	45·2	12·25
Mean	42·2	11·67
C.D. = (5%)	4·539	2·647

Effect of cultural factors on spore germination of *Glomerella cingulata*

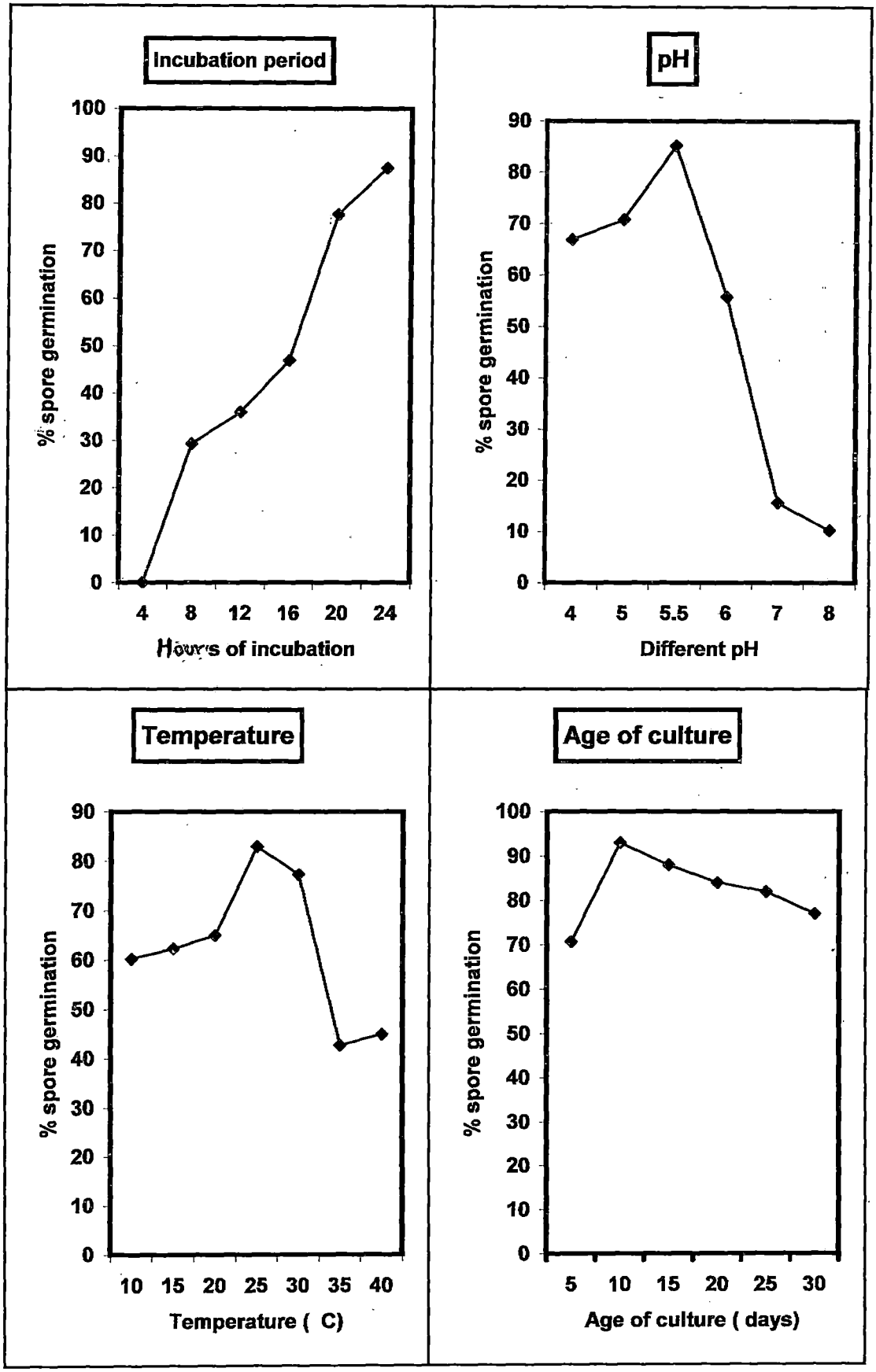


Fig.15

4.8.2. Seasonal difference

The inherent phenol content in the nine varieties was estimated during three different seasons - i.e. summer (April), rainy (July) and autumn (October). Results (Table 32, Fig. 16) revealed that maximum phenol accumulation occurred during rainy season. Both total and orthodihydroxy phenol were relatively lesser during summer and autumn.

Table 32 : Total and orthodihyroxy phenol contents in different tea varieties during in different season.

Varieties	Phenol content (mg/gm tissue)					
	Total phenol			Orthodihyroxy phenol		
	Summer[a]	Rainy[b]	Autumn[c]	Summer[a]	Rainy[b]	Autumn[c]
TV-30	37·8	46·2	37·50	13·00	10·00	8·25
TV-29	37·5	47·5	27·5	8·25	11·75	4·7
TV-26	28·7	46·2	37·75	18·60	19·5	7·5
TV-25	36·25	38·7	31·50	10·00	8·25	7·2
TV-23	25·00	41·2	27·50	9·60	9·25	8·87
TV-22	27·5	35·5	30·50	10·37	12·87	8·00
TV-18	27·2	43·7	18·75	6·82	10·87	3·75
Teenali 17	40·0	46·0	33·75	8·75	14·37	12·75
TV-9	41·2	45·2	35·25	8·50	12·25	8·50

[a] Summer - April
[b] Rainy - July
[c] Autumn - October

4.8.3. Age of leaves

Significant difference in phenol accumulation were observed between young and older tea leaves Maximum phenol contents were recorded in the young tea leaves and minimum in the older ones. (Table 33, Fig. 17). Leaves in between young and older ones also had phenol content in between the young and the old leaves.

Phenol content of leaves of different tea varieties during different seasons

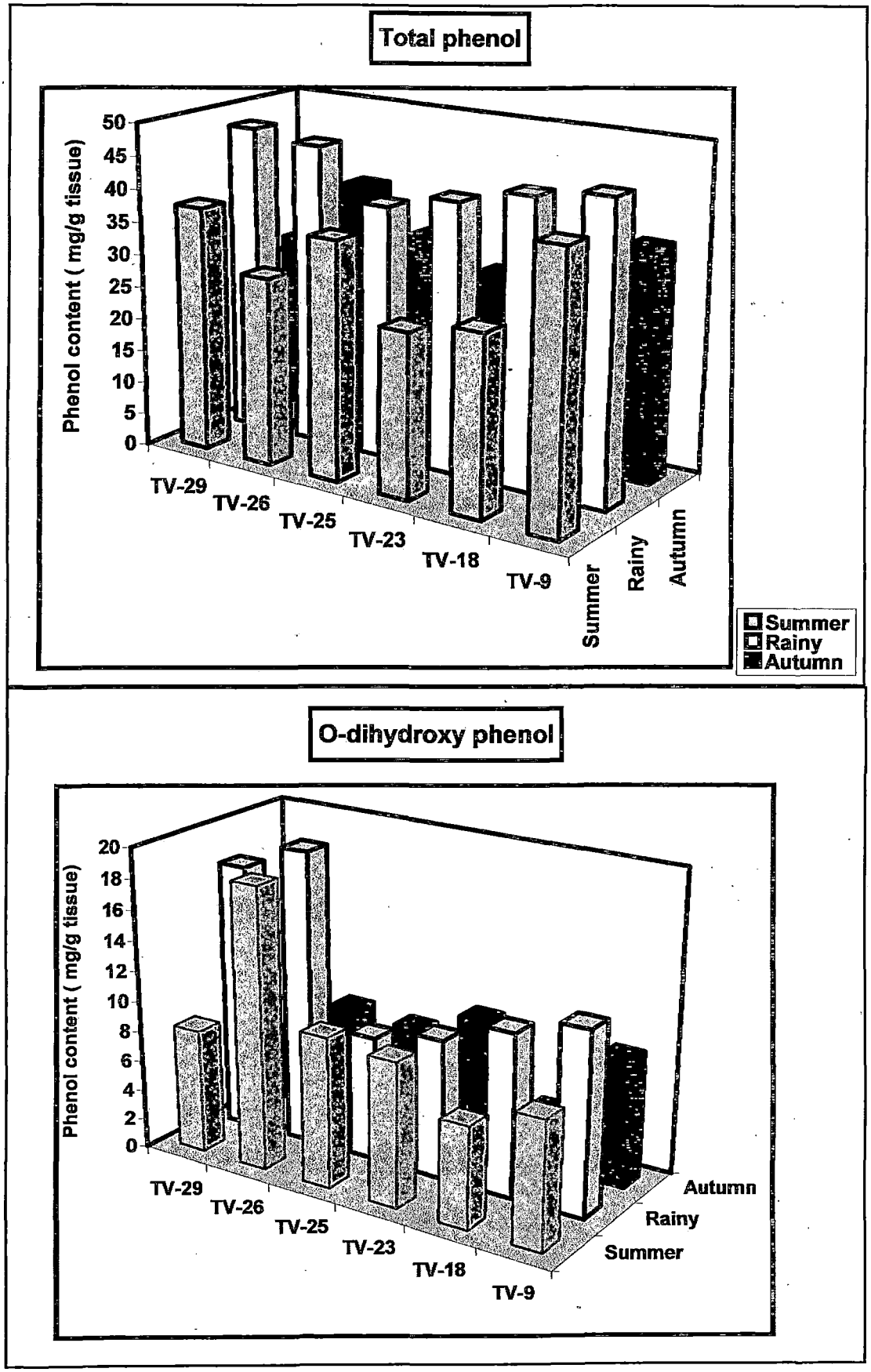


Fig.16

Table 33 : Total and orthodihydroxy phenol content in different tea varieties.

Varieties	Phenolic content (mg/gm tissue)		
	Age of leaves*	Total Phenol	Orthodihydroxyphenol
TV-30	Y	46·2	10·0
	M	40·0	7·5
	O	16·25	3·7
TV-29	Y	47·5	7·75
	M	30·0	6·75
	O	20·37	4·50
TV-26	Y	46·20	19·50
	M	45·0	16·75
	O	38·7	16·25
TV-25	Y	28·7	8·25
	M	18·7	6·50
	O	10·7	4·62
TV-23	Y	41·2	9·25
	M	25·0	8·87
	O	13·7	5·00
TV-22	Y	35·5	12·87
	M	27·5	12·25
	O	22·5	8·37
TV-18	Y	43·7	10·87
	M	32·5	9·87
	O	22·5	8·50
TV-9	Y	45·2	12·25
	M	27·5	10·00
	O	17·5	6·25
Teenali-17	Y	46·0	14·37
	M	27·5	10·25
	O	16·0	4·00

* Y = Young leaves; M= Mature leaves; O = Old leaves.

Phenol content of tea leaves of different ages and varieties

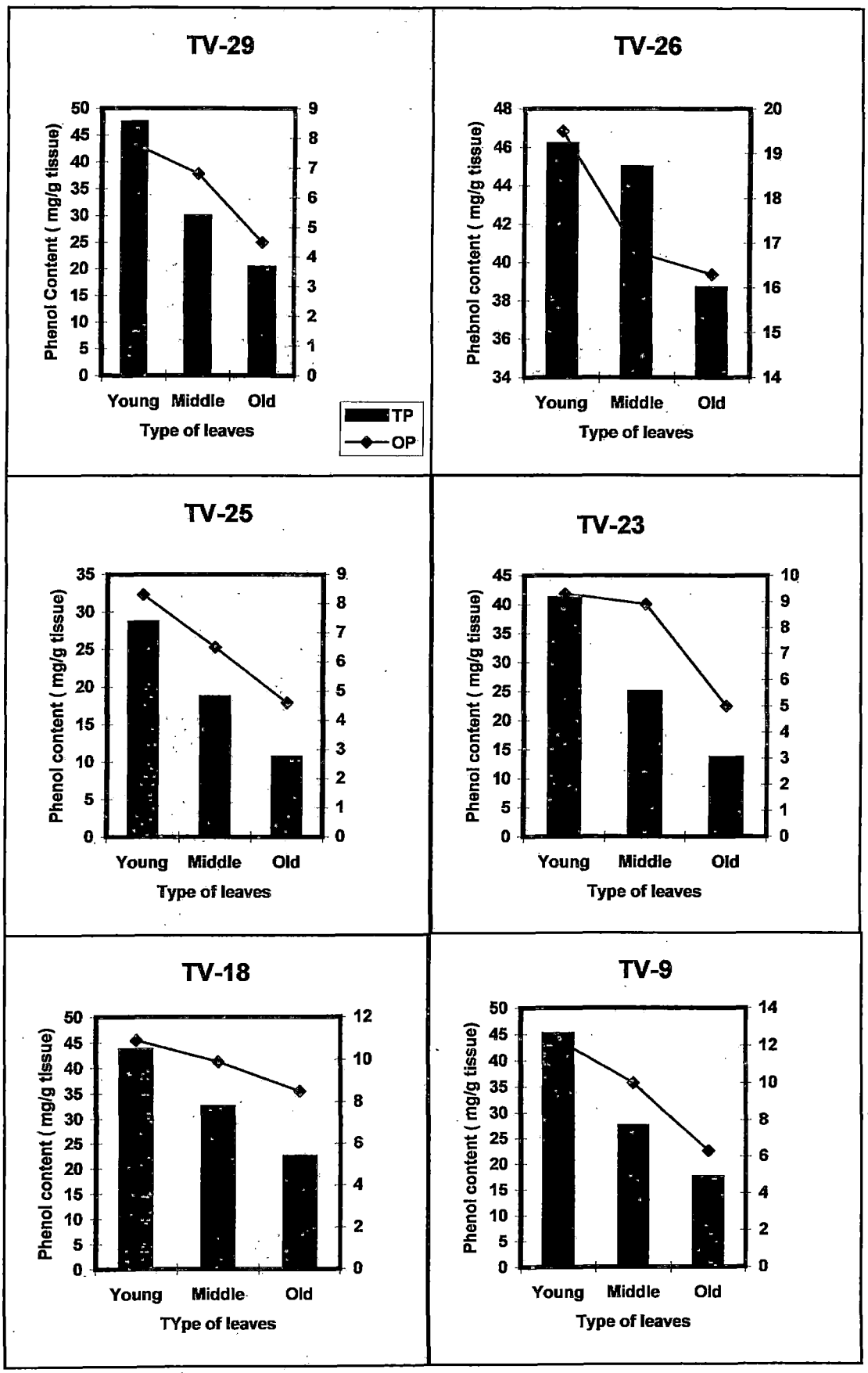


Fig.17

4.8.4. Inoculation with *G. cingulata*

In order to determine the effect of inoculation with *G. cingulata* on phenol contents on six varieties three susceptible and three resistant were selected. Phenol contents were estimated in these six varieties after 48 and 72h. of inoculation. Results (Table 34, Fig. 18) revealed that in all varieties phenol content increased following inoculation. Maximum increased was observed in the most resistant variety (TV 26). The increase was evident in the leaves of all ages. Orthodihydroxy phenol also increased following inoculation in all tested varieties (Table 35).

4.9. Phenol contents of diffusible compounds of tea leaves

Diffusible compounds are very often important in determining the initial response of a plant to attack by a pathogen. Since the previous experiment showed that phenolic contents are involved in the defense reaction of tea leaves of *G. cingulata,* diffusible phenols were estimated. In order to collect the diffusible compounds leaves were inoculated with spore suspension of *G. cingulata* or with sterilled distilled water. After 48h. of inoculation the drops were collected and centrifuged and phenol contents estimated. Total phenol content of the diffusate (optained after inoculation with spore suspension) was significantly higher than that in the exudates (obtained after inoculation with steriled distilled water) and was maximum in the resistant variety (TV-26). Result also revealed that maximum phenolics in both exudates and diffusates was obtained from the middle leaves (Table 36). In case of diffusates, minimum phenol contents was evident in the younger leaves, whereas in the exudates both young and old leaves had varying amounts.

Table 34 : Total phenol content in different tea varieties of healthy and artificially inoculated leaves by *G. cingulata.* inoculated tea leaves.

Varieties	Age of leaves[a]	Phenolic content (mg/gm tissue)			
		48h*		72h*	
		H	I	H	I
TV-18	Y	35·00	40·00	33·75	42·50
	M	27·50	31·25	27·00	36·25
	O	26·25	27·25	25·00	30·00
TV-23	Y	30·50	33·75	28·75	34·25
	M	27·50	26·60	26·50	30·00
	O	23·75	25·00	23·15	26·50
TV-25	Y	32·50	35·00	33·75	38·75
	M	28·50	32·00	27·50	35·60
	O	18·25	20·00	20·00	26·75
TV-29	Y	38·75	42·50	35·00	40·00
	M	31·25	38·00	29·50	34·00
	O	20·25	26·25	18·50	25·00
TV-9	Y	31·50	36·25	30·75	37·75
	M	28·75	32·00	26·25	35·00
	O	20·00	25·00	21·25	28·00
TV-26	Y	40·75	52·50	40·00	59·00
	M	32·50	36·25	33·00	40·00
	O	24·25	30·50	25·25	31·00

* House after inoculation.

Y = Young leaves; M = Mature leaves; O = Old leaves.

Phenol content of healthy and infected tea leaves of different varieties

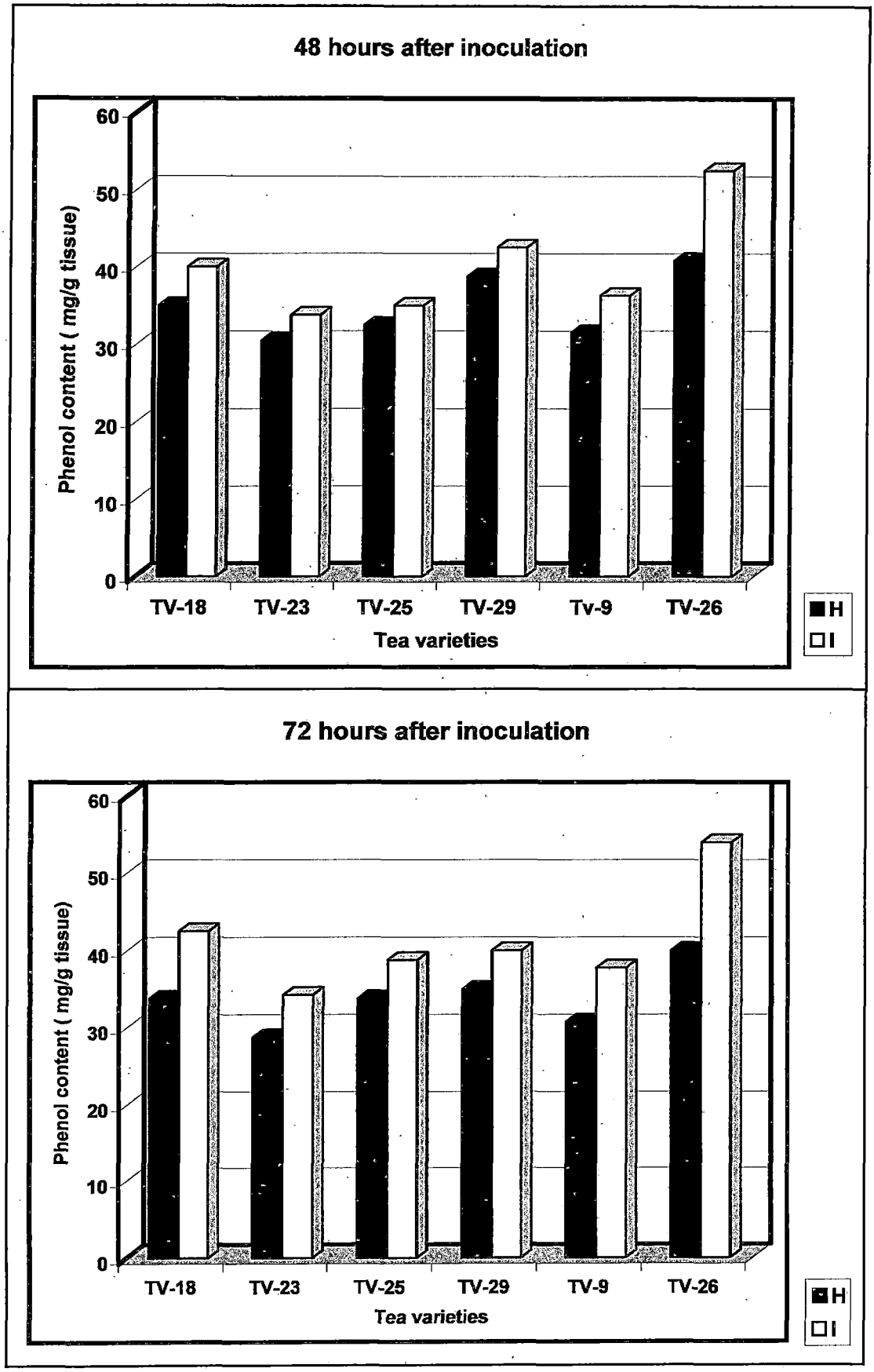


Fig.18

Table 35 : Orthodihydroxy phenol content in different tea varieties of healthy and *G. cingulata.* inoculated tea leaves.

Varieties	Age of leaves[a]	Phenol content (mg/gm tissue)			
		48h*		72h*	
		H	I	H	I
TV-18	Y	9·5	10·3	9·3	12·4
	M	8·0	9·1	8·6	9·9
	O	6·3	6·0	6·5	8·3
TV-23	Y	8·8	11·2	10·5	13·4
	M	8·4	8·8	8·5	10·0
	O	5·0	6·9	6·2	9·
TV-25	Y	11·9	12·5	12·0	14·2
	M	9·2	10·1	9·0	11·3
	O	7·1	8·2	7·3	9·6
TV-29	Y	14·3	16·2	13·9	16·9
	M	10·1	12·3	10·0	14·0
	O	7·2	8·4	7·5	8·5
TV-9	Y	16·2	19·5	16·0	20·9
	M	11·3	14·2	11·2	15·3
	O	8·2	9·6	8·5	11·3
TV-26	Y	18·4	24·3	18·0	27·4
	M	14·1	16·4	13·1	17·1
	O	10·2	14·5	11·0	15·2

[A]Y = Young leaves; M = Mature leaves; O = Old leaves.

* House after inoculation.

Table 36 : Phenol contents of exudates and diffusates of tea leaves of different varieties.

Varieties	Age of leaves	Total phenol (µg/ml)	
		Exudate	Diffusate
TV-18	Y	7·50	20·00
	M	10·00	32·50
	O	12·50	30·00
TV-29	Y	7·50	22·50
	M	10·00	27·50
	O	5·00	30·00
TV-26	Y	12·50	37·50
	M	17·50	40·00
	O	7·50	35·00
TV-25	Y	7·50	20·00
	M	10·50	32·50
	O	10·00	27·50
TV-23	Y	7·50	20·00
	M	10·60	35·00
	O	12·50	30·00
TV-9	Y	7·50	15·00
	M	10·00	17·50
	O	8·00	16·00

*Y = Young leaves; M = Mature leaves; O = Old leaves.

4.10.Studies on chlorophyll content of tea leaves

Chlorophyll is very important to a plant as biomass production depends on photosynthesis which is again dependent on the chlorophyll content. Variation of chlorophyll content as influenced by different factors were studied.

4.10.1. Different varieties

Total chlorophyll, chlorophyll a and chlorophyll b contents were estimated in six of the selected varieties. Total chlorophyll content varied from 637-713 ug/gm tissue but no correlation was present between chlorophyll content and disease resistance. Chlorophyll a and b contents also varied along with total chlorophyll (Table 37).

Table 37 : Chlorophyll content of leaves of different varieties.

Variety	Chlorophyll content (µg/g)		
	Total Chlorophyll	Chlorophyll a	Chlorophyll b
TV-29	693·00	273·00	420·00
TV-26	637·42	278·42	358·00
TV-25	686·42	251·72	434·70
TV-23	713·72	255·78	457·94
TV-18	654·92	259·84	395·08
TV-9	686·95	286·44	400·51
TV-22	658·14	255·50	402·64
TV-30	660·52	259·14	401·38
Teenali-17	558·60	262·92	295·68
Mean	661·07	264·75	396·13
C.D (5%)	22·82	4·47	24·213

4.10.2.Age of leaves

In all tested varieties maximum chlorophyll content was present in the older leaves followed by young leaves and least in middle leaves (Table38, Fig.19).

Table 38 : Chlorophyll content of tea leaves of different ages.

	Chorophyll content (µg/g)								
Varieties	Young leaves			Middle leaves			Old leaves		
	Total	Chla	Chlb	Total	Chla	Chlb	Total	Chla	Chlb
TV-29	558·74	272·72	286·02	428·68	270·76	157·92	693·0	273·00	420·00
TV-26	493·78	262·08	231·70	477·26	269·92	207·34	637·42	278·42	358·00
TV-25	538·58	265·58	273·00	477·26	269·92	207·34	686·42	251·72	434·70
TV-23	302·54	210·42	92·12	279·02	194·18	84·84	713·73	255·78	457·99
TV-18	419·58	256·48	163·10	259·80	239·40	120·40	654·92	259·84	395·08
TV-9	493·50	271·60	221·90	530·60	257·18	273·43	686·95	286·44	400·51
TV-30	494·65	263·48	231·17	651·38	254·34	397·04	660·52	259·14	401·38
TV-22	455·70	270·76	184·94	632·10	253·12	378·98	658·14	255·50	402·64
Teenali17	494·20	267·68	226·52	500·92	268·66	232·26	558·60	262·92	295·68

Chlorophyll content of tea leaves of different ages and varieties

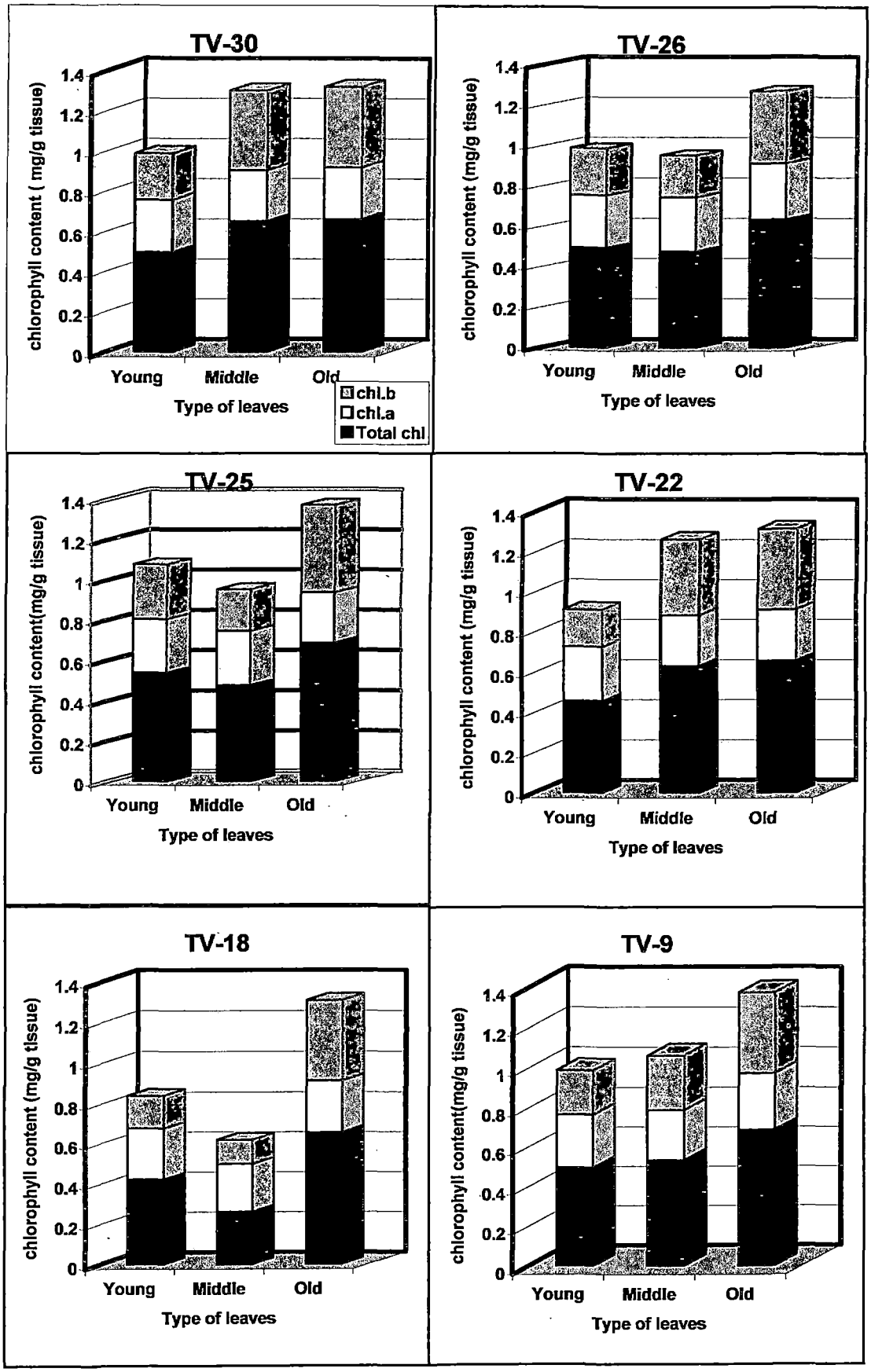


Fig.19

4.10.3. Natural infection

Significant reduction in chlorophyll content was obtained due to infection by *G. cingulata* (Table 39, Fig. 20). While in the healthy leaves chlorophyll content ranged from 637-713 ug/gm. in the infected leaves, it varied from 279-315 ug/gm. Thus the percentage reduction in chlorophyll accumulation was approximately about 50-60%.

Table 39 : Chlorophyll content of healthy and infected tea leaves of different varieties.

Varieties	Chorophyll content (µg/g)					
	Healthy			Infected		
	Total Chloro-phyll	Chloro-phyll a	Chloro-phyll b	Total Chloro-phyll	Chlorophyll a	Chlorophyll b
TV-26	637·42	278·42	358·00	307·57(-51·7)	132·48(-52·41)	175·07(-51·09)
Teenali17	693·00	273·00	420·00	315·00(-54-54)	134·47(-50·74)	180·53(-57·0)
TV-25	686·42	251·72	434·70	302·47(-55·78)	110·00(-56·30)	192·50(-55·71)
TV-23	713·72	255·78	457·94	289·70(-59·4)	131·04(-48·76)	158·66(-63·35)
TV-18	654·94	259·84	395·08	279·44(-57·33)	126·70(-51·23)	152·74(-61·33)
TV-9	686·95	286·44	400·51	313·32(-51·71)	131·81(·53·98)	181·51(·54·68)

Figures in parentheses indicate % decrease in relation to healthy leaves.

4.11. Determination of epicuticular wax content in tea leaves

The penetrating ability of a pathogen into leaves is dependent to a certain extent on the physical barriers it encounters on the leaf surface. Epicuticular wax is important as it provides impermeable layer on the leaf surface. Experiments were conducted to determine the epicuticular wax in leaves of different ages and following infection.

4.11.1. Different varieties

Epicuticular wax (ECW) content varied from .533 mg/ cm^2 leaf area in TV-22 to .937 in TV-26. Maximum wax content was thus present in the most resistant variety (Table 40).

Chlorophyll content of healthy and infected tea leaves of different varieties

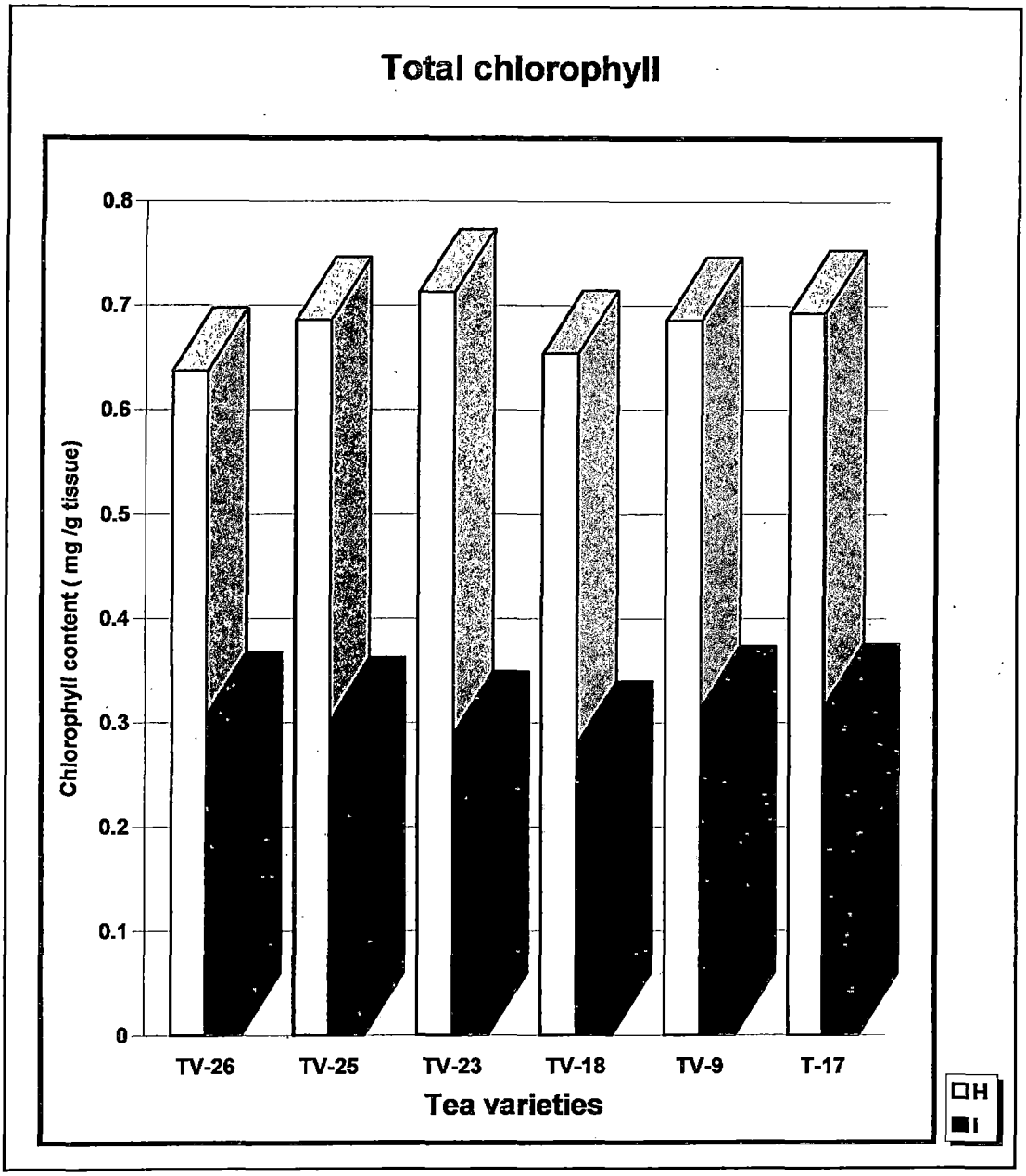


Fig.20

Table 40 : Epicuticular wax contents of leaves of differnt tea varieties.

Variety	Wax content (mg/cm²)
TV-30	·714
TV-29	·733
TV-26	·937
TV-25	·666
TV-23	·700
TV-22	·533
TV-18	·600
TV-9	·680
Teenali	·700
Mean	·695
C.D. (5%)	0·080

4.11.2. Different ages of leaves

ECW content was maximum in the older leaves as compared to younger ones (Table 41, Fig 21). In all varieties tested, minimum ECW was obtained in the younger leaves and maximum in the older ones followed by leaves of middle size.

Table 41 : Epicuticular wax contents of tea leaves of different ages.

Variety	Epicuticular wax content (mg/cm²)		
	Age of leaves		
	Young	Middle	Old
TV-30	·466	·571	·714
TV-29	·600	·666	·783
TV-26	·683	·786	·937
TV-25	·333	·533	·666
TV-23	·416	·538	·700
TV-22	·339	·416	·533
TV-18	·316	·426	·600
TV-9	·348	·466	·680
Teenali 17	·333	·533	·700
Mean	·426	·548	·701
C.D. 5%	·052	·055	·057

Epicuticular wax content of tea leaves of different ages and varieties

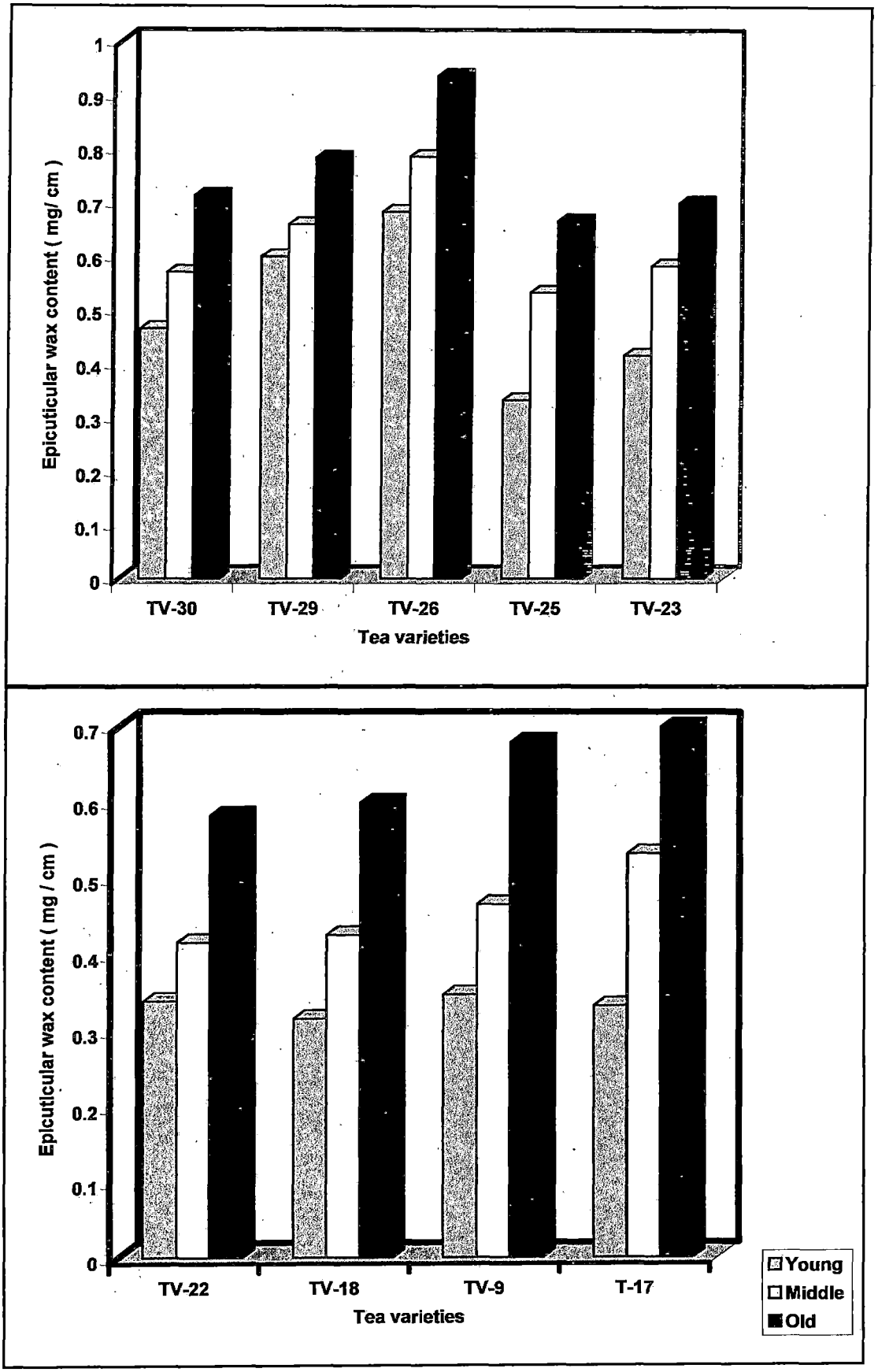


Fig.21

4.11.3. Natural infection

ECW was estimated from healthy and naturally infected leaves of six varieties. Results showed that ECW content was significantly lesser in the infected leaves in comparison to the healthy ones (Table 42).

Table 42 : Wax content of leaves of healthy and infected leaves of different varieties.

Variety	Epicuticular wax content (mg/cm²)	
	Healthy	Infected
TV-18	·666	·333
TV-26	1·00	·666
TV-25	·733	·466
TV-23	·800	·562
Teenali 17	·800	·466
TV-9	·860	·566
Mean	·809	·509
C.D. (5%)	·0693	·0565

Pearson's correlation coefficient was computed between the tested biochemical constituents (phenol, chlorophyll and epicuticular wax) in different varieties with occurrence of disease. Significant negative correlation (-.901) was obtained in case of ECW content and disease, but no significant correlation could be obtained between chlorophyll content or phenol content and occurrence of disease (Table 43). Thus the chlorophyll and phenol contents could not be said to be present in higher amounts in resistant or susceptible varieties, whereas ECW was higher in those varieties in the resistant ones. However, phenol content of healthy leaves increased significantly following inoculation, whereas chlorophyll content decreased.

Epicuticular wax content of healthy and infected tea leaves

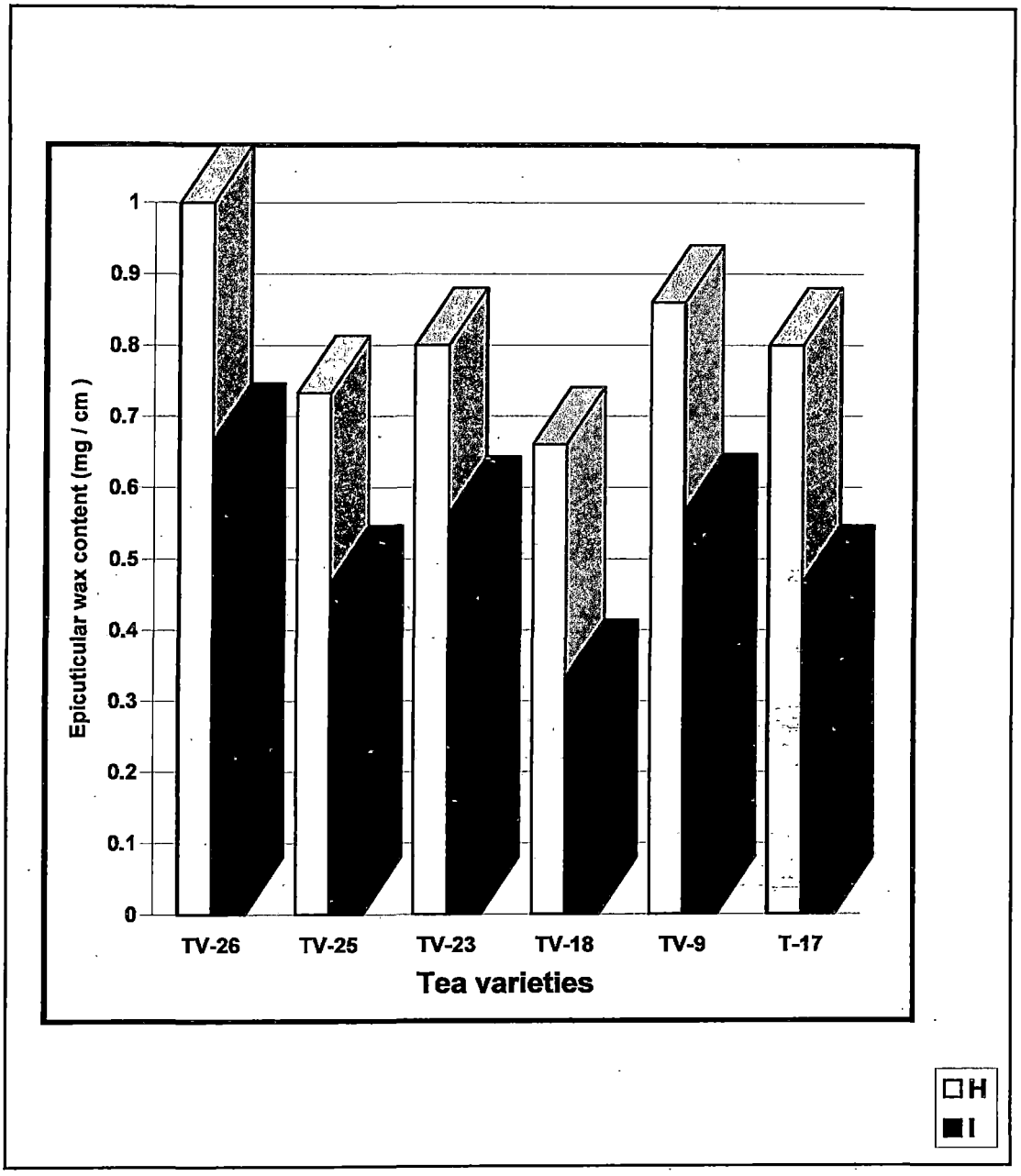


Fig.22

Table 43: Karl Pearson's corelation coefficient matrix showing correlation between occurrence of disease and biochemical constituents in six different tea varieties (2- tailed significance).

	Disease	Chlorophyll	ECW	Phenol
Disease	1·000	·290	-·901*	-·464
Chlorophyll	·290	1·00	-·455	-·250
ECW	-·901*	-·455	1·000	·348
Phenol	-·464	-·250	·348	1·000

* Significant at 1% level.

Values are 'r' values.

4.12. Studies on anatomical features

Cross section of tea leaves on different varieties were cut, stained observed under microscope. Varieties did not show much difference in anatomical features. Hair was present on the lower surface in the most varieties (Plates XII and XIII) Difference were observed only in the spongy parenchyma tissue which in some of the varieties (TV-25, 23 and 29) was interrupted by thick wall structures. Thick cuticle was evident in the upper surface.

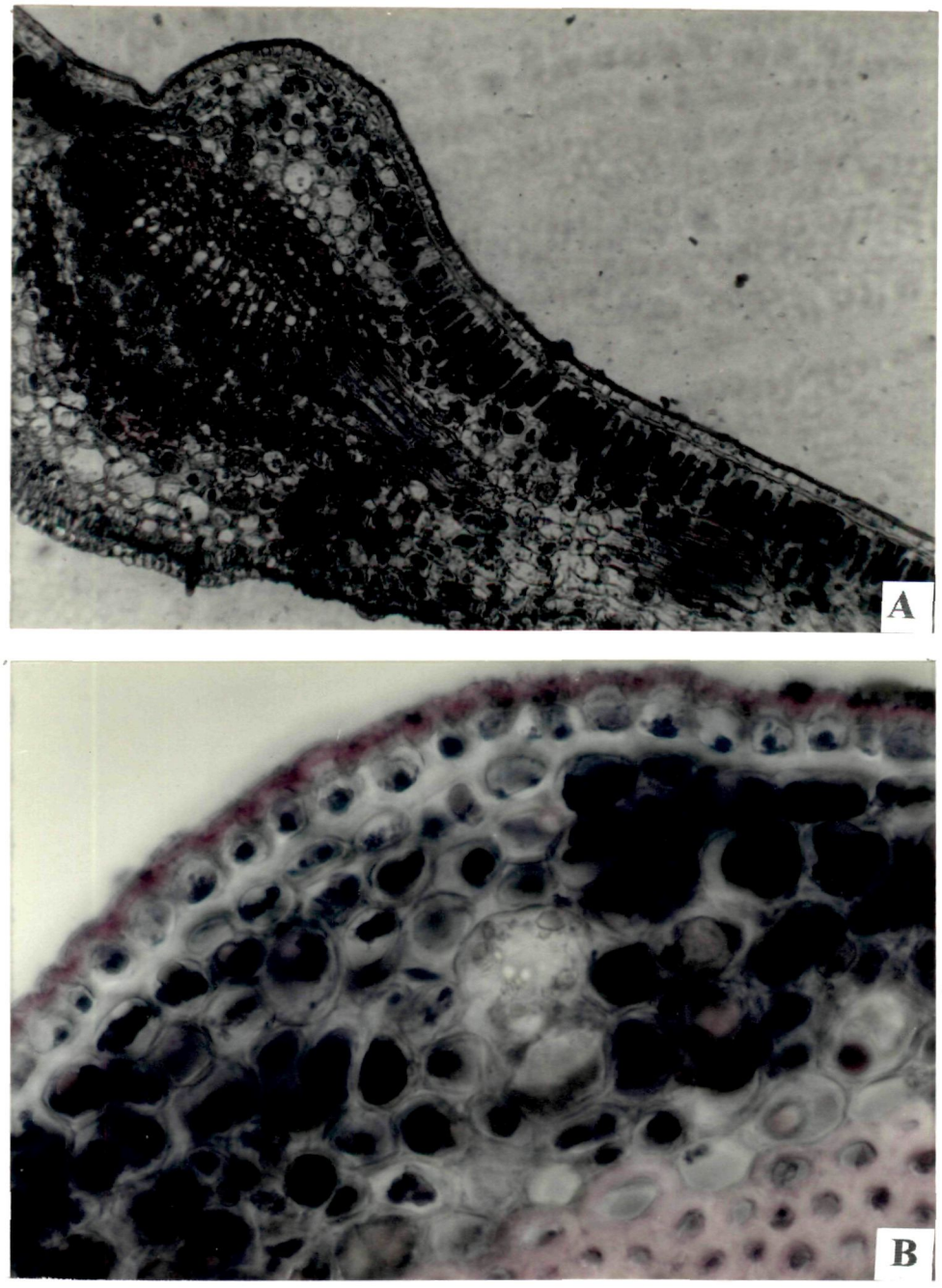


Plate XII (figs. A & B) - Transverse section of leaf of TV-18 (A) and a portion magnified (B)

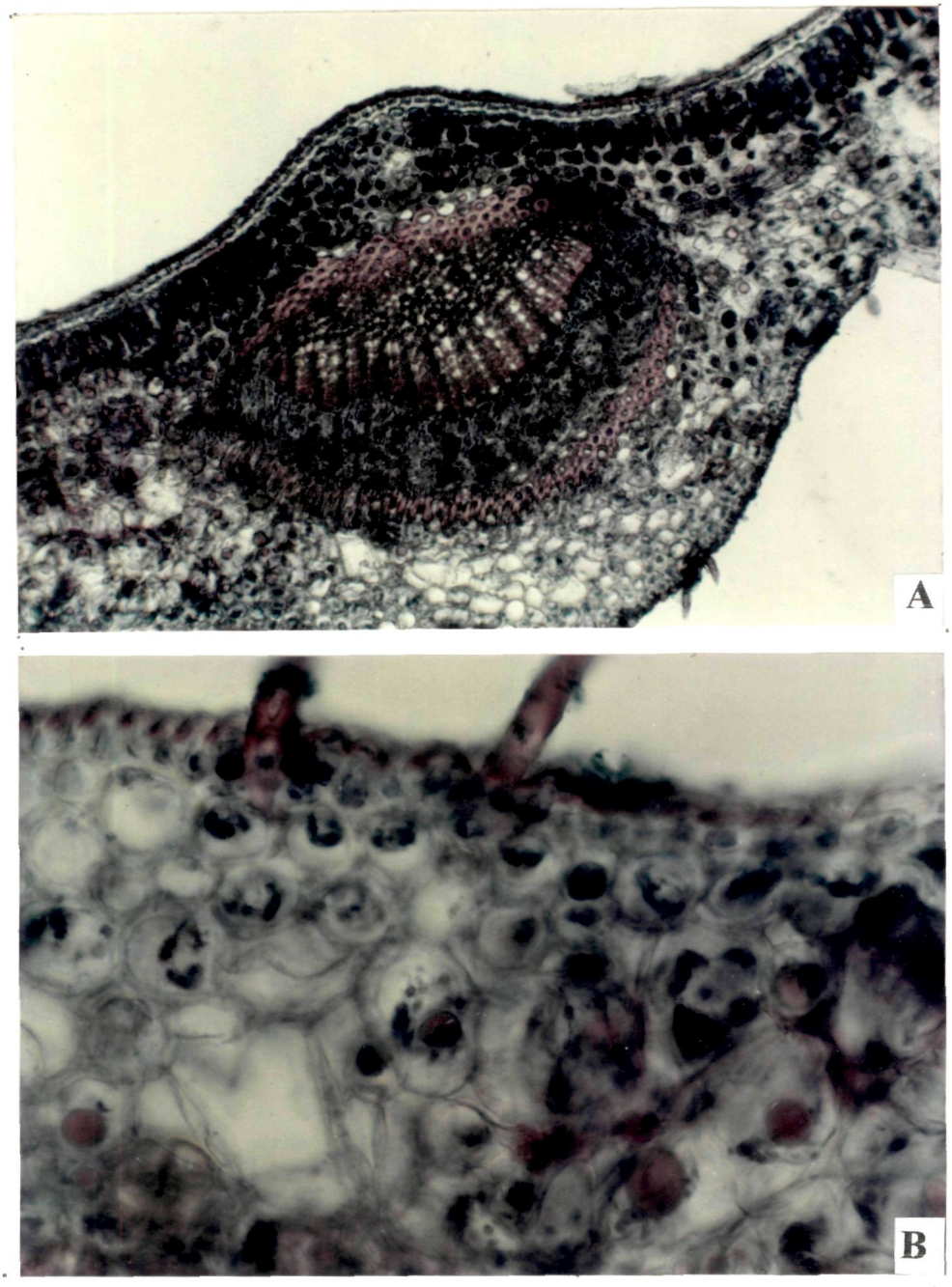


Plate XIII (figs. A & B) - Transverse section of leaf of TV-26 (A) and a portion magnified (B)

Discussion

Plant pathogen interaction is a highly complex series of events triggerred by the deposition of pathogen inoculum on the host. This is followed by the invasion of host tissue by the pathogen which inturn initiates the complex and variable developmental pattern between host and parasite which continues throughout their course of coexistence. Differences in physiological responses and morphological structures of various host genotype affect their susceptibility or resistance in invasions and its consequences while similar variation in pathogens influence their growth rate and virulence (Loomis and Adams, 1983). The host genotype or the biochemical make up of the host alone can not determine the course of disease development which is also dependent to a great degree on the environmental conditions prevailing in a particular area. Thus, in nature complex series of interaction between the environment, the host and the pathogen, finally determines whether the pathogen will be successful in establishing disease or the host will be successful in warding off the pathogen.

At the beginning of this study, nine varieties of tea were screened for resistance against *Glomerella cingulata* under laboratory conditions. TV-18 was most susceptible followed by TV-23, and TV-26 the most resistant followed by TV-29 under both detached and cutshoot inoculations.

Disease incidence under natural conditions in the field also revealed TV-23 and TV-18 to be most susceptible and TV-26 to be most resistant. Result of the present study clearly indicate that tea varieties exhibited differential resistance to *G. cingulata*. Such result have been obtained by previous workers. Gupta *et al.* (1996) screened several sorghum germplasm (Multicut) against important foliar diseases i.e. downy mildew (*Peronosclerospora sorghi*), sooty strip (*Ramulispora sorghicola*), zonate leaf spot (*Gloeocercospora sorghi), grey leaf spot (Cercospora sorghi*) and anthracnose (*Colletotrichum graminicola*). They reported that during 1993 five genotypes i.e. IS 3266, 32407, 70742, 71664 and 6018 exhibited resistance to all five diseases and only one i.e. IS 3266 showed such resistance in 1994. Evaluation of carnation germplasm for resistance against *Alternaria* blight showed Yellow Dusty, Harvest Moon, Leena and shocking pink to be highly susceptible to *Alternaria* blight and scania to be least susceptible (Meeta *et al.* 1996). Resistant linseed genotype against *Alternaria* blight and powdery mildew were also screened by Kaur and Lenka (1998). No genotype was fully resistant while a number exhibited moderate resistance or susceptibility. Chambol and Subhra were

highly susceptible to *Alternaria* blight and LCK 9325 to powdery mildew. Kumar *et al.* (1999) screened 128 varieties/ lines against *Drechslera graminia* and obtained 53 highly resistant, 11 resistant, 6 moderately resistant, 11 moderately susceptible, 6 susceptible and rest of the varieties highly susceptible. Studies by Singh (1999) also revealed that different sugarcane varieties exhibited large variation in their disease reaction to whip smut caused by *ustilago scitaminaea.* Out of twenty five varieties, four were found to be resistant and there highly resistant. Sharma and Badiyala (1998) screened 21 Cultivars of mango for susceptibility to *Collectorichum gloeosporoidis* during different seasons. They reported that none of the cultivars and seedling selection of mango was resistant to anthracnose disease. Higher numbers of spots (62.66) developed on cultivar Amrapali followed in order by Totapari, Lala Da Amb, Safeda and Mallika and these five cultivars were rated as highly susceptible whereas eight others were moderately susceptible. Maximum disease developed on leaves during rainy season followed by spring and minimum in post rainy inoculated leaves. These results are in conformity with those of the present study where maximum disease was obtained during rainy season followed by summer and autumn, both under artificial inoculation and natural conditions. Besides different, seasons, disease development was also affected by other factors like age of culture of inoculum, spore concentration (inoculum density) and available light. Ten days old culture with a concentration of $1.6x10^5$ spore / ml under diffused light conditions were most favourable for brown blight development. Singh and Thapliyal (1998) reported that the pathogenic potential of *Sclerotium rolfsli* causing seedling rot of soybean increased with increased inoculum density. Soybean cultivar PK 327 was slightly tolerant and Bragg highly susceptible to pre-and post emergence rot.

The habitat of pathogen which invades the aerial parts of the plants is immediately and profoundly influenced by weather. These pathogens usually reproduce abundantely and with the onset of favourable conditions spread rapidly from a minimum amount of initial inoculum (Rotem, 1978). The ability of a pathogen to survive during periods of adverse conditions enables it to carry over from one season to another. Atmospheric parameters influencing disease development are usually temperature, relative humidity, rainfall, plant density etc. In the present investigation relationship between occurrence of brown blight disease and weather factors like temperature, humidity, rainfall and sunshine hours were determined in six varieties for three consecutive years. Positive correlation of disease intensity was obtained with minimum

temperature, relative humidity and rainfall and negative correlation with hours of sunshine. Most favourable condition for brown blight development appeared to be a humid atmosphere with a minimum temperature of 24^{0}C, heavy rainfall and lesser hour of sunshine. Such weather conditions are generally present. during rainy season and hence disease development was maximum in rainy season. Several previous workers have worked extensively on the relation of weather conditions to disease development. Rolando *et al.* (1989) reported that the level of infection of *Eucalyptus* by *Puccinia psidii* varied with temperature, leaf wetness period and photo period. Higher disease intensity was observed at 20-25^{0}C after 24 hours of wetness of leaf surface and disease was inversely correlated with leaf exposure during incubation period. Detailed analysis of the effect of rainfall variable on the epidemiology of *Phytophthora* blight of pepper was carried out by Bowers *et al.* (1990). They obtained largest absolute direct effect by the cumulative amount of rainfall while the cumulative number of days with rainfall, the cumulative daily average temperature and chronological time had far lesser effect. Temperature and duration of wetness period also influenced the development of shot hole disease on almond leaves caused by *Willsonomyes carpolophilus* (Shaw *et al.* 1990). A functional relationship between anthracnose of bottle gourd and meterological factor was established by Gandhi *et al.* (1997).

Temperature and relative humidity showed significant effect on disease epidemiology. Rainfall leading to high R.H and less sunshine hours had an important role in disease development. These results are in confirmility with those of the present study. In the present investigation disease was found to occur during most part of the year other than the winter months (November, December, January). Extreme cold condition was not suitable for disease development.

Age of leaves was also seen to be correlated with the degree of disease intensity. Under laboratory conditions younger leaves were more susceptible to disease development than the older leaves. However when survey of disease was conducted in the field it was observed that disease symptoms were visible only on the lower leaves. The apparently contradictory observation on field and laboratory condition can be explained by the fact that in the field the young tea leaves are continuously plucked and hence the time period necessary for inoculum deposition and spore germination is hardly available. The older leaves which remain for longer periods get wounded sooner or later following which the pathogen enter and disease development occurs. This is because the

brown blight pathogen can normally gain entry into the leaf only through wounds (Bertus, 1974; Dickens and Cook, 1989). Dubey (1997) reported that ground nut plants were susceptible to *Thanotophoras cucumaris* at early stage of growth though susceptibility increased with increasing plant age upto 30 days. Maximum disease development was also favoured by a temperature of 25-28^{0}C and relative humidity of 98-100%. Atmospheric temperature and relative humidity were also shown to play a determining role in the initiation and subsequent development of powdery mildew of mango (Verma and Kaur, 1998); They observed that minimum atmospheric temperature plays more prominent role in the initiation of fresh infection besides cloudiness along with muggy and warm weather. Initiation of red rot infection of sugarcane was correlated to climate factors (Kumar *et al.* 1998). Red rot initiation was significantly and negatively correlated with maximum temperature while the reasons of minimum temperature was non significant. Contribution of the rest of the weather variables in red rot initiation was non significant. Roger *et at.* (1999 a and b) conducted detailed study to determine the effects of temperature and moisture and interrupted wet periods on the development of *Mycosphae rella pinodes* on pea. Disease severity and the number of pycnia formed on leaves increased with temperature from 5-20^{0}C and then decreased between 20-30^{0}C. Pycinidiosores were reported to survive in dry periods up to 21 days of inoculation with the infection capacity being restored following re-wetting. Results of the present study along with those of a number of previous workers establish the definite role of environmental factors on disease development as well as initiation of disease.

Climate influences disease development by its effect on the plants as well as on the pathogens (Coakley;1998). Plant factors associated with disease included plants own resistance and susceptibility, age of plant, plant density etc. The growth of the pathogen in the host is dependent to a large degree on these factors as well as on environmental factors. Fungal plant pathogens invade host plant cells with a variety of specialised infection structures of which pressorium is, in many cases, the most important structure formed in preparation of host colonization (Hoch and Staples, 1987). It must be positioned at appropriate site on the host in the most advantage way for subsequent infection to occur and it must be able to withstand adverse environmental conditions. For *G. cingulata* which is able to infect only through wounds, the positioning of appressorium assumes great significance. Ando and Hamaya (1986) reported that the anthracnocse fungus *Gloeosporium thea sinesis* could infect tea only through the trichomes of young leaves.

In the present study, therefore a series of experiments were conducted to determine the various factors affecting spore germination, appressoria formation and mycelial growth of *G. cingulata invitro.* Spore germination, appresioria formation and myceliel growth were affected by different factors like temperature period of incubation, pH, spore concentration, source of nutrient etc. Spore germination and appresoria formation of *G. cingulata* were maximum at 25^{0}C, pH 5.5, diffused light and after 24 hours of incubation using a spore concentration of 1.6x10^5 spores / ml. An incubation period of 10-12 days at pH 6.5-7 with maltose and KNO_3 as carbon and nitrogen sources respectively were optimum for mycelial growth.

Studies of previous authors on various pathogenic fungi established the optimum conditions for spore germination and mycelial growth of these. Jordan *et al.* (1990) found isolates of *Cladosporium allii* and *C. allii cepae* to grow best at 20^{0}C and 15-20^{0}C respectively on 2.5% malt extract agar. Both species tolerated a wide range of pH with maximum sporulation at 5-8.5. Al least 8h. of darkness was necessary for optimum germination of both fungi. Conidia of *Cercospora* species were found to germinate within 2h and 100% germination was recorded by Raghuram and Mallavia (1996) within 8h of incubation. They reported that the conidia of *Pseudo cercospora* species took relatively longer time to germinate than *Cercospora* species. Species of both the above fungi sporulated at r.h. levels between 66-100%. The role of temperature and relative humidity on spore formation of *Uncinula necator,* the causal agent of powdery mildew of grape was determined by Chavan *et al.* (1995). Temperature in the range of 22.2-30.1^{0}C and R.H greater than 57.4% favoured spore production by the pathogen . Among ten carbon sources tested for their effect on growth and sporulation of *Fusarium sp.* causing sheath rot of rice, sucrose, xylose and fructose supported the maximum growth of *F. graminearum, F. moniliforme* and *F. avenaceum* respectively. Sucrose induced maximum sporulation in *F. avenaceum* (Singh and Devi,1996). L-histidine, D. L-asparatic acid and D, L-alanine supports maximum growth of *F. avenaccum, F. moniliforme* and *F. graminearum* respectively. *Colletotrichum truncatum* was reported to have an optimum temperature of 20^{0}C for germination with 3h light followed by 9 h dark (Kaushal *et al.* 1998). Ahmed and Mir (1998) reported that all carbon and nitrogen compounds tested supported vegetative growth of *Willsonomyces carpophyllus* though maltose proved best carbon source followed by mannitol, glucose and suerose. Similar results have also been obtained in the present study where maltose was the best carbon source.

W. carpohyllus also exhibited maximum mycelial growth in medium containing asparagine as the nitrogen source. The above studies clearly point out that the fungi differ greatly in their requirements for growth and sporulation. Not only do the optima for temperature, pH etc. vary but the different fungi also utilize nutrients differently. A particular carbon or nitrogen source which may support good growth of one fungus may not be suitable for another.

Other than environmental conditions, in plants, their biochemical make up is responsible for the particular response shown by plant to a pathogen. The biochemical constituents are again influenced by existing environmental conditions. A particular biochemical component may vary according to the variety of the plant, age of the plant / plant parts, season etc. Accumulation of phenols in plants is one of the most well studied aspect with respect to host pathogen interaction. Many of the phenols in plants occur constitutively and are associated with resistance (Cole, 1984; Baker *et al.* 1989, Chakrarborty *et al.* 1995). In the present study accumulation of phenols in different tea varieties has been thoroughly investigated. No significant correlation between phenol content and resistance and susceptibility of tea leaves to *G. cingulata* could be discerned. Maximum phenol accumulation however was obtained in rainy season. Nosolillo *et al.*(1989) reported that accumulation of phenolic substance in pine seedlings was pronounced throughout October and November. Total phenolics showed a gradual increase over the season. In the present study the phenolic contents were seen to be maximum in the young leaves. Inoculation with *G. cingulata* resulted in an increased accumulation of both total and Orthodihydroxy phenols in resistant genotype of pea was reported by Sharma *et al.* (1996). Sindhan and Parashar (1996) also reported that the total phenol was higher in the resistant cultivars i relation to susceptible ones and that accumulation of phenols was greater in resistant varieties of ground nut infected by early and late leaf spot pathogen than in susceptible cultivars. However, Mitter *et al.* (1997) reported that though chickpea genotypes resistant to gray mould had higher total phenols, the phenols decreased after inoculation by *B. cinerea,* Gularia *et al.* (1998) also reported that the resistant cultivars of pea had higher phenol contents but inoculation with *Erysiphe polygonii* led to a marked increase in the O-dihydroxy phenol only. In most of the studies therefore resistant cultivars have been shown to have higher phenol content than the susceptible ones, which increased following infection by a pathogen. In the present study, though no significant difference in resistant and susceptible varieties of tea could be discerned, a greater

accumulation of phenols following inoculation in the resistant varieties was seen, which is in confirmily with the reports of the previous workers. Besides total phenol, higher levels of diffusible phenolic compounds was obtained in the resistant cultivar. Concentration of diffusible phenol was 2-3 times greater following inoculation of tea leaves. It seems probable that rapid accumulation of phenol at the infection site may result in the effective isolation of pathogen or nonpathogen at the original site of ingress. Since polyphenols are the major constituents of tea leaves, their involvement in the defense mechanism can be expected. It has been reported by Chakraborty *et al.* (1995) that among several phenolic detected in tea leaves catechin was antifungal in nature.

Chlorophyll is undoubdtedly the most important plant pigment since it controls photosynthetic activity of a plant and thereby determines the plant's productivity. Any factor affecting chlorophyll accumulation is therefore of prime importance to the plant. In the present study, chlorophyll accumulation was considered to be one of the biochemical parameters to be investigated and changes, in chlorophyll content due to varietial difference, age of the leaves and following infection by *G. cingulata* were determined. Chlorophyll content varied among the different varieties tested and was higher in the older leaves. Infection with *G. cingulata* resulted in a reduction in chlorophyll content which was quite marked. De Silva and Sivapalan (1982) also studied the chlorophyll content of different tea clones and reported differences among them. Maximum chlorophyll accumulation was obtained by them in the third leaf. They suggested that the chlorophyll content of tea plants varied with shade, season, geographical location and clones. Wide variation in chlorophyll a, b and total chlorophyll were observed among 36 T.R.A. Garden series clones by Bera *et al.* (1997). They grouped these clones into 3 categories - low, medium and high, according to their pigment contents. Studies on effect of infection by pathogen on chlorophyll content have been done by previous workers who also reported a reduction in chlorophyll accumulation following infection (Bhavani *et al.* 1998, Sutha *et al.* 1998, Singh *et al.* 1998). Thus these results are in conformity with those of the present study. The reduction in chlorophyll contents might be due to stimulating of enzyme chlorophyllase which degraded chlorophyll (Kaur and Deshmukh, 1980) or inhibition of chlorophyll synthesis caused by the pathogen (White and Brakke, 1983).

Epicuticular wax is the outermost layer in the leaves of the plant and plays a fundamental role as a barrier between the leaf and the environment-most specifically attack by the pathogens. The amount of wax on leaf surface will

also be one of the factors determining a plant's resistance or susceptibility. Among the varieties tested maximum ECW content was obtained in resistant cultivar and the older leaves had higher ECW than the younger leaves. Mohammad *et al.* (1986) also reported that the wax content was higher in drought resistant tea clone them in the susceptible ones. Very significant differences were also reported in ECW content among sorghum cultivars by Ebercon *et al.* (1977). In the present investigation it was also observed that infected leaves had significantly lower ECW content them the healthy ones. It seems provable that during the course of infection the fungus was successful in degrading epicuticular wax. In a study conducted on significance of epicuticular wax in specificity of blast fungus to rice varieties by Kumar and Sridhar (1987), they found that ECW from resistant cultivar inhibited appressoria formation of *Pyricularia oryzae* while ECW of susceptible cultivar stimulated appressoria formation. They suggested that resistance or susceptibility may in part be governed by this mechanism.

Results of present detailed study on brown blight disease of tea caused by *G. cingulata* have shown that growth of pathogen and its subsequent establishment in the host is governed by a number of environmental factors as well as by genetic make up of each variety causing it to be either susceptible or resistant. Environmental factors are very important as they not only influence the growth of the pathogen and disease development but also influence the growth of the host as well as biochemical components. Such changes of these biochemical components also are involved in regulating the plants response to the pathogen. Thus it can be generalised that the final appearance of disease on host is dependent on a multitude of factors - both external and internal.

Summary

1. Pathogenicity test of *G. cingulata* was tested on 9 varieties of tea (TV-30, TV-29, TV-26, TV-25, TV-23, TV-22, TV-18, Teenali-17, and TV-9) by detached leaf and cutshoot inoculation techniques. TV-18 was most susceptible followed by TV-25, while TV-26 was most resistant followed by TV-29.
2. Ten day old culture of *G. cingulata* had maximum infective capacity and produced maximum lesion on detached leaves.
3. Optimum spore conccentration for disease produciton was $1.6x10^5$ spores/ ml in all varieties.
4. Disease development was maximum under diffused light conditions and during rainy season.
5. Occurrence of brown blight disease was surveyed on 6 varieties of tea (TV-26, 25, 23, 22, 9 and Teenali-17). Symptoms of brown blight disease in the field were common on the lower leaves. TV-26 was most resistant and 25 susceptible.
6. Meteorological data of three years — 1996, 97, 98 were collected which included maximum and minimum temperature, % relative humidity, hours of sunshine and monthly rainfall.
7. Mean maximum temperature ranged from 22-30^{0}C and minimum ranged from 9^{0}C - 25^{0}C.
8. Relative humidity ranged from 87-95% in the morning and from 42% to 75% in the afternoon.
9. Hours of sunshine was recorded ranged from 4.5 in January to 8.7 in May; rainfall ranged from trace to 1090 mm.
10. Positive significant correlation of disease occurrence was obtained with minimum temperature, relative humidity and rainfall, while negative correlation was obtained with hours of sunshine. Maximum temperature had no significant correlation with disease.
11. The growth and the sporulation of *G. cingulata* was observed on nine media of which maximum growth and sporulation was observed in Richard's agar medium (solid) and Richard's medium (liquid).
12. Maximum growth of *G. cingulata* occured at pH7 and minimum at pH4.

13. Maltose and KNO_3 were the best carbohydrate and nitrogen sources for the growth of *G. cingulata.*
14. Maximum spore germination and appressoria formation was observed at 24 hours of incubation. Germtube length was also maximum at 24 hours.
15. Spore germination & appressoria formation at pH4 was found to be high and temperature of 25^0C was optimum.
16. Diffused light was most conducive for spore germination. Phenol content was determined among the 9 varieties of tea. Total phenol content among the 9 varieties varied from 28mg/g tissue to 46 mg/gm tissue and orthodihydroxy phenol content varied from 8-20mg/gm tissue.
17. Maxmimum phenols were recorded in the rainy season and in the young leaves. Inoculation with *G. cingulata* resulted in increase in phenol content, which was maximum, in the most resistant variety (TV-26).
18. This was also observed in the leaves of all ages. Orthodihydroxy phenol also increased after inoculation in all tested varieties.
19. Diffusible phenolics were elicited to the greatest by spore of *G. cingulata* on the resistant variety.
20. Maximum chlorophyll content was present in the older leaves, but showed no significant difference among the varieties. Chlorophyll content was reduced in the leaves following infection.
21. Maximum epicuticular wax content was obtained in the older leaves and minumum in the younger leaves.
22. Epicuticular wax content was higher in the resistant varieties.
23. No major anatomical differences were observed among different varieties.

Reference

Ahuja, S. (1991). Epidemiology of Jowar rust incidence. *Ind. Phytopathol., 45* : 120.

Ando, Y. (1991) . Formation of acervulus free conidia by the tea *(Thea sinensis)* brown blight fungus *Glomerella cingulata* and mass production method. *Plants and tea serirs B. (Japan), 4* : 17 - 23

Ando, Y. and Hamaya, E. (1986). Defence reaction of tea plant against infection of the tea anthracnose fungus. *Study of Tea, 69* : 25 - 43.

Arnows, E.L. (1933).Colorimetric determination of the component of 2, 4 - dihydroxyphenyl - alanine - tyrosinc mixture. *J. Biol. Chem., 118* : 513 - 537.

Asalmon, M.N., Sen, B. and Awasthi, J. (1991). Role of temperature and pH in antagonism of *Aspergillus niger* and *Trichoderma viride* against *Fusarium solani.Ind. Phytopathol.* (Suppl.), *44* : 106

Baker, C.J., O' Neill, N.R., Tomerlin, J.R. (1989). Accumulation of phenolic compounds incompatible clone/race interaction of *Medicaga sativa* and *colletotrichum trifolii. Physiol. Mol. Plant Pathol., 35* : 231-241.

Bakshi, S. and Chauhan, R.K.S. (1986). Change in phenolic contents of sheshum leaf exudates in response to infection with *Phyllactinia dalbergiae. Acta Mycol., 18* : 145 - 148.

Balamurli Krishnan M. and Jeyarajan R. (1997). Effects of weather factors on powdery mildew incidence in Grapeine. *Ind. J. Mycol. Plant Pathol.,27* :225 -226.

Banerjee, B. (1993). Tea production and processing. Oxford and IBH publishing Co. Pvt. Ltd., pp. 336.

Baxter, L.W. (1974). Studies on the twig blight, canker and dieback of *Camellia. American Camellia Year book, 2* : 63 - 75.

Bera, B., Saikia, H. and Singh, I.D. (1997). Biochemical Investigation of tea cultivars chlorophyll and carotenoids. *Two and bud, 44* : 11 - 15.

Bertolini, P. and Tion, S.P. (1997). Effect of temperature of production of *Botrytis allii* conidia on their pathogenicity to harvested white onion bulbs. *Plant pathol., 46* : 55-57.

Bertus, A.L. (1974). Fungicidal Control of *Camellia* dieback. *J. Hort. Sci., 49*:167-169.

Bezbaruah, H.P. and Singh, I.D. (1988). Advances in the use of planting material. In *Field Management in Tea.* Eds. S.K. Sarkar, H.P. Bezbaruah, Tocklai Experimental Station, Jorhat, India, pp.11-23.

Biggs, A.R. and Northouer, J. (1988). Influence of temperature and wetness duration on infection of peach and sweet cherry fruit by *Monilinia fructicola. Phytopathology, 78* : 1352 - 1256.

Bhargava, P.K. and Khare, M.N. (1988a). Epidemiology of *Alternaria* blight of chickpea. *Ind. Phytopathol., 41*:195-198.

Bhargava, P.K. and Khare, M.N. (1988b). Factors influencing mechanism of resistance in *Altanaria* blight of chickpea. *Ind. Phytopathol., 41* : 363-366.

Bhavani, U., Subbaiah, K. Rao V., A Sudhakara, A and SaiGopal, D.V.R. (1998). Studies on mosaic disease of sunflower.Biochemical changes and growth parameters. *Ind. Phytopathol., 54* : 357-358.

Borpujari, N. and Banerjee, M.K. (1994). Management of Young Tea In : *Field Management in Tea* (Ed. J. Chakravartee). Tocklai Experimental Station, Jorhat, Assam. pp. 50 - 57.

Boskovic, J. (1988). The investigation of epidemiology of four spring wheat pathogens in hilly mountain region of Bosnia and Merzegovina *Zast. Bilj., 39* : 323 - 333.

Bowers J.H, Sonoda R.M., and Mitchell, D.J. (1990). Path co-efficient analysis of the effect of rainfall variables on the epidemiology of *Phytophthora* blight of pepper caused by *Phytophthora. Phytopathology., 80* : 1439 - 1446.

Boyle, C. Boyle, H. and Friedrich, S. (1995). Effect of humidity upon turgor, germtube growth appressorium formation *in vitro* and infectivity of conidia from *Blumeria graminis* f. sp. *tritici. Ind. Phytopathol., 48* : 427 - 432.

Brar, J.S. and Bedi, P.S. (1991). Factors affecting growth of *Rhizoctonia bataticola* inciting root rot disease of okra in Punjab. *Ind. Phytopath, 45* : 83.

Bray, H.G., Thorpe, W.V. (1954). Analysis of phenalic compounds of interest in metabolism. *Methods Biochem. Anal., 1*: 27-52.

Carver, T.L., Thomas, W.B.J., Gerson Morri, S.M.T., Roaderick, M.W. (1990). The role of the leaf surface waxes of *Lolium* spp. in resistance to *Erysiphe graminis. Plant Pathol.,* **39** *:573-583.*

Chakraborty, B.N. and Saha, A. (1994a). Accumulation of antifungal compound in tea leaf tissue infected with *Bipolaris carbonum. Folia Microbiol., 39* : 409 - 414.

Chakraborty, U., Das, G. and Chakraborty, B.N. (1995).Factors influencing spore germination, appressoria formation and disease development in *Camellia sinesis* by *Glomerella cingulata. Folia Microbiol. 40* :159-164.

Chavan, S.B., Khandeg, S.V., Varshency, M.C. and Patil, J.P. (1995). Influence of weather parameters on conidia formation in powdery mildew of grape. *Ind. Phytopathol., 48* : 40- 49.

Chee, K.H. (1988).Studies on sporulation, pathogenicity and epidemiology of *Corynespora cassiicola* on *Hevea* rubber. *J. Nat. Rubber Res., 3* : 21 - 29.

Chaudhury, A.K. (1995). Biochemical changes associated with induction of resistance in ground nut plants to *Puccinia arachidis* by seed treatment with non conventional chemicals. *Plant Pathol., 25* : 231 - 234.

Chaudhury, R.G. and Dhar, V. (1988). Epidemiology of rice blast and effect of date of sowing under upland conditions of Arunachal Pradesh. *Ind. Phytopathol., 41* : 552-557.

Chaudhuri, S. and Nair, L.N. (1991). Changes in protein and polyphenol contents in *Dolichos lablab* and *phaseolus vulgaris* induced by *Rhizobium* infection. *Ind. Phytopathol., 45* : 117.

Coakley, M.S. (1998).Variation in climatic and prediction of disease in plants. *Annu. Rev. Phytopathal., 26* : 163 - 181.

Coakley, S.M., Scherm, H. and Chakraborty, S.(1999). Climatic change and plant disease management *Annu. Rev. Phytopathol., 37* : 399 - 426.

Cortes, N. J.A., Casas A. T. and Jemenez Diaz, R.M. (1998).Influence of relative humidity and temperature on development of *Didymella rabici* on chickpea debris *Plant Pathol., 47* :138-141.

Datar, V.V. (1995). Pathogenicity and effects of temperature on six fungi causing fruit rot of chilli. *Ind. J. Plant Pathol, 25* : 195 - 197.

Davydov, E.P., Kvasnyuk, T.F. and Palenkova, S.I. (1989). Effect of various source of C and N on *Septoiatritci* Rob. et. Desm. *Mikol. Phyto pathol., 23* : 422-424.

De-Silva, M.J. and Sivapalan, K. (1982). The chlorophyll content of tea clones in relation to their yield classification and geographical location. *Tea Q. 51* : 54 - 57.

Dickens, J.S.W. and Cook, R.T.A. (1989) : *Glomerella cingulata* on *Camellia. Plant Pathol., 38* : 75 - 85.

Dillard, H.R. (1988). Influence of temperature, pH and osmotic potential and fungicide sensitivity on germination of conidia and growth from sclerotia of *Colletotrichum coccoides in vitro. Phytopathology, 78* : 1357 - 1361.

Dubey, S.C. (1997). Influence of age of plants, temperature and humidity on web blight development in ground nuts. *Indian Phytopathol., 50* : 119 - 120.

Dwivedi, R.P., Dubey, S.C. and Mishra Prasant (1995). Epidemiology and control of Web blight of moth bean caused by *Thanatephorus cucumeris. Ind. Plant Pathal., 25* : 72.

Ebercon, A., Blum, A. and Jordan W.R. (1997). A Rapid colorimetric method for epicuticular wax content of sorghum leaves. *Crop Sci., 17* : 179 - 180.

Eberhard, K. (1991). Influence of cultural factors on the development of mildew in winter Barley. *Arch. Phytopathol. Pflanzen. Schutz, 27* : 143 - 152.

Fiyueroa, L., Fitt, B.D.L., Shaw, M.W., Cariney, M.D. M.C. and Welham, S.J. (1995) . Effect of temperature on the development of light leaf spot (*Pyrenopeziza brassica*) on oil seed rape. *Plant Pathol., 44* : 36 - 38.

Gandhi, S.K., Mehta, N. and Maheshwari S.K. (1997). Functional relationship between anthracnose of bottle ground and meterological factors. *J. Mycol. Plant Pathol., 27* : 152 - 156.

Gasparotto, L., Zambolim, L., Riberio de vale, F.X., Maffia, L.A. and Junqucitra, N.T.V..(1989). Epidemiology of the South American leaf blight of rubber tree. *Fitopathol. Bras., 14* : 65 - 70.

Guleria, S., Paul, B. and Bajaj, K.L. (1998). Level of phenols and phenol. metabolizing enzymes in powdery mildew (*Erysiphe polygoni* DC.) of pea. *Plant Dis. Res.,13* : 181 - 183.

Gupta, G.K. and Singh. D. (1999). Influence of environmental factors on downy mildew development in pearl Millet under arid climate. *J. Mylcol Plant Pathol., 29* : 1 - 4.

Gupta, P.P., Yadav, R., Grewal, R.P.S., and Yadav, D.K. (1976). Screening of sorghum germplasm (Multicut) against important foliar diseases. *Plant Dis. Res., 11* : 1 - 8.

Gupta S.K., Gupta, P.P. and Kaushik, C.D. (1995). Changes in leaf peroxidase, polyphenol oxidase, catalase and total phenols due to *Alternaria* leaf blight in *Brassica* species. *Ind. J. Mycol. Plant Pathol. 25* : 175 - 180.

Hiremath, R.V., Padagarnur, G.M. and Basavaraj, M.K. (1988). Epidemiology of *Alternaria* leaf spot of cotton in transition zone of Karnataka. *Ind. Phytopathol., 41* : 586 - 589.

Hoch, H.C. and Staples, R.C. (1987). Chemical changes among the rust fungi during appressorium development. *Annu. Rev. Phytopathal.,* **25** : 231-247.

Hywel, J. N.L. and Gillespie, A.T. (1990). Effect of temperature on spore germination in *Metarhizium anisopliae* and *Beauveria brassiana. Mycol. Res., 94* : 389 - 392.

Inoune, M. and Takio, C.T. (1990). Optimum pH range for mycelial growth of *Pythium* spp. estimated by MES-2-(N - morpholino) ethanesulfonic acid. *Trans. Mycol. Soc.*, JPN., *31*:191-196.

Jain, N.K. (1991). Indian Tea in Restrospect and Prospect and the impact of R. and D. *In World International symposium on Tea Science,* Japan, 45 - 57.

Janghan I.K., Seong B.K., Wol, S.K., Myong D.C. and Myong S. Y. (1989). Some factors affecting the occurrence of *Alternaria* leaf spot on apple tree (*Malus domestica* Burkh). *Res. Rep. Rural Dev Adm. (Suweon), 31* :16-20.

Jang, P. and Safeeula, K.M (1990). Factors affecting germination of *Peronospora parasitica* in radish. *Proc Indian Acad. Sci. Plant Sci., 100* : 113-116.

Jordan, M. M., Burchill, R.I. and Maude, R.B. (1990). Epidemiology of *Cladosporium allii* and *Cladosporium allii cepae* blotch pathogens of leek and onion *Ann. Appl. Biol., 117* : 313 - 326.

Kapoor K.S., Gill,H.S. and Sharma, S.R. (1991). Epidemiology of *Sclerotinia* stalk rot of cabbage seed plants.*Ind. Phytopathol., 44* : 56.

Kar, A.K. and Lenka, D. (1998). Occurrence of *Alternaria* blight and powdery mildew on linseed genotypes in Orissa. *Plant Dis. Res., 13* : 160-161.

Kaur, J. and Dhillon, M. (1988).Pre-infectional anatomical defence mechanism of ground nut leaf against *cercosporidium personatum Ind. Phytopathol, 41* : 376-380.

Kaur, M. and Deshmukh, K.B. (1980).Phytosynthetic activities of cowpea plants infected with *Erysiphe polygoni. Ind. Phytopathol., 33* : 344 - 345.

Kaushal, R.P., Kumar, A. and Tyagi, P.D. (1998). Role of light, temperature and R.H. on germinationof *Colletotrichum truncatum* and soybean pod infection under laboratory conditions. *J. Mycol. Plant Pathol., 28* : 1-4.

Khare, N., Lakpale, N. and Agrawal, K.C. (1998) : Epidemiloogy of powdery mildew of mungbean in Chhttisgarh Region of Madhya Pradesh. *J. Mycol. Plant Pathol, 28* : 5 - 10.

Kiranmal G., Sreenivasulu, P. and Nayudu, M.V. (1998) . Epidemiology of cucumber mosaic cucumovirus isolates naturally infecting three solanaceous vegetable crops around Tirupati. *Ind. Phytopathol., 51* : 315 - 318.

Klavis, S. (1989). Epidemialogical studies regarding mildew infection of spring barley varieties in mix stands. *Arch. Phytopathol. Pflanzen. Chutz, 25*:49 - 62.

Kotasthane, A.S. and Vyas, S.C. (1990). Phenol content as influenced by fungicides and by different mode of application on mustard plant. *Ind. Phytopathol. (Suppl), 43 & 48* : 6

Kumar, A.S. and Niwas, R. (1998). Relationship between climatic factors and initiation of red rot infection in sugarcane. *Ind. Phytopathol. 51* : 370 - 372.

Kumar, G.R., Ganesh, R., and Pathak, V.N. (1990). Some biochemical changes in the shoot of pearl millet infected by downy mildew. *Curr. Sci., 59* :521 - 522.

Kumar, R. and Singh, S.B. (1996). Influence of weather factor on *Alternaria* leaf spot development in sunflower. *Ind. J. Mycol. Plant Pathol.,26* :196 - 198.

Kumar, S. and Sridhar, R. (1987).Significance of epicuticular wax in the specificity of blast fungus to rice varieties. *Int. J. Crop. Plant Dis.,5*:131- 139.

Kumar, V., Hooda, I. and Sindhan, G.S. (1999). Role of seed exudates in differential behaviour of barley varieties to *Drechslera graminea* infection. *J. Mycol. Plant Pathol., 29* : 121 - 122.

Kumar, V., Kaushik, C.D. and Gupta P.P. (1995). Role of various fctors in development of white rust disease of rapeseed mustard. *Ind. J. Mycol. Plant Pathol, 25* : 145 - 148.

Loomis, R.S. and Adams, S.S. (1983).Integrative analyses of host pathogen relations. *Annu. Rev. Phytopathol., 21* : 241 - 362.

Lukosic, C. M., Kadvani, D.L., Patel, M.H. and Pethani, K.V. (1999). Impact of excess rainfall and drought on downy mildew disease in pearl Millet. *J. Mycol. Plant Pathol., 29* : 398 - 401.

Luthra, Y.P.; Joshi, U.N., Gandhi, S.K. and Arora, S.K. (1998) : Biochemical alterations in downy mildew infected lucerne leaves. *Ind. Phytopathol., 41* : 100 - 106.

Madhu, M., Malhotra R. and Parmar Y.S. (1995) .Epidemiology and management of *Alternaria* blight of carnation (*Dianthus caryophylus L.) Ind. Phytopathol.,* 48 : 76

Mahadevan, A. and Sridhar, R. (1982). Methods in physiological plant pathology. 2nd Edn. Madras : Sivakami Publication, India.

Mandavia, M.K., Andharia J.H., Khandar, R.R. and Parameswaran, M. (1995). Role of phenols in resistance of *Fusarium* wilt in chickpea. *Ind. J. Mycol. Plant Pathol.,25*:107.

Mayee, C.D. (1995). Epidemiology of fungal diseases of major oil seeds. *Ind. J. Mycol. Plant Pathol., 25* : 122.

Mayee, C.D. and Kokate V.N. (1987). Environment in relation to incubation Period of ground nut rust. *Ind. Phytopathol., 40* : 491 - 494.

Meeta, M., Shyam., K.R. and Pathania, N.S. (1996). Evaluation of carnation germplasm for resistance to *Alternaria* blight. *Plant Dis. Res., 11* : 76 - 78.

Mends, B.M.J. and Filho, A.B. (1989). Influence of temperature, wetness duration and leaf type on the quantification of monocyclic parameters of bean rust. *J. Phytopathol. (Berl.), 126* : 183 - 189.

Mishra A. and Gupta, O. (1994) Influence of environment on growth and sporulation of *Colletotrichum dematium. Ind. Mycol. plant Pathol.,24:85-87.*

Mittal, R.K. (1999).Cultural management of foliar disease of black gram in hills of U.P. *J. Mycol. Plant Pathol.,* **29** : 128 - 130.

Mitter, N., Grewal J.S. and Pal, M. (1997) . Biochemical Change in chickpea genotypes resistant and susceptible to grey mould. *Ind. Phytopathol., 50*:490 498.

Mohamed, M. T., Weerasinghe, D.K. and Wickremasinghe, V. (1986). Chemistry of tea (*camellia sinensis)* leaf surface. *S.L.J. Tea. Sci, 55* : 36 - 43.

Mora, G.D., Teliz, M. Rosas, M. and Garcia, R. (1988). Epidemeology of *Phytophthora cinnamoni* in the root system of the avocado. *Rev. Mex. Fitopatol., 6* : 218 - 223.

Moses, G.J. (1995). Disease of Ber (*Zizyphus* spp.). Their epidemiology and management in Andhra Pradesh, India. *Ind. J. Mycol. Plant Pathol., 25* : 75.

Muller, K.O. (1958). Studies of phytoalexins I. The formation and the immunological significance of Phyto alexin produced by *phaseolus vulgaris* in response to infection with *Sclerotinia fructicola* and *phytophthora infestans. Aust. J. Biol. Sc., 11* : 275 - 300.

Mukai, T.,Kalogiri, M., Ando Y., Hakamata, K. and Hara, Y. (1989) . Inhibitory effect of catechins on spore germination of *Cochliobolus miyabeanus. Plants and Tea series (Japan), 3* : 75 - 80.

Munshi, G.D. and Singh T. (1994) : Effect of temperature and R.H. on development of *Uncinula necator* on grapes. *Ind. J. Mycol. Plant Pathol., 24*: 179-183.

Murrary, T. D. and **Campbell, W.C.** (1991). Influence of pH and matric potential on sporulation of *Cepalosporium graminum. Phytopathology, 81*: 79-84.

Naik, M.K., Hiremath, P.C. and Hiremath, S.V. (1988). Post infectional changes in the betal vine leaves infected with *Colletotrichum gloeosporioides. Ind. Phytopathol., 41 :* 370-372.

Nawal, A. and Ho, Y.W. (1990). Effect of temperature and pH on growth pattern of *Ganoderma boninense* from oil palm in peninsular Malaysia. *Pertanika, 13: 303-308.*

Ng'etich, W.K. (1997). Responses of tea to environment *Tea, 18* : 149-155.

Nisar, A., Khan, A.M.W and Muheet, A. (199). Effect of some factor on growth and sporulation of *Alternaria brassicae* causing *Alternaria* blight of rape seed and mustard. *Acta.Bot. Ind., 17*: 49-53.

Nozzolillo, C., Isabelle, P. and Das, G. (1989). Seasonal changes in the phenolic constituents of Jack pine seedling (*Pinus banksiana)* in relation to the purpling phenomenon. *Can.J.Bot.,* **68** : 2010-2017.

Okhovat, M. and Hedjaroude, G.A. (1991). Effect of climatic condition on sporulaiton of *pyricularia oryzae. Phytopathology, 81* : 48-52.

Onsando. J.M. and Owom, P.O. (1990). The toxicity of polyhydroxy phenol and other related compounds on growth of *Hypoxylon serpens* (pers.ex Fr.) kicks, *in vitro. Tea, 11* : 104-108.

Padmanabhan, P., Alexander, K.C. and Shanmugam, R. (1988). Some metabolic changes induced in sugarcan by *Ustilago scitaminea. Ind. Phytopathol., 41*: 225-235.

Panwar M.S., Kumar, R. and Rathi, A.S. (1995). Epidemiology of erogot of bajra caused by *claviceps fusiformis* loveless and its management. *Ind. J. Mycol. Plant Pathol., 25* : 71.

Park, R.F. (1990). The role of temperature and rainfall in the epidemiology of *Puccima striiformis* f.sp. *tritici* in the summer rainfall area of Eastern Australia. *Plant Pathol., 39*: 416-423.

Payne, G.A., Thompson, D.L., Lillehoj, E.B., Zuder, M.S. and Adkins C.R. (1988). Effect of temperature on the preharvest infection of maize kernels by *Aspergillus flavus. Phytopathology, 78* : 1376-1380.

Penaranda, M., Crestina, F.B., Cruz M., Sergio, M.,Lidcoy S.,Merrera, I. and Galanted, E. (1988). Effect of temperature of upon growth and pathogenic activity of *Phytophthora nicotianae* var. *Parasitica* on tomato. *Cent. Agri. C.*, ***15*** : 10-16.

Piza, S.M.T. and Ribeiro, I.J.A. (1989). Influence of temperature, kinds of water and incubation period of germination of uredospores of *Puccinia psidii. Summa Phytopathol.*, ***15*** : 222-226.

Prakash, S. and Saharan, G.S. (1999). Factors affecting conidial germination of *Peronospora trigonella. J. Mycol. Pl. Pathol.*, ***29*** : 368-374.

Prakash, V. and Jhooty, J.S. (1987). Epidemiology of powdery mildew of *Zizyphus mauritiana* caused by *Microsphaera alphitoides* F.SP. 2I3I PHI. *Indian Phytopathol.*, ***40*** : 491-494.

Prasad M.M. and Kumari, S. (1994). Changes in level of protein and phenol contents in banana fruits under pathogenesis. *Ind. Phytopathol.*, ***45***: 319.

Prashar, A. and Sood, A.K. (1994). Effect of temperature and duration of leaf wetness on incubation period of brown spot of soybean caused by *Septoria glycinea. Ind. Phytopathol.*, ***45*** : 307.

Pretorius, Z. and leroux, L. (1988). Occurrance and pathogenicity of *Puccinia recondita.* F. sp. *tritici* on wheat. *Phytophylactia, **20*** : 349-352.

Ragazzi A., parrini, C. Mesturino, L and Dellavalle, F. I. (19987). Epidemiology of *Verticillium dahliae* kleb on olive trees. *Riv. Patol.veg., **23*** : 132-139.

Raghuram, M. and Mallaidh, K.V. (1996). Effect of relative humidity on sporulation and germination of spore of *Cercospora* and *Pseudo cercospora* spp. on infection of legumes. *Ind. Phytopathol.*, ***49*** : 290-293.

Ramiah, R., Sudha. M. and Rajappan K. (1998). Effect of tomato spotted wilt virus infection on photosynthetic pigments in tomato. *Plant Dis. Res.*, ***13***:138-140.

Ranganathan, V. and Natesan, S.(1987) . Manuring of Tea - Revised Recommendation. *Hand Book of Tea culture*, section, ***11*** : 1 - 27.

Rao, B. M.; Shetty, M.S. and Saifulla, K.M. (1987) . Effect of temperature on conidial germination and *germ tube* length growth of different isolates of *Pesonosclerospora sorghi* from. *Ind. Phytopathol.*, ***40*** : 352 - 355.

Rao, K.S., Prasad, S. and Paruathi, V. (1986). Changes in phenolic constituents of rice in response to *Rhizoctonia solani* infection. *Ind. J. Bot.* ***9*** : 43-44.

Rao, R. and Kumar, T.B.A. (1991). Phenol metabolism in cowpea plants to infection of powdery mildew. *Ind. phytopathol., (Suppl.).,* ***16*** : 43 - 44.

Reddy, A. N., Vanaja, M. and Rao, P. R. (1995). *In Vitro* study of spore germination of *Colletrotrichum capasici* on leaf leachates of cultivrs of *Curcuma longa. Ind. J. Mycol. Plant Pathol.*, ***25*** : 90.

Rewal, N. and Grewal, J.S. (1989). Effect of temperature, light and relative humidity on conidial germination of three strains of *Botrytis cinerea* infecting chickpca. *Ind. Phytopathol.*, ***42*** : 79-83.

Rey M.E.C. and Garnett H.M (1988). Epidemmiological studies of *Puccinia digitariae* and *Phyllactaria paspalicala* on grass at Nylsvleg nature reserve. *Safr. J. Bot.*, ***54*** : 551-557.

Roger, C. Tivoli, B and Huber L. (1999a). Effects of temperature and moisture on disease and fruit body development of *Mycosphaerella pinodes on pea (pisum sativum). Plant pathol.*, ***48*** : 1-9.

Roger, C., Tivoli, B. and Huber, L. (1999b). Effects of interrupted wet periods and different temperature on the development of *Ascochyta* blight caused by *Mycosphaerella pinodes* on pea (*Pisum sativum*) seedlings. *Plant Pathology,* ***48*** : 10-18.

Rolando A.R., Alfenas Conto Acelino, A.C., Francico, F.A. and Francisco, D.R.X. (1989). Influence of temperature, leaf wetness period, photoperiod and light intensity on the infection of *Puccinia psidii* in *Eucalyptus. Fitopatol. Bros.,14.* 55-61.

Rotem J. (1978). Climatic and weather influences on epidemis. In *Plant Disease - Plan Disease* — An Advanced Treatise. *2*: (Eds. J.G. Horsfall and E.B. Cowling), pp-317-338, Academic Press New York.

Sahran, G.S. and Meel. M (1994). Epidemiology of *Alternaria* blight of sunflower. *Ind. Phytopathol.*, *45* : 306-307.

Saifulla, M. and Ranganathan K.G. (1990). Studies on the cultural behavior of anthracnose of sorghum caused by *Colletotrichum graminicola* (les) wills. *J. Agri. C. Sci., 24* : 52-56.

Salamati, S. and Tronsmo, A.M. (1997) : Pathogencity of *Rhyn chosporium secalis* isolates from Norway on 30 cultivars of Barley. *Plant pathol., 46* :123 125.

Sandhu, A. and Singh. R.D. (1999). Factors influcing susceptibility of cowpea to *Macrophomina phaseolina. J. Mycol. Plant Pathol., 29* : 421-424.

Saxena, D.R. and Khare M.N. (1988). Factors influencing vascular wilt of lentil. *Ind. Phytopathol., 41* : 69-74.

Shahzad, A. and Mir, N.A. (1998). Effect of different carbon and nitrogen sources on the growth and sporulation of *Wilsonomyces carpophilus. Plant Dis. Res., 13* : 147-148.

Shajahan, A.K.M., Ahmed, H.U., Sharma, N.R. and Miah, S.A. (1990). Epidemiological studies of sheath blight of rice caused by *Rhizoctonia solani kuhn. Bangladesh J. Bot., 19* : 125-134.

Sharma, C.L. and Kapoor, K.S. (1997). Some epidemiological aspects of white rot of pea. *Ind. Phytophathol., 50* : 342-349.

Sharma, I.M. and Badiyala, S.D. (1998). Screening of mango cultivars for susceptibility to *Colletotrichum gloeosporioides* during different season. *Ind. Phytopathol., 51* : 199-200.

Sharma, J.N. and Kaul J.L. (1995). Phenolics and their metabolism in scab resistant and susceptible apple cultivars. *Ind. J. Mycol. Plant Pathol.,25* : 60.

Sharma, K. K. and Gupta, V. K. (1991). Effects of temperature and relative humidity on conidial germination and germtube length of *Podosphaera lecucotricha* on glass slide. *Ind. phytopathol.* (suppl.), *44* : 39.

Sharma, S., Kaur, S., Dhillon, T.S., Singh. M. and Bajaj, K.L. (1996). Studies on phenolic contents in pea genotypes in relation to powdery mildew disease. *Plant Dis. Res., 11* : 159-161.

Shaw, D.A., Adaskoiveg, J.E. and Ogawa, J.M. (1990). Influence of wetness perioid and temperature on infection and development of shot hole disease of almond caused by *Wilsonomyces carpophilus. Phytopathology, 80* : 749-756.

Shaw, M.W. (1990). Effects of temperature, leaf wetness & cultivar on the latent period of *Mycosphaerella graminicola* on winter wheat. *Plant Pathol., 39* : 255-268.

Sidhartha, S., Singh, D.V., Srivastava, K.D. and Agarwal, R. (1995). Some epidemiological aspects of karnal bunt of wheat. *Ind. Phytopathol., 48* : 419-426.

Sindhan, G.S. and Parashar, R.D. (1996). Biochemical changes in ground nut leaves due to infection by early and late leaf spot pathogen. *Ind. J. Mycol. Plant Pathol., 25* : 210-212.

Singh, I., Nandwal, A.S., Kuhad, M.S. and Singh, N. (1998a). Biochemical changes occuring during development of leaves emerged at 3 different stage in pigeonpea. *Ind. J. Plant Physiol., 3.* 89-93.

Singh, K. and Pande, B.N. (1991). Epidemiology of bajra *Pyricularia* leaf spot in relatin to weather condition., *Ind. Phytopathol., 45* : 110.

Singh, M. I. and Tombisana, D. R. K. (1996). Effect of different carbon sources and aminoacids on growth and sporulation of *Fusarium* species causing sheath rot of rice. *Plant Dis. Res., 11* : 139-142.

Singh, M.J., Singh, J. and Cheema, S.S. (1998b). Effect of cucumber mosaic virus on chlorophyll content and mineral elements in chilli. *Plant Dis. Res., 13*: 135-138.

Singh, R., Pandya, R.K. and Khare, M.N. (1998c). Biochemical changes in sunflower leave infected by rust (*Puccinia calcitrapae* var *centarreae). Ind. J. Mycol. Plant Pathol., 28* : 164-167.

Singh, S.D., Gopinath, R. and Pawar, M.N. (1987). Effects of environmental factors on asexual sporulation of *Sclerospora graminicola. Ind. Phytopathol., 40* : 186-193.

Singh, S.N. (1999). Evalution of sugarcane varieties for resistance to smut in Madhya Pradesh. *J. Mycol. Plant Pathol., 29* : 141-142.

Singh, U. and Thapliyal, P.N. (1998). Effect of inoculum density, host cultivars and seed treatement on the seed and seedlings rot caused by *Sclerotium rolfsii. Ind. Phytopathol., 53* : 244-246.

Smith, E.F. and Backman, P.A. (1989). Epidemiology of soyabean stem canker in the south eastern U.S.A. : Relationship between time of exposure to inoculum & disease severity. *Plant Dis. Res., 73* :464-468.

Smith M.C., Holt., J., Kenyon, L. and Foot, C. (1998). Quantitiative epidemiology of banana bunchy top virus disease and its control. *Plant Pathol., 47* : 177-187.

Thakur, M.P. and Agarwal, K.C. (1995). Epidemiological studies on powdery mildew of mungbean and urdbean. *Ind. J. Plant Pathol., 25* : 73-74.

Themts, M. and Ogawa, J. M. (1989). Effect of high temperature on the survival and pathogenicity of propagules of *Mucor piriformis*. *Phytopathology, 79* : 547-554.

Trivedi, N. and Sinha, A.K. (1976). Resistance induced in rice plant against *Helminthosporium* infection by treatment with various fungal fluids. *Phytopathology, 86* : 335.

Vale, F.X., Ribeirodo, X., Zambolim., L. and Chaves, G.M. (1990). Effect of temperature and wetness duration on the infection of soyabean by *Phakospora pachyrhizi. Fitopathlogica Bras., 15* : 200-202.

Verma, K.S. and Kaur, D. (1998). Seasonal carryover and epidemiology of *Oidium mangifera,* the incitant of mango powdery mildew. *Plant Dis. Res., 13* : 119-124.

Verma, K.S. (1991). Effect of carbon, nitrogen and vitamin nutrition on growth and sporulation of some isolates of *Corticium salmonicolor. J. Res. Punjab Agric. Univ., 26* : 608-613.

Verma, S., Gupta, S., Singh, R.V. and Abidi, A.B. (1991). Changes in biochemical constituents of beal fruits infected with *Aspergillus species. Ind. Phytopathol., 44* : 405.

Vidic, M. and Jashic, S. (1988). Contribution to investigation of *Diaporthe phasedorum* var *caulivora* epidemiology on soyabean. *Zast, Bilj., 39* : 297-310.

White, J.L. and Brakke, M.K. (1983). Protein changes in wheat infected with wheat streak mosaic virus and in barley infected with barely stripe mosaic virus. *Physiol. Plant. Pathol., 22* : 87-100.

Xu, X.M. (1996). On estimation of non linear response of fungal development under fluctuating temperature. *Plant Pathol., 45* : 163-171.

Yanase, Y. and Takeda, Y. (1987). Methods for testing the resistance to tea grey blight disease caused by *Pestalotia longiseta* spegazzini in tea breeding. In *Bulletin of the National Research Institute of Vegetables, Ornamental Plants & Tea Series B. (Kanaya). 1* : 1-19.

North Bengal University
Library
Siliguri, Raja Rammohunpur