STUDIES ON SOME FOLIAR FUNGAL DISEASES OF YOUNG TEA (*Camellia sinensis* (L.) O. KTZE) PLANTS

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

Sourish Das Gupta, M.Sc

DEPARTMENT OF BOTANY
UNIVERSITY OF NORTH BENGAL
INDIA
2004

UNIVERSITY OF NORTH BENGAL
Department of Botany

Dr. Aniruddha Saha
M. Sc., Ph. D.
Senior Lecturer,

P. O. - N.B.U.,
SILIGURI-734430
WEST BENGAL, INDIA
Phone: 91 353 2582106 (O)
91 353 2580257 (R)
E-mail: drishtanta @ yahoo.com
as_bot @ rediffmail.com

This is to certify that Mr. Sourish Das Gupta, M.Sc. has worked under my supervision at the Department of Botany, University of North Bengal for Ph. D. thesis entitled **"Studies on some foliar fungal diseases of young tea (*Camellia sinensis* (L.) O. KTZE) plants"**. I am forwarding his thesis for the Ph. D. degree (science) of the University of North Bengal. I recommend that he has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in his thesis.

Aniruddha Saha

(DR. ANIRUDDHA SAHA)

Supervisor & Senior Lecturer,
Department of Botany, N. B. U.

Acknowledgement

I have much pleasure in expressing my deepest sense of gratitude to my supervisor Dr. Aniruddha Saha, Senior Lecturer, Department of Botany, University of North Bengal, for his inspiration, encouragement and guidance throughout the execution of this work. I am grateful to Dr. (Mrs.) Dipannwita Saha, Research Associate, for her ungrudging help and advice at all the times. I am also indebted to Dr. (Mrs.) U. Chakraborty (Head, Department of Botany, University of North Bengal), Prof. A. P. Das, Prof. B. N. Chakraborty, Prof. P. K. Sarkar, Dr. R. Chakraborty, Dr. A. Sen and Mr. P. Mandal of Department of Botany, University of North Bengal for their valuable advice and encouragement throughout the course of this work.

Special thanks are due to Prof. B. N. Chakraborty for helping me in fluorescence-photomicrography, Dr. U. Chakraborty for ELISA-microplate reading and Dr. J. Pal of Department of Zoology, University of North Bengal for providing some laboratory facilities.

I am thankful to Dr. P. N. Chowdhury, ITCC, IARI, Pusa, New Delhi for identifying some of my organisms and I am also thankful to Dr. A. Chowdhury, UBKV for supplying me one fungal culture. I am also thankful to Mr. Partha Roy, Assistant Manager, Mohurgong-Gulma tea estate. Thanks are also due to the authorities of Mohurgong and Gulma tea estate, Matigara Tea Estate, Taipoo Tea Estate and to the people of the different nurseries in Kharibari and other parts of North Bengal for permitting me to do various field experiments. Thanks are also due to Mr. Babul Biswas for scanning of the photographs.

I also express my thanks to my colleagues Mr. Parimal Mandal, Md. Mehebub Isha, Mrs. Chandrani Mandal, Mr. Narayan Kundu and Mr. Surojit Sen. I am also thankful to Mr. Mayukh Sarkar, Mr. Pradipta Bhattacharya, Mr. Bhaskar Bhadra and Mr. Rajeeb Ghosh for their help and encouragement in various times.

Finally, I express my heartfelt gratitude and respect to my beloved parents Mr. Bankim Dasgupta and Mrs. Reba Dasgupta for their endless inspiration, affection and encouragement. Thanks are also due to my sister Ms. Suchandra Dasgupta for her help and encouragement.

Sourish Das Gupta

(SOURISH DAS GUPTA)

Abbreviations

^0C	Degree Celsius
BSA	Bovine Serum Albumin
CD	Critical Difference
CDA	Czapek Dox Agar
cm	centimetre
CRA	Cross Reactive Antigens
ELISA	Enzyme Linked Immunosorbent Assay
FITC	Fluorescein Isothiocyanate
g	gram
h	hours
IAA	Indole Acetic Acid
IARI	Indian Agricultural Research Institute
IBA	Indole Butyric Acid
IgG	Immunoglobulin G
ITCC	Indian Type Culture Collection
JA	Jasmonic Acid
lt	litre
M	Molar
MEA	Malt Extract Agar
MIC	Minimum Inhibitory Concentration
ml	millilitre
N	Normal
OMA	Oat Meal Agar
PBS	Phosphate Buffer Saline
PCA	Potato Carrot Aga.
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PR	Pathogenesis Related
PVP	Poly Vinyl Pyrrollidone
R_f	Run front.
RM	Richards' Medium.
rpm	rotation Per Minute.
SA	Salicylic Acid.
SAR	Systemic Acquired Resistance
TLC	Thin Layer Chromatography.
TV	Tocklai Variety.
UBKV	Uttar Banga krishi Vishwavidyalay
UPASI	United Planters Association of South India
µg	microgram
µl	microlitre

Contents

Introduction

Tea occupies an important position among the plantation crops, in view of its popularity worldwide. Tea originated in China and is one of the world's oldest known prepared beverages. The earliest use of tea by the Chinese is said to have been in the 27th century BC (Yamanishi, 1991). Globally, among some fifty countries growing tea, about a dozen could be regarded as the major producers. India is one of the largest producer while the other countries substantially contributing to global production includes China, Sri Lanka, Kenya, Russia, Japan, Argentina and Uganda.

The commercially cultivated tea plants are derived from *Camellia sinensis* (L.) O. Kuntze, the short leaved 'china' plants: *Camellia assamica* (Masters) Wight, the broad leaved 'Assam' plants: *C. assamica lasiocalyx* (Planchon ex Watt) Wight, the 'Combod' plants and the numerous hybrids among them. These plants prefer a warm humid climate, well-distributed rainfall and long sunshine hours. Presently tea is grown from Georgia, 43 ^{0}N latitude to Nelson (south Island) in New Zealand, 42 ^{0}S latitude and from sea level to 2300 m above mean sea level. Tea, cultivated near the equator produces almost the same yield every month, but further from equator, harvest in winter gradually declines and at latitude beyond about 16 ^{0}, there is complete winter dormancy, the length of dormant period increases progressively with increasing distance from equator (Barua, 1989).

With the introduction of different improved (in view of the production quantity, quality and environmental suitability) varieties and with the expansion of the plantation of the newer areas, the problems of diseases have also increased many folds. To keep pace with the huge demand for the planting material a large number of young plants are required every year. These plants are commonly propagated by clonal propagation, though seed propagation is also being attempted in certain varieties.

Historically, tea was cultivated in India by seed from the very beginning of the tea industry around 1830 and it remained in use for over 120 years. The use of clones started in fifties after the release of Tocklai clones in 1949, although the economic method of vegetative propagation was established around 1938.

Seed populations are highly heterogenous as a result of free outcrossing among themselves from which superior clones have been selected (Bezbaruah

and Singh, 1988). Barua (1963) mentioned that the basic difference between clones and seed populations is one of adaptibility. A seed population in tea is composed of a large number of genetically distinct genotypes, is elastic and can be fitted into a wide range of environmental and cultural conditions, without much change in its overall performance. Contrary to seed populations, thousands of bushes of a clone separated widely in space and time behave in most ways as a single bush. Consequently, a clone lacks elasticity which makes a clone more selective of environment and cultural treatments (Bezbaruah and Singh, 1988). When a clone is forced to grow in some other environment, they becomes more susceptible to diseases. A considerable number of plants suffer within 6 months of their propagation in the clonal nurseries due to damages made by pests and diseases. In addition, many plants are affected in the field just after plantation. Since It is of utmost importance to keep young tea plants free from any pest and disease infestations (Barbora, 1988), intensive management of pest and diseases are essential during the years of early establishment.

It has been noticed that fungi often attack young tea plants. These fungal attacks either in the nurseries/seedbaries or in the new plantation areas may be primary or secondary in nature. It is important to know about a fungus present in a plant, their possible harmful effects, and their interaction with the host and control strategies of the pathogen for proper management of the disease. Interaction of plants and phytopathogenic fungi have become one of the most interesting and rapidly moving sciences. Several workers have worked or reported on different fungal diseases of tea plants (Sarmah, 1960; Chakraborty, 1987). However, it was considered worthwhile to find answers to the following questions: a) what are the different important fungi associated with the planting materials and young tea plants? b) what are the disease reactions? and c) how the diseases could be managed? In the last two decades much has been achieved in understanding the plant-pathogen interaction with reference to the action of genes involved. Infection may be prevented by means of well-determined and genetically managed defense mechanisms in the plant. The process includes constitutive defense barriers or their formation is induced by the attacking pathogen. It means that the defense reaction expression is proceeded by recognition of the pathogen by the plant.

Defense is possible through basic resistance i.e., presence of genetic determinant for host resistance. Cultivars of specific resistance represent a highly selective defense reaction of host plants against one particular pathogen or certain races of it. Some plant shows high resistance to some fungi while some cultivars are highly susceptible. As soon as the pathogen attacks the host, the result of signal transduction events that affect activation of genes synthesizing proteins or enzymes engaged in defense reaction play significant role. Hypersensitive defense reaction of the cells are affected by the pathogen and occurs very rapidly terminating with plant- cell death while inducible defense reaction develops more slowly in the surrounding and surviving plant cells is called a sensitive defense reaction (Klement, 1982).

Induced resistance has been demonstrated in many cultivated plants including vegetable crops, cereals, fruit yielding plants and others (Kuć, 1982; Kessmann *et al.*, 1994; Okey and Sreenivasan, 1996; Uknes *et al.*, 1992 and Lyon *et al.*, 1996). It appears that the first infecting pathogen or an injury immunizes the plants against further infections by homologous pathogens, even though the plant may not carry gene determining cultivar specific resistance. The readiness of the plant to repel subsequent pathogen attacks spread through the whole plant is called systemic acquired resistance (SAR).

The concept of common antigenic relationship between host and parasite has received much attention during the last two decades or so. A pathogen and its host share antigens, which play an important role in the determination of compatible interaction. The absence of the common antigens leads to incompatible interaction. Even within the compatible interactions the degree of compatibility might be determined by the sharing of the common antigens.

Biological control is the reduction of amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man. The formulation technology of fungal antagonists is the effective implementation of biocontrol of various crop diseases (Prasad and Rangeswaran, 1999).

The social and environmental cost of synthetic pesticides and fungicides are very high. Hence there is an all-round compulsion among the multinational

companies and other agencies to go in for biorational alternative arsenals which can be eco-friendly and benign to environment. Out of 2,50,000 higher plant species, which are believed to exist on earth only relatively few, have been thoroughly studied for their therapeutic potential. (Deans and Svoboda, 1990).

Currently plant products are targeted, as they comprise a rich storehouse of biochemicals that could be trapped for use as pesticides. There are 10,000 secondary metabolites such as tarpenoids, alkaloids, phenols, tannins etc. which are very effective in the control of phytopathogenic fungi. In India no such product is registered for the control of phytopathogenic fungi (Narasimhan and Masilamani, 2002). Hence, at the onset of the present work a thorough survey of the tea nurseries of North Bengal were done (Plate I). Following this, isolation of pathogens were also performed randomly. During the survey, six pathogens were isolated from the leaves of young tea plants. A young clone generally raised from a cutting of approximately 10 cm long twig having a large maintenance leaf and a leaf bud. However, both the new and the old leaf (maintenance) are susceptible to attack by fungal pathogens. In all the cases of isolation, it was found that the old maintenance leaf was attacked by *Pestalotiopsis theae*, *Colletotrichum camelliae*, *Corticium theae* and *Fusarium oxysporum*. As maintenance leaf is shed after 5-6 months, hence the damage done by the four above mentioned fungi are not so important in nurseries for developing healthy plants, unlike the *Botryodiplodia theobromae* and *Curvularia eragrostidis* which severely attack the new emerging shoots. Out of these two pathogens, *B. theobromae* can attack both tender stems as well as young leaf and leaf bud while *C. eragrostidis* attack mostly the fully expanded young leaves. *C. eragrostidis* is a new record in tea (Saha *et al.*, 2001) but *B. theobromae* was recorded by Sarmah (1960) and was described as a virulent pathogen of young tea plants.

Hence, in the present study, it was considered to study different aspects of host-parasite interaction with special reference to *C. sinensis* -*B. theobromae* and *C. sinensis* -*C. eragrostidis*. The basic objectives of this study are: -

- To isolate the different foliar fungal pathogens of nursery tea plants.
- Identification of the pathogens after completion of Koch's postulates.

- To find out the susceptible and the resistant varieties of tea plants against *B. theobromae* and *C. eragrostidis* following pathogenicity test.
- To study the morphological and physiological characteristics of *B. theobromae* and *C. eragrostidis.*
- To determine the serological relationship between *C. eragrstidis* and tea varieties as well as *B. theobromae* and tea varieties.
- To study whether the disease reactions could be altered in susceptible tea varieties by chemical treatment and by known SAR inducers.
- To control diseases by eco-friendly plant extracts, chemical fungicides and by biocontrol agents.

Plate I

Fig. 1 : Tea plantation at Taipoo Tea Estate, Kharibari, West Bengal.
Fig. 2 : *Curvularia eragrostidis* affected field at Matigara Tea Estate, Siliguri,West Bengal.
Fig. 3 : *Botryodiplodia theobromae* affected field at Kharibari, Siliguri, West Bengal.

Literature-Review

Any plantation programme needs fresh healthy seedlings or clonal cuttings. The seedlings in nurseries face several disease problems. To control diseases of plants effectively, it is necessary to know about host-parasite interactions. Pathogenesis and disease resistance are closely related to each other because they treat host-parasite interactions from different points of view. Pathogenesis is related to compatible interactions while resistance related to incompatible ones. At the onset of the present study it was considered to review the works of the previous workers in a selective manner. The observations of the different workers in concord with the present line of investigations are being presented briefly in the following paragraphs, however, for convenience the observations have been divided into some groups or aspects. The different aspects of this review are:

- Diseases.
- Growth and physiology of the pathogens.
- Common antigenic relationship.
- Plant disease alteration by chemical treatment.
- Disease control by fungicides.
- Disease control by botanicals.
- Disease control by antagonistic organisms.

Diseases

Like any other cultivated crop, tea plants [*Camellia sinensis* (L.) O. Kuntze.] in northeast India are prone to a number of serious diseases (Fig.1 & 2). The fact that it is a perennial crop grown remote from its natural habitat over vast areas as a homogenous mass under varying soil and climatic conditions makes it a happy hunting ground for many fungi and other disease causing organisms. All the common and economically important diseases of tea with the exception of 'red rust' are incited by fungal pathogens. As early as in the sixties, Agnihothrudu (1964) listed 385 species of fungi on tea plants. After a thorough revision, Chen and Chen (1989) reported that a total of 507 fungi had been recorded on tea. Barua (1989) reported that out of 385 species of fungi occurring on tea all over the world, 190 were recorded in tea in northeast India alone (Fig. 2).

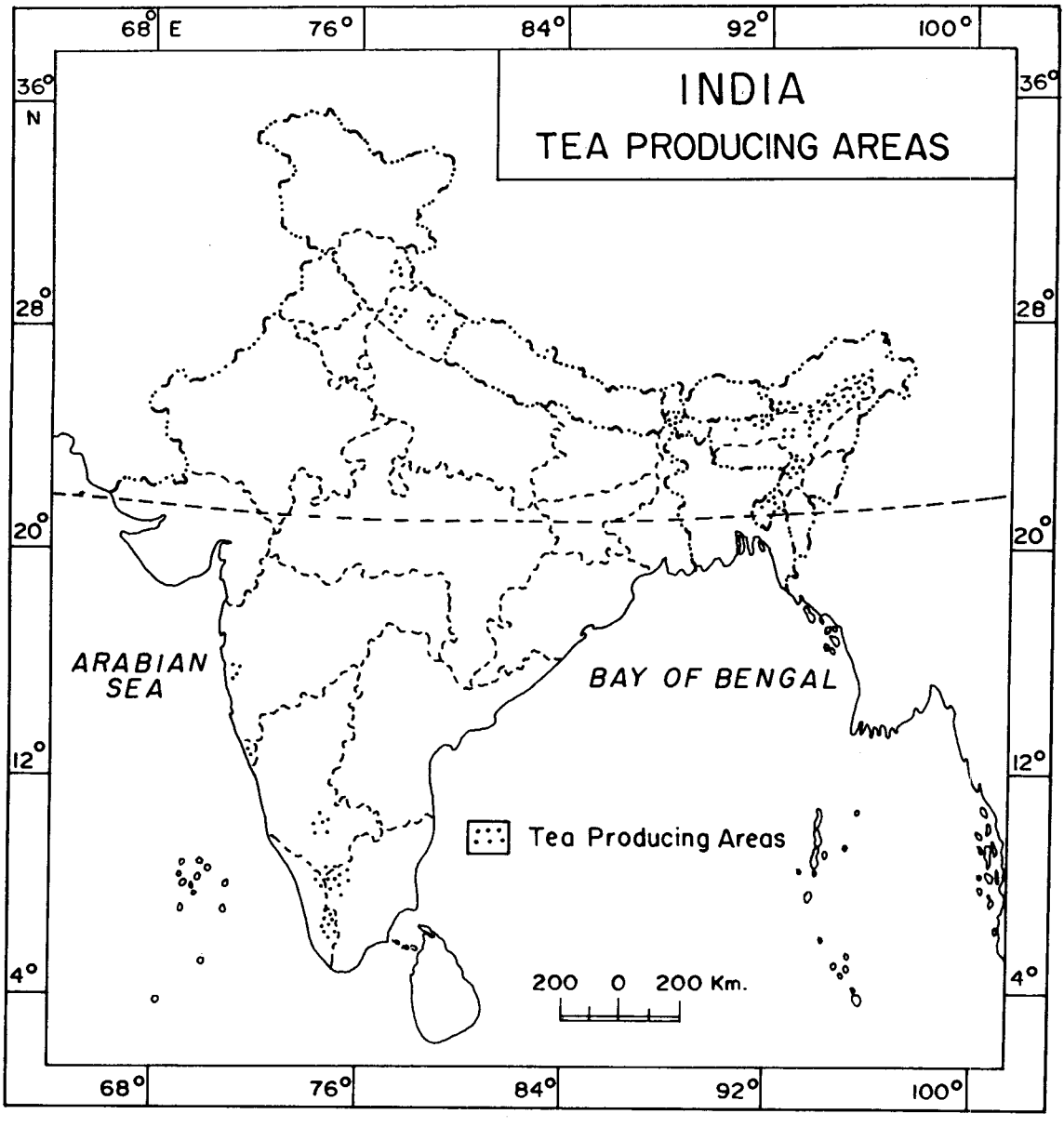

INDIA
TEA PRODUCING AREAS
68° E
76°
84°
92°
100°
36° N
28°
20°
12°
4°
ARABIAN SEA
BAY OF BENGAL
Tea Producing Areas
200 0 200 Km.

Fig.1

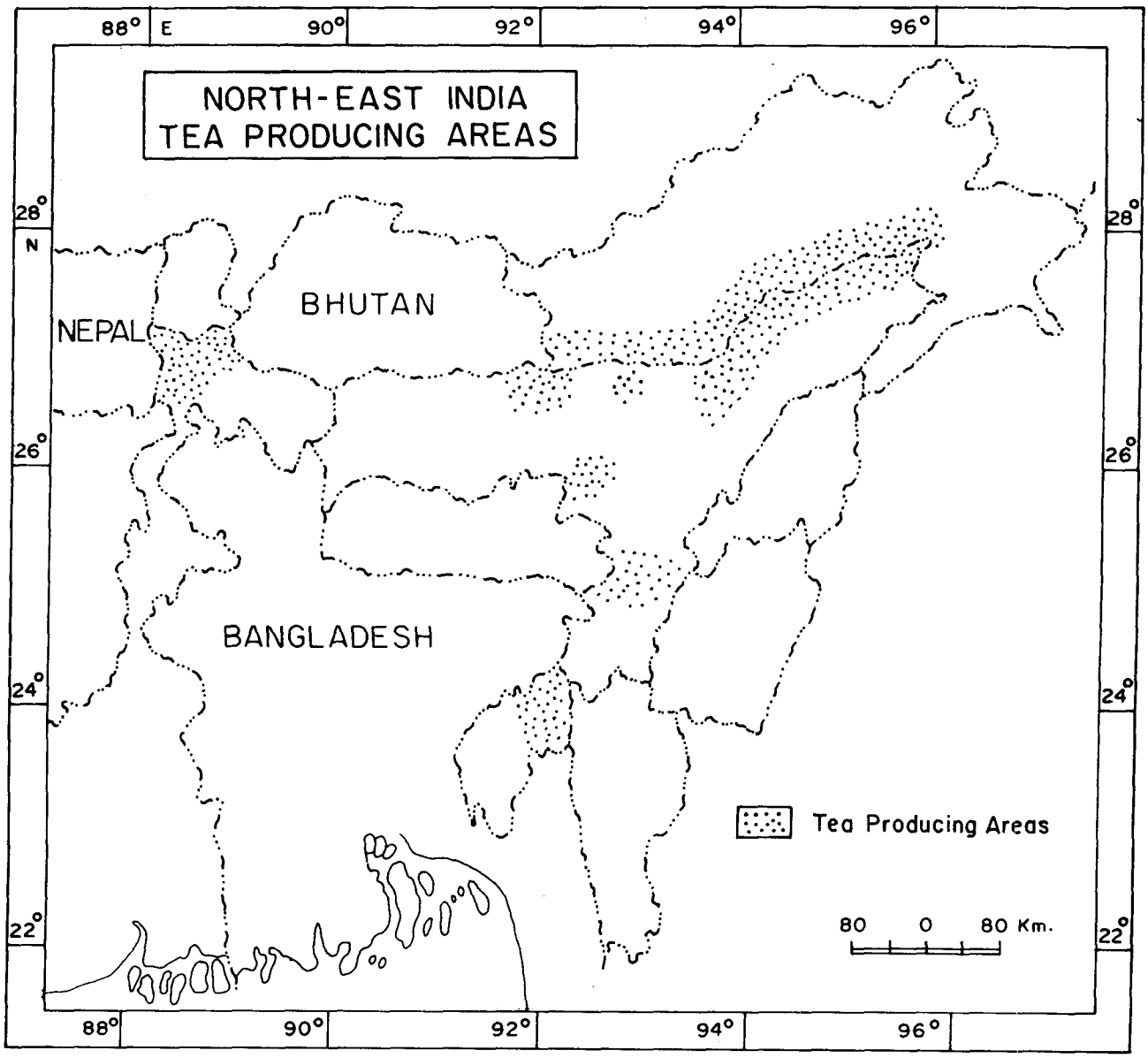

NORTH-EAST INDIA
TEA PRODUCING AREAS
88° E
90°
92°
94°
96°
28° N
26°
24°
22°
NEPAL
BHUTAN
BANGLADESH
Tea Producing Areas
80 0 80 Km.

Fig. 2

Diplodia disease caused by *Botryodiplodia theobromae* Pat. is probably the commonest of all the fungi recorded on tea in northeast India. The pathogen can attack any part of the plant, young or old, only when the plant is debilitated by other causes. Although the disease may be produced in the leaves, roots and stems, the symptoms are of different types; in case of leaves and collar regions it is grayish-black to coal-black, hairy cussions, giving a shooty appearance. The infection is believed to take place through air borne spores. Practically all woody parts, dying roots and twigs show *Botryodiplodia* infection. Sarmah (1960) suggested that improving general health of the plants, by subsoil irrigation, manurial and cultural practices would greatly help in protecting the plants from diplodia root disease. Venkata Ram (1960) observed the predisposing factors like low starch reserves; high soil temperature and low soil moisture were essential as a requisite for fungal invasion to cause diplodia disease. Chandra Mouli (1988) reported that diplodia root disease (a secondary root disease of tea), caused by *B. theobromae* was very common and occurred both in north India as well as south India.

Beside *Botryodiplodia*, several other diseases are reported to affect the tea plants throughout the world. Arpon and Supachi (1980) reported that blister blight of tea caused by *Exobasidium vexans* Massee., was the most serious disease in many tea plantations on the mountains. Saha *et al.* (1980) observed that red rust of tea caused by *Cephaleuros parasiticus* Karst. is the most important and lone algal disease of tea. Smit and Devis (1989) reported about crown and root rot disease caused by *Macrophomina phaseolina* (MP) and *Neocosmospora vesinfecta* (NV) which occurred in rooibos tea throughout the main production areas. Tissue infected by MP was gray with numerous black sclerotia when broken open, and the branches were characteristically twisted. Tissue infected by NV was typically maroon to black with superficial orange to red perithecia. Disease developed on plants inoculated during summer when subjected to moisture stress, but not on plants inoculated during winter. Number of propagules recovered from the soil varied greatly from field to field with the height levels being 32 viable MP sclerotia and 315 visible ascospores per gram of soil.

Wang *et al.* (1990) described diseases of tea in 6 zones of Zheijiang province of Peoples Republic of China from 1985 to 1988. Symptoms and biology

of pathogens of 20 diseases were described. Among them, 5 of the 20 diseases caused by *Colletotrichum camelliae*, *Monochaetia camelliae*, *Pestalotia guepini*, *Phyllosticta theicola* and *Fusarium ventricosum* were distributed more widely and cause more severe damages and those caused by *Pestalotia algeriensis*.

Barthakur (1994) observed that among the common diseases of tea in the hills of Darjeeling, West Bengal, India blister blight caused by *Exobasidium vexans* was the most serious; root diseases caused by *Ustulina zonata*, *Fomes lamaoensis*, *Rosellina arcnata* and *Armillaria mellea* and stem diseases caused by *Tunstallia aculeate* and *Poria hypobrunnea* were also common in Darjeeling gardens but they were difficult to control.

Park (1995) reported that in May and June, 1992-1994 tea with white scab symptoms, i.e., numerous small, circular, reddish or yellowish brown spots on young tea leaves was observed in a plantation in Boseung, Chonnum Province, Korea Republic. At the late growth stages the center of the spot became light gray. The causal agent was identified as *Sphaceloma theae*. Similar symptoms occurred on leaves 5-6 days after inoculation with *S. theae*. Park *et al.* (1996) reported the occurrence of gray blight of tea in several tea plantations in Boseung, Chonnam Province, Korea Republic, during 1992-94 after harvesting and pruning of the second crop. Circular to irregularly shaped dark brown spots developed in concentric rings on leaves and black, dot-like acervuli formed in concentric rings on the lesions. The pruned twigs were blackened and killed and acervuli formed randomly on them. The causal fungus of gray blight was identified as *Pestalotiopsis longiseta*. Typical symptoms by *P. longiseta* appeared 11 days after inoculation.

Khodaparast and Hedjaroude (1996) reported that during 1991-1993, several tea plantations were surveyed in the north of Iran in order to determine the main fungal diseases of tea. The result of the survey and pathogenicity tests showed that *Botrytis* sp., *Glomerella cingulata*, *Fusarium solani*, *Botryodiplodia theobromae*, *Pestalotiopsis longiseta*, *P. nattrassii*, *P. theae*, *Phyllosticta theacearum* and *Corticium rolfsii* were pathogens of tea.

Mouli (1997) gave a brief account on abiotic and biotic problems in the nurseries in India, and suggested several remedial measures. The diseases covered

included stalk rot caused by *Pestalotia theae* and *Colletotrichum camelliae*, root rot by *Pythium* spp., *Cylindrocladium* spp. or *Fusarium* spp., blister blight caused by *Exobasidium vexans* and leaf spot caused by *Cercosporella theae*.

Onsando *et al.* (1997) reported that among the 12 tea growing districts of Kenya, *Armillaria* root rot disease was most severe in the districts east of rift valley. Investigation showed that infection of tea bushes required primarily the mycelial growth from residual tree roots and from infected tea roots rather than from rhizomorphs. Inoculum from residual tree in debris in the soil was the most important source of infection in plantations of seed origin.

Yamaguchi *et al.* (1992) reported the superiority of Fushan variety of green tea over Yabukita (green tea) based on productivity, disease and pest resistance. Hu-ShuXia (1996) found two highly resistant cultivars to *Pestalotiopsis theae* among the 18 cultivars tested in Anhui Province of Peoples Republic of China.

Verma and Balasundaran (1990) investigated shoot-die back in cashew. In addition to feeding injury caused by *Helopeltis antonii* Sign. A fungus viz. *Botryodiplodia theobromae* was also isolated consistently from the dead tissues. The primary cause for entry and establishment of the pathogen seemed to be infestation of the insects. Controlled experiment revealed that die back occurred only when the fungus was inoculated in the lesion caused by feeding of *H. antonii* Sign.

Roux *et al.* (2001) reported the survey of diseases of *Eucalyptus* plantations (mostly *E. grandis*) in southern Uganda during June, 1999. They collected root, stem and leaf samples from the trees ranging in age from a few months to approximately 10 years. The most commonly isolated pathogen was *Lasiodiplodia theobromae* (*Botryodiplodia theobromae*), which was frequently associated with stem cankers and die back. Bacterial wilt, caused by *Ralstonia solanacearum*, was the most common cause of death of trees less than two years old, in the warmer areas around Kampala. In the eastern part of Uganda, the wilt pathogen *Ceratocystis fimbriata* was isolated from dying *Eucalyptus grandis* and, together with *Lasiodiplodia theobromae*, was considered the greatest threat to *Eucalyptus* plantations in Uganda.

Growth and physiology of the pathogens

Pathogens are attaining increasing importance in various economic fields like agriculture and industries. A thorough understanding of the physiological processes of pathogens is also of immense use in understanding the host-parasite relationship and mechanism of pathogenicity. Physiological studies are also helpful in chemotherapy and other control measures.

Saha and Chakraborty (1990) reported the effect of some environmental factors on spore germination of *Bipolaris carbonum* Nelson, a pathogen of tea. Under identical humid condition, the optimal concentration of spores, temperature, and pH for spore germination were recorded to be 11.2 X 10^5 spores.ml^{-1}, 32 ^{0}C and pH 6.75 respectively. Temperature pretreatment at 50 ^{0}C for 20 minutes significantly reduced spore germination, whereas pretreatment at 0 ^{0}C for even 12 hours had no effect on spore germination and germ tube elongation. Light condition and age of the conidia did not affect the spore germination.

Chakraborty *et al.* (1995) shown that factors associated with conidial germination and appressoria formation of *Glomerella cingulata* causing the brown blight disease of tea were studied *in vitro.* Spore germination and appressoria formation were optimum at a temperature of 25 ^{0}C, pH 5.0, a 7 hours light/day regime and a 24 hours incubation period. At a concentration of conidia of 1200/10 days old culture, *G. cingulata* exhibited a maximum germination and appressoria formation. Maximum production of lesions was also evident on detached tea leaves at this spore concentration and in diffuse light. Diffusates of phenolic nature collected from tea varieties susceptible and resistant to *G. cingulata* inhibited spore germination and appressoria formation. Diffusates from resistant varieties were more fungitoxic than from susceptible varieties.

Achar (2000) reported that the mycelial growth of three isolates of *Stenocarpella maydis* from maize seeds increased progressively from 15 ^{0}C to a maximum of 30 ^{0}C. The maximum number of conidia were produced by all three isolates after 8 days of incubation at temperatures ranging from 22 ^{0}C to 30 ^{0}C.

The effects of temperature and pH on the growth and sporangial production of isolates from each of the four known races of *Phytophthora clandestina* Taylor,

Pascoe & Greenhalgh were investigated. Mycelial growth occurred at temperatures from 10 °C-30 °C and pH 3.5-9.0 with highest growth rates of all isolates being at 25 °C with a pH of 6.0-6.5. Sporangial production was greatest between 20 °C-25 °C and pH 5.0-7.0 with all races. However, sporulation occurred over a temperature range from 10 to 30°C and from pH 4.0-9.0 with all isolates. There were no consistent differences between the four pathogenic races of *P. clandestina* in their relative growth rate or extent of sporangial production over a range of temperatures and pH values (Harden *et al.*, 2002).

Conidial germination *in vitro* and foliar lesion expansion were studied for *Sphaerotheca macularis* f. sp. *fragariae*. Detached strawberry (*Fragaria* × *ananassa*) leaves were inoculated, then held in controlled environments of constant temperatures (4 °C-36 °C) and relative humidity (RH, 32-100%) representing the range of these variables observed under California commercial production conditions. Percent germination and lesion expansion rate were determined by destructive sub sampling over time. Conidia germinated at all temperatures by 6 hours and reached a maximum by 48 hours, with the optimum near 20 °C. Lesions were marked with the aid of a microscope and measured by computer-assisted image-analysis to determine expansion rate. Maximal rates occurred at 25 °C. Several growth models were fit to the expansion rate data with high significance. Predicted optima from these models ranged from 22 °C-27 °C and/or 17-27 mm Hg VP (water @ 100% RH). Neither RH, partial vapor pressure of water (VP (water)), nor vapor pressure deficit (VPD) correlated with lesion expansion rate, adding to studies minimizing the importance of RH and VPD as determinants of asexual phase powdery mildew growth other than specifically at spore germination. (Miller *et al.*, 2003).

Common antigenic relationship

It has been evidenced by several workers that the similarity and disparity of the antigenic determinants of a host and a parasite determines the resistance and susceptibility of the host plant (DeVay and Adler, 1976; Chakraborty, 1988; Purkayastha, 1989; Chakraborty and Saha, 1994). Cross-reactive antigens, have also been suggested, to be involved in determining host-parasite compatibility (Alba *et al.*, 1983; Alba and DeVay, 1985; Chakraborty and Saha, 1994).

Ala-El-Dein and El-Kady (1985) used crossed immunoelectrophoresis (CIE) techniques to show that the tested isolates of *Botrytis cinerea* were serologically different; some antigens were specific for each isolate, *Botrytis cinerea* isolate no.1 had four specific antigens; these antigens were absent in other isolates. At least sixteen antigens were common in the isolates tested. Some isolates were serologically similar when tested by double gel diffusion test while they were distinguishable when CIE techniques were used. Numbers of precipitin peaks obtained with CIE techniques were more than double the number of precipitin lines detected with double gel diffusion test. Results revealed that CIE techniques could be used as valuable analytical tools in resolving the spectrum of antigens present in *Botrytis cinerea* isolates. Antigenic structures of *B.cinerea*, *B.tulipae*, *B.paeoniae* and *B.allii* isolates were also compared by using CIE techniques. Antisera against antigens of these isolates gave 24, 15, 20 and 15 precipitin peaks respectively, when analysed in homologous reactions. CIE with an intermediate gel and CIE with antibody absorption *in situ* reacted that each isolate was serologically different from the other and had species-specific antigens. Eight antigens distinguished *B. cinerea* from the other species of *Botrytis*, these were present only in the former species. *B. allii* had less common antigens than the other species.

Antiserum obtained against the mycelial proteins of a strain of *Phytophthora fragariae* could detect 11 different strains of *P. fragariae* in pure culture and pathogen in naturally infected or inoculated roots. The antiserum failed to react with 18 fungal species isolated from underground parts of strawberry but reacted with some strains of *P. cactorum*, which parasitized only rhizomes but not roots. In inoculated strawberry roots, *P fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed (Amouzon-Alladaye *et al.*, 1988)

Evaluation of antisera raised against pooled mycelial suspensions from five isolates (Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of *Phytophthora fragariae* for detecting the red core disease of strawberries by enzyme-linked immunosorbent assay (ELISA) was done by Mohan (1988). Cross-reactivity of antiserum raised against *P. fragariae* with other *Phytophthora* as a

genus detecting antiserum has also been discussed by Mohan (1989). Antiserum of *P. fragariae* isolates (Anti-PfM) reacted strongly with antigens from several *Phytophthora* species. Some cross-reaction with antigens from *Pythium* species was decreased by fractionating on an affinity column of sepharose 4 B bound to extracts of *Fragaria vesca* roots infected with *P. fragariae*. The affinity purified anti-PfM retained its high cross-reactivity with the various *Phytophthora* species. Anti-PfM could not be made specific for *P. fragariae* because it was raised against components shown to be antigenically similar in all *Phytophthora* species tested. However, immunobloting with the affinity purified anti-PfM produced distinct patterns for *P. fragariae*, *P. erythroseptica* and *P cactorum*.

Kitagawa *et al.* (1989) has also developed competitive types of two novel enzyme-linked immunosorbent assays (ELISA) for specific detection of *Fusarium oxysporum* f.sp. *cucumerinum* as well as for general detection of ten strains of common *Fusarium* species that show specific pathogenicities to different plants. Antiserum against a strain of *Fusarium oxysporum* f. sp. *cucumerinum* (F 504) was elicited in rabbits and a highly specific, sensitive and accurate ELISA for the homologous strain was developed by using the antiserum with β- D-galactosidase-labelled anti-rabbit IgG as a secondary antibody and cell fragments of the strain attached to amino-Dylark balls as the solid-phase antigens. This assay was specific for strain F 504 and showed little cross-reactivity with nine other strains of *Fusarium* species including strain F 501 of *F. oxysporum* f.sp. *cucumerinum* (FO). F 501 possess pathogenicity against cucumber similar to that of strain F 504, although slight differences have been observed between these two strains regarding their spore formation and pigment production. Cell fragments of strain F 501 absorbed on amino-Dylark balls possessed sufficient immune activity against anti-FO antibody to use in a heterologous ELISA for general detection of ten *Fusarium* species with high sensitivity.

Common antigenic relationships between soybean and *Colletotrichum dematium* var. *truncata* was also studied by Purkayastha and Banerjee (1990) using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Cross-reactive antigens were detected between susceptible soybean cultivars and the virulent strain of *C. dematium* var. *truncata* but no cross-reactive antigen was

detected between soybean cultivars and avirulent pathogen (*C. dematium*) of non-pathogen *C. corchori*. Results of immunodiffusion and immunoelectrophoresis showed absence of common antigen between resistant cultivars (UPS M-19) and the pathogen, while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. They compared antigenic patterns of untreated and cloxacillin treated soybean leaves which induced resistance of soybean against anthracnose disease. The disappearance of one antigen from cloxacillin treated leaves of susceptible soybean cv. "Soymax" was correlated with alternation of disease reaction.

Polyclonal antiserum raised against mycelial extracts of the rot fungus *Phialophora mutabilis* reacted strongly with its homologous antigen and cross-reacted strongly to moderately with six other *Phialophora* soft rot spp. in ELISA (Daniel and Nilsson, 1991). With the help of an indirect ELISA technique, Ricker *et al.* (1991) showed that increase in cross-reactivity in late bled antiserum (anti-Bc IgG), raised against water soluble antigens from *Botrytis cinerea* corresponded with an increase in the overall serum titres for anti-Bc IgG to antigens of *B. cinerea*. Sundaram *et al.* (1991) reported that polyclonal antiserum of mycelial proteins of *Verticillium dahliae* reacted positively with 11 of 12 isolates of *V. dahliae* from potato, cotton and soil but negatively with one isolate from tomato in indirect ELISA. He also found positive results in detecting *V. dahliae* and *V. allo-atrum* from infected roots and stems of potato in an double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA).

Results of conventional isolation techniques for *Pythium violae* were compared with the assay of cavity spot lesions using polyclonal antibodies raised to *P. violae* or *P. sulcatum* in competition ELISA (Lyons and White, 1992). A double antibody sandwich ELISA test was developed for the detection of *Pseudocercosporella herpotrichoides* using a highly specific monoclonal antibody pH 10 as the capture antibody and genus specific polyclonal rabbit antisera as test antibody. The assay recognized extracts from plants both artificially and naturally infected with *P. herpotrichoides*, at least three-fold higher absorbance values with extracts of *P. herpotrichoides* infected tissue than with extracts from healthy tissues. The high molecular weight fraction of immunogen (mycelial extracts) was shown

to contain cross-reactive antigens: it induced antiserum in mice that cross-reacted with the other stem base fungi even at high dilution (Priestley and Deway, 1993).

Polyclonal antibodies (PAbs) produced against culture filtrates and mycelial extracts immunogen preparations from the soybean (*Glycine max*) and fungal pathogen *Phomopsis longicolla* were purified to the immunoglobulin fraction and tested in indirect ELISA and in direct DAS-ELISA (Brill *et al.*, 1994). The PAbs raised to culture filtrate were more specific but less active in binding to members of Diaparthe-Phomopsis complex than were those to mycelial extract immunogen preparation. DAS-ELISA was more specific and 100-fold more sensitive in detecting members of the complex than was indirect ELISA. Variability in specificity between different PAbs was lower in DAS-ELISA compared to indirect ELISA. Janaux and Spire (1994) used double DAS-ELISA to screen the cross-reactivity of soluble mycelial extracts of *Sclerotinia sclerotiorum*, an important pathogen of rapeseed with other cross-reacting fungal species such as *Botrytis cinerea*, a pathogen commonly present in rapeseed petals using a polyclonal anti-*B. cinerea* serum. An extensive cross reaction was found when two monoclonal and three polyclonal antisera, raised against the cell wall/membrane fractions of *Pythium violae* and *P. sulcatum* screened with a collection of 40 isolates of the genus *Pythium* including 20 species and the H-S group. However, when the binding of the antibodies was assessed in an enzyme-linked immunosorbent assay (ELISA) using cytoplasmic fraction antigens, the combined recognition patterns produced profiles unique to each species (White *et al.*, 1994).

Wakeham and White (1996) raised polyclonal antisera against whole (coded: 16/2) and sonicated (coded: 15/2) resting spores of *Plasmodiophora brassicae* as well as soluble components prepared by filtration and ultracentrifugation (coded: SF/2), cross-reactivity of all three antisera with a range of soil fungi, including *Spongospora subterranean* was low. Test formats including western blotting, dipstick, dot blot, indirect ELISA and indirect immunofluorescence were assessed for their potential to detect resting spores of *P. brassicae* in soil. Dot blot was least sensitive, with a limit of detection level of $1X10^7$ resting spores/ g in soil. With western blotting the lower limit of detection with antiserum 15/2 was $1X10^5$. This antiserum showed the greatest sensitivity in a dipstick assay, indirect

ELISA and indirect immunofluorescence, for all of which there was a limit of detection of $1X10^2$. Of the assays tested, indirect immunofluorescence appears to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *P. brassicae*.

Das and Mitra (1999) produced monoclonal antibodies (MAbs) produced against clover phyllody phytoplasma which were used in immunofluorescence test for the detection of brinjal little leaf phytoplasma in periwinkle. The monoclonal antibodies reacted with the antigen from little leaf infected periwinkle plants, but not with that from healthy plants, indicating a close serological relationship between these two phytoplasmas.

Besides fungus, virus (Petrunak *et al.*, 1991; Abou-Jawdah *et al.* 2001; Hema *et al.*, 2001) and bacterial (Mazarei and Kerr, 1990) pathogens of plants could be successfully detected by various ELISA formats. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and direct antigen coating (DAC)-ELISA tests were evaluated for detection of sugarcane streak mosaic virus (SCSMV-AP), a new member of Tritimovirus genus in the family Potyviridae, in leaf extracts, sugarcane juice and purified virus. The virus was detected up to 1/ 3125 and 1/625 dilutions in infected sugarcane leaf, 5 mul and 10 mul/well in sugarcane juice, 1/3125 and 1/3125 dilutions in infected sorghum leaf and 10 ng and 50 ng/ml for purified virus in DAS-ELISA and DAC-ELISA tests, respectively (Hema *et al.*, 2001). Abou-Jawdah *et al.* (2001) in a survey detected potato virus Y (PVY), potato virus A (PVA), potato virus X (PVX), potato virus M (PVM), potato virus S (PVS) and potato leaf roll virus (PLRV) by ELISA from potato fields in the two main production areas of Lebanon, the Bekaa and Akkar plains.

Plant disease alteration by chemical treatment

Several agents both physical and chemical have been used for alteration of disease reaction. The agents mostly include X-rays, UV-rays and biological agents. Chemicals of diverse nature without any direct toxic action were used in the past. Systemic acquired resistance (SAR) of plants against pathogens is a widespread phenomenon with respect to the underlined signalling pathways as well as to its potential use in plant protection. Plants respond with a salicylic-

dependent signalling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance (Heil and Bostock, 2002).

Yalpini *et al.* (1991) suggested salicylic acid as an endogenous signaling molecule that mediates systemic acquired resistance (SAR) in several host-pathogen systems. Pautot *et al.* (1991) studied the differential expression of tomato proteinase inhibitor I (Pin I) and proteinase inhibitor II (Pin II) genes during infection by *Pseudomonas syringe* PV tomato, the causal agent of bacterial speck disease in tomato. Inoculation of *P. syringe* PV tomato to resistant and susceptible tomato leaves resulted in the accumulation of Pin I mRNA and Pin II mRNA more rapidly in disease resistant variety than in disease-susceptible plants.

Artemisia tridentate (sagebrush), a plant shown to possess methyl jasmonate in leaf surface structures when incubated at chambers with tomato plants, proteinase inhibitor accumulation is induced in tomato leaves, demonstrating that interplant communication, through airborne methyl jasmonate can occur from leaves of one species of plants to leaves of another species and simultanaeously the defensive genes are also expressed (Farmer and Ryan, 1992). Schneider-Muller *et al.* (1994) reported Ca^{2+} ions playing an important role in the induced production of salicylic acid and chitinase, one of the pathogenesis-related proteins.

Leroux (1996) reported that SAR can be accomplished by the exogenous application of aspirin or its derivatives. This biorational approach has been the basis of the development of a new fungicide, BTH (CGA 245704) by Ciba Geigy. BTH has been shown to offer prophylactic protection when applied @ 20-30 $g.ha^{-1}$ at early tillering stage against powedering mildew in wheat, rice blust in rice and blue mould in tobacco.

A single spray of solutions of 0.005 M H_3BO_3, 0.0025 M $CuSO_4$ and 0.0025M $MnCl_2$, on the upper surface of the first true leaf of cucumber plants 2 hours before inoculation with a conidial suspension of *Sphaerotheca fuliginea*, induced systemic protection against powdery mildew in leaves 2 and 3 without causing any damage on the induced leaf (first leaf). A similar level of systemic protection was observed when plants were induced by a variety of micronutrients or microelements with various concentrations, 2, 24 and 72 hours before challenge with *S. fuliginea* (Renveni *et al.*, 1997).

Induced systemic resistance is mediated by a jasmonate/ethylene sensitive pathway and does not involve expression of PR proteins (Pieterse *et al.*, 1998 and VanLoon *et al.*, 1998). Ding *et al.* (1999) reported that pretreatment of tomato fruit with low concentration (0.01 mM) of methyl jasmonate (MeJA) or methyl salicylate (MeSA) induces the synthesis of some stress proteins such as PR proteins which leads to increased tolerance to chilling temperature and resistance to pathogens, thereby decreasing the incidence of decay. Dombrowski *et al.* (1999) indicated that prosystemin and/or large fragment of prosystemin can be active inducers of defense responses in both tomato leaves and suspension cultured cells.

Besides chemicals several virulent and avirulent pathogens, nonpathogens, herbivore attack and wounding might induce resistance. Hammerschmidt (1999) reported the local or systemic induction of disease resistance in the treated plant to subsequent pathogen attack.

Signal transduction pathways that operate both at the site of wounding and undamaged distal leaves regulate plant defense responses to wounding and herbivore attack. Genetic analysis in tomato indicates that systemin and its precursor protein prosystemin are upstream components of a wound-induced inter-cellular signaling pathway that involves both the biosynthesis and action of jasmonic acid. Activation of jasmonate biosynthetic in response to wounding or prosystemin is required for the production of a long distance signal, whose recognition in distal leaves depends on jasmonate signaling, which may act as a transmissible wound signal (Lil *et al.*, 1999). Systemin, an 18-amino acid polypeptide wound signal activate defense gene in wounded leaves of young tomato plants and induces alkalinization of media containing suspension-cultured *Lycopersicon peruvianum*. (Schere and Ryan, 1999).

Jasmonic acid, oligopeptide systemin, oligosaccharides and other phytohormones such as ABA and ethylene as well as physical factors such as hydraulic pressure or electrical pulses have been proposed to play a role in wound signalling. Components of different jasmonic acid dependent and independent wound signalling transduction pathways are mostly similar to those implicated in other signalling cascades in eukaryotes and include reversible protein

phosphorylation steps, calcium/calmodulin-regulated events and production of active oxygen species (Leon *et al.*, 2001).

A central signalling molecule in induced responses against herbivores is jasmonic acid (JA) (Creelman and Mullet, 1997; Wasternack and Parthier, 1997). In response to wounding and/or insect feeding, linolenic acid is released from membrane lipids and then converted enzymatically into JA. JA, in turn, causes the transcriptional activation of genes encoding proteinase inhibitors (PIs) and of enzymes involved in the production of volatile compounds, or of secondary compounds such as nicotine and numerous phenolics, and other defence-related compounds (Creelman and Mullet, 1997; Karban and Baldwin, 1997; Wasternack and Parthier, 1997; Boland *et al.*, 1999). Oligosaccharides (Bishop *et al.*, 1981) and oligogalacturonides (Doares *et al.*, 1995; Norman *et al.*, 1999) released from damaged cell walls might play a role in the elicitation of the general wound response, but specific elicitors such as systemin have also been reported (Pearce *et al.*, 1991). Systemin is an 18-amino acid polypeptide that is released upon wounding from a 200-amino acid precursor ('prosystemin') and that leads to the release of linolenic acid. This activates the octadecanoid signalling cascade (Ryan, 2000). Both JA (Zhang and Baldwin, 1997) and systemin (Ryan, 2000) can be transported in the phloem and thus might act as systemic signals. To date, systemin has been described for tomato only, and not even for other solanaceous plants such as tobacco (Ryan, 2000; León *et al.*, 2001). The importance of cell wall fragments in elicitation was supported by the finding that cellulysin, a mixture of several cell wall-degrading enzymes from the plant parasitic fungus *Trichoderma viride*, can induce several JA-responsive volatiles in lima bean (*Phaseolus lunatus*) (Piel *et al.*, 1997). The action of cellulysin is followed by a rapid increase in endogenous JA (Koch *et al.*, 1999).

Disease control by fungicides

Chemical control continues to play an important role in the integrated control of tea diseases (Muraleedharan and Chen, 1997). Several workers have suggested chemical control of tea diseases. Some of the previous works are being included in this review.

Sulphur compounds were not seriously considered as they imparted undesirable taint to tea (De Jong, 1954). Mulder (1961a,b) reported that nickel chloride when used either alone or in combination with Zineb controlled blister blight satisfactorily. Of the various nickel salts tested nickel chloride was found to be the best (Venkata Ram, 1962). De Silva (1965) stated that nickel chloride though gave satisfactory control was found to be less effective than perenox. In tea recovering from pruning nickel chloride can not be used due to phytotoxicity (Chandra Mouli and Venkata Ram, 1979). Pasaribu and Sinaga (1981) reported the usefulness of nickel chloride in controlling disease in Indonesia.

A combination of nickel chloride with copper oxychloride offered better disease control to either of them sprayed alone (Venkata Ram, 1966). Systemic fungicides like derivatives of 1,4-oxathins, carboxin and oxycarboxin controlled the disease when tested in potted plants (Venkata Ram, 1969). However, these systemic fungicides had poor retention under severe wet weather conditions and they could not be used during periods of heavy rainfall (Venkata Ram, 1977).

Copper fungicide formulations containing 50% metallic copper either as copper oxychloride or cuprous oxide have been the best choice for protective control of blister blight disease. Of the two copper formulations, the latter had an edge over the former (Venkata Ram and Chandra Mouli, 1981). Nickel sulphate was useful in controlling blister blight (Tzong- Maochen and Shin-funchen, 1982). Three different concentrations of copper chloride and two different concentrations of nickel chloride and three different concentrations of the combination of the two chemicals were used to check *Exobasidium vexans* (Venkata Ram and Chandra Mouli, 1983).

Out of many protectant-therapeutant organic fungicides used as alternatives to copper oxychloride and nickel chloride, only chlorothalonil and dithianon provided satisfactory control (Chandra Mouli and PremKumar, 1986). In case of red rust (caused by *Cephaleuros parasiticus*) of tea, copper fungicides having 50% metallic copper are most effective with 1:400 dilution in four rounds of hand spray (Barua, 1988).

Application of copper-antibiotic treatment was inferior to copper plus nickel combination in tea under regular plucking (Venkata Ram and Chandra Mouli, 1979;

Chandra Mouli and Premkumar, 1989). Onsando and Langat (1989) reported the wood rot of tea caused by *Hypoxylon serpens*. The study showed that the normal surgical prunes caused comparatively lower disease incidences while medium prune aggrevated the incidence and severity of wood rot and reduced yields. Among the fungicides tested, only benlate significantly reduced disease incidence and severity.

Hypoxylon wood rot, caused by *Hypoxylon serpens* is one of the major disease of tea in which the pathogen has been found to exist in both sexual and asexual states. Superficial fructifications of stromata of the fungus that appear as irregular dark-grey or black slightly raised patches of various sizes, and gradual decline that culminates in the death of the plant are symptoms of infection of tea bush by *Hypoxylon serpens*. Surgical removal of all infected bushes and treating the resultant wounds with copper oxychloride in raw linseed oil and use of systemic fungicides were suggested in control of the disease (Otieno, 1993). Otieno *et al.* (1994) screened five fungicides and three pruning modes for management of *Hypoxylon* wood rot of tea. The screening results revealed that normal and surgical prune were superior to medium prune in suppressing spread of the disease. The use of benomyl, a systemic fungicide also lowered disease incidence to levels sufficiently different from that of the other fungicides.

Several chemicals have also been used against pathogens of different plants. Matheron and Porchas (1999) compared the *in vitro* activity of azoxystrobin, dimethomorph, and fluazinam on growth, sporulation, and zoospore cyst germination of *Phytophthora capsici, P. citrophthora,* and *P. parasitica* to that of fosetyl-Al and metalaxyl. The 50% effective concentration (EC(50)) values for inhibition of mycelial growth of the three pathogens usually were lowest for dimethomorph and metalaxyl, ranging from < 0.1 to 0.38 µg/ml. However, the 90% effective concentration (EC(90)) levels for dimethomorph always were lower than the other four tested compounds, with values ranging from 0.32 to 1.6 µg/ml. Mycelial growth of *P. capsici, P. citrophthora,* and *P. parasitica* was least affected by azoxystrobin and fluazinam, with estimated EC(90) values > 3,000 µg/ml. Reduction of sporangium formation by *P. capsici, P. citrophthora,* and *P. parasitica* in the presence of dimethomorph at 1 µg/ml was significantly greater than that recorded for the same concentration of azoxystrobin, fluazinam, and fosetyl-Al. For the three

species of *Phytophthora*, zoospore motility was most sensitive to fluazinam (EC(50) and EC(90)) values of < 0.001 µg/ml) and least sensitive to fosetyl-Al, with EC(50) and EC(90) values ranging from 299 to 334 and 518 to 680 µg/ml, respectively. Germination of encysted zoospores of *P. capsici, P citrophthora,* and *P. parasitica* was most sensitive to dimethomorph (EC(50) and EC(90)) values ranging from 3.3 to 7.2 and 5.6 to 21 µg/ml, respectively), intermediate in sensitivity to fluazinam (EC(50) and EC(90) from 18 to 108 and 67 to > 1,000 µg/ml, respectively) and metalaxyl (EC(50) and EC(90)) from 32 to 280 and 49 to 529 µg/ml, respectively), and lowest in sensitivity to azoxystrobin and fosetyl-Al (EC(50) and EC(90) from 256 to > 1,000 µg/ml). The activity of azoxystrobin, dimethomorph, and fluazinam on one or more stages of the life cycle of *P. capsici, P. citrophthora,* and *P parasitica* suggested that these compounds potentially could provide *Phytophthora* spp. disease control comparable to that of the established fungicides fosetyl-Al and metalaxyl.

The control of *Fusarium* head blight (FHB) of wheat using fungicides was investigated in two field trials by Cromey *et al* (2001). The first trial examined the effects of tebuconazole applied at a range of crop growth stages around flowering, whereas the second trial compared nil fungicide, tebuconazole, carbendazim, and azoxystrobin, applied at full ear emergence or mid anthesis. Moderate FHB levels were recorded in untreated plots in both trials. In the first trial, FHB incidence was reduced by up to 90% and yield increased by 14% following two applications of tebuconazole. Levels of *Fusarium* in harvested grain were not affected but mycotoxin levels were reduced by some treatments. In the second trial FHB incidence was decreased and grain weight increased with all fungicides at one or both application stages. High levels of *Fusarium* were recorded in harvested grain in the nil fungicide treatment. Levels of both *Fusarium* and resulting mycotoxins were substantially reduced following treatment with tebuconazole or carbendazim but were not affected by treatment with azoxystrobin.

In a series of experiments, fungicides with different modes of action to the commonly used phenylamide-based products were examined against Downy mildew of rose (*Rosa spp.*) and blackberry (*Rubus fructicosus*), caused by *Peronospora sparsa*. Cymoxanil + mancozeb + oxadixyl and fluazinam gave good

downy mildew control on both rose and blackberry. On outdoor, container-grown rose, high volume sprays of fosetyl-aluminium were also effective, but on young micropropagated blackberry plants, application as a drench treatment was better than as a spray. Good control was also achieved on blackberry with chlorothalonil and with metalaxyl in formulation with either thiram or mancozeb. (O' Neill *et al.*, 2002).

Disease control by botanicals

There are certain advantages in the deployment of botanical pesticides. These are biodegradable, safe to non-target organisms, renewable and suit to sustainability of local ecology and environment. Several authors have demonstrated the use of botanicals to control the plant diseases.

Synergestic enhancement by 2- to 73- folds of antifungal activity of wheat thionins when combined with 2 S albumins of radish or rape was noticed being effective against filamentous fungi and some gram-positive bacteria. Permeabilization of the hyphal plasmalemma of thionins has been shown to be the mode of action (Terras *et al.* 1993). Soil amendments with crop residues lead to build up of allelochemicals and plant nutrients. In a comparative study, Prew *et al.*, (1995) shown that incorporation of straw was found more effective than burning of straw in containing the symptoms of eye spot disease (*Pseudocercosporella herpotrichiodes*) and sharp eye spot disease (*Rhizoctonia cerealis*) of wheat.

Evaluation of rape and Indian mustered as companion crop showed that the latter was more effective in minimizing the incidence not only of take-all disease of wheat but also *Rhizoctonia solani*, *Pythium* and *Cochliobolus sorokiniana*. The tissue extract of Indian mustard was equally effective and hence the role of volatile isothiocyanates is implied (Kirkewaad *et al.*, 1996). Certain phytochemicals like gallic acid and abscicic acid have been shown to be antifungal. For instance, abscicic acid was shown to inhibit mycelial growth and sporidial formation and also germination of teliospores (Singh *et al.*, 1997).

The hexane and methanol extracts of sixteen plants of the family Caesalpiniaceae, collected around Karachi, Pakistan, were phytochemically screened and tested for their antibacterial and antimicrobial activity. As compared

to hexane extracts, the methanol extracts of all the examined plants showed stronger growth inhibitions againstboth bacteria and fungi, *Cassia* species being the biologically more active plants (Ali *et al.*, 1999). Carpinella *et al.* (1999) reported that the ethanol extract of *Melia azedarach* ripe fruits showed fungistatic (MIC 50-300mg/ml) and fungicidal (MFC60-500mg/ml) activity against *Aspergillus flavus*, *Fusarium moniliforme*, *Microsporum canis* and *Candida albicans*.

The antimicrobial activities of *Valex* (the extract of valonia), the extracts of mimosa bark, gallnut powders, *Salvia ancheri* Benthum. var. *ancheri* and *Phlomis bourgei* Boiss. were studied. The results of the study indicated that mimosa bark extracts had the greatest antibacterial activity, followed by the *Valex*, gullnut powders, *Salvia ancheri* var. *ancheri* and *Phlomis bourgeie* extracts, respectively. Furthermore, it was found that gullnut powders andthe extracts of mimosa bark contained high amounts of tannins and showed antifungal activity (Digrak *et al.*, 1999).

Two hundred and four species of traditional Chinese herbal medicines belonging to 80 families were collected from Yunnan Province in People's Republic of China and tested for antifungal activities using a *Pyricularia oryzae* bioassay. Twenty-six herbal medicines from 23 families were active against *P oryzae* and the ethanol extract of *Dioscorea camposita* (dioscoreaceae) exhibited the most bioactivity among all the tested samples (Ke Hu *et al.*, 1999).

The antibacterial activity of ethanol extracts of 15 plant species used in the traditional medicine in Jordan and other middle east countries were tested. Extracts of certain parts of these plants were tested *in vitro* against 14 pathogenic bacterial species and strains using the agar diffusion methods. Three plants exhibited broad spectrum antibacterial activity: *Punica Granatum* L., *Quercus infectoria* Olive., and *Rhus coriaria* L. The most susceptible bacteria were *Pseudomonas aeruginosa*, *Bacillus cereus* and *Streptococcus pyogenes* (ATCC 12351), and the most resistant species were *Escherichia coli* (ATCC 25922 and clinical isolates), *Klebsiella pneumoniae, Shigella dysentriae* (ATCC 49345), and *Yersinia enterocolitica* (ATCC 9610) (Nimri *et al.*, 1999).

Three thiosulfinates with antimicrobial activity were isolated from oil-macerated garlic extract and their structures were identified by Yoshida *et al.* (1999a)

as 2-propene-1-sulfinothioic acid S-(Z,E)-1-propenyl ester [AllS(O)SPn-(Z,E)], 2-propenesulfinothioic acid S-methyl ester [AllS(O)SMe], and methane sulfinothioic acid S-(Z,E)-1-propenyl ester [MeS(O)SPn-(Z,E)]. Antimicrobial activities of AllS(O)SPn-(Z,E) and AllS(O)SMe against gram-positive and negative bacteria and yeasts were compared with 2-propene-1-sulfinothioicacids-2-propenylester[AllS(O)SAll,allicin]. Antimicrobial activity of All S(O) S Me and All S(O)S Pn-(Z,E) were comparable and inferior to that of allicin, respectively. In another study, an organosulfur compound was isolated and identified from oil-macerated garlic extract by silica gel column chromatography and preparative TLC. The antimicrobial activity of isoE-10-DA was inferior to those of similar oil-macerated garlic extract compounds such as E-ajoene, Z-ajoene and Z-10-DA (Yoshida *et al.*, 1999b).

The leaves of five *Betula* species, *B. pendula*, *B. browicziana*, *B. medwediewii*, *B. litwinowii* and *B. recurvata* collected from different parts of Turkey were hydrodistilled to yield the consequent essential oils. The essential oils showed antifungal activity against various phytopathogenic fungi like *Cephalosporium aphidicola*, *Drechslera sorokiniana*, *Fusarium solani* and *Rhizoctonia cereals* (Demirci *et al.*, 2000).

Rao *et al.* (2000) reported about Limonene, the major constituents of essential oil of exocarpic part of *Citrus sinensis* which possessed strong and broad-spectrum antifungal activity against important fungal pathogens of sugarcane. The mycelial growth of *Ceratocystis paradoxa* at 2000ppm and that of *Fusarium moliniforme* and *Curvularia lunata* at 3000ppm concentration of limonene were completely inhibited. It proved fungistatic at minimum inhibitory concentration and exhibited non-phytotoxicity or germination and growth of sugarcane.

Analysis of methanol extracts from leaves, stem bark, root bark, fruits and seed kernels of *Butyrospermum. pradoxum* (*Vitellaria paradoxa*) by Ogunwande *et al.* (2001) revealed the presence of alkaloids (in leaves and stem barks), flavones (in stem and root bark), saponins (in root bark), steroids (in stem bark, fruits and seed kernels) and tannins (in leaves and root bark) which have antimicrobial activity against different bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella*

typhi, *Staphylococcus aureas*, *Ralstonia solanacearum* and *Bacillus cereus*) and *fungi* (*Fusarium oxysporum* and *Candida albicans*).

Control of *Botrytis cinerea* Pers. leaf colonisation and bunch rot in grapes with oils was studied in laboratory and field tests. In detached lateral experiments, the essential oils from thyme (*Thymus vulgaris* L.) and clove (*Syzygium aromaticum* L.), as well as massoialactone (derived from the bark of the tree Cryptocarya massoia R.Br.) were not phytotoxic on leaves at concentrations of 0.33% or less. B. cinerea sporulation on artificially induced necrotic leaf lesions was significantly reduced by thyme (Thyme R) and massoialactone oils at 0.33%. A single application at veraison (1997/98) of either compound at concentrations of 0.33% controlled bunch rot and necrotic leaf lesion colonisation by *B. cinerea* compared with Botrytis control treatments. Spray applications of Thyme R oil (0.33%) at 8–10 day intervals (1998/99) from flowering to harvest controlled B. cinerea bunch rot but also caused floral tissues to senesce (Jaspers *et al.*, 2002).

Crude extract of the bulb of *Eucomis autumnalis* showed *in vitro* mycelial growth inhibition of *Mycosphaerella pinodes*, the cause of black spot or *Ascochyta* blight, in peas. The control of *Ascochyta* blight by different concentrations of the crude *E. autumnalis* extract was followed *in vivo* by leaf symptoms over a 6 day period at 20°C in a growth cabinet. The crude extract prevented *M. pinodes* spore infection of the leaves when the leaves were inoculated with spores both before or after treatment with the extract, confirming complete inhibition of spore germination. The crude *E. autumnalis* extract showed no phytotoxic reaction on the leaves even at the highest concentration applied (Pretorius *et al.*, 2002).

Disease control by antagonistic organisms

To develop biological control strategies of any disease, a thorough knowledge of life cycle of the pathogen(s), their mode of survival, the plant pathogen interaction processes starting from, the physical relationship of the pathogen to its host during pathogenesis, and the time and factors leading to infection and disease development are needed.

Leach (1939) showed that tea bush prunings left on the soil surface for a while to permit their colonization by airborne saprophytes were then protected

from colonization by *Armillaria mellea* when subsequently buried This resulted in lesser *Armillaria* inoculum and hence less root rot of tea. Kerr (1980) showed that the K84 strain of *Agrobacterium radiobacter* produces a bacteriocin, agrocin, achieves a population higher than that of the pathogen and prevents transfer of the tumour inducing (Ti) plasmid (TDNA) from the pathogen to the host. Ayers and Adams (1981) reported Deuteromycetes fungus, *Coniothyrium minitans*, a biocontrol agent produces several enzymes which cause lysis and kill upto 99% of the sclerotia of *Sclerotinia sclerotiorum*. Upadhyay and Rai (1985) reported that *Trichoderma viride* and *Trichoderma harzianum* significantly suppressed *Fusarium udum* in soil and roots of pigeon pea.

Panday *et al.* (1997) reported that a large number of bacteria and fungi were isolated from the rhizosphere of established tea bushes over a period of one year. Fiftyone of these bacterial isolates were tested for their antifungal activity against 12 test fungi, which include 9 minor and 3 major pathogens of tea. The bacterial isolates exhibiting the highest antifungal activity were also the most dominant bacterial species in the rhizosphere.

Similarly, several pathogens of other crops were also controlled by several workers such as mycostop was a biofungicide that had been effectively used to control a number of soil and seed-borne pathogens like *Botrytis cinerea, Rhizoctonia solani* etc. and seed borne foot rot disease of wheat and barley (Tahvonen and Lahdenperä, 1988; Tahvonen and Avikainen, 1990). The active component of mycostop was the spores and mycelium of *Streptomyces griseoviridis*. The product has been used successfully in seed treatment, soil drench, drip irrigation and as a transplant dip to control various disease causing fungi (Lahdenperä, 1987; Lahdenperä *et al.*, 1990 and Mohammadi, 1992). Suleman *et al.* (2002) used Mycostop at the rate of 0.35g/l or greater which reduced spore germination, plasmolysed germlings and reduced sporulation of *C. radicicola*. In essence, it reduced the inoculum potential of *C. radicicola*.

The antagonistic effect of *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* was well established as reported by several workers. Wu *et al.*, (1986) and Vinod (1988) reported the antagonistic effect of *A. niger*. The antagonistic property of *A. flavus* against many pathogenic microorganisms has been reported

by Massoor and Chandra (1987) and Deb (1990) though not specific against *Pythium* spp. The hyphal coiling and production of inhibitory substances by different species of *Trichoderma*, resulting in dieback and disintegration of *Pythium* spp were reported by Raju (1991) and Vinod *et al.* (1991). Mukherjee and Sen (1992) observed that the culture filtrate of *A. fumigatus* ihibited the growth of *Macrophomina phaseolina* and sclerotial germination.

Mukhopadhyay *et al.* (1992) found good control of chickpea wilt complex when seeds were treated with *Gliocladium virens* (10^7 conidia/ml) and carboxin 0.1%. Ghizalberti and Rowland (1993) shown that alkylpyrones, cycloneroditol etc.,the metabolites isolated from *Trichoderma harzianum*, were active against take-all disease of wheat. Similarly, 2,14-diacetyl phloroglucinol, isolated from fluorescent Pseudomonads, was responsible for controlling the take-all disease of wheat.

Several authors have shown considerable potential of *Trichoderma* and *Gliocladium* in controlling disease caused by *Sclerotium rolfsii* in snapbean, sugarbeet, tomato, chickpea and cotton in greenhouse and field studies (Elade *et al.*, 1983; Upadhyay and Mukhopadhyay, 1983; Henis, 1984; Punja, 1985; Papavizus and Lewis, 1989; Wokocha, 1990; Ciccarese *et al.*, 1992 and Latunda Dada 1993).

The fungus *Ulocladium atrum* Preuss, indigenous to Netherlands, was found to exclude *Botrytis cinerea* from necrotic plant tissue moreeffectively under field conditions than other antagonists such as Gliocladium catenulatum Gilman and E.Abbott, *Aureobasidium pullulans* (deBary) Arnaud or *Chaetomium globosum* Kunze: Fr. (Köhl *et al.*, 1995). Sutton (1995) and Lima *et al.* (1997) reported that biological control of various filamentous fungi and yeasts is one of the methods that has shown good perspectives for non-chemical management of gray mould in strawberry.

Different isolates of *Trichoderma harzianum* showed differential antagonistic potential as biocontrol agent against *Sclerotium rolfsii* as reported by Maity and Sen (1985) and Biswas (1999).

Filonow (1998) observed that three antagonistic yeasts competed successfully for sugars since their uptake was faster and higher than that of *Botrytis*

cinerea. He concluded from this that high competitiveness plays a central role in antagonism. The antagonistic yeast *Cryptococcus albidus* were highly effective when applied to the strawberry fruits after harvest by colonizing the wounds and competing for nutrients or space and thereby reducing the infection (Droby *et al.*, 1989; Benbow and Sugar, 1999 and Helbig, 2002).

Pandey and Upadhyay (1999) reported the comparative performance of chemical, biological and integrated control of wilt of pigeonpea caused by *Fusarium udum*. In chemical control,bavistin was found highly effective, while *Trichoderma viride* and *T. harzianum*-C isolates were found best among biocontrol agents. Integration of biocontrol agents with bavistin was not beneficial. However, integration of the bioagents with thiram reduced wilt incidence significantly. Thus seed coating with bioagents proved better and safe for the management of wilt of pigeonpea.

Prasad *et al.* (1999) tested 14 isolates of *Trichoderma* and *Gliocladium* species were tested in vitro against *Sclerotium rolfsii*, the causal organism of root/ collar rot of sunflower. Two isolates of *T. viride*, four isolates of *T. harzianum*, one each of *T.hamatum*, *T. koningii*, *T.polysporum*, *G.virens*, *G.deliquescens* and *G.roseum* inhibited mycelial growth of the pathogen significantly. Among *Trichoderma* species, *T. harzianum*, isolates PDBCTH2 gave 61.4% inhibition of mycellial growth followed by PDBCTH 8 (55.2%) and PDBCTH 7 (54.9%). Among *Gliocladium* isolates, *G.virens* gave maximum inhibition (39.9%) of mycelial growth. Suppression of sclerotial production by antagonists ranged from 31.8 to 97.8%. Complete inhibition of sclerotial germination was obtained with the culture filtrates of *T. harzianum* (PDBCTH 2, 7 and 8), *T. pseudokoningii* and *G. deliquescens*. The three *T. harzianum* isolates and the *T. viride* isolate (PDBCTV 4) were superior under greenhouse conditions with PDBCTH 8 showing maximum disease control (66.8%) followed by PDBCTH 7 (66.0%), PDBCTV 4 (65.4%), PDBCTH 2 (61.6%) and were even superior to fungicide captan. *G. deliquescens* gave maximum (55.7%) disease control among *Gliocladium* spp.

A modified granular formulation containing powdered wheat bran, kaolin, acacia powder and biomass of isolates of *Trichoderma harzianum* (PDBCTH 10 and PDBCTH 8), *T. virens* (PDBCTV$_S$ 3 and ITCC 4177) and *Gliocladium deliquescens* (ITCC 3450) were evaluated (Prasad and Rangeshwaran, 1999) for

their effect on the reduction of chickpea damping off caused by *Rhizoctonia solani*, reduction of pathogen inoculum and proliferation of the bioagents in the soil. Granules with all isolates of bioagents significantly reduced damping off. At 4 weeks, PDBCTH 10 and PDBCTH 8 isolates treatments have recorded better plant stands (63 and 53%) than fungicide (captan) treatment (43%). But none of the isolates of bioagents have recorded plant stands comparable to non-infested control (83%). The above two *T. harzianum* isolates were more effective in reducing saprophytic growth of the pathogen compared to other bioagents.

Native microorganisms were isolated from the rhizosphere of healthy ginger plants, in the rhizome rot affected fields and screened *in vitro* for their antagonistic effects against the pathogen *Pythium aphanidermatum* by dual culture and cell free culture filtrate studies. *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* were found to be potential antagonists (Shanmugam and Sukunara Verma, 1999).

The antagonistic potential of eight isolates of *Trichoderma harzianum* against four fungal pathogens of betelvine (*Phytophthora parasitica*, *Colletotrichum capsici*, *Sclerotium rolfsii* and *Rhizoctonia solani*) were shown. Isolates T_1, T_2 and T_3 had highest promise under *in vitro* conditions (D'Souza *et al.*, 2001).

Saprophytic fungus *Ulocladium atrum* Preuss was a promising biological control agent for control of *Botrytis cinerea* in strawberry and other crops (Boff *et al.* 2002).

The microbial basis of specific suppression to four diseases, Fusarium wilts, potato scab, apple replant disease, and take-all, was reported by Weller et al. (2002). One of the best-described examples occurs in take-all decline soils. In Washington State, take-all decline results from the buildup of fluorescent *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol. Producers of this metabolite may have a broader role in disease-suppressive soils worldwide.

During the past ten years, over 80 biocontrol products have been marketed worldwide. A large percentage of these have been developed for greenhouse crops. Products to control soilborne pathogens such as *Sclerotinia*, *Pythium*, *Rhizoctonia*

and *Fusarium* include *Coniothyrium minitans*, species of *Gliocladium*, *Trichoderma*, *Streptomyces*, and *Bacillus*, and nonpathogenic *Fusarium*. Products containing *Trichoderma*, *Ampelomyces quisqualis*, *Bacillus*, and *Ulocladium* are being developed to control the primary foliar diseases, *Botrytis* and powdery mildew. The development of *Pseudomonas* for the control of *Pythium* diseases in hydroponics and *Pseudozyma flocculosa* for the control of powdery mildew was also reported. (Paulitz and Bélanger, 2002).

Candida guilliermondii (strains 101 and US 7) and *C. oleophlla* (strain I-182) were screened for biocontrol activity (BA) against *Botrytis cinerea*. *In vivo* application of both *C. oleophila* (strain I-182) and *C. guilliermondii* (strains 101 and US 7) gave significant control of *B. cinerea* (Saligkarias *et al.*, 2002).

Materials & Methods

3.1. Plant materials

3.1.1. Host plant

3.1.1.1. Selection of suitable varieties

Several tea varieties (21 Tocklai varieties, 2 garden series clones approved by Tocklai Experimental Station, Jorhat, Assam and 1 clonal variety of UPASI) were used during the present study. All the varieties except the one of UPASI, mentioned above were selected for plantation in the experimental garden, Department of Botany, University of North Bengal, based on their growing suitability as observed under field conditions and environmental aspects, over the years at Tocklai Experimental Station, Jorhat, Assam, India and was recommended by Bezbaruah and Singh (1988). Amongst them 21 Tocklai varieties, used during the work are enlisted in the Table 1 with their general characteristics.

3.1.1.2. Procurement of selected varieties

The nursery tea plants of selected varieties were procured from tea nurseries (Plate II) of Kharibari, Siliguri, West Bengal and were planted in the experimental garden. Twigs of different tea varieties used for cut shoot experiment, were collected from the nursery tea plants of the clone house of Mohurgong and Gulma tea estate, Siliguri, West Bengal. Some twigs were also collected from the experimental garden during the work.

3.1.1.3. Plantation of selected varieties

In order to suppress the weed from the experimental plot/field, Simazine (75 g/20 lt water) as pre-emergent and Glyphosate (1:200) against thatch type of grasses, as described by Barbora (1988) were applied before plantation of selected tea varieties. Several pits of 40 cm^3 were dug at approximately 60 cm and 90 cm intervals between plant-to-plant and row-to-row respectively. Planting mixtures per pit were formulated in the following ratios: well-rotten dry cattle manure: super phosphate: rock phosphate: thimate :: 4000:25:25:2.5. Procedures for mixing are as follows. Rock phosphate was placed at the bottom of the pit following which half portion was covered with cattle manure-soil mixture. Thimate was mixed with a portion of excavated soil and was applied with upper part of the pit. Super phosphate was placed approximately 5 cm below the ground level. Finally, plantation

Table 1

Tocklai released clonal cuttings used in the study and their general characteristics*

Clone no.	Leaf style /size	Pube-scence	Rooting	Yield rating	Quality	Drought resistance
TV-1	Erect/Medium	Medium	Very good	Good	Good	Good
TV-2	Erect/Medium	High	Good	Above average	Above average	Poor
TV-3	Erect/Medium	Low	Fair	Average	Above average	Poor
TV-6	Erect/Medium	High	Fair	Above average	Above average	Poor
TV-8	Erect/Medium	Low	Good	Good	Average	Poor
TV-9	Erect/Medium	Low	Very good	Very good	Average	Good
TV-11	Flat/Medium	High	Good	Above average	Above average	Fair
TV-12	Flat/Medium	Medium	Good	Above average	Above average	Fair
TV-16	Erect/Medium	Medium	Good	Above average	Good	Good
TV-17	Erect/Medium	Medium	Good	High	Good	Good
TV-18	Erect/Medium	Low	Very good	High	Average	Good
TV-19	Flat/Large	Low	Very good	High	Above average	Good
TV-20	Flat/Large	Low	Good	High	Good	Good
TV-22	Flat/Large	Low	Good	High	Above average	Poor
TV-23	Flat/Large	Low	Good	High	Average	Poor
TV-25	Erect/Medium	Low	Very	High	Average	Fairly resistant
TV-26	Erect/Medium	Low	Very good	High	Average	Fairly resistant
TV-27	Erect/Small	Medium	Very good	Above average	Above average	Tolerant
TV-28	Erect/Small	Medium good	Very average	Above average	Above	Tolerant
TV-29	Erect/Small	Low	Excellent	Very high	Average	-
TV-30	Erect/Small	Low	Excellent	Very high	Average	-

*Characteristics noted received from Tocklai Experimental Station source and our field studies. - = Not known.

Plate II

Fig. 1: Different tea clonal varieties are being raised in the nurseries.
Fig. 2: Maintenance of nursery tea plants.
Fig. 3: Nursery tea plants ready for plantation (in plastic sleeves) and/or experimental purpose.

was done with a total of 120 young tea plants of all 24 varieties. Some of the young tea plants of selected varieties were also grown in earthen pots (1 plant per pot, 25 cm diameter) each containing 4.5 kg of soil: planting mixture (1:1).

3.1.1.4. Clonal propagation

Cuttings of tea plants approximately 10 cm long having one mature leaf and one auxillary leaf bud was introduced in soil in plastic sleeves. The cuttings were kept for few days with light watering. The buds developed to form twigs that in turn produced new leaves. The original mature leaves served as maintenance leaves. After the development of new leaves and roots the plants grew normally. The mature leaves were either shed or remain attached until nine months.

3.1.1.5. Maintenance of planted varieties

During the work, the tea plants in the experimental garden were maintained with great care. Plants were grown in the garden under the natural conditions of daylight with mean monthly temperature ranging from maximum 31.0 ^{0}C to minimum 10.4 ^{0}C (Jain, 1991). All the plants were watered as and when required using ordinary tap water. The average rainfall in the region of our study is 3956.7 mm (Jain, 1991).

3.1.2. Collection of plants for extraction of botanicals

Twenty-one plant materials (leaf, bark, stem, root, rhizome etc. as applicable) were collected from foothills of Eastern Himalayas (sub Himalayan West Bengal). The plants were selected on the basis of easy availability in the growing areas of sub Himalayan West Bengal. The plants were collected, identified and voucher specimens have been deposited in the Departmental herbarium of the Department of Botany, University of North Bengal.

3.2. Fungal culture

3.2.1. Source of fungal culture

Fungal cultures used during the work were either isolated from the field or procured were also collected from Indian Type Culture Collection, IARI, Pusa, New Delhi. The details of the source of the fungal cultures are given in the Table 2 and Table 3.

Table 2
List of fungal cultures isolated from tea nurseries of North Bengal

Fungal Culture	Source	Identified By	Identification No., if any
Botryodiplodia theobromae *Patoulliard.**	Naturally infected leaves and collar regions of nursery tea plants of Kharibari, Siliguri, West Bengal.	Dr.P.N.Chowdhury, IARI, New Delhi	4151.2K
Curvularia eragrostidis (P. Hennings) Meyer.	Naturally infected leaves of nursery tea plants of Matigara tea estate, Siliguri, West Bengal.	Dr.P.N.Chowdhury, IARI, NewDelhi	4150.2K
Pestalotiopsis theae (Saw.) Stey.	Naturally infected leaves of nursery tea plants of Mohurgong and Gulma tea estate, Siliguri, West Bengal.	Identified by Dr. A. Saha, Department of Botany, University of North Bengal.	–
Colletotrichum camelliae Mass.	Naturally infected leaves of nursery tea plants of Mohurgong and Gulma tea estate, Siliguri, West Bengal.	Identified by Dr. A. Saha, Department of Botany, University of North Bengal.	–
Corticium theae Bernard.	Naturally infected leaves of nursery tea plants of Kharibari, Siliguri, West Bengal.	Identified by Dr. A. Saha, Department of Botany, University of North Bengal.	–
Fusarium oxysporum Schlecht.	Naturally infected leaves of nursery tea plants of Mohurgong and Gulma tea estate, Siliguri, West Bengal.	Identified by Dr. A. Saha, Department of Botany, University of North Bengal.	–

**Botryodiplodia theobromae* presently known as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.

Table 3
List of cultures of fungal antagonists used during the study

Name of the fungal antagonists	Source	Number
Trichoderma viride Persoon Ex S. F. Grey.	IARI, New Delhi	ITCC-2109
Trichoderma koningii Oudemans.	IARI, New Delhi	ITCC-2170
Trichoderma harzianum Rifai.	IARI, New Delhi	ITCC-4572
*Gliocladium virens** (Isolate I)	*IARI, New Delhi*	*ITCC-4177*
Gliocladium virens (Isolate II)	*Dr.Apurba Chowdhury,* UBKV, West Bengal	_

* *Gliocladium virens* presently known as *Trichoderma virens*

3.2.2. Verification of Koch's Postulates

In order to fulfill the Koch's postulations fresh young tea leaves of nursery tea plants collected from Kharibari, Siliguri, West Bengal and Mohurgong and Gulma tea estate, Siliguri, West Bengal were used. At first, the leaves were inoculated with conidial suspensions of *B. theobromae*, *C. eragrostidis*, *P. theae*, *C. camelliae*, *C. theae* and *F. oxysporum* separately following detached leaf inoculation technique (Dickens and Cook, 1989) and after 72 hours of inoculation, the pathogen in each case was reisolated from infected tea leaves. Before isolation, the infected portions were cut into small pieces, washed thoroughly with sterile distilled water, surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 1-3 minutes, washed several times again with sterile distilled water and finally transferred aseptically into sterile PDA slants. These isolates were examined after two weeks of inoculation and the identity of the organisms were confirmed after comparing them with the respective stock cultures.

3.2.3. Maintenance of stock cultures

Freshly prepared sterile slants of PDA medium were used for maintenance and preservation of fungal cultures. After two weeks of subculturing, the fungal pathogens grown on sterile PDA slants were stored in two different conditions, viz. at low temperature in refrigerator (5 ^{0}C) except the culture of *C. theae* and at room temperature. Apart from weekly transfer for experimental purpose, the cultures were also examined at regular intervals to test their pathogenicity. The cultures of three different species of *Trichoderma* (*T. viride*, *T. koningii* and *T. harzianum*) and two isolates (isolate I and isolate II) of *Gliocladium virens* were also maintained at 5 ^{0}C and at room temperature.

3.3. Major Chemicals used

In addition to the common laboratory reagents, following chemicals were used during the work :

Chemicals	Company
Acetic acid glacial	E. Merck (India) Ltd., Mumbai, India
Adjuvant complete Freund	Difco Laboratories, Detroit, Michigan, USA
Adjuvant incomplete Freund	Difco Laboratories, Detroit, Michigan, USA
Agarose	SRL Pvt. Ltd., Mumbai, India
Asparagine	SRL Pvt. Ltd., Mumbai, India
Barbituric acid	HiMedia Laboratories Ltd., Mumbai, India
Bovine serum albumin	Sigma Chemicals Co., USA
Bromophenol blue	HiMedia Laboratories Ltd., Mumbai,
Cadmium chloride	SRL Pvt. Ltd., Mumbai, India
Chloroform	E. Merck (India) Ltd., Mumbai, India
Coomassie brilliant blue	SRL Pvt. Ltd., Mumbai, Indi
Di ethyl ether	SRL Pvt. Ltd., Mumbai, India
Folin ciocalteau reagent	Ranbaxy Laboratories Ltd., India
Goat anti-rabbit IgG FITC conjugate	Bangalore Genei Pvt. Ltd., Bangalore, India
Goat anti-rabbit IgG horseradish peroxidase conjugate	Bangalore Genei Pvt. Ltd., Bangalore, India
Indole-3- acetic acid	E. Merck (India) Ltd., Mumbai, India

Chemicals	Company
Indole-3-butyric acid	E. Merck (India) Ltd., Mumbai, India
Jasmonic acid	Sigma Chemicals Co., USA
Mannitol	Qualigens, Glaxo India Ltd., Mumbai, Indi
Mercury (II) chloride	E. Merck (India) Ltd., Mumbai, India
Methanol	SRL Pvt. Ltd., Mumbai, India
Nickel chloride	Sigma Chemicals Co., USA
Polyethylene glycol (PEG)	SRL Pvt. Ltd., Mumbai, India
Polyvinyl pyrrollidone	SRL Pvt. Ltd., Mumbai, India
Salicylic acid	SRL Pvt. Ltd., Mumbai, India
Sea sand	Hi Media Laboratories, Mumbai, India
Silica Gel G	E. Merck (India) Ltd., Mumbai, India
Sodium azide	Loba Chemie Pvt. Ltd., Mumbai, India
Sodium molybdate	E. Merck (India) Ltd., Mumbai, India
Sodium selenite	SRL Pvt. Ltd., Mumbai, India
Tetramethyl benzidine/hydrogen peroxide	Bangalore Genei Pvt. Ltd., Bangalore, India
Tris (Tri hydroxy methyl methylamine)	Qualigens, Glaxo India Ltd., Mumbai, India
Tween 20	Hi Media Laboratories, Mumbai, India
Zinc sulphate	E. Merck (India) Ltd., Mumbai, India

3.4. Chemical fungicides used

Trade name	Chemical name
Bavistin	Carbendazim [2-(methoxycarbamoyl)-benzimidazole]
Baynate	Thiophanate methyl
Calixin	Tridemorph-tridecyl-2, 6-dimethyl morphine]
Captan	Cis N-trichloromethylthio-4-cyclohexen-1, 2-dicarboximide
Indofil	Mancozeb
Roko	Thiophanate methyl (70% WP)

3.5. Composition of media and solutions used

Some of the media/solutions used during the work are listed below along with their standard compositions and/or modifications, if any.

POTATO DEXTROSE BROTH (PDB)

Peeled potato	:	40 g
Dextrose	:	2 g
Distilled water	:	100 ml

(Required amount of peeled potato was boiled in distilled water. The potato broth was taken by straining through cheesecloth and required amount of dextrose was added. Finally, the medium was sterilized at 15 lb p.s.i. for 15 minutes).

POTATO DEXTROSE AGAR (PDA)

Potato dextrose agar was prepared by adding 2% agar powder to the final potato dextrose broth solution. The agar was melted by heating the media before sterilization.

MALT EXTRACT AGAR (MEA)

Malt extract	:	20 g
Agar agar	:	20 g
Distilled water	:	1000 ml

(Required amount of malt extract was dissolved by boiling in distilled water. Then, required amount of agar powder was added and boiled with constant shaking till the agar was dissolved. Finally, the medium was sterilized at 15 lb p.s.i. for 15 minutes).

OAT MEAL AGAR (OMA)

Oat meal	:	40 g
Agar agar	:	15 g
Distilled water	:	1000 ml

(Powdered oat was boiled in distilled water in a water bath, occasionally stirred and strained through cheese cloth. Then required amount of agar powder was added to it and boiled with constant shaking till the agar was dissolved. Finally, the medium was sterilized at 15 lb p.s.i. for 15 minutes).

POTATO CARROT AGAR (PCA)

Grated Potato	:	20 g
Grated Carrot	:	20 g
Agar agar	:	20 g
Distilled water	:	1000 ml

(Grated potato and grated carrot were mixed and boiled with distilled water. After cooling, the broth was strained through cheese cloth and required amount of agar powder was added and boiled to dissolve the agar. Finally, the medium was sterilized at 15 lb p.s.i. for 15 minutes).

CZAPEK DOX AGAR (CDA)

$NaNO_3$	:	3 g
K_2HPO_4	:	1 g
KCl	:	0.5 g
$MgSO_4$, $7H_2O$	:	0.5 g
$FeSO_4$	:	0.01 g
Sucrose	:	30 g
Agar agar	:	15 g
Distilled water	:	1000 ml.

(Initially, all the ingredients except agar and K_2HPO_4 were dissolved. Next, agar was added and dissolved by steaming. Finally K_2HPO_4 was added to the molten solution, mixed thoroughly and sterilized at 15 lb p.s.i. for 15 minutes).

RICHARD'S SOLUTION / MEDIUM (RM)

KNO_3	:	10 g
KH_2PO_4	:	5 g
$MgSO_4$, $7H_2O$	:	2.5 g
$FeCl_3$	:	0.02 g
Sucrose	:	50 g
Distilled water	:	1000 ml

(Required amount of all the constituents were taken and mixed with required distilled water. All the constituents were dissolved by stirring and sterilized at 15 lb p.s.i. for 15 minutes).

HOAGLAND AND KNOP'S SOLUTION

KNO_3	:	0.61 g
$Ca(NO_3)_2, 4H_2O$	:	0.95 g
$MgSO_4, 7H_2O$	:	0.49 g
$NH_4(H_2PO_4)$	:	0.12 g
$MnSO_4, 4H_2O$	:	3.0 g
$ZnSO_4, 7H_2O$	:	0.5 mg
H_3PO_3	:	0.5 ml
$CuSO_4, 5H_2O$	:	0.025 mg
$Na_2MoO_4, 2H_2O$	:	0.025 mg
H_2SO_4	:	0.5 µl
$FeC_6O_5H_7, 5H_2O$	:	0.2 g
Distilled water	:	1000 ml

(Required amount of all the constituents were taken and they were mixed thoroughly in distilled water)

3.6. Inoculation technique

3.6.1. Detached leaf inoculation

Pathogenicity test was done by artificial inoculation of detached leaf with test pathogens following the detached leaf inoculation technique proposed by Dickens and Cook (1989). To perform the experiment fresh young fully expanded and detached nursery tea leaves were placed on plastic trays lined with moist blotting papers. The leaves in the trays were inoculated with mycelial plugs as well as spore suspensions of the two pathogens separately. Initially two wounds (light scratch of 2 mm length) were made on the adaxial surface of each leaf with the help of a sterile, sharp needle. Mycelial plugs (2 mm in diameter) bearing the conidia of the test pathogens of 15 d old cultures were then aseptically placed on the wounds. Sterile PDA plugs were used to inoculate control sets. Similarly, 20 µl (24 drops/leaf) of conidia or spore suspensions bearing approximately $1X10^6$ conidia/ml of test pathogens (prepared from 15 d old cultures) were placed on the wounds of each leaf with a hypodermic syringe in a separate set of experiment. 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 gelly in order to maintain

the required moistures inside the trays during incubation. Fifty leaves of each variety were inoculated in each treatment.

3.6.2. Cut shoot inoculation

The inoculation technique described by Yanase and Takeda (1987) was followed for cut shoot inoculation. Twigs with 3-4 leaves of nursery tea plants grown in the clone house of Mohurgong and Gulma Tea Estate and of Taipoo Tea Estate were cut carefully with a sharp blade and immediately introduced into glass vials containing sterile tap water and transferred to the laboratory. Some of the twigs were also collected from the Experimental garden, Department of Botany, University of North Bengal. Leaves were inoculated with the mycelial plugs (2 mm diameter) bearing conidia of test pathogens as described by Dickens and Cook (1989). The mycelial plugs were wrapped with absorbent cotton wool moistened with sterile distilled water and sterile PDA plugs were used as controls. For each treatment sixteen cut shoots of a variety were placed into the holes of Styrofoam board, and the board was floated on the modified Hoagland and Knop's solution in a glass chamber. The glass chambers were incubated for a week. The chambers were covered with transparent cellophane papers. The cellophane papers were perforated with a needle for aeration.

3.6.3. Nursery tea plant inoculation

Following the whole plant inoculation method of Dickens and Cook (1989), nursery tea plants (raised through clonal cuttings) were treated with the test pathogens. Inoculation was done by spraying conidial suspensions ($1X10^6$ conidia/ ml) prepared from 15 d old cultures of test pathogens grown on sterile PDA slants. In control sets, plants were sprayed with sterile distilled water. The plants were kept for 48 hours in transparent polythene chamber to maintain high humidity. Twenty plants of each variety were inoculated in each treatment.

3.7. Disease assessment

3.7.1. Detached leaf

Symptoms (brown necrotic lesions) produced at the inoculation sites were termed 'lesions' and the number of lesions produced out of the total number of spore suspension drops/mycelial plugs multiplied by 100 gave the percentage of

lesions produced. Percentage of lesion production and diameter of each lesion were calculated after 24, 48 and 72 hours of inoculation. All calculations were based on the average of three separate treatments, each treatment comprising of 50 inoculated leaves.

3.7.2. Cut shoot

The number of lesions developed on the leaves after 24, 48 and 72 hours of inoculation were counted and diameters of each lesion were measured. The results were computed following the method of Sinha and Das (1972). The diameters were categorized into four groups and a value was assigned to each group as follows:

- Very small-restricted lesions of 1-2 mm diameter : 0.1
- Lesions with sharply defined margins of 2-4 mm diameter : 0.25
- Slow spreading lesions of 4-6 mm diameter : 0.5
- Spreading lesions of variable size (beyond 6 mm in diameter) with diffused margin : 1.0

The number of lesions 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 observations on sixteen cut shoots per treatment.

3.7.3. Nursery tea plant

Both number and sizes of lesions produced on the leaves were assessed after 4, 8, 12 and 16 days of inoculation as described by Sinha and Das (1972). On the basis of visual observations, lesions were graded into four size groups viz. very small, small, medium and large with respective values of 0.1, 0.25, 0.5 and 1.0 assigned to them to give an approximate idea of their relative size. Number of lesions in each group was multiplied by the value assigned to it and the sum total of such values for all the leaves gave the disease index for a plant. Results were always computed as the mean of observations on twenty plants per treatment.

3.8. Growth and physiology of the pathogens

3.8.1. Assessment of mycelial growth

A number of different solid and liquid media were used to assess the mycelial growth of isolated fungal pathogens. Initially, each fungus was grown in sterile petriplates containing 20 ml of different sterile test medium and was incubated for a maximum of 8 days at 28±2 °C. Radial growth of mycelia were measured after regular intervals to assess the mycelial growth in solid medium.

To assess the mycelial growth in different liquid media, similar petriplates with sterile PDA medium were inoculated with the test fungi and incubated for only 4 days at 28±2 °C. From the advancing zone of the cultures, agar blocks of 4 mm diameter containing the mycelia were cut aseptically by a cork borer and transferred to Ehrlenmayer flasks of 250 ml, each containing 50 ml of sterilized different liquid medium and incubated at 28±2 °C. After a minimum of 25 days of incubation, the growing fungal mycelia were strained through double-layered cheese cloth, blotted and dried at 60 °C. Finally, they were cooled and weighed.

3.8.2. Assessment of germination of spores

3.8.2.1. Preparation of spore suspension

Fifteen days old sporulated fungal culture was taken and approximately 3-5 ml sterile distilled water was poured in the culture tube aseptically, Gentle scrapping was done by an inoculating needle on the agar surface. After the scrapping, the tube was shaken and the resultant mixture was strained through cheese cloth. The filtrate was used as spore suspension. The concentration of the spores in the suspension was adjusted by adding sterile distilled water following hemocytometer count.

3.8.2.2. Spore germination bioassay

Assessment of spore germination of the test fungus was done under various conditions as required. Spore suspensions of test pathogens were prepared and placed (30 µl) on clean, grease free glass slides with sterile distilled water/test solution (30 µl). The slides were incubated at 28±2 °C in a humid chamber. After the desired incubation period, the slides were stained with cotton blue-lactophenol and observed under microscope. Finally, the percent spore germination and average germ tube length in each case were calculated.

3.9. Collection and maintenance of Rabbits

Six New Zealand male white rabbits were used in the study for raising polyclonal antibody (Plate III : 1). The rabbits were bought from the local rabbit seller and brought to the laboratory at least a month before starting any immunization programme for proper acclimatization. The body weight of the rabbits was approximately 1.5 kg and their age was around 10 months. The rabbits were kept in separate cages (60 cm X 60 cm X 70 cm) attached with metal trays at the bottom and placed in a well-ventilated room. The room was cleaned regularly with permitted room freshening solutions. Each rabbit was supplied with sufficient quantities of vegetables like carrot (*Daucus carrota* L.), soaked gram (*Cicer arietinum* L.), common grass [*Cynodon dactylon* (L.) Pers.], lettuce leaves (*Lactuca sativa* L.), Cabbage leaves (*Brassica oleraceae* L. var. *capitata*), Cauliflower leaves (*B. oleraceae* L. var. *botrytis*) etc. as food along with clean water. The quantity of food was adjusted only after a thorough consultation with local veterinary doctor. Routine health checking of the rabbits was also done. After one month, the immunization program was started after ascertaining their complete body fitness.

3.10. Preparation of antigens

3.10.1. Antigen from tea leaf

The protein extraction procedure of Alba and DeVay (1985) and Chakraborty and Saha (1994) was followed in order to extract leaf antigens from young tea leaves. Fresh, young leaves of the required varieties of tea were collected from the clone house of Mohurgong and Gulma Tea Estate, Siliguri, West Bengal; washed thoroughly with cold water and kept for 2 hours at -20 ^{0}C. The cold leaves (20 g fresh weight in each case) were grounded in prechilled mortar at 4 ^{0}C with 20 g of insoluble polyvinyl pyrrollidone (PVP). The leaf paste was suspended in cold 0.05 M sodium phosphate buffer (pH 7.0) containing 0.85% sodium chloride and 0.02 M ascorbic acid. The leaf slurry was strained through muslin cloth and centrifuged at 4 ^{0}C for 30 minutes at 12,100 g. The supernatants were collected and ammonium sulphate was added at 4 ^{0}C to 100% saturation under constant stirring, kept overnight 4 ^{0}C and finally centrifuged at 4 ^{0}C for 15 minutes at 12,100 g. The precipitate obtained was dissolved in cold 0.05 M sodium phosphate buffer (pH

7.0) and was dialysed against 0.005 M sodium phosphate buffer (pH 7.0) for 24 hours at 4 °C with 10 changes. After dialysis, the preparation was centrifuged at 4 °C for 15 minutes at 12,100 g and supernatant was stored at -20 °C until required.

3.10.2. Antigen from fungal mycelia

The mycelial antigens were prepared following the method as described by Chakraborty and Saha (1994) with some modifications. Mycelial discs (4 mm diameter) were transferred to 10 Ehrlenmayer flasks of 250 ml capacity, each containing 50 ml of sterilized PDB medium and incubated at 28±2 °C. After 15 days, the fungal mycelia were harvested, washed with 0.2% sodium chloride and rewashed with sterile distilled water. Washed mycelia (25 g fresh weight) were homogenized with 0.05 M sodium phosphate buffer (pH 7.4) containing 0.85% sodium chloride in a mortar and pestle with sea sand and kept overnight at 4 °C. The homogenates were then centrifuged at 4 °C for 30 minutes at 12,100 g. The supernatant was equilibrated to 100% saturated ammonium sulphate under constant stirring and again kept overnight at 4 °C. Next, the mixtures were centrifuged at 4 °C for 30 minutes at 12,100 g. The supernatants were discarded and precipitates were dissolved in cold 5ml 0.05 M sodium phosphate buffer (pH 7.4). The preparations were dialyzed for 24 hours at 4 °C against 0.005 M sodium phosphate buffer (pH 7.4) with 10 changes. After dialysis, the preparations were centrifuged at 4 °C for 15 minutes at 12,100 g and supernatant was stored at -20 °C until further use.

Protein content of both the plant and fungal antigens were determined following the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard.

3.11. Raising and collection of blood sera through immunization

3.11.1. Raising of antisera

Before immunization, normal sera were collected from each rabbit (Plate III : 3). Antisera against antigens of host (*C. sinensis*) and pathogens (*B. theobromae and C. eragrostidis*) were raised in separate rabbits by giving intrarmuscular injections (1 ml) of antigens emulsified with equal volume of Freund's complete adjuvant (Plate III : 2). The doses were repeated at 7 days intervals with Freund's

Plate III

Fig. 1 : New Zealand white male rabbit.(Approximately 10 month old &1.5 kg.body weight)
Fig. 2 : Administration of intramuscular injection in rabbit.
Fig. 3 : Collection of blood from rabbit following marginal ear vein puncture.

incomplete adjuvant for 6 consecutive weeks. Final protein concentration was 2 mg/ml in the emulsion. On the 4th day after the last injection, the blood samples were collected and antisera were prepared.

3.11.2. Collection of sera

Blood from rabbits was taken by puncturing the marginal ear vein (Plate III: 3). At first the rabbits was taken out from the cage, placed on a table and the hairs were removed from the vein of the ear with a sterilized blade and was disinfected with rectified spirit. After irritation of the ear with xylene, an incision was made with a sharp sterilized blade on the border vein of the ear and about 10 ml blood was collected in a sterile glass graduated tube. After taking the desired quantity of blood precautions (adhesive surgical tapes etc.) were taken to stop the flow of the blood from the punctured area of the ear. The blood samples were kept undisturbed for an hour at 35-37 ^{0}C for clotting. In order to avoid the loss of serum included within the clot, it was loosened from the glass surface by turning a sterile wooden stick around the glass near the glass wall. Finally normal sera (pre-bleed sera) as well as antisera were clarified by centrifugation at 4 ^{0}C for 10 minutes at 3000 g and were distributed in sterile cryo vials and stored at -20 ^{0}C until required.

3.12. Determination of titre value

Titres of antisera against the homologous antigens and titres of antigens against homologous antisera were determined following immunodiffusion technique as described by Ouchterlony (1967) and Clausen (1969). A constant amount (5 µl) of undiluted antiserum or antigen was placed in the central well, while diluted antigens or antisera (diluted with normal saline with ratios 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128, respectively) were pippeted into the outer well. Diffusion was allowed for 48-72 hours at 25 ^{0}C in a humid chamber. Titre was expressed as the reciprocal of the highest dilution of antiserum or antigen that reacted with antigen or antiserum giving precipitin lines.

3.13. Immunological experiments

3.13.1. Immunodiffusion

Agar gel double diffusion test was performed following the method of Ouchterlony (1958). Barbital buffer (50 ml, 0.05 M, pH 8.6) was taken in a

100 ml Ehrlenmayer flask and was placed in a boiling water bath. Agarose (0.4g) was mixed with hot barbital buffer. The buffer-agarose mixture was carefully placed on water bath. Finally a clear molten agarose solution was prepared. The solution was then mixed with 0.1% (w/v) sodium azide (a bacteriostatic agent). The agarose solution was dispensed carefully in clean and dry square and rectangular glass plates of 6 cm X 6 cm and 6 cm X 3 cm respectively so that no air bubble was trapped in the agarose medium to avoid asymmetrical diffusion. Before dispensing, the glass plates were washed with extran solution and water and then the glass plates were serially dipped in 90% ethanol, ethanol:ethyl ether (1:1, v/v) and ether for removal of grease, if any. The glass plates were placed inside sterile petridishes. When the agarose solution was solidified, 3 to 6 wells of 5 mm diameter were cut by a sterile cork borer. The distances of the peripheral wells from the central wells were 5-7 mm. The antigens, normal sera and undiluted antisera were placed with a micropippette directly into the appropriate wells and diffusion was allowed to continue in a humid chamber at 25 ^{0}C for 48-72 hours. The precipitation reaction was observed after proper staining of the slides only in cases where common antigens were present.

3.13.2. Immunoelectrophoresis

3.13.2.1. Preparation of agarose coated glass slides

For immunoelectrophoresis rectangular glass pieces (8 cm X 3.5 cm) were made grease free as mentioned earlier in section 3.13.1. The slides were dried and placed on a clean surface. Thin and uniform layers (2 mm thick) of fluid agarose medium (0.8%), containing 0.1% sodium azide in 0.05 M barbital buffer (pH 8.6) were dispensed on each slide, taking care so that no air bubbles were trapped in the agarose medium. This was necessary in order to avoid irregularity that may cause asymmetrical migration and diffusion during later stages. Finally, the slides were stored at 4 ^{0}C in petridishes until use.

3.13.2.2. Electrophoresis

The agarose coated slides were placed in the middle compartment of the electrophoretic platform and two central wells of 4 mm diameter were dug out from each slide. The anode and cathode chambers were filled with 0.05 M barbital

buffer (pH 8.6). Different antigens were placed into separate wells. Bromophenol blue was used as marker for tracing the electrophoretic movement of the antigens. Filter paper (Whatman-1) strips were soaked in buffer and placed on both ends of the slides, which connected the buffer solution of anode and cathode compartments with the agarose surface of the slides. An electric current of 2.5 mAmp/slide; 10 V/ cm was passed through the slides for two hours in cold (4 ^{0}C) conditions. When the bromophenol blue marker reached near the short edge of the glass slides the current was discontinued and the glass slides were taken out.

3.13.2.3. Diffusion

A longitudinal trough parallel to the long edge of the slides was cut between the two central wells of the agarose surface and undiluted antiserum was placed into the trough. Diffusion was allowed to continue for 24-48 hours in a moist chamber at 25 ^{0}C.Precipitation arcs if formed were recorded.

3.13.3. Washing, Drying and Staining of slides

After immunodiffusion and immunoelectrophoresis the glass slides were washed with 0.9% aqueous sodium chloride carefully for 48 hours to remove all the unreacted antigens and antisera widely dispersed in the agarose surface. Following that the slides were washed with distilled water for three hours to remove the sodium chloride and dried at 40 ^{0}C for 30 minutes. Next, the slides were stained either with 0.5% coomassie blue or 0.5% amido black (0.5g coomassie blue/amido black, 5 g $HgCl_2$ and 5 ml glacial acetic acid dissolved in 95 ml distilled water) for 30 minutes at room temperature, washed thrice with 2% v/v acetic acid for three hours (one hour each time) to remove the excess stain. Finally the slides were washed with distilled water and dried at 40 ^{0}C for 30 minutes.

3.13.4. Indirect Enzyme Linked Immunosorbent Assay (Indirect ELISA)

Combining the methods of Koeing and Paul (1982) and Talbot (2001), indirect ELISA was performed. At first antigens were diluted with coating buffer [carbonate buffer (0.1 M), pH 9.6] and 100 µl of each diluted antigens were placed on the wells of a flat bottomed micro titre ELISA plate (Tarsons) except one well which was considered as blank. The plate was incubated overnight at 4 ^{0}C in

refrigerator. After incubation, the plate was taken out and each well of the plate was flooded with phosphate buffer saline (PBS)-Tween (0.15 M PBS + 0.8% NaCl + 0.02% KCl + 0.05% Tween 20) and washed thoroughly for four to five times. After washing the plate was dried in air. Following this, 100 µl of PBS-BSA (0.15 M PBS containing 1% BSA) was added to each well to coat all the unbound sites. The plate was incubated for 2 hours at room temperature. After incubation, the plate was again washed with PBS-Tween and air-dried. After this, 100 µl of diluted antisera (diluted with PBS-Tween) were added to each well except the blank and the control wells where normal sera was added (serially diluted with PBS-Tween containing 0.5% BSA). The plate was incubated overnight at 4 °C. In the next day, thorough washing of the plate was done with PBS-Tween. After washing and drying, 100 µl (1:10000) goat-anti rabbit IgG-Horse radish peroxidase conjugate was added to each well except the blank and the plate was incubated for 2 hours at 30±2 °C. After incubation, the plate was again washed with PBS-Tween and shaken dry. Then 100 µl (1:20) of tetramethyl benzidine/ hydrogen peroxide (TMB/H_2O_2), a chromogenic substrate was added to each well except the blank. After addition of substrate, a blue colour was produced due to the reaction between the enzyme and the substrate. Finally, the reaction was terminated after 30 min by adding 100 µl of 1(N) H_2SO_4 to each well except blank. Absorbance values were recorded in an ELISA reader (Mios Junior, Merck) at 492 nm.

3.13.5. Immunofluorescence

3.13.5.1. Fluorescence staining of mycelia of pathogens

The technique of Merz *et al.* (1969) was followed with some modifications. The mycelia of the test pathogens (used as antigens) were placed on sterile microscopic slides containing albumin. After adhering, the mycelia were treated with 95% ethanol-ethyl ether (1:1,v/v) for 10 minutes at room temperature for fixing. Next, the slides were treated with 95% ethanol for 20 minutes at 37 °C. The fixed mycelia were then flooded with normal sera or with ten-fold dilution (diluted with 0.01M potassium phosphate buffer, pH 7.0, containing 0.14 M sodium chloride) of appropriate antisera. The slides were then incubated for 30 minutes within a moist chamber. After incubation, the slides were washed with PBS (pH 7.2) for two to

three times and air-dried. Following that, the diluted (diluted with carbonate-bicarbonate buffer, pH 9.6 in 1:40 dilution) goat-anti rabbit IgG conjugated with fluorescein isothiocyanate (FITC) was added and the slides were kept in the dark. After this all processings were done in a dark room. After 30 minutes, the slides were again washed twice with PBS and once with distilled water and air-dried. Finally, the slides were mounted in a glycerol based mounted medium (Hardham *et al.*, 1986) and a cover glass was placed on the mycelia and sealed. The slides were observed in a fluorescence microscope (Leitz, German).

3.13.5.2. Fluorescence staining of leaf sections of the host

The technique as described by DeVay *et al.* (1981) was followed. The cross sections through the midrib portions of the leaves were cut, placed into the slides and flooded with 100 µl normal serum and/or with ten-fold dilution (diluted with 0.01 M potassium phosphate buffer, pH 7.0, containing 0.14 M sodium chloride) of appropriate antisera. The slides were then incubated for 30 minutes at 27 °C. After proper incubation, the antisera treated leaf sections were washed by shaking the sections with 4 ml of 0.01 M potassium phosphate buffer, pH 7.0, containing 0.14 M sodium chloride for 15 minutes. Following that, 5 µl of diluted (diluted with carbonate-bicarbonate buffer, pH 9.6 in 1:40 dilution) goat-anti rabbit IgG conjugated with FITC was added and the slides were kept for incubation in a dark moist chamber at 27 °C. Henceforth, all processings were done in a dark room. After 30 min the leaf sections were again washed by shaking the sections with 4 ml of 0.01 M potassium phosphate buffer, pH 7.0, containing 0.14 M sodium chloride for 15 minutes at 27 °C. Finally, the slides were mounted in a glycerol based mounted medium (Hardham *et al.*, 1986), and observed in a fluorescence microscope as mentioned earlier (section 3.13.5.1). Photographs were taken with a Leica Wild MPS 48 camera on Fuji 800 ASA photographic film.

3.14. Application of various chemicals in selected tea varieties

Nine different chemicals (nickel chloride, salicylic acid, jasmonic acid, indole acetic acid, cadmium chloride, sodium selenite, sodium molybdate, sodium azide and indole butyric acid) were sprayed on the cut shoots of selected tea varieties at different concentrations. Fresh aqueous leaf extracts from jasmine plants at desired

concentrations were also sprayed on the cut shoots in a closed chamber. The control set was sprayed with sterile distilled water. Inoculation technique as described by Dickens and Cook (1989) was followed for inoculation of the sprayed twigs with the test pathogens. Results were taken and expressed as mean disease index/shoot following the procedure of Sinha and Das (1972).

3.15. Extraction and estimation of ortho-dihydroxy phenol and total phenol from the leaves of the twigs ofselected tea varieties after chemical treatment

3.15.1. Extraction

Tea leaves (1g) were immersed into absolute alcohol (10 ml) and boiled for 5 minutes. After boiling, the leaves were cooled and crushed with 80% alcohol, strained through cheesecloth and the final volume was made up to 5 ml.

3.15.2. Estimation of ortho-dihydroxy phenol

Sample (0.1ml) was mixed with distilled water (0.9 ml). Two milliliters of 0.5 (N) HCl, 1 ml of Arnow's reagent and 2ml of 1(N) NaOH were added sequentially in the sample. A blank containing all the reagents minus Arnow's was used to adjust the absorbance to zero. Quantity of phenol was estimated following the standard curve made from caffeic acid in a digital photoelectric colorimeter (Electronics India, Model No. 312) at 520 nm.

3.15.3. Estimation of total phenol

One-milliliter sample (0.1ml of leaf extract + 0.9 ml distilled water) was added to 1ml half-diluted folin-ciocalteau reagent and 2 ml Na_2CO_3 solution (20%). The mixture was boiled in water bath for 1 minute and cooled under running tap water. The mixture was then diluted up to 25 ml by adding distilled water. A blank was prepared with all reagents except Folin Ciocalteau's to adjust the zero reading. Quantity of total phenol was estimated with caffeic acid standard curve in a digital photoelectric colorimeter (Electronics India, Model No. 312) at 520 nm.

3.16. Bioassay of common chemical fungicides by poisoned food technique

Bioassay of fungicides was done by taking five different concentrations of six different fungicides following poisoned food technique as described by Suleman *et al.* (2002). Nine ml of sterilized PDA was mixed with 1 ml of prepared fungicide solution (made in sterile distilled water), and poured into sterilized petriplates of 70 mm diameter under aseptic condition. After solidification of media, the plates were inoculated at the center with 4 mm discs from 15 days old cultures of test pathogens. Control plates (without any fungicides) were also inoculated. The plates were incubated at 28±2 °C. After the required incubation period, the radial growth of the mycelia was measured and percent inhibition over control was calculated. Minimum inhibitory concentration (MIC) values of the test fungicides were also determined.

3.17. Screening, extraction and bioassay of botanicals

3.17.1. Preparation of plant extracts

Following the method of Mahadevan and Sridhar (1982) with some modifications, the extracts of the plant parts were made. Fresh plant materials were collected and washed thoroughly with sterile distilled water and allowed to dry at room temperature. After drying the materials were weighed, ground and extracted separately with sterile distilled water and ethanol (0.5 g/ml). The extracts were filtered through double-layered cheese cloth and centrifuged at 10,000 rpm for 15 minutes. The supernatants of the aqueous extracts were sterilized by passing through a Millipore filter (0.2 µm). All extracts were stored at 4 °C. The extracts were used for spore germination bioassay and TLC plate bioassay.

3.17.2. Spore germination bioassay

For screening of inhibitory effects of botanicals against test pathogens, the technique as described by Suleman *et al.* (2002) was followed. Aqueous plant extract (30 µl) was placed on a clear grease free microscopic slides. In control, 30 µl sterile distilled water was placed. Then 30 µl of spore suspension was added to 30 µl plant extract or 30 µl distilled water. In case of ethanol extract of plants 30 µl of ethanol extract was placed and in the control 30 µl of ethanol was placed. Spore

suspension (30 µl) was added after ehanol was evaporated from both the ethanol extract and ethanol from the slides. The slides were then incubated at 28±2 ^{0}C in a humid chamber. Humid chamber was prepared inside petridishes of 90 mm diameter. At first two small glass rods (60-70 mm in length) were placed in a petridish and a slide was placed on the rods in an uniformly balanced positions. Then the petridish was filled with sterile distilled water so that the bottom of the slide remains just above the water surface. The petridish was then covered. After 24 hours, the slides were stained with lacto phenol-cotton blue mixture and observed under the microscope. Approximately, 500 spores were observed for germination and length of 50 germ tubes were measured.

3.17.3. Preparation of TLC plates

Glass plates (20 cm X 10 cm) were dipped in chromic acid and then washed in running tap water. The plates were dried in hot air oven. After cooling the plates were placed in a platform of 100 cm X 20 cm. Silica gel G Merck (70 g) was dissolved in a conical flask containing 100 ml double distilled water, shaken vigorously and poured in a TLC applicator. A coat of silica gel (1 mm thick) was applied on the glass plates. The plates were allowed to dry and kept for future use.

3.17.4. Developing of TLC plates

TLC plates were activated at 70 ^{0}C for 30 minutes in a hot air oven. After cooling, sample extracts were applied on the plates by a capillary tube, 1 cm away from the basal end and the distance of two different spots was 1.5-2 cm. The sample-loaded plates were developed in a glass chamber containing solvent (chloroform: methanol:: 9:1). The solvent was allowed to run up to 15 cm. Following this the plates were taken out, air dried and used for chromatographic analysis or bioassay.

3.17.5. TLC plate bioassay

Air dried TLC plates were sprayed with spore suspension diluted with Richard's media (spore suspension: liquid Richard's media:: 1:1). The mixture was sprayed with an automyzer and then placed in a humid chamber keeping the coated side down on two parallel glass rods placed 16 cm apart. The humid chamber was

made by pouring mild hot water in a tray (25 cm X 15 cm). Precaution was taken so that the coated side do not touch the water surface. The whole set was incubated at 25±1 °C for 3 days. After three days, the plates were taken out and the diameters of the inhibition zones were noted, if any. The R_f values of the zones were recorded.

3.18. Application of biocontrol agents

3.18.1. Dual culture technique

Screening of potential antagonist was done following the dual culture method of Johnson and Curl (1982). In this technique, 20 ml of sterilized PDA medium in 100 ml Ehrlenmeyer flask was prepared. They were then transferred to sterile petriplates of 90 mm diameter, each containing 20 ml of PDA. Discs (4 mm) were cut from 15 d old cultures of a pathogen and of a biocontrol agent. The two discs were placed aseptically into the peripheral region of the petriplates in a straight line but opposite to each other. The plates were then incubated at 28±2 °C. Radial growth of the pathogen and the biocontrol agent were measured and percent inhibition in comparison to control (where no biocontrol agent was placed) was determined for each treatment.

3.18.2. Bioassay of cell free culture filtrate by poisoned food technique

Poisoned food technique as described in section 3.16 was followed with some modifications. PDA (9 ml) was prepared in 100 ml Ehrlenmeyer flask and sterilized. Membrane filtered culture filtrate (1ml) was added to the molten PDA medium, mixed well and poured in sterile petridishes (70 mm diameter) under aseptic conditions. After solidification of the media, the plates were inoculated with the pathogens, incubated for desired period and radial growth was measured following the methods described earlier (section 3.16).

3.19. Statistical analysis

Statistical analysis was done with the help of Smith's statistical package (version 2.5), developed by Dr. Gray Smith, Pomona College, Claremont-91711,. USA.

Experimental

4.1. Pathogenicity test of *B. theobromae* and *C. eragrostidis* in different tea varieties

Pathogenicity of two fungal pathogens (*B. theobromae* and *C. eragrostidis*) were tested in three different ways. These were the detached leaf inoculation technique, cut shoot inoculation technique and nursery tea plants inoculation technique. Detached leaf inoculation technique (Plate IV) and cut shoot inoculation technique (Plate V) have been discussed in details in the materials and methods (Section 3.6 & 3.7). Experiments following the above mentioned two techniques were performed on 24 different tea varieties viz. TV-1, TV-2, TV-3, TV-6, TV-8, TV-9, TV-11, TV-12, TV-16, TV-17, TV-18, TV-19, TV-20, TV-22, TV-23, TV-25, TV-26, TV-27, TV-28, TV-29, TV-30, Teenali 17/1/54, MG-1 and Sundaram. The nursery tea plants inoculation technique was done on 10 different varieties. The varieties were TV-2, TV-9, TV-11, TV-12, TV-18, TV-22, TV-25, TV-26, TV-28 and TV-30. Tocklai Experimental Station, Jorhat, Assam, India certify all the varieties except Sundaram mentioned above. United Planters Association of South India (UPASI) has released the Sundaram variety. The disease assessment procedures and details of incubation periods and temperatures etc. have been mentioned in the materials and methods (Section 3.6 & 3.7).

4.1.1. Pathogenicity test following detached leaf inoculation technique

Pathogenicity of *B. theobromae* and *C. eragrostidis* were performed on detached leaves of 24 different varieties of tea mentioned above. The results presented in the Table 4 and Table 5 represents percentage of lesion formed and mean diameter of lesions after 24, 48 and 72 hours of incubation. Fig. 3 and Fig. 4 shows a graphical representation of percent lesion after 72 h of incubation. The leaves were mounted with spore suspension droplets and the brown necrotic infected sites were termed as lesions (Plate IV : 1-5). The lesions were counted and diameters of the lesion were measured. The percentages of lesions formed in three separate experiments (i.e., replicates) were taken. The mean values of the three experiments were tabulated in Table 4 and Table 5 respectively for the two pathogens.

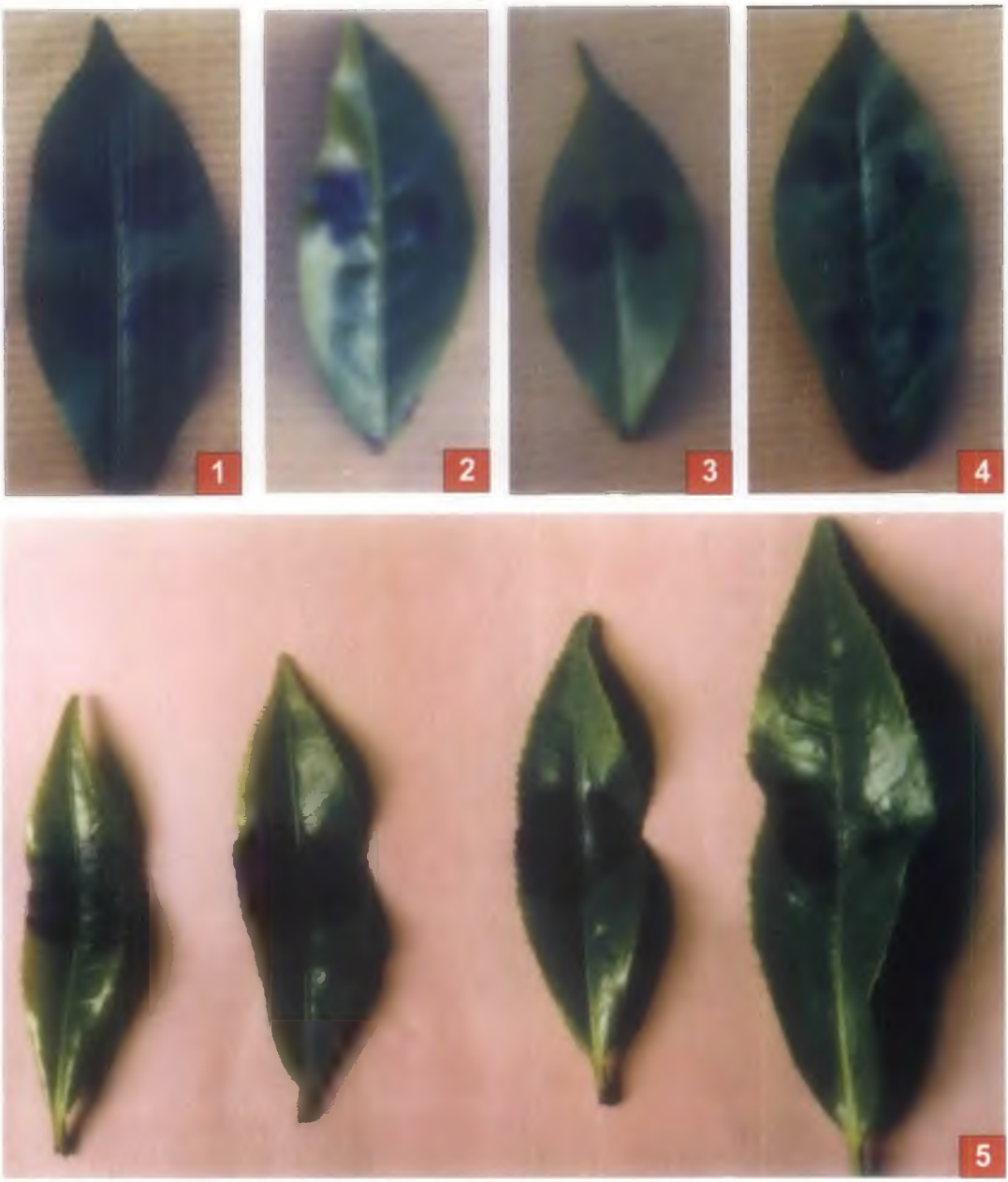


Plate IV

Artificially infected leaves of susceptible varieties from twelve month old nursery tea plants.

Fig.1-4 : Tea leaves (TV-12) artificially infected with *Curvularia eragrostidis* following detached leaf inoculation technique.

Fig.5 : Tea leaves (TV-11) artificially infected with *Botryodiplodia theobromae* following detached leaf inoculation technique.

Plate V

Healthy and infected cut shoots from young tea plants.

Fig.1: Healthy cut shoots of TV-11 (Control). **Fig.2 :** Cut shoots of TV-11 infected with *Botryodiplodia theobromae*. **Fig. 3 :** Healthy cut shoots of TV-12 (Control). **Fig.4 :** Cut shoots of TV-12 infected with *Curvularia eragrostidis*.

On the basis of the data presented in the Table 4, it is evident that TV-11, TV-12, TV-9 and TV-2 are susceptible against *B. theobromae* because they produced 90.50%, 87.50%, 84.40% and 81.30% lesions respectively. A variety was considered highly susceptible when it produced more than 80% lesion and resistant when the lesion production was less than 25% after 72 h of incubation. Accordingly, TV-26, TV-6 and TV-30 were resistant against *B. theobromae* and produced 12.50%, 16.66% and 20.60% lesions respectively.

On the basis of the data presented in the Table 5, it was evident that TV-12, TV-11, TV-27 and TV-29 were susceptible against *C. eragrostidis* because they produced 92.00%, 89.00%, 88.00% and 87.50% lesions respectively, while TV-25, TV-26 and TV-8 were resistant against *C. eragrostidis* and produced 22.00%, 25.00% and 25.00% lesions respectively after 72 h of incubation.

4.1.2. Pathogenicity test following cut shoot inoculation technique

Twigs with 3-4 leaves of nursery tea plants of 24 different varieties already mentioned above were selected for pathogenicity test of B. *theobromae* and *C. eragrostidis*. Details of the experimental set up has been discussed in the materials and methods (Section 3.6 & 3.7). The results were computed following the method of Sinha and Das (1972) and presented in the Table 6 and Table 7 respectively for the two pathogens.

On the basis of the data presented in the Table 6 and from Fig. 5(A), it was evident that TV-11 was the most susceptible and TV-26 was the most resistant tea varieties against *B. theobromae*. After 72 h of inoculation, mean disease index/shoot value was 1.69 (maximum) in the shoots belonging to TV-11. TV-12 (also considered as susceptible variety against *B. theobromae*) showed the mean disease index/shoot value of 1.63 after 72 h. The mean disease index/shoot value was 0.62 in case of TV-26 while other resistant varieties like TV-6 and Sundaram showed the mean disease index/shoot values of 0.81 and 0.78 respectively after 72 h of inoculation. The tea varieties, which showed the mean disease index/shoot values between 1.00 and 1.40 after 72 h of inoculation, were considered as moderately resistant tea varieties against *B. theobromae.* When mean number of lesions were considered, it was found that mean number of lesions / shoot value was1.62 in

Table 4
Pathogenicity test of *B. theobromae* on detached leaves of different tea varieties

Tea Varieties	[a] Percentage of lesion formed			[b] Mean diameter of lesion (mm)		
	Incubation periods (Hours)			Incubation periods (Hours)		
	24 h	48 h	72 h	24 h	48 h	72 h
TV-1	33.30±0.25	53.00±0.44	66.60±0.66	1.50±0.21	3.70±0.36	6.10±0.51
TV-2	10.60±0.40	45.80±0.55	81.30±0.73	0	2.00±0.31	5.20±0.38
TV-3	0	12.50±0.29	37.50±0.50	0	3.00±0.28	5.10±0.36
TV-6	4.90±0.35	8.33±0.67	16.66±0.83	1.00±0.39	1.00±0.44	3.00±0.32
TV-8	25.00±0.46	50.00±0.58	62.50±0.81	1.20±0.42	3.50±0.38	5.00±0.49
TV-9	24.30±0.36	46.00±0.29	84.40±0.50	0	2.80±0.22	5.70±0.28
TV-11	64.00±0.53	87.50±0.76	90.50±0.96	2.70±0.46	3.40±0.40	6.80±0.53
TV-12	25.00±0.25	61.80±0.41	87.50±0.76	1.00±0.24	4.50±0.28	6.70±0.43
TV-16	0	0	30.00±0.69	0	0	4.30±0.35
TV-17	5.30±0.25	12.50±0.50	25.00±0.59	1.00±0.29	1.30±0.30	1.80±0.21
TV-18	0	37.50±0.76	50.00±0.76	0	4.30±0.42	5.20±0.48
TV-19	25.00±0.29	37.50±0.29	50.00±0.50	2.50±0.37	3.30±0.47	3.50±0.41
TV-20	25.00±0.54	50.00±0.50	55.00±0.77	1.50±0.33	4.30±0.40	6.00±0.24
TV-22	0	19.00±0.60	37.50±0.90	0	5.30±0.42	6.40±0.50
TV-23	31.00±0.45	50.00±0.59	62.50±0.96	2.10±0.22	2.50±0.20	3.00±0.25
TV-25	0	37.50±0.50	50.00±0.68	0	2.00±0.23	5.70±0.27
TV-26	0	0	12.50±0.50	0	0	4.00±0.41
TV-27	0	27.60±1.15	50.00±1.26	0	1.50±0.12	4.80±0.20
TV-28	0	0	33.00±0.70	0	0	4.70±0.20
TV-29	0	20.30±0.50	41.60±0.81	0	3.00±0.47	6.20±0.31
TV-30	0	12.80±0.29	20.60±0.55	0	4.00±0.47	5.40±0.43
MG-1	36.60±0.56	48.40±0.77	63.30±1.02	1.70±0.15	3.40±0.26	6.40±0.24
Teenali 17/1/54	25.00±0.55	39.00±0.65	61.60±0.87	2.00±0.22	4.30±0.28	5.80±0.46
Sun-daram	0	8.33±0.36	25.00±0.70	0	2.00±0.32	5.30±0.44

[a] Mean of 3 replications.
[b] Mean of 50 lesions.
Data after ± represent standard error values.

Table 5

Pathogenicity test of *C. eragrostidis* on detached leaves of different tea varieties.

Tea Varieties	[a] Percentage of lesion formed			[b] Mean diameter of lesion (mm)		
	Incubation periods (Hours)			Incubation periods (Hours)		
	24 h	48 h	72 h	24 h	48 h	72 h
TV-1	50.0±0.20	71.6±0.41	71.6±0.75	2.58±0.40	4.60±0.42	8.00±0.52
TV-2	75.5±0.35	87.5±0.60	87.5±0.82	2.70±0.34	5.00±0.46	7.70±0.60
TV-3	0	0	30.5±0.60	0	0	4.30±0.50
TV-6	0	0	33.3±0.41	0	0	4.70±0.43
TV-8	0	25.0±0.41	25.0±0.63	0	2.00±0.44	2.70±0.46
TV-9	12.9±0.21	37.5±0.35	37.5±0.52	2.50±0.46	3.30±0.42	5.50±0.46
TV-11	75.0±0.43	75.0±0.61	89.0±0.84	2.60±0.43	4.00±0.51	9.00±0.64
TV-12	87.5±0.52	90.0±0.66	92.0±0.96	2.70±0.24	4.70±0.26	9.10±0.58
TV-16	37.5±0.61	50.0±0.70	87.5±0.64	2.30±0.28	4.60±0.34	7.60±0.45
TV- 17	52.5±0.40	62.5±0.51	87.5±0.80	3.90±0.26	4.20±0.28	7.30±0.50
TV-18	25.0±0.51	25.0±0.46	37.5±0.73	2.50±0.34	3.00±0.46	5.00±0.42
TV-19	0	37.5±0.55	87.5±0.92	0	3.10±0.24	6.70±0.46
TV-20	25.0±0.43	37.5±0.62	37.5±0.81	3.50±0.12	3.70±0.23	5.00±0.50
TV-22	0	25.0±0.44	50.0±0.66	0	1.30±0.11	4.70±0.20
TV-23	25.0±0.50	50.0±0.69	87.5±0.93	3.50±0.22	5.20±0.52	7.30±0.28
TV-25	0	0	22.0±0.60	0	0	1.50±0.22
TV-26	0	0	25.0±0.55	0	0	1.80±0.26
TV-27	66.6±0.61	83.3±0.82	88.0±1.22	2.10±0.20	2.80±0.38	6.00±0.52
TV-28	16.6±0.40	25.0±0.63	50.0±0.76	2.00±0.23	2.50±0.46	6.10±0.48
TV-29	33.3±0.30	69.0±0.61	87.5±0.95	2.70±0.46	2.90±0.48	6.40±0.52
TV-30	42.5±0.41	62.5±0.64	76.0±0.69	2.30±0.50	3.50±0.47	6.00±0.53
MG-1	16.6±0.51	50.0±0.81	66.6±1.15	1.50±0.42	3.40±0.40	8.30±0.64
Teenali 17/1/54	25.0±0.40	33.3±0.66	33.3±0.82	2.00±0.32	5.00±0.44	8.60±0.62
Sun-daram	0	0	25.5±0.60	0	0	3.30±0.46

[a] Mean of 3 replications.
[b] Mean of 50 lesions.
Data after ± represent standard error values.

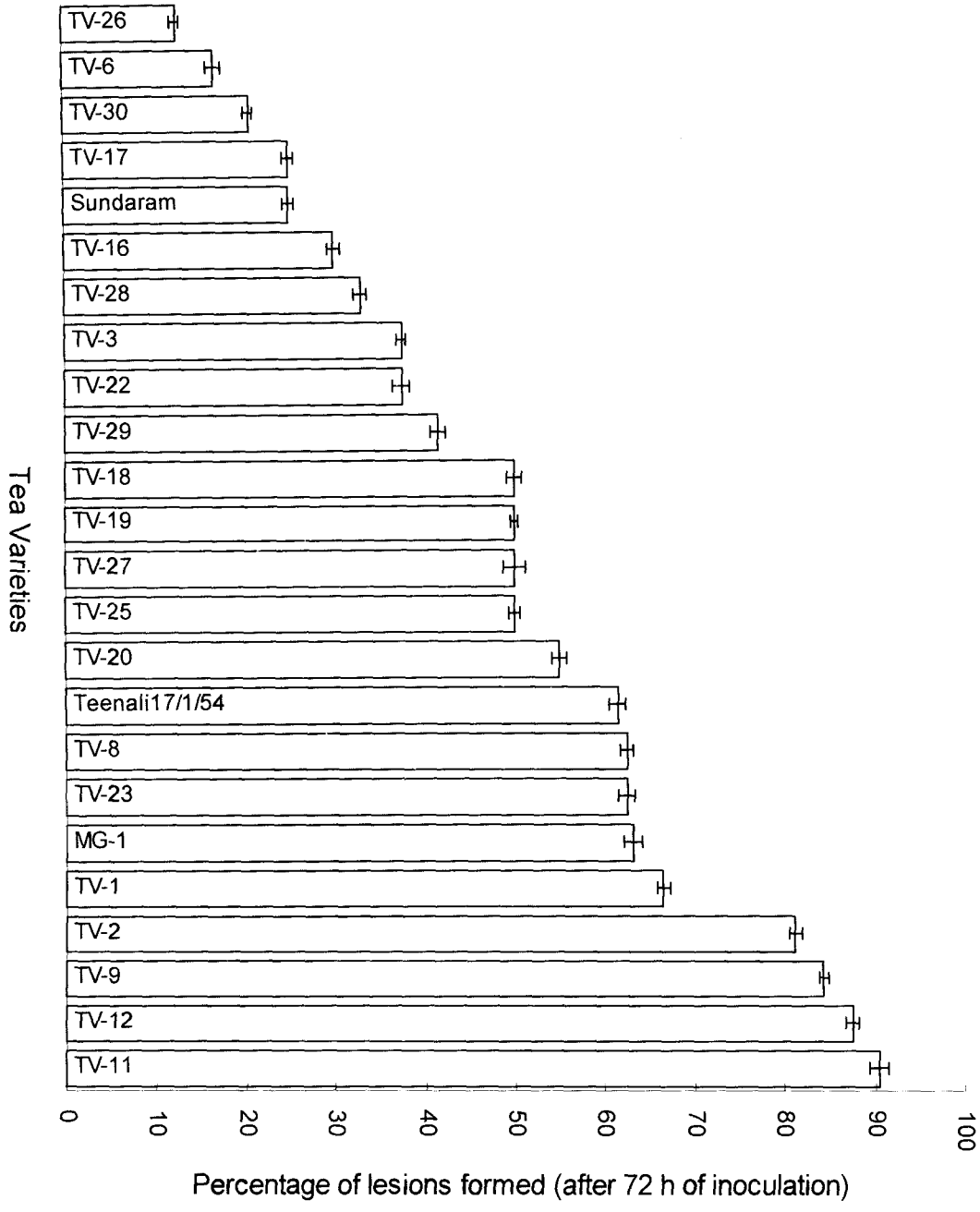


Fig.3: Pathogenicity of *B. theobromae* on detached leaves of different tea varieties

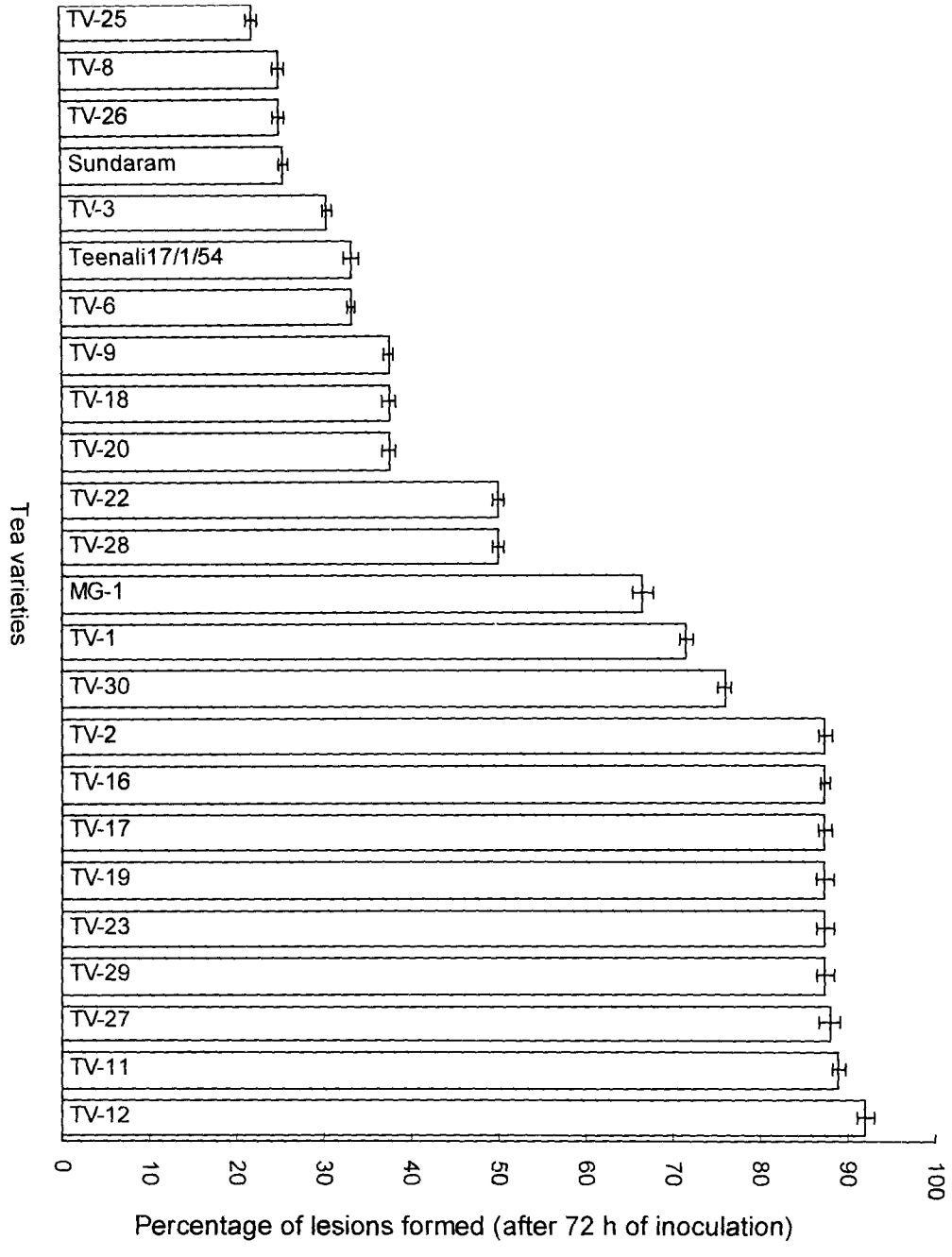


Fig.4: Pathogenicity of *C. eragrostidis* on detached leaves of different tea varieties

case of TV-11 against B. theobromae after 72 h of inoculation while in case of another susceptible variety TV-12, the value was 1.58. In resistant varieties (TV-26, TV-6 and Sundaram), the mean numbers of lesions/shoot values were 0.44, 0.46 and 0.52 respectively after 72 h of inoculation. In case of C. eragrostidis, the twigs [Table 7, Fig. 5 (B)] of TV-12 showed maximum disease occurrence among the varieties tested (mean disease index after 72 h = 1.71). Hence TV-12 was considered as the most susceptible variety. In case of TV-11, the mean disease index/shoot value was 1.63 after 72 h and it was considered as another susceptible variety against C. eragrostidis.

The mean disease index/shoot value was 0.27 in case of TV-25 which was considered as the most resistant variety. Other resistant varieties like TV-26, Sundaram, TV-8 and TV-3 showed the mean disease index/shoot values of 0.85, 0.88, 0.97 and 0.98 respectively after 72 h of inoculation. The tea varieties, which showed the mean disease index values between 1.00 and 1.40 after 72 h of inoculation, were considered as moderately resistant tea varieties against *C. eragrostidis*.

The mean number of lesions/shoot value was 2.56 in case of TV-12 against *C. eragrostidis* after 72 h of inoculation while in case of another susceptible variety TV-11, the value was 2.38. In resistant varieties (TV-25, TV-26, Sundaram, TV-8 and TV-3), the mean numbers of lesions/shoot values were 0.72, 0.81, 1.06, 1.15 and 1.18 respectively after 72 h of inoculation.

4.1.3. Pathogenicity test following nursery tea plant inoculation technique

The whole plant inoculation method as described by Dickens and Cook (1989) was applied to perform the pathogenicity test of nursery tea plants of 10 different clonal varieties (already mentioned above) against the two pathogens *B. theobromae* and *C. eragrostidis*. The methodologies are described in materials and methods (Section 3.6.3 & 3.7.3). The results of the experiments were calculated and computed following the procedure of Sinha and Das (1972) and summarized in Table 8 and Table 9 respectively for *B. theobromae* and *C. eragrostidis*.

Table 6
Pathogenicity test of *B. theobromae* on cut shoot of different tea varieties

Tea Varieties	Incubation periods (Hours)					
	24 h		48 h		72 h	
	Mean No. of lesions/ shoot*	Mean disease index/ shoot	Mean No. of lesions/ shoot	Mean disease index/ shoot	Mean No. of lesions/ shoot	Mean disease index/ shoot
TV-1	0.78±0.07	0.25±0.03	0.88±0.06	0.73±0.07	1.22±0.07	1.27±0.05
TV-2	0.76±0.03	0.23±0.03	0.84±0.06	0.69±0.06	1.34±0.08	1.31±0.08
TV-3	0.36±0.09	0.13±0.04	0.44±0.03	0.42±0.05	0.62±0.03	1.10±0.03
TV-6	0.28±0.05	0.13±0.02	0.34±0.04	0.42±0.07	0.46±0.03	0.81±0.07
TV-8	0.68±0.03	0.22±0.03	0.76±0.09	0.68±0.03	1.04±0.08	1.32±0.02
TV-9	0.88±0.08	0.36±0.05	0.96±0.05	0.83±0.05	1.44±0.08	1.42±0.03
TV-11	0.98±0.09	0.43±0.03	1.16±0.07	0.95±0.06	1.62±0.09	1.69±0.07
TV-12	0.92±0.08	0.41±0.04	1.12±0.08	0.96±0.05	1.58±0.06	1.63±0.08
TV-16	0.32±0.06	0.11±0.02	0.40±0.02	0.37±0.04	0.56±0.08	1.00±0.06
TV- 17	0.32±0.02	0.13±0.05	0.36±0.03	0.37±0.05	0.58±0.04	0.86±0.08
TV-18	0.75±0.04	0.16±0.02	0.82±0.06	0.52±0.06	0.98±0.07	1.38±0.09
TV-19	0.70±0.03	0.21±0.04	0.72±0.02	0.54±0.05	1.00±0.03	1.25±0.09
TV-20	0.72±0.04	0.14±0.06	0.80±0.04	0.46±0.06	1.02±0.03	1.28±0.07
TV-22	0.40±0.08	0.18±0.03	0.48±0.03	0.58±0.05	0.68±0.04	1.12±0.08
TV-23	0.68±0.01	0.19±0.02	0.78±0.04	0.61±0.07	1.12±0.02	1.21±0.07
TV-25	0.60±0.02	0.32±0.03	0.62±0.04	1.02±0.04	0.92±0.03	1.30±0.05
TV-26	0.25±0.08	0.11±0.07	0.32±0.05	0.33±0.09	0.44±0.07	0.67±0.06
TV-27	0.62±0.02	0.33±0.04	0.64±0.06	1.02±0.07	0.94±0.09	1.25±0.07
TV-28	0.36±0.09	0.12±0.05	0.42±0.02	0.38±0.07	0.58±0.04	1.05±0.08
TV-29	0.56±0.06	0.30±0.02	0.58±0.08	1.00±0.04	0.88±0.08	1.15±0.06
TV-30	0.58±0.07	0.24±0.03	0.62±0.09	0.72±0.04	0.86±0.09	1.12±0.04
Teenali 17/1/54	0.76±0.03	0.25±0.04	0.78±0.05	0.81±0.04	1.06±0.07	1.36±0.04
MG-1	0.82±0.05	0.31±0.03	0.98±0.07	1.06±0.06	1.18±0.09	1.25±0.07
Sun-daram	0.30±0.03	0.11±0.03	0.34±0.07	0.35±0.07	0.52±0.08	0.78±0.08

* Mean of 3 replications.
Data after ± represent standard error values.

Table 7

Pathogenicity test of *C. eragrostidis* on cut shoots of different tea varieties

Tea Varieties	Incubation periods (Hours)					
	24 h		48 h		72 h	
	Mean No. of lesions/ shoot*	Mean disease index/ shoot	Mean No. of lesions/ shoot*	Mean disease index/ shoot	Mean No. of lesions/ shoot*	Mean disease index/ shoot
TV-1	0.90±0.09	0.35±0.04	1.44±0.02	0.73±0.06	1.72±0.07	1.21±0.08
TV-2	1.08 ±0.06	0.39 ±0.05	1.66±0.04	0.81±0.08	2.16±0.06	1.41±0.08
TV-3	0.58 ±0.08	0.31 ±0.06	0.74±0.03	0.65±0.06	1.18±0.07	0.98±0.07
TV-6	0.56 ±0.07	0.29 ±0.06	0.80±0.09	0.67±0.07	1.34±0.09	1.11±0.09
TV-8	0.62 ±0.06	0.29 ±0.08	0.96±0.09	0.64±0.08	1.15±0.06	0.97±0.05
TV-9	0.86 ±0.03	0.38 ±0.04	1.15±0.06	0.75±0.07	1.38±0.07	1.19±0.05
TV-11	1.28 ±0.07	0.45 ±0.06	1.96±0.08	0.98±0.08	2.38±0.09	1.63±0.09
TV-12	1.36 ±0.02	0.46 ±0.06	2.18±0.05	1.14±0.07	2.56±0.06	1.71±0.05
TV-16	0.96 ±0.08	0.39 ±0.03	1.52±0.09	0.82±0.04	1.98±0.06	1.36±0.06
TV- 17	0.95 ±0.05	0.38 ±0.03	1.46±0.07	0.92±0.04	1.92±0.08	1.39±0.07
TV-18	0.84 ±0.04	0.36 ±0.02	1.20±0.06	0.79±0.03	1.54±0.07	1.21±0.06
TV-19	0.68 ±0.03	0.35 ±0.04	1.38±0.04	0.71±0.05	1.74±0.05	1.30±0.08
TV-20	0.72 ±0.03	0.37 ±0.06	1.04±0.05	0.86±0.09	1.41±0.06	1.13±0.05
TV-22	0.76 ±0.06	0.36 ±0.05	1.12±0.06	0.75±0.08	1.43±0.08	1.15±0.07
TV-23	0.75 ±0.05	0.36 ±0.02	1.28±0.07	0.75±0.03	1.62±0.09	1.33±0.05
TV-25	0.46 ±0.06	0.21 ±0.05	0.58±0.09	0.23±0.07	0.72±0.09	0.27±0.09
TV-26	0.52 ±0.03	0.24±0.02	0.66±0.06	0.53±0.03	0.81±0.07	0.85±0.06
TV-27	1.16 ±0.03	0.44±0.03	1.72±0.05	0.87±0.05	2.21±0.06	1.38±0.08
TV-28	0.70 ±0.04	0.32±0.05	1.21±0.05	0.78±0.07	1.52±0.07	1.13±0.07
TV-29	1.06 ±0.08	0.43±0.04	1.62±0.09	0.91±0.07	2.05±0.09	1.39±0.08
TV-30	0.94 ±0.05	0.32±0.03	1.48±0.04	0.73±0.03	1.82±0.05	1.27±0.05
Teenali 17/1/54	0.78 ±0.06	0.33±0.03	1.05±0.03	0.69±0.04	1.34±0.05	1.14±0.06
MG-1	0.82 ±0.08	0.28±0.04	1.32±0.05	0.81±0.05	1.66±0.06	1.15±0.07
Sun-daram	0.62 ±0.04	0.28±0.06	0.88±0.06	0.61±0.08	1.06±0.07	0.88±0.09

*Mean of 3 replications.

Data after ± represent standard error values.

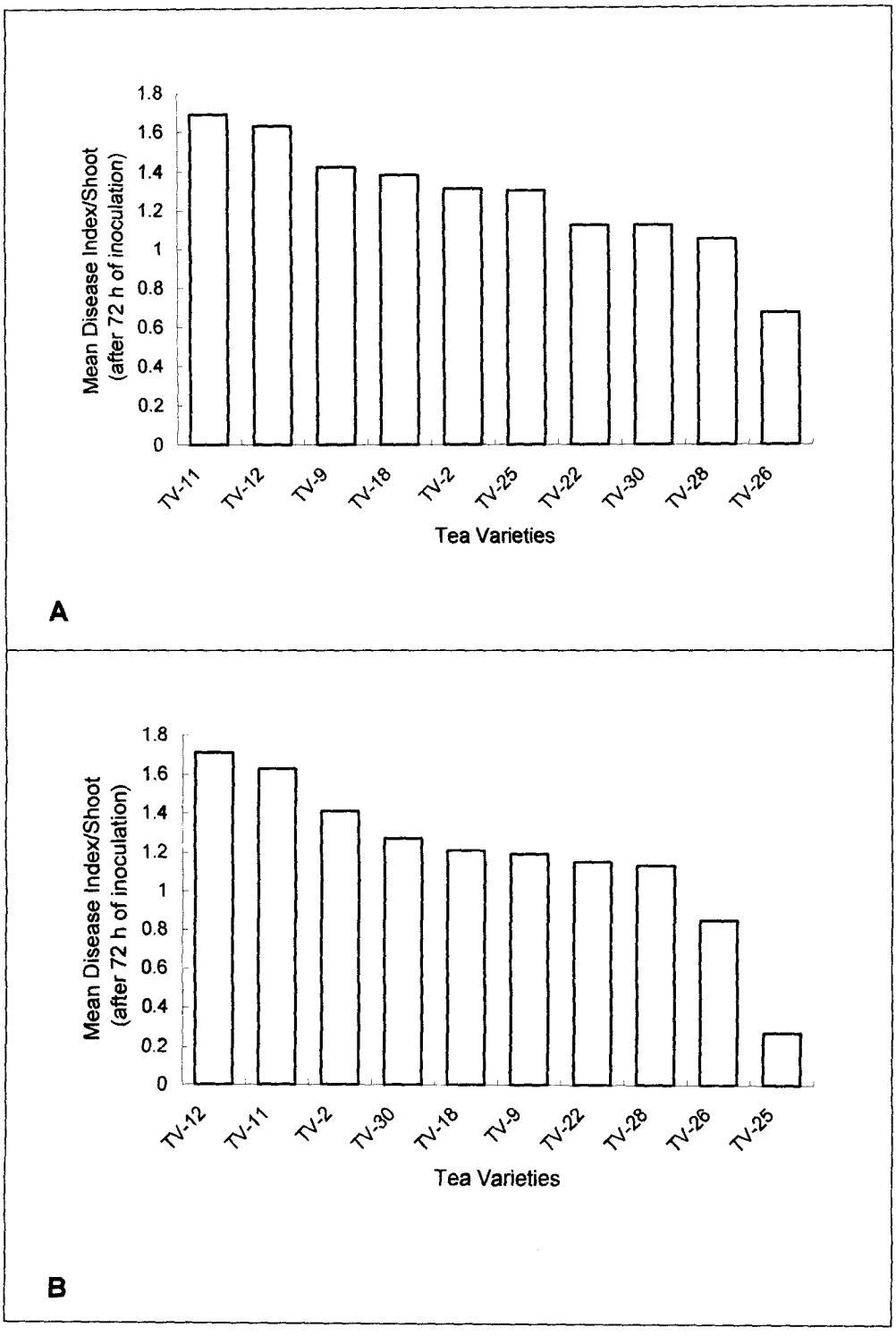


Fig. 5 (A & B): Pathogenicity of *B. theobromae* (A) and *C. eragrostidis* (B) on cut shoots of different tea varieties

From the data represented in the Table 8 and from Fig. 6, it was quite clear that the nursery tea plants of TV-11 showed maximum disease development (mean disease index/plant was 13.02) after 16 d of inoculation. Therefore, tea plants of TV-11 could be considered as the most susceptible variety against *B.theobromae* among the varieties tested. The nursery tea plants belonging to varieties like TV-12, TV-9 and TV-2 were also considered as susceptible tea varieties as the disease occurrence in these varieties were similar to TV-11 and they showed the mean disease index/plant values of 11.10, 10.80 and 9.91 respectively after 16 d of inoculation. On the other hand, TV-26 was the most resistant one as it produced minimum value of mean disease index/plant (3.71) among all the varieties tested after 16 d of inoculation.

The lesion production was maximum (mean number of lesions/plant is 13.50) in case of the most susceptible variety TV-11 against *B.theobromae* after 16 d of inoculation. In other susceptible varieties the mean number of lesions/plant were 13.10 (TV-12), 12.50 (TV-9) and 11.23 (TV-2). The most resistant variety TV-26 showed the minimum disease occurrence as the mean number of lesions/plant was 4.73 after 16 d of inoculation.

The mean disease index/plant values of nursery tea plants belonging to TV-12, TV-11, TV-2 and TV-30 were 9.56, 9.21, 8.90 and 8.35 after 16 d of inoculation with *C. eragrostidis* as evident from Table 9 and Fig.7 and they were considered as susceptible varieties against *C. eragrostidis*. TV-22, TV-18 and TV-28 were moderately resistant as the mean disease index/plant values were 7.01, 6.81 and 6.58 respectively. The remaining three varieties TV-25, TV-26 and TV-9 were the resistant as the mean disease index/plant values were 3.57, 5.11 and 5.25 respectively after 16 d of inoculation.

From the results it was evident that among the nursery tea plants of ten different clonal varieties tested against *C. eragrostidis*, TV-25 was the most resistant variety. The lesion production was maximum (mean number of lesions/ plant =14.80) in case of TV-12, the most susceptible variety against *C. eragrostidis* after 16 d of inoculation. In other susceptible varieties like TV-11, TV-2 and TV-30 the mean number of lesions/plant were 13.25, 11.50 and 8.60 respectively. The most resistant variety, TV-25, showed minimum disease occurrence as the mean number of lesions/plant was 4.08 after 16 d of inoculation.

Table 8
Pathogenicity test of *B. theobromae* on nursery tea plants of ten different tea varieties.

Tea Varieties	Incubation periods							
	4 days		8 days		12 days		16 days	
	Mean disease index/ plant[a]	Mean No.of lesions/ plant[b]	Mean disease index/ plant	Mean No. of lesions/ plant	Mean disease index/ plant	Mean No. of lesions/ plant	Mean disease index/ plant	Mean No. of lesions/ plant
TV-2	0.69 ±0.04	1.60 ±0.22	3.72 ±0.36	4.81 ±0.20	7.34 ±0.17	8.61 ±0.06	10.8 ±0.15	11.23 ±0.29
TV-9	0.85 ±0.03	3.04 ±0.14	3.91 ±0.21	5.23 ±0.17	7.01 ±0.30	9.92 ±0.58	11.10 ±0.70	12.50 ±0.29
TV-11	1.58 ±0.04	3.60 ±0.09	5.44 ±0.39	5.10 ±0.25	9.70 ±0.30	8.88 ±0.48	13.02 ±0.21	13.50 ±0.40
TV-12	0.66 ±0.03	3.00 ±0.05	3.59 ±0.35	6.20 ±0.15	6.64 ±0.32	9.25 ±0.80	9.82 ±0.17	13.10 ±0.31
TV-18	0.88 ±0.04	1.32 ±0.09	2.34 ±0.14	2.95 ±0.19	3.10 ±0.31	4.88 ±0.16	5.25 ±0.14	6.50 ±0.23
TV-22	1.00 ±0.06	2.80 ±0.07	2.12 ±0.20	3.24 ±0.25	4.44 ±0.09	4.24 ±0.18	6.52 ±0.31	6.91 ±0.09
TV-25	0.76 ±0.03	1.12 ±0.05	2.08 ±0.21	1.71 ±0.20	4.41 ±0.44	3.51 ±0.29	6.01 ±0.29	5.20 ±0.25
TV-26	0.45 ±0.06	1.00 ±0.05	1.32 ±0.12	1.59 ±0.09	2.50 ±0.24	2.91 ±0.05	3.71 ±0.42	4.73 ±0.44
TV-28	1.28 ±0.05	2.40 ±0.11	3.28 ±0.15	3.90 ±0.35	5.20 ±0.43	5.62 ±0.36	6.81 ±0.44	7.10 ±0.06
TV-30	0.48 ±0.04	1.44 ±0.12	1.48 ±0.07	2.06 ±0.27	3.08 ±0.25	4.21 ±0.12	5.21 ±0.19	6.10 ±0.13
CD at 5%	0.25	0.63	1.57	1.58	2.16	2.42	2.32	1.68
CD at 1%	0.22	0.56	1.39	1.40	1.91	2.14	2.06	1.49

[a] Mean of 3 replications.
[b] Average of 50 lesions.
Data after ± represent standard error values.

Table 9
Pathogenicity test of *C. eragrostidis* on nursery tea plants of ten different tea varieties.

Tea Varieties	Incubation periods							
	4 days		8 days		12 days		16 days	
	Mean disease index/ plant[a]	Mean No.of lesions/ plant[b]	Mean disease index/ plant	Mean No. of lesions/ plant	Mean disease index/ plant	Mean No. of lesions/ plant	Mean disease index/ plant	Mean No. of lesions/ plant
TV-2	1.48 ±0.72	3.36 ±1.39	3.16 ±1.86	5.21 ±1.43	4.62 ±1.97	8.61 ±1.66	8.90 ±2.16	11.50 ±1.86
TV-9	1.38 ±0.75	2.90 ±0.92	2.91 ±1.28	4.80 ±1.10	3.50 ±1.36	5.62 ±1.37	5.25 ±1.51	7.63 ±1.81
TV-11	1.52 ±0.35	3.44 ±1.58	3.20 ±1.48	5.52 ±1.62	4.76 ±2.59	9.80 ±1.81	9.21 ±2.61	13.25 ±2.10
TV-12	1.56 ±0.70	3.32 ±1.26	2.92 ±1.26	6.80 ±1.40	4.89 ±2.37	10.10 ±1.53	9.56 ±2.57	14.80 ±1.78
TV-18	1.44 ±0.36	3.04 ±1.09	3.08 ±1.44	5.10 ±1.29	4.60 ±1.54	6.25 ±1.42	6.81 ±1.82	7.80 ±1.57
TV-22	1.32 ±0.68	2.56 ±0.62	2.76 ±1.12	3.89 ±0.99	4.56 ±1.23	5.90 ±1.30	7.01 ±1.48	7.83 ±1.58
TV-25	0.84 ±0.22	1.50 ±0.85	1.23 ±1.09	1.84 ±1.09	2.30 ±1.29	2.88 ±1.30	3.57 ±1.36	4.08 ±1.62
TV-26	0.95 ±0.36	2.40 ±0.72	2.12 ±0.89	3.85 ±1.12	3.40 ±0.94	4.59 ±1.44	5.11 ±1.18	5.88 ±1.79
TV-28	1.30 ±0.62	2.80 ±1.16	3.12 ±0.78	5.40 ±1.36	4.52 ±0.81	6.08 ±1.69	6.58 ±1.15	8.20 ±2.01
TV-30	1.28 ±0.32	3.12 ±1.21	1.81 ±1.41	4.58 ±1.45	4.60 ±1.52	6.21 ±1.71	8.35 ±1.71	8.60 ±2.15
CD at 5%	1.57	1.32	1.17	1.14	0.79	0.56	0.65	1.54
CD at 1%	1.39	1.17	1.03	1.02	0.70	0.49	0.57	1.36

[a] Mean of 3 replications.
[b] Average of 50 lesions.
Data after ± represent standard error values.

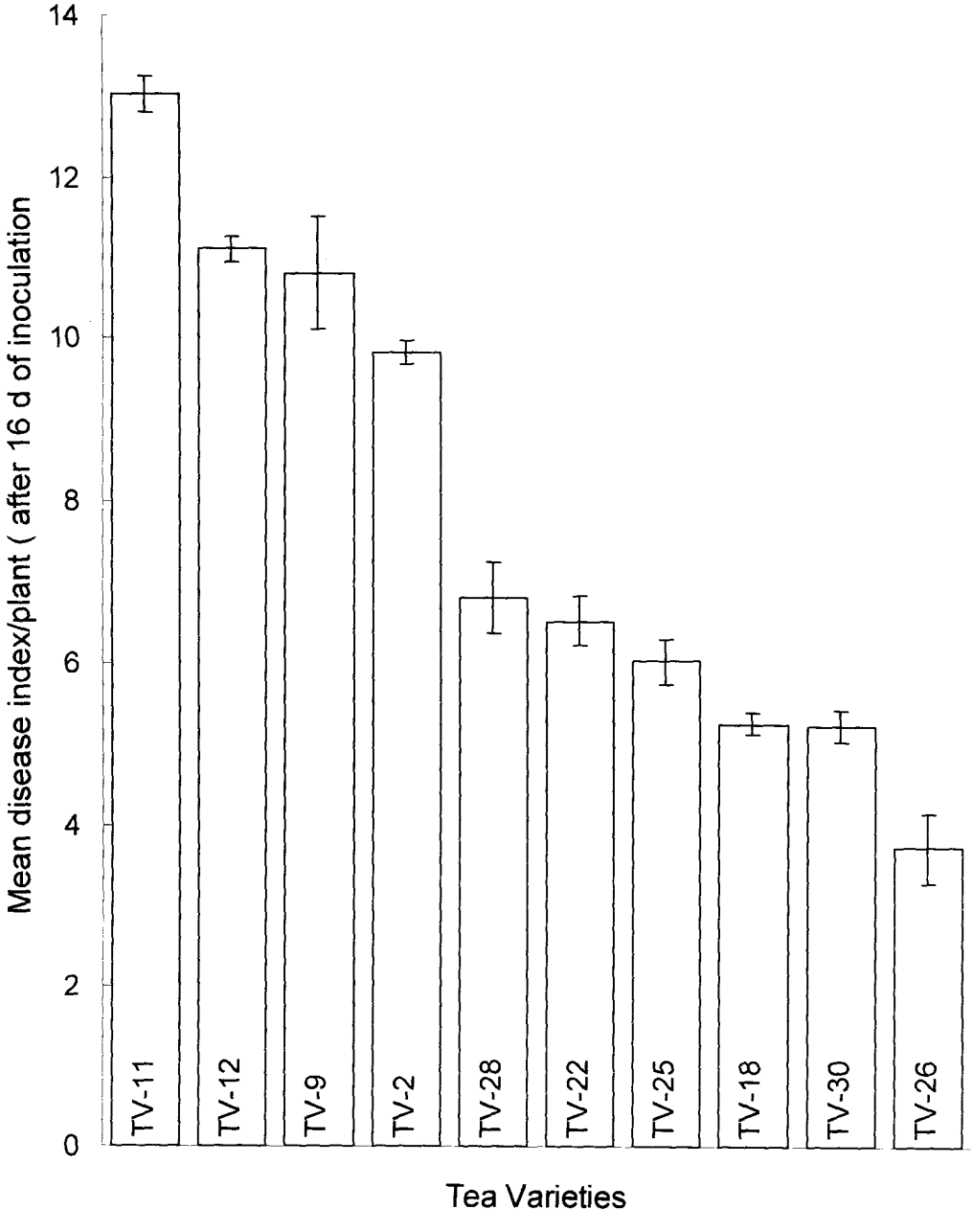


Fig.6: Pathogenicity of *B. theobromae* on nursery tea plants of different varieties

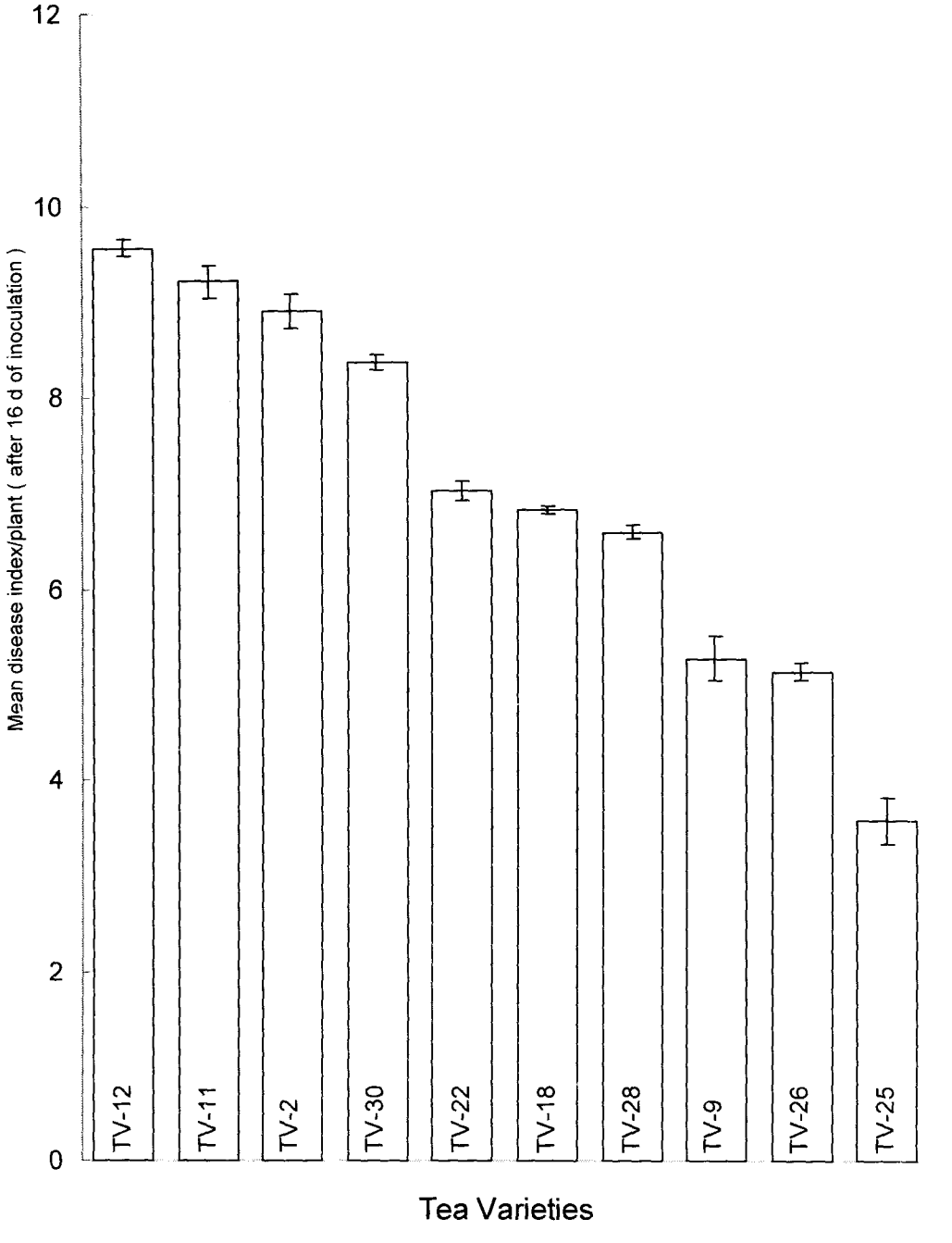


Fig.7: Pathogenicity of *C. eragrostidis* on nursery tea plants of different varieties

4.2. Observation on morphology of *B. theobromae* and *C. eragrostidis*

The morphology of *B. theobromae* and *C. eragrostidis*, the two isolated pathogens were observed in PDA (slants and plates) and In PDB (Ehrlenmayer flask). When cultured in PDA or PDB, the mycelia of *B. theobromae* was white in colour which gradually turned brown and further darker to deep brown (Plate VI : 1, 3, 4 & 5). When *C. eragrostidis* was cultured in PDA, the mycelia was white which gradually turned gray and finally turned to black (Plate VII : 1, 2 & 3). From the pure cultures of the two isolated pathogens *B. theobromae* and *C. eragrostidis*, the mycelia were taken and the slides were stained using cotton blue-lactophenol. The slides were mounted with cover glass, sealed and observed under microscope.

Mycelium and conidiophores of *B. theobromae* were dark coloured. Two to three conidia were found associated with conidiophore. The length and breadth of the mature conidia were 10-15 µm and 8-12 µm respectively. The mature conidia were dark, two-celled and hyphae were septate, the diameter of the mature hyphae was between 4-7 µm (Fig. 8; Plate VI : 2, 6).

In case of *C. eragrostidis*, mycelium and conidiophores were gray, conidia were black in colour. Two to three conidia were found associated with conidiophores. Diameter of the conidiophores was 6-8 µm. The length and breadth of the mature conidia were 20-27 µm and 10-15 µm respectively. Conidia were four-celled, hyphae were septate, hyaline and diameter ranged from 1-4 µm. Branching of hyphae was at different angles. Conidia when allowed for germination on glass slides showed germination of spores from one side only. In no case germination was found from two poles (Fig.9; Plate VII : 4, 5).

4.3. Culture conditions affecting growth and sporulation of *B. theobromae* and *C. eragrostidis*

4.3.1. Mycelial growth of *B. theobromae* and *C. eragrostidis* in different solid media

In order to assess the vegetative growth of *B. theobromae* and *C. eragrostidis* in solid media, six different media viz. PDA, MEA, CDA, OMA, PCA and RA were used. The radial growth of the two fungi were measured and noted in Table 10 and Table 11.

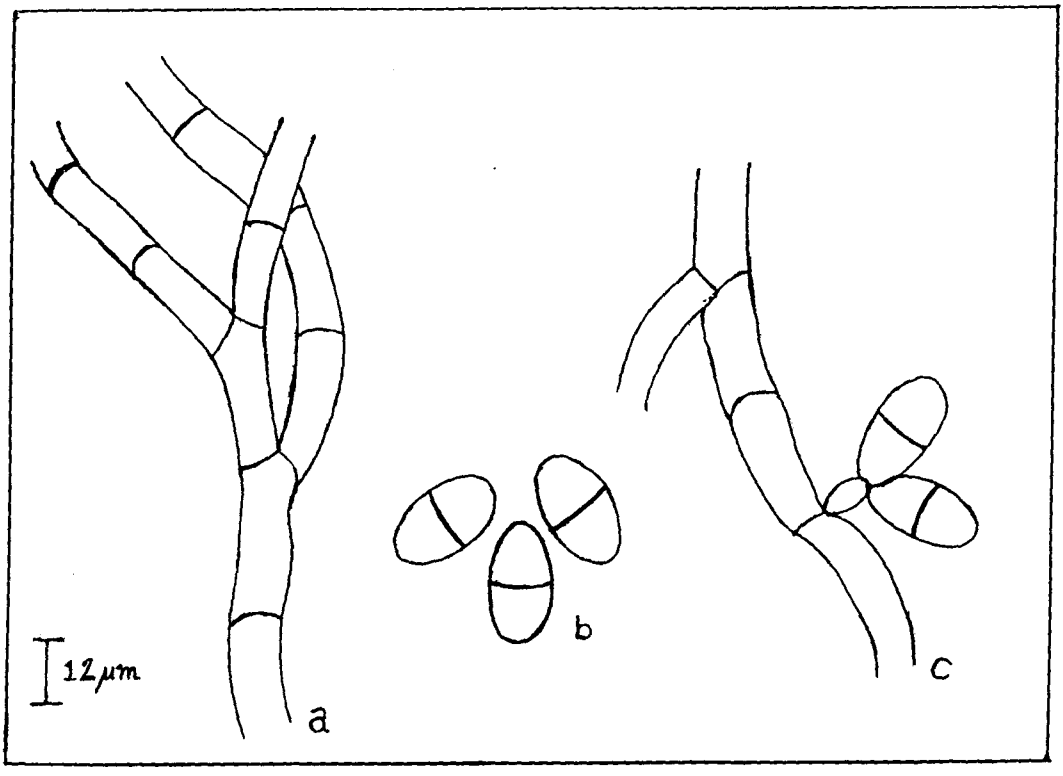


Fig. 8 (a-c): *Botryodiplodia theobromae*
a, Hypha with septa and branching; **b,** Conidia;
c, Conidia with conidiophore attached with the hyphae

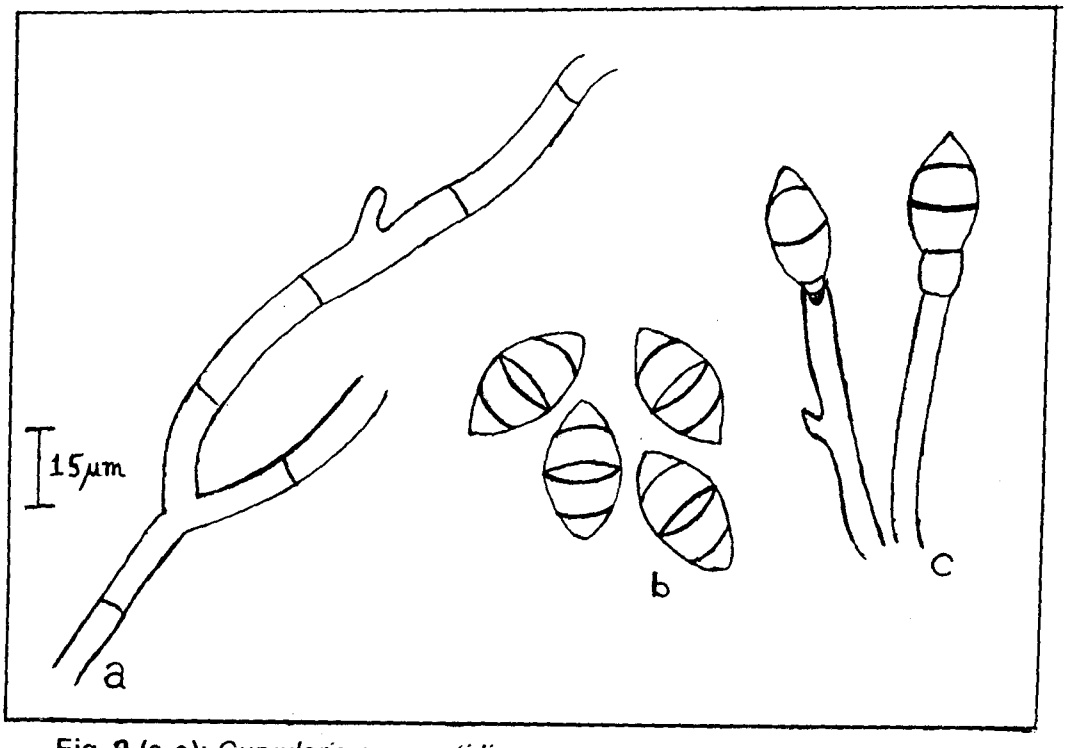
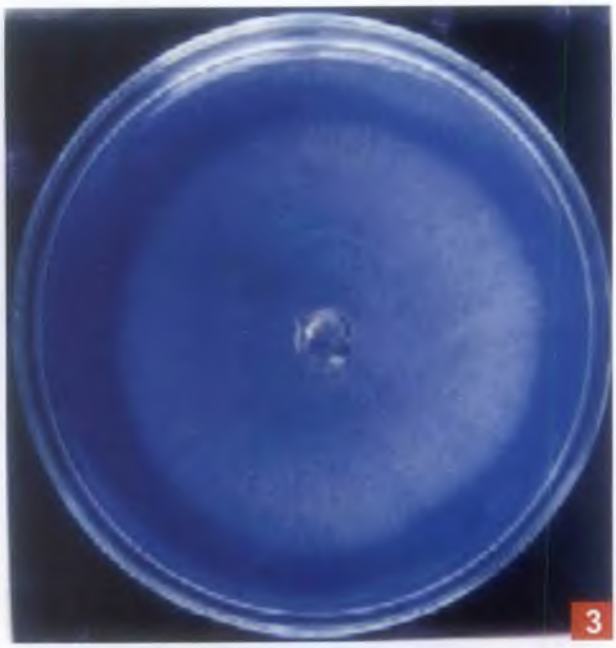
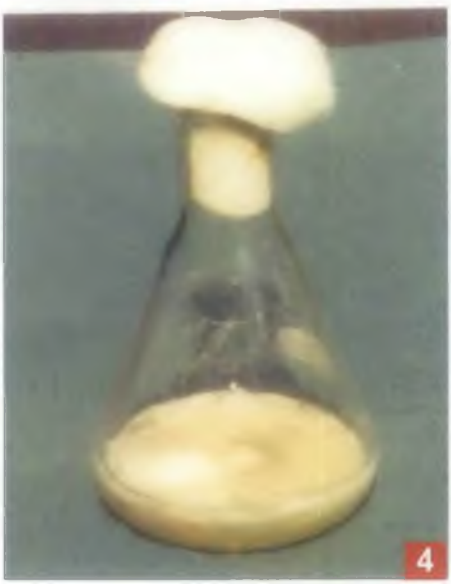


Fig. 9 (a-c): *Curvularia eragrostidis*
a, Hypha with septa and branching; **b,** Conidia;
c, Conidia with conidiophore attached with the hyphae





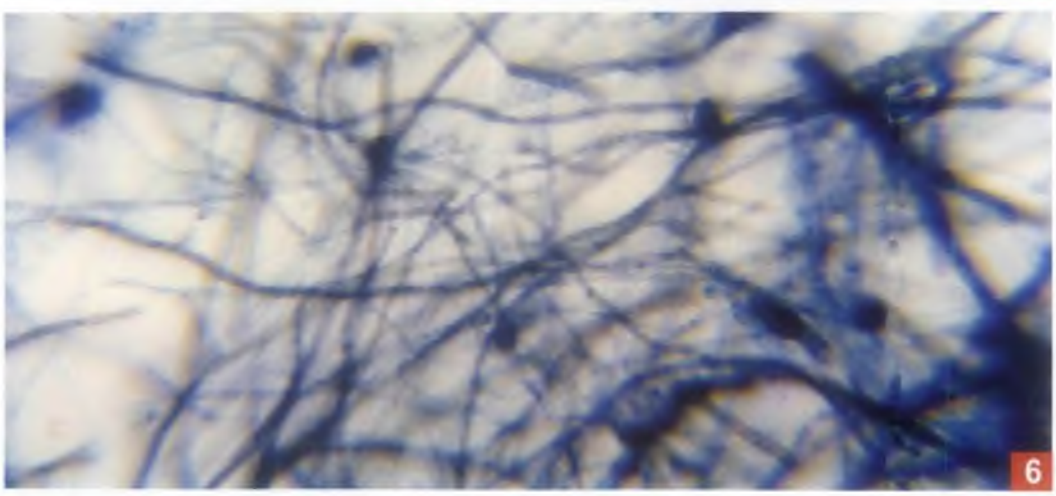
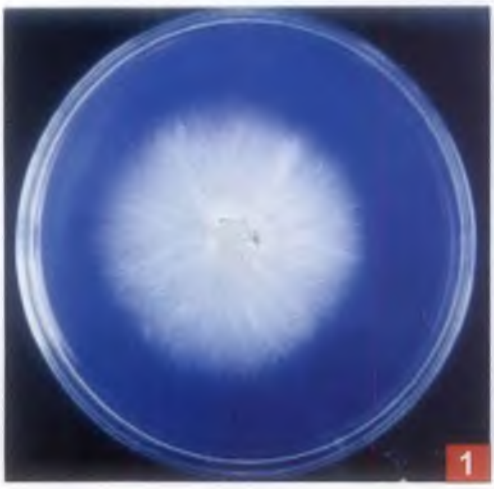
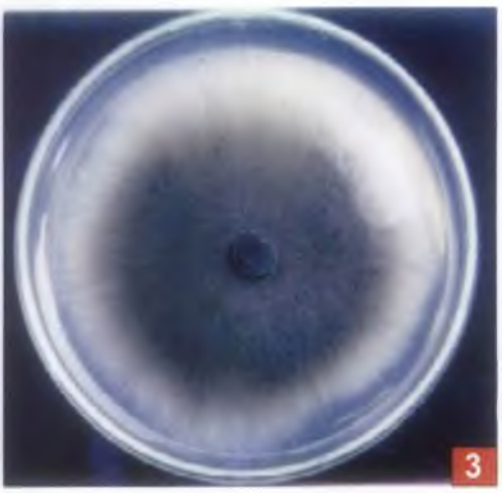


Plate VI

Fig. 1 : Culture of *Botryodiplodia* theobromae in PDA slant. **Fig. 2 :** Spores of *B. theobromae*.
Fig. 3 : Culture of *B. theobromae* in Petridish. **Fig. 4 :** Culture of *B. theobromae* in liquid medium (PDB).
Fig.5 : Sporulated culture of *B. theobromae*. **Fig. 6 :** Spores of *B. theobromae* intermingled with hyphae.





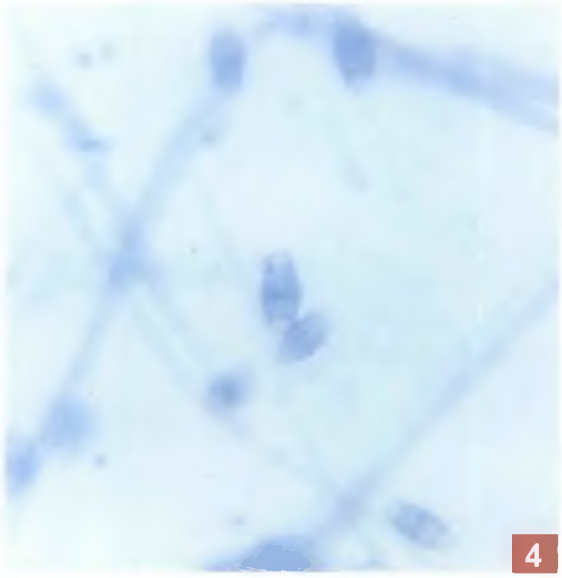


Plate VII

Fig. 1 : Culture of *Curvularia eragrostidis* in Petridish.
Fig. 2 : Culture of *C. eragrostidis* in PDA slant.
Fig 3 : Sporulated culture of *C. eragrostidis.*
Fig. 4 & 5 : Spores of *C. eragrostidis.*

From Table 10 and Fig.10 (A), it was evident that *B. theobromae* can grow in all the media tested but among the six media, malt extract agar was the best growth medium for *B. theobromae* because it showed 90 mm radial growth (maximum measurable growth in 90 mm petridish) after 2 d of inoculation. However, *C. eragrostidis* recorded much slower growth and it required 8 d to reach 89 mm radial growth in PCA. A similar pattern of growth was recorded in all the other media tested [Table 11 & Fig.10 (B)].

Table 10
Mycelial growth of *B. theobromae* in different solid media

Medium of growth	Radial growth (mm)*		
	Day 1	Day 2	Day 3
PDA	38±0.26	87±0.66	90±0.00
MEA	39±0.32	90±0.00	90±0.00
CDA	38±0.57	86±0.26	90±0.00
OMA	31±0.55	63±0.15	90±0.00
PCA	29±0.21	58±0.26	88±0.20
RA	36±0.66	87±0.10	90±0.00
CD at 5%	3.59	2.70	0.66
CD at 1%	3.13	2.35	0.57

*Mean of 3 replications.
Data after ± represent standard error values.

Table 11
Mycelial growth of *C. eragrostidis* in different solid media

Medium of growth	Radial growth (mm)*			
	Day 2	Day 4	Day 6	Day 8
PDA	28±0.26	44±0.49	74±0.86	81±0.58
MEA	32±0.10	45±0.53	73±0.15	86±0.61
CDA	35±0.30	48±0.40	65±1.15	80±0.47
OMA	32±0.45	44±0.43	67±0.90	87±1.05
PCA	27±0.15	51±0.15	66±0.20	89±0.00
RA	21±0.20	40±0.85	63±0.10	77±0.20
CD at 5%	2.12	4.11	5.78	4.72
CD at 1%	1.85	3.59	5.04	4.12

*Mean of 3 replications.
Data after ± represent standard error values.

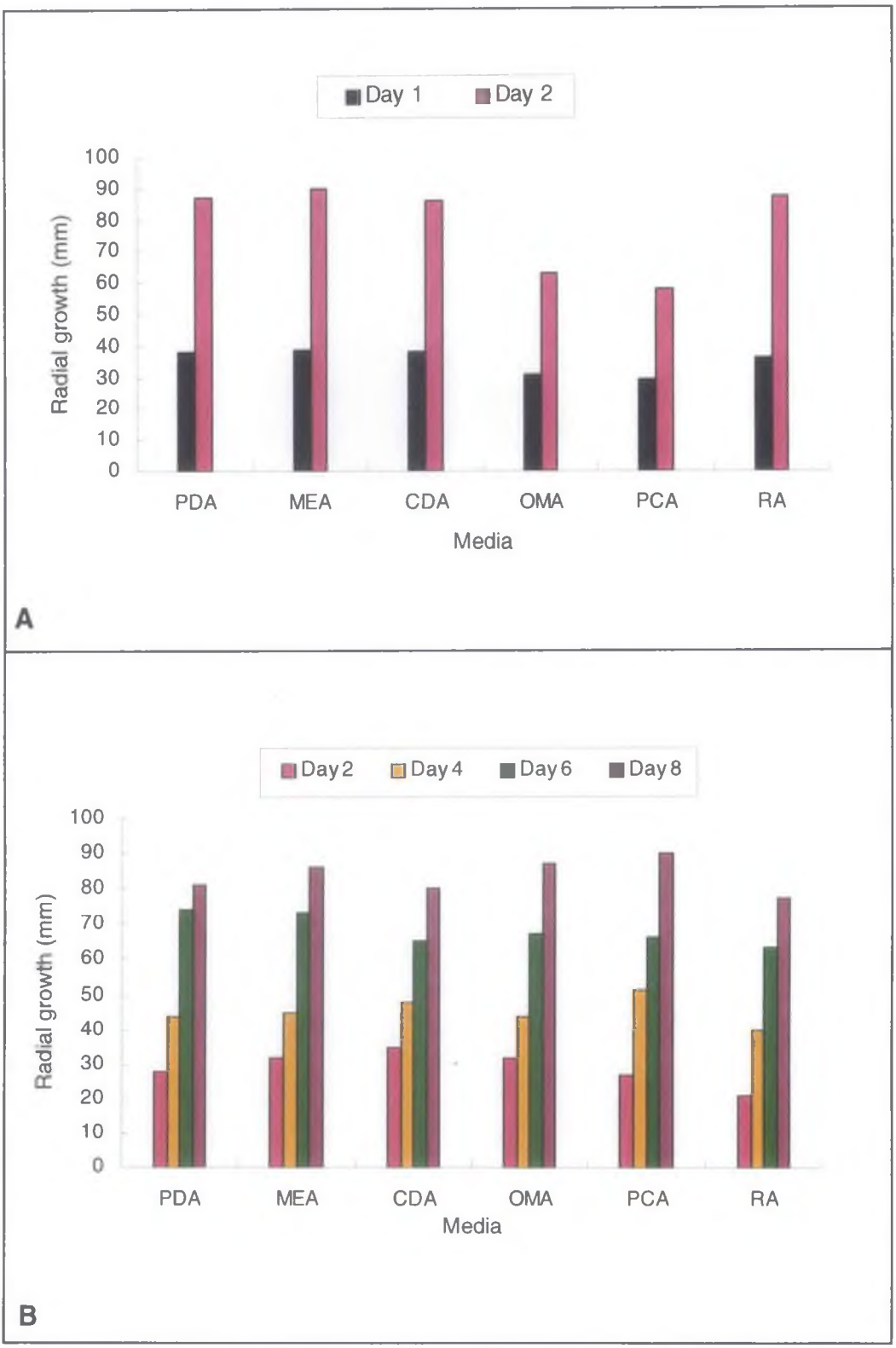


Fig. 10 (A & B) : Mycelial growth of *B. theobromae* (A) and *C. eragrostidis* (B) in different solid media.

[PDA= Potato Dextrose Agar; MEA= Malt Extract Agar; CDA= Czapek Dox Agar; PCA= Potato Carrot Agar; OMA= Oat Meal Agar; RA= Richard's Agar]

4.3.2. Mycelial growth of *B. theobromae* and *C. eragrostidis* after different periods of incubation

Growth of *B. theobromae* and *C. eragrostidis* was assessed after different periods of incubation at 28±2 °C. The test fungi were inoculated in sterile PDB according to the method described in section 3.8.1. After 5, 10, 15, 20 and 25 d of incubation, the fungal mycelia were harvested by strained through cheese cloth, blotted and dried at 60 °C. Finally the dried mycelia were cooled and mycelial dry weights were taken and noted in Table 12 and Table 13.

B. theobromae showed maximum growth (210.0 mg) after 15 d of incubation. From the Table 12 and Fig.11 (A), it was evident that maximum increase in growth took place within 5 d. The rate of growth decreased after 5 d and continued up to 15 d. After 15 d, the mycelial weight declined. In case of *C. eragrostidis*, similar result was obtained. It recorded maximum growth (193.0 mg) after 15 d of incubation and maximum increase in growth took place within 5 d [Table 13 & Fig.11 (B)]. The rate of growth decreased after 5 d and after 15 d mycelial dry weight declined.

Table 12
Effect of different incubation periods on the growth of *B. theobromae*

Medium of growth	Incubation periods (days)	Mycelial dry weight (mg)*
PDB	5	140.0±0.61
	10	185.0±0.57
	15	210.0±0.62
	20	185.0±0.35
	25	148.0±0.40
CD at 5%		2.89
CD at 1%		2.49

*Mean of 3 replications.
Data after ± represent standard error values.

Table 13
Effect of different incubation periods on the growth of *C. eragrostidis*

Medium of growth	Incubation periods (days)	Mycelial dry weight (mg)*
PDB	5	155.6±0.26
	10	176.0±0.20
	15	193.0±0.82
	20	171.8±0.25
	25	140.0±0.71
CD at 5%		3.91
CD at 1%		3.37

*Mean of 3 replications.
Data after ± represent standard error values.

4.3.3. Mycelial growth of *B. theobromae* and *C. eragrostidis* at different pH

Sterilized PDB medium (50 ml in 250 ml Ehrlenmayer flask) with pH 5.0, pH 5.5, pH 6.0 and pH 6.5 were prepared and the two test fungi (*B. theobromae* and *C. eragrostidis*) were inoculated separately according to the method described in materials and methods (Section 3.8.1) and incubated at 28±2 ^{0}C. The pH of the medium was adjusted adding 1 (N) NaOH or 1 (N) HCl drop-wise into the medium before sterilization. The values of mycelial dry weights after 5, 10, 15, 20 and 25 d of incubation were noted in Table 14 and Table 15.

From the Table 14 and Fig.12 (A), it was evident that the mycelial dry weight of *B. theobromae* was maximum (246.0 mg) at pH 6.0 and after 15 d of incubation and lowest (113.0 mg) at pH 5.0 after 5 d of incubation. When different pH were considered, it was found that after 15 d of incubation the mycelial dry weights were maximum in all cases except pH 5.0. At pH 5.0 the maximum mycelial dry weight was recorded after 20 d of incubation. In case of C. eragrostidis [Table 15 & Fig.12 (B)], maximum mycelial dry weight (235.0 mg) was recorded at pH 6.0 after 15 d of incubation and lowest (150.0 mg) at pH 5.5 after 5 d of incubation. When different pH were considered, mycelial dry weights were maximum after 15 d in all the cases.

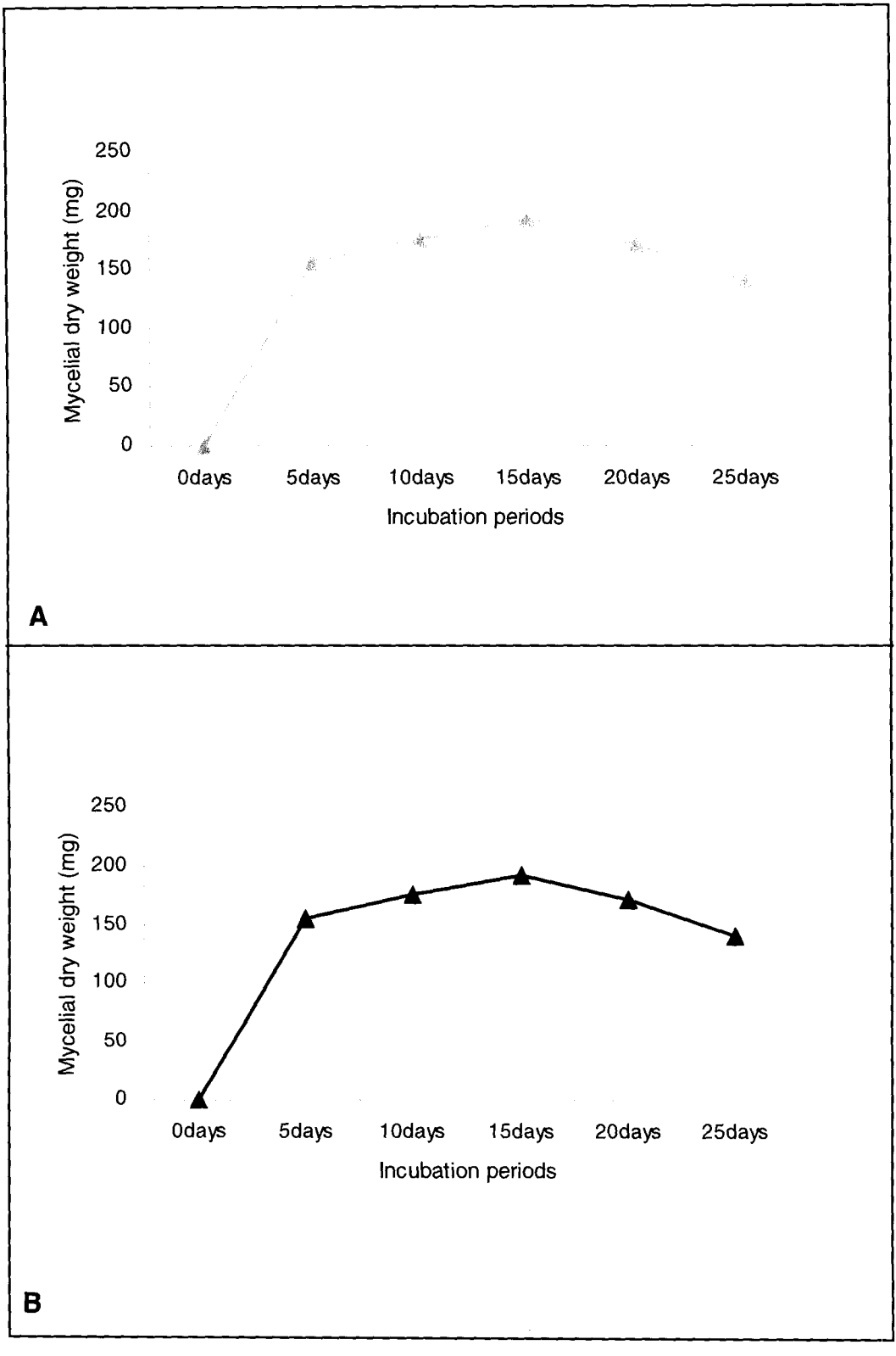


Fig.11 (A & B) : Growth of *B. theobromae* (A) and *C. eragrostidis* (B) in potato dextrose broth (PDB) medium in different days of incubation

Table 14
Effect of different pH on the growth of *B. theobromae*

Medium of growth	pH	Mycelial dry weight (mg)*				
		5 days	10 days	15 days	20 days	25 days
PDB	5.0	113±0.26	157±0.42	188±0.45	200±0.87	180±0.80
	5.5	140±0.30	184±0.53	215±0.20	198±0.92	173±1.05
	6.0	182±0.29	210±0.26	246±0.15	225±0.38	205±0.38
	6.5	179±1.05	208±0.36	238±0.68	214±0.25	192±0.50
CD at 5%		5.41	2.71	3.29	3.37	8.93
CD at 1%		4.59	2.30	2.79	2.86	7.58

Mean of 3 replications.
Data after ± represent standard error values.

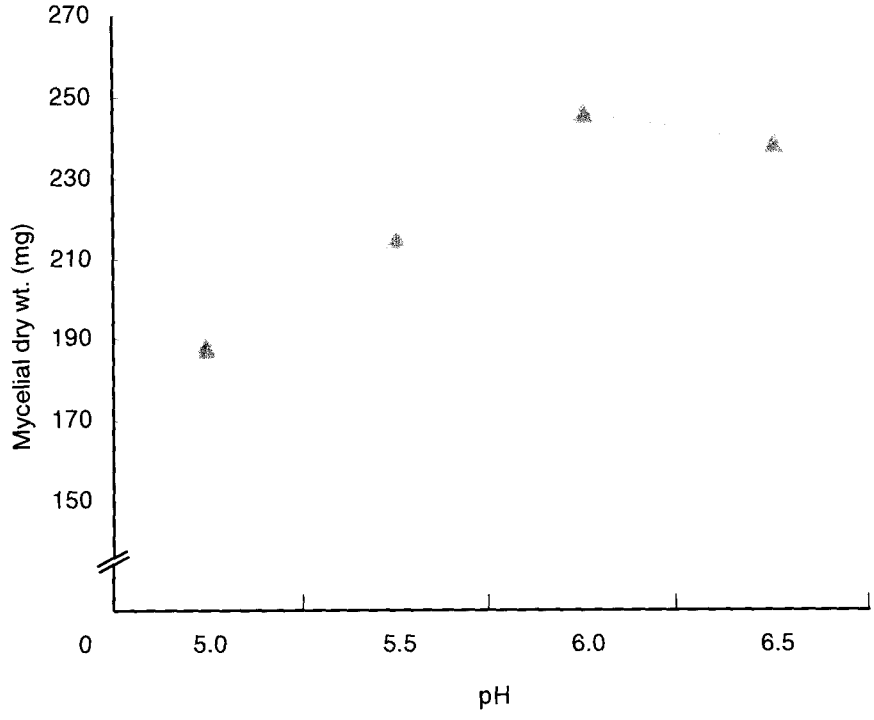
Table 15
Effect of different pH on the growth of *C. eragrostidis*

Medium of growth	pH	Mycelial dry weight (mg)*				
		5 days	10 days	15 days	20 days	25 days
PDB	5.0	156±0.53	160±0.85	195±0.32	172±0.26	140±0.71
	5.5	150±0.64	180±0.80	205±0.60	190±0.58	175±0.72
	6.0	154±0.20	210±0.40	235±0.30	220±0.26	199±0.58
	6.5	160±1.11	183±0.11	213±0.56	198±0.26	185±0.60
CD at 5%		6.44	5.44	4.18	3.20	5.69
CD at 1%		5.46	4.62	3.55	2.71	4.82

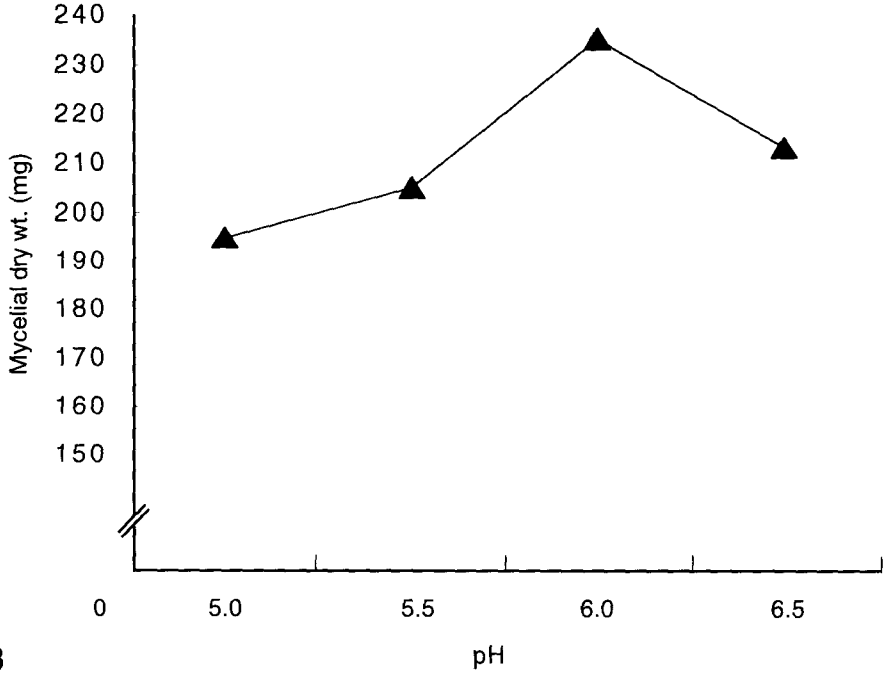
*Mean of 3 replications.
Data after ± represent standard error values.

4.3.4. Assessment of mycelial growth of *B. theobromae* and *C. eragrostidis* on different carbon sources

The nutritional requirements of a pathogen are of considerable interest as it may help in understanding the physiology of disease development. As no published report is yet available on the nutritional physiology of the two tea pathogens (*B. theobromae* and *C. eragrostidis*), it was considered worthwhile to study different carbon sources for the optimum growth and sporulation of *B. theobromae* and *C. eragrostidis*. A basal medium (Glucose 1%; Asparagine 0.2%; KH_2PO_4 0.1%; $MgSO_4$, $7H_2O$ 0.05%; Zn^{++}, Mn^{++} and Fe^{++} 2 µg / ml) was used for the purpose. The different carbon sources tested were glucose, sucrose, mannitol and galactose. The equivalent amount of carbon present in 1% glucose



A



B

Fig. 12 (A & B) : Growth of *B. theobromae* (A) and *C. eragrostidis* (B) in potato dextrose broth (PDB) in different pH after 15 days of incubation

was used as standard and added separately to the basal medium. The medium was simultaneously sterilized by autoclaving at 15 lb.p.s.i. for 15 minutes and after cooling, the two pathogens were inoculated and incubation was allowed for 5, 10, 15, 20 and 25 days. For this study, Ehrlenmayer flasks of 250 ml capacity were used where each flask contained 50 ml of sterilized media. In control sets, no carbon sources were used in the basal medium. After incubation for the specified time periods, the mycelia were harvested, dried at 60 ^{0}C and weighed. The results were tabulated in Table 16 and Table 17. After each incubation period, the sporulations were also recorded in five different grades on the basis of visual observations.

From the results [Table 16 and Fig. 13(A)], it was evident that *B. theobromae* showed a gradual increase in growth up to 15 d when glucose and sucrose were used as carbon sources but in case of mannitol and galactose, there were gradual increase in growth up to 20 d of incubation. Mycelial dry weight was maximum (302.5 mg) after 15 d of incubation when glucose was used as carbon source. When mannitol was used as carbon source, the growth was minimum among the carbon sources tested. Glucose was found as the best carbon source among the different carbon sources tested when overall growth pattern of *B. theobromae* was observed. In mannitol, *B. theobromae* showed minimum (186 mg) growth. Sporulation was excellent in glucose and sucrose after 15 and 20 d of incubation respectively. In all the other cases, sporulation was graded as good, fair, poor and nil. Generally good sporulation was found after 15 d of incubation. In case of mannitol, good sporulation was observed after 20 d of incubation. After 5 d of incubation sporulation started in case of sucrose but no sporulation was observed after 5 d in case of glucose, mannitol and galactose. In control set, insignificant growth without any sporulation was observed.

Glucose and sucrose were found satisfactory carbon sources in case of *C. eragrostidis* [Table 17 and Fig.13 (B)]. The fungus showed maximum growth (310 mg) after 15 d when glucose was used as carbon source and minimum (115 mg) in galactose after 5 d of incubation. Among the carbon sources tested, *C. eragrostidis* showed a gradual increase in growth up to 15 d. Sporulation was found excellent in case of glucose after 15 d of incubation. After 5 d of incubation, sporulation started in all the carbon sources tested. In control, *C. eragrostidis* showed insignificant growth which increased up to 25 d and no sporulation was observed.

Table 16
Effect of different carbon sources on the growth and sporulation of *B. theobromae*

Carbon sources	Incubation periods									
	5 days		10 days		15 days		20 days		25 days	
	Mwt (mg)*	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Glucose	220.0 ±0.81	-	280.6 ±0.72	++	302.5 ±1.04	+++	288.8 ±0.43	++++	266.9 ±1.00	++++
Sucrose	205.2 ±0.61	+	255.4 ±0.80	++	300.0 ±0.76	++++	292.5 ±0.87	++++	274.0 ±0.50	++++
Mannitol	70.0 ±1.15	-	125.1 ±0.58	+	156.3 ±0.65	++	186.0 ±0.58	+++	175.0 ±0.87	+++
Galactose	115.0 ±0.80	-	176.8 ±0.99	++	228.0 ±1.15	+++	251.0 ±1.03	+++	244.8 ±0.92	+++
Control***	8.3 ±0.43	-	10.6 ±0.30	-	13.5 ±0.76	-	17.8 ±0.61	-	20.6 ±0.30	-
CD at 5%	10.35		5.68		8.22		4.41		6.76	
CD at 1%	8.94		4.90		7.09		3.81		5.84	

* Mwt (mg)= Mycelial dry weight in mg, Mean of 3 replications.
** Spn= Sporulation, - = Nil. + = Poor. ++ = Fair. +++ = Good. ++++ = Excellent.
*** Basal medium without any carbon source.
Data after ± represent standard error values.

Table 17
Effect of different carbon sources on the growth and sporulation of *C. eragrostidis*

Carbon sources	Incubation periods									
	5 days		10 days		15 days		20 days		25 days	
	Mwt (mg)*	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Glucose	254.0 ±0.50	+	292.5 ±0.81	++	310.0 ±0.61	++++	296.0 ±1.15	++++	272.0 ±1.00	++++
Sucrose	215.5 ±0.76	++	266.7 ±0.87	++	289.0 ±1.00	+++	270.0 ±0.81	+++	246.0 ±0.58	+++
Mannitol	124.0 ±0.58	+	176.3 ±0.65	++	206.0 ±0.72	+++	188.0 ±0.58	+++	156.0 ±0.23	+++
Galactose	115.0 ±0.53	+	150.0 ±0.92	+	169.0 ±0.81	++	145.0 ±0.87	++	121.5 ±0.76	++
Control***	9.4 ±0.92	-	12.0 ±1.15	-	15.8 ±0.42	-	18.0 ±0.55	-	23.5 ±0.87	-
CD at 5%	5.69		4.37		6.66		5.07		6.76	
CD at 1%	4.91		3.77		5.75		4.38		5.84	

* Mwt (mg)= Mycelial dry weight in mg, Mean of 3 replications.
** Spn= Sporulation, - = Nil. + = Poor. ++ = Fair. +++ = Good. ++++ = Excellent.
*** Basal medium without any carbon source.
Data after ± represent standard error values.

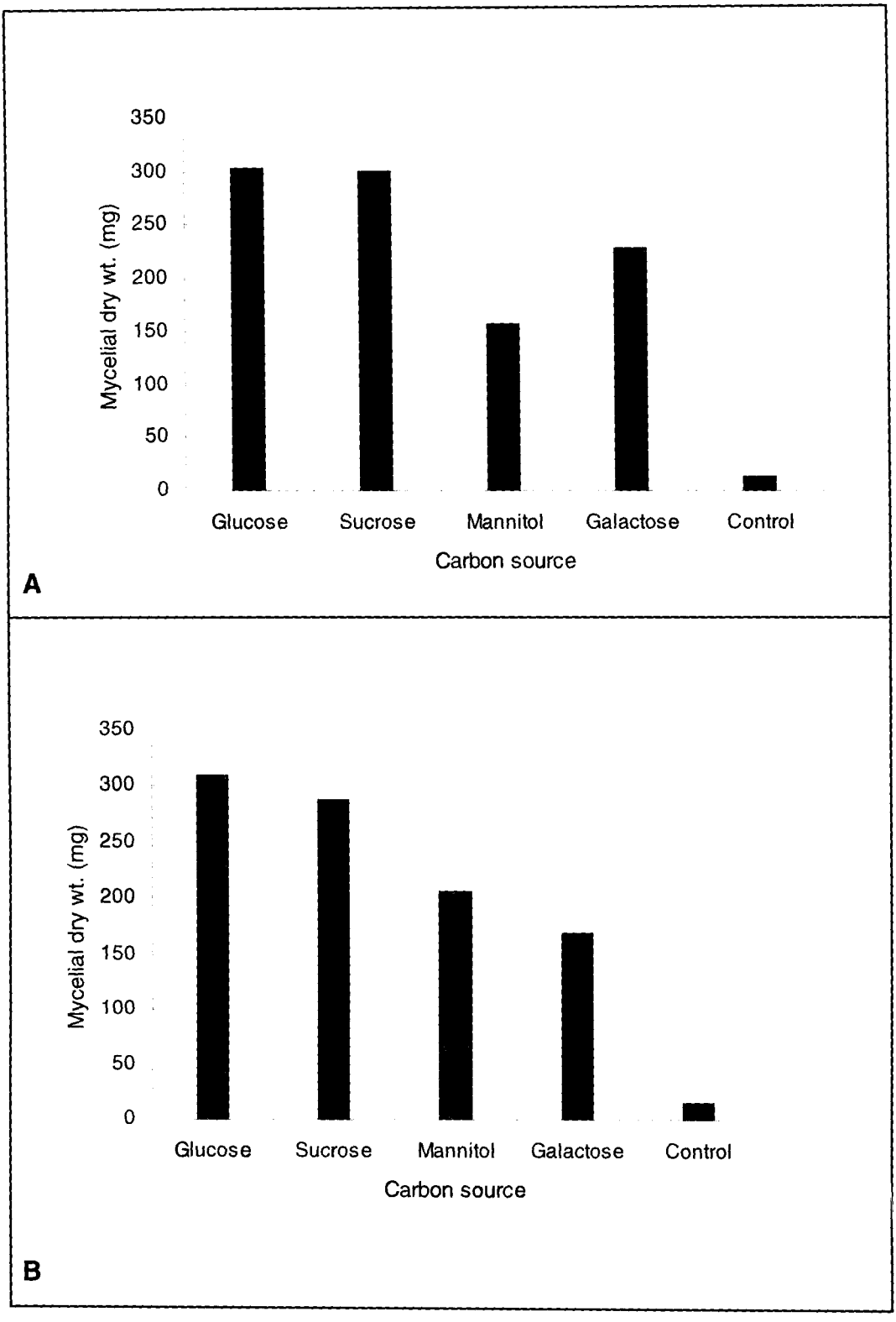


Fig. 13 (A & B): Effect of different carbon sources on the growth of *B. theobromae* (A) and *C. eragrostidis* (B) after 15 days of incubation.

4.3.5. Assessment of mycelial growth of *B. theobromae* and *C. eragrostidis* on different nitrogen sources

To assess the mycelial growth and sporulation of *B. theobromae* and *C. eragrostidis* on different nitrogen sources (both inorganic and organic), modified Asthana and Hawker's medium 'A' (glucose 10 g; KNO_3 3.5 g; KH_2PO_4 1.75 g; $MgSO_4$, $7H_2O$ 0.75 g; agar agar 20 g and distilled water 1 lt) without agar agar was used as basal medium. The quantity of various nitrogen sources was so adjusted to give the same amount of nitrogen as furnished by 3.5 g KNO_3 in the basal medium. The medium with required nitrogen sources was prepared and dispensed 50 ml each in 250 ml Ehrlenmayer flasks, sterilized by autoclaving at 15 lb.p.s.i. for 15 minutes and after cooling, the test fungi were inoculated and incubated for 5, 10, 15, 20 and 25 days at 28±2 °C temperature. In control, no nitrogen source was used in the basal medium. After specified incubation periods, the mycelia were harvested, dried at 60 °C and weighed. The results were tabulated in Table 18 and Table 19. After each of the different incubation periods, the sporulations were also recorded in five different grades based on visual observations.

From the results represented In Table 18 and Fig. 14, *B. theobromae* showed satisfactory growth in peptone (maximum 347 mg and minimum 252 mg) and beef extract (maximum 328 mg and minimum 213 mg), both of which were organic nitrogen source. Among the inorganic nitrogen sources tested, potassium nitrate showed best results (maximum 215 mg and minimum 144 mg). Media containing NH_4NO_3 as nitrogen source showed minimum (135 mg) mycelial growth. In all cases, maximum increase in growth was observed within first 5 d of inoculation and it declined after 15 or 20 d. Sporulation was found good after 15 d of incubation in case of potassium nitrate and yeast extract whereas in beef extract good Sporulation was observed after 20 d of incubation. In control, insignificant growth was observed without any sporulation.

Organic nitrogen source peptone was proved to be an excellent nitrogen source (maximum 384.5 mg and minimum 269.0 mg) among the different nitrogen

Table 18
Effect of different nitrogen sources on growth and sporulation of *B. theobromae*

Nitrogen source	Incubation periods									
	5 days		10 days		15 days		20days		25 days	
	Mwt (mg)*	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Inorganic										
Potassium nitrate	155.0 ±1.00	+	206.5 ±0.76	++	261.2 ±0.30	+++	268.1 ±0.38	+++	255.0 ±1.15	+++
Sodium nitrate	135.2 ±0.94	+	171.1 ±0.58	+	190.4 ±0.99	++	199.0 ±0.82	++	188.2 ±0.62	++
Ammonium nitrate	90.4 ±0.67	-	128.3 ±0.62	+	165.0 ±0.40	++	180.0 ±0.80	++	175.6 ±0.76	++
Ammonium sulphate	120.0 ±0.68	-	165.0 ±0.96	++	190.8 ±0.42	++	195.3 ±0.40	++	186.5 ±0.76	++
Organic										
Peptone	286.0 ±0.40	-	318.0 ±0.80	+	347.0 ±1.13	++	305.0 ±0.61	++	252.0 ±0.50	++
Yeast extract	180.0 ±0.53	+	239.0 ±0.50	++	272.0 ±0.64	+++	221.0 ±0.53	+++	190.6 ±0.58	+++
Beef extract	213.0 ±0.76	+	276.6 ±0.42	++	328.0 ±0.58	++	310.0 ±0.69	+++	281.0 ±0.50	+++
Control***	5.7 ±0.91	-	8.2 ±0.61	-	10.6 ±0.87	-	11.8 ±0.42	-	15.3 ±0.38	-
CD at 5%	4.40		2.63		5.01		4.13		5.32	
CD at 1%	3.87		2.32		4.42		3.64		4.69	

* Mwt (mg)= Mycelial dry weight in mg, Mean of 3 replications.
** Spn= Sporulation, - = Nil. + = Poor. ++ = Fair. +++ = Good. ++++ = Excellent.
*** Basal medium without any nitrogen source.
Data after ± represent standard error values.

sources tested against *C. eragrostidis* (Table 19 & Fig. 15). It was followed by another organic nitrogen source beef extract (maximum 355.0 mg and minimum 210.0 mg). In case of inorganic nitrogen sources, potassium nitrate showed the best results followed by ammonium sulphate (maximum 201.0 mg and minimum 135.0 mg), sodium nitrate (maximum 199.0 mg and minimum 120.0 mg) and ammonium nitrate (maximum 175.0 mg and minimum 105.0 mg). Sporulation was not good in most of the cases. It was found good after 15 d of incubation when ammonium nitrate, ammonium sulphate and peptone were used as nitrogen

Table 19
Effect of different nitrogen sources on growth and sporulation of *C. eragrostidis*

Nitrogen source	Incubation periods									
	5 days		10 days		15 days		20days		25 days	
	Mwt (mg)*	Spn**	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn	Mwt (mg)	Spn
Inorganic										
Potassium nitrate	144.0 ±1.00	+	172.0 ±0.64	+	215.0 ±0.53	++	199.0 ±0.72	++	187.0 ±0.53	++
Sodium nitrate	120.0 ±0.58	-	155.0 ±0.58	+	189.0 ±0.72	++	199.0 ±0.71	+++	185.0 ±1.08	+++
Ammonium nitrate	105.0 ±0.71	+	133.0 ±0.92	++	175.0 ±0.90	+++	169.0 ±0.53	+++	150.0 ±0.90	+++
Ammonium sulphate	135.0 ±1.02	-	172.0 ±0.58	+	201.0 ±0.50	+++	188.0 ±1.15	+++	175.0 ±0.78	+++
Organic										
Peptone	315.0 ±0.49	+	358.0 ±1.15	++	384.5 ±0.87	+++	336.0 ±0.58	+++	269.0 ±0.60	+++
Yeast extract	155.0 ±0.75	+	224.0 ±0.58	+	265.0 ±0.50	++	207.5 ±0.76	++	144.0 ±0.82	++
Beef extract	210.0 ±0.95	+	290.2 ±0.42	+	355.0 ±0.69	++	329.0 ±0.81	++	272.0 ±0.79	++
Control***	4.4 ±0.81	-	8.1 ±0.66	-	11.1 ±1.21	-	14.4 ±0.70	-	20.6 ±0.87	-
CD at 5%	5.99		8.06		5.92		5.78		5.81	
CD at 1%	5.28		7.11		5.22		5.09		5.12	

Mwt (mg)= Mycelial dry weight in mg; Spn= Sporulation.
* Mean of 3 replications.
** - = Nil. + = Poor. ++ = Fair. +++ = Good. ++++ = Excellent.
*** Basal medium without any nitrogen source.
Data after ± represent standard error values.

sources. Only in case of sodium nitrate, sporulation was found good after 20 d of incubation. Control set did not show significant growth or sporulation.

4.3.6. Spore germination and germ tube elongation of *B. theobromae* and *C. eragrostidis* after different periods of incubation

Spore germination and germ tube elongation of *B. theobromae* and *C. eragrostidis* were studied after different periods of incubation. The results were tabulated in Table 20 and Table 21.

In case of *B. theobromae* germination of spores started after 2 hours of incubation as observed from the results [Table 20 & Fig. 16 (A)]. The percent germination of spores and germ tube length increased with increasing time interval.

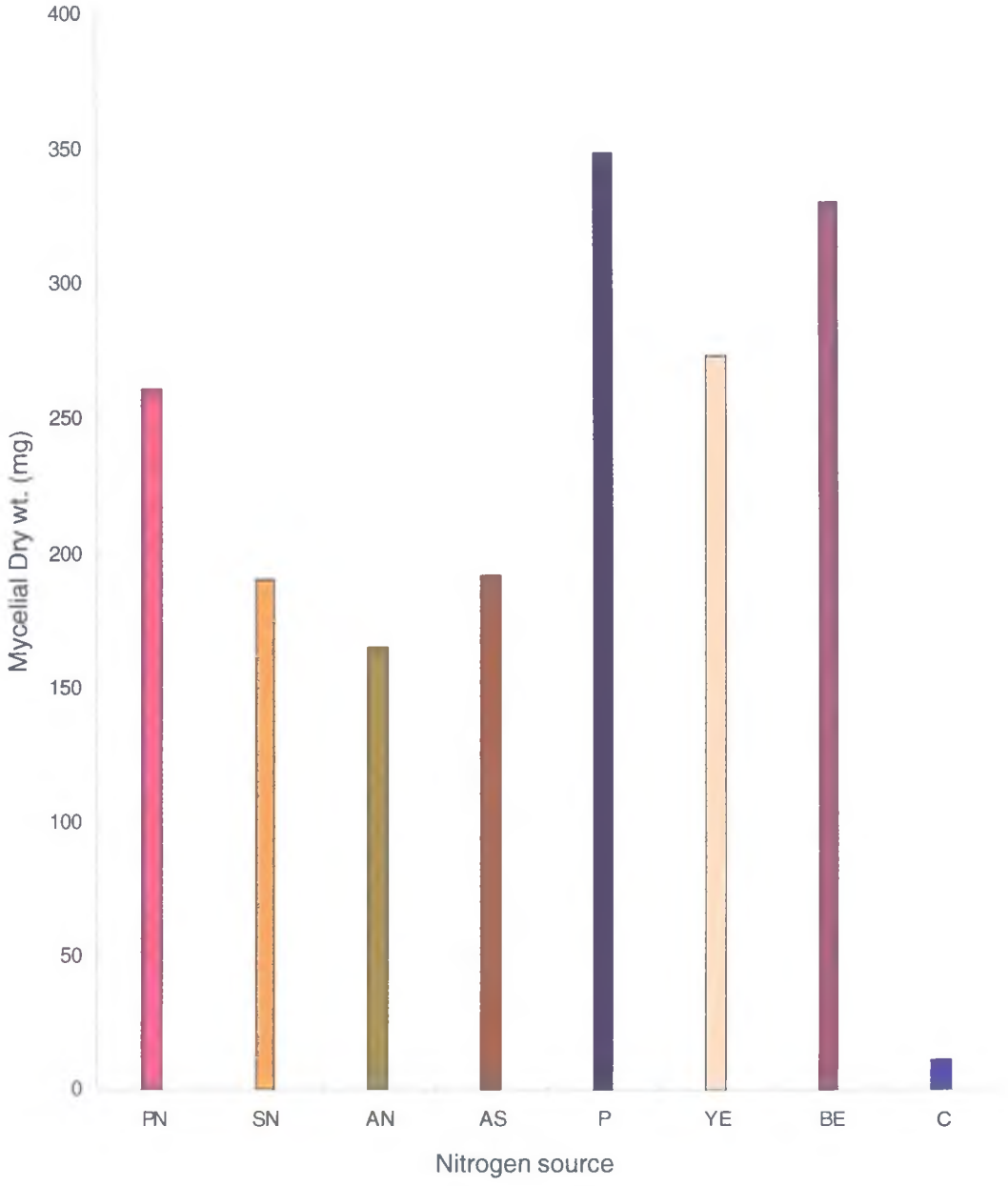


Fig. 14: Effect of different nitrogen sources on the growth of *B.theobromae* after 15 days of incubation

Abbreviations : PN = Potassium Nitrate SN = Sodium Nitrate
AN = ammonium Nitrate AS = Ammonium Sulphate
P = Peptone YE = Yeast Extract
BE = Beef Extract C = Control

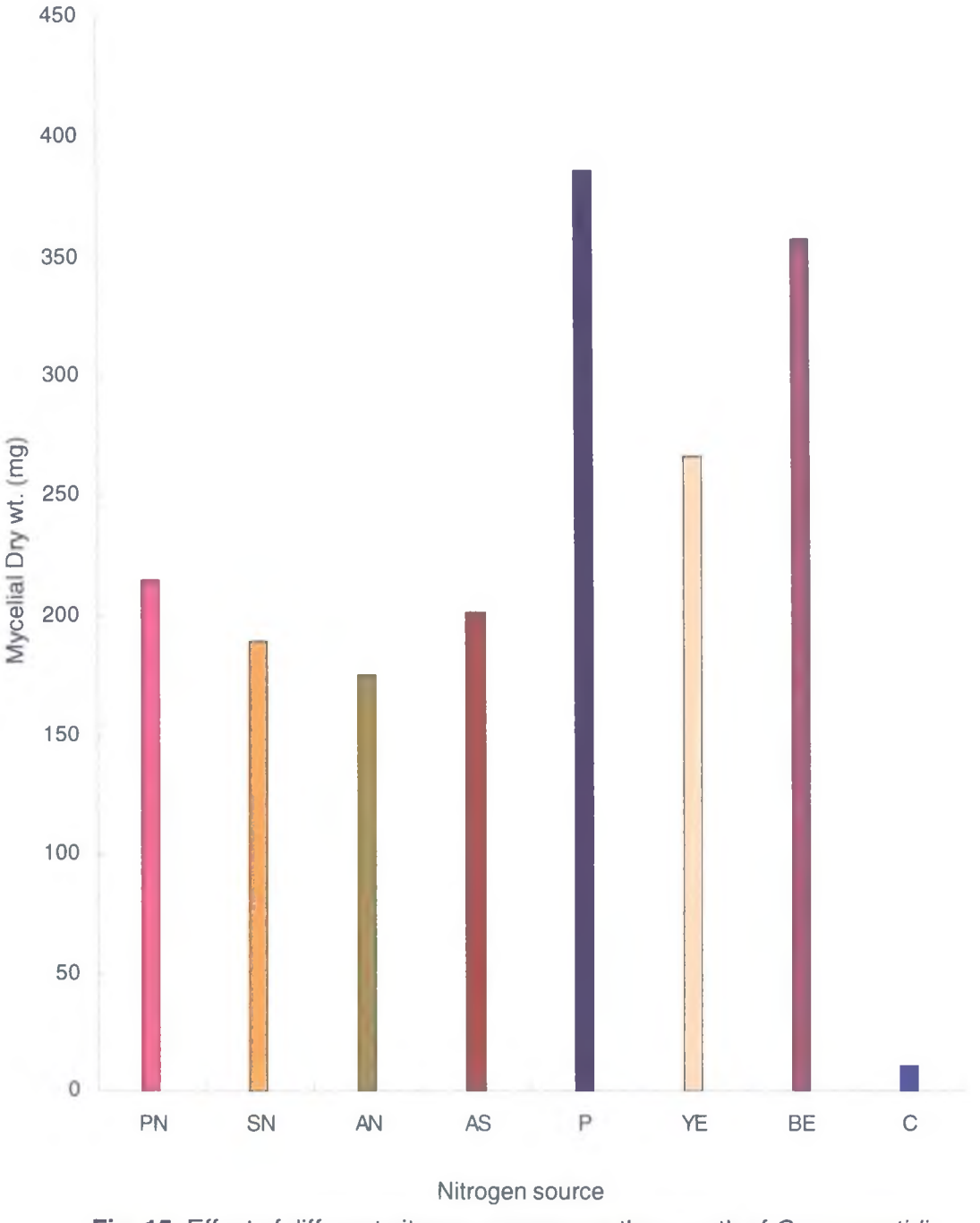


Fig. 15: Effect of different nitrogen sources on the growth of *C. eragrostidis* after 15 days of incubation

Abbreviations : PN = Potassium Nitrate SN = Sodium Nitrate
AN = ammonium Nitrate AS = Ammonium Sulphate
P = Peptone YE = Yeast Extract
BE = Beef Extract C = Control

Table 20
Effect of different incubation periods on spore germination and germ tube elongation of *B. theobromae*

Incubation period (hours)	Percent germination*	Germ tube length (µm)
2	0	0
4	39.14±0.25	24.46±0.29
6	61.46±0.23	39.33±0.34
8	69.25±0.29	60.89±0.15
10	72.81±0.17	72.18±0.22
12	76.55±0.21	81.25±0.51
14	76.81±0.26	91.38±0.69
16	77.15±0.19	97.46±0.29
18	77.62±0.18	108.11±0.13
20	78.51±0.30	115.68±0.15
22	79.28±0.27	119.30±0.17
24	80.18±0.24	124.00±0.50
CD at 5%	1.49	2.32
CD at 1%	1.32	2.06

*Mean of 3 replications.
Data after ± represent standard error values.

After 24 h, spore germination and germ tube length was recorded to be 80.18% and 124.00 µm respectively.

Spores of *C. eragrostidis* on the other hand started germination within 2 hours of incubation and percent germination and germ tube length continued to increase with increasing time interval [Table 21 & Fig. 16 (B)]. After 24 h *C. eragrostidis* showed maximum (88.80%) spore germination and its germ tube length was also maximum (46.40 µm).

4.3.7. Spore germination and germ tube elongation of *B. theobromae* and *C. eragrostidis* at different pH

Solution of different pH values of 4.0 6.0, 6.5, 6.75, 7.25 and 9.0 were prepared by mixing 0.01M K_2HPO_4 and KH_2PO_4 in required ratios. The spores of the test fungi

Table 21
Effect of different incubation periods on spore germination and germ tube elongation of *C. eragrostidis*

Incubation period (hours)	Percent germination*	Germ tube length (µm)
2	11.21±0.16	08.70±0.15
4	42.35±0.22	13.65±0.12
6	61.44±0.22	18.77±0.13
8	75.18±0.19	21.38±0.19
10	81.89±0.27	25.71±0.13
12	84.25±0.18	32.55±0.13
14	84.66±0.21	33.33±0.12
16	84.91±0.25	36.18±0.10
18	85.11±0.20	37.50±0.29
20	85.81±0.34	40.38±0.09
22	87.49±0.15	44.25±0.09
24	88.80±0.20	46.40±0.15
CD at 5%	0.77	0.53
CD at 1%	0.68	0.47

*Mean of 3 replications.
Data after ± represent standard error values.

were suspended in the solutions of different pH and allowed to germinate on glass slides for 24 hours. Slide germination technique as mentioned in the materials and methods (Section 3.17.2) were followed. The results presented in the Table 22 and Table 23 showed that the percent germination sharply declined (17.8%) at pH 9.0. Similarly, in case of C. eragrostidis germination was highest (83.15%) at pH 7.25 and lowest (26.6%) at pH 9.0.Among the different pH tested against *B. theobromae*, germ tube length was maximum (106.00 µm) at pH 7.25 and minimum (62.24 µm) was recorded at pH 9.0 [Table 22 & Fig. 17 (A)]. Germ tube length was maximum (50.40 µm) at pH 7.25 and minimum (29.20 µm) at pH 9.0 [Table 23 and Fig. 17 (B)].

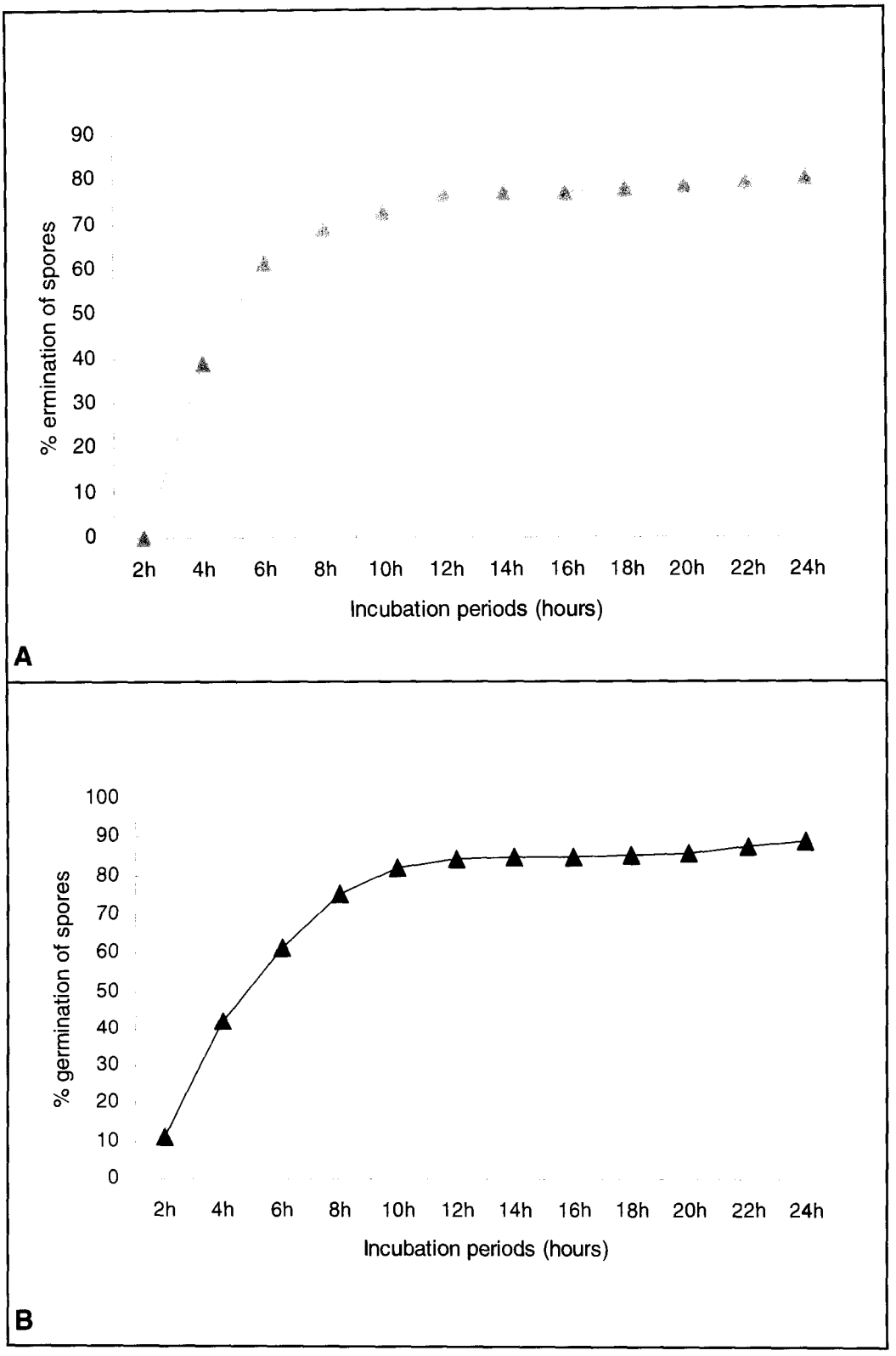


Fig.16 (A & B): Effect of different incubation periods (hours) on the germination of spores of *B. theobromae* (A) and *C. eragrostidis* (B)

Table 22

Effect of different pH on spore germination and germ tube elongation of *B. theobromae* after 24 hours of incubation

pH	Percent germination when control raised to 100*	Germ tube length (µm)
4.00	46.20±0.76	80.20±0.43
6.00	52.00±0.53	84.40±0.56
6.50	57.30±0.85	98.80±0.43
6.75	75.33±0.40	99.00±0.25
7.25	80.00±0.53	106.6±0.83
9.00	17.80±0.42	62.24±0.38
CD at 5%	4.77	3.83
CD at 1%	4.17	3.34

*Mean of 3 replications.
Data after ± represent standard error values.

Table 23

Effect of different pH on spore germination and germ tube elongation of *C. eragrostidis* after 24 hours of incubation

pH	Percent germination when control raised to 100*	Germ tube length (µm)
4.00	40.90±0.97	30.00±0.50
6.00	44.23±0.62	46.40±0.70
6.50	47.16±0.58	47.50±0.36
6.75	55.55±0.29	49.20±0.40
7.25	83.15±0.44	50.40±0.30
9.0	26.60±0.83	29.20±0.42
CD at 5%	4.83	2.23
CD at 1%	4.21	1.94

*Mean of 3 replications.
Data after ± represent standard error values.

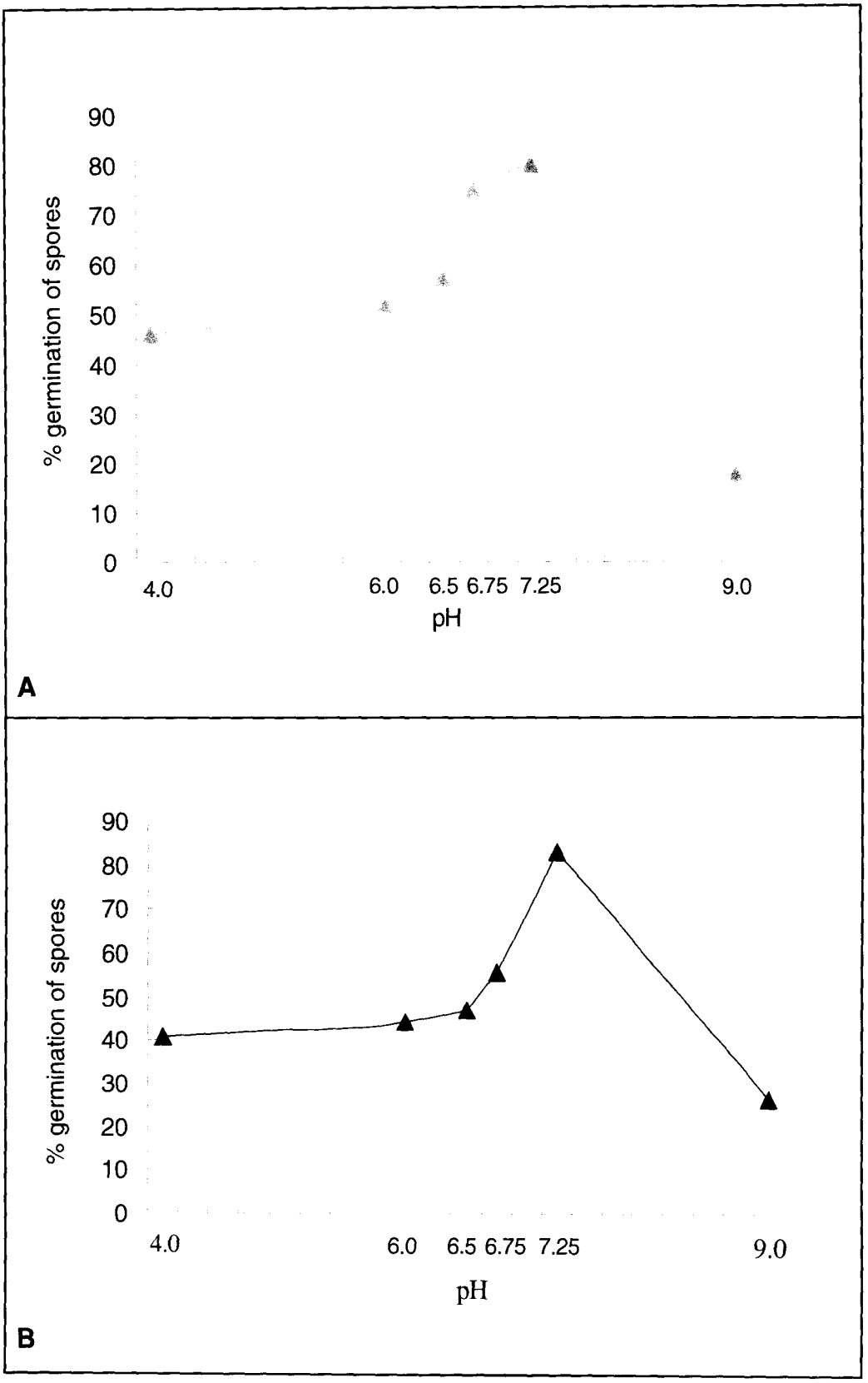


Fig.17 (A & B): Effect of different pH on the germination of spores of *B. theobromae* (A) and *C. .eragrostidis* (B).

4.3.8. Spore germination and germ tube elongation of *B. theobromae* and *C. eragrostidis* at different temperature treatment.

Spore suspension of the test fungi were pretreated with 20 °C, 25 °C,28 °C, 30 °C, 40 °C, 45 °C and 50 °C temperature for ten minutes, and subsequently the spores were allowed to germinate on glass slides following slide germination technique at 28±2 °C as mentioned in materials and methods section (Section 3.17.2.). The results were noted in Table 24 and Table 25.

In case of *B. theobromae* germination was optimum when pretreated at 28 °C but no germination was observed when pretreated at 40 °C and above temperatures [Table 24 and Fig.18 (A)].

Table 24

Effect of different temperature on spore germination and germ tube elongation of *B. theobromae* after 24 hours of incubation

Temperature (°C)	Percent germination*	Germ tube length (µm)
20°C	72.69±0.34	99.61±0.56
25°C	81.78±0.29	118.25±0.63
28°C	82.23±0.65	124.00±0.64
30°C	80.92±0.51	121.96±0.16
35°C	64.45±0.32	113.26±0.25
40°C	0	0
45°C	0	0
50°C	0	0
CD at 5%	3.72	4.23
CD at 1%	3.21	3.66

*Mean of 3 replications.
Data after ± represent standard error values.

C. eragrostidis showed maximum values for both spore germination (76.23%) and germ tube elongation (53.00 µm) at 28 °C and minimum values for both (05.62% and 11.34 µm respectively) at 40 °C. At 45 °C and higher temperatures spore germination of *C. eragrostidis* was completely inhibited [Table 25 and Fig.18 (B)].

Table 25

Effect of different temperature on spore germination and germ tube elongation of *C. eragrostidis* after 24 hours of incubation

Temperature (^{0}C)	Percent germination*	Germ tube length (µm)
20^{0}C	59.42±0.27	36.25±0.14
25^{0}C	72.68±0.57	48.00±0.43
28^{0}C	76.23±0.39	53.00±0.58
30^{0}C	70.81±0.40	46.81±0.70
35^{0}C	22.95±0.42	29.38±0.33
40^{0}C	05.62±0.29	11.34±0.19
45^{0}C	0	0
50^{0}C	0	0
CD at 5%	2.63	3.29
CD at 1%	2.34	2.87

*Mean of 3 replications.
Data after ± represent standard error values.

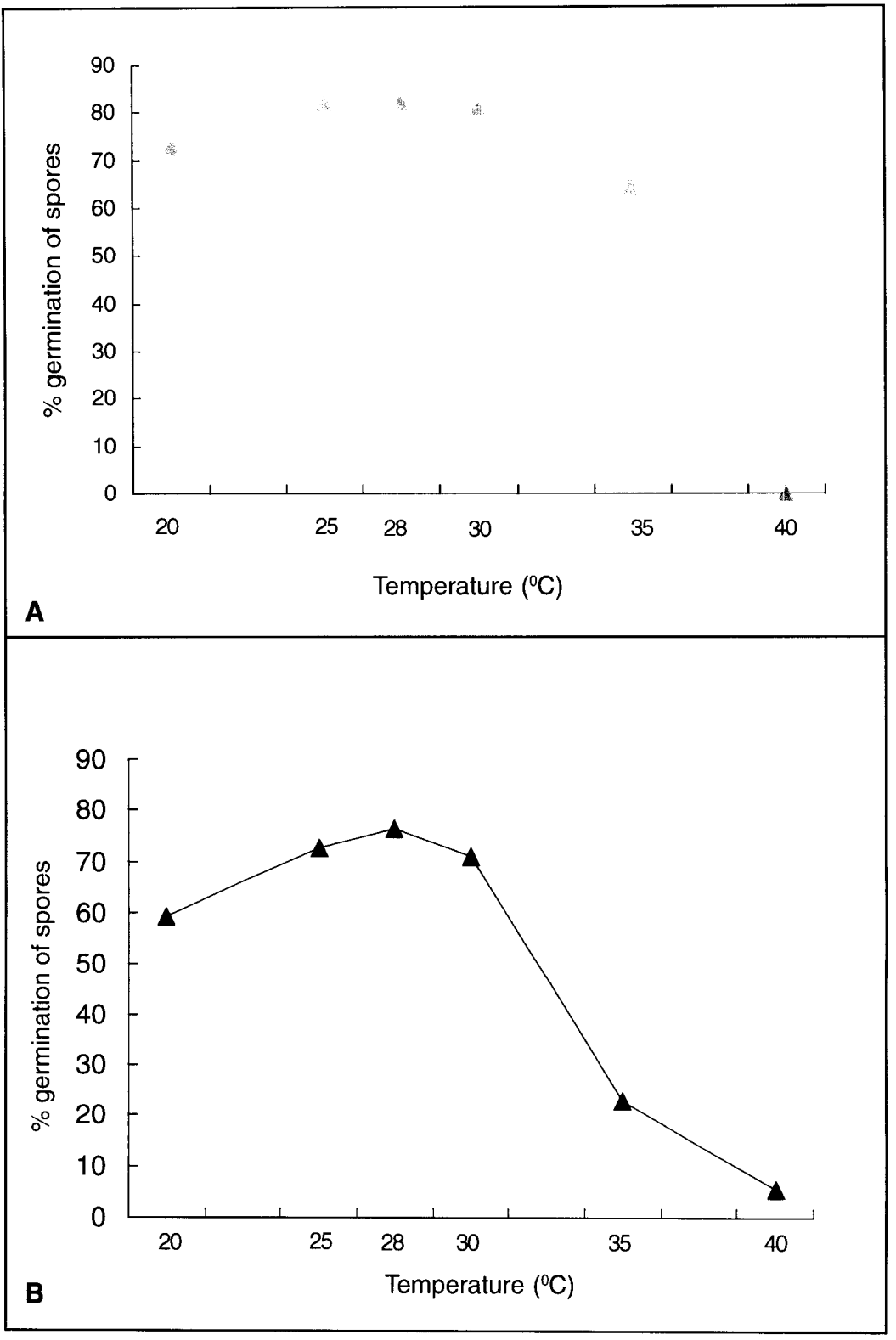


Fig.18 (A & B): Effect of different incubation periods (temperature) on the germination of spores of *B. theobromae* (A) and *C. .eragrostidis* (B)

4.4. Experiments on immunological studies

4.4.1. Serological relationship between different tea varieties and test pathogens (*B. theobromae* & *C. eragrostidis*) by agar gel double diffusion

The standard method as described by Ouchterlony (1958) was followed to determine the serological relationship between host and pathogens by agar gel double diffusion. The antigens of the two pathogens *B. theobromae* and *C. eragrostidis*, antigen of non-pathogen *Gliocladium virens* (isolate II), leaf antigens of three susceptible tea varieties like TV-11, TV-12 and TV-18 as well as leaf antigens of three resistant varieties like TV-25, TV-26 and TV-30 were used in the experiments. In addition to the above leaf antigens, the antigens of TV-3, TV-6, TV-8 and Sundaram varieties were also tested in some cases. The antisera used in the experiments were antisera of *B. theobromae* and *C.eragrostidis* and antisera of leaves of TV-11, TV-12, TV-25 and TV-26. Preparation of antigens, antisera and details of the procedure has already been discussed in the materials and method (Section 3.13.1). The results are shown in Table 26 and Table 27 respectively for the two pathogens *B. theobromae* and *C. eragrostidis*.

From the results noted in Table 26, it was observed that common antigenic relationship were present not only in cases of homologous reactions i.e. between antisera and antigens of *B. theobromae* (Plate VIII : 6 & Plate IX : 3), TV-11 (Plate VIII : 1 & Plate IX : 1) and TV-26 (Plate VIII : 2) but also in cross reactions between antisera of *B. theobromae* and antigens from leaves of TV-11 (Plate IX : 3), TV-12 (Plate IX : 3) and TV-18. No precipitation bands were observed when antigens of TV-26, TV-30 and TV-6 (Plate IX : 3) and *G. virens* (isolate II) were used against antisera of *B. theobromae*. Common precipitin bands were also found in reactions between the antisera of TV-11 and antigens of *B. theobromae* (Plate IX : 1), TV-12 (Plate IX : 1 & 4), TV-18, TV-26 (Plate IX : 4) and TV-30. No precipitin band was found between the antigen of *G. virens* (isolate II) and antisera of TV-11 (Plate IX : 4). When the antisera of TV-26 were used, common precipitin bands were found where the antigens were of TV-11, TV-12, TV-18, TV-6, TV-26 and TV-30. No precipitation band was observed when an antigen of *G. virens* (isolate II) was treated with the antisera of TV-26.

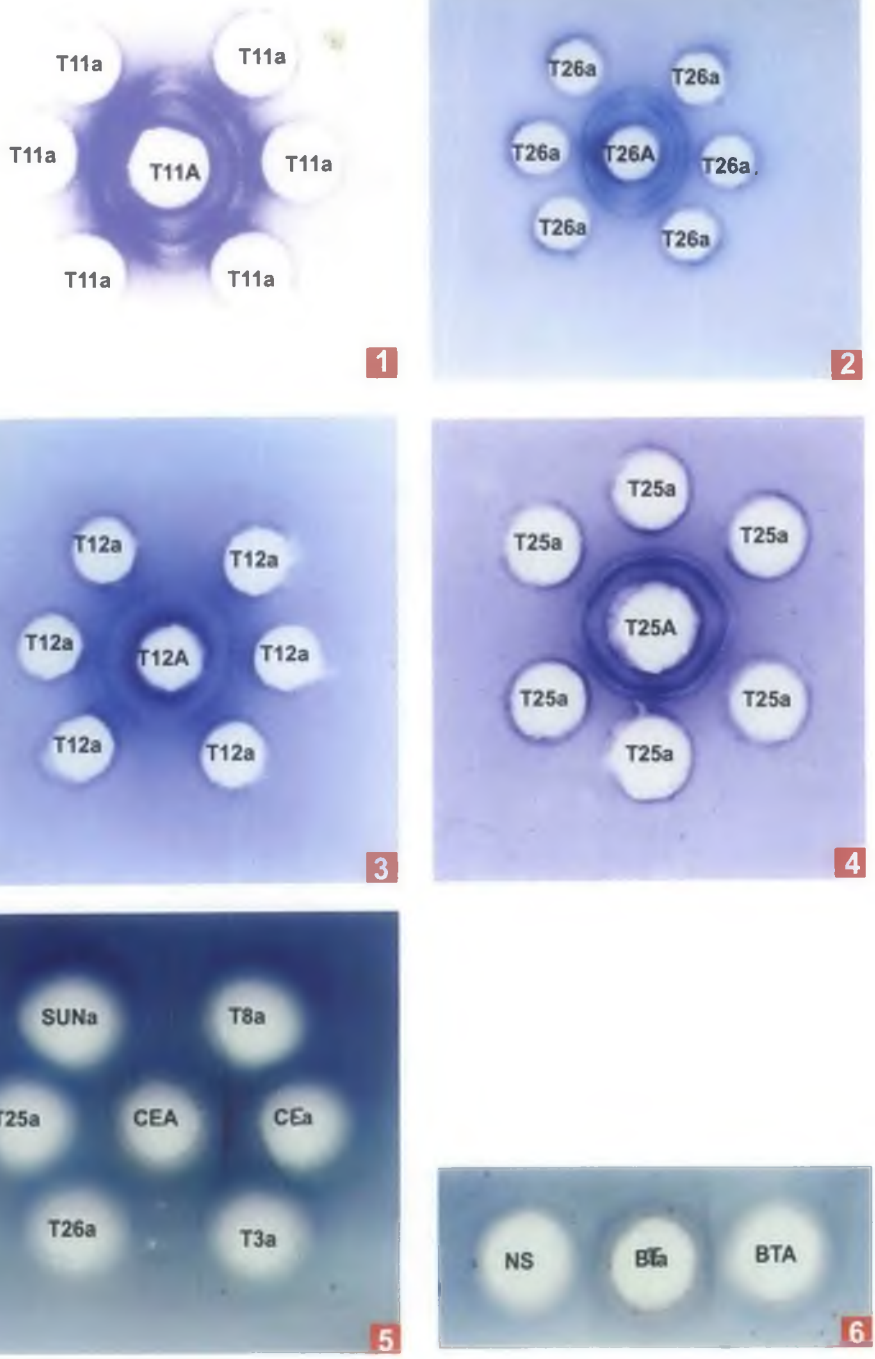


Plate VIII

Agar gel double diffusion test using different antigens and antisera.

Fig.1 : Leaf antigen of TV-11 (T11a) were used in peripheral wells while central well contained antisera of TV-11 (T11A).

Fig. 2 : Leaf antigen of TV-26 (T26a) were used in peripheral wells while central well contained antisera of TV-26 (T26A).

Fig. 3 : Leaf antigen of TV-12 (T12a) were used in peripheral wells while central well contained antisera of TV-12 (T12A).

Fig. 4 : Leaf antigen of TV-25 (T25a) were used in peripheral wells while central well contained antisera of TV-25 (T25A).

Fig. 5 : Leaf antigens of Sundaram variety (SUNa), TV-25 (T25a), TV-26 (T26a), TV-3 (T3a) and TV-8 (T8a) and fungal antigen of *Curvularia eragrostidis* (CEa) were used in peripheral wells while central well contained antisera of *C. eragrostidis* (CEA)

Fig. 6 : Normal sera (NS) and antisera of *Botryodiplodia theobromae* (BTA) were used in peripheral wells while central well contained antigen of *B. theobromae* (BTa).

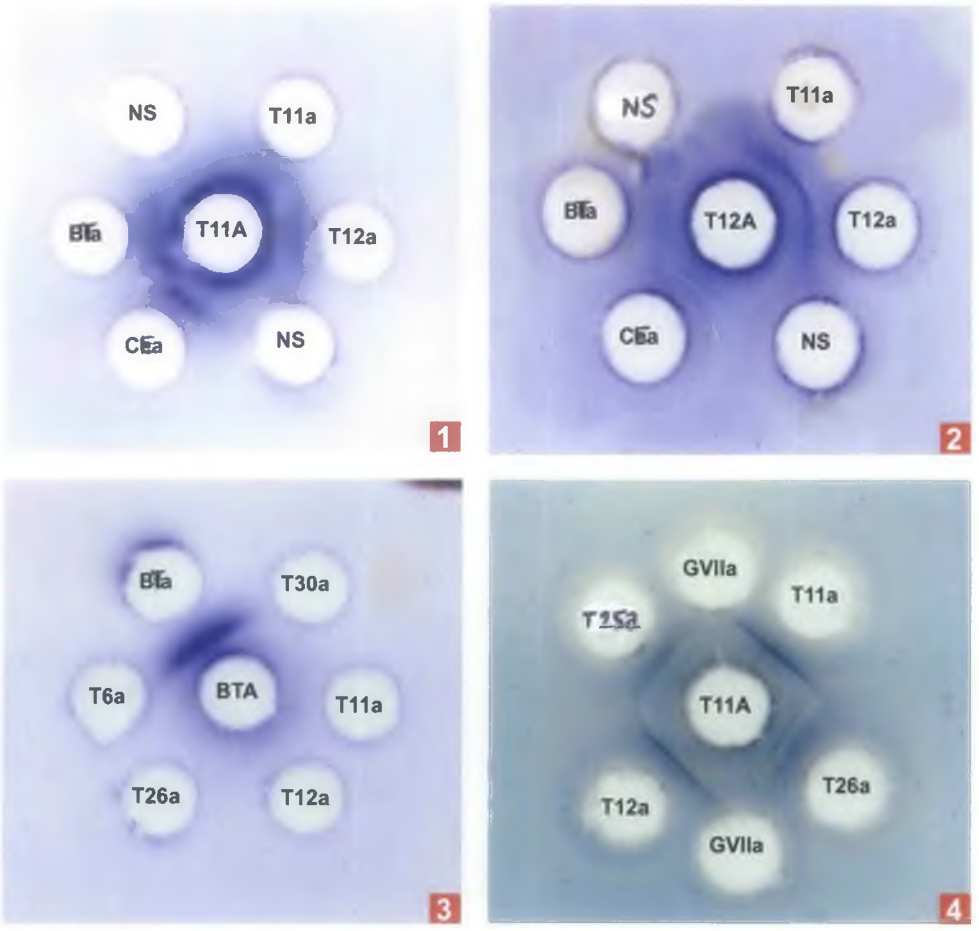


Plate IX

Agar gel double diffusion test using different antigens and antisera.

1: Leaf antigens of TV-11 (T11a), TV-12 (T12a);fungal antigens of *Botryodiplodia theobromae* (BTa), *Curvularia eragrostidis* (CEa) and normal sera (NS) were used in peripheral wells while central well contained antisera of TV-11(T11A).

2: Leaf antigens of TV-11 (T11a), TV-12 (T12a);fungal antigens of *B. theobromae* (BTa), *C.eragrostidis* (CEa) and normal sera (NS) were used in peripheral wells while central well contained antisera of TV-12 (T12A).

3: Leaf antigens of TV-6 (T6a), TV-11 (T11a), TV-12 (T12a), TV-30 (T30a), TV-26 (T26a) and fungal antigen of *B. theobromae* (BTa) were used in peripheral wells while central well contained antisera of *B. theobromae* (BTA).

4: Leaf antigens of TV-11 (T11a), TV-26 (T26a), TV-12 (T12a), TV-25 (T25a) and antigens of non pathogen *Gliocladium virens,* isolate II (GVIIa) were used in peripheral wells while central well contained antisera of TV-11 (T11a).

Table 26
Common antigenic relationship between tea varieties and *B. theobromae* (Based on agar gel double diffusion test)

Antigens of pathogen, host and non-pathogen	Antisera of pathogen and host		
	B.theobomae (BTA)*	TV-11 (T11A)	TV-26 (T26A)
Pathogen			
B. theobromae (BTa)	+	+	-
Susceptible varieties			
TV-11 (T11a)	+	+	+
TV-12 (T12a)	+	+	+
TV-18 (T18a)	+	+	+
Resistant varieties			
TV-6 (T6a)	-	+	+
TV-26 (T26a)	-	+	+
TV-30 (T30a)	-	+	+
Non-pathogen			
G. virens-isolate II (GV IIa)	-	-	-

Common precipitin band present (+).
Common precipitin band absent (-).
*The codes of different antigens and antisera are in the parenthesis.

From the results noted in Table 27, it was observed that common antigenic relationship were present not only in cases of homologous reactions i.e. between antisera and antigens of *C. eragrostidis* (Plate VIII : 5), TV-12 (Plate VIII: 3 & Plate IX : 2) and TV-25 (Plate VIII : 4) but also in cross reactions between antisera of *C. eragrostidis* and antigens from leaves of TV-11, TV-12 and TV-18. No precipitation bands were observed when antigens of TV-3, TV-8, TV-25, TV-26, Sundaram and *G. virens* (isolate II) were used against antisera of *C. ergrostidis*. Common precipitin bands were also found in reactions between the antisera of TV-12 and antigens of *C. ergrostidis* (Plate IX : 2), TV-11 (Plate IX :2), TV-18, TV-25 and TV-26. No precipitin reaction between the antigen of *G. virens* (isolate II) and antisera of TV-12. When the antisera of TV-25 was used, common precipitin bands were observed with antigens of TV-11, TV-12, TV-18, TV-3, TV-8, TV-25, TV-26 and Sundaram. No precipitation band was observed when an antigen of *G. virens* (isolate II) was reacted with the antisera of TV-25.

Table 27

Common antigenic relationship between tea varieties and *C. eragrostidis* (Based on agar gel double diffusion test)

Antigens of pathogen, host and non-pathogen	Antisera of pathogen and host		
	C. eragsostidis (CEA)*	TV-12 (T12A)	TV-25 (T25A)
Pathogen			
C. eragrostidis (CEa)	+	+	-
Susceptible varieties			
TV-12 (T12a)	+	+	+
TV-11 (T11a)	+	+	+
TV-18 (T18a)	+	+	+
Resistant varieties			
TV-3 (T3a)	-	+	+
TV-8 (T8a)	-	+	+
TV-25 (T25a)	-	+	+
TV-26 (T26a)	-	+	+
Sunadaram (SUNa)	-	+	+
Non-pathogen			
G. virens-isolate II (GV IIa)	-	-	-

Common precipitin band present (+).
Common precipitin band absent (-).
*The codes of different antigens and antisera are in the parenthesis.

4.4.2. Serological relationship between different tea varieties and test pathogens (*B. theobromae* & *C. eragrostidis*) by immunoelectrophoresis

Following standard methods, immunoelectrophoresis was done using the antisera and antigens of *B. theobromae*, *C. eragrostidis*, TV-11, TV-12, TV-25 and TV-26. The detail of the procedure has already been described in the materials and method (Section 3.13.2). The results of the experiments were noted in Table 28 and Table 29 for *B. theobromae* and in Table 30 and Table 31 for *C. eragrostidis*.

From Table 28, it was revealed that the antigen of *B. theobromae* and TV-11 share one precipitin arc in both the cases with antisera of *B. theobromae* and with antisera of TV-11 while antigen of TV-11 and TV-26 share two precipitin arcs between them when both were reacted with the antisera of TV-26. When overall

Table 28

Immunoelectrophoretic test of antigens and antisera of tea varieties and *B. theobromae*

Antigens of pathogen, host and non-pathogen	Antisera of *B. theobromae* (BTA)*				Antisera of TV-11(T11A)		Antisera of TV-26 (T26A)		
	Precipitin arcs				Precipitin arcs		Precipitin arcs		
	1st	2nd	3rd	4th	1st	2nd	1st	2nd	3rd
Pathogen									
B. theobromae (BTa)	+	+	+	+	+	-	-	-	-
Susceptible variety									
TV-11 (T11a)	+	-	-	-	+	+	+	+	-
Resistant variety									
TV-26 (T26a)	-	-	-	-	-	+	+	+	+
Non-pathogen									
G. virens-isoiate II (GV IIa)	-	-	-	-	-	-	-	-	-

Common precipitin band present (+).

Common precipitin band absent (-).

*The codes of different antigens and antisera are in the parenthesis.

results were summarized in the Table 29, it was found that the antigens of *B. theobromae* showed four precipitin arcs in homologous reaction with the antisera of *B. theobromae*, but only one precipitin arc was found between the antigens of *B. theobromae* and antisera of TV-11. No precipitin arc was observed in the immunoelectrophoretic test between the antigen of TV-26 and antisera of *B. theobromae* and also in reciprocal reaction [Plate X : 1-8 & Fig. 19 (A-N)].

Table 29 also revealed that the antigen of TV-11 showed one precipitin arc with antisera of *B. theobromae* [Plate X : 4 & Fig. 19 (C)], two precipitin arcs with both the antisera of TV-11 [Plate X : 6 & Fig. 19 (B & M)] and the antisera of TV-26 [Fig. 19 (L)]. The antigen of TV-26 on the other hand showed no precipitin arcs with antisera of *B. theobromae* [Plate X : 7 & Fig. 19 (E)], one precipitin arc with antisera of TV-11 [Fig. 19 (B)] and three precipitin arcs in homologous test with antisera of TV-26 [Fig. 19 (N)]. When antigen of non-pathogen *G. virens* (isolate II) was tested with the antisera of *B. theobromae*, TV-11 and TV-26, no precipitin arc was observed.

Table 29

Comparison of precipitation arcs found in immunoelectrophoresis of tea varieties (susceptible and resistant), pathogen (*B. theobromae*) and non-pathogen

Antigens of pathogen, host and non-pathogen	Total No. of precipitin arcs		
	Antisera of pathogen and host		
	B. theobromae (BTA)*	TV-11 (T11A)	TV-26 (T26A)
Pathogen			
B. theobromae (BTa)	4	1	0
Susceptible variety			
TV-11 (T11a)	1	2	2
Resistant variety			
TV-26 (T26a)	0	1	3
Non-pathogen			
G. virens-isolate II (GV IIa)	0	0	0

*The codes of different antigens and antisera are in the parenthesis.

Table 30 revealed that the antigen and antisera of *C. eragrostidis* showed 4 precipitin arcs when reacted. Antisera of *C. eragrostidis* when reacted with antigen of TV-12 showed one precipitin arc which is comparable to the 4th arc of the homologous reaction [Fig. 19 (I)] while antigen of TV-12 and TV-25 share two precipitin arcs when both were reacted with the antisera of TV-25 [Fig. 19 (H & J)]. When overall results were summarized in the Table 31, it was found that the antigens of *C. eragrostidis* showed four precipitin arcs in homologous reaction with the antisera of *C. eragrostidis* [Fig. 19 (G)], but only one precipitin arc was found between the antigens of *C. eragrostidis* and antisera of TV-12 [Fig. 19 (D)]. No precipitin arc was observed in the immunoelectrophoretic test between the antigen of TV-25 and the antisera of *C. eragrostidis* (Plate X: 8) and also in reciprocal reaction [Fig. 19 (H)].

From the Table 31, it was revealed that the antigen of TV-12 showed one precipitin arc with antisera of *C. eragrostidis* [Plate X : 3 & Fig. 19 (I)], three precipitin arcs with the antisera of TV-12 [Plate X : 5 & Fig. 19 (D & F)] and two precipitin arcs with the antisera of TV-25 [Fig. 19 (H)]. The antigen of TV-25 on the other hand showed no precipitin arc with antisera of *C. eragrostidis* [Plate X : Fig.

8 & Fig. 19 (K)], two precipitin arcs with antisera of TV-12 [Fig. 19 (F)], and four precipitin arcs in homologous test with antisera of TV-25 [Fig. 19 (J)]. When antigen of non-pathogen *G. virens* (isolate II) was treated with the antisera of *C. eragrostidis*, TV-12 and TV-25 respectively, no precipitin arc was observed.

Table 30

Immunoelectrophoretic test of antigens and antisera of tea varieties and *C. eragrostidis*.

Antigens of pathogen, host and non-pathogen	Antisera of *C. eragrostidis* (CEA)*				Antisera of TV-12 (T12A)			Antisera of TV-25(T25A)			
	Precipitin arcs				Precipitin arcs			Precipitin arcs			
	1st	2nd	3rd	4th	1st	2nd	3rd	1st	2nd	3rd	4rt
Pathogen											
C. eragrostidis (CEa)	+	+	+	+	+	-	-	-	-	-	-
Susceptible variety											
TV-12 (T12a)	-	-	-	+	+	+	+	+	+	-	-
Resistant variety											
TV-25 (T25a)	-	-	-	-	+	+	-	+	+	+	+
Non-pathogen											
G. *virens*-isolate II (GV IIa)	-	-	-	-	-	-	-	-	-	-	-

Common precipitin band present (+); Common precipitin band absent (-).
*The codes of different antigens and antisera are in the parenthesis.

Table 31

Comparison of precipitation arcs found in immunoelectrophoresis of tea varieties (susceptible and resistant), pathogen (*C. eragrostidis*) and non-pathogen.

Antigens of pathogen, host and non-pathogen	Total No. of precipitin arcs		
	Antisera of pathogen and host		
	C. eragrostidis (CEA)*	TV-12 (T12A)	TV-25 (T25A)
Pathogen			
C. eragrostidis *(CEa)*	4	1	0
Susceptible variety			
TV-12 (T12a)	1	3	2
Resistant variety			
TV-25 (T25a)	0	2	4
Non-pathogen			
G. virens-isolate II (GV IIa)	0	0	0

*The codes of different antigens and antisera are in the parenthesis.

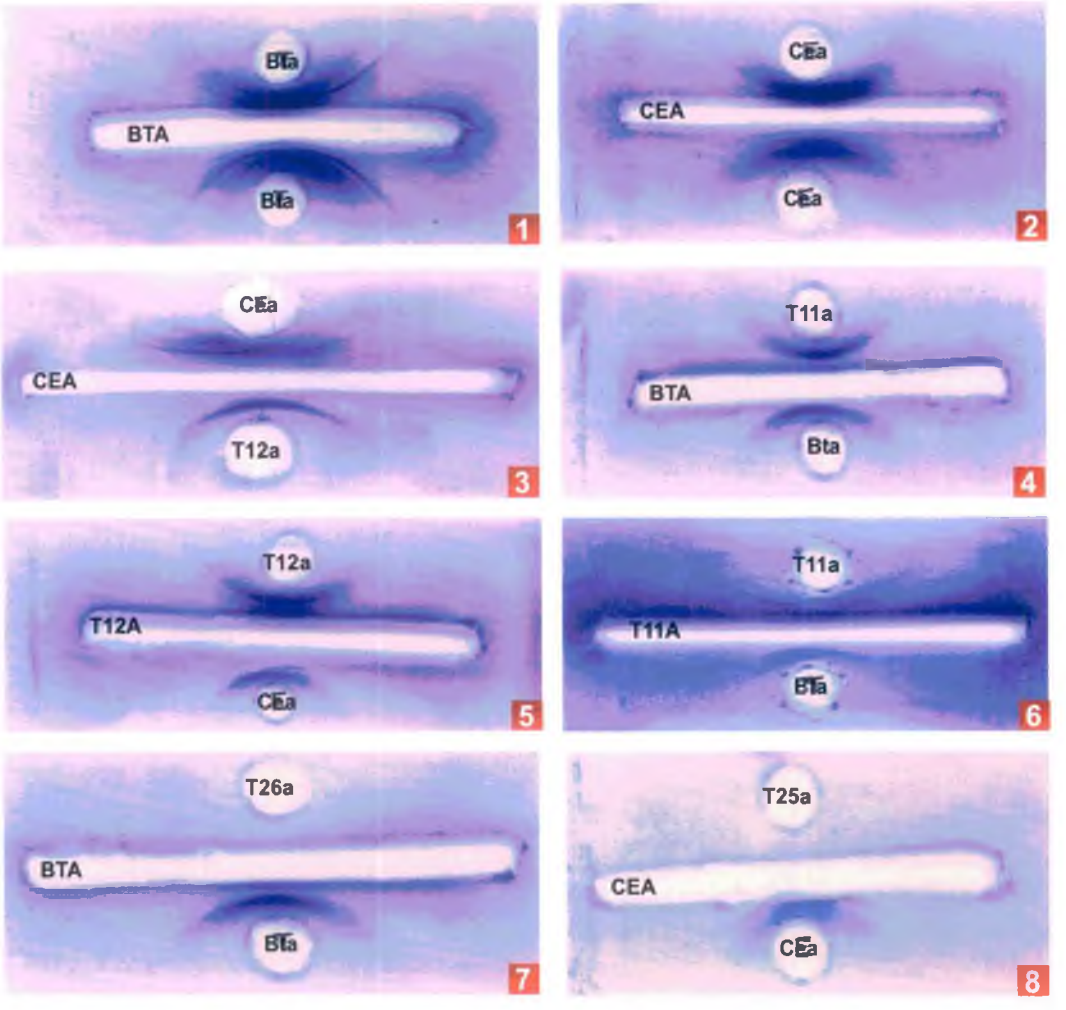


Plate X

Fig.1, 2, 3, 4, 5, 6, 7 & 8 : Immunoelectrophoresis of antigens and antisera. Different antigens used in the wells are of TV-12 (T12a), TV-11 (T11a), TV-26 (T26a), TV-25 (T25a), *Botryodiplodia theobromae* (BTa) and *Curvularia eragrostidis* (CEa). Different antisera used in the central rectangular trough are of *B. theobromae* (BTA),*C. eragrostidis* (CEA), TV-12 (T12A) and TV-11 (T11A).

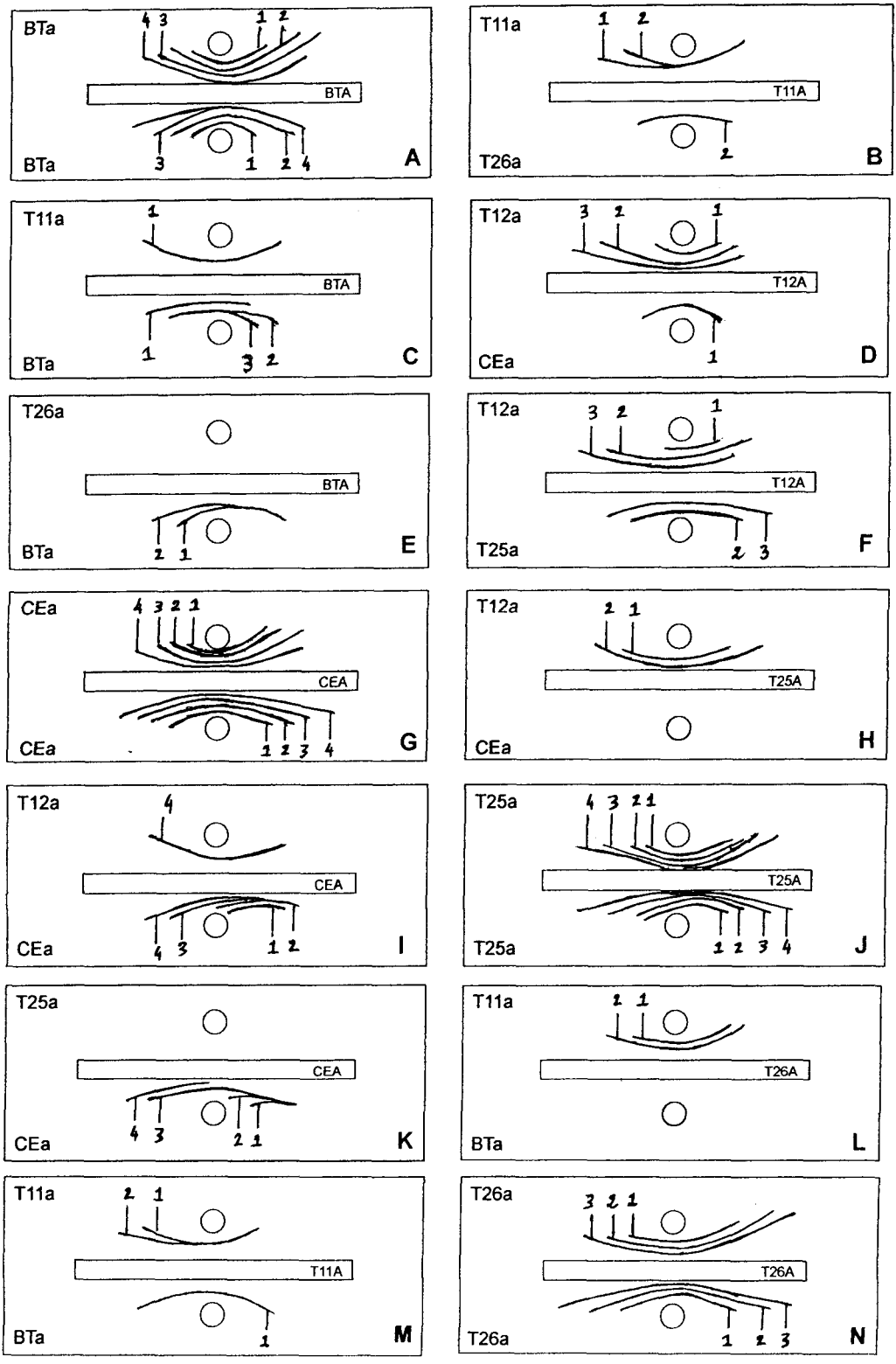


Fig.19(A-N): Immunogram showing immunoelectrophoretic patterns of antigens and antiisera.Different antigens used in the wells are of TV-11 (T11a), TV-12 (T12a),TV-25 (T25a), TV-26 (T26a), *Botryodiplodia theobromae* (BTa) and *Curvularia eragrostidis* (CEa). Different antisera used in the central rectangular trough are of *B.theobromae* (BTA), *C.eragrostidis* (CEA), TV-11(T11A) and TV-12 (T12A).

4.4.3. Immunosorbent assays for detection of cross-reactive antigen

4.4.3.1. Indirect enzyme linked immunosorbent assays (ELISA) between (I) *B. theobromae* and tea varietiers and (II) *C. eragrostidis* and tea varieties

The leaf antigens of twenty-four tea varieties (TV-1, TV-2, TV-3, TV-6, TV-8, TV-9, TV-11, TV-12, TV-16, TV-17, TV-18, TV-19, TV-20, TV-22, TV-23, TV-25, TV-26, TV-27, TV-28, TV-29, TV-30, Teenali 17/1/54, MG-1 and Sundaram), mycelial antigens of the two pathogens (*B. theobromae* and *C. eragrostidis*) and antisera of four tea varieties (TV-11, TV-12, TV-25 and TV-26) as well as the antisera of the two pathogens (mentioned above) were used to perform indirect ELISA. Two different dilutions (1/125 and 1/250) of each antisera and normal sera were tested against three different concentrations (5 μg ml^{-1}, 10 μg ml^{-1} and 20 μg ml^{-1}) of each antigen separately. The preparation of antigens and antisera as well as the procedure of indirect ELISA has already been discussed in details in materials and methods section. The absorbance of all the combinations at 492 nm was determined by an ELISA reader. The results were given in Table 32 and Table 33 respectively for the two pathogens *B. theobromae* and *C. eragrostidis*.

From the Table 32, it was evident that all the three concentrations of the antigens of *B. theobromae* showed higher absobance values when tested with two different dilutions of the antisera of TV-11 than when tested with two different dilutions of the antisera of TV-26. The reciprocal test of this combination also showed higher absorbance values in case of the antigens of TV-11 tested with antisera of *B. theobromae* than in case of the antigens of TV-26 tested with the antisera of *B. theobromae*. These clearly indicated that the cross-reactivity was higher between *B. theobromae* and TV-11 than between *B. theobromae* and TV-26. Results obtained in different assays showed that in all combinations, the absorbance values of normal serum control were lower than the corresponding test values.

From the Table 33, it was evident that all the three concentrations of the antigens of *C. eragrostidis* showed higher absobance values when tested with two different dilutions of the antisera of TV-12 than when tested with two different

Table 32

Indirect ELISA (A_{492}) results of different combinations of antigens (antigens of twenty-four tea varieties and of *B. theobromae*) against three different antisera (antisera of TV-11, TV-26 and *B. theobromae*)

Antigen of host/pa-thogen	µg protein /ml	Normal sera (NS) and antisera (AS) of susceptible and resistant tea varieties and pathogen								
		TV-11 (T11A)			TV-26 (T26A)			*B. theobromae* (BTA)		
		NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250
TV-11 (T11a)	20	0.041	2.986	2.715	0.041	2.257	2.212	0.041	1.494	1.370
	10	0.037	2.929	2.699	0.037	2.238	2.129	0.037	1.406	1.351
	5	0.033	2.867	2.660	0.033	2.180	1.959	0.033	1.342	1.305
TV-12 (T12a)	20	0.040	2.951	2.664	0.040	2.281	2.240	0.040	1.398	1.258
	10	0.036	2.914	2.645	0.036	2.247	2.161	0.036	1.362	1.235
	5	0.032	2.854	2.613	0.032	2.193	2.062	0.032	1.324	1.192
TV-9 (T9a)	20	0.039	2.932	2.643	0.039	2.317	2.266	0.039	1.269	1.239
	10	0.035	2.902	2.606	0.035	2.273	2.188	0.035	1.219	1.191
	5	0.031	2.831	2.579	0.031	2.244	2.094	0.031	1.171	1.146
TV-2 (T2a)	20	0.038	2.915	2.620	0.038	2.351	2.275	0.038	1.207	1.181
	10	0.034	2.867	2.592	0.034	2.335	2.221	0.034	1.183	1.152
	5	0.030	2.820	2.558	0.030	2.296	2.120	0.030	1.137	1.109
TV-1 (T1a)	20	0.041	2.887	2.601	0.041	2.413	2.292	0.041	1.115	1.065
	10	0.038	2.865	2.571	0.038	2.350	2.248	0.038	1.049	0.973
	5	0.035	2.801	2.534	0.035	2.310	2.159	0.035	1.016	0.925
MG-1 (MG1a)	20	0.040	2.863	2.575	0.040	2.442	2.317	0.040	0.969	0.895
	10	0.037	2.840	2.540	0.037	2.416	2.260	0.037	0.928	0.871
	5	0.034	2.792	2.512	0.034	2.397	2.177	0.034	0.896	0.808
TV-23 (T23a)	20	0.039	2.831	2.549	0.039	2.479	2.349	0.039	0.941	0.881
	10	0.036	2.795	2.506	0.036	2.435	2.303	0.036	0.899	0.860
	5	0.033	2.766	2.471	0.033	2.424	2.236	0.033	0.856	0.799
TV-8 (T8a)	20	0.038	2.799	2.513	0.038	2.512	2.376	0.038	0.924	0.876
	10	0.035	2.780	2.475	0.035	2.474	2.347	0.035	0.881	0.856
	5	0.032	2.734	2.445	0.032	2.449	2.268	0.032	0.835	0.783

Table 32 (Contd.....)

Table 32 (Contd.....)

Indirect ELISA (A_{492}) results of different combinations of antigens (antigens of twenty-four tea varieties and of *B. theobromae*) against three different antisera (antisera of TV-11, TV-26 and *B. theobromae*)

Antigen of host/pa-thogen	µg protein/ ml	Normal sera (NS) and antisera (AS) of susceptible and resistant tea varieties and pathogen								
		TV-11 (T11A)			TV-26 (T26A)			*B. theobromae* (BTA)		
		NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250
Teenali 17/1/54 (TLIa)	20	0.037	2.770	2.489	0.037	2.544	2.401	0.037	0.873	0.822
	10	0.034	2.758	2.450	0.034	2.493	2.373	0.034	0.754	0.808
	5	0.031	2.701	2.426	0.031	2.471	2.279	0.031	0.824	0.771
TV-20 (T20a)	20	0.036	2.745	2.461	0.036	2.599	2.423	0.036	0.773	0.722
	10	0.033	2.724	2.429	0.033	2.533	2.408	0.033	0.754	0.718
	5	0.030	2.683	2.396	0.030	2.513	2.281	0.030	0.724	0.671
TV-25 (T25a)	20	0.041	2.716	2.426	0.041	2.628	2.462	0.041	0.738	0.685
	10	0.039	2.682	2.390	0.039	2.554	2.435	0.039	0.715	0.661
	5	0.037	2.666	2.365	0.037	2.536	2.314	0.037	0.686	0.649
TV-27 (T27a)	20	0.040	2.683	2.393	0.040	2.639	2.486	0.040	0.689	0.647
	10	0.038	2.636	2.361	0.038	2.604	2.465	0.038	0.665	0.630
	5	0.036	2.608	2.338	0.036	2.551	2.393	0.036	0.625	0.596
TV-19 (T19a)	20	0.039	2.647	2.360	0.039	2.654	2.515	0.039	0.661	0.636
	10	0.037	2.624	2.333	0.037	2.624	2.481	0.037	0.633	0.611
	5	0.035	2.590	2.302	0.035	2.562	2.426	0.035	0.608	0.580
TV-18 (T18a)	20	0.038	2.608	2.345	0.038	2.686	2.549	0.038	0.640	0.626
	10	0.036	2.566	2.296	0.036	2.626	2.510	0.036	0.611	0.592
	5	0.034	2.543	2.254	0.034	2.599	2.440	0.034	0.594	0.573
TV-29 (T29a)	20	0.037	2.573	2.321	0.037	2.716	2.587	0.037	0.621	0.607
	10	0.035	2.558	2.286	0.035	2.684	2.531	0.035	0.588	0.572
	5	0.033	2.505	2.247	0.033	2.621	2.465	0.033	0.576	0.560
TV-22 (T22a)	20	0.036	2.554	2.309	0.036	2.758	2.603	0.036	0.593	0.573
	10	0.034	2.533	2.230	0.034	2.701	2.540	0.034	0.584	0.541
	5	0.032	2.470	2.185	0.032	2.638	2.518	0.032	0.562	0.508

Table 32 (Contd.....)

Table 32 (Contd.....)

Indirect ELISA (A_{492}) results of different combinations of antigens (antigens of twenty-four tea varieties and of *B. theobromae*) against three different antisera (antisera of TV-11, TV-26 and *B. theobromae*)

Antigen of host/pa-thogen	µg protein/ ml	Normal sera (NS) and antisera (AS) of susceptible and resistant tea varieties and pathogen								
		TV-11 (T11A)			TV-26 (T26A)			*B. theobromae* (BTA)		
		NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250
TV-3 (T3a)	20	0.035	2.531	2.269	0.035	2.786	2.620	0.035	0.575	0.551
	10	0.033	2.492	2.209	0.033	2.712	2.592	0.033	0.558	0.520
	5	0.031	2.431	2.176	0.031	2.651	2.541	0.031	0.541	0.495
TV-28 (T28a)	20	0.034	2.503	2.251	0.034	2.803	2.651	0.034	0.549	0.532
	10	0.032	2.480	2.180	0.032	2.762	2.618	0.032	0.537	0.512
	5	0.030	2.429	2.149	0.030	2.691	2.580	0.030	0.515	0.478
TV-16 (T16a)	20	0.038	2.478	2.237	0.038	2.817	2.684	0.038	0.533	0.508
	10	0.037	2.439	2.175	0.037	2.789	2.650	0.037	0.492	0.471
	5	0.036	2.399	2.119	0.036	2.724	2.616	0.036	0.457	0.440
TV-17 (T17a)	20	0.032	2.452	2.198	0.032	2.850	2.721	0.032	0.527	0.481
	10	0.031	2.422	2.164	0.031	2.817	2.702	0.031	0.484	0.462
	5	0.030	2.362	2.115	0.030	2.771	2.633	0.030	0.447	0.380
Sundar am (SUNa)	20	0.041	2.435	2.162	0.041	2.902	2.768	0.041	0.515	0.468
	10	0.040	2.399	2.131	0.040	2.839	2.723	0.040	0.467	0.448
	5	0.039	2.329	2.106	0.039	2.813	2.654	0.039	0.416	0.361
TV-30 (T30a)	20	0.039	2.412	2.130	0.039	2.941	2.780	0.039	0.498	0.451
	10	0.037	2.360	2.092	0.037	2.907	2.751	0.037	0.435	0.419
	5	0.035	2.310	2.059	0.035	2.888	2.695	0.035	0.399	0.373
TV-6 (T6a)	20	0.038	2.378	2.098	0.038	2.986	2.803	0.038	0.466	0.412
	10	0.036	2.341	2.041	0.036	2.937	2.772	0.036	0.421	0.395
	5	0.035	2.288	2.017	0.035	2.891	2.721	0.035	0.385	0.361
TV-26 (T26a)	20	0.040	2.346	2.053	0.040	2.995	2.813	0.040	0.392	0.365
	10	0.038	2.315	2.010	0.038	2.941	2.784	0.038	0.318	0.299
	5	0.035	2.267	1.962	0.035	2.902	2.750	0.035	0.298	0.257
B. t. (BTa)	20	0.035	2.170	2.008	0.035	0.559	0.468	0.035	2.281	2.191
	10	0.033	1.884	1.761	0.033	0.518	0.436	0.033	2.180	2.170
	5	0.030	1.845	1.699	0.030	0.470	0.401	0.030	2.158	2.141

B. t. = *B. theobromae*.

*Codes of antigens and antisera are in the parenthesis.

dilutions of the antisera of TV-25. The reciprocal test of this combination also showed higher absorbance values in case of the antigens of TV-12 tested with antisera of *C. eragrostidis* than in case of the antigens of TV-25 tested with the antisera of *C. eragrostidis*. These clearly indicated that the cross-reactivity was higher between *C. eragrostidis* and TV-12 than between *C. eragrostidis* and TV-25. Results obtained in different assays showed that in all combinations, the absorbance values of normal serum control were lower than the corresponding test values.

4.4.3.2. Immunofluorescence between (i) *B. theobromae* and tea varieties and (ii) *C. eragrostidis* and tea varieties

It was evident from the earlier serological experiments like immunodiffusion, immunoelectrophoresis and indirect enzyme linked immunosorbent assay (indirect ELISA) that cross-reactive antigens (CRA) were present between tea clonal varieties and two pathogens (*B. theobromae* and *C. eragrostidis*). To find out tissue and cellular location of CRA shared by the two pathogens and tea leaves, immunofluorescence studies were performed. Leaf sections (cut through midrib) of TV-11 and TV-26 (susceptible and resistant respectively against *B. theobromae*); TV-12 and TV-26 (susceptible and resistant respectively against *C. eragrostidis*) and mycelia of *B. theobromae* and *C. eragrostidis* were used as antigens. The antisera of *B. theobromae*, *C. eragrostidis*, TV-11, TV-12, TV-25 and TV-26 were used in the experiments. To determine the exact location of CRA, both leaf section and fungal mycelia were treated with antisera labeled indirectly with fluorescein isothiocyanate (FITC). The details of the procedure has been discussed in the materials and method (Section 3.13.5.2).

From the observation of unstained leaf sections under UV-fluorescence microscope, it was revealed that all the leaf sections showed faint natural autofluorescence in the outer walls of the epidermal cells. Similar results were observed when the leaf sections were treated with normal serum followed by FITC labeling.. When the leaf sections were treated with the antisera of respective leaf varieties (i.e. with homologous antisera) and indirectly labeled with FITC, they showed bright fluorescence in the epidermal regions, mesophyll tissues and xylem elements of the leaf (Plate XI : 1 & 3; Plate XII : 1 & 3). In heterologous treatments

Table 33

Indirect ELISA (A_{492}) results of different combinations of antigens (antigens of 24 tea varieties and of *C. eragrostidis*) against three different antisera (antisera of TV-12, TV-25 and *C. eragrostidis*)

Antigen of host/pa-thogen	pg protein/ ml	Normal sera (NS) and antisera (AS) of susceptible and resistant tea varieties and pathogen								
		TV-12 (T12A)			TV-25 (T25A)			*C. eragrostidis* (CEA)		
		NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250
TV-12 (T12a)	20	0.040	2.997	2.776	0.040	2.260	2.225	0.040	1.585	1.492
	10	0.036	2.936	2.701	0.036	2.248	2.136	0.036	1.536	1.445
	5	0.032	2.871	2.682	0.032	2.185	1.961	0.032	1.491	1.401
TV-11 (T11a)	20	0.041	2.969	2.675	0.041	2.289	2.241	0.041	1.558	1.469
	10	0.037	2.920	2.648	0.037	2.251	2.163	0.037	1.527	1.421
	5	0.033	2.853	2.620	0.033	2.196	2.071	0.033	1.472	1.386
TV-27 (T27a)	20	0.040	2.930	2.652	0.040	2.328	2.275	0.040	1.502	1.410
	10	0.038	2.894	2.611	0.038	2.271	2.190	0.038	1.471	1.383
	5	0.036	2.850	2.595	0.036	2.239	2.102	0.036	1.456	1.359
TV-29 (T29a)	20	0.037	2.921	2.637	0.037	2.347	2.282	0.037	1.481	1.361
	10	0.035	2.860	2.599	0.035	2.325	2.233	0.035	1.459	1.327
	5	0.033	2.817	2.551	0.033	2.299	2.125	0.033	1.426	1.299
TV-23 (T23a)	20	0.039	2.891	2.621	0.039	2.381	2.297	0.039	1.450	1.358
	10	0.036	2.862	2.580	0.036	2.348	2.254	0.036	1.421	1.319
	5	0.033	2.815	2.539	0.033	2.316	2.162	0.033	1.405	1.285
TV-19 (T19a)	20	0.039	2.865	2.581	0.039	2.435	2.319	0.039	1.399	1.306
	10	0.037	2.849	2.551	0.037	2.426	2.268	0.037	1.361	1.268
	5	0.035	2.790	2.526	0.035	2.386	2.190	0.035	1.325	1.217
TV-17 (T17a)	20	0.032	2.847	2.561	0.032	2.486	2.351	0.032	1.368	1.277
	10	0.031	2.801	2.517	0.031	2.440	2.306	0.031	1.331	1.236
	5	0.030	2.710	2.488	0.030	2.431	2.237	0.030	1.310	1.196
TV-16 (T16a)	20	0.038	2.800	2.521	0.038	2.513	2.381	0.038	1.304	1.210
	10	0.037	2.776	2.482	0.037	2.481	2.349	0.037	1.287	1.176
	5	0.036	2.741	2.444	0.036	2.458	2.269	0.036	1.262	1.128

Table 33 (Contd.....)

Table 33 (Contd.....)

Indirect ELISA (A_{492}) results of different combinations of antigens (antigens of 24 tea varieties and of *C. eragrostidis*) against three different antisera (antisera of TV-12, TV-25 and *C. eragrostidis*)

Antigen of host/pa -thogen	µg protein/ ml	Normal sera (NS) and antisera (AS) of susceptible and resistant tea varieties and pathogen								
		TV-12 (T12A)			TV-25 (T25A)			*C. eragrostidis* (CEA)		
		NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250
TV-2 (T2a)	20	0.038	2.796	2.508	0.038	2.551	2.402	0.038	1.273	1.191
	10	0.034	2.762	2.489	0.034	2.504	2.373	0.034	1.247	1.142
	5	0.030	2.735	2.450	0.030	2.486	2.281	0.030	1.218	1.115
TV-30 (T30a)	20	0.039	2.767	2.498	0.039	2.606	2.423	0.039	1.245	1.148
	10	0.037	2.758	2.475	0.037	2.543	2.418	0.037	1.209	1.109
	5	0.035	2.721	2.441	0.035	2.517	2.287	0.035	1.186	0.976
TV-1 (T1a)	20	0.041	2.725	2.466	0.041	2.630	2.470	0.041	1.213	1.110
	10	0.038	2.703	2.451	0.038	2.566	2.444	0.038	1.180	0.983
	5	0.035	2.697	2.435	0.035	2.542	2.315	0.035	1.135	0.951
MG-1 (MG1a)	20	0.040	2.694	2.460	0.040	2.645	2.491	0.040	1.182	0.980
	10	0.037	2.667	2.443	0.037	2.611	2.473	0.037	1.148	0.948
	5	0.034	2.649	2.425	0.034	2.558	2.368	0.034	1.116	0.926
TV-28 (T28a)	20	0.034	2.658	2.453	0.034	2.661	2.525	0.034	1.145	0.958
	10	0.032	2.642	2.435	0.032	2.631	2.496	0.032	1.111	0.906
	5	0.030	2.631	2.416	0.030	2.566	2.399	0.030	0.988	0.871
TV-2 (T2a)	20	0.038	2.646	2.440	0.038	2.687	2.561	0.038	1.108	0.899
	10	0.034	2.637	2.426	0.034	2.640	2.521	0.034	0.999	0.859
	5	0.030	2.621	2.412	0.030	2.571	2.426	0.030	0.953	0.815
TV-20 (T20a)	20	0.036	2.626	2.399	0.036	2.721	2.585	0.036	1.064	0.835
	10	0.033	2.611	2.377	0.033	2.679	2.542	0.033	1.005	0.806
	5	0.030	2.596	2.351	0.030	2.623	2.447	0.030	0.932	0.762
TV-18 (T18a)	20	0.038	2.599	2.358	0.038	2.768	2.599	0.038	1.006	0.796
	10	0.036	2.580	2.333	0.036	2.723	2.587	0.036	0.915	0.747
	5	0.034	2.558	2.296	0.034	2.640	2.469	0.034	0.867	0.716

Table 33 (Contd.....)

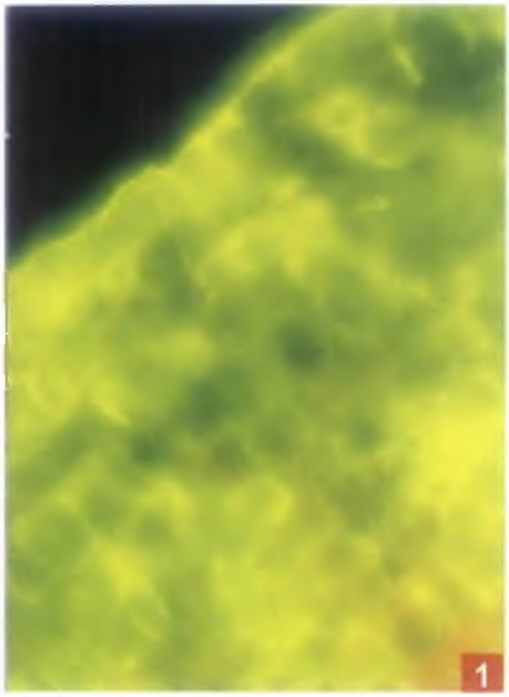
Table 33 (Contd.....)

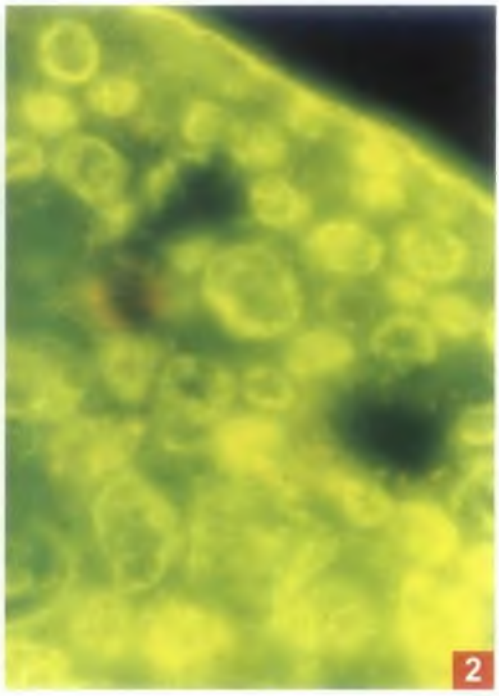
Indirect ELISA (A_{492}) results of different combinations of antigens (antigens of 24 tea varieties and of *C. eragrostidis*) against three different antisera (antisera of TV-12, TV-25 and *C. eragrostidis*)

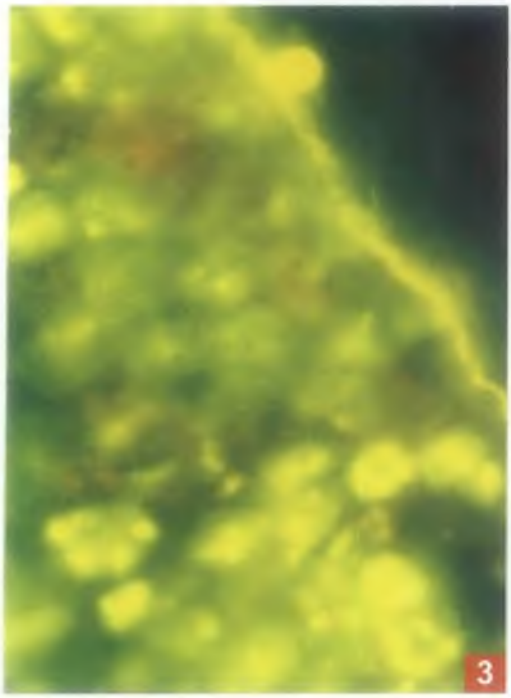
Antigen of host/pa-thogen	µg protein/ ml	Normal sera (NS) and antisera (AS) of susceptible and resistant tea varieties and pathogen								
		TV-12 (T12A)			TV-25 (T25A)			*C. eragrostidis* (CEA)		
		NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250	NS 1/125	AS 1/125	AS 1/250
TV-9 (T9a)	20	0.039	2.561	2.325	0.039	2.795	2.619	0.039	0.945	0.735
	10	0.035	2.545	2.291	0.035	2.751	2.601	0.035	0.866	0.692
	5	0.031	2.522	2.265	0.031	2.661	2.488	0.031	0.818	0.648
TV-6 (T6a)	20	0.038	2.537	2.300	0.038	2.823	2.655	0.038	0.836	0.696
	10	0.036	2.510	2.269	0.036	2.765	2.626	0.036	0.720	0.637
	5	0.035	2.490	2.236	0.035	2.689	2.532	0.035	0.677	0.602
Teenali 17/1/54 (TLIa)	20	0.037	2.496	2.266	0.037	2.847	2.681	0.037	0.713	0.628
	10	0.034	2.435	2.230	0.034	2.792	2.661	0.034	0.661	0.590
	5	0.031	2.401	2.205	0.031	2.726	2.572	0.031	0.624	0.543
TV-3 (T3a)	20	0.035	2.459	2.241	0.035	2.870	2.709	0.035	0.675	0.583
	10	0.033	2.422	2.208	0.033	2.821	2.687	0.033	0.631	0.531
	5	0.031	2.391	2.185	0.031	2.757	2.601	0.031	0.596	0.494
Sundar am (SUNa)	20	0.041	2.425	2.210	0.041	2.899	2.752	0.041	0.619	0.529
	10	0.040	2.390	2.188	0.040	2.845	2.710	0.040	0.572	0.482
	5	0.039	2.363	2.149	0.039	2.792	2.639	0.039	0.458	0.449
TV-26 (T26a)	20	0.040	2.394	2.187	0.040	2.922	2.781	0.040	0.546	0.475
	10	0.038	2.366	2.138	0.038	2.866	2.742	0.038	0.465	0.421
	5	0.035	2.331	2.095	0.035	2.815	2.665	0.035	0.416	0.383
TV-8 (T8a)	20	0.038	2.365	2.158	0.038	2.939	2.805	0.038	0.476	0.390
	10	0.035	2.318	2.116	0.035	2.878	2.767	0.035	0.405	0.339
	5	0.032	2.286	2.075	0.032	2.839	2.692	0.032	0.358	0.298
TV-25 (T25a)	20	0.041	2.329	2.126	0.041	2.964	2.848	0.041	0.401	0.348
	10	0.039	2.281	2.092	0.039	2.896	2.795	0.039	0.343	0.286
	5	0.037	2.262	2.046	0.037	2.861	2.727	0.037	0.291	0.221
C.e (CEa)	20	0.035	2.170	2.008	0.035	0.559	0.468	0.035	2.281	2.191
	10	0.033	1.884	1.761	0.033	0.518	0.436	0.033	2.180	2.170
	5	0.030	1.845	1.699	0.030	0.470	0.401	0.030	2.158	2.141

C. e. = *C. eragrostidis.*

*Codes of antigens and antisera are in the parenthesis.







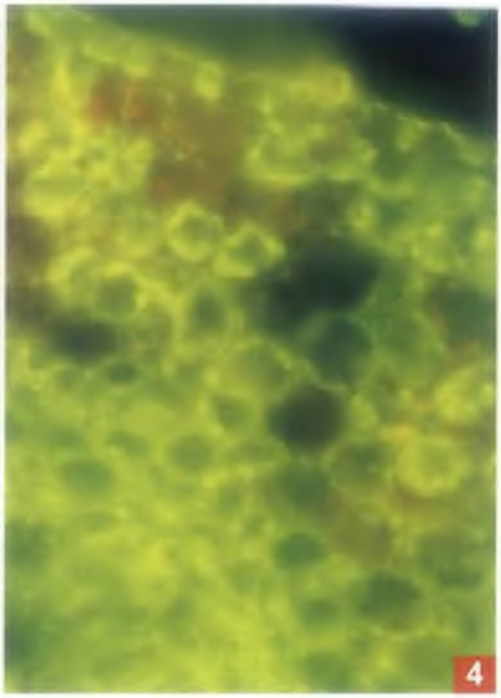


Plate XI

Fluorescein isothiocyanate (FITC)-antibody staining of tea leaf tissues for detection of cross-reactive antigens and homologous antigens.

Fig.1 : Leaf section (TV-11) treated with antisera of TV-11 (T11A) and FITC-antirabbit goat antisera conjugate.

Fig. 2 : Leaf section (TV-11) treated with antisera of *Botryodiplodia theobromae* (BTA) and FITC-antirabbit goat antisera conjugate.

Fig. 3 : Leaf section (TV-26) treated with antisera of TV-26 (T26A) and FITC-antirabbit goat antisera conjugate.

Fig. 4 : Leaf section (TV-26) treated with antisera of *B. theobromae* (BTA) and FITC-antirabbit goat antisera conjugate.

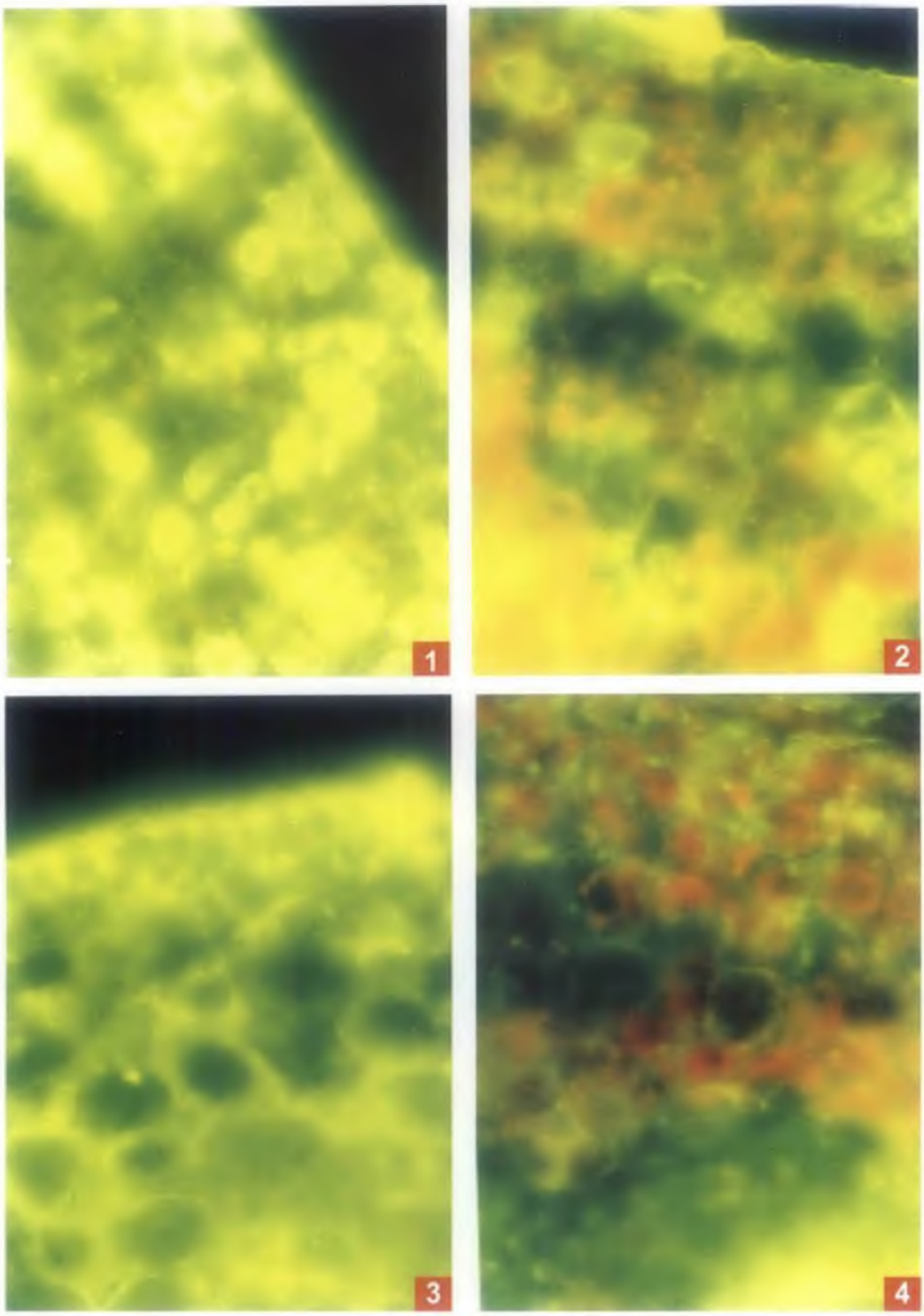


Plate XII

Fluorescein isothiocyanate (FITC)-antibody staining of tea leaf tissues for detection of cross-reactive antigens and homologous antigens.

Fig.1 : Leaf section (TV-12) treated with antisera of TV-12 (T12A) and FITC-antirabbit goat antisera conjugate.

Fig.2 : Leaf section (TV-12) treated with antisera of *Curvularia eragrostidis* (CEA) and FITC-antirabbit goat antisera conjugate.

Fig.3 : Leaf section (TV-25) treated with antisera of TV-25 (T25A) and FITC-antirabbit goat antisera conjugate.

Fig.4 : Leaf section (TV-25) treated with antisera of *C. eragrostidis* (CEA) and FITC-antirabbit goat antisera conjugate.

i.e. when the leaf sections of susceptible variety (TV- 11) were treated with antisera of *B. theobromae* and indirectly labeled with FITC, strong fluorescence was visible (Plate XI : 2). Comperatively less fluorescence was visible when the leaf section of the resistant variety (TV-25) were treated with antisera of *B. theobromae* and indirectly labeled with FITC (Plate XI : 4). Similarly, strong fluorescence was visible when leaf section of TV-12 (susceptible variety) was indirectly labeled with FITC following *C. eragrostidis* antisera treatment (plate XII :2). Very faint fluorescence was observed when leaf section of TV-25 (resistant variety) was indirectly labeled after *C. eragrostidis* antisera treatment (Plate XII :4) The CRA observed in heterologous reactions, were mainly concentrated around the epidermal cells. No fluorescence was observed when fungal mycelia and conidia (of *B. theobromae* and *C. eragrostidis*) were observed under fluorescence microscope. When fungal mycelia was treated with normal sera and FITC conjugate, no fluorescence was observed. But when *B. theobromae* was treated with its antisera and labeled with FITC conjugate, strong fluorescence was observed around the hyphae and conidia. In cross-reactions, when the fungal mycelia were treated with antisera of susceptible varieties indirectly labeled with FITC, fluorescence was observed in the hyphal tips and conidia but the fluorescence was not so strong when the mycelia were treated with the antisera of resistant varieties and labeled with FITC.

4.5.. Alteration of disease reaction (disease index) following application of various chemicals in selected tea varieties against *B. theobromae* and *C. eragrostidis*

4.5.1. Induction of resistance by treating with various chemicals

Several chemicals have been recorded to induce systemic acquired resistance (SAR) which alters the disease reaction so that the whole plant resists pathogen attack. Hence as a strategy to control the disease caused by *B. theobromae* and *C. eragrostidis*, the following work was undertaken. Nickel chloride, salicylic acid, jasmonic acid, indole acetic acid, cadmium chloride, sodium selenite, sodium molybdate, sodium azide and indole butyric acid were selected for spraying at three different concentrations (10^{-3}M, 10^{-4}M and 10^{-5}M) as described in materials and methods (section 3.14). Twigs with 3-4 leaves of TV-11 (most susceptible

against *B. theobromae*) and TV-12 (most susceptible against *C. eragrostidis*) were collected and they were sprayed with all the three concentrations of the chemicals mentioned. Along with the above chemicals commercially available jasmonic acid at 10^{-3} M concentration were sprayed in a closed chamber. Fresh aqueous leaf extracts (2 g fresh weight/10 ml of distilled water) from *Jasminum jasminoides* were also sprayed on the twigs. After 24 hours of spraying, twigs of TV-11 were inoculated with *B. theobromae* and the twigs of TV-12 were inoculated with *C. eragrostidis* by cut shoot inoculation technique as suggested by Dickens and Cook (1989). Sterile distilled water was sprayed on the control twigs. Results were taken as described in materials and methods (Section 3.14) and listed in Table 34 and Table 35 respectively for the two pathogens *B. theobromae* and *C. eragrostidis*.

From Table 34, it was evident that disease induction (mean disease index/ shoot) by *B. theobromae* in the cases where twigs were sprayed with indole acetic acid, cadmium chloride and sodium selenite (in all the three concentrations) were very much similar with the control (where no chemicalswere sprayed) after 24, 48 and 72 h of inoculation. In case of sodium molybdate, sodium azide and indole butyric acid, mean disease index/shoot was found to be higher than control in all the three concentrations. But when the twigs were sprayed with the three different concentrations of nickel chloride and salicylic acid, low concentration of jasmonic acid and with fresh aqueous leaf extract of *J. jasminoides* the mean disease index/ shoot values were less than the control.

In case of *C. eragrostidis,* the mean disease index were found less compare to control after 24, 48 and 72 h of inoculation only when the twigs of TV-12 were sprayed with the three different concentrations of nickel chloride and salicylic acid, with low concentration of jasmonic acid and with fresh aqueous leaf extract of *J. jasminoides* as evident from the Table 35. It was also revealed from the Table 35 that the mean disease index/shoot was maximum when the twigs were sprayed with higher concentration of the chemicals but minimum when the concentrations of the corresponding chemicals were low.

From the results given in Table 34 and Table 35, it was quite clear that the disease occurrence were less compared to control against the two pathogens *B. theobromae* and *C. eragrostidis* when the susceptible twigs were sprayed with

Table 34

Induction of disease resistance in susceptible tea variety (TV-11) against *B. theobromae* following treatment of nine different chemicals and one plant extract

Chemical Treatment	Concentration	Mean disease index/shoot*		
		Incubation period		
		24 hours	48hours	72 hours
Nickle Chloride	10^{-3} M	0.33±0.044	0.41±0.043	0.45±0.040
	10^{-4} M	0.39±0.031	0.55±0.030	0.70±0.028
	10^{-5} M	0.39±0.037	0.68±0.025	0.82±0.030
Salicylic Acid	10^{-3} M	0.25±0.030	0.63±0.026	0.83±0.036
	10^{-4} M	0.31±0.030	0.69±0.043	0.91±0.035
	10^{-5} M	0.39±0.038	0.82±0.047	1.08±0.032
Jasmonic Acid	10^{-3} M	0.29±0.032	0.35±0.030	0.58±0.034
Jasminum jasminoides	E	0.32±0.066	0.41±0.021	0.63±0.055
Indole Acetic Acid	10^{-3} M	0.41±0.036	0.75±0.055	1.25±0.041
	10^{-4} M	0.45±0.036	0.86±0.031	1.31±0.045
	10^{-5} M	0.46±0.051	0.91±0.021	1.45±0.048
Cadmium Chloride	10^{-3} M	0.36±0.036	0.62±0.030	0.96±0.046
	10^{-4} M	0.41±0.055	0.70±0.036	1.28±0.029
	10^{-5} M	0.42±0.017	0.72±0.038	1.35±0.025
Sodium Selenite	10^{-3} M	0.30±0.025	0.69±0.030	1.09±0.033
	10^{-4} M	0.37±0.027	0.77±0.043	1.26±0.040
	10^{-5} M	0.38±0.061	0.85±0.030	1.40±0.045
Sodium Molybdate	10^{-3} M	0.65±0.030	1.30±0.036	1.58±0.028
	10^{-4} M	0.96±0.038	1.49±0.030	1.62±0.045
	10^{-5} M	1.00±0.021	1.58±0.036	1.69±0.030
Sodium Azide	10^{-3} M	0.55±0.031	0.99±0.030	1.32±0.038
	10^{-4} M	0.70±0.038	1.05±0.021	1.46±0.028
	10^{-5} M	0.81±0.021	1.40±0.025	1.60±0.039
Indole Butyric Acid	10^{-3} M	0.56±0.026	1.07±0.049	1.29±0.052
	10^{-4} M	0.68±0.021	1.35±0.032	1.46±0.047
	10^{-5} M	0.77±0.064	1.44±0.021	1.59±0.035
Control (Untreated-inoculated)		0.46±0.053	1.05±0.055	1.70±0.058

E = Fresh aqueous extract of leaves from *Jasminum jasminoides* (2 g fresh weight / 10 ml distilled water).

* Mean of 3 replications.

Data after± represent standard error values.

Table 35

Induction of disease resistance in susceptible tea variety (TV-12) against *C. eragrostidis* following treatment of nine different chemicals and one plant extract

Chemical Treatment	Concentration	Mean disease index/shoot* Incubation period		
		24 hours	48hours	72 hours
Nickle Chloride	10^{-3} M	0.23±0.040	0.34±0.042	0.39±0.051
	10^{-4} M	0.28±0.035	0.39±0.025	0.42±0.036
	10^{-5} M	0.36±0.021	0.41±0.027	0.43±0.034
Salicylic Acid	10^{-3} M	0.21±0.025	0.39±0.031	0.86±0.042
	10^{-4} M	0.28±0.035	0.42±0.028	0.89±0.051
	10^{-5} M	0.32±0.030	0.51±0.047	0.94±0.035
Jasmonic Acid	10^{-3} M	0	0.19±0.025	0.26±0.028
Jasminum jasminoides	E	0.12±0.015	0.21±0.019	0.29±0.024
Indole Acetic Acid	10^{-3} M	0	0.34±0.051	0.96±0.058
	10^{-4} M	0.30±0.040	0.55±0.061	1.25±0.065
	10^{-5} M	0.75±0.048	0.95±0.058	1.29±0.070
Cadmium Chloride	10^{-3} M	0	0.52±0.042	1.25±0.053
	10^{-4} M	0	0.60±0.035	0.32±0.057
	10^{-5} M	0.40±0.025	0.65±0.057	1.40±0.063
Sodium Selenite	10^{-3} M	0.30±0.028	0.70±0.030	1.20±0.055
	10^{-4} M	0.35±0.035	0.75±0.046	1.30±0.061
	10^{-5} M	0.41±0.021	0.92±0.057	1.45±0.072
Sodium Molybdate	10^{-3} M	0.30±0.060	0.70±0.045	1.20±0.075
	10^{-4} M	0.40±0.064	0.70±0.056	1.36±0.081
	10^{-5} M	0.40±0.069	0.75±0.048	1.50±0.070
Sodium Azide	10^{-3} M	0.40±0.033	0.75±0.048	1.70±0.075
	10^{-4} M	0.55±0.057	1.30±0.040	1.80±0.081
	10^{-5} M	0.80±0.061	2.20±0.028	2.75±0.084
Indole Butyric Acid	10^{-3} M	0.30±0.033	0.70±0.036	1.00±0.066
	10^{-4} M	0.32±0.030	0.70±0.046	1.30±0.060
	10^{-5} M	0.55±0.042	1.20±0.050	2.20±0.070
Control (Untreated-inoculated)		0.46±0.051	0.46±0.060	0.71±0.068

E = Fresh aqueous extract of leaves from *Jasminum jasminoides* (2 g fresh weight/ 10 ml distilled water).

* Mean of 3 replications.

Data after± represent standard error values.

nickel chloride, salicylic acid and jasmonic acid. Therefore it could be concluded that these chemicals could induce resistance in susceptible twigs against the two pathogens. Keeping this in mind it was considered worthwhile to select these chemicals for further experiments. Nickel chloride, salicylic acid and jasmonic acid at a concentration of 10^{-3} M were prepared and sprayed on the twigs of TV-11, TV-12, TV-25 and TV-26. After 24 h of spraying, twigs of TV-11and TV-26 (most susceptible and resistant varieties respectively against *B. theobromae)* were inoculated with *B. theobromae* and twigs of TV-12 and TV-25 (most susceptible and resistant varieties respectively against *C. eragrostidis)* were inoculated with *C. eragrostidis*. In control sets, the twigs were inoculated with the pathogens without pretreatment with the chemicals. The inoculation was done following the cut shoot inoculation technique of Dickens and Cook (1989) as described in materials and method (Section 3.14) and mean disease index/shoot after 24, 48 and 72 h of inoculation following the method of Sinha and Das (1972) was calculated. The results were given in Table 36 and Table 37 respectively for the two pathogens *B. theobromae* and *C. eragrostidis*.

Table 36 and Fig. 20 showed that the mean disease index/shoot was 0.45 after 72 h of inoculation with *B. theobromae* when the susceptible variety (TV-11) was sprayed with nickel chloride but under similar conditions the value was 0.31 in case of resistant variety (TV-26). When salicylic acid was sprayed, mean disease index/shoot was recorded to be 0.79 after 72 h when sprayed to the susceptible variety but the value was recorded to be 0.13 incase of resistant variety. In case of jasmonic acid, mean disease index/shoot of the susceptible variety was found to be 0.62 after 72 h and that of the resistant variety was found to be 0.20.From the results it was evident that the values of mean disease index/shoot in all the cases were lower than the mean disease index/shoot values of control sets after 24, 48 and 72 h of inoculation.

Table 37 and Fig. 21 shows that the mean disease index/shoot was 0.63 after 72 hours of inoculation with *C. eragrostidis* when the susceptible variety (TV-12) were sprayed with nickel chloride but under similar conditions the mean disease index/shoot was 0.19 in case of resistant variety (TV-25). Similarly, the mean disease index/shoot was recorded to be 0.98 after 72 h of inoculation when salicylic acid

Table 36

Disease incidence in susceptible and resistant tea varieties (TV-11 and TV-26 respectively) against *B. theobromae* following treatment of three selected chemicals

Chemical treatment (10^{-3} M concentration)	Clonal variety	Mean disease index/shoot*		
		Incubation period		
		24 hours	48 hours	72 hours
Nickel Chloride	TV-11	0.32±0.055	0.42±0.055	0.45±0.057
	TV-26	0.13±0.047	0.24±0.072	0.31±0.046
Salicylic Acid	TV-11	0.29±0.075	0.61±0.060	0.79±0.049
	TV-26	0.08±0.040	0.12±0.036	0.13±0.043
Jasmonic Acid	TV-11	0.34±0.083	0.48±0.058	0.62±0.049
	TV-26	0.12±0.047	0.16±0.049	0.20±0.055
Control (Untreated-inoculated)	TV-11	0.68±0.057	0.93±0.049	1.70±0.049
	TV-26	0.15±0.038	0.26±0.055	0.38±0.053
CD at 5%		0.36	0.35	0.18
CD at 1%		0.32	0.31	0.16

* Mean of 3 replications.
Data after± represent standard error values.

was sprayed to the susceptible variety but the same was recorded to be 0.16 when sprayed to the resistant variety. When jasmonic acid was sprayed to the susceptible variety, mean disease index/shoot was found to be 0.84 after 72 h of inoculation but the value was found to be 0.33 when sprayed to the resistant variety. In control sets, the mean disease index/shoot was lower than the treated sets both in susceptible and resistant varieties after 24, 48 and 72 h of inoculation.

4.5.2. Changes in orthodihydroxy phenol and total phenol contents of healthy leaves and leaves inoculated with *B. theobromae* and *C. eragrostidis*

Accordingly, twigs of TV-11(most susceptible to *B. theobromae*), TV-12 (most susceptible to *C. eragrostidis*), TV-25 (most resistant to *C. eragrostidis*) and TV-26 (most resistant to *B. theobromae*) were collected and sprayed with 10^{-3} M nickel chloride, salicylic acid and jasmonic acid. After 24 h of spraying, twigs of TV-11and TV-26 were inoculated with *B. theobromae* and twigs of TV-12 and TV-25 were inoculated with *C. eragrostidis* and they were categorized as inoculated-treated twigs. In another set, the twigs were sprayed with the chemicals in a similar way but were not inoculated. This was categorized as the uninoculated-treated

Table 37

Disease incidence in susceptible and resistant tea varieties (TV-12 and TV-25 respectively) against *C. eragrostidis* following treatment of three selected chemicals

Chemical treatment (10^{-3} M concentration)	Clonal variety	Mean disease index/shoot		
		Incubation period		
		24 hours	48 hours	72 hours
Nickel chloride	TV-12	0.22±0.038	0.53±0.055	0.63±0.049
	TV-25	0.17±0.047	0.19±0.040	0.19±0.049
Salicylic acid	TV-12	0.25±0.043	0.46±0.041	0.98±0.047
	TV-25	0.12±0.041	0.13±0.049	0.16±0.060
Jasmonic acid	TV-12	0.47±0.049	0.66±0.047	0.84±0.049
	TV-25	0.18±0.049	0.23±0.060	0.33±0.047
Control (Untreated-inoculated)	TV-12	0.53±0.047	0.83±0.051	1.66±0.049
	TV-25	0.20±0.051	0.25±0.047	0.40±0.046
CD at 5%		0.25	0.28	0.15
CD at 1%		0.22	0.24	0.13

* Mean of 3 replications.
Data after± represent standard error values.

twigs. In control sets, the twigs were inoculated without any chemical treatment. The orthodihydroxy phenol and total phenol were extracted from leaves and estimated in mg g^{-1} fresh weight tissue after 24, 48 and 72 h of incubation. The details of the phenol extraction and estimation procedures has discussed in the materials and methods (Section 3.15). The results of the experiments where *B. theobromae* was the pathogen are given in the Table 38 and those where *C. eragrostidis* was the pathogen are listed in Table 39.

The results showed that the orthodihydroxy phenol contents were maximum in inoculated-treated leaves of resistant varieties (TV-26 against *B. theobromae* and TV-25 against *C. eragrostidis*) and minimum in inoculated-treated leaves of susceptible varieties (TV-11 against *B. theobromae* and TV-12 against *C. eragrostidis*) after 24, 48 and 72 h of inoculation when the twigs were sprayed with nickel chloride, salicylic acid and jasmonic acid. Uninoculated-treated leaves of resistant varieties showed a higher orthodihydroxy phenol content than susceptible varieties in all the treatments.

From the results it was also evident that orthodihydroxy phenol contents were higher in inoculated-treated and uninoculated-treated leaves of both

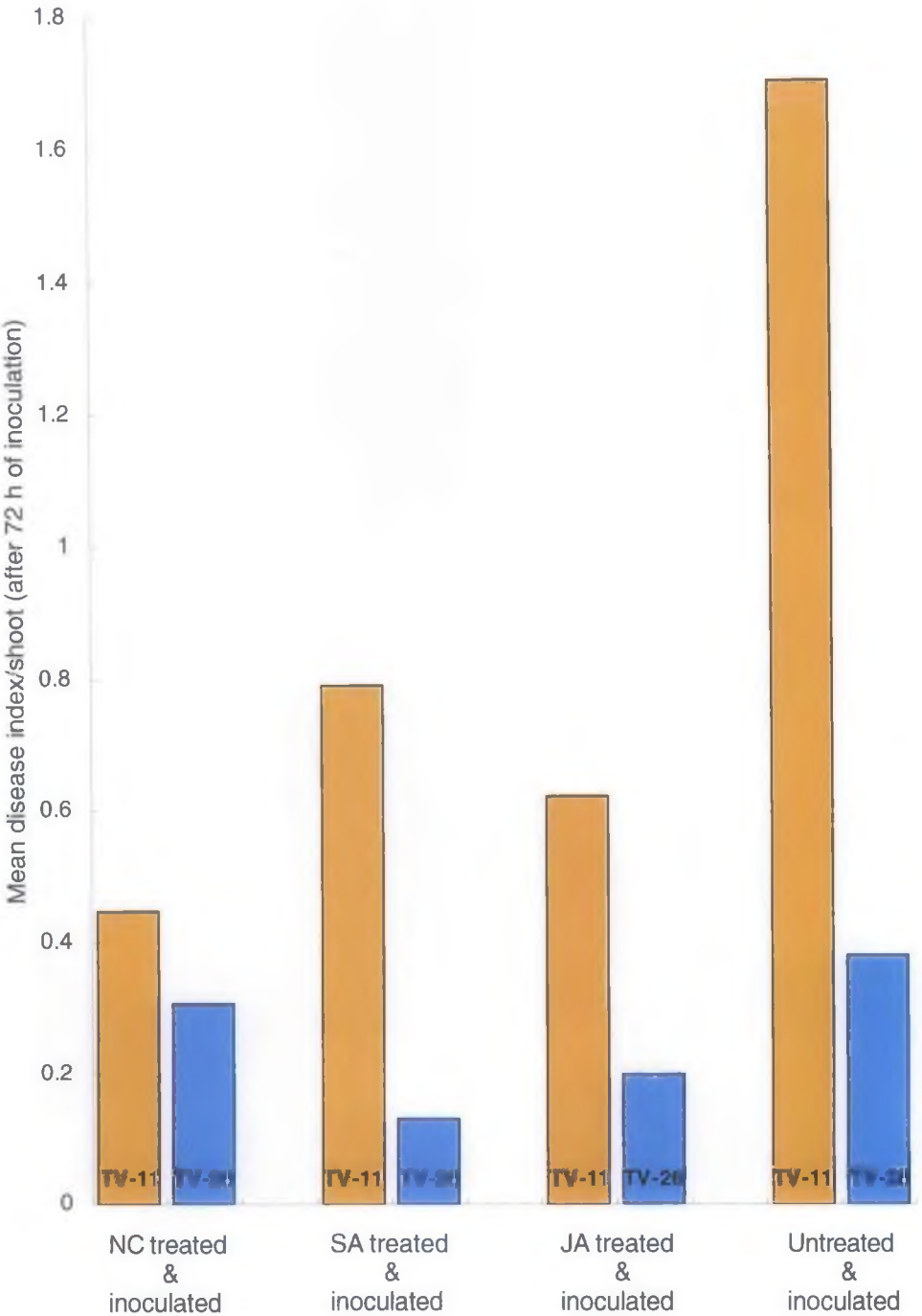


Fig. 20 : Disease occurrence in susceptible (TV-11) and resistant (TV-26) tea varieties against *B. theobromae* following treatment with 10^{-3} M nickel chloride (NC), salicylic acid (SA) and jasmonic acid (JA)

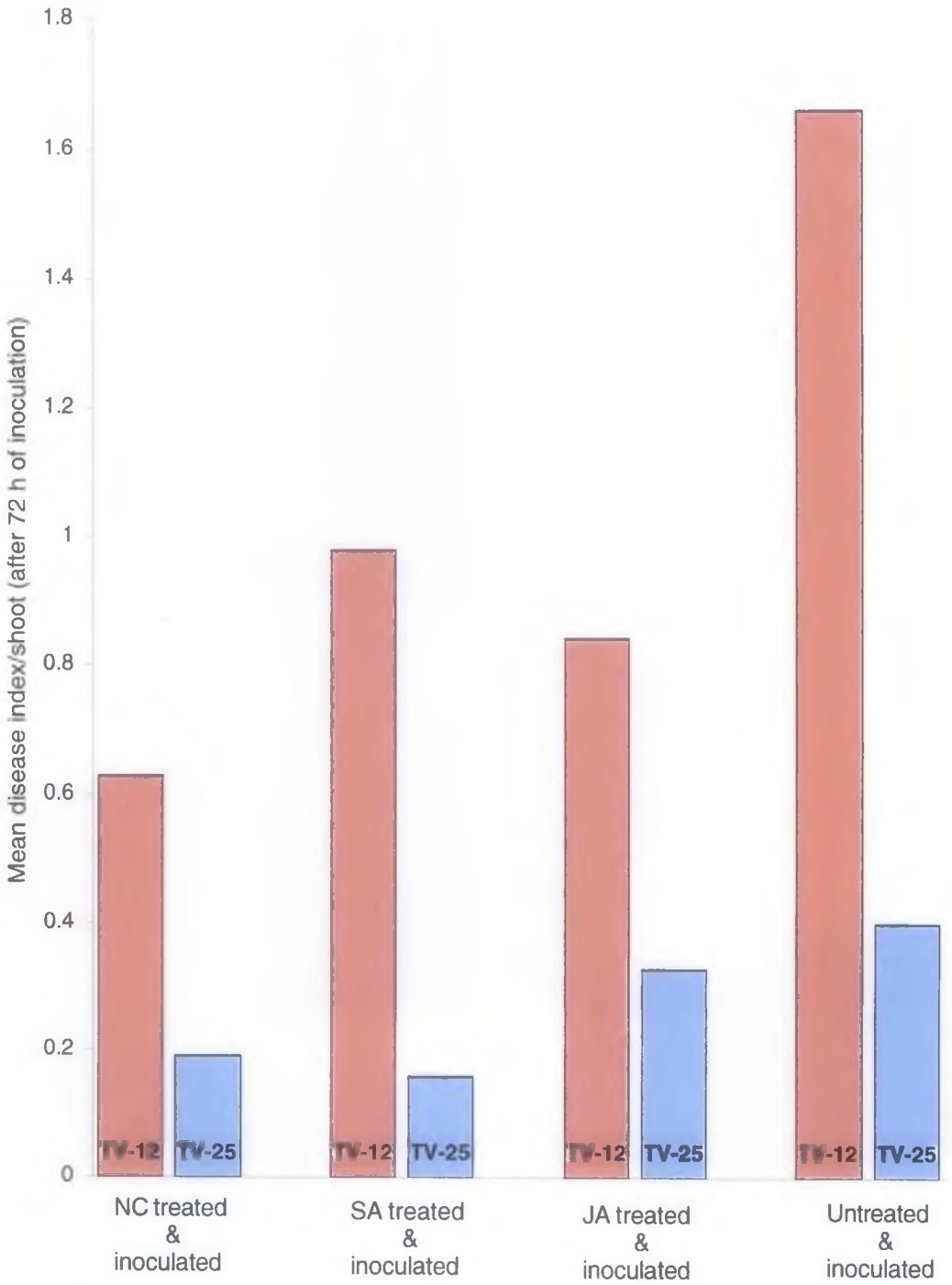


Fig. 21: Disease occurrence in susceptible (TV-12) and resistant (TV-25) tea varieties against *C. eragrostidis* following treatment with 10^{-3} M nickel chloride (NC), salicylic acid (SA) and jasmonic acid (JA)

Table 38

Orthodihydroxy phenol and total phenol content in healthy and *B. theobromae*-infected tea leaves of resistant and susceptible varieties (TV-11 and TV-26 respectively) following treatment with three selected chemicals

Chemical treatment	Fungal inoculation	Tea variety	Orthodihydroxy phenol (mg/g)			Total phenol (mg/g)		
			24h	48h	72h	24h	48h	72h
Nickel Chloride	Inoculated	TV-11	1.02	1.08	1.10	4.25	4.60	6.12
		TV-26	2.24	2.30	2.42	5.85	6.62	7.25
	Uninoculated	TV-11	1.07	1.16	1.26	5.32	5.92	6.30
		TV-26	2.25	2.75	2.87	5.87	6.62	7.37
Salicylic Acid	Inoculated	TV-11	1.08	1.19	1.29	3.87	4.75	5.50
		TV-26	2.00	2.75	2.93	5.62	6.52	7.50
	Uninoculated	TV-11	0.97	1.06	1.18	5.60	6.57	7.87
		TV-26	1.37	2.43	2.62	6.25	7.87	8.20
Jasmonic Acid	Inoculated	TV-11	1.00	1.19	1.28	6.06	6.40	7.50
		TV-26	1.75	2.25	2.65	6.41	7.56	7.98
	Uninoculated	TV-11	1.12	1.22	1.32	6.15	6.66	6.75
		TV-26	1.25	1.75	2.50	6.60	7.93	8.25
Control (Untreated-inoculated)		TV-11	0.60	0.75	0.87	3.75	3.84	3.96
		TV-26	1.37	1.50	1.62	5.00	5.56	6.12

susceptible and resistant varieties when compared with control. Similar results were also found when total phenol contents were estimated. Maximum values of total phenol content were recorded in inoculated-treated leaves of resistant varieties and minimum in inoculated-treated leaves of susceptible varieties after 24, 48 and 72 h of inoculation following treatment with all the three chemicals. Uninoculated-treated leaves of resistant varieties showed higher total phenol content than susceptible varieties in all cases. When compared to control, total phenol contents were higher in inoculated-treated and uninoculated-treated leaves of both susceptible and resistant varieties.

Table 39

Orthodihydroxy phenol and total phenol content in healthy and *C. eragrostidis*-infected tea leaves of resistant and susceptible varieties (TV-12 and TV-25 respectively) following treatment with three selected chemicals

Chemical treatment	Fungal inoculation	Clonal variety	Orthodihydroxy phenol (mg/g)			Total phenol (mg/g)		
			24h	48h	72h	24h	48h	72h
Nickel chloride	Inoculated	TV-12	0.75	1.07	1.28	4.81	5.12	5.30
		TV-25	2.12	2.37	2.50	5.90	6.06	6.22
	Uninoculated	TV-12	0.87	1.26	1.60	4.90	5.35	5.58
		TV-25	2.26	2.79	2.80	6.15	6.47	6.80
Salicylic acid	Inoculated	TV-12	0.60	1.09	1.36	4.87	5.50	5.75
		TV-25	1.62	2.00	2.12	6.00	6.17	6.31
	Uninoculated	TV-12	0.78	1.17	1.41	4.99	5.62	5.91
		TV-25	1.75	2.18	2.51	6.02	6.33	6.67
Jasmonic acid	Inoculated	TV-12	0.45	1.12	1.48	5.62	6.15	6.60
		TV-25	2.25	2.50	2.69	6.50	6.78	7.12
	Uninoculated	TV-12	0.61	1.25	1.49	5.81	6.24	6.81
		TV-25	1.81	2.37	2.90	6.65	7.05	7.60
Control (Untreated-inoculated)		TV-12	0.42	0.68	0.90	4.25	4.37	4.75
		TV-25	1.50	1.92	2.15	5.75	5.81	6.16

4.6. *In vitro* bioassay of some commonly used fungicides against *B. theobromae* and *C. eragrostidis*

Some commonly used fungicides were tested for their efficacy against the two fungal pathogens. Bioassay was done taking five different concentrations (50 μgml^{-1}, 125 μgml^{-1}, 250 μgml^{-1}, 500 μgml^{-1} and 1000 μgml^{-1}) of six different fungicides (Roko, Indofil, Captan, Calixin, Bavistin and Baynate) following poisoned food technique of Suleman *et al.*, 2002 as described in the materials and methods (Section 3.16). Initially five different concentrations (500 μgml^{-1}, 1,250 μgml^{-1}, 2,500 μgml^{-1}, 5,000 μgml^{-1} and 10,000 μgml^{-1}) of all the fungicides were made. Then 9 ml of sterilized PDA medium was prepared and mixed with 1 ml of prepared fungicide solution (made in sterile distilled water) so that the final concentrations became 50 μgml^{-1}, 125 μgml^{-1}, 250 μgml^{-1}, 500 μgml^{-1} and 1000 μgml^{-1}.

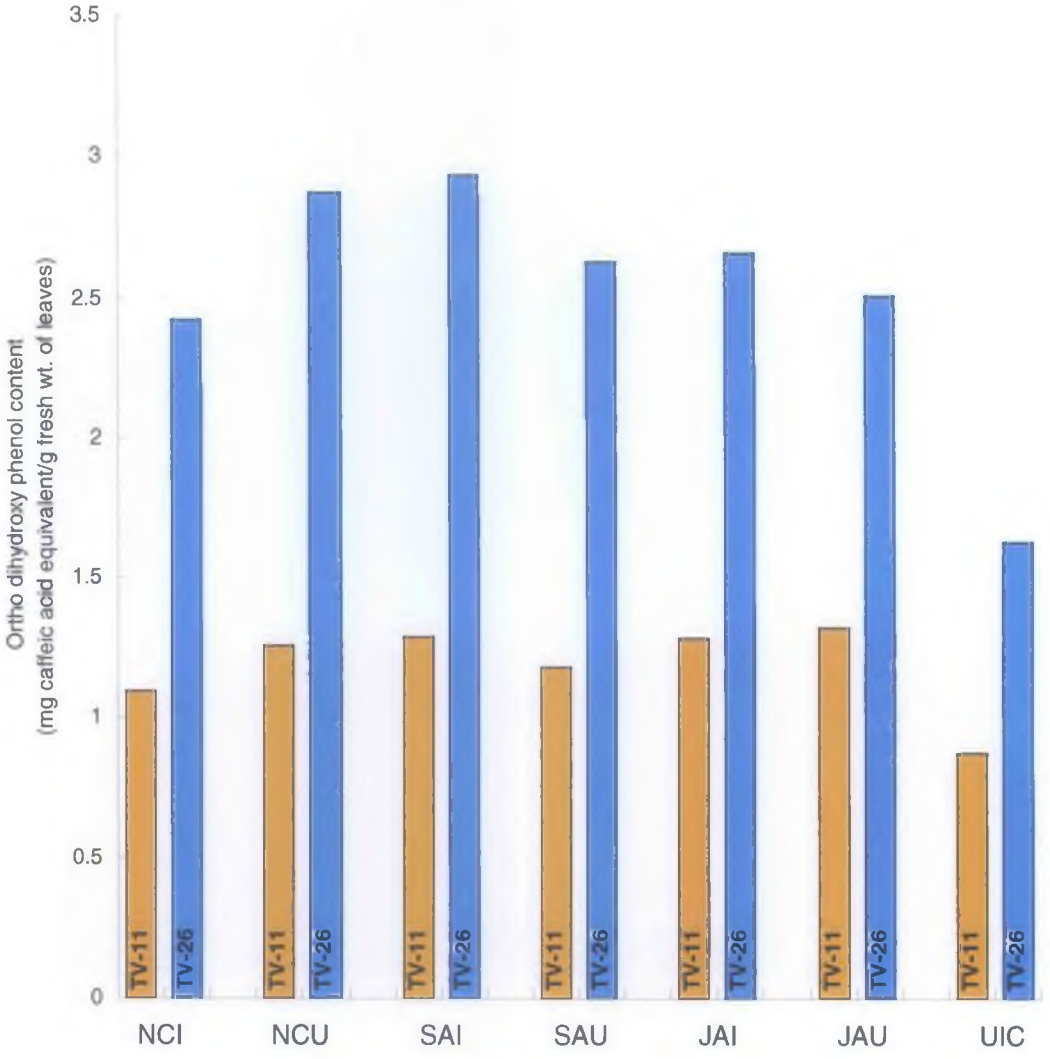


Fig.22: Ortho dihydroxy phenol content in healthy and *B. theobromae*-infected tea leaves of susceptible (TV-11) and resistant (TV-26) varieties following treatment with 10^{-3} M nickel chloride, jasmonic acid and salicylic acid

Abbreviations:
NCI = Nickel chloride treated & inoculated
NCU = Nickel chloride treated & uninoculated
SAI = Salicylic acid treated & inoculated
SAU = Salicylic acid treated & uninoculated
JAI = Jasmonic acid treated & inoculated
JAU = Jasmonic acid treated & uninoculated;
UIC = Untreated & inoculated control

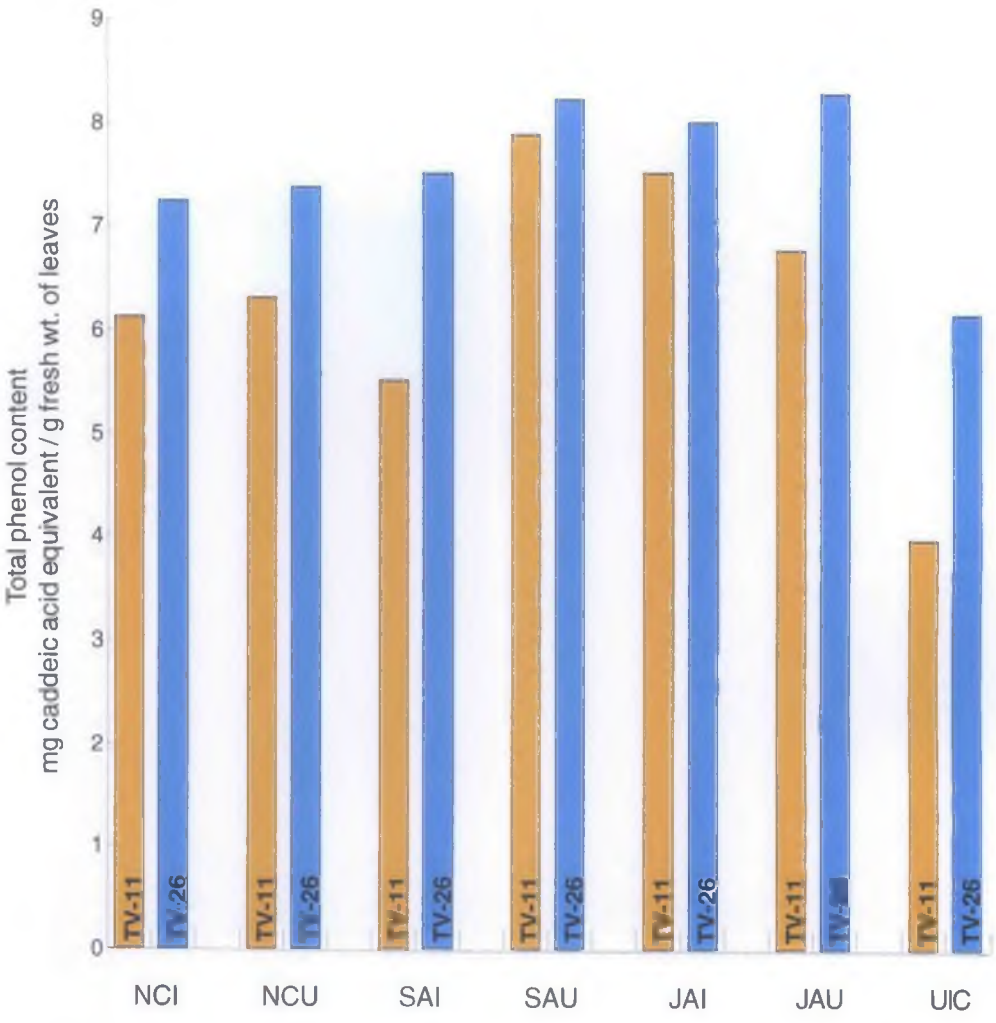


Fig.23: Total phenol content in healthy and *B. theobromae*-infected tea leaves of susceptible (TV-11) and resistant (TV-26) varieties following treatment of 10^{-3} M concentration of Nickel chloride, Jasmonic acid and Salicylic acid

Abbreviations:
NCI = Nickel chloride treated & inoculated
NCU = Nickel chloride treated & uninoculated
SAI = Salicylic acid treated & inoculated
SAU = Salicylic acid treated & uninoculated
JAI = Jasmonic acid treated & inoculated
JAU = Jasmonic acid treated & uninoculated;
UIC = Untreated & inoculated control

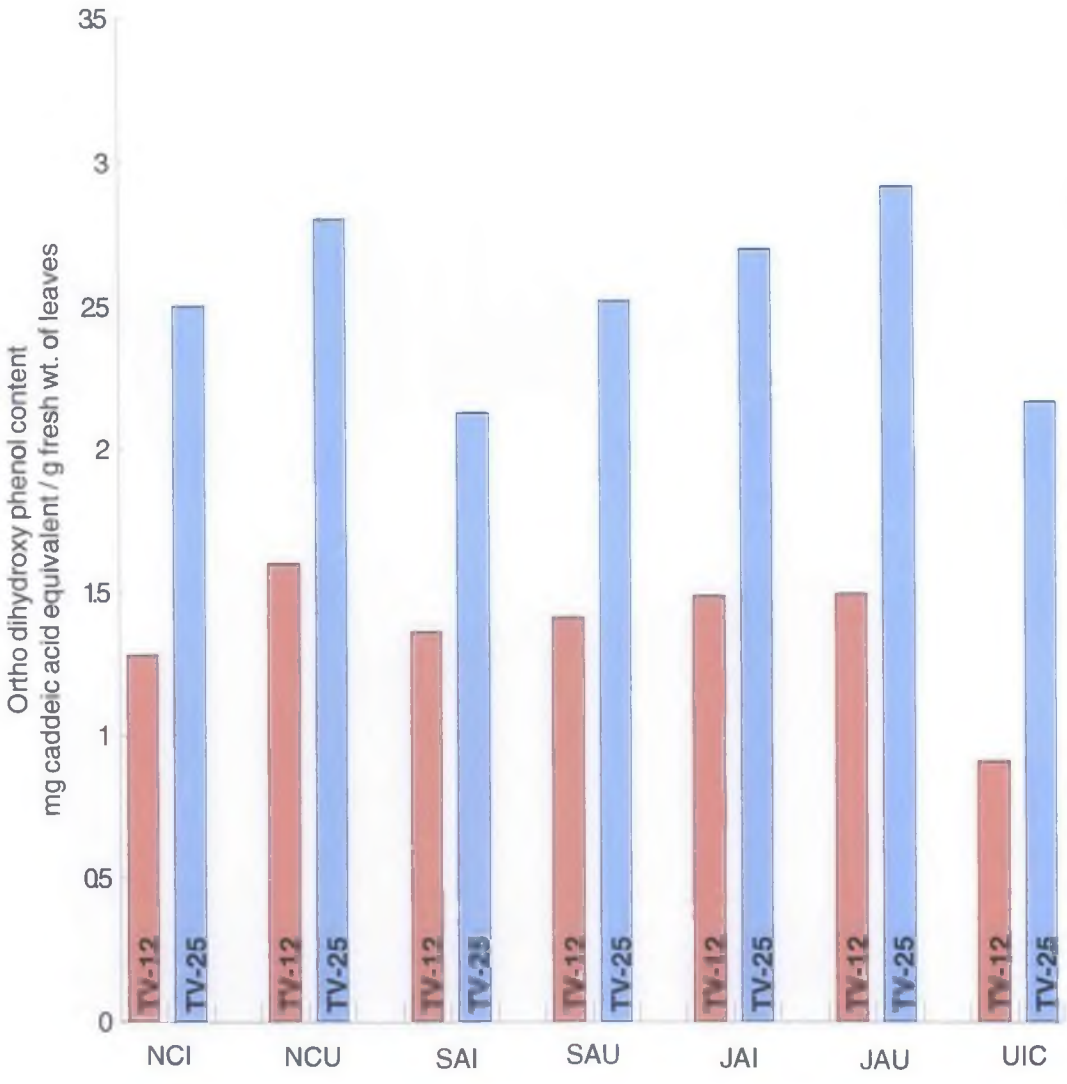


Fig.24: Ortho dihydroxy phenol content in healthy and *C. eragrostidis*-infected tea leaves of susceptible (TV-11) and resistant (TV-26) varieties following treatment of 10^{-3} M concentration of Nickel chloride, Jasmonic acid and Salicylic acid

Abbreviations:
NCI = Nickel chloride treated & inoculated
NCU = Nickel chloride treated & uninoculated
SAI = Salicylic acid treated & inoculated
SAU = Salicylic acid treated & uninoculated
JAI = Jasmonic acid treated & inoculated
JAU = Jasmonic acid treated & uninoculated
UIC = Untreated & inoculated control

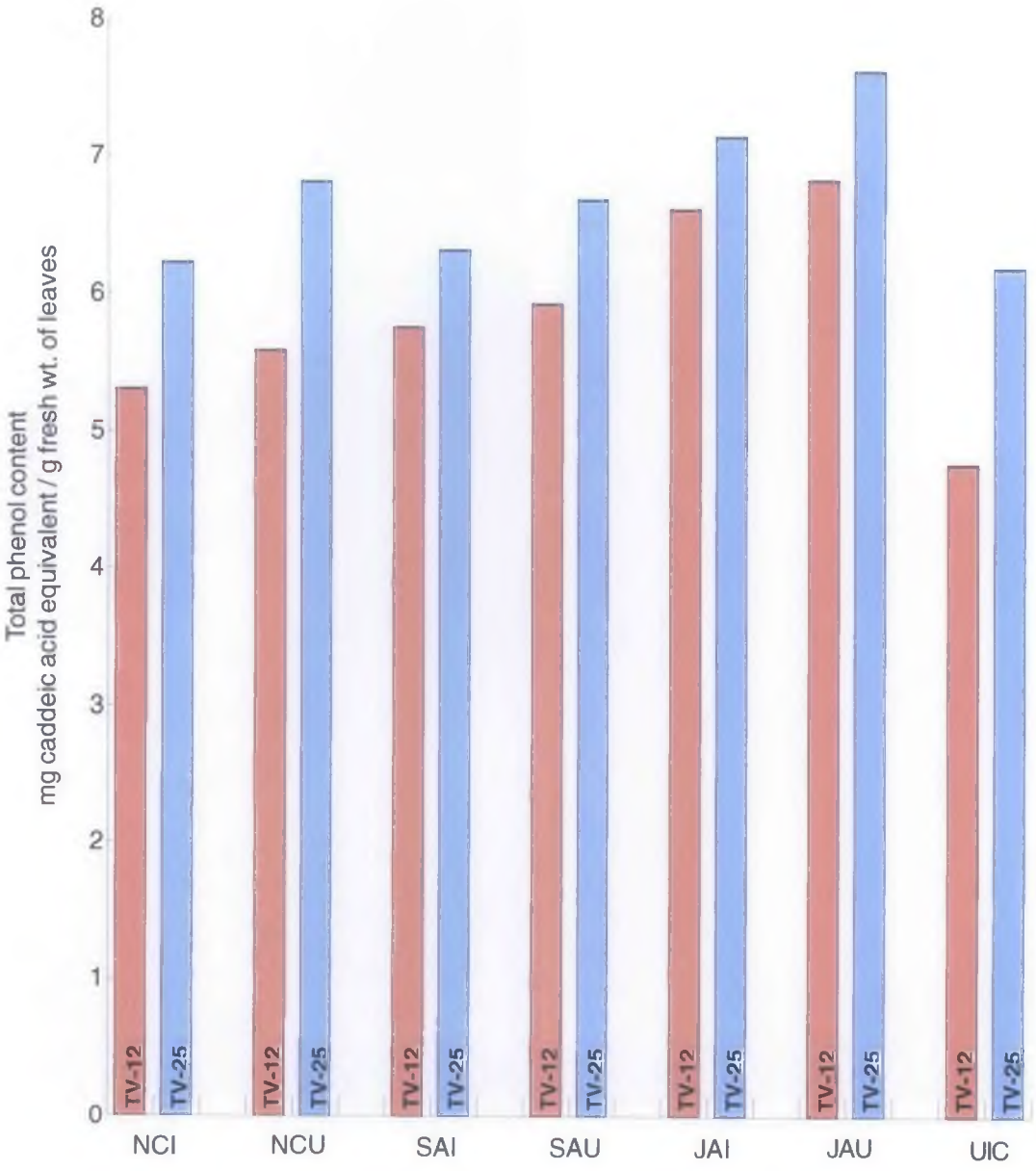


Fig.25 : Total phenol content in healthy and *C. eragrostidis*-infected tea leaves of susceptible (TV-12) and resistant (TV-25) varieties following treatment with 10^{-3} M nickel chloride, jasmonic acid and salicylic acid

Abbreviations:
NCI = Nickel chloride treated & inoculated
NCU = Nickel chloride treated & uninoculated
SAI = Salicylic acid treated & inoculated
SAU = Salicylic acid treated & uninoculated
JAI = Jasmonic acid treated & inoculated
JAU = Jasmonic acid treated & uninoculated
UIC = Untreated & inoculated control

Bioassay studies (Table 40) against the *B. theobromae* with the above-mentioned fungicides showed that Calixin at 1000 μgml^{-1} concentration completely inhibited the growth while 50 μgml^{-1} of Calixin showed inhibition of 75.71% over control (Plate XIII : 2-5). Roko and Bavistin showed complete inhibition of growth even at 50 μgml^{-1} while Indofil and Baynate showed complete inhibition in all the concentrations except 50 μgml^{-1}. At 50 μgml^{-1} concentration of Indofil and Baynate, inhibitions were 32.85% and 90.00% respectively. Although Captan was ineffective at 50 μgml^{-1} but inhibited the growth of *B. theobromae* to some extent at 125 μgml^{-1} (20.00%) and 250 μgml^{-1} (50.00%). However at 500 μgml^{-1} and 1000 μgml^{-1} concentrations, it showed 100% inhibition. From the results it was concluded that four of the six fungicides (Roko, Indofil, Bavistin and Baynate) were effective while others were effective only at higher concentrations.

From the results tabulated in Table 41, it was evident that among all the fungicides tested against C. eragrostidis, only Calixin was found to be 100% effective over control at all concentrations. However, at high concentrations (500 μgml^{-1} and 1000 μgml^{-1})all the fungicides showed complete inhibition. Complete inhibition was also recorded by Baynate, Indifil and Captan at 250 μgml^{-1} concentration. At the lowest concentration tested (50 μgml^{-1}) percentage inhibition ranged from 5.71 (Roko) to 55.71 (Captan).

Following the above experiment it was considered worthwhile to determine the MIC value of the fungicides tested against the two pathogens. Table 40 and Table 41 indicated the range of MIC values for each fungicide against B. theobromae and C. eragrostidis. Accordingly, ten different concentrations of all the fungicides were prepared by serial dilution and supplemented to the media to get the desired concentrations. Poisoned food technique (Suleman *et al.*, 2002) was followed according to the procedure as described in materials and methods (Section 3.16).

Table 42 summerized the MIC values of the six fungicides (Roko, Indofil, Captan, Calixin, Bavistin and Baynate) against *B. theobromae* and *C. eragrostidis*. From the results it was evident that MIC values of Roko, Indofil, Captan, Calixin, Bavistin and Baynate against *B. theobromae* were 45 μgml^{-1}, 100 μgml^{-1}, 400 μgml^{-1}, 280 μgml^{-1}, 35 μgml^{-1} and 60 μgml^{-1} respectively. On the other hand MIC values of Roko, Indofil, Captan, Calixin, Bavistin and Baynate against *C. eragrostidis* were 430 μgml^{-1} , 225 μgml^{-1}, 250 μgml^{-1}, 40 μgml^{-1}, 300 μgml^{-1} and 150 μgml^{-1} respectively.

Table 40

Effect of different fungicides on the growth of *B. theobromae* (Poisoned food technique)

Fungicides	Concentration (µg/ml)	*B. theobromae*	
		Colony diameter (mm) after 4 days of inoculation*	Percent inhibition over control**
Roko	50	0	100
	125	0	100
	250	0	100
	500	0	100
	1000	0	100
Indofil	50	47.00±0.56	32.85±0.71
	125	0	100
	250	0	100
	500	0	100
	1000	0	100
Captan	50	70.00±0.39	0
	125	56.00±0.48	20.00±0.55
	250	35.00±0.51	50.00±0.63
	500	0	100
	1000	0	100
Calixin	50	17.00±0.64	75.71±0.35
	125	12.00±0.69	82.85±0.44
	250	07.00±0.72	90.00±0.69
	500	0	100
	1000	0	100
Bavistin	50	0	100
	125	0	100
	250	0	100
	500	0	100
	1000	0	100
Baynate	50	7.00±0.29	90.00±0.36
	125	0	100
	250	0	100
	500	0	100
	1000	0	100

*Mean of 3 replications.

** Control diameter= 70 mm.

Data after± represent standard error values.

Table 41

Effect of different fungicides on the growth of *C. eragrostidis* (Poisoned food technique)

Fungicides	Concentration (µg/ml)	*C. eragrostidis*	
		Colony diameter (mm) after 7 days of inoculation*	Percent inhibition over control**
Roko	50	66.00±0.25	05.71±0.36
	125	52.00±0.50	25.71±0.71
	250	30.00±0.29	57.14±0.41
	500	0	100
	1000	0	100
Indofil	50	58.00±0.11	17.14±0.16
	125	42.00±0.56	40.00±0.62
	250	0	100
	500	0	100
	1000	0	100
Captan	50	31.00±0.75	55.71±0.61
	125	23.00±0.68	67.14±0.73
	250	0	100
	500	0	100
	1000	0	100
Calixin	50	0	100
	125	0	100
	250	0	100
	500	0	100
	1000	0	100
Bavistin	50	56.00±0.18	20.00±0.48
	125	48.00±0.26	31.42±0.51
	250	26.00±0.35	62.85±0.30
	500	0	100
	1000	0	100
Baynate	50	46.00±0.41	34.28±0.48
	125	22.00±0.56	68.57±0.50
	250	0	100
	500	0	100
	1000	0	100

*Mean of 3 replications.

** Control diameter= 70 mm.

Data after± represent standard error values.

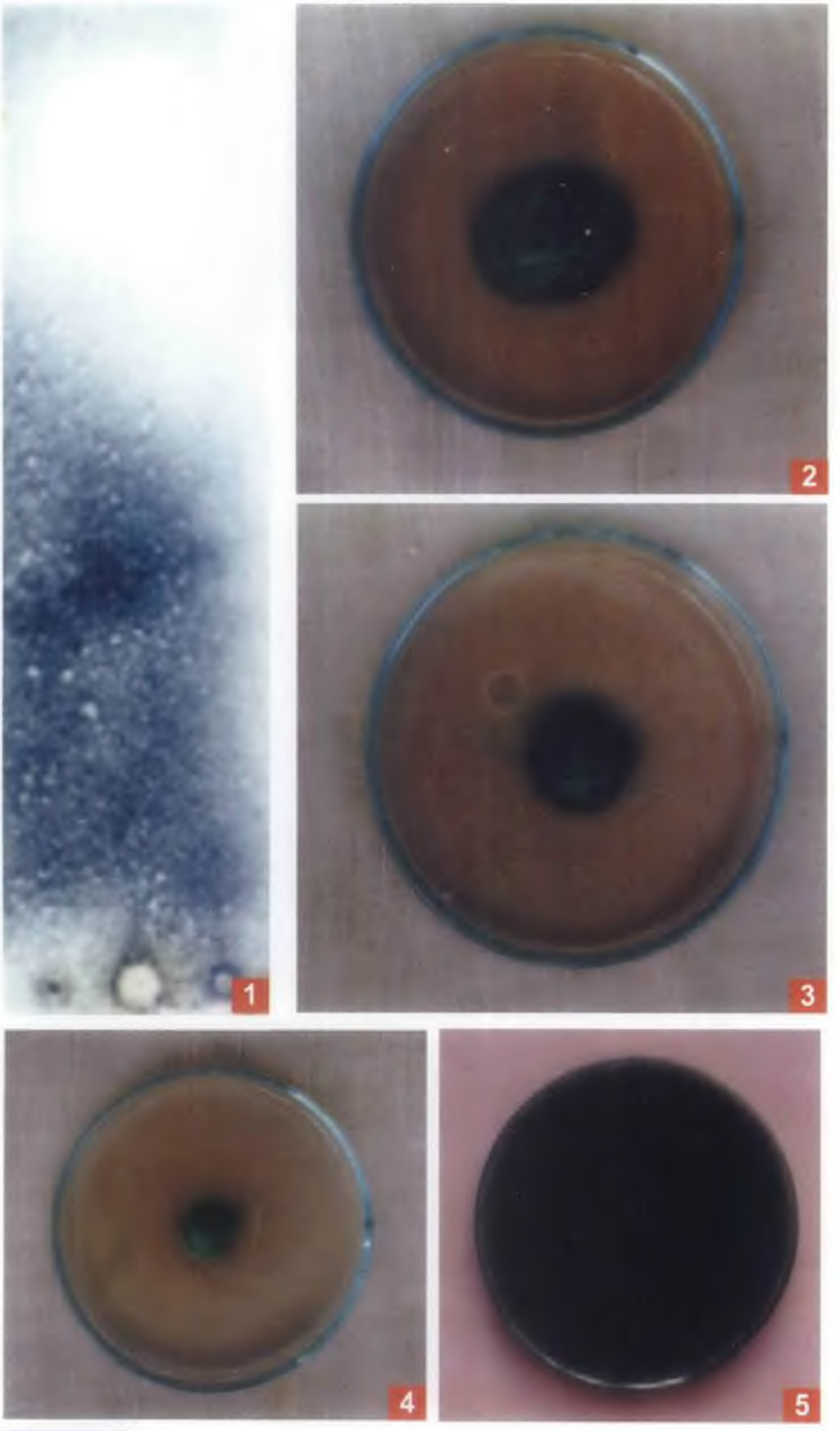


Plate XIII

Fig.1 : Thin Layer Chromatograms sprayed with *Curvularia eragrostidis* spores. Zonesof inhibition shown by aqueous extract of bulb of *Allium sativum*.

Fig.2-4 : Growth of *Botryodiplodia theobromae* at concentration of 50 μgml^{-1}, 125 μgml^{-1} and 250 μgml^{-1} of fungicide (Calixin) respectively following poisoned food thechnique.

Fig. 5 : Growth of *B. theobromae* without any fungicide (Control).

Table 42

MIC values of fungicides for radial growth of *B. theobromae* and *C. Eragrostidis* after poisoned food technique

Fungicides	MIC of *B. theobromae* (μgml^{-1})	MIC of *C. eragrostidis* (μgml^{-1})
Roko	45	430
Indofil	100	225
Captan	400	250
Calixin	280	40
Bavistin	35	300
Baynate	60	150

4.7. Screening of potential antifungal properties from different plant extracts against *B. theobromae* and *C. eragrostidis.*

Extracts (both aqueous and ethanol) of 21 plants were used for screening of their antifungal properties against *B. theobromae* and *C. eragrostidis.* A slide germination technique was followed for the screening. The procedure of preparation of both aqueous and ethanol extracts of the plants and details of the slide germination bioassay has been discussed in the materials and methods (Section 3.17.1 & 3.17.2). The percent germination and percent inhibition of spores against respective plant extracts as well as the average length of the germ tubes were calculated and the results were tabulated in the Table 43 and Table 44 respectively for *B. theobromae* and *C. eragrostidis* respectively.

Among the aqueous extracts of the 21 plants tested against *B. theobromae*, the leaf extract of *Melia dubia* showed complete inhibition of the spore germination while bulb extract of *Allium cepa* and leaf extract of *Clerodendrum viscosum* inhibited more than 90.00% spore germination (Table 43). Ethanol extract of the leaves of *M. dubia* and *Azadirachta indica* showed above 90.00% inhibition. Aqueous and ethanol extracts of *M. dubia* leaves were found most effective against *B. theobromae.* The average lengths of the germ tubes were found less in comparison to control when the spores were allowed to germinate in presence of these extracts.

Table 43

Effect of different plant extracts on the spore germination of *B. theobromae*

Plant Species tested (Family of the plant)	Aqueous extract			Ethanol extract		
	Percent germination	Percent inhibition	Average germ tube length (µm)	Percent germination	Percent inhibition	Average germ tube length (µm)
Allium cepa L. (Alliaceae)	3.60±0.46	96.40±0.40	32.00±1.00	44.90±0.21	55.10±0.56	50.64±0.65
Allium sativum L. (Alliaceae)	62.10±0.45	37.90±0.30	51.00±1.00	70.50±0.75	29.50±0.69	77.32±0.86
Andrographis paniculata (Burm. f.) Wall. Ex Nees (Acanthaceae)	44.20±0.23	55.80±0.36	55.00±0.54	72.60±0.67	27.40±0.77	63.32±0.69
Azadirachta indica A. Juss. (Meliaceae)	69.30±0.40	30.70±0.30	61.80±1.03	5.60±0.83	94.40±0.70	35.80±0.93
Bougainvillea spectabilis Willd. (Nyctaginaceae)	11.00±0.34	89.00±0.40	43.80±0.79	42.20±0.69	57.80±0.79	81.32±0.72
Cascabela thevatea (L.)Lippold (Apocynaceae)	24.50±0.40	75.50±0.35	44.00±0.83	84.20±0.62	15.80±0.81	65.32±0.57
Cassia tora L. (Caesalpinoidae)	70.10±0.29	29.90±0.40	63.50±0.78	51.40±0.70	48.60±0.74	71.56±0.68
Catheranthus roseus (L.) G. Don (Apocynaceae)	44.70±0.41	55.30±0.36	54.60±0.74	64.30±0.45	35.70±0.65	66.64±0.84
Clerodendrum viscosum Vent. (Verbanaceae)	6.20±0.46	93.80±0.45	35.60±0.59	45.00±0.60	55.00±0.58	95.32±0.78
Cymbopogon pendulus (Nees ex Steud.) J. F. Wats. (Graminaceae)	61.80±0.40	38.20±0.35	55.60±0.70	57.50±0.65	42.50±1.15	21.32±0.63
Datura metel L. (Solanaceae)	32.60±0.23	67.40±0.39	52.10±0.95	24.50±0.63	75.50±0.75	38.48±0.57

Table 43 (Contd....)

Table 43 (Contd...)

Effect of different plant extracts on the spore germination of *B. theobromae*

Plant Species tested (Family of the plant)	Aqueous extract			Ethanol extract		
	Percent germination	Percent inhibition	Average germ tube length (µm)	Percent germination	Percent inhibition	Average germ tube length (µm)
Dryopteris filix-mas (L.) Schott (Polypodiaceae)	7.00±0.41	93.00±0.41	41.00±0.84	19.80±0.59	80.20±0.56	41.43±0.77
Emblica officinalis Gaertn. (Euphorbiaceae)	37.90±0.46	62.10±0.50	56.00±0.60	40.90±0.87	59.10±0.62	28.39±0.80
Euphorbia hirta L.. (Euphorbiaceae)	94.40±0.35	5.60±0.48	79.00±0.63	87.50±0.60	12.50±0.65	56.00±0.79
Hyptis suaveolens (L.) Poit. (Labiatae)	91.60±0.23	8.40±0.46	84.20±0.40	50.50±0.85	49.50±0.69	24.61±0.39
Jatropha curcas L. (Euphorbiaceae)	75.00±0.46	25.00±0.10	71.20±0.55	63.00±0.66	37.00±0.64	73.80±0.52
Lantana camara L. (Verbanaceae)	96.50±	3.50±0.15	80.00±0.79	33.70±0.31	66.30±0.66	96.70±0.67
Melia dubia Cav. (Meliaceae)	0	100.00	0	8.70±0.52	91.30±0.75	33.33±0.72
Polyalthia longifolia(Sonnerat) Thwaites (Annonaceae)	72.20±0.45	27.80±0.30	58.00±0.30	18.20±0.64	81.80±0.90	36.71±0.75
Tagetes erecta L. (Compositae)	89.60±0.52	10.40±0.24	66.00±0.60	88.50±0.77	11.50±0.81	36.00±0.66
Zingiber officinale Roscoe (Zingiberaceae)	31.00±0.53	69.00±0.98	48.00±0.51	37.60±0.71	62.40±0.59	80.00±0.65
Control	100.00	0	115.80±0.71	100.00	0	98.00±0.58

Data after ± represent standard error values.

In case of *C. eragrostidis*, it was evident from the results noted in Table 44 that the aqueous extracts of the bulb of *Allium sativum* completely inhibited the germination of spores. Aqueous extracts from the leaves of *Cascabela thevatea, Dryopteris filix-mas* and *Emblica officinalis* exhibited more than 90.00% inhibition of spore germination. In case of ethanol extracts, the leaf extract of *Polyalthia longifolia* showed 90.60% inhibition of spore germination bulb extract of *A. sativum* showed 86.00% inhibition. Aqueous and ethanol extracts of the bulb of *A. sativum* were found to be most effective against *C. eragrostidis.* However, in case of all the plant extracts, germ tube lengths were less in comparison to control.

4.7.1. TLC plate bioassay for detection of antifungal properties of selected plant extracts

It was evident from the results tabulated in Table 43 that aqueous leaf extracts of *Melia dubia*, *Clerodendrum viscosum*, aqueous bulb extracts of *Allium cepa* and ethanol extracts of *Azadirachta indica* were effective against *B. theobromae*. Similarly against *C. eragrostidis*, aqueous extracts of the bulb of *A. sativum*, leaf extracts of *C. thevatea, D. filix-mas*, *E. officinalis* and ethanol extract of the leaves of *P. longifolia* were effective (Table 44). Therefore these extracts were selected for bioassay on thin layer chromatograms to detect presence of specific antifungal components which would produce inhibition zones at specific locations (R_f). Accordingly, these extracts were spotted on pre-activated TLC plates, developed in a solvent, and dried and bioassay was done following procedures described in materials and methods (Section 3.17.3, 3.17.4 & 3.17.5). After completion of the experiments, the R_f values of the inhibition zones were recorded and zone diameters were measured (Table 45). Aqueous leaf extract of *M. dubia* showed a large (10 mm) antifungal zone with an R_f value of 0.8 when sprayed with spores of *B. theobromae*. Inhibition zone against *C. eragrostidis* was produced by aqueous extract of *A. sativum* bulbs which had a diameter of 38 mm and R_f was recorded to be 0.98 (Plate XIII : 1).

Table 44

Effect of different plant extracts on the spore germination of *C. eragrostidis*

Plant Species tested (Family of the plant)	Aqueous extract			Ethanol extract		
	Percent germination	Percent inhibition	Average germ tube length (µm)	Percent germination	Percent inhibition	Average germ tube length (µm)
Allium cepa L. (Alliaceae)	67.70±0.91	32.30±0.33	40.00±1.08	34.10±0.63	65.90±0.45	17.64±1.18
Allium sativum L. (Alliaceae)	0	100.00	0	14.00±0.89	86.00±0.20	41.32±0.18
Andrographis paniculata (Burm. f.) Wall. Ex Nees (Acanthaceae)	75.00±0.58	25.00±0.36	10.80±0.82	83.30±0.90	16.70±0.86	22.64±1.02
Azadirachta indica A. Juss. (Meliaceae)	43.70±0.38	56.30±0.79	24.80±0.43	31.30±0.33	68.70±0.80	25.32±0.33
Bougainvillea spectabilis Willd. (Nyctaginaceae)	35.80±1.10	64.20±0.38	35.48±0.77	55.40±0.27	54.70±0.41	40.00±0.46
Cascabela thevatea (L.)Lippold (Apocynaceae)	7.40±0.65	92.60±0.30	28.00±0.60	56.50±0.33	43.50±1.02	41.32±0.33
Cassia tora L. (Caesalpinoidae)	43.10±0.55	56.90±0.34	19.26±0.46	93.70±0.96	6.30±0.32	18.68±0.98
Catheranthus roseus (L.) G. Don (Apocynaceae)	14.70±0.35	85.30±0.46	8.00±1.08	73.20±1.02	26.80±1.19	25.64±0.39
Clerodendrum viscosum Vent. (Verbanaceae)	28.10±0.63	71.90±0.50	34.00±0.46	93.80±0.69	6.20±1.14	40.64±0.60
Cymbopogon pendulus (Nees ex Steud.) J. F. Wats. (Graminaceae)	85.80±0.52	14.20±0.69	25.20±1.03	64.50±0.25	35.50±0.78	42.00±0.25
Datura metel L. (Solanaceae)	52.70±0.49	47.30±1.05	21.66±0.50	34.00±1.05	66.00±1.00	39.32±0.60

Table 44 (Contd....)

Table 44 (Contd....)

Effect of different plant extracts on the spore germination of *C. eragrostidis*

Plant Species tested (Family of the plant)	Aqueous extract			Ethanol extract		
	Percent germination	Percent inhibition	Average germ tube length (µm)	Percent germination	Percent inhibition	Average germ tube length (µm)
Dryopteris filix-mas (L.) Schott (Polypodiaceae)	3.70±0.92	96.30±0.65	11.25±0.76	19.40±0.22	80.60±0.82	29.36±0.63
Emblica officinalis Gaertn. (Euphorbiaceae)	5.70±0.35	94.30±1.17	15.80±0.90	15.00±0.64	85.00±1.14	12.64±0.85
Euphorbia hirta L.. (Euphorbiaceae)	89.80±0.68	10.20±0.89	44.00±0.52	82.60±0.51	17.40±0.64	25.78±0.99
Hyptis suaveolens (L.) Poit. (Labiatae)	70.60±0.46	29.40±0.86	45.44±0.78	87.30±0.70	12.70±0.35	28.00±1.08
Jatropha curcas L. (Euphorbiaceae)	87.50±0.84	12.50±0.78	40.18±0.99	85.20±0.85	14.80±0.40	15.61±0.95
Lantana camara L. (Verbanaceae)	44.70±0.85	55.30±0.60	22.71±0.21	57.40±0.75	42.60±0.30	33.67±0.40
Melia dubia Cav. (Meliaceae)	43.10±0.71	56.90±0.77	32.85±0.96	46.30±1.18	53.70±1.03	19.38±0.34
Polyalthia longifolia(Sonnerat) Thwaites (Annonaceae)	30.60±1.08	69.40±0.28	32.00±0.50	9.40±0.60	90.60±0.38	12.64±1.02
Tagetes erecta L. (Compositae)	50.50±0.78	49.50±0.75	36.27±0.83	81.60±0.55	18.40±0.44	42.64±0.85
Zingiber officinale Roscoe (Zingiberaceae)	22.40±0.86	77.60±0.39	20.00±1.04	44.40±0.36	55.60±0.94	29.12±0.31
Control	100.00	0	46.00±0.75	100.00	0	45.40±0.66

Data after ± represent standard error values.

Table 45

TLC plate bioassay of extracts of two different plants against *B. theobromae* and *C. eragrostidis*

Plant extracts	*B. theobromae*		*C. eragrostidis*	
	Inhibition zone		Inhibition zone	
	Diameter (mm)	R_f	Diameter (mm)	R_f
Melia dubia (Aqueous leaf extract)	10	0.8	-	-
Allium sativum (Aqueous bulb extract)	-	-	38	0.98

4.8. *In vitro* screening of some known biocontrol agents for controlling the *B. theobromae* and *C .eragrostidis*.

Poisoned food technique and dual culture technique as described in materials and methods (Section 3.18.1 & 3.18.2) were followed to screen the antagonistic potentiality of some known biocontrol fungi on the growth of *B. theobromae and C. eragrostidis*. In case of poisoned food technique, cell free culture filtrates of *Trichoderma viride*, *T. koninigii*, *T. harzianum*, *Gliocladium virens* (isolate I) and *G. virens* (isolate II) were tested against *B. theobromae and C. eragrostidis* and radial growth of the test fungi were measured after three days of inoculation and simultanaeously percent inhibitions (with respect to control) were calculated in each case. The results were noted in Table 46 and Table 47 respectively for *B. theobromae and C. eragrostidis*. Similarly, in case of dual plate culture, radial growth and percent inhibitions over control were calculated after three days of inoculation in each case. The results were summarized in Table 48 and Table 49 respectively for *B. theobromae and C. eragrostidis*.

From the results [Table 46 and from the Fig. 26 (A)], it was evident that *Gliocladium virens* (isolate II) showed maximum (45.71%) inhibition of *B. theobromae* followed by *G. virens* (isolate I) and *Trichoderma viride* (40.00% and 27.14% respectively). The other two biocontrol fungi (*T. koningii* and *T. harzianum*) showed less than 25% inhibition. In case of *C. eragrostidis*, the cell-free culture filtrate of *T. harzianum* showed maximum (55.40%) inhibition followed by *G.virens*

(Isolate II) [Table 47 & Fig. 26 (B)]. The other three biocontrol fungi *T. viride*, *T. koningii* and *G. virens* (isolate II) showed 21.62%, 30.81% and 40.54% inhibitions respectively.

Table 46

Effect of cell-free culture filtrates of antagonists on the growth of *B. theobromae*

Antagonists	Radial growth (mm) of *B. theobromae* after 3 days of inoculation*	Percent inhibition over control**
Trichoderma viride	51.00±0.50	27.14±0.71
T. koningii	64.00±0.25	08.75±0.36
T. harzianum	55.00±0.11	21.42±0.16
Gliocladium virens (Iisolate I)	42.00±0.55	40.00±0.79
G. virens (Isolate II)	38.00±0.29	45.71±0.41
CD at 5%	2.54	3.62
CD at 1%	2.19	3.12

* Mean of 3 replications ; ** Control diameter = 70 mm
Data after ± represent standard error values.

Table 47

Effect of cell-free culture filtrates of antagonists on the growth of *C. eragrostidis*

Antagonists	Radial growth (mm) *of C. eragrostidis* after 3 days of inoculation*	Percent inhibition over control**
Trichoderma viride	29.00±0.23	21.62±0.62
T. koningii	25.60±0.11	30.81±0.31
T. harzianum	16.50±0.17	55.40±0.47
Gliocladium virens (Iisolate I)	22.00±0.30	40.54±0.82
G. virens (Isolate II)	19.00±0.29	48.64±0.78
CD at 5%	1.06	2.88
CD at 1%	0.92	2.49

* Mean of 3 replications ; ** Control diameter = 37 mm
Data after ± represent standard error values.

From Table 48 and Fig. 27(A), it was evident that in case of dual plate culture the maximum inhibition (51.51%) was shown by *G. virens* (isolate II) and the least (10.60%) by *T. koningii* against *B.theobromae*. All the other antagonistic

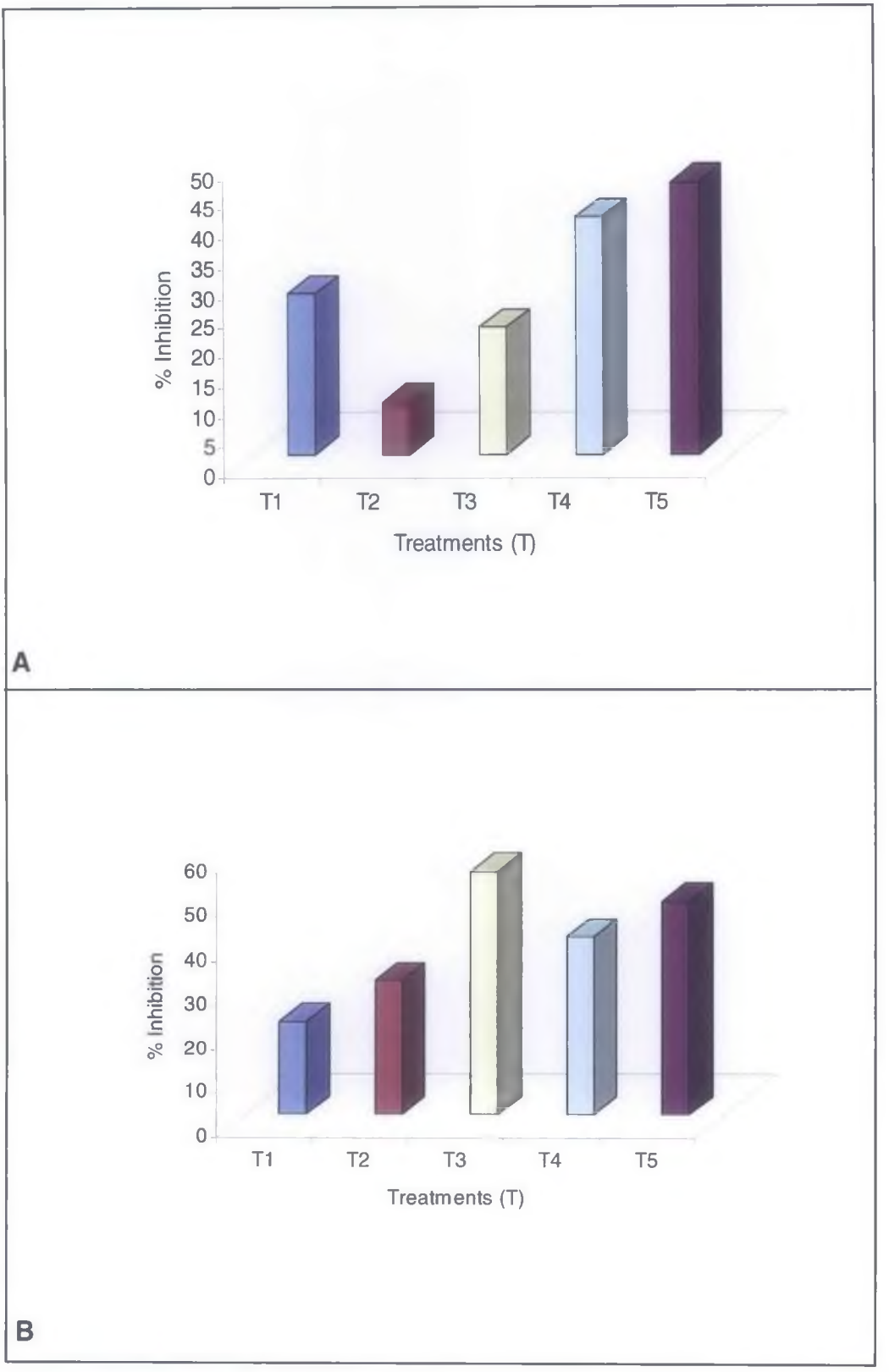


Fig. 26 (A & B): Effect of five different antagonistic fungi on the percent inhibition of the growth of *B. theobromae* (A) and *C. eragrostidis* (B) after poisoned food technique.

[T1 = Culture filtrate of *Trichoderma viride* ; T2 = Culture filtrate of *T. koningii* ; T3 = Culture filtrate of *T. harzianum* ; T4 = Culture filtrate of *Gliocladium virens* (isolate I) ; T5= Culture filtrate of *G. virens* (isolate II)]

fungi tested against *B. theobromae* showed less than 50% inhibition. *C. eragrostidis*, on the other hand was least inhibited (18.47%) by *T. viride* while *T. harzianum* showed maximum inhibition (45.65%) when dual culture technique was performed [Table 49 & Fig. 27 (B); Plate XIV & XV].

Table 48

Effect of antagonists on the growth of *B. theobromae*

Antagonists	Colony diameter (mm) of *B. theobromae* after 3 days of inoculation*	Percent inhibition over control**
Trichoderma viride	45.00±1.00	31.81±1.52
T. koningii	59.00±1.00	10.60±1.52
T. harzianum	51.50±0.87	21.96±1.31
Gliocladium virens (Iisolate I)	37.50±0.87	43.18±1.31
G. virens (Isolate II)	32.00±0.76	51.51±1.15
CD at 5%	2.12	3.23
CD at 1%	1.83	2.78

* Mean of 3 replications ; ** Control diameter = 66 mm
Data after ± represent standard error values.

Table 49

Effect of antagonists on the growth of *C. eragrostidis*

Antagonists	Colony diameter (mm) of *C. eragrostidis* after 3 days of inoculation*	Percent inhibition over control**
Trichoderma viride	37.50±0.86	18.47±0.73
T. koningii	34.50±0.77	25.00±1.14
T. harzianum	25.00±0.96	45.65±0.97
Gliocladium virens (Iisolate I)	30.00±1.00	34.78±1.16
G. virens (Isolate II)	28.00±1.53	39.13±1.11
CD at 5%	4.79	4.51
CD at 1%	4.14	3.89

* Mean of 3 replications ** Control diameter = 46mm.
Data after ± represent standard error values.

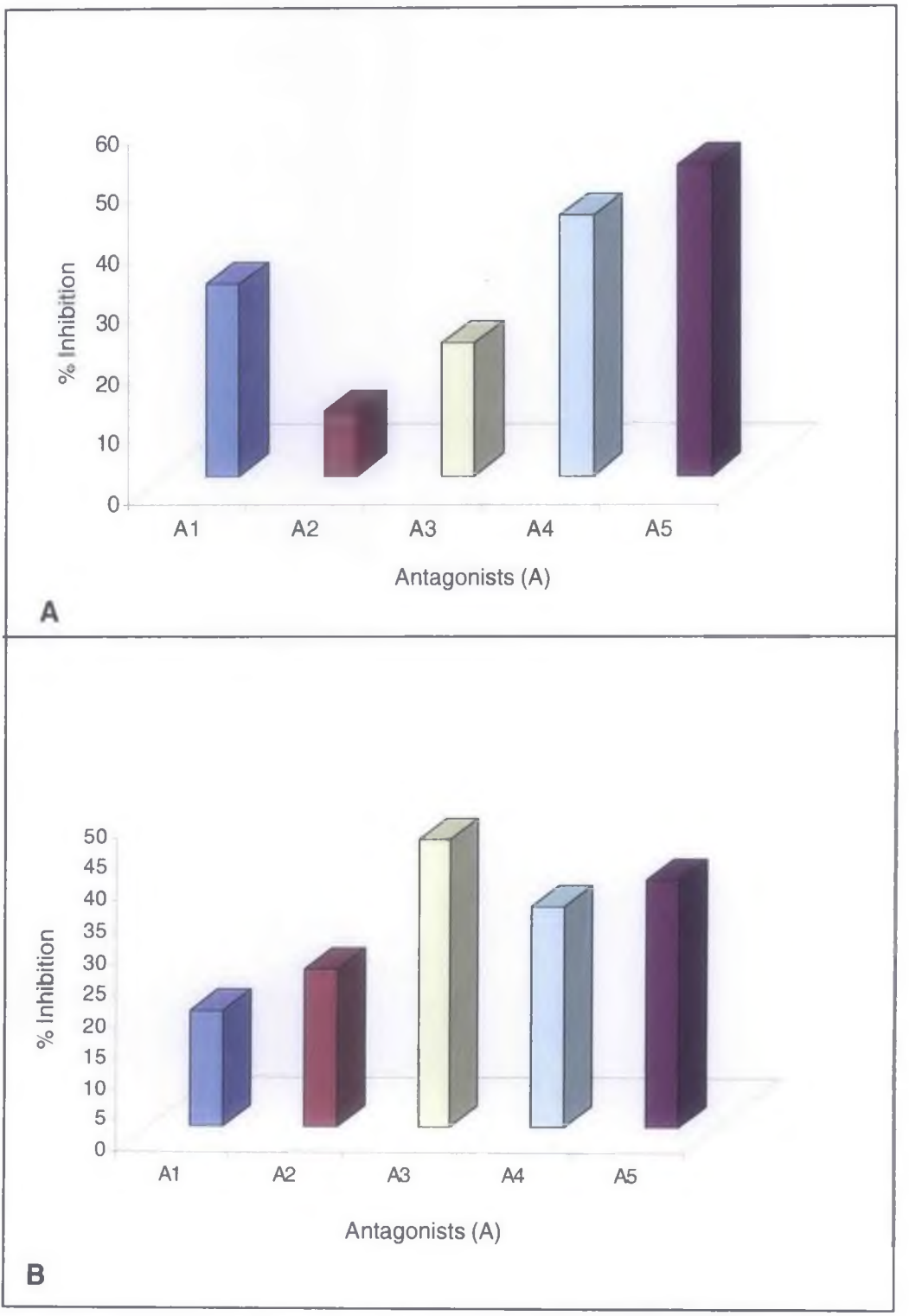
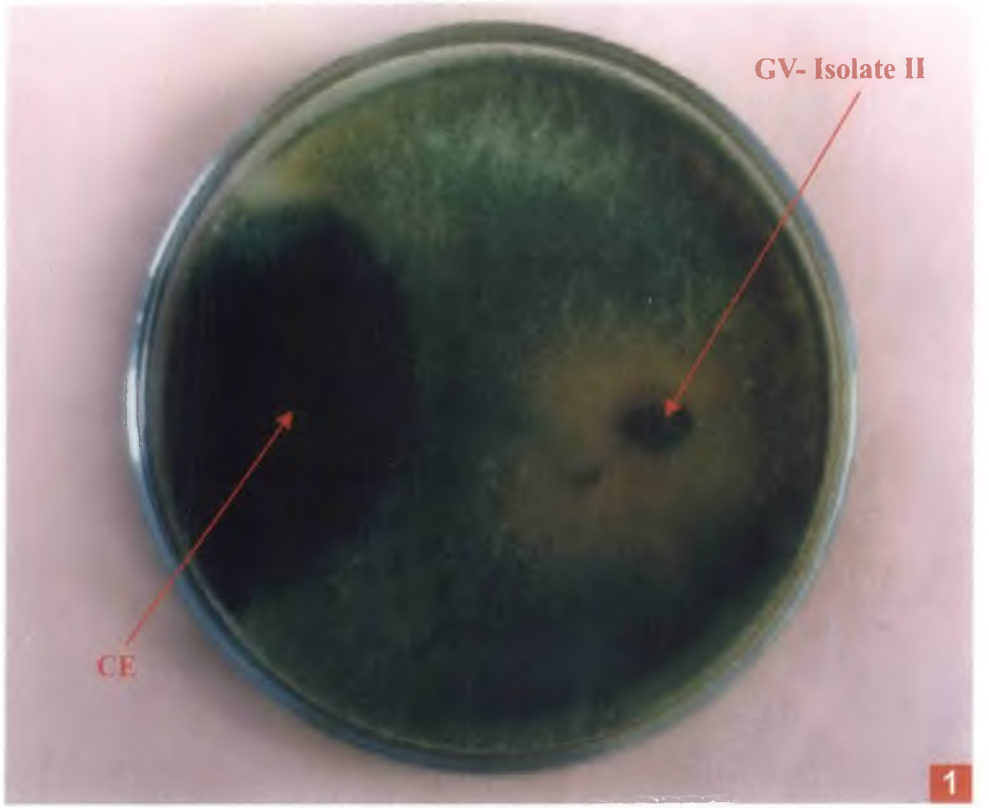


Fig. 27 (A &B): Effect of five different antagonistic fungi on the percent inhibition of the growth of *B. theobromae* (A) and *C. eragrostidis* (B) after dual culture technique.

[A1 = *Trichoderma viride* ; A2 = *T. koningii* ;A3 = *T. harzianum* ; A4 =*Gliocladium virens* (isolate-I) ; A5 = *G. virens* (isolate-II)]



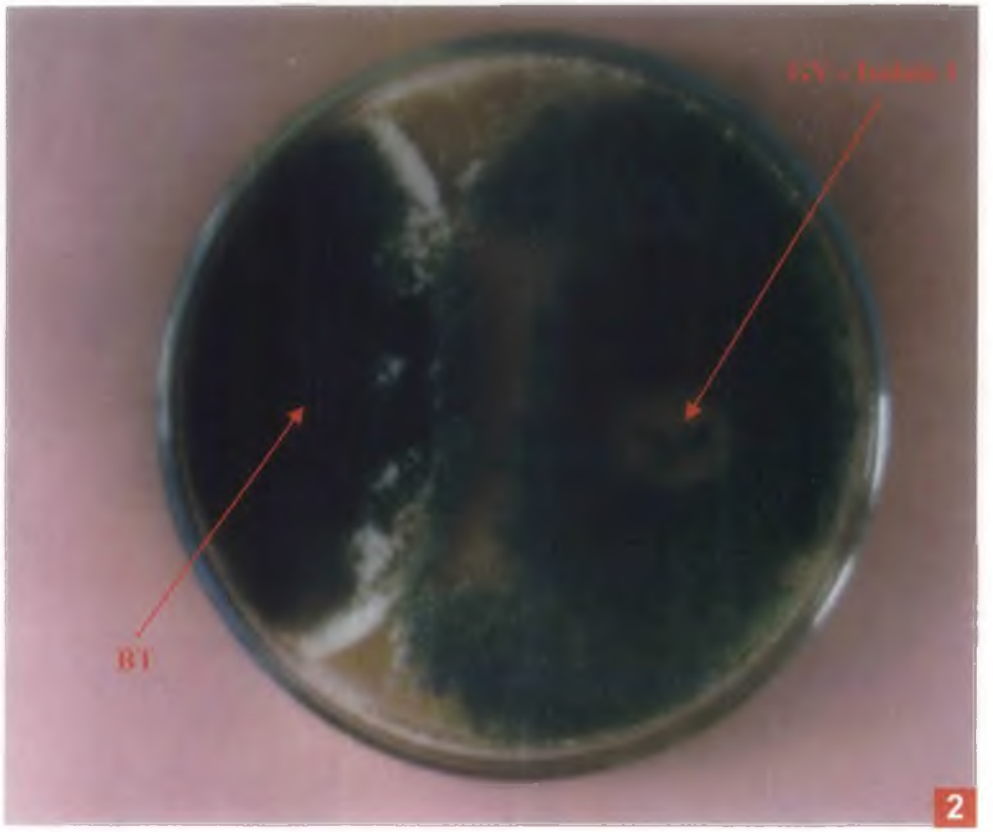


Plate XIV

Fig. 1: Dual culture of *Gliocladium virens* (GV-Isolate II) & *Curvularia eragrostidis* (CE)
Fig. 2: Dual culture of *G.virens* (GV-Isolate I) & *Botryodiplodia theobromae* (BT)

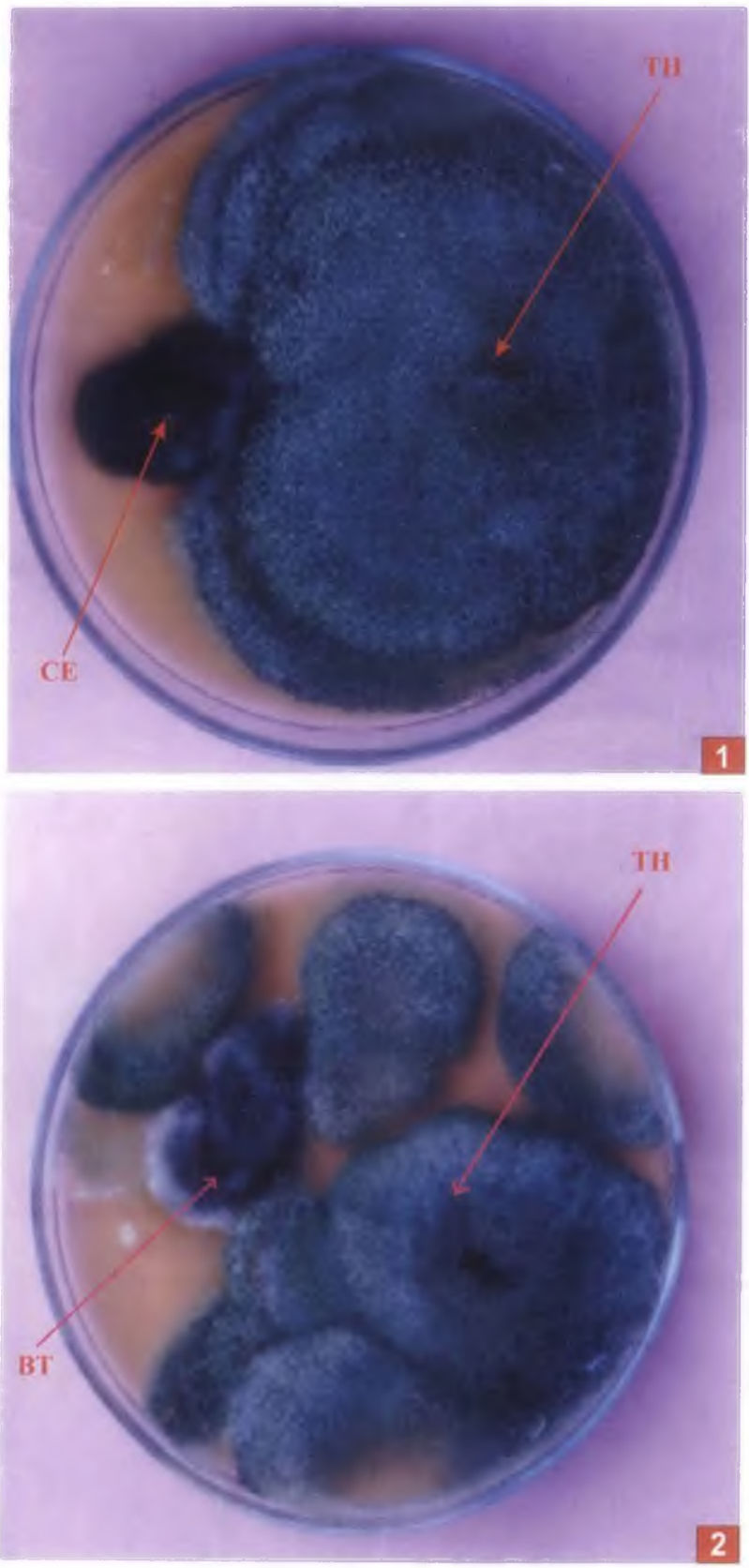


Plate XV

Fig. 1: Dual culture of *Trichoderma harzianum* (TH) & *Curvularia eragrostidis* (CE).
Fig. 2: Dual culture of *T. harzianum* (TH) & *Botryodiplodia theobromae* (BT).

4.9. Foliar application of plant extract, fungal antagonists and fungicides for controlling the foliar diseases of nursery tea plants caused by *B. theobromae* and *C. eragrostidis*.

The nursery tea plants of susceptible variety (TV-11) were sprayed (in separate sets) with the crude aqueous extract of leaves of *M. dubia* (2 g fresh weight/10 ml distilled water), fungicide calixin (500 μgml^{-1}) and spore suspension of two biocontrol fungi *T. harzianum* and *G. virens*-isolate II. After 24 hours of spraying, the plants in each set were inoculated with *B. theobromae* following the whole plant inoculation method of Dickens and Cook (1989) [Section 3.6.3]. In control set, the plants were only inoculated with *B. theobromae* without any spraying.

Mean disease index/plant was calculated following the procedure of Sinha and Das (1972) and noted in Table 50 and graphically presented in Fig. 28. Similarly, in another experiment crude aqueous extract of bulbs of *A. sativum*, fungicide calixin (500 μgml^{-1}) and spore suspension of two biocontrol fungi, *T. harzianum* and *G. virens*-isolate II were sprayed (in separate sets) on the nursery tea plants of TV-12 and after 24 h each set was inoculated with *C. eragrostidis*. In control set no spraying was done but the plants were inoculated with *C. eragrostidis*. Mean disease index/plant was noted in the Table 51 and represented graphically in Fig. 29.

From the results represented in Table 50, it was quite clear that among the spray-treatment, the mean disease index/plant (when the plants were inoculated with *B. theobromae*) was maximum (3.95) when the plants were sprayed with fungicide calixin and minimum (2.64) when the plants were sprayed with crude aqueous leaf extracts of *M. dubia*, after 16 days of inoculation. It was also evident from the results (Fig. 28) that the disease occurrences were significantly reduced in each spraying set from the untreated-inoculated control.

In *C. eragrostidis* inoculated experimental sets, mean disease index/plant was maximum (4.91) when the plants were sprayed with spore suspension of *T. harzianum* and minimum (2.68) when the plants were sprayed with crude aqueous bulb extracts of *A. sativum*, after 16 days of inoculation (Table 51). In control set, disease occurrence was always found to be higher than experimental sets after any of the specified time period (Fig. 29).

Table 50

Control of nursery tea plants (TV-11) against foliar disease caused by *B. theobromae* by foliar treatment with crude plant extract, fungal antagonists and fungicide

Treatment	Mean disease index/plant*			
	Incubation periods			
	4days	8days	12 days	16days
Crude aqueous leaf extract of *M. dubia*	0.65 ±0.040	1.78 ±0.066	1.98 ±0.086	2.64 ±0.047
Spore suspension of *G. virens* (Isolate II)	0.78 ±0.055	2.09 ±0.102	3.26 ±0.047	3.70 ±0.048
Spore suspension of *T. harzianum*	1.59 ±0.056	2.51 ±0.045	3.48 ±0.052	3.76 ±0.068
Calixin at 500 µg ml^{-1} concentration	0.36 ±0.063	2.81 ±0.059	3.18 ±0.034	3.95 ±0.047
Control (untreated-inoculated)	0.68 ±0.057	3.61 ±0.073	6.50 ±0.075	8.81 ±0.035
CD at 5%	0.11	0.18	0.15	0.11
CD at 1%	0.09	0.16	0.13	0.09

* Mean of 3 replications ; Data after ± represent standard error values.

Table 51

Control of nursery tea plants (TV-12) against foliar disease caused by *C. eragrostidis* by foliar treatment with crude plant extract, fungal antagonists and fungicide

Treatment	Mean disease index/plant*			
	Incubation periods			
	4days	8days	12 days	16days
Crude aqueous bulb extract of *A. sativum*	0.98 ±0.090	1.23 ±0.051	2.45 ±0.066	2.68 ±0.061
Spore suspension of *G. virens* (Isolate II)	1.08 ±0.070	2.85 ±0.043	3.52 ±0.055	4.39 ±0.072
Spore suspension of *T. harzianum*	1.25 ±0.049	3.11 ±0.055	4.04 ±0.034	4.91 ±0.047
Calixin at 500 µg ml^{-1} concentration	0.59 ±0.055	1.09 ±0.087	2.21 ±0.058	3.66 ±0.081
Control (untreated-inoculated)	1.50 ±0.052	3.29 ±0.070	4.82 ±0.085	7.01 ±0.086
CD at 5%	0.18	0.20	0.21	0.15
CD at 1%	0.15	0.17	0.19	0.14

* Mean of 3 replications ; Data after ± represent standard error values.

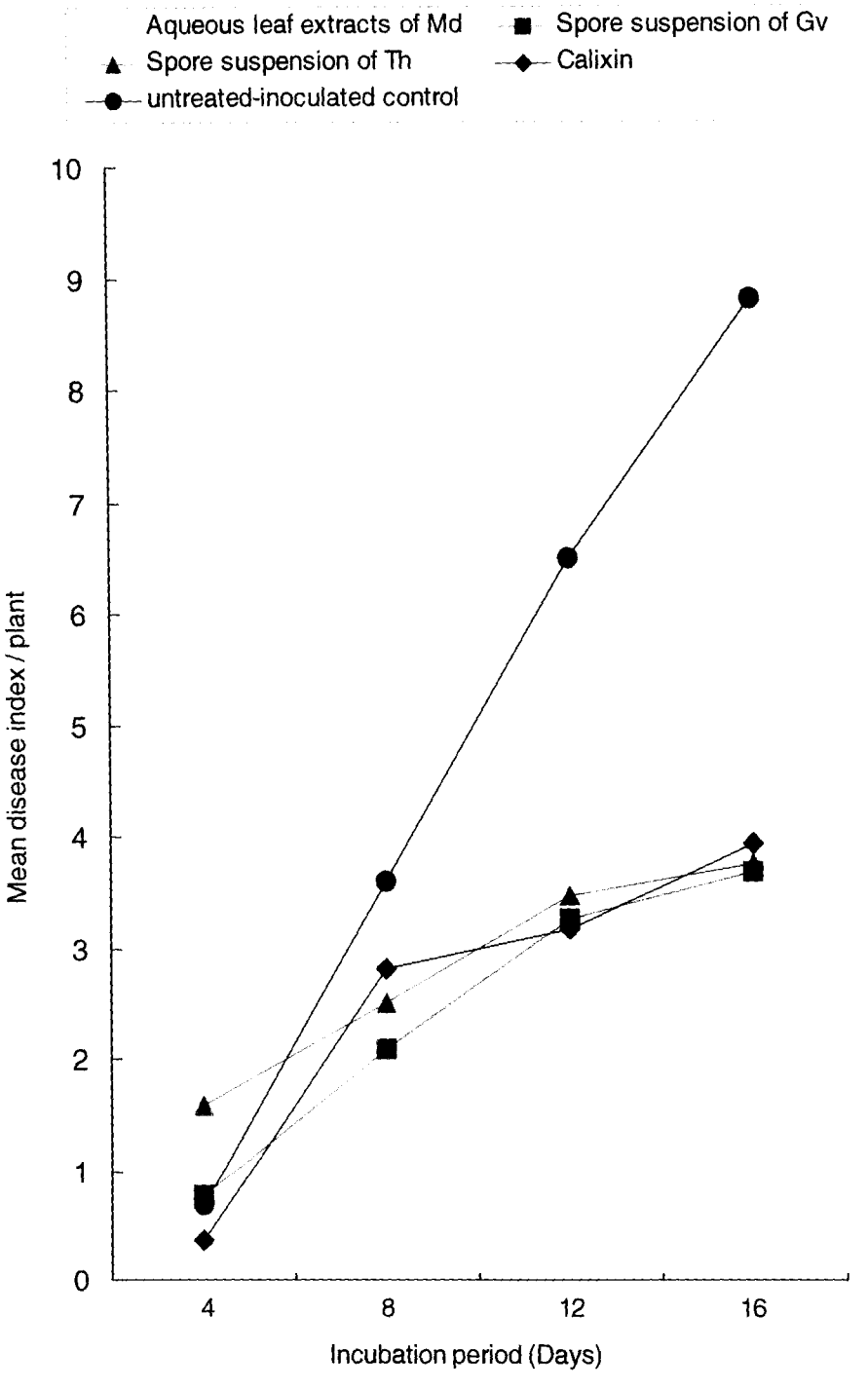


Fig. 28: Effect of plant extract, fungicide and biocontrol fungi on nursery tea plants (TV-11) against foliar disease caused by *B. theobromae.*

[Md = *M. dubia*; Th = *T. harzianum*; Gv = *G. virens* (isolate II)].

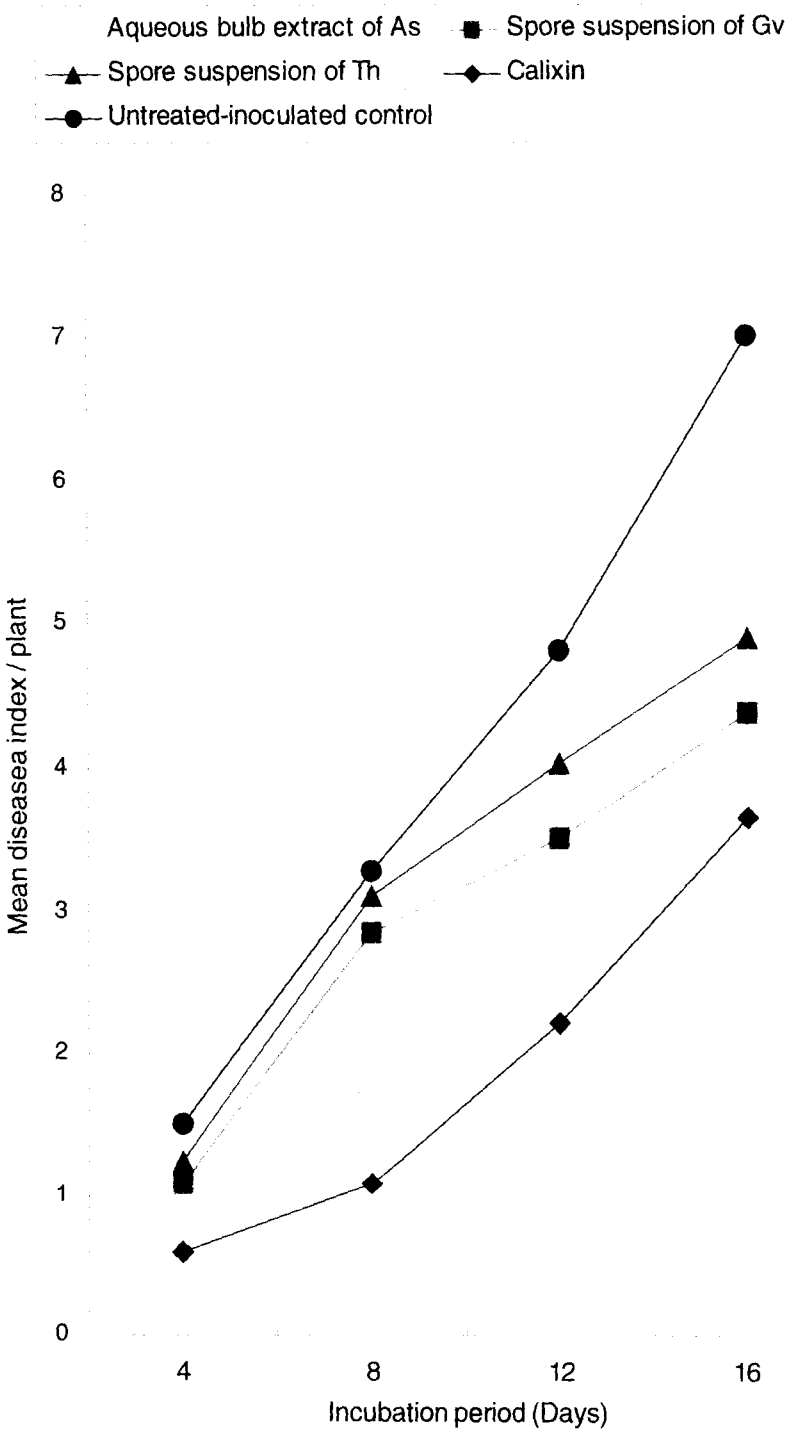


Fig. 29: Effect of plant extract, fungicide and biocontrol fungi on nursery tea plants (TV-12) against foliar disease caused by *C. eragrostidis*

[As = *A. sativum*, Th = *T. harzianum*; Gv = *G. virens* (isolate II)]

Discussion

In India cultivation of tea started around 1830 when tea plants were raised from the seeds. With the huge demand of the planting materials for the plantation industry the economic method of vegetative propagation by clonal cuttings was established around 1938 but this type of propagation became essential after the release of Tocklai clones in 1949 (Bezbaruah and Singh, 1988). To get healthy tea plants, the importance of healthy seedlings is also essential. Presently several clones are available for raising tea plants. The clonal cuttings are raised in the nurseries. Proper disease management is required for raising healthy seedlings. Plant disease management is an important aspect of successful cultivation of any crop. The applications of broad-spectrum chemical fungicides are the common practice in most of the nurseries. However, this is hazardous to the environment. Hence, it is essential to adopt ecofriendly methods to control fungal diseases in the nurseries. With these observations the present work was undertaken and it is likely that the results will broaden the scientific base upon which total control of foliar fungal diseases of nursery tea plants may be established.

At the beginning, several fungi were isolated from the plant parts of young tea plants in the nurseries. These are *B. theobromae*, *C. eragrostidis*, *C. cammelliae*, *P. theae*, *C. theae*, *F. oxysporum* etc. After isolation their pathogenesis were tested through verification of Koch's postulations. It was found that six different fungi (*B. theobromae*, *C. eragrostidis*, *C. cammelliae*, *P. theae*, *C. theae*, *F. oxysporum*) were producing diseases in the nurseries in North Bengal. Out of six fungi, *B. theobromae* and *C. eragrostidis* were consistently found to be associated with the young seedlings. Out of the six organisms found, *C. cammelliae* and *P. theae* were mostly isolated from the mature maintenance leaves. This finding is very much in conformity with that of Sarmah (1960). Sarmah (1960) reported that *C. camelliae* and *P. theae* were extremely common in old tea leaves. After development of the new shoots upto 2 to 6 leaves stages *C. cammelliae* and *P. theae* were not found in the isolations made from the young leaves. In one isolation, *F. oxysporum* was also found associated with the mature leaves but the involvement of *F. oxysporum* was not found frequently. *F. oxysporum* was reported as a pathogen of tea by previous workers (Debnath and Barthakur, 1994; Pandey *et al.*, 2000). Another pathogen *C. theae* was found in cases of heavy rainfall followed by prevailing

humid weather for prolonged period generally in the month of July and August in North Bengal. But this was also found in few cases of isolations made from young tea plants raised from clonal cuttings. The fungi like *B. theobromae and C. eragrostidis* were very common in the nurseries of North Bengal and were found in the isolations frequently. Out of the two pathogens *C. eragrostidis* is a new report in tea (Saha *et al.*, 2001). *B. theobromae* may attack any part of the tea plant, young or old. In our study also, it was isolated from all the parts of young tea plants but the tender stem and leaves of nursery plants were more prone to attack by the fungi. *C. eragrostidis* (P. Hennings) Meyer. was originally isolated from naturally infected leaves of nursery tea plants of Matigara tea estate, Siliguri, West Bengal. After verification Koch's postulations the fungus was identified in the laboratory and was also sent to ITCC, IARI, New Delhi for identification. The two pathogens have major role for the failure of seedling growth in the tea nurseries.

Differential pathogenicity of a fungus to different varieties gives us information about the degree of susceptibility or resistance of a particular variety or pathogenicity of different fungi to a particular plant variety gives us information about differernt pathogen's different infecting ability. Pathogenicity of the isolated fungi, *B. theobromae* and *C. eragrostidis*, was tested following three different techniques, viz. detached leaf inoculation, cut shoot inoculation and intact nursery tea plant inoculation. Results obtained from studies following different techniques were in agreement with each other. Dickens and Cook (1989) also used all these three methods to detect resistance and susceptibility of *Camellia* plants against *Glomerella cingulata*. Brennan *et al.* (2003) examined the pathogenicity of five different fungal species (*Fusarium areenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *Macrodochium nivale*) following the *in vitro* coleoptile growth rate of wheat seedlings (cv. Falstaff). Yanase and Takada (1987) followed only the cut shoot method for determining the resistance of tea plants to gray blight disease caused by *Pestalotia longiseta* in his laboratory.

The pathogenicity results distinctly showed that TV-11 was the most susceptible and TV-26 was the most resistant against *B. theobromae* among the varieties tested. TV-12 and TV-25 were found to be the most susceptible and resistant varieties respectively when tested against *C. eragrostitis*. Saha (1992)

observed that TV-26, TV-25 and TV-16 were resistant and TV-18, TV-9 and TV-17 were succeptable varieties when tested against *Bipolaris carbonum*, a foliar fungal pathogen of tea. Pathogenicity of other foliar tea pathogens like *Pestalotiopsis theae* and *Colletotrichum cammelliae* were studied by Chakraborty *et al.* (1995) who found that TV-18 was highly susceptible and TV-9 was moderately resistant among different clonal and seed varieties. Hu-Shu Xia (1996) studied the pathogenicity of *P. theae* in eighteen tea cultivers in Anhui province, China and found that two cultivers were highly resistant. The results of the present study is in conformity with that of the studies of earlier workers. Thus, the identity of a tea variety may inform us about its degree of susceptibility or resistance towards foliar fungal pathogens. Such information might be helpful in disease management specially during multiple pathogen attack.

Once a fungal pathogen is isolated and identified, a thorough understanding on the morphological and physiological features becomes necessarry. It forms the basis of further studies on understanding disease development, host-pathogen interaction and control of the disease caused by the pathogen. These basic knowledge becomes more essential when a fungus is newly recorded to be a pathogen. Since, one of the pathogens isolated during the present work is being reported as the tea leaf pathogen for the first time, any work on defense responses of the plant to pathogen attack or control strategies of the disease caused by the pathogen could not be worthwhile without basic knowledge on the morphological and physiological aspects of the fungal pathogen. Hence, a thorough microscopic observation of the morphological characters of mycelia and spores along with studies on growth conditions and nutritional requirements of both the pathogens were undertaken.

In the present study, six different solid media (PDA, MEA, CDA, OMA, PCA and RA) were used to study the growth of *B. theobromae* and *C. eragrostidis*. Among these, malt extract agar (MEA) was found to be the best growth medium for *B. theobromae* while *C. eragrostidis* showed maximum growth in potato carrot agar (PCA) although both the fungi showed satisfactory growth in all the solid media tested. However, differences in their radial growth pattern was observed when *C. eragrostidis* recorded maximum radial growth after 8 days of incubation while

B. theobromae showed the same after the second day. Rapid increase in growth was observed within the first 5 days after which the rate of growth decreased. After 15 days mycelial dry weight declined due to autolysis and depletion of media. There was no significant difference in the growth of the two fungi when mycelial dry weights were considered though the radial growth pattern differed. During growth, mycelia of *B. theobromae* adhered to the surface of the agar media and grew rapidly extending the radius while *C. eragrostidis* showed vertical extended fluffy growth and extended the radius at a much slower rate.

Studies on the nutritional requirements of the pathogens revealed that for *B. theobromae* glucose and sucrose were the best carbon sources for growth and sporulation respectively. For *C. eragrostidis* glucose was the best carbon source for both growth and sporulation. However, in sucrose, sporulation started earlier in case of both pathogens. Sucrose was also found to be the best source for growth and sporulation of *B. carbonum* by Saha (1992). Peptone was the best nitrogen source for both growth and sporulation of *C. eragrostidis*. For *B. theobromae*, peptone was best for growth but sporulation was recorded better when potassium nitrate and yeast extract were the nitrogen sources. Saha (1992) recorded that ammonium nitrate was the best nitrogen source for growth of *B. carbonum* and sodium nitrate was best for sporulation among the inorganic nitrogen sources tested while peptone was the best among the organic nitrogen sources tested for both growth and sporulation of the pathogen.

Wu and Wu (2003) found that *Alternaria protenta*, a pathogen of sunflower showed abundant sporulation on glucose peptone agar and leonien agar but not on dextrose nitrate agar.

The influence of various carbon and nitrogen sources of fungal metabolism has been studied by several workers (Jamaluddin, 1977; Roy, 1977; Devadath and Padmanabhan, 1977). In a study, potassium nitrate was found to be a satisfactory nitrogen source for the growth of *Aspergillus flavus* by Jamaluddin (1977).

Since spore germination is a determining factor at the onset of host colonization by a fungal pathogen, several studies were undertaken to evaluate the influence of environmental factors like pH, temperature, incubation periods etc. on

the germination of spores *in vitro*. *C. eragrostidis* showed slightly quicker and better germination than *B. theobromae* but germ tubes of *B. theobromae* were much longer than *C. eragrostidis* when their spores were allowed to germinate on microscopic slides. Germination started within 2 hours in case of *C. eragrostidis* and between 2-4 hours for *B. theobromae.* Saha and Chakraborty (1990) also observed that germination of *B. carbonum* begun between 2-4 hours *in vitro*. During the present study, the best temperature for germination was found to be 28 ^{0}C. Pretreatment of spores of both *B. theobromae* and *C. eragrostidis* at 45 ^{0}C and above totally inhibited their germination. Saha and Chakraborty (1990) observed that spore germination of *B. carbonum* was reduced to 27% when pretreated at 50 ^{0}C but pretreatment at 0 ^{0}C for even 12 hours had no effect on germination and germ tube elongation. Flett and Wehner (1989) observed that high temperature reduced the viability of spores. In another study, Achar (2000) observed that conidia from *Stenocarpella maydis* exposed at temperatures below 22 ^{0}C germinated only after 17 hours of incubation and rate of germination increased from 22-27 ^{0}C, afterwhich the germination rate declined.

The prevailing temperatures in areas covered during the present study and also in other tea growing areas do not reach above 40 ^{0}C. Hence, spores are never exposed to temperatures which may be as high to reduce germination. Both the pathogens under study may persist in the soil or leaves for extended time periods and germinate rapidly in the presence of a potential host. Studies of effect of pH on spore germination showed that pH 7.25 was the best pH for both germination and germ tube elongation. Saha and Chakraborty (1990) reported that pH 6.75 was best for germination of spores of *B. carbonum* while pH 7.2 was best for germ tube elongation. Callaghan (1974) reported that more than 97% of conidia in *Basidiobolus ranarum* germinated between pH range 7-9.

Common antigens generally show precipitin arc in the agarose gel. Clausen (1969) demonstrated immunodiffusion in semi-solid matrices (i.e., precipitin reactions of antigens and antibodies) as a major tool in serology. Three basic immunological techniques are radial immunodiffusion, immunoelectrophoresis and agar gel double diffusion. Several authors have successfully used these techniques while demonstrating cross-reactive antigens (Michael and James, 1981; Benhamou

et al., 1986), detection of plant pathogens (Burrell *et al.*, 1966; Ishizaki *et al*, 1981; Iannelli *et al.*, 1982) and common antigenic relationship (Burrell *et al.*, 1966; Chard *et al.*, 1985; Alba and DeVay, 1985; Purkayastha and Ghosal, 1987; Purkayastha and Banerjee, 1990; Chakraborty and Saha, 1994;Ghosh and Purkayastha, 2003). Enzyme-linked immunosorbent assay (ELISA) and direct and indirect fluorescent antibody staining were also reported as important techniques for detecting pathogen, cross-reactive antigens and establishment of common antigenic relationship (Warnock, 1973; Gendloff *et al.*, 1983; Geric *et al.*, 1987; Arie *et al.*, 1988; Fuhrmann *et al.*, 1989; Watabe, 1990). Chakraborty *et al.* (1997) demonstrated that the presence of cross-reactive antigens between *Exobasidium vexans* and the susceptible tea varieties were evident in immunodiffusion, indirect ELISA and indirect immunofluorescence tests. Using similar techniques, cross-reactive antigens were found between *Bipolaris carbonum* and susceptible tea varieties (Chakraborty and Saha, 1994) and also between *Pestalotiopsis theae* and susceptible tea varieties (Chakraborty *et al.*, 1995).

In the present study, the leaf antigens of susceptible and resistant varieties of tea were cross reacted separately with antisera of *B. theobromae* and *C. eragrostidis*. The mycelial antigens of *B. theobromae* and *C. eragrostidis* were also cross reacted with antisera of susceptible and resistant tea varieties. Serological comparisons were made in all the tests. Serological comparisons were also made by a non pathogen of tea viz. *Gliocladium virens* (isolate II). No common antigen could be detected in the agar gel double diffusion test when antigens of resistant varieties of tea were cross reacted with the antisera of the pathogens respectively. But the susceptible varieties of tea showed presence of common antigens cross reacted with the antisera of the pathogens. In the reciprocal reactions also common antigens were detected in the form of precipitin arcs. No precipitin arcs could be detected when antisera of tea varieties and antigen of *Gliocladium virens* (non pathogen to tea) was allowed to react.

Cross-reactive antigens has been detected in a number of host parasite combinations. Some of the combinations are as follows, soybean and *Macrophomina phaseolina* (Chakraborty and Purkayastha, 1983); Jute and *Colletotrichum corchori* (Bhattacharya and Purkayastha, 1985); soybean and

Colletotrichum dematium var. *truncata* (Purkayastha and Banerjee, 1990); groundnut and *Macrophomina phaseolina* (Purkayastha and Ghosal, 1987); soybean and *Myrothecium roridum* (Ghosh and Purkayastha, 1990).

Chakraborty and Saha (1994) in a study showed that two precipitin arcs were common when leaf antigens of susceptible tea variety reacted with antiserum of pathogen *Bipolaris carbonum*. No precipitin arc was detected in the agar gel double diffusion tests when resistant tea variety antigen reacted with the antiserum of *B. carbonum* and also in reciprocal reaction. Their finding suggests that common antigens play an important role in the compatibility of the host-pathogen interaction. Till date a number of host-pathogen/nonpathogen interactions have been studied by workers which include several plants, and their pathogen and nonpathogens.

Ghosh and Purkayastha (2003) done immunodiffusion tests using polyclonal antibodied and antigens of different ginger cvs, virulent (SR 2) and avirulent strains (IR) of *Pythium aphanidermatum* and a nonpathogen of ginger, *Colletotrichum capsici*. Cross reactive antigens (CRAS) were not detected between antigens of infected rhizome or nonpathogen and antiserum of avirulent strains of *P. aphanidermatum* SR 2, but CRA was easily detected when antigens of heavily infected ginger (cv. Mahima) were cross-reacted with antiserum of the pathogen, which was confirmed by immunoelectrophoretic and cross immunoelectrophoretic tests.

In all the cases the common antigens could be detected between susceptible varieties and pathogens. No common antigenic relationship was found between resistant varieties and pathogen. Similarly no antigenic relationship could be established between antigens of nonpathogens and antisera of the plants of interest. In the light of earlier studies the present finding is very much significant and in conformity with the earlier works.

The results of agar gel double diffusion were substantiated by immunoelectrophoretic studies. Antisera of *B. theobromae* when reacted of its own, it showed four precipitin arcs. Out of the four precipitin arcs only one precipitin arc was common with leaf antigen of susceptible variety (TV-11). No precipitin arc was found between antigen of resistant variety (TV-26), and antisera of *B. theobromae*.

In homologous reaction antiserum of TV-26 showed three precipitin arcs but in cross reaction with *B. theobromae* showed no precipitin arc. Similarly, when antiserum of *C. eragrostidis* reacted with antigen of its own, four precipitin arcs were found. Only one was common between the antiserum of *C. eragrostidis* and antigen of susceptible variety (TV-12). No precipitin arc could be detected between antigen of resistant variety (TV-25) and antisera of *C. eragrostidis.* No common relationship between host (*C. sinensis*) and nonpathogen was observed.

The results of the present study are in agreement with the works of the earlier workers. The results of the immunodiffusion and immunoelectrophoretic tests clearly indicate that there is no common antigenic relationship between hosts and nonpathogens and common antigenic relationship exists between pathogen and susceptible hosts. Absence of common antigenic relationship between pathogens and resistant hosts.

At a very low concentration, enzyme-linked immunosorbent assay (ELISA) is one of the most specific and rapid methods for detecting cross-reactive antigen (CRA) and identifying fungal diseases (Alba and DeVay, 1985; Mohan, 1988; Dewey and Brasier, 1988; Lyons and White, 1992; Linfield, 1993; Chakraborty and Saha, 1994; Chakraborty *et al.*, 1995, 1997). Croft (2002) used an enzyme-linked immunoassay for rating sugarcane cultivars for resistance to ratoon stunting disease caused by *Leifsonia* (*Clavibacter*) *xyli* subsp. *Xyli.* ELISA has also been used for early detection of pathogens by several workers (Chakraborty and Saha, 1994; Ghosh and Purkayastha, 2003).

In this study, CRA was detected in indirect ELISA using very low concentrations of antigens and antisera. The higher ELISA values in cross reactions indicated presence of more CRA and also indicated susceptibility and lower ELISA values indicated lower amount of CRA and also indicated resistance. It is to note that the degree of resistance and susceptibility results (by ELISA values) were in conformity with the results of the pathogenicity. The three concentrations of the antigens of *B. theobromae* showed higher absorbance values when tested with antisera of susceptible variety (TV-11) than when tested with antisera of resistant variety (TV-26). The reciprocal test of this combination also showed higher

absorbance values in case of the antigens of susceptible variety (TV-11) tested with antisera of *B. theobromae* than in case of the antigens of resistant variety (TV-26) tested with the antisera of *B. theobromae*. These clearly indicated the presence of maximum cross-reactivity between *B. theobromae* and susceptible variety (TV-11) than the other combinations of the antigens and antisera. In the similar way, all the three concentrations of the antigens of *C. eragrostidis* showed higher absorbance values when tested with two different dilutions of the antisera of susceptible variety (TV-12) than when tested with two different dilutions of the antisera of resistant variety (TV-25). The reciprocal test of this combination also showed higher absorbance values in case of the antigens of susceptible variety (TV-12) tested with antisera of *C. eragrostidis* than in case of the antigens of resistant variety (TV-25) tested with the antisera of *C. eragrostidis*. These clearly indicated the presence of cross-reactivity was maximum between *C. eragrostidis* and susceptible variety (TV-12) than the other combinations of antigens and antisera.

Chakraborty and Saha (1994) also detected presence of CRA between *Bipolaris carbonum* and susceptible tea varieties in indirect ELISA. They used polyclonal antibodies and goat-antirabbit IgG conjugate. In semipurified mycelial preparation of concentrations 5-25 µg/ml were cross reacted with antisera dilution of 1/125 and 1/250. Antigenic preparations of *B. carbonum* (isolate BC 1) exhibited higher absorbance value in cross reaction with antiserum of susceptible tea variety than the reaction with antiserum of resistant variety.

Alba and DeVay (1985) detected CRA in crude as well as in purified preparations of mycelia of *Phytophthora infestans* race 4 and race 12.3.47 with antisera of potatoes (cv. King Edward and cv. Peutland Dell) by using indirect ELISA. Mohan (1988) detected homologous soluble antigens at protein concentration of 2 µg/ml using antisera of *Phytophthora fragariae* in indirect ELISA.

Chakraborty *et al.* (1996) detected CRA in semipurified mycelial preparations at concentrations ranging from 5-25 µg/ml with antiserum dilution of 1/125. Antigenic preparations from Glomerella cingulata exhibited higher absorbance value (>2) when cross reacted with antiserum of susceptible variety than resistant one.

In the present study, polyclonal antibodies (raised in rabbits) indirectly labeled with fluorescein isothiocyanate conjugate (conjugated with antirabbbit globulin-specific goat antiserum) were used to locate CRA in the leaf sections of tea and mycelial cells of the fungal pathogens. When the leaf sections were treated with the antisera of respective leaf varieties i.e. with homologous antisera indirectly labeled with fluorescein isothiocyanate (FITC), bright fluorescence was observed. Fluorescence was observed in the epidermal regions, mesophyll tissues and xylem elements of the leaf. Leaf sections in heterologous treatments (i.e. when the leaf sections were treated with antisera of the two pathogens and then reacted with FITC) showed comparatively more fluorescence in susceptible varieties than the resistant one. The CRA observed in heterologous reactions, were mainly concentrated around the epidermal cells. In homologous treatments of the fungal mycelia, intense fluorescence was observed around the hyphae and conidia. In cross-reactions, when the fungal mycelia were treated with antisera of susceptible varieties indirectly labeled with FITC, fluorescence was observed in the hyphal tips and conidia but the fluorescence was not so strong when the mycelia were treated with the antisera of resistant varieties and FITC.

In indirect immunofluorescence test, strong fluorescence was observed in the hyphae and in the conidia of both the pathogens *B. theobromae* and *C. eragrostidis* in both homologous and heterologous treatments. Intense fluorescence was also found in the regions of epidermal cells and mesophyll tissues of the leaf sections of susceptible varieties treated with homologous and heterologous antisera indirectly labeled with FITC-antirabbit IgG conjugate indicating the tissue and cellular location of CRA. Similar tissue and cellular location of CRA in cross-sections of cotton roots (DeVay *et al.*, 1981a), potato leaves (DeVay *et al.*, 1981b) and tea leaves (Chakraborty and Saha, 1994; Chakraborty *et al.*, 1995, 1997) using fluorescein isothiocyanate (FITC) labeled antibodies have been reported.

Chakraborty and Saha (1994) showed locations of CRA in tea leaf sections using antibodies (raised against *B. carbonum*) indirectly labeled with FITC. CRA between TV-18 and fungal cells (*B. carbonum*) were mainly present in hyphal tips and in patch like areas on conidia, on mycelium and mainly around epidermal cells and mesophyll tissues of leaves. Cross reactions of young cotton (Acala-2) roots

inoculated with antiserum to *Fusarium oxysporum* f. sp. *vasinfectum* and stained with FITC-conjugated, antirabbit globulin-specific goat antiserum exhibited strong fluorescence at the epidermal and cortical cells and the endodermis and xylem tissues indicating a general distribution of the CRA determinants in roots (DeVay *et al.*, 1981a). DeVay *et al.* (1981b) also showed the interaction of *Phytophthora infestans* and potato plants.

The onset of systemic acquired resistance (SAR) correlated with the systemic induction of genes, especially genes encoding pathogenesis-related proteins (Lamb *et al.*, 1989; Ryals *et al.*, 1996). The basic concept of disease development requires a susceptible host plant, a virulent pathogen and a suitable environment (Agrios, 1997). It may be assumed that a change in any of these three factors can result in less or no disease. Changes in the physical environment can often have a profound effect on the physiology of the plants as it adapts to the change (Thomashow, 1999) Plant resistance is usually of the passive type; most plants are non-host for most pathogenic fungi. However, when plants are infected by fungal parasites, a number of situations are encountered ranging from very weak to no defense to very intense or active defense ultimately leading to resistance (Klarzynski and Fritig, 2001). Natural plant defence reactions gained high attention both in 'reasoned agriculture' and fundamental research. These studies were focused on plant-pathogen recognition, signal transduction and induction of resistance (SAR). It has been demonstrated that the activation of resistance in plants is generally initiated by host recognition of elicitors directly or indirectly released from the invading pathogen. Despite various chemical composition, many elicitors of plant defence reactions share a signaling pathway that coordinates the plant defences (Ebel and Cosio, 1994; Ebel and Mithöfer, 1998). Plant defense primarily depends on some need based dynamic responses to attempted infection, mostly an inducible phenomenon, its qualitative and quantitative aspects being regulated by signals from the invading pathogens, from phytohormones, salicylic acid and jasmonic acid (Cohen *et al.*, 1993; Durner *et al.*, 1997; Howe *et al.*, 1996; McConn *et al.*, 1997).

It was evident from the results that out of the nine different chemicals and one plant extract only three (nickel chloride, salicylic acid and jasmonic acid) were

found effective in controlling the disease caused by *B. theobromae* and *C. eragrostidis*. The mean disease indexes/shoot in the twigs treated with nickel chloride, salicylic acid and jasmonic acid were less in comparison to untreated twigs (control) after 24, 48 and 72 hours of inoculation. Similarly, nickel chloride (10^{-3} M), salicylic acid (10^{-3} M) and commercially available jasmonic acid (10^{-3} M) were sprayed on the twigs of both susceptible and resistant tea varieties and then were inoculated with *B. theobromae* and *C. eragrostidis*. It was observed from the results that the disease occurrence was much reduced following application of nickel chloride, salicylic acid and jasmonic acid.

Several previous workers have also reported that certain chemicals are capable of inducing resistance in plants. Chakraborty and Purkayastha (1987) induced resistance by using sodium azide (100 μgml^{-1}) in the susceptible soybean cultivar (Soymax) against *Mcrophomina phaseolina*, a pathogen of charcoal rot disease. Gibberelic acid (100 μgml^{-1}) was used by Ghosal and Purkayastha (1987) and showed increased resistance to sheath rot disease in susceptible rice cultivar (Jaya). Similarly, cloxacillin was used for induction of resistance in susceptible soybean variety against *Colletotrichum dematium* var. *truncata* (Purkayastha and Banerjee, 1990).

Systemic action of triazole compound was reported in clonal tea plants (TES-34) which are highly susceptible to *Exobasidium vexans*, a pathogen causing blister blight in tea (Premkumar *et al.*, 1998). SAR was induced by exogenous application of salicylic acid or synthetic compounds such as CGA-245704 (a benzothiadiazole derivative) and CGA-41396 (2,6-dichloroisonicotinic acid) were used by Kessmann *et al.* (1994); Lawton *et al.* (1996) and Lyon *et al.* (1997). Mohr and Cahill (2001) pretreated soybean hypocotyls with norflurazone and inoculated with a compatible race of *Phytophthora sojae*. The treatment displayed pathogen restriction in *planta*.

Taguchi *et al.* (1998) showed PAL A mRNA peaked up after 6-8 hours in tobacco cultured cells following treatment with methyl jasmonate. They showed methyl jasmonate activated defense through increased PAL (phenyl alanine ammonia lyase) activity. Cohen *et al.* (1993) used jasmonic acid and its methyl

esters as inducer of resistance in potato and tomato plants against *Phytophthora infestans*. Kato *et al.* (1984) used probenazole as a systemic compound to induce systemic protection against *Pyricularia oryzae*, a pathogen of rice.

A number of defined chemicals and extracts have been proposed (by different authors) with resistance inducing activity includes various inorganic salts, silicon, oxalate, phosphate, 2-thiouracil, polyacrylic acid, L-lysine but according to Kessmann *et al.* (1994) those compounds could not fulfill the criteria for SAR inducers. They explained possibly those compounds resulted in a local necrosis, which trigger as SA-dependent pathway like that induced by pathogen infection.

From the results of our study it may be concluded that nickel chloride, salicylic acid and jasmonic acid have some role in inducing defense reactions in tea plants. Our findings are also supporting the works of the earlier workers

Role of phenolics in the disease resistance in plants is proved beyond doubt (Daniel, 1995). Accumulation of phenolic compounds following infection with plant pathogens have been reported by several authors (Mahadevan, 1991, Mandavia *et al.*, 1997). Vidhyasekaran (1988) considered phenolic compounds to play an important role in disease resistance. Phenolic compounds are known to impart resistance to fungal disease. There are reports of an increase of total phenols in response to tikka disease in groundnut and of chlorogenic acid, caffeic acid and catechol in response to damping off in groundnut (Mandavia *et al.*, 1997).

In our study also the concentrations of orthodihydroxy phenols and total phenols extracted from the leaves of both susceptible and resistant twigs were measured. The concentrations of orthodihydroxy phenol were always maximum in the leaves of resistant twigs in comparison to susceptible one in both inoculated (inoculated with *B. theobromae* or *C. eragrostidis*) and uninoculated plants when they were treated with nickel chloride, salicylic acid and jsmonic acid. But in comparison to untreated-inoculated twigs, the orthodihydroxy phenol contents were found more in the treated leaves of both resistant and susceptible twigs (both inoculated and uninoculated).

When total phenol contents were measured, the concentrations of total phenol were always maximum in the leaves of resistant twigs in comparison to

susceptible one in both inoculated (inoculated with *B. theobromae* or *C. eragrostidis*) and uninoculated plants when they were treated with nickel chloride, salicylic acid and jsmonic acid. But in comparison to untreated-inoculated twigs, the total phenol contents were found more in the treated leaves of both resistant and susceptible twigs (both inoculated and uninoculated).

From the above discussions, it may be concluded that jasmonic acid, salicylic acid and nickel chloride have the ability to induce systemic resistance in tea plants probably through phenyl propanoid pathway as evidenced by the significant changes in the phenolic compounds.

Chemical control continues to play an important role in the integrated control of tea diseases. In the present study, minimum inhibitory concentration (MIC) of bavistin was maximum among the six fungicides tested against *B. theobromae*. The MIC value of calixin was minimum among the six fungicides when tested against *C. eragrostidis*.

Introduction of systemic fungicides like pyracarbolid and tridemorph showed good performance against blister blight (Venkata Ram, 1974, 1975; Venkata Ram and Chandra Mouli, 1976). Presently, ergosterol biosynthesis inhibiting fungicides such as cyproconazole, bitertanol and hexaconazole were found effective against *Exobasidium vexans* causing blister blight of tea, even at very low concentration (Agnihothrudu and Chandra Mouli, 1990). Combination of chlorothalonil and benomyl was used to control anthracnose of tea in Japan (Horikawa, 1988). Benomyl and thiophanate were combined and used to control *Colletotrichum cammalliae* causing brown blight of tea. Benomyl and thiophanate also controlled white scab in China (Chen and Chen, 1990). Fungicides with diverse chemical nature were evaluated against blister blight disease and a few fungicides belonging to morpholine (tridemorph) and triazole (bitertanol, hexaconazole and propiconazole) groups were found promising. Recommendations were made to tea industry on the usage of tridemorph (Venkata Ram, 1974), bitertanol (Chandra Mouli, 1993), hexaconazole (Chandra Mouli, 1993; Premkumar and Muraleedharan, 1997) and propiconazole (Premkumar, 1997). The cost effectiveness of various schedules has also been reported (Premkumar and Baby, 1996).

Indiscriminate use of fungicides is not only harmful to human beings but adversely affect the microbial population present in the ecosystem. A number of plant species have been reported to possess some natural substances in their leaves and bulb which were toxic to many fungi causing plant diseases (Biswas *et al.*, 1995).

In the present study, among the 21 plant extracts (both aqueous and ethanol) tested against *B. theobromae*, aqueous extract of *Melia dubia* showed significant antifungal activity by inhibiting spore germination of *B. theobromae*. Aqueous bulb extracts of *Allium cepa* and aqueous leaf extract of *Clerodendrum viscosum* also inhibited germination of spores of *B. theobromae*. Ethanol extract of M. dubia leaves and *Azadirachta indica* were also effective in controlling germination of *B. theobromae* significantly. Spore germination of the other pathogen *C. eragrostidis* was also inhibited by the aqueous bulb extract of *A. sativum*, *Cascabela thevatea*, *Dryopteris filix-mas* and *Embelica officinalis*. Ethanol extract of *A. sativum* bulb and *Polyalthia longifolia* leaves were also effective significantly in controlling germination of spores of *C. eragrostidis*. On the basis of the antifungal activity *M. dubia* leaf extracts and *A. sativum* bulb extract were selected for on the 'chromatogram inhibition assay' (TLC plate bioassay) respectively against *B. theobromae* and *C. eragrostidis*. The inhibition zone on the chromatogram was found at R_f 0.8 against *B. theobromae* when *M. dubia* leaf extract was used for antifungal activity. Similarly, at R_f 0.98 inhibition zone was formed against *C. eragrostidis* when aqueous bulb extract of *A. sativum* was used for antifungal activity. Our results are in conformity with the previous workers.

Several investigators evaluated crude extracts prepared from the plants collected either randomly or based on known ethnomedical use. Such studies have focused on screening plant materials for antimicrobial (Al-Shamma and Mitscher, 1979; Khatibi *et al.*, 1989; Navarro *et al.*, 1996; Rao, 1996), anthelmitic (Naqvi *et al.*, 1991), antiviral (Vlietinck *et al.*, 1995), cytotoxicity and muagenicity (Alkofahi *et al.*, 1996, 1997), molluscidal activity (Nick *et al.*, 1995), as well as for general pharmacological effects (Nick *et al.*, 1995). Biological evaluations have also been conducted on plants from different regions including India (Naqvi *et al.*, 1991), Jordan (Alkofahi *et al.*, 1996, 1997), Kenya (Githinji and Kokwaro, 1993), New Zealand (Bloor,

1995), Pakistan (Rizvi *et al.*, 1987), Papua New Guinea (Rao, 1996), Saudi Arabia (Khatibi *et al.*, 1989) and Somalia (Samuelsson *et al.*, 1992). The essential oils of *Origanum syriacum* exhibited a strong antifungal actions against *Fusarium oxysporum*, *Aspergillus niger* and *Penicillium* spp (Daouk *et al.*, 1995).

Chouksey and Srivastava (2001) screened the compound I from *Terminalia arjuna* which has antifungal activity against the fungi *Aspergillus niger*, *Candida albicans* and *Bacillus oryzae* at 25 and 50 ppm. The extract of mimosa bark and gullnat powder inhibited the development of *Alternaria alternata*, having inhibition zones of 21 and 15 mm, respectively. However, they were effective against *Penicillium italicum*, *Fusarium equiseti* and *Candida albicans* as reported by Digrak *et al.* (1999). Among the 11 plants of Mimoaceae from Pakistan were tested for their antimicrobial activity, the methanol extract of *Acacia nilotica* was found active against fungi (Ali *et al.*, 2001). The leaf extract of *Clerodendrum viscossum* completely checked the radial growth of the test fungus, *Curvularia lunata*. The leaf extract (1:10 dilution was the most successful for the inhibition of the test fungus in terms of its growth (Parimelazhagan and Francis, 1999).

Several authors have reported antifungal activity in different crops (Mittal *et al.*, 2002; Yasmin and Saxena, 1990; Sharma *et al.*, 2002; Appleton and Tansey, 1975; Barone and Tansey, 1977; Singh *et al.*, 1979; Yoshida *et al.*, 1987; Singh *et al.*, 1990; Reimers *et al.*, 1993; Singh *et al.*, 1995; Parimelazhagan and Francis, 1999; Ali *et al.*, 2001).

Biological control of plant diseases involves the use of one organism to control or eliminate a pathogenic organism. Biological control has attracted a great interest in plant pathology because the unnecessarily frequent use of pesticides and fungicides is increasingly causing concern in modern society in terms of human toxicity and hazardous effects on natural environments (Goto, 1990). Search for effective biocontrol agents for the management of plant diseases has been intensified in recent years to reduce the dependence on ecologically hazardous chemicals (Sharma *et al.*, 2001). Several microorganisms with high activity have been identified (Tronsmo and Raa, 1977; Janisiewicz, 1988; Peng and Sutton, 1991; Droby *et al.*, 1992). Biological control has been reported only in very few cases of tea diseases,

in comparison to pests. Application of beneficial microorganisms has been attempted in the control of tea root diseases. *Trichoderma viride* Pers. & Fr. and *T harzianum* Pers. & Fr. showed inhibitory activity against *Poria hypobrunnea* (Barua *et al.*, 1989). Horikawa (1988) isolated *Streptomyces roseosporus* from the gray blight spots on tea leaves which parasitized *P. longiseta* spores. After three days of inoculation, the conidia of *P. longiseta* and *P. theae* were destroyed by *S. roseosporus*.

Antagonistic nature of several species of the genus *Trichoderma* have been studied (Papavizas, 1985; Chet, 1987). Researches on *T. harzianum* as a biocontrol agent also showed differential antagonistic potential among isolates (Maity and Sen, 1985; Biswas, 1999). Eight isolates of *T. harzianum* Rifai isolated from soils of different betelvine plantations of West Bengal were investigated against four major fungal pathogens (*Phytophthora parasitica*, *Sclerotium rolfsii*, *Rhizoctonia soiani* and *Colletotrichum capsici*) of betelvine. (D'Souza *et al.*, 2001).

In the present study, *Gliocladium virens* (isolate II) inhibited *B. theobromae* significantly in dual culture technique and poisoned food technique. In dual culture technique *Gliocladium virens* (isolate II) directly inhibited the pathogen but in case of poisoned food technique cell-free culture filtrate showed the inhibition. It clearly showed that the inhibiting activity lies in the extracellular fluid. Similarly, *Trichoderma harzianum* showed significant inhibition of *C. eragrostidis* in both the tests (Dual culture technique and Poisoned food technique) performed. Cell free culture filtrates have been used to demonstrate the rate of antibiosism a mechanism of biological control (Khara and Hadwan, 1990; Tu, 1992; Naik and Sen, 1992). Antibiotic substances such as cell free culture filtrates have been emphasized (Robinson, 1969; Fravel, 1988). As going for biocontrol methods is normally cost effective compared to chemical control of plant diseases, Shanmugam and Sukunara Varma (1999) clearly established the efficacy of the antagonists *Aspergillus niger*, *A. fumigatus*, *A. flavus* and *Trichoderma viride* in inhibiting the rhizome rot pathogen.

Despite the progress in biocontrol research biocontrol products is often less consistent than chemical control. However a greater understanding of the ecology of the organisms involved, as well as the epidemiology of the system, will

help us to develop ecologically rational approaches to disease management. More efficient and effective ways of growing and formulating biocontrol organisms are needed in many cases in order to make biocontrol economically viable (Fravel, 1999).

Potential plant extracts (*M. dubia* and *A. sativum*), fungicide (Calixin) and biocontrol fungi (*T. harzianum* and *G. virens* – isolate II) reduced disease significantly in the nursery tea plants of the susceptible varieties (TV-11 and TV-12) against *B. theobromae* and *C. eragrostidis*

Pretorius *et al.* (2002) reported the *In vivo* control of *Mycosphaerella pinodes* on pea leaves by a crude bulb extract of *Eucomis autumnalis*. The crude extract prevented *M. pinodes* spore infection of the leaves when the leaves were inoculated with spores both before or after treatment with the extract, confirming complete inhibition of spore germination. The crude *E. autumnalis* extract showed no phytotoxic reaction on the leaves even at the highest concentration applied. Premkumar *et al.* (1998) studied the systemic activity and field performance of triazole fungicides against blister blight pathogen of tea and established the antisporulant actions of the triazole fungicides.

The present study has confirmed and also extended some of the findings of early workers. *C. eragrostidis* have been reported as a new pathogen of tea. This study also reveals certain new facts of fundamental importance. The significance of antigenic relationship with regard to compatible interaction between the hosts and the pathogens (*C.sinensis-B.theobromae* and *C.sinensis-C.eragrostidis*) has been demonstrated by various serological techniques. The pathogenicity of different tea varieties have been done in three different ways and also correlated with that of indirect ELISA. Major cross-reactive antigens were located in the cells of tea and pathogens by staining with fluorescence isothiocyanate conjugated with antibodies. Resistance was induced in susceptible tea plants by the application of some chemicals. However, more works need to be done before formulating a definite defense inducers although this investigation has provided an insight into the control of diseases caused by pathogens. Suitable control measures may be designed from the present study at least for the nursery tea plants.

Summary

Summary

A brief review of the literature related to the present lines of investigation has been presented. The review mainly includes diseases of tea plants, growth and physiology of the pathogens, common antigenic relationship, plant disease alteration by chemical treatment, and disease control by chemical fungicides, botanicals and antagonistic organisms.

Different experimental procedures and techniques used during the present study have been described in details in the materials and methods section.

At the onset of the present work a thorough survey of the tea nurseries of north Bengal were done. During the survey, 6 pathogens were isolated from the leaves of young tea plants. These were *B. theobromae*, *C. eragrostidis*, *C. cammelliae*, *P. theae*, *C. theae*, *F. oxysporum*. Out of these two fungi *B. theobromae and C. eragrostidis* were consistently found associated with the leaves of young seedlings of the nurseries of North Bengal. *C. eragrostidis* is a new report of this study.

Pathogenicities of two fungal pathogens (*B. theobromae* and *C. eragrostidis*) were determined on 24 different tea varieties following detached leaf inoculation technique and cut shoot inoculation technique. Pathogenicity were also tested on 10 different varieties following nursery tea plant inoculation technique. From the results of pathogenicity TV-26 and TV-11 were selected as resistant and susceptible variety respectively against *B. theobromae*. Similarly, TV-25 and TV-12 were resistant and susceptible variety respectively against *C. eragrostidis.*

Different solid media, different incubation periods (days) and different pH on growth of *B. theobromae* and *C. eragrostidis* were investigated. The rate of growth of both the fungi increased after 5 days and continued up to 15 days and then declined. Among the different pH (pH 5.0, pH 5.5, pH 6.0 and pH 6.5) tested for growth, pH 6.0 was the best pH for growth of both *B. theobromae* and *C. eragrostidis.*

Among the four different carbon sources, glucose was revealed as an excellent carbon source when overall growth pattern of *B. theobromae* was investigated. Sporulation of *B. theobromae* was excellent in glucose and sucrose. *C. eragrostidis* on the other hand showed satisfactory growth when glucose and sucrose were

used as carbon sources. Sporulation was excellent when glucose was used as carbon source. Among the seven different nitrogen sources, only potassium nitrate, peptone and beef extract were satisfactory nitrogen sources for the growth of *B. theobromae*. Sporulation was found good after 15 days of incubation in case of potassium nitrate and yeast extract. Peptone was an excellent organic nitrogen source for growth of *C. eragrostidis*. Sporulation was found good after 15 days of incubation when ammonium nitrate, ammonium sulphate and peptone were used as nitrogen sources.

Effect of different incubation periods, different pH and different temperatures on the germination of spores of both *B. theobromae* and *C. eragrostidis* were studied. Both the pathogens showed maximum germination of their spores after 24 hours of incubation. The spore germination of both *B. theobromae* and *C. eragrostidis* was maximum at pH 7.25 and minimum at pH 9.0. At 28 ^{0}C, spore germination was maximum in case of *B. theobromae* but at 40 ^{0}C no spores germinated. *C. eragrostidis* showed maximum spore germination at 28 ^{0}C but did not show germination at 45 ^{0}C.

During the present study the antigens of the two pathogens (*B. theobromae* and *C. eragrostidis)*, antigen of non-pathogen (*Gliocladium virens* isolate II), leaf antigens of three susceptible tea varieties (TV-11, TV-12 and TV-18), three resistant tea varieties (TV-25, TV-26 and TV-30) and leaf antigens of TV-3, TV-6, TV-8 and Sundaram were used in different combinations with the antisera of *B. theobromae*, *C. eragrostidis*, TV-11, TV-12, TV-25 and TV-26 for determining the degree of cross reactivity. For this several techniques like immunodiffusion, immunoelectrophoresis, indirect ELISA and indirect immunofluorescence microscopy were performed.

In agar gel double diffusion test when antigens of *B. theobromae*, TV-6, TV-11, TV-12, TV-18, TV-26 and TV-30 were treated with the antisera of *B. theobromae*, TV-11 and TV-26, common precipitin bands were observed in homologous treatments (i.e. between antisera and antigens of *B. theobromae*, TV-11 and TV-26) as well as in cross-reactions between antisera of *B. theobromae* and antigens from leaves of TV-11, TV-12 and TV-18. Common precipitin bands were also found in reactions between the antisera of TV-11 and antigens of *B. theobromae*, TV-6, TV-12, TV-18,

TV-26 and TV-30. Common precipitin bands were found between the antisera of TV-26 and the antigens from leaves of TV-6, TV-11, TV-12, TV-18, TV-26 and TV-30. Precipitation bands were not found when the antigen of non-pathogen *G. virens* (isolate II) was allowed to diffuse against the antisera of *B. theobromae*, TV-11 and TV-26 in agar gel double diffusion test. Similarly, when antigens of *C. eragrostidis*, TV-3, TV-8, TV-11, TV-12, TV-18, TV-25, TV-26 and Sundaram were treated with the antisera of *C. eragrostidis*, TV-12 and TV-25, common precipitin bands were observed in homologous treatments (i.e. between antisera and antigens of *C. eragrostidis*, TV-12 and TV-25) as well as in cross-reactions between antisera of *C. eragrostidis* and antigens from leaves of TV-11, TV-12 and TV-18. Common precipitin bands were also found in reactions between the antisera of TV-12 and antigens of *C. eragrostidis*, TV-3, TV-8, TV-11, TV-18, TV-25, TV-26 and Sundaram. Common precipitin bands were found between the antisera of TV-25 and the antigens from leaves of TV-3, TV-8, TV-11, TV-12, TV-18, TV-25, TV-26 and Sundaram. Precipitation bands were not found when the antigen of non-pathogen *G. virens* (isolate II) was treated with the antisera of *C. eragrostidis*, TV-12 and TV-25.

In immunoelectrophoresis, the antigens of *B. theobromae* showed four precipitin arcs in homologous reaction with the antisera of *B. theobromae*, but only one precipitin arc was found between the antigens of *B. theobromae* and antisera of TV-11. No precipitin arcs were observed in the immunoelectrophoretic test between the antigens of *B. theobromae* and the antisera of TV-26. On the other hand, the antigen of TV-11 showed one precipitin arc with antisera of *B. theobromae*, two precipitin arcs with both the antisera of TV-11 and of TV-26. The antigen of TV-26 showed no precipitin arcs with antisera of *B. theobromae*, one precipitin arc with antisera of TV-11 and three precipitin arcs in homologous test with antisera of TV-26. When antigen of non-pathogen *G. virens* (isolate II) was tested with the antisera of *B. theobromae*, TV-11 and TV-26 separately, no precipitin arc was observed. The antigens of *C. eragrostidis* showed four precipitin arcs in homologous reaction with the antisera of *C. eragrostidis*, but only one precipitin arc was found between the antigen of *C. eragrostidis* and antisera of TV-12. No precipitin arcs were observed in the immunoelectrophoretic test between the antigens of *C. eragrostidis* and the antisera of TV-25. The antigen of TV-12 showed one precipitin

arc with antisera of *C. eragrostidis*, three precipitin arcs with the antisera of TV-12 and two precipitin arcs with the antisera of TV-25. The antigen of TV-25 on the other hand showed no precipitin arcs with antisera of *C. eragrostidis*, two precipitin arcs with antisera of TV-12 and four precipitin arcs in homologous test with antisera of TV-25. When antigen of non-pathogen *G.virens* (isolate II) was tested with the antisera of *C. eragrostidis*, TV-12 and TV-25 respectively, no precipitin arc was observed.

The leaf antigens of 24 different tea varieties, mycelial antigens of the two pathogens and antisera of 4 tea varieties (TV-11, TV-12, TV-25 and TV-26) and of the two pathogens were used to perform indirect ELISA. The higher ELISA values in cross reactions indicated susceptibility and lower ELISA values indicated resistance. TV-11 showed maximum ELISA value (1.494) against antisera of *B. theobromae* at 20 $\mu g\ ml^{-1}$concentration. The TV-26 showed minimum ELISA value (0.257) against antisera of *B. theobromae* at 5 $\mu g\ ml^{-1}$ concentration. The two varieties were susceptible and resistant respectively. Similarly, TV-12 showed maximum ELISA value (1.585) against antisera of *C. eragrostidis* at 20 $\mu g\ ml^{-1}$ concentration. The TV-25 showed minimum ELISA value (0.221) against antisera of *C. eragrostidis* at 5 $\mu g\ ml^{-1}$ concentration. The two varieties were susceptible and resistant respectively.

In indirect immunofluorescence, when the leaf sections were treated with the antisera of respective leaf varieties i.e. with homologous antisera indirectly labeled with FITC, bright fluorescence was observed. Leaf sections in heterologous treatments (i.e. when the leaf sections were treated with antisera of the two pathogens and then reacted with FITC) showed comparatively more fluorescence in susceptible varieties than the resistant one. The CRA observed in heterologous reactions, were mainly concentrated around the epidermal cells. In homologous treatments of the fungal mycelia, intense fluorescence was observed around the hyphae and conidia. In cross-reactions, when the fungal mycelia were treated with antisera of susceptible varieties indirectly labeled with FITC, fluorescence was observed in the hyphal tips and conidia but the fluorescence was not so strong when the mycelia were treated with the antisera of resistant varieties and FITC.

Nine different chemicals and the aqueous leaf extract of *Jasminum jasminoides* were sprayed on the cut shoots of susceptible tea varieties before they

were inoculated with the two pathogens *B. theobromae* and *C. eragrostidis* separately. Three chemicals (nickel chloride, salicylic acid and jasmonic acid) were found effective in controlling the diseases caused by *B. theobromae* and *C. eragrostidis*. Nickel chloride, salicylic acid and jasmonic acid at 10^{-3} M concentrations were found to be effective in reducing the disease occurrence (caused by *B. theobromae* and *C. eragrostidis*). The concentrations of orthodihydroxy phenol were found always to be maximum in the leaves of resistant twigs compared to susceptible one in both inoculated (inoculated with *B. theobromae* and *C. eragrostidis* separately) and uninoculated one when they were treated with the three chemicals separately. But in comparison to untreated-inoculated twigs, the orthodihydroxy phenol contents were found more in the leaves of both resistant and susceptible twigs (in both inoculated and uninoculated sets) when they were treated with the three chemicals. Similar results were also observed when the concentrations of total phenol were estimated in inoculated-treated, uninoculated-treated and untreated-inoculated leaves of susceptible and resistant twigs.

Some common fungicides were tested for the control of the two pathogens and the MIC of the fungicides were determined following poisoned food technique. Bavistin was the best fungicide which controlled *B. theobromae* while Calixin was the best fungicide which controlled *C. eragrostidis*.

In the present study, both aqueous and ethanol extracts of 21 different species of plants were screened for their potential antifungal properties against *B. theobromae* and *C. eragrostidis* following slide germination technique. *Melia dubia*, *Azadirachta indica*, *Allium sativum*, *Clerodendrum viscosum*, *Dryopteris filix-mas*, and *Polyalthia longifolia* showed promising antifungal activity in slide germination bioassay as well as in TLC plate bioassay.

Antagonistic potentiality of some known biocontrol fungi (*T. viride*, *T. koningii*, *T. harzianum*, isolate I and isolate II of *G. virens*) on the growth of *B. theobromae and C. eragrostidis* were tested. *G. virens* (isolate II) showed maximum inhibition of *B. theobromae* while *T. harzianum* were found effective against *C. eragrostidis*.

In vitro control experiments with crude aqueous leaf extracts of *M. dubia*, crude aqueous bulb extracts of *A. sativum*, different concentrations of calixin and

two biocontrol fungi (*T. harzianum* and isolate II of *G. virens*) showed reduced growth of *B. theobromae* and *C. eragrostidis* at different stages of the study. When the crude aqueous leaf extracts of *M. dubia*, calixin at 500 μgml^{-1} concentrations and spore suspensions of *T. harzianum* and isolate II of *G. virens* were sprayed separately on nursery plants of susceptible tea variety (TV-11) and inoculated with *B. theobromae*, there was reduced disease development in comparison to control. Similarly, when the crude aqueous bulb extracts of *Allium sativum*, calixin at 500 μgml^{-1} concentrations and spore suspensions of *T. harzianum* and isolate II of *G. virens* were sprayed separately on nursery plants of susceptible tea variety (TV-12) and inoculated with *C. eragrostidis*, there was reduced disease development in comparison to control.

Implications of the results have also been discussed in the discussion section.

References

Abou-Jawdah, Y.; Sobh, H. and Sad, A. (2001) Incidence of potato virus diseases and their significance for a seed certification program in Lebanon. *Phytopathol. Mediterr.* **40**: 113-118.

Achar, P. N. (2000) Effect of temperature on hyphal growth, conidial production and germination of *Stenocarpella maydis* from maize seeds. *Geobios.* **27**: 3-8.

Agnihothrudu, V. (1964) A world list of fungi reported on tea (*Camellia* spp.). *J. Madras Univ.* **B 34**: 155-217.

Agnihothrudu, V. and Chandra Mouli, B. (1990) Blister blight of tea and its control. *Planters' Chron.* **85**: 205-217.

Agrios, G. N. (1997) Plant Pathology (4th ed.). Academic Press. New York, USA.

Ala-El-Dein, O. and El-Kady, S. (1985) Crossed immunoelectrophoretic studies on *Botrytis cinerea* Pers. and other *Botrytis* species. *Acta. Phytopath Aung.* **20**: 291-301.

Alba, A. P. C. and DeVay, J. E. (1985) Detection of cross-reactive antigens between *Phytophthora infestans* (Mont.) de Barry and *Solanum* species by indirect enzyme-linked immunosorbent assay. *Phytopathologische Zeitsschrift.* **112**: 97-104.

Alba, A. P. C.; Guzzo, S. D.; Mahlow, M. F. P. and Moraes, W. B. C.(1983). Common antigens in extracts of *Hemileia vastaxix* Berk, et Br. urediniospores and *Coffea arabica* L. leaves and roots. *Fitopatho.* **8**. 473-483.

Ali, M. S.; Azhar, I.; Ahmad, F.; Ahmad, V. U. and Usmanghani, K. (2001) Antimicrobial screening of Mimoaceous Plants. *Pharm. Biol.* **39**: 43-46.

Ali, M. S.; Azhar, I.; Ahmad, V. U. and Usmanghani, K. (1999) Antimicrobial screening of some Caesalpiniaceae. *Fitoterapia.* **70**: 299-304.

Alkofahi, A.; Batshoun, R.; Owais, W. and Najib, N. (1996) Biological activity of some Jordanian medicinal plant extracts. *Fitoterapia.* **67**: 435-442.

Alkofahi, A.; Batshoun, R.; Owais, W. and Najib, N. (1997) Biological activity of some Jordanian medicinal plant extracts. Part II. *Fitoterapia.* **68**: 163-168.

Al-Shamma, A. and Mitscher, L. A. (1979) Comprehensive survey of indigenous Iraqi plants for potential economic value. 1. Screening results of 327 species for alkaloids and antimicrobial agents. *J. Nat. Prod.* **42**: 633-642.

Amouzon-Alladaye, E.; Dune, J. and Clarjean, M. (1988). Immunoenzymatic detection of *Phytophthora fragariae* in infected strawberry plants. *Phytopathol.* **78**:1022-1026

Appleton, J. A. and Tansey, M. R. (1975) Inhibition of growth of zoopathogenic fungi by garlic extract. *Mycologia*. **67**. p. 882.

Arie, T., Namba, S., Yamashita, S., and Doi, Y. (1988) Detection of resting spores of *Plasmodiophora brassicae* Waron. from soil and root by Fluorescent antibody technique. *Phytopath. Soc.* Japan **54**:242-245

Arpon, T. and Supachi, La (1980) Tea diseases (blister blight caused by *Exobasidium vexans*, red rust caused by *Cephaleurus* sp. and others) in Thailand. 2nd South East Asian Symp. on Plant Dis. in the Tropics. p. 81 (Abstr.).

Ayers, W. A. and Adams, P. B. (1981) Mycoparasitisms and its application to biological control of plant diseases. *In* Biological control of crop production Ed. G. C. Papavizas. Beltsville symp. in Agricultural research *5*. Granada: Allen held, Osmum publishing. pp. 91-105.

Barbora, B. C. (1988). Latest concept on management of young tea. *In* Field management in tea. Ed. S. K. Sarkar and H. P. Bezbaruah. Tocklai Experimental Station, Jorhat, Assam. pp. 50-62.

Barone, F. E. and Tansey, M. R. (1977) Isolation, purification, synthesis and kinetics of activity of the anticandidal component of *Allium sativum* and a hypothesis for its mode of action. *Mycologia*. **69**. p. 793.

Barthakur, B. K. (1994) Disease problem of tea in Darjeeling. *Two and A Bud.* **41**: 12-14.

Barua, D. N. (1963) Use and abuse of clones. *Two and A Bud.* **10**: 3-6.

Barua, D. N. (1989) Science and Practice in Tea Culture. Tea Research Association, Calcutta. p. 509.

Barua, K. C.; Barthakur, B. K.; Dutta, P. and Begum, R. (1989) Biocontrol of *Poria* stem disease of Tea (*Camellia sinensis* (L.) O. Kuntze) caused by *Poria hypobrunnea* Petch.. Natl. Symp. on Recent Trends in Plant Disease control. North eastern Hill University, Nagaland (India). p. 226 (Abstr.).

Benbow, J. M. and Sugar, D. (1999) Fruit surface colonization and biological control of post harvest diseases of pear by preharvest yeast applications. *Plant Dis.* **83**: 839-844.

Benhamou, N., Onellette, G. B. Gardiner, R. B. and Day, A, W. (1986). Immunolocyto chemical localization of antigen binding site in the cell surface of two arcomycte fungi using antibodies produced against fimbriae from *Ustilago violacea* and *Rhodotorula rubra. Can. J. Microbiol.* **32**: 871-833.

Bezbaruah, H. P. and Singh, I. D. (1988) Advances in the use of planting materials. *In* Field management in tea. Ed. S. K. Sarkar and H. P. Bezbaruah. Tocklai Experimental Station, Jorhat, Assam. pp.11-23.

Bhattacharyya, B. and Purkayastha, R. P. (1985) Occurrence of common antigens in jute and *Colletotrichum corchori. Curr. Sci.* **54**: 251-252.

Bishop, P. D.; Makus, D. J.; Pearce, G. and Ryan, C. A. (1981) Proteinase inhibitor-inducing factor activity in tomato leaves resides in oligosaccharides enzymatically released from cell walls. *Proc. of the Natl. Acad. of Sci. of the USA.* **78**: 3536–3540.

Biswas, K. K. (1999) Screening of isolates of *Trichoderma harzianum* Rifai for their relative biocontrol efficacy against *Fusarium oxysporum* f. sp. *udum* and *Rhizoctonia solani* Küun. *Ann. Pl. Prot. Sci.* **7**:125-30.

Biswas, S.; Das, N. K.; Wuadri, S. M. H. and Sarachandra, B. (1995) Evaluating different plant extracts against three major diseases of mulberry. *Indian Phytopathol.* **48**: 342-346.

Bloor, S. J. (1995) A survey of extracts of New Zealand indigenous plants for biological activities. *New Zealand J. Bot.* **33**: 523-540.

Boff, P.; Kohl, J.; Gerlagh, M. and Dekraker, J. (2002) Biocontrol of grey mould by *Ulocladium atrum* applied at different flower and fruit stages of strawberry. *Biocontrol.* **47**: 193-206.

Boland, W.; Koch, T.; Krumm, T.; Piel, J. and Jux, A. (1999) Induced biosynthesis of insect semiochemicals in plants. *In* Insect-plant interactions and induced plant defence. Eds. D. J. Chadwick and J. A. Goode. Chichester, New York. Wiley. pp. 110-131.

Brennan, J. M.; Fagan, B.; van Maanen, A. ; Cooke, B. M. and Doohan, F. M. (2003) Studies on *in vitro* Growth and Pathogenicity of European *Fusarium* Fungi. *European J. Pl. Pathol.* **109**: 577-587.

Burrell, R. G.; Clayton, G. W.; Gallegly, M. E. and Killy, V. G. (1966) Factors affecting the antigenicity of the mycelium of three species of *Phytophthora*. *Phytopathol.* **56**: 522-526.

Callaghan, A. A. (1974) Effect of pH and light on conidium germination in *Basidiobolus ranarum*. *Trans.Brit.Mycol.Soc.* **63** :13-18.

Carpinella, M. C.; Herraro, G. G.; Alonso, R. A. and. Palacios, S. M. (1999) Antifungal activity of *Melia azedarach* fruit extract. *Fitoterapia.* **70**: 296-298.

Chakraborty, B. N. (1987) A new record of leaf disease of tea (*Camellia sinensis* (L.) O. Kuntze.) *Curr. Sci.* **56**: 900.

Chakraborty, B. N. (1988). Antigenic disparity. *In* Experimental and Conceptual Plant Pathology. Eds. R. S. Singh; U. S. Singh; W. M. Hess and D. J. Weber. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. pp. 477-484.

Chakraborty, B. N. and Purkayastha, R. P. (1983) Serological relationship between *Macrophomina phaseolina* and soybean cultivars. *Physiol. Pl. Pathol.* **23**: 197-205.

Chakraborty, B. N. and Purkayastha, R. P. (1983) Serological relationship between *Macrophomina phaseolina* and soybean cultivars. *Physiol. Pl. Pathol.* **23**: 197-205.

Chakraborty, B. N. and Purkayastha, R. P. (1987) Alteration of glyceollin sy nthesis and antigenic patterns after chemical induction of resistance in soybean to *Macrophomina phaseolina*. *Can. J. Microbiol.* **33**:835-840.

Chakraborty, B. N. and Saha, A. (1994) Detection and cellular location of cross-reactive antigens shared by *Camellia sinensis* and *Bipolaris carbonum*. *Physiol. and Mol. Pl. Pathol.* **44**: 403-416.

Chakraborty, B. N., Basu, P., Das, R., Saha, A. and Chakraborty, U. (1995) Detection of cross-reactive antigens between *Pestalotiopsis theae* and tea leaves and thelr cellular location. *Annals of Applied Biol.* **127**: 11-21.

Chakraborty, B. N., Chakraborty, U., Saha, A., Das, R. and Basu, P. (1997) Detection and management of blister blight of tea. *In* Management of Threatening Plant Diseases of National Importance. Eds. V. P. Agnihotri; A. K. Sarbhoy and D. V. Singh, Malhotra Publishing House. New Delhi. pp. 227-235.

Chakraborty, U.; Basu, P.; Das, R.; Saha, A. and Chakraborty, B. N. (1996) Evaluation of antiserum raised against *Pestalotiopsis theae* for detection of grey blight disease of tea by ELISA. *Folia Microbiol.* **41**: 413-418.

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

Chandra Mouli, B. (1988) Root diseases of tea and their control. *J. Coffee Res.* **18**: 27-37.

Chandra Mouli, B. (1993) Blister blight control-New Recommendations. *Handbook of tea Culture. Section 13.* United Planters' Association of Southern India. p. 8.

Chandra Mouli, B. and Premkumar, R. (1986) Control of tea blister blight (*Exobasidium vexans* Massee) with organic fungicides. *J. Plantation Crops.* **16**: 249-253.

Chandra Mouli, B. and Premkumar, R. (1989) Evaluation of foliar fungicides for tea blister blight control. 1987. *Fungic. Nematic. Tests*. American Phytopathological Society. **44**: 280-281.

Chandra Mouli, B. and Venkata Ram, C. S. (1979) Superiority of copper-antibiotic combination to copper oxychloride alone in blister blight control. *Proc. Placrosym* II: 163-171.

Chard, J. M.; Gray, T. R. G. and Frankland, J. C. (1985) Use of an anti-Mycene galopus serum as an immunofluoresence seagert. *Trans. Br. Mycol. Soc.* **84**: 243-249.

Chen, Z. M. and Chen, X. F. (1989) A world list of pathogens reported on tea plant. *J. Tea Sci.* **9**: 73-88.

Chen, Z. M. and Chen, X. F. (1990) The diagnosis of tea diseases and their control (In Chinese). Shanghai Scientific and Technical Publishers, Shanghai. p. 275.

Chet, I. (1987) *Trichoderma*: application, mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi. *In* Innovative Approaches to Plant Disease Control. Ed. I. Chet. Wiley, N. Y. pp. 137-160.

Chouksey, B. K. and Srivastava, S. K. (2001) New constituent from the roots of *Terminalia arjuna*: Antifungal agent. *Indian J. Chem.* **40B**:354-356.

Ciccarese, F.; Frisullo, S.; Amenduni, M. and Cirulli, M. (1992) Biological control of *Sclerotium rolfsii* root rot of sugarbeet with *Trichoderma harzianum*. *In* Biological Control of Plant Diseases: Progress and Challenge for the Future. Eds. E. C. Tjamos; G. C. Papavizas and R. J. Cook. Plenum Press, New York. pp. 243-248.

Clausen, J. (1969) Immunochemical techniques for the identification and estimation of macromolecules. *In* Laboratory Techniques in Biochemistry and Molecular Biology, vol. 1. Eds. T. S. Work and E. Work. North Holland Publishing Company,

Cohen, Y.; Gisi, U. and Niderman T. (1993) Local and systematic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid jasmonic methyl ester. *Phytopathol.* **83**: 1054-1062.

Creelman, R. A. and Mullet, J. E. (1997) Biosynthesis and action of jasmonates in plants. *Ann. Rev. Pl. Physiol. and Pl. Mol. Biol.* **48**: 355-381.

Croft, J. B. (2002) A method for rating sugarcane cultivars for resistance to ratoon stunting disease based on an enzyme linked Immunoassay. *Aus. Pl. Pathol.* **31**: 63-66.

Cromey, M. G.; Lauren, D. R.; Parkes, R. A.; Sinclair, S. C.; Shorter, S. C. and Wallace, A. R. (2001) Control of *Fusarium* head blight of wheat with fungicides. *Aus. Pl. Pathol.* **30**: 301-308.

D'souza, A.; Roy, J. K.; Mohanti, B. and Dasgupta, B. (2001) Sereening of *Trichoderma harzianum* against major fungal pathogens of betelvine. *Indian Phytopathol.* **54**: 340-345.

Daniel, G., and Nilrson, T. (1991) Antiserum to the fungus *Phialophora antabilis* and its use in enzyme linked immunosorbent assays for the detection of soft rot in preservative treated and untreated wood. *Phytopathol.* **81**: 1319-1325.

Daniel, M. (1995) Phytoalexins and other post-infectional compounds of some economically important plants of India. *In* Handbook of Phytoalexin metabolism and action. Eds. M. Daniel and R. P. Purkayastha. pp. 533-553.

Daouk, R. K.; Dagher, S. M. and Sattout, E. J. (1995) Antifungal activity of the essential oil of *Origanum syriacum* L. *J. Food Prot.* **58**: 1147-1149.

Das, A. K. and Mitra, D. K. (1999) Detection of Brinjal leaf Phytoplasma by Monoclonal Antibodies. *J. Mycol. Pl. Pathol.* **29**: 48-51.

De Jong, P. (1954) Report of the Chief Scientific Officer, II. Research and Advisory. *Ann. Rep. UPASI Sci. Dep.* Tea Sect. for 1953-1954. pp. 4-7.

De Silva, R. L. (1965) Investigations with new fungicides for the control of blister blight (*Exobasidium vexans* Massee.) on tea. *Tea Quart.* **43**: 140-146.

Deans S. G. and Svoboda, K. P. (1990) Biotechology and bioactivity of culinary and medicinal plants. *Ag. Biotech. News and Information.* **2**: 211-216.

Deb, P. R. (1990) *In vitro* inhibitory activity of some rhizosphere fungi of soybean against *Sclerotium rolfsii* Sacc. growth. *Acta. Botanica. India.* **18**: 159-162.

Debnath, S. and Barthakur, B. K. (1994) Mortality of tea cuttings infected by *Fusarium oxysporum* Schlecht. *Two and A Bud.* **41**: 44-46.

Demirci, F.; Demirci, B.; Baser, K. H. C. and Güven, K. (2000) The composition and antifungal bioassay of the essential oils of different *Betula* species growing in Turkey. *Chem. Nat. Com.* **2**: 126-130.

Devadath, S. and Padmanabhan, S. Y. (1977) Nutritional requirements of *Xanthomonas oryzae*, the incitant of bacterial blight of rice. *In* Physiology of Microorganisms. Ed. K. S. Bilgrami. Today and Tomorrow's Printers and Publishers, New Delhi, India. pp. 25-38.

DeVay, J. E. and Adler, H. E. (1976) Antigens common to host and parasites. *Ann. Rev. Microbiol.* **30.** p. 147.

DeVay, J. E., Wakeman, R. J., Kavanagh, J. A. and Charudattan, R. (1981a). The tissue and cellular location of major cross-reactive antigens shared by cotton and soil borne fungal parasites. *Physiol. Pl. Pathol.* **18**: 59-66.

DeVay, J. E., Wilson, U. E., Wakeman, R. J. and Kavanagh, J. A. (1981b). Occurrence of common antigenic determinant between potato and *Phytophthora infestans* in relation to host parasite compatibility. *In* Proc. Internatl. Symp. on *Phytophthora*: Its Biology. Riverside, CA: University of California.

Dewey, F. M. and Brasier, C. M. (1988) Development of ELISA for *Ophiostoma ulmi* using antigen coated wells. *Plant Pathol.* **37**: 28-35.

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

Digrak, M.; Alma, M. H.; Ilcim, A. and Sen, S. (1999) Antibacterial and antifungal effects of various commercial plant extracts. *Pharm. Biol.* **37**: 216-220.

Ding, C. K.; Wang, Y.; Gross, K. C. and Smith, D. L. (1999) Jasmonate and salicylate induced the expression of pathogenesis-related protein genes and increase resistance to chilling injury in tomato fruit. *Plant physiol.* **112**: 853-860.

Doares, S. H.; Syrovets, T.; Weiler, E. W. and Ryan, C. A. (1995) Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. Proc. Natl. Acad. Sci. USA. **92**: 4095-4098.

Dombrowski, J. E.; Pearce, G. and Ryan, C. A. (1999) Prosystemin is the 200-amino acid precursor of systemin. *Plant Cell.* **13**: 1467-1475.

Droby, S.; Chalutz, E.; Wilson, C. L. and Wisniewski, M. E. (1989) Characterization of biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* on grape-fruit. *Can. J. Microbiol.* **35**: 794-800.

Droby, S.; Chalutz, E.; Wilson, C. L. and Wisniewski, M. E. (1992) Biological control of postharvest diseases: a promising alternative to the use of synthetic fungicides. *Phytoparasitica* **20**: 149-153.

Durner, J.; Shah, J and Klessig, D. F. (1997) Salicylic acid and disease resistance in plants. *Trends In Pl. Sci.* **2**: 266-274.

Ebel, J. and Cosio, E. (1994) Elicitors of plant defence responses. *Internatl. Rev. Cytology.* **148**: 1-36.

Ebel, J. and Mithöfer, A. (1998). Early events in the elicitation of plant defence. *Planta.* **206**: 335-348.

Elade, Y.; Chet, I.; Byle, P. and Henis, Y. (1983) Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii* scanning electron microscopy and fuorescent microscopy. *Phytopathol.* **73**: 85-88.

Farmer, E. E. and Ryan, C. A. (1992) Interplant Communication: Airborne Methyl Jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Plant Cell Physiol.* **75**: 997-999.

Filonow, A. B. (1998) Role of competition of sugars by yeasts in the biocontrol of gray mold of apple. *Biocont. Sci. and Tech.* **8**: 243-256.

Flett, B. C. and Wehner, F. C. (1989) The effect of temperature and relative humidity on *Stenocarpella maydis* spore survival. Proc. in *Phytophylactica*. **21**. p.100. (Abstr.)

Fravel, D. (1999) Hurdles and bottlenecks on the road to biocontrol of plant pathogens. *Aus. Pl. Pathol.* **28**: 53-56.

Fravel, D. R. (1988) Role of antibiosis in the biocontrol of plant disease. *Ann. Rev. Phytopathol.* **26**: 75-91.

Fuhrmann, B.; Rquebert, M. F.; Hoegaerden, M. V. and Strasberg, A. D. (1989) Immunological differentiation of *Penicillium* species. *Can. J. Microbiol.* **35**: 1043-1047.

Gendloff, E. H.; Ransdell, D. C. and Burton, C. L. (1983) Fluorescent antibody studies with *Eutypa armeniaceae*. *Phytopathol.* **73**: 760-764.

Gerik, J. S.; Lommel, S. A. and Huisman, O. C. (1987) A specific serological staining procedure for *Verticillium dahliae* in cotton root tissue. *Phytopathol.* **77**: 261-265.

Ghosh, R. and Purkayastha, R. P. (2003) Molecular diagnosis and Induced systemic protection against rhizome rot disease of ginger caused by *Pythium aphanidermatum*. *Curr. Sci.* **85**: 1782-1783.

Ghosh, S. and Purkayastha, R. P. (1990) Analysis of host-parasite cross-reactive antigens in relation to *Myrothecium*-infection of soybean. *Indian J. Exp. Biol.* **28**: 1-3.

Githinnji, C. W. and Kokwaro, J. O. (1993) Ethnomedicinal study of major species in the family Labiatae from Kenya. *J. Ethnopharmacol.* **39**: 197-203.

Goto, M. (1990) Fundamentals of bacterial Plant pathology. Academic Press. New York, USA.

Hammerschmidt, R. (1999) Induced disease resistance: how do induced plants stop pathogens?. *Physiol. And Mol. Pl. Pathol.* **55**: 77-84.

Harden, T. J.; Simpfendorfer, S. and Murray, G. M. (2002/2001) Effect of temperature and pH on the growth and sporulation of *Phytophthora clandestine*. *Aus. Pl. Pathol.* **30**: 1-5.

Hardham, A. R.; Suzaki, E. and Perkin, J. L. (1986) Monoclonal antibodies to isolate-, species- and genus- specific components on the surface of zoospores and cysts of the fungus *Phytophthora cinnamoni*. *Can. J. Bot.* **64**: 311-321.

Heil, M. and Bostock, R. M. (2002) Induced Systemic Resistance (ISR) against pathogens in the context of Induced Plant Defences. *Annals of Bot.* **89**: 503-512.

Helbig, J. (2002) Ability of the antagonistic yeast *Cryptococcus albidus* to control *Botrytis cinerea* in strawberry. *Biocontrol.* **47**: 85-89.

Hema, M.; Savithri, H. S. and Sreenivasulu, P. (2001) Antibody and nucleic acid probe-based techniques for detection of sugarcane streak mosaic disease of sugarcane in India. *Curr. Sci.* **81**: 1105-1108.

Henis, Y. (1984) Interaction between *Sclerotium rolfsii* and *Trichoderma* spp.: relationship between antagonism and disease control. *Soil Biol. Biochem.* **16**: 391-395.

Horikawa, T. (1988) Occurrence of tea grey blight and tea shoot blight caused by *Pestalotia longiseta* Spegazzini and control. *In* Proc. Internatl. Symp. on Recent Developments in tea production. Taiwan. Tea Experimental Station, Taiwan. pp. 181-195.

Howe, G. A.; Lightner, J.; Browse, J. and Ryan, C. A. (1996) An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *The Pl. Cell.* **8**:2067-2077.

Hu Shu Xia (1996) A preliminary evaluation of tea germplasm resource resistance to tea grey blight disease in Anhui Province, China. *Crop Gen. Resources.* **2**: 41-42.

Iannelli, D.; Capparelli, R; Cristinzio, G.; Marziano, F.; Scala, F. and Navicello,C. (1982) Serological differentiation among *Formae speclales* and physiological races of *Fusrium oxysporum*. *Mycologia*. **74**: 313-319.

Ishizaki, H.; Nakammra, Y. and wheat, R. W. (1981) Serological cross reactivity between *Sporotheix schenckii* and various unrelated fungi. *Mycopathologia*. **73**: 65-68.

Jain, N. K. (1991) Indian Tea in Retrospect and Prospect and the imopact of Research and Development. *In* World Internatl. Symp. on Tea Sci. Japan. pp. 45-57.

Jamaluddin (1977) Nitrogen nutrition of some Aspergilli. *In* Physiology of Microorganisms. Ed. K. S. Bilgrami. Today and Tomorrow's Printers and Publishers, New Delhi, India. pp. 59-67.

Janisiewicz, W. J. (1988) Biocontrol of postharvest diseases of apples with antagonist mixtures. *Phytopathol.* **78**: 194-198.

Jaspers, M. V.; Walter, M.; Eade, K. Frampton, C. M. and Stewart, A. (2002) Control of *Botrytis cinerea* in grape using thyme oil. *Aus. Pl. Pathol.* **30**: 21-25.

Johnson, L. F. and Curl, E. A. (1972) *Methods for research on the ecology of soilborne plant pathogen*. Burgess Publishing Company. Minneapolis.

Karban, R. and Baldwin, I. T. (1997) *Induced responses to herbivory*. Chicago and London: University of Chicago Press.

Kato, T.; Yamaguchi, Y.; Harano, T.; Yokohama, T. and Uyehara, T (1984) Unsaturated hydroxy fatty acids, the self defensive substance in rice plant against rice blust disease. *Chem. Lett.* 409-412.

Ke, Hu; Dong, A.; Liu, H.; Feng, H.; Sun, Q. and Yao, X. (1999) Bioactivity of traditional Chinese herbal medicines against *Pyricularia oryzae*. *Pharm. Biol.* **37**: 225-230.

Kerr, A. (1980) Biological control of crown gall through production of agrocin 84. *Plant Dis.* **64**: 25-30.

Kessmann, H.; Staub, T.; Hofmann, C.; Maetzke, T.; Herzog, J.; Ward, E.; Uknes, S. and Ryals, J. (1994) Induction of systemic acquired resistance in plants by chemicals. *Ann. Rev. Phytopathol.* **32**: 439-459.

Khara, H. S. and Hadwan, H. A. (1990) *In vitro* studies on antagonism of *Trichoderma* sp. against *Rhizoctonia solani* the causal agent of damping off of tomato. *Plant Dis. Res.* **2**: 144-147.

Khatibi, A.; Shah, A. H.; Ageel, A. M.; Ahmad, M. S.; Yahya, M. A. and Tariq, M. (1989) Saudi folk medicine: phytochemical and antimicrobial screening. *Pakistan J. Pharm. Sci.* **2**: 29-34.

Khodaprast, A.S. and Hedjaroude, G.A. (1996) Fungal pathogen of tea plant in northern Iran. *Iranian J. Pl. Pathol.* **32**: 233-243.

Kirkegaard, J. A.; Wong, P. T. W. and Desmarchelier, J. M. (1996) *In vitro* suppression of fungal root pathogens of cereals by *Brassica* tissues. *Plant Pathol.* **45**: 593-603.

Kitagawa, T.; Sakamoto, K. and Ogura, H. (1989) Novel enzyme immunoassays for specific detection of *Fusarium oxysporum* f. sp. *cucumerinum* and for general detection of various *Fusarium* species. *Phytopathol.* **79**: 162.

Klarzynski, O. and Fritig, B. (2001) Stimulation of plant defense responses. C. R. Acad. Sci. Paris. Sciences de la vie. *Life sciences.* **324**: 953-963.

Klement, Z. (1982) Hypersensitivity in phytopathogenic prokaryotes. Eds. M. S. Mount and G. H. Lacy. Academic press. Inc. New York. pp. 149-177.

Koch, T.; Krumm, T.; Jung, V.; Engelberth, J. and Boland, W. (1999) Differential induction of plant volatile biosynthesis in the lima bean by early and late intermediates of the octadecanoid-signaling pathway. *Plant Physiol.* **121**: 153-162.

Koenig, R. and Paul, H. L. (1982) Variants of ELISA in plant virus diagnosis. *J. Virol. Method.* **5**: 113-125.

Köhl, J.; Malhoek, W. M. L.; VanderPlas, C. H. and Fokkema, N. J. (1995) Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field conditions. *Phytopathol.* **85**: 393-401.

Kuć, J. (1982) Induced immunity to plant disrase. *BioScience*. **32**: 854-860.

Lahdenperä, M. L. (1987) The control of *Fusarium* wilt on carnation with a *Streptomyces* preparation. *Acta. Hort.* **216**: 85-92.

Lahdenperä, M. L.; Simon, E and Uoti, J. (1990) Mycostop-a novel biofungicide based on *Streptomyces* bacteria. *In* Biotic Interaction of Soil-borne Diseases. Eds. A.B.R. Beemstar *et al.* Elsivier, Amsterdum. pp. 258-263.

Lamb, J. A.; Lawton, M. A.; Dron, M. and Dixon, R. A. (1989) Signals and transduction mechanisms for activation of plant defences against microbial attack. *Cell.* **56**: 215-224.

Latunde Dada, A. O. (1993) Biological control of southern blight disease of tomato caused by *Sclerotium rolfsii* with simplified mycelial formulations of *Trichoderma koningii*. *Plant Pathol.* **42**: 522-529.

Lawton, K. A.; Friedrich, L.; Hunt, M.; Weymann, K.; Delaney, T.; Kessmann, H.; Staub, T. and Ryals, J. (1996) Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *The Pl. J.* **10**: 71-82.

Leach, R. (1939) Biological control and ecology of *Armillaria mellea* (Vahl.) Fr. *Trans. Brit. Mycol. Soc.* **23**: 320-329.

Leon, J.; Rojo, E. and Sanchez-Serrano, J. J. (2001) Wound signaling implants. *Plant Cell.* **98**: 784-786.

Leroux, P. (1996) Recent Developments in the mode of Action of Fungicides. *Pestic. Sci.* **47**: 191-197.

Lil, L.; Lee, G. I. and Howe, G. A. (1999) Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *Biochem. and Mol. Biol.* **16**: 728-731.

Lima, G.; Ippolito, A.; Nigro, F. and Salerno, M. (1997) Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against post harvest strawberry rots. *Postharv. Biol. and Technol.* **10**: 169-178.

Linfield, C. A. (1993). A rapid serological test for detecting *Fusarium oxysporum* f.sp. *narcissi* in Narcissus. *Annals of Applied Biol.* **123**: 685-693.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L. and Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

Lyon, G. D.; Forrest, R. S. and Newton, A. C. (1996) SAR-The potential to immunize plants against infection. *In* Proc. Brighton Crop Prot. Conf. 1996. No.3. British Crop Protection Council. pp. 939-946.

Lyons, N. F. and White, J. G. (1992). Detection of *Pythium violae* and *Pythium sulcatum* in carrots with cavity spots using competition ELISA. *Annals of Applied Biol.* **120**: 235-244.

Mahadevan, A. (1991) Post-infectional Defence Mechanisms. Biochemical aspects of plant disease resistance. Vol. 2. Today and Tomorrow's Printers and Publishers. New Delhi. p. 871.

Mahadevan, A. and Sridhar, R. (1982). *Methods in Physiological Plant Pathology.* 2nd Edn. Sivakami Publications, Madras.

Maity, D. and Sen, C. (1985) Integrated biocontrol of *Sclerotium rolfsii* with nitrogenous fertilizer and *Trichoderma harzianum. Indian J. Agric. Sci.* **55**: 464-467.

Mandavia, M. K.; Patel, C. M.; Maravia, G. V. and Parameswaran, M. (1997) Role of Phenolic Compounds in Resistance to *Fusarium* Wilt in Chickpea. *Indian. J. Agric. Biochem.* **10**: 11-13.

Mandavia, M. K.; Patel, C. M.; Maravia, G. V. and Parameswaran, M. (1997) Role of Phenolic Compounds in Resistance to *Fusarium* Wilt in Chickpea. *Indian J. Agric. Biochem.* **10**: 11-13.

Masroor, M. K. and Chandra, S. (1987) Antagonism between *Aspergillus* spp and *Xanthomonas campestris* pv *citri* (Hasse) Daye, incitant of citrus canker. *Natl. Acad. Sci. Lett.* **10**: 233-236.

Matheron, M. E. and Porchas, M. (1999) Impact of Azoxystrobin, Dimethomorph, Fluazinam, Fosetyl-Al, and Metalaxyl on Growth, Sporulation, and Zoospore Cyst Germination of Three *Phytophthora* spp. *Plant Dis.* **84**:454-458.

Mazarei, M. and Kerr, A. (1990) Distinguishing pathovers of pseudomonas syringae on peas: nutritional, pathogenicity and serological tests. *Plant Pathol.* **39**. p. 278.

McConn, M.; Creelman, R. A.; Bell, E.: mullet, J. E. and Browse, J. (1997) Jasmonate is essential for insect defense in *Arabidopsis. Proc. Natl. Acad. Sci. USA.* **94**: 5473-5477.

Merz, W. G.; Burrell, R. G. and Gallegly, M. E. (1969) A serological comparison of six heterothallic species of *Phytophthora. Phytopathol.* **59**: 367-370.

Michael, R. and James, E. P. (1981) Immunochemical identification of *Fusarium moniliformae* ribosomes fom diseased corn (*Zea mays* L.) stalk tissue. *Physiol. Pl. Pathol.* **19**: 277-288.

Miller, T. C.; Gubler, W. D.; Geng, S. and Rizzo, D. M. (2003) Effects of Temperature and Water Vapor Pressure on Conidial Germination and Lesion Expansion of *Sphaerotheca macularis* f. sp. *fragariae. Plant Dis.* **87**: 484-492.

Mittal, M.; Sharma, R. and Paul, M. S. (2002) Antifungal properties of some fern from north west Himalayas. *Geobios.* **29**: 244-246.

Mohammadi, O. (1992) Mycostop biofungicide-present status. *In* Biological Control of Plant Diseases. Eds. E. S. Tjamos *et al.* Plenum Press, New York. pp. 207-210.

Mohan, S. B. (1989) Cross reactivity of antiserum raised against *Phytophthora fragariae* with other *Phytophthora* species and its evaluation as a genus-detecting antiserum. *Plant Pathol.* 39:352-363.

Mohan, S.B. (1988). Evaluation of antisera raised against *Phytophthora fragariae* for detecting the red core disease of strawberries by immunosorbent assay (ELISA). *Plant Pathol.* **37**: 206-216.

Mohr, P. G. and Cahill, D. M. (2001) Relative roles of glyceollin, lignin and the hypersensitive response and the influence of ABA in compatible and incompatible interactions of soybeans with *Phytophthora sojae*. *Physiol. and Mol. Pl. Pathol.* **58**: 31-41.

Mukherjee, B. and Sen, C. (1992) *Aspergillus* and *Penicillium* species: Potential agents for biocontrol of *Macrophomina phaseolina*. *Indian Phytopathol.* **45**: 39-43.

Mukhopadhyay, A. N., Shreshtha, S. M. and Mukherjee, P. K. (1992) Biological seed treatment for control of soil borne plant pathogens. *F. A. O. Plant Prtot. Bull.* **40**: 21-30.

Mulder, D. (1961a) How to economise on blister blight control. The devision to spray based on sunshine records. *Tea quart.* **32**: 88-90.

Mulder, D. (1961b) Report of the plant pathologist for 1961. *Rep. Tea Res. Inst.* Ceylon. pp. 95-99.

Muraleedharan, N. and Chen, Z. M. (1997) Pests and diseases of tea and their management. *J. Plantation Crops.* **25**: 15-43.

Naqvi, S. A. H.; Khan, M. S. Y. and Vohora, S. B. (1991) Anti-bacterial, anti-fungal and anthelminthic investigations of Indian medicinal plants. *Fitoterapia*. 62: 221-228.

Narasimhan, S. and Masilamani, S. (2002). Biocontrol of phytopathogenic fungi. Asian Congress of Mycology and Plant pathology, University of Mysore. p.152 (Abstr.).

Navarro, V.; Villarreal, M. L.; Rojas, G. and Lozoya, X. (1996) Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. *J. Ethnopharmacol.* **53**: 143-147.

Nick, A.; Rali, T. and Sticher, O. (1995) Biological screening of traditional medicinal plants from Papua New Guinea. *J. Ethnopharmacol.* **49**: 147-156.

Nimri, L. F.; Meqdam, M. M. and Alkofahi, A. (1999) Antibacterial activity of Jordanian medicinal plants. *Pharm. Biol.* **37**: 196-201.

Norman, C.; Vidal, S. and Palva, E. T. (1999) Oligogalacturonide-mediated induction of a gene involved in jasmonic acid synthesis in response to the cell-wall-degrading enzymes of the plant pathogen *Erwinia carotovora*. *Mol. Pl. Microbe Interact.* **12**: 640-644.

Ogunwande, I. A.; Bello, M. O.; Olawore, O. N. and Muili, K. (2001) A Phytochemical and antimicrobial studies on *Butyrospermum paradoxum*. *Fitoterapia*. **72**: 54-56.

Okey, E. N. and Sreenivasan, T. N. (1996) Salicylic acid: A factor in systemic resistance of cocaoto *Phytophthora pulmivora*. *In* Proc. Brighton Crop Prot. Conference. 1996. No. 3. British Crop Protection Council. pp. 955-960.

O'Neill, T. M.; Pye, D. and Locke, T. (2002) The effect of fungicides, irrigation and plant density on the development of *Peronospora sparsa*, the cause of downy mildew in rose and blackberry. *Annals of Applied biol.* **140**: 207-214.

Onsando, J. M.; Wargo, P. M. and Waudo, S. W. (1997) Distribution, severity and spread of *Armillaria* root disease in Kenya plantation. *Plant Dis.* **81**: 133-137.

Onsando, J. M.and Langat, J. K. (1989) Management of wood rot of tea (*Hypoxylon serpens* Pers. ex. Fri.) in Kenya. *Tea Research Foundation* (Kenya) **10**: 180-186.

Otieno, W. (1993) *Hypoxylon* wood rot of tea [*Camellia sinensis* (L.) O. kuntze.]: the causal agent , symptoms and control-a review. *Tea Board of Kenya* (Kenya). **14**: 61-64.

Otieno, W.; Sudoi, V.; Othiena, C. O. and Langat, J. K. A. (1994) Management of *Hypoxylon* wood rot of tea [*Cammellia sinensis* (L.) O. Kuntze.] using sanitation practices and fungicides. *Tea Board of Kenya* (Kenya). **15**: 110-112.

Ouchterlony, O. (1958) Diffusion in gel methods for immunological analysis. *Progress in Allergy*.**5**: 1-78.

Ouchterlony, O. (1967) Immunodiffusion and immunoelectrophoresis. *In* Handbook of Experimental Immunology. Ed. D. M. Weir. Blackwell Scientific Publications, Oxford and Edinburgh.

Panday, A.; Palni, L. M. S. and Coulomb, N. (1997) Antifungal activity of bacteria isolated from the rhizosphere of established tea bushes. *Microbiological Res.* **152**: 105-112.

Panday, K. K. and Upadhyay, J. P. (1999) Comparative study of chemical, biological and integrated approach for management of *Fusarium* wilt of pigeonpea. *J. Mycol. Pl. Pathol.* **29**: 214-216.

Pandey, A.; Palni, L. M. S. and Bag, N. (2000) Biological hardening of tissue culture raised tea plants through rhizosphere bacteria. *Biotech. Lett.* **22**: 1087-1091.

Papavizas, G. C. (1985) *Trichoderma* and *Gliocladium* : Their biology, ecology and potential for biocontrol. *Ann. Rev. Phytopathol.* **23**: 23-54.

Papavizas, G. C. and Lewis, J. A. (1989) Effect of *Gliocladium* and *Trichoderma* on damping-off of and blight of snapbean caused by *Sclerotium rolfsii. Plant Pathol.* **38**: 277-286.

Parimelazhagan, T. and Francis, K. (1999) Antifungal activity of *Clerodendrum viscosum* against *Curvularia lunata* in rice seeds. *J. Mycol. Pl. Pathol.* **29**: 139-141.

Park, S. K. (1995) Diseases of tea trees II. White scab of tea tree caused by *Sphaceloma theae. Korean J. Pl. Pathol.* **11**: 383-385.

Park, S. K.; Park, K. B. and Chao, K. H. (1996) Diseases of tea trees III. Grey blight of tea tree caused by *Pestalotiopsis longiseta. Korean J. Pl. Pathol.* **12**: 463-465.

Pasaribu, E. H. and dan Sinaga, J. S. (1981) Effectivity of several organic and inorganic fungicides against blister blight. *Warta BPTK.* **7**: 177-182.

Paulitz, T. C. and Bélanger, T. C. (2001) Biological control in greenhouse systems. *Annu. Rev. Phytopathol.* **39**: 103-133.

Pearce, G.; Strydom, D.; Johnson, S. and Ryan, C.A. (1991) A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science.* **253**: 895-898.

Peng, G. and Sutton, I. C. (1991) Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Can. J. Pl. Pathol.* **13**: 247-257.

Petrunak, D. M.; Gildow, F. E. and Christ, B. J. (1991) Incidence and distribution of six viruses infecting potato in Pennsylvania. *Plant Dis.* **75**. p. 644.

Prasad, R. D. and Rangeshwaran, R. (1999) Granular formation of *Trichoderma* and *Glioclodium* spp. in biocontrol of *Rhizoctonia solani* of Chickpea. *J. Mycol. Pl. Pathol.* **29**: 222-226.

Prasad, R. D.; Rangeshwaran, R. and Sreerama Kumar, P. (1999) Biological control of root and collar rot of sunflower. *J.Mycol. Pl. Pathol.* **29**: 184-188.

Premkumar, R. (1997) Tea: blister blight control-recommendations on the usage of propiconazole. *Planters' Chron.* **92**. p. 169.

Premkumar, R. and Baby, U. I. (1996) Cost effective blister blight control. *Bull. UPASI Tea Sci. Dep.* **49**: 47-54.

Premkumar, R. and Muraleedharan, N. (1997) Tea blister blight control: recommendations on the usage of hexaconazole in tea recovering from pruning. *Handbook of Tea Culture*, Section 13. United Planters' Association of Southern India. p.1.

Premkumar, R.; Baby, U. I. and Chandra Mouli, B. (1998) Systemic activity and field performance of triazole fungicides against blister blight pathogen of tea. *Developments in Plantation Crops Res.* pp. 279-283.

Pretorius, J. C.; Craven, P. and Van Der Watt E. (2002) *In vivo* control of *Mycosphaerella pinodes* on pea leaves by a crude bulb extract of *Eucomis autumnalis. Annals of Applied biol.* **141**: 125-131.

Pretorius, J. C.; Zietsman, P. C. and Eksteen, D. (2002) Fungitoxic properties of selected South African plant species against plant pathogens of economic importance in agriculture. *Annals of Applied biol.* **141**: 117-124.

Prew, R. D.; Ashby, J. E.; Bacon, T. G.; Christian, D. G.; Gutteridge, R. J.; Jenkyn, J. F.; Powell, W. and Todd, A. D. (1995) Effects of incorporating or burning straw, and of different cultivation systems, on winter grown on two soil types, 1981-91. (Cambridge). *J. Agric. Sci.* **124**: 173-194.

Priestley, A. and Dewey, F. M. (1993) Development of a monoclonal antibody immunassay for eye spot pathogen *Pseudocercosporella herpotrichoides. Plant Pahtol.* **42**: 403-412.

Punja, Z. K. (1985) The biology, ecology and control of *Sclerotium rolfsii. Ann. Rev. Phytopathol.* **23**: 97-127.

Purkayastha, R. P. (1989) Specificity and disease resistance in plants. Presidential address, Section Botany, 76th Session of the Indian Science Congress. Madurai.

Purkayastha, R. P. and Banerjee, R. (1990) Immunoserological studies on cloxacillin induced resistance of soybean against anthracnose. *J. Pl. Dis. Prot.* **97**: 349-359.

Purkayastha, R. P. and Ghosal, A. (1987) Immunoserological studies on root rot of groundnut (*Arachis hypogea* L.). *Can. J. Microbiol.* **33**: 647-651.

Raju, C. A. (1991) Survey of antagonism in *Trichoderma* species against *Pythium aphanidermatum*. Natl. Sem. on Biological Control in Plantation Crops. 27-28th June 1991, Rubber Res. Inst. India, Kottayam, India. p.25. (Abstr.).

Rao, G. P.; Sharma, S. R. and Singh, P. K. (2000) Fungitoxic and insect repellent efficacy of limonene against sugarcane pets. *J. Essential Oil-bearing Plants.* 3: 157-163.

Rao, K. S. (1996) Antibacterial activity of some medicinal plants of Papua New Guinea. *Internatl. J. Pharmacognosy.* **34**: 223-225.

Reimers, F.; Smolka, S. E.; Werres, S; Schumacher, K. P.; and Wagner, K. G. (1993) Effect of ajoene, a compound derived from *Allium sativum*, on phytopathogenic and epiphytic microorganisms. *J. Plant. Dis. Prot.* **100**. p. 622.

Renveni, M.; Agapov,V. and Renveni, R. (1997) A foliar spray of micronutrient solutions induces local and systemic protection against powedery mildew (*Sphaerotheca fuliginia*) in cucumber plants. *European J. Pl. Pathol.* **103**: 581-588.

Ricker, R. W.; Marois, J. J.; Dlott, J. W.; Bostock, R. M. and Morrison, J. C. (1991) Immunodetection and quantification of *Botrytis cinerea* on harvested wine grapes. *Phytopathol.* **81**: 404-411.

Rizvi, H. A.; Yasmeen, A.; Modan, M. and Dadar, Y. (1987) Screening of higher plants for antibacterial activity. *Pakistan J. Scientific and Industrial Res.* **30**: 215-220.

Robinson, P. M. (1969) Aspects of staling in liquid culture and fungi. *New Phytol.* **68**: 351-357.

Roux, J.; Coutinho, T. A.; Byabashaija, D. M. and Wingfield, M. J. (2001) Diseases of plantation Eucalyptus in Uganda. *South African J. Sci.* **97**: 16-18.

Roy, A. K. (1977) The influence of carbon sources on the aminoacid composition of four pathogenic isolates of *Colletotrichum dematium*. *In* Physiology of Microorganisms. Ed. K. S. Bilgrami. Today and Tomorrow's Printers and Publishers, New Delhi, India. pp. 69-74.

Ryals, J. A.; Neuenschwander, U. H.; Willits, M. G.; Molina, A.; Steiner, H. Y. and Hunt, M. D. (1996) Systemic acquired resistance. *The Pl. Cell.* **8**: 1809-1819.

Ryan, C.A. (2000) The systemin signaling pathway: differential activation of plant defensive genes. *Biochimica et Biophysica Acta.* **1477**: 112–121.

Saha, A. (1992) Studies on the resistance of tea [*Camellia sinensis* (L.) O. KTZE.] to *Bipolaris carbonum* Nelson. Ph. D. Thesis. University of North Bengal, West Bengal, India.

Saha, A.; Dasgupta, S. and Saha, D.(2001) Discovery of *Curvularia eragrostidis* on Tea (*Camellia sinensis* (L.) O. Ktze.) leaves from clonal-cutting nurseries in North Bengal. *Environ. and Ecol.* **19**: 846-848.

Saha, A. and Chakraborty, B. N. (1990) Spore germination of *Bipolaris carbonum* Nelson. causing tea leaf disease. *Indian Botanical Contactor.* **7**: 131-133.

Saha, D. L.; Ali, M. A. and Haque, M. I. (1980) Red rust of tea and its control. Bangladesh Association of the Advancement of Science (BAAS). p. 20

Saligkarias, I. D. ; Gravanis, F. T. and Epton, H. A. S. (2002) Biological control of *Botrytis cinerea* on tomato plants by the use of epiphytic yeasts *Candida guilliermondii* strains 101 and US 7 and *Candida oleophila* strain I-182: I. *in vivo* studies. *Biological Cont.* **25**: 143-150.

Samuelsson, G.; Farah, M. H.; Claeson, P.; Hagos, M.; Hedberg, O.; Warfa, A. M.; Hassan, A. O.; Elmi, A. H. and Abdurahman, A. D. (1992) Inventory of plants used in traditional medicine of Somalia. II. Plants of the families Combretaceae to Labiatae. *J. Ethnopharmacol.* **37**: 47-70.

Sarmah, K. C. (1960) Disease of tea and associated crops in North East. Memorandum No.26. Indian Tea Association, Tocklai Experimental Station.

Scherer, J. M. and Ryan, C. A. (1999) A 160kD systemin receptor on the surface of *Lycopersicon peruvianum* suspension-cultured cells. *Plant Biol.* **84**: 1125-1128.

Schneider-Muller, S.; Kurosaki, F. and Nishi, A. (1994) Role of salicylic acid and intracellular Ca^{2+} in the induction of chitinase activity in carrot suspension culture. *Physiol. and Mol. Pl. Pathol.* **45**: 101-109.

Shanmugum, V. and Sukunara Verma, A. (1999) Effect of native antagonists against *Pythium aphanidermatum*, the causal organism of rhizome rot of ginger. *J.Mycol. Pl. Pathol.* **29**: 375-379.

Sharma, K.; Shukla, S. D.; Mehta, P. and Bhatnagar, M. (2002) Fungistatic activity of *Semecarpus anacardium* Linn. f nut extract. *Indian J. Experimental Biol.* **40**: 314-318.

Sharma, S. D. ; Mishra, A.; Pandey, R. N. and Patel, S. J. (2001) Sensitivity of *Trichoderma harzianum* to fungicides. *J. Mycol. Pl. Pathol.* **31**. 251-253.

Singh, P. P.; Basra, A. S. and Pannu, P. P. S. (1997) Abscisic Acid is a potent inhibitor and Sporidial formation in *Neovossia indica* Cultures: Dual mode of action via loss of Polyamines and Cellular Turgidity. *Phytoparasitica*. **25**: 111-116.

Singh, U. P.; Pandey, V. N.; Wagner, K. G. and Singh, K. P. (1990) Antifungal activity of ajoene, a constituent of garlic (*Allium sativum* L.). *Can. J. Bot.* **68**. p. 1354.

Singh, U. P.; Pathak, K. K.; Khare, M. N. and Singh, R. B. (1979) The effect of aqueous garlic leaf extract on two pathogens, *Fusarium oxysporum* f. sp. Ciceri and *Sclerotinia sclerotiorum*. *Mycologia*. **71**. p. 556.

Singh, U. P.; Prithiviraj, B.; Wagner, K. G. and Schumacher, K. P. (1995) Effect of ajoene, a constituent of garlic (*Allium sativum*) on powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*). *J. Plant. Dis. Prot.* **102**. p. 399.

Sinha, A. K. and Das, N. C. (1972) Induced resistance in rice plants to *Helminthosporium oryzae*, *Physiol. Pl. Pathol.* **2**. 401-410.

Smit, W. A. and Devis, P. S. (1989) Crown root rot of rooibos tea caused by *Macrophomina phaseolina* and *Neocosmospora vesinfecta*. *Phytophylactica*. **81**: 291-296.

Suleman, P.; Al-Musallam, A. and Menezes, C. A. (2002) The effect of biofungicide Mycostop on *Ceratosystis radicicola,* the causal agent of black scorch on date palm. *Biocontrol.* **47**: 207-216.

Sundarm, S.; Plasencia, J. and Banttari, E. E. (1991) Enzyme linked immunosorbent assay for detection of *Verticillium* spp. Using antisera produced to *V. dahliae* from potato. *Phytopathol.* **81**: 1485-1489.

Sutton, J. C. (1995) Evaluation of microorganisms for biocontrol: *Botrytis cinerea* and strawberry, a case study. *In* Advances in Plant Pathology. Eds. J. H. Andrews and I. C. Tommerup. Academic Press. San Diego. USA. pp. 173-190.

Taguchi, G.; Sharan, M.; Gonda, K.; Yanagisawa, K.; Shimosaka, M; Hayashida, N. and Okazaki, M. (1998) Effect of methyl jasmonate and elicitor on PAL gene expression in tobacco cultured cells. *J. Pl. Biochem. and Biotechnol.* **7**: 79-84.

Tahvonen, R. and Avikainen, H. (1990) Effect of *Streptomyces* sp. on seed-borne foot rot disease of wheat and barley. I Pot experiments. *Ann. Agric. Fenn.* **29**: 187-194.

Tahvonen, R. and Lahdenperä, M. L. (1988) Biological control of *Botrytis cinerea* and *Rhizoctonia solani* in lettuce by *Streptomyces* sp. *Ann. Agric. Fenn.* **27**: 107-116.

Talbot, N. (2001) Molecular and cellular biology of filamentous fungi. Oxford University Press. pp. 236-238.

Terras, F. R. G.; Schoofs, H. M. E.; Thevissen, K.; Osborn, R. W.; Vanderleyden, J.; Cammue, B. P. A. and Broekaert, W. F. (1993) Synergistic Enhancement of the Antifungal Activity of Wheat and Barley Thionins by Radish and Oil Seed Rape 2 S Albumins and by Barley Trypsin Inhibitors. *Plant Physiol.* **103**: 1311-1319.

Thomashow, M. F. (1999) Plant cold accumulation: freezing tolerance genes and regulatory mechanisms. *Ann. Rev. Pl. Physiol. and Pl. Mol. Biol.* **50**: 571-599.

Tronsmo, A. and Raa, J. (1977) Antagonistic action of Trichoderma pseudokoningii against the apple pathogen *Botrytis cinerea*. *Phytopathol.* **89**: 216-220.

Tu, J. C. (1992) Biological control of Sclerotinia sclerotium. *In* Recent Developments in Biocontrol of Plant Diseases. Eds. K. G. Mukherjee; J. P. Tewari; D. K. Arora and G. Saxena. Aditya Books Pvt. Ltd. New Delhi. pp. 24-32.

Tzong-Maochen and Shin-Funchen (1982) Diseases of tea and their control in People's Republic of China. *Plant Dis.* **66**: 961-965.

Uknes, S.; Mauch-Mani, B.; Moyer, M.; Potter, S.; Williams, S.; Dincher, S.; Chandler, D.; Slusarenko, A.; Ward, E. and Ryals, J. (1992) Acquired resistance in *Arabidiopsis. Plant Cell.* **4**: 645-656.

Upadhyay, J.P. and Mukhopadhyay, A. N. (1983) Effect of non-volatile and volatile antibiotics of *Trichoderma harzianum* on growth of *Sclerotium rolfsii. Indian J. Mycol.Pathol.* **13**: 232-233.

Upadhyay, R. S. and Rai, B. (1985) Antagonistic behaviour of root region microfungi of pigeonpea against *Fusarium udum. In* Ecology and Management of Soil-borne Plant Pathogens. American Phytopathological Society, St.Paul, Minnesota. p. 133.

Van Loon, L. C.; Bakker, P. A. H. M. and Pieterse, C. M. J. (1998) Systemic resistance induced by rhizosphere bacteria. *Ann. Rev. Phytopathol.* **36**: 453-483.

Venkata Ram, C. S. (1960) A reassessment of diplodia root disease of tea plants. UPASI Sci. Dep. Bull. Tea Sect. **19**: 18-28.

Venkata Ram, C. S. (1962) A study of the comparative efficiency of nickel salts against blister blight disease of tea. *Ann. Rep. UPASI Sci. Dep.* Tea Sect. for 1961-62. pp. 8-14.

Venkata Ram, C. S. (1966a) Report of the plant pathological Ann. Rep. UPASI Sci. Dep. for 1965-66 pp. 24-37.

Venkata Ram, C. S. (1969) Systemic control of *Exobasidium vexans* on tea with 1, 4-oxathin derivatives. *Phytopathol.* **59**: 125-128.

Venkata Ram, C. S. (1974) Calixin, a systemic fungicide effective against blister blight (*Exobasidium vexans*) of tea plant. *Pesticides.* **8**: 21-25

Venkata Ram, C. S. (1975) Systemic activity and field performance of pyracarbolid in the control of blister blight pathogen of tea. *Z. Pfl. Krankh. Pfl. Schutz.* **82**: 65-76.

Venkata Ram, C. S. (1977) Laboratory and field evaluation of carboxin and oxycarboxin against *Exobasidium vexans* on tea. *Indian Phytopathol.* **30**: 449-455.

Venkata Ram, C. S. and Chandra Mouli, B. (1976) Systemic fungicides for integrated blister blight control. *UPASI Tea Sci. Dep. Bull.* **33**: 70-87.

Venkata Ram, C. S. and Chandra Mouli, B. (1979) *Ann. Rep. UPASI Sci. Dep.* **53**: 98-114.

Venkata Ram, C. S. and Chandra Mouli, B. (1981) *Ann. Rep. UPASI Sci. Dep.* **55**:105-109.

Venkata Ram, C. S. and ChandraMouli, B.(1983) Interaction of dosage, spray interval and fungicide action in blister blight disease control in tea. *Crop Prot.* **2**: 27-36.

Verma, R. B. and Balasundaran, M. (1990) Tea mosquito (*Halopeltis antonii*) feeding as a predisposing factor for entry of wood pathogens in cashew. *Entomon.* **15**:247-252.

Vidhyasekaran, P. (1988) Phenolics and disease resistance. *In* Physiology of Disease Resistance in Plants, I. Ed. P. Vidhyasekaran. CRC Press, Boca Raton. pp. 49-81.

Vinod, P. B. (1988) Antibiotic producing and antagonistic microorganisms in the forest soils of Kerala. M.Sc. (Ag.) thesis, Kerala Agricultural University. Vellanikkara. p.156.

Vinod, P. B.; Verma, A. S. and Cheeran, A (1991) Antagonistic and antibiotic properties of *Bacillus subtilis* isolated from the forest soils of Kerala. Nat. Sem. on Biol. Cont. Plantation Crops. 22-28th June 1999, Rubber Res. Inst.India Kottayam, India. p. 26. (Abstr.).

Vlietinck, A. J.; Van-Hoof, L.; Totte, J.; Lasure, A.; Berghe, D. V.; Rwangabo, P. C. and Mvukiyumwami, J. (1995) Screening of one hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J. Ethnopharmacol.* **46**: 31-47.

Wakeham, A. J. and White, J. G. (1996) Serological detection in soil of *Plasmodiophora brassicae* resting spores. *Physiol. Mol.Plant Pathol.* **48**:189-303.

Wang, J.; Dian, X. and Zhang, B. (1990) The diseases of *Camellia* in Zhejiang Province. *Acta. Phytopathologica Sci.* **20**: 89-92.

Wasternack, C. and Parthier, B. (1997) Jasmonate-signalled plant gene expression. *Trends In Pl. Sci.* **2**: 302–307.

Watabe, M. (1990) Immunofluoresencent antibody technique for detecting *Phytopathora* in soil . *Phytopahtol .Soc.* Japan. **56**: 169-272.

Weller, M. D.; Raaijmakers, J. M.; Gardener, M. and Thomashow, L. S. (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Ann. Rev. Phytopathol.* **40**:309-348.

White, J. G.; Lyons, N. F.; Wakeham, A. J.; Mead, A. and Green, J. R. (1994) Serological profiling of fungal genus *Pythium. Physiol. Mol. Plant Pathol.* **44**:349-361.

Wokocha, R. C. (1990) Integrated control of *Sclerotium rolfsii* infection of tomato in the Nigerian savanna : Effect of *Trichoderma viride* and some fungicides. *Crop. Prot.* **9**: 231-234.

Wu, H. C. and Wu, W. S. (2003) Sporulation, pathogenicity and chemical control of *Alternaria protenta*, a new seedborne pathogen on sunflower. *Aus. Pl. Pathol.* **32**: 309-312.

Wu, W. S.; Liu, S. D.; Chang, Y. C. and Tschen, J. (1986) Hyperparasitic relationships between antagonists and *Rhizoctonia solani. Plant Prot. Bull.* Taiwan. **28**: 91-100.

Yalpini, N.; Silverman, P.; Wilson, T. M. A.; Kleir, D. A. and Raskin, I. (1991) Salicylic acid is a systemic signal and inducer of pathogenesis-related proteins in virus-infected tobacco. *Plant Cell.* **3**: 809-818.

Yamaguchi, S.J.; Takyu, T.; Ikeda, N.; Takeda, Y.; Watanabe, A. and Yanase, Y. (1992) A new cultivar (*Camellia sinensis*) "Fushun" for green tea. *Bulletin of National Research.* Institute of vegetables, ornamental plants and tea Series (No.5). pp. 1-13.

Yamanishi, T. (1991) Flavour characteristics of various Teas. *In* The Proc. World Tea, Internat. Symp. on Tea Sci. Shizouka, Japan. pp. 1-11.

Yanase, Y. and Takeda, Y. (1987) Method for testing the resistance to tea grey blight caused by *Pestalotia longiseta*. Spegazzini in tea breeding. *Bulletin of the National Research Institute of Vegetables, Ornamental plants and tea*, B (Kenya) **1**:1-4.

Yasmin, P. and Saxena, S. K. (1990) Effect of fern extracts on growth and germination of fungi. *Curr. Sci.* **59**: 16-25.

Yoshida, H.; Katsuzaki, H.; Ohta, R.; Ishikawa, K.; Fukuda, H.; Fujino, T. and Suzuki (1999b) An organosulfur compound isolated from oil-macerated garlic extract and its antimicrobial effect. *Biosci. Biotechnol. Biochem.* **63**: 588-590.

Yoshida, H.; Katsuzaki, H.; Ohta, R.; Ishikawa, K.; Fukuda, H.; Fujino, T. and Suzuki, A. (1999a) Antimicrobial activity of the thiosulfinates isolated from oil-macerated garlic extract. *Biosci. Biotechnol. Biochem.* **63**: 591-594.

Yoshida, S.; Karuga, S.; Hayashi, N.; Ushiriguchi, T.; Matsurra, H. and Nakagawa, S. (1987) Antifungal activity of ajoene derived from garlic. *Applied Environ. Microbiol.* **53**: 615-617.

Zhang, Z. P. and Baldwin, I. T. (1997) Transport of (2-14C) jasmonic acid from leaves to roots mimics wound-induced changes in endogenous jasmonic acid pools in *Nicotiana sylvestris*. *Planta* **203**: 436-441.