STUDIES ON RED ROT DISEASE OF SUGARCANE CAUSED BY *Colletotrichum falcatum* Went

A THESIS SUBMITTED TO
THE ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, BHUBANESWAR
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

(PLANT PATHOLOGY)

BY

Asish Kumar Sahoo

Department of Plant Pathology,
COLLEGE OF AGRICULTURE
Orissa University of Agriculture and Technology
BHUBANESWAR
1993

THESIS ADVISOR **Dr. C. M. DAS**

DEDICATED TO
MY BELOVED PARENTS

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C E R T I F I C A T E

Certified that the thesis entitled **"STUDIES ON RED ROT DISEASE OF SUGARCANE CAUSED** BY **Colletotrichum falcatum** Went submitted in partial fulfilment for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** is a faithful record of the bonafide research work carried out by **Sri Asish Kumar Sahoo** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance & help received during the course of this investigation has been duly acknowledged.

Bhubaneswar

(DR. C.M. DAS)

Dated: 1st, **August, 1994.**

1.8.94

A C K N O W L E D G E M E N T

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Asish Kumar Sahoo

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Bhubaneswar,

Dated : 1st **August 1994.**

C O N T E N T S

LIST OF TABLES

LIST OF FIGURES

A B S T R A C T

Field susceptibility to red rot irrespective of the different sugarcane cultivars grown was mainly governed by soil, water, pH of soil, atmospheric temperature and rain fall. The disease was more severe from September to October under partially submerged condition, soil pH of 5.5 to 6.5, atmospheric temperature of 25-30°C accompanied with rain splashed wind for about 15 days preceeding the incidence. Media supplemented with glucose supported maximum growth and sporulation of Colletotrichum falcatum. The pathogen could grow and sporulate from 15-35°C and optimum being 25-35°C. The optimum pH for maximum growth and sporulation was at 6.5. Reactions of five different cultivars of sugarcane to the present isolate of C. falcatum were intermediate and were either moderately susceptible except for variety V-186 which was moderately resistant. Trichoderma viride was found antagonistic and hyperparasitic on C. falcatum in dual culture. Turmeric rhizome extract followed by neem oil, sonflower oil and garlic bulb extract showed antifugal activity against C. falcatum. The in vitro growth of pathogen was completely inhibited at 4% concentration of turmeric. Carbendazim, Thiophenate methyl, metalaxyl, oxycarboxin compounds were most effective at 500 ppm concentration in inhibiting the in vitro growth.

CHAPTER I

INTRODUCTION

I N T R O D U C T I O N

Sugarcane is known to have been cultivated since 6000 B.C in many South eastern countries. Its native place has been reported to be Polynesian and Melanesian islands in the region of Malaya Archepelago (in particular in New-Guinea Islands) in the pacific (Brandes, 1957). It is believed to have existed in the home yards of the Melanesian natives earlier than 12000 B.C. This was mainly grown for requirements of sugar alongwith other sugar producing plants like wild date palm, Palmyra palm & other palms from South America. However, presently sugarcane (Saccharum sp.) and sugar beet (Beta vulgaris) ware used as the only two sources accounting for the wide sugar production in the world. Sugarcane dominates the tropical and sub tropical areas, while sugarbeet is grown mostly in temperate areas.

In India cultivation of sugarcane dates back to earlier than 1000 B.C. for production of "Sarkara" & "Jaggery" particularly in the Gangetic Valley of India, as has been known from ancient writtings particularly from "Atharva-veda". At present India is the largest producer of sugarcane and only next to Cuba.

Earlier, sugarcane cultivation was restricted to districts of Ganjam & Puri in Orissa and rather was sporadic in river basins & few fertile lands. But at present it has been extended to different districts because of installations of sugar factories at different areas of the state. Presently, the area has been extended upto 50,550 hectars with annual production of 3549,000 tonnes (Source -" Orissa Agricultural Statitics", 1991-92).

Out of various diseases affecting sugarcane crop, "Red rot" known as "Cancer of sugarcane" is the most destructive disease. It is incited by Colletotrichum falcatum Went (= Glomerella tucumanensis (Speg.) Von Arx and Muller). The first authentic report of the disease in India was furnished by Barber (1901), when the exotic variety "Red Mauritius" was affected on large scale in Godavari Delta of Andhra Pradesh, and Ganjam district of Orissa.

The impact of this disease was severe in eastern Uttar Pradesh and nothern Bihar which have become hot spot for this disease. Even at present, the disease is considered as one of the major constraints in the profitable cultivation of sugarcane. This disease has forced many wonder varieties like CO 213, CO 312, CO 419, CO 453, CO 1148, BO 3, BO 17, BO 54 etc. to got out of cultivation. The present loss in terms of cane yield has been estimated to be ranging from 12 to 44%, particularly more under water logged conditions (Agnihotri & Singh, 1993).

In Orissa presently the disease has gained its importance because of the increase in the areas under large scale cultivation of sugarcane. The extent of yield loss in cane production during last two years have been found to exceed upto 50% in individual fields in coastal low lying areas.

The author has found severe occureence of red rot in low lying areas of district of Jagatsinghpur & Banki area of Cuttack district during field visits during the months of August to October, 1993. The disease was seen to spread so severely in individual fields, that farmers were busy in harvesting the immature canes even during the month of September, expecting total loss due to the disease if it would be left upto usual

period of harvest. The disease was observed to spread more quickly under partial or fully submerged conditions particularly with poor sanitation.

In view of the seriousness of the disease at present in Orissa, the present work was undertaken. Although, studies on various aspects of the disease & the pathogen have been investigated earlier by several authors in India, the variability in the varietal reactions to the pathogenic races and exposer of different varieties to new areas have resulted into complex problems of management of this disease.

This investigation deals with certain studies related to its field occurence, physiological requirements of the pathogen, varietal reactions of some commonly adapted varieties in Orissa where the disease has been in severe form, studies on efficacy of some plant products and fungicides in inhibiting the in vitro growth of the pathogen and studies on antagonistic effect of candidate microflora, (Trichoderma viride) on the test fungus (Colletotrichum falcatum).

The present study, would definitly add to the knowledge of understanding this disease and its pathogen particularly with reference to the present context of its wide spread occurence in different areas of the state of Orissa.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Studies on this disease have been undertaken extensively in past in India & presently by Central Sugarcane Research Institute located at Lucknow, Sugarcane Breeding Research Station at Coimbatore and substations located in different States, and by various Agricultural Universities in the country. Therefore, literature on various aspects of the disease is extensive and the major research findings have been on varietal breeding for high yield as well as on disease resistance. Studies on other aspects of the disease like, epidemiology, racial variation of the pathogen and on management of the disease have been extensively worked out by different workers which have been reviewed below. Inspite of extensive work done earlier on this disease, the problem, faced by farmers due to this disease seems to remain fully unsolved, because of extensive continuous cultivation of particular varieties, drastic change in the agroclimatic condition, and more obviously due to racial variations amongst pathogenic races.

2.1 MARPHOLOGY OF CAUSAL ORGANISM:

The causal organism was first described by Went (1893) from Java (Indonesia)& named the pathogen as Colletotrichum falcatum Went. He has described the acervulus, with hyaline, closely packed short conidiophores; setae which were brown, septate, slightly bulbous at the base and tappering towards apex and measuring 100-200 µm long. The condia were hyaline, one celled, falcate or sickle shaped, sometime fusoid with one end rounded and other slightly pointed, granular, containing usually one oil globule.

The perfect state or the ascigerous state was first described in Argentina by spegazzini (1896) as Physalospora tucumanensis speg. This species was not recognised as the perfect stage until Carvajal & Edgerton (1943) reported it in Louisiana. After examining the specimen from Louisiana, Von Arx & Muller (1954) placed the perfect stage under the genus Glomerella. Both the name Physalospora tucumanensis speg. & Glomerella tucumanensis (speg.) Arx & Muller are in usage at present.

The perfect stage was first described from India by Chona & Srivastava (1952) from culture at IARI New Delhi. Chona & Bajaj (1953) reported the perfect stage occuring on sugarcane leaves. Edgerton (1943) and Von Arx (1954) described that the red rot fungus is homothallic.

2.2 PATHOTYPES AND PHYSIOLOGICAL SPECIALIZATION:

The existence of physiological races of Glomerella tucumanensis was established by Abbott (1933). Abbott (1938) distinguished two cultural races, "light" & "dark". The "light" race produced white to light grey, cottony mycleium and the "dark" one produced compact, valvety, dark, grey mycleium. He found many isolates which were intermediate of these two races. The "light" race generally produced abudant spores and proved more virulent than the drak races as has been reported by Rafay and Padmanabhan, 1941; Rafay, 1950; Chattopadhyay and Sarkar, 1960; Chona and Srivastav, 1960; Prakasham and Venkata Reddy, 1961.

Rafay and Padmanabhan (1941) & chona (1954) have differentiated the races on the basis of cultural characters and on the basis of spore masses by Ahmed (1972).

Lo (1947) described two types of conidia and setae of C falcatum and identified two strains of the fungus on the basis of size of condia and setae.

Ling(1950) suggested that the size of perithecia occuring in vitro and in vivo was attributed to different races.

Carvalo (1958) suggested the possibility of racial variation in Colletotrichum falcatum through heterokaryosis. He damonstrated that crosses of marker defficiency mutant resulted in the formation of heterokaryotic colonies. The fusion occured in hyphae and germ tubes with nuclear migration and constituent formation of heterokaryotic mycelium.. The appressoria were formed after hyphal fusion and were multinucleate.

Singh et al. (1968) observed hyphal fusion of various types (e.g., side to side, end to end, peg to peg). They have also reported fusion of two conidia as well as fusion of condium with appressorium.

Sandhu et al. (1974) and Lewin et al. (1978) suggested that virulence of strains to be main criterion for race identification. They observed that isolates causing stalk rot were also capable of midrib infection where as the isolates, infecting midrid were unable to cause stalk rot.

Khirbat et al. (1980) reported three strains on the basis of disease reactions on COL.9, CO 62399, Co 312.

Satyanarayan and Achutaramarao (1984), and Gupta & Uppadhaya (1980) reported three races of the pathogen on the basis of varietal reaction. The former worker reported two physiologically different strains in light race strains of Andhar Pradesh.

2.3 PHYSIOLOGICAL STUDIES:

A large number of studies on the Physiological requirements of C. falcatum Went, have been conducted earlier by various workers as evidence from the earlier literatures. However, references of such workers felt desirable for the present study on Physiological pathology of causal organism have only been enlisted here particularly highlighting the studies conducted during last ten years.

2.3.1 Growth & sporulation in different culture media:

Singh, & Kamal (1979) reported that, out of different media containing juices of different sugarcane varieties, the growth of Colletotrichum falcatum was greater in solid and liqued media containing juice of susceptible cultivars than in media containing extracts from resistant varieties.

Media supplemented with glucose supported maximum growth of C falcatum followed by fructose, maltose, and galactose as has been reported by Rajak (1981). He had further reported that pentoses were poor sources of carbohydrates for growth of the fungus. Maximum sporulation occured on maltose, sucrose, and raffinose while,

xylose, glucose and starch did not help for satisfactory sporulation.

Ghosal & Nerganta (1987) observed that, red pigmented extracts from sugar cane plant infected by C. falcatum inhibited the radial mycelial growth as well as germination of spores. They suggested that the inhibition caused by the pigmented juice was due to the presence of growth stimulatoy factor like luteolinidin in sugarcane extract. While in vitro spore germination was inhibited by the pigment, spore exposed to extract from uninfected sugarcane stalks nearly completed germination within 14 hours. They have further reported that the first pigment component in infected tissue did't appear until 24 hours after infection, indicating that the pigment components might functioned to slow down the growth & spread of the mycelium in vivo but might not play role in controlling spore germination in vivo. They further pointed out that, concentration of phenolic compounds in the cane tissues could't be correlated with resistance against C falcatum (= G. tucumanensis).

Possible correlation between sporulation and virulence in some isolates of C. falcutum was studied by Jhamaria and Ghemawat (1991) who concluded that, tests with single spore isolates of C. falcacum from infected sugarcane showed a possible correlation between sporulation on seven agar media and virulence on seven sugarcane cultivars, differing in resistance to the pathogen.

Dattamajumdar et al. (1991) studied the production, maintainance and germinability of C. falcatum condia by putting longitudinally splited canes in flasks filled up with sterile distilled water and inoculated with sterile spore suspension. The flasks were incubated at 27° C for 2 days in open diffused sunlight. They found abundant sporulation after 15 days and 5 sporulating pieces of canes

which yielded 1 litre of spore suspension with 10^6 condia/ml. Germinability of conidia from stalk pieces was higher than that of conidia from Oat meal agar culture. Darkness did not induce sporulation.

2.3.2 Effect of temperature and light intensity on *in vitro* growth & sporulation and *in vivo* disease development:

Singh (1973) suggested that higher light intensity reduced condial germination & increased appressoria formation in *C. falcatum* during sporulation. Conidia produced at temperature above optimum germinated better and formed pure appressoria. Increased germination was observed in conidia obtained from inoculated sugarcane leaves as well as in those produced in culture. The light type virulent isolates were more sensitive to these treatments than the dark less virulent isolates.

Ahmed and Divinagracia (1974) had reported the optimum temperature of 25° - 30°C for growth of *C. falcatum* and maximum sporulation occured at 25°C, while the fungus could grow within the range of 10° - 35°C temperature and could sporulate within the temperature range of 20°-30°C.

Ghose (1975) studied the effect of temperature on mycelial growth and sprulation and observed that, *C. falcatum* and *C. graminicolum* could grow at 10°- 35°C with maximum sporulation at 30°C.

Rajak (1981) found that the optimum temperature for growth of C. falcatum to be 20°C followed by 30°C while it could grow within the temperature range within 30°C. There was no growth at 8°, 10°, 40°C. He reported maximum sporulation at 25°C and the fungus did't sporulate at 8°, 10° & 40°C.

Singh et al. (1988) studied the effect of atmospheric temperature on susceptibility and resistance of different cultivars to red rot of sugarcane caused by C. falcatum (G. tucumanensis). They had found that, under lower temperature condition (5.1° to 18.7°C), Susceptible cultivars behaved as intermediate (moderately susceptible or resistant), and intermediate ones as resistant under warm conditions (16.5° to 35°C) favourable to disease development as in month of September. The cultivars showed their reaction to the pathogen within 15 days of inoculation. The varieties tested showed variable reactions to different four races of the pathosen as regards their susceptibility and resistance. However, no appreciable differences in rating of cultivars against a particular isolate was observed in the months of September, November and February because of the consistency in the grades of two important parameters, i.e., lesion width and white spot.

Beniwal & Satyavir (1991) observed that sugarcane red rot incidence was decreased with a decrease in average temperature from 31 to 21°C following plug inoculation method. The decrese was more pronounced in clones, CO 3802, S 77/467, CO 1158, COJ 64, compared with resistant clone CO 7314 and highly suscepatible clones, COL 29 &

S 78/560. Development of red rot was optimum at 29.4° to 31°C during the month of August and first week of September under Hissar condition.

2.3.3 Effect of soil moisture on disease development:

Narendra Singh & Lal (1987) presented that water plays an important role in the dissemination of inoculum and predisposed the host to penetration by the pathogen. They have studied on the susceptible sugarcane cultivar COLK 7701 by placing the stalks in glass jars containing soil, water and inoculum of C. falcatum (G. tucumanensis) which showed that the pathogen remained viable in stagnant water upto 60 days & for 7-8 months in infected tissue on the surface of damp soil.

During investigation of water logging condition on the pathogenicity of C. falcatam to sugucare, Dattamajumdar et al.(1990) found that conidial fusion occured under partially submerged condition. After fusion, the resultant conidium had the potential of high virulence to resistant sugarcane genotypes.

2.3.4 Effect of pH on in vitro growth and sporulation:

Ahmed and Divinagracia (1974) found pH range of 5.5 to 6.5 to be optimum for in vitro growth and sporulation of C. falcatum.

Rajak (1981) reported the racial variation in pH requirements of the pathogen. The isolate with which he was studying could tolerate a wide range of pH from 3.0 to 10.0. However, he found that

the isolate could attain best growth at 6.5, a medium very near to neutrality. He recorded good to excellent sporulation between 5.0 to 8.0 while the fungus did not sporulate at pH 3.0.

2.3.5 Survival of Pathogen in Soil:

Dastur (1946) reported the survival of C. falcatum on crop debries incorporated in Soil upto 6 months in fallow land, while chona and Naraini (1952) found that in natural soil or in manured soil, the fungus survived for 3-4 months only.

Singh et al.(1977) noticed that C. falcatum survived in soil for 63 days in winter and for 34 days in autumn and suggested that the resting structure of the pathogen (viz.appressoria, Chlamydospores, thick walled hyphae and setae) play vital role in its survival.

Wariach (1985) found that infected sugarcane debries put in Soil had no effect on set germination, but in late planting (Oct.-Nov.), the germination was reduced. They had reported that the pathogen survived in soil for 90 days in winter and 60 days in summer under Punjab condition.

2.3.6 Other Physiological aspects:

Several workers have worked on correlation of Phenolics and degree of resistance of varieties of sugarcane to red rot pathogen. Singh et al (1976) found variations in the degree of resistance found in different cultivars and isolates of the pathogen in relation to level of total Phenols. However, they did't found any correlation between

degree of resistance and total phenols found in healthy or inoculated stalk of varieties CO 312, CO 997, CO 1148, CO 62399 and COL 9.

Singh and Wariach (1977) reported increase in total soluble salt, titrable acidity, gum content and reducing sugar in the Juice of canes inoculated with C. falcatum before 4 months of study compared to juice of healthy canes. But there was a decrease in pH, soucrose, and purity coefficient. Varieties, CO 312, & COL 29 were susceptible while CO 7101, COJ 64 & COJ 67 were moderately resistant based on linear spread of infection & metabolic changes.

Carvalho et al. (1979) got variants in culture induced by saltation which occured when specific vitamins and amino acids were added to a minimal medium. They have studied the morphological differences in these sub-races as well as reported variations in the utilisation of different sources of carbon among the variants. They also observed differences in their pathogenicity by inoculating these variants separately on three different cultivars.

Olufolaji and Bamgboye (1986) reported the production of a toxin from physalospora tucumanensis which could induce red rot symptoms on sugarcane, similar to those caused by the fungus. They reported the toxin as an anthraquinone, soluble in water and in most organic solvents. This toxin was yellow with RF value of 0.78 in thin layer chromatography. The minimum amount of toxin needed to induce symptom was 5 microgram and the minimum time required was 24 hours.

Biochemical changes with respect to total carbohydrates and DNA, RNA, were recorded by Agnihotri et al. (1989) in 8 genotypes infected by C.

falcatum. The total carbohydrate decreased in all the cultivars tested by them with increasing RNA where the amount of DNA did't change appreciably.

Verma et al. (1991) reported marked decrease in total chlorophyll and carotenoids after 15 days of inoculation in varieties COS 89266, COS 767 & VP 12, while there was slight increase in BO 91 cultivar. Nitrate reductase activity and total organic nitrogen were decreased in COS 89226, UP 12, BO 91 but remained unchanged in cultivars like UP 12, COS 767 & BO 91, and decreased in COS 89226. They concluded that metabolites and photosynthetic pigments were influenced during early pathogenesis of infected sugarcane leaves.

Brinker and Seiglar (1991) reported the presence of phytoalexin (piceatannol) from infected sugarcane by C. falcatum which was not found either in healthy or wounded sugarcane. This compound inhibited both spore germination and germtube growth in vitro.

Madan et al (1991) found high activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonialyase (TAL) associated with resistant of two resistant cultivars tested against red rot, while low activity of each enzyme was noticed in susceptible cultivars.

2.4 VARIETAL RESISTANCE:

Sarwar and Khoker (1988) suggested that inoculation of C. falcatum spore suspension into sugarcane setts by a hypodermic syringe was the most effective method for artificially developing the disease. The fungus multiplied in streaks in the vascular tissues of the stem and highest concentration of red spot occured near the nodal end.

Ramji et al. (1989) studied the response of 8 sugarcane cultivars to 5 different isolates of C. falcatum (G. tucumanensis). The setts were inoculated using plug, nodal and whorl methods of inoculation. They had reported varied responses of isolates with different cultivars. They found that Baragua (Saccharum officinarum) and Khakai (Saccharum sinense) were resistant to moderately resistant, while CO 997, CO 312, were suscepatible or highly susceptible to all 5 isolates tested. They have concluded that, different isolates of red rot pathogen pressent in different parts of India extensively differed in their pathogenicity. They suggested that screening, would be only effective, if the varieties would be tested with large number of isolates for their resistance and susceptibility.

Beniwal et al. (1989) studied the reaction of 10 isolates collected from different varieties & areas of Haryana on sugarcane clones CO 7717, CO 1148 by using plug, nodal, nodal injury and whorl inoculation method. They have differentiated 10 isolates into three pathotypes - 1(RR_1, RR_9, RR_{10}), pathotype-2 (RR_2, RR_3, RR_5, RR_7, RR_8) & pathotype-3 (RR_4 & RR_6). pathotype-2 was reported to show a resistant reaction on CO 7717, Pathotype-3 showed resistant reaction on CO 1148, and Pathotype-1 showed resistant reaction on both.

Kalaimani & Muthuswamy (1989) tested 4 isolates of C. falcatum on their degree of Pathogenicity with different methods of inoculation. When they inoculated the cultivars i.e. COC 671 & COC 8001 by plug method, the cultivar COC 671 showed resistance while COC 8001 was moderately resistant to isolates I, II, III of C. falcatum (G. tucumanensis), but they were susceptible and highly susceptible respectively to isolate IV. They also found that in nodal inoculation, COC 671 was resistant and COC 8001 was

moderately resistant to isolate I,II,III and moderately susceptible to isolate IV. They also found COC 8001 to be affected severly by isolate IV but only moderately affected by the other three isolates when inoculated in the whorl. Finally they concluded that isolate IV was more virulent than the other 3 isolates, generally causing more disease in the test cultivar by all three inoculation techniques.

Beniwal et al. (1990) could find significant development of disease in late planted canes than canes planted in usual time when inoculated with nodal method in varieties, COS 802, CO 1158 and COJ 64. However, there was no significant differences in disease development between late planting and normal planting when the canes were inoculated with plug method.

2.5 BIOLOGICAL CONTROL:

Narendra Singh (1983) studied the effect of different rhizosphere soils collected from the root regions of 8 important sugarcane cultivars on the lysis of in vitro mycelial growth of C. falcatum. Lysis occured in rhizosphere soil collected from CO 1148 and CO 1158 and lysis was maximum in soil collected from C 38 fields. He had also studied the antagonistic effect of 3 isolates of Trichoderma and 2 isolates of Aspergillus and found them to be highly antagonistic to 11 isolates of C. falcatum tested by him.

The antagonistic effect of streptomyces rochei on C. falcatum and Colletotrichum gleosporoides was proved by Sharma and Gupta (1982). Sharma and Sinha (1982) found reduction in the incidence of red rot lesions on stalks inoculated simultaneously with streptomyces dayalbachenis and C. falcatum or a week later with the antagonistics. However, the development of the disease was reduced maximum (average length of

lesions) when the antagonistic was applied a week before the pathogen.

The antagonistic activity of Trichoderma sps. has been domonstrated by several workers (Morshed 1985, on C lindemuthiarum; Albonso and cruez, 1987 on C. falcatum; Sesan, 1988 on C lindemuthianum; Kanapathipillai et al. 1988 on C masarum).

Albonso and cruez (1987) further reported the inhibition of spore germination of C. falcatum in culture filtrates of Trichoderma.

2.6 EFFECT OF PLANT PRODUCTS:

In vitro evaluation of oils of Tagetis Patula, sunflower, clove, cardamon, and eucalyptus was found to significantly reduce the in vitro growth of C. falcatum alongwith Helminthosporium sativum and Fusarium oxysporum as reported by Singh (1976).

Singh et al. (1977) reported the inhibition of in vitro growth of C falcatum, H Sativum, and F oxysporum by aqueous extracts Araucaria bidwillii and Lindsaya sp. (Ferns).

Kishore et al. (1982) found the leaf extracts of Allmanda carthartica & Artabotrys hexapetal , Polyalthia longifolia to be highly effective in completely inhibiting the in vitro growth of C. falcatum.

Rao et al. (1992) evaluated the effect of essential oil extract from seeds of Cuminum cyminum, dry flower bud of cloves (Syzigium aromaticum) against in vitro growth of C. falcatum. The in vitro growth of C. falcatum was significantly reduced at 1000-3000 ppm of the three oils. These were fungistatic at 1000 ppm and fungicidal at 2000 ppm and 3000 ppm. The

fungitoxicity of the oil remained unchanged even after autoclaving and on storage upto six months at room temparature. These oils were more effective than some commonly used fungicides including carbendazim, mancozeb, tiophenate-methyl, & copper oxychloride against in vitro growth of C. falcatum, Curvularia pallscens, & Periconia atropurpurea.

The aldehyde fraction of C. cinimon oil and Phenolic fraction of S. aromaticum oil contained the main fungitoxic constituent. The other effective oils were seed oil of Foneculum vulgare & Eupatorium capillifolium leaf oil at 3000 ppm and 2000 ppm respectively could completely inhibit the mycelial growth of G. tucumanensis. Leaf extract of Ocicum basallicum at 3000 ppm could also check the growth of above fungi.

2.7 FUNGICIDES:

S.L. Sharma et al. (1957) reported that perenox could effectively control the secondary spread of red rot.

Kar and Verma (1963) reported that reduction in mid rib lesion by spraying Blitox and Dithane Z-78 (100-200 ppm).

Anzalone (1970) reported Benomyl to be one of the best seed treating chemical for control of red rot of sugarcane.

T.N. Srivastava et al. (1972) demonstrated that diphenyltin dichloride was the most active agent among the diaryltin dichlorides against C. falcatum.

Chand et al. (1974) recommended that preplant treatment of sugarcane setts with benomyl (0.25%) & vitavax (0.05%) increased germination by 50% & controlled C. falcatum infection.

Pan and Sen (1976) found tridemorph to be effective against leaf infection as well as soil and seed borne C. falcatum.

Lewin et al. (1976) reported that a good control of the disease even with lower dose (0.25%) and shorter treatment duration (dip & out) with Bavistin.

O.K. Sinha et al. (1979) explained spray of Blitox-50 (0.3%) and Carbendazim (0.1%) on the standing crop proved ineffective in checking secondary spread.

Waraich (1983) reported 0.5% Bavistin (carbendazim) as effective in control of seed material naturally infected with C. falcatum (G. tucumanensis).

S.K. Khirbat et al. (1984) reported that out of 14 fungicides, benzimidazoles performed best but Plantvax (oxycarboxin) & Hoe 6053 (pyracarbolid) also effectively inhibited spore germination of C. falcatum (G. tucumanensis).

However, Padmanabhan et al. (1990) found that none of the chemical dips or foliar spray of carbendazim or copper oxychloride) was effective in controlling red rot (G. tucumanensis) in the field.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation deals with studies on various aspects of Red rot of sugarcane caused by Colletotrichum falcatum went. The various "Materials and Methods" followed during the investigation have been described below and the references of methods used by earlier workers and followed here have also been mentioned wherever felt necessary.

3.1 COLLECTION OF DISEASED SPECIMENS:

The work was started during the month of August, 1993 and disease samples were collected from various locations of district of Jagatsinghpur such as Basantpur, Sanalahanka, Adhanga, Praharajpur & Samantapur of Biridi block. Samples of infected leaves showing mid-rib symptoms and infected rinds showing external reddening & internal tissue infection were carefully collected and kept separately in clean paper packets and labelled properly with respect to various field symptoms, date of collection, field condition, name of varieties from which the specimens were collected and intercultural operations from land preparation, till collection of samples.

3.2 MICROSCOPIC EXAMINATION:

The samples from different locations were separately examined for tentative identification of causal organism. For this, temporary slides were prepared with lactophenol cotton blue from teasing materials of infected areas as well as sections of diseased midribs. The respective slides were examined under microscope. Various microscopic morphological characters of vegetative & reproductive structures were noted along with measurement of such structures using ocular micrometer previously calibrated with

stage micrometer.

3.3 ISOLATION OF CAUSAL ORGANISM INTO PURECULTURE:

For isolation of causal organism, Potato dextrose agar medium (PDA) was used. Tissue isolation method and pour plate/dilution plate technique were also used. The pure culture obtained were further purified by using single spore isolation method (Riker & Riker, 1936). The pure culture thus obtained was examined under the microscope inorder to match it with the characters of the pathogen examined from diseased samples.

3.4 PROVING OF PATHOGENICITY:

The pathogenicity test was done in vitro conditions following methods used by Khirbat et al., 1980; Sarwar & Khoker, 1988; Ramji et al., 1989. For in vitro studies, Uniform sized healthy setts of varieties CO. 6304 and CO 85061 showing maximum field susceptibility were selected for this purpose. Such setts were surface sterilised with 1:1000 $Hgcl_2$ solution by suspending the setts in the said solution for 2 minutes and then ringed in sterile distilled water several times and air dried.

The above setts were grouped into three lots with three setts in each lot which were kept separately in moist polythene chambers where sufficient sterile water was sprayed several times by a hypodermic syringe as well as sufficiently soaked Cotton squabs were put in each polythene chamber to keep it humid during the entire period of study. The setts in different lots were inoculated separately by cork borer method, hypodermic syringe method and split method.

For cork borer method, equal sized tissue of individual cane sett was removed by a sterilised cork borer. Equal sized fungal disc of a 8 day old pureculture was suspended into the hole made in the setts & then it was plugged by the same removed tissue by help of cork borer. All the three setts of the lot were inoculated in the same manner. Control without inoculation was kept separately.

The other lot of three setts were inoculated by spore suspension (2×10^4 conidia/ml) prepared from a sporulating 8 day old culture of the test fungus by means of a sterilized hypodermic syringe in which 2 cc of spore suspension was injected near the eye bud of individual setts. Control was kept separately by injecting sterile distilled water only.

The third lot of cane setts were inoculated by mycelial discs of same size put & smeared in the internal rind tissues, by splitting open the canes & then a thin layer of sterile wet cotton squab was rapped on the inoculated tissue & the splitted inoculated canes were tightly tagged.

Observations on the incidence of the disease resulting from different methods of inoculation were taken after 10 days of inoculation.

3 .5 SCREENING FOR VARIETAL RESISTANCE:

A Preliminary screening of different varieties commonly grown by farmers of Orissa was carried out under in vitro condition following procedures described by (Khibat et al., 1980). For this healthy sugarcane setts of varieties CO 62175, CO 6304, CO 7219, CO 8021 and V 186 were selected for the purpose. Inoculation of setts was done by all three methods followed for testing pathogenicity which have been described earlier.

Observations on the degree of incidence of the disease (extent of reddening of rind tissue & its spread, type of growth of fungal fructification and extent of rotting of rind tissues) weretaken after 10 days of inoculation. Triplicates were used for each treatment for each method of inoculation) and for each variety tested. Control for each such treatment was left without inoculating the setts and providing all other conditions mentioned for inoculated lots.

For scoring the disease rating in different varieties inoculated in the above mentioned three methods, various criteria used by Srinivasan & Bhat (1961) & Khirbat et al. (1980) were taken into consideration. For each criterion, numerical rating are given in the following & the disease index was calculated by adding all numerical values of three inoculated canes from each variety per treatment and finally the average disease index was worked out.

Rating:

'O' = No external symptom, internal reddening very sparse, restricted at the point of inoculation, no fungal fructification present.

'1' = Reddening at the point of inoculation extending upto 1/3 rd or less width of the cane, very sparse whitish grey fungal fructification restricted only at the point of inoculation.

'2' = Reddening extending longitudinally in both sides of the point of inoculation covering 2/3rd of the cane width, whitish grey fungal fructification restricted to the pith area.

"3" = External as well as internal reddening seen extending through out the length of the cane, rotting of internodal tissue, 3/4th of the cane width was affected, with greyish black mycelial growth covering the entire internal rind tissue amongst compact greyish to black mycelial growth.

The disease index ratings were classified as,

Resistant	=	(0 - 1.0)
Intermediate	=	(1.1 - 2.0)
Susceptible	=	(2.1 - 3.0)

3.6 GROWTH AND SPORULATION IN DIFFERENT MEDIA:

In order to select the best natural and synthetic media supporting maximum growth & sporulation of the causal organism, seven different commonly used culture media such as Potato dextrose agar, Host extract agar, Oat meal agar, (natural media) Sabauraud's medium (Semi-synthetic), Czapek's medium and Richard's medium (synthetic) were selected.

(a) Constituent of different media,

(i) Czapek's Sucrose agar medium

Sodium nitrate	-	2gm.
Potassium dibasic phosphate	-	1gm.
Potassium chloride	-	0.5gm.
Magnesium sulphate	-	0.5gm.
Ferrous sulphate	-	0.01gm.
Sucrose	-	30gm.
Agar	-	20gm.
Distilled water	-	1 liter

(ii) Richard's agar medium

Potassium nitrate	-	10gm.
Potassium monobasic phosphate	-	5gm.
Megnesium sulphate	-	2.5gm.
Ferric chloride	-	0.02gm.
Sucrose	-	50gm.
Agar	-	20gm.
Distilled water	-	1 litre

(iii) Sabauraud's agar medium

Peptone	-	10gm.
Glucose	-	40gm.
Agar	-	15gm.
Distilled water	-	1 litre

(iv) Potato Dextrose agar medium

Peeled potato pieces	-	200gm.
Dextrose	-	20gm.
Agar	-	20gm.
Distilled water	-	1 litre

(v) Oat meal agar medium

Oat meal	-	100gm.
Agar	-	20gm.
Distilled water	-	1 litre

(vi) Host leaf extract agar medium

Host leaf (green)	-	250gm.
Agar	-	20gm.
Distilled water	-	1 litre

(vii) Host juice (cane) agar medium

Juice (freshly crushed)	-	1 litre
Agar	-	20gm.

The procedures and the ingredients used for preparation of different media were followed as for the discription of Riker & Riker (1936).

Glass wares used for preparation of media were thoroughly cleaned by potassium dichromate solution (60gm. $K_2Cr_2O_7$ dissolved in 1000 mlof distilled water to which 60ml concentric H_2SO_4 was added slowly), ringed several times in water and then used. All the media were kept separately in 250 ml capacity conical flasks, plugged with non-absorbant cotton plugs and sterilized in 15 lb pressure for 20 minutes except for media containing sucrose which were sterilised at 10 lb pressure for 20 minutes. Then 15 ml of each medium was separately poured and spread uniformly in sterilized petriplates under aseptic condition and then allowed to settle down. Each petriplate was inoculated at the centre of the medium with equal sized fungal disc taken from a 7 day old culture.

Observation on the rate of growth was taken after 3 days of inoculation at 2 days intervals by measuring the diameter of linear colony. The observation was continued till the linear growth of the fungus in any of the media taken was covering the entire petriplate. The rate of suporulation was calculated from a 10 day old culture. The spore suspension was prepared from different media by suspending 5 numbers of equal sized fungal discs from different media and suspending them separately in 15 ml of sterile water. The number of spores present in each microscopic field were counted in each case. Average of five microscopic fields in each case was taken to represent the rate of sporulation

(-)	=	no sporulation
+	=	for 10 numbers of spores

++	=	(11 - 20) spores
+++	=	(21 - 30) spores
++++	=	(31 - 40) spores
+++++	=	41 & above spores

3.7 EFFECT OF TEMPERATURE ON GROWTH & SPORULATION:

Inorder to determine the optimum temperature for growth & sporulation Czapek's medium was selected as the basal medium as it had supported maximum growth of the fungus out of two synthetic media tried. Each sterilized petriplate of equal size was filled up with 15cc of sterilised basal medium under aseptic condition and inoculated with equal sized fungal discs, only after the medium was solidified in the petriplates. All these were done using a "Laminar flow". The inoculated petriplates were incubated in different temperatures viz. 10°, 15°, 20°, 25°, 30° & 35°C. Quadriplicates were maintained for each treatment. The observation on the rate of linear growth of the fungus in different treatments were taken at 2 days interval upto 10 days of inoculation. The rate of sporulation was counted only after 10 days of the inoculation.

3.8 EFFECT OF pH ON GROWTH & SPORULATION:

The basal liquid medium was adjusted to different pH i.e.,3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, by using Buckman's pH meter. Each of the 100ml of the adjusted medium was poured into 250ml capacity conical flask separately. The conical flasks were plugged tightly by nonabsorbant cotton & sterilised at 15 lb pressure for 20 minutes. The flasks along with media were inoculated by putting fungal discs (4 numbers) in each conical flasks. Triplicates were used for each & the inoculated flasks were incubated at room temperature of ±28°C. The

mycelial mat obtain after 15 days of incubation were harvested on Wattman (No.1) filter paper after filtering in each case. The dry weight of the mycelial mat was taken after oven drying at 60°C for 1 hour.

3 .9 STUDIES ON GERMINATION OF SPORES:

Cavity slide method (Shervelle , 1961) was adopted for the purpose. Spore suspensions were made with spores obtained from a 10(ten) day old culture grown on PDA slants in sterile distilled water. A drop of spore suspension was examined under microscope and found to contain 30-40 spore per microscopic field. A drop of spore suspension was taken in each sterile cavity slide and replicated for 3 times. Cavity slides were kept inside sterilised petriplates which were kept moist by wet blotting papers to keep the slides in humid conditions. The petriplates were kept at room temperature 28 - 30°C.

This methods was also adopted for studying the effect of different factors on spore germination.

3.10 BIOLOGICAL CONTROL:

Here the pathogen (C. falcatum) and candidate microflora (Trichoderma viride) were taken and the test was done by dual culture methods & spore germination method.

3 .10.1 Dual culture method:

This method was undertaken as per the procedure adopted by Bineeta Sen (1992).

(a) Bit inoulation method:

This method was earlier described by Wood (1951).

Fungal discs(5mm) of the pathogen were placed at 4 equidistant points near the periphery of the culture plates and the same sized disc of antagonist (Trichoderma viride)was inoculated at the centre. A reverse method was also adopted by taking pathogen at the centre and the antagonist at 4 equidistant point near the periphery. Control was taken without antagonist separately.

Observation on the per cent inhibition of test fungus by candidate microflora (T. viride) was taken after 7 days of inoculation. The per cent inhibitation was calculated by the formula following Mathur (1987).

(i) $$\frac{dc - dt\ (T_1+T_2+T_3+T_4)/4}{dc} \times 100$$

Where dc = Colony diameter of test fungus in control.

dt = average colony diameter of test fungus in treatments (T_1, T_2, T_3, T_4).

(ii) $$\frac{dc - dt}{dc} \times 100$$

Where dc = colony diameter of test fungus in control.

dt = colony diameter of test fungus in treatment.

(b) Streak method:

This method was adopted by following the description given by Buxton & Richards, 1955 & Bineeta Sen, 1992. Spore suspension of a 7 day old culture of C. falcatum (test fungus) was inoculated by making a streak by means of an sterilized inoculated needle on one side of the petriplate containing potato dextrose agar. Similarly spore suspension from a 7 day old culture of candidate microflora (T. viride) was inoculated similarly

at the other end of the petriplate parallel to the test fungus at 40 mm distance. Triplicates were used and control without candidate microflora and inoculated with test fungus only in two parallel streaks each at 40 mm distance were kept to serve as control. All the inoculated petriplates were incubated at room temperature of ± 25°C.

Observation on the percent inhibition of test fungus inoculated along with candidate microflora compared to growth in control was calculated after 7 days of incubation.

Calculation: $\frac{wc - wt}{wc} \times 100$

Where, wc = width of the growth of test fungus* in control.

wt = width of the growth of test fungus* in treatment.

* The width of the fungal growth for each replication is the average of 3 readings of different length of the colony.

3.10.2 Spore germination method:

(i) Preparation of sterile culture filtrates of antagonists:

For preparation of culture filtrates of antagonists Czapek's liquid medium was used, which was prepared in the previously described manner. Clean conical flasks of 250 ml capacity were taken & 50ml of the medium was poured into each flask. After plugging by non-absorbant cotton, these flasks were sterilised at 15 lbs pressure/sq.inch for 20 minutes. Each flask containing the sterilised medium was aseptically inoculated with an agar disc of each antagonist, from a 7 day old petridish culture. The mouth of each flask was replugged immediately after inoculation. The flasks were incubated at 28± 1°C for 11 days. On 12th day, medium of each flask along with mycelial mat was twice filtered through sterile wattman No.1 filter paper in sterile flask to get cell-free culture filtrate.

(ii) Effect of culture filtrate of Trichoderma in spore germination of test fungi:

For this experiment spore suspension of test fungus was prepared. A 5mm diameter agar disc of mycelial mat, from a 10day old petridish culture of the test fungus was put in a sterile culture tube containing 1 ml sterile Czapek's liquid medium and shaked well. From the spore suspension, one drop was put on the surface of a clean glass slide. The number of spores was counted & standardized to 40-50 spores per microscopic field by adding more Czapek's liquid medium or one more agar disc from the test fungus culture, as the case may be. Finally one drop of standardized spore suspension was put on a microscopic slide to which one drop of antagonist's culture filtrate was added and gently mixed. For control, one drop of sterile water was mixed with one drop of standardised spore suspension.

3.11 EFFICACY OF DIFFERENT PLANT EXTRACT ON GROWTH & SPORULATION OF THE FUNGUS:

Plant products of five different plants such as Neem (Azadirachta indica) Turmeric (Curcuma longa), Ginger (Zingiber officinale), Garlic (Allium sativum) Sunflower (Helianthus annus) were taken for this purpose. In case of Neem, three types of products such as leaf extracts, seed extracts, and oil were used at concentration of 2%, 4%, 6%, 8% & 10% for each such product. The extracts of rhizomes and bulbs were taken in case of turmeric, ginger and garlic respectively. Sunflower oil was taken at above concentrations.

The extraction of different concentrations were prepared in sterilised distilled water (W/V) following the method described by Singh et al. 1980 & Thakur & Khune, 1991. All these extracts except oils were first filtered through sterilised muslin cloth, and then refiltered through double layered Wattman (No.1) filtered paper by using a power operated suction filter pump. The basal medium was poisoned with different plant products so as to maintain different concentrations mentioned above & Then they were sterilised by autoclave at 10 lb pressure for 30 minutes. Equal amount of prepared poisoned basal media at different concentrations were poured in each sterilized petriplate separately which were inoculated with equal sized fungal disc of a 7 days old culture of test fungus under aseptic condition. Control with only unpoisoned basal medium was maintained separately for each treatment. Observations on the rate of inhibition of linear growth were taken after 7 days of inoculation.

3.12 EVALUATION OF EFFICACY OF FUNGICIDES ON GROWTH OF THE FUNGUS:

Poison food technique followed by Bagchi and Das (1968) was followed here for evaluating the inhibition of linear growth of the fungus in different concentration of seven fungicides i.e , Kava ch, Bavistin, Indofil.M-45, Ridomil, Sutox, Plantvax, Topsin-M,. Then the sterilised basal medium was poisoned by each fungicide separately at concentration of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm,& 2500 ppm, & 15ml of such poisoned medium was poured into each petriplate separately, then allowed to solidfy and inoculated with equal sized fungal discs from a 7 day old culture. Triplicates were used in each case & control was maintained in each case without poisoning the medium with fungicides.

Observations were taken on the linear growth of the fungus of measuring the diameter of the colony after 7 days of incubation. The data obtained were represented in tabular form for analysis of results.

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 FIELD OBSERVATION OF THE DISEASE & COLLECTION OF DISEASE SPECIMEN:

Severe occurence of red rot in varieties viz. CO 6304, V-186 CO 85061 were observed in the farmers field located in different areas of district of Jagatsinghpur of Orissa state, during August to September 1993. The author has visited different locations & it was observed that in some fields with above mentioned varieties, the canes were affected most severely with 50 per cent loss of canes in individual fields particularly in fields where the above varieties were grown. Recent reports on losses in cane yield upto the extent of 40% in different varieties have been reported by several workers (Singh & Singh, 1989; Agnihotri, 1990).

It was further observed that, the most affected fields were water logged with about 6" to 1' standing water & the farmers did not maintain the sanitation in the field & as a result of which affected fields had maximum population of weeds. The disease was said to progress very quickly within 15 days and the farmers were seen to have been engaged in harvest of the immatured canes in order to avoid total loss. Most of the fields had clay loam soil with nearly neutral PH(5.5 - 6.5).

Field observations on different types of symptoms revealed that rind infection was maximum in variety CO 85061 in the field with water logging condition & maximum weed population, where as, the same variety had very less rind infection and maximum midrib and leaf infection were observed in another location where the concerned farmer had taken care of field sanitation and drainage of water.

During this period, there was intermitant heavy showers with rain splashed wind for about 15 days preceeding the incidence and this condition was still continuing during the period of visit. The average day and night temperature during that period was found to be in an around 30°C & 25°C respectively.

It was therefore concluded that, the above field conditions coupled with the above mentioned environmental conditions were responsible for epiphytotic nature of the disease. It was further observed that the varieties CO 7219, CO 8021, CO 62175, were less affected with about 30 per cent plants in the field were affected (Moderately susceptible). However, none of the varieties were found to be either moderately resistant or resistant.

It is therefore concluded that, varietal resistance or susceptability seemed to have been governed on environmental conditions including soil, moisture, pH and atmospheric temperature rather than varietal differences. The possibilities of pathogenic race variations as regards virulence and avirulence nature of the pathogen can not be ruled out which needs further studies. Narendra Singh et al. (1987) studied the effect of soil moisture on incidence of red rot on sugarcane & had similar views. Duttamajumda et al. (1990) pointed out that, even the resistant varieties soccumb to the disease under partially submerged condition & suggested that, water logged condition might affect the biology of red rot pathogen, thereby changing the pathogenic behaviour to a more virulent form. In vitro studies done earlier on pH requirement of substratum for growth of C. falcatum indicated that a pH level from 5.5 - 6.5 was the

optimum range for better growth and survival of the pathogen (Rajak, 1981 & Ahmed & Divinagarcia, 1974).

Atmospheric temparature of 25° - 30°C was most favourable for development & progress of the disease as found during the present field observation is in confirmity with the earlier reports of Singh et al. (1988) who had reported that under low atmospheric temparature (5.1 - 18.7°C), the susceptible cultivars behaved as moderately resistant, while moderately resistant cultivars as resistant under warmed condition (16.5° - 35°C).

4.2 SYMPTOMATOLOGY:

The characterstic field symptoms revealed the appearance of reddish grey lesions at different places of midrib of leaves which sometimes coalesce to cover the entire midrib. The individual spots were blackish-grey at the centre surrounded by redish margin with black pin headed acervuli on the blackish grey central portion. Tiny reddish brown specks were infrequently observed on the upper surface of the lamina, particularly confined to tip or middle portion. The spots were linear & varied from (2-3.5 mm) in length and 0.5 mm in width. Acervuli are produced over the spots and severe lamina infection alongwith infection in the mid rib resulted in drying and drooping of leaves. In some cases discoloration of 3rd & 4th leaves from the tip & margin and finally resulting into withering and drooping of leaves were also observed.

In latter stages the infection spreads to the rind with characterstic external reddening of the rind particularly near the node. As the disease progressed, the affected internodes shrivelled, the rind sank and became longitudinally wrinkled. Such canes were lighter in weight and

broken easily. When such canes were splitted open, then internodal tissues were found to become dull red, interupted by transversed white patches. In advanced stage of rind infection, dirty brown to whitish grey mycelia and frutfication of the fungus were observed on cavities of the pith (Plate I, fig.1). These field observations of the symptom were typically of red rot disease caused by C. falcatum and similar to those described by earlier workers (Kishan Singh et al., 1993).

4.3 MICROSCOPIC EXAMINATION OF THE AFFECTED TISSUE:

The microscopic examination of the midrib lesion and affected rind tissues revealed the presence of several bodies of acervuli in the microscopic field. The acervulus consisted of conidiogenous cells (condidiophores and conidia). The conidiophores were short, hyaline, aseptate and measured 18 µm x 7 µm and conidia were hyaline, single celled, falcate, measured 23 - 35 µm x 4 - 6 µm with a large oil globule at the centre (Plate I, fig. 3 & 5). Setae were absent in the acervulus. From the above characters the causal agent was identified as Colletotrichum falcatum Went; Arch.Java.Suikerindus, 1, 265, 1893 (= Glomerella tucumanensis (speg.) Arx & Muller; Beitrge Zur Kryptogamen flora der Schweiz, 11, 195, 1954. Characterstic morphological features of the pathogen matched well with those earlier described by several authors except for absense of setae in the present isolate and slight variations in the size of conidia. Slight variation in the size of conidia have been reported amongst races of the pathogen by Khirbat et al. (1980).

Absence of setae in the acervulus has often been refered as one of the few characters coined with related genus Gloeosporium. However, the stability of presence or absence of setae in the acervulus of Colletotrichum sps. and in Gloeosporium sps. for delimiting both the taxa is still left as

PLATE I

Fig. 1. Symptom on affected rind showing internal reddening and rotting of tissue with greyish white fructification in the pith.

Fig. 2. A 12 day old culture of C. falcatum showing charecters of dark isolate.

Fig. 3. Photomicrograph of conidia of C. falcatum.

Fig. 4. A 6 day old slant culture showing whitish mycelial growth at the early stage.

Fig. 5. Photomicrograph of conidia of C. falcatum showing oil globule.

controversial. The presence or absence of setae to distinguish these two genera was rejected since the formation of these structures depends upon external conditions and can be controlled by varying the relative humidity alone (Frost, 1964). So all the species which were formerly included in the genus Gloeosporuim have been included in the genus Colletotrichum (Sutton, 1973).

4.4 ISOLATION OF THE PATHOGEN:

The pathogen was isolated into pure culture in potato dextrose agar medium (PDA) and Oat meal agar by tissue isolation method and the pure culture obtained was further purified by single spore isolation. Whitish mycelial growth was found in PDA as well as in Oat meal agar medium after 3 days of isolation. The colour of the colony gradually changed to dull grey and latter to deep grey to dull black in colour (Plate I, fig. 2 & 4). Microscopic examination of the pure culture on morphological characters along with measurement of conidiophores and conidia of the pathogen revealed that, the pure culture was of C. falcatum similar to the morphological characters of the pathogen found from affected tissue.

4.5 PATHOGENICITY:

The pathogenic potential of the fungus (C. falcatum) was successfully proved by different methods of inoculation described in "Materials & Methods".

In case of setts inoculated by hypodermic syringe method, setts did't show any remarkable external symptoms even after 10 days of inoculation except for pressence of little mycelial growth outside the setts at the nodal region i.e. near the point of inoculation. On splitting open the

setts reddening of internal rind tissues near the point of inoculation was observed. Inoculation following plug method revealed the appearance of typical internal rind infection of red rot. Reddening of internal tissue extended longitudinally in both the directions and whitish grey fungal fructification found at the point at inoculation. Inoculated canes with split method, revealed reddening of the internode throughout the length of the cane setts. There was whitish grey mycelial growth and black dot like structures, the acervuli of the fungus were found in the cavity of the pith region. The pathogen was reisolated from the artificially inoculated canes and microscopic examination revealed similar morphological features, typical of C. falcatum. Thus the pathogenicity was successfully proved following Koch's postulate.

The above methods of inoculation was done following methods described by Khirbat et al., 1980; Sarwar & Khokar, 1988; Ramji et al., 1989, who have successfully proved the pathogenicity with similar characterstic development of symptoms.

4.6 GROWTH AND SPORULATION ON DIFFERENT SOLID MEDIA:

To determine the best medium for growth and sporulation of C. falcutum, different solid media were tested as per the procedures mentioned in "Materials & Methods". The data obtained are presented in Table-1.

It may be seen from table 1 that maximum rate of growth as well as sporulation was obtained in host leaf extract agar followed by sugarcane juice agar. The growth rate was same in both the medium upto 3 days after inoculation but after 7 days the grwoth rate was significantly more in host leaf extract agar than juice agar. Other media tried were significantly inferior to host leaf extract as well as to juice agar in respect to rate of

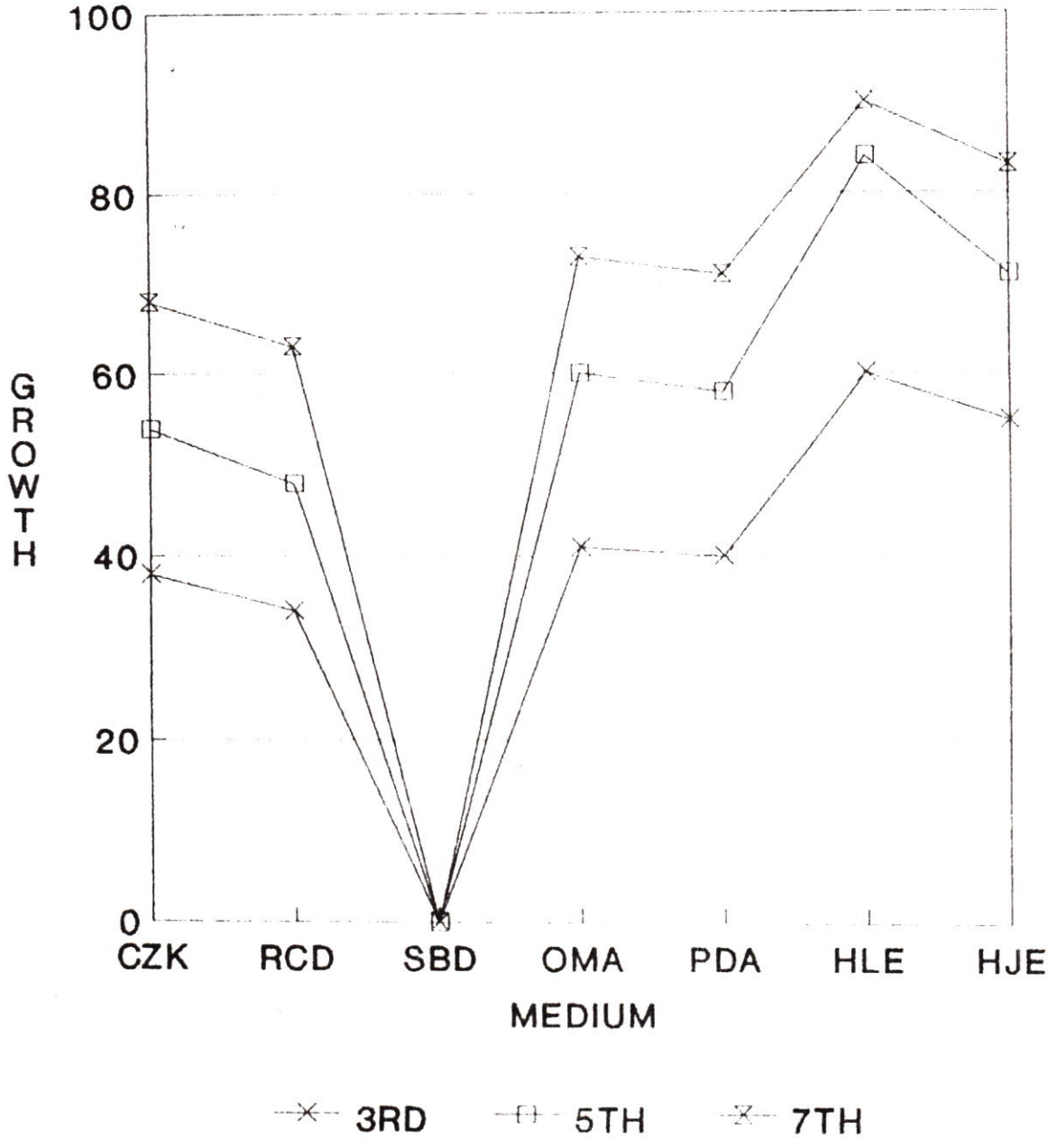
Table 1. Linear growth of C. falcatum on different solid media

Medium	Colony diameter[a] in (mm)			Sporulation
	3rd day	5th day	7th day	
Czapek's sucrose agar medium	38 (6.17)*	54 (7.4)	68 (8.26)	++++
Richard's agar medium	34 (5.87)	48 (6.94)	63 (7.99)	+++
Sabauraud's agar medium	0 (0.71)	0 (0.71)	0 (0.71)	-
Oat meal agar medium	41 (6.49)	60 (7.79)	73 (8.55)	+++++
Patato dextrose agar	40 (6.38)	58 (7.63)	71 (8.45)	+++++
Host Leaf extract agar medium	60 (7.79)	84 (9.20)	90 (9.51)	+++++
Sugarcane juice agar medium	55 (7.42)	71 (8.47)	83 (9.15)	+++++
SEm ±	0.11	0.08	0.12	
CD (at 0.05)	0.34	0.25	0.37	

* Transformed values in parenthesis are values from original value by the formula $\sqrt{x + 0.5}$

a Each reading is an average of 3 replication for each treatment.

LINEAR GROWTH OF C.falcatum ON DIFFERENT MEDIA



CZK - Czapek's **RCD** - Richard's **SBD** - Sabauraud's

OMA - Oat meal agar PDA - Patato dextrose agar

HLE - Host leaf extract HJE - Host juice extract

growth and there was no significant difference in growth rate among rest of media tried except for Sabauraud's medium where the pathogen neither could grow nor could sporulate.

The pathogen could sporulate excellently in host leaf extract agar, juice agar, oat meal agar and potato dextrose agar followed by Czapek's and Richard's agar. The growth rate was at par in Czapek's and Richard's agar but the sporulation was significantly more in Czapek's for which Czapek's medium was selected as the basal medium out of the above two synthetic media tried. Singh and Kamal (1979) have also obtained identical results & reported that growth of C. falcatum was greater in media containing juice of sugarcane.

It may be further seen that media supplemented with monosaccharides (fructose and dextrose) as well as disaccharide in form of sucrose supported good growth and sporulation of C. falcatum except for sabauraud's medium supplemented with glucose where the fungus could not grow nor could sporulate. These findings are similar to the findings of Rajak (1981) regarding maximum utilization of monosaccharides by C. falcatum but absence of growth in sabauraud's agar supplemented with glucose in the present finding is a deviation. This might be due to the fact that, the utilization of different forms of sugar by fungi not only depends on the configuration of specific sugar but also on the differences in the ability of utilizing sugar along with the other specific compounds by different pathogenic races.

The colony characters of the pathogen were similar in all the media tested. Initially the colony was white, gradually changing to light grey after 7 days of growth and finally to dark grey with reddish

orange, shining minute droplets oozing out on the compact valvety mycelial colony towards the middle part of the culture after about 15 days of inoculation.

From the above observations, it may be concluded that this isolate is a dark cultural race of C. falcatum depending on the findings of Abbott, 1933; Rafay and Padmanabhan, 1941 ; Chona and Srivastava 1960; Kishan Singh et al., 1993.

4.7 EFFECT OF TEMPERATURE ON GROWTH & SPORULATION:

The data obtained from observation on the effect of temperature on growth & sporulation have been presented below in Table 2.

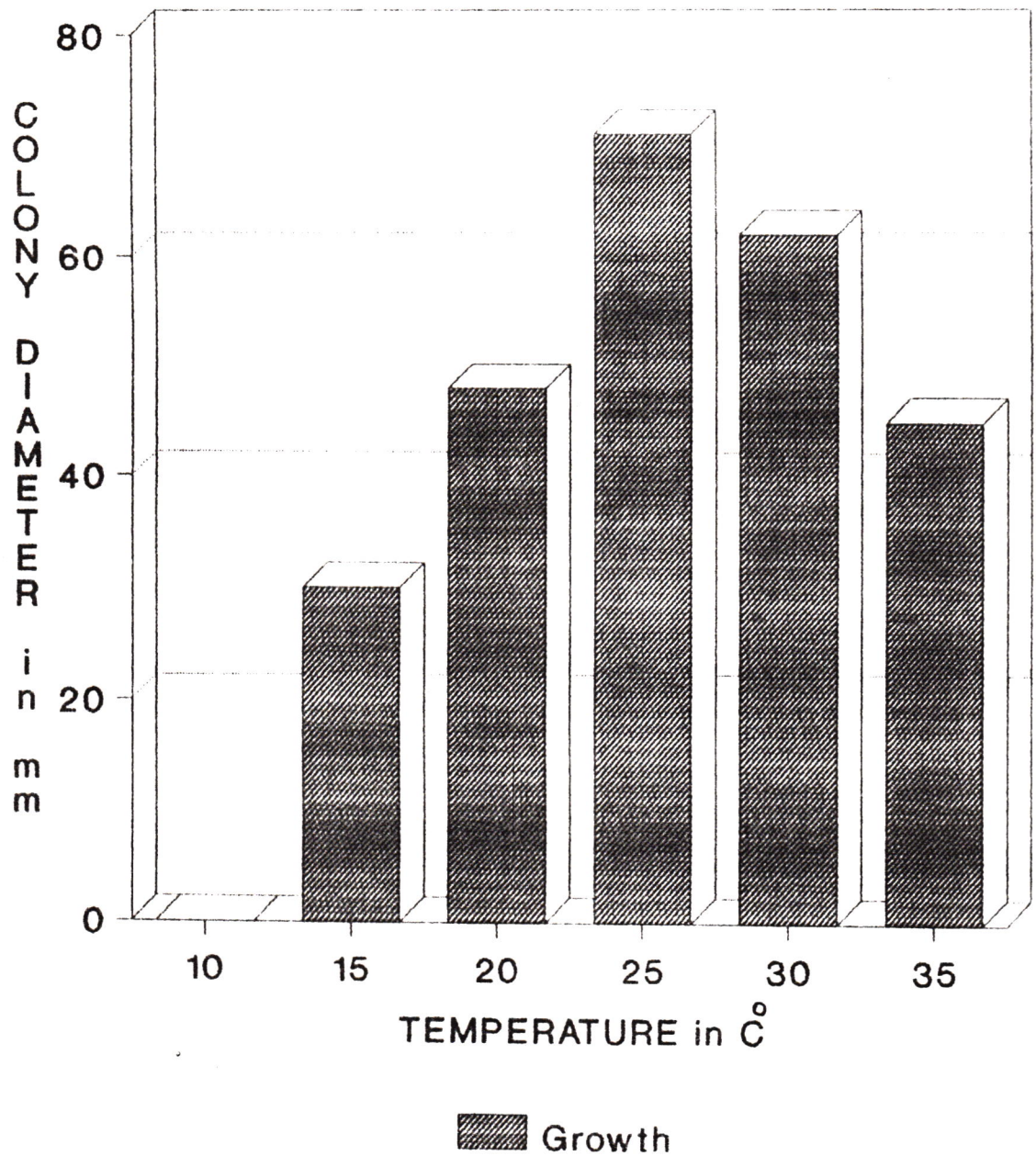
Table 2 Effect of temperature on growth and sporulation of C. falcatum.

Temperature (C°)	Colony diameter[a] in (mm)	Sporulation
10	0 (0.71)*	-
15	30 (5.54)	+
20	48 (6.97)	+++++
25	71 (8.43)	+++++
30	62 (7.90)	+++
35	45 (6.72)	++
SEm ±	0.12	
CD(at 0.05)	0.36	

* Figure in parenthesis is transformed by the formula $\sqrt{x + 0.5}$ where 'x' is the original value.

a Each reading is an average of 4 replication for each treatment.

EFFECT OF TEMPERATURE ON GROWTH OF C.falcatum



In vitro study of rate of growth and sporulation of C. falcatum as influenced by different range of temperatures revealed that the fungus could grow and sporulate within 15° - 35°C and the optimum range of temperature for growth as well as sporulation was 25° - 30°C. The rate of linear growth and sporulation was gradually decreasing when the level of temperature either increased or decreased from 25°C. Optimum temperature for sporulation was 20° - 30°C and it was maximum at 25°C. The pathogen could neither grow nor sporulate at 10°C.

The present finding is identical with the findings of Ahmed and Divinagracia (1974). Various workers (Ghose, 1975,; Rajak, 1981 and Singh et al., 1988) have reported similar of optimum temperature for growth and sporulation of C. falcatum except slight variations probably could be attributed due to racial variations of the pathogen.

4.8 EFFECT OF DIFFERENT PH ON GROWTH AND SPORULATION:

This study was done as for the procedure mentioned under "Materials and Methods". The dry weight of mycelial mat and rate of sporulation from the broth cultures at different pH level were taken after 15 days of inoculation and data obtained were presented in Table 3.

Table 3 Effect of pH on growth & sporulation of C. falcatum

pH	**Weight[a] of mycelial mat in (mg)**	**Sporulation**
3.5	320	+
4.5	420	+++
5.5	520	+++++
6.5	580	+++++
7.5	510	+++++
8.5	410	++++
9.5	330	++
10.5	180	-
SEm ±	10.72	
CD(at 0.05)	33.04	

a Each reading is an average of 3 replication for each treatment.

The tabulated data on growth rate and rate of sporulation revealed that C. falcatum could grow and tolerate a wide range of pH i.e., 3.5 - 9.5 and the optimum pH level for in vitro growth and sporulation was from 5.5 to 7.5. There was maximum growth as well as sporulation at pH 6.5. There was very sparse or no growth and the pathogen did not sporulate at pH 10.5.

The present founding is in confirmity with earlier findings of Ahmed and Divinagracia (1974) and Rajak (1981) who have reported the optimum pH requirement of C. falcatum to be 5.5 - 6.5 and 6.5 respectively for its excellent growth and sporulation.

4.9 SCREENING OF VARIETIES:

This study was undertaken under in vitro condition in order to find out a quick result on respond of some commonly grown varieties of sugarcane in Orissa to the single isolate of C. falcatum isolated from most susceptible location. The result obtained here is of preliminary in nature and can only be confirmed after the study is undertaken under natural conditions in the fields.

Artificial inoculation of canes and rating for disease index were done following the methods used earlier by Khirbat et al. (1980), Sarwar and Khoker (1988); Ramji et al. (1989) and Beniwal et al. (1989). The procedures have been mentioned under"Materials and Methods" and rating of disease development in different varieties was done and the calculated values of disease index after 10 days of inoculation have been presented in Table 4 and depicted in Plate II and fig. 1 to 6.

Table 4 Disease index in different varieties inoculated with 7 day old culture of C. falcatum

Sl.No.	Variety	Disease rating[a] in different methods			Total	Average	Remark*
		Syringe	Plug	Split			
1.	CO 8021	2	6	7	15	1.66	Intermediate
2.	CO 7219	3	6	8	17	1.88	Intermediate
3.	V - 186	2	4	4	10	1.11	Intermediate
4.	CO 62175	3	5	8	16	1.77	Intermediate
5.	CO 6304	1	4	9	14	1.55	Intermediate

a Each reading is an average of 3 replication of each treatment

* Disease index rating

(0 - 1.0) - Resistant

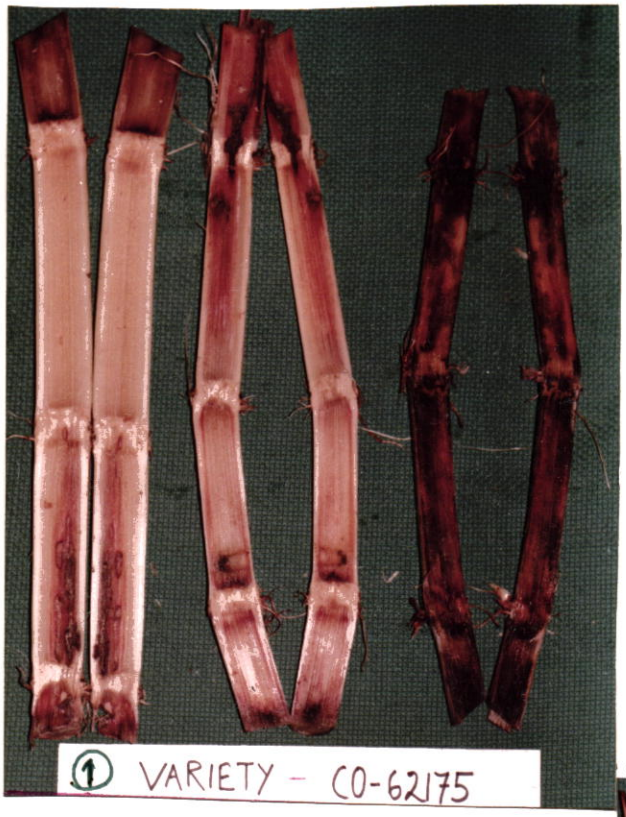
(1.1 - 2.0) - Intermediate

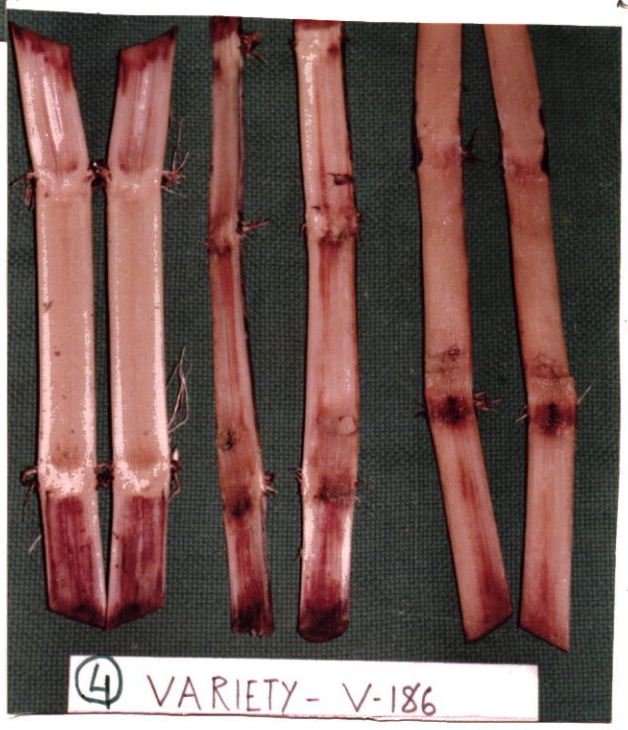
(2.1 - 3.0) - Susceptible

PLATE II

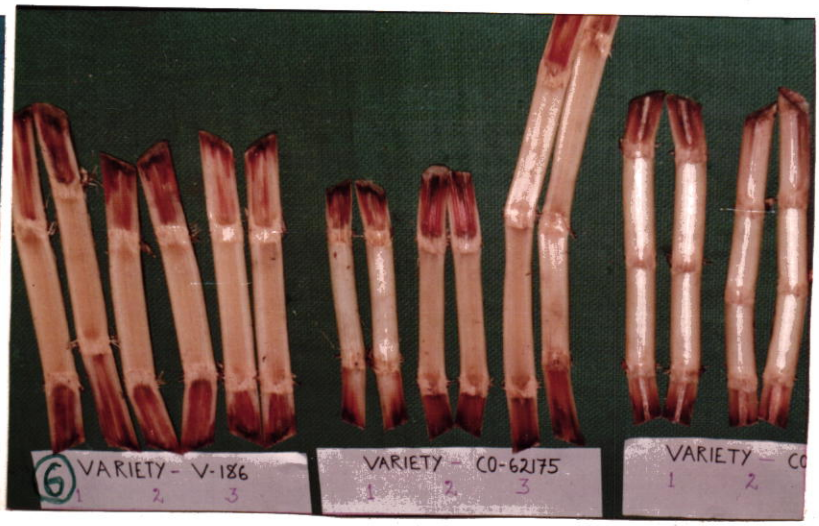
Fig. 1 to 5 Development of the disease in different varieties of canes, artificially inoculated by different methods.

Fig. 6 Control with inoculation.


1 VARIETY - CO-62175


4 VARIETY - V-186


5 VAR.CO-7219


6 VARIETY - V-186
1 2 3
VARIETY - CO-62175
1 2 3
VARIETY - CO
1 2

From the table 4, it may be seen that all the 5 varieties tested against the present isolate of C. falcatum were intermediate in reaction and might be either moderately susceptible (CO 7219, CO 62175, CO 8021 and CO 6304) or moderately resistant (V-186). The resistance and susceptibility of sugarcane cultivars to red rot under in vivo conditions are much influenced by standing water, soil pH, atmospheric temperature and relative humidity and rainfall which have already been discussed earlier (Narendra Singh et al. 1987). Variability among isolates under specific environmental condition also influences upon resistance and susceptibility of a cultivar. Dattamajumdar et al. (1990) had the view that, even the resistant varieties succumb to the disease under partial submerged condition.

The present finding might be helpful in localities from which the present isolate of the pathogen was isolated and where the above varieties are usually grown by the farmers.

4.10 ANTAGONISTIC EFFECT OF Trichoderma viride ON THE TEST FUNGUS:

4.10.1 Dual culture technique:

The antagonistic potential of T_8 isolate of Trichoderma viride was studied by dual culture method as described by Buxton & Richards 1955; Bineeta Sen ,1992 and detail of the procedure has been mentioned in "Materials & Methods". The per cent inhibitions of the test fungus, C. falcatum grown with Trichoderma viride in two different methods of dual culture have been presented in Table 5 and depicted in Plate 3 and fig. 1 & 2.

PLATE III

Fig. 1. Antagonistic effect of Trichoderma viride against C. falcatum in dual culture after 3 days of inoculation.

(i) Test fungus at the centre.

(ii) Candidate microflora at the centre.

(iii) Streaking method.

Fig. 2. Antagonistic effect of T. viride against test fungus after 7 days of inoculation.

PLATE III

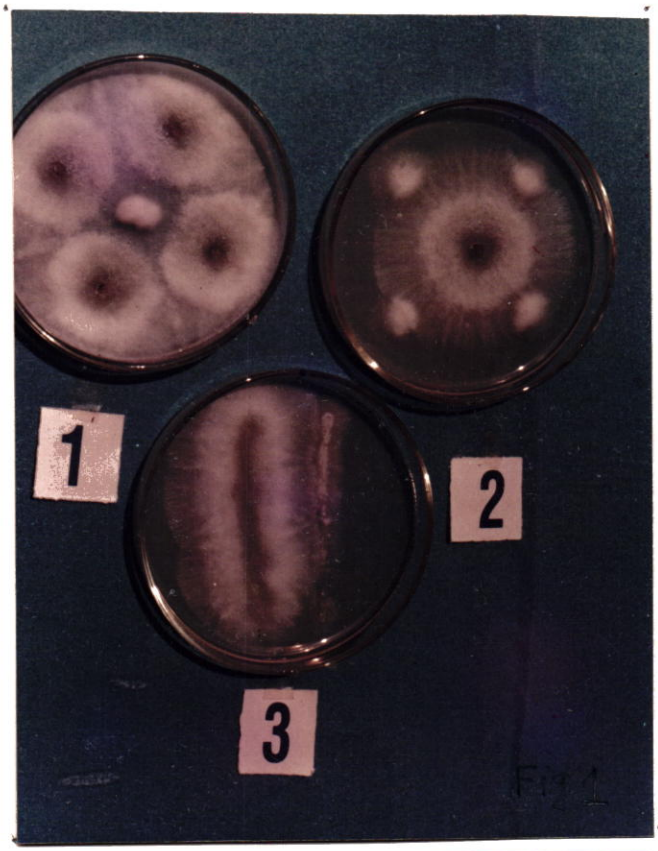


Fig 1

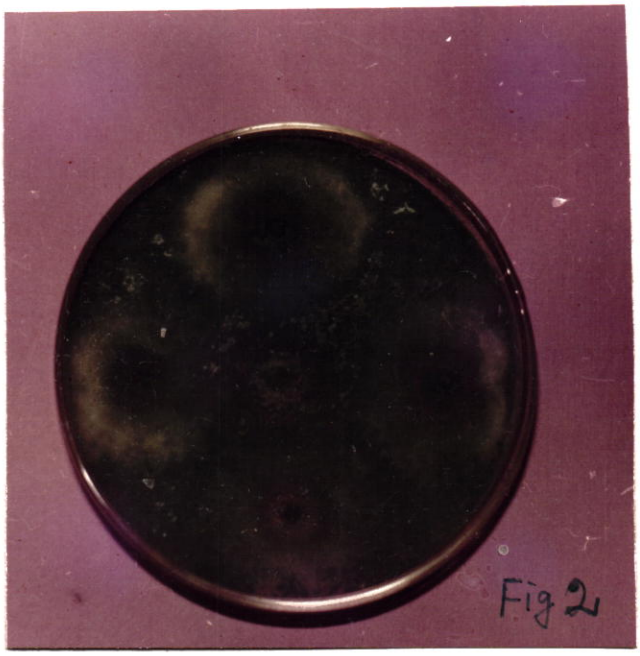


Fig 2

Table 5 Per cent inhibition of *in vitro* growth of *C. falcatum* by *Trichoderma viride* in dual culture

Test fungi	Percentage of surface growth inhibition[a]
C. falcatum	1. 84.28
	2. 80.21
	3. 82.27

a Each reading is an average of 3 replication.

It may be seen from the above table that, the candidate microflora *Trichoderma viride* (T_8) could inhibit the growth of the test fungus (*C. falcatum*) in all the different treatments of dual culture and therefore proved to be antagonistic to the test fungus.

The antagonistic activity of *Trichoderma sps.* on species of *Colletotrichum* has been demonstrated by several workers, viz., Morshed, 1958, on *C. lindemuthianum;* Albonso and Cruez, 1987 on *C. falcatum*; Sesan, 1988 on *C. lindemuthianum* and Kanapathipillai *et al.*, 1988 on *C. musarum.*

4.10.2 *In vitro* inhibition of conidial germination of *C. falcatum* in culture filtrate of T_8 isolate of *T. viride.*

Culture filtrate of *T. viride* was collected from a 11 day old culture and sterile spore suspension of the test fungus was suspended to the filtrate of candidate microflora in cavity slides as for the procedure mentioned in "Materials and Methods". The data on per cent

inhibition of germination of test fungus after 24 hours of incubation has been presented in Table 6.

Table 6 Rate of spore germination C. falcatum in Trichoderma culture filtrate.

Treatments	Percentage of spore germination[a] after 24 hours.
T_8	6
Control	88

a Each reading is an average of 3 replications.

It may be seen from the above table that Trichoderma viride culture filtrate had drastically reduced the germination of conidia compared to strile spore suspension in water. Further it was observed that conidial germination started after 8 hours of incubation insterile water, while it was started only after 16 hours of incubation in the antagonistic culture filtrate.

Similar results have earlier been reported by Albonso and Cruez (1987) for C. falcatum.

4. 11 EFFICACY OF SOME PLANT PRODUCTS ON in vitro GROWTH OF C. falcatum:

This study was undertaken by supplementing different concentrations of plant products separately in the basal medium by poisoned food technique as for the procedures mentioned under "Materials and Methods" following procedures described by Singh et al., 1977; Kishore et al., 1982 and Rao et al., 1992. The observations for

per cent inhibition of radial growth in different treatments were taken after incubating for 9 days and the data obtained were presented in Table 7 and depicted in Plate 4 & fig.1.

Table 7 Percent inhibition[a] of growth of C. falcatum on Czapek's agar medium poisoned with different plant extracts.

Name of the different Plant extracts.	Concentration				
	2%	4%	6%	8%	10%
Neem Leaf	47.94 * (43.80)	49.49 (44.96)	55.55 (48.16)	59.59 (55.50)	64.64 (53.50)
Neem seed	22.22 (28.10)	27.77 (31.70)	31.30 (33.96)	38.38 (37.26)	43.42 (41.23)
Neem oil	70.19 (56.90)	73.22 (58.83)	75.24 (60.10)	79.29 (62.93)	81.30 (64.38)
Sunflower oil	63.63 (52.93)	63.13 (52.60)	66.66 (54.71)	69.69 (56.60)	72.21 (58.20)
Turmeric (rhizome)	77.77 (61.86)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Ginger (rhizome)	27.77 (31.76)	28.78 (32.40)	32.31 (34.90)	34.69 (36.03)	39.89 (39.13)
Garlic (bulb)	30.80 (33.66)	51.51 (45.80)	59.09 (50.30)	68.17 (55.63)	72.72 (58.53)
SEm ±	1.16	0.95	0.89	1.06	0.92
CD(at 0.05)	3.57	2.92	2.74	3.27	2.83

* Values in parenthesis are the angular values.

a Each reading is an average of 3 replication for each treatment after 7 days of inoculation.

The data presented in the above table revealed that out of different products, rhizome extract of turmeric (curcuma longa) was very effective and completely inhibited the in vitro growth of C. falcatum at

PERCENT INHIBITION OF GROWTH OF C.falcatum WITH DIFFERENT PLANT EXTRACTS

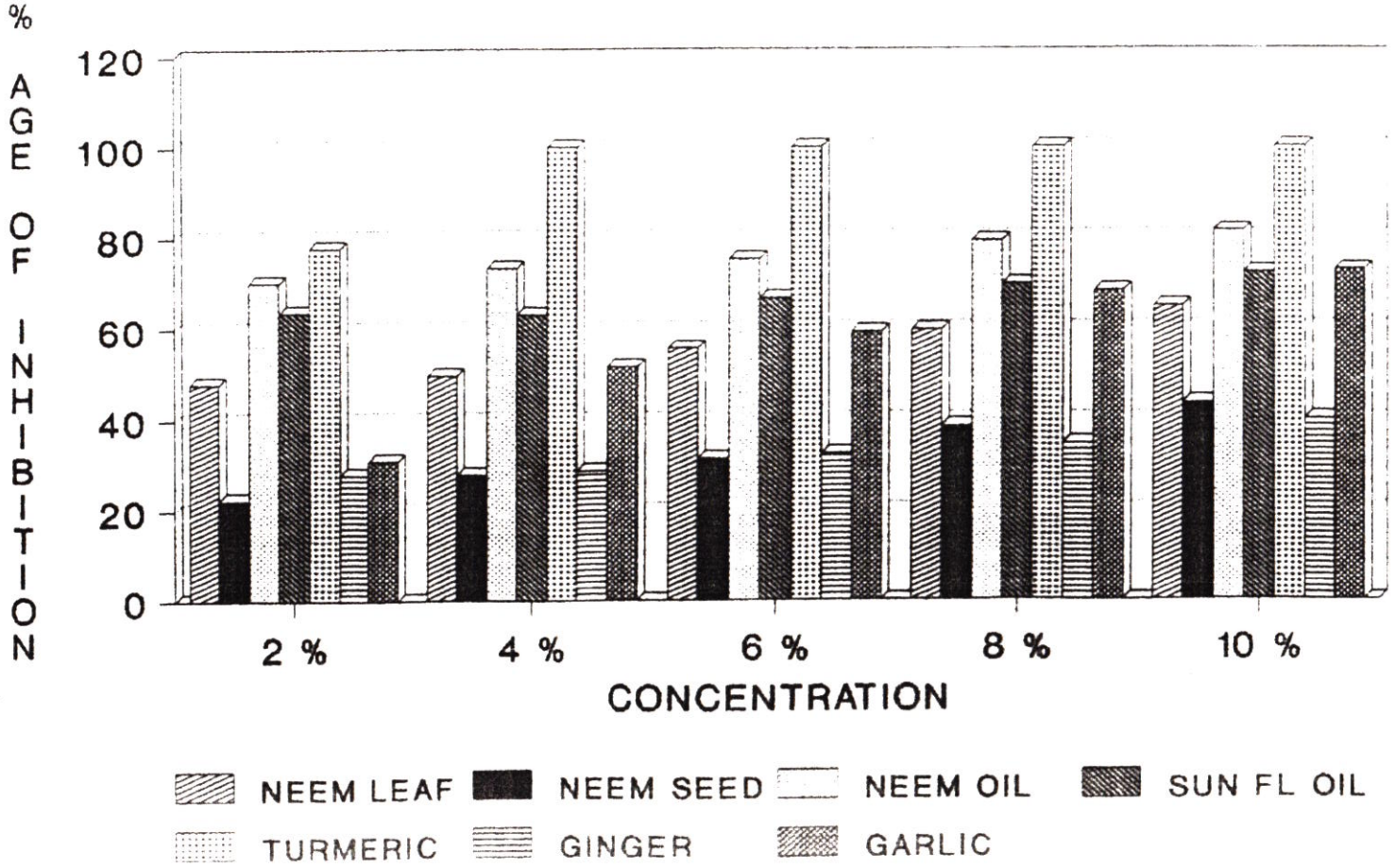


PLATE IV

Fig. 1. *In vitro* efficacy of neem oil & turmeric rhizome extract against *C. falcatum.*

Fig. 2. *In vitro* efficacy of indofil M-45 & Bavistin on *C. falcatum.*

PLATE IV

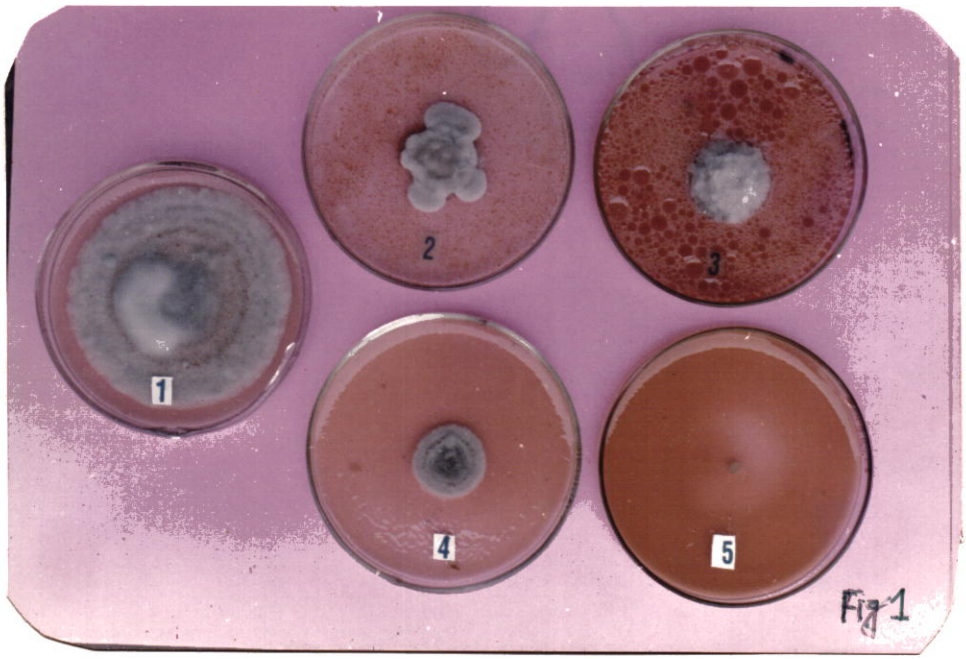


Fig 1

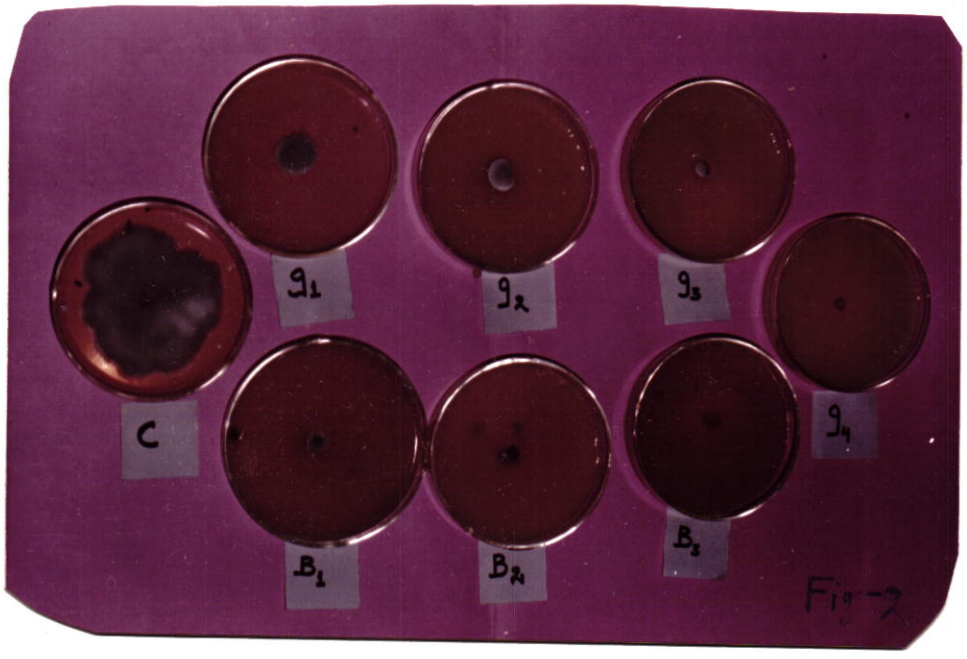


Fig 2

at 4-10 per cent concentrations followed by neem oil, sunflower oil, and garlic extract. Termeric extract was significantly superior to neem oil in all the concentrations used. All other products were inferior to turmeric extract, neem oil, sunflower oil & garlic extract at all the concentrations used but were significantly superior to control. However, there was no significantly difference between sunflower and garlic juice at higher concentrations but the former was significantly superior to the later at 2, 4 & 6 per cent concentrations in inhibiting the in vitro growth of C. falcatum.

The two primary quality determinants for turmeric are the content of the pigments and of the volatile oil. The pigment is known as curcumin (one of the non-steam volatile diferuloyl methane derivatives). The bitter after taste is due to some as yet uncharacterized bitter principles (Ferrara, 1958 & Luckner et al., 1967). The pigment consisted of desmethoxycurcumin & bis-desmethoxycurcumin (Srinivasan, 1953). This non-steam-volatile principle of the pigment seems to act as fungistatic or antifungal against C. falcatum & might be the cause of inhibition of its in vitro growth.

The active principle of garlic extract having antifungal activities seems to be due to presence of allicin (diallyl thiosulfinate), a heat labile and acid tolerant compound and inhibitor of sulfhydryl metabolic exzymes. Garlic extracts have been reported to have antifungal properties against number of pathogenic fungi (Singh et al., 1979; Singh & Singh, 1980; Ahmed et al., 1984).

Although the efficacy of neem products have not been evaluated earlier particularly against C. falcatum, it has been reported to inhibit the in vitro growth of C. papayae (Ahmed et al., 1977). The antifungal activities of neem products were due to presence of nimbidin and sulphur (Singh et al., 1980).

4.12 EFFICACY OF SOME COMMERCIAL FUNGICIDES AGAINST in vitro GROWTH OF C. falcatum:

For evaluating a comparative efficacy of commonly used fungicides with that of selected plant products, seven fungicides were assessed as regards their efficacy in inhibiting in vitro growth of the test fungus. The experiment was conducted as per the procedure mentioned in "Materials & Methods". The data obtained are presented in Table 8 and depicted in Plate 4 & fig. 2.

It may be seen from the Table 8 that all the fungicides tested could significantly inhibit the growth of C. falcatum in all the concentrations used. Among the fungicides tested, Bavistin, Topsin-M Plantvax and Ridomil could completely inhibit the growth in all the concentrations and were at par followed by Indofil M-45 & Kavauch. Minimum inhibition of growth was seen in sutox.

Effevtiveness of carbendazim against C. falcatum has been reported by several workers (Waraich, 1983; Lewin et al., 1976; Chand et al., 1974; Khirbat et al., 1984). Poor efficacy of copper oxychloride

PERCENT INHIBITION OF C.falcatum WITH DIFFERENT COMMERCIAL FUNGICIDES

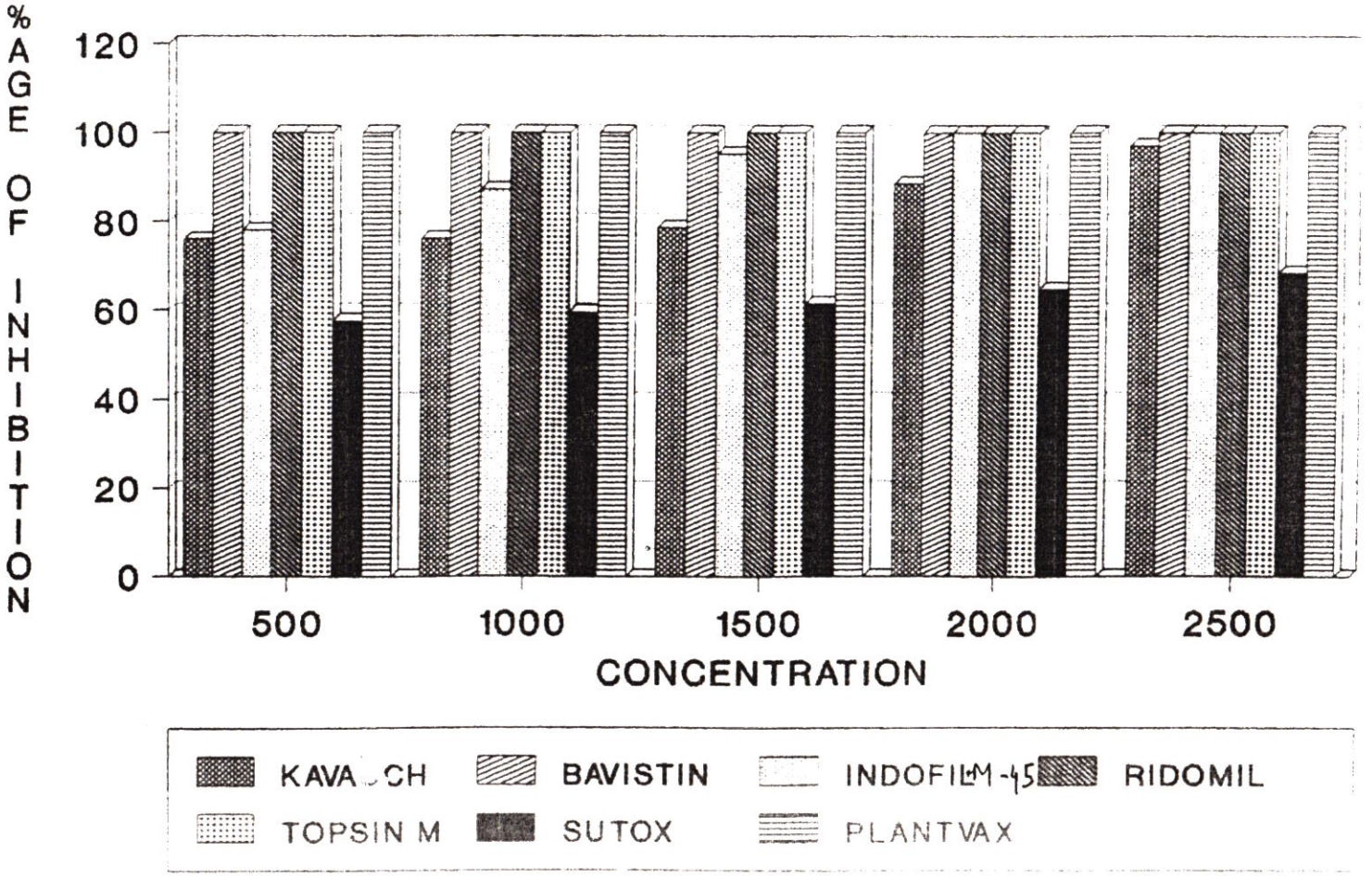


Table 8 Percent inhibitation[a] of C. falcatum on poisoned basal medium with different commercial fungicides.

Name of the commercial fungicides	Concentration				
	500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm
Kavach chlorothanil	76.02 (60.70)*	76.18 (60.66)	78.56 (62.43)	88.57 (70.20)	97.14 (82.00)
Bavistin (carbendazim)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Indofil M-45 (Mancozeb)	78.09 (62.06)	87.14 (68.93)	95.23 (77.33)	100 (90.00)	100 (90.00)
Ridomil (metalaxyl)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Topsin-M (thiophanate methyl)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
Sutox (copper oxychloride)	57.61 (49.36)	59.52 (50.43)	61.42 (51.56)	64.57 (53.56)	68.28 (55.73)
Plantvax (oxycarboxin)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
SEm ±	1.81	0.54	1.89	1.33	5.45
CD (at 0.05)	5.58	1.66	5.82	4.10	16.80

* Angular value in parenthesis against original value.
a Each reading is an average of 3 replication for each treatment after 7 days of inculation.

in controlling red rot has been reported by Sinha et al., 1979; and Padmanabha et al ., 1990. These earlier findings were similar to the present findings regarding efficacy of different fungicides against C. falcatum.

CHAPTER V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Severe out break of red rot in varieties CO 6304, V-186, CO 85061 were observed in different areas of districts of Jagatsinghpur in Orissa. Field observations revealed that, the disease progressed very quickly and 50 per cent loss of canes in individual fields were observed under partially submerged condition during the months of September to October. The soil pH of the affected ffields were determined to be 5.5 to 6.5. It was further observed that there was intermintent heavy showers with rain splashed wind for about 15 days preceeding the incidence with average day and night temperature being 30°C & 25°C respectively and this condition was still continuing during the period of visit which might have been responsible for the quick development and spread of the disease.

Varieties like CO 85061 & V-186 were severely affected under water logging condition and lack of sanitation, where as the same varieties remained free of infection in well drained & well maintained fields. It was therefore, concluded that varietal resistance or susceptability seemed to have been governed by environmental conditions including temperature, rainfall, soil pH & moisture rather than varietal differences. The role of variations in pathogenic races as regards virulence and avirulence nature of the pathogen can't be ruled out which needs further study.

The pathogen was brought into pure culture and the studies on cultural characters revealed that the present isolate is a dark strain of

the pathogen as described by Abbott (1938).

The causal agent was identified as Colletotrichum falcatum Went (= Glomerella fucumanensis) (speg). Von Arx & Muller). The pathogenicity test under in vitro conditions was proved successfully by different methods of inoculation. The morphological characters of the pathogen matched in all respect with the earlier description of the species.

Reactions of five different varieties viz CO 6304, CO 62175, CO 7219, V-186, CO 8021 grown under Orissa conditions against the present race of the pathogen revealed that all the varieties showed intermediate reaction with V-186 as moderately resistant and the rest of the varieties were moderately susceptible to the present race.

The pathogen could grow as well as sporulate maximum when the culture medium was supplemented with glucose followed by sucrose except for sabauraud's agar medium where glucose was supplemented along with peptone.

Colletotrichum falcatum could grow & sporulate within the temperature limit from 15° - 35°C and the optimum being 25° - 30°C. The optimum pH for its maximum growth & sporulation was at pH 6.5 while it could grow & sporulate well from pH 5.5 to 7.5.

Studies on antagonistic effect of to isolate of Trichoderma viride gave excellent positive result. The in vitro growth of test fungus C. falcatum was inhibited by 82% in dual culture with the candidate microflora. Similarly the spore germination of test fungus was found to

be inhibited in culture filtrate of T. viride.

Evaluation of different plant products indicated that the in vitro growth of the pathogen was completely inhibited in all the concentrations used (4%, 6%, 8%, 10%) by turmeric extract followed by neem oil, sunflower oil and garlic extract.

Out of different commercial fungicides tested in the present study, carbendazim products (Bavistin), Thiophenate methyl (Topsin-M), oxycarboxin (Plantvax) & metalaxyl (Ridomil) were the most effective in inhibiting the in vitro growth of the pathogen upto 100 per cent even in 500 ppm concentration. Mancozeb (Indofil M-45) was the next in order of preference.

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