ETIOLOGY AND MANAGEMENT OF RHIZOME ROT DISEASE OF BANANA

By

USHA. N. K.

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2003

DECLARATION

I hereby declare that the thesis entitled **"Etiology and Management of Rhizome Rot Disease of Banana"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara
3-5-03

USHA.N.K

CERTIFICATE

Certified that this thesis entitled "**Etiology and Management of Rhizome Rot Disease of Banana**" is a record of research work done independently by Mrs. Usha N.K under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Vellanikkara
3.5.03

3/5/03

Dr. K. Anita Cherian

(Major Advisor, Advisory Committee)
Assistant Professor
Department of Plant Pathology
Banana Research Station
Kannara.

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Mrs. Usha. N.K.,** a candidate for the degree of **Master of Science in Agriculture** with major field in **Plant Pathology,** agree that the thesis entitled **"Etiology and Management of Rhizome Rot Disease of Banana"** may be submitted by Mrs. Usha. N.K., in partial fulfilment of the requirement for the degree.

19/7/03

Dr. K. Anita Cherian
(Chairperson, Advisory Committee)
Assistant Professor
Department of Plant Pathology
Banana Research Station
Kannara

19/7/2003

Dr. Koshy Abraham
(Member, Advisory Committee)
Associate Professor and Head,
Department of Plant Pathology
College of Horticulture
Vellanikkara, Thrissur

19.7.03

Dr. A. Sukumara Varma
(Member, Advisory Committee)
Associate Dean
College of Horticulture
Vellanikkara, Thrissur

19/7/03

Dr. Rema Menon
Associate Professor and Head
Banana Research Station
Kannara

19/7/03

EXTERNAL EXAMINER

Dr. V. PRAKASAM, Ph. D.,
Professor of Plant Pathology,
Tamil Nadu Agrl. University,
COIMBATORE-641003.

ACKNOWLEDGEMENT

I humbly bow my head before the god Almighty, who blessed me with the will power and courage to complete this endeavour. I submit this small venture before god for his grace in providing me with health and strength through out the study.

With deep respect, I express my heartful gratitude and unforgettable indebtedness to my guide Dr. Anita Cherian. K, for her gracious guidance, valuable suggestions, constructive criticism, constant encouragement and above all extreme patience, understanding and whole hearted co-operation rendered through out the course of the study. I am indeed honoured to submit my thesis under her guidance.

I wish to extend my whole hearted gratitude to Dr. Koshy Abraham, Associate Professor and Head, Department of Plant Pathology and member of my advisory committee for his precious suggestions and ever willing help rendered through out the course of research work.

I am respectfully thankful to Dr. A. Sukumara Varma, Associate Dean and former Head of Department of Plant Pathology, and member of my advisory committee for his valuable suggestions and support and critical analysis of the thesis.

I am deeply obliged to Dr. Rema Menon, Associate Professor and Head, Banana Research Station Kannara and member of my advisory committee for her guidance, warm and friendly nature in rendering help and critical analysis of the thesis.

I record my sincere thanks to Dr. S. Beena and Dr. D. Girija, Assistant Professors, and Dr. Sheela Paul, Associate Professor, Department of Plant Pathology for their valuable suggestions, guidance and support through out the period of research work.

I am deeply obliged to Dr. M.V. Rajendran Pillai, Associate Professor, Department of Plant Pathology for his help in the preparation of photographs and valuable help throughout the venture.

I express my deep sense of gratitude to all the staff members of the Department of Plant Pathology for their help and encouragement during the entire course of study.

I am extremely grateful to Sri. S. Krishnan and Smt. Soudamini, Associate Professors, Department of Agricultural Statistics for their whole hearted help through out the statistical analysis of the data.

I am grateful to Dr. Suma, Associate Professor and Mrs Sakunthala Nair, Assistant Professor and all other staff members of Banana Research Station, Kannara for all the help rendered to me during the entire course of the study.

My special thanks to Dr. A. Augustin Antony, Associate Professor, Department of Biochemistry, AICRP(M & AP) for helping me and allowing me to do the biochemical tests in his lab.

A special note of thanks to the labourers of Department of Plant Pathology, College of Horticulture And Banana Research Station, Kannara for their co-operation and assistance rendered to me during the conduct of research work.

My special thanks to Mr. Santhosh and Jeo for all their help and support in doing the computer works.

I offer my sincere thanks to my dear most friend Mini, for her whole hearted support and relentless help through out this endeavour.

I am extremely grateful to my dear most friends Suma, Girija, Sujatha, Smitha, Hani, Lekha, Sheena, Teena, Jyothi, Rajesh, Shinoj and Sherin for their sincere support all throughout.

I am thankfully obliged to my dear classmate friends Reshmy and Nandakumar for their timely help and support.

I express my sincere thanks to all my batch mates, juniors and seniors (especially Mole P. Cherian, Maya, Thanuja and Annie Mathew). I express my heartfelt thanks to Byju, Amjad, Sangeetha and Anita at GKVK Campus, Bangalore for rendering me all the help at UAS, Bangalore

Words can't express my profound gratitude to my friends Pratheesh and Deepa James for their overfilling help and support given to me in time of need.

The award of Junior Fellowship of the KAU is thankfully acknowledged.

I dedicate this small piece of work, as a token of love to my beloved sister who is not with us today, but I can feel the fragrance of her love through out my life.

I am in dearth of words to express my gratitude and indebtedness to my loving husband for his constant support, help and love in all my endeavours.

Lastly but not least, I owe my profound gratitude to my dear most father, mother, brother, sister-in law, father and mother in law, brother in law, Achootty, valiammas, achamma and all my dear and near for their boundless affection, constant prayers, moral support and unfailing inspiration all along the study. Without them, it would have been impossible to complete my study.

With sincere gratitude, I dedicate this thesis to all those who have helped me to present this endeavour in this form.

Usha. N.K.

Affectionately Dedicated To My

Beloved Parents

&

Loving Husband

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Introduction

1. INTRODUCTION

Banana is one of the most accepted and versatile fruit crops across the globe and this every day fruit is nearly the staple source of energy for the rural poor in the tropical and subtropical regions of the world. In India, it is the most important fruit crop contributing 37 per cent of the total food production, with an annual production of 16.91 million tonnes from 490.70 thousand hectares (Singh, 2002). The crop, which has a diverse use with high export potential, plays a significant role in the socio- economic and cultural heritage of our country.

Banana is the most important economically profitable fruit crop grown in Kerala, mainly by small and marginal farmers, providing a vital source of income. However, the productivity of this crop is not very appreciable.

Among the many constraints, the incidence of various diseases is the most significant factor for its low productivity. In recent years, rhizome rot disease has become a serious problem to the commercial cultivation of banana in southern states of India like Kerala, Karnataka, and Tamil Nadu (Lakshmanan and Mohan, 1991; Khan and Nagaraj, 1998; KAU, 1999; Nagaraj *et al.*, 2002a,b; Rani *et al.*, 2002).

In Kerala, Nendran (*Musa* AAB) is the most widely cultivated variety of banana and it is found to be highly susceptible to this disease. Further, it was noticed that the disease is more seen in banana cultivated in paddy fields. Since the area under the cultivation of banana in paddy fields in Kerala is rapidly increasing, there is every chance that this disease may emerge as a major threat to the production of banana, if suitable remedial measures were not taken at the appropriate time.

A perusal of literature revealed that not much work has been done on the etiology and management of rhizome rot disease of banana in Kerala. Hence, the present investigation was carried out with the following objectives,

- Isolation of the pathogen.
- Studying the etiology and symptomatology of the disease
- Cultural and biochemical characterisation of the pathogen.
- Evolving an effective management practice against the disease.

Review of literature

2. REVIEW OF LITERATURE

Bacterial rhizome rot of banana also referred to as bacterial head rot or tip over disease was first observed in the Aguan Valley of Honduras in 1948 (Stover, 1959). A watery decay of the banana rhizomes resulted in the toppling over of the banana plant. He found that a bacterium was associated with the disease. Later the disease was reported from Panama (Loos, 1962). Hildreth (1963) reported the occurrence of rhizome rot on banana variety GrosMichel in Central America causing an annual loss of 10 -15 per cent. Volcani and Zutra (1967) detected bacterial soft rot of banana plants caused by *Erwinia carotovora* for the first time in Israel. Llanos and Carmen (1967) recorded a new bacterial disease on banana characterised by rot and collapse of the pseudostem since 1964. Since then the disease has been reported from Columbia (Fernandez-Borrero, 1967), Central America (Wardlaw, 1972), Latin America (Stover, 1972), India (Edward *et al.*, 1973), Venezuela (Ordosgoitty *et al.*,1974), Jamaica (Shillingford, 1974), Cuba (RiveraDocando, 1978), Papua New Guinea (Tomlinson *et al.*, 1987), Tarjon (Choi *et al.*, 1988), Brazil (Periera and Nunes,1988) and from Iran (Hassanzadeh, 1990). Guzman and Sandoval (1996) reported a high incidence of pseudostem soft rot in the banana hybrids FHIA-01 and FHIA-02 in Corbana. Recently, the disease has been reported from Iconozo in Columbia (Gomez-Caicedo *et al.*, 2001)

In India, the first occurrence of this disease was reported from Allahabad on banana variety Basrai (Edward *et al.*, 1973) and later from West Bengal on Cavendish banana variety, Giant Governor (Chattopadhyay and Mukherjee, 1986). In West Bengal, the disease became a threat to the cultivation of this Cavendish variety (Chattopadhyay, 1987). Lakshmanan and Mohan (1986) reported rhizome rot disease affecting Cavendish banana grown in Tamil Nadu with nearly 40-60 per cent loss and later recorded a disease incidence of 60-80 per cent. (Lakshmanan and Mohan, 1991). Khan and Nagaraj (1998) reported the incidence of tip over disease of banana for the first time in Kolar and Bangalore districts of Karnataka. The disease was reported from South Gujarat by Solanky *et al.* (2001). Tip over disease of banana occurred widely during 1999-2000 in almost all banana growing tracts of South India. (Nagaraj *et al.*, 2002a,b; Rani *et al.*, 2002).

In Kerala, rhizome rot disease of banana is occurring from 1987 onwards (KAU, 1990) and has become a serious problem to the commercial cultivation of banana variety, Nendran. Now the disease has been reported in other commercial varieties like Rasthali (AAB) and Robusta(AAA) (KAU, 1999). The disease is more prevalent on banana grown in paddy fields.

2.1 FACTORS FAVOURING DISEASE DEVELOPMENT

Several workers have carried out studies on the factors favouring the development of the disease.

Stover (1959) stated that the disease was most serious in areas previously flood fallowed for 4 to 6 months and in methyl bromide fumigated or steam sterilized soil in the green house. Newly planted rhizomes and plants less than 6 months old were frequently destroyed and no seasonal trend in the incidence was apparent, nor the disease occured consistently in all areas.

Loos (1962) opined that the predisposing factors for the incidence of bacterial rhizome rot were the type of heart bud of the planting material, strain virulence of the bacterium, soil and climatic factors. All these contributed equally to the disease severity.

Hildreth (1963) reported that seed wounding, undersized seed, immature seed, excessive exposure to sun and drying, excessive planting depth, favouring of apical bud growth over lateral bud growth, trimming nematode lesions and use of contaminated tools contributed to the disease development.

According to Shillingford (1974) water logging and poor drainage conditions favoured the development of the disease. Chattopadhyay and Mukherjee (1986) in their study found that the spread of the pathogen was through infected planting material.

Tomlinson *et al.* (1987) observed that the disease outbreak occurred during the wet season in a period of prolonged wet weather. The pathogen, *E.*

chrysanthemi persisted in the soil where the diseased plant material was present. The newly planted suckers frequently rotted soon after heavy rainfall.

Thwaites *et al.* (2000) reported that the bacteria probably invaded the rhizome directly after planting through the wounds or decaying leaf sheaths, around the central bud. The disease was common in wet weather, particularly if the ground was poorly drained and water logged.

According to the investigations conducted at Banana Research Station, Kannara, it was found that the disease was prevalent on banana grown in paddy fields where water logged condition prevailed (KAU, 1999).

Nagaraj *et al.* (2002a) conducted studies on the mode of transmission of the pathogen under field conditions and found that the most effective mode of transmission of the pathogen was through infected rhizomes and infected plantlets. The highest incidence of the disease was noticed when pared and injured rhizomes were used for planting

The effect of the attack of rhizome borer (*Cosmopolites sordidus*) on the incidence of rhizome rot was also investigated by several workers.

Stover (1959) reported that the banana root borer, *C. sordidus* was frequently associated with the rot. Observations on the tip over disease of banana in Allahabad showed that severe damage occurred in cases where weevil attack was severe (Edward *et al.*, 1973). Shillingford (1974) observed that in Robusta banana, severe tunneling due to borer activity accelerated the bacterial rot. Borer tunnels were surrounded in many instances by typical bacterial rot.

2.2 SYMPTOMATOLOGY

Wardlaw (1935) mentioned about a banana disease in Jamaica called black head, which referred to any disease of the rhizome showing superficial, or internal brown or dark water soaked patches. Hord and Flippin (1953) described the symptoms of rhizome head rot of banana found in Honduras as watery decay of the

rhizome, which resulted in the toppling over of the plant. But the etiology of the disease was first reported by Stover (1959) and he had detailed the symptomatology of the disease. He reported that the field symptoms were manifested at three stages of growth *viz*., rotting of newly planted rhizomes which failed to sprout, stunting and poor growth of young plants and toppling over of the mature plants at shooting stage. Regions of dark to pale brown water soaked tissue developed from rhizome surface invaded through cortex and stele. These water soaked areas became internally pocketed with dark peripheral rings. The inner areas frequently decayed leaving cavities surrounded by dark spongy matter. As the rot progressed young plants became yellowish stunted and were easily pushed over. Mature plants seldom exhibited outward symptoms but are easily blown or pushed over particularly at the bunching stage. When the pseudostem of a mature diseased plant toppled, the rhizome got split and a portion of the infected tissue adhered to the pseudostem, the remainder of the rhizome stayed anchored by the roots. In severe cases, the young rhizomes of the suckers emerging around the mature rhizome were also infected.

Llanos and Carmen (1967) reported that the symptoms of the disease were characterized by rot and collapse of the pseudostem.

Lacy (1971) found that the most striking and characteristic feature of the disease was the presence of a large basal cavity extending upto the centre of the plant rhizome and originating directly from the old planting piece. He also suggested that, those cavities might mark another point of entry of the pathogen.

Stover (1972) reported that the disease usually affected only large non-fruiting plants but not the daughter suckers. Edward *et al.* (1973) described the symptoms of tip over disease of banana occurred in Allahabad. The important symptoms comprised of discolouration and soft rot of rhizomes and suckers, scanty roots with dark brown lesions and necrotic tips. The pseudostem in many cases tipped over breaking across the much-rotted part of the stem especially at the soil level. Severely affected plants were stunted, developed water soaked discoloured leaf sheath bases and yellowing of leaf blades that eventually had scorched appearance. Both young and mature plants showed the symptoms.

Ordosgoitty *et al.* (1974) reported that plants with yellowish translucent spots on the stem turned reddish brown and spread over the basal parts. A foetid liquid oozed out of the stem lesions and the plants weakened and fell.

Symptoms described by Shillingford (1974) about the bacterial rhizome rot in Jamaica were same as that described by Lacy (1971). In more advanced stages, the tissue rot to a black soggy mass, leaving deep cavities around the base of the pseudostem. The disease did not appear to have any obvious symptoms on the foliage. Bunches from severely affected plants were small and deformed, with twisted fingers. Plants with bunch toppled easily. The rhizome broke and a portion of the infected tissue remained adhered to the pseudostem. The part of the rhizome stayed in the ground, anchored by roots. Growth of the suckers slowed down and leaves turned yellow.

Chattopadhyay and Mukherjee (1986) noticed that the symptoms appeared on the leaves, which turned pale to yellowish lusterless, withering very slowly. The rotting of rhizome resulted a mashy soft tissue emitting disagreeable odour. Chattopadhyay (1987) reported that the disease generally appeared at the very primary stage of establishment of plantain and caused a serious setback in the initial stages. The soft rot of the rhizome progressed up the pseudostem destroying the growing point and causing internal decay, often with vascular discolouration. Yellowing and wilting of the leaves were characteristic symptoms.

Periera and Nunes (1988) noted that the disease was characterized by massive soft rot of the rhizome with a typical odour like that of caused by *E. carotovora* The rot progressed up the pseudostem, destroying the growing point causing internal decay and plant death. External symptoms consisted of yellowing, wilting and collapse of the leaves.

Robinson and Manicom (1991) observed that the infected plants toppled over during the fruit development stage in cultivars Cavendish and Valery.

Lakshmanan and Mohan (1991) reported that newly planted rhizomes and the plants less than six months old were frequently destroyed.

Buddenhagen (1994) opined that the disease might cause poor sprout emergence, stunting and yellowing of young plants or later snapped off at fruiting. Yellow to brownish water soaked areas with black borders developed mainly in the cortex. These areas later became cavities resembling those caused by banana root borer (*C. sordidus.)*

Guzman and Sandoval (1996) observed the symptoms of pseudostem soft rot in banana hybrids FHIA-01 and FHIA-02 and reported that the most characteristic symptom of the disease were cigar leaf necrosis, soft rot and unpleasant odour of the pseudostem internal area.

In Karnataka, the disease generally appeared in plants of age six to ten months. The chief symptoms were massive soft rot accompanied by odorous rot of the centre or a portion of the rhizome. The leaves turned pail to yellow, lusterless, wilting very slowly and eventually appeared scorched (Khan and Nagaraj, 1998; Nagaraj *et al.*, 2002a,b; Rani *et al.*, 2002).

Thwaites *et al.* (2000) describing the disease symptoms reported that the internal appearance of the affected banana plants depended on the age of the banana plant and the severity of the infection. Newly planted rhizomes failed to sprout, and seedlings exhibited stunted growth and yellowing. External symptoms in the mature plants were often absent, but where the decay was extensive, the rhizome got weakened by a wind or even by a fruit weight. Internally the cortex contained regions of brown or yellow water soaked material with well-defined large margin. If the decay became wide spread the emerging daughter suckers might be affected. Wilting of older leaves is said to be a distinguishing feature of the disease.

Gomez-Caicedo *et al.* (2001) reported that the symptoms of the disease consisted of chlorosis in the lower leaves followed by wilting at the petiole then ascending wilt that finally affected all the leaves of the plant. A cross section of an infected pseudostem about one metre above the ground level reveals watery rot and an unpleasant smell. In addition, the colour of the inner leaf sheaths ranged from brown to dark brown.

2.3. THE ETIOLOGY

Stover (1959) reported that the causal organism of rhizome rot disease of banana to be a motile gram negative rod shaped bacteria that forms dull greyish white colonies on nutrient agar. In 1962, Loos reported that the rhizome rot disease, which occurred in Panama, was due to *Erwinia carotovora* ssp. *carotovora* earlier known as *Pectobacterium carotovorum*. Hildreth (1963) reported that the rhizome rot disease of banana variety Grosmichel in Central America was caused by *Pectobacterium carotovorum (E. carotovora).*Volcani and Zutra (1967) found that the bacterial soft rot of banana in Israel was caused by *E. carotovora*. Fernandez-Borrero and Lopez-Duque Selma (1970) reported that the causal agent of watery rot of pseudostem of banana was *Erwinia paradisiaca*. Stover (1972) reported that the bacteria *Erwinia chrysanthemi* and *E.carotovora* were the causal agents of the disease. Wardlaw (1972) reported that *E. carotovora* was the causal agent of tip over disease of banana.

Edward (1973) found that the causal agent of tip over disease of banana in Allahabad was *E.carotovora*. Ordosgoitty *et al.* (1974) isolated *E. carotovora* var. *carotovora* from diseased banana plants in Venezeula. Shillingford (1974) found that the bacterial rhizome rot of banana in Jamaica was caused by *E. chrysanthemi*. RiveraDocando (1978) reported that the bacterial corm rot of banana in Cuba was caused by *E. carotovora* ssp. *carotovora*.

Dickey (1979) in a comparative study of phenotype properties of *E. chrysanthemi* from several hosts studied Stover's strains and isolated further five strains from Panama and four from Honduras. All these isolates from Cavendish cultivars (AAA) were identified as *E. chrysanthemi* based on a number of bio chemical tests. The pathogen causing soft rot of the banana was identified as *E. chrysanthemi* p.v. *paradisiaca* by Dickey and Victoria (1980).

In West Bengal, the identity of the causal organism of pseudostem rot of banana was confirmed as *E. chrysanthemi* p.v. *paradisiaca* (Chattopadhyay and Mukherjee, 1986). Choi *et al.* (1988) in Tarjon identified the pathogen isolated

from the soft rotten banana fruits as *E.carotovora* ssp. *carotovora*. Rivera and Ezavin (1989) found that the corm disease in *Musa accuminata* cultivars (AAA) in Venezuela was caused by *E. chrysanthemi*. Cedeno *et al.* (1990) reported that the bacterium *E.carotovora* ssp.. *atroseptica* was the pathogen of soft pseudostem rot in Harton plantation in the Southern Maracaibo lake area. Hassanzadeh (1990) characterized the soft rot causing bacteria in banana on the basis of biochemical and pathogenity tests and found that the isolates were intermediate between *E.carotovora* and *E. chrysanthemi*. In 1990, Hao *et al.* isolated a new species of *Erwinia* infecting banana, tomato and cucumber as *Erwinia percinus*. Reports from Kerala revealed that, the rhizome rot of banana is caused by a Gram-negative bacteria (KAU, 1990). Lakshmanan and Mohan (1991) found that the rhizome rot and tip over disease of Cavendish bananas in Tamil Nadu is caused by *E.carotovora*.

Jimenez and Cordoves (1992) reported *E. chrysanthemi* as the causal agent of plantain corm necrosis. Buddenhagen (1994) reviewed that the pathogen causing rhizome rot was considered either *E.carotovora* (Jones) Bergy *et al.* or *E. chrysanthemi* Burkholder *et al.* and is a soil borne wound invader. Guzman and Wang (1998) reported that the finger soft rot of banana in CostaRica was caused by *Erwinia* spp. Gomez-Caicedo *et al.* (2001) reported that the causal agent of the bacterial rot of pseudostem of Harton plantain in Columbia was found as *Erwinia* spp. In South Gujarat, the rhizome rot disease of banana was found associated with a soil borne pathogenic bacteria, *Erwinia* spp. (Solanky *et al.*, 2001). Based on the morphological, physiological and biochemical studies and also the pathogenicity tests *E.carotovora* ssp. *carotovora* was identified as the causal organism of tip over disease of banana in Karnataka (Nagaraj *et al.*, 2002a; Rani *et al.* 2002).

2.4 METHODS OF ISOLATION OF THE PATHOGEN.

There are many chemically defined media suitable for the isolation of bacteria. The media are well defined based on their nutritional requirements. In order to differentiate the various kinds of bacteria certain reagents are incorporated in to the culture media, thus forming differential medium. Generally bacteria are isolated on nutrient agar medium (Bradbury, 1970)

Stover (1959) macerated the disinfected diseased rhizome bits and the suspension was streaked on nutrient agar (NA) plates to yield grayish white colonies of the bacterium.

The sections of the corm or pseudostem showing soft rot were washed thoroughly and small sections of the edge of the rotting tissue were removed aseptically, washed in running tap water and dried with tissue paper. Sections were then crushed in 2-3 ml sterile distilled water and the resulting suspension was streaked on Crystal Violet Pectate medium (Tomlinson *et al.*, 1987).

Gomez-Caicedo *et al.* (2001) macerated the disinfected diseased rhizome samples in a mortar containing one ml sterile distilled water. Luria Bertani (LB) medium was added to 50 µl of this macerated preparation. The petridishes were then incubated at 28°C for 48 hrs.

Isolation of *E.carotovora* ssp. *carotovora* from infected banana rhizomes was done on NA to yield creamy yellow, mucoid, shiny, irregular and small colonies. (Nagaraj *et al.*, 2002a; Rani *et al.*, 2002)

Erwinia spp has been isolated from diseased samples of crops other than banana. Dickey and Kelman (1981) isolated *Erwinia carotovora* from the infected potato tissues. Disinfected soft rotten potato tissues were crushed with a sterile glass rod and the resultant suspension was allowed to stand for 5-30 minutes. Removed loop of this suspension or either added to a sterile water blank (1-2ml) and mixed or directly streaked on Crystal Violet Pectate medium.

In another method, small pieces of (1-2mm) of potato stem tissue from near the margin of the root area were placed in 3ml sterile distilled water. The tissue was teased apart with sterile needles or macerated with sterile scalpel and allowed to stand for 15 minutes. A loopful of suspension was streaked on to dry plates of Nutrient agar or selective media and incubated at 25°C (Cother and Sivasithamparam, 1983)

Isolation of *Erwinia carotovora* var. *carotovora* was done from onion bulbs showing soft rot symptoms. The infected soft portion of the bulbs after surface sterilization were punctured using an inoculation needle and streaked on the petri dish containing selective medium (Senthilvel, 2000).

Several methods were adopted by different workers for the isolation of soft rotting *Erwinia* spp. from the soil. Logan (1963) developed a selective medium (in which sodium acetate was added to the common basal medium as the carbon source) for the isolation of *E. artoseptica* causing black leg of potato from the soil. He found that the medium was also effective for the isolation of *E.carotovora* and *E. aroideae* from soil.

Schaad and Brenner (1977) attempted isolation of *E. chrysanthemi* causing bacterial wilt and root rot of sweet potato from the soil.

Tomlinson *et al.* (1987) attempted the isolation of the pathogens (causing rhizome rot of banana) *viz. E chrysanthemi* and *E.carotovora* ssp. *carotovora* from the rhizosphere of the infected plants. The former was isolated from all diseased samples taken and from the soil surrounding the rotting corms. The later organism was occasionally isolated along with *E. chrysanthemi* from the diseased corms, but not from the soil. Neither bacterium was isolated from the soil away from the root system of the plants.

2.5 PATHOGENICITY STUDIES

Stover (1959) proved Koch's postulates and standardized the method of inoculation to determine the pathogenicity of the bacterium causing soft rot of banana. Healthy banana rhizomes were inoculated with bacterial culture grown on nutrient agar using cork boring method and were planted in sterile soil or vermiculite. Control was also maintained. The rhizomes were removed 25 days after planting, cut open and examined. The inoculated rhizomes exhibited symptom of typical rhizome rot around the area of inoculation. In most cases the rot had spread about 3.5 cm from the point of inoculation. Isolation from the outer periphery of the rotted zone yielded pure cultures of the bacterium inoculated.

Disease symptoms above ground appeared about 8-10 weeks after the inoculated rhizome were planted in pots.

Fernandez-Borrero(1967) proved the pathogenicity of *Erwinia* sp causing wet rot of pseudo stem in Columbia.

The pathogenicity of the rhizome rot bacterium was established by inoculating healthy banana rhizomes with the bacterium and the characteristic internal symptoms developed. It was confirmed that, the bacterium was related to the group which were the causal agents of numerous soft rots of storage tissues since the bacterium caused soft rot in tomato, potato, carrots and onions. (Shillingford, 1974). Dickey and Victoria (1980) demonstrated the pathogenicity of the *Erwinia* strains from *Musa paradisiaca* and the strains caused tissue degradation of plantain pseudostem pieces and seedlings. AngelesRamos *et al.* (1982) proved the pathogenicity and were able to reisolate the bacteria from rotten areas produced on the inoculated leaf sheath tissues.

Cother and Sivasithambaram(1983) reported that the time and temperature of incubation required to induce the rotting depends on the organism present. *E. chrysanthemi* will cause extensive rotting after three days at 30°C. *E.carotovora* p.v. *carotovora* and p.v *atroseptica* may require 10 days at 20-25°C. Chattopadhyay and Mukherjee (1986) confirmed the pathogenicity of the pathogen *E. chrysanthemi* p.v. *paradisiaca* in Cavendish banana.

Tomlinson *et al.* (1987)) tested individual isolates of *Erwinia* spp. for pathogenicity by inoculating them into plants by smearing a sterile dissecting needle, with a 48h old culture of the test bacterium on sucrose peptone agar and stab inoculating young banana suckers. Development of softening and discolouration spreading from the site of inoculation and reisolation of the test organism from such tissue was taken as evidence of pathogenicity. Results of the pathogenicity tests using *E. chrysanthemi* ranged from localized softening and discolouration around the point of inoculation to extensive rotting and splitting of pseudostem within 14 days of inoculation. With *E.carotovora* ssp. *carotovora*

never produced more than a localized discolouration around the point of inoculation.

Hassanzadeh (1990) conducted pathogenicity tests on banana in Iran, indicating that the pathogen isolates were intermediate between *E.carotovora* and *E. chrysanthemi*.

Pathogenicity of *E.carotovora* was confirmed on Cavendish banana during the studies on tip over disease of banana (Lakshmanan and Mohan, 1991)

The bacterium could reproduce the rhizome rot when an agar plug containing the inoculum was placed inside the rhizome of healthy banana (Thwaites *et al.*, 2000).

Standardisation of inoculation technique was done while doing the pathogenicity tests of *Erwinia carotovora* on banana. Of the different methods tried, pseudostem injection and root dip methods were found to be showing profound effect on disease development (Nagaraj *et al.*, 2002b).

Pathogenicity tests were confirmed with *E.carotovora* ssp. *carotovora* in banana during the investigations on tip over disease of banana in Karnataka (Rani *et al.*, 2002).

2.6 CHARACTERISATION AND IDENTIFICATION OF THE PATHOGEN

The pathogen associated with different diseases could be identified based on the morphological, cultural, biochemical and molecular characterisation.

2.6.1. Morphological characterization

Stover (1959) observed that rhizome rot pathogen was a motile Gram-negative rod shaped bacteria. Edward *et al.* (1973) reported that the bacterial ooze from the infected parts contained Gram-negative short rod shaped bacteria.

Many researchers reported that the pathogen causing the rhizome rot disease to be a motile Gram-negative small rod shaped bacteria (Dickey, 1979; Gomez-Caicedo *et al.*, 2001; Rani *et al.*, 2002). Studying the causal organism causing wilt and root rot of sweet potato, Schaad and Brenner (1977) observed that *E. chrysanthemi* cells were gram negative, peritrichous rods.

Studies showed that the bacteria, *Erwinia* spp (both *E.carotovora* var *carotovora* and *E. chrysanthemi*) were Gram negative rods occurring singly in pairs or sometimes in short chains and were motile and facultative aerobic (Lelliot, 1974; Bradbury, 1977a,b; Starr, 1981; Cother and Sivasithamparam, 1983; Cappuccino and Sherman, 1992).

2.6.2 Cultural characterisation

Stover (1959) reported that the rhizome rot pathogen of banana, *E.carotovora* produced greyish white colonies on nutrient agar medium.

Dickey (1979) studied the colony characteristics and pigment production of rhizome rot causing bacterium on Difco NA, YDC and modified YDC (Yeast extract Dextrose Calcium carbonate Agar). The bacterial colonies on NA after 24h at 27^{0}C were convex, slightly to moderately irregular and undulate, pale cream coloured and butyrous and averaged 1.5 –2.5mm in diameter. On YDC, the colonies were convex, somewhat umbonate, irregular, undulate lighertan, butyrous and 2.5-3.5mm in diameter (Dickey and Victoria, 1980). AngelesRamos *et al.* (1982) found that the pathogen produced colonies on NA and YDC, which were creamy white, smooth and slightly mucoid.

According to Chattopadhyay and Mukherjee (1986), the bacterium produced convex, slightly irregular, undulate pale cream coloured colonies on NA at 27^{0}C. Tomlinson *et al.* (1987) studied the colony characteristics and pigment production of the rhizome rot pathogen on potato dextrose agar (PDA) and sucrose peptone agar (SPA) and found that *E. chrysanthemi* produced blue pigment on PDA, while *E.carotovora* did not. Isolations made from the affected rhizome

yielded cream coloured, convex, irregular fluidal colonies on NA (Khan and Nagaraj, 1998).

Gomez-Caicedo *et al.* (2001) cultured the pathogen on LB (Luria–Bertani) medium at 28°C for 48 hours and found that the colonies were creamish, buttery and had a foetid odour.

Nagaraj *et al.* (2002a,b) and Rani *et al.* (2002) found that the colonies of *E.carotovora* ssp. *carotovora* appeared pale cream coloured, convex, irregular, shiny, mucoid and fluidal on nutrient agar.

Erwinia chrysanthemi produced greyish white to creamy white smooth colonies, round with margins becoming undulate to feathery, but grows flat to slightly raised after 3-6 days growth on Potato Dextrose agar at pH 6.5, colonies were reminiscent of fried eggs being umbonate with undulate margin. (Bradbury1977 b; Lelliot, 1974)

On YDC (Yeast extract Dextrose Calcium carbonate agar) many isolates of *E. chrysanthemi* produced a characteristic dark insoluble pigment after 5-10 days at 27°C (Bradbury, 1977a) *E. carotovora* var. *carotovora* produced greyish white colonies, smooth, round and glistening, slightly raised in NA within 24 hours at 25-30°C (Bradbury, 1977a).

Schaad and Brenner (1977) examined the bacterial colonies of *E. chrysanthemi* for size, colour, consistency and elevation on YDC, NA and Muller and Scroth agar. On YDC the colonies were slightly convex, finely granular, undulate white to light tan in colour and averaged 5mm in diameter. On NA, colonies were effuse to low-convex, transparent, entire, colourless and averaged 4 mm in diameter. On MS media colonies were slightly raised, smooth, slightly undulate and clear with an orange centre.

Schaad and Brenner (1977) tried six media *viz.*, Yeast Extract Dextrose agar (YDC), Nutrient agar (NA), Miller and Scroth agar (MS), Bioquest XLD agar (XLD) Bact Brilliant Green Agar (BG) and Bacto m Endo agarLES (mEndo) for the growth of *E. chrysanthemi.* They found that the growth on YDC agar and NA

were rapid with visible colonies present after 24 hrs at 30°C. After 72 hrs, colonies on YDC were slightly convex, finely granular, undulate white to light tan colour and averaged 5 mm in diameter. On NA the colonies were effuse to low convex, transparent entire colourless and averaged 4 mm in diameter. Growth on MS agar was slower than YDC with visible colonies present after 48-72 h. Colonies on MS agar averaged 2-3 mm after 72 h at 30°C and were convex slightly undulate and orange with colourless margins and were mucoid. Colonies growing in mass were blue. The bacterium was distinguished from other *Erwinia* by its characteristic growth and reaction on MS agar, XLD agar, Bact BG agar and mEndo agar. On XLD the colonies were yellow and medium. On Bacto BG they were red and medium but variation was there and on bacto m Endo the growth was very poor. *E. carotovora* colonies on MS agar were undulate on XLD agar and on BG agar the colonies were yellow and medium. In m Endo agar the colonies were red and medium.

A differential medium for *Erwinia* developed by Logan (1966) to distinguish E.carotovora p.v *atroseptica*, E.carotovora p.v *carotovora* and *E. chrysanthemi* cultures were streaked on plates with the media to obtain single well sepearted colonies and incubated for 24 hours at 27° C. *E.carotovora* p.v. *carotovora* reduced tetrazolium in the medium to insoluble red formazan and colonies (about 1.5 mm in diameter) developed a pink to purple centre. Single colonies of p.v. *atroseptica* remained colourless and less than 0.5 mm diameter. Colonies of *E. chrysanthemi* were larger than p.v. *carotovora* (about 2 mm) and completely red/purple. (Fahy and Hayward, 1983)

Formation of the unusual blue (bipyridiyl) pigment, indigoidine on YDC seemed to distinguish *E. chrysanthemi* from other soft rot *Erwinias* (Starr *et al.*, 1966).

Dickey (1981) found that all streams of *E. chrysanthemi* grow in peptone water medium + bromocresol purple without an additional carbon source. All strains of *E. cypripedii*, *E. chrysanthemi* and *Erwinia* spp.grew poorly in Ayers Rupp and Johnson basal broth medium without carbon source.

2.6.3 Ecological characterization

With regard to the pH requirement, no work has been done specifically on *Erwinia*. In the case of other plant pathogenic bacteria, Vaughan (1944) observed optimum growth of *Pseudomonas solanacearum* in culture between pH 6 and 8.5. According to Kelman (1953), the optimum pH requirement of *P.solanacearum* was 6.6 to 6.7. No studies have been made on the growth of *Erwinia* spp. at different pH level so far. Jyothi (1992) studied the growth of *P.solanacearum* causing bacterial wilt of chillies at different pH levels ranging from 5 to 9.5 at 0.5 intervals and found that maximum growth was obtained between pH ranges 6.5-7.5.

In the case of temperature requirement, while characterizing the rhizome rot pathogen, Dickey (1979) observed that *Erwinia* sp. pathogen grew well at 37°C and 39°C. Same results had been reported by Dickey and Victoria (1980) and Tomlinson *et al.* (1987). All the isolates of *Erwinia* grew well at 36-37°C (Nagaraj *et al.*, 2002a). *E.carotovora* var. *carotovora* and *E. chrysanthemi* grew well at temperature ranging from 37-40°C. The maximum temperature for growth of *E. carotovora* var. *carotovora* is 35°C (Bradbury, 1977 a, b). The optimum temperature of growth of *Erwinia* spp. ranged from 27-30°C. (Bradbury, 1977 a. b) and maximum temperature for growth varied from 32°C- 40°C or more. *E.carotovora* var. *atroseptica* failed to grow at 37°C (Bradbury, 1977 a, b). Perombelon and Hyman (1986) developed a rapid method for identifying and quantifying three major classes of soft rot *Erwinias viz. E.carotovora* p.v. *carotovora* , *E. chrysanthemi* and *E. atroseptica* directly from the plant materials based on their temperature tolerances and sensitivity to erythromycin. They found that their growth temperature requirements were different. All these bacteria grew at temperatures below 36°C on nonselective media, but *E. atroseptica* could not grow at higher temperatures. *E. chrysanthemi* strains can grow at 39°C but most *E.carotovora* var. *carotovora* strains could not do so.

2.6.4 Biochemical and physiological characterization

Biochemical tests are carried out to detect the presence of certain enzymes like cytochrome–C, oxidase, catalase, arginine dihydrolase and lipase, pigment production, levan production, gas production from nitrate, glucose oxidation etc. which help in the characterization and identification of the pathogen isolates.

Suslow *et al.* (1982) developed a simple test i.e. solubility in 3per cent KOH solution and its result correlated well with the staining reaction. Gomez-Caicedo *et al.* (2001) observed the formation of viscous mucilaginous suspension on addition of drop of 3per cent KOH and confirmed that the rhizome rot pathogen was gram negative.

Based on the presence or absence of cytochrome oxidase, Kovacs (1956) classified aerobic or facultative anaerobic bacteria into two groups, oxidase positive and negative. He attempted to differentiate between *Pseudomonas* and classified *Pseudomonas solanacearum* as oxidase positive.

The bacterium *E.carotovora* p.v. *atroseptica, E.carotovora* p.v. *carotovora* and *E. chrysanthemi* were found to be oxidase negative. (Lelliot, 1974; Bradbury, 1977 a, b; Dickey, 1979; Dickey and Victoria, 1980; Dickey and Kelman, 1981; AngelesRamos *et al.*, 1982; Bradbury, 1986; Cappuccino and Sherman, 1992; Nagaraj *et al.*, 2002 a, b; Rani *et al.*, 2002).

Erwinia spp. answered positive in catalase reaction (Lelliot, 1974; Bradbury, 1986; Cappuccino and Sherman, 1992). Both *E.carotovora* var. *carotovora* and *E. chrysanthemi* were found to be catalase positive. (Bradbury, 1977a,b). Dickey (1979) studying the physiological and biochemical properties of *E. chrysanthemi* found that the strains were catalase positive. *Erwinia* strains from *Musa paradisiaca* were catalase positive (Dickey and Victoria, 1980). Gomez-Caicedo *et al.* (2001) found that the causal organism of vascular rot and wilt in plantain *(Musa* ABB Simmonds) belonged to *Erwinia* spp. and showed a positive reaction for catalase test. The rhizome rot pathogen was found to be catalase positive (Nagaraj *et al.*, 2002a,b and Rani *et al.*, 2002)

The absence of pigment production by *E. carotovora* and *E. chrysanthemi* on King's medium had been reported by many workers (Dickey, 1979; Cappuccino and Sherman, 1992;Gomez-Caicedo *et al.*, 2001). The bacteria also failed to produce brown diffusible melanin pigment.

According to CMI description of plant pathogenic fungi and bacteria, *E.carotovora* var. *carotovora* and *E. chrysanthemi* were found to hydrolyse gelatin rapidly, but not starch. The former hydrolysed casein but the reaction was found to be variable with the latter. Both the species of *Erwinia* hydrolysed gelatin, but not starch. (Dickey, 1979;Bradbury, 1986; Tomlinson *et al.*, 1987; Cappuccino and Sherman, 1992; Gomez-Caicedo *et al.*, 2001;Nagaraj *et al.*, 2002a,b)

Dickey and Victoria (1980) found that the strains of *Erwinia* isolated from *Musa paradisiaca* failed to liquefy gelatin. Senthilvel (2000) observed that *E.carotovora* var. *carotovora* infecting onion bulbs hydrolysed gelatin and casein, but not starch.

Utilization of sodium salts of various organic acids by species of *Erwinia* (*E.carotovora* and *E. chrysanthemi*) was studied by several workers. Cother and Sivasithamparam (1983) found that *E.carotovora* failed to utilize sodium malonate and tartrate, whereas *E. chrysanthemi* utilized them. Studies had proved that both the species utilized organic salts like formate, acetate, citrate, gluconate, fumarate, lactate, malate and succinate, but not oxalate, propionate and benzoate as carbon and energy yielding sources. (Lelliot, 1974;Bradbury, 1977a,b; Dickey, 1979; Dickey and Victoria, 1980; Bradbury, 1986; Cappuccino and Sherman, 1992)

Both the species of *Erwinia viz., E.carotovora* and *E. chrysanthemi* produced H_2S from cysteine medium. (Bradbury, 1977a,b; Bradbury, 1986; Cappuccino and Sherman, 1992). Many workers in their studies on the characterisation of the pathogen causing rhizome rot of banana observed that the pathogen (both *E.carotovora* and *E. chrysanthemi)* produced H_2S from cysteine in peptone water medium (Dickey, 1979; Dickey and Victoria, 1980;Tomlinson *et al.*, 1987)

According to CMI decription of plant pathogenic bacteria, the reaction of *E. carotovora* var *carotovora* to MR test was variable and *E. chrysanthemi* gave negative reaction. In general, *Erwinia* group of bacteria gave negative response to MR test (Cappuccino and Sherman, 1992). Tomlinson *et al.* (1987) found that both the pathogen isolates they characterised (*E. chrysanthemi*) gave a negative MR test.

E.carotovora ssp. *carotovora* did not produce indole, whereas *E. chrysanthemi* produced indole. (Bradbury, 1977a,b; Cother and Sivasithamparam, 1983; Bradbury, 1986). Tomlinson *et al.* (1987) found that *E. chrysanthemi* isolate 1 was indole positive while isolate 2 was negative. Dickey (1979) found that *E.carotovora* did not produce indole, whereas the reaction was variable with *E. chrysanthemi*. Dickey and Victoria (1980) and Chattopadhyay and Mukherjee (1986) found that the soft rot pathogen strains produced indole. The pathogen failed to produce indole (Nagaraj *et al.*, 2002a,b)

Erwinia group of pathogenic bacteria is poor hydrolysers of urea. Urease enzyme is not produced by *E.carotovora* and *E. chrysanthemi* (Lelliot, 1974; Bradbury, 1977a,b; Dickey, 1979; Bradbury, 1986; Tomlinson *et al.*, 1987;Cappuccino and Sherman, 1992; Nagaraj *et al.*, 2002 a,b).

Both the species of *Erwinia viz.*, *E.carotovora* and *E. chrysanthemi* were found to be excellent utilizers of sodium citrate in Simmon's Citrate Agar. (Bradbury, 1977a,b; Cappuccino and Sherman, 1992). Scientists had also confirmed the above result (Dickey, 1979; Dickey and Victoria, 1980; Tomlinson *et al.*, 1987) with soft rot pathogen of banana. Gomez-Caicedo *et al.* (2001) observed that the pathogen causing vascular rot and wilt of plantain failed to produce levan from sucrose.

In general, *Erwinia* spp. ferment fructose, galactose, sucrose, β methyl glucoside, d- methanol, D-mannose, ribose and D-sorbitol but rarely adonitol, dextrin, dulcitol and melizitose. Gas production from D-glucose was positive in the

case of *E. chrysanthemi* and doubtful in case of *E. carotovora* (Cappuccino and Sherman, 1992)

E. carotovora p.v. *carotovora* produced acid from 1per cent concentrations of arabinose, ribose, xylose, glucose, galactose, mannose,fructose, lactose, raffinose, sorbitol, mannitol, β methyl glucoside, but not from glycerol, dulcitol, inulin,adonitol, dextrin or starch. *E. chrysanthemi* differed from *E. carotovora* in that it failed to produce acid from lactose and maltose. But acid was produced in lactose in 7-28 days in many strains (Bradbury, 1977a,b;Cother and Sivasithamparam, 1983; Bradbury, 1986)

Both the species of *Erwinia viz. E. carotovora* and *E. chrysanthemi* which caused the rhizome rot disease of banana were found to produce NH_3 from peptone medium. (Bradbury, 1977a,b; Tomlinson *et al.*, 1987; Gomez-Caicedo *et al.*, 2001)

E. carotovora p.v. *carotovora* could tolerate NaCl upto 5-7 per cent and grow in the medium whereas *E. chrysanthemi* had a maximum level tolerance of NaCl 3-5 per cent (Bradbury,1977a,b)

Characterizing the soft rot bacteria in banana, Dickey (1979) found that the pathogen successfully grew in the medium with 5 per cent NaCl. Similar result was obtained by Shillingford (1974) and Tomlinson *et al.* (1987) in their study.

Gomez-Caicedo *et al.* (2001) found that the vascular wilt pathogen of plantain grew well at 3 per cent and 4 per cent NaCl.

The rhizome rot pathogen of banana failed to utilize arginine by the production of the dihydrolase enzyme. (Dickey, 1979; Tomlinson *et al.*, 1987; Gomez-Caicedo *et al.*, 2001)

Both the species of *Erwinia* causing rhizome rot and wilt of banana were *(E. carotovora and E. chrysanthemi*) found to reduce nitrate to nitrite. ((Dickey, 1979; Tomlinson *et al.*, 1987)

Lipase activity of the rhizome rot causing bacteria has not been studied so far. In general *E. carotovora* was found to show a negative lipase activity, whereas *E. chrysanthemi* gave a positive reaction (Cappuccino and Sherman, 1992).

In general, *E. carotovora* did not produce reducing substances from sucrose, but in the case of *E. chrysanthemi* the reaction varied in different strains (Bradbury, 1977a,b; Cother and Sivasithamparam ,1983). Tomlinson *et al.* (1987) found that of the two isolates of the rhizome rot pathogen, which they characterized, one produced reducing substances from sucrose, while the other did not.

In general, *E. carotovora* was found to be resistant to erythromycin, whereas *E. chrysanthemi* was found to be sensitive (Bradbury, 1977a,b). Among the three classes of soft rot bacteria *viz. E. chrysanthemi, E. carotovora* p.v. *carotovora* and *E. carotovora* p.v. *atroseptica, E. chrysanthemi* alone is sensitive to erythromycin (Graham, 1972; Perombelon and Hyman, 1986). Dickey (1979) reported that *E. chrysanthemi,* the causal agent of rhizome rot of banana was sensitive to (15µg) erythromycin but the sensitivity to penicillin G varied with strains. The pathogen was found to be sensitive to both erythromycin (15µg) and penicillin (2U) (Dickey and Victoria, 1980).

Of the two isolates of *E. chrysanthemi* characterized by Tomlinson *et al.* (1987) both were sensitive to kanamycin (1000µg) but were not sensitive to rifampicin (15µg), colistin (10µg) and vancomycin (5µg). One of the isolates was sensitive to erythromycin (60µg) and penicillin (2 units), but the other was not.

Gomez-Caicedo *et al.* (2001) found out that *Erwinia* was sensitive to tetracycline and streptomycin, but resistant to penicillin.

Both these species of *Erwinia viz. E. carotovora* p.v. *carotovora* and *E. chrysanthemi* were found to produce soft rotting on potato and carrot slices (Lelliot, 1974;Bradbury, 1977a,b; Cother and Sivasithamparam, 1983). Shillingford (1974) tested the pathogenicity of the bacterium on tomatoes, potatoes, carrots and onions. Tomatoes rotted completely within 48 hrs leaving the

skin more or less intact. Potatoes also rotted rather quickly. But tissue breakdown in carrots and onions was slow. It was confirmed therefore that the bacteria was related to the group, which are causal agents of numerous soft rots of storage tissues. *Erwinia* strains from *Musa paradisiaca* gave positive potato soft rot test. (Dickey and Victoria, 1980). The pathogen causing tip over disease of banana caused soft rotting on potato. (Nagaraj *et al.*, 2002a,b).

2.6.5 Molecular characterization

Advances in the fields of molecular biology like plasmid isolation and profiling, Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) and Randomly Amplified Polymorphic DNA (RAPD) could be applied successfully in the detection and identification of plant pathogenic bacteria (Singh, 1998).

Plasmids are autonomously replicating genetic elements existing in multicopies in the bacterial cells in a stable form under non-selective conditions. Plasmids have now been reported in all genera of plant pathogenic bacteria. In plant pathogenic bacteria, the important traits like virulence, production of toxins, enzymes, resistance to antibiotics and host range are coded by plasmids. These traits help plant pathogen in adapting to their environment and competing with other micro flora. Most studies to date have only reported the presence of plasmid DNAs and very little is known about their genetics (Coplin *et al.*, 1982)

One to several resident plasmids have been detected in each of several *Erwinia* sp *viz. E. amylovora, E. chrysanthemi, E. herbicola* (Chatterjee and Starr, 1980) and *E. carotovora* var. *carotovora* (Daughtery, 1978)

Covalently closed circular (CCC) plasmid DNA was isolated from lysates of *E. herbicola* (pBR 322 strain) transformants by equilibrium centrifugation in cesium chloride – ethidium bromide gradients. The CCC DNA of *E. herbicola* (pBR 322) was found by electron microscopy and gel electrophoresis to contain a DNA species the same size as that of pBR322. Electron microscopy of open circular plasmid DNA from putative pBR322 transformants of L-322 revealed that

size classes of molecules. Measurement of these molecules in relation to nicked Col El plasmid DNA (4.2 megadaltons)(Md) which was added to the sample just before electro microscopy showed that a dense band corresponding to a large plasmid of about 26.8±0.6 (n=26) Md predominated along with several rare species of plasmid molecules. Two of these were 40.6±11.1 (n=6) and 6.8±0.1 (n=3) Md (Sparks and Lacy, 1979).

Two cryptic plasmids were purified from cell lysates of *E. chrysanthemi* NCPPB-377, an organism isolated from maize (*Zea mays*) causing bacterial stalk rot by two methods *viz.* Sarkosyl method and method suggested by Guerry *et al.* (1973). Electron microscopy of purified supercoiled plasmid DNA revealed a large PLS1 (50.4±1.9) mega daltons and a small PLS2 (4.8±0.3) mega daltons plasmid. These plasmids were separated by centrifugation on linear log sucrose gradients and were characterized by restriction endonucleases ECoR1, pst1, Sal 1,Hind III and Hind II (Sparks and Lacy, 1980). Rani *et al.* (2002) conducted PCR analysis using specific primers for *Erwinia* for the precise identification of tip over disease pathogen, *E. carotovora* p.v. *carotovora.*

2.7 HOST RANGE OF THE PATHOGEN

Rivera *et al.* (1980) reported that the isolates of *Erwinia* from both banana and maize were pathogenic to maize, potato, pineapple, *Diffenbachia*, *Pennisetum pupureusm*, and *Brachiaria mutica.* The banana strains also infected plantain, Canna, *Capsicum, Poinsettia* and *Begonia.*

Dickey (1981) tested six plant species *viz. Chrysanthemum moriflorum*, *Dieffenbechia amonea, D. maculate, Philodendron, panduriforae, P. sellorum* and *Syngonium podophyllum* by standardized methods for reaction to 383 strains of *E. chrysanthemi* from 35 plant species. Among them, 229 strains of *E. chrysanthemi* and 93 strains of *Erwinia* sp. failed to produce a positive reaction for *Syngonium podophyllum.* Some *Syngonium* strains and the *Caladium* strains of *E. carotovora* ssp. *carotovora* also were positive for *P. panduriforae*, and *P. sellorum*.. Seventy-three strains of the *E. chrysanthemi* from 14 hosts caused slightly positive reactions on the corn leaves.

E. carotovora p.v. *carotovora* found to infect *Canna* sp., *Capsicum* sp., *Brassica* sp., *Colocasia* sp., *Daucus carota, Dahlia* sp., *Gladiolus* sp., *Solanum tuberosum, Xanthosoma sagittifolius, Zea mays, Zingiber officinalis* etc (Bradbury, 1986).

Lakshmanan and Mohan (1991) tested twelve intercrops *viz. Cajanas cajan, Phaseolus vulgaris, Dolichos lab lab, Vigna sinensis, Phaseolus aureus,Phaseolus mungo, Arachis hypogea, Lycopersicon esculentum, Beta vulgaris, Allium cepa, Brassica oleraceae* var. *capitata, D. carota* and screened them for their reaction to the bacterium. Among the intercrops *L. esculentum, B. vulgaris, A. cepa, B. oleraceae,* var *capitata, D. carota* were readily infected with in 10 days after inoculation.

Nanda *et al.* (1994) observed the occurrence of a bacterial soft rot in ginger caused by *Erwinia carotovora.*

Thwaites *et al.* (2000) found that the isolates of *E. chrysanthemi* were not specific to banana and under experimental conditions caused rotting on potato, tomato and onion, although the natural host ranges of the organism were not known.

The pathogen causing tip over disease of banana was found to cause infection on potato, tomato, carrot, radish, cabbage and mandarin, but the time taken to express symptoms varied (Nagaraj *et al.*, 2002a)

2.8 SURVIVAL OF THE PATHOGEN

Wardlaw (1935) found that the soft rot pathogen occurred in many countries in quite different soils and was apparently able to remain alive saprophytically for years.

First attempt to study the soil survival of soft rotting *Erwinia* sp. was done by Logan (1963). He examined the survival of *E. atroseptica* in soil, where black leg

of potato occurred, by doing dilution plate techniques on a selective medium containing sodium citrate as the carbon source.

Tomlinson *et al.* (1987) found that the rhizome rot pathogen *E. carotovora* var. *carotovora* survived in the soil surrounding the rotting corms, but E. *chrysanthemi* did not. Neither bacterium was isolated from soil away from the root system of diseased plants.

The survival of *E. chrysanthemi* causing pseudostem watery rot on banana, was studied in five soil series *viz.* 3 alluvial, black clay and red clay inoculated with diseased plant residue under laboratory conditions in Cuba during 1984-85. Relationships were found between *E. chrysanthemi* survival on plant residues incorporated in soil, total soluble salts and pH values. The bacteria survived in moderately saline soils with neutral pH for 110-112 days. The pathogen population was reduced with reduction in salt content and pH slightly basic (Rodriguez *et al.*, 1991).

Senthilvel (2000) studied the survival of the soft rot bacterium of onion in artificially inoculated sterilized and unsterilized soil, infected leaves, bulbs and its combinations. The maximum colony counts were observed in the combination of sterilized soil, leaves and bulb sources while the minimum was recorded in sterilized soil ($32X10^6$ cfu ml^{-1}) indicating the role of natural antagonists in suppression of the pathogen.

Nagaraj *et al.* (2002a) reported that the saprophytic survival of plant pathogenic bacteria resulted in their persistance in soil in the absence of growing plants or plant residues. In *in vivo* studies conducted to know the survival of *E. carotovora* ssp. *carotovora* in soil and plant debris at different soil moisture regimes revealed that the bacteria survived for 195 days and 180 days in unsterilized and sterilized soils maintained at 50 per cent Water Holding Capacity (WHC) of the soil. The bacterium survived for 135 days in infected rhizomes incorporated in soil maintained at 50 per cent WHC compared to 105 days under dry conditions.

2.9 VARIETAL SCREENING FOR HOST RESISTANCE

Stover (1959) reported that the incidence of rhizome rot up to 50 per cent in Gros Michel (AAA) variety. In general he found that *Musa* AAB and ABB cultivars were more resistant than AAA varieties. Loos (1962) reported that banana variety Lacatan was immune to the disease while Gros Michel was susceptible. Lacy (1971) reported that Highgate, a dwarf mutant of Gros Michel was susceptible to the disease. Cultivars in AAA Cavendish sub group were generally less affected (Stover, 1972). Shillingford (1974) conducted an experiment to determine the relative pathogenicity of the rhizome rot bacteria on commercial Cavendish cultivars Valery, Robusta and Lacatan. He found that the disease incidence and severity were highest for Robusta, followed by Valery whereas Lactan rhizome was unaffected.

Chatopadhyay (1987) found that the disease seriously affected the Cavendish clone Giant Governor.

Tomlinson *et al.* (1987) screened about ten cultivars maintained in Papua New Guinea Biological Foundation Banana collection and they observed that the diploid cultivars showed significantly high levels of soft rot than the triploid cultivars. The cultivars with ABB genome were virtually unaffected by bacterial soft rot while cultivars with AAA genome showed an intermediate level of disease incidence between triploid ABB and diploid AA cultivars

In Tamil Nadu, the Cavendish bananas alone was found to be suceptible. Among the nine commonly grown banana cultivars screened *viz.* Robusta (AAA), Dwarf Cavendish (AAA), Poovan (AAB), Rasthali (AAB), Monthan (AAB), Virupakshi (AAB), Nendran (AAB), Neyvannan (AAB) and CO-1 (AAB) (Lakshmanan and Mohan, 1991).

Robinson and Manicom (1991) observed that the cultivars, Grand Nain, and Valery were susceptible to *E. chrysanthemi*. Williams and Dwarf Cavendish did not seem to be similarly susceptible.

In South Africa, upto 30 per cent of the plants of Grand Nain (AAA) or Valery (AAA) raised from suckers were affected by *E. chrysanthemi*, whereas tissue cultured plants were not. Cavendish (AAA) and Williams (AAA) were less affected. AA clones were affected more than AAA clones and these more than ABB clones (Buddenhagen, 1994).

Guzman and Sandoval (1996) observed that the hybrids FHIA-01 and FHIA -02 were susceptible to pseudostem soft rot.

Thwaites *et al.* (2000) reported that clones containing the B genome were rarely affected by soft rot. Of the cultivars in the AAA genome group, Lactan (Cavendish sub group) is less susceptible than Valery (Cavendish sub group) and Gros Michel.

2.10 MANAGEMENT OF THE DISEASE

2.10.1. *In vitro* sensitivity of the pathogen to plant protection chemicals, botanicals and bioagents

Many workers had studied about the *in vitro* sensitivity of the rhizome rot pathogen to various plant protection chemicals.

Dickey (1979) reported that *E. chrysanthemi*, the causal organism of rhizome rot disease, was sensitive to (15µg) erythromycin. But the sensitivity to pencillin G varied with strains.

Of the two isolates of *E. carotovora* characterised by Tomlinson *et al.* (1987) both the isolates were sensitive to kanamycin (1000µg). One of the isolates was sensitive to erythromycin (60µg) and pencillin (2 units) but the others were not. Jimenez and Cordoves (1992) isolated three strains of *Pseudomonas* in the rhizophere of apparently healthy plantains. These bacteria were antagonistic *in vitro* to the isolates of *E. chrysanthemi* and *E. carotovora*.

Limon *et al.* (1999) found that the *Trichoderma* could inhibit the pathogen via its antibiotic substances or by breaking down bacterial cell walls by means of enzymes such as chitinases, β 1,3 gluconases, protease, mannases and hydrolases. Gomez-Caicedo *et al.* (2001) studied the *in vitro* sensitivity of the pathogen causing rot and wilt in plantain to various antibiotics using readymade antibiotic discs. He found that the pathogen was sensitive to tetracycline and streptomycin, but resistant to pencillin. Investigations on various management strategies for tip over disease of banana revealed that of the various antibiotics evaluated under *in vitro*, methoxy ethyl mercuric chloride @2000ppm.Copper sulphate $CuSO_4$ @4000 ppm Streptocycline @750ppm and Norfloxin @750ppm were effective in inhibiting the growth of the pathogen (Nagaraj *et al.*, 2002b)

Allirani (1994) studied the effect of water extract of *Ocimum* spp., garlic and cowdung each at concentration of 10, 15, 25 and 50 gl^{-1} in inhibiting the the growth of *Pseudomonas solanacearum* causing the bacterial wilt of ginger, using standard filter paper disc method. Maximum inhibition was obtained in water extract of *Ocimum* at 50 gl^{-1} followed by garlic 50 gl^{-1}.

Garlic extract *(Allium sativum)* was found to inhibit the growth of *E. carotovora* p.v. *carotovora* causing onion wilt and bulb rot (Alice and Sivaprakasam, 1996). Krebs and Jaggi (1999) tested essential oils and water extracts of hemp flowers against *E. carotovora*. The essential oil inhibited the soft rot pathogen in some cases.

2.10.2 Field management of the disease

Stover (1959) reported that antibiotic Agrimycin was not effective in controlling rhizome rot disease under field conditions.

Chattopadhyay (1987) found that the banana corm soft rot caused by *E. chrysanthemi* p.v. *paradisiaca* could be controlled by drenching soil and plants with bleaching powder (2g/litre)or dipping the corms in chlorine water.

Lakshmanan and Mohan (1986) found that of the six fungicides tested against the tip over disease of Cavendish banana under field conditions, Methoxy ethyl mercuric chloride at 0.1 per cent applied to the soil 5-6 times at monthly intervals gave the best control of the disease.

Salazar and Duque (1994) reported that Streptomycin sulphate at 0.5 g and iodine at 6ml l^{-1} water gave better control of *E. chrysanthemi* p.v. *paradisiaca* on plantain than copper oxy chloride at 4g l^{-1} and commercial (veterinary) iodine at 5g l^{-1}.

While investigating the various management strategies for the tip over disease of banana, Nagaraj *et al.* (2002b) conducted two field trials to evaluate the efficacy of various bactericides and antibiotics. They found that antibiotics alone or in combination with copper sulphate or carbendazim were very effective in controlling the disease. Soil drenching with streptocycline either alone or in combination with $CuSO_4$ or K-cycline plus $CuSO_4$ completely suppressed the disease and increased the yield by 143.37per cent. In another field trial, norfloxin plus $CuSO_4$ gave very good control of disease (100per cent) followed by streptocycline plus $CuSO_4$.

Rani *et al.* (2002) conducted a field trial to evaluate the efficacy of various biocontrol agents in controlling the tip over disease pathogen and found that *Bacillus subtilis* was very effective in reducing the disease incidence (100per cent) and increasing yield followed by *Pseudomonas fluorescens.* Water extracts of Citronella (*Cymbopogan* sp) was also effective in controlling the disease. An organic formulation *viz.* modified panchagavya was moderately effective in controlling the disease.

Materials and Methods

3. MATERIALS AND METHODS

The present study, "Etiology and Management of Rhizome rot disease of Banana", was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara, during the period 2001-2002. The pot culture and the field experiments were carried out at Banana Research Station, Kannara.

3.1 ISOLATION OF THE PATHOGEN

3.1.1 Collection of the diseased samples

The infected parts of the diseased plants showing typical symptoms of rhizome rot were collected from the farmers' fields and from the fields of Banana Research Station, Kannara. The affected rhizomes in the initial stages of rotting were collected in sterile polythene bags and were brought to the laboratory. Along with the infected samples, the rhizosphere soil of the infected plants was also collected in sterile polythene bags.

3.1.2 Isolation from the infected plant samples

The collected diseased samples (rhizome bits of banana var. Nendran) were subjected to ooze test to find out the presence of the bacterium. The infected portions with profuse were cut into bits and these bits were surface sterilized with 0.1per cent mercuric chloride for one minute and then washed in three changes of sterile distilled water. These bits were then placed on sterilized glass slide in a drop of sterile distilled water and teased apart to obtain a milky bacterial suspension. This suspension was streaked on solid NA medium in sterile petridishes. The dishes were incubated at room temperature (28 ± 1^{0}C) for 48h to get well-isolated colonies of the bacteria. These single colonies obtained were repeatedly streaked on the same medium to get pure culture of the bacteria.

The culture obtained was streaked on Logan's differential medium for *Erwinia* and incubated at room temperature (28 ± 1^{0}C) for 48h. The colony

characters and morphological characters were studied. The composition of the Logan's media is given in the Appendix 1.

3.1.3 Isolation of the pathogen from the soil

Soil samples were collected from the rhizosphere of the infected plants. The bacterium was isolated from the collected samples using serial dilution plate technique (Johnson and Curl, 1972) using Logan's differential medium for *Erwinia*. The dishes were incubated at room temperature (28±1^{0}C) for 48h. The purple centered bacterial colonies developed were counted and expressed as the number of colony forming units (cfu) per gram of the sample. This is used to estimate the population of the pathogen in the soil. Single colonies obtained were further streaked on the same medium and purified.

3.2 PATHOGENICITY STUDIES

Pathogenicity studies were conducted under laboratory and under field conditions, in order to prove the Koch's postulates.

3.2.1 Pathogenicity tests under laboratory conditions

Healthy suckers of banana variety Nendran were taken, washed well and surface sterilized with 0.1 per cent mercuric chloride for one minute and then washed with three changes of sterile distilled water. A plug of 1cm diameter rhizome tissue was removed to a depth of 2.5cm with a sterile cork borer. A disc of bacterial culture (1 cm diameter) was inoculated into the wound and the removed tissue was inserted. The wound was then covered with moist sterile cotton. In the control, a disc of nutrient agar alone was inserted into the wound, without bacterial inoculum. Three replications were maintained. The inoculated suckers along with the control were incubated in moist chamber in order to maintain the humidity. The inoculated suckers were observed for the development of the symptom of rhizome rot. Reisolation of the pathogen was done on NA medium.

3.2.2 Standardization of the inoculation technique

A pot culture study was carried out to standardize the inoculation technique. Healthy banana suckers were artificially inoculated by different methods and planted in earthen pots of diameter 25 cm containing sterile potting mixture.

T_1 - *Inoculation by Cork boring*

Wounds of about 2.5 cm depth were made on healthy suckers and discs of bacterial growth on nutrient agar medium was inoculated into these wounds and the removed tissue was inserted and covered with wet sterile cotton by spreading a thin film over the wound. These inoculated suckers were planted in pots filled with sterilized potting mixture.

T_2 - *Pinpricking followed by dipping in bacterial suspension*

Pinpricks were made on healthy suckers using a sharp sterile needle and the suckers were dipped in bacterial suspension (O.D value 0.732 at 620nm wavelength) for one hour. These inoculated suckers were planted in pots filled with sterilized potting mixture.

T_3 - *Pouring natural inoculum - bacterial ooze*

Naturally infected rhizomes of banana var. Nendran were collected from the field, cut into pieces and put in a bucket filled with 10 l sterile water for 1hr. Bacterial ooze coming out from the cut pieces was collected and this inoculum was poured around the suckers planted in the pots.

T_4 - *Applying infected rhizome bits around the planted suckers*

Naturally infected rhizome bits collected from the field were cut into small pieces and put around the suckers planted in the pots filled with sterile soil.

T5 - *Control*

Control was maintained without inoculation and each treatment was replicated four times. The inoculated suckers planted in the pots were kept inside the greenhouse and high humidity was maintained. The plants were observed for the development of the symptoms for six months from May to October. Biometric observations were also taken fortnightly.

The method of inoculation, which recorded maximum infection, was selected for further studies. The pathogen was re-isolated from the artificially inoculated host plant, which showed typical symptoms of rhizome rot. Morphological characters of the reisolated colonies of the isolates were compared with that of the original isolates to confirm the identity.

3.3 SYMPTOMATOLOGY

Symptomatology of the disease was studied both under natural and artificial conditions.

3.4 CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

Characterization and identification of the pathogen was done according to the methods recommended by "The Manual of Microbiological Methods" published by the Society of American Bacteriologists (1957) and by Laboratory Methods in Microbiology (Harrigan and Mc Cance, 1966). The composition of the media used for various tests are given in the Appendix I.

3.4.1 Morphological and cultural characterization

The colony morphology was studied from a 48h old culture of the bacterium grown on nutrient agar medium. The cells were stained for gram reaction using Hucker's modified method of gram staining (Hucker and Corn, 1923).

Motility of the bacterium was tested using cavity slide. A drop of the bacterial suspension was placed on a clean cover slip. The cover slip was then carefully inverted and placed over the cavity slide such that the drop of bacterial suspension was hanging into the cavity of the slide. It was observed under the microscope to observe the motility of the bacterium.

3.4.1.1 Growth of the bacterium on Logan's differential medium for Erwinia

The nature and extent of growth, colour, shape, type of margin and slime production of the colonies were studied on Logan's differential medium for *Erwinia* (Logan, 1966). A loopful of the dilute suspension of 48 h old culture of the bacterium was streaked on Logan's media and incubated for 48h at room temperature (28 ± 1^{0}C). Isolated colonies were observed for the following characters like size, pigmentation, form, margin, elevation etc. (Cappuccino and Sherman, 1992).

1. Size – Pinpoint, small, moderate or large
2. Pigmentation -Colour of the colony
3. Form – Shape of the colony
    a. Circular – Unbroken peripheral edge
    b. Irregular – Indented peripheral edge
    c. Rhizoid – Root like spreading growth
4. Margin – The appearance of the outer edge of the colony
    a. Entire – Sharply defined, even
    b. Lobate - Marked indentations
    c. Undulate – Wavy indentations
    d. Serrate – Tooth like appearance
    e. Filamentous – Thread like spreading edge
5. Elevation – The degree to which colony growth is raised on the agar surface
    a. Flat – Elevation not discernible
    b. Raised – Slightly elevated
    c. Convex – Dome shaped elevation
    d. Umbonate - Raised with elevated, convex central region
6. Mucoid nature – Amount of slime produced
    a. Mucoid b. Non-mucoid

3.4.1.2 Growth of bacterium in nutrient broth

For studying the growth of the bacterium in liquid medium, 5 ml of sterilized nutrient broth taken in a test tube was inoculated with a loopful of 48 h old bacterial culture grown on nutrient agar medium. Uninoculated broth was maintained as the control. The inoculated medium was shaken daily. The growth of the isolates was measured after 48 h using a 'Spectronic 20' colorimeter adjusted to a wavelength of 620 nm.

3.4.1.3 Growth of the bacterium on different solid media

Nature of growth colour, size, shape, and type of margin, slime production and fluidity of the bacterial colonies were studied on eight different solid media. The media used and their compositions are given in the Appendix 1. A loopful of the dilute suspension of the bacterial isolate was streaked on different solid media and incubated at room temperature. Observations were taken on mean diameter of single colonies after 24, 48, 72 and 96 h of inoculation at room temperature (28 ± 1^{0}C). The media used were Potato Dextrose Agar medium (PDA), Yeast Extract Dextrose Calcium Carbonate Agar medium (YDC), Nutrient Agar (NA), King's B medium (KB), Sucrose Peptone Agar medium (SPA), Thornton's Agar medium (TA), Meat Extract Agar medium (MEA) and Logan's medium. Three replications were maintained.

3.4.2. Physiological characterization

3.4.2.1 Growth of the bacterium at different pH

Nutrient broth with varying pH *viz.*, 2,3,4,5,6,7,8 and 9 were used for the study. The bacterium was inoculated into the broths adjusted to the above mentioned pH levels. The growth of the isolate was measured in terms of optical density of the broth (OD value) after 24, 48, 72 and 96 h using Spectronic 20 colorimeter adjusted to a wavelength of 620 nm. Three replications were maintained and an uninoculated broth served as the control.

3.4.2.2 Growth of the bacterium at different temperatures

The growth of the bacterium was studied at varying temperatures of 5,10,15,20,28,37 and 40^{0}C. A loopful of the 48 h old bacterial culture was inoculated into nutrient broth and incubated at different temperatures mentioned above. The growth in terms of the optical density of the broth (O.D value) was measured after 24, 48,72 and 96h of incubation using Spectronic 20 colorimeter adjusted to a wavelength of 620 nm. Three replications were maintained and an uninoculated broth served as the control.

3.4.2.3 Oxygen requirement

To determine whether the bacterium is aerobic or anaerobic, nutrient agar columns containing bromocresol purple in test tubes were inoculated by stabbing with 48 h old bacterium culture using a straight inoculation needle. To create an anaerobic condition, the agar surface in one of the tubes was sealed with sterile liquid paraffin oil to a depth of 1cm. All the tubes were incubated at room temperature and observations were recorded.

3.4.3 Biochemical characterization

3.4.3.1 Solubility in 3 per cent KOH

Two drops of 3 per cent KOH were placed on the centre of a glass slide. A loopful of 48 h old bacterial growth was mixed with the alkali kept on the slide with rapid circular agitation. After five to eight seconds, the loop was alternately raised or lowered first off the slide surface to detect the presence or absence of viscous strand formation. (Suslow *et al*, 1982).

3.4.3.2 Kovac's oxidase test

Ready to use oxidase disc from Hi media, Mumbai were used for this test. A disc was placed at the centre of the glass slide. A loopful of 48 h old bacterial

culture was taken and ruffed on the disc. Time taken for the development of purple colour, if any was noted (Kovacs, 1956).

3.4.3.3 Catalase test

To detect the production of catalase by the bacterium, a loopful of 48 h old culture was smeared on the glass slide and covered with few drops of 3 per cent hydrogen peroxide and immediately observed for any gas production. The production of gas bubbles indicated catalase positive reaction.

3.4.3.4 Pigment production

The production of the water-soluble pigment by the bacterium was studied on King's B agar (King *et al*, 1954) containing 1 per cent tyrosine. The isolate was streaked on the medium plated on sterile petridishes and incubated at room temperature (28 ± 1^{0}C). After 48 h, the culture was examined under UV light for flourescent or brown melanin pigmentation.

3.4.3.5 Starch hydrolysis

The ability of the bacterium to hydrolyse starch was tested using starch medium containing 0.2 per cent soluble starch. The 48 h old bacterial culture was spot inoculated on the medium plated on sterile petridishes. After four days of incubation at room temperature, (28 ± 1^{0}C) hydrolysis was tested by pouring Lugol's iodine over the plate. A colourless zone around the bacterial growth in contrast to the blue background of the medium indicated positive starch hydrolysis.

3.4.3.6 Utilization of organic acids

Sodium salt of five organic acids *viz* sodium acetate, sodium benzoate, sodium citrate, sodium succinate and sodium oxalate were used for the study. One per cent of the sodium salts of the organic acids were added to the basal medium for utilization of organic acids with bromothymol blue as indicator. The isolate of the bacterium (48 h old) was inoculated in slants in triplicate and incubated at

room temperature (28±1^{0}C). Uninoculated control was also maintained. Observations were recorded at regular intervals for a period of one month.

3.4.3.7 Production of hydrogen sulphide

The ability of the bacterium to produce hydrogen sulphide was tested using peptone water medium. Five ml of the medium was dispensed in test tubes and autoclaved. Lead acetate paper strips of 5x50mm size were prepared by soaking them in super saturated solution of lead acetate. These strips were inserted aseptically between the plug and inner wall of the tubes hanging just above the broth. The tubes inoculated with 48 h old culture of bacterium were incubated at room temperature and observations were recorded up to 14 days. Blackening of the lead acetate impregnated strips indicated liberation of hydrogen sulphide.

3.4.3.8 Methyl red test (MR test)

Methyl red broth was used for the test. The medium was dispensed in five ml aliquots in tubes and sterilized by steaming for 30 minutes for three successive days. The tubes were inoculated with 48h old culture of the bacterium for MR test. The tubes were incubated for seven days at room temperature.

A few drops of methyl red (0.1g dissolved in 300 ml of 95 per cent ethyl alcohol and diluted to 500 ml with distilled water) was added to the culture tubes. A distinct red colour indicated positive methyl red reaction and an yellow colour with the methyl red indicator is regarded as negative reaction.

3.4.3.9 Gelatin liquefaction

Nutrient gelatin medium was used for the purpose. Stab method was used for the test. Gelatin was mixed together with all the other ingredients and heated over a water bath until the gelatin was dissolved. The medium was dispensed in test tubes to a depth of about 5 cm and sterilized at 4.5 Kg pressure for 20 minutes. Observing it for 2 days checked the sterile condition of the medium. These gelatin columns were stabbed with a straight inoculation needle charged with 48 h old

culture of the bacterium. The tubes were incubated and observed for the liquefaction of the get column at regular intervals up to one month.

3.4.3.10 Production of indole

Tryptone broth medium was used for this test. The medium was dispensed in tubes and autoclaved. Gnedza oxalic acid test strips were used for detecting indole production. Filter paper strips of size 5x50mm were soaked in warm saturated solution of oxalic acid and cooled. When the strips got covered with oxalic acid crystals, they were dried at room temperature and used without sterilizing.

The tryptone broth tubes were inoculated with the bacterium in triplicate and oxalic acid strips were inserted into the tube, by the side of the plug, incubated and observed regularly for 14 days. Change in colour of oxalic acid crystals on test strip to pink or red indicated indole production.

3.4.3.11 Caesin hydrolysis

Test medium containing skimmed milk powder was prepared and sterilized. Sterilized medium was then poured in petridishes and single line streak of the bacterial culture was made on the medium. Control was maintained without inoculation and the results were recorded (Aneja, 1993). The presence of a clear zone around the bacterial growth indicated positive reaction.

3.4.3.12 Urease production

The medium of Christensen's agar (Christensen, 1946) was used for this test. Ninety ml aliquots of the medium were dispensed in 250 ml conical flasks and autoclaved. To each flask, 10 ml of 20 per cent sterile urea solution was added. The urea solution was sterilized by steaming it for half an hour. Five ml of the media was dispensed into sterile test tubes and slants were prepared. The slants were then inoculated with 48h old test culture and observations on colour change

were recorded for 15 days at regular intervals. Colour change of the medium from yellow to red gave positive indication of urease activity.

3.4.3.13 Citrate utilization

Simmon's citrate agar as used for the study. Hundred ml of the media was prepared and poured into test tubes (about 5 ml in each tube) and stabbed with the test culture. The stabbed cultures were incubated at room temperature for 48 h. Observations on colour change were taken for seven days. A change in colour from green to blue indicated positive reaction.

3,4.3.14 Production of levan

Single colonies of 48 h old bacterial culture were streaked on King's B agar plates supplemented with five per cent sucrose. After incubation at room temperature for 48 h, they were observed for levan production as indicated by large, white, domed and mucoid colonies (Hayward, 1964).

3.4.3.15 Production of reducing substances from sucrose

Two solutions were used for the test. Solution A was prepared with 10g peptone and 17g agar in 800ml distilled water. Solution B with 40g sucrose in 200ml distilled water. Solution A was autoclaved and solution B was steam sterilized for half an hour. Later solution B was added to cooled solution A. The medium was poured into sterile petri plates. The inoculum was transferred to the medium by spot inoculating with a loop. The plates were incubated for 48 h at 28 ± 1^{0}C. After 48 h of incubation each plate was flooded with 10ml of Benedict's reagent. Incubated at 60^{0}C for 30-45 minutes. Positive reactions produced an orange zone on a blue background.(Dickey and Kelman, 1980).

3.4.3.16 Production of ammonia

Peptone water medium was used for this test. The bacterium was inoculated into the medium and incubated for 48 h. The accumulation of ammonia was

detected by adding 2-3 drops of Nessler's reagent, which gave a brown to yellow precipitate with ammonia.

3.4.3.17 Nitrate reduction test

Five ml of nitrate reduction test medium was dispensed to test tubes and autoclaved at 121^{0}C for 15 minutes. The 48 h old bacterial culture was inoculated into the medium and was incubated at room temperature (28±1^{0}C) for 48h. The observation on gas production was noted (Schaad, 1992).

3.4.3.18 Carbohydrate Utilization

Modified Hugh and Leifson's method (Hugh and Leifson, 1953 and Hayward, 1964) was used for the preparation of the basal medium. To the basal medium 1 per cent carbohydrate solution steamed in pressure cooker for half an hour was added and the molten media was poured @ 5 ml per test tube and 48h old bacterial culture was inoculated into the media and incubated at room temperature. To provide anaerobic condition, a set of the inoculated tubes was sealed with sterile liquid paraffin oil to a depth of 1cm. Observations were taken at 24h intervals upto one week. Change in the colour of the medium to yellow indicated positive utilization of the carbon compounds with acid production. The sugars used were maltose, dulcitol, glycerol, ribose, mannitol, lactose, sucrose, galactose, glucose and sorbitol. Observation on gas production was also noted..

3.4.3.19 Lipase activity

Sierra's medium (Sierra, 1957) was used for the study. Ten per cent stock solution of sterilized Tween 80 was added @ 10 ml per 100ml of molten basal medium. It was then poured into sterile petriplates. Using a loopful of 48h old inoculum, four radial streaks were made on the medium on the plates. Plates were incubated at room temperature for seven days, examined daily for the formation of a dense precipitate around the bacterial growth, which indicated positive lipase activity.

3.4.3.20 Arginine dihydrolase reaction

Thornley's semisolid medium (Thornley, 1960) was used for this test. Five ml of the medium was dispensed in test tubes and autoclaved. The medium was stabbed with 48 h growth of the test bacterium and sealed with sterile liquid paraffin to a depth of 1cm. The tubes were incubated for 1 week at room temperature (28 ± 1^{0}C) and observed daily for colour change. A change in the colour of the medium to red indicated positive arginine dihydrolase activity.

3.4.3.21 Sensitivity to antibiotics

Nutrient agar medium with 1 per cent dextrose was prepared and sterilized. The medium was poured in sterile petridishes and was seeded with 48h bacterial culture. Sterile filter paper discs dipped in antibiotic solution of required concentrations were placed on the surface of the seed layer and incubated at room temperature (28 ± 1^{0}C) for 48h. Observations were recorded. Erythromycin, penicillin and streptomycin at 25, 50 and 100 ppm were tried. A control was also maintained.

3.4.3.22 Growth in sodium chloride (NaCl)

Peptone water with 3 per cent and 4 per cent NaCl respectively were used for the test. The medium was dispensed in tubes autoclaved and inoculated with 48h old culture of the bacterium incubated at room temperature for 48 h and observations on the growth were recorded and measured in terms of optical density of the broth (OD value) using Spectronic-20 colorimeter adjusted to a wavelength of 620 nm. Uninoculated broth served as the control.

3.4.4 Potato and carrot soft rot test

Lelliot's method (Lelliot, *et al,* 1966) was employed for the test. Slices of washed, peeled and alcohol flamed potato tubers and washed alcohol flamed carrot tubers were placed in sterile petriplates. The surface of the slices was immediately covered with sterile distilled water till the slices were half immersed. A portion of

potato dextrose agar medium with 48 h old good growth of the bacterial isolate was placed in a nitch made at the centre of each slice. The slices were observed for rotting and the uninoculated slices served as the control.

3.4.5 Molecular Characterization by plasmid profiling

The bacterium was characterized by the molecular techniques like plasmid profiling. Composition of buffers and solutions used for the various molecular tests are given in the Appendix I.

Plasmid DNA of the bacterial isolate was extracted by using the following protocol (Santha, 2001)

Procedure

2 ml LB medium containing proper antibiotic inoculated with a single bacterial colony

↓

Overnight incubation on a shaker at 200 rpm at 37^0C

↓

Centrifugation at 13,000 rpm for 1 min.

↓

Supernatant removed carefully.

↓

Cell pellet resuspended in 100ml resuspension buffer.

↓

Treatment with 100 ml lysis buffer and gentle mixing at room temperature.

↓

120 ml of neutralization buffer added, contents mixed gently for 3 min at room temperature.

↓

Centrifugation at 13,000 rpm for I min, supernatant transferred to a fresh 1.5 ml centrifuge tube.

↓

200 ml isopropanol added and mixed for 1 min.

↓

Centrifugation at 13,ooo rpm for 1 min to get DNA pellet.

↓

70*per cent* ethanol (500 ml) wash of DNA pellet followed by air drying.

↓

DNA dissolved in 100ml sterile water. 10ml of plasmid DNA sample was mixed with 5ml of gel loading dye and loaded for gel electrophoresis along with the marker.

3.5. HOST RANGE OF THE PATHOGEN

Investigations on the host range of the pathogen were done on five different host plants. The five hosts selected were those commonly intercropped in banana gardens of Kerala and the wild, related species of banana. The hosts selected were ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) colocasia (*Colocasia* sp.) canna (*Canna* sp.) and heliconia (*Heliconia* sp.). These host plants were planted in earthen pots of 2.5 cm diameter filled with sick soil mixed with bits of infected rhizomes. There were three replications. The plants were observed for six months from the date of planting for the development of any symptoms associated with the rhizome rot disease.

Parallel to the pot culture experiment the host plants were also planted in a rhizome rot sick field maintained at Banana Research Station, Kannara, along with the susceptible banana variety Nendran. Three replications were maintained. In both experiments, the inoculum (infected rhizome bits and other infected plants portions) was applied at the base of the host plants to increase the inoculum pressure. These plants were then observed for six months from the date of planting for the development of any symptoms associated with the disease.

3.6 SURVIVAL OF THE PATHOGEN

A pot culture experiment was conducted to study the survival of the pathogen in the soil. Sterile potting mixture filled in earthen pots of size 25 cm diameter was inoculated with the pathogen by applying natural inoculum and infected plant material. Four replications were maintained. Soil samples were collected form each pot from five cm depth and monthly serial dilution was done in order to find out the viable population of the pathogen in the soil for six consecutive months after inoculation. Logan's differential medium was used for the isolation of the pathogen from the soil.

3.7 REACTION OF BANANA GERMPLASM TO RHIZOME ROT DISEASE

Two hundred and forty accessions of banana maintained in the field gene bank at Banana Research Station, Kannara were screened for the natural occurrence of the disease. The screening was done in the crop planted in 2001 and 2002. Disease incidence was recorded at fortnightly intervals and the percentage infection for each variety was calculated.

3.8 MANAGEMENT OF THE DISEASE

3.8.1 *In vitro* evaluation of different plant protection chemicals against the pathogen

In vitro efficacy of commonly used antibiotics and fungicides in inhibiting the growth of the bacterium was tested by standard filter paper disc method. The details of the chemicals used and their concentration are given below.

Table 1. Fungicides

Sl.No.	Fungicide	Active ingredients	Concentration (per cent)
1	Bordeaux mixture	Copper sulphate and lime	0.5, 1.0, 1.5
2	Calixin 5per cent	Tridemorph	0.05, 0.1, 0.15

3	Bleaching powder	Chlorine	0.5, 1.0, 1.5
4	Fytolan 50WP	Copper oxychloride	0.2, 0.3, 0.4
5	Bavistin 50WP	Carbendazim	0.05, 0.1, 0.15
6	Indofil M – 45 80WP	Mancozeb (Dithiocarbamate)	0.2, 0.3, 0.4
7	Kocide 77WP	Copper hydroxide	0.1, 0.2, 0.3

Table 2. Antibiotics

Sl.No	Antibiotic	Concentration (ppm)
1	Streptocycline	100, 200, 300
2	Erythromycin	25, 50, 100
3	Tetracycline	25, 50, 100
4	Kanamycin	25, 50, 100
5	Ambistryn S	25, 50, 100
6	Ampicillin	25, 50, 100
7	Chloramphenicol	25, 50, 100
8	Bacterimycin	100, 200, 300
9	Gentamycin	25, 50, 100
10	Rifampicin	25, 50, 100
11	Nalidixic acid	25, 50, 100
12	Penicillin	25, 50, 100

The different concentrations of the chemicals were prepared in sterile distilled water. Sterile filter paper discs of 10mm diameter were dipped in the solution and placed over potato dextrose agar in petriplates seeded with 48h old culture of the bacterium. Sterile filter paper discs dipped in sterile water served as control. The plates were incubated at room temperature ($28^{\pm}1^{0}$C) and observations were recorded after 48h.

3.8.2 *In vitro* evaluation of different botanicals and cow dung against the pathogen

The following botanicals were evaluated against the pathogen in *in vitro* by standard filter paper disc method.

Leaves of tulsi, citronella, neem and garlic bulbs were washed in sterile distilled water. The leaves and garlic bulbs were disinfected by keeping under U.V light for about one hour by keeping it upside down every 15 min. Cow dung was autoclaved. Water extract of cow dung (both sterilized and unsterilized) was prepared at 10 per cent, 50 per cent and 100 per cent concentrations. The required concentrations of the botanicals were prepared as given in the table below.

Table 3. Botanicals.

Sl.No.	Botanical	Scientific name	Concentration (percent)
1	Tulsi	*Ocimum sanctum*	10, 50, 100
2	Citronella	*Cymbopogan winferianus*	10, 50, 100
3	Neem	*Azadiracta indica*	10, 50, 100
4	Garlic	*Allium sativum*	10, 50, 100

Sterile filter paper discs of 10mm diameter dipped in water extracts of the above mentioned botanicals and cow dung were placed over PDA seeded with 48h old culture of the bacterium. Sterile filter paper discs dipped in sterile water served as control. The plates were incubated at room temperature and observations were recorded after 48h.

3.8.3 *In vitro* evaluation of different bio agents against the pathogen

The *in vitro* efficacy of two bio agents *viz., Pseudomonas flourescens* and *Trichoderma harzianum* in inhibiting the growth of the bacterium was evaluated.

a. Pseudomonas flourescens

A lawn of the pathogen was prepared on Potato Dextrose Agar medium by pour plate method. *P. flourescens* was spot inoculated on this. The culture was incubated at room temperature for 48h to check whether the bioagent inhibited the growth of the pathogen by producing a clear zone of inhibition around the inoculated area. Control without inoculating the bioagent was also maintained.

b. Trichoderma harzianum

A small disc of PDA along with good growth of *T. harzianum* was made with a sterile cock borer and placed on the lawn of pathogen prepared on PDA in sterile petridishes. The dishes were incubated at room temperature (28±1°C) for three to four days and observed for inhibition zone around the growth of the bioagent. Control with the pathogen alone was also maintained.

In the second method, a small disc of PDA with good growth of *T.harzianum* was made using a sterile cock borer and placed on PDA in a sterile petridish. After two days of incubation at room temperature, (i.e. when the fungus grew and spread the centre of the dish) the pathogen was streaked at the sides surrounding the growth of the fungus and the dishes were kept for incubation for 48h at room temperature to see whether the fungus inhibited the growth of the pathogen. A control without the bio agent was also maintained.

3.8.4 Management of the disease under field condition (*in vivo*)

A pot culture experiment was conducted during May and November 2002, at Banana Research Station, Kannara to study the effect of various treatments on the management of the disease under the field conditions. Healthy banana suckers after inoculating with the pathogen by the standardised inoculation technique (application of natural inoculum) were planted in sterile soil in cement pots of 50 cm diameter. The soil was sterilized by fumigation with 40per cent formaldehyde diluted to 1:25 ratio with water for four days. The experiment was laid out in Completely Randomised Design with seven treatments. The fungicide, antibiotic,

botanical and bio agent, which performed the best under the *in vitro* management study were selected for the management of the disease under field conditions. Along with these treatments, bleaching powder @ 8g plt^{-1}, *Pseudomonas flourescens* (commercial formulation) @ 7g plt^{-1}, lime @ 500g plt^{-1} were also applied to study their effect in the disease management. The details of the experiment are given below.

Design	-	Completely Randomised Design
Variety	-	Nendran
Treatments	-	Seven
Replications	-	Three
No. of plants/ replication	-	Four

Treatments

T_1 - Bleaching powder 8g plt^{-1}

T_2 - *Pseudomonas flo*urescens (Commercial formulation) 7g plt^{-1}

T_3 - Lime 500g plt^{-1}

T_4 - Fytotan 0.4per cent

T_5 - Streptocycline 300ppm

T_6 - Garlic 100per cent

T_7 - Control

The suckers were artificially inoculated with the pathogen by standardised method of inoculation (application of natural inoculum. The treatments were imposed one and three months after planting. Observations on disease incidence (ie. foliage symptoms and extent of rhizome rot based on rhizome rot index and biometric characters were taken upto 6-7 months. The rhizome rot index scale developed by Loos (1962) was followed.

Table 4. Rhizome rot index scale

Score	Symptom
1	No rot
2	Slight rot
4	Fair degree of rot (twice index 2)
8	Heavy degree of rot (twice index 4)
16	Whole rhizome rotted

Biometric characters like number of leaves, collar girth and height of the plant were recorded at monthly intervals. Foliage symptoms like yellowing and flaccidity were also noted. Destructive sampling was done after 6 months and extent of rot of each rhizome was recorded.

3.9 STATISTICAL ANALYSIS

Observations under each experiment were tabulated and analyzed statistically using MSTAT C package (Freed, 1986).

Results

4. RESULTS

The results of the study on "Etiology and Management of the Rhizome rot disease of Banana" undertaken in the Department of Plant Pathology, College of Horticulture, Vellanikkara and Banana Research Station, Kannara are presented below.

4.1. ISOLATION OF THE PATHOGEN

4.1.1. Isolation from the infected plant samples

The infected rhizomes of banana var. Nendran showing typical symptoms of rhizome rot disease were collected from the farmers fields and fields of Banana Research Station, Kannara. These rhizome bits produced bacterial ooze when subjected to ooze test confirming the presence of the bacterium. Standard method of the isolation of the bacteria *viz.*, tissue maceration followed by streaking yielded bacteria, which was isolated on NA medium. Small, pale white colonies were selected and purified by repeated streaking on the same medium. The culture obtained was maintained by sub culturing at fortnightly intervals.

The bacterial culture was then streaked on plates with Logan's differential medium for *Erwinia* and purple centered single colonies of size of about 1.5 mm diameter were produced which confirmed the bacterium to be *Erwinia carotovora.*

4.1.2 Isolation of the pathogen from the soil

The pathogen was isolated from the rhizosphere of the infected banana plants by serial dilution plate technique using Logan's differential medium for *Erwinia.* Purple centered colonies with mean diameter of about 1.5 mm were obtained after incubation at room temperature (28 ± 1^{0}C) for 48h. The colonies were counted and further streaked on the medium and purified.

4.2. PATHOGENICITY STUDIES

4.2.1. Pathogenicity tests under laboratory conditions

Under laboratory conditions, the artificially inoculated banana suckers showed rotting symptoms after eight days of inoculation. The area surrounding the point of inoculation became discoloured, rotten and emitted a foul smell. The rhizome eventually became internally sunken with dark brown or yellow water soaked areas surrounded by dark rings (Plate 1). No symptom of decay was observed in the control. Reisolation of the pathogen from the inoculated tissue was done on NA medium. The isolated bacterium was further streaked on Logan's differential medium and purple centered colonies, similar to the original isolate, were obtained.

4.2.2.Standardisation of the inoculation technique

Koch's postulates were successfully proved on banana variety Nendran. Before inoculation, the inoculation technique was standardized. The inoculated plants showed typical disease symptoms *viz.* flaccidity, wilting and yellowing two months after inoculation. The pathogen was isolated from the inoculated plants and was found to be same as the original isolate used for inoculation.

Inoculation technique was standardized on banana variety, Nendran by conducting different inoculation techniques *viz.*, cork boring, pinpricking, application of natural inoculum (bacterial ooze) and infected rhizome bits around the planted suckers. The results of the experiment are given in the Table 5. The biometric observations of the inoculated plants and healthy plants taken for two months after planting at fortnightly intervals and compared to study the influence of infection on the growth of banana.

The results of the experiment revealed that the infection and mortality were found to be more in plants inoculated by natural inoculum (bacterial ooze), infected rhizome bits and pinpricking. These three methods were found equally

Table.5 Effect of inoculation on the disease incidence and biometric characters of banana variety, Nendran

Sl. No.	Method of Inoculation	Incubation Period (DAI**)	Percentage Disease incidence	Yellowing	Flaccidity	Biometric characters (Mean)		
						Height (cm)	Collar girth (cm)	Number of leaves
1	Cork boring	60 ^{b}	25 ^{b}	-	+	37.30 ^{c}	11.21 ^{bc}	4.62 ^{ab}
2	Pin pricking	60 ^{b}	50 ^{a}	+	+	39.83 ^{bc}	9.77 ^{b}	4.65 ^{ab}
3	Natural inoculum	53 ^{a}	50 ^{a}	+	+	42.62 ^{b}	13.37 ^{b}	4.00 ^{bc}
4	Infected rhizome bits	56 ^{ab}	50 ^{a}	+	+	43.06 ^{b}	12.15 ^{b}	4.50 ^{b}
5	Control	-	-	-	-	54.12 ^{a}	17.82 ^{a}	5.80 ^{a}

Each observation is a mean of four replications.
The values with different superscripts differ significantly.
** DAI- Days after inoculation.

good to be used as the methods of inoculation. Among these, the application of natural inoculum into the soil after planting was found the most effective since the incubation period (53 days) was the least. Next to this method, application of infected rhizome bits was the best in terms of incubation period (56 days). Both the cork boring and pin pricking methods took 60 days for symptom expression.

The biometric observations clearly revealed the influence of the disease on the growth and development of banana suckers. The height and the collar girth of the control plants were significantly superior (54.12 cm and 17.82 cm respectively) when compared to the inoculated ones. The number of leaves was also less in inoculated plants when compared to the healthy control even though statistically on par. The results revealed that the pathogen has got a negative influence on the normal growth and development of the host plant (Plate 2).

4.3 SYMPTOMATOLOGY

Symptomatology was studied both under natural and artificial conditions on banana var. Nendran. Naturally infected banana plants in the field were observed for the development of disease symptoms. The development of symptoms depended on the stage of growth of the plant and the severity of infection. Newly planted rhizomes when infected failed to sprout and young infected plants exhibited stunted growth and yellowing. In mature plants, the disease was noticed when the plants are 5-6 months old. The first visible symptom was the flaccidity of leaves followed by yellowing and buckling of the leaves. The rhizome showed discolouration, soft rotting and emitted a foul smell from the rotten tissue (Plate 3, 4, 5). When the decay became extensive, the pseudostem became weakened, and it broke at the corm region by wind or even by the bunch weight. In some cases, the emerging daughter suckers were also affected. The above ground symptoms appearing on the plant could be very well correlated with the extent and region of rotting of the rhizome.

In the case of artificially inoculated plants, about 25per cent of suckers rotted immediately after planting. Typical disease symptoms *viz*. yellowing and flaccidity of leaves were produced after 1 1/2 – 2 months after inoculation.

Yellowing was first noticed on lower leaves. The cigar leaf failed to open and became flaccid. The pseudostem became weakened and was detached from the corm area by a gentle push (Plate 6). The transverse section of the rhizome showed that, the rhizome was completely rotten, with water-soaked light and dark brown areas with a dark brown border. The decayed rhizomes emitted a foul smell. Fifty per cent of the inoculated plants died within two months after inoculation.

4.4 CHARACTERIZATION AND IDENTIFICATION OF THE PATHOGEN

4.4.1. **Morphological characterization**

On NA medium, the growth of the bacterium was observed after 48 h of incubation at room temperature. Pale white circular colonies were observed. The colonies were slightly raised and mucoid.

The bacteria were gram-negative short rods when viewed under the oil immersion objective of a microscope.

The motility of the bacterial cells were observed under the microscope using cavity slide.

4.4.2. Cultural characterization

4.4.2.1.Growth of the bacterium on Logan's differential medium for Erwinia

The bacterium was streaked on petriplates with Logan's differential medium for *Erwinia* and the growth of the bacterium was observed after 48h of incubation at room temperature (28 ± 1^{0}C). Small circular colonies with purple centre were produced. These colonies were slightly raised with a little slime production. The mean diameter of the individual colonies was 1.5 mm. (Plate 7)

Plate 1. Pathogenicity test (*in vitro*)-Symptom on suckers

Plate 2. Pathogenicity studies – Comparison of growth characters
1.Cork boring 2.Pin pricking 3.Natural inoculum
4.Infected rhizome bits C. Control

Plate 3. Aerial symptoms of rhizome rot disease

Plate 4. Flaccidity and yellowing of the leaves

Plate 5. Cross section of a rotten rhizome

Plate 6. Disease symptoms on artificially inoculated banana

4.4.2.2 Growth of the bacterium in liquid broth

Growth of the bacterium in nutrient broth at pH 7 was measured by recording the optical density of the broth after incubating at room temperature (28±1°C) for 48h .The optical density was measured in terms of OD value read using 'Spectronic 20' colorimeter adjusted to a wavelength of 620nm. Uninoculated broth served as control. The mean OD value of the isolate was 0.731.

4.4.2.3.Growth of the bacterium on different solid media

The growth of the bacterium on eight different solid media was compared and the results are presented in the Table 6. The growth was measured in terms of diameter of three isolated colonies from each replication. Observations were recorded after 24,48,72 and 96 h of incubation at room temperature (28±1°C).

Among the eight different media tried, maximum growth was recorded on sucrose peptone agar (4.9 mm) after 96 h of incubation at room temperature, which was on par with the growth recorded on King's B medium (4.7 mm). The next best medium was potato dextrose agar (Plate 8). The least diameter was recorded on NA medium. Slime production and fluidity was excellent on King's B, PDA and YDC. Slime production was moderate in SPA and Logan's medium and less in Thorton's medium and Meat Extract Agar medium. As the incubation period increased the colony diameter also increased.

4.4.3 Physiological characterization

4.4.3.1 Growth of the bacterium at different pH

Growth of the bacterium in nutrient broth adjusted to different pH levels was recorded by measuring the optical density of the broth in comparison with uninoculated control after 24,48,72 and 96h of incubaton, using Spectronic 20 colorimeter at a wavelength of 620nm. The results of the study are given in the Table.7.

Table 6.Effect of the different solid media on the growth of the bacterium

Medium	Size and form	Slime / fluidity	Colour	Diameter of isolated colonies in mm after			
				24h	48h	72h	96h
PDA	Medium Circular	++++	Pale white	2.6 ^{f}	3.3 ^{cd}	3.9 ^{bc}	4.4 ^{abc}
YDC	Medium circular	++++	Creamy white	1.6 ^{jk}	2.2 ^{i}	3.0 ^{de}	3.8 ^{bc}
NA	Small circular	++	Pale white	1.5 ^{jk}	2.4 ^{gh}	3.0 ^{de}	3.6 ^{bcd}
KB	Medium circular	++++	Pale white	2.6 ^{f}	3.6 ^{bcd}	4.3 ^{abc}	4.7 ^{ab}
SPA	Medium circular	+++	Creamy white	2.5 ^{fg}	3.4 ^{ed}	4.5 ^{abc}	4.9 ^{a}
TT	Small circular	++	Pale white	2.2 ^{i}	2.9 ^{de}	3.1 ^{de}	3.7 ^{bcd}
ME	Small circular	++	Creamy white	2.0 ^{i}	2.4 ^{gh}	3.4 ^{cd}	3.9 ^{bc}
LDM	Small circular	+++	Purple	1.8 ^{ij}	2.0 ^{i}	2.8 ^{de}	3.1 ^{de}

The values with different superscripts differ significantly.

Slime/ fluidity- +: very little, ++: little, +++: medium,++++: high.

PDA- Potato Dextrose Agar; YDC- Yeast extract Dextrose Calcium carbonate Agar; NA- Nutrient Agar;KB- King's B Agar; SPA-Sucrose Peptone Agar;TT-Thornton's Agar; ME- Meat Extract Agar;LDM-Logan's Differential Medium.

Each observation is a mean of 3 replications.

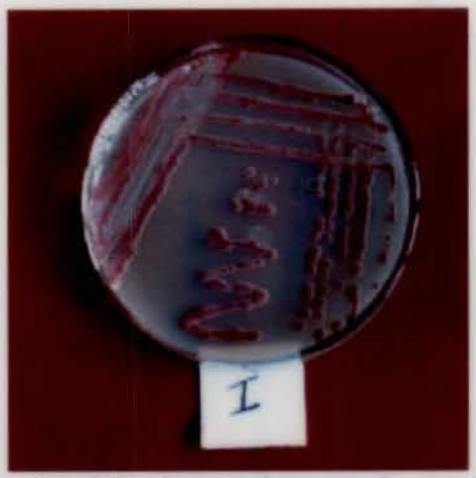


Plate 7. Growth of the bacterium on Logan's medium

Plate 8. Growth of the bacterium on SPA, King's B, PDA (Left to right)

Plate 9. Utilization of organic acids A. Succinate B. Acetate. D. Citrate C. Control

Among the different pH levels tested the maximum growth was recorded at neutral pH 7, which was followed. by pH 6, 8 and 5.The trend was similar at 24, 48, 72 and 96 h of incubation. The growth of the bacterium was very poor at pH below 5 and above 8.

Table. 7. The effect of different pH on the growth of the bacterium

pH	O.D. value of the broth after			
	24h	48h	72h	96h
2	0.013 ^{mn}	0.027 ^{kl}	0.059 ^{jk}	0.060 ^{jk}
3	0.021 ^{lm}	0.056 ^{jkl}	0.058 ^{jk}	0.060 ^{jk}
4	0.083 ^{ij}	0.197 ^{hij}	0.206 ^{hi}	0.210 ^{hi}
5	0.231 ^{h}	0.306 ^{fg}	0.321 ^{efg}	0.333 ^{ef}
6	0.542 ^{cd}	0.640 ^{abcd}	0.681 ^{abc}	0.697 ^{abc}
7	0.612 ^{bcd}	0.718 ^{ab}	0.762 ^{a}	0.774 ^{a}
8	0.332 ^{ef}	0.430 ^{e}	0.452 ^{de}	0.466 ^{cde}
9	0.017 ^{lm}	0.028 ^{kl}	0.058 ^{jk}	0.063 ^{jk}

Each observation is an average of 3 replications.

The values with different superscripts differ significantly.

4.4.3.2 Growth of the bacterium at different temperature

Growth of the bacterium at different temperature was studied and the results are presented in the Table.8.The maximum growth was recorded at 28^{0}C (OD value 1.367) after 96 h of incubation, which was followed by the growth at 28^{0}C at 72h (1.222) which was on par with the growth at 28^{0}C at 48h (1.168). The growth at 37^{0}C at 24, 48, 72 and 96 h of incubation were 0.660, 0.802, 0.854 and 0.951 respectively. The growth was very poor at lower temperatures of 5^{0}C and 10^{0}C.

Table 8. The effect of different temperature on growth of the bacterium

Temperature ^{0}C	O.D. value of the broth after			
	24h	48h	72h	96h
5	0.272 ^{mn}	0.289 ^{mn}	0.310 ^{mn}	0.321 ^{mn}
10	0.235 ^{n}	0.270 ^{mn}	0.346 ^{mn}	0.389 ^{m}
15	0.604 ^{kl}	0.742 ^{ghij}	0.775 ^{ghi}	0.794 ^{gh}
20	0.697 ^{hijkl}	0.822 ^{gh}	0.983 ^{de}	1.083 ^{cd}
28	0.691 ^{hijkl}	0.168 ^{bc}	1.222 ^{b}	1.367 ^{a}
37	0.660 ^{ijkl}	0.802 ^{gh}	0.854 ^{fg}	0.951 ^{ef}
40	0.573 ^{l}	0.613 ^{jkl}	0.652 ^{ijkl}	0.716 ^{hijk}

Each observation is the mean of 3 replications.
The values with different superscripts differ significantly.

4.4.3.3 Oxygen requirement

The pathogen grew and changed the colour of nutrient dextrose agar medium from purple to yellow at a slow and fast rate in tubes with and without molten agar seal indicating that it can grow both aerobically and anaerobically.

4.4.4. Biochemical characterization

4.4.4.1 Solubility in 3 per cent KOH

When the bacterial suspension was mixed with the alkali and the loop was just raised and lowered just off the slide, viscous strand formation was noticed confirming the gram-negative nature of the bacterium.

4.4.4.2 Kovac's Oxidase test

A loopful of inoculum was taken and rubbed on oxidase disc. There was no purple colour development, proving that the pathogen was negative for oxidase.

4.4.4.3 Pigment production

The bacterium did not produce flourescent pigment on King's B medium containing one per cent tyrosine. The isolate also did not produce brown diffusible melanin pigment on it. Thus the pathogen failed to produce any water soluble pigment on King's B medium.

4.4.4.4 Catalase test

When mixed with hydrogen peroxide the pathogen produced gas bubbles indicating the catalase positive reaction of the isolate.

4.4.4.5 Starch hydrolysis

The bacterium did not show the ability to hydrolyse starch as indicated by the absence of a colourless zone around the bacterial growth in contrast to the blue background of the medium.

4.4.4.6 Utilization of organic acids

Of the five sodium salts of organic acids tested *viz.* sodium citrate, sodium acetate, sodium succinate, sodium oxalate and benzoate the pathogen utilized sodium citrate, acetate and succinate, while failed to utilize sodium oxalate and benzoate as the source of carbon (Plate 9).

4.4.4.7 Production of Hydrogen sulphide

Filter paper strips, soaked in warm saturated lead acetate, were inserted into the test tubes containing broth inoculated with the bacterium with the lower end of the strip just above the broth. Blackening of the lead acetate impregnated strips was noticed. This indicated the liberation of hydrogen sulphide by the pathogen.

4.4.4.8 Methyl red test (MR test)

Negative reaction for MR test was noticed with the bacterial isolate as evidenced by the absence of distinct red colour in the culture tube when few drops of 0.02 per cent methyl red in fifty per cent alcohol was added.

4.4.4.9 Gelatin liquefaction

The bacterial isolate utilised gelatin and the liquefaction of the gel column was observed within a fortnight indicating positive gelatin hydrolysis reaction.

4.4.4.10 Production of indole

The bacterial isolate was inoculated into the broth and oxalic acid strips were inserted into the tubes by the side of the plug and suspended over the broth and observed for the colour change of the strips. The oxalic acid crystals on the test strips did not turn pink or red. This showed that the bacterium failed to produce indole.

4.4.4.11 Caesin hydrolysis

The isolate was inoculated into the test medium taken in a petridish by making a single line streak on the medium. Following inoculation and incubation, no clear zone of proteolysis was obtained surrounding the bacterial growth, indicating that the isolate failed to hydrolyse casein

4.4.4.12 Urease production

The slants containing Christensen's agar medium was inoculated with the test bacterium. The bacterium showed negative urease activity as indicated by the absence of colour change of Christensen's urea agar from yellow to red.

4.4.4.13 Citrate utilization

Simmon's Citrate agar in the test tube was stabbed with the test culture and observed for the colour change. The colours of the culture medium changed from green to blue. This indicated that pathogen could utilize citrate.

4.4.4.14 Production of levan

The bacterium failed to produce levan from sucrose as indicated by the absence of raised, convex colonies on King's B agar supplemented with five per cent sucrose.

4.4.4.15 Production of reducing substances from sucrose

The bacterium failed to produce reducing substances from sucrose. This was indicated by the absence of orange zone in the culture on the blue background of the Benedict's reagent.

4.4.4.16 Production of ammonia

The accumulation of ammonia in the medium was detected by using Nessler's reagent, which gave a brown to yellow precipitate indicating that the bacterium could produce ammonia (Plate 10).

4.4.4.17 Nitrate reduction test

Nitrate reduction test medium was dispersed into test tubes and the bacterial culture was inoculated into it and observed for gas production. No gas production was observed in the test medium. This indicated negative nitrate reduction reaction by the bacterium.

4.4.4.18 Carbohydrate utilization

Of the ten carbon compounds tested the pathogen produced acid from glucose, ribose, maltose, glycerol, mannitol, lactose, galactose and sorbitol both in aerobic and anaerobic conditions. The isolate failed to utilize dulcitol. The results are given in the Table 9. Gas production was noticed from sorbitol, galactose, maltose, sucrose and ribose as indicated by the breakage of the agar column inside the tube. Acid production from glycerol and lactose was poor and slow (Plate 11).

Table 9. Utilization of carbohydrates by the bacterium

Sugar	Intensity of colour change and gas production after				
	24h	48h	72h	96h	1 wk
Maltose	+	+++	+++	+++	++++
Sorbitol	++	++	+++	++++	++++
Galactose	++	+++	+++	++++	++++
Glucose	+	+	++	++	+++
Mannitol	+	++	+++	+++	+++
Sucrose	++	+++	+++	+++	++++
Ribose	++	+++	++++	++++	++++
Lactose	-	+	+	++	++
Dulcitol	-	-	-	-	-
Glycerol	-	+	+	+	++

Colour intensity: '-'greenish blue; '+' olive green to yellow; '++' yellowish; '+++' slight orange; '++++' bright orange.

4.4.4.19 Lipase activity

Sierra's medium poured into sterile petri dishes was inoculated with the test culture. A dense precipitate around the bacterial growth was observed after 24h of incubation. This showed that the bacterium showed lipase activity (Plate 12).

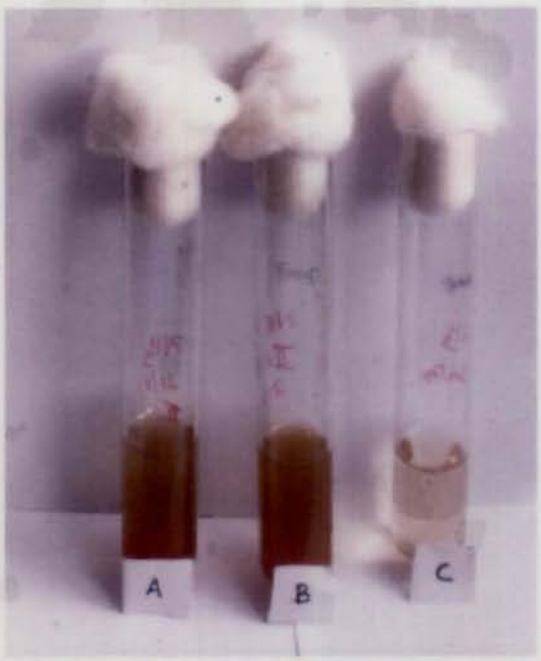


Plate 10. Production of ammonia by the bacterium. C. Control

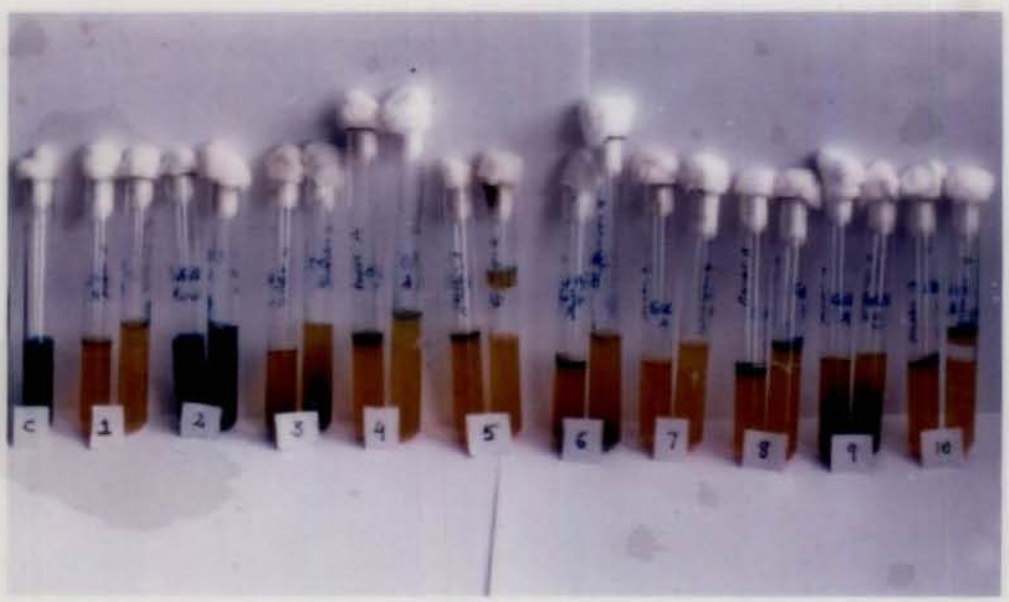


Plate 11. Carbohydrate utilization
C. Control 1. Maltose 2. Dulcitol
3. Glycerol 4. Ribose 5. Mannitol
6. Lactose 7. Sucrose 8. Galactose
9. Glucose 10. Sorbitol
Left tube Aerobic Right tube Anaerobic

4.4.4.20 Arginine dihydrolase reaction

The bacterium failed to turn the colour of Thornley's semi solid Arginine medium to red indicating its ability to hydrolyse arginine.

4.4.4.21 Sensitivity to erythromycin

The isolate was found to be insensitive to erythromycin at 25,50 and 100 ppm indicated by the absence of clear zone around the antibiotic kept on the culture medium seeded with the bacterium.

4.4.4.22 Sensitivity to streptomycin

The isolate was sensitive to streptomycin at 25,50,100 ppm indicated by the presence of clear zone around the antibiotic disc kept on the culture medium seeded wit the bacterium.

4.4.4.23 Sensitivity to penicillin

The isolate was found to be insensitive to penicillin at 25,50,100 ppm indicated by the absence of clear zone around the antibiotic disc, kept on the culture medium seeded with bacterium.

4.4.4.24 Growth in Sodium chloride (NaCl)

Growth was observed in the medium inoculated with the bacterium. The turbidity was measured using 'Spectronic 20' at 620nm wavelength. The O.D value for the isolate was 0.258 after 48h of incubation with 3 per cent NaCl. With 4 per cent NaCl it was 0.223. The results showed that the pathogen could tolerate the salt concentration of 3 and 4 per cent levels.

4.4.5. Molecular characterization by plasmid profiling

Molecular characterization of the pathogen was carried out by plasmid profiling.

Two plasmids were purified from the cell lysates of the isolate of the pathogen. The electrophoresis of the purified, plasmid DNA when viewed through alpha imager revealed that there were two plasmids .One with a molecular weight slightly above that of a linear molecular marker weighing 21 kilo basepairs and the other was on par with a linear molecular marker with 21 kilo basepairs molecular weight (Plate 18).

4.4.6 Potato and carrot soft rot test

The inoculation of the isolate of the pathogen caused blackening and rotting of the potato slices within three to four days of inoculation emitting a foul smell. The pathogen was reisolated from the rotten material. Similarly the carrot slices inoculated with the isolate showed rotting and emitted a foul smell within 3-4 days. The pathogen was reisolated from the rotten carrot slices (Plate 13 and 14).

Table 10. Summary of the morphological, cultural, physiological and bio-chemical characters of the pathogen

Sl.No.	Characters studied	Result
1.	Gram staining	-ve
2.	Potato soft rot test	+ve
3.	Carrot soft rot test	+ve
4.	Motility	+ve
5.	Pigment production	
	a) Water soluble	-ve
	b) Water unsoluble	-ve
6.	Oxygen requirement	
	a) aerobic	+ve
	b) anaerobic	+ve
7.	Solubility in *3per cent* KOH	+ve
8.	Oxidase	-ve
9.	Catalase test	+ve
10.	Starch hydrolysis	-ve
11.	Utilization of organic acids	
	a. Sodium acetate	+ve

	b. Sodium benzoate	-ve
	c. Sodium citrate	+ve
	d. Sodium succinate	+ve
	e. Sodium oxalate	-ve
12.	Production of H_2S	+ve
13.	MR test	-ve
14.	Gelatin liquifaction	+ve
15.	Production of indole	-ve
16.	Caesin hydrolysis	-ve
17.	Urease production	-ve
18.	Citrate utilization	+ve
19.	Levan from sucrose	-ve
20.	Reducing substances from sucrose	-ve
21.	Production of NH_3	+ve
22.	Nitrate reduction test	-ve
23.	Carbohydrate utilization (both aerobic & anaerobic)	
	Maltose	+ve
	Glycerol	+ve
	Ribose	+ve
	Mannitol	+ve
	Dulcitol	-ve
	Lactose	+ve
	Sucrose	+ve
	Glucose	+ve
	Sorbitol	+ve
	Galactose	+ve
24.	Lipase activity	+ve
25.	Arginine dihydrolase reaction	-ve
26.	Sensitivity to antibiotics	
	a. Erythromycin	-ve
	b. Streptomycin	+ve
	c. Penicillin	-ve
27.	Growth at 3 and 4 per cent NaCl	+ve
28	Growth at 37°C	+ve

On the basis of the growth and colony characters on NA medium and Logan's differential medium and various biochemical, physiological and pathogenicity tests, the pathogen causing rhizome rot disease of banana var. Nendran could be identified as *Erwinia carotovora*. Further the characteristics of the bacteria were compared with those described for Erwinia carotovora in Bergy's Manual of Determinative Bacteriology (1953), Schaad (1992) and CMI

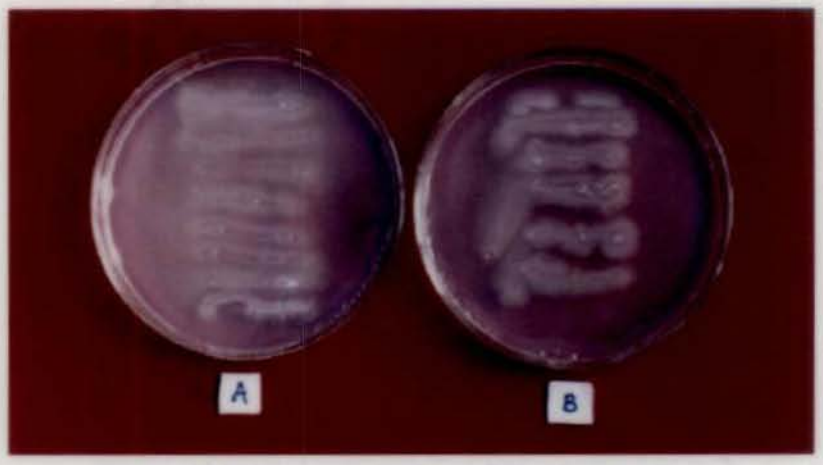


Plate 12 Lipase activity of the bacterium

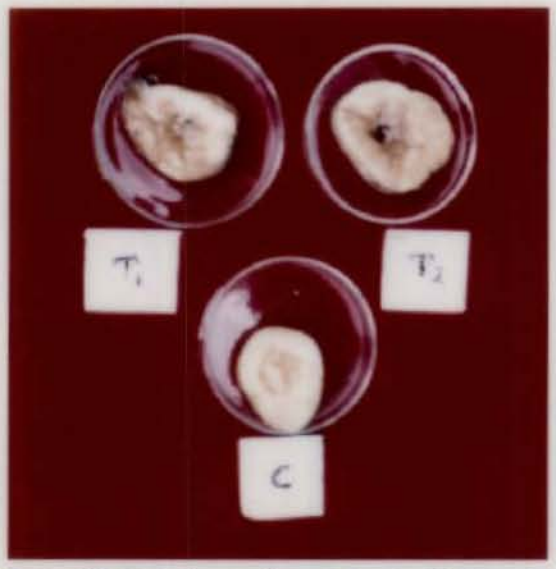


Plate 13 Potato soft rot test C Control

Plate 14 Carrot soft rot test C Control

descriptions of Plant Pathogenic Fungi and Bacteria, thus confirming the identity of the pathogen.

4.5 HOST RANGE OF THE PATHOGEN

The five hosts inoculated with the pathogen, *viz* heliconia (*Heliconia* sp.), canna (*Canna* sp.), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*) and colocasia (*Colocasia* sp.) did not show any symptoms of infection. This revealed that these plants did not act as the host of the pathogen.

4.6 SURVIVAL OF THE PATHOGEN

A pot culture experiment was conducted to study the survival of the pathogen in the soil. Soil samples collected from each pot were subjected to serial dilution for six months at monthly interval from June to November and the population of the pathogen in the soil was estimated. The results are given in the Table 11.

The population of the bacteria was found to be fluctuating depending on the climatic conditions and soil moisture level. Maximum inoculum was found during the month of August followed by June. Least number of colony forming units per gram of the infected soil was found during the month of November. As a whole, the population showed an increase during the rainy season (June-August) and then showed a steep decline in September. Again, it showed slight increase during October and again declined thereafter.

4.8 SCREENING OF BANANA VARIETIES FOR DISEASE RESISTANCE.

Two hundred and forty accessions of banana maintained in the germplasm at BRS Kannara were observed at weekly intervals for disease incidence and mortality due to the disease. The data was collected during 2001 and 2002. Of the 240 accessions screened only 11 were found to be infected by the disease and the percentage loss of the accessions of banana screened are tabulated. (Table 12 and Figure 1).

Table 11. Pathogen population in the soil – soil survival

Month (2002)	Number of c.f.u (colony forming units) per gram of the soil sample (x 10^5)				
	R1	R2	R3	R4	Mean
June	23.60	21.60	29.60	16.60	22.85^{ab}
July	18.00	19.60	18.30	16.60	18.12^{b}
August	25.60	21.00	31.30	24.30	25.55^{a}
September	7.60	10.30	6.60	5.00	7.37^{bcd}
October	12.00	17.30	8.30	11.00	12.15^{bc}
November	4.33	8.66	5.33	9.33	6.91^{cd}

Each observation is an average of 3 replications.

The values with different superscripts differ significantly.

Among the 11 varieties infected, the variety Poppoulu (AAB) was found to be the most susceptible one and recorded cent per cent loss. The varieties Nendran (AAB) and Nedu Nendran (AAB), Gros michel (AAA), Mottakunnan (AAB) recorded 40 per cent mortality.

Table 12. Screening of banana germplasm against rhizome rot disease of banana

Sl No	Varieties infected	Genome	Percent mortality
1	Matti	AA	20
2	Manjeri Nendran	AAB	20
3	Poppoulu	AAB	100
4	Sugandhi	AAB	20
5	Zansibar	AAB	20
6	Padalimariyan	AAB	20
7	Gros Mischel	AAA	40
8	Mottakunnan	AAB	40
9	Nendran	AAB	40
10	Nedu Nendran	AAB	40
11	Robusta	AAA	20

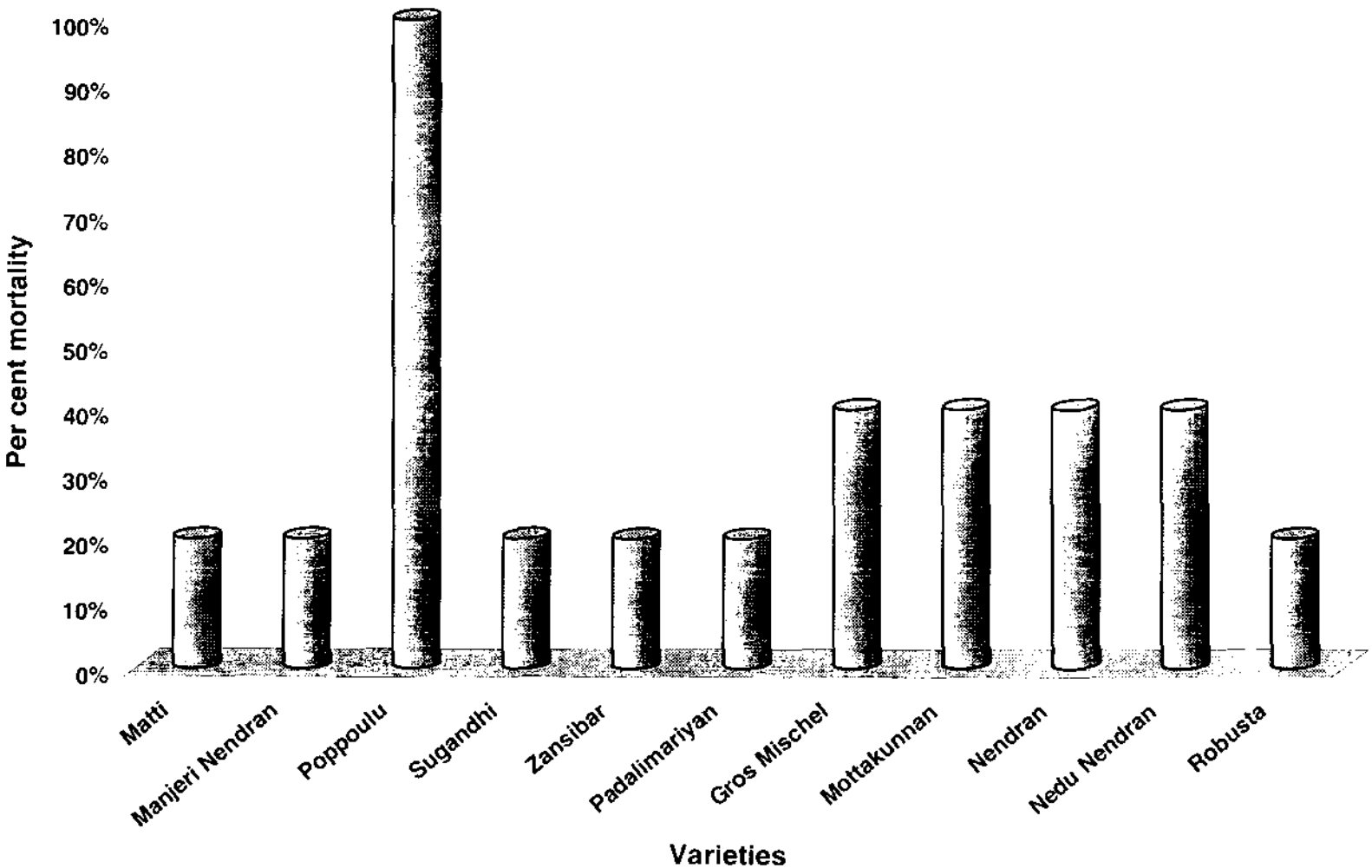
Results showed that the disease was more predominant in accessions with AAB genome.

4.8 MANAGEMENT OF THE DISEASE.

4.8.1 *In vitro* evaluation of different plant protection chemicals against the pathogen.

This study was aimed to assess the efficacy of antibiotics and fungicides in inhibiting the growth of the pathogen.

Fig.1. Per cent plant loss in banana varieties due to rhizome rot disease incidence



a. Effect of Fungicides.

Bordeaux mixture, fytolan, calixin, bavistin, indofil M-45, bleaching powder and kocide at three different concentrations were tested for their effectiveness in inhibiting the growth of the bacterium. The results are presented in the Table 13. Among the fungicides tested, fytolan at 0.4 per cent recorded the maximum inhibition among the fungicides tried (8.7 mm)(Plate 15). This was followed by fytolan 0.3per cent (8.2 mm) followed by concentration 0.2per cent (7.8 mm).

Indofil M-45 and kocide showed inhibition only at higher concentrations (7.0 mm and 7.5 mm respectively). Fytolan and bordeaux mixture were found to inhibit the bacterium at all concentrations tried. Calixin, bavistin and bleaching powder did not show any inhibition on the growth of the bacterium.

Table 13. *In-vitro* evaluation of fungicides against the pathogen

Fungicide	Concentration (percent)	Inhibition zone (mm)
Bordeaux mixture	0.5	06.0^{de}
	1.0	5.8^{ed}
	1.5	6.6^{bcd}
Fytolan	0.2	7.8^{abc}
	0.3	8.2^{ab}
	o.4	8.7^{a}
Calixin	0.05	0
	0.10	0
	0.15	0
Bavistin	0.05	0
	0.10	0
	0.15	0
Indofil M-45	0.2	0
	0.3	0
	0.4	7.0^{bcd}
Bleaching powder	0.5	0
	1.0	0
	1.5	0
Kocide	0.1	0
	0.2	0
	0.3	7.5^{abcd}
Control	Sterile water	0

The values with different superscripts differ significantly.

Each observation is a mean of 3 replications.

b. Effect of Antibiotics.

The effect of twelve antibiotics in inhibiting the growth of the pathogen was studied by standard filter paper disc method. The results are presented in the Table 14. The antibiotics except tetracycline, erythromycin rifampicin, ampicillin and penicillin showed varying levels of inhibition on the growth of the bacterium. Streptocycline at 300 ppm was found to be the best treatment (17.5 mm)(Plate 15), followed by streptocycline at 200 ppm (15.7 mm). Kanamycin 100 ppm (14.6 mm) and streptomycin 100 ppm (14.5 mm) were on par and showed inhibition next to the above two treatments. Chloramphenicol at 100 ppm recorded the least inhibition among the effective antibiotics.

Table 14. *In vitro* evaluation of antibiotics against the pathogen

Antibiotic	Concentration (ppm)	Inhibition zone (mm)
Streptocycline	100	13.6 [bc]
	200	15.7 [ab]
	300	17.5 [a]
Erythromycin	25	0
	50	0
	100	0
Tetracycline	25	0
	50	0
	100	0
Ampicillin	25	0
	50	0
	100	0
Rifampicin	25	0
	50	0
	100	0
Nalidixic acid	25	10.6 [de]
	50	5.0 [e]
	100	0
Kanamycin	25	11.0 [cd]
	50	14.3 [abc]
	100	14.6 [abc]
Streptomycin	25	4.7 [e]
	50	11.6 [cd]
	100	14.5 [abc]
Bacterimycin	100	8.0 [de]
	200	11.0 [cd]
	300	11.6 [cd]

Gentamycin	25	13.0 [bcd]
	50	12.6 [bcd]
	100	13.3 [bc]
Chloramphenicol	25	0
	50	5.3 e
	100	4.6[e]
Penicillin	25	0
	50	0
	100	0
Control	Sterile water	0

The values with different superscripts differ significantly.

Each observation is a mean of 3 replications.

4.8.2 *In vitro* evaluation of different botanicals and cow dung against the pathogen

Water extracts of neem, garlic, citronella, tulsi and cowdung (sterilized and unsterilised) were used at 10 per cent, 50 per cent and 100 per cent concentrations for the study. Among these, only garlic and citronella showed inhibitory effect on the growth of the pathogen. Garlic showed inhibition at 50 and 100 per cent concentrations (13.7 mm and 15.0 mm respectively) where as citronella showed inhibition only at 10per cent concentration (7.6 mm).Garlic at 100 per cent gave the maximum inhibition (Plate 16). The results of the experiment are given in the Table 15 Neem, tulsi and cowdung did not show any inhibitory effect on the growth of the pathogen.

Table 15. In vitro evaluation of botanicals and cow dung against the pathogen

Botanical	Concentration (percent)	Inhibition zone (mm)
Neem	10	0
	50	0
	100	0
Garlic	10	0
	50	13.7[a]
	100	15.0[a]
Citronella	10	7.6[b]
	50	0
	100	0
Cowdung (sterilized)	10	0
	50	0
	100	0

Cowdung(unsterilized)	10	0
	50	0
	100	0
Tulsi	10	0
	50	0
	100	0
Control	Sterile water	0

The values with different superscripts differ significantly.

Each observation is a mean of 3 replications

4.8.3 *In vitro* evaluation of different bioagents against the pathogen

Two bioagents namely, *Pseudomonas flourescens* and *Trichoderma harzianum* were evaluated for their efficacy in inhibiting the growth of the pathogen. Both the bio control agents did not show any inhibitory effect on the growth of the pathogen under *in vitro* conditions.

4.8.4 Management of the Rhizome rot disease under field conditions *(in vivo)*

A pot culture experiment in Completely Randomized Design was conducted to investigate in to the management strategies to control the rhizome rot disease of banana. The observations made on the disease incidence and biometric characters like height, collar girth and number of leaves were recorded. The results are presented in the Table 16 and 17 (Plate 17).

Observations on the disease incidence showed that the coefficient of infection was nil in the case of treatments T4 (fytolan 0.4per cent) and T5 (streptocycline 300 ppm) and was maximum incase of the control (56.24) showing that T4 (fytolan 0.4per cent) and T5 (streptocycline 300 ppm) were the most effective treatments and were statistically on par. None of the plants were infected in these two treatments. Garlic extract was also effective which showed a disease incidence of only 25 per cent (CI 4.54), which was on par with T1 (Bleaching powder 8 g plt^{-1}) and T2 (*Pseudomonas flourescens).* In the case of control the disease incidence as 100 per cent (Figure 2) and CI 56.24.The disease severity was maximum in the control (56.24) and nil in the case of T4 andT5.

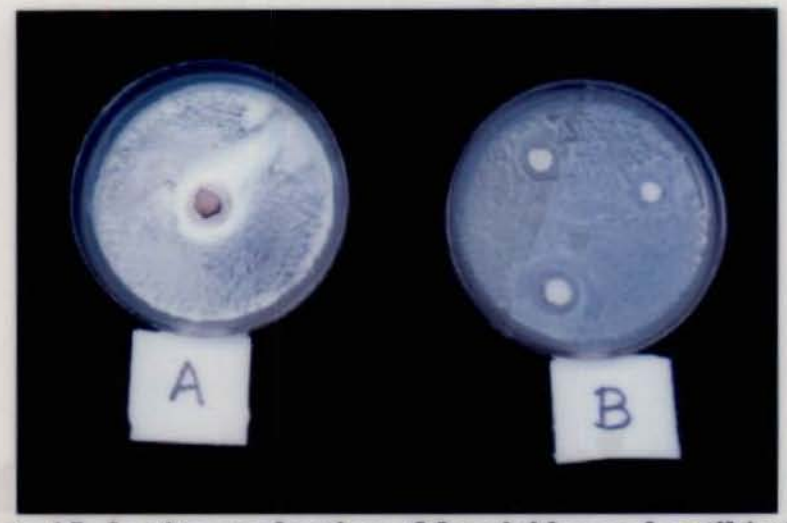


Plate 15. *In vitro* evaluation of fungicides and antibiotics against the pathogen
A Fytolan(0.4%) B Streptocycline(300ppm)

Plate 16 *In vitro* evaluation of botanicals against the pathogen Garlic (100%)

Plate 17 Management of Rhizome rot disease of banana under field conditions

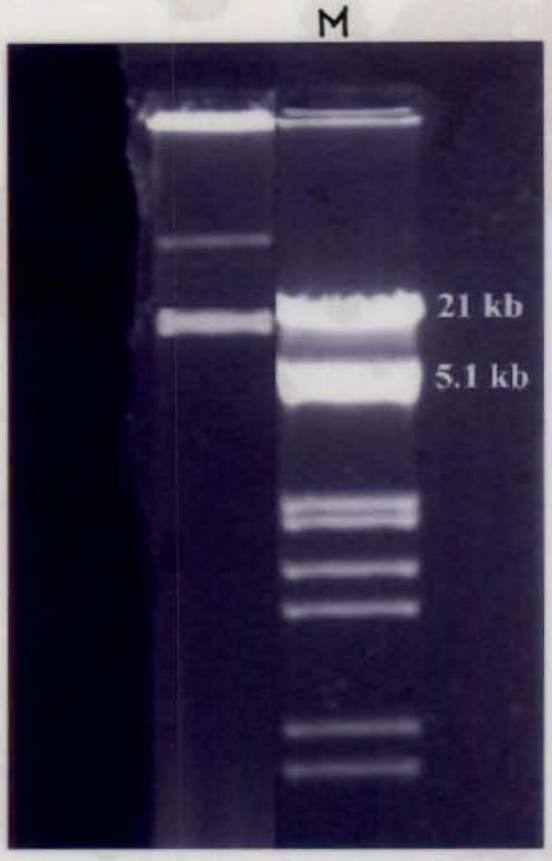


Plate 18 Isolation of the plasmids of the pathogen

M- Molecular weight marker ⟶ λ DNA / Eco R1 + Hind III

Table 16. Effect of different treatments on the incidence of Rhizome rot disease of banana var. Nendran

Treatment	Percent disease severity	Percent disease incidence	Coefficient of infection (CI)
T1	18.19	41.66	7.64^{bcd}
T2	23.43	58.33	13.90^{bc}
T3	15.60	50.00	19.1^{b}
T4	0.00	0.00	0.00^{d}
T5	0.00	0.00	0.00^{d}
T6	13.01	25.00	4.54^{cd}
T7	56.24	100.00	56.24^{a}

The values with different superscripts differ significantly.

Each value is the mean of monthly observations.

T1: Bleaching powder 8g plt^{-1}

T2: *Pseudomonas flourescens* 7g plt^{-1}

T3: Lime 500g plt^{-1}

T4: Fytolan 0.4 per cent (Best among the fungicides)

T5: Streptocycline 300 ppm (Best among the antibiotics)

T6: Garlic 100 per cent (Best among the botanicals)

T7: Control

Table 17. Effect of the different treatments used for the management of Rhizome rot disease on the biometric characters of banana variety Nendran

Treatment	Height (cm)	Collar girth (cm)	No. of leaves
T1	74.60^{c}	20.30^{bc}	7.53^{c}
T2	82.18^{bc}	21.41^{abc}	7.43^{c}
T3	89.57^{abc}	22.51^{abc}	8.58^{bc}
T4	99.14^{ab}	24.47^{ab}	9.76^{ab}
T5	104.00^{a}	24.67^{a}	10.46^{a}
T6	90.08^{abc}	22.47^{abc}	8.77^{bc}
T7	77.32^{c}	18.05^{c}	7.80^{c}

Each value is the mean of monthly observations.

The values with different superscripts differ significantly.

T1: Bleaching powder 8g plt^{-1}

T2: *Pseudomonas flourescens* 7g plt^{-1}

T3: Lime 500g plt^{-1}

T4: Fytolan 0.4 per cent

T5: Streptocycline 300 ppm

T6: Garlic 100 per cent

T7: Control

Table 18. Effect of different management treatments on the severity of rhizome rot disease of banana variety, Nendran

Treatment	Rhizome rot index score	Yellowing (+ve / -ve)	Flaccidity (+ve / -ve)
T1	4	+ve	+ve
T2	4	+ve	+ve
T3	8	+ve	+ve
T4	1	-ve	-ve
T5	1	-ve	-ve
T6	2	-ve	+ve
T7	16	+ve	+ve

Each score value is a mode of 3 replications.

T1: Bleaching powder 8g plt^{-1}

T2: *Pseudomonas flourescens* 7g plt^{-1}

T3: Lime 500g plt^{-1}

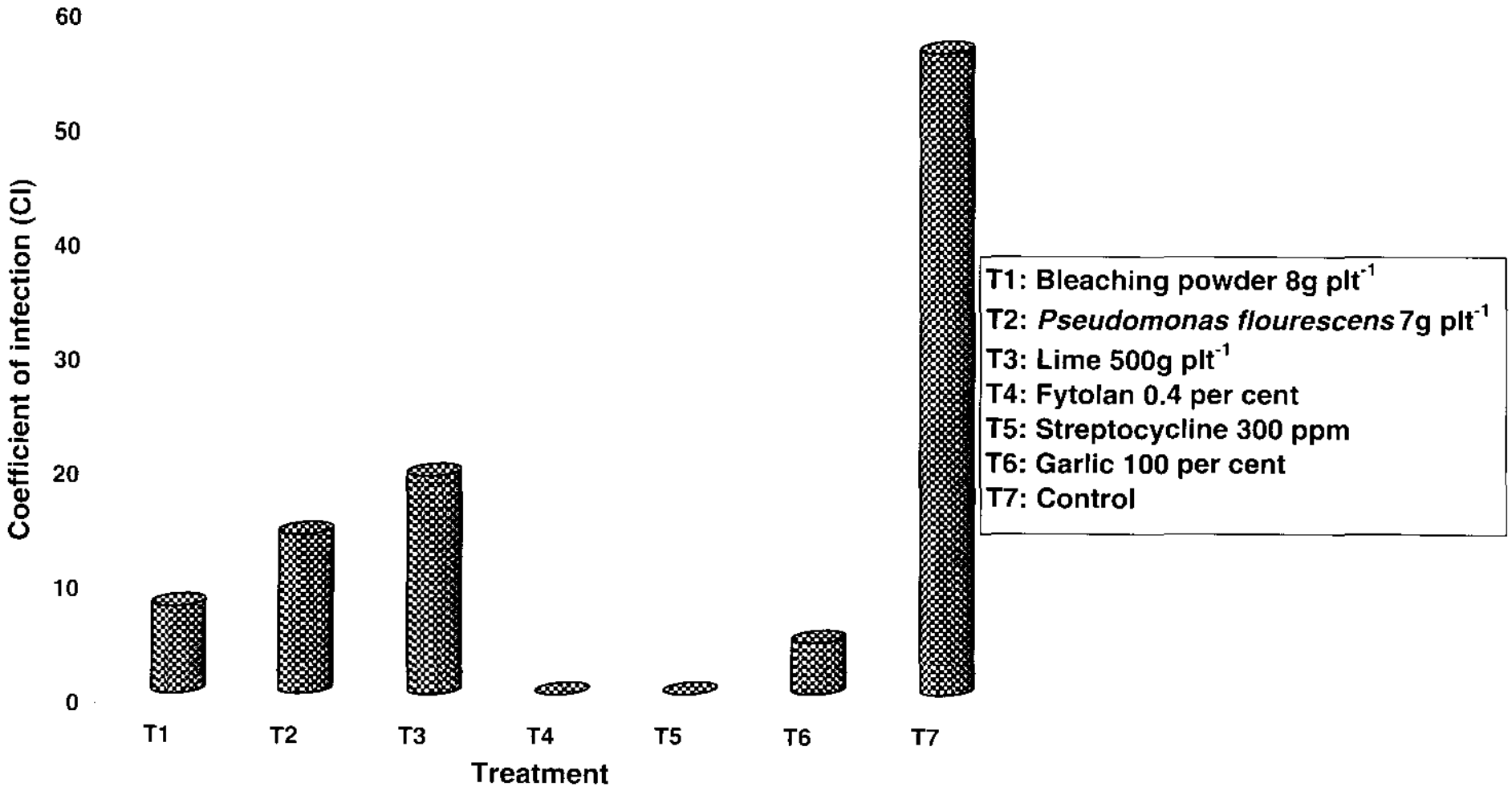
T4: Fytolan 0.4 per cent

T5: Streptocycline 300 ppm

T6: Garlic 100 per cent

T7: Control

Fig. 2. Effect of different management treatments on the disease incidence



Disease scoring was done according to the rhizome rot index scale proposed by Loos (1962). The observations revealed that the maximum rhizome rot index score (16) was seen in the control pots (T7) i.e. whole rhizome was rotten. The minimum score (1) was obtained in the pots receiving the treatments streptocycline 300 ppm (T5) and Fytolan (T4) i.e. no rot. The treatments T3, T2 and T1 showed scores 4, 4 and 8 respectively i.e., showing fair and heavy degree of rot. Yellowing and flaccidity was observed in all pots except T4 and T5.

With regard to the growth parameters, the height of the plant was maximum in T5 (steptocycline 300 ppm)(104 cm), which was on par with T4 (fytolan 0.4 per cent) (99.14 cm). The collar girth was maximum in T4 and T5 (24.47 and 24.67 respectively). Maximum number of leaves was produced in plants receiving T5 treatment (10.46) followed by T4 (9.76). In the case of the control treatments, the height, collar girth and the number of leaves were 77.32,18.05 and 7.80 respectively.

The results of the experiment on the management of the disease showed that fytolan at 0.4per cent and streptocycline at 300 ppm were equally good in controlling the rhizome rot disease of banana.

Discussion

5. DISCUSSION

Banana is one of the most important tropical fruit crops of the world. In most of the developing countries, it is also an essential staple food of millions of people. The successful commercial cultivation of banana is hindered mainly due to the incidence of diseases. Nowadays rhizome rot or tip over disease has emerged as one of the important diseases of banana reducing the productivity of the crop in different parts of the world. (Stover, 1959; Loos, 1962; Fernandez-Borrero, 1967; Shilling ford, 1974; Tomlinson *et al.* 1987; Gomez-Caicedo *et al.*, 2001).

In India, this disease is now assuming alarming proportions in many banana growing areas especially in southern states like Kerala, Karnataka and Tamil Nadu (Edward *et al., 1973;*Chattopadhay and Mukherjee, 1986; Lakshmanan and Mohan, 1986; Khan and Nagaraj, 1998; Nagaraj *et al.* 2002 a,b; Rani *et al.*, 2002).

In Kerala, the disease was reported from 1987 onwards and has now become a serious threat to the commercial cultivation of banana variety Nendran. (KAU, 1990; KAU, 1999). Hence the present study was undertaken to investigate into the etiology, symptomatology and management of this disease.

The rhizome rot causing pathogen *Erwinia* sp. was isolated from the infected rhizomes of banana variety Nendran. Repeated isolations done from the samples collected from farmer's field as well as from the fields of Banana Research Station, Kannara yielded the same type of bacterial colonies on nutrient agar medium which were pale white, slightly mucoid and raised with undulate margin. Isolation was also done in Logan's differential medium specific for *Erwinia* sp. Stover (1959), Dickey and Victoria (1980), Angeles-Ramos *et al.* (1982) and Chattopadhyay and Mukherjee (1986) isolated similar bacterial colonies on nutrient agar medium from rhizome rot infected samples of banana. In the present study, standard method of isolation *viz.*, tissue maceration followed by streaking was followed. In both the cases, similar bacterial colonies were obtained. Several researchers successfully tried isolation of the pathogen from infected rhizome bits by tissue maceration method. (Stover, 1959; Tomlinson *et al.*, 1987; Gomez-Caicedo *et al.* 2001; Nagaraj *et al.* 2002 a, b).

A part from isolating the pathogen from the infected plant samples, attempts were also made to isolate the pathogen from the rhizosphere soil of the infected banana plants. In this case, the isolation was done on Logan's differential medium for *Erwinia*, in order to confirm that the bacterium isolated is not any other saprophytic bacteria, but soft rot pathogen *Erwinia*. The isolation of the bacterium from rhizosphere soil also confirmed the soil borne nature of the pathogen. This factor has to be taken care while formulating the management strategies against the disease. Tomlinson *et al.*, (1987) and Nagaraj *et al.* (2002a) also attempted to isolate the pathogen from the rhizosphere of the infected banana plants.

5.1. Pathogenicity studies

Pathogenicity can be explained as the capacity of any pathogen to induce malfunction or interfere with the physiological activities of the plant. In the present investigation, the method of inoculation was standardized prior to pathogenicity studies. The standardization of the inoculation technique was done by conducting a pot culture experiment involving different methods of inoculation of the pathogen *viz.*, inoculating agar plugs with the bacterial culture into the healthy sucker tissue by corkboring, pinpricking the suckers and dipping in bacterial culture suspension, applying natural inoculum (bacterial ooze collected from diseased samples) around the planted suckers and applying infected rhizome bits. Of these the application of natural inoculum, rhizome bits, and pinpricking followed by dipping in bacterial culture suspension were found equally good as inoculation methods since, the extent of loss due to the disease was similar in these three methods. However the application of natural inoculum (bacterial ooze collected from diseased samples) showed the least incubation period of 6 weeks (56 days). Hence, this method was standardized as the best method of inoculation for further studies.

Many workers have standardized different inoculation methods. Stover (1959) found that rhizome inoculation by boring a plug of about 3/8 inch diameter to a depth of 3.5cm could induce the disease symptoms within 25 days of inoculation. Wardlaw (1972) succeeded in inducing the disease by inoculating the bacterial culture to the rhizome. Rahman *et al.* (1994) while working with *Erwinia*

carotovora ssp. *carotovora* causing soft rot of carrot found that inoculating the bacterial pathogen by injection, pin prick and cork borer method to tubers was more effective in causing the disease.

Under artificially inoculated conditions, the inoculated banana suckers showed typical rotting symptom after eight days of inoculation. The portion around the injury became discoloured and emitted a foul smell. Stover (1959) also reported similar results.

After standardizing the method of inoculation, Koch's postulates were successfully proved on Nendran variety of banana, the variety in which the disease was most prevalent under natural conditions. The pathogen was reisolated and the identity was confirmed to be similar to that of the original isolate used for inoculation. Similar to the present investigation, the pathogenicity of this bacteria (causing rhizome rot disease) has been confirmed by several workers earlier. (Stover, 1959; Chattopadhyay and Mukherjee; 1986; Tomlinson *et al.*, 1987; Lakshmanan and Mohan, 1991; Gomez Caicedo *et al.*, 2001; Rani *et al.*, 2002).

The incubation period taken under *in vitro* conditions is very less compared to that of the *in vivo* conditions, which might be due to the very high humidity provided during incubation. Stover (1959) and Loos (1962) also reported a long incubation period while conducting pathogenicity tests in pot culture.

The biometric observations taken during the study clearly revealed that the pathogen showed a negative influence on the normal growth and development of the host plant. The height, collar girth, and number of leaves of the control plants were significantly superior (54.12 cm, 17.82 cm and 5.8 respectively) when compared to the diseased suckers.

5.2. Symptomatology

Investigations on the development of symptoms produced under natural field conditions on banana variety Nendran revealed that the disease was prevalent in two to six months old plants. The first visible symptom of the disease was

flaccidity and yellowing of lower leaves. The infected plants appeared weak. Such weak plants-toppled down within a week or two. When the rhizomes of these plants were examined, they showed extensive rotting, tissues becoming soft with a foul smell. The internal cortex showed regions of brown or yellow water soaked region with well-defined dark margins. Cavities were also observed in the rotted portions of the rhizome, which may be confused for the infestation of rhizome weevils. The above ground symptoms of the disease could be correlated with the extent of rotting of the rhizomes. The infected plant toppled down only when the rhizome rot became severe. At this stage the plant could not be saved. So the control measure should be taken at the initial stage of the disease when the plant shows a flaccidity and loss of luster of the leaves. Hence this aspect of the study on the development of symptoms is of utmost practical importance to take up timely management practice to save the crop.

In the case of artificially inoculated plants, 25 per cent of suckers rotted immediately after planting. Typical disease symptoms *viz.* yellowing and flaccidity of leaves were produced in the inoculated plants after six to eight weeks of inoculation. The lower leaves showed yellowing first. The rhizome portion was completely rotten with a foul smell.

Similar symptoms were observed in rhizome rot affected banana by earlier workers throughout the world including India (Stover, 1959; Edward *et al.*, 1973; Shilling ford, 1974; Tomlinson *et al.*, 1987; Chattopadhyay, 1987; Gomez-Caicedo *et al.*, 2001; Rani *et al.*, 2002). Lakshmanan and Mohan (1991) also reported the occurrence of pseudostem rotting in Tamil Nadu and the disease severely affected three to five months old plants. Khan and Nagaraj (1998) recorded similar symptoms in rhizome rot diseased plants seen in various banana growing areas of Karnataka.

5.3 Characterization and identification of the pathogen

The characterization and identification of the pathogen was carried out based on the cultural, morphological and biochemical characters using standard procedures (Lelliott, 1974 and Schaad, 1992).

Growth of the bacterium was observed after 48h of incubation at room temperature. Pale white circular colonies of small size were observed. The colonies were slightly raised and mucoid.

Growth of the pathogen was observed on Logan's differential medium for *Erwinia* after 48 h of incubation at room temperature. Small, circular, convex, undulate colonies with purple centre having mean diameter of 1.5-2 mm were obtained.

Studies conducted by several workers revealed that the pathogen, *Erwinia* produced pale white, convex, slimy mucoid, circular colonies on nutrient agar (Stover, 1959; Dickey, 1979; Tomlinson *et al.*, 1987; Nagaraj *et al.* 2002 a, b; Rani *et al.*, 2002). According to CMI Descriptions of Plant Pathogenic Bacteria and Fungi, the bacteria belonging to *Erwinia* sp. *viz.*, E. *chrysanthemi* and *E. carotovora* produced greyish white colonies, smooth, round, glistening, slightly raised on nutrient agar. These characters match with the characters of the bacteria isolated in the present study.

A differential medium for *Erwinia* developed by Logan (1966) to distinguish *Erwinia* at the species level. *E. carotovora* produced colonies (about 1.5 mm diameter) with a pink to red purple centre. Single colonies of *E.atroseptica* remained colourless and less than 0.5 mm diameter. Colonies of *E. chrysanthemi* were large than that of *E. carotovora* (about 2 mm) and were completely red or purple (Fahy and Hayward, 1983). The results obtained in the present study revealed that the colony characters observed were similar to those of *E.carotovora*.

The pathogen grew well in nutrient broth at pH 7 after incubation at room temperature for 48 h. Similar result was obtained by Dickey (1981) in his study with the rhizome rot pathogen.

The growth of the isolate on eight different solid media showed some variations. Of the eight different media tried, Sucrose Peptone Agar (SPA) and King's B medium were found equally good as the best media to support the growth of the pathogen, followed by Potato Dextrose Agar (PDA) medium. Slime

production and fluidity were excellent in King's B, PDA and Yeast extract Dextrose Calcium carbonate Agar (YDC). Several workers have grown the pathogen on different solid media (Stover, 1959; Dickey, 1979; Tomlinson *et al.*, 1987; Nagaraj *et al.*, 2002 a, b; Rani *et al.*, 2002). The results obtained by the earlier workers are in agreement with he present study. Schaad and Brenner (1977) studied the growth of *E.chrysanthemi* on YDC, NA, MS, Bioquest XLD agar, BG, M Endo agar and compared the growth on these media. The growth obtained on YDC and NA was similar to that of the pathogen in this study.

The isolate of the pathogen recorded good growth between pH 6 and 7, the maximum being recorded at pH 7 after 96h of incubation at room temperature. No studies have been made on the growth of rhizome rot pathogen at different pH levels, so far. Workers have made similar studies on *Pseudomonas solanacearum* and found that good growth was obtained between pH ranging from 6.5-7.5 (Kelman, 1953; Jyothi, 1992).

The isolate of the pathogen was found mesophilic in nature, with good growth between 20^0C and 40^0C, the maximum growth being recorded at 28^0C. Growth was observed at 37^0C. These results are in agreement with that of Dickey (1979), Tomlinson *et al.*, (1987) and Nagaraj *et al.* (2002 a, b). According to the CMI Descriptions of Plant Pathogenic Bacteria and Fungi, both the species of *Erwinia viz., E. carotovora* and *E.chrysanthemi* (rhizome rot pathogens) grew well at temperatures ranging from 37-40^0C. Perombelon and Hyman (1986) in their studies found that both the species of *Erwinia viz., Erwinia carotovora* and *Erwinia chrysanthemi* grew well at 37^0C.

The isolate of the pathogen was gram-negative short rods when viewed under the microscope. They were found to be motile. Similar results have been obtained by several workers (Stover, 1959; Dickey, 1979; Gomez-Caicedo *et al.*, 2001; Rani *et al.*, 2002).

The isolate used in the present study failed to produce any pigments on King's B medium as reported by many workers (Dickey, 1979; Tomlinson *et al.* 1987; Cappuccino and Sherman, 1992; Gomez Caicedo *et al.* 2001; Nagaraj *et al.*

2002 a, b). The isolate grew and changed the colour of nutrient dextrose agar medium with bromocresol purple from purple to yellow at a slow and fast rate in tubes with and without agar seal indicating that the isolate could grow both aerobically and anaerobically. This facultative anaerobic nature of the pathogen was established by many workers (Lelliott, 1974; Dickey, 1979; Tomlinson *et al.*, 1987; Gomez Caicedo *et al.*, 2001;Nagaraj *et al.*, 2002 a, b)

The pathogen produced viscous strands from the bacterial suspension when mixed with 3 percent KOH, confirming the gram-negative nature as reported by Gomez- Caicedo *et al.* (2001).

The bacterial isolate was found to be oxidase negative and catalase positive. It was found to hydrolyse gelatin, but not starch and casein. These results are in accordance with the reports of many workers (Lelliott, 1974; Bradbury, 1977a,b; Dickey and Victoria, 1980; Dickey nd Kelman, 1981; Bradbury, 1986; Tomlinson *et al.*, 1987; Gomez Caicedo, *et al.* 2001; Senthilvel, 2000)

The pathogen utilized sodium citrate, sodium acetate, sodium succinate but did not utilize oxalate and benzoate as reported by Bradbury, 1977a; Dickey, 1979; Dickey and Victoria, 1980; Cother and Sivasithamparam, 1983; Tomlinson *et al.*, 1987.

The bacterial isolate liberated H_2S and produced NH_3 from peptone, but failed to produce indole, levan from sucrose. It was methyl red negative, urease negative and did not reduce nitrate and did not hydrolyse arginine. All these results agreed with those obtained by many workers, while characterizing the rhizome rot pathogen. (Dickey, 1979; Dickey and Victoria, 1980; Dickey and Kelman, 1981; Bradbury, 1986; Tomlinson *et al.*, 1987; Gomez Caicedo *et al.*, 2001; Nagaraj, 2002 a, b; Rani *et al.*, 2002)

The isolates failed to produce reducing substances from sucrose. Similar result was obtained by Tomlinson *et al.* (1987) with one of the isolates of the pathogen. Generally *E. carotovora* did not produce reducing substances from

sucrose, but in the case of *E.chrysanthemi* the reaction varied with different strains (Bradbury, 1977 a, b; Cother and Sivasithamparam, 1983).

The isolate of the pathogen was found to show positive lipase activity. Lipase activity of the rhizome rot causing bacteria has not been confirmed so far.

Of the ten carbon compounds tested the isolate of the bacterium produced acid from glucose, ribose, maltose, glycerol, mannitol, lactose, galactose and sorbitol, both in aerobic and anaerobic conditions. It did not utilize to dulcitol. Gas production was noticed from maltose, sucrose, sorbitol, galactose and ribose. Acid production from glycerol and lactose was poor and slow. Similar results were obtained by several workers while characterizing the rhizome rot pathogen (Dickey, 1979; Dickey and Victoria, 1980; Tomlinson *et al.*, 1987; Gomez Caicedo *et al.*, 2001; Nagaraj *et al.*, 2002 a, b; Rani *et al.*, 2002).

The isolate of the pathogen was found to grow well at a temperature of 37^0C and in the medium with 3 per cent and 4 per cent NaCl which were characterising features of *Erwinia* sp. (Bradbury, 1977 a, b). Results obtained are in agreement with the reports of Dickey, (1979), Tomlinson *et al.*, (1987) and Gomez Caicedo *et al.*, (2001).

The bacterial isolate was found to be sensitive to streptomycin, but were resistant to erythromycin and penicillin. In general, E. *carotovora* was found to be resistant to erythromycin where as *E. chrysanthemi* was found to be sensitive (Bradbury, 1977 a, b). Dickey (1979) reported that the rhizome rot pathogen, *E. chrysanthemi* was sensitive to erythromycin. But of the isolates characterized by Tomlinson *et al.* (1987) one was resistant to erythromycin.

The sensitivity of the pathogen to penicillin varied but was sensitive to streptomycin (Dickey, 1979; Tomlinson *et al.*, 1987). Gomez Caicedo *et al.* (2001) in his study found that the pathogen was sensitive to streptomycin, but resistant to penicillin. Based on this character also the pathogen under present study could be confirmed as *Erwinia carotovora*.

Plasmids were isolated and purified from the cell lysates of the isolates of the pathogen. Two plasmids were isolated. Of the two plasmids isolated one was on par with the molecular weight of a linear plasmid marker with 21 kilo base pair (kb) weight, while the other weighed slightly above 21kb. The results obtained in the present study were in agreement with previous studies. (Daughtery, 1978; Chatterjee and Starr, 1980; Sparks and Lacy, 1980).

Inoculation of the bacterium caused blackening and rotting of the potato slices and carrot slices. Similar observations were recorded by Lelliott, (1974), Dickey (1979), Tomlinson *et al.* (1987), Gomez Caicedo *et al.* (2001), Nagaraj *et al.* (2002 b) and Rani *et al.* (2002).

The potato soft rot test and carrot rotting test showed the pectin degrading ability of the pathogen.

5.4. Host range studies

Host range studies conducted to find out ability of the bacterium to cause disease on five different hosts *viz.*, Heliconia (*Heliconia* sp.) Canna (*Canna* sp.) ginger (*Zingiber officinale*) Turmeric (*Curcuma longa*) and Colocasia (*Colocasia* sp.) revealed that the pathogen failed to produce symptoms in them. Earlier reports reveal that the rhizome rot pathogen was found to infect plants other than banana like *Chrysanthemum*, *Diffenbachia*, *Philodendron*, *Canna* sp., *Capsicum* sp., *Zingiber officinale*, *Colocasia* sp., *Xanthosoma* sp., *Zea mays*, tomato, potato, carrot, radish etc. But the reaction depended on the strain of the pathogen, host conditions and conditions of infection. (Shilling ford, 1974; Dickey, 1979; Dickey, 1981; Bradbury, 1986; Lakshmanan and Mohan, 1991; Nanda *et al.*, 1994; Thwaites *et al.*, 2000; Nagaraj *et al.*, 2002a).

The results of the various tests conducted to confirm the identity of the pathogen were in agreement with the reports of Dickey, 1979; Dickey and Kelman, 1981; Bradbury, 1977a,b; Tomlinson *et al.*, 1987; Nagaraj *et al.*, 2002 a, b; Rani *et al.*, 2002). Thus on the basis of the morphological, cultural, physiological and

biochemical characteristics and the pathogenicity tests, the pathogen causing rhizome rot disease of banana was identified as *Erwinia carotovora*.

5.5 Survival of the pathogen

Saprophytic survival of plant pathogenic bacteria results in their persistence in soil. The plant pathogenic bacteria surviving in saprophytic phase seems to be in hypo biotic state with markedly reduced metabolic rates. Therefore understanding the occurrence and duration of soil borne phase of soft rot bacteria is basic to develop control measures. *Erwinia carotovora* ssp. *carotovora* has been considered to survive in soil (Goto, 1990). In the present study, it was found that the pathogen survived in sterilized soil in the presence of diseased plant remnants for 180-185 days. The population was found to be fluctuating depending on the weather conditions and moisture level of the soil. The results were in accordance with the reports of Goto (1990) who stated that *Erwinia* couldn't survive for long time beyond six months and the reports of Rodriguez *et al.* (1991). The results of the investigations conducted by Nagaraj *et al.* (2002 a) revealed that the pathogen causing tip over disease of banana could not survive beyond 195 days, as a matter of fact that the survival of the bacteria was greatly influenced by moisture status.

5.6. Varietal screening

Screening of large number of lines/types of a crop with considerable genetic diversity is a method for locating resistant lines/types against the disease, which could be further, utilized for the development of resistant varieties with desirable characters. With this idea, a large number of accessions maintained in the germplasm of Banana Research Station, Kannara were evaluated for host resistance against the rhizome rot disease of banana under field conditions from May to October 2001 and 2002. The study revealed that, out of the 240 accessions of banana evaluated, only 11 varieties were found to be infected by the disease. The results of the disease incidence and percentage mortality of the accessions of banana screened showed that the variety Poppoulu (AAB) was the most susceptible variety and recorded 100 percent loss. The disease was more predominant in accessions with AAB genome.

5.7. Management of the disease

In order to find out a suitable chemical, botanical or bioagent for reducing the incidence of the disease, an *in vitro* study was conducted using seven fungicides, twelve antibiotics, five botanicals and two bioagents. Among the fungicides tested, bordeaux mixture and fytolan were found to inhibit the bacterium at all the concentrations tried. Fytolan at 0.4 per cent recorded maximum inhibition, which was followed by Fytolan at 0.3 per cent. All the antibiotics tried, except tetracycline, erythromycin, ampicillin, rifampicin and penicillin showed varying levels of inhibition on the growth of the bacterium. streptocycline at 300 ppm was found to be the most superior one followed by streptocycline at 200 ppm. Among the botanicals tried, garlic and citronella showed inhibition on the growth of the pathogen. Garlic at100per cent concentration showed maximum inhibition followed by 50 per cent concentration. Citronella showed inhibition only at 10 per cent concentration.

Both the biocontrol agents tried *viz. Pseudomonas flourescens* and *Trichoderma harzianum* did not show any inhibition on the growth of the pathogen under *in vitro* conditions. The results of *in vitro* management of the pathogen using different antibiotics agreed with those of the studies conducted by earlier workers (Dickey, 1979;Tomlinson *et al.*, 1987; Gomez-Caicedo *et al.* (2001). Nagaraj *et al.* (2002 b) found that methoxy ethyl mercuric chloride @ 2000 ppm, $CuSO_4$ @ 4000 ppm, streptocycline @ 750 ppm and norfloxin @ 750 ppm were found effective in inhibiting the growth of the pathogen.

On the contrary, to the present results, earlier workers reported that the pathogen could be inhibited by bioagents like *Pseudomonas* sp. (Jimenez and Cordoves, 1992) and *Trichoderma* sp. (Limon *et al.*, 1999) under *in vitro* conditions. The failure of *P. flourescens* in inhibiting the growth of the pathogen under *in vitro* conditions may be due to the variation in the strain of the bio agent used. Even though it did not show any inhibitory effect under *in vitro* conditions, it was effective in controlling the disease under field conditions. Similar to the

present results Rani *et al.* (2002) reported that the pathogen could be controlled by water extract of citronella.

A pot culture experiment was conducted to investigate the management strategies for rhizome rot disease of banana. Six treatments along with a control without any management treatment were maintained in a Completely Randomized Design. The treatments include the best fungicide (fytolan @ 0.4 per cent) the best antibiotic (streptocycline @ 300 ppm), the best botanical (garlic extract @ 100 per cent) under *in vitro* studies, Bleaching powder @ 8g plt^{-1} lime 500g plt^{-1} and the commercial formulation of *P. flourescens*. Based on the observations made on the disease incidence and biometric characters, it was found that fytolan at 0.4 per cent and streptocycline at 300 ppm were found to be the most superior ones in controlling the disease and these were statistically on par. None of the plants were infected in these two treatments. Garlic extract was also effective in controlling the disease. In the case of control the disease incidence was 100 percent. The effect of antibiotics like streptomycin sulphate, bleaching powder, copper sulphate formulations and Carbendazim in suppressing the disease similar to the present study has been reported by several workers (Lakshmanan and Mohan, 1986; Chattopadhyay, 1987; Salazar and Duque, 1994; Nagaraj *et al.*, 2002 b).

Summary

6. SUMMARY

Rhizome rot disease of banana is emerging as one of the important diseases of banana reducing the productivity of the crop in different parts of the world including India. Hence, the present investigation was undertaken to study the etiology, symptomatology and to evolve a suitable management practice for the disease.

The pathogen, *Erwinia carotovora* was isolated from the infected rhizomes of banana var. Nendran and its pathogenicity was established after standardizing the method of inoculation. Symptomatology was studied under natural field conditions and under artificially inoculated controlled conditions. The first visible symptom of the disease was flaccidity and yellowing of leaves. The infected plants appeared weak and such plants toppled down with in a week or two. When the rhizome of these plants was examined, they showed extensive rotting, tissues becoming soft with a foul smell. The internal cortex showed brown or yellow water soaked regions with well-defined dark margins.

The isolated bacteria produced pale white colonies of small size slightly raised with undulate margin on nutrient agar medium. On Logan's differential medium, a specific medium for *Erwinia*, the isolate produced small circular colonies with purple centre slightly raised with a mean diameter of 1.5 mm.

Growth of the pathogen was studied on eight different solid media. It was found that maximum growth was obtained on Sucrose Peptone Agar and King's B medium, while the slime production was maximum on King's B medium, PDA and YDC. The bacterial isolate recorded good growth between pH range of 6 and 7, the maximum being at pH 7. Among the different temperatures tested, the isolate showed maximum growth at 28°C.

The pathogen was found to be gram negative, motile small rods when viewed under the microscope. It could not produce any water soluble or insoluble pigments. It was found to be facultatively anaerobic and produced viscous strands when mixed with 3 per cent KOH. The pathogen was found to be oxidase negative,

catalase positive and could hydrolyse gelatin but not starch and casein. The pathogen utilized organic acids *viz.* sodium citrate, sodium acetate and sodium succinate, but not oxalate and benzoate. The isolate liberated H_2S and produced ammonia from peptone, but failed to produce indole and levan from sucrose. It showed methyl red negative, urease negative reaction and could not reduce nitrate and hydrolyse arginine. The isolate failed to produce reducing substances from sucrose. The bacterial culture was found to show positive lipase activity.

Of the ten carbon compounds tested, the isolate of the bacterium produced acid from glucose, ribose, maltose, glycerol, mannitol, lactose, galactose and sorbitol both in aerobic and anaerobic conditions. It did not utilize dulcitol. The bacteria caused rotting of potato tubers and carrot indicating its pectin degrading ability.

The isolate of the pathogen was found to grow well at a temperature of 37^0C and in the medium with 3 per cent and 4 per cent sodium chloride. It was sensitive to streptomycin, but was resistant to erythromycin and penicillin. The isolate was characterised at the molecular level by doing the plasmid isolation and studying the plasmid profile. On the basis of the morphological, physiological, biochemical, and molecular characteristics the pathogen causing rhizome rot disease of banana variety Nendran was identified as *Erwinia carotovora*.

The host range studies of the pathogen on five different hosts *viz.* heliconia, canna, ginger, turmeric and colocasia under field conditions revealed that the pathogen did not infect these hosts. But it produced typical soft rot symptoms when inoculated on potato tubers and carrot under *in vitro* conditions. The pathogen survived in sterilized soil in the presence of infected rhizome for 180-185 days.

Out of the 240 accessions of banana screened for the resistance to the rhizome rot disease, 11 varieties were found to be infected by the disease under natural field conditions. The results of the disease incidence and percent mortality of the accessions of banana screened showed that the genome AAB is the most

susceptible among the genomes screened and the variety Poppoulu (AAB) was the most severely infected one.

An *in vitro* study was conducted to find out the inhibitory effect of twelve different antibiotics, seven fungicides, five botanicals and two bioagents against the pathogen. Among the fungicides tried, fytolan at 0.4 per cent concentration gave the maximum inhibition on the growth of the pathogen followed by fytolan at 0.3 per cent. Among the antibiotics tried, streptocycline at 300ppm was found to be the most effective one in controlling the pathogen. Among the botanicals tried garlic extract at 100 per cent concentration gave the maximum inhibition on the growth of the bacteria.

A pot culture experiment was conducted on the management of rhizome rot disease of banana var. Nendran. The treatments were evaluated based on the observations on the disease incidence and biometric characters. The treatments *viz.*, fytolan at 0.4 per cent and streptocycline at 300 ppm were found to be the most effective ones which were statistically on par. Garlic extract at 100 per cent concentration was found to be the next best one in controlling the disease which was on par with *Pseudomonas flourescens* (T2) and bleaching powder (T1).

References

REFERENCES

*Alice, D. and Sivaprakasam, K.1996. Fungicidal, bactericidal and nematological effect of garlic clove extract. *J.Ecobiol.* 8: 99-103

Allirani, G. 1994. Management of bacterial wilt of Ginger (*Zingiber officinale* Rose) incited by *Pseudomonas solanacearum* (Smith) Smith. M.Sc.thesis. Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India, p.92.

Aneja, K.R. 1993. *Experiment in Microbiology, Plant Pathology and Tissue culture*. Wishwa Prakashan, New Delhi, pp. 117-195.

*AngelesRamos, L., Strider, D.L. and Tzeing, K.C. 1982. Species of *Erwinia* associated with soft rot disease of plant in Taiwan. *Proceedings of Fifth International Conference on Plant Pathogenic Bacteria*. (ed: Lozano, J.C) CIAT, Cali, Columbia, pp. 9-18.

Bradbury, J. F. 1970. Isolation and preliminary study of bacteria from plants. *Rev. Pl. Path.* 49: 213-218.

Bradbury, J.F . 1977a. *Erwinia carotovora* var *carotovora*. *CMI Descriptions of Pathogenic Fungi and Bacteria* (No. 552). Common Wealth Mycological Institute Kew, Surrey, England.

Bradbury, J.F. 1977b. *Erwinia chrysanthemi*. *CMI Descriptions of Pathogenic Fungi and Bacteria* (No. 553). Common Wealth Mycological Institute, Kew, Surrey, England.

Bradbury, J.F. 1986. *Guide to Plant Pathogenic Bacteria*. CAB International Mycological Institute, Kew, Surrey, England. pp.61-73.

Buddenhagen, T.W. 1994. Banana diseases caused by bacteria. *Compendium of Tropical Fruit Diseases*. The American Phytopathological Society. St.Paul, Minnesota.

Cappuccino, J.G. and Sherman, N.1992. *Microbiology-A laboratory manual.* The Benjamin/Cummings Publishing Company, Inc., NewYork.

*Cedeno, M., L.R., Nieves, B.M.M. and Palacios, E.L. 1990. *Erwinia carotovora* sub sp. *atroseptica* causante de la pudricion blanda del platano "Harton" (*Musa* AAB) in Venezuela. *Fitopatologia Venezolana* 3:6-9.

Chatterjee, A.K. and Starr, M.P. 1980. Genetics of *Erwinia* species. *A. Rev. Microbiol.* 34: 645-676.

Chattopadhyay, P.K. 1987. Soft rot- A threat to banana cultivation in West Bengal. *Ind. Hort.* 31:9.

Chattopdhyay, P.K. and Mukherjee, N. 1986. A pseudostem rot of banana due to *Erwinia chrysanthemi* .p.v .*paradisiaca. Curr. Sci. India.* 55: 780-790.

*Choi, J.E., Park, J.S., Kang, H.W. 1988. Bacterial soft rot of banana fruit caused by *Erwinia carotovora* sub sp. *carotovora* and *Pseudomonas cichorii. Korean J. Pl. Path.*4 : 202-206.

Christensen, W.B. 1946. Urea deposition as a measure of differentiating *Proteus* and *Paracolon* cultures from each other and from *Salmonella* and *Shigella* types. *J.Bacteriol.* 52:461.

Cother, E.J. and Sivasithamparam, K. 1983. Erwinia: The "Carotovora" group. *Plant bacterial diseases: a diagnostic guide* (eds. Fahy, P.C. and Persley, G.J) Academic Press, Australia. pp.87 – 106.

*Coplin, D.L., Frederick, R.D., Tindal, M.H.and Mc Cammon, S.L.1982. *Proceedings of International Conference on Plant Pathogenic Bacteria.* (5th). Cali, Columbia. pp.379-388.

*Daughtery, M.L.1978. Native extra chromosomal deoxyribonucleic acid in *Erwinia carotovora* var *carotovora*. M.Sc thesis, University of Massachusetts, Amherst. p.64.

Dickey, R.S. 1979. *Erwinia chrysanthemi*: a comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. *Phytopathology*. 69: 324-329.

Dickey, R.S. 1981. *Erwinia chrysanthemi*: reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. *Phytopathology*. 71: 23-29.

Dickey, R.S. and Kelman, A. 1981. *Erwinia*: The soft rot or "carotovora" group. *Laboratory guide for Identification of Plant Pathogenic Bacteria* (ed. Schaad, N.W). American Phytopathological Society, St. Paul, Minnesota.pp. 31-35.

Dickey, R.S. and Victoria, J.I. 1980. Taxonomy and emended description of strains of *Erwinia* isolated from *Musa paradisiaca* Linnaeus. *Int. J. Syst. Bacteriol.* 30:129-134 .

Edward, J.C., Tripathi, S.C. and Singh, K.P. 1973. Observations on a tip over disease of banana in Allahabad. *Curr. Sci.* 42:696-697.

Fahy, P.C. and Hayward, A.C. 1983. Media and methods for isolation and diagnostic tests. *Plant bacterial disease; a diagnostic guide* (eds. Fahy, P.C. and Persley, G.J) Academic Press, Australia, pp 337-378.

*Fernandez-Borrero, O. 1967. Wet rot of the pseudostem of the banana caused by *Erwinia* sp. *Cenicafe*. 18:39-46.

*Fernandez-Borrero, O. and Lopez-duque Selma. 1970. Watery rot of the pseudostem of banana caused by *Erwinia paradisiaca*. *Cenicafe*. 21:3-4.

Freed, R. 1986. MSTAT C *Version 1.2*.Department of Crop and Soil Sciences, Michigen State University, USA.

Gomez-Caicedo, L.E., Echeverry, N.E. and Gonzalez, R.S. 2001. Evaluation of cultural, chemical and biological control of vascular rot and wilt in plantain (*Musa* AAB Simmonds). *Infomusa*. 10: 17-20.

*Goto, M. 1990. *Fundamentals of Bacterial Plant Pathology*. Academic press, Inc. Harcourt Brace Jovanovich Publishers, Tokyo, p.342.

Graham, D.C. 1972. Identification of soft rot coliform bacteria. *Proceedings of the Third International Conference on Plant Pathogenic Bacteria*. 1971. Wageningen, The Netherlands: Center for Agricultural Publishing and Documentation .pp 273-279.

*Gurrey, P., Le blanc, D. J. and Falkow, S. 1973. General Method for the Isolation of Plasmid deoxy ribonucleic acid. *J. Bacteriol.* 116: 1064-1066.

*Guzman, M. and Sandoval, J. 1996. Sintomas de la pudricion suave del pseudotallo en ls hibridos FHIA-02. *Corbana* .46: 145-150.

*Guzman, M. and Wang, A. 1998. Symptoms description, identification of causal agent and inoculum source determination of the fungi soft rot in banana. (*Musa* AAA). *Corbana*. 23:109-124.

*Hao, M.V., Brenner, D.J., Steigerwalt, A.G., Kosako, Y. and Komagata, K. 1990. *Erwinia persicinus*, a new species isolated from plants. *Int. J. Syst. Bacteriol.* 40:379-383.

Harrigan, W.F. and Mc. Cance, M.E. 1966. *Laboratory Methods in Microbiology*. Academic Press, London and New York, p.343

*Hassanzadeh, N. 1990. Characterization of a new soft rot *Erwinia* to banana in Iran. *Iranian J. Pl. Path.* 26: 13-17.

Hayward, A.C. 1964. Characteristics of *Pseudomonas solanacearum*. *J.Appl. Bacteriol.* 27 : 265 – 277.

Hildreth, R.C.1963. Studies on bacterial rhizome rot of bananas. *Rev. Pl. Path.* 42:79.

Hord, H.V.V. and Flippin, R.S. 1953. Head rot of banana rhizomes in Honduras. *Pl. Dis. Reptr.*37 : 326-327.

Hucker, G.J. and Conn, H.J. 1923. Methods of Gram staining, *N.Y. State Agr. Expt. Sta. Tech. Bull.* p.129.

Hugh, R. and Leifson, E.1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *J.Bacteriol.* 66:24-26.

*Jimenez, G. and Cordoves, N. 1992. Caracterizacion de bacterias de la rizofera del platano antagonicas a *Erwinia chrysanthemi* Burk. *Proteccion de Plantas* . 2:67-75.

Johnson,L.F. and Curl, E.A.1972. Isolation of groups of micro organisms from soil. *Methods for Research on the Ecology of Soil Borne Plant Pathogens.* Burgees Publishing Company. New York, p.63.

Jyothi, A.R. 1992. Characterization and management of bacterial wilt of chillies caused by *Pseudomonas solanacearum* E.F.Smith. MSc thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India, p.87.

K.A.U. 1990. *Annual Research Report*, Banana Research Station, Kerala Agricultural University, Kannara. p.91

K.A.U. 1999. *Annual Research Report*. Banana Research Station, Kerala Agricultural University, Kannara. p.91

*Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. *N.C.Agr. Expt. Sta Tech Bull.* 99. p 194.

Khan, A.N.A and Nagaraj, M.S. 1998. Occurrence of a new bacterial disease on banana in Karnataka. *Annual Meeting and Symposium on Integrated Disease Management and Crop loss assessment.* Indian Phytopathological Society (southern chapter), U.A.S, Bangalore. pp73.

*King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of pyocyanim and flourescein. *J.Lab. Clin. Medicine*. 44. 301-307.

Kovacs, N.1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature*. 178:703.

*Krebs, H. and Jaggi, W.1999. Effect of plant extracts against the bacterial soft rot of potatoes: *Erwinia carotovora*. *Agrar forschung*. 6: 17 – 20.

*Lacy, D.S. 1971. *Erwinia* head rot of bananas in Jamaica. *Proceedings of the Association for Co-operation in Banana Research in the Caribbean and Tropical America.* Jamaica. pp 104 – 110.

Lakshmanan, P. and Mohan, S. 1986. Control of tip over disease of Cavendish banana. *S.Indian Hort.* 34:336-337.

Lakshmanan, P. and Mohan, S. 1991. Studies on rhizome rot and tip over disease of banana. *Madras agric. J.* 79: 56-57.

Lelliott, R.A., EveBilling, and Hayward, A.C. 1966. A determinative scheme for the fluorescent plant pathogenic *Pseudomonads*. *J.Appl. Bacteriol.* 29: 470 – 489.

Lelliott, R.A. 1974. Genus XII *Erwinia*. Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920. *Bergey's Manual of Determinative Bacteriology* (eds.Buchanan,R.E. and Gibbons, N.E) 8th edition. Williams and Wilkins Co, Baltimore. pp 332-339.

*Limon, M.C., Pintor-Toco, J.A., Benitez, T.1999. Increased antifungal activity of *Trichoderma harzianum* transformants that over express a 33-K Da chitinase . *Phytopathology*. 89: 254-261.

*Llanos, and Carmen, 1967. A new disease of banana in the Cauca Valley: bacteriosis. *Agricultura trop*. 23:806-812.

Logan, C. 1963. A selective medium for the isolation of soft rot coliforms from soil. *Nature* (London). 199:623.

Logan, C. 1966. Simple method of differentiating *Erwinia carotovora* variety "*atro septica*" from *E.carotovora* and *E.carotovora* variety "*aroidae*". *Nature* (London). 212: 1584-1589.

Loos, C.A. 1962. Factors affecting incidence of rhizome rot caused by *Erwinia carotovora* of Grosmichel and Lactan bananas. *Phytopathology*. 52:110-114.

Nagaraj,M.S., Khan, A.N.A., Ravikumar, M.R. and Amarananjundeswara,H. 2002a. Management of Tip- over disease of banana. Global Conference on Banana and Plantain, 28-31 October 2002. U.A.S, Bangalore, India. *Abstract*: 166.

Nagaraj, M.S., Khan, A.N.A., Ravikumar, M.R.and Amarananjunderwara, H. 2002b. Survival and transmission of *Erwinia carotovora* subsp. *carotovora* causing Tip-over disease of banana. Global Conference on Banana and Plantain, 28-31 October 2002. UAS, Bangalore, India. *Abstract*: 167.

Nanda, M. Dhal, N.K. and Rath, G.C. 1994. Occurrence of bacterial soft rot of ginger in Orissa- a new report. *Orissa.J.Agric.Res.* 7: p73.

*Ordosgoitty, F.A., Santos, P.R. and Haddad, G.O. 1974. Watery rot of the pseudostem of plantain and its presence in three plantain growing regions of Venezuela. *Agronomia Tropical*. 24:247-258.

*Pereira, L.V and Nunes, R.A.S. 1988. Soft rot of the rhizome and pseudostem of banana (*Musa acuminata*) *Fitopathologia Brazil*. 13: 70-71.

Perombelon, M.C.M. and Hyman, L.J. 1986. A rapid method to identify and quantify soft rot Erwinias directly from plant material based on their temperature tolerances and sensitivity to erythromycin. *J.Appl. Bacteriol.* 60: 61-66.

Rahman, M.N., Dey, T.K., Rahaman, M.Z., Haque, M.S. and Zakaria, M. 1994 Comparative effectiveness of inoculation methods and tuber resistance against soft rot bacterium. *Bangladesh J. Pl.Pathol.* 10:21-22.

Rani, A.S ., Nagaraj, M.S., Khan, A.N.A. and Reddy, K. 2002. Studies on etiology and biological control of Tip-over disease of banana in Karnataka. Global Conference on Banana and Plantain, 28-31 October 2002. UAS, Bangalore, India *Abstract*: 169.

*Rivera, N. and Ezavin, M. 1989. Necrosis del cormo del platano causada por *Erwinia chrysanthemi. Ciencia y Tecnica en la Agricultura*. 12: 59-70.

*Rivera,N., Garcia,A. and Gil, C.L. 1980. Pathotype characterization of *Erwinia chrysanthemi* from banana and maize. *Cienc. Tecnia. Agricul. Prot. Plant.* 3:47-60.

*RiveraDocando, N. 1978. Comparative study of two new bacterial diseases in banana growing area of Cuba. *Agrotecnia de Cuba*. 10: 35-44.

*Robinson, J.C. and Manicom, B.Q. 1991. Rhizomes of new banana cultivars are susceptible to *Erwinia* corm rot. *Inlightings bulletin Navorsinsinstitvut vir sitrus en Subtropiere Vrugte*. 228: 1-2.

*Rodriguez, A.M., Iznaga, R.F. and Hinojosa, D. 1991. Survival of *Erwinia chrysanthemi* in different soil series. *Proteccion de Plantas*. 1:59-67.

*Salazar, P.H. and Duque, S. 1994. Cultural and chemical control of pseudostem pit *(Erwinia chrysanthemi p.v paradisiaca)* in *Musa* spp. *Fitopatalogia Columbiana*. 18: 9-13.

Santha, I.M. 2001. *Laboratory Manual on Plant Genomic Library Construction and Screening*. IARI. New Delhi. pp: 33-34.

Schaad, N.W. 1992. *Laboratory guide for identification of Plant Pathogenic Bacteria*. Second edition. International Book Distributing Co, Lucknow. pp 44-58.

Schaad, N.W and Brenner, D.1977. A bacterial wilt and root rot of sweet potato caused by *Erwinia chrysanthemi*. *Phytopathology*. 67: 302-308.

Senthilvel, V.2000. Studies on the bacterial soft rot of onion bulbs caused by *Erwinia carotovora* (Jones). M.Sc thesis. Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, p.133.

Shillingford, C.A. 1974. Bacterial rhizome rot of banana in Jamaica. *Pl. Dis. Reptr.* 58: 214-218.

Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro organisms and some observations on the influence of the contact between cells and fatty substrates. *J.Microbiol. Serol.* 23. 15-22.

Singh, B.D.1998. *Biotechnology*. Kalyani Publishers, New Delhi, pp. 33-40.

Singh,H.P. 2002. Indian Bananas-Issues and Strategies. Global Conference on Banana and Plantain, 28-31 October 2002. UAS, Bangalore, India *Abstract* : 1.

Society of American Bacteriologists. 1957. *Manual of Microbiological methods*. Mc Graw Hill Book Co. Inc., New York, U.S.A.

Solanky, K.V., Mehta, B.P., Sabalpara, A.N., Joshi, K.R. and Vala, D.G. 2001. Control of rhizome rot of banana through chemicals under South Gujarat conditions. *J.Mycol. Pl. Pathol.* 31:108-109.

Sparks, R.B, Jr. and Lacy, G.H. 1979. Transformation of *Erwinia herbicola* with plasmid pBR 322 deoxyribonucleic acid. *Phytopathology*, 63 : 1293 – 1297.

Sparks, R.B, Jr. and Lacy, G.H. 1980. Purification and characterization of cryptic plasmids pLS 1 and pLS 2 from *Erwinia chrysanthemi*. *Phytopathology*. 70 : 369 – 372.

Starr, M.P. 1981. The Genus *Erwinia*. *The Prokaryotes* (ed.Starr,M.P., Stolp, H., Truper, H.G., Balows, A. and Schlegel, H.G.) Springer Verlag, Berlin. pp 1261-1267.

Starr, M.P., Cosens, G. and Knackmuss, H.J. 1966. Formation of blue pigment indigoidine by phytopathogenic *Erwinia*. *Appl. Microbiol.* 14: 870-872.

Stover, R.H. 1959. Bacterial rhizome rot of bananas. *Phytopathology*, 49: 290-292.

Stover, R.H. 1972. *Banana, Plantain and Abaca Diseases*. Common Wealth Mycological Institute, Kew, Surrey, England. p 316.

Suslow, T.V., Schroth, M.N. and Isaka, M. 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathology*, 72 : 917 – 918.

Thornley, M.J.1960. The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. *J.Appl. Bacteriol.* 23:37-52.

Thwaites, R., Eden-green, S.J. and Black, R. 2000. Diseases caused by bacteria. *Diseases of Banana, Abaca and Enset* (ed. Jones, D.R.). CAB International, Surrey, England. pp 213-239.

Tomlinson, D.L., King, G.A. and Ovia, A. 1987. Bacterial corm and rhizome rot of banana (*Musa* spp.) in Papua New Guinea caused by *Erwinia chrysanthemi*. *Trop. Pest Mangmt.* 33:196-199.

Vaughan, E.K.1944. Bacterial wilt of tomato caused by *Phytomonas solanacearum*, *Phytopathology*. 34:443 – 458.

Volcani, Z. and Zutra, D.1967. Bacterial soft rot: *Erwinia carotovora* in Israel. *Rev. Appl. Mycol.*46 : 228.

*Wardlaw, C.W. 1935. *Diseases of the banana and the Manila hemp plant*. Mac Millan, London. p 651.

Wardlaw, C. W. 1972. *Banana Diseases*. 2nd edition. Longman, London. p. 878.

* Originals not found.

Appendix

APPENDIX – I

A. Composition of media

1. King's B medium

Peptone	-	2.0 g
Glycerol	-	1.0 g
$K_2 HPO_4$	-	0.15g
$MgSO_4$	-	0.15g
Distilled water	-	100ml
pH	-	7.2 – 7.4
Agar	-	2.0g

2.Thornley's semi solid medium

Bacto peptone	-	1.0g
NaCl	-	5.0g
K_2HPO_4	-	0.3.g
Phenol red	-	0.0Lg
L – Arginine Hydrochloride	-	10.0g
Bacto Agar	-	3.0g
Distilled water	-	1l
pH	-	7.2

3. Hayward's semi solid medium

$NH_4 H_2 PO_4$	-	1.0g
KCl	-	0.2g
$Mg SO_4. 7 H_2O$	-	0.2g
Bacto peptone	-	1.0g
Bromothymol blue	-	0.08g
Distilled water	-	1l
pH	-	7.0-7.1
Agar	-	3.0g

4. Sierra's medium

Bacto peptone	-	10.0g
NaCl	-	5.0g
$CaCl_2.H_2O$	-	0.1g
Bacto agar	-	17.0g
Distilled water	-	1l

5. Luria Bertani (LB)medium

Tryptone	-	10.0g
Yeast Extract	-	5.0g
NaCl	-	5.0.g
Glucose	-	1.0g
Distilled water	-	1l
pH	-	7

6. Nutrient Agar medium

Peptone	-	10.0g
Yeast Extract/Beef extract	-	5.0g
Agar	-	20.0g
Distilled water	-	1l
pH	-	6.8

7. Thornton's Standardised agar

Mannitol	-	1.0g
Asparagine	-	0.5g
K_2HPO_4	-	1.0g
KNO_3	-	0.5g
$MgSO_4$	-	0.2g
$CaCl_2$	-	0.1g
NaCl	-	0.1g
Ferric Chloride	-	0.002g
Agar	-	20.0g
Distilled water	-	1l
pH	-	7.4

8. Basal medium for Organic acids

Peptone	-	1.0g
$NH_2 H_2 PO_4$	-	1.0g
KCl	-	0.2g
Mg SO_4 $7H_2O$	-	0.2g
Bromothymol blue	-	0.03g
Agar	-	3.0g
Distilled water	-	1l
pH	-	7.0

9.Starch medium

Peptone	-	10.0g
Beef Extract	-	5.0g
Starch solution	-	2.0g
Agar	-	20.0g
Distilled water	-	1l
pH	-	7.0

10. Peptone water medium

Peptone	-	10.0g
NaCl	-	5.0g
Distilled water	-	1l
pH	-	7.0

11. MR broth

Proteose peptone	-	5.0g
Glucose	-	5.0g
$K_2H PO_4$	-	5.0g
Distilled water	-	1l
pH	-	7.0

12. Gelatin medium

Peptone	-	10.0g
Beef Extract	-	5.0g
Gelatin	-	120.0g
Distilled water	-	1l
pH	-	7.0

13. Tryptone broth medium

Tryptone/ Caesin digest	-	10.0g
NaCl	-	5.0g
Distilled water	-	1l
pH	-	7.0

14.Simmon's citrate agar

Ammonium dihydrogen Phosphate	-	1.0g
Dipotassium Phospate	-	1.0g
NaCl	-	5.0g
Sodium citrate	-	2.0g
Mg SO_4	-	0.2G
Agar	-	15.0g
Bromothymol blue	-	0.08g

15. Logan's medium

Nutrient agar	-	28.0g
Yeast Extract	-	5.0g
Glucose	-	5.0g
0.5% 2,3,5-triphenyl Tetrazolium chloride	-	10ml
Distilled water	-	1l
pH	-	7.0

16. Potato Dextrose agar

Potato	-	200.00g
Dextrose	-	20.0g
Agar	-	15.0g
Distilled water	-	1l
pH	-	7.0

17. Yeast Extract Dextrose Calcium Carbonate agar

Yeast Extract	-	10.0g
Glucose/Dextrose	-	20.0g
$CaCO_3$	-	20.0g
Agar	-	15.0g
Distilled water	-	1l

18. Urease medium

Peptone	-	1.0g
NaCl	-	5.0g
KH_2PO_4	-	2.0g
Glucose	-	1.0g
Agar	-	20.0g
Phenol red	-	6ml
Distilled water	-	1l
pH	-	6.8

19. Sucrose Peptone Agar

Sucrose	-	1.0g
Peptone	-	0.5g
K_2HPO_4	-	0.05g
$MgSO_4.7H_2O$	-	0.03g
Agar	-	1.5g
Distilled water	-	1l

20. Meat Extract Agar

Meat Extract	-	1.5g
Yeast Extract	-	3.5g
Peptone	-	6.0g
Agar	-	15.0g
Distilled water	-	1l
pH	-	7.0

21. Nitrate reduction test

KNO3 (nitrite free)	-	1.0g
Peptone	-	10.0g
Beef extract	-	5.0g
Distilled water	-	1l
pH	-	7.0

B. Buffers and Solutions

1. Resuspension buffer

50 mM Tris Cl pH 8.0
10 mM EDTA
0.2 mg/ml RNase A

2. Lysis Buffer

200 mM NaOH
1% SDS

3. Neutralization buffer (for plasmid DNA isolation)

3 M Potasium Acetate pH 4.8

4. Gel loading dye

Glycerol	-	60%
TAE buffer	-	30%
1% Bromophenol blue	-	10%

ETIOLOGY AND MANAGEMENT OF RHIZOME ROT DISEASE OF BANANA

By

USHA. N. K.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2003

ABSTRACT

Rhizome rot disease of banana is one of the most serious diseases of banana in Kerala.The pathogen was isolated from infected rhizomes of banana var. Nendran and the pathogenicity was established after standardising the method of inoculation.. The symptomatology of the disease was studied in detail under natural field conditions and under artificially inoculated controlled conditions. The salient symptoms of the disease were flaccidity and yellowing of leaves and soft rotting of the rhizome. Based on the morphological, cultural, biochemical, physiological and molecular characters, coupled with its pathogenicity, the pathogen causing rhizome rot disease of banana var. Nendran was identified as *Erwinia carotovora*.

Studies were conducted on the host range of the pathogen on five hosts *viz.* heliconia, canna, ginger, turmeric and colocasia under field conditions. The results revealed that the pathogen failed to infect these hosts. Studies on soil survival of the pathogen revealed that the pathogen survived in the sterilized soil in the presence of diseased rhizomes for 180-185 days.

Out of the 240 accessions of banana screened for resistance to rhizome rot disease under natural field conditions, the genome AAB is found to be the most susceptible one among the genomes screened. The variety Poppoulu (AAB) was found to be the most severely infected one. The commercial variety of Kerala *viz.*, Nendran was also found to be highly susceptible to the disease.

In vitro inhibitory effect of antibiotics, fungicides, botanicals, and bioagents on the growth of the pathogen was tested. Among the fungicides, fytolan at 0.4 per cent concentration gave the maximum inhibition followed by fytolan at 0.3 per cent. Among the antibiotics tried, streptocycline at 300 ppm was found to be the most superior one. Garlic extract at 100 per cent concentration was the best botanical for inhibiting the pathogen.

Pot culture experiment on the management of the disease revealed that among the six treatments given, fytolan at 0.4 per cent and streptocycline at 300

ppm were found to be the most effective treatments in controlling the rhizome rot disease of banana. Garlic extract at 100 percent concentration was also found to be effective in managing the disease under field conditions, which was on par with *Pseudomonas flourescens* (T2) and bleaching powder (T1).