BIOLOGICAL MANAGEMENT OF *Phytophthora* POD ROT OF COCOA

By

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THESIS

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2004

DECLARATION

I, hereby declare that this thesis entitled **"Biological management of *Phytophthora* pod rot of cocoa"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Biological management of *Phytophthora* pod rot of cocoa"** is a record of research work done independently by **Mr. R. Bhavani** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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CONTENTS

CHAPTER	TITLE	PAGE NO
1	INTRODUCTION	1 – 2
2	REVIEW OF LITERATURE	3 - 25
3	MATERIALS AND METHODS	26 – 43
4	RESULTS	44 - 93
5	DISCUSSION	94 - 111
6	SUMMARY	112 - 116
	REFERENCES	117 - 150
	APPENDICES	
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
3.1	List of *Phytophthora* isolates from different locations of Thrissur district	27
4.1	Cultural characteristics of *Phytophthora* isolates on Carrot Agar medium	45
4.2	Morphological characteristics of *Phytophthora* isolates	47
4.3	Dissimilarity matrix of *Phytophthora* isolates based on morphological characters	49
4.4	Grouping of *Phytophthora* isolates based on dissimilarity index of morphological characters	49
4.5	Comparative virulence of different *Phytophthora* isolates on detached cocoa pods	50
4.6	Similarity matrix of *Phytophthora* isolates based on protein profiles	52
4.7	Grouping of *Phytophthora* isolates based on similarity index of protein profiles	52
4.8	*In vitro* sensitivity of *Phytophthora* isolates to fungicides and antibiotics	54
4.9	Reaction of cocoa types against *Phytophthora* pod rot	56
4.10	Epiphytic microflora on cocoa pod surface	60
4.11	*In vitro* screening of fungal isolates against *P. palmivora*	62
4.12	*In vitro* screening of bacterial isolates against *P.palmivora*	64

4.13	Effect of selected antagonists against *P.palmivora* on detached cocoa pods	66
4.14	Cultural and biochemical characters of bacterial antagonists	69
4.15	Compatibility of *Trichoderma* spp. with fungicides	72
4.16	Compatibility of *Trichoderma* spp. with insecticides	74
4.17	Compatibility of *Trichoderma* spp. with fertilizers	76
4.18	Compatibility of *Pseudomonas fluorescens* isolates with fungicides	78
4.19	Compatibility of *Pseudomonas fluorescens* isolates with insecticides	81
4.20	Compatibility of *Pseudomonas fluorescens* isolates with fertilizers	83
4.21	*In vitro* sensitivity of *P. palmivora* to fungicides	85
4.22	*In vitro* sensitivity of *P. palmivora* to insecticides	86
4.23	*In vitro* sensitivity of *P. palmivora* to fertilizers	87
4.24	Effect of various treatments on the incidence of PPR in cocoa garden (After first spraying)	90
4.25	Effect of various treatments on the incidence of PPR in cocoa garden (After second spraying)	91
4.26	Effect of various treatments on the incidence of PPR in cocoa garden (After third spraying)	92

LIST OF FIGURES

Figure No.	Title	Between Pages
4.1	Unweighted pair group average method (UPGMA) dendrogram based on morphological characters of *Phytophthora* isolates	48-49
4.2	Unweighted pair group method (UPGMA) dendrogram based on protein profiles of *Phytophthora* isolates	51-52
4.3	Reaction of cocoa types against *Phytophthora* pod rot on detached pods	58-59
4.4	Compatibility of *Trichoderma viride* (20 F) with fungicides	72-73
4.5	Compatibility of *Trichoderma harzianum* with fungicides	72-73
4.6	Compatibility of *Trichoderma viride* (20F) with insecticides	74-75
4.7	Compatibility of *Trichoderma harzianum* with insecticides	74-75
4.8	Compatibility of *Trichoderma viride* (20F) with fertilizers	76-77
4.9	Compatibility of *Trichoderma harzianum* with fertilizers	76-77
4.10	Compatibility of *Pseudomonas fluorescens* (23B) with fungicides	78-79
4.11	Compatibility of *Pseudomonas fluorescens* (24B) with fungicides	78-79
4.12	Compatibility of *Pseudomonas fluorescens* (K) with fungicides	78-79

4.13	Compatibility of *Pseudomonas fluorescens* (23B) with insecticides	81-82
4.14	Compatibility of *Pseudomonas fluorescens* (24B) with insecticides	81-82
4.15	Compatibility of *Pseudomonas fluorescens* (K) with insecticides	81-82
4.16	Compatibility of *Pseudomonas fluorescens* (23B) with fertilizers	83-84
4.17	Compatibility of *Pseudomonas fluorescens* (24B) with fertilizers	83-84
4.18	Compatibility of *Pseudomonas fluorescens* (K) with fertilizers	83-84
4.19	Effect of fungicides on *P. palmivora*	87-88
4.20	Effect of insecticides on *P. palmivora*	87-88
4.21	Effect of fertilizers on *P. palmivora*	87-88
4.22	Effect of various treatments on PPR incidence	92-93

LIST OF PLATES

Plate No.	Title	Between Pages
1	Different stages of *Phytophthora* pod rot infection on cocoa	1-2
2	Cultural and morphological characters of *Phytophthora palmivora*	47-48
3	Protein profile of *P. palmivora* isolates	51-52
4	*Trichoderma* spp. and *P. palmivora* in dual culture	64-65
5	Photomicrographs of *T. viride* and *T. harzianum*	64-65
6	*Pseudomonas* spp. and *P. palmivora* in dual culture	64-65
7	Mycoparasitism of *P. palmivora* by *T. harzianum*	69-70
8	Mycoparasitism of *P. palmivora* by *T. viride*	69-70
9	Effect of $FeCl_3$. $6H_2O$ on siderophore production	70-71

Introduction

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) ranks third as a beverage crop in the world next to Tea and Coffee. It belongs to the family Sterculiaceae, originated millions of years ago in South America. The largest cocoa producing countries are Cote d' Ivoire, Ghana and Indonesia. The world production of cocoa beans account about 2853.4 thousand tones in 2000-01 (ICCO, 2002-03). In India, commercial cultivation of cocoa was started in early 1960's. Currently 18,000 hectares are under cultivation, with dry bean production of 10,000 tonnes. Kerala is the principal cocoa growing state of India accounting for more than 50 per cent area under cultivation. Cocoa cultivation confined to Kerala few years back is now spreading to Karnataka, Andra Pradesh and Tamil Nadu.

Cocoa is prone to the attack of many diseases. Among the various diseases, *Phytophthora* pod rot (PPR) is the most serious one. PPR commonly known as 'Black pod' disease was reported to cause 44 per cent of the global crop losses (Vander vossen, 1997). In India, this disease was first reported in 1965 (Ramakrishnan and Thankappan, 1965) and incidence of the disease has been found to vary from 12.93 to 29.78 per cent depending upon locality (Chandramohanan, 1985). Several *Phytophthora* species have been reported to cause the disease from different geographic regions of the world. Among these, *P. palmivora* is the most predominant one. In India, involvement of *P.capsici* (Chowdappa *et al.*, 1993) and *P. citrophthora* (Chowdappa and Chandramohanan, 1996) in PPR of cocoa was reported in addition to *P. palmivora* (Ramakrishnan and Thankappan, 1965). This pathogen is also known to cause seedling blight, stem canker, chupon blight and twig die back in cocoa.

A line of chemical fungicides, especially Bordeaux mixture was reported to be very effective in the management of PPR of cocoa provided proper cultural practices

are taken up. Often, it was observed that improper preparation and untimely application of this fungicide failed to give the desired effect. Further, indiscriminate use of fungicides in the cocoa ecosystem may pose many potential ecological problems. Hence, now a days thrust is being given to develop ecofriendly disease management practices utilizing potential microbes, which are harmful to the pathogen. So it is ideal to explore the possibility of exploiting the potential of antagonistic epiphytic microflora found on the surface of cocoa pods in the integrated disease management of PPR of cocoa. The efficiency of epiphytic microbes for the control of this disease has been pointed out by Krauss and Soberanis (1999).

One of the components of integrated plant disease management in the utilization of resistant/tolerant genotypes if any, for reducing the losses. Earlier studies revealed availability of resistant genotypes against the PPR of cocoa (Lawrence, 1978; Chandramohanan, 1982; Abraham *et al.*, 2001). Thus, in view of the above facts and serious nature of this disease, the present investigation was carried out with the following objectives.

1. Isolation and identification of the pathogen/s from different cocoa growing tracts of Thrissur district
2. Screening of cocoa genotypes against PPR disease.
3. Isolation of the epiphytic antagonistic microorganisms of the pathogen.
4. Assessment of efficacy of selected antagonists against the pathogen.
5. Studies on mechanism of antagonism.
6. Field evaluation of antagonists against PPR disease.

Review of literature

2. REVIEW OF LITERATURE

The *Phytophthora* pod rot (PPR) disease of cocoa was first noticed in Guyana and West Indies by Jenman and Harrison (1897). The disease was named as 'black pod' caused by *Phytophthora* by Massee (1899). In India, the disease was reported by Ramakrishnan and Thankappan (1965). Occurrence of *Phytophthora* infection on cocoa was observed from all cocoa growing countries by Thorold (1975). In India, *P. palmivora* was reported to cause various diseases in cocoa like PPR (Ramakrishnan and Thankappan, 1965), canker (Chandramohanan, 1978), seedling die back (Chandramohanan, 1979) and twig die back and chupon blight (Chandramohanan *et al.*, 1979). Chandramohanan and Kaveriappa (1981) noted that among the diseases of cocoa occurring in South India, black pod was observed in 93.4 per cent of gardens in Kerala, Karnataka and Tamil Nadu.

Blencowe and Wharton (1961) reported an annual crop loss of 19 per cent in *P. palmivora* infected areas. In India, the incidence of PPR of cocoa has been found to vary from 12.93 to 29.78 per cent depending on locality (Chandramohanan, 1985). According to Vandervossen (1997), among the cocoa diseases PPR was the most important one worldwide and caused about 44 per cent crop loss. According to Dakwa (1973) and Akrofi *et al.* (1997) *Phytophthora* incidence and crop losses of cocoa fluctuated with the seasons and locality.

2.1. CAUSAL ORGANISM

World wide, black pod, stem canker, chupon blight, twig die back and seedling blight of cocoa were known to be caused by *Phytophthora* spp.

The first record of a fungus associated with black pod of cocoa was from Ceylon and named the pathogen as *Peronospora corda* (Carruthers, 1898). Massee

(1899) after examining infected pods from Trininad, identified the causal agent as *Phytophthora omnivora* de Bary. The fungus associated with the black pod disease in Cameroon was studied by Faber (1909) and noted the difference in characters that reported for *P. omnivora.* However, he retained only the generic name to this fungus. Coleman (1910) described the fungus associated with the disease in Ceylon as *Phytophthora theobromae* Coleman. Reinking (1919) reported that the fungus causing bud rot of coconut was identical with *P. theobromae*. Butler (1925) confirmed that *P. theobromae* was morphologically identical with *Phytophthora palmivora* (Butl.) Butl. the causal organism of bud rot of palmyrah palm (Butler, 1907; 1919). Orellana (1954; 1959) suggested the name as *Phytophthora palmivora* var. *theobromae* for cocoa strain as it differed in physiological and cultural characters from other strains occurring on palms, rubber etc.

Several *Phytophthora* species have been reported to cause the pod rot disease of cocoa in different geographic regions of the world. These include *P. palmivora* with world wide distribution (Gregory, 1974), *P. megakarya* in West Africa (Griffin, 1977), *P. capsici* in Central and South America and some Caribbean countries (Zentmyer, 1988b) and India (Chowdappa *et al.*, 1993), *P. citrophthora*, in Brazil (Campello and Luz, 1981) and India (Chowdappa and Chandramohanan, 1996), *P. megasperma* in Venezuela (Zentmyer, 1988b), *P. katsurae* in Cote d' Ivoire (Liyanage and Wheeler, 1989) and *P. nicotianae* in Malaysia (Tey and Bong, 1990).

Tucker (1931) and Waterhouse (1974) recognized two groups of *P. palmivora* isolates and referred as 'typical' and 'atypical' forms, which were distinguishable on the basis of sporangial morphology. During the Rothamsted cocoa *Phytophthora* workshop in 1976, it was agreed that there were at least three and possibly more distinct morphological forms within the *P. palmivora*, which were temporarily designated as MF_1, MF_2, MF_3 and MF_4 'other types' and 'pepper forms'

(Griffin, 1977). The important characteristics of these four morphological forms are given below.

Characteristics	MF_1	MF_2	MF_3	MF_4
Cultures on carrot agar (growth pattern)	Stellate striate with aerial mycelium	Stellate or Striate	Cottony aerial mycelium	Petaloid with moderate amount of mycelium
Shape of sporangia	Near spherical to ovate - elongate with round base	Near spherical to ovate - elongate with round base and deciduous	Near spherical ovate - elongate with round base and deciduous	Elongate and boat shaped base usually tapered towards pedicel
Pedicel length	< 5µm	> 5µm	10-15µm	> 15µm
L/B ratio	1.2 to 1.8	-	1.2 - 1.6	1.9 - 2.3
Compatibility type	Predominantly A_2 (A_1 un common)	-	Predominantly A_1 (A_2 rare)	A_1 and A_2

Based on the pedicel length, Zentmyer *et al.* (1977) and Kaosiri *et al.* (1978) classified the cocoa isolates of *Phytophthora* into four groups.

Group I : Short and thick sporangial stalks with average length < 5 µm

Group II : Thin sporangial stalks intermediate in length (average 5-15 µm)

Group III : Unusual and characteristically long stalks with an average length of >15µm

Group IV : Non-caducous sporangia

The first three groups correspond to MF_1, MF_3 and MF_4 of the cocoa isolates of *P. palmivora* (Griffin, 1977). The fourth group with non-caducous sporangia were found to be *P. citrophthora* (Campello and Luz, 1981; Kellam and Zentmyer, 1986). Importance of sporangial stalk length as a valuable taxonomic criterion for identifying the isolates in the *P. palmivora* complex of cocoa was reported by Waterhouse (1974), Kaosiri *et al.* (1978), Al-Hedaithy and Tsao (1979) and Zentmyer *et al.* (1979).

According to Newhook *et al.* (1978), Ho (1981), Waterhouse *et al.* (1983) and Stamps *et al.*(1990) the size, shape and length to breadth ratio of sporangia were frequently considered as an important characteristic feature in identifying *Phytophthora* species. Kaosiri *et al.* (1978) reported that caducity of sporangia was a useful taxonomic feature for some *Phytophthora* species, where caducous sporangia with short or long stalks were produced by all cocoa isolates on carrot agar that previously had been identified as *P. palmivora* and *P. capsici* respectively. Kellam and Zentmyer (1986) examined *P. citrophthora* isolates from cocoa, which produced non-caduons, irregular shaped and papillate sporangia and was found morphologically similar to *P. citrophthora* isolates from citrus.

Alizadeh and Tsao (1985) and Zentmyer (1988a) considered sporangial ontogeny as an important taxonomic criterion in distinguishing *Phytophthora* species. The formation of sporangia of *P. palmivora* and *P. megakarya* of cocoa has been reported as typical sympodial (Idosu and Zentmyer, 1978; Brasier and Griffin, 1979; Zentmyer, 1988a). The umbellate type of sporangial formation in *P. capsici* was observed by Zentmyer (1988a).

The morphological and physiological characteristics of 950 isolates of *Phytophthora* collected from all the major cocoa growing areas of the world were studied by Brasier and Griffin (1979). They reported that majority of the isolates

belonged to either one of the three main groups *viz.*, MF_1, MF_3 and MF_4. Therefore, MF_1 and MF_2 forms were redesignated as *P. palmivora*, whereas MF_3 was described as a new species *P. megakarya*. Waller (1981) reported that *P. palmivora* MF_4 closely resembles with *Phytophthora* isolates from pepper. Later it was renamed as *P. capsici*.

Kaosiri *et al.* (1980) reported that *P. palmivora* isolates from different geographical origins could be grouped on the basis of five different colony patterns. The differences in colony pattern were complemented by differences in their growth rates. Further, difference in morphological characters was also recorded for isolates from different countries. The cluster analysis based on morphological, physiological and pathological characters of *Phytophthora* spp. was reported by Ho (1982), which formed separate clusters based on species of *Phytophthora* infecting cocoa. The pedicel length appeared to be most stable morphological characters.

2.1.1 SDS - PAGE

Clare (1963) first provided the evidence that protein banding patterns may be a useful taxonomic determinant for pythiaceous fungi. The use of electrophoretic protein profiles for distinguishing species and sub groups within the species of *Phytophthora* was highlighted by various workers (Kaosiri and Zentmyer, 1980; De vallavieille and Erselius, 1984; Hansen *et al.*, 1986; Bielenin *et al.*, 1988; Chowdappa and Chandramohanan, 1995; Appiah *et al.*, 2003). Oudemans and Coffey (1991) reported that there were no wide variations in cultural and morphological characters among the cocoa isolates of *P. palmivora* occurring in India. Further, according to them *P. palmivora* has been found to be highly homogenous species on isozyme analysis.

Comparative studies on *Phytophthora* isolates of cocoa revealed that *P. palmivora, P. megakarya, and P. capsici* could be resolved into three distinct groups based on protein banding patterns (Kaosiri and Zentmyer, 1980; Chowdappa and Chandramohanan, 1995). Electrophoresis of soluble proteins from mycelia helps in distinguishing both interspecific and intraspecific variation in *Phytophthora* spp. from cocoa (Chowdappa and Chandramohanan, 1995). Chowdappa and Sarma (2003) opined that protein banding patterns might be useful in separating morphological species. The variation within a species seems to be very little taxonomic value.

2.2 EPIDEMIOLOGY AND SPREAD OF DISEASE

Epidemiological studies revealed that the pathogen *P. palmivora,* infecting various crops were most active in warm tropical regions receiving higher amounts of rainfall (Coleman, 1910, Sundaraman and Ramakrishnan, 1924; Rao, 1927). Braudeaux (1974) noticed that the high rainfall and humidity in cocoa growing areas were favourable for development of cryptogamic disease, particularly *P. palmivora.* Wood (1974) confirmed Dade's (1927) assertion that atmospheric humidity was probably the most important factor affecting the incidence of black pod disease. Alvim and Kozlowsky (1977) observed that high humidity within the plantation, adequate rainfall and thick vegetative cover over the soil were conducive for the disease development. Further, they reported that the spores of *P. palmivora* were dispersed through rain, which readily germinate in water droplets on the pod surface to initiate fresh infections.

Gorenz and Okaisabor (1971) reported that the layer of dead leaves in the soil and plant debris like pod husks, in which *P. palmivora* thrives saprophytically, serves as source of primary inoculum. Gregory (1981) hypothesized that cocoa root infection by *Phytophthora* might serve as a site of fungal survival in the soil environment where chlamydospores and oospors served as inoculum for infecting the

roots of cocoa seedlings. Kellam and Zentmyer (1986) noticed that the mortality rate of seedlings was higher for *P. palmivora* and *P. citrophthora* than for *P. capsici*. They also reported that the broken pods in husk pits that hold water and less exposed to direct sunlight were more suited for fungal survival.

2.3 SYMPTOMATOLOGY

The symptomatology of *Phytophthora* disease in cocoa was studied by many workers. According to Ramakrishnan and Thankappan (1965), cocoa pods of all ages were affected and the disease appeared as a brown discolouration in the initial stages both from the apical and pedicel end of the pods, which later spread rapidly occupying the entire area of the pod surface. A white web of mycelium was also seen on the surface of infection, whereby the infected tissue shrunk and became dark brown in colour and corky in texture. The internal tissues of the fruit and sometimes the beans also turned dark brown.

Gregory (1974) observed that the major symptoms produced by *P. palmivora* on cocoa plant includes seedling blight, trunk canker, dieback of twigs, blight and necrosis of leaf and petiole and rotting of fruit. Firman (1974) noticed that *P. palmivora* attacks all major organs of cocoa including pods, stems, leaves and roots. Pereira *et al.* (1980) reported that the first indication of infection was the presence of water soaked spots, which turned necrotic and enlarged, eventually covering the whole pod surface.

Abraham *et al.* (1992) reported that during the rainy season, abnormal symptoms were noticed on immature pods infected by *P. palmivora*, which were characterized by concentric rings in the sub-epidermal region of the infected portion of the pod, cementing together of the beans with placenta and husk and watery consistency of the kernel in infected beans.

2.4 SCREENING FOR HOST RESISTANCE

Asare-Nyako and Amponsah (1973) described the glasshouse method for screening of the cocoa seedlings against black pod. Out of 51 cultivars of cocoa screened, nine cultivars such as EET 59, EET 376, Pound 7, UF 713, UF 715, SCA 6, SCA 12, Catongo and Diamantes 800 showed a promising degree of resistance. Pods were used to test the susceptibility of cultivars to the disease by inoculating them with spore suspension (Lawrence, 1978 and Phillips-Mora and Galindo, 1989).

Screening of cocoa types for PPR resistance using detached cocoa pods was carried out by many other workers which resulted in the identification of moderately resistant types like EET 59,EET 376, SCA 6, DR 16, C 78 (Lawrence, 1978; Chandramohanan, 1982). The average diameter of the lesions 9 days after inoculation due to *P. capsici* was 1/2 and 1/3 of those due to *P. palmivora* and *P. citrophthora* respectively (Luz and Yamada, 1984).

From East Java, clones DR 16, SCA 6, SCA 12 and ICS 6 were reported to be resistant to black pod pathogen *P. palmivora* (Sri-Sukamato and Mawardi, 1986). Out of 82 cocoa genotypes screened, seven were resistant to *P. capsici*, 21 to *P. palmivora* and only two to *P. citrophthora* (Luz *et al.*, 1996). Phillips-Mora (1996) reported that out of 350 cocoa cultivars evaluated seven per cent were resistant, 29 per cent moderately resistant, 34 per cent moderately susceptible and 31 per cent susceptible.

Screening on detached pods of different cocoa types against *P. palmivora* at Cadbury KAU Co-operative Cocoa Research Project, College of Horticulture, Vellanikkara showed that the cocoa type G VI 14 recorded the lowest percentage of pod area infection (KAU, 1994; 2000). Abraham *et al.* (2001) evaluated the reaction

of 166 cocoa types to PPR and reported that four types showed moderately resistant reaction.

2.5 MANAGEMENT OF THE DISEASE

2.5.1 Chemical control

2.5.1.1 Laboratory studies

2.5.1.1.1 Fungicides

According to Martin (1968), Okaisabor (1970) and Filani (1973) copper fungicides were highly fungicidal and inhibitory to zoospore germination of *P. palmivora* under laboratory conditions. Filani (1976) noticed restricted growth of *P. palmivora* with cuprous oxide, copper sulphate, copper oxychloride, copper hydroxide and copper carbonate at all concentrations tested. Chandramohanan *et al.* (1979) observed that Captafol (0.2 per cent), copper oxychloride, Guazatire and Fenfuram each at 0.3 per cent inhibited the lesion development on detached cocoa pods. Figueiredo and Lellis (1981) noticed the effect of copper oxychloride in inhibiting the growth of *P. palmivora* under *in vitro* conditions. Reddy and Chandramohanan (1984) observed that Bordeaux mixture (0.75per cent) Fytolan and Thiram (0.5per cent), Dithane M45 (0.4per cent), Difolatan (0.3per cent) and Captan (0.5per cent) completely inhibited the growth of *P. palmivora in vitro*. According to Coffey and Bower (1984) *P. palmivora* was very sensitive to metalaxyl even at lower concentrations of 0.1 $\mu l\ ml^{-1}$.

McGregor (1984) observed that copper sandoz (50per cent cu^{++}) was superior to all other formulations tested against sporulation of *P. palmivora*. Also the mycelial growth of *P. capsici* and *P. citrophthora* was completely inhibited by Ridomil (metalaxyl) and curzate at 25 ppm. *P. palmivora* was killed by 75 ppm copper sandoz, curzate and Gofex (copper oxychloride + metallic copper) (Campello *et al.*, 1984). Tey and wood (1984) tested the *in vitro* effect of eight fungicides

against *P. palmivora* and found that cychloheximide and mancozeb were highly toxic even at low concentrations. Metalaxyl, etridiazole and cyprofuram were highly inhibitory to mycelial growth, sporangium and chlamydospore production of cocoa isolates (Chan and Kwee, 1986). Further, Ramachandran *et al.* (1988) noticed the sensitiveness of different isolates of *Phytophthora* from various crops to metalaxyl.

Raghu and Chandramohanan (1993) reported that Ridomil MZ (0.2 and 0.3 per cent) Fytolan, Blitox, Foltaf, Dithane M45 and Captaf (each at 0.3 per cent concentrations) were fungicidal to *P. palmivora* under *in vitro* conditions. Further, they also found that Ridomil MZ, Foltaf and Captaf inhibited *P. palmivora* infection on detached cocoa pods. Prem (1995) noticed that among seven fungicides tested, Fytolan, Captaf, Bordeaux mixture, Akomin and Ridomil each at 0.1, 0.2 and 0.3 per cent concentrations completely inhibited the growth of *P. palmivora* causing seedling blight of cocoa. May and Kimati (2000) found that metalaxyl was the most efficient fungicide for inhibiting the mycelial growth of *P. parasitica*. Vijayaraghavan (2003) tested, nine fungicides and reported the inhibitory effect of Bordeaux mixture (0.5, 1 and 1.5 per cent), Indofil M45, Ridomil MZ and Fytolan (0.2, 0.3 and 0.4 per cent), Akomin (0.3 and 0.4 per cent), Captaf and Kocide (0.1, 0.2 and 0.3 per cent) on the growth of *P. capsici*.

2.5.1.1.2 Antibiotics

Not much work has been conducted on the *in vitro* effect of antibiotics against *Phytophthora* spp. However, Somani and Patel (1970) noticed that Aureofungin gave good control of *P. nicotianae* var. *nicotianae*. Treatment of tuber slices with chloramphenicol (50 to 800 ppm) or streptomycin (3.125 - 50 ppm) inhibited the growth of *P. infestans* in potato (Barna *et al.*, 1972; Ersen *et al.*, 1972). Reddy and Chandramohanan (1984) observed complete inhibition of the growth of black pod pathogen *P. palmivora* by Aureofungin. Prem (1995) studied *in vitro*

inhibitory effect of six antibiotics *viz.*, streptomycin sulphate, Streptocycline, terramycin, Aureofungin, amoxycillin and chloramphenicol each at 200, 400 and 500 ppm concentrations against *P. palmivora*. He found that chloramphenicol and terramycin at 400 and 500 ppm completely inhibited the growth of the pathogen.

2.5.1.1.3 Fertilizers

A perusal of literature revealed that only very few work has been conducted on the *in vitro* effect of fertilizers against *Phytophthora* spp. However, Lilly and Barnett (1951) reported that, in general, nitrate nitrogen favoured the mycelial growth of many fungi. Cameroon and Milbrath (1965) and Pal (1974) observed that ammonium nitrate acted as the best nitrogen source for the growth of *Phytophthora* spp. According to Jain *et al.* (1982) among the inorganic nitrogen salts, ammonium nitrate supported the growth of *P. parasitica* var. *nicotianae* followed by ammonium sulphate and ammonium chloride. The effect of six nitrogen sources *viz.*, ammonium sulphate, ammonium chloride, ammonium nitrate, sodium nitrate, potassium nitrate and peptone on growth of *P. capsici* was studied by Jayasekhar and Muthusamy (2000). They found that ammonium nitrate was the best source for growth of the pathogen. Vijayaraghavan (2003) noticed that among fertilizers tested against *Phytophthora capsici*. Factomphos and ammonium sulphate partially inhibited the growth of the fungus.

2.5.1.2 Field studies

The need to control PPR of cocoa with chemical fungicides depends on the magnitude of attack and loss of crop as well as yield (Asare-Nyako, 1974; Muller, 1974). Gorenz (1971) noticed effectiveness of Bordeaux mixture in controlling PPR of cocoa. Bordeaux mixture gave the best control followed by Captan, Lonacol, Fytolan and Hinosan (Menon *et al.*, 1973). Effect of Kocide against black pod of

cocoa caused by *P. palmivora* was reported by Rocha *et al.* (1973). Kueh (1980) observed that the pod infection by *P. palmivora* was reduced by Etridiazole and metalaxyl. McGregor (1982) conducted field and detached pod test with Ridomil and Mordox (Cuprous oxide) and observed substantial reduction of infection by *P. palmivora* on cocoa. McGregor (1983) stated metalaxyl as a best available systemic fungicide against black pod of cocoa. Significant reduction in black pod incidence of cocoa with metalaxyl, chlorothanil and fentin acetate was observed by Kueh (1984). Field studies conducted by Reddy and Chandramohanan (1984) with five fungicides indicated the efficacy of copper based fungicides against *P. palmivora* of cocoa. According to Sreenivasan *et al.* (1990) distribution of copper deposits on bark and pods of cocoa reduced the incidence of *P. palmivora*.

Trunk injections of phosphonates, such as potassium phosphonate and Aliette CA, provided effective and durable control of *Phytophthora* diseases (Pegg *et al.*, 1985). Ventilborgh (1987) reported the effectiveness of copper fungicides and Ridomil 72 plus against *P. palmivora*, the causal agent of PPR of cocoa. Trunk injection with potassium phosphonate provided significant protection against infection of pods with *Phytophthora* for at least one year (Anderson *et al.*, 1989). However, Holderness (1990; 1992) observed that foliar sprays with potassium phosphonate gave poor control of both canker and pod rot of cocoa.

Copper containing materials like copper oxide and copper oxychloride gave promising results against *Phytophthora* diseases of cocoa (Manalo and Tangonan, 1992). Guest *et al.* (1994) stated that trunk injection with potassium phosphonate (8 or 16 g a.i/tree every 6 months) was effective against black pod and stem canker of cocoa. Spraying of either metalaxyl and copper-I-oxide (Ridomil 72 plus) or cuprous oxide (Nordox 75) combined with cultural practices were found effective against *P. megakarya* on cocoa (Akrofi and Appiah, 1995). Opoku *et al.* (1998) observed systemic fungicide, Foli-R-Fos 400 (Commercial Potassium

phosphonate 40per cent a.i) was effective than contact copper fungicides in the control of both *P. megakarya* and *P. palmivora*. The package of cultural practices was an essential part in PPR management on cocoa and also enhancing the effectiveness of chemical fungicides (Akrofi, 2000; Akrofi *et al.*, 2003). According to Chandramohanan (2002) combination of cultural practices and application of Bordeaux mixture was very effective in managing the PPR of cocoa.

2.5.2 Biological control

Sanford and Broadfoot (1931) were the pioneers to introduce the term biological control in Plant Pathology and conducted the first experiment on biological control of plant pathogens with antagonists. Baker and Cook (1974) defined biological control as "the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms, accomplished naturally or through manipulation of environment, host or antagonist or by mass introduction of one or more antagonists".

Coniothyrium, Gliocladium, Trichoderma, Latesaria, Sporodesmium, Aspergillus and *Fusarium* and several bacteria and actinomycetes are known for their potential biocontrol activities against pathogens including several species of *Phytophthora* (Malajezuk, 1983; Adams, 1990; Naik and Sen, 1992). The surface of cocoa pods provides a habitat for epiphytic microorganisms and interfering with the pathogenic activities of *P. palmivora*, the causal agent of black pod (Galindo, 1992). He isolated microorganisms from the surface of healthy cocoa pods from trees in abandoned farms with a high incidence of PPR. Further, he found success against PPR of cocoa with *Pseudomonas fluorescens*.

The antagonistic nature of epiphytic bacteria isolated from healthy cocoa pods towards *P. palmivora* was also observed by Dennis *et al.* (1995). Moreover,

Natarajan and Manibhushanarao (1996) reported that using fungal antagonists against fungal pathogens had gained considerable attention and appears to be promising as a viable supplement to chemical control. Usefulness of native fungal antagonists especially *Trichoderma* spp. isolated from healthy cocoa pods by baiting technique to reduce the pod rot incidence of cocoa was demonstrated by Krauss *et al.* (1998). Konam (1999) opined that for a sustainable and ecofriendly PPR disease management, planting resistant/tolerant varieties, application of natural antagonists and following proper cultural practices were essential. Krauss and Soberanis (1999) observed that timely application of biocontrol agents and regulating the level of shade increased the yield of cocoa upto 160 per cent. The effect of antagonistic epiphytic micro flora from cocoa pods in reducing the pod rot disease was also highlighted by Chadramohanan (2002).

2.5.2.1 Fungal antagonists

Several studies suggested the potential use of *Trichoderma* as an effective biocontrol agent against *Phytophthora* diseases of crop plants. Liu and Baker (1980) reported that the genus *Trichoderma* to be a potential biocontrol agent against plant pathogenic fungi. Galindo (1992) observed that *T. harzianum* reduced pod rot infection caused by *Phytophthora* sp. on cocoa. Krauss *et al.* (1998) too reported that certain species of *Gliocladium* (*Trichoderma*) reduced frosty pod rot. Chowdappa (2000) noticed the effectiveness of *T. harzianum* as a potential biocontrol agent and recommended for its included in the integrated pod rot disease management of cocoa. Further, Hoopen *et al.* (2003) observed that *Gliocladium roseum* provided the greatest hope for efficient biocontrol against *P. palmivora* on cocoa.

2.5.2.1.1 Mechanism of antagonism of Trichoderma spp.

Weinding (1934) reported that antibiotics produced by *Trichoderma* spp. were involved in biocontrol activities. Pyke and Dietz (1966) described dermadine, a major volatile antibiotic produced by *Trichoderma*. *Trichoderma* spp. were known to produce large quantities of fungitoxic metabolites such as trichodermin, dermin, trichoviridin, trichobrachin etc. (Yomono *et al.*, 1970). Dennis and Webster (1971) suggested that the inhibitory effect of antagonists against pathogens under *in vitro* conditions might be due to the production of inhibitory volatile metabolites.

Several workers studied the mechanism of antagonism of *Trichoderma* spp. like competitive saprophytic ability, antibiotic production, direct parasitism and lysis (Ayers and Adams, 1981; Bell *et al.*, 1981). Further, several strains of *Trichoderma* spp. produced cellulase and other cell wall degrading enzymes against pathogens (Chet and Baker, 1981; Elad *et al.*, 1982). Elad *et al.* (1983) reported that *Trichoderma* bring about mycoparasitism by enzymatic lysis of pathogenic fungal hyphae through the production of enzymes like ß, 1-3 glucanase, chitinase, cellulose and protease.

Ridout *et al.* (1986) and Claydon *et al.* (1987) noted that mycoparasitism by *T. harzianum* was due to the production of cell wall degrading enzymes, volatile alkyl pyrones and antibiotics. Harman *et al.* (1993) purified chitinolytic enzymes produced by *T. harzianum*. D'Ercole *et al.* (1993) revealed that *T. harzianum* and *T. viride* produced volatile compounds like caprillic, caprinic, capronic acid, ethylene and formic aldehydes. Schirmbock *et al.* (1994) and Delacruz *et al.* (1995) noticed that the enzymes chitobiohydrolase, endochitinase or ß-1,3 glucanase produced by *T. harzianum* inhibited spore germination and hyphal elongation of phytopathogenic fungi. Sivasithamparam and Ghisalberi (1998) listed 43 substances produced by *Trichoderma* spp. that have antibiotic activity and of these alkyl pyrones, isonitriles,

polyketides, peptaibols, diketopiperazines, sesquiterpenes and steroids had frequently been associated with biocontrol activity of some species and strains of *Trichoderma*.

Bhai (2000) observed overgrowth of *Trichoderma* spp. on *Phytophthora* and parasitized the hyphae when both were grown on agar media. She also noticed hyphal lysis, penetration and coiling of the parasite besides the production of volatile compounds. Rey *et al.* (2001) reported that the mutant of *T. harzianum* strain performed better than the wild type by overgrowing, sporulating and killing the pathogen faster. Rocha-Ramirez *et al.* (2002) and Markovich and Kononova (2003) stated that *Trichoderma atroviride* produced hydrolytic enzymes and coiled around the host fungal hyphae.

2.5.2.1.2 Field efficacy of Trichoderma spp.

The large scale use of *Trichoderma* as a biological fungicide in control of plant diseases was reported by Cates (1990). Several strains of *Trichoderma* sp. were reported to be effective against many soil borne plant pathogenic fungi both under green house and field conditions (Chet and Inbar, 1994)

Pechprome and Soytong (1996) emphasized the need for field testing of biocontrol agents to prove their efficacy. The role of *Trichoderma* spp. in reducing the pod rot of cocoa was observed by Krauss *et al.* (1998). The mixture of *Trichoderma* and *Cheatomium* mycofungicides significantly controlled the root and stem rot of black pepper *P. palmivora* and *P. parasitica* root rot of sweet orange (Sodsa-art and Soytong, 1999; Soytong *et al.*, 1999). Elad *et al.* (1999) observed that some *Trichoderma* strains induced SAR by production of chitinases and peptidases in both root and leaf tissue of treated plants.

According to Krauss and Soberanis (1999 timely application of biocontrol agents not only reduced PPR but also increased the yield. Bong *et al.* (2000) noticed the efficacy of *Trichoderma* and *Gliocladium virens* alone and in combinations for the control of black pod of cocoa. Different *Trichoderma* spp. like *T. aureoviride, T. harzianum, T. pseudokoningi, T. polysporum, T. longibrachiatum* and *T. koningi* overgrew and lyse the mycelia of *P. capsici* of black pepper to varying degree (Saju *et al.*, 2002).

2.5.2.2 Bacterial antagonists

Epiphytic microflora comprises resident and transient microorganisms. The main components of the flora were bacteria, yeasts and filamentous fungi (Baker and Cook, 1974). Galindo (1992) noticed the epiphytic bacterial populations were highest during periods of precipitation and high relative humidity and lowest during dry periods.

The inhibitory effect of *Pseudomonas aeruginosa* to *Phytophthora palmivora* under *in vitro* condition was reported by Attafuah (1965). Galindo (1992) reported that the epiphytic microflora like *P. fluorescens* and *P. aeruginosa* were antagonistic to cocoa pod rot pathogen. Dennis *et al.* (1995) also reported that the epiphytic bacteria isolated from healthy pods of severely infected cocoa plantations were antagonistic to the development of *P. palmivora*. Sharifuddin (2000) identified nine potential antagonistic bacteria *viz.*, *Enterobacter* sp., *Pseudomonas aeruginosa, Serratia marcescens, Burholderia cepacia* and five isolates of *Bacillus* sp against *P. palmivora* and *P. nicotianae* from cocoa rhizosphere based on *in vitro* screening.

2.5.2.2.1 *Mechanism of antagonism of Pseudomonas* spp.

Howell and Stipanovic (1980) reported that *Pseudomonas* spp. inhibited the growth of Oomycetes, including plant pathogens in the genus *Pythium*. Howell and Stipanovic (1980) stated that the production of pyoluteorin and siderophores as the main mechanisms of antagonism. *P. fluorescens* produced a suite of antibiotics including pyrrolnitrin, pyoluteorin and 2,4 diacetyl phloroglucinol. Moreover, it also produced hydrogen cyanide and the siderophores like pyochelin and pyoverdin (Stutz *et al.*, 1986). Several workers also described the mode of action of pseudomonads as production of antibiotics or by nutrient competition (Janisiewiez and Roitman, 1988). Dowling and O'Gara (1994) found that *P. fluorescens* induced the production of plant growth regulators like gibberellin, cytokinins and indole acetic acid thus, enhancing plant growth and increasing disease resistance.

Numerous other modes of action have been reported for the antagonistic effects of *P. fluorescens* in controlling disease, which include synergistic effects observed on fungal pathogen with a combination of antifungal compounds, competition for nutrients, production of cell wall type enzymes and induced systemic resistance (Dalisay and Kuc, 1995; Dunne *et al.*, 1998; Singh *et al.*, 1999; Nandakumar *et al.*, 2001). The PGPR strains may stimulate the production of biochemical compounds associated with host defense (M'piga *et al.*, 1997). Demeyer and Hofte (1997) reported that the salicylic acid and lipopolysaccharides produced by *P. fluorescens* may act as local and systemic signal molecules in inducing resistance in plants. Several other antibiotics produced by *P. fluorescens* like 2,4-diacetyl phloroglucinol, phenazines, oomycin, pyoluteorin, pyrrolnitrin and viscosinamide were also effective against various phytopathogens (Nielsen *et al.*, 1998; Ligon *et al.*, 2000; Raaijmakers and Weller, 2001).

2.5.2.2.2 *Field efficacy* of *Pseudomonas* spp.

Several researchers recognized *P. fluorescens* as a biological control agent in suppressing many diseases (Kloepper, 1991; Xu and Gross, 1996; Keel *et al.*, 1996; Sharifi-Tehrani *et al.*, 1998; McSpadden-Gardener *et al.*, 2000).

For the survival of epiphytic microorganisms like bacteria on the surface of aerial parts there must be film of water or relative humidity above 95 per cent in cacao plantations (Alvim, 1977). Jimenez *et al.* (1986) reported the differential interaction between *P. fluorescens* isolates and the cocoa cultivars under field conditions. Loper (1988) reported that fluorescent pseudomonads comprised a large proportion (33 to 100 per cent) of the effective biocontrol strains screened against *Pythium* damping off in green house conditions. Galindo (1992) noticed *P. fluorescens* isolates from the surface of healthy cocoa pods were antagonistic to *P. palmivora* under field condition and also observed lower disease incidence. Moreover, the bacterial populations fluctuated between 8 x 10^4 and 7 x 10^6 cfu/cm^2 and the highest levels was observed during periods of precipitation and high humidity and the lowest during dry periods. Fluorescent pseudomonads were typically among the most effective antagonists selected for suppression of both soil borne and aerial diseases of plants (Loper *et al.*, 1997)

2.5.2.3 Compatibility of antagonists with fungicides

T. harzianum Rifai, tolerant to most of the fungicides and was used for the integrated control of many plant diseases (Henis *et al.*, 1979; Papavizas and Lewis, 1981; Papavizas, 1982). Papavizas (1985) observed that integrated approach could be successful only if antagonists were compatible with fungicides and biopesticides. According to Mukhopadhyay *et al.* (1986) *T. harzianum* could tolerate fungicides *viz.*, Metalaxyl (0.1 per cent) and carbendazim (0.0065 per cent). Similar results were

reported by Mukherjee *et al.* (1989). Kay and Stewart (1994) observed *T. harzianum* C 52 was insensitive to thiram and mancozeb. Krishnamoorthy and Bhaskaran (1994b) found that Captan was fungicidal to *T. viride*, while it had little effect on *T. harzianum*. They also observed that in copper oxychloride poisoned medium *T. harzianum* showed normal growth and sporulation. Mondal *et al.* (1995) noticed inhibition of mycelial growth of all the *Trichoderma* spp. to a greater extent with the addition of 200 and 500 ppm carbendazim in culture medium. *T. harzianum* was inhibited to 63 per cent with 500 ppm of Dithane M45 after 3 days of incubation (Singh *et al.*, 1995). Shanmugham (1996) reported that Bordeaux mixture completely inhibited the growth of *T. viride*. The compatibility of potassium phosphonate to *Trichoderma* spp. was reported by Rajan and Sarma (1997).

Sarma and Anandaraj (1999) observed that copper fungicides were toxic to *Trichoderma* spp. and *Gliocladium virens*. Paciulyte *et al.* (2000) tested the sensitivity of thirteen fungi to copper sulphate and copper oxychloride and found that both the fungicides were active against fungi and it was also found that *Trichoderma* spp. were more sensitive to copper oxychloride than copper sulphate. Among several contact and systemic fungicides tested, metalaxyl at all tested rates (1, 10, 100 $\mu g\ l^{-1}$) did not interfere with the mycelial growth of *Trichoderma* (May and Kimati, 2000). Akbari and Prakhia (2001) reported that Thiram, mancozeb, tridemorph, metalaxyl MZ and Fosetyl-Al were non-inhibitory to *T. harzianum, T. viride* and *G. virens* at all concentrations tested. Carbendazim inhibited the growth of antagonists at all concentrations tested. McLean (2001) tested *in vitro* sensitivity of spores of *T. harzianum* to eight fungicides commonly applied to onions and indicated that *T. harzianum* was least sensitive to procymidone and Captan and most sensitive to mancozeb, tebucohazole and Thiram.

Sharma *et al.* (2001) found that among two systemic and six non-systemic fungicides tested, tolerance of *T. harzianum* for metalaxyl was seven times higher

than carbendazim. Jayakumar *et al.* (2003) noticed that *Trichoderma* sp. was compatible with metalaxyl MZ even at 1000 µg/ml. Vijayaraghavan and Abraham (2003) reported that Bordeaux mixture at all concentrations completely inhibited the growth of *T. harzianum, T. viride* and *T. longibrachiatum* while, Ridomil MZ, Akomin-40, Indofil M-45 and Anthracol at different concentrations showed varying degrees of inhibition.

2.5.2.4 Compatibility of antagonists with insecticides

Sharma and Mishra (1995) studied the compatibility of the biocontrol agent *T. harzianum* with aldicarb, phorate and carbofuran applied for management of nematodes and mealy bugs in black pepper. The study indicated that these insecticides were less toxic. Jebakumar *et al.* (2000) found that phorate and chlorphyriphos could be safely applied with *T. harzianum*.

T. harzianum strain T-22 was compatible with Diazinon, Lindane, Lorsban, Malathion, Marathon, Methoxychlor and Orthene Sevin. Sushir and Pandey (2001) opined that among the four insecticides tested *in vitro*, chlorpyriphos (Durret 20 EC) was found more safer as it has no adverse effect on radial growth upto 2000 µl ml^{-1} concentration, whereas endosulfan (Thiodan 35 EC) and triazophos (Hostathion 40 EC) were found to be more toxic even at 50 µl ml^{-1}, which showed growth inhibition of 55.55 and 57.77 per cent respectively.

Sarma (2003) reported that *Trichoderma* spp. is compatible with potassium phosphonate and chlorpyriphos and therefore indicated their potential for IDM with dual mode of action in suppressing both pathogenic fungi and plant parasitic nematodes. Vijayaraghavan (2003) found that three species of *Trichoderma viz., T. viride, T. harzianum* and *T. longibrachiatum* were compatible with phorate and carbofuran at all concentrations tested. Monocrotophos and Quinolphos were

incompatible with these antagonists, Chlorpyriphos, Endosulfan, Dimethoate and Cypermethrin were showed varying degree of inhibition at different concentrations.

2.5.2.5 Compatibility of antagonists with chemical fertilizers

In general, nitrate nitrogen was most favourable for mycelial growth of many fungi (Lilly and Barnett, 1951). Kaufman and Williams (1965) and Rajan and Singh (1974) found that nitrogen significantly influenced fungi antagonistic to soil borne plant pathogens. They also reported that application of fertilizers increased the population levels of soil saprophytes leading to increased antibiosis and competition. *Trichoderma* spp. seemed to be a versatile class of fungi, capable of utilizing a wide range of nitrogen sources (Danielson and Davey, 1973).

Further, Danielson and Davey (1973) reported that ammonium nitrate was found as the best nitrogen source for three species of *Trichoderma* tested. They stated that minerals like Mg, P and K are essential for sporulation of *Trichoderma* spp. Jackson *et al.* (1991) noticed Urea as a good source of nitrogen for *T. harzianum*, which was not only supportive, but also stimulated growth and sporulation. Neelamegam (1992) observed better growth of *T. viride* when ammonical form of nitrogen was incorporated into the medium.

Krishnamoorthy and Bhaskaran (1994a) noticed a significant increase in population of *T. viride* in soil following application of nitrogen and phosphate fertilizers. Muriate of potash was appreciably tolerated by the bioagent, whereas zinc sulphate was highly toxic to the fungus (Jayaraj, 1995; Sharma and Mishra, 1995). Jayaraj and Ramabadran (1997) studied the influence of three nitrogenous fertilizers (urea, ammonium sulphate and ammonium chloride) on survival and competitive saprophytic ability of *T. harzianum*. It was found that all the nitrogenous fertilizers favoured the growth and survival of *T. harzianum* in soil.

Jayaraj and Ramabadran (1998) also studied the efficacy of various nitrogen salts on the *in vitro* growth, sporulation and production of cellulase and antifungal substances of *T. harzianum*. They reported that among nitrogen sources, ammonium nitrate, ammonium sulphate and sodium nitrate influenced the maximum growth, sporulation, production of cellulases and antifungal substances, while urea and calcium nitrate recorded the least growth. Vijayaraghavan (2003) evaluated fertilizers like Urea, Rajphos, MOP, Factomphos and ammonium sulphate at various concentrations for compatibility with three antagonists *viz., T. harzianum, T. viride* and *T. longibrachiatum*. The results revealed that all the fertilizers except Factomphos were compatible with the antagonists to various extents.

Very few workers have examined the compatibility of bacterial antagonists with chemicals or fertilizers. Elkins and Lindow (1999) reported that mancozeb had no detrimental effect on *P. fluorescens* A506 when applied at least five days before or after application of antagonist. However, the studies elsewhere also showed that there was no obvious detrimental effect of fertilizers and chemicals on survival and establishment of *Serratia entomophila* (Townsend *et al.*, 2003).

Materials and Methods

3. MATERIALS AND METHODS

3.1 ISOLATION OF THE PATHOGEN

The pathogen causing *Phytophthora* pod rot (PPR) disease of cocoa was isolated from naturally infected pods collected from cocoa garden of CCRP farm, College of Horticulture, Vellanikkara. After collection, the infected pods were washed in tap water and the infected areas along with healthy portion were cut into small bits. These bits were surface sterilized with 0.1 per cent mercuric chloride solution for 30-60 seconds and washed in three changes of sterile water. The sterilized bits were placed on Potato Dextrose Agar (PDA) (Appendix I) medium in Petri dishes and were incubated at room temperature. When mycelial growth was visible, small bits of the growth were transferred to PDA slants. Similarly 15 isolates of the pathogen were isolated from different cocoa growing tracts of Thrissur district (Table.3.1). The isolates were purified by hyphal tip method. The cultural and morphological characters *viz.*, rate and pattern of growth, width of hyphae, length of sporangiophore, pedicel length and L/B ratio of the 15 isolates grown on Carrot Agar were studied following standard protocols.

3.2. PATHOGENICITY

The pathogenicity of the 15 isolates obtained from different locations was proved by artificial inoculation on detached cocoa pods. For this, pods of half maturity were taken and a hole of 10 mm diameter was made on the middle portion of the pod using a sterile cork borer to a depth of 3 mm. Mycelial discs (10 mm diameter) of *Phytophthora* isolates grown on Carrot Agar (Appendix I) medium were taken from seven day old culture and were placed in the hole and covered with cotton moistened with sterile water. The inoculated pods were incubated in polythene bags with a pad of cotton wetted with sterile water in order to provide high humidity.

Observations were recorded on the development of characteristic symptoms. The isolates of the pathogen were reisolated from the artificially inoculated pods and their cultural and morphological characters were compared with that of original isolates. These isolates were maintained in Carrot Agar slants for further studies.

Table 3.1. List of *Phytophthora* isolates from different locations of Thrissur district

S. No.	Isolates	Locations
1	C_1	VSDTH Block, CCRP, Vellanikkara
2	C_2	FCVSD Block, CCRP, Vellanikkara
3	C_3	PT I Block, CCRP, Vellanikkara
4	C_4	PT IV Block, CCRP, Vellanikkara
5	C_5	CYT I Block, CCRP, Vellanikkara
6	C_6	CYT IV Block, CCRP, Vellanikkara
7	C_7	G VI Block, CCRP, Vellanikkara
8	C_8	G VI S I Block, CCRP, Vellanikkara
9	C_9	S IV H Block, CCRP, Vellanikkara
10	C_{10}	SOP Block, CCRP, Vellanikkara
11	Ka_1	Kannara
12	Ch_1	Cherumkuzhi
13	Pa_1	Pattikad
14	Al_1	Alpara
15	Av_1	Avinisheri

3.3 STUDIES ON VARIABILITY AMONG *Phytophthora* ISOLATES OF *COCOA*

Morophological, virulence, protein profile and sensitivity pattern towards fungicides and antibiotics of the15 *Phytophthora* isolates were studied to find out the variation, if any, existing among them.

3.3.1 Morphological characters

The morphological characters of 15 *Phytophthora* isolates studied were analysed with Euclidean co-efficient and was clustered by the Unweighed Pair Group Method (UPGMA: Sneath and Sokal, 1973) using NTSYS pc 2.02 software to produce grouping. The genetic dissimilarity matrix was also computed.

3.3.2 Virulence

Fifteen *Phytophthora* isolates obtained from different locations were tested to study the variations in their virulence. For this half matured pods of a variety from CCRP farm, Vellanikkara were collected and were inoculated with isolates of the pathogen. The inoculation procedure was the same as mentioned above 3.2. Three replications were maintained for each isolate. Observations on the length and breadth of lesions developed were taken daily for seven days. Based on this the per cent pod area infection was calculated as follows.

$$\text{Percentage of pod area infection} = \frac{\text{Length x breadth of lesion}}{\text{Length x breadth of pod}} \times 100$$

3.3.3 Protein profile analysis

3.3.3.1 SDS-PAGE

The mycelial protein banding pattern of 15 different isolates of *Phytophthora* was done by the method suggested by Sardev *et al.* (1998). One gram of each mycelial sample of 15 isolates was extracted with equivalent quantity of denaturing buffer (Appendix II) at 4°C. The homogenate was centrifuged at 15000 rpm for 15 min. at 4°C and 100µl of the supernatant was taken and mixed with 200µl acetone in a microfuge tube and stored at sub zero temperature for 15 min. After this, the samples were subjected to centrifugation at 5000 rpm for 5 min. at 4°C.

The sample extracts were mixed with tracking dye (Bromophenol blue) in 9:1 ratio and 50 µl of samples containing equal amount of proteins were loaded into the wells of polyacrylamide gels (Sigma-Aldrich Techware System, Sigma, USA). The stock solutions used for preparation of gel columns were given in Appendix II. The medium ranged molecular weight marker of protein (Bangalore Genei, India) was used. Electrophoresis was carried out at constant voltage of 100 volts. The gels were stained with 0.2 per cent Coomassie brilliant blue (R 250) (Appendix II) solution for 30 min. at room temperature. The staining solution was decanted and the excess stain was removed by addition of destaining solution (Appendix II). When the stained protein bands were visible, the gel was subjected to documentation.

For each *Phytophthora* isolate, presence of band was scored as 1 and its absence as 0. Pooled data from all 15 isolates were taken for cluster analysis. The analysis was performed using NTSYS pc 2.02 software. Analysis of mycelial protein profiles of isolates were done with Jaccard's co-efficient (Jaccard, 1908) and was clustered by the unweighed pair group method (UPGMA) to produce grouping. The genetic similarity matrix was also computed.

3.3.4 Sensitivity of *Phytophthora* isolates to fungicides and antibiotics

The differential reaction of 15 isolates of the pathogen to selected fungicides and antibiotics were tested following poisoned food technique (Riker and Riker, 1936). The details of fungicides and antibiotics used for the study and their concentrations are given below.

S. No.	Chemical Name	Trade Name	Concentration (per cent)
1	Copper oxychloride	Fytolan 50 WDP	0.2
2	Metalaxyl + Mancozeb	Master 72 WP	0.125
3	Mancozeb	Indofil-M45 75 WP	0.3
4	$CuSO_4$ + lime + H_2O	Bordeaux mixture	1.0
5	Potassium phosphonate	Akomin-40	0.3
6	Streptomycin sulphate	Ambistryn-S	0.03
7	Chloromycetin	Chloramphenicol	0.03

The quantity of fungicides and antibiotics needed to get the desired concentration was added to 100 ml sterilized melted PDA medium, mixed well and poured into sterile Petri dishes at the rate of 15-20 ml per plate. After solidification, mycelial discs of 6 mm diameter of actively growing isolates of the pathogen were cut and placed at the center of each plate. The control plates consisted of PDA medium alone inoculated with different isolates of *Phytophthora*. Three replications were maintained for each isolates. The inoculated dishes were incubated at room temperature and the mycelial growth of *Phytophthora* spp. was taken daily till the control plates showed full growth. The per cent inhibition of mycelial growth was calculated using the following formula

$$I = \frac{C - T}{C} \times 100$$

Where, I - Per cent inhibition of growth of the pathogen

C - Growth of the pathogen in control (mm)

T - Growth of the pathogen in treatment (mm)

3.4 SCREENING OF COCOA TYPES AGAINST *Phytophthora* POD ROT DISEASE

All the available pod bearing cocoa genotypes maintained in the Germplasm VI of Cadbury - KAU Co-operative Cocoa Research Project, Vellanikkara were screened for their reaction to the disease. Detached cocoa pods of half maturity from 225 cocoa types were used for the screening. The most virulent *Phytophthora* isolate selected from earlier study was used for the inoculation. The inoculation procedure was same as mentioned above 3.3.2. Three replications were maintained for each cocoa genotype. Observations on the length and breadth of lesion developed were recorded daily for eight days. The per cent pod area infection was calculated as mentioned in 3.3.2. Based on the percentage of pod area infected, the cocoa types were grouped into four categories.

R - Resistant - < 25 per cent pod area infected

MR - Moderately resistant - > 25 to < 50 per cent pod area infected

MS - Moderately susceptible - >50 to < 75 per cent pod area infected

S - Susceptible - > 75 per cent pod area infected

3.5. ISOLATION OF EPIPHYTIC MICROFLORA FROM COCOA PODS

The healthy pods available in severely infected cocoa gardens were collected during the month of July 2003, from different locations of CCRP farm, Vellanikkara and Kannara of Thrissur district. Then the pod husk was cut (3mm deep) into pieces of 2.5 cm^2 size and 10 such pieces were placed in 100 ml sterile water. The contents were shaken for 30 min in a rotatory shaker. The total epiphytic microflora were quantitatively estimated by serial dilution plate technique. Martins Rose Bengal streptomycin agar, Thornton's standardized agar, King's B medium and Kenknights agar media (Appendix I) were used for estimating fungi, bacteria, fluorescent pseudomonads and actinomycetes at dilutions of 10^1, 10^2, 10^2 and 10^2 respectively. Three replications were maintained and observations on the total population of the each microflora were recorded.

3.5.1 *Fungi*

The single colonies of fungi were transferred to Petri dishes containing PDA. Pure cultures of fungi were obtained by hyphal tip isolation method and were maintained on PDA slants. The fungi were identified upto the generic level.

3.5.2 Bacteria

The bacterial colonies developed in the dilution plates were streaked on Nutrient Agar (NA) to get single colonies. The pure cultures were maintained on NA slants.

3.5.3 Fluorescent pseudomonads

The bacterial colonies developed in the dilution plates on King's B medium was observed under UV transilluminator for the presence of greenish yellow fluorescent colonies. Only the fluorescent colonies were selected for streaking on King's B medium. Later, they were purified and pure cultures were maintained on King's B slants.

3.5.4 Actinomycetes

The single colonies of actinomycetes developed on Kenknights Agar medium were transferred to test tube slants of the same medium and maintained as pure culture.

3.6 *In vitro* SCREENING OF EPIPHYTIC MICROFLORA AGAINST THE PATHOGEN

3.6.1 Screening of epiphytic fungal isolates

The sixteen fungal isolates were tested for their antagonistic action against *P. palmivora* by dual culture method (Skidmore and Dickinson, 1976). The organisms were inoculated on dual cultures after giving due consideration of their growth rate. Mycelial disc (6 mm diameter) of the pathogen from eight day old culture grown on Carrot Agar medium was inoculated aseptically on one side of a Petri dish and incubated at room temperature for two days. After this, 6 mm diameter mycelial discs of the fungal antagonists were inoculated in the same PDA plate 4 cm away from the pathogen disc and incubated. Three replications were maintained for

each isolate. The pathogen and the fungal antagonists grown in monoculture served as control.

The growth measurements (mm) were taken daily for seven days after 24 h of inoculation of antagonists. The standard culture of *Trichoderma harzianum* was also tested for its antagonism to *P. palmivora*. The per cent inhibition of mycelial growth of the pathogen was calculated using the formula suggested by Vincent (1927).

$$I = \frac{C - T}{C} \times 100$$

Where, C - Growth of the pathogen in control (mm)

T - Growth of the pathogen in dual culture (mm)

The nature of antagonistic action of epiphytic fungi against the pathogen was assessed (Purkayastha and Bhattacharya, 1982) and assigned to four categories.

A - Overgrowth : *P. palmivora* overgrown by test organism

B - Homogenous : Free intermingling of hyphae

C - Cessation of growth : Cessation of growth at line of contact

D - Aversion : Development of clear zone of inhibition.

Based on the per cent inhibition of mycelial growth of the pathogen, the efficient antagonists were selected for further studies.

3.6.2 Screening of epiphytic bacterial isolates

Twenty four bacterial isolates including two epiphytic fluorescent pseudomonads and two standard cultures of *Pseudomonas fluorescens* were tested for

their antagonistic effect against *P. palmivora* by dual culture method, using PDA. Mycelial disc of 6 mm diameter of the pathogen was inoculated at 2.5 cm away from edge of the Petri dish and incubated for seven days. After this, the bacterial isolates were inoculated as a line of streak at 4 cm away from the pathogen disc. The Petri dishes with *P. palmivora* alone served as control. Three replications were maintained for each isolate. The plates were incubated at room temperature. Observations on the growth of the pathogen (mm) were taken daily for seven days. The per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.6.1.

3.6.3 Screening of epiphytic actinomycetes isolates

Five isolates of actinomycetes were tested for their antagonistic effect by dual culture method. Due to slow growing nature of actinomycetes, these isolates were inoculated at 2 cm away from the edge of Petri dish two days prior to inoculation of the pathogen. The 6 mm size mycelial disc of the pathogen was inoculated 4 cm away from the point of actinomycete inoculation. Petri dishes with *P. palmivora* alone served as control. Three replications were maintained for each isolate. Observations on the growth of pathogen and actinomycetes were taken daily for 10 days. The per cent inhibition of mycelial growth of the pathogen was calculated as mentioned in 3.6.1.

3.7 EFFECT OF SELECTED ANTAGONISTS AGAINST THE PATHOGEN ON DETACHED PODS

Eight fungal and seven bacterial isolates, which were found more inhibitory to the pathogen under *in vitro*, were selected for this study. The fungal antagonists were cultured on PDA medium and the bacterial antagonists on Nutrient Agar (NA). Spore or bacterial suspension of the culture of the promising antagonists were prepared in sterile water having a concentration of 10^6 - 10^8 cfu/ml. Half

matured pods of uniform size from a cocoa type (G VI 172) from CCRP farm, Vellanikkara were used for the study. The pods were washed in tap water and air dried. A well of 10 mm diameter was made using a sterile cork borer to a depth of 3 mm at the middle portion of the pods. The culture suspension of each antagonists were sprayed on the entire surface of the detached pods and air dried for one hour. Mycelial disc of 10 mm size from actively growing eightday old culture of *P. palmivora* grown on Carrot Agar (CA) was cut along with medium and inoculated in the hole and covered with cotton moistened with sterile water. In another method, the culture suspension of each antagonist was sprayed on the surface of the detached pods and air dried for one hour. Mycelial disc of the pathogen was inoculated at the surface of the pods without any injury and covered with cotton moistened with sterile water. The inoculated pods were incubated in polythene bags, with a pad of cotton moistened with sterile water to provide high humidity. They were then incubated at room temperature. The pods inoculated *P. palmivora* alone served as control. Observations on the lesion development were taken at 24 h intervals for a period of 10 days. The per cent pod area infection was calculated using the formula as given in section 3.3.2.

Based on the per cent reduction of infection by *P. palmivora* on detached pods, five most efficient antagonists were selected for field evaluation. This included two native bacterial, one native fungal and standard culture each of *T.harzianum* and *P.fluorescens* (K).

3.8 IDENTIFICATION OF EFFICIENT ANTAGONISTS

3.8.1 Fungi

An attempt was made to identify the native fungal isolate, which showed higher per cent reduction of infection. The cultural characters of the antagonists like

growth, colony colour and pigmentation were studied in PDA. The morphological characters were studied by slide culture technique (Riddle, 1950). Observations were made on the mycelium, branching pattern of conidiophore, length and breadth of phialide and also shape, size, colour and shape of spore.

3.8.2 Bacteria

3.8.2.1 Characterization of bacterial isolates

Two promising bacterial antagonists selected from the study were characterized and identified based on the cultural and biochemical characters.

The cultural characters like Gram reaction, production of fluorescent pigments, growth at 4° and 41°C and biochemical characters like Catalase activity, Oxidase test, Levan production, Starch hydrolysis, Nitrate reduction, Urease activity and Arginine hydrolase activity were tested following the procedures of Hucker and Conn (1923), King *et al.* (1954), Cappucino and Sherman (1992), Kovacs (1956), Hayward (1964), Cappucino and Sherman (1992), Hayward *et al.* (1990), Christensen (1946) and Thornley (1960) respectively.

3.9 MECHANISM OF ANTAGONISM OF SELECTED ANTAGONISTS ON *P. palmivora*

3.9.1 Fungi

To study the mechanism of antagonism on *P. palmivora*, dual culture technique of Dennis and Webster (1971) was used. In sterile Petri dishes, PDA medium was poured and allowed to solidify. Sterilized cellophane discs of 90 mm

diameter were placed over this so as to lie flat on medium using a pair of sterile forceps. Agar discs of 6 mm diameter containing the mycelium of *P. palmivora* taken from an actively growing culture was inoculated at one end of the Petri plate 48 h prior to inoculation of the antagonists, which was placed two centimeter away from the pathogen. The plates were incubated at room temperature and observations were taken at regular intervals until there was some hyphal intermingling. Microscopic observation for hyphal interaction was done by cutting out one sq. cm portion of cellophane containing intermingling hyphal growth of antagonists and pathogen and mounting in cotton blue lactophenol.

3.9.2 Bacteria

The ability of two selected epiphytic bacterial antagonists and standard culture of *P.fluorescens* to the production of siderophore and HCN was studied.

3.9.2.1 Siderophore production

Siderophore production by the bacterial isolates was detected by the method of Kloepper *et al.* (1980). The King's B medium was amended with two concentrations of $FeCl_3$. 6 H_2O @ 1 and 10 mg l^{-1}. The sterilized medium was poured into each Petri plate. The test cultures were streaked on the surface of the medium. Three replications were maintained. The inoculated plates were incubated at room temperature for 48 h. Observations on production of greenish yellow fluorescent pigment were recorded.

3.9.2.2 HCN production

HCN production by the bacterial isolates were detected by the method of Bakker and Schipper (1987). The King's B medium was amended with 4.4 g l^{-1}

glycine and sterilized. The sterile medium was poured into dishes and allowed to solidify and the isolates were inoculated. Whatman No.1 filter paper disc (90 mm diameter) was soaked in 0.5 per cent picric acid in 2 per cent sodium carbonate and was placed on the lid of each Petri plate. The Petri plates were sealed with parafilm and incubated at 28 ± 2°C for four days. The uninoculated plates served as control. Three replications were maintained for each isolate. Observations on colour change of filter paper from deep yellow to orange brown were recorded.

3.10 COMPATIBILITY OF SELECTED ANTAGONISTS TO PLANT PROTECTION CHEMICALS AND CHEMICAL FERTILIZERS USED IN COCOA GARDENS

The *in vitro* compatibility of the three selected epiphytic antagonists and the standard cultures of *T. harzianum* and *P. fluorescens* to plant protection chemicals and chemical fertilizers used in cocoa gardens was studied.

3.10.1 Fungicides

The fungicides listed below were used for *in vitro* evaluation by poison food technique.

S. No.	Chemical Name	Trade Name	Concentration (Per cent)
1	Copper oxychloride	Fytolan 50 WDP	0.2, 0.3, 0.4
2	Copper hydroxide	Kocide 77 WP	0.1, 0.2, 0.3
3	Mancozeb	Indofil-M45 75 WP	0.2, 0.3, 0.4
4	Carbendazim	Bavistin 50 WP	0.05,0.1,0.2
5	$CuSO_4$ + Lime + H_2O	Bordeaux mixture	0.5, 1.0, 1.5
6	Potassium phosphonate	Akomin-40	0.2, 0.3, 0.4

3.10.2 Insecticides

The insecticides listed below were used for *in vitro* evaluation.

S. No.	Chemical Name	Trade Name	Concentration (per cent)
1	Carbaryl	Sevin 50 WDP	0.05,0.1,0.2
2	Quinalphos	Ekalux 25EC	0.05,0.1,0.2
3	Endosulfan	Excel 35 EC	0.04,0.08,0.15
4	Monocrotophos	Nuvacron 36 EC	0.04,0.08,0.15
5	Phorate	Phorate 10 G	0.003,0.0045,0.006

3.10.3 Fertilizers

The list of fertilizers given below was used for *in vitro* evaluation.

S. No.	Name	Concentration (Per cent)
1	Urea	1.0, 1.5, 2.0
2	Ammonium chloride	2.0, 2.5, 3.0
3	Ammonium sulphate	2.0, 2.5, 3.0
4	Rajphos	2.0, 2.5, 3.0
5	Muriate of potash	2.0, 2.5, 3.0

3.10.1.1 Fungal antagonists

The quantity of fungicides, insecticides and fertilizers needed to get the desired concentration was added to 100 ml sterilized, molten PDA medium, mixed well and poured into sterilized Petri dishes at the rate of 15-20 ml per plate. To avoid contamination, the fertilizers were exposed to U.V. light in the laminar flow for a

period of 30 minutes before adding it into the medium. After solidification of the medium, mycelial discs of 6 mm diameter from actively growing fungal antagonists were cut and placed at the centre of each Petri dish. Control Petri dishes consisted of PDA medium alone inoculated with the antagonist. Three replications were maintained for each concentration. The inoculated plates were incubated at room temperature and observations on mycelial growth of the fungal antagonists were taken when the control plates showed full growth. The per cent inhibition of growth of the antagonists was calculated as given in the section 3.3.4

3.10.1.2 Bacterial antagonists

Sterile filter paper discs of 6 mm diameter were soaked in different concentrations of fungicides, insecticides and fertilizers. The discs were placed at the center of Petri dishes containing King's B medium seeded with 48 h old culture of the three different isolates of the bacterium. Control Petri dishes consisted of filter paper disc soaked in sterile water. Three replications were maintained. The inoculated plates were incubated at room temperature and the observations on inhibition zone were recorded after 48 h. The per cent inhibition of growth of bacterial antagonists was calculated as given in the section 3.3.4.

3.11 *In vitro* EVALUATION OF PLANT PROTECTION CHEMICALS AND CHEMICAL FERTILIZERS AGAINST THE PATHOGEN

All the plant protection chemicals and fertilizers used for *in vitro* compatibility test against the antagonists were selected for this study also. The method of testing was as described in section 3.10.1.1 Three replications were maintained and observations on the mycelial growth were recorded until the control

plates showed full growth. The per cent inhibition of growth of the pathogen calculated as given in the section 3.3.4.

3.12 FIELD EVALUATION OF SELECTED ANTAGONISTS AGAINST *Phytophthora* POD ROT

A field experiment was laid out to study the efficacy of three selected antagonists and standard cultures of *T. harzianum* and *P. fluorescens* along with recommended fungicides against the PPR disease of cocoa. The experiment was carried out during July, 2004 at CCRP farm, College of Horticulture, Vellanikkara. The details of the experiment are as follows:

Design	- CRD
Treatment	- 8
No. of plants/treatment	- 5
Replications	- 3

Treatments,

T_1 - Control

T_2 - *P. fluorescens* (24B)

T_3 - *T. viride* (20F)

T_4 - *Trichoderma harzianum*

T_5 - *Pseudomonas fluorescens* (K)

T_6 - *P. fluorescens* (23B)

T_7 - Bordeaux mixture 1%

T_8 - Potassium phosphonate 0.3%

The treatments were given over and above the cultural management practices recommended for PPR disease.

The fungal antagonists were multiplied in PDA medium and spore suspension were prepared using sterile water and was filtered through the double layered muslin cloth to remove mycelial bits. The bacterial antagonists were multiplied on King's B medium, after 48 h cultures were scrapped and cell suspension was prepared with sterile water. The concentration of the antagonist in the spray solution was adjusted to 10^6-10^8 cfu ml $^{-1}$.

The antagonists and fungicides were applied as spray starting from second week of July, 2004 and repeated twice at fortnightly intervals. The spraying was directed towards the main stem and all pod bearing twigs of the plant. Observations on the total number of pods and number of pods infected were recorded at weekly intervals till two weeks after third spraying and the per cent disease incidence worked out.

3.13 STATISTICAL ANALYSIS

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986). Multiple comparisons among treatment means were done using DMRT.

Results

4. RESULTS

The results of the studies on biological management of *Phytophthora* pod rot (PPR) of cocoa are presented in this chapter.

4.1 ISOLATION OF THE PATHOGEN

Fifteen isolates of the pathogen causing PPR of cocoa were isolated from naturally infected pods collected from cocoa gardens of Cadbury KAU Co-operative Cocoa Research Project, Vellanikkara and other locations of Thrissur district. These isolates were purified by hyphal tip method and maintained on CA slants by periodic sub culturing.

4.2 PATHOGENICITY

Pathogenicity of the isolates was tested on detached half matured cocoa pods by artificial inoculation as described in Materials and Methods. All the isolates produced water soaked circular lesions within 48 h. Later, the lesions enlarged in size and turned to chocolate brown in colour. Whitish growth of the fungus consisting mycelia and sporangia were produced over the dark brown area immediately behind the advanced border. Re-isolation from artificially inoculated pods yielded pathogen having similar characters as that of original ones.

4.3 CULTURAL AND MORPHOLOGICAL CHARACTERS OF THE PATHOGEN

The pathogenic fungal isolates were subjected to cultural and morphological studies and the results are presented in Table 4.1 & 4.2. It was evident from the data that slight variations existed on the rate and pattern of growth of the

Table 4.1. Cultural characteristics of *Phytophthora* isolates on Carrot Agar medium

S.No	Isolates	Mean diameter of colony (mm)								Growth pattern	Margin
		Days after inoculation									
		2	3	4	5	6	7	8	9		
1	C_1	25	34	46	56	64	73	82	90	Slightly stellate with aerial mycelium	Uniform
2	C_2	26	37	52	62	72	80	90	90	Stellate striated with aerial mycelium	Irregular wavy
3	C_3	27	40	51	62	70	76	85	90	Slightly stellate with aerial mycelium	Irregular
4	C_4	29	42	54	66	77	86	90	90	Stellate striated with aerial mycelium	Uniform wavy
5	C_5	26	38	51	60	68	79	87	90	Cottony mycelium	Irregular wavy
6	C_6	27	40	53	66	74	84	90	90	Slightly stellate with aerial mycelium	Uniform wavy
7	C_7	26	39	53	63	72	82	90	90	Fluffy cottony mycelium	Irregular wavy
8	C_8	30	42	54	74	79	88	90	90	Stellate striated with aerial mycelium	Irregular wavy
9	C_9	28	40	59	75	81	86	90	90	Stellate striated with aerial mycelium	Irregular wavy
10	C_{10}	29	42	54	70	79	87	90	90	Fluffy cottony mycelium	Uniform
11	Ka_1	28	39	51	67	79	90	90	90	Stellate striated with aerial mycelium	Uniform wavy
12	Ch_1	34	46	62	77	90	90	90	90	Slightly stellate with aerial mycelium	Uniform
13	Pa_1	37	52	67	81	85	90	90	90	Fluffy cottony mycelium	Uniform
14	Al_1	30	41	63	72	81	90	90	90	Cottony mycelium	Irregular wavy
15	Av_1	41	49	68	80	88	90	90	90	Slightly stellate with aerial mycelium	Irregular

Mean of three replications

isolates. They showed growth patterns like slightly stellate with aerial mycelium (C_1, C_3, C_6, Ch_1, Av_1), stellate striated with aerial mycelium (C_2, C_4, C_8, C_9, Ka_1), fluffy cottony mycelium (C_7, C_{10}, Pa_1) and cottony mycelium (C_5, Al_1). The margins of colonies were uniform, irregular, uniform wavy and irregular wavy. On carrot agar medium, the isolates took 6-9 days for full growth in 90 mm Petri dishes. The isolate Ch_1 from Cherumkuzhi took only six days for full growth. Among the remaining, four, seven and three isolates took seven, eight and nine days respectively for attaing full growth.

The mycelium of different isolates was branched, hyaline and coenocytic. Somatic hyphae ranged from 3.22-6.45 µm in breadth. Among 15 isolates, eight recorded more than 5 µm in mean width and the rest below 4.73 µm. Sporangiophores arose from the somatic hyphae and their tip became swollen, later developed into sporangia (Table 4.2). Sporangiophores were indeterminate and measured 46.2-129 µm in length. In majority of the isolates, the mean sporangiophore length was more than 70 µm. The maximum and the minimum length of sporangiophore were observed with isolate Pa_1 and C_4 respectively. Young sporangia were more or less spherical with less dense protoplasm (Plate 2). At maturity, the protoplasm become dense, granular and differentiated into zoospores within the sporangium itself. The apical portion of the maturing sporangium developed into a papilla and it became pronounced, when fully matured (Plate 2). Matured sporangia of isolates measured 25.8 - 64.5 x 17.2 - 38.7 µm. Among the 15 isolates, eight showed more than 40 µm in mean sporangial length and rest below that. Majority of the isolates produced sporangia with mean width in between 25 to 30 µm. L/B ratio of the sporangia of isolates ranged from 1.11 to 2.0. Six isolates showed mean L/B ratio of more than 1.5. The least ratio was recorded the isolate C_6. The sporangia were borne terminally on the sporangiophores (Plate 2) in a simple sympodial fashion and were caducous. Decidious sporangia had short and thick pedicel, the length of which ranged from 3.15 to 4.3 µm. The mean pedicel length of most of the isolates was

Table 4.2. Morphological characteristics of *Phytophthora* isolates

S.No	Isolates	Width of hyphae (µm)		Length of Sporangiophore (µm)		Pedicel length (µm)		Sporangial dimensions					
								Length (µm)		Width (µm)		L/B ratio	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1	C_1	4.08	3.22-4.30	78.26	68.8-86.0	3.65	3.22-4.30	33.97	25.8-38.7	22.36	17.2-25.8	1.52	1.20-1.80
2	C_2	5.60	5.25-6.30	68.60	54.6-84.0	3.88	3.15-4.20	39.06	29.4-50.4	25.62	21.0-33.6	1.52	1.28-1.83
3	C_3	4.08	3.22-4.30	86.00	64.5-103.2	3.76	3.22-4.30	36.55	30.1-38.7	27.09	21.5-30.1	1.36	1.00-1.80
4	C_4	5.59	4.30-6.45	60.00	51.6-68.8	3.65	3.22-4.30	43.00	34.4-51.6	27.95	21.5-30.1	1.54	1.28-1.83
5	C_5	4.51	3.22-6.45	80.84	64.5-103.2	3.76	3.22-4.30	42.57	30.1-64.5	28.81	21.5-34.4	1.46	1.16-1.87
6	C_6	5.59	4.30-6.45	72.24	51.6-94.6	3.22	3.22-4.30	37.41	25.8-43.0	27.95	21.5-34.4	1.34	1.14-1.50
7	C_7	4.73	4.30-6.45	87.72	68.8-98.9	3.22	3.22-4.30	34.83	25.8-38.7	25.80	21.5-30.1	1.36	1.14-1.80
8	C_8	5.01	4.30-6.45	65.93	51.6-81.7	3.76	3.22-4.30	44.29	30.1-60.2	27.52	21.5-34.4	1.60	1.25-2.00
9	C_9	5.59	4.30-6.45	84.29	64.5-116.1	3.87	3.22-4.30	49.45	38.7-55.9	30.96	25.8-38.7	1.59	1.25-2.00
10	C_{10}	4.73	4.30-6.45	87.70	77.4-98.8	3.33	3.22-4.30	38.27	30.1-51.6	27.09	25.8-34.4	1.41	1.16-1.71
11	Ka_1	5.37	4.30-6.45	97.12	46.2-126	3.62	3.22-4.30	43.00	30.1-60.2	29.02	21.5-34.4	1.48	1.00-1.83
12	Ch_1	3.87	3.22-4.30	62.78	47.3-94.6	3.55	3.22-4.30	36.98	25.8-47.3	26.23	21.5-34.4	1.44	1.11-2.00
13	Pa_1	5.16	4.30-6.45	113.52	94.6-129	3.76	3.22-4.30	43.00	38.7-47.3	30.96	30.1-34.4	1.38	1.12-1.57
14	Al_1	5.16	4.30-6.45	82.56	64.5-98.9	3.76	3.22-4.30	41.71	34.4-51.6	27.50	21.5-30.1	1.51	1.33-1.71
15	Av_1	4.08	3.22-4.30	93.31	77.4-120.4	3.44	3.22-4.30	44.29	34.4-55.9	32.25	30.1-34.4	1.37	1.12-1.62

Mean of 25 observations

A B C

D E F

Plate 1. Different stages of *Phytophthora* pod rot infection on cocoa

A. Healthy pods B - E. Different stages of pod infection F. Leaf infection of *Phytophthora*

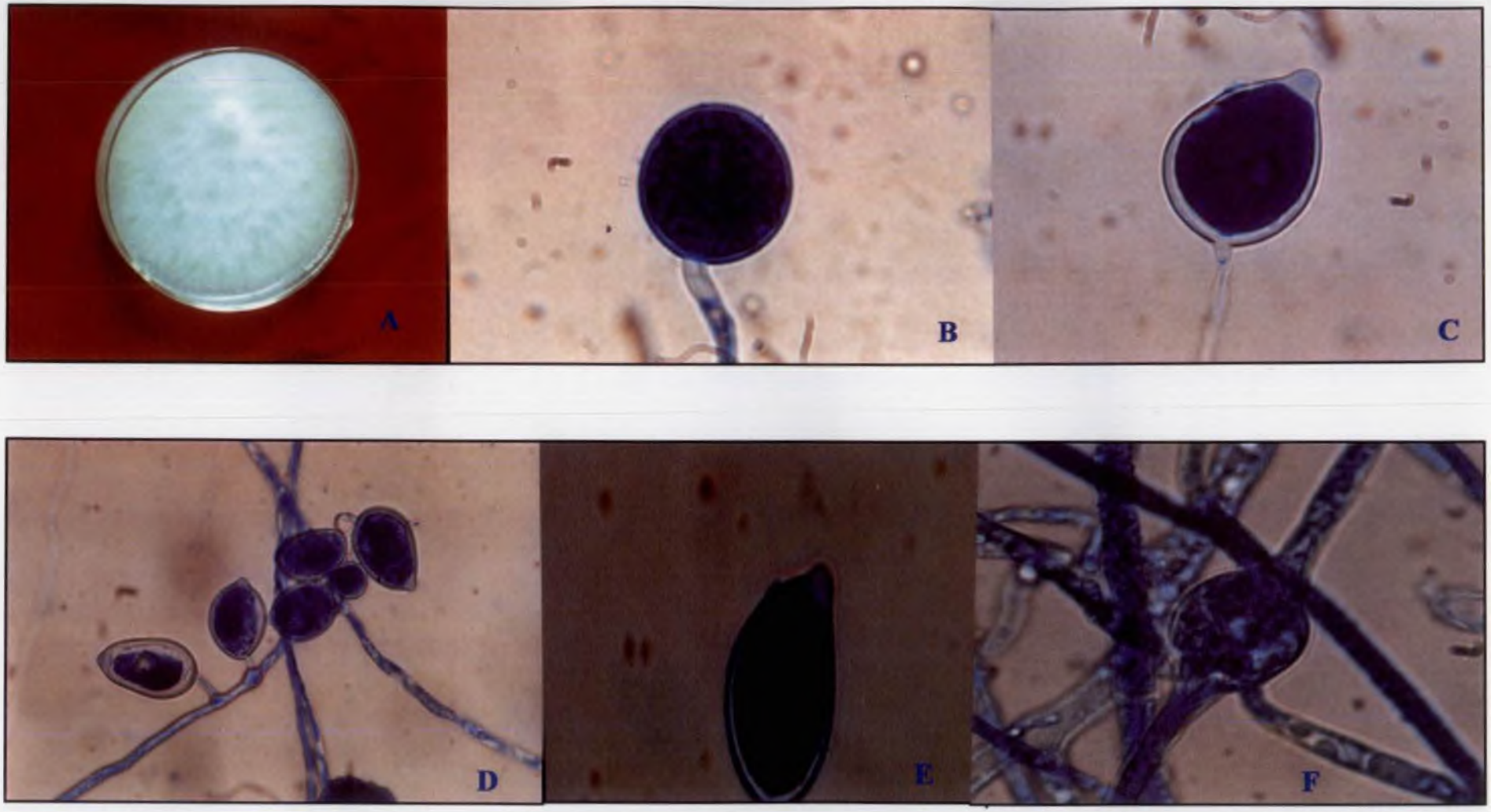


Plate 2. Cultural and morphological characters of *Phytophthora palmivora*

A. Culture of *P. palmivora* B. Young sporangium C. Terminal sporangium
D. Mycelia with sporangia E. Mature sporangium F. Chlamydospore

more than 3.5 µm and the least length was recorded in the isolates C_6 and C_7. The fungus produced abundant chlamydospores in old Carrot Agar medium. They were borne intercalary and more or less spherical (Plate 2). Based on the cultural and morphological characters, all the 15 fungal isolates were identified as *Phytophthora palmivora* Butler (Butler).

4.4 STUDIES ON VARIABILITY AMONG *Phytophthora* ISOLATES

4.4.1 Morphological characters

Genetic dissimilarity index (DI) of *Phytophthora* isolates was computed from morphological characters as Euclidean co-efficient using NTSYS pc 2.02 software (Table.4.3). The dendrogram was constructed by using unweighed pair group method (UPGMA) as shown in Fig.4.1. The lowest dissimilarity of 1.23 was observed between isolate C_7 and C_{10} followed by 1.32 between C_5 and Al_1. Highest dissimilarity of 5.84 was observed between C_7 and C9 followed by 5.76 between C_1 and C_9. The isolates were grouped into two clusters based on the morphological characters (Table.4.4). The cluster A was divided further into four sub clusters A_1, A_2, A_3 and A_4. In A_3 subcluster, isolates C_7 and C10 had the lowest dissimilarity index (1.23), while, in subcluster A_2, C_3 and Ch_1 had a dissimilarity index of 2.15. The cluster B was also divided into three sub clusters *viz.*, B_1, B_2 and B_3. In subcluster B_1, isolate C_5 and Al_1 were placed with the lowest DI (1.32).

4.4.2 Virulence

For this study, detached pods from a cocoa type were artificially inoculated with the 15 isolates of the pathogen as described in Materials and Methods. Observations on the per cent pod area infection recorded at different

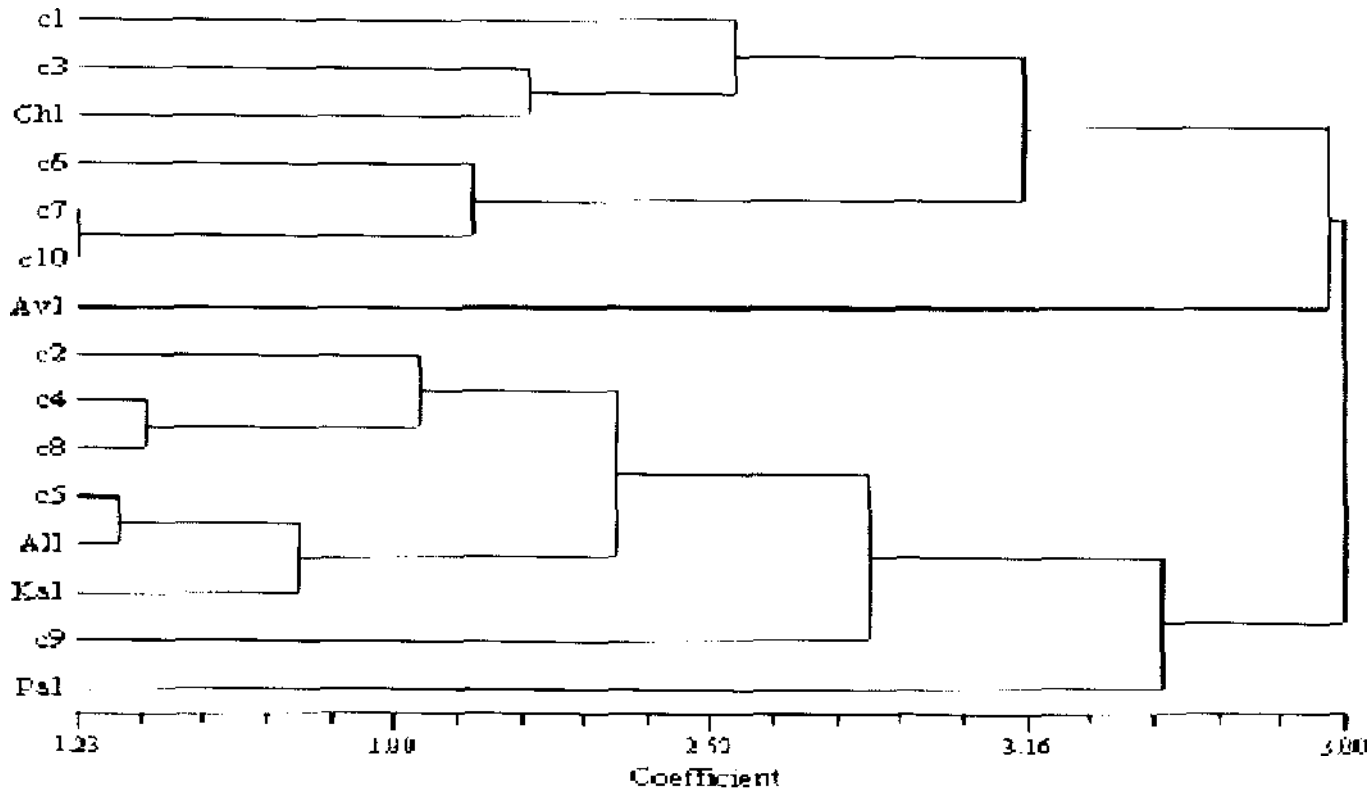


Fig 4.1. Unweighed pair group average method (UPGMA) dendrogram based on morphological characters of *Phytophthora* isolates

Table 4.3. Dissimilarity matrix of *Phytophthora* isolates based on morphological characters

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	Ka_1	Ch_1	Pa_1	Al_1	Av_1
C_1	0														
C_2	3.25	0													
C_3	2.84	3.42	0												
C_4	4.14	1.81	3.10	0											
C_5	3.50	2.62	2.10	2.50	0										
C_6	4.47	3.81	3.60	3.41	3.59	0									
C_7	3.28	4.14	2.76	4.27	3.55	2.05	0								
C_8	3.79	2.04	3.89	1.37	2.18	4.34	4.66	0							
C_9	5.76	3.54	4.98	2.83	2.98	5.23	5.84	2.48	0						
C_{10}	3.16	3.46	2.33	3.36	2.46	2.02	1.23	3.63	4.66	0					
Ka_1	4.24	2.91	3.17	2.73	1.90	3.31	3.48	2.79	2.60	2.39	0				
Ch_1	2.30	3.33	2.15	3.39	2.53	3.46	2.88	3.30	5.21	2.49	3.87	0			
Pa_1	5.33	3.47	4.06	2.64	3.07	3.73	4.91	3.32	4.11	4.19	4.04	3.62	0		
Al_1	3.32	1.65	2.74	1.85	1.32	3.46	3.58	1.68	2.63	2.54	1.46	3.03	3.42	0	
Av_1	5.21	4.96	3.19	4.36	2.56	3.83	3.76	4.32	4.27	2.85	2.89	3.81	4.17	3.52	0

Table 4.4. Grouping of *Phytophthora* isolates based on dissimilarity index for morphological characters

Cluster	Sub cluster	Isolates
A	A_1	C_1
	A_2	C_3, Ch_1
	A_3	C_6, C_7, C_{10}
	A_4	Av_1
B	B_1	C_2, C_4, C_8, C_5, Al_1, Ka_1,
	B_2	C_9
	B_3	Pa_1

Table 4.5. Comparative virulence of different *Phytophthora* isolates on detached cocoa pods

S.No.	*Phytophthora* isolates	Per cent pod area infection			
		Days after inoculation			
		2	4	6	8
1	C_1	2.98 ^{bc}	14.33 ^{c}	34.10 ^{bcd}	70.88 ^{abc}
2	C_2	6.09 ^{a}	27.69 ^{a}	71.24 ^{a}	96.29 ^{a}
3	C_3	4.42 ^{ab}	25.78 ^{ab}	63.81 ^{abc}	100.0 ^{a}
4	C_4	2.92 ^{bc}	20.14 ^{abc}	67.63 ^{a}	97.99 ^{a}
5	C_5	3.50 ^{bc}	19.59 ^{abc}	49.69 ^{abcd}	79.49 ^{abc}
6	C_6	4.38 ^{ab}	20.78 ^{abc}	55.48 ^{abcd}	88.91 ^{ab}
7	C_7	6.12 ^{a}	28.34 ^{a}	80.07 ^{a}	98.48 ^{a}
8	C_8	3.08 ^{bc}	19.10 ^{abc}	54.74 ^{abcd}	86.69 ^{ab}
9	C_9	4.43 ^{ab}	19.77 ^{abc}	63.02 ^{abc}	82.61 ^{ab}
10	C_{10}	1.92 ^{c}	15.22 ^{c}	32.89 ^{cd}	59.38 ^{ab}
11	Ka_1	4.45 ^{ab}	25.34 ^{ab}	64.97 ^{ab}	93.81 ^{abc}
12	Ch_1	3.32 ^{bc}	22.16 ^{abc}	62.31 ^{abc}	93.43 ^{abc}
13	Pa_1	3.17 ^{bc}	18.06 ^{bc}	50.51 ^{abcd}	86.52 ^{abc}
14	Al_1	4.05 ^{abc}	21.02 ^{abc}	56.62 ^{abcd}	92.25 ^{bc}
15	Av_1	1.90 ^{c}	5.30 ^{d}	27.34 ^{d}	53.37 ^{c}

Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

intervals revealed significant difference among the isolates in their virulence (Table.4.5). After two days of inoculation, isolate C_7 followed by C_2 produced the maximum infection. However, these two were on par with other five isolates with respect to the pod area infection. Isolates Av_1 and C_{10} caused the least infection. Four days after inoculation, isolate C_7 followed by C_2 produced the maximum infection and were on par with other nine isolates. The minimum infection was caused by isolates Av_1 and C_1.

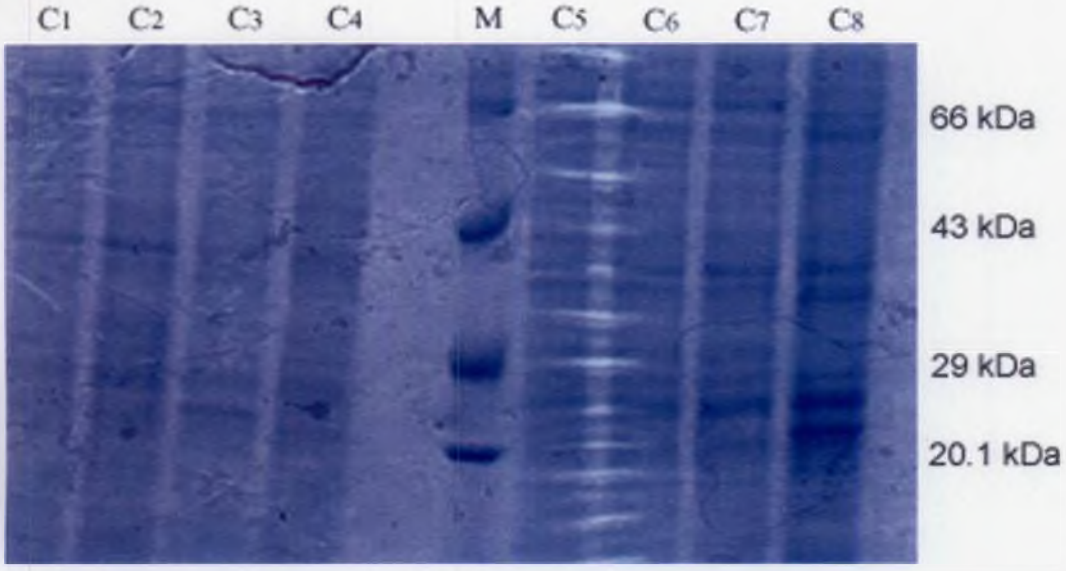
After six day of inoculation also, isolate C_7 followed by C_2 produced the maximum infection and these two were on par with other 10 isolates. Isolates Av_1 and C_{10} recorded the least infection. However, on the eighth day of inoculation, isolate C_3 recorded cent per cent pod area infection closely followed by C_7. The least infection was recorded in isolates Av_1 and C_{10}. Even though, isolate C_3 fully infected the pod on the last observation, isolate C_7 showed a consistent increase in infection at different intervals of observation indicating its more virulence and hence this was selected for further studies.

4.4.3 Protein profile analysis

4.4.3.1 SDS-PAGE

The mycelial protein banding pattern of 15 isolates of the *Phytophthora* were studied by using SDS-PAGE (Plate 3). The results revealed that protein banding pattern had not much variation among the isolates.

The genetic similarity index (SI) calculated from the protein profile data was used to estimate the genetic relatedness among 15 isolates of *Phytophthora*. The genetic distances based on protein profile data were used to construct a dendrogram as shown in Fig.4.2. Which indicated that the isolates belonged to only one cluster.



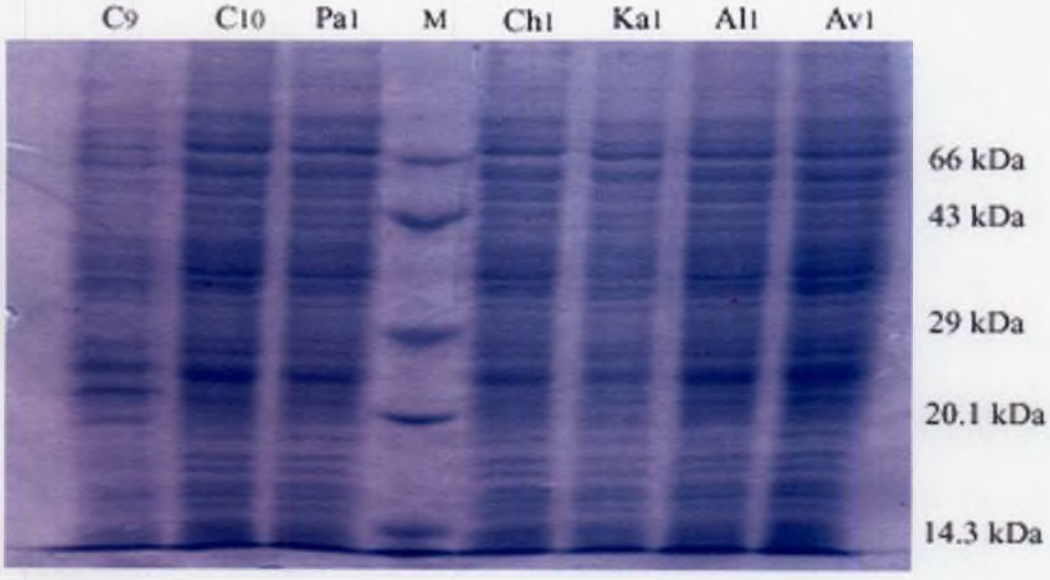


Plate 3. Protein profile of *P. palmivora* isolates

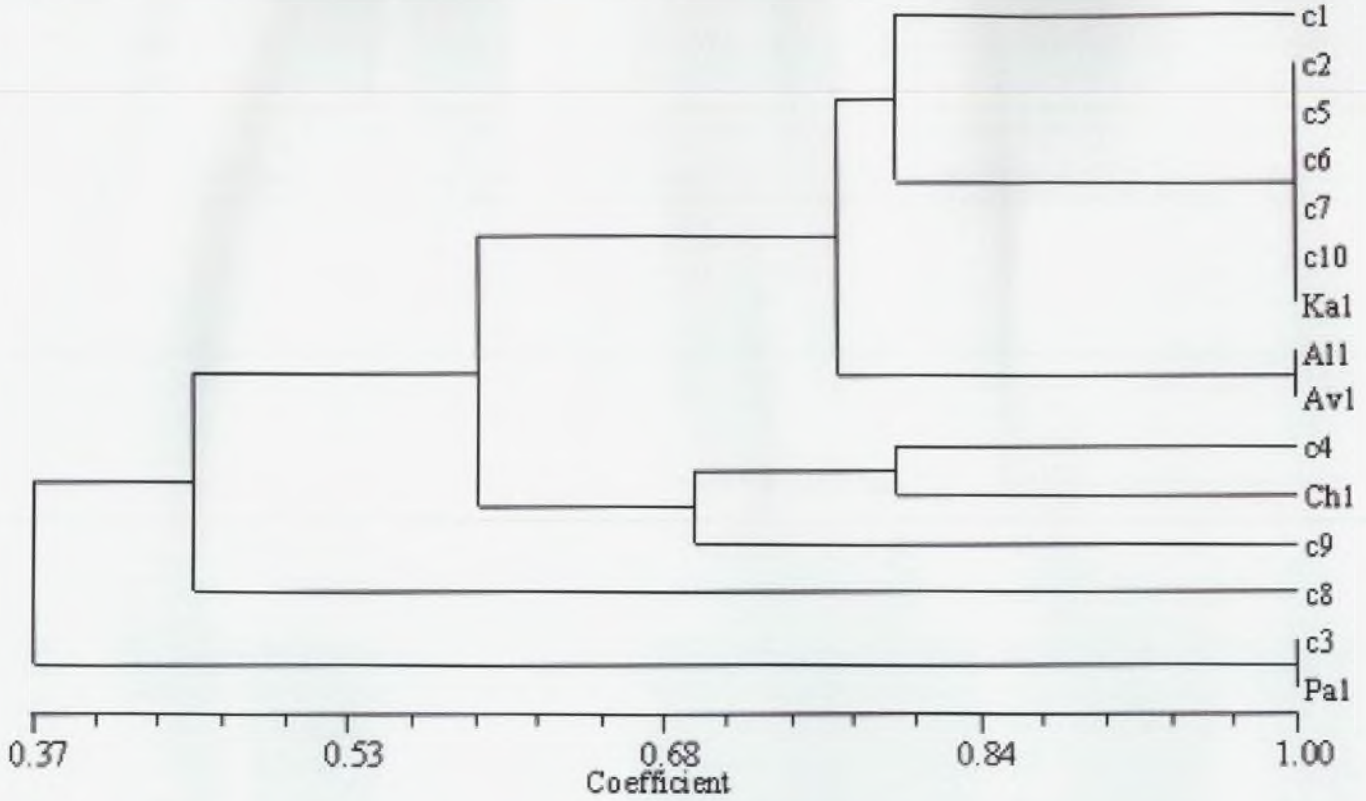


Fig 4.2. Unweighed pair group method (UPGMA) dendrogram based on protein profiles of *Phytophthora* isolates

Table 4.6. Similarity matrix of *Phytophthora* isolates based on protein profiles

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	Ka_1	Ch_1	Pa_1	Al_1	Av_1
C_1	1.0														
C_2	0.8	1.0													
C_3	0.2	0.4	1.0												
C_4	0.6	0.8	0.6	1.0											
C_5	0.8	1.0	0.4	0.8	1.0										
C_6	0.8	1.0	0.4	0.8	1.0	1.0									
C_7	0.8	1.0	0.4	0.8	1.0	1.0	1.0								
C_8	0.6	0.4	0.2	0.2	0.4	0.4	0.4	1.0							
C_9	0.2	0.4	0.6	0.6	0.4	0.4	0.4	0.6	1.0						
C_{10}	0.8	1.0	0.4	0.8	1.0	1.0	1.0	0.4	0.4	1.0					
Ka_1	0.8	1.0	0.4	0.8	1.0	1.0	1.0	0.4	0.4	1.0	1.0				
Ch_1	0.4	0.6	0.4	0.8	0.6	0.6	0.6	0.4	0.8	0.6	0.6	1.0			
Pa_1	0.2	0.4	1.0	0.6	0.4	0.4	0.4	0.2	0.6	0.4	0.4	0.4	1.0		
Al_1	0.6	0.8	0.2	0.6	0.8	0.8	0.8	0.6	0.6	0.8	0.8	0.8	0.2	1.0	
Av_1	0.6	0.8	0.2	0.6	0.8	0.8	0.8	0.6	0.6	0.8	0.8	0.8	0.2	1.0	1.0

Table 4.7. Grouping of *Phytophthora* isolates based on similarity index of protein profiles

Cluster	Sub cluster	Isolates
A	A_1	C_1, C_2, C_5, C_6, C_7, C_{10}, Ka_1, Al_1, Av_1
	A_2	C_4, Ch_1, C_9
	A_3	C_8
	A_4	C_3, Pa_1

As a whole, the 15 isolates were classified into four sub clusters (Table.4.7). In A_1 sub cluster, isolates C_2, C_5, C_6, C_7, C_{10} and Ka_1 had 100 per cent SI (Table.4.6). But, isolate C_1 showed 80 per cent SI with these six isolates. Further, the isolate Al_1 and Av_1 were found to have 100 per cent SI, while isolate C_1 have 60 per cent SI with these two isolates. Isolates C_4 and Ch_1 belonged to subcluster A_2 with 80 per cent SI. Isolate C_9 had 60 and C_4 80 per cent respectively with C_4 and Ch_1. The subcluster A_3 was formed by a single isolate C_8. However, the isolate C_3 and Pa_1 had 100 per cent SI which formed a separate subcluster A_4.

4.4.4 Sensitivity of *Phytophthora* isolates to fungicides and antibiotics

4.4.4.1 Fungicides

Five fungicides, *viz.*, Indofil M-45 (0.3%), Akomin-40 (0.3%), Master (0.125%), Fytolan (0.2%) and Bordeaux mixture (1%) were tested for their inhibitory effect against 15 isolates of *P. palmivora*. Among the fungicides tested, Bordeaux mixture, Fytolan and Master completely inhibited the growth of all isolates. Indofil M-45 and Akomin-40 incorporated media supported the growth of the fungal isolates (Table.4.8). But, there was no significant difference in sensitivity among the isolates towards these two fungicides. All the isolates except C_{10} and Pa_1 showed mean diameter of growth of more than 30 mm with the maximum with isolate C_8 in Indofil M45 incorporated medium. In Akomin-40 incorporated medium, all 15 isolates recorded mean diameter of growth of less than 15 mm with the maximum with isolate Av_1 and the minimum by isolates C_1 and C_2.

4.4.4.2 Antibiotics

The *in vitro* inhibitory effect of two antibiotics *viz.*, streptomycin sulphate and chloramphenicol each at 300 ppm concentration was tested against the 15

Table 4.8. *In vitro* sensitivity of *Phytophthora* isolates to fungicides and antibiotics

S.No.	*Phytophthora* isolates	Fungicides		Antibiotics	
		Concentration (0.3%)		Concentration (300ppm)	
		Indofil-M 45	Akomin-40	Strept. sulphate	Chloramphenicol
		Mean diameter of colony (mm)	Mean diameter of colony (mm)	Mean diameter of colony (mm)	Mean diameter of colony (mm)
1	C_1	32.00 [a]	13.00 [a]	30.33 [a]	17.67 [abc]
2	C_2	32.00 [a]	13.00 [a]	31.67 [a]	17.67 [abc]
3	C_3	32.33 [a]	13.33 [a]	32.33 [a]	18.33 [abc]
4	C_4	35.33 [a]	13.67 [a]	20.33 [a]	16.33 [abc]
5	C_5	33.67 [a]	13.67 [a]	20.33 [a]	15.67 [abc]
6	C_6	34.67 [a]	14.00 [a]	20.33 [a]	15.67 [abc]
7	C_7	35.33 [a]	14.00 [a]	20.00 [a]	14.33 [bc]
8	C_8	37.33 [a]	13.67 [a]	21.00 [a]	14.00 [bc]
9	C_9	35.33 [a]	14.33 [a]	20.67 [a]	14.33 [bc]
10	C_{10}	29.33 [a]	13.67 [a]	20.33 [a]	14.33 [bc]
11	Ka_1	32.00 [a]	13.33 [a]	20.00 [a]	13.67 [c]
12	Ch_1	30.33 [a]	13.67 [a]	20.33 [a]	14.33 [bc]
13	Pa_1	29.00 [a]	14.33 [a]	18.00 [a]	21.67 [a]
14	Al_1	34.67 [a]	14.33 [a]	20.00 [a]	20.00 [ab]
15	Av_1	32.33 [a]	14.67 [a]	19.00 [a]	21.33 [a]

Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT

Phytophthora isolates. Statistical analysis revealed no significant difference in the colony diameter of the fungal isolates grown in streptomycin sulphate ammended media. The maximum growth was recorded by the isolate C_3, followed by C_2. Isolate Pa_1 showed the minimum growth (Table.4.8).

The isolates differed significantly in their sensitivity towards 300 ppm of chloramphenicol. The isolate Ka_1 exhibited more sensitivity and it was on par with other 10 isolates. Isolate Pa_1 was less sensitive and was on par with eight others.

4.5 SCREENING OF COCOA TYPES AGAINST *Phytophthora* POD ROT DISEASE

The results on the reaction of 225 cocoa types maintained in Germplasm VI of Cadbury - KAU Co-operative Cocoa Research Project, Vellanikkara against PPR are presented in Table.4.9.

After four day of inoculation, all the cocoa types screened except G VI 122 recorded less than 50 per cent pod area infection. Further, it was noted that 163 cocoa types showed less than 25 per cent pod area infection. Among these, 18 exhibited less than 10 per cent pod area infection with minimum in type G VI 14. A per cent pod area infection between 25-50 was recorded on other 61 cocoa types.

Data on the per cent pod area infection on the eighth day after inoculation showed that the cocoa types G VI 14, G VI 73, G VI 77, G VI 124, G VI 138, G VI 154, G VI 279 and G VI 284 recorded less than 50 per cent pod area infection with the minimum of 33.59 per cent in type G VI 14. A per cent pod area infection between 50-75 was observed in 64 cocoa types. Among them, 15 types recorded pod area infection of less than 60 per cent with least values of 50.24 and 50.28 per cent in

Table 4.9. Reaction of cocoa types against *Phytophthora* pod rot

S.No	Cocoa type	Per cent pod area infection			S.No	Cocoa type	Per cent pod area infection		
		4 DAI	8 DAI	Reaction			4 DAI	8 DAI	Reaction
1	G VI 2	25.49	100.0	S	43	G VI 51	19.69	89.39	S
2	G VI 3	35.45	92.0	S	44	G VI 52	18.22	91.33	S
3	G VI 4	6.20	55.54	MS	45	G VI 53	8.85	55.28	MS
4	G VI 5	7.77	77.84	S	46	G VI 54	20.83	100.0	S
5	G VI 6	28.52	80.24	S	47	G VI 55	11.29	73.13	MS
6	G VI 8	25.95	85.36	S	48	G VI 56	16.51	88.07	S
7	G VI 9	20.22	93.31	S	49	G VI 57	20.28	90.25	S
8	G VI 10	24.76	80.42	S	50	G VI 60	18.64	72.78	MS
9	G VI 11	23.57	100.0	S	51	G VI 61	36.64	100.0	S
10	G VI 13	27.34	100.0	S	52	G VI 64	28.24	92.5	S
11	G VI 14	6.10	33.59	MR	53	G VI 67	20.13	100.0	S
12	G VI 16	17.71	89.82	S	54	G VI 68	25.34	88.78	S
13	G VI 17	24.04	73.61	MS	55	G VI 71	27.29	82.50	S
14	G VI 19	22.93	79.88	S	56	G VI 73	8.87	46.52	MR
15	G VI 20	19.60	83.89	S	57	G VI 75	22.80	81.21	S
16	G VI 21	21.66	89.96	S	58	G VI 77	7.17	47.02	MR
17	G VI 22	25.80	57.50	MS	59	G VI 78	22.54	76.67	S
18	G VI 24	29.12	91.66	S	60	G VI 79	25.73	88.53	S
19	G VI 25	13.67	62.01	MS	61	G VI 80	22.07	78.26	S
20	G VI 26	19.31	73.13	MS	62	G VI 82	9.45	63.87	MS
21	G VI 27	19.45	87.35	S	63	G VI 84	19.61	100.0	S
22	G VI 28	18.52	70.24	MS	64	G VI 85	10.26	85.27	S
23	G VI 29	25.92	79.84	S	65	G VI 86	25.26	91.64	S
24	G VI 30	13.41	66.86	MS	66	G VI 87	21.42	72.28	MS
25	G VI 31	14.89	100.0	S	67	G VI 89	16.97	73.29	MS
26	G VI 33	12.93	91.39	S	68	G VI 92	13.67	52.86	MS
27	G VI 34	19.45	90.02	S	69	G VI 94	15.77	90.62	S
28	G VI 35	43.28	83.87	S	70	G VI 96	18.32	86.18	S
29	G VI 36	27.37	100.0	S	71	G VI 98	32.10	84.98	S
30	G VI 37	21.86	96.80	S	72	G VI 100	10.49	99.40	S
31	G VI 38	24.55	62.45	MS	73	G VI 101	20.64	72.98	MS
32	G VI 39	19.39	90.30	S	74	G VI 102	17.40	70.31	MS
33	G VI 40	18.27	89.32	S	75	G VI 103	28.24	89.01	S
34	G VI 41	21.14	100.0	S	76	G VI 104	18.36	74.27	MS
35	G VI 42	15.16	84.14	S	77	G VI 105	16.88	79.58	S
36	G VI 43	19.90	100.0	S	78	G VI 106	13.30	90.25	S
37	G VI 44	22.45	60.21	MS	79	G VI 107	25.68	100.0	S
38	G VI 45	10.33	57.47	MS	80	G VI 108	11.06	75.98	S
39	G VI 46	19.95	92.77	S	81	G VI 109	25.97	81.41	S
40	G VI 48	32.54	76.66	S	82	G VI 110	22.08	89.73	S
41	G VI 49	25.01	100.0	S	83	G VI 111	16.73	73.77	MS
42	G VI 50	11.48	72.04	MS	84	G VI 112	14.90	76.50	S

85	G VI 113	32.68	100.0	S	133	G VI 164	18.98	86.35	S
86	G VI 114	24.23	100.0	S	134	G VI 165	21.72	100.0	S
87	G VI 115	36.26	100.0	S	135	G VI 167	15.54	62.91	MS
88	G VI 117	27.82	86.25	S	136	G VI 168	17.53	81.60	S
89	G VI 118	10.66	63.43	MS	137	G VI 169	16.54	71.14	MS
90	G VI 120	17.10	89.14	S	138	G VI 170	16.92	57.97	MS
91	G VI 122	64.10	100.0	S	139	G VI 171	24.94	88.54	S
92	G VI 123	18.02	70.34	MS	140	G VI 172	18.43	67.85	MS
93	G VI 124	8.58	41.06	MR	141	G VI 173	19.94	79.12	S
94	G VI 125	16.85	67.18	MS	142	G VI 174	23.43	100.0	S
95	G VI 126	39.30	100.0	S	143	G VI 175	17.92	82.24	S
96	G VI 127	22.29	90.17	S	144	G VI 176	23.48	92.31	S
97	G VI 128	20.41	100.0	S	145	G VI 177	12.03	92.01	S
98	G VI 129	14.12	73.73	MS	146	G VI 178	14.12	71.39	MS
99	G VI 130	26.80	85.35	S	147	G VI 181	16.66	60.71	MS
100	G VI 131	14.26	84.69	S	148	G VI 182	16.61	88.72	S
101	G VI 132	19.44	65.46	MS	149	G VI 183	29.00	100.0	S
102	G VI 133	31.29	76.91	S	150	G VI 185	20.65	100.0	S
103	G VI 134	25.21	69.92	MS	151	G VI 186	20.93	84.05	S
104	G VI 135	23.51	100.0	S	152	G VI 187	16.49	81.32	S
105	G VI 136	19.05	88.01	S	153	G VI 188	18.34	74.99	MS
106	G VI 137	15.00	57.04	MS	154	G VI 189	25.18	100.0	S
107	G VI 138	6.72	48.24	MR	155	G VI 190	17.21	67.46	MS
108	G VI 139	13.23	66.74	MS	156	G VI 191	31.48	91.45	S
109	G VI 140	13.61	71.74	MS	157	G VI 192	29.02	88.32	S
110	G VI 141	23.57	91.84	S	158	G VI 193	20.84	82.98	S
111	G VI 142	15.34	83.95	S	159	G VI 194	19.14	84.28	S
112	G VI 143	15.47	82.13	S	160	G VI 195	12.67	79.98	S
113	G VI 144	21.71	91.84	S	161	G VI 196	18.03	79.23	S
114	G VI 145	17.95	68.09	MS	162	G VI 197	16.68	78.00	S
115	G VI 146	13.38	50.28	MS	163	G VI 198	9.32	59.66	MS
116	G VI 147	10.32	56.81	MS	164	G VI 199	10.43	54.30	MS
117	G VI 148	26.25	100.0	S	165	G VI 202	19.21	100.0	S
118	G VI 149	21.99	88.82	S	166	G VI 204	16.81	73.87	MS
119	G VI 150	17.30	70.89	MS	167	G VI 205	49.74	100.0	S
120	G VI 151	18.41	68.16	MS	168	G VI 214	33.97	92.12	S
121	G VI 152	39.53	95.81	S	169	G VI 224	21.69	62.36	MS
122	G VI 153	34.61	91.09	S	170	G VI 225	20.71	76.51	S
123	G VI 154	11.18	49.51	MR	171	G VI 227	14.09	83.97	S
124	G VI 155	26.66	70.56	MS	172	G VI 229	21.72	100.0	S
125	G VI 156	19.12	73.06	MS	173	G VI 232	23.29	65.05	MS
126	G VI 157	12.94	83.65	S	174	G VI 234	24.51	67.61	MS
127	G VI 158	44.26	100.0	S	175	G VI 235	8.71	57.01	MS
128	G VI 159	15.36	80.99	S	176	G VI 236	6.18	76.28	S
129	G VI 160	31.76	95.50	S	177	G VI 237	7.83	80.79	S
130	G VI 161	31.39	89.09	S	178	G VI 239	34.58	96.79	S
131	G VI 162	23.09	82.21	S	179	G VI 241	15.01	84.37	S
132	G VI 163	26.39	80.06	S	180	G VI 242	31.00	95.01	S

181	G VI 243	20.45	87.95	S	204	G VI 268	13.82	76.15	S
182	G VI 246	28.73	95.09	S	205	G VI 269	34.68	100.0	S
183	G VI 247	18.55	53.05	MS	206	G VI 270	15.10	83.75	S
184	G VI 249	32.45	87.53	S	207	G VI 271	12.80	85.22	S
185	G VI 250	17.28	74.34	MS	208	G VI 272	22.67	100.0	S
186	G VI 251	8.18	54.76	MS	209	G VI 273	17.14	91.30	S
187	G VI 252	38.97	82.75	S	210	G VI 274	12.77	70.96	MS
188	G VI 253	46.02	100.0	S	211	G VI 275	8.83	50.24	MS
189	G VI 254	27.63	87.78	S	212	G VI 276	33.11	94.35	S
190	G VI 255	13.14	99.36	S	213	G VI 277	19.30	100.0	S
191	G VI 256	26.81	73.47	MS	214	G VI 279	12.20	48.21	MR
192	G VI 257	9.85	73.48	MS	215	G VI 280	15.46	100.0	S
193	G VI 258	9.87	70.69	MS	216	G VI 281	15.68	94.99	S
194	G VI 259	10.77	92.32	S	217	G VI 282	29.99	91.39	S
195	GVI 260	25.96	100.0	S	218	G VI 283	24.79	83.81	S
196	GVI 261	16.72	78.07	S	219	G VI 284	16.98	48.02	MR
197	GVI 262	26.13	90.11	S	220	G VI 286	25.02	86.24	S
198	GVI 264a	16.43	66.60	MS	221	G VI 287	10.18	75.68	S
199	GVI 264b	9.95	66.96	MS	222	G VI 288	43.39	100.0	S
200	GVI 265a	9.62	90.54	S	223	G VI 289	45.26	100.0	S
201	GVI 265b	21.96	96.83	S	224	G VI 291	35.89	93.28	S
202	GVI 266	8.64	67.98	MS	225	G VI 295	26.66	95.56	S
203	G VI 267	22.67	92.40	S					

Mean of three replications

DAI - Days after inoculation

R - Resistant - < 25% pod area infected

MR - Moderately resistant - > 25 to < 50% pod area infected

MS - Moderately susceptible - >50 to < 75% pod area infected

S - Susceptible - > 75% pod area infected

G VI 275 and G VI 146 respectively. More than 75 per cent pod area infection was observed in 153 cocoa types. Among them, 38 types showed cent per cent infection. Hence, based on the reaction the 225 cocoa types screened they were grouped into four categories as follows (Fig.4.3).

Resistant	- Nil
Moderately resistant	- 8
Moderately susceptible	- 64
Susceptible	- 153

4.6 ISOLATION OF EPIPHYTIC MICROFLORA FROM COCOA PODS

The healthy pods from severely infected cocoa gardens from various locations of CCRP farm and Kannara were used for isolation of epiphytic microflora. The epiphytic microflora *viz.*, fungi, bacteria, actinomycetes and fluorescent pseudomonads were isolated from these samples by serial dilution plate technique and the results are presented in Table.4.10.

The population of epiphytic microflora varied in pods collected from different locations. Population of bacteria was more than that of fungi and actinomycetes. Fluorescent pseudomonads were isolated from only from two locations. The highest count of fungi (37.33 x 10^1 cfu/25 cm^2) was from the of pods collected from VSDTH block of CCRP farm, followed by that collected from G IV block of CCRP farm. The least count was from pods from G VI block of CCRP farm. The highest epiphytic count of bacteria and actinomycetes were from pods of G IV block of CCRP farm. The least population of bacteria and actinomycetes were from pods collected from Kannara and CYT I block of CCRP farm respectively. The fluorescent pseudomonads were isolated from pods of CCRP farm *viz.*, G VI block and CYT I block with more population in the former.

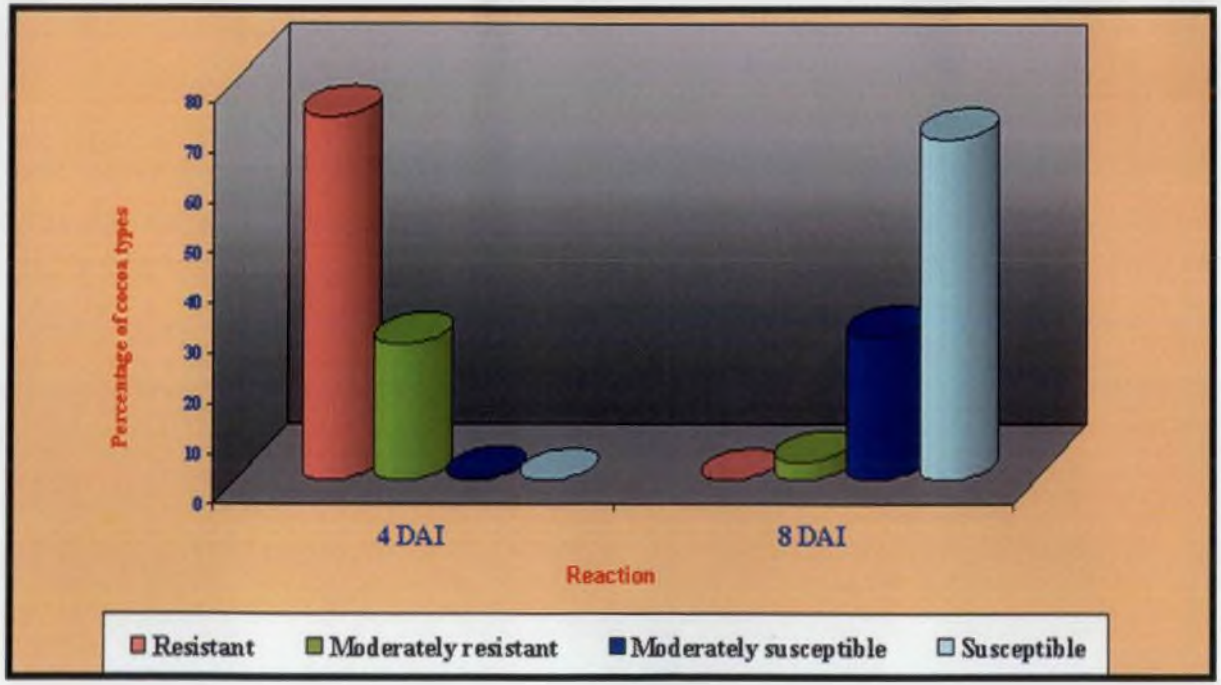


Fig 4.3. Reaction of cocoa types against *Phytophthora* pod rot on detached pods

Table 4.10. Epiphytic microflora on cocoa pod surface

S.No	Locations	Fungi (x 10^1 cfu / 25 cm^2)	Bacteria (x 10^2 cfu / 25 cm^2)	Actinomycetes (x 10^2cfu / 25 cm^2)	Fluorescent Pseudomonads (x 10^2cfu / 25 cm^2)
1	VSDTH block, CCRP farm	37.33	58.33	17.66	0
2	CYT I block, CCRP farm	27.00	34.66	13.66	6.33
3	G IV block, CCRP farm	36.66	71.00	22.00	0
4	GVI block, CCRP farm	17.66	49.00	17.00	9.33
5	Kannara	31.66	34.00	16.66	0

Mean of three replications

The representative colonies of epiphytic microbes were selected and used for further studies. Thus, 16 fungi, 22 bacteria and five actinomycetes were selected. Attempts to identify the fungal isolates upto their generic level revealed that out of 16 fungal isolates, four each belonged to the genus *Trichoderma* and *Aspergillus*, two to *Penicillium* and the rest remains unidentified.

4.7 *In vitro* SCREENING OF EPIPHYTIC MICROFLORA FOR THEIR ANTAGONISTIC REACTION AGAINST THE PATHOGEN

4.7.1 Screening of fungal isolates

Sixteen epiphytic fungal isolates and a standard culture of *T. harzianum* were screened for their antagonistic effect against *P. palmivora*. The per cent inhibition of the pathogen by the fungal isolates and their antagonistic reaction were worked out and the results are presented in Table.4.11.

After four days of inoculation of the antagonists, all the fungal isolates tested exerted varying levels of inhibition of the pathogen ranging from 3.29 to 100 per cent. Out of the total 17 fungal isolates, six *viz.*, 1F, 8F, 10F, 20F, 22F and *T. harzianum* showed cent per cent inhibition of the pathogen. The per cent inhibition of the pathogen by the antagonists seven days after inoculation ranged from 22.54 to 100 per cent (Plate 4). Among the isolates, eight of them showed cent per cent inhibition, which included four isolates of *Trichoderma* spp. (1F, 8F, 10F, 20F), three unidentified cultures (4F, 9F, 22F) and the standard culture of *T. harzianum*. Below 50 per cent inhibition of the pathogen was exhibited by three isolates.

Table 4.11. *In vitro* screening of fungal isolates against *P. palmivora*

S.No	Fungal isolates	Per cent inhibition		
		4 DAI	7 DAI	Antagonistic reaction
1	1F (*Trichoderma* sp.)	100.0	100.0	A
2	3F (*Penicillium* sp.)	10.73	26.13	B
3	4F (Unidentified)	29.41	100.0	A
4	5F (*Aspergillus niger*)	11.13	41.45	C
5	6F (*Penicillium* sp.)	22.90	54.05	B
6	7F (Unidentified)	24.19	64.86	B
7	8F (*Trichoderma* sp.)	100.0	100.0	A
8	9F (Unidentified)	26.82	100.0	A
9	10F (*Trichoderma* sp.)	100.0	100.0	A
10	12F (Unidentified)	7.21	57.67	D
11	14F (*Aspergillus niger*)	30.74	70.27	C
12	17F (*Aspergillus flavus*)	11.13	85.59	C
13	20F (*Trichoderma* sp.)	100.0	100.0	A
14	21F (*Aspergillus flavus*)	5.88	58.56	C
15	22F (Unidentified)	100.0	100.0	A
16	25F (Unidentified)	3.29	22.54	D
17	*T. harizanum*	100.0	100.0	A

Mean of three replications

DAI - Days after inoculation of antagonists

A - Over growth

B - Homogenous

C - Cessation of growth

D - Aversion

Among the fungi which exerted cent per cent inhibition all of them overgrew the pathogen. Four isolates *viz.*, 5F, 14F, 17F and 21F showed cessation of the growth at the point of contact with the pathogen. The remaining ones exhibited either homogenous or aversion type of reaction.

4.7.2 Screening of bacterial isolates

Twenty two epiphytic bacterial isolates and two standard cultures of *P. fluorescens* were evaluated for their antagonistic reaction against *P. palmivora*. Observations on the per cent inhibition of the pathogen were recorded four days after inoculation of the antagonists. All the bacterial isolates exhibited varying levels of inhibition of growth of the pathogen (Table.4.12). Among them, the standard culture Pf (K) recorded the maximum inhibition of 64.44 per cent followed by other standard culture, Pf (T). Five isolates of epiphytic bacteria (10B, 21B, 22B, 23B, 24B) from pods exhibited more than 40 per cent inhibition with the maximum by isolate 23B (Plate 6).

After seven days of inoculation the bacterial isolates exhibited per cent inhibition ranging from 4.77 to 78.11. Here also, the standard cultures [Pf (T)] and KAU [Pf (K)] were more inhibitory with more than 75 per cent inhibition. Further, these two isolates were found to be more effective than epiphytic isolates. Among the epiphytic isolates, five (3B, 15B, 21B, 23B, 24B) showed more than 60 per cent inhibition, with the maximum by isolate 21B. Less than 10 per cent inhibition was recorded by three isolates (2B, 14B, 16B).

4.7.3 Screening of actinomycetes isolates

None of the actinomycetes were inhibitory to the pathogen.

Table 4.12. *In vitro* screening of bacterial isolates against *P.palmivora*

S.No	Bacterial isolates	Per cent inhibition	
		4 DAI	7 DAI
1	1B	23.52	55.15
2	2B	3.29	9.50
3	3B	23.52	61.21
4	4B	18.98	58.78
5	5B	10.00	25.71
6	6B	12.62	21.91
7	7B	2.22	10.48
8	10B	42.50	57.14
9	11B	3.73	30.48
10	12B	8.17	27.62
11	13B	18.53	33.21
12	14B	2.22	8.57
13	15B	22.22	60.00
14	16B	1.00	4.77
15	17B	34.84	43.82
16	18B	24.44	42.85
17	19B	37.77	46.68
18	20B	15.50	32.40
19	21B	40.38	63.16
20	22B	40.00	52.85
21	23B	48.17	62.85
22	24B	40.38	60.52
23	Pf (T)	58.53	78.11
24	Pf (K)	64.44	77.14

Mean of three replications

DAI - Days after inoculation

Plate 4. *Trichoderma* spp. and *P. palmivora* in dual culture

A. *T. viride* **B. *T. harzianum***

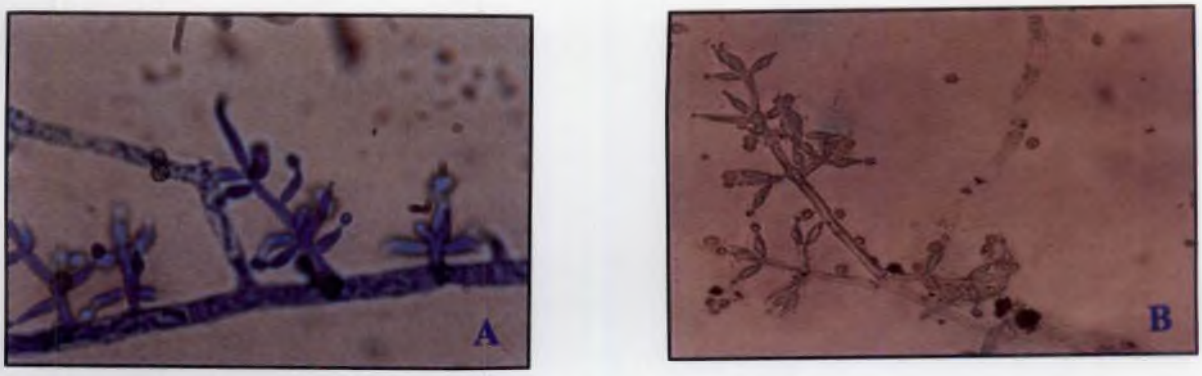


Plate 5. Photomicrographs of *T. viride and T. harzianum*

A. *T. viride* **B. *T. harzianum***

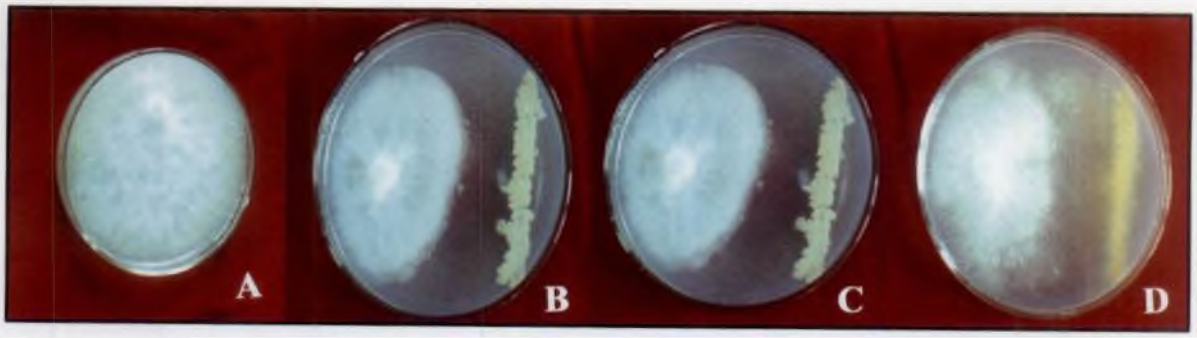


Plate 6. *Pseudomonas* spp. and *P. palmivora* in dual culture

A. Monoculture of *P. palmivora* **B. *P. fluorescens* (23B)**
C. *P. fluorescens* (24B) **D. *P. fluorescens* (K)**

4.7.4 Selection of fungal and bacterial antagonists for further screening

Promising isolates of fungi and bacteria were selected based on *in vitro* evaluation. Seven epiphytic fungal isolates (1F, 4F, 8F, 9F, 10F, 20F, 22F) and a standard culture of *T. harzianum*, which showed cent per cent inhibition of the pathogen were selected. Among the bacterial antagonists, which exerted more than 60 per cent inhibition of the pathogen, five epiphytic isolates (3B, 15B, 21B, 23B, 24B) and two standard *P. fluorescens* cultures were selected for further screening on detached cocoa pods.

4.8 EFFECT OF SELECTED ANTAGONISTS AGAINST *P. palmivora* ON DETACHED PODS

Fifteen promising antagonists selected based on their *in vitro* efficacy were evaluated for their ability in checking the *Phytophthora* infection on injured and uninjured detached cocoa pods. The per cent pod area infection was recorded five and 10 days after inoculation. It was observed that the per cent pod area infection was more on pods inoculated with injury than those inoculated without injury. Further, none of the antagonists tested were absolutely effective in preventing lesion development. Observations on the fifth day after inoculation on pods with injury indicated that the bacterial antagonist 23 B exerted the maximum per cent reduction of infection (47.62) (Table.4.13). The least effect was with isolate 4 F (12.77).

On pods inoculated without injury, the per cent pod area infection ranged from 3.60 to 25.71 after five days of inoculation. Six isolates of the antagonists tested showed more than 60 per cent efficacy over control in reducing infection with the

Table 4.13. Effect of selected antagonists against *P.palmivora* on detached cocoa pods

S.No.	Antagonists	Per cent pod area infection							
		5 DAI				10 DAI			
		With injury	Per cent reduction of infection over control	Without injury	Per cent reduction of infection over control	With injury	Per cent reduction of infection over control	Without injury	Per cent reduction of infection over control
1	1F	24.78	20.12	16.16	37.15	81.12	18.88	72.91	27.09
2	4F	27.06	12.77	17.36	32.48	74.11	25.89	67.51	32.49
3	8F	26.78	13.67	21.94	14.67	97.80	2.20	80.02	19.98
4	9F	25.65	17.32	8.06	68.66	86.80	13.20	67.49	32.51
5	10F	21.90	29.41	20.08	21.90	72.50	27.50	78.64	21.36
6	20F	24.25	21.83	3.60	86.00	89.34	10.66	58.51	41.49
7	22F	24.41	21.31	14.00	45.55	97.00	3.00	61.44	38.56
8	*T. harzianum*	26.39	14.93	9.39	63.48	97.40	2.60	66.58	33.42
9	3 B	25.71	17.12	19.79	23.03	91.07	8.93	74.37	25.63
10	15 B	25.53	17.70	16.04	37.62	80.46	19.54	73.44	26.56
11	21B	22.76	26.63	12.01	53.29	79.62	20.38	80.12	19.88
12	23B	16.25	47.62	9.70	62.28	53.92	46.08	35.95	64.05
13	24B	22.12	28.70	11.40	55.66	82.26	17.74	53.24	46.76
14	Pf (T)	25.56	17.61	6.30	76.55	82.90	17.10	65.59	34.41
15	Pf (K)	24.48	21.09	7.88	69.36	70.71	29.21	65.45	34.55
16	Control	31.02	-	25.71	-	100.0	-	100.0	-

DAI - Days after inoculation

maximum effect by isolate 20 F followed by Pf (T). The least efficiency was recorded with 8 F (14.67).

Ten days after inoculation, the minimum infection was on injured pods treated with isolate 23 B (53.92), which also showed the maximum per cent reduction of infection compared to control. All the other antagonists showed a per cent reduction of infection over control ranging from 2.20 to 29.21.

In the case of pods inoculated without injury, those treated with three antagonists *viz.*, 20 F, 23 B and 24 B recorded less than 60 per cent pod area infection and the minimum with isolate 23 B (35.95). Treatment with isolate 23 B had more than 60 per cent efficacy in reducing the infection.

4.8.1 Selection of efficient antagonists for field evaluation

For field evaluation, selection of the most promising antagonists was done based on the efficacy of isolates in reducing the infection on detached pods. Thus, among the epiphytic micro flora, the fungal isolate 20 F and bacterial isolates 23 B and 24 B, which exhibited maximum per cent efficacy over control in checking the disease were selected. The standard cultures of *T. harzianum* and *P. fluorescens* (K) were also selected for the field experiment.

4.9 IDENTIFICATION OF SELECTED ANTOGONISTS

4.9.1 Fungal antagonists

The cultural and morphological characters of the selected *Trichoderma* isolate 20 F were studied following standard procedures

4.9.1.1 *Trichoderma* sp. *(20 F)*

Colonies were dark green, fast growing, formed smooth surfaced sparse mycelial mat, which later became hairy and formed loose scanty aerial hyphae, which made the colonies some what whitish and emitted typical 'coconut odour' in old culture. Mycelium hyaline, smooth walled, septate and much branched. Conidiophores arise in compact or loose tuft, main branches produced several side branches in groups of two to three, all branches stand at wide angles. Phialides in false or irregular whorls, usually 2-3 phialides 8-14 x 2-3 µm in size at each whorl (Plate 5), curved, pin shaped, narrower at the base, wider above the middle, attenuated into long neck. Phialospores globose or short obovoid, 3-5 µm in size with minute roughing of their walls (spiny). Phialospores were pale green, accumulated at the tip of each phialide singly. Based on these characters, the isolate 20 F was identified as *Trichoderma viride* Per ex. S.F. Gray.

4.9.2 Bacterial antagonists

4.9.2.1 Characterization of bacterial antagonists

The cultural and biochemical characters of the bacterial isolates 23 B, 24 B and Pf (K) are presented in Table.4.14. The isolates were Gram negative short rods, produced greenish yellow fluorescent pigments, showed growth at 4°C, failed to grow at 41°C. The isolates were positive in catalase activity, oxidase reaction, levan production, starch hydrolysis, urease activity, arginine hydrolase and nitrate reduction reaction. Based on the cultural and biochemical characters of the bacterial isolates studied, the isolates (23 B, 24 B) were tentatively identified as *Pseudomonas fluorescens* Pelleroni.

Table. 4.14. Cultural and biochemical characters of bacterial antagonists

Cultural and biochemical characters	Bacterial isolates		
	23 B	24 B	Pf (K)
Gram reaction	-	-	-
Pigmentation	Greenish yellow	Greenish yellow	Greenish yellow
Growth at 4°C	+	+	+
Growth at 41°C	-	-	-
Catalase activity	+	+	+
Oxidase reaction	+	+	+
Levan production	+	+	+
Starch hydrolysis	+	+	+
Urease activity	+	+	+
Arginine hydrolase	+	+	+
Nitrate reduction	+	+	+

+ - Positive reaction

- - Negative reaction

4.10 MECHANISM OF ANTAGONISM OF SELECTED ANTAGONISTS AGAINST *P. palmivora*

4.10.1 Fungal antagonists

The selected antagonist of *T. viride* 20 F and standard culture of *T. harzianum*, proved to be efficient parasites of *P. palmivora*. The hyphae of the pathogen were found tightly held by coiling with hyphae of the antagonists (Plate 7&8). In addition, the hyphae of the antagonists penetrated the host hyphae at several points and grew to its inner cavity. Besides over growing and coiling, the antagonists

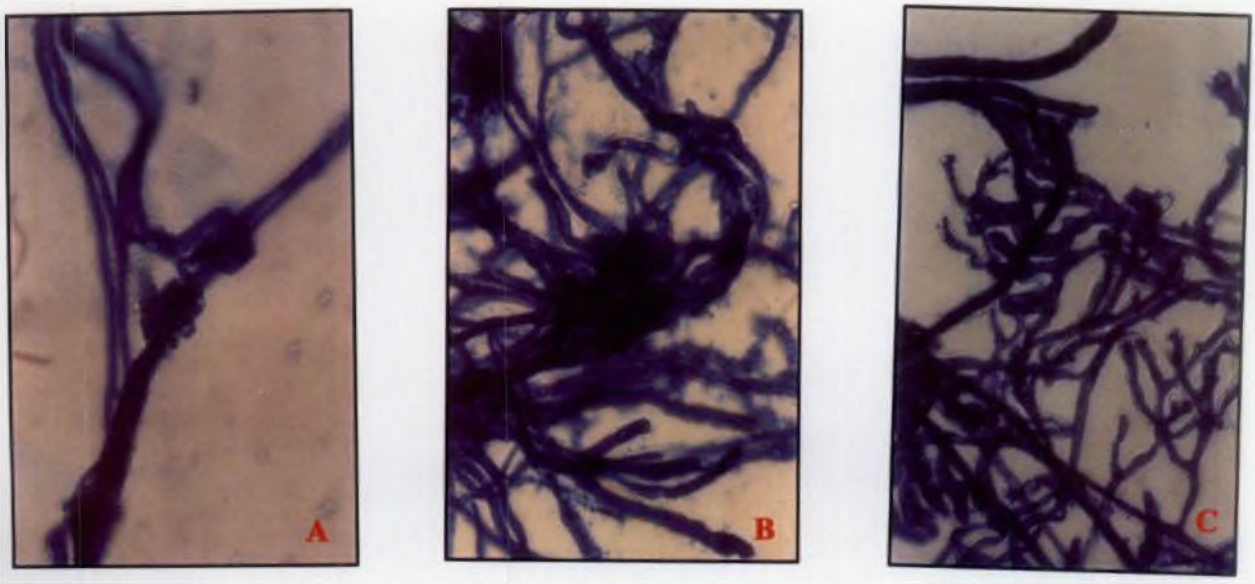


Plate 7. Mycoparasitism of *P. palmivora* by *T. harzianum*

A. Coiling **B. Disintegration** **C. Hyphal malformation**

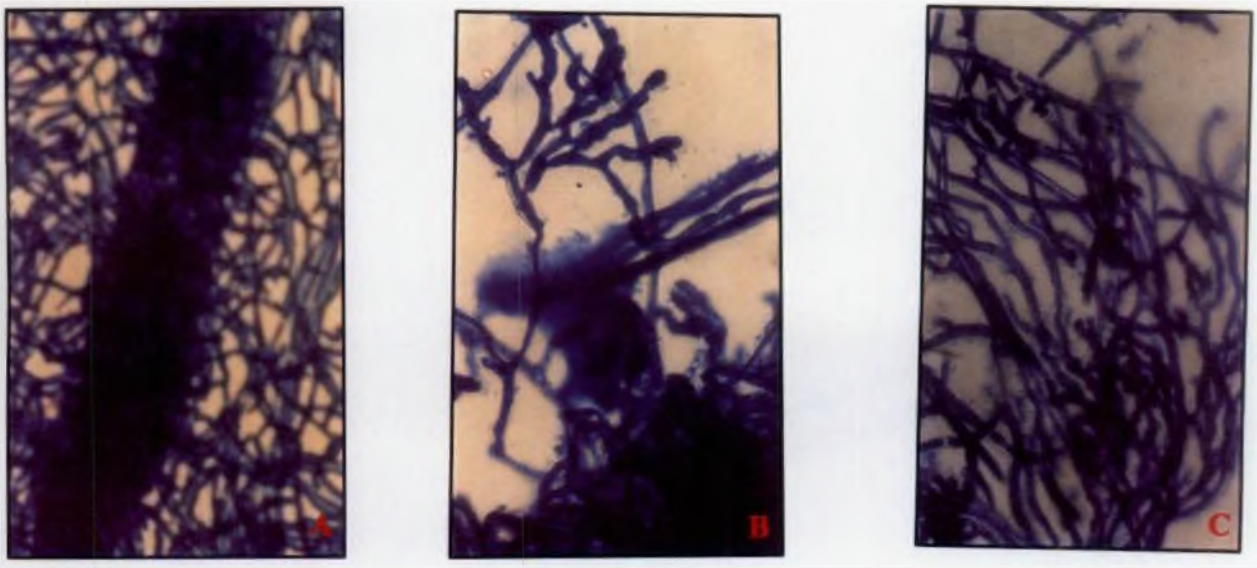


Plate 8. Mycoparasitism of *P. palmivora* by *T. viride*

A. Coiling **B. Disintegration** **C. Hyphal malformation**

also caused disintegration (lysis) of the pathogen hyphae (Plate 7&8). It was also noticed that free intermingling of antagonist hyphae with pathogen hyphae caused hyphal malformations in host (Plate 7&8).

4.10.2 Bacterial antagonists

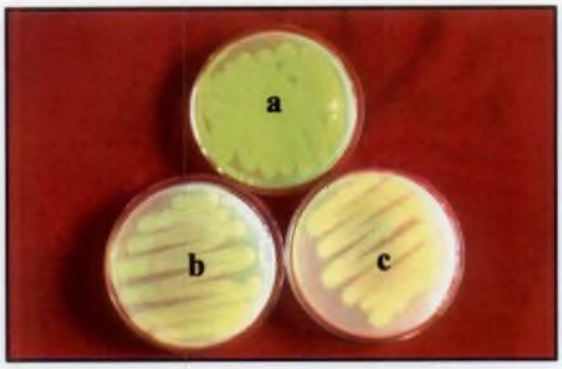
The selected *P. fluorescens* isolates (23 B, 24 B) and standard culture of *P. fluorescens* Pf (K) was studied for their ability to produce siderophore and HCN.

4.10.2.1 Siderophore production

The epiphytic bacterial isolates and standard culture of *P. fluorescence* were grown on King's B medium supplemented with $FeCl_3.6H_2O$ at different concentrations. It was observed that all *P. fluorescens* isolates produced abundant fluorescent pigments in medium not supplemented with $FeCl_3.6H_2O$. However, the rate of production of fluorescent pigments was less in medium incorporated with $FeCl_3.6H_2O$. Further, as the concentration of $FeCl_3.6H_2O$ increased, there was a decrease in the production of fluorescent pigments indicating siderophore production by isolates of *P. fluorescens* tested (Plate 9).

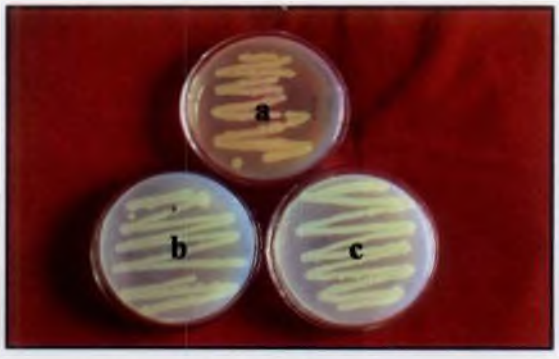
4.10.2.2 HCN production

The epiphytic (23B and 24B) and standard *P. fluorescens* isolates produced HCN as evidenced by the change in colour of the filter paper soaked in 0.5 per cent picric acid in two per cent sodium carbonate from deep yellow to orange brown.



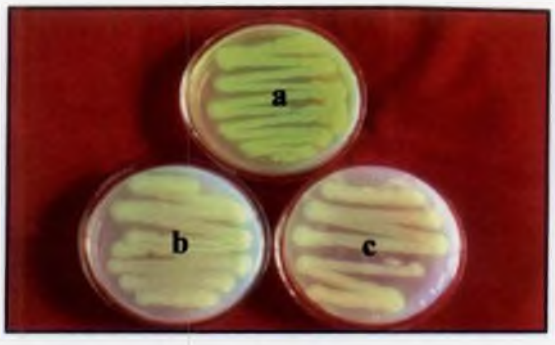
9A. *Pseudomonas fluorescens* (23B)

a. Control
b. 1 mg $FeCl_3$. 6 H_2O l^{-1}
c. 10 mg $FeCl_3$. 6 H_2O l^{-1}



9B. *Pseudomonas fluorescens* (24B)

a. Control
b. 1 mg $FeCl_3$. 6 H_2O l^{-1}
c. 10 mg $FeCl_3$. 6 H_2O l^{-1}



9C. *Pseudomonas fluorescens* (K)

a. Control
b. 1 mg $FeCl_3$. 6 H_2O l^{-1}
c. 10 mg $FeCl_3$. 6 H_2O l^{-1}

Plate 9. Effect of $FeCl_3$. 6 H_2O on siderophore production

4.11 COMPATIBILITY OF SELECTED ANTAGONISTS TO COMMON PLANT PROTECTION CHEMICALS AND CHEMICAL FERTILIZERS USED IN COCOA GARDENS

Different fungicides, insecticides and chemical fertilizers commonly used in cocoa gardens were evaluated at various concentrations to know their compatibility with the selected *T. viride* (20 F), *P. fluorescens* (23 B and 24 B) and standard cultures of *T. harzianum* and *P. fluorescens* (K).

4.11.1 Fungal antagonists

4.11.1.1 Fungicides

Six fungicides *viz.*, Fytolan, Kocide, Indofil M-45, Bavistin, Bordeaux mixture and Akomin-40 each at three concentrations were evaluated for this study (Table.4.15). The fungal antagonists did not show any inhibition at 0.2 per cent Akomin-40. In general, it was noticed that as the concentration of fungicides increased, there was a corresponding increase in the inhibition of the antagonists. The two isolates of *Trichoderma* were compatible with Akomin-40 and Indofil M-45 and partial compatibility with Fytolan. However, these were incompatible with Bordeaux mixture, Bavistin and Kocide.

Regarding the response of *T. viride* to fungicides at various concentrations it differed significantly (Fig.4.4). This fungus showed no inhibition with 0.2 per cent Akomin-40. Akomin-40 (0.3 per cent) and Indofil M-45 (0.2 per cent) recorded an inhibition of 11.85 per cent and these were followed by Indofil M-45 (0.3 per cent), Akomin-40 (0.4 per cent) and Indofil M-45 (0.4 per cent) recording per cent inhibition of 14.07, 16.30 and 16.66 respectively. Cent per cent inhibition of the fungus was noticed with Bordeaux mixture and Bavistin at all concentrations tested.

Table 4.15. Compatibility of *Trichoderma* spp. with fungicides

S.No	Fungicides	Concentration (per cent)	*Trichoderma viride* (20F)		*Trichoderma harzianum*	
			Mean diameter of colony (mm)	Per cent inhibition over control	Mean diameter of colony (mm)	Per cent inhibition over control
1	Fytolan	0.2	67.33 (8.24) [c]	25.18	54.00 (7.37) [f]	40.00
		0.3	65.00 (8.09) [c]	27.77	48.67 (7.01) [g]	45.92
		0.4	56.67 (7.59) [d]	37.03	44.67 (6.72) [g]	50.36
2	Kocide	0.1	19.67 (4.49) [e]	78.14	20.33 (4.56) [h]	77.41
		0.2	15.67 (4.02) [ef]	82.58	16.67 (4.41) [h]	81.47
		0.3	12.33 (3.58) [f]	86.30	11.00 (3.39) [i]	87.77
3	Indofil-M 45	0.2	79.33 (8.94) [b]	11.85	72.67 (8.55) [c]	19.25
		0.3	77.33 (8.82) [b]	14.07	67.00 (8.22) [d]	25.55
		0.4	75.00 (8.69) [b]	16.66	60.67 (7.82) [e]	32.58
4	Bavistin	0.05	0 (0.71) [g]	100.0	0 (0.71) [j]	100.0
		0.1	0 (0.71) [g]	100.0	0 (0.71) [j]	100.0
		0.2	0 (0.71) [g]	100.0	0 (0.71) [j]	100.0
5	Bordeaux Mixture	0.5	0 (0.71) [g]	100.0	0 (0.71) [j]	100.0
		1.0	0 (0.71) [g]	100.0	0 (0.71) [j]	100.0
		1.5	0 (0.71) [g]	100.0	0 (0.71) [j]	100.0
6	Akomin-40	0.2	90.00 (9.51) [a]	0.00	90.00 (9.51) [a]	0.00
		0.3	79.33 (8.94) [b]	11.85	80.00 (8.97) [b]	11.11
		0.4	75.33 (8.71) [b]	16.30	76.33 (8.77) [bc]	15.18
7	Control	-	90.00 (9.51) [a]	-	90.00 (9.51) [a]	-

Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

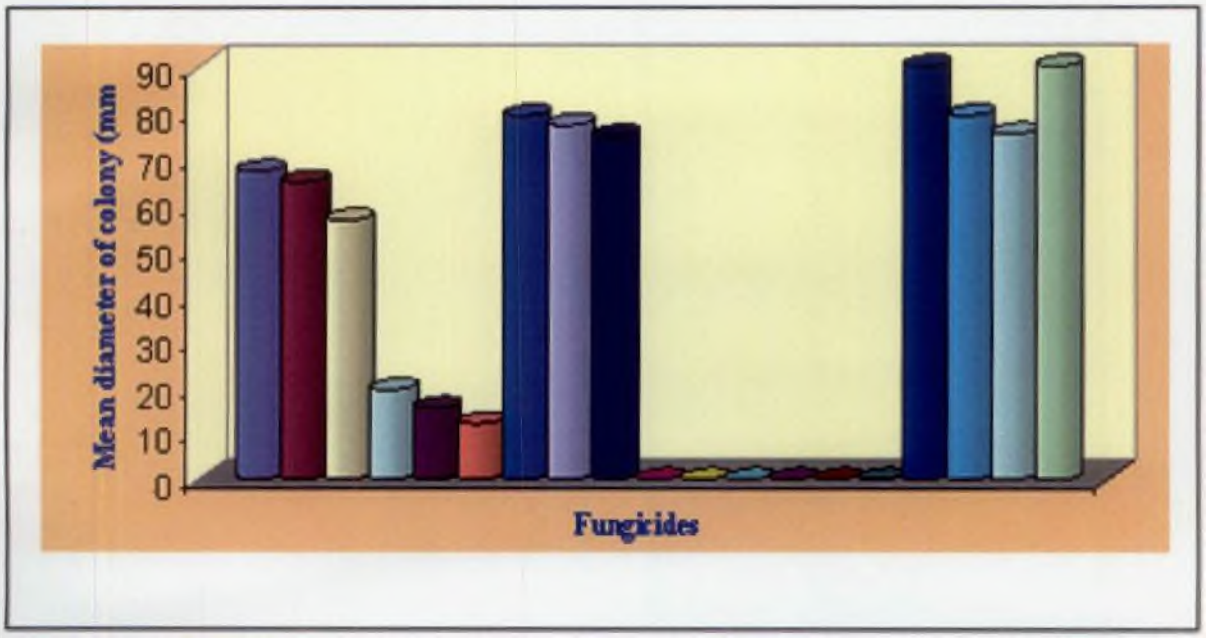


Fig 4.4. Compatibility of *Trichoderma viride* (20 F) with fungicides

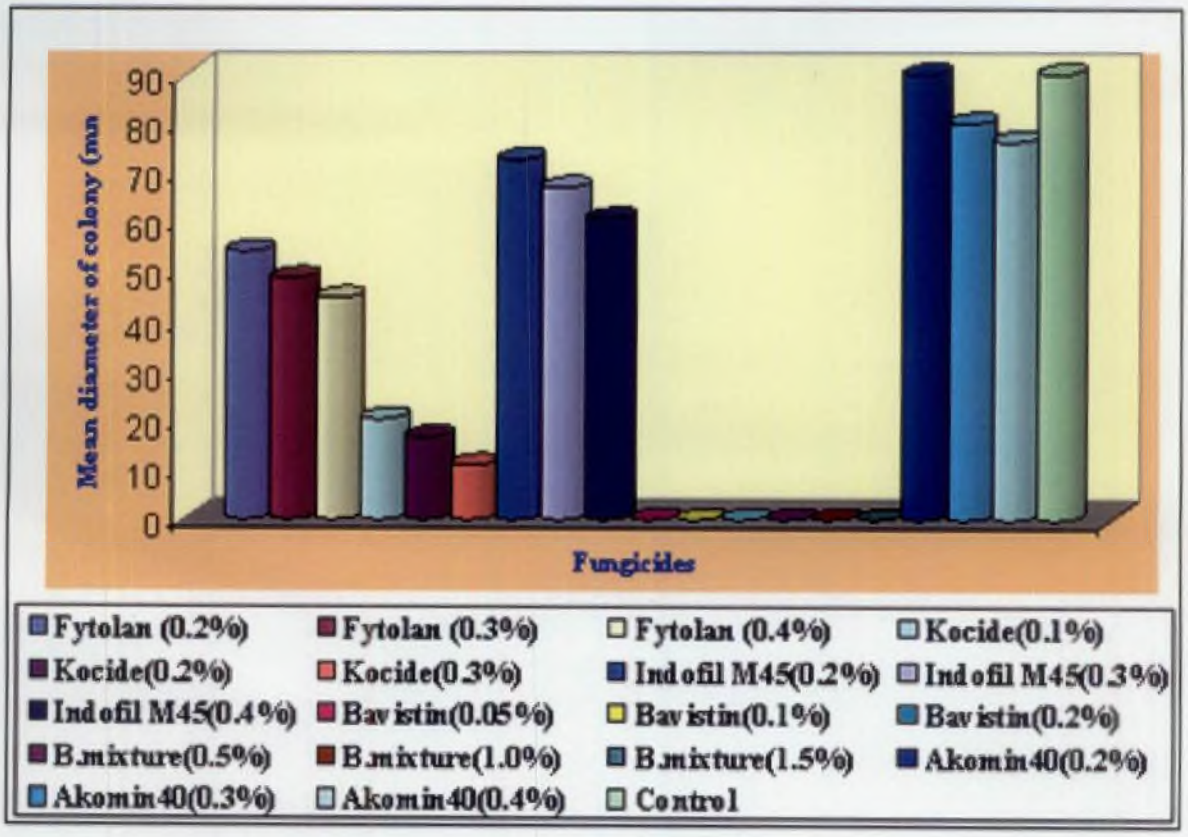


Fig 4.5. Compatibility of *Trichoderma harzianum* with fungicides

Fytolan at all concentrations were comparatively less inhibitory to the fungus and its two lower concentrations were on par with each other and recorded per cent inhibition ranging from 25.18 to 37.03. The response of *T. viride* to Kocide was very poor indicating its incompatibility at all concentrations.

T. harzianum also differed significantly in its response to fungicides at various concentrations (Fig.4.5). It showed no inhibition with 0.2 per cent Akomin-40. A per cent inhibition of 11.11 was recorded with Akomin-40 (0.3 per cent), which was closely followed by Akomin-40 (0.4 per cent) and Indofil M-45 (0.2 per cent). Cent per cent inhibition was exhibited with Bordeaux mixture and Bavistin at all concentrations. Indofil M-45 (0.3 and 0.4 per cent) showed a per cent inhibition of 25.55 to 32.58 respectively. Different concentrations of Fytolan recorded a per cent inhibition of 40 to 50.36. Kocide at all concentrations were more inhibitory to the fungus and recorded a per cent inhibition upto 87.77 per cent. It was also observed that Fytolan and Indofil M-45 were more inhibitory to this fungus compared *T.viride*.

4.11.1.2 Insecticides

The *in vitro* sensitivity of five insecticides *viz.*, Sevin, Ekalux, Endosulfan, Nuvacron and Phorate each at three concentrations were tested against two *Trichoderma* spp.

It was evident from the data (Table.4.16) that the antagonists exhibited varying levels of sensitivity to the different insecticides. The two species of *Trichoderma* were compatible with Phorate at all concentrations tested. Nuvacron, Ekalux and Endosulfan were incompatible with the antagonists.

The response of *T. viride* differed significantly to insecticides at various concentrations (Fig.4.6). *T. viride* was compatible with Phorate at all concentrations

Table 4.16. Compatibility of *Trichoderma* spp. with insecticides

S.No	Insecticides	Concentration (per cent)	*Trichoderma viride* (20F)		*Trichoderma harzianum*	
			Mean diameter of colony (mm)	Per cent inhibition over control	Mean diameter of colony (mm)	Per cent inhibition over control
1	Sevin	0.05	62.67 (7.94) [b]	30.81	63.67 (8.01) [b]	29.25
		0.1	44.00 (6.67) [c]	51.11	45.33 (6.77) [c]	49.63
		0.2	40.67 (6.41) c	54.81	39.00 (6.29) [d]	56.66
2	Ekalux	0.05	14.33 (3.83) [f]	84.07	12.00 (3.53) [g]	86.66
		0.1	0 (0.71) [g]	100.0	8.33 (2.97) [h]	90.74
		0.2	0 (0.71) [g]	100.0	0 (0.71) [i]	100.0
3	Endosulfan	0.04	25.67 (5.12) [d]	71.47	30.33 (5.55) [e]	66.30
		0.08	19.00 (4.42) [e]	78.88	24.67 (5.02) [f]	72.58
		0.15	11.33 (3.44) [f]	87.41	11.67 (3.48) [g]	87.03
4	Nuvacron	0.04	0 (0.71) [g]	100.0	0 (0.71) [i]	100.0
		0.08	0 (0.71) [g]	100.0	0 (0.71) [i]	100.0
		0.15	0 (0.71) [g]	100.0	0 (0.71) [i]	100.0
5	Phorate	1 kg a.i ha^{-1}	90.00 (9.51) [a]	0	90.00 (9.51) [a]	0
		1.5 kg a.i ha^{-1}	90.00 (9.51) [a]	0	90.00 (9.51) [a]	0
		2 kg a.i ha^{-1}	90.00 (9.51) [a]	0	90.00 (9.51) [a]	0
6	Control	-	90.00 (9.51) [a]	-	90.00 (9.51) [a]	-

Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

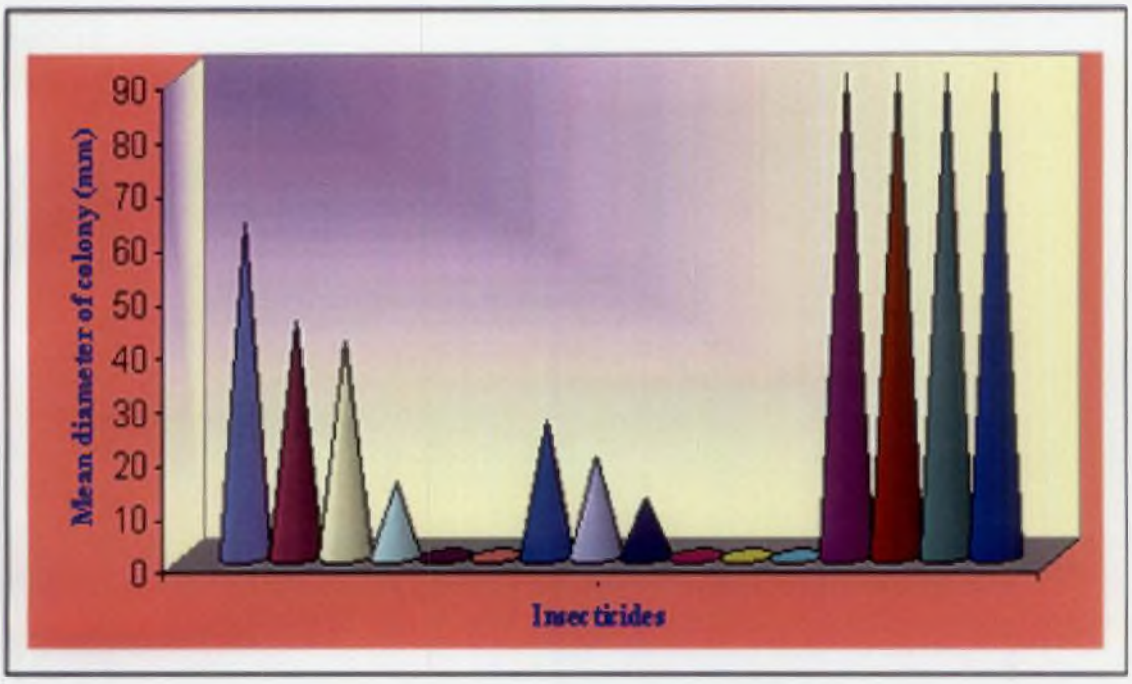


Fig 4.6. Compatibility of *Trichoderma viride* (20F) with insecticides

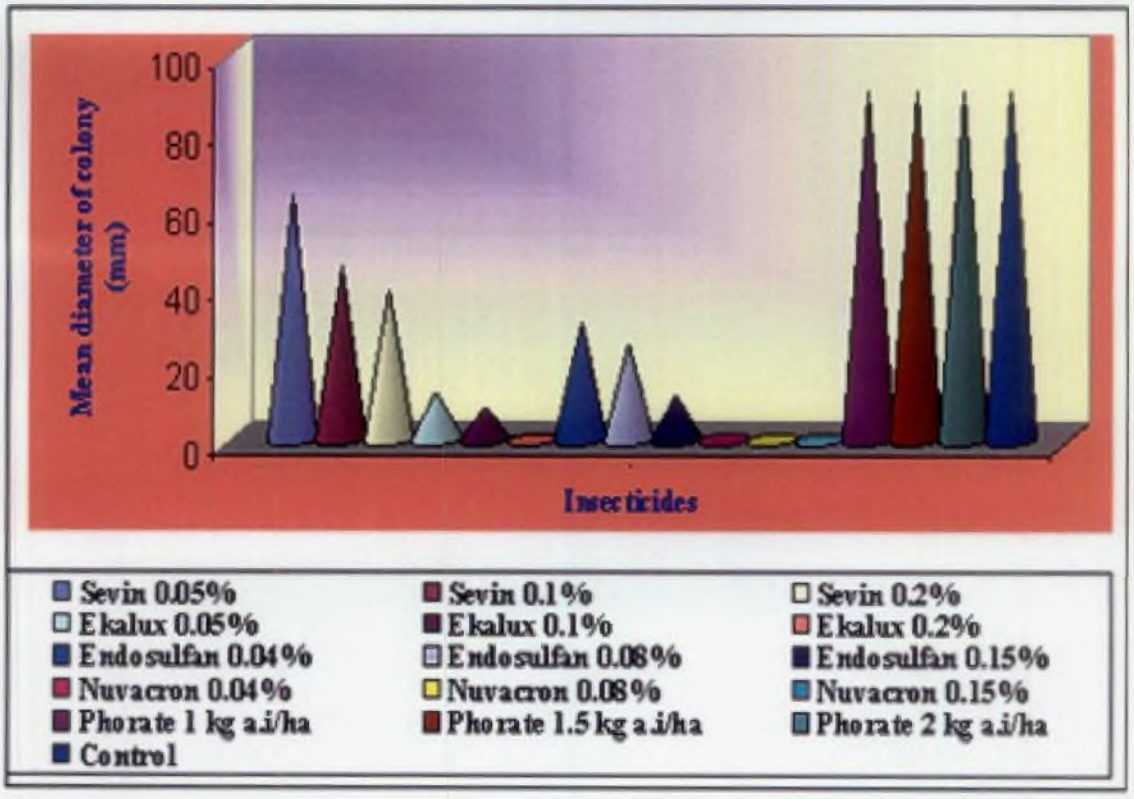


Fig 4.7. Compatibility of *Trichoderma harzianum* with insecticides

as there was no inhibition of the growth. Sevin at different dosages caused an inhibition ranging from 30.81 to 54.81 per cent. Nuvacron at all concentrations and Ekalux at 0.1 and 0.2 per cent recorded cent per cent inhibition of the fungus. In addition to these, Endosulfan at all concentrations, Ekalux 0.05 per cent recorded per cent inhibition ranging from 71.47 to 87.41 indicating the incompatibility of these insecticides to the antagonist.

Nuvacron at all concentrations and Ekalux 0.2 per cent were completely inhibitory to *T. harzianum*, and were followed by Ekalux 0.1 per cent exhibiting an inhibition of 90.74 per cent (Fig.4.7). More than 80 per cent inhibition was observed with Endosulfan 0.15 per cent and Ekalux 0.05 per cent. Phorate was compatible with the fungus. Sevin at all concentrations showed lesser compatibility than Phorate, which recorded percent inhibition of 29.25 to 56.66.

4.11.1.3 Fertilizers

Urea, ammonium chloride, ammonium sulphate, Rajphos and Muriate of potash (MOP) at various concentrations were evaluated for their compatibility to the two fungal antagonists. In general, it was noticed that as the concentration of fertilizers increased there was more inhibition. The study indicated that ammonium chloride, Rajphos and MOP were compatible with antagonists to various extents (Table.4.17).

T. viride proved to be compatible with all concentrations of MOP. The higher two concentrations of MOP and lower two concentrations of Rajphos and 2 per cent ammonium chloride were on par with each other and recorded a per cent inhibition upto 13.33 per cent (Fig.4.8). A partial inhibition of growth of the fungus was noticed at the highest concentration of Rajphos. This was followed by higher two concentrations of ammonium chloride, which recorded per cent inhibition ranging

Table 4.17. Compatibility of *Trichoderma* spp. with fertilizers

S.No.	Fertilizers	Concentration (Per cent)	*Trichoderma viride* (20F)		*T. harzianum*	
			Mean diameter of colony (mm)	Per cent inhibition over control	Mean diameter of colony (mm)	Per cent inhibition over control
1	Urea	1.0	65.33 (8.11) ^{fg}	27.41	66.67 (8.20) ^{f}	25.92
		1.5	47.67 (6.94) ^{i}	49.25	56.67 (7.56) ^{i}	37.03
		2.0	29.67 (5.48) ^{j}	67.03	31.00 (5.61) ^{k}	65.55
2	Ammonium chloride	2.0	79.33 (8.94) ^{b}	11.85	71.67 (8.50) ^{e}	20.36
		2.5	70.33 (8.42) ^{de}	21.85	64.67 (8.07) ^{fg}	28.14
		3.0	67.00 (8.22) ^{ef}	25.55	63.00 (7.97) ^{gh}	30.00
3	Ammonium sulphate	2.0	61.33 (7.86) ^{g}	68.52	61.67 (7.88) ^{h}	72.96
		2.5	54.33 (7.40) ^{h}	39.63	41.33 (6.47) ^{j}	54.07
		3.0	28.33 (5.37) ^{j}	31.85	24.33 (4.98) ^{l}	31.47
4	Rajphos	2.0	82.67 (9.12) ^{b}	8.14	81.00 (9.03) ^{c}	10.00
		2.5	78.00 (8.86) ^{bc}	13.33	74.00 (8.63) ^{d}	17.77
		3.0	74.33 (8.65) ^{cd}	17.41	66.67 (8.20) ^{f}	25.92
5	MOP	2.0	89.33 (9.48) ^{a}	0.74	90.00 (9.51) ^{a}	0
		2.5	82.33 (9.10) ^{b}	8.52	86.33 (9.32) ^{b}	4.07
		3.0	78.33 (8.88) ^{bc}	12.96	85.00 (9.25) ^{b}	5.55
6	Control	-	90.00 (9.51) ^{a}	-	90.00 (9.51) ^{a}	-

Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are $\sqrt{x+0.5}$ transformed value

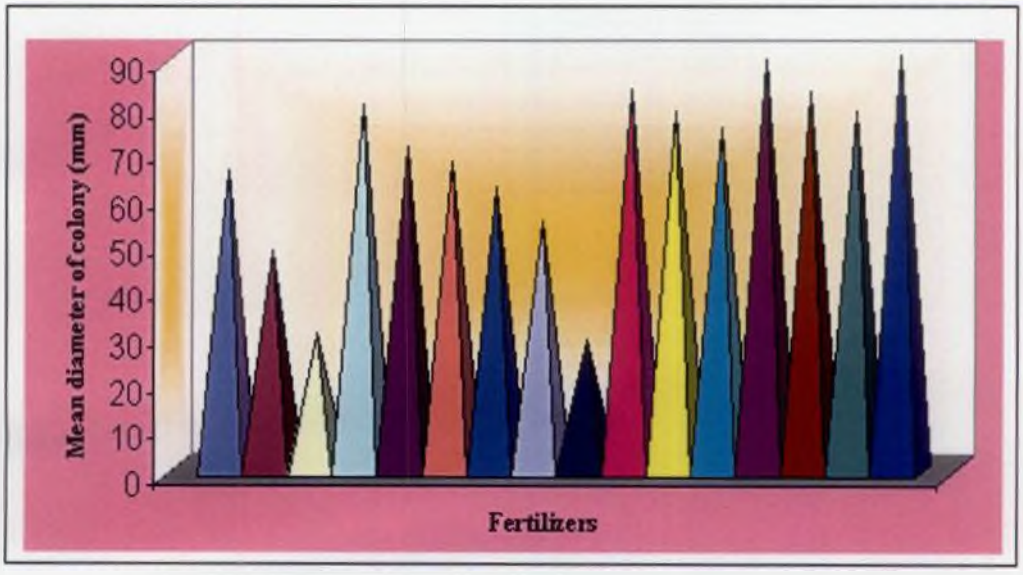


Fig 4.8. Compatibility of *Trichoderma viride* (20F) with fertilizers

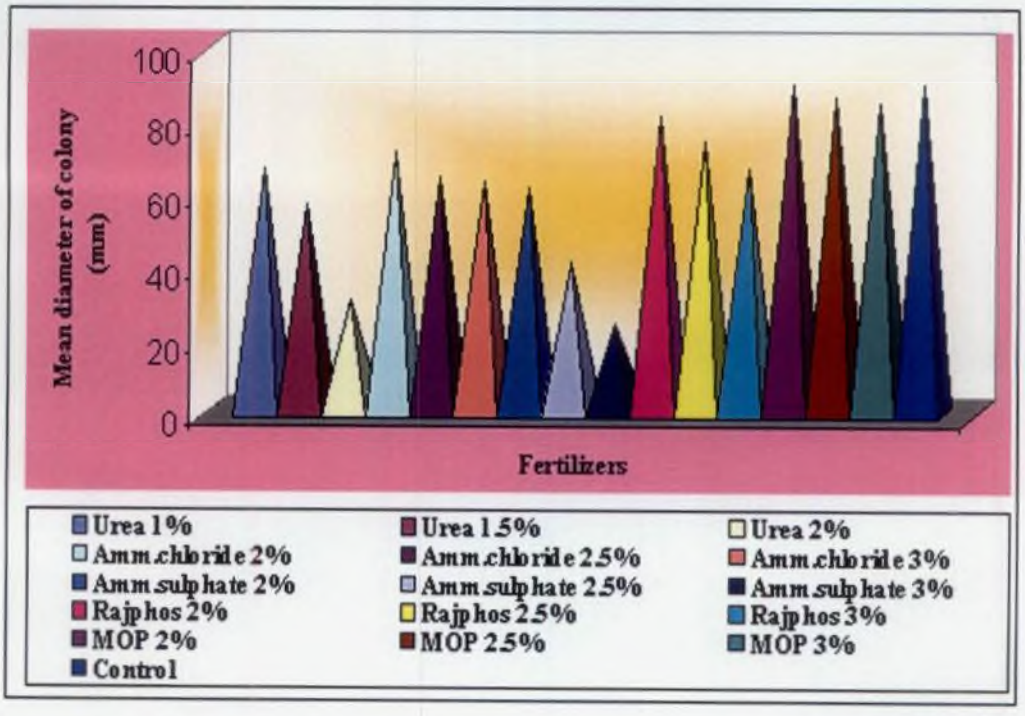


Fig 4.9. Compatibility of *Trichoderma harzianum* with fertilizers

from 17.41 to 25.55. The response of *T. viride* to all concentrations of Urea and ammonium sulphate tested were comparatively poor. The maximum per cent inhibition was recorded with ammonium sulphate (3 per cent) followed by (2 per cent) urea, which recorded 68.52 and 67.03 per cent inhibition respectively.

In the case of *T. harzianum*, full growth of the fungus was noticed in lowest concentration of MOP (Fig.4.9). This was followed by higher two concentrations of MOP, which recorded a per cent inhibition 4.07 and 5.55 respectively indicating their compatibility with the fungus. A partial inhibition of growth of the fungus was noticed with all concentrations of Rajphos and lowest concentration of Urea and ammonium chloride with per cent inhibition ranging from 10 to 25.92. Comparatively poor growth of *T. harzianum* was noticed at all concentrations of ammonium sulphate and highest two concentrations of Urea and ammonium chloride tested. Among these, the maximum per cent inhibition was recorded with 3 per cent ammonium sulphate followed by 2 per cent of urea, which recorded an inhibition of 72.96 and 65.55 per cent respectively.

4.11.2 Bacterial antagonists

4.11.2.1 Fungicides

Bordeaux mixture, Fytolan, Kocide, Akomin-40, Indofil M-45 and Bavistin each at three concentrations were tested *in vitro* to study their compatibility with the two native bacterial antagonists and standard culture of *P. fluorescens* (K). The results are presented in Table.4.18.

In general, fungicides at different concentrations showed varying percentage of inhibition. Bordeaux mixture at tested concentrations showed

Table 4.18. Compatibility of *Pseudomonas fluorescens* isolates with fungicides

S.No	Fungicides	Concentration (per cent)	*P. fluorescens* (23B)		*P. fluorescens* (24B)		*P. fluorescens* Pf (K)	
			Mean diameter of inhibition zone (mm)	Per cent inhibition over control	Mean diameter of inhibition zone (mm)	Per cent inhibition over control	Mean diameter of inhibition zone (mm)	Per cent inhibition over control
1	Fytolan	0.2	7.00 (2.74) ^{f}	7.77	7.00 (2.74) ^{c}	7.77	20.33 (4.56) ^{abcd}	22.59
		0.3	9.66 (3.19) ^{ef}	10.37	9.33 (3.14) ^{c}	10.37	20.67 (4.60) ^{abcd}	22.97
		0.4	9.66 (3.19) ^{ef}	11.11	10.67 (3.34) ^{c}	11.86	23.00 (4.84) ^{abc}	25.56
2	Kocide	0.1	10.00 (3.24) ^{ef}	11.11	0 (0.71) ^{d}	0	16.67 (4.41) ^{bcde}	18.52
		0.2	12.33 (3.58) ^{de}	13.70	7.00 (2.74) ^{c}	7.77	19.00 (4.42) ^{abcd}	21.11
		0.3	15.66 (4.02) ^{cd}	17.40	8.66 (3.03) ^{c}	9.62	23.67 (4.91) ^{abc}	26.30
3	Indofil-M 45	0.2	0 (0.71) ^{g}	0	10.33 (3.29) ^{c}	11.48	0 (0.71) ^{f}	0
		0.3	8.66 (3.03) ^{ef}	9.62	10.67 (3.34) ^{c}	11.86	9.00 (3.08) ^{de}	10.00
		0.4	10.00 (3.24) ^{ef}	11.11	12.33 (3.58) ^{bc}	13.70	12.00 (3.54) ^{cde}	13.33
4	Bavistin	0.05	0 (0.71) ^{g}	0	6.66 (2.68) ^{c}	7.40	0 (0.71) ^{f}	0
		0.1	7.00 (2.74) ^{f}	7.77	7.00 (2.74) ^{c}	7.77	0 (0.71) ^{f}	0
		0.2	7.00 (2.74) ^{f}	7.77	8.66 (3.03) ^{c}	9.62	7.67 (2.86) ^{e}	8.52
5	Bordeaux Mixture	0.5	20.33 (4.56) ^{bc}	22.59	21.00 (4.64) ^{ab}	23.33	27.67 (5.30) ^{ab}	30.74
		1.0	25.33 (5.08) ^{ab}	28.14	25.33 (5.08) ^{a}	28.14	31.00 (5.61) ^{ab}	34.44
		1.5	29.00 (5.43) ^{a}	32.22	26.67 (5.21) ^{a}	29.63	32.67 (5.76) ^{a}	36.30
6	Akomin-40	0.2	0 (0.71) ^{g}	0	0 (0.71) ^{d}	0	0 (0.71) ^{f}	0
		0.3	0 (0.71) ^{g}	0	0 (0.71) ^{d}	0	0 (0.71) ^{f}	0
		0.4	9.66 (3.19) ^{ef}	10.73	0 (0.71) ^{d}	0	0 (0.71) ^{f}	0
7	Control	-	0 (0.71) ^{g}	-	0 (0.71) ^{d}	-	0 (0.71) ^{f}	-

Mean of three replications In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

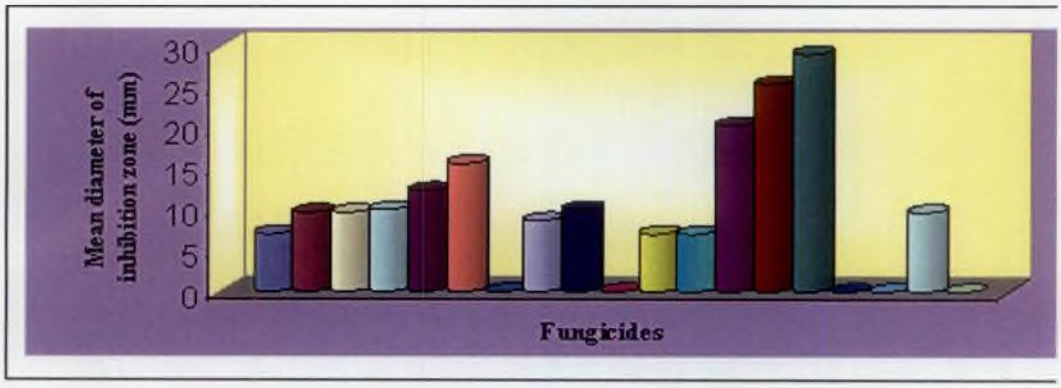


Fig 4.10. Compatibility of *Pseudomonas fluorescens* (23B) with fungicides

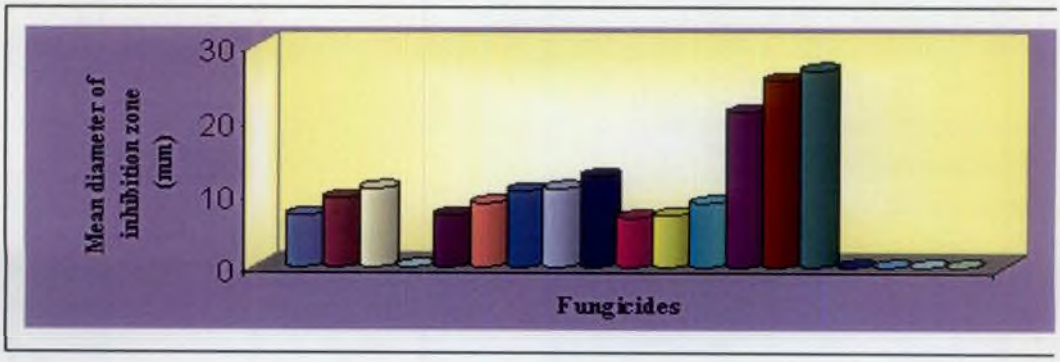


Fig 4.11. Compatibility of *Pseudomonas fluorescens* (24B) with fungicides

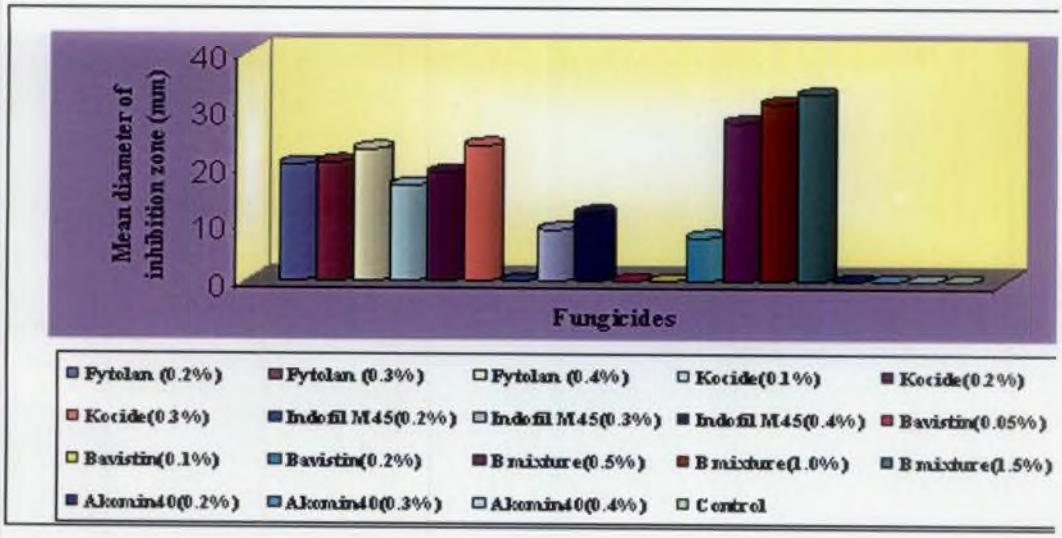


Fig 4.12. Compatibility of *Pseudomonas fluorescens* (K) with fungicides

comparatively higher inhibitory effect against the bacterial antagonists, followed by two other copper fungicides.

The response of *P. fluorescens* 23 B to different fungicides at various concentrations differed significantly (Fig.4.10). The lowest concentration of Indofil M-45, Bavistin and two lower concentrations of Akomin-40 were not inhibitory to this isolate. The two higher concentrations of Bavistin, Indofil M-45 and all concentrations of Fytolan showed comparatively less inhibitory effect. Bordeaux mixture and Kocide were highly inhibitory to the growth of the bacteria with the former exerted the maximum effect.

P. fluorescens 24 B also reacted differently to various fungicides at different concentrations tested (Fig.4.11). The lowest concentration of Kocide and all concentrations of Akomin-40 tested did not show any inhibition of growth of this antagonist. All the concentrations of Fytolan, Indofil M-45, Bavistin and two higher concentrations of Kocide showed inhibitory effect against this isolate. Here also, Bordeaux mixture at all the three concentrations was highly inhibitory to the growth of the bacteria, which were on par with each other.

In the case of *P. fluorescens* (K) the different fungicides differed significantly in their inhibitory effect (Fig.4.12). All concentrations of Akomin-40, two lower concentrations of Bavistin and the lowest concentration of Indofil M-45 did not show any inhibition of growth of this culture. All other fungicides tested at various concentrations were inhibitory to this bacteria at various extents. Here, the copper fungicides Bordeaux mixture, Fytolan and Kocide in that order exerted the maximum zone of inhibition. Bordeaux mixture at all concentrations exerted more than 30 per cent inhibition of growth of this isolate.

4.11.2.2 Insecticides

The *in vitro* sensitivity of Sevin, Ekalux, Endosulfan, Nuvacron and Phorate each at three concentrations was tested against the three *P. fluorescens* isolates.

The data indicated that the antagonists exhibited significant difference in their sensitivity to the insecticides tested (Table.4.19). The native *P. fluorescens* isolate 23 B did not show any inhibition of growth with the three concentrations of Ekalux (Fig.4.13). Same results were observed with two lower concentrations of Sevin and Nuvacron and also with the lowest dose of Endosulfan. Phorate at all concentrations exerted inhibitory effect to this isolate and were on par with Sevin, Endosulfan and Nuvacron at their inhibitory dosages. The maximum inhibition of growth was noticed with 0.15 per cent Endosulfan followed by the highest concentrations of Phorate and Nuvacron.

Endosulfan at 0.04 per cent was not inhibitory to the native *P. fluorescens* isolate 24 B (Fig.4.14). The higher two concentrations of Endosulfan and the other insecticides at different concentrations exhibited inhibitory effect, which ranged from 7.78 to 12.22 and were on par with each other. The maximum inhibition was recorded with 0.15 per cent Endosulfan and the least with lowest dosage of Phorate, Sevin and Nuvacron.

The lowest two concentrations of Ekalux, Nuvacron (0.04 per cent), Endosulfan (0.04 per cent) and phorate at 1kg ai.ha^{-1} did not show any zone of inhibition on standard culture of *P. fluorescens* (K) (Fig.4.15). Other insecticides tested at varying concentrations and the highest dosage of Ekalux recorded inhibitory effect against this antagonist and effect was on par with each other. The highest

Table 4.19. Compatibility of *Pseudomonas fluorescens* isolates with insecticides

S.No	Insecticides	Concentration (*ppm*)	*P. fluorescens* (23B)		*P. fluorescens* (24B)		*P. fluorescens* Pf (K)	
			Mean diameter of inhibition zone (mm)	Per cent inhibition over control	Mean diameter of inhibition zone (mm)	Per cent inhibition over control	Mean diameter of inhibition zone (mm)	Per cent inhibition over control
1	Sevin	0.05	0 (0.71) [c]	0	7.00 (2.74) [a]	7.78	8.66 (3.03) [a]	9.62
		0.1	0 (0.71) [c]	0	7.33 (2.80) [a]	8.14	10.33 (3.29) [a]	11.48
		0.2	8.33 (2.97) [ab]	9.26	7.66 (2.86) [a]	8.51	11.66 (3.49) [a]	12.96
2	Ekalux	0.05	0 (0.71) [c]	0	7.33 (2.80) [a]	8.14	0 (0.71) [b]	0
		0.1	0 (0.71) [c]	0	7.66 (2.86) [a]	8.51	0 (0.71) [b]	0
		0.2	*0 (0.71)* [c]	*0*	*9.66 (3.19)* [a]	*10.73*	*8.33 (2.97)* [a]	*9.26*
3	Endosulfan	0.04	0 (0.71) [c]	0	0 (0.71) [b]	0	0 (0.71) [b]	0
		0.08	8.66 (3.02) [ab]	9.62	7.33 (2.80) [a]	8.14	9.33 (3.14) [a]	10.37
		0.15	12.00 (3.54) [a]	13.33	11.00 (3.39) [a]	12.22	13.33 (3.72) [a]	14.81
4	Nuvacron	0.04	0 (0.71) [c]	0	7.00 (2.74) [a]	7.78	0 (0.71) [b]	0
		0.08	0 (0.71) [c]	0	7.66 (2.86) [a]	8.51	11.00 (3.40) [a]	12.22
		0.15	9.30 (3.14) [ab]	10.33	9.66 (3.19) [a]	10.73	12.66 (3.63) [a]	14.07
5	Phorate	1 kg a.i ha^{-1}	7.00 (2.74) [b]	7.78	7.00 (2.74) [a]	7.78	0 (0.71) [b]	0
		1.5 kg a.i ha^{-1}	8.00 (2.91) [ab]	8.89	8.66 (3.03) [a]	9.62	7.00 (2.74) [a]	7.78
		2 kg a.i ha^{-1}	9.66 (3.19) [ab]	10.73	8.66 (3.03) [a]	9.62	7.66 (2.86) [a]	8.51
6	Control	-	0 (0.71) [c]	-	0 (0.71) [b]	-	0 (0.71) [b]	-

Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

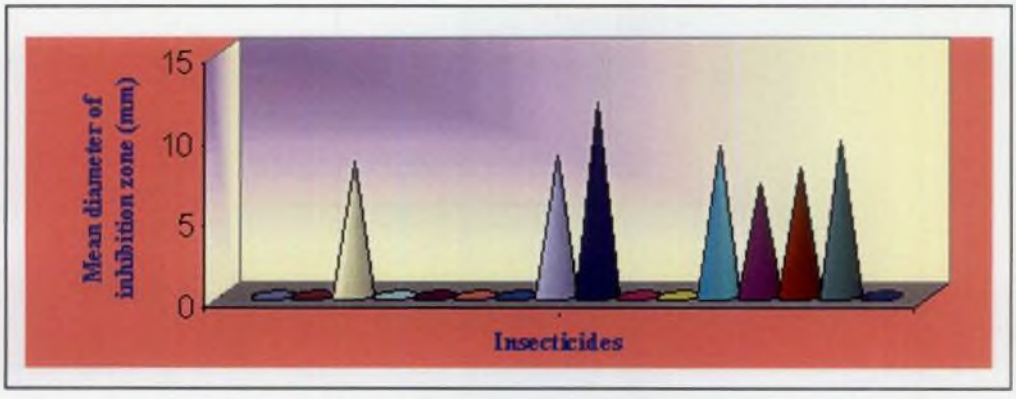


Fig 4.13. Compatibility of *Pseudomonas fluorescens* (23B) with insecticides

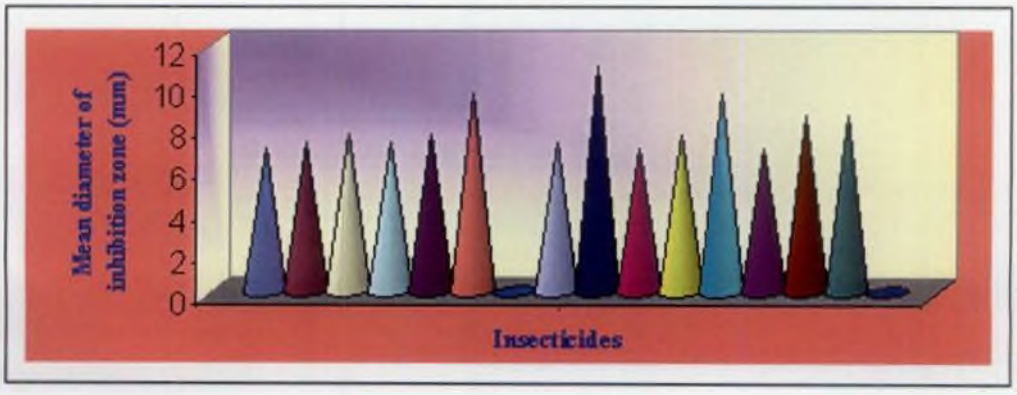


Fig 4.14. Compatibility of *Pseudomonas fluorescens* (24B) with insecticides

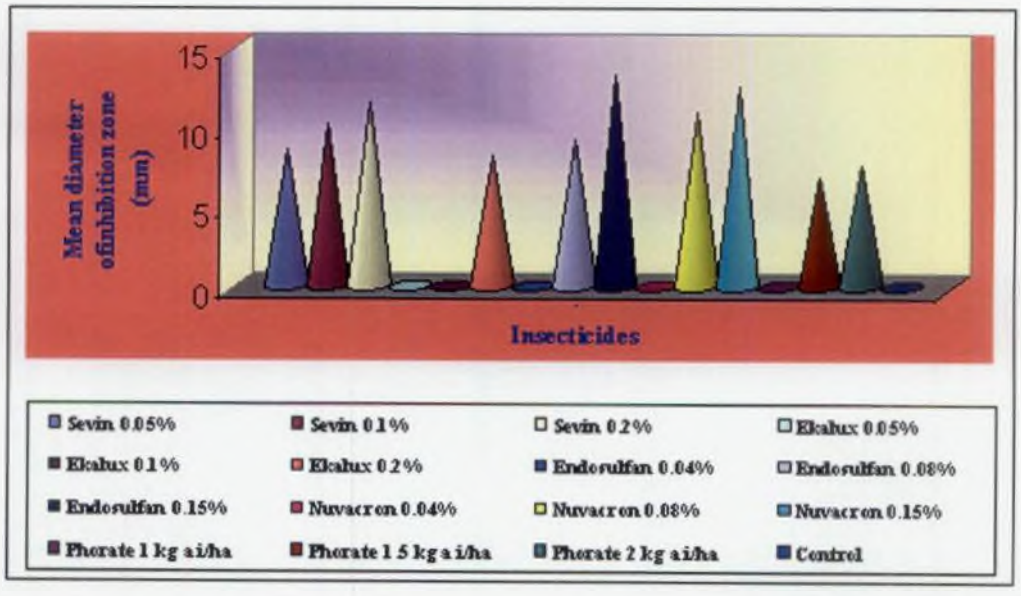


Fig 4.15. Compatibility of *Pseudomonas fluorescens* (K) with insecticides

inhibitory effect was with Endosulfan 0.15 per cent and the lowest with Phorate at 1.5 kg a.i ha^{-1}.

4.11.2.3 Fertilizers

Urea, ammonium chloride, ammonium sulphate, Rajphos and MOP at various concentrations were evaluated for their inhibitory effect against the three bacterial antagonists.

The data presented in Table.4.20 indicated significant difference in the inhibitory effect of fertilizers towards the antagonists. *P. fluorescens* isolate 23 B was compatible with Rajphos and MOP at all concentrations tested. Urea at 1.5 and 2 per cent concentrations exhibited the maximum zone of inhibition (Fig.4.16). This was followed by 3 per cent ammonium chloride. In general, ammonium sulphate recorded comparatively less inhibition.

Rajphos and MOP were not inhibitory to *P. fluorescens* isolate 24 B (Fig.4.17). The remaining fertilizers at varied concentrations recorded inhibition ranging from 8.14 to 12.96 per cent and were on par with each other. Among these the maximum and the minimum per cent inhibition was recorded by 2 per cent urea and ammonium sulphate respectively.

In the case of *P. fluorescens* (K), no inhibition of growth was noticed with lower two concentrations of ammonium sulphate and MOP and with 2 per cent Rajphos (Fig.4.18). All other fertilizers at varied concentrations showed less than 11 per cent inhibition over the control with an inhibition zone ranging from 7.0 to 9.66 mm. However, their inhibitory effect were on par with each other.

Table 4.20. Compatibility of *Pseudomonas fluorescens* isolates with fertilizers

S.No	Fertilizers	Concentration (per cent)	*P. fluorescens* (23B)		*P. fluorescens* (24B)		*P. fluorescens* Pf (K)	
			Mean diameter of inhibition zone (mm)	Per cent inhibition over control	Mean diameter of inhibition zone (mm)	Per cent inhibition over control	Mean diameter of inhibition zone (mm)	Per cent inhibition over control
1	Urea	1.0	7.66 (2.86) ^{bc}	8.51	7.66 (2.86) ^{a}	8.51	8.33 (2.97) ^{a}	9.26
		1.5	18.0 (4.30) ^{a}	20.00	10.33 (3.29) ^{a}	11.48	9.33 (3.13) ^{a}	10.37
		2.0	19.33 (4.45) ^{a}	21.48	11.66 (3.49) ^{a}	12.96	9.33 (3.13) ^{a}	10.37
2	Ammonium chloride	2.0	8.00 (2.92) ^{bc}	8.89	8.00 (2.92) ^{a}	8.89	9.00 (3.08) ^{a}	10.00
		2.5	9.00 (3.08) ^{bc}	10.00	9.33 (3.14) ^{a}	10.37	9.33 (3.13) ^{a}	10.37
		3.0	12.33 (3.58) ^{b}	13.70	10.33 (3.29) ^{a}	11.48	9.66 (3.19) ^{a}	10.73
3	Ammonium sulphate	2.0	7.00 (2.74) ^{c}	7.78	7.33 (2.80) ^{a}	8.14	0 (0.71) ^{b}	0
		2.5	7.33 (2.80) ^{c}	8.14	7.66 (2.86) ^{a}	8.51	0 (0.71) ^{b}	0
		3.0	8.00 (2.92) ^{bc}	8.89	9.00 (3.08) ^{a}	10.00	8.33 (2.97) ^{a}	9.26
4	Rajphos	2.0	0 (0.71) ^{d}	0	0 (0.71) ^{b}	0	0 (0.71) ^{b}	0
		2.5	0 (0.71) ^{d}	0	0 (0.71) ^{b}	0	7.00 (2.74) ^{a}	7.78
		3.0	0 (0.71) ^{d}	0	0 (0.71) ^{b}	0	8.00 (2.92) ^{a}	8.89
5	MOP	2.0	0 (0.71) ^{d}	0	0 (0.71) ^{b}	0	0 (0.71) ^{b}	0
		2.5	0 (0.71) ^{d}	0	0 (0.71) ^{b}	0	0 (0.71) ^{b}	0
		3.0	0 (0.71) ^{d}	0	0 (0.71) ^{b}	0	8.33 (2.97) ^{a}	9.26
6	Control	-	0 (0.71) ^{d}	-	0 (0.71) ^{b}	0	0 (0.71) ^{b}	-

Mean of three replications
In each column figures followed by same letter do not differ significantly according to DMRT
Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

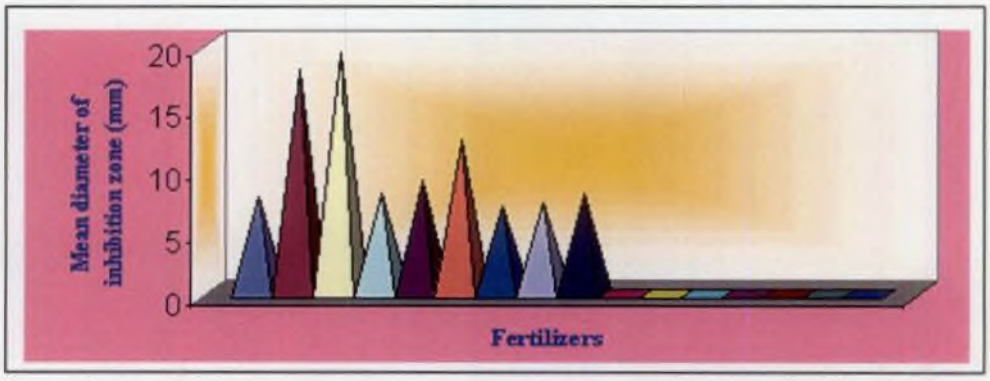


Fig 4.16. Compatibility of *Pseudomonas fluorescens* (23B) with fertilizers

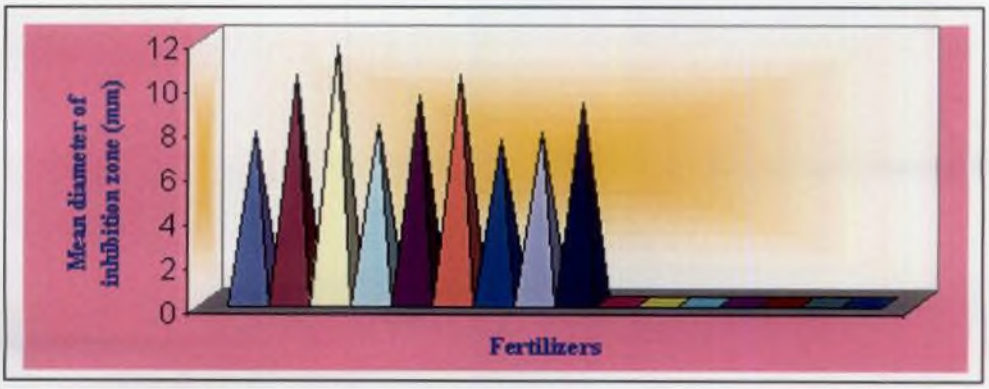


Fig 4.17. Compatibility of *Pseudomonas fluorescens* (24B) with fertilizers

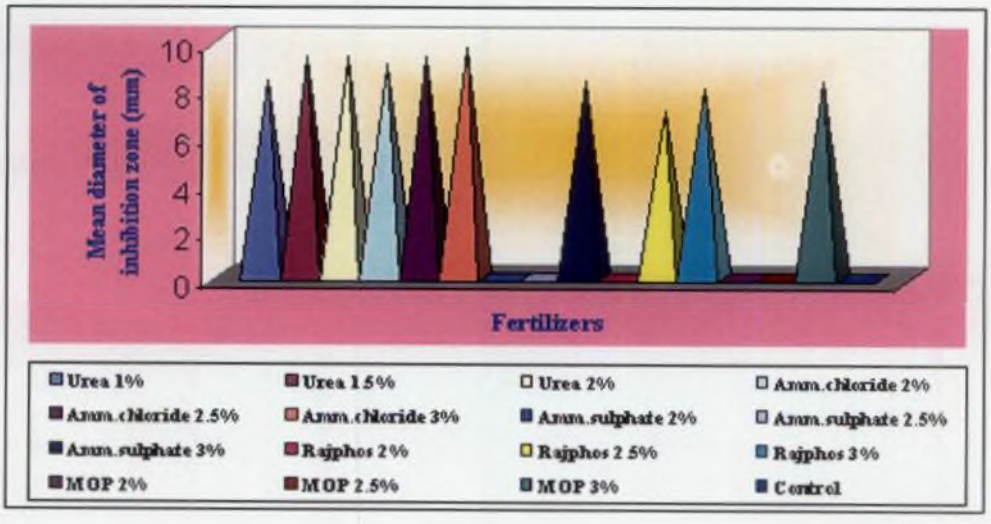


Fig 4.18. Compatibility of *Pseudomonas fluorescens* (K) with fertilizers

4.12 EVALUATION OF FUNGICIDES, INSECTICIDES AND CHEMICAL FERTILIZERS AGAINST *P. palmivora*

The *in vitro* sensitivity of the pathogen, *P. palmivora* to fungicides, insecticides and chemical fertilizers commonly recommended for use in cocoa gardens were tested.

4.12.1 Fungicides

The data on the *in vitro* sensitivity of *P. palmivora* to different fungicides are presented in Table.4.21. There was significant difference among the fungicides at all concentrations tested in inhibiting the pathogen (Fig.4.19). The fungus showed no inhibition with all concentrations of Bavistin tested. Fytolan, Kocide and Bordeaux mixture at all concentrations completely inhibited the growth. Though, the highest concentration of Akomin-40 showed complete inhibition of the pathogen, its two lower concentrations permitted only slight growth and recorded 79.25 and 83.70 per cent efficacy over control. The various concentrations of Indofil M-45 significantly differed in their inhibitory effect with the percentage efficacy over control varied from 54.44 to 72.22.

4.12.2 Insecticides

The pathogen showed significant difference in its *in vitro* sensitivity to the five insecticides tested (Table.4.22).

Nuvacron at all concentrations completely inhibited the growth of the fungus (Fig.4.20). Moreover, 0.08 and 0.15 per cent Endosulfan and 0.2 per cent Ekalux also exhibited complete inhibition of the pathogen while their lower

Table 4.21. *In vitro* sensitivity of *P. palmivora* to fungicides

S.No.	Fungicides	Concentration (per cent)	*P. palmivora*	
			Mean diameter of colony (mm)	Per cent inhibition over control
1	Fytolan	0.2	0 (0.71) [g]	100.0
		0.3	0 (0.71) [g]	100.0
		0.4	0 (0.71) [g]	100.0
2	Kocide	0.1	0 (0.71) [g]	100.0
		0.2	0 (0.71) [g]	100.0
		0.3	0 (0.71) [g]	100.0
3	Indofil M45	0.2	41.00 (6.44) [b]	54.44
		0.3	33.33 (5.82) [c]	62.96
		0.4	25.00 (5.05) [d]	72.22
4	Bavistin	0.05	90.00 (9.51) [a]	0
		0.1	90.00 (9.51) [a]	0
		0.2	90.00 (9.51) [a]	0
5	Bordeaux mixture	0.5	0 (0.71) [g]	100.0
		1.0	0 (0.71) [g]	100.0
		1.5	0 (0.71) [g]	100.0
6	Akomin 40	0.2	18.67 (4.38) [e]	79.25
		0.3	14.67 (3.89) [f]	83.70
		0.4	0 (0.71) [g]	100.0
7	Control	-	90.00 (9.51) [a]	-

Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Table 4.22. *In vitro* sensitivity of *P. palmivora* to insecticides

S.No.	Insecticides	Concentration (per cent)	*P. palmivora*	
			Mean diameter of colony (mm)	Per cent inhibition over control
1	Sevin	0.05	19.00 (4.42) [c]	78.88
		0.1	15.00 (3.94) [f]	83.33
		0.2	11.00 (3.39) [g]	87.77
2	Ekalux	0.05	21.00 (4.64) [e]	76.66
		0.1	14.00 (3.81) [f]	84.44
		0.2	0 (0.71) [h]	100.0
3	Endosulfan	0.04	14.00 (3.81) [f]	84.44
		0.08	0 (0.71) [h]	100.0
		0.15	0 (0.71) [h]	100.0
4	Nuvacron	0.04	0 (0.71) [h]	100.0
		0.08	0 (0.71) [h]	100.0
		0.15	0 (0.71) [h]	100.0
5	Phorate	1 kg a.i ha^{-1}	44.67 (6.72) [b]	50.36
		1.5 kg a.i ha^{-1}	39.00 (6.29) [c]	56.66
		2 kg a.i ha^{-1}	25.33 (5.08) [d]	71.85
6	*Control*	-	90.00 (9.51) [a]	-

Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

Table 4.23. *In vitro* sensitivity of *P. palmivora* to fertilizers

S.No.	Fertilizers	Concentration (Per cent)	*P. palmivora*	
			Mean diameter of colony (mm)	Per cent inhibition over control
1	Urea	1.0	83.67 (9.17) [b]	7.03
		1.5	80.67 (9.01) [bc]	10.36
		2.0	78.33 (8.88) [cd]	12.96
2	Ammonium chloride	2.0	71.67 (8.49) [e]	20.36
		2.5	46.00 (6.82) [j]	48.88
		3.0	40.00 (6.36) [k]	55.55
3	Ammonium sulphate	2.0	61.33 (7.86) [g]	31.85
		2.5	46.67 (6.87) [j]	48.14
		3.0	40.67 (6.42) [k]	54.81
4	Rajphos	2.0	68.00 (8.27) [f]	24.44
		2.5	58.00 (7.65) [h]	35.55
		3.0	51.67 (7.22) [i]	42.58
5	MOP	2.0	82.67 (9.12) [b]	8.14
		2.5	78.00 (8.86) [cd]	13.33
		3.0	76.00 (8.75) [d]	15.55
6	Control	-	90.00 (9.51) [a]	-

Mean of three replications

In each column figures followed by same letter do not differ significantly according to DMRT

Figures in parenthesis are $\sqrt{x+0.5}$ transformed values

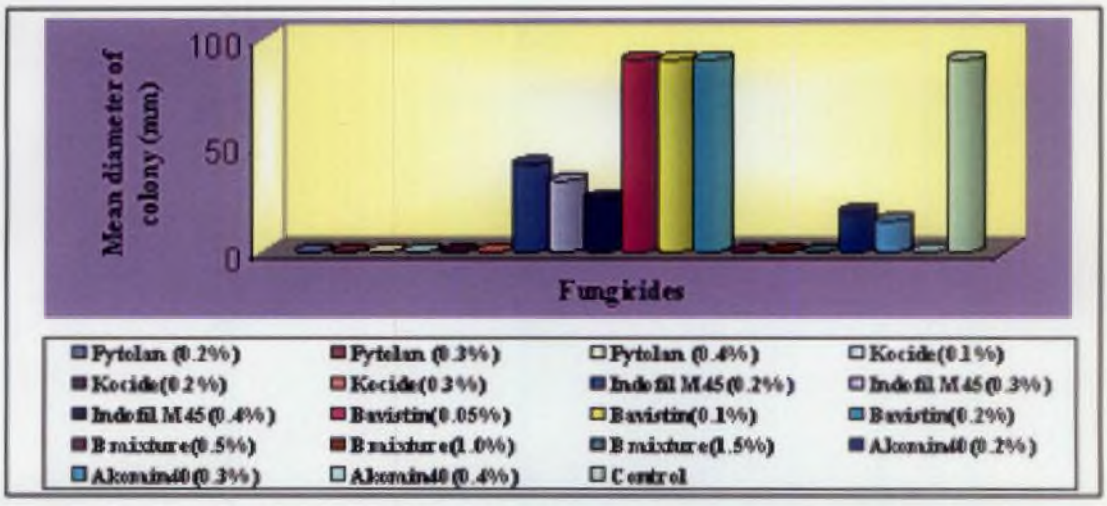


Fig 4.19. Effect of fungicides on *P. palmivora*

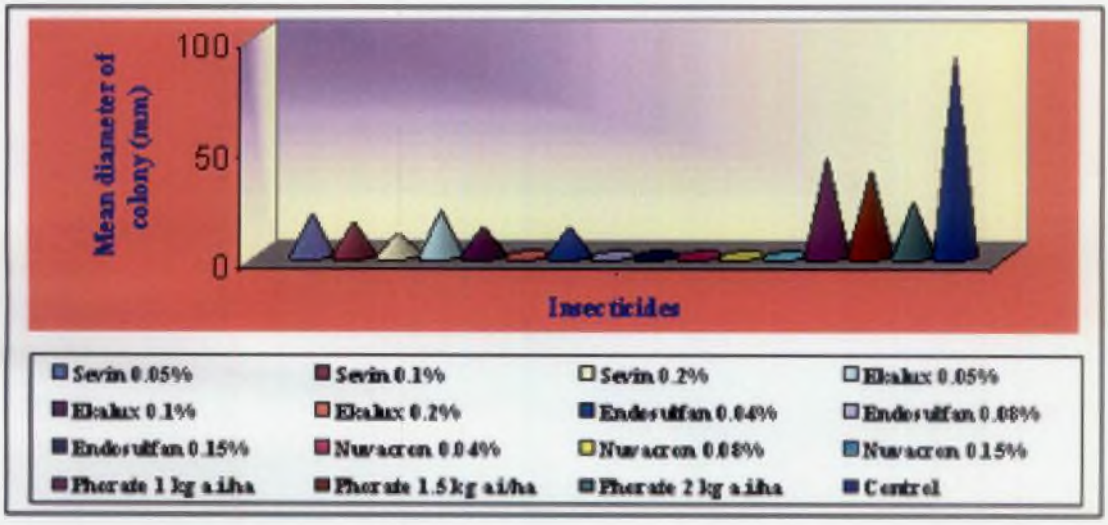


Fig 4.20. Effect of insecticides on *P. palmivora*

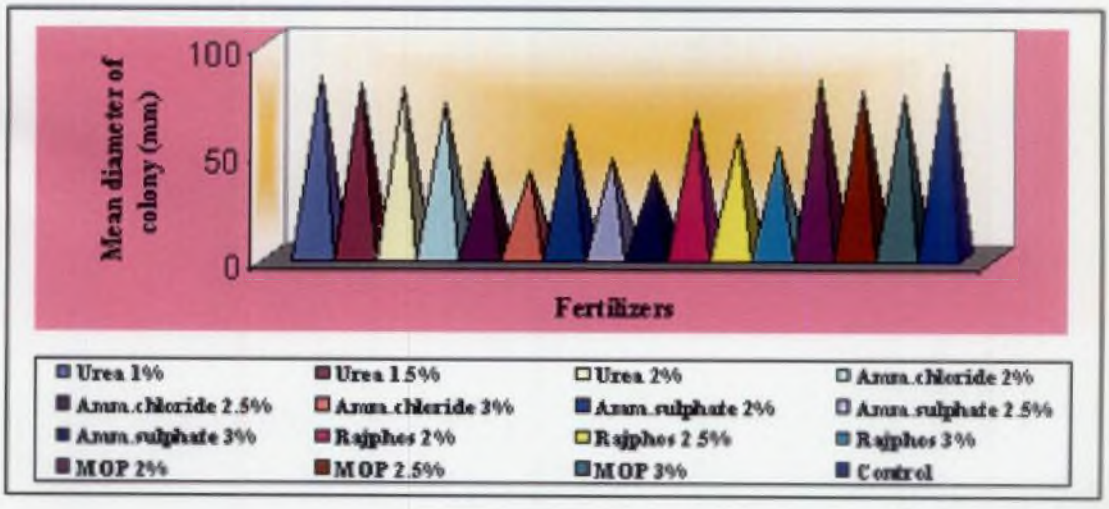


Fig 4.21. Effect of fertilizers on *P. palmivora*

concentrations supported only slight growth. Various concentrations of Sevin also showed higher inhibitory effect. Phorate at different dosages supported comparatively good growth of the pathogen.

4.12.3 Fertilizers

The results furnished in Table.4.23 indicated that the fertilizers tested had a significant effect on the growth of the pathogen.

In general, Urea followed by MOP supported good growth of fungus at the tested concentrations (Fig.4.21) showing only 7.03 to 15.55 per cent inhibition. Though significantly different, the lowest concentrations of ammonium chloride and Rajphos also recorded less inhibitory effect against the pathogen. The higher concentrations of ammonium chloride and Rajphos and all concentrations of ammonium sulphate showed higher inhibitory effect ranging from 31.85 to 55.55 per cent.

4.13 FIELD EVALUATION OF SELECTED ANTAGONISTS AGAINST *Phytophthora* POD ROT

A field experiment was carried out to study the efficacy of three selected epiphytic antagonists *viz.*, *T. viride* 20 F, *P. fluorescens* 23 B and *P. fluorescens* 24 B along with standard cultures of *T. harzianum* and *P. fluorescens* (K) for the management of PPR of cocoa. The experiment was conducted as described in Materials and Methods. Observations on the disease incidence were recorded at weekly intervals for six weeks and the percentage efficacy of the treatments over control worked out.

4.13.1 Effect of various treatments on the incidence of PPR in cocoa garden

Data on disease incidence on the seventh day after first spraying revealed no significant difference among the treatments in reducing the PPR (Table.4.24). The least percentage of disease incidence (1.39) was recorded with the treatment T_6 (*P. fluorescens* 23 B) followed by T_8 (Potassium phosphonate 0.3%). However, the maximum disease incidence of 4.78 per cent was recorded in plants in treatment T_3 (*T. viride* 20 F) followed by T_4 (*T. harzianum*). Analysis of the data on disease incidence on the 15th day after first spraying also revealed no significant difference among the treatments (Table.4.24). The minimum disease incidence was noticed in treatment T_6 (*P. fluorescens* 23 B) followed by T_5 (*P. fluorescens*). Plants in treatment T_3 (*T. viride* 20 F) recorded maximum disease incidence (10 per cent).

Observations on disease incidence recorded during seventh day after second spraying showed significant difference among the treatments (Table.4.25). During the period of observation, the minimum disease incidence (5.13 per cent) was observed in plants in treatment T_6 (*P. fluorescens* 23 B). This was followed by the treatment T_8 (Potassium phosphonate 0.3%) and was on par with treatments T_2 (*P. fluorescens* 24 B), T_5 (*P. fluorescens*) and T_7 (Bordeaux mixture 1%). The maximum percentage of disease incidence was noted with treatment T_4 (*T. harzianum*) and was on par with T_1 (Control) and T_3 (*T. viride* 20 F). Observations on the 15th day of second spraying (Table.4.25) also showed significant difference among the treatments (Fig.4.22). The minimum percentage of disease incidence was noticed in plants in treatment T_6 (*P. fluorescens* 23 B) followed by T_7 (Bordeaux mixture 1%) and were on par with three other treatments. The maximum disease incidence was observed in T_1 (Control) followed by T_4 (*T. harzianum*). Among the various treatments T_2 (*P. fluorescens* 24 B), T_5 (*P. fluorescens*), T_6, T_7, and T_8 (Potassium phosphonate 0.3%) showed more than 50 per cent efficiency over control in checking the disease with the maximum in T_6.

Table 4.24. Effect of various treatments on the incidence of PPR in cocoa garden (After first spraying)

S.No	Treatment	Per cent disease incidence (PDI)			
		7 days after 1st spraying	Per cent efficacy of the treatment over control	15 days after 1st spraying	Per cent efficacy of the treatment over control
1	T_1	4.40 [a]	-	6.71 [a]	-
2	T_2	2.83 [a]	35.68	7.41 [a]	-10.43
3	T_3	4.78 [a]	-8.64	10.0 [a]	-49.03
4	T_4	4.71 [a]	-7.05	8.98 [a]	-33.83
5	T_5	3.03 [a]	31.14	4.77 [a]	28.91
6	T_6	1.39 [a]	68.41	2.61 [a]	61.10
7	T_7	3.16 [a]	28.18	6.53 [a]	2.68
8	T_8	2.07 [a]	52.95	4.92 [a]	26.68

In each column figures followed by same letter do not differ significantly according to DMRT

T_1. Control

T_2. *P. fluorescens* (24B)

T_3. *T. viride* (20F)

T_4 - *T. harzianum*

T_5. *P. fluorescens* (K)

T_6. *P. fluorescens* (23B)

T_7. Bordeaux mixture 1%

T_8. Potassium phosphonate 0.3%

Table 4.25. Effect of various treatments on the incidence of PPR in cocoa garden (After second spraying)

S.No	Treatment	Per cent disease incidence (PDI)			
		7 days after 2nd spraying	Per cent efficacy of the treatment over control	15 days after 2nd spraying	Per cent efficacy of the treatment over control
1	T_1	13.30 ^{ab}	-	25.01 ^{a}	-
2	T_2	9.68 ^{abc}	27.22	12.28 ^{bc}	50.90
3	T_3	13.78 ^{a}	-3.61	17.79 ^{ab}	28.87
4	T_4	14.72 ^{a}	-10.68	23.16 ^{a}	7.40
5	T_5	8.63 ^{abc}	35.09	11.44 ^{bc}	54.26
6	T_6	5.13 ^{c}	61.42	7.84 ^{c}	68.65
7	T_7	8.35 ^{abc}	37.22	9.02 ^{bc}	63.93
8	T_8	6.23 ^{bc}	53.16	11.24 ^{bc}	55.06

In each column figures followed by same letter do not differ significantly according to DMRT

T_1 - Control

T_2 - *P. fluorescens* (24B)

T_3 - *T. viride* (20F)

T_4 - *T. harzianum*

T_5 - *P. fluorescens* (K)

T_6 - *P. fluorescens* (23B)

T_7 - Bordeaux mixture 1%

T_8 - Potassium phosphonate 0.3%

Table 4.26. Effect of various treatments on the incidence of PPR in cocoa garden (After third spraying)

S.No	Treatment	Per cent disease incidence (PDI)			
		7 days after 3rd spraying	Per cent efficacy of the treatment over control	15 days after 3rd spraying	Per cent efficacy of the treatment over control
1	T_1	34.85 [a]	-	43.53 [a]	-
2	T_2	14.67 [cd]	57.91	21.05 [c]	51.64
3	T_3	23.34 [bc]	33.03	27.09 [bc]	37.77
4	T_4	30.48 [ab]	12.54	35.17 [ab]	19.21
5	T_5	15.20 [cd]	56.38	20.66 [c]	52.54
6	T_6	11.62 [d]	66.66	24.03 [bc]	44.80
7	T_7	12.83 [d]	63.19	17.01 [c]	60.92
8	T_8	14.27 [cd]	59.05	18.68 [c]	57.09

In each column figures followed by same letter do not differ significantly according to DMRT

T_1. Control

T_2. *P. fluorescens* (24B)

T_3. *T. viride* (20F)

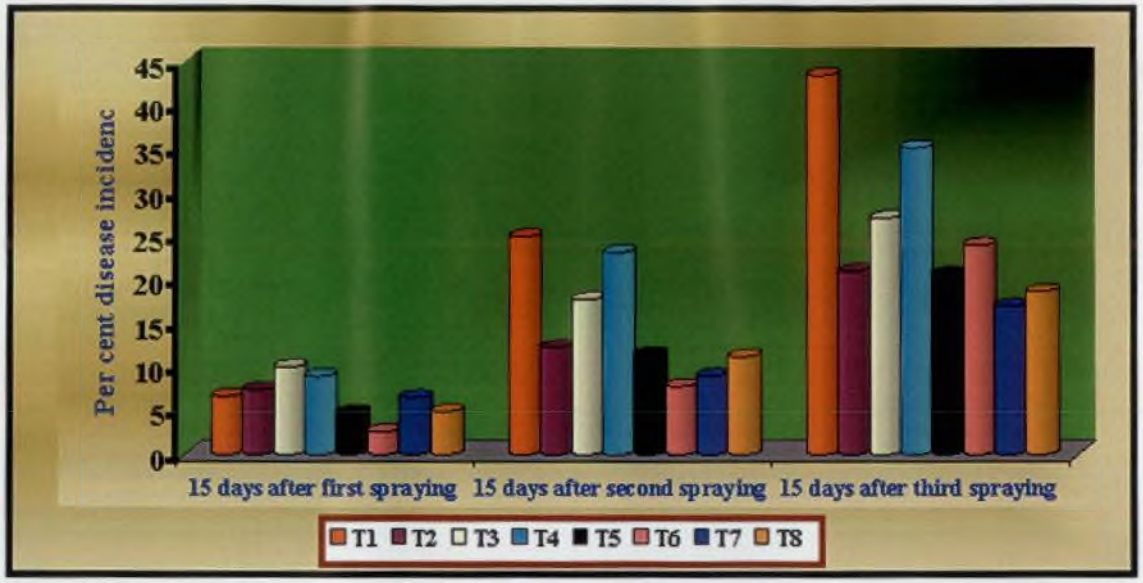
T_4 - *T. harzianum*

T_5. *P. fluorescens* (K)

T_6. *P. fluorescens* (23B)

T_7. Bordeaux mixture 1%

T_8. Potassium phosphonate 0.3%



T 1 - Control, T 2 .-*P. fluorescens* (24B), T 3 .-*T. viride* (20F), T 4 - *T. harzianum*, T 5 .-*P. fluorescens* (K)

T 6 - *P. fluorescens* (23B), T 7 - Bordeaux mixture 1%, T 8 .-Potassium phosphonate 0.3%

Fig 4.22. Effect of various treatments on PPR incidence

On the seventh day after third spraying, significantly less disease incidence was noticed with the treatment T_6 (*P. fluorescens* 23 B) closely followed by T_7 (Bordeaux mixture 1%). However, these were on par with treatment T_2 (*P. fluorescens* 24 B), T_5 [*P. fluorescens* (K)] and T_8 (Potassium phosphonate 0.3%). All other treatments recorded higher per cent disease incidence ranging from 23.34 in T_3 (*T. viride* 20 F) to 34.85 in T_1 (Control). It was noticed that, five treatments (T_2, T_5, T_6, T_7 and T_8) showed more than 50 per cent efficiency over control in checking the disease with the maximum value with the treatment T_6 (*P. fluorescens* 23 B). Analysis of the data on disease incidence on the 15th day after third spraying also revealed significant difference among the treatments (Table.4.26) (Fig.4.22). Here, the minimum disease incidence was noticed in plants in treatment T_7 (Bordeaux mixture 1%) followed by T_8 (Potassium phosphonate 0.3%), T_5 [*P. fluorescens* (K)] and T_2 (*P. fluorescens* 24 B) and these were on par with T_6 (*P. fluorescens* 23 B) and T_3 (*T. viride* 20 F). The maximum per cent disease incidence was observed in T_1 (Control) followed by T_4 (*T. harzianum).* More than 50 per cent efficacy over control in reducing the disease incidence was recorded by treatments T_2, T_5, T_8 and T_7 with the latter showing the maximum efficiency.

Discussion

5. DISCUSSION

Fungal diseases act as one of the major constraints in the cultivation of cocoa in Kerala. Among them, *Phytophthora* pod rot (PPR) is the most serious one, inflicting heavy crop losses. Since, cocoa is grown as a mixed crop, this disease assumes serious proportions during monsoon periods due to obvious reasons, if proper disease management practices are not adopted regularly in time. Successful control of this disease has been noticed with cultural practices like proper shade regulation and pruning coupled with application of chemical fungicides. Bordeaux mixture is widely accepted as an effective fungicide for the control of PPR. Often, improper preparation and untimely application of this fungicide failed to give the desired effect. Further, continuous use of fungicides in cocoa gardens especially in homesteads, may give rise to many ecological problems. To ward off the deleterious effects to the ecosystem due to the use of chemical fungicides, ecofriendly disease management practices utilizing potential microbial antagonists are gaining much importance in recent years. Introduction of a well adapted effective antagonist into the cropping system where cocoa is cultivated, will help in its multiplication and establishment, thereby repeated application can be avoided. With this view, the present study was undertaken to harness the potential of antagonistic epiphytic microflora from cocoa pods for the management of PPR of cocoa. Efforts were also made to evaluate the reaction of cocoa types against the pathogen to locate any resistant/tolerant ones.

Occurrence of PPR of cocoa has been reported from various cocoa growing tracts of the world (Thorold, 1975). In India, the disease was first reported by Ramakrishnan and Thankappan (1965) and the causal organism was identified as *Phytophthora palmivora*. Association of other species of *Phytophthora* like *P. megakarya* (Griffin, 1977), *P. capsici* (Zentmyer, 1988b), *P. citrophthora* (Campello and Luz, 1981), *P. megasperma* (Zentmyer, 1988b), *P. katsurae* (Liyanage and

Wheeler, 1989) and *P. nicotianae* (Tey and Bong, 1990) with causation of this disease were also reported. In India, *P. capsici* and *P. citrophthora* (Chowdappa *et al.*, 1993; Chowdappa and Chandramohanan, 1996) were found in association with the disease in addition to *P. palmivora*. So, in the present investigation, efforts were made to find out the involvement of *Phytophthora* species other than *P. palmivora* in causing pod rot. For this, isolations of the pathogen/s from 15 different locations of cocoa growing tracts of Thrissur district were carried out and established their pathogenicity.

5.1 IDENTIFICATION OF THE PATHOGEN

The cultural and morphological characters of 15 isolates of *Phytophthora* causing pod rot of cocoa were studied in detail. Many workers considered colony morphology on carrot agar medium as a reliable character for differentiating *Phytophthora* spp. (Griffin, 1977; Idosu and Zentmyer, 1978; Brasier and Griffin, 1979; Kellam and Zentmyer, 1986). Isolates of the pathogen showed variation in growth patterns like slightly stellate to striate with fluffy to cottony mycelium on carrot agar medium and took 6-9 days for full growth. The mycelium was hyaline, coenocytic, measuring 3.22-6.45 µm in width. Similar observations were reported by Waterhouse (1974), Prem (1995) and Appiah *et al.* (2003) while studying isolates of *P. palmivora* from cocoa.

Sphorangiophores were distinguishable, indeterminate in growth and measured 42.6-129 µm in length. The young sporangia of the isolates were more or less spherical with less dense protoplasm. At maturity, the protoplasm becomes dense, granular and finally differentiated into zoospores. The sporangia of the fungal isolates were borne terminally in a sympodial fashion, which support the earlier works (Waterhouse, 1963; 1974; Idosu and Zentmyer, 1978; Brasier and Griffin,

1979; Zentmyer, 1988a; Prem, 1995). They considered sporangial ontogeny as criteria for distinguishing *Phytophthora* spp.

Sporangial morphology is considered as another important character for identifying *Phytophthora* spp. (Newhook *et al.*, 1978; Ho, 1981; Brasier *et al.*, 1981; Waterhouse *et al.*, 1983; Stamps *et al.*, 1990). The matured sporangia of the fungal isolates causing PPR of cocoa were spherical to ovoid with round base, papillate and caducous measured 25.8-64.5 x 17.2-38.7 µm with L/B ratio ranging from 1.11 to 2.00. Majority of the isolates recorded L/B ratio of more than 1.5. Thus, the present observations are in conformity with those reported by Griffin (1977), Brasier and Griffin (1979), Prem (1995) and Appiah *et al.* (2003). According to them sporangia of *P. palmivora* causing PPR of cocoa were ellipsoid to ovoid, papillate and caducous with L/B ratio ranging from 1.2-2.2.

The sporangia of isolates of PPR pathogen were caducous, with short thick stalk ranging from 3.15 to 4.3 µm length. The mean pedicel length of most of the isolates were more than 3.5 µm. Based on the pedicel length, Zentmyer *et al.* (1977) and Kaosiri *et al.* (1978) classified *Phytophthora* isolates of cocoa into four groups *viz.*, Group I (<5 µm), Group II (5-15 µm), Group III (>15 µm) and Group IV (non-caducous sporangia). The first three groups correspond to *P. palmivora*, *P. megakarya* and *P. capsici* respectively (Kaosiri *et al.*, 1978; Brasier and Griffin, 1979; Waller, 1981) and Group IV *P. citrophthora* (Campello and Luz, 1981; Kellam and Zentmyer, 1986). It was also reported that pedicel length is a stable character under normal conditions (Waterhouse, 1974). In the present study, pedicel length of the isolates correspond to the original description of Group I of Zentmyer *et al.* (1977) and Kaosiri *et al.* (1978), which was subsequently identified as *P. palmivora* in cocoa (Brasier and Griffin, 1979; Zentmyer, 1988a).

Hence, based on the colony and morphological characters like width of somatic hyphae, sporangiophore length, sporangial morphology, L/B ratio and pedicel length, all the 15 fungal isolates used in the present investigation were identified as *Phytophthora palmivora* (Butler) Butler. However, Chowdappa *et al.* (1993) and Chowdappa and Chandramohanan (1996) observed the association of *P. capsici* and *P. citrophthora* in addition to *P. palmivora* in the causation of PPR of cocoa. Therefore, more studies are required by collecting *Phytophthora* isolates from different agro climatic zones of Kerala for confirmation.

5.2 VARIATION AMONG *Phytophthora* ISOLATES

To find out variations among the isolates of *P. palmivora*, the morphological, virulence, protein profile characteristics of 15 isolates were taken into consideration besides their sensitivity pattern towards fungicides and antibiotics. The different morphological characters of the isolates studied were subjected to cluster analysis. Clustering was done as per UPGMA method of Sneath and Sokal (1973) and the clustering pattern of the isolates revealed a degree of variability. The 15 isolates were grouped under two clusters, A and B. The cluster A consisted of four subclusters and the cluster B with three subclusters. The lowest dissimilarity index (DI) was between isolates C_7 and C_{10}, while the highest with C_7 and C_9. Ho (1982) also observed similar findings while studying the isolates of *Phytophthora* spp. infecting cocoa.

The study on the virulence of the pathogen indicated some differences among the isolates. The isolates C_7 and C_2 were more virulent than the other isolates, whereas isolate Av_1 and C_{10} were less virulent. Such type of difference in the virulence of *Phytophthora* isolates from cocoa was reported by Ho (1982) and Luz and Campello (1985).

Considering the fact that analysis of protein profile is relatively one of the easy means of identifying fungi at species level and even upto sub-species level. The genetic similarity of the 15 isolates was also computed from their protein profiles using Jaccards co-efficient. The UPGMA cluster analysis grouped the 15 *Phytophthora* isolates into a single major cluster A with four subclusters. Cent per cent similarity was observed for isolates C_2, C_5, C_6, C_7, C_{10} and Ka_1 and also between Al_1 and Av_1, which formed separate subculture A_1. Further, the isolate C_3 and Pa_1 were also found to have cent per cent similarity and it formed subcluster A_4. It is pertinent to note that protein profile analysis did not show much variation among the isolates of the pathogen in contrast to that noticed for their morphological and virulence characters. Chowdappa and Sarma (2003) studied the protein profiles of *Phytophthora* spp. and opined that protein banding patterns may be useful in separating morphological species. However, they noticed little variation within the species.

Studies on sensitivity pattern of isolates of *P. palmivora* to fungicides and antibiotics revealed that all isolates were highly sensitive to Bordeaux mixture, Fytolan and Master. Indofil M-45 and Akomin-40 were less inhibitory. The isolates showed slight variation in their sensitivity to Indofil M-45. Isolate Pa_1 and C_{10} were more sensitive to Indofil M-45 than others. The least sensitivity was noticed with isolate C_8, which formed a separate subcluster in protein profile analysis. In Akomin-40 incorporated media, only slight variation in sensitivity was noticed among the isolates. These findings are in agreement with protein profile analysis, which indicated not much variation among isolates.

The isolates showed significant difference in their sensitivity towards antibiotics. Chloramphenicol was more inhibitory than streptomycin sulphate. Among the isolates, Ka_1, Ch_1, C_7, C_8 and C_{10} were more sensitive to chloramphenicol of which Ka_1 showed maximum sensitivity. Remaining ones were comparatively less

sensitive. Isolate Pa_1 was the least sensitive one and it formed a separate subcluster in protein profile analysis. Moreover, isolate Pa_1 was also found to be highly sensitive to streptomycin sulphate. The isolate C_1, C_2 and C_3 were less sensitive compared to other isolates and the least sensitivity was observed with C_3. It is to be noted that the less sensitive ones formed separate subclusters in protein profile analysis. Luz and Campelo (1985) observed difference in sensitivity towards fungicides with different species of *Phytophthora* of which, *P. capsici* was found to be more sensitive towards fungicides tested than *P. palmivora* and *P. citrophthora*. Thus, the investigation revealed that the isolates showed variations with respect to their morphological, virulence and sensitivity towards antibiotics. However, there were not much variations with regard to protein profiles and sensitivity to fungicides. Therefore, further studies using molecular markers, which are useful for characterization of microorganisms should be carried out so as to come to a definite conclusion.

5.3 SCREENING OF COCOA TYPES AGAINST PPR DISEASE

Identification and utilization of host resistance is one of the most effective and practical ways of reducing losses due to plant diseases. This is particularly true for diseases of cocoa like PPR, where it is often difficult to manage the disease during southwest monsoon period. Thus, an investigation was carried out for locating a source of resistance against PPR among the available genetic resources of cocoa maintained in Germplasm VI of Cadbury - KAU Co-operative Cocoa Research Project, College of Horticulture, Vellanikkara.

The study revealed that none of the 225 cocoa types screened against the disease were immune to the disease. However, they reacted differently with the pathogen. Eight cocoa types *viz.*, G VI 14, G VI 73, G VI 77, G VI 124, G VI 138, G VI 154, G VI 279, G VI 284 showed less than 50 per cent pod area infection with the minimum value in G VI 14. Based on the criterion used in this study, these eight

types were categorized as moderately resistant. Between 50-75 per cent pod area infection was observed in 64 cocoa types and they were grouped as moderately susceptible. The remaining 153 types recorded more than 75 per cent pod area infection, thus exhibiting highly susceptible reaction.

Differential reactions of cocoa types towards *P. palmivora* have been reported from different countries. Resistant/moderately resistant reaction of many selections of cocoa like EET 59, EET 376, UF 713, UF 715, SCA 6, SCA 12, Pound 7, Catongo, Diamantes 800 (Asare-Nyako and Amponsah, 1973), DRC 16, SCA 6, SCA 12, IC 6 (Sri-Sukamato and Mawardi, 1986), EET 59, EET 376, SCA 6, DRC 16, C 78 (Lawrence, 1978), K 5, K 6, K 51 (Thrower, 1960), BE 5 and EEG 8 (Pinto *et al.*, 1999) were reported. From India, Chandramohanan (1982) noticed tolerant reaction of a cocoa type C 78 against *P. palmivora*. Abraham *et al.* (2001) screened 166 types and noticed moderately resistant reaction in four cocoa types against PPR. As none of the cocoa types evaluated in the present study showed absolute resistance, the moderately resistant types can be utilized for large scale multiplication and distribution in the endemic areas, provided they possess desirable agronomic traits. Moreover, these types can be used for further genetic improvement following appropriate manipulation tools.

5.4 ISOLATION AND EVALUATION OF EPIPHYTIC ANTAGONISTS

The aerial parts of plants are the habitat of many epiphytic microflora, which may be harmful or beneficial to the plant. Some of these beneficial microflora may be actively antagonistic thereby protecting the plant from invasion by the harmful organisms. But compared to antagonists isolated from soil, aerial antagonists are reported to be less efficient due to obvious reasons inherent in their respective niche. Inspite of this, efforts are being made to exploit the potential of natural epiphytic antagonistic microflora for the management of many plant diseases. With

this aim, the epiphytic microflora associated with healthy pods from heavily infected cocoa gardens were enumerated and studied. On pod surface, the population of bacteria was more than that of fungi and actinomycetes. Fluorescent pseudomonads were isolated only from two locations. Galindo (1992) also observed abundance of bacteria on the cocoa pod surface and concluded that high humidity prevailing in cocoa gardens was responsible for this. Further, the leachates on the surface of pods may have also contributed to the higher number of bacteria.

Based on the cultural characters of isolated epiphytic microbes, representative cultures were selected for further studies. Thus, 16 isolates of fungi, 22 isolates of bacteria, five isolates of actinomycetes and two isolates of fluorescent pseudomonads were selected to study their antagonistic action against *P. palmivora* in comparison with standard cultures of *T. harzianum* and *P. fluorescens* (K). All fungal isolates including *T. harzianum* tested were antagonistic to *P. palmivora* in varying degrees. Among them, eight isolates showed cent per cent inhibition of the pathogen while others recorded intermediary reactions. Majority of the isolates screened, overgrew the pathogen within a short period of time while, the remaining ones showed cessation, homogenous and aversion type of antagonistic action as reported by Purkayastha and Bhattacharya (1982). Such antagonistic nature of fungal isolates obtained from healthy cocoa pods against PPR pathogen of cocoa was noticed by Krauss *et al.* (1998). It is pertinent to note that, five out of eight isolates which exhibited cent per cent inhibition belonged to the genus *Trichoderma*. The potential of *Trichoderma* spp. as an effective biocontrol agent against *P. palmivora* of cocoa was elucidated by many workers (Galindo, 1992; Krauss *et al.*, 1998; Bong *et al.*, 2001; Hoopen *et al.* 2003).

All the bacterial isolates tested including two standard cultures of *P. fluorescens* were found antagonistic to the pathogen. The two standard cultures were more inhibitory than other epiphytic ones. Five epiphytic bacterial isolates consisting

of two fluorescent pseudomonads recorded more than 60 per cent inhibition. None of the actinomycetes were antagonistic to the pathogen. The usefulness of fluorescent pseudomonads against many plant diseases including PPR of cocoa is well established. Attafuah (1965) and Galindo (1992) evaluated the antagonistic nature of epiphytic bacteria against *P. palmivora* from cocoa. They observed that many epiphytic bacteria including *P. fluorescens and P. aeruginosa* were inhibitory to the pathogen.

Based on the results of the above studies, the effectiveness of eight fungal and seven bacterial isolates including standard cultures having maximum inhibitory effect against *P. palmivora* were further evaluated on detached cocoa pods. For this, one hour after application of the antagonist on the pods, the pathogen was inoculated with and without injury. The study revealed that none of the isolates tested were effective in checking the disease incidence. However, it was observed that application of antagonist had an effect in reducing the infection. The antagonists 20F, 23B and 24B exerted more than 40 per cent efficiency over control on uninjured pods. But, with injury only the isolate 23B showed more than 40 per cent efficiency. The standard cultures of antagonists recorded 33-35 per cent efficiency. Effectiveness of epiphytic *P. fluorescens* on detached cocoa pods against *P. palmivora* infection was illustrated by Jimenez *et al.* (1986) and Galindo (1992). Hence, the fungal isolate 20F and bacterial isolates 23B and 24B, which exhibited maximum efficiency in checking the disease on detached pods were selected for field evaluation.

5.5 IDENTIFICATION OF SELECTED ANTAGONISTS

An attempt was made to characterize and identify the three potential antagonists selected for field evaluation. The cultural and morphological characters of the fungal isolate 20F were studied. The fungus produced whitish colonies, which on maturity turned dark green and emitted typical 'coconut odour'. The mycelium was

septate, smooth walled and hyaline with dendroid conidiophores branching, phialides formed in false or irregular whorls mostly with less than four phialides (8-14 x 2-3 µm) in each whorl, phialospores globose or short obovoid, 3-5 µm with minute spines on walls. These characters were in confirmity with that reported by Webster (1964) for *Trichoderma viride* and hence, this isolate was identified as *Trichoderma viride* Pers. ex. S.F. Gray.

Bacterial antagonists 23B and 24B were identified based on cultural and biochemical characters. The isolates were Gram negative short rods, produced greenish yellow fluorescent pigments, grew at 4°C but failed to grow at 41°C They were positive in catalase activity, oxidase test, levan production, starch hydrolysis, urease activity, arginine hydrolase and nitrate reduction reaction. These characters were almost in agreement with that reported for *P. fluorescens*. Accordingly, the bacterial antagonists 23B and 24B were tentatively identified as *Pseudomonas fluorescens* Pelleroni.

5.6 MECHANISM OF ANTAGONISM

The antagonistic organisms bring out action on the pathogen by various mechanisms. Accordingly, studies were conducted to understand some of the mechanisms of antagonistic action of the selected epiphytic antagonists and standard cultures against *P. palmivora*. *Trichoderma* spp. are known to exert many diversified mechanisms of antagonism like mycoparasitism, production of antifungal metabolites, cell wall degrading enzymes as reported by Dennis and Webster (1971), Sivasithamparam and Ghisalberi (1998), Claydon *et al.* (1987) and Ridout *et al.* (1986). They are also known to produce large quanities of fungistatic metabolites such as trichodermin, dermin, trichoviridin and trichobrachin (Yomono *et al.*, 1970; Bruckner *et al.*, 1990), which are active against many soil borne plant pathogens. In the present study, selected *T. viride* and standard *T. harzianum* overgrew the

pathogen and showed interactions like coiling, penetration and disintegration of the host hyphae, which ultimately led to death of the pathogen. Such type of antagonistic reactions was reported by D'Ercole *et al.* (1993) and Vijayaraghavan (2003) with *Trichoderma* spp. on various plant pathogens including *Phytophthora* spp. It was observed that the fungal antagonists were fast growing and overgrew the slow growing *P. palmivora* colony within four days and this type of growth of *Trichoderma* was comparable to that reported by Mukherjee *et al.* (1989) and Bhai (2000) on pathogens that belonged to Pythiaceae.

Similarly, numerous modes of action have been postulated and demonstrated for the antagonistic effects of *P. fluorescens* like competition for nutrients (O'sullivan and O'Gara, 1992), production of cell wall lytic enzymes and many toxic metabolites (Singh *et al.*, 1999). So, the ability of the three *P. fluorescens* isolates {23B, 24B and Pf (K)} in producing HCN and siderophores was assessed. It was found that the isolates produced HCN and siderophores, which is in agreement with the findings of Kloepper *et al.* (1980). They reported that *Pseudomonas* strains supplies essential iron needed for the production of antifungal metabolites proving their ability as biocontrol agents. They also opined that though more than one mechanism may operate in suppressing the pathogens, the relative importance of a particular mechanism may vary with different conditions in the given situation. Thus, more detailed investigations on the various other mechanisms involved in the antagonistic action are needed to be carried out in different situations for better understanding of ways by which the bacterial isolates brings action against the pathogen.

5.7 COMPATIBILITY STUDIES

In any integrated plant disease management programme, its components must be compatible with each other. Otherwise, the desired output of the programme

may not be achieved. This is more pertinent when biocontrol agents are used as one of the components in the integrated disease management strategy. So in the present study, the compatibility of selected *T. viride* (20 F) and standard culture *T. harzianum* with six fungicides recommended for use in cocoa gardens were evaluated. It was found that both the antagonists were compatible with Akomin-40 and Indofil M45 and incompatible with Bordeaux mixture, Kocide and Bavistin. Fytolan was partially compatible with both the antagonists. These findings are in tune with that by many workers (Mondal *et al.*, 1995; Shanmugham, 1996; Rajan and Sarma, 1997; Sarma and Anandaraj, 1999; Paciulyte *et al.*, 2000; McLean *et al.*, 2001; Vijayaraghavan and Abraham, 2003). Thus, it can be concluded that both the fungal antagonists can be safely incorporated, if necessary, with Akomin-40 and Indofil M 45 in the integrated management of PPR of cocoa. Fytolan can also be used in time of need in the IDM PPR of cocoa, as it showed partial compatibility with antagonists tested.

Insect pests like mealy bugs are problems in cocoa cultivation. Many farmers resort to insecticides application to ward of the insect pests. In this context, the *in vitro* compatibility of insecticides with *T. viride* (20 F) and *T. harzianum* was studied. It revealed that Ekalux, Nuvacron and Endosulfan were incompatible with the antagonists. Similar findings were reported by Vijayaraghavan (2003) while studying the compatibility of antagonistic *Trichoderma* spp. with insecticides. In phorate incorporated media, full growth of antagonists was observed indicating their compatibility. However, Sevin showed partial compatibility. Compatibility of phorate with *Trichoderma harzianum* (Sharma and Mishra, 1995) and *T. viride* (Vijayaraghavan, 2003) was reported. According to Jebakumar *et al.* (2000) Phorate being systemic has no effect on direct contact with *T. harzianum*. Sushir and Pandey (2001) had conducted similar studies and reported that Endosulfan was more toxic even at 50 µl ml^{-1}. Hence, the study point out that Endosulfan, Nuvarcon and Ekalux, that are effective against mealy bugs on cocoa, should not be applied when antagonist were used for PPR management.

The compatibility of two *Trichoderma* spp. with fertilizers *viz.*, urea, ammonium chloride, ammonium sulphate, Rajphos and Muriate of potash (MOP) were studied. Ammonium chloride, Rajphos and MOP supported comparatively good growth of antagonists. For the fungal antagonists, ammonium sulphate and urea at all concentrations recorded comparatively higher inhibition. Krishnamoorthy and Bhaskaran (1994a) found an increase in population of *T. viride* due to application of nitrogenous and phosphatic fertilizers. Compatibility of Rajphos to the two species of *Trichoderma* may be attributed to the utilization of phosphorus for growth and sporulation of *Trichoderma*. Moreover, according to Sharma and Mishra (1995) Muriate of Potash was also tolerated by the bioagent. But the compatibility of *Trichoderma* spp. to ammonium chloride may be due to the presence of ammonical form of nitrogen that is more preferred and most favourable for mycelial growth of the antagonists (Neelamegam, 1992). Jayaraj and Ramabhadran (1997) also observed the enhancement of growth and survival of *T. harzianum* with nitrogenous fertilizers. Partial inhibition of the antagonists observed in case of ammonium sulphate may be due to the presence of sulphur in the fertilizer as reported by Sharma and Mishra (1995). They found that zinc sulphate was highly toxic to *T. harzianum* due to presence of sulphur.

The *in vitro* compatibility of fungicides, insecticides and fertilizers to the selected bacterial antagonists (23B and 24B) and standard culture of *P. fluorescens* (K) were also studied. The same plant protection chemicals and fertilizers, which were used for testing the compatibility with the fungal antagonists, were used for this study also. In general, the standard *P. fluorescens* (K) was more sensitive to fungicides than the two native *P. fluorescens*. However, Akomin 40, Indofil M 45 and Bavistin were found compatible with the three bacterial antagonists, though there was some difference in their sensitivity with various concentrations. The variation in response of *P. fluorescens* isolates to the above fungicides observed during the study

might be due to the variation among the isolates of the antagonists. Among the copper fungicides, Bordeaux mixture was more sensitive to the antagonists followed by Kocide and Fytolan and were found incompatible with the bacterial antagonists. Elkins and Lindow (1999) found that mancozeb had no detrimental effect on *P. fluorescens* A 506 when applied at least five days before or after application of antagonist. Hence, due to the detrimental effect, copper fungicides should not be recommended along with antagonists. Alternatively the compatible ones may be incorporated in the IDM of PPR of cocoa.

The *in vitro* compatibility of insecticides with the three bacterial antagonists also revealed more sensitive nature of standard *P. fluorescens* (K) than the other two. The lower two concentrations of Sevin, Ekalux, Nuvacron and Endosulfan were compatible with the bacterial antagonists though, there was some difference in their sensitivity with their concentrations. The highest concentration of these insecticides was incompatible with the antagonists. However, phorate at all concentrations was partially compatible with the antagonists. Thus, the lower concentrations of these insecticides could be recommended for insect control without much adverse effect against bacterial antagonists. Therefore, it is evident that there lies the potential for biocontrol agents to be used along with plant protection chemicals so as to obtain a synergistic action and as components of integrated control packages.

With regard to compatibility of fertilizers with bacterial antagonists, it was found that Rajphos and MOP were compatible with the bacterial antagonists. Among the nitrogenous fertilizers, urea was more inhibitory to the growth of antagonists indicating their incompatibility. Ammonium chloride and ammonium sulphate showed varying levels of inhibition of growth indicating their partial compatibility. Very few studies have been carried out on the compatibility of biocontrol agents with chemicals or fertilizers (Burges and Jones, 1998). Studies elsewhere showed that

there was no obvious detrimental effect of fertilizers and chemicals on survival and establishment of *Serratia entomophila* at least when applied separately to the soil (Townsend *et al.*, 2003).

5.8 INHIBITORY EFFECT AGAINST THE PATHOGEN

Another study was conducted to find out the *in vitro* inhibitory effect of fungicides, insecticides and fertilizers used in cocoa gardens on the growth of *P. palmivora*. The results revealed that among the different fungicides tested, Bordeaux mixture, Fytolan and Kocide at different concentrations completely inhibited the growth. Though, the highest concentration of Akomin-40 showed complete inhibition of the pathogen, its lower two concentrations permitted slight growth. More than 50 per cent inhibition of the pathogen was observed with Indofil M45. Evidently, Bavistin was not at all inhibitory to the pathogen. *In vitro* inhibitory effect of cuprous oxide, copper sulphate, copper oxychloride, copper hydroxide, copper carbonate and potassium phosphonate against *P. palmivora* on cocoa was well documented by many workers (Filani, 1976; Figueiredo and Lellis, 1981; McGregor, 1984; Reddy and Chandramohanan, 1984; Manalo and Tangonan, 1992; Prem, 1995). Further, Reddy and Chandramohanan (1984) and Tey and Wood (1984) also documented the inhibitory effect of mancozeb against *P. palmivora* of cocoa. Thus, it is possible that in addition to Bordeaux mixture, chemicals like potassium phophonate, copper oxy chloride, Kocide and to a certain extent mancozeb, can also be used to control *P. palmivora* of cocoa. However, the field efficacy of these fungicides has to be tested before recommending for large scale application.

All insecticides tested *in vitro* showed inhibitory effect on the pathogen. Nuvacron at all concentrations, the highest concentration of Ekalux and two higher concentrations of Endosulfan recorded complete inhibition. Remaining insecticides at different concentrations exerted inhibition ranging from 50.36 to 87.77 per cent. Such

inhibitory effects of insecticides to *P.capsici* were reported by Vijayaraghavan (2003). As many of these insecticides like Ekalux are regularly used to control insect pests of cocoa, the present investigation revealed that the application of these insecticides had an indirect effect in checking the PPR of cocoa. However, their

istic or any other effect with fungicides need to be assessed before coming to a

bitory effect of common fertilizers against the pathogen was

found that the fertilizers like urea and MOP had not much

inhibitory ... fungus. A partial inhibition of mycelial growth was noticed with ammonium chloride, ammonium sulphate and Rajphos. Less inhibitory effect of urea and MOP might be due to the utilization of N and K by the pathogen. Similar findings have been reported by Cameroon and Milbrath (1965), Pal (1974) and Jain *et al.* (1982) for *Phytophthora* spp. The inhibition of growth of pathogen with ammonium sulphate, Rajphos and ammonium chloride may be due to the effect of sulphur, phosphorous and chlorine present in these fertilizers. Since, the pathogen is soil borne, application of fertilizers with some inhibitory effect, may help in reducing the inoculum level in the soil.

5.9 FIELD EVALUATION OF ANTAGONISTS AGAINST PPR DISEASE

Though, many antagonistic microflora performs well in controlled conditions, they may not be effective in actual field conditions. Several reasons are attributed for the poor performance of such potential antagonists obtained in *in vitro* like poor adaptability in the field due to various environmental and soil conditions. Hence, the promising antagonists selected under controlled conditions have to be tested in the actual field condition to know their potential in managing the disease. This is especially true with epiphytic antagonistic microbes. With this aim, a field experiment was carried out at Cadbury - KAU Co-operative Cocoa Research Project,

College of Horticulture to test the efficacy of selected epiphytic *T. viride* (20F) and two isolates of *P. fluorescens* (23B and 24B). The efficacy of these antagonists was compared with the standard cultures of *T. harzianum* and *P. fluorescens* (K) and two fungicides. The antagonists and fungicides were applied as spray and repeated twice at fortnightly intervals. Observations on disease incidence were recorded at weekly intervals.

It was observed that the disease incidence during the initial two periods of observations did not show significant difference among the treatments. However, the treatments differed significantly during the subsequent periods. Observations on seven and 15 days after first spraying revealed the efficacy of *P. fluorescens* (23 B) (T_6) in checking the incidence of PPR. Observations on seven and 15 days after second spraying also indicated that this treatment was significantly superior showing maximum efficiency over control. On the seventh day after third spraying the same results was observed. It is also to be noted that during these periods of observations application of fungicides and other *P. fluorescens* isolates exerted good effect in reducing the incidence of the disease. However, 15 days after third spraying the treatment T_7 (Bordeaux mixture 1%), T_8 (Potassium phosphonate 0.3%) and T_5 [*P. fluorescens* (K)] in that order recorded the minimum incidence of disease and maximum efficiency over control. Similar to the results obtained in the present study many workers also reported the efficacy of Bordeaux mixture and potassium phosphonate in the management of PPR of cocoa (Menon *et al.*, 1973; Reddy and Chandramohanan, 1984; Sreenivasan *et al.*, 1990; Chandramohanan, 2002). Effectiveness of biocontrol agents like *Trichoderma* spp. and fluorescent pseudomonads against PPR of cocoa was also established (Attafuah, 1965; Jimenez *et al.*, 1986; Galindo, 1992; Krauss *et al.*, 1998; Krauss and Soberanis, 1999; Sharifuddin, 2000; Bong *et al.*, 2001). However, in the present study, the fluorescent pseudomonads especially the native one (*P. fluorescens* 23B) performed well in checking PPR of cocoa, while, the reverse was observed with fungal antagonists. This

may be due to the better colonization of bacterial antagonists on the pod surface and their effect in inducing systemic resistance in the host plant. Poor performance of fungal antagonists may be due their inefficient colonization on pods that were formed on the main stem and branches of the tree under shade. Better colonization of *Trichoderma* on cocoa pods exposed to direct sunlight than those under shade was noticed by Hoopen *et al.* (2003)

Thus, the field experiment clearly revealed the usefulness of antagonistic fluorescent pseudomonads in reducing the PPR of cocoa, the efficacy of which is comparable with that of fungicides. However, the performance of these bioagents in different cocoa growing tracts is to be ascertained before recommending to the farming community as an ecofriendly management practice against the disease.

Summary

6. SUMMARY

Phytophthora pod rot (PPR) is one of the serious diseases of cocoa in Kerala. Though, the disease is amenable to cultural and chemical management practices, excessive use of chemical fungicides often results in many ecological problems. In this context, the present investigation was taken up to harness the potential of epiphytic antagonists from cocoa pods for the management of PPR disease. Further, the ability of different cocoa types in resisting the disease was also evaluated. Fifteen isolates of the pathogen causing PPR of cocoa were isolated from different locations and their pathogenicity established. The cultural and morphological characters of the 15 isolates were studied. On Carrot Agar medium, isolates showed variation in growth pattern and took 6-9 days for full growth. Sporangiophores were indeterminate in growth and measured 42.6-129 µm in length. The sporangia were borne terminally on sporangiophores in a sympodial fashion. The sporangia of isolates were spherical to ovoid with round base, papillate, caducous and measured 25.8-64.5 x 17.2-38.7 µm with *L/B* ratio ranging from 1.11 to 2.00. The pedicel length ranged from 3.15- 4.3µm. Based on these characters, the isolates of the pathogen were identified as *Phytophthora palmivora* (Butler) Butler.

To find out the variation existing among the isolates of *Phytophthora palmivora,* the morphological, virulence and protein profile characteristics of 15 isolates were studied in addition to their sensitivity pattern towards fungicides and antibiotics. Cluster analysis of morphological characteristics revealed a degree of variability among the isolates. They also showed variation in virulence. The isolates C_7 and C_2 were more virulent, and Av_1 and C_{10} less virulent. Analysis of the data on protein profiles of isolates was subjected to cluster analysis, which did not show much variation among the isolates. Regarding the sensitivity pattern of fungicides and antibiotics, it was found that all isolates were highly sensitive to Bordeaux mixture, Fytolan and Master. Indofil M45 and Akomin 40 were less inhibitory and the isolates

showed slight variation in their sensitivity. The isolates did not show much difference in their sensitivity towards streptomycin sulphate. However, with chloramphenicol they showed significant difference in their sensitivity.

None of 225 cocoa types screened against the PPR of cocoa were immune to *P. palmivora*. All cocoa types showed varying percentage of pod area infection. Eight cocoa types *viz.*, G VI 14, G VI 73, G VI 77, G VI 124, G VI 138, G VI 154, G VI 279, G VI 284 showed minimum per cent infection and these were categorized as moderately resistant and 64 types were moderately susceptible and the rest susceptible.

The epiphytic microflora associated with healthy cocoa pods from heavily infected cocoa gardens were isolated and it was observed that bacterial population were more than that of fungal and actinomycetes. Fluorescent pseudomonads were isolated from two locations. From the isolated microflora, 16 fungi, 22 bacteria and five actinomycetes and two fluorescent pseudomonads were selected. The antagonistic effect of these epiphytic microbes against *P. palmivora* was tested by dual culture method in comparison with standard *T. harzianum* and *P. fluorescens*. The study revealed that all fungal and bacterial isolates were antagonistic towards the pathogen in varying degrees. Actinomycetes were not antagonistic. Among the fungal isolates eight showed cent per cent inhibition of the pathogen while others recorded intermediary reactions. Among bacterial isolates, five epiphytic isolates including two pseudomonads showed more than 60 per cent inhibition.

The eight fungal and seven bacterial isolates including standard cultures showing maximum inhibitory effect against *P. palmivora* were further tested on detached cocoa pods. It was observed that none of the isolates tested were absolutely effective in checking the incidence of the disease. However, application of antagonists had an effect in checking the infection. Based on the study, the

antagonists 20F, 23B and 24B, which exerted more than 40 per cent efficiency over control were selected for field evaluation.

The cultural and morphological characters of the efficient fungal isolate (20F) were studied and it was identified as *Trichoderma viride* Pers.ex.S.F.Gray. The efficient bacterial isolates (23B and 24B) were identified as *Pseudomonas fluorescens* Pelleroni.

The mechanism of antagonism of the selected *T. viride* (20F) and *P. fluorescens* (23B and 24B) were studied in comparison with standard *T. harzianum* and *P. fluorescens* (K). Both the *Trichoderma* species overgrew the pathogen and showed interactions like coiling, penetration and disintegration of the host hyphae. Three *P. fluorescens* isolates [23B, 24B and Pf (K)] produced HCN and siderophores.

The compatibility of the selected fungal antagonist *T. viride* (20F) and *T. harzianum* to six fungicides commonly used in cocoa gardens was assessed. It was found that Bordeaux mixture and Kocide were incompatible with the antagonists, while Indofil M-45 and Akomin 40 were compatible. However, Fytolan was partially compatible with the fungal antagonists.

Studies on *in vitro* compatibility of insecticides with above antagonists revealed that Ekalux, Nuvacron and Endosulfan were incompatible, while phorate was compatible. Sevin showed partial compatibility.

The compatibility of two *Trichoderma* spp. with fertilizers *viz.*, urea, ammonium chloride, ammonium sulphate, Rajphos and Muriate of potash were studied. Ammonium chloride, Rajphos and MOP supported good growth of the antagonist. Ammonium sulphate and urea were incompatible with these antagonists.

The compatibility of fungicides, insecticides and fertilizers to the efficient three bacterial antagonists [23B, 24B and Pf (K)] were studied. The standard *P. fluorescens* (K) was more sensitive to fungicides than the two native *P. fluorescens*. Akomin 40, Indofil M-45 and Bavistin were compatible with bacterial antagonists. Among copper fungicides, Bordeaux mixture was more inhibitory to the antagonists followed by Kocide and Fytolan.

With regard to insecticides, the standard *P. fluorescens* (K) was more sensitive than the other two native ones. The lower two concentrations of Sevin, Ekalux, Nuvacron and the Endosulfan were compatible with the bacterial antagonists. However, the higher concentrations of these insecticides were incompatible. Phorate at all concentrations was partially compatible.

With respect to the compatibility of bacterial antagonists with fertilizers, it was found that Rajphos and MOP were compatible with the antagonists. Among the nitrogenous fertilizers, urea was more inhibitory to the growth of antagonists indicating their incompatibility. Ammonium chloride and ammonium sulphate showed varying levels of inhibition of growth indicating their partial compatibility.

Another study was conducted to find out the *in vitro* inhibitory effect of fungicides, insecticides and fertilizers used in cocoa gardens on the growth of *P. palmivora*. Bordeaux mixture, Fytolan and Kocide at different concentrations completely inhibited the growth of pathogen. The lower two concentrations of Akomin 40 permitted slight growth but there was no growth at 0.4 per cent. Only partial inhibition of the pathogen was observed with Indofil M-45. Bavistin was not inhibitory to the pathogen.

All insecticides tested *in vitro* showed inhibitory effect on the pathogen. Nuvacron at all concentrations and the highest concentration of Ekalux and two

higher concentrations of Endosulfan recorded cent per cent inhibition. Remaining insecticides at different concentrations exerted varying levels of inhibition.

The efficacy of common fertilizers applied in cocoa gardens were assessed against the pathogen and it was found that the fertilizers like urea and MOP had no much inhibitory effect on the growth of the fungus. Partial inhibition of the mycelial growth of the fungus was noticed with all concentrations of ammonium chloride, ammonium sulphate and Rajphos.

A field experiment was carried out at Cadbury - KAU Co-operative Cocoa Research Project, College of Horticulture, Vellanikkara to test the efficacy of selected epiphytic *T. viride* (20F) and two isolates of *P. fluorescens* (23B and 24B). The efficacy of these antagonists was compared with the standard cultures of *T. harzianum* and *P. fluorescens* and two recommended chemicals. The antagonists and fungicides were applied as spray starting from second week of July 2004 and repeated twice at fortnightly intervals. Observations on the per cent disease incidence were recorded at weekly intervals.

Results revealed that there was no significant effect among the treatments on the per cent disease incidence during the first two intervals of the observations. However, significant difference was observed during the next four periods of observations. Observations after seven and 15 days of first and second spraying revealed that the application of *P. fluorescens* (23B) was more effective in checking the incidence of PPR compared to other treatments. On the seventh day after third spraying, the same trend was observed. However, on 15 days after third spraying, the treatment T_7 (Bordeaux mixture 1%), T_8 (potassium phosphonate 0.3%) and T_5 [*P. fluorescens* (K)] in that order recorded the minimum incidence of disease and maximum efficiency over control.

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* Originals not seen

Appendices

APPENDIX – I

MEDIA COMPOSITION

(Ingredients per litre)

1. **CARROT AGAR**

Carrot	: 200.0 g
Agar	: 20.0 g

2. **POTATO DEXTROSE AGAR**

Potato	: 200.0 g
Dextrose	: 20.0 g
Agar	: 20.0 g

3. **MARTIN'S ROSE BENGAL STREPTOMYCIN AGAR**

Dextrose	: 10.0g
Peptone	: 5.0 g
KH_2PO_4	: 1.0 g
$MgSO_4$	: 0.5 g
Agar	: 20.0 g
Rose Bengal	: 0.03 g
Streptomycin	: 30 mg

4. NUTRIENT AGAR MEDIUM

Glucose	: 5.0g
Peptone	: 5.0g
Beef extract	:3.0g
NaCl	: 5.0g
Agar	: 20.0g
p^H	: 6.5 to 7.5

5. THORNTON'S STANDARDISED AGAR

Mannitol	: 1.0 g
Asparagine	: 0.5 g
K_2HPO_4	: 1.0 g
KNO_3	: 0.5 g
$MgSO_4$	: 0.2 g
$CaCl_2$	: 0.1 g
NaCl	: 0.1 g
$FeCl_3$. 6 H_2O	: 0.002g
Agar	: 20.0 g
p^H	: 7.4

6. KING'S B MEDIUM

Peptone	: 20.0 g
Glycerol	: 10.0 ml
K_2HPO_4	: 10.0 g
$MgSO_4.7H_2O$	: 1.5 g
Agar	: 20.0 g
pH	: 7.2

7. KEN KNIGHTS AGAR MEDIUM

Dextrose	: 1.0g
KH_2PO_4	: 0.1 g
$NaNO_3$	: 0.1 g
KCl	: 0.1g
$MgSO_4$	: 0.1g
Agar	: 20.0g
p^H	: 7

APPENDIX – II

(Stock solutions used for SDS – PAGE)

1. **Acrylamide stock (30%)**

   Acrylamide : 29.2 g

   Bis- acrylamide : 0.8 g

2. ***Separating (resolving) gel buffer stock***

   1.5 M Tris-HCl : pH 8.8

   (Dissolve 18.45g Tris-base/HCl in 50 ml deionized water. Adjust to pH 8.with

   6N HCl and makeup 100 ml with deionized water and store at 4°C)

3. **Stacking gel buffer stock**

   0.5 M Tris-HCl : pH 6.8

   (Dissolve 6 g Tris-base/HCl in 60 ml deionized water. Adjust to pH 6.8 with

   6N HCl and makeup 100 ml with deionized water and store at 4°C)

4. **Sample buffer (SDS-reducing buffer)**

Deionized water	: 2.6 ml
0.5 M Tris-HCl pH 6.8	: 1.0 ml
2 - Mercaptoethanol	: 0.8 ml
Glycerol	: 1.6 ml
20% (w/v) SDS	: 1.6 ml
0.5% Bromophenol blue (optional)	: 0.4 ml
Total volume	: 8.0 ml

5. Electrode (running) buffer

Tris-base/HCl	: 3.0 g
Glycine	: 41.4 g
SDS	: 1.0 g
Make with deionized water	: 1.0 litre

6. Staining solution

Coomassie brilliant blue (R 250)	: 0.2 g
Methanol	: 30 ml
Acetic acid	: 10 ml
Deionized water	: 60 ml

7. Destaining solution

Methanol	: 30 ml
Acetic acid	: 10 ml
Deionized water	: 60 ml

8. Separating gel (12%) preparation

Deionized water	: 6.7 ml
1.5 M Tris-HCl pH 8.8	: 5.0 ml
10% SDS	: 0.2 ml
Acrylamide stock	: 8.0 ml
Ammonium persulphate (APS)	: 0.1 ml
TEMED	: 0.01 ml
Total volume	: 20.0 ml

9. Stacking gel (4%) preparation

Deionized water	: 6.1 ml
0.5 M Tris-HCl pH 6.8	: 2.5 ml
10% SDS	: 0.1 ml
Acrylamide stock	: 1.3 ml
Ammonium persulphate (APS)	: 0.05 ml
TEMED	: 0.01 ml
Total volume	: 10.0 ml

BIOLOGICAL MANAGEMENT OF *Phytophthora* POD ROT OF COCOA

By

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ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

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Kerala Agricultural University

Department of Plant Pathology

COLLEGE OF HORTICULTURE

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ABSTRACT

Phytophthora pod rot (PPR) is one of the serious diseases of cocoa in Kerala. Fifteen isolates of the pathogen were isolated from different locations of Thrissur district and their pathogenicity established.

The cultural and morphological characters of 15 isolates of the pathogen like growth on Carrot Agar, mycelial width, length of sporangiophore, sporangial ontogeny, length and breadth of sporangia, L/B ratio, and pedicel length were studied. Based on these characters, the different isolates were identified as *Phytophthora palmivora* (Butler) Butler.

To find out the variations among the isolates of *P. palmivora* the morphological, virulence and protein profile characteristics of 15 isolates were studied in addition to their sensitivity towards fungicides and antibiotics. Analysis of data on protein profiles and sensitivity towards fungicides did not show much variation among the isolates. But the isolates showed some variations with regard to their morphology, virulence and sensitivity to antibiotics.

Among 225 cocoa types screened for host resistance, eight cocoa types *viz*., G VI 14, G VI 73, G VI 77, G VI 124, G VI 138, G VI 154, G VI 279, G VI 284 showed moderately resistant reaction. Sixty three types were moderately susceptible and rest highly susceptible.

Quantitative estimation of epiphytic microflora from cocoa pods yielded more bacteria than fungi and actinomycetes. The epiphytic fungi and bacteria showed antagonistic activity against *P. palmivora*. Actinomycetes were not antagonistic to the pathogen. Eight fungal and seven bacterial antagonists including standard cultures of *T. harzianum* and *P. fluorescens* [Pf (K) and Pf (T)] exhibited maximum inhibition on the pathogen. Studies on the detached pods revealed the efficacy of epiphytic fungal isolate 20F and two bacterial isolates

23B and 24B against the pathogen. The efficient fungal isolate 20F was identified as *T. viride* Pers. ex. S.F. Gray and the bacterial isolates 23B and 24B as *P. fluorescens* Pelleroni.

The mechanism of antagonism of epiphytic *T. viride* and standard *T. harzianum* was studied. The antagonists overgrew the pathogen and showed interactions like coiling, penetration, hyphal malformations and disintegration of host hyphae. Three *P. fluorescens* isolates including Pf (K) produced HCN and siderophores.

T. viride (20F) and *T. harzianum* were compatible with Akomin 40 and Indofil M45. They were incompatible with Bordeaux mixture, Bavistin and Kocide. Fytolan was partially compatible. The two fungal antagonists were compatible with insecticide phorate, while Sevin was partially compatible. Ekalux, Nuvacron and Endosulfan were incompatible. Ammonium chloride, Rajphos and MOP were compatible with two *Trichoderma* spp. while ammonium sulphate was incompatible.

Two native *P. fluorescens* (23B and 24B) and standard Pf (K) were compatible with Akomin 40, Indofil M45 and Bavistin and incompatible with Bordeaux mixture, Kocide and Fytolan. With regard to insecticides, the lower two concentrations of Sevin, Ekalux, Endosulfan and Nuvacron were compatible with the bacterial antagonists, while higher concentration was incompatible. Phorate was partially compatible. Fertilizers like Rajphos and MOP were compatible with antagonists. Urea was incompatible. Ammonium sulphate and ammonium chloride were partially compatible.

Among different fungicides screened, Bordeaux mixture, Fytolan and Kocide completely inhibited *P. palmivora*. The lower two concentrations of Akomin 40 and Indofil M45 at all concentrations permitted slight growth. Bavistin was not inhibitory to the fungus. Nuvacron at all concentrations, the

highest concentration of Ekalux and two higher concentrations of Endosulfan were inhibitory to the pathogen. Sevin and phorate exerted varying levels of inhibition. Urea and MOP were not much inhibitory to the pathogen. Ammonium chloride, ammonium sulphate and Rajphos showed partial inhibition.

Field evaluation of isolates of *P. fluorescens* [23B, 24B, Pf (K)] and two *Trichoderma* spp. revealed the efficacy of fluorescent pseudomonads in reducing the incidence of PPR of cocoa. The efficacy was comparable with the recommended fungicides.