FOLIAR FUNGAL PATHOGENS ASSOCIATED WITH YELLOWING DISEASE OF COCONUT

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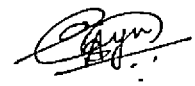
DECLARATION

I hereby declare that this thesis entitled **"Foliar Fungal Pathogens Associated with Yellowing Disease of Coconut"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other university or society.



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LIST OF ABBREVIATIONS

%	per cent
CD	Critical difference
cm	centimeter
cm^2	square centimeter
Fig.	Figure
g	gram
h	hour
ha	hectare
kg	kilogram
l	litre
M	Molar
m	meter
mg	milligram
ml	millilitre
mM	milli molar
mm	millimeter
mm^2	square millimeter
N	Normal
nm	nanometer
°C	degree Celcius
ppm	Parts per million
PR protein	Pathogenesis related protein
var.	Variety
µm	micrometer
µg	microgram

Introduction

1. INTRODUCTION

Coconut palm (*Cocos nucifera* L.) is a versatile tree, popularly known as 'King of Palms' and 'Kalpavriksha', which means "tree that gives all that is necessary for living". It is the most useful tropical tree in the world and is cultivated in more than 92 countries in an area of approximately 12.16 million ha (APCC, 2008) and provides subsistence for more than 10 million people globally. It is grown as a traditional plantation crop in India for the past 3000 years and now is grown in Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Goa, Maharashtra, Gujarat, West Bengal, Orissa, Tripura, and Islands of the Andaman and Nicobar and Lakshadweep with an area of 1.8952 million ha and production of 10824.3 million nuts (Kumar *et al.*, 2011).

In Kerala, coconut occupies first place in the area under cultivation of crops and Kerala ranks top in area (788000 ha) and production (3992 million nuts) of coconut in India (Kumar *et al.*, 2011). However the productivity (5066 nuts ha^{-1}) is below the national average (5711 nuts ha^{-1}) and that of neighboring Tamil Nadu state (9467 nuts ha^{-1}) and Union Territory Pondicherry (9524 nuts ha^{-1}). Serious incidence of pests and diseases, unproductive and senile palms, low use of production inputs, adverse climatic conditions, etc. retards the productivity of coconut and often causes huge losses. Among these the century old non-lethal, debilitating Root (wilt) disease is recognized as the most critical production constraint threatening coconut cultivation in Kerala. The disease was first noticed in the erstwhile princely state of Travancore around 1874 and appeared in a serious manner after the great floods of 1882 in Erattupetta area of Meenachil Taluk in Kottayam District of Kerala (Butler, 1908) and now the disease is reported to be present in all districts of Kerala and many of the adjoining districts of Tamil Nadu and Karnataka, and also from Goa and Orissa (Mishra *et al.*, 1989; Koshy, 1999; Thomas, 2009). The annual loss due to the disease was estimated to be about 968 million nuts (CPCRI, 1985a). Very often leaf

rot disease caused by a complex of fungi, is frequently superimposed on Root (wilt) affected palms and it further devitalizes the already weakened palm. It is presumably estimated that a loss of 461 million nuts is caused due to leaf rot disease alone annually (CPCRI, 1985a).

Recently Yellowing Disease of coconut characterized by rapid mid whorl yellowing, abnormal shedding of immature and mature nuts, flowers, drying of inflorescence and spadix, intense leaf spots or blights on symptomatic leaves, etc. is spreading in Thiruvananthapuram and nearby areas. The palms became barren within few months after the appearance of initial symptoms and the affected palms often die within a short period. The foliar pathogens associated with Yellowing Disease further aggravate the disease greatly. It is not clearly known whether the pathogen associated with Yellowing disease is the same as that of Root (wilt). No elaborate studies have been so far taken up to elucidate the etiology of Yellowing Disease and to compare it with that of Root (wilt) disease. However the palms affected by both these diseases are also found to be affected by a manifold of foliar pathogens. Although several fungal pathogens are reported to be associated with Root (wilt)-leaf rot disease complex, no such studies have so far been taken up to identify the various foliar fungal pathogens found to be attacking the foliage of Yellowing Disease affected palms. Hence the present study was taken up with the following objectives:

- Field survey on the incidence and intensity of Yellowing Disease of coconut in comparison with Root (wilt) disease at the Instructional Farm, College of Agriculture, Vellayani.
- Detailed symptomatology.
- Isolation of foliar fungal pathogens associated with the symptomatic leaves.
- Pure culturing, characterization, identification and testing the pathogenicity of the associated fungal pathogens.

- Biochemical analysis with respect to total sugars, total soluble proteins and phenylalanine ammonia lyase of leaves of the diseased palms in comparison with healthy ones.
- *In vitro* evaluation of efficacy of newer fungicides against major foliar fungal pathogens identified in the study.

Review of literature

2. REVIEW OF LITERATURE

2.1 OCCURRENCE AND DISTRIBUTION OF ROOT (WILT) DISEASE OF COCONUT

Among the devastating diseases of coconut reported from India, Root (Wilt) Disease (RWD) is the most wide spread, debilitating, destructive and age old disease, first noticed in the erstwhile princely state of Travancore around 1874 and appeared in a serious manner after the great floods of 1882 in Erattupetta area of Meenachil Taluk in Kottayam District of Kerala (Butler, 1908; Menon and Pandalai, 1958; Koshy, 1999; Sasikala *et al.*, 2005). By around 1907, the disease was reported from Kaviyoor and Kalloopara area of Tiruvalla Taluk and Kattanam near Kayamkulam of Karthikappally Taluk in Alappuzha district (Varghese, 1934; Koshy, 1999; Sasikala *et al.*, 2005). Since then the disease has spread in all directions from the original foci of infection and now the disease is reported to be present in all districts of Kerala and many of the adjoining districts of Tamil Nadu and Karnataka, and also from Goa and Orissa (Mishra *et al.*, 1989; Koshy, 1999; Thomas, 2009).

The disease was spreading far and wide and by around 1951, it was widely present from Quilon in the south to Malayattoor in the north (Menon and Nair, 1951). The total area affected by the disease was estimated to be around 40,000 ha (Menon and Pandalai, 1958). By 1972 the total area affected by RWD in Kerala was increased to 0.25 million ha and the disease was further found to spread to Thiruvananthapuram and Thrissur districts (Pillai *et al.*, 1973). The malady affected 34 per cent of the area under coconut cultivation causing an annual loss of about 340 million nuts (Pillai *et al.*, 1980). By 1981-82 the disease was observed in certain pockets of Palghat (Radha *et al.*, 1981), Malappuram and Kozhikode districts (Radha, 1983; Jayasankar, 1983). According to a comprehensive survey conducted under the leadership of CPCRI in 1984 - 85, the disease was found to be prevalent in

more or less contiguous manner in 0.41 million ha in eight southern districts of Kerala and in isolated pockets in the districts of Palakkad, Malappuram, Kozhikode and Kannur. The percent disease incidence ranged from 1.5 per cent in Thiruvananthapuram district to a maximum of 75.6 per cent in Kottayam district. The survey further revealed that the annual loss due to the disease was approximately 968 million nuts in Kerala (CPCRI, 1985a). However according to a survey of 1996-97 there was an overall average reduction in disease incidence in Kerala from 32.37 per cent in 1984 to 24.05 per cent in 1996 chiefly due to the removal of diseased palms in the highly infected areas and replanting with quality seedlings. The percentage incidence ranged from 2.09 per cent in Thiruvananthapuram district to 48.03 per cent in Alappuzha district (Anon., 1997). During 1999, root (wilt) affected palms were observed in the East and West Eleri Krishi Bhavan area and Kasaragod in Kasaragod district (Solomon *et al.*, 2001).

One of the recent investigations of Coconut Development Board, Kochi indicated that the incidence and severity of the disease has greatly increased from that of 1996 survey in certain districts of Kerala such as Thiruvananthapuram (2.09 to 26.89 %), Kollam (25.97 to 60 %) and Thrissur (6.19 to 43.95 %) (Anon., 2009).

In Tamil Nadu RWD was first observed during 1971 in Sengottai area on 5 to 6 years old seedlings. Infected coconut trees were observed in certain pockets of Kanyakumari District very soon (SubbaRaja and Ahmed, 1975; Sadanandan *et al.*, 1980). Later the disease was spread to various districts such as Coimbatore, Tirunelveli and Theni (Srinivasan *et al.*, 2000; Srinivasan and Sasikala, 2001; Thomas, 2009; ChandraMohanan, 2010).

Mild to advanced stages of RWD were also reported from Sullia Taluk of Dakshina Kannada District of Karnataka (Sasikala *et al.*, 2005).

Root (wilt) affected palms are predisposed to and 'super infected' with leaf rot pathogens (Varghese, 1934; Radha and Lal, 1968; George and Radha, 1973; Pillai,

1975; Mathai, 1980; Srinivasan, 1991; Srinivasan *et al.*, 1998). The leaf rot disease presumably was in existence in the erstwhile states of Travancore and Cochin since 1880 (Menon, 1935; Menon and Nair, 1948, 1952). Many of the early observations indicated leaf rot disease as a part of root (wilt) disease (Butler, 1908; McRae, 1916, Menon, 1935; Menon and Nair, 1948, 1951, 1952). Leaf rot disease incidence was also noticed in apparently healthy palms, but such palms expressed RWD symptoms subsequently (Radha and Lal, 1968). Incidence of leaf rot disease was severe in RWD infected seedlings in field conditions (CPCRI, 1986). On an average 65 per cent of RWD affected palms were super infected with leaf rot disease (Srinivasan, 1991).

The recently observed yellowing disease (mid whorl yellowing/ quick yellow decline) of coconut which is fast spreading in the southern districts of Kerala had not been recorded as such earlier. However instances of diseased palms having quite healthy outer leaves and yellowed leaves in the inner whorls were noticed during the early investigations of root (wilt) disease (Menon, 1937). While describing the symptomatology of root (wilt) disease it was categorically mentioned that mid whorl yellowing symptom was very much associated with root (wilt) disease on certain infected palms at certain locations (Koshy, 1999; Anithakumari *et al.*, 2003). Satyarajan *et al.* (1988) while describing important diseases of coconut and their management reported about the occurrence of quick yellow decline in certain pockets of Kerala.

Coconut is reported to be affected by similar phytoplasmal maladies in North America, Africa and few other Asian countries. Coconut Lethal Yellowing Disease was first reported from Grand Cayman Islands in the Caribbean region in 1834 (Myrie *et al.*, 2006) and is now considered as the most dreadful coconut disease known to date which killed millions of coconut palms in the Caribbean islands, east coast of North America, Central America, West and East African countries (Seymour *et al.*, 1972; Ashburner *et al.*, 1996; Mpunami *et al.*, 1999; Harries *et al.*, 2001; Roca

de Doyle, 2001; Harrison *et al.*, 2002; Mejía *et al.*, 2004; Oropeza *et al.*, 2005; Martinez, *et al.*, 2007; Foissac and Wilson, 2010). Recently lethal yellowing of coconut was also noticed in the Madang Province in Papua New Guinea in South Pacific Ocean (Kelly *et al.*, 2011).

Lethal yellowing like diseases of coconut were also reported from different tropical countries under a variety of names such as Cape Saint Paul wilt in Ghana (Leather, 1959; Dabek *et al.*, 1976; Nipah *et al.*, 2007), Kain-cope disease in Togo (Nienhaus and Steiner, 1976), Kribi disease in Cameroon (Dollet *et al.*, 1977; Ohler, 1999), Awka wilt in Nigeria (Ekpo and Ojomo 1990; Omamor, 2011), Lethal disease in East African countries and Natuna wilt in South East Asia (Eden-Green, 1997), all of which are causing enormous damage to coconut palms in the respective regions and economic loss to the countries. Recently a 'Kerala wilt' like disease has been reported on coconut from Srilanka which is described as Weligama Coconut Leaf Wilt disease. It is fast spreading from Weligama area in the South to northwards (Pathiraja *et al.*, 2010).

2.2 DISEASE ASSESSMENT

Measurement of disease intensity in quantitative terms is a critical component to have the realistic information on resultant crop losses. George and Radha (1973) developed a scoring system taking into account the symptom expression for quantifying the disease severity of RWD of coconut. The major foliar symptoms associated with the RWD of coconut are flaccidity, yellowing and necrosis. On the basis of their frequency of occurrence and intensity, due weightage and grade points were assigned to each symptom. The percentage of palms showing flaccidity, yellowing and necrosis were rounded to 50, 30 and 20 for adult palms and 75, 15 and 10 for young palms, were taken as the weights for the respective symptom. The grade points assigned to the different symptom vary from 0 to 5 for flaccidity (F), 0 to 3 for yellowing (Y) and 0 to 2 for necrosis (N) according to the intensity and no of

leaflets showing the symptom. Each leaf had to be graded separately for each symptom for working out the disease index of a palm. Disease index (I) for the palm was calculated separately for adult palms and young palms using different formulae. Hence, the method was further simplified by Nambiar and Pillai (1985) by rating the leaves in any of the five spirals in the crown of the palm.

2.3 SYMPTOMATOLOGY

Flaccidity, yellowing and necrosis of leaflets of central and outer whorls of the infected palm are considered to be the typical foliar symptoms of root (wilt) disease (Varghese, 1934; Menon and Nair, 1951; Menon and Pandalai, 1958; Radha and Lal, 1972; Pillai, 1975; SubbaRaja and Ahmed, 1975; Koshy, 1999; Srinivasan *et al.*, 2000; Solomon *et al.*, 2001; Srinivasan and Sasikala, 2001; Sasikala *et al.*, 2005; ChandraMohanan and Peter, 2008). General yellowing and drooping of outer whorl of leaves, sickly pale yellow colour of the inner leaves, curling of leaflets and flaccidity of leaves, shedding of immature nuts, reduction in the number and size of leaves and rotting of roots were also described as the associated symptoms in RWD affected coconut palms (Menon and Nair, 1951). Dwivedi *et al.* (1979) considered softening and whitening of leaflets of the spindle to be the initial symptoms of the disease. In some palms the distal ends of leaves at the fourth or fifth position from the spindle curl (1-1.5 m below the leaf tip), break, hang down, become yellow, dry and drop off (Koshy, 2000; ChandraMohanan, 2010).

A detailed account on the progression of symptoms in diseased palms was provided by various workers. In disease advanced palms, the leaves may shed in quick succession and general growth rate of the palm is retarded. The production of female flowers is curtailed; the spathe becomes much smaller in size accompanied by premature nut fall and the yield gradually reduced. The spathes appear stunted and fail to open normally. The inflorescences may become black, rotten and totally sterile in very severe cases. The quality of the nuts is adversely affected, the husk

gets thinner, the fibers weaker and the shell does not harden. In some cases the kernel or endosperm becomes thinner and turns to rubbery copra when dried (Varghese, 1934; Varkey and Davis, 1960; Pillai, 1975). Shedding of immature nuts even before the appearance of other visual symptoms or after is another important characteristic of the disease (Koshy, 1999).

Some of the earlier workers quoted rotting of roots as the most important symptom associated with RWD (Butler, 1908; Menon and Nair, 1949; Michael, 1964).

In general, the symptoms of RWD develop very slowly so that an affected tree may continue its life for 10 to 15 years, and give reasonable yield by adopting appropriate management practices after the first appearance of symptoms (Pillai, 1975).

Root (wilt) affected palms are predisposed to and superimposed with leaf rot pathogens (Varghese, 1934; Radha and Lal, 1968; George and Radha, 1973; Pillai, 1975; Mathai, 1980; Srinivasan, 1991; Srinivasan *et al.*, 1998). The early symptom of leaf rot disease was found to occur on the unopened spindle leaf as minute water soaked lesion like brown spots at the distal ends and margins of the leaflets or intermittently scattered on the laminar area. The rotting of the affected portion extends into the interior of the spindle resulting in the extensive decay of the upper portions of the spindle (McRae, 1916; Sundararaman, 1925; Varghese, 1934; Menon and Nair, 1948, 1951, 1952; Menon and Pandalai, 1958; Radha and Lal, 1968; Lily, 1981; Srinivasan and Gunasekaran, 1992). Often the rotten leaflets of the spindle cement together. When the leaflets of such spindle gradually unfurled, the rotten portions dried up and blown off by wind and the broken portions and mid-vein became blackish and shriveled while the leaflets of the basal part of the spindle were not infected and opened normally producing the typical 'fan leaf' like symptom (Menon and Nair, 1951; Menon and Pandalai, 1958; Joseph and Rawther, 1991;

Srinivasan, 1991; Koshy, 1999, Vrinda, 2002). Lesions of the disease were also seen on petiole, midrib and mid-veins (CPCRI, 1996; Srinivasan and Gunasekaran, 2000). Leaf rot pathogens generally infect only root (wilt) affected palms (Srinivasan, 1991; Srinivasan and Gunasekaran, 1996a, 2000).

Great difference in symptom expression in certain RWD affected palms was documented by some workers. Sudden appearance of bright yellowing of 3-4 leaves in the middle whorl, followed by the appearance of large number of brown spots of various shapes with a halo around on all leaflets of yellowed leaves was the first symptom of RWD in certain cases. The lesions further coalesced resulting in severe blighting of the leaf lamina (CPCRI, 1985b). In the beginning, yellowing was sometimes restricted from the leaf tip to the middle of the leaf. Shedding of buttons/ immature nuts and inflorescence necrosis were the other prominent symptoms in these palms. These yellowed leaves were dried up faster and shed while leaves of lower whorls remained green. Very bright yellowing and more incidence of mid whorl yellowing were noticed in the variety Chowghat Orange Dwarfs, their segregants and their hybrids (Koshy, 1999; ChandraMohanan, 2010). These palms succumbed to leaf rot and inflorescence necrosis within two months of the appearance of initial symptoms of yellowing, immature nut fall and abnormal button shedding (Koshy, 1999).

On the contrary, general symptoms of lethal yellowing disease of coconut were premature nut fall, necrosis or blackening of inflorescence, chlorosis or yellowing of leaves, defoliation and bud death. The first obvious symptom was premature drop of most or all nuts. Nut fall was followed by floral necrosis, which was most readily observed on newly matured flowers and no fruit was set. At this stage foliar discoloration began to appear. In tall varieties, the older leaves turned yellow, which further progressed inwards until the entire crown was discolored. Sometimes, the discoloration started from the middle leaves of the crown. As leaf yellowing was advanced, the spear leaf collapsed and hung down in the crown. Death of the apical

meristem usually occurred when one-half to two-thirds of the crown were yellowed. Eventually, the entire crown of the palm was withered and toppled, leaving a bare and barren trunk. Infected palms usually died within 3 to 5 months even though in some cultivars it was extended up to two years after the first appearance of symptoms (Plavsic-Banjac *et al.*, 1972; Mpunami, 1999; Harrison *et al.*, 2002; Myrie *et al.*, 2006; Roca *et al.*, 2006; Howard and Harrison, 2007; Nipah *et al.*, 2007; Harrison *et al.*, 2008). Sometimes affected leaves appeared flaccid giving an overall wilted appearance to the palm canopy (Harrison and Elliott, 2008).

2.4 ISOLATION OF FOLIAR FUNGAL PATHOGENS ASSOCIATED WITH LEAF SPOTS ON SYMPTOMATIC LEAVES

In RWD affected palms the natural resistance is broken and the foliage is susceptible to the attack by secondary pathogens such as fungi and bacteria that cause severe leaf rot or leaf blight (Pillai, 1975).

The major foliar diseases of RWD affected palms were caused by fungal pathogens causing leaf rot, leaf spot or leaf blight. Leaf rot disease of coconut was superimposed on more than 65 per cent of the root (wilt) diseased palms and contributed to the rapid decline and reduction in yield of the affected palms (Srinivasan, 1991).

The involvement of microorganisms with leaf rot disease of coconut was thoroughly investigated by several workers (McRae, 1916; Sundararaman, 1925; Varghese, 1934; Menon and Nair, 1948, 1951, 1952; Lily, 1963; Radha and Lal, 1968; Lily, 1981). These investigations identified the involvement of diverse fungal pathogens such as *Helminthosporium halodes, Fusarium* spp., *Gloeosporium* spp., *Gliocladium* spp., *Pestalotia* spp., *Thielaviopsis paradoxa, Rhizoctonia* spp. and *Rhizoctonia solani.* Sathiarajan *et al.* (1988) isolated *Helminthosporium halodes, Diplodia* spp., *Fusarium* spp., *Gloeosporium* spp., *Gliocladium roseum, Pestalotia palmarum* and *T. paradoxa* from leaf rot disease specimen. In leaf rot infected

coconut palms in Orissa *H. halodes, Fusarium* spp., *Gloeosporium* spp., *Gliocladium roseum* and *Rhizoctonia* state of *Macrophomina phaseolina* were found (Mishra *et al.*, 1989).

The complex fungal etiology of leaf rot disease was thoroughly investigated by Srinivasan and Gunasekaran over a period of time. The fungi previously identified as *Helminthosporium* (*Bipolaris*) *halodes, Gloeosporium* spp., and *G. roseum* were re-identified as *Exserohilum rostratum, Colletotrichum gloeosporioides* and *Gliocladium vermoeseni* as per the international standards (Srinivasan and Gunasekaran, 1993, 1994a, 1996b, 1996c). Other fungal pathogens such as *T. paradoxa* (Srinivasan and Gunasekaran, 1993, 1996b; Srinivasan *et al.*, 1995), *Rhizoctonia solani* (Srinivasan and Gunasekaran, 1996b; Srinivasan *et al.*, 1995), *Mortierella elongata* (Srinivasan *et al.*, 1995), *Curvularia* spp., *Acremonium* spp., *Thielavia microspora, T. terricola, Chaetomium brasiliense* (Srinivasan and Gunasekaran, 1994b), *Fusarium solani, F. moniliforme* var. *intermedium* (Srinivasan *et al.*, 1995; Srinivasan and Gunasekaran, 1996b, 1996c, 1999a) and *Cylindrocladium scoparium* (Srinivasan and Gunasekaran, 1995) were also isolated from leaf rot affected spindle leaves. Several fungal pathogens such as *C. gloeosporioides, E. rostratum, G. vermoeseni, F. solani, F. moniliforme* var. *intermedium, T. paradoxa, R. solani, M. elongata, C. scoparium* and *P. palmarum* could be isolated from older leaves as well (Srinivasan *et al.*, 1995). Vrinda (2002) obtained *C. gloeosporioides, E. rostratum, F. solani, F. moniliforme, F. oxysporum, Cephalosporium sacchari, Gliocladium roseum, Scytalidium* spp. and *Curvularia* spp. from leaf rot specimens collected from southern districts of Kerala during three different seasons. Ramesh *et al.* (2004) reported the association of four fungal species *viz. Chalaropsis thielavioides, Botryodiplodia theobromae, Fusarium* spp. and *Pestalotia* spp. on the leaf rot affected spindle leaves of coconut in Goa. The frequent association of more than one fungus in diseased palms confirmed the fungal

complex nature of leaf rot disease (Srinivasan, 2002; Srinivasan *et al.*, 2002; Vrinda, 2002).

In mid-whorl yellowed leaves of coconut palms, *Colletotrichum gloeosporioides* and *Fusarium* spp. were found associated with blighted tissues (Srinivasan and Gunasekaran, 1999b).

Leaf blight or Grey leaf spot is caused by *Pestalotiopsis palmarum* which invades leaf tissues through injuries or when the palms growing under extremely unfavourable conditions. The pathogen was first isolated from decaying coconut leaves in Bengal by Cooke in 1875. The disease was known to occur in Kerala as early as 1925 (Sundararaman, 1925; Varghese, 1934). Later *P. palmarum* was isolated from coconut leaf spots by several workers in different parts of world (Bertus, 1927; Chowdhury, 1946; Ramakrishnan and Subramanian, 1952; Sivaprakasam *et al.*, 1969; Brown, 1975; Francis, 1977; Fernando and Mahindapala, 1977; Obazee and Ikozun, 1985; Ram, 1989; Anupama, 1997; Karthikeyan and Bhaskaran, 1999; Praveena, 1999; Subramanyan, 2003). A leaf spot disease of coconut incited by *Pestalotiopsis guepinii* was reported from Brazil (Anjos *et al.*, 2000; Cardoso *et al.*, 2003).

Occurrence of leaf blight due to *Botryodiplodia theobromae* on coconut have been reported from India and other countries such as Brazil, Mexico, Malaysia and Srilanka (Ram, 1989; Noriega *et al.*, 1991; Warwick *et al.*, 1991, 1993). The pathogen was reported to cause lethal leaf blight of coconut in Coimbatore district in Tamil Nadu (Nakkeeran *et al.*, 1998). During 2006 a new lethal disease was observed in coconut palms in Thanjavur district in Tamil Nadu and the associated fungi include *Botryodiplodia*, *Trichoderma* and *Aspergillus* spp. (Bhaskaran *et al.*, 2007b).

Leaf spots or blights caused by various other pathogens have been reported on coconut such as *Curvularia* leaf spot in root (wilt) affected coconut palms (Radha

and Menon, 1954), *Curvularia maculans* leaf spot in Malaysia (Chan, 1974), *Phomopsis cocoina* leaf spot (Punithalingam, 1975), *Curvularia eragrostidis* leaf spot, *Curvularia tuberculata* leaf spot and blight (Mishra and Singh, 1987), *Alternaria alternata* leaf spot (Rao and Subramanyam, 1975), *A. tenuissima* leaf spot (Mishra, 1987), *Exosporium* leaf spots (Mitra, 1929), *Cercospora apii* leaf spot (Rao and Renukumar, 1989), *Pestalosphaeria elaeidis* leaf blight (Sathiarajan and Govindan, 1989) and *Bipolaris incurvata* leaf blight (Kamalakannan *et al.*, 2006).

2.5 PATHOGENICITY STUDIES OF ISOLATED FUNGAL PATHOGENS FROM COCONUT FOLIAGE

Many of the fungi isolated from leaf rot diseased specimen were found to be pathogenic. *H. halodes, Gloeosporium* spp., *G. roseum* and *Pestalotia* spp. produced typical symptoms associated with leaf rot disease during the pathogenicity trials by Menon and Nair (1948, 1951). Among these the most virulent pathogen was *H. halodes* which produced infection within 12 hours. Subsequent studies by various investigators also confirmed the etiological role of *H. halodes* (*Bipolaris halodes*) (Lily, 1963; Radha and Lal, 1968; Lily, 1981; Sathiarajan *et al.*, 1988; Mishra *et al.*, 1989). Later during the extensive studies on the etiology of leaf rot disease by Srinivasan and Gunasekaran (1996a, 1998, 1999a) it have been proved that *C. gloeosporioides, E. rostratum, G. vermoeseni, F. solani, F. moniliforme* var. *intermedium, C. scoparium, T. paradoxa, R. solani, M. elongata* and *Curvularia* spp. are pathogenic in causing leaf rot disease. In RWD affected palms these fungi produced severe rotting but in healthy palms they produced restricted lesions. *C. gloeosporioides* and *E. rostratum* were aggressive and produced intense lesions on RWD palms (Srinivasan and Gunasekaran, 1996a). Apart from *C. gloeosporioides, E. rostratum, F. solani, F. moniliforme* and *Curvularia* spp., *F. oxysporum, C. sacchari, G. roseum* and *Scytalidium* spp. were found to be pathogenic in causing leaf rot disease (Vrinda, 2002). Leaf rot of coconut was also reported to be caused by *C. thielavioides* and *B. theobromae* in Goa (Ramesh *et al.*, 2004).

Bertus (1927) inoculated *P. palmarum* on wounded coconut leaves and pathogenicity was proved. Subsequently pathogenicity of *P. palmarum* on coconut leaves was confirmed by several workers (Chowdhury, 1946; Sivaprakasam *et al.*, 1969; Brown, 1975; Francis, 1977; Obazee and Ikozun, 1985; Anupama, 1997; Praveena, 1999; Subramanyan, 2003). The pathogenicity of *P. guepinii* on coconut leaves was also confirmed by artificial inoculation (Anjos *et al.*, 2000; Cardoso *et al.*, 2003).

Ram (1989) isolated *P. palmarum* and *B. theobromae* from leaf blight affected coconut palms but pathogenicity was confirmed only for *B. theobromae* under the conditions tested.

2.6 CHARACTERIZATION OF THE ORGANISMS ISOLATED FROM COCONUT FOLIAGE

Menon and Nair (1948) isolated *H. halodes* from leaf rot specimen in Travancore area. The measurement of conidia of this fungus was 36-98 x 10-18 µm. Later Shanmughom (1963) had given the characteristics of the fungus isolated from coconut leaves. On PDA medium color of the colony was yew green with hyaline growing region when young, becoming more or less black with age. The aerial growth consisted of sporophore and spores. The mycelium was hyaline when young, changed to dark brown with aging. Hypha was profusely branched and septate. The conidiophores arised mainly from thicker hyphae were single, straight or curved and flexuous, often prominently geniculate with dark scars on geniculations where the conidia were born on conidiophores. Conidia were borne apically and laterally, younger conidia usually being on the apex. They were born in clusters of 4 to 10 or more, in racemose arrangement. Conidia were straight or curved, cylindrical to elliptical. The immature spores were lighter in color while mature spores were brownish yellow to cocoa brown or deeper. These were mostly with 1 to 17 septa, accentuated end septa and subhyaline end cells and 16.2-198.8 x 6.5-22.7 µm sized.

The acuminate basal end regularly showed a prominent hilum 1.5 to 2 µm long and 2 to 3 µm wide. A short sporophore proliferated from the apex of the mature conidium produced 1 to 10 secondary spores on the geniculations. These were smaller in size and lighter in color.

Srinivasan and Gunasekaran (1994a) re-identified *H. halodes* (=*Bipolaris halodes*) as *E. rostratum* and the characteristics were described. On PDA *E. rostratum* colonies were slow growing, effuse, dark greyish to olivaceous brown, and velvety. Conidiophore was simple, septate, olivaceous brown and geniculate conidia straight or curved, brown to olivaceous, thick walled except in subhyaline region in the apex, hilum protruding from the basal cell, basal septum darker and thicker and upto 18 distoseptate.

E. rostratum colonies on PDA medium were black in colour and appeared as felty growth. Conidiophores were single, brown and measured 26.4-33.0 x 6.6 µm. The conidia were straight, cylindrical, 4 to 9 septate, end cells cut off by a thick dark septa and measured 33-69.3 x 13.2- 19.8 µm (Vrinda, 2002).

Srinivasan and Gunasekaran (1994a) characterized *C. gloeosporioides* isolated from coconut leaves infected with leaf rot. The colonies were fast growing on PDA medium, greyish white with abundant aerial mycelium in concentric rings. The fungus consistently developed fructifications in the medium, acervuli with dark brown long setae. Conidia were hyaline, aseptate, straight to slightly bent, slightly narrower in the middle and abtuse at the apices. *C. gloeosporioides* isolated from leaf rot affected coconut leaves on Czapek's medium was light grey to dark grey or dirty white coloured. The acervuli were spherical to saucer shaped, setose or non-setose. Conidia were mostly cylindrical with rounded ends, aseptate 10.23-14.03 x 3.3-4.01µm sized (Vrinda, 2002).

Colonies of *G. vermoeseni* isolated from leaf rot affected coconut leaves produced whitish colonies on PDA which turned to salmon or pink later. The colony

reverse was olivaceous black on ageing. Conidiophores were hyaline, simple, septate and penicillately branched terminating on phialides. Conidia were hyaline, unicellular, oval to elliptical and pinkish or rose in masses (Srinivasan and Gunasekaran, 1994a).

Vrinda (2002) isolated *G. roseum* from leaf rot specimen. The colonies produced on PDA medium were fast growing, granular and salmon coloured from above. Conidiophores were with divergent verticillium like branches measured 9.9 – 16.5 µm, phialides in whorls of three to four and 9.9 x 3.3 µm in size. Conidia were in columns with slightly asymmetrical apex and obliquely round base.

One of the most predominantly isolated pathogens from coconut leaf rot affected leaves was *F. moniliforme* var. *intermedium* as reported by Srinivasan and Gunasekaran (1999a). The organism was slow growing with dense aerial mycelium, delicately white, vinaceous, and floccose with a powdery appearance and in yellowish pink shades. Microconidia were aseptate, clavate and formed in chains on simple conidiophores. Macroconidia were formed less frequently, 3-5 septate, straight to falcate and the septa usually indistinct. Chlamydospores were absent (Srinivasan and Gunasekaran, 1999a). Vrinda (2002) also characterised *F. moniliforme*. The fungus produced aerial mycelium on PSA medium with pinkish conidial mass turned to dark blue later. Clavate microconidia were produced abundant in chains. Macroconidia were 20.17 x 3.3 µm sized having conical apical cell. Chlamydospores were absent.

Colonies produced by *F. solani* isolated from root (wilt) affected coconut palm were fast growing, aerial mycelium striate, dense, floccose and grayish white. Abundant cylindrical to oval microconidia were produced from lateral phialides. Macroconidia developed from branched conidiophores, inequilterally fusoid, with the widest point above the centre and commonly 1-9 septate. Chlamydospores present were globose to oval and terminal or intercalary (Srinivasan and

Gunasekaran, 1999a). On PSA medium the pathogen produced aerial mycelium with cream coloured conidial mass. Macroconidia were 25.5 x 3.3 µm sized with conical apical cell. Microconidia were rod shaped. Chlamydospores were present in chains (Vrinda, 2002).

Vrinda (2002) isolated *F. oxysporum* from coconut leaf rot specimen which produced light pink colonies with aerial mycelium on PSA medium. The macroconidia were 18.3 x 3.3 µm sized and had conical apical cell. Rod shaped microconidia and chlamydospores were present.

Vrinda (2002) isolated *C. sacchari* from coconut leaf rot specimen. The fungi produced light pinkish coloured colonies on PDA medium. Hyaline, oblong, aseptate conidia (6.6-9.9 x 3.3 µm) were produced from the tip of the ultimate branches and many aggregated to form 'heads' which were easily shed.

Scytalidium spp. isolated from leaf rot specimens by Vrinda (2002) produced fast growing, effuse and black colonies on PDA medium. Some of the hyphae were smooth, narrow, cylindrical and colourless while others were thick, pale to mid-brown with occasional darker swollen cells and often with thick dark septa. The hypahe often laid closely adpressed forming bundles. Setae and hyphopodia were absent. Conidiophores were micronematous, mononematous or sometimes synnematous, branched or unbranched, straight or flexuous, colourless or brown and smooth. Conidiogenous cells were fragmented and formed arthroconidia which were integrated, intercalary, determinate and cylindrical, dolliform or ellipsoidal. Conidia were catenate, separating, simple, smooth and aseptate. The colourless thin walled cylindrical conidia were 9.9-13.2 x 3.3-6.6 µm sized while the broader, dark brown, thick walled, oblong conidia were 9.9 x 5-6.6 µm sized.

Colonies of *Curvularia* spp. isolated from leaf rot affected coconut palms were black, velvety, conidia four celled and measured 19.8-29.01 x 9.9-13.2 µm, middle cell large, broad and dark (Vrinda, 2002).

Francis (1977) isolated *P. palmarum* from grey leaf spot affected coconut leaves. The mycelium produced on PDA medium was cottony white when young, turning pale yellow on aging and distinct zonations present. The spore was 20.90-41.50 x 8.75-10.50 µm sized and had 10.50-35.50 µm long appendages. Similar morphological characters of the colony were also given by Subramanyan (2003). The conidia of the fungus were five celled, the intermediate cells constricted at the dividing septa, upper and lower cells hyaline, while the middle cells were olive green in colour. The upper cell had three long slender colourless simple appendages. The size of the spore was in the range 27.21-30.14 x 6.31-7.54 µm and the appendage was 10.13-12.97 µm long (Subramanyan, 2003).

Lasiodiplodia theobromae causing post-harvest basal rots of coconut was characterized by Viana *et al.* (2002). The young pycnidia were with hyaline, unicellular, conidia having thin double cell wall which became dark and glandular when ripe measuring about 24.5 x 13.0 µm.

L. theobromae isolates from Eriophyid mite infested coconuts showed marked variation in their morphological characters. The colony colour varied between dark grey, greyish black and white. Majority of the isolates of dark grey and greyish black type were exhibited good to fast growth and abundant sporulation. All white type isolates were good to moderately fast in growth with very poor sporulation. The size of the conidia of dark grey type was in the range 19.98 - 26.64 x 9.99 - 13.32 µm, greyish black type was in the range 19.98 -24.97 x 9.99 - 13.22 µm and that of white type was 11.65 - 19.98 x 6.66 – 9.99 µm (Venugopal *et al.*, 2008).

2.7 BIOCHEMICAL ANALYSIS

Phytoplasmas affected phloem function, impairing carbohydrate translocation and subsequently causing the accumulation of soluble sugars in source leaves (Lepka *et al.*, 1999). They found higher levels of reducing sugars and sucrose in source leaves of infected plants than in healthy ones. In roots, concentration of sugars was

low and seemed not to be affected by the phytoplasma infection. Sucrose levels appeared to be similar to those of healthy plants, but variations, depending on the virulence of the phytoplasma isolate and the host/ phytoplasma association, were reported.

Mathew (1977) analysed the carbohydrate content of the root (wilt) affected palms and showed that total, reducing and non-reducing sugars were significantly higher in the leaves of infected palms. He inferred this as due to impaired translocation to the roots in affected palms. In the case of lethal yellowing affected coconut palms also the total sugars were higher in the leaves compared to that of healthy palms. Concentration of total sugars increased slowly in the recently expanded leaves of coconut palms with the development of the disease before decreasing at the later stages of lethal yellowing. In the intermediate leaves sugar concentrations increased more rapidly with the advance of the disease before decreasing in the later stages (Maust *et al.*, 2003). The total sugars in the leaf rot affected tender leaves were higher than that of the healthy leaves (CPCRI, 1981, 1982). After inoculation with *P. palmarum* the total sugar contents in coconut leaves were found to be augmented up to eight days and subsequently the levels decreased (Karthikeyan and Bhaskaran, 1997). Subramanyan (2003) also observed an increased sugar content in *P. palmarum* infected leaves.

Usually plants infected by pathogens show high protein content (Agrios, 1997). An increase in the total amount of proteins has been found in maize bushy stunt phytoplasma infected maize plants (Junqueira *et al.*, 2004). In particular, resistant hybrids accumulated higher protein content than susceptible ones, supporting the hypothesis that accumulation of PR-proteins contributed to the increase of total proteins in infected tissues. Contradictory results have been obtained in maize plants infected with different Mollicutes, in tomato plants affected by stolbur phytoplasma (Favali *et al.*, 2001), in grapevine affected by bois noir phytoplasma (Bertamini *et al.*, 2002b) and in apple trees affected by apple

proliferation (Bertamini *et al.*, 2002a), where a decrease in total soluble proteins has been observed.

A decrease in protein fractions was observed in the leaf tissues of root (wilt) affected coconut palms (Pillai and Shanta, 1965). The soluble protein content was adversely affected in Thanjavur wilt diseased coconut palms in the east coast region of Tamil Nadu (Vijayaraghavan and Ramachandran, 1988). The leaf protein content reduced in lethal yellowing affected palms from the foliar discolouration phase. At this stage the protein content dropped to 45 per cent of its original level (Leon *et al.*, 1996).

An altered phenol metabolism is generally characteristic of all plant diseases. Romanazzi and Landi (2011) reported that grapevines, both affected by bois noir and recovered from the disease, showed an upregulation of phenylalanine ammonia-lyase (PAL), as compared with healthy plants.

The levels of phenylalanine ammonia lyase was higher in root (wilt) affected coconut palms (Joseph and Jayasankar, 1980). The lowest PAL activity was recorded in palms having zero disease indexes. PAL activity increased from healthy to apparently healthy and then it decreased with increase in intensity of the disease. However in diseased palms the PAL activity was markedly higher than that of healthy palms (Joseph, 1983). An increase in enzyme activity was noticed immediately after contraction of disease and in the later stages of the disease the levels of enzyme activity were decreased but significantly higher than the levels found in healthy palms/ seedlings (Mayilvaganan and Jacob, 2008).

There was significant increase in PAL activity in coconut roots infected with *Ganoderma lucidum* after treatment with biocontrol agents *Pseudomonas fluorescens*, *Trichoderma viride* and *T. harzianum* and also with application of chitin (Karthikeyan *et al.*, 2006).

2.8 *IN VITRO* EVALUATION OF FUNGICIDES AGAINST FUNGAL PATHOGENS ISOLATED FROM COCONUT LEAVES

In an earlier work by Menon and Nair (1951) copper sulphate, mercuric chloride and phenol were tested against leaf rot fungi among which mercuric chloride and phenol were found to be lethal at various concentrations tested. Later Bordeaux mixture (1%), Fungimar copper (0.3 %) and Kirti copper (0.5%) were tested against the leaf rot pathogen, *H. halodes* and complete inhibition of mycelial growth was obtained (Prasannakumari *et al.*, 1960). Srinivasan and Gunasekaran (1998) tested Indofil M-45, Fytolan, Captan, Thiram, Contaf, Calixin and Aureofungin-Sol against *C. gloeosporioides*, *E. rostratum*, *G. vermoeseni*, *F. solani*, *F. moniliforme* and *T. paradoxa* by poisoned food technique out of which Contaf (hexaconazole) completely inhibited the growth of pathogens, Indofil M-45 completely inhibited *E. rostartum* while Fytolan caused 100 per cent suppression of growth of *C. gloeosporioides*. Vrinda (2002) tested five different fungicides *viz.*, copper oxychloride, mancozeb, carbendazim, hexaconazole and propiconazole against the major leaf rot pathogens such as *C. gloeosporioides*, *E. rostratum* and *F. solani* and found that hexaconazole and propiconazole completely inhibited the growth of all pathogens while carbendazim produced 100 per cent inhibition of growth of *C. gloeosporioides* and *F. solani*.

Kudalkar *et al.* (1991) studied the effect of Bavistin, Dithane M-45, Dithane Z-78, Blitox, Fytolan and Sulfex against *P. palmarum* causing grey blight of coconut and found that carbendazim (Bavistin) was the most effective (100% inhibition) fungicide among the different fungicides tested. Mancozeb (Dithane) was also found effective in this study which inhibited growth by 78-86 per cent. In another study eleven fungicides were screened against *P. palmarum*, and the mycelial growth of the fungus was completely inhibited by carbendazim, thiophanate-methyl and tridemorph at all concentration tried (Selvan *et al.*, 1993). Karthikeyan and Bhaskaran (1998) conducted an *in vitro* evaluation of twelve fungicides against *P.*

palmarum and found that carbendazim, thiophanate methyl and mancozeb completely inhibited the mycelial growth of the fungus at 500 ppm concentration and the fungicides thiram (1000 ppm), tridemorph (3000 ppm) and ziram (3000 ppm) were also effective in suppressing the growth of the fungus. Further *in vitro* experiments consisting of 9 different fungicides *viz.*, carbendazim, difenconazole, hexaconazole, propiconazole, triademefon, tridemorph, mancozeb, copper oxychloride and chlorothalonil was conducted to evaluate their efficacy against *P. palmarum* and found that mycelial growth of the fungus was completely inhibited by mancozeb (0.2 % and 0.3 %) while propiconazole gave maximum mycelial growth inhibition (97.2%) among the different systemic fungicides tested (Praveena and Kachapur, 2002).

Field evaluation of fungicides was conducted against *Lasiodiplodia theobromae* causing leaf blight of coconut in Brazil in which the efficacy of cyproconazole (40 ml/l of water), difenoconazole (20 ml/20 l); propiconazole (40 ml/20 l) and tebuconazole (40 ml/20 l) were evaluated. The maximum control of leaf blight was obtained with tebuconazole, reducing the disease incidence by 37 per cent, while the other fungicides had little effects (Warwick and Abakerli, 2001). Laboratory screening of six fungicides *viz.*, tridemorph, carbendazim, hexaconazole, propiconazole, copper oxychloride and mancozeb was performed against *Lasiodiplodia theobromae* causing lethal leaf blight of coconut in Tamil Nadu. The fungicides hexaconazole and propiconazole gave 100 per cent inhibition of mycelial growth at 100 ppm while carbendazim, tridemorph and copper oxychloride gave 100 per cent inhibition at 500 ppm and mancozeb gave 100 per cent inhibition at 1000 ppm (Bhaskaran *et al.*, 2007a).

Materials and methods

3. MATERIALS AND METHODS

3.1 SURVEY

A detailed survey was conducted to study the extent of incidence and intensity of Root (Wilt) Disease (RWD) and Yellowing Disease (mid whorl yellowing) of coconut at the Instructional Farm, College of Agriculture, Vellayani. Incidence and intensity of the disease on the palms were recorded in all the six blocks of the Farm.

3.1.1 Disease incidence

The per cent disease incidence was calculated by recording the number of palms showing symptoms of root (wilt) and Yellowing Disease (mid whorl yellowing) out of the total number of palms in each block.

3.1.2 Disease intensity

The disease intensity of RWD affected palms was calculated by estimating the disease index using the simplified method described by Nambiar and Pillai (1985). According to this method one spiral having five leaves at different developmental stages was chosen for assessing the disease index of RWD affected coconut palms. Flaccidity, yellowing and necrosis were identified as the prominent symptoms for calculating the disease index.

For flaccidity, the score values varying from zero to five were assigned based on the extent of the flaccidity and the per cent of leaflets in a particular leaf showing the symptom. The following were the score values assigned -

0 - No disease

1 - Less than 20 per cent of leaflets of a leaf showing the symptom

2 - 20 to 40 per cent of leaflets of a leaf showing the symptom

3 - 40 to 60 per cent of leaflets of a leaf showing the symptom

4 - 60 to 80 per cent of leaflets of a leaf showing the symptom

5 - Above 80 per cent of leaflets of a leaf showing the symptom

For yellowing, the score values varying from zero to three were assigned based on the intensity of yellowing and the number of leaflets in a particular leaf showing the symptom. The score values assigned were as follows -

0 - No disease

1 – Less than one third of leaflets showing yellowing

2 - One third to two third of leaflets showing yellowing

3 - Above two third of leaflets showing yellowing

For necrosis, the score values varying from zero to two were assigned based on the intensity of necrosis and the per cent of leaflets in a particular leaf showing the symptom. The following score values were assigned -

0 - No necrosis

1 - Less than 50 per cent leaflets showing necrosis

2 - More than 50 per cent leaflets showing necrosis

Each leaf was graded separately for flaccidity, yellowing and necrosis. Disease index (DI) for the palm was worked out using the formula,

$$DI = \frac{\text{Sum } (F+Y+N)}{L} \times 10$$

where 'F', 'Y' and 'N' are the grade points assigned to a leaf for flaccidity, yellowing and necrosis and 'L' indicates total number of leaves in one spiral of the palm.

The disease index varies from zero to 100, where zero represents the total absence of all the symptoms indicating that the palm is healthy and 100 means the presence of all the symptoms in the most acute stage on all the leaves. If the disease index is below 20, the palm is categorized as at mild stage of infection,

between 20 and 50, at moderate stage of infection and above 50, at advanced stage of infection.

For palms showing mid whorl yellowing symptoms a modified scale was evolved, since the symptom expression is drastically different. Unlike Root (Wilt) affected palms, here flaccidity, yellowing and necrosis are equally conspicuous and important on the leaves showing the symptoms. These symptoms were first and most intensely exhibited on the leaves at the mid whorl region of the crown, which may later progresses upwards or downwards or both as disease advances. Such palms also exhibited severe drying and necrosis of inflorescence and abnormal shedding of buttons, immature nuts followed by mature nuts. Hence a modified scale is necessary to fully describe and to quantify the severity of foliar symptoms, inflorescence necrosis and shedding of buttons and nuts or loss of productivity. Severity of foliar symptoms was estimated by providing equal weights to flaccidity, yellowing and necrosis of the five leaves showing the symptom in the third spiral. Following were the grades assigned for flaccidity, yellowing and necrosis:-

Flaccidity

0 – No flaccidity

1 – About one third leaflets showing mild to moderate flaccidity

3 – One third to two third leaflets showing moderate to severe flaccidity

5 – More than two third leaflets exhibit severe flaccidity

Yellowing

0 – No yellowing

1 – About one third leaflets showing yellowing

3 – One third to two third leaflets showing yellowing

5 – More than two third leaflets exhibit severe yellowing

Necrosis

0 – No necrosis

1 – About one third leaflets showing mild necrosis

3 – One third to two third leaflets showing moderate necrosis

5 – More than two third leaflets exhibit severe necrosis

The disease index (DIF) was calculated using the formula,

$$DIF = \frac{Sum\ (F+Y+N)}{3 \times m \times n} \times 100$$

where 'F', 'Y' and 'N' are the grade points assigned to a leaf for flaccidity, yellowing and necrosis, 'm' represents the maximum score assigned and 'n' is the number of leaves in one spiral.

Severity of inflorescence dieback/ drying was calculated by assessing the extent of drying of six younger most inflorescences of the infected palm. The following were the scores assigned for inflorescence dieback/ drying:

0 - No inflorescence dieback/ drying

1 - About one third of inflorescence showing drying/ dieback

3 - One third to two third of inflorescence showing drying/ dieback

5 - More than two third of inflorescence showing drying/ dieback

Severity of inflorescence dieback (DIID) was calculated using the formula,

$$DIID = \frac{Sum\ of\ scores\ of\ inflorescence\ dieback}{m \times n} \times 100$$

where 'm' represents maximum score value assigned and 'n' represents number of inflorescences observed.

Based on the number of buttons or immature nuts and mature nuts remaining in the palm the severity of shedding of nuts/ buttons and loss of productivity was arbitrarily determined. The following categorizations were assigned to evaluate the severity of nut/ button shedding and loss of productivity:-

Mild shedding and loss in productivity - More than 25 nuts remaining on the palm

Moderate shedding and loss in productivity - Between 10 to 25 nuts remaining on the palm

Severe shedding and loss in productivity - Less than 10 nuts remaining on the palm

The disease index for foliar symptoms of the palms showing mid whorl yellowing varies from zero to 100. If the disease index for foliar symptoms is less than 20 per cent, the palm is at mild stage of infection, if it is between 20 and 50 per cent the palm is at moderate stage of infection and if it is above 50 per cent the palm is at severe stage of infection. The severity of inflorescence dieback varies from zero to 100 and if this index is less than 25 per cent, the palm is at mild stage of infection, if it is between 25 and 50 per cent, the palm is at moderate stage of infection and if it is above 50 per cent it is at severe stage of infection. If the number of nuts per palm is more than 25 it is considered as at mild stage of infection, between 10 and 25 nuts, the palm is at moderate stage of infection and if it is below 10 nuts, the palm is at severe stage of infection. If any one or more of the above indices is in the severe category, the palm is classified as severely infected and such palm is to be cut and destroyed.

3.2 SYMPTOMATOLOGY

Symptoms exhibited by the palms were recorded during the survey. The selected palms showing mild, moderate and severe symptoms were regularly monitored for eighteen months to study the variation in symptom expression.

3.3 ISOLATION OF FOLIAR FUNGAL PATHOGENS ASSOCIATED WITH SYMPTOMATIC LEAVES OF COCONUT PALMS

Disease specimen of leaflets of symptomatic leaves were collected from the selected diseased palms (Purposive sampling) in the Instructional Farm, College of

Agriculture, Vellayani during the period from October 2009 to July 2010. The fungal pathogens associated with the disease were isolated from infected outer whorl, middle whorl, inner whorl of leaves and spindle leaves by routine isolation method.

The infected portions of leaves were cut into small bits and washed with water. The pieces were then surface sterilized with 0.1 per cent mercuric chloride solution for one minute and washed in three changes of sterile distilled water. These bits were placed on solidified Potato Dextrose Agar (PDA) medium (four pieces per plate) in sterilized petri dishes. The dishes were incubated at room temperature. When the growth of the fungus was visible mycelial bits were transferred aseptically to PDA slants and labelled. The slants were incubated at room temperature.

3.4 PURE CULTURING

All the fungal pathogens isolated from infected leaves of coconut were purified by single spore isolation technique (Johnston and Booth, 1983; Kotasthane and Agrawal, 2010). Dilute spore suspension was prepared in sterile distilled water from sporulating culture (five to twelve days old culture). One ml of the spore suspension was dispersed across the bottom of sterile petri dishes to which ten ml of melted; cooled water agar (two per cent) was added. These plates were incubated at room temperature for 12 h. The plates were examined under microscope to discern single isolated germinated spore and the suitable spores were marked with ink on the surface of the petri dishes. A cut about 2 mm square was made in the agar around the selected spore under aseptic conditions. The agar block containing germinating spore was then transferred by means of a sterile inoculation needle to PDA slants and incubated at room temperature. These pure cultures were maintained for further studies.

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The infected portions of leaves were cut into small bits and washed with water. The pieces were then surface sterilized with 0.1 per cent mercuric chloride solution for one minute and washed in three changes of sterile distilled water. These bits were placed on solidified Potato Dextrose Agar (PDA) medium (four pieces per plate) in sterilized petri dishes. The dishes were incubated at room temperature. When the growth of the fungus was visible mycelial bits were transferred aseptically to PDA slants and labelled. The slants were incubated at room temperature.

3.4 PURE CULTURING

All the fungal pathogens isolated from infected leaves of coconut were purified by single spore isolation technique (Johnston and Booth, 1983; Kotasthane and Agrawal, 2010). Dilute spore suspension was prepared in sterile distilled water from sporulating culture (five to twelve days old culture). One ml of the spore suspension was dispersed across the bottom of sterile petri dishes to which ten ml of melted; cooled water agar (two per cent) was added. These plates were incubated at room temperature for 12 h. The plates were examined under microscope to discern single isolated germinated spore and the suitable spores were marked with ink on the surface of the petri dishes. A cut about 2 mm square was made in the agar around the selected spore under aseptic conditions. The agar block containing germinating spore was then transferred by means of a sterile inoculation needle to PDA slants and incubated at room temperature. These pure cultures were maintained for further studies.

3.5 TESTING THE PATHOGENICITY

Detached healthy leaflets of spindle leaf and mature leaf of coconut were used for the study. The leaflets were kept in moistened polythene tube with the basal portion covered using moist cotton wad. Mycelial bit from seven days old culture of the pathogen was inoculated on the leaflet with injury by pinprick. The mycelial bits were covered with moist cotton wool. Leaflets with pinprick injury inoculated with plain agar disc and covered using moist cotton wool served as control. The inoculated leaflets were incubated under room temperature. Symptom development and spread of lesions were noted. The pathogens were re-isolated from the lesions developed by artificial inoculation.

3.6 CHARACTERISATION AND IDENTIFICATION OF FUNGAL PATHOGENS

3.6.1 Colony characters

Five mm discs taken from actively growing cultures of all the fungal pathogens were inoculated at the centre of 9 cm petri dishes with sterile PDA (Appendix - I) and incubated at room temperature. Isolates of *Fusarium* spp. were also grown on PSA and CLA (Appendix - I) for elaborate studies. CA, MEA, PDA and CDA (Appendix - I) were used to study the characteristics of *Chalara fimbriata.* Colony colour and characters were recorded.

3.6.2 Conidial characters

The morphological characters were studied by using the slide culture technique as described by Riddel (1950). Two glass rods were placed on filter paper-lined bottom of petri dish and a clean glass slide was placed on top of it. A cover slip was also kept inside it and sterilized. Sterile plain agar medium (2%) was poured in sterilized petri dishes and after solidification one cm^2 blocks of agar discs were cut out using a sterile needle. One agar block was placed on the sterile slide and the four sides were inoculated with mycelial bits of the pathogen. A

cover slip was placed on the top of the inoculated agar block. After incubation at room temperature for desirable growth, the cover slip was lifted off and mounted on another slide using lactophenol-cotton blue (Appendix - II) stain. The agar block was removed from the culture slide and another mount was prepared on it. The slides were then examined under microscope and measurements of conidia were taken.

3.6.3 Identification of fungal pathogens

The morphological and cultural characteristics of the fungal isolates obtained from symptomatic leaves of coconut palms were compared with the original description. For confirmation of the identification of the pathogens based on morphological characters the cultures were sent to Agharkar Research Institute, Pune - 411 004.

3.7 BIOCHEMICAL ANALYSIS OF LEAVES

Changes in total sugars, total soluble proteins and activity of phenylalanine ammonia lyase in three different whorls of leaves *viz.* outer whorl, middle whorl and inner whorl were studied at four different stages of infection in the palm, early stage, moderate stage and severe stage and compared with that of healthy palms.

3.7.1 Estimation of total sugars

Total sugar content in the infected and healthy leaves was estimated by Anthrone method (Hedge and Hofreiter, 1962). Leaf samples of 100 mg each were weighed out and hydrolyzed with 5 ml of 2.5 N hydrochloric acid in a boiling water bath. The hydrolyzate was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made upto 100 ml and centrifuged at 5000 rpm for 15 minutes. From the supernatant 0.5 ml aliquot was taken and made upto 1.0 ml by adding distilled water. To this 4 ml anthrone reagent (Appendix - III) was added and heated for eight minutes in a boiling water bath. This was cooled rapidly and absorbance was measured at 630 nm in spectrophotometer.

Amount of total sugars was calculated from standard graph prepared using glucose and expressed in terms of milligrams of glucose equivalent per gram of leaf tissue on fresh weight basis.

3.7.2 Estimation of total soluble proteins

Total soluble protein content of infected and healthy leaves was estimated as per the procedure described by Bradford (1976). One gram of leaf sample was homogenized in 10 ml 0.1 M sodium acetate buffer (Appendix - IV) and centrifuged at 5000 rpm for 15 minutes at 4°C. The supernatant was saved for the estimation of soluble protein. The reaction mixture consisted of 0.5 ml enzyme extract, 0.5 ml distilled water and 5 ml of five times diluted dye (Appendix - V) solution. The absorbance was read at 595 nm in a spectrophotometer against reagent blank. Bovine serum albumin was used as the protein standard.

The protein content was expressed as microgram albumin equivalent of soluble protein per gram on fresh weight basis.

3.7.3 Estimation of phenylalanine ammonia lyase

Phenylalanine ammonia lyase activity in the infected and healthy leaves was analysed based on the procedure described by Dickerson *et al.* (1984). The enzyme extract was prepared by homogenizing one gram leaf sample in 5 ml of 0.1M sodium borate buffer (pH 8.8) (Appendix - IV) containing a pinch of polyvinyl pyrrolidone using chilled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was used for the assay of phenylalanine ammonia lyase activity. The reaction mixture contained 3 ml of 0.1 M sodium borate buffer, 0.2 ml enzyme extract and 0.1 ml of 12 mM L-phenylalanine prepared in the same buffer. The blank contained 3 ml of 0.1 M sodium borate buffer and 0.2 ml enzyme extract. The reaction mixture and blank was incubated at 40°C for 30 minutes and reaction was stopped by adding 0.2 ml of 3N hydrochloric acid. The absorbance was read at 290 nm in a spectrophotometer.

The phenylalanine ammonia lyase activity was expressed as micrograms *trans*-cinnamic acid formed per minute per gram on fresh weight basis.

3.8 *IN VITRO* EVALUATION OF FUNGICIDES AGAINST THE PREDOMINANT FOLIAR FUNGAL PATHOGENS IN COCONUT PALMS AFFECTED BY YELLOWING DISEASE

In vitro evaluation of fungicides against the predominant foliar fungal pathogens was performed using poisoned food technique (Nene and Thapliyal, 1982).

The fungicides used for *in vitro* bioassay were listed in Table 1.

Table 1. Fungicides and their concentrations used for *in vitro* evaluation

Fungicides	Trade Name	Recommended Dose
Propiconazole	Tilt	1 ml l^{-1}
Tebuconazole	Folicur	1.5 ml l^{-1}
Flusilazole	Nustar	0.3 ml l^{-1}
Hexaconazole	Contaf	0.5 ml l^{-1}
Carbendazim	Bavistin	1 g l^{-1}
Mancozeb	Dithane M-45	3 g l^{-1}
Copper Hydroxide	Kocide 101	2.5 g l^{-1}
Hexaconazole + Zineb	Avtar	1.5 g l^{-1}
Captan + Hexaconazole	Taqat	2 g l^{-1}

The required quantity of fungicides for 100 ml medium was added into a 250 ml 'conical flask containing 100 ml of molten, cooled, sterile PDA and mixed thoroughly. The medium was poured in sterile petridishes. After solidification of the medium each plate was inoculated with 6 mm diameter mycelial discs of seven day old culture of the respective pathogens by placing it at the centre of the dish. Plates containing media without fungicides served as control. Three replications were maintained for each fungicide. The petridishes were incubated at room

temperature and fungal colony diameter was measured daily until fungal growth was complete in the control plates.

The per cent inhibition of mycelial growth was calculated using the formula

$$I = \frac{100\ (C - T)}{C}$$

where I – Inhibition of mycelial growth, C – Growth of the pathogen in control plates (cm) and T – Growth of the pathogen in treatment plates (cm) (Vincent, 1927).

Results

4. RESULTS

4.1 SURVEY

Results of the survey conducted on the incidence and intensity of RWD and Yellowing Disease of coconut at the Instructional Farm, College of Agriculture, Vellayani during the period from November 2009 to February 2010 are presented in Tables 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13.

4.1.1 Disease Incidence

The incidence of both RWD and Yellowing Disease were observed in all the six blocks of the Instructional Farm to varying extends (Table 2).

Table 2. Incidence of RWD and Yellowing Disease

Block	RWD affected palms	Yellowing Disease affected palms	Total no. of affected palms	Per cent disease incidence
A	72 (8.08 %)	19 (2.12 %)	91	10.2 %
B	202 (14.00 %)	56 (3.88 %)	258	17.9 %
C	67 (5.87 %)	7 (0.61 %)	74	6.5 %
D	124 (10.04 %)	23 (1.86 %)	147	11.9 %
E	64 (9.37 %)	15 (2.20 %)	79	11.6 %
F	32 (4.51 %)	7 (0.99 %)	39	5.5 %
Total	561 (9.19 %)	127 (2.08 %)	688	11.3 %

Among the 6107 coconut palms grown in the six different blocks of the Instructional Farm, 688 palms (11.3 %) were affected by either RWD or Yellowing Disease. Among them 561 palms (9.19 %) had shown typical symptoms of RWD while 127 palms (2.08 %) were showing the characteristic symptoms of Yellowing Disease. When both the diseases were taken together, the highest disease incidence was noticed in the B block (17.9 %) and the lowest disease incidence was in F block (5.5 %). The highest incidence of RWD was in B block (14 %) while the lowest incidence was in F block (4.51 %). In the case of Yellowing Disease the highest

incidence was observed in B block (3.88 %) and the least incidence was in C block (0.61 %). Out of the total infected palms 98.26 per cent (676 palms) were yielding palms while only 1.74 per cent (12 palms) were at the pre-bearing stage. Five palms at pre-bearing stage were affected by RWD while seven palms were affected by Yellowing disease.

Leaf rot disease was observed on 42 per cent (234 palms) of RWD affected palms and 52.5 per cent (63 palms) of the Yellowing Disease affected palms.

4.1.2 Disease intensity

4.1.2.1 Disease intensity of Root (Wilt) Disease affected palms

Results of the survey on disease intensity of RWD affected palms indicated that the palms were at different stages of infection in different blocks. There were 111 palms (19.8 %) at an advanced stage of infection (disease index above 50) (Table 3), 397 palms (70.8 %) at moderate stage of infection (disease index between 20 and 50) (Table 4) and 53 palms (9.4 %) at mild stage of infection (disease index below 20) (Table 5). Among the five infected palms (0.89 %) at pre-bearing stage, four palms were at moderate stage of infection and one palm was at advanced stage of infection.

Altogether 110 palms (19.78 %) of the RWD affected palms had a total loss of productivity among which 61 palms (10.97 %) were at the advanced stage of infection (Table 3), 47 palms (8.45 %) were at moderate stage of infection (Table 4) and two palms (0.36 %) were at mild stage of infection (Table 5). The data further indicated that altogether 127 infected palms (22.8 %) had a productivity of less than 10 nuts/ palm/ year, among which 29 palms (5.2 %) were at advanced stage of infection (Table 3) while it was 94 palms (16.9 %) in the moderate stage of infection category (Table 4) and only four palms (0.7%) in the mild stage of infection category (Table 5).

Table 3. Disease intensity of RWD affected palms at advanced stage of infection

Sl. No.	Tree No.	DI	No. of harvestable nuts
1	A4	58	6
2	A8	58	>10
3	A23	52	0
4	A28	62	0
5	A33	54	0
6	A35	56	0
7	A36	54	0
8	A42	58	0
9	A48	56	>10
10	A49	86	0
11	A70	68	10
12	B2	54	2
13	B4	60	0
14	B6	62	0
15	B9	72	0
16	B17	54	0
17	B18	58	0
18	B46	68	0
19	B49	68	0
20	B59	62	0
21	B64	62	0
22	B68	50	0
23	B72	62	0
24	B83	52	8
25	B84	72	4
26	B85	62	10
27	B87	50	2
28	B97	50	0*
29	B101	50	6
30	B111	58	0
31	B113	58	9
32	B117	66	10
33	B128	100	0
34	B143	50	4
35	B144	54	7
36	B171	56	6
37	B182	100	0
38	B183	54	0
39	B194	58	1
40	C8	76	0
41	C15	78	0
42	C21	60	0
43	C22	58	0
44	C35	50	7
45	C44	64	0
46	C48	62	0
47	C52	100	0
48	C55	56	0
49	C59	64	0
50	D12	76	0
51	D13	62	0
52	D38	50	4
53	D44	62	0
54	D49	56	0
55	D50	54	0
56	D55	58	0
57	D72	72	0
58	D81	68	0
59	D87	64	0
60	D90	74	0
61	D92	70	0
62	D102	60	0
63	E3	56	0
64	E5	62	6
65	E6	78	0
66	E8	50	>10
67	E9	52	0
68	E10	54	0
69	E13	74	0
70	E14	62	>10
71	E16	74	0
72	E17	68	1
73	E19	52	>10
74	E20	62	>10
75	E22	62	>10
76	E25	72	0
77	E28	54	>10
78	E29	56	0
79	E30	70	0
80	E31	76	0
81	E32	58	2
82	E33	50	>10
83	E34	58	0
84	E35	62	2
85	E37	80	1
86	E38	74	1
87	E40	52	4
88	E44	50	>10
89	E45	58	0
90	E46	52	10
91	E47	76	7
92	E48	58	0
93	E51	58	1
94	E52	58	8
95	E54	66	0
96	E55	54	>10
97	E58	78	2
98	E61	52	10
99	E62	62	>10
100	E63	80	0
101	E64	68	2
102	F1	54	>10
103	F2	76	6
104	F4	76	6
105	F6	78	2
106	F7	54	>10
107	F8	62	>10
108	F10	50	4
109	F22	62	0
110	F24	50	0
111	F29	50	0

*palm at pre-bearing stage

Table 4 continued

Sl. No.	Tree No.	DI	No. of harvestable nuts
130	B100	26	0*
131	B102	34	>10
132	B103	28	>10
133	B104	32	1
134	B105	36	>10
135	B106	36	>10
136	B107	44	>10
137	B108	40	>10
138	B109	34	5
139	B110	38	>10
140	B112	42	>10
141	B114	48	1
142	B115	46	0
143	B116	42	>10
144	B118	38	>10
145	B119	34	>10
146	B120	38	>10
147	B121	34	>10
148	B122	24	>10
149	B123	44	>10
150	B124	26	>10
151	B125	26	1
152	B126	44	>10
153	B127	44	6
154	B129	44	4
155	B130	42	3
156	B131	24	>10
157	B132	40	7
158	B133	38	>10
159	B134	42	2
160	B135	26	>10
161	B136	46	5
162	B137	44	11
163	B138	30	>10
164	B139	22	8
165	B140	36	>10
166	B141	20	>10
167	B142	40	>10
168	B145	34	1
169	B146	36	2
170	B147	34	0
171	B148	38	1
172	B149	42	11
173	B150	40	>10
174	B151	38	0
175	B152	36	2
176	B153	40	>10
177	B154	42	7
178	B155	34	>10
179	B156	32	4
180	B157	32	>10
181	B158	48	0
182	B159	26	>10
183	B160	42	0
184	B161	32	>10
185	B162	34	>10
186	B163	44	>10
187	B164	38	>10
188	B165	24	>10
189	B166	26	>10
190	B167	22	>10
191	B168	34	>10
192	B169	34	0*
193	B170	38	1
194	B172	32	0
195	B173	20	>10
196	B174	36	>10

197	B175	38	>10
198	B176	42	>10
199	B177	36	>10
200	B178	26	>10
201	B179	44	>10
202	B180	48	7
203	B181	36	>10
204	B184	46	3
205	B185	34	>10
206	B186	42	0
207	B187	46	0
208	B188	38	>10
209	B189	32	0
210	B190	24	>10
211	B191	48	>10
212	B192	42	>10
213	B193	24	3
214	B195	40	>10
215	B196	32	>10
216	B197	34	>10
217	B198	36	3
218	B199	28	>10
219	B200	28	>10
220	B201	28	>10
221	B202	40	3
222	C1	24	>10
223	C2	32	>10
224	C3	40	>10
225	C4	30	>10
226	C5	26	0
227	C6	34	>10
228	C7	28	8
229	C9	38	>10
230	C10	24	>10
231	C11	44	5

232	C12	38	>10
233	C13	24	>10
234	C14	30	>10
235	C20	30	>10
236	C23	28	5
237	C24	30	5
238	C25	32	>10
239	C26	30	>10
240	C27	22	7
241	C28	28	8
242	C29	28	7
243	C30	26	>10
244	C31	28	10
245	C32	46	2
246	C33	28	>10
247	C36	28	>10
248	C37	28	>10
249	C38	28	8
250	C39	22	>10
251	C41	32	>10
252	C42	24	>10
253	C43	30	6
254	C45	44	3
255	C46	20	>10
256	C47	20	>10
257	C49	32	>10
258	C50	22	>10
259	C51	34	4
260	C53	34	0
261	C54	36	>10
262	C56	40	0
263	C57	38	>10
264	C58	40	0
265	C60	28	>10
266	C61	20	>10

Continued on page 40

Table 4 continued

Sl. No.	Tree No.	DI	No. of harvestable nuts
267	C62	24	>10
268	C63	22	0*
269	C64	30	0*
270	C65	32	>10
271	C66	28	0
272	C67	22	>10
273	D3	32	>10
274	D4	20	>10
275	D5	28	>10
276	D6	28	>10
277	D7	20	>10
278	D9	44	>10
279	D10	20	>10
280	D11	22	>10
281	D14	22	>10
282	D16	20	>10
283	D30	40	>10
284	D31	24	>10
285	D32	22	>10
286	D33	30	>10
287	D34	46	0
288	D36	32	7
289	D37	30	3
290	D39	40	0
291	D41	34	0
292	D42	42	>10
293	D43	30	>10
294	D45	26	>10
295	D47	44	0
296	D48	20	>10
297	D51	36	>10
298	D52	22	>10
299	D53	32	>10
300	D54	38	>10
301	D56	42	0
302	D57	28	>10
303	D58	26	>10
304	D59	26	>10
305	D63	26	>10
306	D65	34	>10
307	D66	28	10
308	D68	30	8
309	D69	28	>10
310	D70	30	>10
311	D71	26	7
312	D73	24	>10
313	D74	38	>10
314	D75	38	>10
315	D76	30	>10
316	D77	26	>10
317	D79	24	>10
318	D80	26	>10
319	D82	40	4
320	D83	32	0
321	D85	34	>10
322	D86	28	1
323	D88	30	5
324	D91	36	10
325	D93	30	>10
326	D94	20	>10
327	D96	26	>10
328	D97	34	>10
329	D98	28	>10
330	D99	20	>10
331	D100	20	>10
332	D101	22	>10
333	D103	34	>10

334	D104	26	0
335	D105	20	>10
336	D106	28	>10
337	D108	20	>10
338	D110	30	1
339	D111	20	>10
340	D112	22	>10
341	D113	34	0
342	D114	22	>10
343	D115	36	0
344	D117	26	7
345	D118	30	>10
346	D120	42	5
347	D121	36	>10
348	D122	30	>10
349	D123	30	>10
350	D124	28	>10
351	E1	28	>10
352	E2	36	>10
353	E4	28	>10
354	E7	48	>10
355	E11	48	>10
356	E12	34	>10
357	E15	42	8
358	E18	46	>10
359	E21	42	>10
360	E23	34	0
361	E24	42	>10
362	E26	26	>10
363	E27	42	9
364	E36	30	>10
365	E39	34	>10
366	E41	40	>10
367	E42	28	4
368	E43	42	>10
369	E49	46	>10
370	E50	42	>10
371	E53	48	>10
372	E56	42	>10
373	E57	26	>10
374	E59	34	>10
375	E60	38	>10
376	F3	36	>10
377	F5	48	>10
378	F9	36	>10
379	F11	46	>10
380	F12	40	>10
381	F13	28	>10
382	F14	28	>10
383	F15	34	>10
384	F16	26	>10
385	F17	36	>10
386	F18	32	>10
387	F19	22	>10
388	F20	34	>10
389	F21	34	>10
390	F23	36	5
391	F25	38	0
392	F26	34	>10
393	F27	44	>10
394	F28	40	>10
395	F30	20	>10
396	F31	22	>10
397	F32	24	>10
*palm at pre-bearing stage			

category, 252 palms (45.3 %) in the moderate stage category and 47 palms (8.5%) in the mild stage category). These results are summarized in Table 6.

Table 6. Productivity of RWD affected bearing palms at different stages of infection

Stage of infection	Palms with total loss of productivity (No.)	Palms with productivity less than 10 nuts/ palm/ year (No.)	Palms with productivity more than 10 nuts/ palm/ year (No.)	Total	Per cent of the total RWD affected palms
Advanced stage	61	29	20	110	19.78 %
Moderate stage	47	94	252	393	70.68 %
Mild stage	2	4	47	53	9.53 %
Total	110	127	319	556	
Per cent of the total RWD affected palms	19.78 %	22.84 %	57.37 %		

4.1.2.2 Disease intensity of Yellowing Disease affected palms

The disease intensity of Yellowing Disease affected palms with respect to foliar symptoms (DIF), inflorescence dieback (DIID) and loss of productivity (Number of harvestable nuts/ palm/ year) are presented in the Tables 7, 8, 9, 10 and 11. The data indicated that out of the total 127 affected palms, 120 palms (94.5 %) were yielding palms (Tables 7, 8, 9 and 10) and seven palms (5.5 %) were at the pre-bearing stage (Table 11). Among the 120 yielding palms, 45 palms (37.5 %) had a total loss of productivity (0 nuts/ palm) (Table 7) and 28 palms (23.3 %) had less than 10 nuts/ palm/ year (Table 8). The results also indicated that 31 palms (25.8%) had yielded nuts in the range of 10 to 25 nuts/ palm/ year and are considered at the stage of moderate loss of productivity (Table 9) and 16 palms (13.3 %) had produced above 25 nuts/ palm/ year and are at the stage of mild loss of productivity (Table 10).

Table 5. Disease intensity of RWD affected palms at mild stage of infection

Sl. No.	Tree No.	DI	No. of harvestable nuts
1	A5	14	>10
2	A16	14	>10
3	A25	14	>10
4	A43	10	1
5	A44	12	10
6	A53	18	10
7	A72	18	0
8	B8	14	>10
9	B10	14	>10
10	B26	16	>10
11	B34	18	>10
12	B54	18	>10
13	B57	16	>10
14	B94	16	>10
15	C16	16	>10
16	C17	16	>10
17	C18	16	>10
18	C19	18	>10
19	C34	16	>10
20	C40	14	>10
21	D1	14	8
22	D2	16	>10
23	D8	16	>10
24	D15	12	>10
25	D17	14	>10
26	D18	10	>10
27	D19	12	0
28	D20	10	>10
29	D21	14	>10
30	D22	14	>10
31	D23	12	>10
32	D24	14	>10
33	D25	12	>10
34	D26	16	>10
35	D27	18	>10
36	D28	10	>10
37	D29	16	>10
38	D35	14	>10
39	D40	16	2
40	D46	18	>10
41	D60	16	>10
42	D61	18	>10
43	D62	16	>10
44	D64	8	>10
45	D67	12	>10
46	D78	18	>10
47	D84	18	>10
48	D89	18	>10
49	D95	12	>10
50	D107	14	>10
51	D109	16	>10
52	D116	18	8
53	D119	18	>10

Results of the data in the Tables 3, 4 and 5 indicated that altogether 242 palms (43%) either had a total loss of productivity or had less than 10 nuts/ palm/ year or at the pre-bearing stage which are to be cut and removed.

Eventhough affected by RWD, 319 palms (57.4 %) had a productivity of 10 nuts or above/ palm/ year out of which 20 palms (3.6 %) were in the advanced stage

category, 252 palms (45.3 %) in the moderate stage category and 47 palms (8.5%) in the mild stage category). These results are summarized in Table 6.

Table 6. Productivity of RWD affected bearing palms at different stages of infection

Stage of infection	Palms with total loss of productivity (No.)	Palms with productivity less than 10 nuts/ palm/ year (No.)	Palms with productivity more than 10 nuts/ palm/ year (No.)	Total	Per cent of the total RWD affected palms
Advanced stage	61	29	20	110	19.78 %
Moderate stage	47	94	252	393	70.68 %
Mild stage	2	4	47	53	9.53 %
Total	110	127	319	556	
Per cent of the total RWD affected palms	19.78 %	22.84 %	57.37 %		

4.1.2.2 Disease intensity of Yellowing Disease affected palms

The disease intensity of Yellowing Disease affected palms with respect to foliar symptoms (DIF), inflorescence dieback (DIID) and loss of productivity (Number of harvestable nuts/ palm/ year) are presented in the Tables 7, 8, 9, 10 and 11. The data indicated that out of the total 127 affected palms, 120 palms (94.5 %) were yielding palms (Tables 7, 8, 9 and 10) and seven palms (5.5 %) were at the pre-bearing stage (Table 11). Among the 120 yielding palms, 45 palms (37.5 %) had a total loss of productivity (0 nuts/ palm) (Table 7) and 28 palms (23.3 %) had less than 10 nuts/ palm/ year (Table 8). The results also indicated that 31 palms (25.8%) had yielded nuts in the range of 10 to 25 nuts/ palm/ year and are considered at the stage of moderate loss of productivity (Table 9) and 16 palms (13.3 %) had produced above 25 nuts/ palm/ year and are at the stage of mild loss of productivity (Table 10).

Most of the Yellowing disease affected palms with total loss of productivity had either 100 per cent inflorescence dieback (27 palms (60 %)) or above 50 per cent inflorescence dieback (11 palms (24.44 %)) while four palms (8.9%) had moderate levels of inflorescence dieback, two palms (4.44 %) had mild inflorescence dieback and only one palm (2.22 %) was free from inflorescence dieback. It was also evident that among the 45 Yellowing Disease affected palms with total loss of productivity 18 palms (40%) also had the severe category scoring for the disease index for foliar symptoms, 24 palms (53 %) had the moderate category of foliar symptom severity and only three palms (7 %) had mild category of foliar symptom severity (Table 7).

Among the 28 palms showing severe loss of productivity(less than 10 nuts/ palm/ year), most of the palms (17 palms (60.7 %)) were having severe inflorescence dieback (either above 50 % or 100%) while eight palms (28.6 %) had moderate levels of inflorescence dieback and three palms (10.7 %) were at mild inflorescence dieback stage. Among these palms most of the palms (17 palms (60.7 %)) had a moderate category scoring for the disease index for foliar symptoms, three palms (10.7 %) had the severe category of foliar symptom severity and eight palms (28.6%) had mild category of foliar symptom severity (Table 8).

Most of the palms showing moderate loss of productivity were with severe (10 palms (32 %)) or moderate (11 palms (36 %)) levels of inflorescence dieback while four palms (13 %) had mild levels of inflorescence dieback and six palms (19 %) were free from inflorescence dieback. Most of these palms (18 palms (58 %)) had a moderate category scoring for the disease index for foliar symptoms, four palms (13%) had the severe category of foliar symptom severity and nine palms (29 %) had mild category of foliar symptom severity (Table 9).

Among the 16 palms showing mild loss of productivity, 13 palms (81.25 %) were free from inflorescence dieback, while only one palm (6.25%) had severe inflorescence dieback and two palms (12.5%) were having mild stage of

inflorescence dieback. With regard to the expression of foliar symptoms nine palms (56.25%) had mild category of foliar symptom severity, six palms (37.5 %) had moderate category of foliar symptom severity and only one palm (6.25 %) had severe category of foliar symptom severity (Table 10).

With regard to the expression of foliar symptoms by Yellowing disease affected palms the results showed that 26 palms (21.7 %) were having severe foliar symptoms (above 50 %), 65 palms (54.2 %) had moderate levels of foliar symptom expression (between 20 and 50 %) and 29 palms (24.1 %) had mild foliar symptom expression (below 20 %) (Tables 7, 8, 9 and 10).

Out of the total 120 Yellowing disease affected yielding palms 66 palms (55%) were having severe inflorescence dieback (above 50 %) or 100 % inflorescence dieback while 23 palms (19.17 %) had moderate levels of inflorescence dieback (between 25 and 50 %) and 11 palms (9.16 %) were at mild inflorescence dieback stage (less than 25 %) and 20 palms (16.67 %) were free from inflorescence dieback symptom (Tables 7, 8, 9 and 10).

The overall effect of Yellowing disease of coconut indicated that 87 palms (72.5 %) had either above 50 per cent foliar symptom severity or above 50 per cent severity in inflorescence dieback or yielded less than 10 nuts/ palm/ year either singly or in combination and hence are to be removed and destroyed (Tables 7, 8, 9 and 10). The survey also indicated that seven palms at pre-bearing stage were also affected by Yellowing disease and showed moderate levels of foliar symptom expression (Table 11). These palms also have to be removed.

The study further indicated that 47 palms (39 %) had yielded more than 10 nuts/ palm/ year even though inflicted by the disease out of which 18 palms (15 %) yielded more than 15 nuts /palm/ year had no inflorescence dieback and six palms (5%) had more than 40 nuts/ palm/ year (Table 9 and 10).

The results of the study were summarized in Table 12 and 13.

Table 7. Disease intensity of Yellowing Disease affected palms showing a total loss of productivity

Sl. No.	Tree No.	DIF (%)	DIID (%)	No. of harvestable nuts
1	AP 3	64	100	0
2	AP 4	80	100	0
3	AP 6	44	100	0
4	AP 7	41	36.6	0
5	AP13	55	100	0
6	AP15	69	100	0
7	AP17	87	100	0
8	BP 2	40	66.6	0
9	BP 3	49.3	100	0
10	BP 4	73.3	93	0
11	BP 5	61	100	0
12	BP 8	28	100	0
13	BP 10	33	50	0
14	BP 11	33	83	0
15	BP 12	37	33.3	0
16	BP 15	24	20	0
17	BP 16	56	50	0
18	BP 23	24	33.3	0
19	BP 30	57.2	100	0
20	BP 39	100	100	0
21	BP 40	55	100	0
22	BP 45	19	66.6	0
23	BP 55	36	100	0
24	BP 56	31	100	0
25	CP 1	39	100	0
26	CP 2	41	100	0
27	CP 3	40	100	0
28	DP 4	52	100	0
29	DP 7	24	100	0
30	DP 8	28	100	0
31	DP11	21	100	0
32	DP14	36	83	0
33	DP15	12	100	0
34	DP16	31	100	0
35	DP19	28	100	0
36	DP22	15	17	0
37	EP 1	44	75	0
38	EP 2	65	100	0
39	EP 5	74	73.2	0
40	EP 8	60	80	0
41	EP 14	52	0	0
42	FP 1	66.4	93	0
43	FP 3	41	100	0
44	FP 4	62	100	0
45	FP 6	44	25	0

Table 8. Disease intensity of yellowing disease affected coconut palms showing severe loss of productivity

Sl. No.	Tree No.	DIF (%)	DIID (%)	No. of harvestable nuts
1	AP 1	17	56	5
2	AP 8	20	10	8
3	AP 9	18.67	6.67	5
4	AP10	21	17	5
5	AP12	49	37	4
6	AP18	32	30	9
7	BP 1	21	100	1
8	BP17	15	50	2
9	BP18	21	83	1
10	BP21	27	33.3	9
11	BP37	32	66.6	3
12	BP38	20	100	1
13	BP42	19	33.3	8
14	BP46	4	83	5
15	BP53	41.3	100	3
16	BP54	13.3	33.3	5
17	CP 7	24	60	4
18	DP 1	23	53	2
19	DP 3	31	100	1
20	DP12	27	66.6	5
21	DP17	19	33.3	9
22	DP20	20	50	6
23	DP23	12	66.6	2
24	EP 4	50.4	75	3
25	EP 6	38.6	42	8
26	FP 2	54.4	100	4
27	FP 5	52	36	4
28	FP 7	21.2	50	2

Table 9. Disease intensity of yellowing disease affected coconut palms showing moderate loss of productivity

Sl. No.	Tree No.	DIF (%)	DIID (%)	No. of harvestable nuts
1	AP 2	90	40	15
2	AP16	45	50	11
3	AP19	23	0	19
4	BP 9	35	0	19
5	BP13	44	50	14
6	BP14	21	33.3	17
7	BP25	31	66.6	16
8	BP26	19	33.3	17
9	BP28	34.4	17	13
10	BP29	10.4	33.3	15
11	BP32	26.4	60	24
12	BP33	24	17	24
13	BP34	30.4	50	16
14	BP36	28	50	12
15	BP41	16	16.6	22
16	BP44	39	33.3	22
17	BP47	20	83	17
18	BP48	23	17	21
19	BP49	19	33.3	13
20	BP51	16	33.3	19
21	DP 2	17.3	33.3	14
22	DP 6	15	50	18
23	DP10	12	0	23
24	DP18	24	0	22
25	EP 3	65	50	12
26	EP 7	40	0	21
27	EP 9	60	42	15
28	EP11	46.4	0	13
29	EP12	14.4	60	12
30	EP13	68	32	17
31	EP15	29.3	32	19

Table 10. Disease intensity of Yellowing Disease affected palms showing mild loss of productivity

Sl. No.	Tree No.	DIF (%)	DIID (%)	No. of harvestable nuts
1	AP 5	10.6	0	26
2	BP 24	57	66.6	42
3	BP 27	16	0	32
4	BP 31	20	0	28
5	BP 35	21.2	17	71
6	BP 43	42.7	16.6	28
7	BP 50	25.3	0	49
8	BP 52	33.3	0	43
9	CP 4	17	0	65
10	CP 5	19	0	31
11	CP 6	24	0	28
12	DP 5	12	0	32
13	DP 9	19	0	26
14	DP13	12	0	29
15	DP21	19	0	44
16	EP 10	12	0	32

Table 11. Disease intensity of Yellowing Disease affected palms at pre-bearing stage

Sl. No.	Tree No.	DIF (%)
1	AP 11	21
2	AP 14	23
3	BP 6	33
4	BP 7	37
5	BP 19	35
6	BP 20	39
7	BP 22	37

DIF - Disease index for foliar symptoms
DIID- Disease index for inflorescence dieback

Table 12. Categorization of Yellowing Disease affected palms based on intensity of foliar symptoms

Category of palms	Intensity of foliar symptoms (No. of palms in each category)			Total	Per cent of the total infected palms
	Severe	Moderate	Mild		
Palms with total loss of productivity	18	24	3	45	37.5 %
Palms with severe loss of productivity	3	17	8	28	23.3 %
Palms with moderate loss of productivity	4	18	9	31	25.8 %
Palms with mild loss of productivity	1	6	9	16	13.3 %
Total	26	65	29	120	
Per cent of the total infected palms	21.6 %	54.2 %	24.2 %		

Table 13. Categorization of Yellowing Disease affected palms based on intensity of inflorescence dieback

Category of palms	Intensity of inflorescence dieback (No. of palms in each category)				Total	Per cent of the total infected palms
	Severe	Moderate	Mild	No symptom		
Palms with total loss of productivity	38	4	2	1	45	37.5 %
Palms with severe loss of productivity	17	8	3	-	28	23.3 %
Palms with moderate loss of productivity	10	11	4	6	31	25.8 %
Palms with mild loss of productivity	1	-	2	13	16	13.3 %
Total	66	23	11	20	120	
Per cent of the total infected palms	55 %	19.2 %	9.2 %	16.6 %		

The disease indices for foliar symptoms and inflorescence dieback and its effect on yield were statistically analyzed using correlation and the results are presented in Table 14.

Table 14. Correlation matrix for foliar symptoms, inflorescence dieback and yield of the palm

Parameters	DIF	DIID	No. of nuts per palm
DIF	1.0000		
DIID	0.4310**	1.0000	
No. of nuts per palm	-0.3254**	-0.6646**	1.0000

** indicates values significant at 1 % level.

DIF - Disease index for foliar symptoms

DIID- Disease index for inflorescence dieback

There was significant positive correlation between disease intensity of foliar symptoms and severity of inflorescence dieback and negative correlation between disease intensity of foliar symptoms and number of nuts/ palm/ year. A highly significant negative correlation exists between severity of inflorescence dieback and number of nuts/ palm/ year.

4.2 SYMPTOMATOLOGY

4.2.1 Symptoms of RWD/ Yellowing Disease

Flaccidity, ribbing (abnormal bending) of leaflets, yellowing and necrosis were the typical foliar symptoms observed on RWD affected coconut palms (Plate 1). The most obvious and diagnostic symptom of the disease was loss of turgor of leaf lamina and the abnormal inward bending of the leaflets which is usually termed as flaccidity. General yellowing and drooping of outer whorls of leaves, pale yellow colour of the inner leaves, and necrosis of the tips and margins of leaflets starting

Plate 1. Root (Wilt) disease affected coconut palm

Plate 2. Initial symptoms of leaf rot

Plate 3. Enlarged, coalesced lesions

from the distal end of the leaves progressing inwards on the yellowed leaves were the other important symptoms. In few of the palms the distal end of leaf at the fourth or fifth position from the spindle was curled at about 1 to 1.5 m below the leaf tip, was broken, hung down, became yellow and dried up just before the appearance of typical foliar symptoms. Shedding of immature nuts and inflorescence dieback were noticed in some of the RWD affected palms. In disease advanced palms, the leaves became progressively smaller in size and crown size was greatly reduced. Reduction in spathe size with few female flowers, drying up of spathes and necrosis of spikelets were also observed in such palms. The stunted spathes failed to open normally. With further advancement of the disease, inflorescence production was ceased. In pre-bearing palms inflorescence was not produced due to infection.

Leaf rot disease was observed in 42 per cent of RWD affected palms. The disease initially appeared as minute water soaked lesions of varying sizes and shapes at the margins and distal ends of leaflets of the unopened spindle leaf (Plate 2). These lesions were turned to varying shades of brown to black, enlarged in size, coalesced and caused extensive rotting which further advanced into the interior of the spindle (Plate 3). On the surface of affected leaflets tan coloured mycelial growth/ sporulation could be observed. As the spindle leaves gradually unfurled, the rotten cemented portions of the leaflets were dried and blown off by wind, leaving a protruding blackened and shrivelled mid vein while the base of the leaflets gradually unfurled normally. This gave a typical 'fan leaf' like appearance to the affected leaf. When the succeedingly emerging spindle leaves were affected by the disease the palms gave a disfigured appearance (Plate 4). In very severe cases the bud was also destroyed causing death of the palm. The lesions were also observed on petiole, mid rib, mid veins and laminar areas of leaflets.

Yellowing Disease (mid whorl yellowing) of coconut was characterized by rapid chlorosis or yellowing and/ or bronzing and flaccidity of a few or all the leaves in the middle whorl of the palm crown (Plate 5). Yellowing initially started on the

Plate 4. Coconut palm affected by Root (wilt)-leaf rot complex

Plate 5. Yellowing and flaccidity of the leaves in the middle whorl of the palm affected by Yellowing Disease

leaflets at the tip of leaves which progressed down to the base. In few instances yellowing followed by necrosis and blighting of the leaves were started from the innermost whorl of leaves (Plate 6). In most cases yellowing/ bronzing and flaccidity were progressed from the middle whorl of leaves upwards and/ or downwards or both as disease advanced. Rapid drying and necrosis of inflorescence (Plate 7) and abnormal shedding of flowers, buttons, immature nuts and mature nuts in succession which occurred either simultaneously or prior to yellowing (Plate 8) were always associated with Yellowing disease. Gradually the palms became barren within a short span of four to eight months time (Plate 9). The newly emerging inflorescence with few or no female flowers showed necrosis of spikelets from tip downwards either before or just after opening. On the yellowed/ chlorotic leaves intense brown to black or grey spots of varying sizes and shapes were developed which were further enlarged, coalesced together and blighted the leaflets (Plate 10). Necrosis was also started from the tips of yellowed leaflets and progressed inwards (Plate 11). These dried up leaves were shed sooner (Plate 12). Sometimes shedding of leaves started from one side of the crown where the first yellowed leaf was observed (Plate 13).

The newly produced leaves were shorter and weaker with chlorosis (Plate 14). The size of the crown was reduced and the growth of the palm was highly retarded. The short leaves in the crown were crowded together resulting in rosette appearance. Inflorescence production was ceased. The spathes if at all produced, were very much reduced in size and turned blackish which failed to open. In adverse cases spindle also got infected, withered and the growing point was rotten leading to death of the palm very rapidly (Plate 15).

Most of the palms affected by Yellowing Disease were also 'superinfected' with leaf rot pathogens sooner than later (Plate 16 and Plate 17). Leaf rot initially appeared as minute water soaked lesions having different sizes, shapes and colours on the margins and distal ends of leaflets of the unopened spindle leaf. The lesions

Plate 6. Symptoms of Yellowing Disease in the inner whorl of the affected palm

Plate 7 A

Plate 7 B

Plate 7. Inflorescence dieback in Yellowing Disease affected coconut palm

Plate 8. Fallen nuts due to abnormal shedding in Yellowing Disease affected palm

Plate 9. Inflorescence dieback with barren crown

Plate 10. Leaf spots and blights on chlorotic/ yellowed leaflets

Plate 11. Necrosis and severe blighting of the yellowed leaves in the middle whorl

Plate 12. Necrosis and shedding of yellowed leaves starting from middle whorl

Plate 13. Shedding of leaves from one side of the crown

Plate 14. Shorter and weaker leaves in the crown

Plate 15. The withered crown

Plate 16. Leaf rot symptoms in Yellowing Disease affected palm

Plate 17. Yellowing Disease affected palm with leaf rot symptoms

enlarged, coalesced, led to extensive rotting and further advanced into the interior of the spindle. On the surface of affected leaflets mould growth could be observed. Further progression of leaf rot disease was similar to that of RWD affected palms.

The Yellowing Disease affected palms were continuously monitored for about eighteen months. It was very often noticed that a few of the healthy palms surrounding the infected palm were also contracted by the disease and show all the typical symptoms (Plate 18).

4.2.2 Symptoms produced by various fungal pathogens upon artificial inoculation on spindle leaves and mature leaves:

All the fungal isolates obtained from coconut leaves produced more or less similar symptoms upon artificial inoculation on detached leaflets of spindle leaves as well as mature leaves. In general pathogens produced tiny brown water soaked lesions on detached spindle and mature leaflets. The lesions on spindle leaflets expanded more rapidly and covered larger area than in the mature leaflets. The lesions were of various shades of brown viz. light brown, brown, reddish brown and dark brown and angular, oval or irregular shaped. On mature leaflets round to oval or irregular brown lesions were produced by most of the pathogens. Detailed description on the symptoms produced upon artificial inoculation by the various fungal isolates are described in para 4.5.

4.3 ISOLATION OF FOLIAR FUNGAL PATHOGENS ASSOCIATED WITH SYMPTOMATIC LEAVES OF COCONUT PALMS

The foliar fungal pathogens associated with the disease were isolated from the symptomatic leaves of infected palms during the period from October 2009 to July 2010 and the results are presented in Table 15.

Plate 18. Adjacent palms showing symptoms of Yellowing Disease

Table 15. Fungal isolates obtained from Yellowing Disease affected coconut palms:

Stage of the palm/ leaf from which pathogens isolated	Fungal isolates obtained
Palms at pre-bearing stage	VA 3, VA 4, VA 5, VA 12, VA 23, VB 1, VB 2, VB 4, VB 5, VB 6, VB 8, VB 14, VB 15, VB 18 (14 Nos.)
Leaf rot affected spindle leaf/ leaf rot affected mature leaves of yielding palms	VA 6, VA 7, VA 8, VA 9, VA 10, VA 14, VA 24, VA 25, VB 9, VB 10, VB 12, VB 13, VB 16, VB 17, VB 20, VB 21, VB 23, VB 24, VB 25, VB 26, VB 27, VB 28, VB 30, VB 31, VC 6, VC 7, VC 8, VC 9, VD 6, VE 1, VE 2, VE 3, VE 4, VE 6, VF 5 (35 Nos.)
Chlorotic/ yellowed leaves of yielding palms	VA 1, VA 2, VA 11, VA 13, VA 15, VA 16, VA 17, VA 18, VA 19, VA 20, VA 21, VA 22, VA 26, VA 27, VA 28, VA 29, VA 30, VA 31, VA 32, VB 3, VB 7, VB 11, VB 19, VB 22, VB 29, VB 32, VB 33, VB 34, VB 35, VC 1, VC 2, VC 3,VC 4, VC 5, VC 10, VD 1, VD 2, VD 3, VD 4, VD 5, VD 7, VD 8, VD 9, VD 10, VD 11, VD 12, VD 13, VD 14, VD 15, VD 16, VE 5, VE 7, VE 8, VF 1, VF 2, VF 3, VF 4, VF 6, VF 7, VF 8, VF 9, VF 10 (62 Nos.)

4.4 PURE CULTURING

All the fungal isolates obtained from symptomatic leaves of coconut palms affected by yellowing disease were purified by single spore isolation technique and maintained on PDA slants for further studies.

Based on the morphological and cultural characteristics the isolates were grouped and the details are furnished in Table 16. In certain groups of isolates sub-grouping such as A, B and C were necessary based on distinctiveness in morphological and cultural characteristics.

Table 16. Fungal isolates from symptomatic leaves of coconut palms

Group	Sub Group	Fungal isolates
FI 1		VA 25, VB 28
FI 2		VA 12, VB 8
FI 3		VA 24, VB 27
FI 4		VA 15, VB 13, VB 21
FI 5	A	VA 28, VA 32, VB 4, VB 5, VB 31, VB 35, VC 5, VC 9, VE 8, VF 9
FI 5	B	VA 3, VA 10, VD 5, VD 16, VE 6, VF 8
FI 6		VB 25, VB 26
FI 7	A	VA 1, VA 4, VA 20, VB 10, VB 17, VC 4, VC 6, VD 4, VF 3
FI 7	B	VA 9, VB 2, VB 3, VD 1, VE 2
FI 8		VA 14, VB 12, VB 16, VD 6
FI 9	A	VA 8, VA 13, VA 30, VA 31, VB 15, VB 34, VD 10, VD 11, VD 13, VD 15, VF 7
FI 9	B	VA 19, VB 19, VC 3, VC 7, VE 3, VF 5
FI 9	C	VB 23, VD 3, VE 5
FI 10	A	VA 2, VA 5, VA 11, VA 27, VB 6, VB 7, VB 20, VB 29, VC 1, VC 8, VD 12, VE 4, VE 7, VF 6
FI 10	B	VD 2, VD 7
FI 11		VA 6, VA 26, VA 29, VB 1, VB 24, VB 32, VC 2, VC 10, VD 14, VE 1, VF 1, VF 10
FI 12		VA 7, VA 16, VA 21, VA 22, VB 11, VB 18, VB 30, VB 33, VD 8, VD 9
FI 13		VA 17, VA 18, VA 23, VB 14, VB 22, VF 2, VF 4
FI 14		VB 9

4.5 TESTING THE PATHOGENICITY

4.5.1 Testing the pathogenicity of the fungal isolates

The pathogenicity of the fungal isolates obtained from the symptomatic leaves were tested by artificial inoculation on detached leaflets of the spindle as well as

mature leaves and subsequent re-isolation from the inoculated leaflets. The results are presented in the following paragraphs as well as in Tables 17 and 18.

Isolate FI 1

Tiny brown water-soaked lesions were produced on detached spindle leaflets on fourth day after inoculation, which expanded and turned to dark brown irregular shaped lesions. The lesions were surrounded by water-soaked area and not restricted by mid-vein. Symptoms produced on spindle leaflets are presented in Plate 19 A (a). On mature leaflets tiny pinprick sized water-soaked lesions appeared, but they did not expand further.

Isolate FI 2

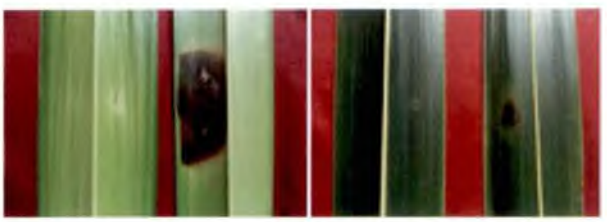
The pathogen produced minute water-soaked lesions on detached spindle and mature leaflets on third day after inoculation. The lesions on spindle leaflets were expanded more rapidly, reddish brown with a light brown area surrounding the spot, beyond which a water-soaked area developed. Mid-vein of leaflets were also turned reddish brown, but the expansion of lesions was slow in the mid-vein area (Plate 19 A (b)). On mature leaflets the pathogen produced minute pinprick sized water-soaked lesions which expanded to a circular, grayish brown lesion surrounded by yellow halo with water-soaking (Plate 19 A (b)). On the underside of lesions it produced brown coloured spores.

Isolate FI 3

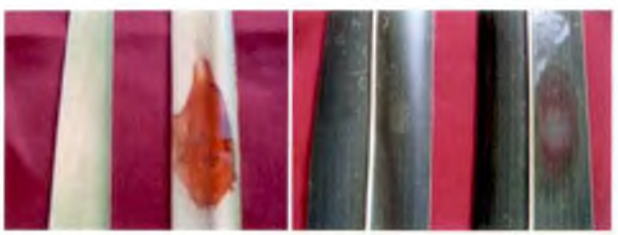
The isolate produced tiny brown water-soaked lesions on fourth day after inoculation on detached spindle leaflets and on fifth day after inoculation on mature leaflets. The tiny lesions expanded to dark brown, oval, angular shaped lesions surrounded by water-soaked region, restricted by mid-vein in the spindle leaflets (Plate 19 A (c)). On mature leaflets oval, dark brown lesions with yellow halo and water-soaked area developed (Plate 19 A (c)).

(a) Isolate FI 1

(b) Isolate FI 2



(c) Isolate FI 3



(d) Isolate FI 4

(e) Isolate FI 5 A

Plate 19 A. Symptoms produced by different fungal isolates on detached leaflets

Isolate FI 4

The pathogen produced tiny brown water-soaked oval shaped lesions on detached spindle leaflets and mature leaflets on second day after inoculation. The lesions were expanded rapidly in the spindle leaflets and were light brown initially which turned to reddish brown later, oval - spindle shaped and not restricted by mid-vein. The lesions were surrounded by large water-soaked area (Plate 19 A (d)). On mature leaflets it produced an oval shaped lesion with a dark brown centre and light brown periphery surrounded by yellow halo and water-soaked area. A brown exudate was visible on the underside of the inoculated area on mature leaflets (Plate 19 A (d)).

Isolate FI 5 A

The pathogen produced minute water-soaked brown lesions of circular to oval shape on second day after inoculation on detached spindle leaflets and on third day after inoculation on mature leaflets. On spindle leaflets the lesions were expanded more rapidly, not restricted by mid-vein and were oval to circular with a water-soaked area (Plate 19 A (e)). On mature leaflets it produced oval shaped dark brown lesion surrounded by yellow halo and water-soaked area (Plate 19 A (e)).

Isolate FI 5 B

The pathogen isolate produced tiny brown water-soaked circular to oval lesions on detached spindle leaflets and mature leaflets on third day after inoculation. On spindle leaflets the lesions were expanded more rapidly, not restricted by mid-vein and were oval to circular with a water-soaked area (Plate 19 B (a)). On mature leaflets it produced oval shaped dark brown lesion surrounded by yellow halo and water-soaked area (Plate 19 B (a)).

Isolate FI 6

Tiny brown water-soaked lesions were produced on detached spindle leaflets on fifth day after inoculation, which were expanded and turned to dark brown,

irregular shaped lesions. The lesions were surrounded by water-soaked area and not restricted by mid-vein eventhough the expansion was slowed down (Plate 19 B (b)). On mature leaflets the pathogen did not produce any symptoms.

Isolate FI 7 A

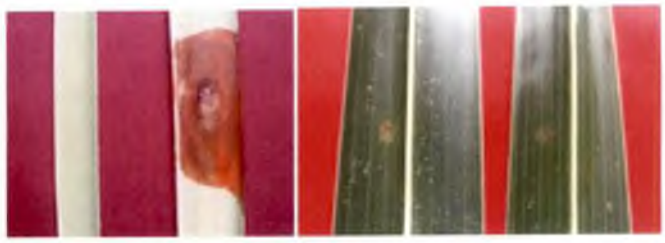
The pathogen produced minute water-soaked light brown lesions of circular to oval shape on third day after inoculation on detached spindle leaflets and mature leaflets. On spindle leaflets the lesions were expanded and not restricted by mid-vein. The expanded lesions were oval shaped with water-soaked area and sporulation underneath (Plate 19 B (c)). On mature leaflets it produced oval shaped dark brown lesion surrounded by chlorotic and water-soaked area. The chlorotic area was later turned to yellow halo. A brown exudate and pink mycelia with spores were visible on the underside of the inoculated area (Plate 19 B (c)).

Isolate FI 7 B

The pathogen produced minute water-soaked light brown lesions of circular to oval shape on third day after inoculation on detached spindle leaflets and mature leaflets. On spindle leaflets the lesions were expanded and not restricted by mid-vein, surrounded by water-soaked area and sporulation beneath (Plate 19 B (d)). On mature leaflets it produced oval shaped dark brown lesion surrounded by chlorotic and water-soaked area. The chlorotic area was later turned to yellow halo. A brown exudate and pink mycelia with spores were visible on the underside of the inoculated area (Plate 19 B (d)).

Isolate FI 8

The fungus produced minute water-soaked light brown lesions of circular to oval shape on fourth day after inoculation on detached spindle leaflets and mature leaflets. The lesions were expanded, turned to reddish brown with an off white area at the centre and mycelial growth beneath (Plate 19 B (e)). On mature leaflets grayish brown circular lesions with small yellow halo and water-soaked region were



(a) Isolate FI 5 B

(b) Isolate FI 6

(c) Isolate FI 7 A

(d) Isolate FI 7 B

(e) Isolate FI 8

Plate 19 B. Symptoms produced by different fungal isolates on detached leaflets

produced. Cream or light brown mycelia were produced on the underside of lesions (Plate 19 B (e)).

Isolate FI 9 A

The pathogen produced tiny brown water-soaked oval shaped lesions on detached spindle leaflets and mature leaflets on third day after inoculation. The lesions were brown coloured, oval to irregular, surrounded by water-soaked area, not restricted by mid-vein and expanded rapidly in the spindle leaflets (Plate 19 C (a)). On mature leaflets it produced an oval shaped dark brown lesion with yellow halo and water-soaked area. A brown exudate was visible beneath the inoculated area on mature leaflets (Plate 19 C (a)).

Isolate FI 9 B

The pathogen isolate produced tiny light brown water-soaked oval shaped lesions on detached spindle leaflets and mature leaflets on third day after inoculation. The lesions were light brown to brown coloured, oval to irregular, surrounded by water-soaked area, not restricted by mid-vein and expanded rapidly in the spindle leaflets (Plate 19 C (b)). On mature leaflets it produced an oval, dark brown lesion with yellow halo and water-soaked area. A brown exudate was visible beneath the inoculated area on mature leaflets (Plate 19 C (b)).

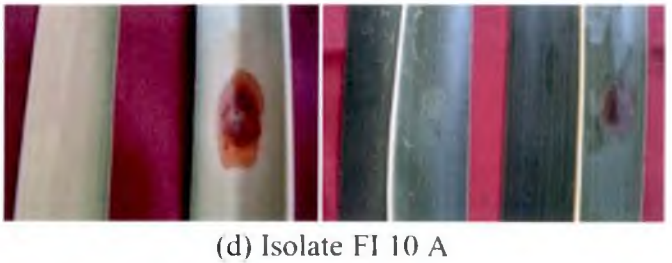
Isolate FI 9 C

The fungal isolate produced tiny light brown water-soaked oval shaped lesions on detached spindle leaflets and mature leaflets on fourth day after inoculation. The lesions were light brown coloured, oval to irregular, surrounded by water-soaked area and restricted by mid-vein in the spindle leaflets (Plate 19 C (c)). On mature leaflets it produced an oval, brown lesion with yellow halo and water-soaked area. A brown exudate was visible beneath the inoculated area on mature leaflets (Plate 19 C (c)).

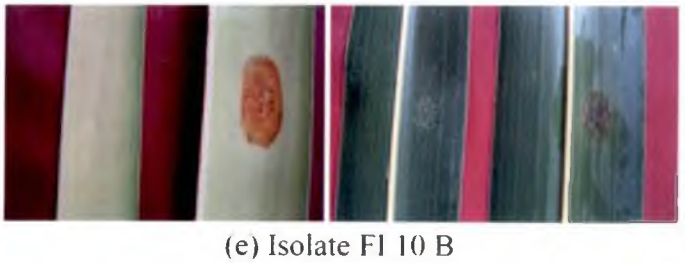
(a) Isolate FI 9 A

(b) Isolate FI 9 B

(c) Isolate FI 9 C



(d) Isolate FI 10 A



(e) Isolate FI 10 B

Plate 19 C. Symptoms produced by different fungal isolates on detached leaflets

Isolate FI 10 A

The pathogen produced minute light brown water-soaked oval shaped lesions on fourth day after inoculation on detached spindle leaflets and on third day after inoculation on mature leaflets. The lesions were light brown, oval to irregular and surrounded by water-soaked area with white mycelia beneath (Plate 19 C (d)). On mature leaflets it produced oval, greyish brown lesion surrounded by brown coloured margin with yellow halo and water-soaked area. Dark brown exudate and whitish mycelia were visible beneath the inoculated area on mature leaflets (Plate 19 C (d)).

Isolate FI 10 B

The pathogen produced minute light brown water-soaked oval shaped lesions on fourth day after inoculation on detached spindle leaflets and on third day after inoculation on mature leaflets. The lesions were light brown, oval shaped and surrounded by water-soaked area with white mycelia beneath (Plate 19 C (e)). On mature leaflets it produced circular, greyish brown lesion surrounded by brown coloured margin with yellow halo and water-soaked area. Dark brown exudate and whitish mycelia were visible underneath (Plate 19 C (e)).

Isolate FI 11

The fungi produced minute light brown water-soaked circular to oval shaped lesions on fifth day after inoculation on detached spindle leaflets and on third day after inoculation on mature leaflets. The lesions were light brown, oval to irregular and surrounded by water-soaked area (Plate 19 D (a)). On mature leaflets it produced greyish circular lesions surrounded by chlorotic water-soaked area. Dark brown exudate and whitish mycelia were visible beneath the inoculated area on mature leaflets (Plate 19 D (a)).

Isolate FI 12

Minute brown water-soaked lesions were produced on detached spindle leaflets on third day after inoculation, which expanded and turned to reddish brown oval

lesions later. The lesions were surrounded by water-soaked area (Plate 19 D (b)). On mature leaflets brown coloured, pin prick sized water-soaked lesions were produced on fourth day after inoculation which were enlarged further became circular to irregular surrounded by yellow halo and water-soaked area. Dark brown exudates were visible underneath (Plate 19 D (b)).

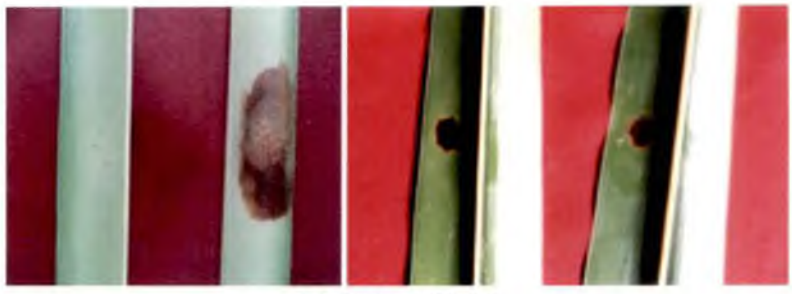
Isolate FI 13

It produced minute dark brown water-soaked circular lesions on detached spindle leaflets and mature leaflets on fourth day after inoculation. The lesions were dark brown, circular to irregular and surrounded by water-soaked area (Plate 19 D (c)). On mature leaflets it produced brown circular lesions with grayish centre surrounded by yellow halo and water-soaked area. Dark brown exudate and brown mycelia were visible on the lower side on mature leaflets (Plate 19 D (c)).

Isolate FI 14

Tiny brown oval water-soaked lesions were produced on detached spindle leaflets on fifth day after inoculation, which expanded and turned to dark brown later. The lesions were surrounded by water-soaked area and not restricted by mid-vein (Plate 19 D (d)). On mature leaflets the pathogen could not produce any symptoms.

(a) Isolate FI 11



(b) Isolate FI 12

(c) Isolate FI 13

(d) Isolate FI 14

Plate 19 D. Symptoms produced by different fungal isolates on detached leaflets

Table 17. Lesion size produced by various pathogen isolates on detached spindle leaflets (cm^2) *

Sl. No.	Pathogen Isolates	Lesion size		Average
		1 WAI (cm^2)	2 WAI (cm^2)	
1	Isolate FI 1	0.47	2.95	1.71
2	Isolate FI 2	0.72	3.59	2.15
3	Isolate FI 3	0.49	1.48	0.99
4	Isolate FI 4	16.39	48.61	32.50
5	Isolate FI 5 A	4.23	21.63	12.93
6	Isolate FI 5 B	1.72	19.90	10.81
7	Isolate FI 6	0.35	3.27	1.81
8	Isolate FI 7 A	2.98	13.34	8.16
9	Isolate FI 7 B	2.62	10.98	6.80
10	Isolate FI 8	0.55	10.31	5.43
11	Isolate FI 9 A	2.71	18.90	10.80
12	Isolate FI 9 B	2.12	15.15	8.64
13	Isolate FI 9 C	0.93	13.10	7.01
14	Isolate FI 10 A	1.18	8.59	4.88
15	Isolate FI 10 B	0.83	9.23	5.03
16	Isolate FI 11	0.32	0.92	0.62
17	Isolate FI 12	0.76	1.54	1.15
18	Isolate FI 13	0.99	13.42	7.21
19	Isolate FI 14	1.96	17.02	9.49

*Mean of three replications

WAI – Week after inoculation

Table 18. Lesion size produced by various pathogen isolates on detached mature leaflets (cm^2) *

Sl. No.	Pathogen Isolates	Lesion size		Average
		1 WAI (cm^2)	2 WAI (cm^2)	
1	Isolate FI 1	0.00	0.00	0.00
2	Isolate FI 2	0.47	2.32	1.39
3	Isolate FI 3	0.04	0.04	0.04
4	Isolate FI 4	8.60	13.95	11.28
5	Isolate FI 5 A	0.48	3.34	1.91
6	Isolate FI 5 B	0.35	0.43	0.39
7	Isolate FI 6	0.00	0.00	0.00
8	Isolate FI 7 A	3.40	5.82	4.61
9	Isolate FI 7 B	1.06	2.36	1.71
10	Isolate FI 8	0.38	0.65	0.52
11	Isolate FI 9 A	2.93	4.60	3.77
12	Isolate FI 9 B	0.46	4.76	2.61
13	Isolate FI 9 C	0.53	0.96	0.75
14	Isolate FI 10 A	3.18	5.62	4.40
15	Isolate FI 10 B	0.39	1.38	0.89
16	Isolate FI 11	0.53	1.78	1.15
17	Isolate FI 12	0.98	1.56	1.27
18	Isolate FI 13	0.88	1.93	1.40
19	Isolate FI 14	0.00	0.00	0.00

*Mean of three replications

WAI – Week after inoculation

4.5.2 Comparative virulence of the fungal isolates

The comparative virulences of different fungal isolates were determined by measuring the time taken for symptom expression and the lesion size on inoculated spindle leaflets and mature leaflets. Based on this the isolates were classified into three categories and results are presented in Tables 19 and 20:

A. On spindle leaflets

1. Highly virulent - time taken for symptom expression was 2 days and lesion size more than 20 cm^2 two weeks after inoculation

2. Moderately virulent - time taken for symptom expression was 3 to 4 days and lesion size between 10 and 20 cm^2 two weeks after inoculation

3. Less virulent - time taken for symptom expression was more than 4 days and lesion size less than 10 cm^2 two weeks after inoculation

B. On mature leaflets

1. Highly virulent - time taken for symptom expression is 2 days and lesion size more than 10 cm^2 two weeks after inoculation

2. Moderately virulent - time taken for symptom expression is 3 to 4 days and lesion size between 4 and 10 cm^2 two weeks after inoculation

3. Less virulent - time taken for symptom expression is more than 4 days and lesion size less than 4 cm^2 two weeks after inoculation

4.5.2.1 Comparative virulence of different fungal isolates based on symptom expression on spindle leaflets

Among the various fungal isolates tested Isolate FI 4 and Isolate FI 5 A produced the lesions on the second day after inoculation on spindle leaflets (Table 19). Isolate FI 4 was found to be more virulent with respect to lesion size (48.6 cm^2 two weeks after inoculation while that of the Isolate FI 5 A was 21.6 cm^2). Eight fungal isolates were found to be moderately virulent which produced the lesions

within 3 to 4 days and the lesion size varied from 10 to 20 cm^2 two weeks after inoculation. Among them the highest lesion size (19.90 cm^2) was produced by Isolate FI 5 B, while the smallest lesion size (10.31 cm^2) was produced by Isolate FI 8. Nine isolates were found to be less virulent, having produced the lesions between 3 to 5 days and their lesion sizes varied from 9.23 cm^2 to 0.92 cm^2, the highest lesion (9.23 cm^2) was produced by FI 10 B and the smallest lesion (0.92 cm^2) was produced by FI 11. Isolate FI 14 although produced the lesion on the 5^{th} day, its lesion size was 17.02 cm^2 (Table 19).

4.5.2.2 Comparative virulence of different fungal isolates based on symptom expression on mature leaflets

Isolate FI 4 was found to be highly virulent, having produced the lesion on second day after inoculation with a lesion size of 13.95 cm^2 (Table 20). Isolate FI 7 A, Isolate FI 10 A, Isolate FI 9 B and Isolate FI 9 A were able to produce symptoms on third day after inoculation having a lesion size of 5.82 cm^2, 5.62 cm^2, 4.76 cm^2, and 4.60 cm^2 respectively two weeks after inoculation. All these isolates were rated as moderately virulent. Eleven isolates were rated as less virulent which were able to produce the symptoms from 3 to 5 days after inoculation and the lesion size varied from 3.34 cm^2 to 0.04 cm^2. Isolate FI 5 A had the maximum lesion size (3.34 cm^2) and Isolate FI 3 had the smallest lesion size (0.04 cm^2). Isolate FI 1, Isolate FI 6 and Isolate FI 14 did not produce any symptoms and were rated as non-pathogenic (Table 20).

Table 19. Comparative virulence of different fungal isolates based on symptom expression on spindle leaflets*

Sl. No.	Fungal Isolate	Category	No. of days taken for symptom expression	Lesion size 2 WAI (cm^2)
1	Isolate FI 4	Highly virulent	2	48.61
2	Isolate FI 5 A	Highly virulent	2	21.63
3	Isolate FI 5 B	Moderately virulent	3	19.90
4	Isolate FI 9 A	Moderately virulent	3	18.90
5	Isolate FI 9 B	Moderately virulent	3	15.15
6	Isolate FI 13	Moderately virulent	3	13.42
7	Isolate FI 7 A	Moderately virulent	3	13.34
8	Isolate FI 9 C	Moderately virulent	4	13.10
9	Isolate FI 7 B	Moderately virulent	3	10.98
10	Isolate FI 8	Moderately virulent	4	10.31
11	Isolate FI 10 B	Less virulent	4	9.23
12	Isolate FI 10 A	Less virulent	4	8.59
13	Isolate FI 2	Less virulent	3	3.59
14	Isolate FI 1	Less virulent	4	2.95
15	Isolate FI 12	Less virulent	4	1.54
16	Isolate FI 3	Less virulent	4	1.48
17	Isolate FI 14	Less virulent	5	17.02
18	Isolate FI 6	Less virulent	5	3.27
19	Isolate FI 11	Less virulent	5	0.92

*Mean of three replications

WAI – Week after inoculation

Table 20. Comparative virulence of different fungal isolates based on symptom expression on mature leaflets*

Sl. No.	Fungal Isolate	Category	No. of days taken for symptom expression	Lesion size 2 WAI (cm^2)
1	Isolate FI 4	Highly virulent	2	13.95
2	Isolate FI 7 A	Moderately virulent	3	5.82
3	Isolate FI 10 A	Moderately virulent	3	5.62
4	Isolate FI 9 B	Moderately virulent	3	4.76
5	Isolate FI 9 A	Moderately virulent	3	4.60
6	Isolate FI 5 A	Less virulent	3	3.34
7	Isolate FI 7 B	Less virulent	3	2.36
8	Isolate FI 2	Less virulent	3	2.32
9	Isolate FI 13	Less virulent	4	1.93
10	Isolate FI 11	Less virulent	3	1.78
11	Isolate FI 12	Less virulent	4	1.56
12	Isolate FI 10 B	Less virulent	3	1.38
13	Isolate FI 9 C	Less virulent	4	0.96
14	Isolate FI 8	Less virulent	4	0.65
15	Isolate FI 5 B	Less virulent	3	0.43
16	Isolate FI 3	Less virulent	5	0.04
17	Isolate FI 1	Non - pathogenic	No symptom	0.00
18	Isolate FI 6	Non - pathogenic	No symptom	0.00
19	Isolate FI 14	Non - pathogenic	No symptom	0.00

*Mean of three replications

WAI – Week after inoculation

4.6 CHARACTERIZATION AND IDENTIFICATION OF FOLIAR FUNGAL PATHOGENS ASSOCIATED WITH YELLOWING DISEASE OF COCONUT

4.6.1 Characterization and identification of foliar fungal pathogens

All the fungal isolates obtained from symptomatic leaves were brought into pure culture and the cultural and morphological characteristics were studied in detail for the identification of the pathogen. These cultures were further sent to Agharkar Research Institute, Pune - 411 004 for validation and confirmation of the identifications made at the Department of Plant Pathology, College of Agriculture, Vellayani. Following are the details of characteristics of the fungal isolates studied.

Isolate FI 1

Initially the colony was dull white with entire margins, reached 9 cm diameter on PDA in 7 days. Profuse aerial mycelium was produced which turned to greenish grey. The reverse side was greyish black. The aerial mycelium was completely absent in aged cultures (Plate 20 A (a)). Conidiophores were short to long, flexuous, septate, hyaline initially turning to golden brown, arising singly measured 75-227.5 µm length. Conidia were obclavate, muriform, dark brown, thick walled; arising singly or in branched or un-branched chains of 6-15 from each conidiophore had 5-7 transverse septa and 0-3 longitudinal septa and of 7.5-17 x 30-77.5 µm. The conidia were beaked and beak measured 5-7.5 x 7.5-62.5 µm (Plate 21 A (a)). The fungal isolate was identified as *Alternaria alternata* (Fr.) Keissler.

Isolate FI 2

The fungus produced fast growing colonies initially whitish, turned to brown from centre when sporulation started (from one day after inoculation), margins smooth, entire and whitish; covered 9 cm on PDA in 4 days (Plate 20 A (b)). Reverse side was cream coloured. The mycelium was fairly loose, white to faintly yellow, largely submerged and had abundant erect conidiophores 12.5-20 x 800-2250 µm bearing conidial structures. Conidiophore was hyaline/ slightly brownish,

smooth walled, with a globose vesicle at tip bearing phialides on its surface producing dark brown spherical conidia of 2.5-4.5 μm diameter (Plate 21 A (b)). The fungal isolate was identified as *Aspergillus niger* Gr.

Isolate FI 3

The fungus was relatively slow growing producing colonies which were initially whitish, turn to yellowish on upside and slight yellow on lower side taking 18 days to cover 9 cm on PDA. Slightly aerial mycelium was produced on PDA which forms ropy strands near the point of inoculation in the centre of the petri dish (Plate 20 A (c)). The colony had relatively flat growth, slightly moist texture and filiform margins which were glabrous initially. The aerial mycelium was diffused towards edges and somewhat denser in the older parts of the colony. Hyaline hyphae produced simple, erect, unbranched phialides tapering towards tip on which hyaline, globose to cylindrical, slightly fusiform, single celled conidia of 1.25-2.5 x 3.75-7.5 μm were produced in slimy heads (Plate 21 A (c)). The fungal isolate was identified as *Cephalosporium* spp.

Isolate FI 4

The colonies were fast growing reaching 9 cm diameter on PDA in two days, grayish white initially with filiform margins, filamentous radiating growth with immersed and aerial mycelium, turning to black, powdery upon abundant sporulation from third day after inoculation into media (Plate 20 A (d)). The fungus produced fruity smell on all the artificial media tried *viz.* CA, MEA, PDA and CDA. The reverse side was grayish white initially turned to black. Phialides were abundant, two types were produced usually *viz.* short type (10-27.5 μm long) that produce short chains of oval, conidia hyaline initially turned to dark brown thick walled one on maturity, 3.5-7 x 10-17.5 μm sized (Plate 21 A (d1)) and long type (55-90 μm long) that produce long chains of cylindrical conidia which were hyaline, 4-5 x 8-15 μm sized (Plate 21 A (d2)). The cylindrical conidia were also darkened, became pale

brown and thick walled (Plate 21 A (d3)). Chlamydospores were produced singly or in chains. The fungal isolate was identified as *Chalara fimbriata* Ellis & Halst.

Isolate FI 5 (FI 5 A and FI 5 B)

The colonies were fast growing, reached 9 cm diameter in 5 days, white to grayish white, raised, with aerial mycelium on PDA (Plate 20 A (e) and Plate 20 A (f)). The reverse side was white initially, turned to greenish black from centre. Colonies had entire, smooth margins. Zonations were present. Mycelium was hyaline, superficial, septate and branched. Spores were abundant and sporulation started from centre in the setose acervuli formed. Sporulation was comparatively lower in the Isolate FI 5 B. Setae were septate, brown, smooth, tapering towards end and measured 3-5 x 37.5-85 µm. Spores were emerged in pinkish orange, slimy spore masses. Conidia were hyaline, aseptate, straight-slightly bent, slightly narrower in the middle, ends rounded, oblong to cylindrical, thin walled, guttulate measured 2.5-4 x 7.5-17.5 µm (Plate 21 A (e) and Plate 21 A (f)).

The fungal isolates were identified as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.

Isolate FI 6

Colonies were grayish white initially turned to grayish black, with aerial mycelium covered 9 cm diameter in 5 days on PDA (Plate 20 A (g)). Reverse black. The margins were smooth, entire and white initially. Hyphae were septate and brown coloured. Conidiophores were brown coloured, simple or branched, septate, exhibited sympodial geniculate growth (bent at the points where the conidia originate). The fungus produced four celled pyriform curved conidia of 7.5-11.5 x 17.5-27.5 µm. The middle cells were larger and brown coloured. The second cell was large, broad and dark brown. The end cells were light brown and small (Plate 21 A (g)). The fungal isolate was identified as *Curvularia* spp.

Isolate FI 7 (FI 7 A and FI 7 B)

On PDA cultures initially had white mycelia which turned to pinkish purple colour from centre and develop violet pigments with age. The colonies were having irregular margins, took 9 days to complete growth in 9 cm petri dish. The surface of the colonies had a veined appearance initially which turned to powdery after sporulation (Plate 20 A (h)). Delicate aerial mycelium was produced. Zonations were observed on the back side and had darker pink turning to purple and violet later. Upon ageing it turned to dark violet or dark magenta which looked almost black. The isolate FI 7 B was producing veined, mucoid colonies with little aerial growth even after sporulation (Plate 20 A (i)). Mycelium was septate. Macroconidia were rarely produced on PDA medium. Macroconidia (produced on CLA) were long and slender 2-3 x 25-35 µm, slightly falcate, thin walled, hyaline, 3-5 sepatate with curved and tapering apical cell and notched basal cell (Plate 21 B (a1)). Microconidia were produced from conidiogenous cells which were usually monophialides but sometimes produced in pairs. These were abundant in aerial mycelium and produced on conidiophores of 3-4 x 17.5-35 µm in long chains. Sometimes false were heads present. The conidia were small, hyaline, non-septate, obovoid and 2-2.5 x 5-10 µm sized (Plate 21 B (a2)). Chlamydospores were not noticed in culture. The fungal isolates were identified as *Fusarium verticillioides* (Saccardo) Nirenberg.

Isolate FI 8

Colonies were fast growing with entire margins, covered PDA/ PSA in 9cm petri dish within 5 days and produced abundant aerial mycelium that initially white, turned to off-white, beige or light brown with age (Plate 20 A (j)). On the lower surface light orange pigmentation was observed which became darker with age. Hyphae were hyaline and septate. Light orange sporodochia was produced in older cultures on PSA and CLA. Macroconidia were 2-3.5 x 30-40 µm, 3-6 septate,

falcate, slender, hyaline, ventral surface relatively straight with curved, tapering apical cell and a foot shaped basal cell (Plate 21 B (b1)). Straight/ fusoid, spindle shaped 2-3.5 x 12.5-15 μm, 3-5 septate, hyaline mesoconidia produced from polyphialides (2-4 x 62.5-70 μm) in single or two in the aerial mycelia and these were most abundant in culture (Plate 21 B (b2)). Microconidia were pyriform to obovate, single septate, 1.85-2.5 x 6-10 μm sized only produced in older cultures (Plate 21 B (b3)). Globose chlamydospores were present either singly or in chains in the hypha, hyaline initially turned to light yellow. The fungal isolate was identified as *Fusarium semitectum* Berk. & Rav.

Isolate FI 9 (FI 9 A, FI 9 B and FI 9 C)

Isolate FI 9 A produced greyish black fast growing colonies which reached 9cm growth 3 days after inoculation on PDA, produced abundant aerial mycelium and spores. Mycelium was initially white, floccose which turned to off white and to grayish black, immersed and superficial, septate (Plate 20 A (k)). Margins wavy. The lower side of agar plate was black. Sporulation started from the fifth day after inoculation. The fungus produced pear shaped, shiny, black, stromatic pycnidia of 145-172.5 x 180-220 μm scattered on the media from which spores were oozing out through the apical ostiole (Plate 21 B (c1)).

Isolate FI 9 B produced grey coloured fast growing colonies which reached 9 cm diameter in 3 days, had abundant aerial mycelium and good sporulation. Mycelium was initially white, floccose which turned to off white and to grey, immersed and superficial, septate (Plate 20 A (l)). Margins wavy. The lower side of agar plate had black colour at centre and rest was dark grey. Pycnidia were produced at the centre and periphery of media.

Isolate FI 9 C covered 9cm diameter in 5 days, had abundant aerial mycelium, cottony white initially turned to off-white later. Septate, Immersed and superficial mycelium was produced (Plate 20 B (a)). Margins wavy. The lower surface of the

agar plate had off-white colour. Sporulation was very poor and started from ninth day after inoculation. Pycnidia were produced at the periphery of media.

Paraphyses were cylindrical, hyaline and of 55-58 µm. Conidia were sub-ovoidal to ellipsoidal, single celled and hyaline with a granular content when immature. On maturity the conidia were melanized and became dark brown coloured, thick walled, ellipsoidal and had a transverse septum and longitudinal striations. The conidia measured 6.5-14 x 15-26.5 µm (Plate 21 B (c2)).

The fungal isolates were identified as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.

Isolate FI 10 (FI 10 A and FI 10 B)

The fungal isolates produced cottony white mycelium with zonations and irregular margin, reverse cream to slightly yellow (Plate 20 B (b) and (Plate 20 B (c)). Diameter reached 9 cm in 6-7 days on PDA. Acervuli (160-200 µm) developed from small clumps of hyphae after five days and produced conspicuous black spore masses. Sporulation was superficial and submerged. The pathogen produced 5 celled, smooth, fusiform conidia, usually straight, slightly constricted at septa, 5-6.5 x 16.5-21 µm sized, upper and lower cells were hyaline, three middle cells were brown coloured, concolourous. The three slender, hyaline apical appendages were 15-20 µm long and the short hyaline pedicel was 2-3.5 µm long (Plate 21 C (a)). The fungal isolates were identified as *Pestalotiopsis maculans* (Corda) Nag Raj.

Isolate FI 11

The fungus produced cottony white mycelium with distinct zonations and irregular margins. Diameter reached 9 cm in 8 days on PDA. The lower surface was cream initially turned to yellow later. Upon ageing the cultures were turned yellow (Plate 20 B (d)). Acervuli developed from small clumps of hyphae after five days and produced conspicuous black spore masses. The pathogen produced 5 celled fusiform conidia, usually straight, slightly constricted at septa, 5.5-7.5 x 19-25 µm

sized, upper and lower cells were hyaline, three middle cells were coloured olivaceous brown, versicolourous (single median cell darker than other ones). The upper cell had three longer, slender, hyaline appendages 20-27.5 µm long. There was a short hyaline pedicel 2-5 µm long below the lower hyaline cell (Plate 21 C (b)). The fungal isolate was identified as *Pestalotiopsis palmarum* (Cooke) Stey.

Isolate FI 12

It produced relatively fast growing white floccose colonies on PDA which cover 9 cm diameter in 4 days. Margins wavy, zonations present, a clear zone around the inoculated area which had only thin flat mycelium, under surface white (Plate 20 B (e)). When it became older, cultures had black sheets and reverse became pale brown. Conidia were produced in pycnidia, hyaline, fusiform-narrow oval, single celled, acute-sub acute at the ends, guttulate, 2-2.5 x 6.5-8.75µm. The fungal isolate was identified as *Phomopsis* spp.

Isolate FI 13

The fungus produced relatively fast growing colonies which cover 9 cm growth on PDA within 4 days. Mycelium was cream-brown, floccose and aerial with zonations (Plate 20 B (f)). Sporulation was noticed massively in the black spots of mycelium where conidia produced from almost hyaline sporodochia. Conidiophores were unbranched, hyaline, smooth, thread like, straight or flexuous, 12.5-27.5 µm long with a swollen base (Plate 21 C (c1)). Conidia was single celled, double walled, hyaline initially, turned to brown when mature, measured 6.5-10 x 9.5-13.5 µm (Plate 21 C (c2)). The fungal isolate was identified as *Arthrinium* spp.

Isolate FI 14

Colonies were slow growing, reaching 7 cm after 21 days in PDA, margins entire, grey to olivaceous black, later often iridescent with encrusted colony centre (Plate 20 B (g)). Reverse side was dark grey. Vegetative hyphae were hyaline to dark brown. In young cultures simple phialides or short, atypical, conidiophores

were formed and after 2-3 weeks normal branched dark brown repeatedly verticillate conidiophores appeared (Plate 21 C (d1)). Phialides were almost hyaline with a constriction at the base and flask shaped with a long narrow neck, 1.25-2.5 x 14.5-32.5 µm. At the apex of the phialides, ellipsoidal or ovoidal, hyaline, 1.0-2.0 x 1.5-3.0 µm sized conidia were aggregating in slimy heads (Plate 21 C (d2)). The fungus was also sporulating within the medium with lighter conidiophores and subglobose conidia, 1.5-2.2 µm. Chlamydospores were absent. The fungus isolate was identified as *Verticillium* spp.

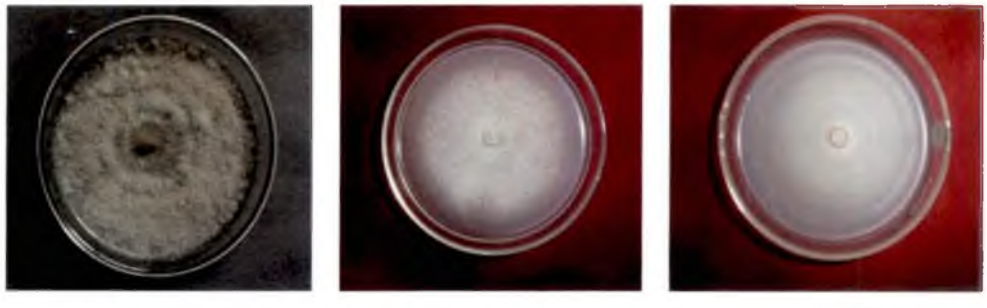
The final consolidated list of fungal isolates and their identifications are presented in Table 21.

Table 21. Foliar fungal pathogens associated with Yellowing Disease of coconut

Sl. No.	Pathogen Isolates	Name of the pathogen
1	Isolate FI 1	*Alternaria alternata* (Fr.) Keissler.
2	Isolate FI 2	*Aspergillus niger* Gr.
3	Isolate FI 3	*Cephalosporium* spp.
4	Isolate FI 4	*Chalara fimbriata* Ellis & Halst.
5	Isolate FI 5 A	*Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc.
6	Isolate FI 5 B	*Colletotrichum gloeosporioides* (Penz.) Penz. And Sacc.
7	Isolate FI 6	*Curvularia* spp.
8	Isolate FI 7 A	*Fusarium verticillioides* (Saccardo) Nirenberg.
9	Isolate FI 7 B	*Fusarium verticillioides* (Saccardo) Nirenberg.
10	Isolate FI 8	*Fusarium semitectum* Berk. & Rav.
11	Isolate FI 9 A	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.
12	Isolate FI 9 B	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.
13	Isolate FI 9 C	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.
14	Isolate FI 10 A	*Pestalotiopsis maculans* (Corda) Nag Raj.
15	Isolate FI 10 B	*Pestalotiopsis maculans* (Corda) Nag Raj.
16	Isolate FI 11	*Pestalotiopsis palmarum* (Cooke) Stey.
17	Isolate FI 12	*Phomopsis* spp.
18	Isolate FI 13	*Arthrinium* spp.
19	Isolate FI 14	*Verticillium* spp.

(a) Isolate FI 1 (b) Isolate FI 2 (c) Isolate FI 3

(d) Isolate FI 4 (e) Isolate FI 5 A (f) Isolate FI 5 B

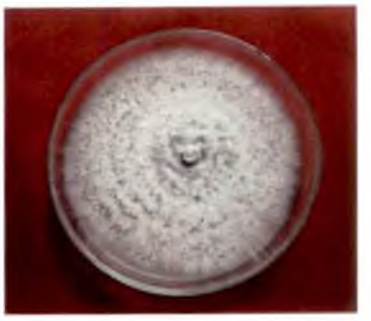


(g) Isolate FI 6 (h) Isolate FI 7 A (i) Isolate FI 7 B

(j) Isolate FI 8 (k) Isolate FI 9 A (l) Isolate FI 9 B

Plate 20 A. Growth of pathogen isolates on culture media

(a) Isolate FI 9 C

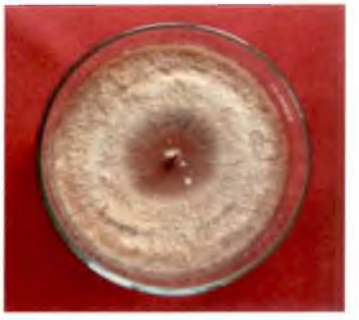


(b) Isolate FI 10 A

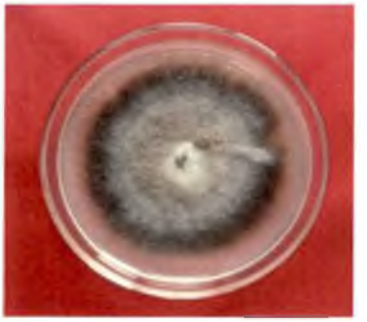
(c) Isolate FI 10 B

(d) Isolate FI 11

(e) Isolate FI 12

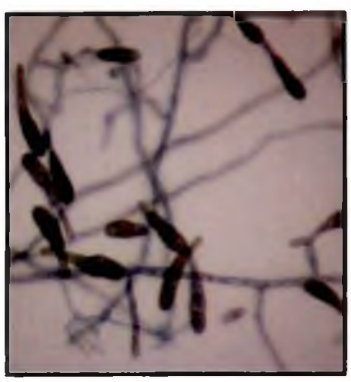


(f) Isolate FI 13



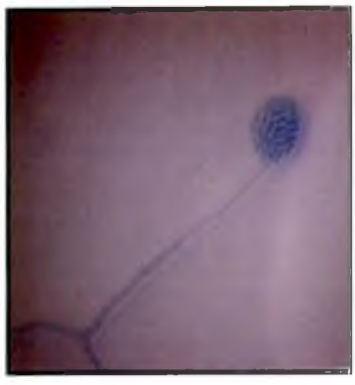
(g) Isolate FI 14

Plate 20 B. Growth of pathogen isolates on culture media



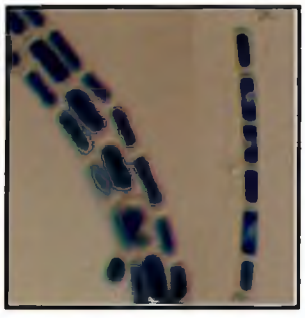
(a) Conidia of Isolate FI 1 (*Alternaria alternata*) (400x)

(b) Conidial head of Isolate FI 2 (*Aspergillus niger*) (400x)

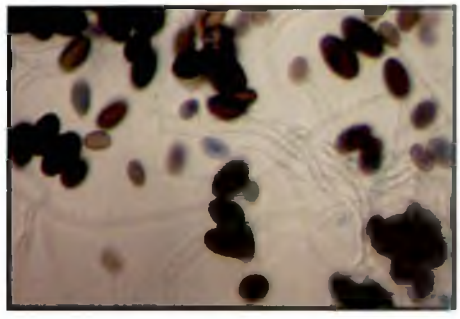


(c) Conidia of Isolate FI 3 (*Cephalosporium* spp.) produced in slimy heads (1000x)

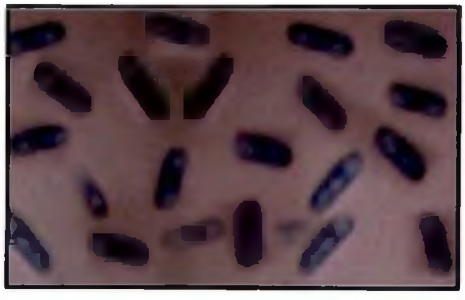
(d1) Immature oval conidia of Isolate FI 4 (*Chalara fimbriata*) (400x)



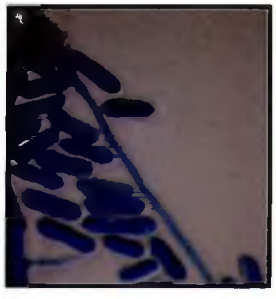
(d2) Immature cylindrical conidia of Isolate FI 4 (*C. fimbriata*) (1000x)



(d3) Mature oval conidia and cylindrical conidia of Isolate FI 4 (*C. fimbriata*) (400x)



(e) Conidia of Isolate FI 5 A (*C. gloeosporioides*) (1000x)

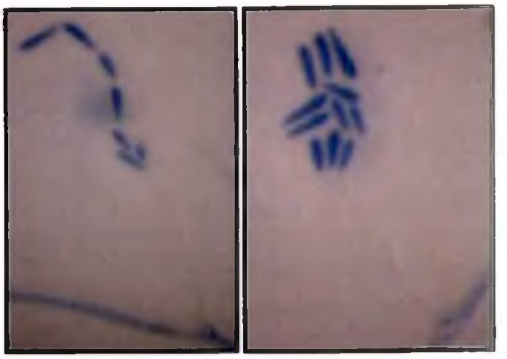


(f) Conidia of Isolate FI 5 B (*C. gloeosporioides*) 1000x

(g) Conidia of Isolate FI 6 (*Curvularia* spp.) (400x)

Plate 21 A. Microphotographs of pathogen isolates

(a1) Macroconidia of Isolate FI 7 A (*Fusarium verticillioides*) (1000x)

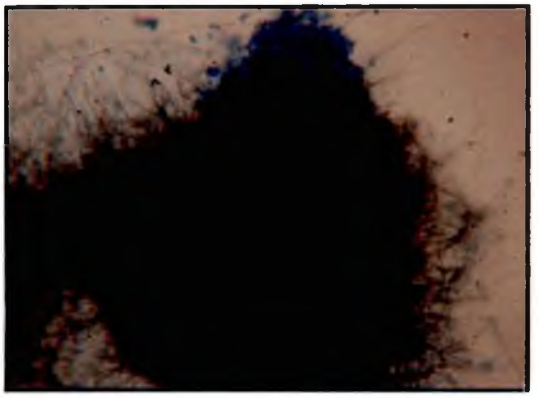


(a2) Microconidia of Isolate FI 7 A (*F. verticillioides*) (1000x)

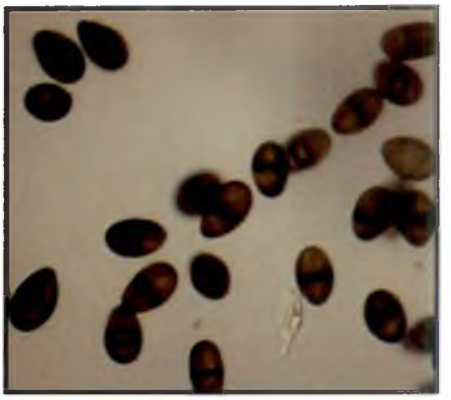
(b1) Macroconidia of Isolate FI 8 (*Fusarium semitectum*) (1000x)

(b2) Mesoconidia of Isolate FI 8 (*F. semitectum*) (1000x)

(b3) Microconidia of Isolate FI 8 (*F. semitectum*) (1000x)



(c1) Pycnidia with immature conidia of Isolate FI 9 A (*Lasiodiplodia theobromae*) (100x)

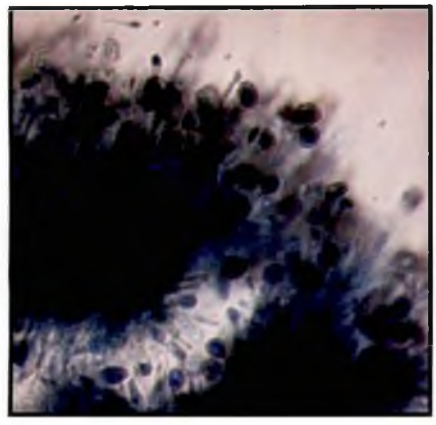


(c2) Conidia of Isolate FI 9 A (*L. theobromae*) (400x)

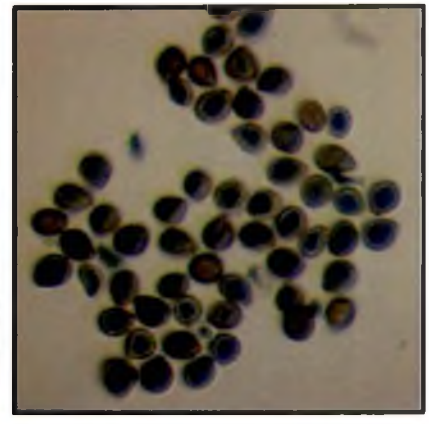
Plate 21 B. Microphotographs of pathogen isolates

(a) Conidia of Isolate FI 10 A (*Pestalotiopsis maculans*) (400x)

(b) Conidia of Isolate FI 11 (*Pestalotiopsis palmarum*) (400x)

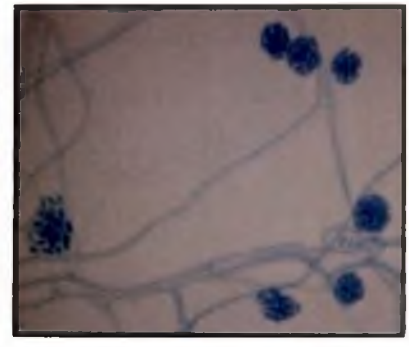


(c1) Sporodochia of Isolate FI 13 (*Arthrinium* spp.) (400x)



(c2) Conidia of Isolate FI 13 (*Arthrinium* spp.) (1000x)

(d1) Phialides bearing aggregates of conidia of Isolate FI 14 (*Verticillium* spp.) (400x)



(d2) Conidia of Isolate FI 14 (*Verticillium* spp.) (1000x)

Plate 21 C. Microphotographs of pathogen isolates

4.6.2 Fungal pathogens associated with yellowing disease of coconut during different seasons

Various fungal pathogens which were isolated from symptomatic leaves of coconut palms during the three seasons namely October-January, March-May, June-July 2009-2010 are identified as illustrated in the Table 22.

The maximum number of pathogens were isolated during the summer season (March-May, 2010). The most extensively occurring fungal pathogens among all the six blocks of the Instructional Farm were *L. theobromae, P. maculans, C.gloeosporioides* and *P. palmarum.* Isolate PA 13 eventhough isolated from only three blocks had an extensive occurrence there. The maximum number of fungal pathogens was isolated from Block A and B. They were *A. alternata, A. niger, Cephalosporium* spp., *C. fimbriata, C. gloeosporioides, F. semitectum, F. verticillioides, L. theobromae, P. maculans, Phomopsis* spp. and *Arthrinium* spp. from Block A. All these pathogens and *Curvularia* spp., *P. palmarum* and *Verticillium* spp. were observed from symptomatic leaves collected from Block B.

During the period from October to January (2009-2010) the pathogens isolated from the different blocks were *C. gloeosporioides, L. theobromae, F. verticillioides, P. maculans, P. palmarum* and *Phomopsis* spp. More number of pathogens were isolated from block A while only one pathogen could be obtained from block F. The most frequently occurring pathogens were *F. verticillioides, P. palmarum* and *C. gloeosporioides.*

During the rainy season (June-July, 2010) the different plant pathogens isolated from different blocks were *C. gloeosporioides, L. theobromae, P. maculans, P. palmarum, C. fimbriata* and *Phomopsis* spp. Among them the most frequently occurring pathogens in all the blocks were *C. gloeosporioides* and *P. maculans* and the least isolated was *C. fimbriata* and *Phomopsis* spp.

Table 22. Foliar fungal pathogens associated with yellowing disease of coconut during different seasons

Block	Pathogens isolated		
	October-January	March-May	June-July
A	*Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Fusarium verticillioides, P. maculans, P. palmarum, Phomopsis* spp.	*Alternaria alternata, Aspergillus niger, Cephalosporium* spp., *Chalara fimbriata, Colletotrichum gloeosporioides, F. verticillioides, Fusarium semitectum, L. theobromae, P. maculans, P. palmarum, Phomopsis* spp., *Arthrinium* spp.	*Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Pestalotiopsis maculans, Pestalotiopsis palmarum*
B	*Colletotrichum gloeosporioides, F. verticillioides, Pestalotiopsis maculans, Pestalotiopsis palmarum*	*A. alternata, A. niger, C. fimbriata, Cephalosporium* spp., *C. gloeosporioides, Curvularia* spp., *F. verticillioides, F. semitectum, L. theobromae, P. maculans, P. palmarum, Phomopsis* spp., *Arthrinium* spp., *Verticillium* spp.	*C. gloeosporioides, C. fimbriata, Lasiodiplodia theobromae, Pestalotiopsis maculans, P. palmarum, Phomopsis* spp.
C	*C. gloeosporioides, P. maculans, F. verticillioides, L. theobromae, P. palmarum*	*C. gloeosporioides, P. maculans, P. palmarum, F. verticillioides, L. theobromae*	*C. gloeosporioides, P. maculans, P. palmarum*
D	*C. gloeosporioides, F. verticillioides, L. theobromae, P. maculans*	*C. gloeosporioides, F. verticillioides, F. semitectum, L. theobromae, P. maculans, Phomopsis* spp.	*C. gloeosporioides, L. theobromae, P. maculans, P. palmarum*
E	*F. verticillioides, P. palmarum*	*F. verticillioides, Lasiodiplodia theobromae, P. maculans, P. palmarum*	*C. gloeosporioides, P. maculans*
F	*Pestalotiopsis palmarum*	*F. verticillioides, L. theobromae, P. palmarum, Arthrinium* spp.	*C. gloeosporioides, L. theobromae, P. maculans, P. palmarum*

4.6.3 Frequency of occurrence of foliar fungal pathogens in symptomatic leaves of coconut palms

L. theobromae was the most frequently isolated pathogen from the spots and blights in the chlorotic/ yellowed leaves and leaf rot affected mature leaves of mid-whorl yellowing affected palms in the Instructional Farm. The next two pathogens isolated most frequently include *C. gloeosporioides* from leaf rot specimen and spots and blights in the chlorotic/ yellowed leaves and *P. maculans* from spots and blights in the chlorotic/ yellowed leaves and leaf rot affected mature leaves (Table 23).

Table 23. Frequency of occurrence of various pathogens

Name of the pathogen		Frequency of isolation	
Alternaria alternata (Isolate FI 1)		2	
Aspergillus niger (Isolate FI 2)		2	
Cephalosporium spp. (Isolate FI 3)		2	
Chalara fimbriata (Isolate FI 4)		3	
Colletotrichum gloeosporioides	(Isolate FI 5 A)	10	16
	(Isolate FI 5 B)	6	
Curvularia spp. (Isolate FI 6)		2	
Fusarium verticillioides	(Isolate FI 7 A)	9	14
	(Isolate FI 7 B)	5	
Fusarium semitectum (Isolate FI 8)		4	
Lasiodiplodia theobromae	(Isolate FI 9 A)	11	20
	(Isolate FI 9 B)	6	
	(Isolate FI 9 C)	3	
Pestalotiopsis maculans	(Isolate FI 10 A)	14	16
	(Isolate FI 10 B)	2	
Pestalotiopsis palmarum (Isolate FI 11)		12	
Phomopsis spp. (Isolate FI 12)		10	
Arthrinium spp. (Isolate FI 13)		7	
Verticillium spp. (Isolate FI 14)		1	

F. verticillioides, P. palmarum and *Phomopsis* spp. were the other pathogens isolated in more frequency. *A. alternata, Cephalosporium* spp., *C. fimbriata, Curvularia* spp., *F. semitectum, Arthrinium* spp. and Isolate *Verticillium* spp. were found associated with symptomatic leaves in lesser number of palms.

4.7 BIOCHEMICAL ANALYSIS OF COCONUT LEAVES

Changes in total sugars, total soluble proteins and activity of phenylalanine ammonia lyase in three different whorls of leaves *viz.* outer whorl, middle whorl and inner whorl were determined at three different stages of infection in the palm *i.e.*, early stage, moderate stage and severe stage and compared with that of healthy palms.

4.7.1 Total sugars

The total sugars in the infected and healthy palms were estimated and the data are presented in Table 24. It was found that the total sugars in the infected palms at all stages of infection were significantly higher than that in the healthy palms. At the early stage of infection the total sugars were increased and reached 72.9, 79.5 and 79.8 mg g^{-1} in the outer, middle and inner whorl respectively. At the moderate stage the values decreased significantly from that of the early stage. Greatest reduction was obtained in the outer whorl (59.1 mg g^{-1}) followed by middle whorl (74.6 mg g^{-1}) and inner whorl (75.4 mg g^{-1}). There was a gradual and significant increase in total sugars in the outer whorl and middle whorl of the palm at severe stage (71.4 mg g^{-1} and 78.5 mg g^{-1} respectively). The gradual increase in the total sugar contents was also observed in the inner whorls (78.4 mg g^{-1}). In the healthy palm the total sugar content was more or less similar in different whorls of the palm. But in the infected palms the total sugars were significantly lower in the outer whorls when compared with the middle and inner whorls. In the middle and inner whorls there was no significant difference with regard to the total sugar content in infected palms at all stages of infection.

Table 24. Changes in total sugars of coconut leaves at different stages of infection*

Health status of the palm	Outer whorl ($mg\ g^{-1}$)	Middle whorl ($mg\ g^{-1}$)	Inner whorl ($mg\ g^{-1}$)	Mean
Healthy (H)	63.0	63.3	64.0	63.4
Disease at early stage (E)	72.9	79.5	79.8	77.4
Disease at moderate stage (M)	59.1	74.6	75.4	69.7
Disease at severe stage (S)	71.4	78.5	78.4	76.1
Mean (Diseased)	67.8	77.5	77.9	

*Mean of three replications

CD values: Health status of palm 5.444

Different whorls of palm 1.752

Health status vs. whorls 3.505

Table 25. Per cent increase or decrease of total sugars over healthy palms

Stage of infection of the palm	Outer whorl (%)	Middle whorl (%)	Inner whorl (%)	Mean (%)
Disease at early stage (E)	15.7	25.6	24.7	22
Disease at moderate stage (M)	-6.2	17.9	17.8	9.8
Disease at severe stage (S)	13.3	24.0	22.5	19.9
Mean	7.6	22.5	21.7	

In the outer whorls the total sugars were widely varied in the infected palms. While there was 15.7 per cent increase at the early stage of infection, it was 13.3 per cent at the severe stage of infection as against a 6.2 per cent reduction at the moderate stage. However these values were very much higher in the middle whorls and inner whorls of the palm. In general the per cent increase of total sugars in the outer whorl of infected palms even though higher than the healthy palms, was much less than that of the middle whorl and inner whorl. In the middle and inner whorls of the palms the total sugars showed almost similar trends at different stages of infection. In the middle and inner whorls of the palms there was 25.6 per cent and

24.7 per cent increase respectively at the early stage of infection, which was 17.9 per cent and 17.8 per cent respectively at the moderate stage and 24 per cent and 22.5 per cent respectively at the severe stage of infection (Table 25).

4.7.2 Total soluble proteins

The results of the study on the total soluble protein in yellowing disease affected palms at different stages of infection are presented in Table 26.

Table 26. Changes in total soluble proteins of coconut leaves at different stages of infection*

Health status of the palm	Outer whorl (mg g^{-1})	Middle whorl (mg g^{-1})	Inner whorl (mg g^{-1})	Mean
Healthy (H)	3.27	3.08	2.83	3.06
Disease at early stage (E)	8.00	7.82	8.47	8.10
Disease at moderate stage (M)	5.48	4.60	4.32	4.80
Disease at severe stage (S)	3.83	3.71	3.91	3.82
Mean (Diseased)	5.77	5.38	5.57	

*Mean of three replications

CD values: Health status of palm 0.312

Different whorls of palm 0.276

Health status vs. whorls 0.553

The total soluble protein contents in the different whorls of the infected palms were found to be significantly higher than that of the healthy palms at all stages of infection. Consequent to infection the total soluble proteins increased to the highest level at early stage of infection in all the whorls of the palm (8.00, 7.82, 8.47 mg g^{-1} in outer whorl, middle whorl and inner whorl respectively as compared to 3.27, 3.08 and 2.83 mg g^{-1} in the healthy palms) and thereafter it decreased to significantly lower levels at moderate stage (5.48, 4.60 and 4.32 mg g^{-1} respectively) and to further lower levels at the severe stage of infection (3.83, 3.71 and 3.91 mg g^{-1}

respectively). There was also significant difference in the total protein contents between middle whorl (7.82 mg g^{-1}) and inner whorl (8.47 mg g^{-1}) at the early stage of infection, while at moderate stage the total soluble protein content was significantly higher in the outer whorl (5.48 mg g^{-1}) as compared to middle and inner whorl (4.60 and 4.32 mg g^{-1} respectively). However at severe stage of infection, there was no significant difference in the total soluble proteins between the different whorls (3.83, 3.71 and 3.91 mg g^{-1} in the outer whorl, middle whorl and inner whorl respectively).

The study further revealed that the increase of total soluble proteins in the infected palms was highest in the inner whorl (199.3 %), followed with that in the middle whorl (153.9 %) and in the outer whorl (144.7 %) at the early stage of infection (Table 27). Subsequently from the peak of increase a gradual reduction in the total soluble proteins was observed in all the different whorls at moderate stage of infection (67.6 %, 49.4 % and 52.7 %) and further these values decreased to still lower levels (17.1 %, 20.5 % and 38.2 %) at severe stage of infection. The least per cent increase was noticed in the outer whorl (17.1 %) at severe stage of infection (Table 27).

Table 27. Per cent increase of total soluble proteins over healthy palms

Stage of infection of the palm	Outer whorl (%)	Middle whorl (%)	Inner whorl (%)	Mean (%)
Disease at early stage (E)	144.7	153.9	199.3	166.0
Disease at moderate stage (M)	67.6	49.4	52.7	56.6
Disease at severe stage (S)	17.1	20.5	38.2	25.3
Mean	76.5	74.6	96.7	

4.7.3 Phenylalnine ammonia lyase (PAL)

The activity of Phenylalnine ammonia lyase (PAL) in the infected palms as compared to healthy palms at different stages of infection in different whorls of the

palms is illustrated in the Table 28. The result showed that the activity of PAL in the infected palms at all stages of infection in different whorls of the palm were significantly higher than that of the healthy palms. There was a very rapid increase in the PAL activity at early stages of infection in all whorls of the palm (54.67 μg g^{-1}, 56.33 μg g^{-1} and 58.22 μg g^{-1} at early stage of infection as compared to 24.78 μg g^{-1}, 24.33 μg g^{-1} and 21.22 μg g^{-1} in the healthy palms). Thereafter these values decreased significantly and reached an average value of 32.15 μg g^{-1} at moderate stage of infection and 28.63 μg g^{-1} at severe stage of infection.

Table 28. Changes in PAL acivity of coconut leaves at different stages of infection*

Health status of the palm	Outer whorl (μg g^{-1})	Middle whorl (μg g^{-1})	Inner whorl (μg g^{-1})	Mean
Healthy (H)	24.78	24.33	21.22	23.44
Disease at early stage (E)	54.67	56.33	58.22	56.41
Disease at moderate stage (M)	29.89	34.22	32.33	32.15
Disease at severe stage (S)	28.44	28.00	29.44	28.63
Mean (Diseased)	37.67	39.52	39.99	

*Mean of three replications

CD values: Health status of palm 4.111

Different whorls of palm 1.706

Health status vs. whorls 3.412

The per cent increase of PAL activity in infected palms at early stage of infection was found to be highest in the inner whorl (174.4 %) followed with that in the middle whorl (131.5 %) and then in the outer whorl (120.6 %). Although decreased subsequently at later stages of infection the same trend of highest increase in the PAL activity was observed in the inner whorl of leaves at moderate stage of infection (52.4 %) and also at severe stage of infection (38.7 %). Least increase in PAL activity was observed in the outer whorl of leaves at all stages of infection

(120.6 % at early stage, 20.6 % at moderate stage and 14.8 % at severe stage) as compared to the inner whorl (174.4 %, 52.4 % and 38.7 % respectively) (Table 29).

Table 29. Per cent increase of PAL activity over healthy palms

Stage of infection of the palm	Outer whorl (%)	Middle whorl (%)	Inner whorl (%)	Mean (%)
Disease at early stage (E)	120.6	131.5	174.4	142.2
Disease at moderate stage (M)	20.6	40.6	52.4	37.9
Disease at severe stage (S)	14.8	15.1	38.7	22.9
Mean (Diseased)	52.0	62.4	88.5	

4.8 *IN VITRO* EVALUATION OF FUNGICIDES AGAINST THE PREDOMINANT FOLIAR FUNGAL PATHOGENS

The results of the *in vitro* evaluation of fungicides to test their efficacy against the predominant fungal pathogens in the leaves of Yellowing Disease affected coconut palms *viz.*, *Lasiodiplodia theobromae* (Plate 22), *Colletotrichum gloeosporioides* (Plate 23), *Pestalotiopsis maculans* (Plate 24) and *Fusarium verticillioides* (Plate 25) are presented in the Table 30.

All the fungicides inhibited the growth of the pathogens tested. Among the different fungicides tested propiconazole, tebuconazole and mancozeb completely inhibited the growth of all the pathogens tested. Carbendazim also completely inhibited the growth of *L. theobromae, P. maculans* and *F. verticillioides.* Flusilazole was also found to be highly effective in completely inhibiting *C. gloeosporioides* and above 88 per cent inhibition of all the other fungi tested. A combination product of captan and hexaconazole resulted in above 87 per cent inhibition of all the fungal pathogens tested. Among the different fungicides tested the least effective fungicide was found to be the combination product of hexaconazole and zineb, followed by copper hydroxide and hexaconazole.

Plate 22. Effect of fungicides on growth of *Lasiodiplodia theobromae*

T 1 Propiconazole; T 2 Tebuconazole; T 3 Flusilazole;
T 4 Hexaconazole; T 5 Carbendazim; T 6 Mancozeb;
T 7 Hexaconazole+Zineb; T 8 Captan+Hexaconazole; T 9C Control

Plate 23. Effect of fungicides on growth of *Colletotrichum gloeosporioides*

T 1 Propiconazole; T 2 Tebuconazole; T 3 Flusilazole; T 4 Hexaconazole;
T 5 Carbendazim; T 6 Mancozeb; T 7 Copper hydroxide;
T 8 Hexaconazole+Zineb; T 9 Captan+Hexaconazole; T 10C Control

Plate 24. Effect of fungicides on growth of *Pestalotiopsis maculans*

T 1 Propiconazole; T 2 Tebuconazole; T 3 Flusilazole; T 4 Hexaconazole;
T 5 Carbendazim; T 6 Mancozeb; T 7 Copper hydroxide;
T 8 Hexaconazole+Zineb; T 9 Captan+Hexaconazole; T 10C Control

Plate 25. Effect of fungicides on growth of *Fusarium verticillioides*

T 1 Propiconazole; T 2 Tebuconazole; T 3 Flusilazole; T 4 Hexaconazole;
T 5 Carbendazim; T 6 Mancozeb; T 7 Copper hydroxide;
T 8 Hexaconazole+Zineb; T 9 Captan+Hexaconazole; T 10C Control

Table 30. Effect of fungicides on radial growth of *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Pestalotiopsis maculans* and *Fusarium verticillioides**

Fungicide	Recommended Dose	Percentage inhibition of growth			
		Lasiodiplodia theobromae#	*Colletotrichum gloeosporioides*	*Pestalotiopsis maculans*	*Fusarium verticillioides*
Propiconazole	1 ml l^{-1}	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Tebuconazole	1.5 ml l^{-1}	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Flusilazole	0.3 ml l^{-1}	88.9 (70.53)	100.0 (90.00)	90.0 (71.54)	88.4 (70.10)
Hexaconazole	0.5 ml l^{-1}	80.0 (63.43)	90.0 (71.54)	88.9 (70.51)	84.6 (66.86)
Carbendazim	1 g l^{-1}	100.0 (90.00)	88.2 (69.86)	100.0 (90.00)	100.0 (90.00)
Mancozeb	3 g l^{-1}	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)
Copper hydroxide	2.5 g l^{-1}	-	92.6 (77.02)	77.8 (61.92)	83.4 (65.95)
Hexaconazole + Zineb	1.5 g l^{-1}	67.1 (54.96)	85.6 (67.65)	88.5 (70.18)	72.3 (58.22)
Captan + Hexaconazole	2 g l^{-1}	88.9 (70.51)	88.2 (69.87)	88.5 (70.18)	87.7 (69.44)
CD		0.989	6.135	1.822	1.246

*Mean of three replications

#Since the pathogen was not found to be sensitive to copper hydroxide it was not included in the experiment.

Number in parenthesis are transformed means in angles

Discussion

5. DISCUSSION

A detailed survey on the incidence and intensity of RWD and Yellowing Disease of coconut was carried out at the Instructional Farm, College of Agriculture, Vellayani. The results indicated that both the diseases were widely prevalent in all the blocks of the Farm. A total of 688 palms (11.3 %) out of 6107 were affected by either RWD or Yellowing Disease and the per cent disease incidence varied from 5.5 to 17.9 in different blocks of the Farm (Fig. 1). The per cent disease incidence of RWD and Yellowing Disease in the Farm were 9.19 per cent (561 palms) and 2.08 per cent (127 palms) respectively out of which 556 (99.1 %) of the RWD affected palms and 120 (94.5 %) of the Yellowing Disease affected palms were yielding palms. RWD had been reported in Thiruvananthapuram district to varying extends such as 1.52 per cent (CPCRI, 1985a), 2.09 per cent (Anon., 1997) and 26.89 per cent (Anon., 2009). However the present investigation showed that the RWD incidence is comparatively lower (9.19%) at Instructional Farm, Vellayani as against the latest Coconut Development Board observation (Anon., 2009).

The occurrence of mid-whorl yellowing was previously described as an associated varied symptom of RWD by earlier investigators (Menon, 1937; CPCRI, 1985b; Koshy, 1999). Yellowing Disease with typical and characteristic mid-whorl yellowing and other associated symptoms observed in the present investigation are different from the characteristic symptoms of RWD. Even though the incidence of the disease was comparatively low (2.08%), in most instances its occurrence resulted in total loss in yield, very often led to death of the palm. This study is the first report of the incidence of Yellowing Disease of coconut in Kerala. The study further indicated that the proportion of incidence of RWD and Yellowing Disease at the Instructional Farm, Vellayani was 4.5:1. Such a very high frequency of occurrence of Yellowing Disease is a matter of serious concern and points to the urgency of tackling Yellowing Disease quite

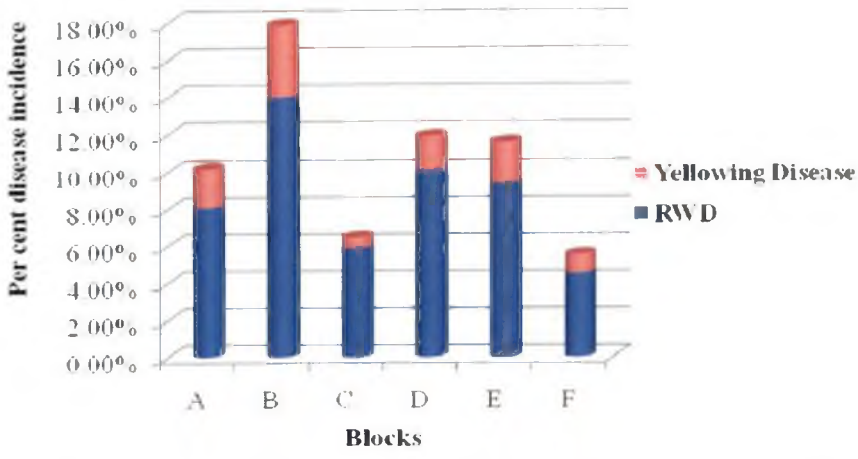


Fig. 1 Incidence of RWD and Yellowing Disease in different blocks of the Instructional Farm

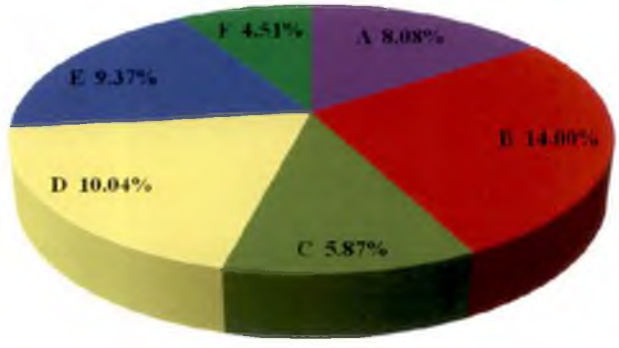


Fig. 2 Incidence of RWD in different blocks of the Instructional Farm

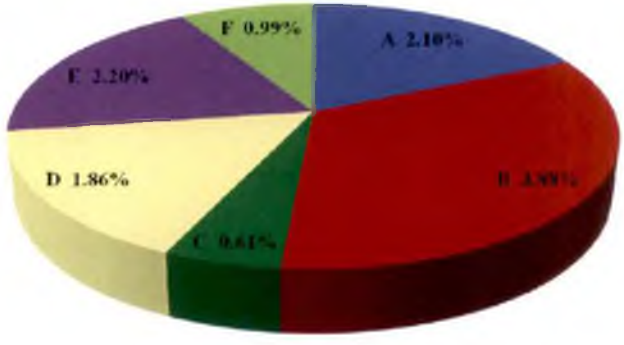


Fig. 3 Incidence of Yellowing Disease in different blocks of the Instructional Farm

different from RWD. More elaborate studies are urgently required to investigate the etiology of the disease and evolve suitable measures to address the problem.

The study showed that there was much variation in the incidence of these two diseases in different blocks of the Instructional Farm with the highest incidence of both the diseases in B block (14 % and 3.88 % respectively) and the least incidence of RWD was in F block (4.51 %) and Yellowing Disease in C block (0.61 %) (Fig. 2 and Fig. 3). The highest incidence observed in B block may presumably be due to a very high plant density, high water table, periodical water-logging and high humidity. Verghese (1960), Pillai *et al.* (1973), Radha *et al.* (1981) and Rethinam *et al.* (1982) also observed relatively higher incidence of RWD in water-logged low-lying areas. Patil and Mathew (1982) opined that RWD was mostly prevalent in places where the water table was high, the depth of the soil was shallow and in places where drainage facilities were meager and unsatisfactory and the symptoms of RWD were more pronounced in those areas with poor soil aeration and water-logged conditions.

Estimation of disease intensity based on foliar symptoms of RWD indicated that the infected palms can be categorized into three groups *viz.*, palms at advanced stage of infection, palms at moderate stage of infection and palms at mild stage of infection (Fig. 4) and further that 111 RWD affected palms (19.8%) were at advanced stage of infection, 397 infected palms (70.8%) were at moderate stage of infection and 53 palms (9.4%) were at mild stage of infection. Among those palms at advanced stage of infection, 61 palms had a total loss of productivity and 29 palms had less than 10 nuts/ palm/ year making the total unproductive palms to 90 (81.8%) out of 110 infected yielding palms. Among the palms at moderate stage of disease 47 palms had total loss of productivity and 94 palms had less than 10 nuts/ palm/ year making the aggregate in that category to 141 palms (35.9 %) out of 393 infected yielding palms. In the case of palms at mild stage of infection only two palms had total loss of productivity and four palms had a productivity of less than 10 nuts/ palm/ year making its total to six unproductive palms (11.3%) out of 53. The data clearly indicated that the

productivity of the palms steadily declined with corresponding increase in disease intensity (81.8% loss of productivity in disease advanced palms, 35.9 per cent loss of productivity in palms at moderate stage of infection and 11.3 per cent loss of productivity in palms at mild stage of infection). Thus visual assessment of disease intensity by measuring the severity of foliar symptoms and by estimating the number of harvestable nuts/ palm/ year, it is possible to decide whether such palms should be maintained further with good management practices to get sustainable yields or to be removed and destroyed from the field to eradicate the source of inoculum and replant. The present study of RWD is in conformity with earlier reports with respect to the relationship of severity of the disease and yield loss (Jayasankar, 1991) and the policy of removal of uneconomical RWD affected palms (Jayasankar, 1991; KAU, 2007; ChandraMohanan and Peter, 2008; Anon., 2009; Krishnakumar *et al.*, 2010). It is also evident from the results that 18.1 per cent (20 palms) of disease advanced palms, 64.1 per cent (252 palms) of the palms at moderate stage of infection and 88.7 per cent (47 palms) of the palms at mild stage of infection had a higher productivity of above 10 nuts/ palm/ year. These palms could be maintained with recommended management practices to obtain sustainable yields (KAU, 2007).

Survey on RWD affected palms in the Farm indicated that out of 556 bearing palms infected 19.8 per cent had a total loss of productivity and 22.8 per cent had productivity of less than 10 nuts/ palm/ year making a total of 237 (42.6%) unproductive palms and hence it has to be cut and removed (Fig. 5). The five palms infected at pre-bearing stage also have to be cut and removed.

The disease intensity of Yellowing Disease affected palms was calculated differently from that of RWD affected palms due to the striking difference in the pattern of symptom expression, speed of progression, extent of damage and pattern of spread. It was calculated with respect to severity of foliar symptoms, inflorescence dieback and loss of productivity based on a disease scale and index developed specifically for the present study. The results of the disease intensity with respect to foliar symptoms indicated that out of the 120 yielding palms

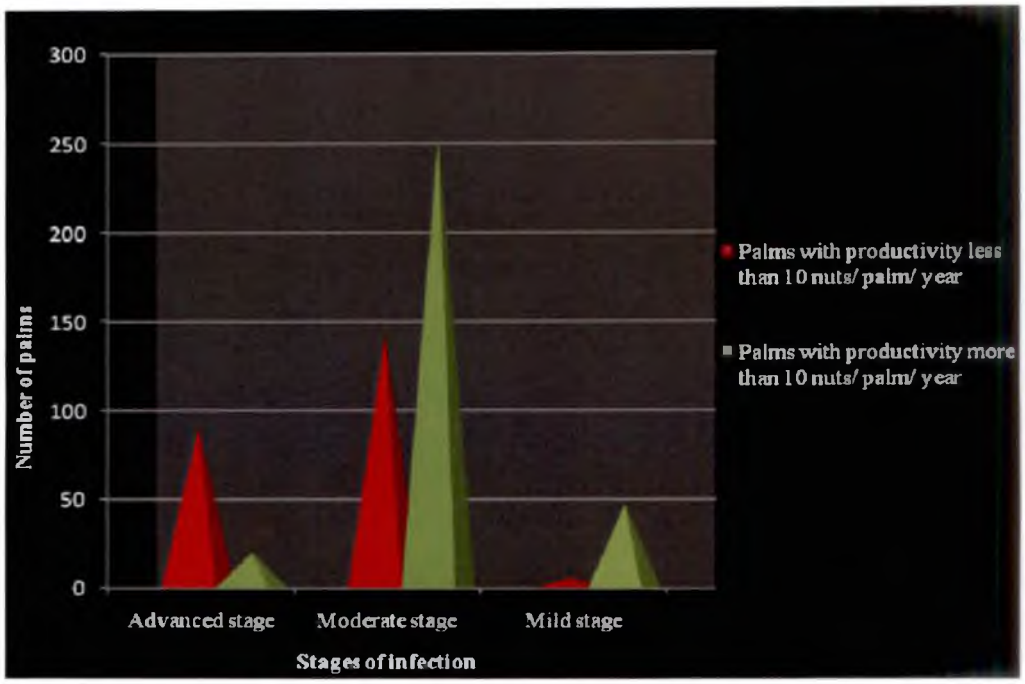


Fig. 4 RWD affected palms at different stages of infection

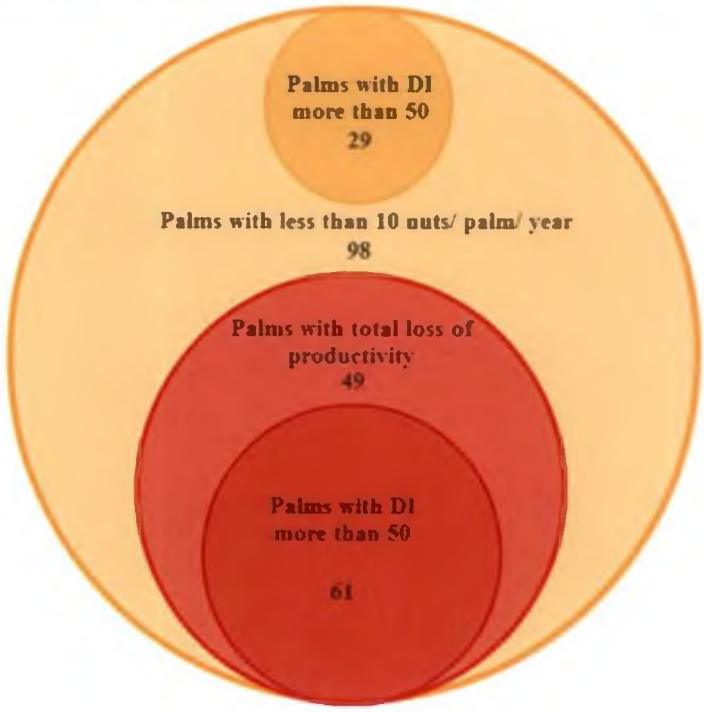


Fig. 5 RWD affected palms recommended to be removed

infected, 26 palms (21.6%) were at severe stage of infection with respect to foliar symptoms, 65 palms (54.2%) were at moderate stage of infection and 29 palms (24.2%) were at mild stage of infection. Among those at severe stage of infection, 18 palms had a total loss of productivity, three palms had less than 10 nuts/ palm/ year making the total unproductive palms to 21 out of 26 (80.8%). Among the palms at moderate stage of infection 24 palms had total loss of productivity and 17 palms had less than 10 nuts/ palm/ year making the total number of unproductive palms to 41 out of 65 (63.1%). In the case of palms at mild stage of infection, three palms had a total loss of productivity and 8 palms produced less than 10 nuts/ palm/ year, making the total unproductive palms in that category to 11 (37.9%) (Fig.6). A comparison of the disease severity based on foliar symptoms and yield loss caused by RWD and Yellowing disease of coconut indicated that in the case of both diseases the percentage of unproductive palms at severe stage of infection is almost equal (81.8% in the case of RWD and 80.8% in the case of Yellowing Disease). But at moderate infection stage there was 35.9 per cent unproductive palms in the case of RWD while it was 63.1 per cent in Yellowing Disease, which is almost double the value of RWD. At mild stage of infection, the percentage of unproductive palms in the case of RWD was 11.3 per cent while it was as high as 37.9 per cent in the case of Yellowing Disease. It is significant to observe that even though there was decline in productivity of palms affected by both the diseases with corresponding increase in disease intensity, productivity deterioration was much faster in Yellowing disease and such palms rapidly become unproductive even when mild symptoms are exhibited in the foliage.

Disease intensity calculated on the basis of severity of inflorescence dieback indicated that out of the 120 yielding palms infected, 66 palms (55%) were at severe stage of inflorescence dieback, 23 palms (19.2%) were at moderate stage of infection, 11 palms (9.2%) were at mild stage of inflorescence dieback and 20 palms (16.6%) were having no inflorescence dieback at all (Fig. 7). Among those palms at severe stage of infection 38 palms had total loss of

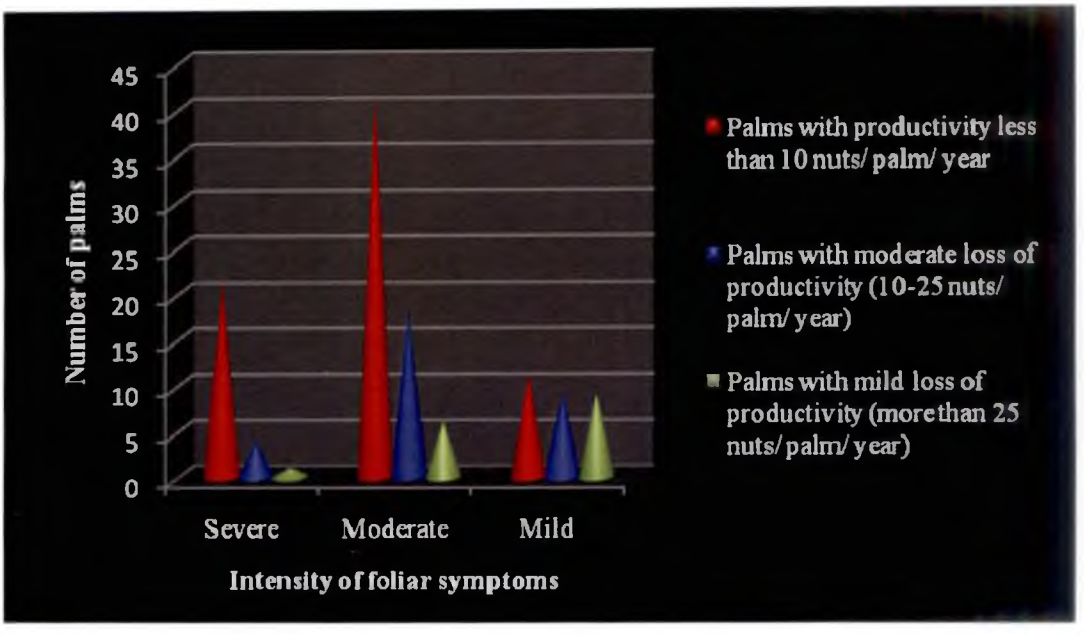


Fig. 6 Intensity of foliar symptoms in Yellowing Disease affected palms

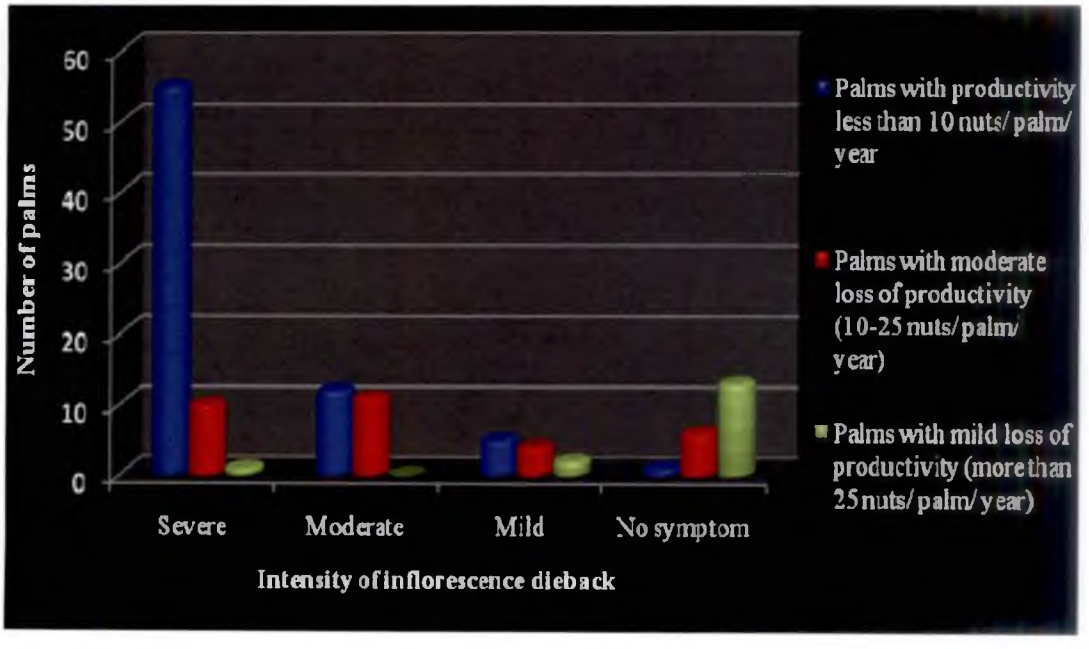


Fig. 7 Intensity of inflorescence dieback in Yellowing Disease affected palms

productivity and 17 palms had less than 10 nuts/ palm/ year, making the total unproductive palms to 55 out of 66 (83.3%). In the case of palms at moderate stage of infection, four palms had total loss of productivity and eight palms produced less than 10 nuts/ palm/ year, making the total unproductive palms to 12 out of 23 (52.2%). Out of the total of 11 palms in the mild infection category, two palms had total loss of productivity and three palms had less than 10 nuts/ palm/ year, making the total unproductive palms to five (45.5%) in that category. Out of the total of 20 palms with no inflorescence dieback only one palm was unproductive (5%). It can be inferred from this observation that the positive relationship between severity of inflorescence dieback and loss of productivity is stronger than that of severity of foliar symptoms and loss of productivity. The correlation analysis of the data confirmed this inference which showed that severity of inflorescence dieback was highly negatively correlated with yield (*i.e.*, high positive correlation with loss of productivity) more than that of the foliar symptoms, eventhough both the parameters are having significant negative correlation with yield of the palm.

Eventhough inflicted by the disease, 39.2 per cent of the palms (47 palms) had more than 10 nuts/ palm/ year and 5 per cent of the palms yielded more than 40 nuts/ palm/ year. When these palms were continuously monitored after the survey, it was observed that many of the them became barren after few months and had shown severe inflorescence dieback. From these observations it is evident that the Yellowing Disease affected palms' health deteriorated faster and it soon became unproductive. It may be due to very high rate of inflorescence dieback and rapid sequential nut fall in Yellowing Disease affected palms. More over the spread of the disease to nearby palms was also much faster. Hence a different management strategy has to be evolved to tackle the problem of Yellowing Disease.

The Yellowing Disease affected palms having above 50 per cent foliar symptom severity or above 50 per cent severity in inflorescence dieback or yield less than 10 nuts/ palm/ year either singly or in combination have to be cut and

destroyed in order to reduce the inoculum build up in the Farm and to prevent further spread of the disease to nearby areas and to replant the sites with tolerant varieties/ seedlings of elite palms. Thus out of the 120 Yellowing Disease affected bearing palms, 87 palms (72.5%) had either above 50 per cent foliar symptom severity or above 50 per cent severity in inflorescence dieback or yielded less than 10 nuts/ palm/ year either singly or in combination (Fig. 8) and hence these palms were recommended to be cut and destroyed. The disease affected pre-bearing palms (7 nos.) also have to be cut and removed.

All the typical symptoms of RWD such as flaccidity, ribbing, yellowing and necrosis observed in the present study are identical to those reported earlier (Varghese, 1934; Menon and Nair, 1951; Menon and Pandalai, 1958; Radha and Lal, 1972; Pillai, 1975; SubbaRaja and Ahmed, 1975; Solomon *et al.*, 1999; Koshy, 1999; Koshy, 2000; Srinivasan *et al.*, 2000; Solomon *et al.*, 2001; Srinivasan and Sasikala, 2001; Sasikala *et al.*, 2005; ChandraMohanan and Peter, 2008; ChandraMohanan, 2010). Leaf rot disease was observed in 42 per cent of RWD affected palms in the Vellayani Campus. The symptoms produced on leaf rot disease affected palms described in para 4.2.1 are in agreement with earlier reports (McRae, 1916; Sundararaman, 1925; Varghese, 1934; Menon and Nair, 1948, 1951, 1952; Menon and Pandalai, 1958; Radha and Lal, 1968; Lily, 1981; Joseph and Rawther, 1991; Srinivasan, 1991; Srinivasan and Gunasekaran, 1992; CPCRI, 1996; Koshy, 1999, Srinivasan and Gunasekaran, 2000; Vrinda, 2002). RWD affected palms are in general predisposed to and superinfected with leaf rot pathogens (Varghese, 1934; Radha and Lal, 1968; George and Radha, 1973; Pillai, 1975; Mathai, 1980; Srinivasan, 1991; Srinivasan *et al.*, 1998) and 30 to 76 per cent of the RWD affected palms were reported to be infected with leaf rot disease also (Anon., 1989; Srinivasan, 1991). On an average 65 per cent of the RWD affected palms were reported to be infected with leaf rot disease (Srinivasan, 1991). However the incidence of leaf rot disease in RWD affected palms at Instructional Farm, Vellayani was only 42 per cent. This indicated that leaf rot disease in Vellayani is not as prevalent as elsewhere.

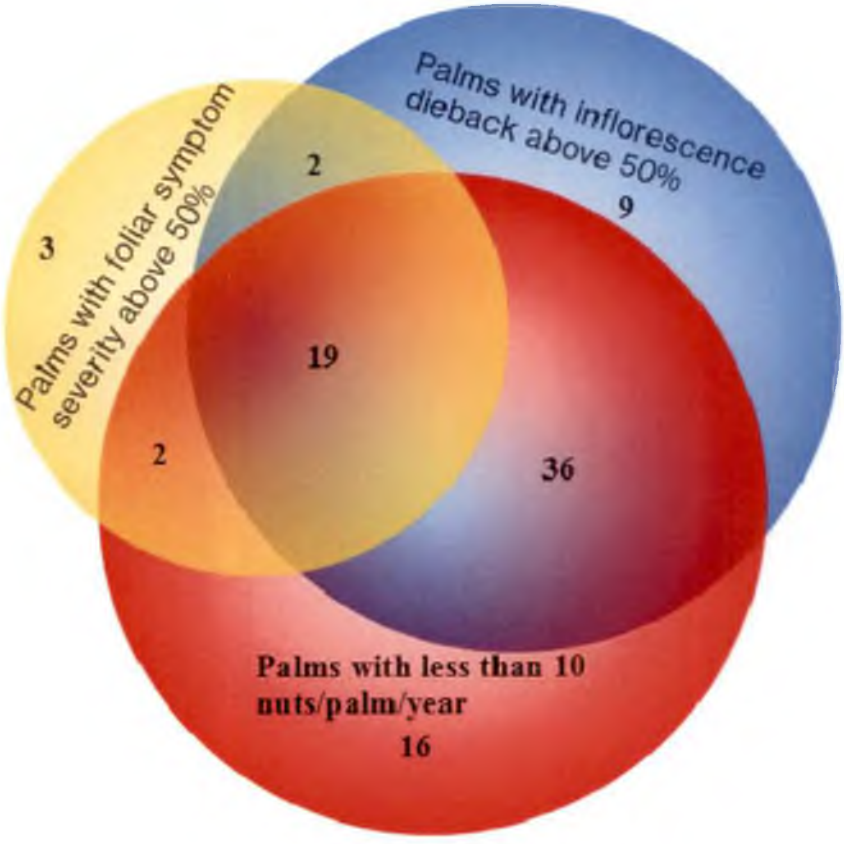


Fig. 8 Yellowing Disease affected palms at severe stage of infection

Yellowing Disease (mid whorl yellowing) of coconut was characterized by rapid chlorosis or yellowing and / or bronzing and flaccidity of a few or all the leaves in the middle whorl of the palm crown. In few instances these symptoms were started from the innermost whorl of leaves. On these yellowed/ chlorotic leaves intense brown to black or grey spots of varying sizes and shapes were developed which were further enlarged, coalesced together and blighted the leaflets. Gradually yellowing/ bronzing progressed upwards and/ or downwards or both and such leaves soon dried up and sometimes might shed from one side of the crown. The newly produced leaves were shorter and weaker with chlorosis. Rapid drying and necrosis of inflorescence and abnormal shedding of flowers, buttons, immature nuts and mature nuts occurred simultaneously or even prior to yellowing and the infected palms soon became barren within a short span of four to eight months time. Ultimately if spindle got infected death of the palm occurred due to rotting and killing of the growing point.

Most of these symptoms are entirely different from that of the RWD affected palms. The characteristic intense yellowing/ browning of leaves initially limited to one or a few or more leaves in the middle whorl of the crown is typical of Yellowing Disease. Unlike RWD the intensity of flaccidity in Yellowing Disease affected leaves was milder. The most pronounced symptom was the rapid drying of inflorescence and falling of buttons, immature nuts and mature nuts in quick succession. Although earlier workers such as Menon (1937), CPCRI (1985b), Koshy (1999), ChandraMohanan (2010) described variability of RWD symptom as described above, they were of the opinion that those were varied symptoms of RWD. The rapid decline in the health of the palm, which very often lead to death of the palm, quick spread of the disease to the nearby palms and the unique and characteristic symptomatology drastically different from the RWD affected palms clearly suggests that Yellowing Disease of coconut could be totally different from RWD at least in symptomatology. More elaborate study is required to establish whether Yellowing Disease is a part of RWD, or a totally different disease incited by member of a different phytoplasmal group. It

is quite pertinent to note at this point that some of the symptoms of Yellowing Disease of coconut such as rapid yellowing or browning, rapid and total nut fall in quick succession are similar to Lethal Yellowing (Plavsic-Banjac *et al.*, 1972; Thomas and Norris, 1980; Romney, 1983; Tsai, 1988; Mpunami, 1999; Harrison *et al.*, 2002; Harrison and Jones, 2003; Myrie *et al.*, 2006; Roca *et al.*, 2006; Howard and Harrison, 2007; Nipah *et al.*, 2007; Harrison *et al.*, 2008: Harrison and Elliot, 2008). As in the case of Lethal Yellowing sometimes yellowing of single leaf in the middle whorl of the crown followed by the upward movement of yellowing was also observed (Thomas and Norris, 1980; Tsai, 1988; Harrison and Jones, 2003). In the case of Lethal Yellowing also sometimes the leaves appear flaccid (Harrison and Elliott, 2008).

Incidence of leaf rot disease was found to be high in Yellowing disease affected palms at the Instructional Farm. It has been categorically established that leaf rot disease occurred only on palms debilitated by RWD. Since the Yellowing Disease affected palms are also greatly debilitated leaf rot pathogens occurred in high frequency and deteriorated the palm further. The symptoms produced by leaf rot were quite similar to that in RWD affected palms.

The detailed isolations conducted over a period of time (October 2009 to July 2010) from the symptomatic leaves of Yellowing Disease affected coconut palms showed that a number of fungi were associated with leaf spots and leaf blights on these leaves. After detailed characterization studies and elaborate pathogenicity tests it was found that *Aspergillus niger, Chalara fimbriata, Colletotrichum gloeosporioides, Fusarium verticillioides, Lasiodiplodia theobromae, Pestalotiopsis maculans, Pestalotiopsis palmarum, Arthrinium* spp. and *Phomopsis* spp. were the pathogens associated with the leaves showing mid-whorl yellowing symptoms. From the leaf rot affected spindle leaf/ mature leaf of the Yellowing Disease affected coconut palms *Alternaria alternata, Cephalosporium* spp., *C. fimbriata, Curvularia* spp., *C. gloeosporioides, F. verticillioides, F. semitectum, L. theobromae, P. maculans, P. palmarum, Phomopsis* spp. and *Verticillium* spp. were isolated. Isolations from outer whorls

of leaves showing yellowing and necrosis indicated that *C. gloeosporioides, F. verticillioides, L. theobromae, P. maculans* and *P. palmarum* were the pathogens associated with leaf spots and leaf blights in the outer whorls of the infected palm. It may be noted that four pathogens *viz., C. gloeosporioides, F. verticillioides, L. theobromae* and *P. maculans* were isolated from all the whorls of the palm, while the pathogens frequently observed both in the middle whorl and leaf rot affected areas were *C. fimbriata, Arthrinium* spp. and *Phomopsis* spp. *A. niger* was isolated only from the middle whorl while *A. alternata, Cephalosporium* spp., *Curvularia* spp., *F. semitectum* and *Verticillium* spp. were occurred only in the leaf rot affected areas. *P. palmarum* was isolated from both middle whorl and outer whorl of the infected palm. These studies clearly showed very high variability in the occurrence of foliage pathogens in the different whorls of the Yellowing Disease affected palms.

The association of pathogens with leaf rot disease such as *C. gloeosporioides* (Srinivasan and Gunasekaran, 1993, 1994a, 1996b, 1996c; Vrinda, 2002), *Curvularia* spp. (Srinivasan and Gunasekaran, 1994b; Vrinda, 2002), *F. moniliforme* var. *intermedium* (Srinivasan *et al.*, 1995; Srinivasan and Gunasekaran, 1996b, 1996c, 1999a), *F. moniliforme* (Vrinda, 2002), *Cephalosporium sacchari* (Vrinda, 2002) and *P. palmarum* (Sathiarajan *et al.*, 1988; Srinivasan *et al.*, 1995) have been well documented and are in agreement with the present investigation. All of the published species descriptions refer to *Fusarium verticillioides* in the broad *F. moniliforme sensu lato* sense (Leslie and Summerell, 2006). Involvement of *Chalaropsis thielavioides* and *L. theobromae* with the leaf rot of coconut also has been reported from Goa (Ramesh *et al.*, 2004). *Thielaviopsis paradoxa* was reported to cause leaf rot (Sathiarajan *et al.*, 1988; Srinivasan and Gunasekaran, 1993, 1996b; Srinivasan *et al.*, 1995) and leaf spot (Protacio, 1964) of coconut. *Phomopsis cocoina* was reported to cause leaf spot of coconut (Ponnappa, 1970; Punithalingam, 1975). In the present study the occurrence of *C. fimbriata, A. alternata, P. maculans, F. semitectum, Phomopsis* spp. and *Verticillium* spp. was observed with leaf rot of coconut among which

Phomopsis spp. and *Verticillium* spp. have not been reported with leaf rot disease previously and this forms the first report of this pathogen with leaf rot disease in Yellowing Disease affected palms. Even though the perfect state of *Chalara fimbriata*, *Ceratocystis fimbriata* was isolated from coconut kernel (Mohan and Swamy, 1986) it was not previously reported from the coconut foliage infected with Yellowing disease. *A. alternata* was reported to cause leaf spot of coconut in India (Rao and Subramanyam, 1975) while *Pestalotiopsis guepinii* was reported to cause coconut leaf spots in Brazil (Anjos *et al.*, 2000; Cardoso *et al.*, 2003) and seedling blight of *Myristica malabarica* from Western Ghats in India (Swapna Priya and Nagaveni, 2010). *P. guepinii* was the former name of *Pestalotiopsis maculans* (Nag Raj, 1985) observed in the present study and it was not previously reported from coconut in India. *F. semitectum* is commonly isolated from soil (Burgess, *et al.*, 1988; Leslie, *et al.*, 1990) and from diverse aerial plant parts in tropical and sub-tropical areas, *e.g.*, banana fruits (Knight *et al.*, 1977; Shillingford and Sinclair, 1980; Wallbridge, 1981; Marin *et al.*, 1996) and palm fronds (Leslie and Summerell, 2006). Even though Booth and Sutton (1984) proposed the name *F. pallidoroseum* for this species, the more accepted name is *F. semitectum* (Subramanian, 1971; Nirenberg, 1990; Leslie and Summerell, 2006). *Arthrinium* spp. is a cosmopolitan saprophytic fungus commonly isolated from decaying plant material and from soil. *Arthrinium* spp. was found to be associated with decline symptoms of grapevines (Mendes *et al.*, 2003). *Arthrinium phaeospermum* was reported to cause Moso basal culm rot of Bamboo (LiQin, 2000) and was also observed from maize seeds (Dawar, 2009).

Among the pathogens isolated from leaf spots or blights in the symptomatic leaves of Yellowing Disease affected coconut palms *A. niger* was only isolated from infected palms at pre-bearing stage while *C. gloeosporioides, F. verticillioides, L. theobromae, P. maculans, P. palmarum, Arthrinium* spp. and *Phomopsis* spp. were observed in the infected palms at yielding stage. *C. fimbriata* was also found associated with the symptoms in the middle whorl of the Yellowing Disease affected yielding palms in the present study. *C.*

gloeosporioides and Fusarium spp. were found associated with blighted tissues of the yellowed leaves in the middle whorl of the RWD affected coconut palm (Srinivasan and Gunasekaran, 1999b). *Aspergillus* spp. and *L. theobromae* were isolated from coconut palms suffering from lethal leaf blight disease in Tamil Nadu recently (Bhaskaran *et al.*, 2007a). The other pathogens observed with leaf spots or blights in the symptomatic leaves in the middle whorl and outer whorl of Yellowing Disease affected coconut palms were in agreement with the previous observations that they were found associated with leaf rot or leaf spots or leaf blights in these leaves.

L. theobromae is a cosmopolitan fungus infecting a wide range of host plants and in coconut this fungus commonly causes fruit rot and nut fall. In Kerala this pathogen was reported previously in the discoloured shell and husk of poor quality nuts from RWD affected palms (Lily and Pillai, 1980) and as causal agent of nut rot of healthy (Gunasekaran and Srinivasan, 2000) as well as eriophyid mite infested coconuts (ChandraMohanan and Baby, 2004; Venugopal and ChandraMohanan, 2006; Venugopal *et al.*, 2008). The pathogen was reported to cause leaf blight on coconut in India and other countries such as Brazil, Mexico, Malaysia and Srilanka (Ram, 1989; Noriega *et al.*, 1991; Warwick *et al.*, 1991, Correia and Costa, 2005). During the last decade it was reported to cause severe leaf blight in coconut in Coimbatore district in Tamil Nadu which led to death of the palm (Nakkeeran *et al.*, 1998). It was also isolated from coconut palms suffering from a lethal disease observed in Thanjavur district in Tamil Nadu (Bhaskaran *et al.*, 2007b). It was reported to cause leaf rot of coconut palms in Brunei, Fiji, Indonesia, Malaysia, Vietnam and India (Goa) (Nambiar, 1994; Ramesh *et al.*, 2004).

P. palmarum was reported as causal agent of leaf blight or grey leaf spot of coconut in different parts of the world by several workers (Sundararaman, 1925; Varghese, 1934; Bertus, 1927; Chowdhury, 1946; Sivaprakasam *et al.*, 1969; Brown, 1975; Francis, 1977; Obazee and Ikozun, 1985; Ram, 1989; Sitepu and Darwis, 1989; Anupama, 1997; Karthikeyan and Bhaskaran, 1999; Praveena,

1999; Subramanyan, 2003). Grey leaf spot caused by *P. palmarum* is a common occurrence in almost all coconut palms and usually appears in the lower whorls first. In the present study the pathogen was isolated from leaves of outer whorl, middle whorl and mature leaves in the inner whorl of the infected palm. It was also isolated from leaf rot affected spindle leaves and mature leaves of RWD affected coconut palms (Srinivasan *et al.*, 1995).

The comparative virulences of 19 pathogenic fungal isolates obtained from the Yellowing Disease affected palms were assessed by artificially inoculating them on the spindle leaflets and mature leaflets of healthy coconut leaves and measuring the time taken for symptom expression as well as lesion size on the inoculated leaflets. It was found that *C. fimbriata* (Isolate FI 4) and *C. gloeosporioides* (Isolate FI 5 A) were the most virulent with respect to both the time taken for symptom expression and the size of the lesion produced in the spindle leaflets. Eight moderately virulent pathogens were identified as *C. gloeosporioides* (Isolate FI 5 B), *L. theobromae* (Isolate FI 9 A, Isolate FI 9 B and Isolate FI 9 C), *Arthrinium* spp., *F. verticillioides* (Isolate FI 7 A and Isolate FI 7 B) and *F. semitectum* (Isolate FI 8). Nine less virulent pathogens identified in the study were *P. maculans* (Isolate FI 10 A and Isolate FI 10 B), *A. niger* (Isolate FI 2), *A. alternata* (Isolate FI 1), *Phomopsis* spp., *Cephalosporium* spp. (Isolate FI 3), *Verticillium* spp. (Isolate FI 14), *Curvularia* spp. (Isolate FI 6) and *P. palmarum* (Isolate FI 11). A perusal of the literature showed that in one of the previous studies *C. gloeosporioides* took about four days for symptom expression while *Cephalosporium* spp. and *Curvularia* spp. took five and six days respectively (Vrinda, 2002). However in the present study, a highly virulent *C. fimbriata* and *C. gloeosporioides* took only two days to produce the symptom, clearly indicating the fact that they are highly pathogenic on coconut leaves under favourable environment. It is also clear from the study that different fungi exhibited greater variability in different regions with regard to their pathogenicity and other characteristics (Pande *et al.*, 1991; Mathur and Totlan, 2001).

It may be noted that *C. fimbriata* was highly virulent when it was tested on the mature leaflets too. The moderately virulent pathogens on mature leaflets were identified as *F. verticillioides* (Isolate FI 7 A), *P. maculans* (Isolate FI 10 A) and *L. theobromae* (Isolate FI 9 A and Isolate FI 9 B) and 11 less virulent pathogens were *C. gloeosporioides* (Isolate FI 5 A and Isolate 5 B), *F. verticillioides* (Isolate FI 7 B), *A. niger* (Isolate FI 2), *Arthrinium* spp. (Isolate FI 13), *P. palmarum* (Isolate FI 11), *Phomopsis* spp. (Isolate FI 12), *P. maculans* (Isolate FI 10 B), *L. theobromae* (Isolate FI 9 C), *F. semitectum* (Isolate FI 8) and *Cephalosporium* spp. (Isolate FI 3). Three fungal isolates namely *A. alternata* (Isolate FI 1), *Curvularia* spp. (Isolate FI 6) and *Verticillium* spp. (Isolate FI 14) failed to produce any symptoms on mature leaflets and hence they are considered as non-pathogens on mature coconut leaves. In one of the previous studies *P. palmarum* took 6-7 days to produce initial symptoms of the disease (Subramanian, 2003). However it took lesser time for symptom expression (3 days and 5 days on mature leaflets and spindle leaflets respectively) in the present study, presumably may due to virulence characteristics of the isolate.

Based on testing the virulence of the isolated pathogens on the coconut leaves, it is inferred that *C. fimbriata* and *C. gloeosporioides* were highly pathogenic and highly virulent in causing leaf rots and leaf blights on younger leaves while *C. fimbriata, F. verticillioides, P. maculans* and *L. theobromae* are highly virulent and moderately virulent in causing leaf spots and blights on mature leaves of Yellowing Disease affected coconut palms.

Detailed investigation on the morphological and cultural characteristics of the various pathogen isolates were conducted to identify the pathogens and these characteristics were compared with the original descriptions of the respective pathogens. These pathogen isolates were sent to Agharkar Research Institute, Pune and the tentative identifications were validated based on their report. Fungal isolate FI 1 had characteristics identical to *Alternaria alternata* described by Simmons (1967), Ellis (1971) and Simmons (2007) and hence identified as *Alternaria alternata*. The characteristics of the fungal isolate FI 4, was compared

with that of Nag Raj and Kendrick (1976) and identified as *Chalara fimbriata*. The characteristics of the Isolates FI 5 A and FI 5 B were similar and upon comparison with the descriptions given by Sutton (1980 and 1992), these were identified as *Colletotrichum gloeosporioides*. The Isolates FI 7 A and FI 7 B had almost similar characteristics which were compared with the descriptions given by Leslie and Summerell (2006) and identified as *Fusarium verticillioides*. Fungal isolate FI 8 had characteristics identical to *F. semitectum* described by Leslie and Summerell (2006) and hence identified as *F. semitectum*. The nomenclatures used for Isolate FI 7 A, Isolate FI 7 B and Isolate FI 8 were according to the recommendations by Seifert *et al.* (2003) and Leslie and Summerell (2006). The cultural and morphological characters of Isolate FI 2, Isolate FI 6, Isolate FI 13 and Isolate FI 14 were found identical to *Aspergillus niger, Curvularia* spp., *Arthrinium* spp. and *Verticillium* spp. described by Ellis (1971) and they were identified as the respective pathogens. Isolate FI 3 had characteristics similar to *Cephalosporium* spp. described by Subramanian (1971) and it was identified as *Cephalosporium* spp. The morphological characteristics of the Isolate FI 9 A, Isolate FI 9 B and Isolate FI 9 C were similar. These isolates were compared with the descriptions given by Punithalingam (1976) and found identical and hence identified as *Lasiodiplodia theobromae*. Isolate FI 11 had characteristics similar to *Pestalotiopsis palmarum* described by Sutton (1980), Nag Raj (1993) and Jeewon (2001) and hence identified as *Pestalotiopsis palmarum*. The characteristics of the Isolate FI 10 A and FI 10 B were identical and upon comparison with the descriptions given by Sutton (1980), Nag Raj (1993) and Jeewon (2001) these were identified as *P. maculans*. The nomenclatures followed for Isolate FI 10 A and Isolate FI 10 B were according recommendations by Nag Raj (1985). The characteristics of the Isolate FI 12 were found identical to those described by Punithalingam (1985) and the isolate was identified as *Phomopsis* spp.

Study on the occurrence of various foliar fungal pathogens on the symptomatic leaves during the three seasons showed that it was during the

summer season (March-May, 2010) that a maximum number of fourteen different pathogens were isolated while only six pathogens could be obtained during the other two seasons. It may be noted that the predominant pathogens infecting the coconut leaves during June-July and October-January seasons were *C. gloeosporioides, L. theobromae, P. maculans* and *P. palmarum.* The frequency of occurrence of *F. verticillioides* was also high during October-January season. When the summer season started, apart from these fungi several other pathogens started infecting the coconut leaves due to favourable environmental conditions such as high temperature, humidity and intermittent rains which might have further supported by the availability of enough decaying organic matter in the crown. Similar observations, higher incidence of saprophytic fungi such as *Fusarium* spp. and *Curvularia* spp. have been reported by several other workers (Srinivasan and Gunasekaran, 1993, 1996b, 2000; Sarhan, 2001; Vrinda, 2002). It is also important to observe that the highest number of fungal infections on the foliage of Yellowing Disease affected palms occurred in Blocks A and B. It might be due to high humidity and poor ventilation in the garden. During the rainy season (June-July, 2010) the most frequently occurred pathogen in all the blocks were *C. gloeosporioides* and *P. maculans.* Many of the earlier works indicated that *C. gloeosporioides* is the major pathogen of leaf rot disease during the rainy season (Srinivasan and Gunasekaran, 1993, 1996b, 2000; Vrinda, 2002). However this is the first report of occurrence of *P. maculans* as a foliar pathogen of coconut in India.

Among the various pathogens isolated from the different whorls of the infected palms *L. theobromae* was the most frequently isolated pathogen followed by *C. gloeosporioides* and *P. maculans* (Fig. 9). Although *C. gloeosporioides* (Srinivasan and Gunasekaran 1996b) and *Fusarium* spp. (Vrinda, 2002) were reported to be the most frequently occurred pathogens in the leaf rot affected parts of RWD palms, in the present study it was *L. theobromae* that most frequently occurred in Yellowing Disease affected palms. However it was isolated at a lesser frequency during June-July (2010) period. It may probably be

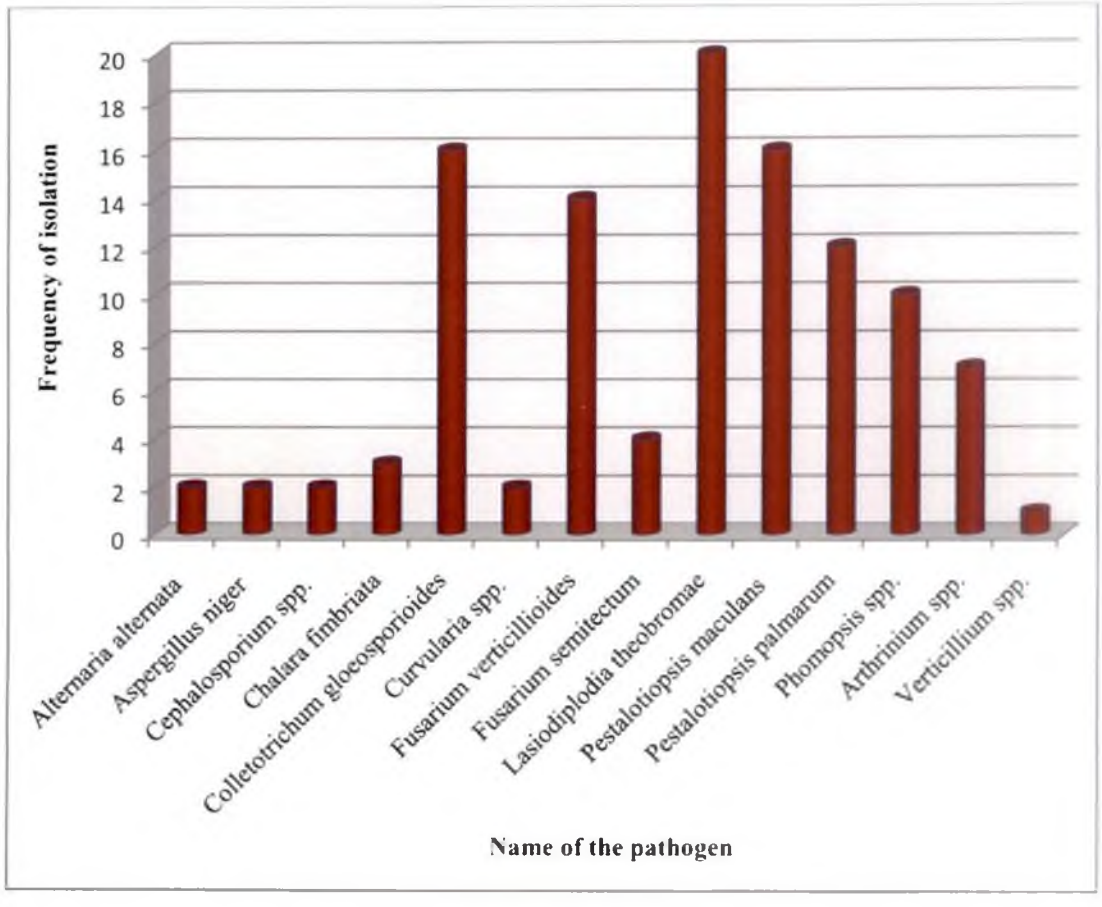


Fig. 9 Frequency of occurrence of different foliar fungal pathogens on Yellowing Disease affected palms

due to the very heavy rainfall occurred during the season. A similar observation was made in Brazil with respect to the conidial dispersal study of the fungus in which there was a positive relationship between high conidial dispersal and rainfall at the range of 25 to 80 mm beyond which the relationship was negative (Correia and Costa, 2005). In coconut this fungus was reported to cause fruit rot and nut fall of healthy (Gunasekaran and Srinivasan, 2000) and eriophyid mite infested coconuts in Kerala (ChandraMohanan and Baby, 2004; Venugopal and ChandraMohanan, 2006; Venugopal *et al.*, 2008), leaf rot (Nambiar, 1994; Ramesh *et al.*, 2004) and leaf blight (Ram, 1989; Noriega *et al.*, 1991; Warwick *et al.*, 1991, Correia and Costa, 2005). Recently it was reported to cause lethal leaf blight of coconut in Tamil Nadu which led to death of the palm (Nakkeeran *et al.*, 1998; Bhaskaran *et al.*, 2007a). Widespread occurrence of this pathogen in the present investigation on Yellowing Disease affected palms and the above mentioned reports indicated its potential of causing very severe damage to coconut palm and hence new strategies are to be evolved to combat the problem.

It is also noteworthy to observe that the frequency of occurrence of *Pestalotiopsis palmarum* and *P. maculans* were highest during the rainy season. It had been reported that grey leaf blight caused by *P. palmarum* was most severe during the rainy season (Suriachandraselvan *et al.*, 1991; Subramanyan, 2003).

Biochemical analysis of the coconut leaves with respect to total sugars showed that it was significantly higher in the leaves of the infected palms at all stages of infection (Fig. 10). At the early stage of infection the total sugars were significantly increased than that of the healthy palms. The values decreased significantly at the moderate stage, but it increased significantly at the severe stage and reached at levels slightly lower than that in the early stage. In the healthy palm the total sugar content was more or less similar in different whorls of the palm. But in the infected palms the total sugars were significantly lower in the outer whorls when compared with the middle and inner whorls. In the middle and inner whorls there was no significant difference with regard to the total sugar content in infected palms at all stages of infection. Mathew (1977) analysed the

carbohydrate content of the RWD affected palms and showed that total, reducing and non-reducing sugars were significantly higher in the leaves of infected palms. He inferred this as due to impaired translocation to the roots in affected palms. In the case of lethal yellowing affected coconut palms also the total sugars were higher in the leaves compared to that of healthy palms. Concentration of total sugars increased slowly in the recently expanded leaves of coconut palms with the development of the disease before decreasing in the later stages of lethal yellowing. In the intermediate leaves sugar concentrations increased more rapidly with the advance of the disease before decreasing in the later stages (Maust *et al.*, 2001, 2003). Lepka *et al.* (1999) found higher levels of reducing sugars and sucrose in source leaves of phytoplasma infected plants than in healthy ones. Phytoplasmas affect phloem function, impairing carbohydrate translocation and subsequently causing the accumulation of soluble sugars in source leaves. These sugars in phloem are a potential food supply. Phytoplasmas contain a minimal genome and lack genes coding for ATP synthases and sugar uptake and use, making them dependent on their host (Christensen *et al.*, 2005). Impaired photosynthesis, the accumulation of carbohydrates in mature leaves and decreased starch content in sink tissues such as roots, often described for phytoplasma infection (Lepka *et al.*, 1999; Tan and Whitlow, 2001; Bertamini *et al.*, 2003; Maust *et al.*, 2003; Choi *et al.*, 2004; Junqueira *et al.*, 2004), seem to be secondary effects and can easily be related to inhibition of phloem transport. The effect on carbohydrate translocation seems to correlate with the virulence of the strain (Lepka *et al.*, 1999; Tan and Whitlow, 2001). Accumulation of carbohydrates in source leaves is generally believed to result in a feedback inhibition of photosynthesis causing chlorosis (Lepka *et al.*, 1999; Tan and Whitlow, 2001; Bertamini *et al.*, 2002b; Maust *et al.*, 2003). Increase in sugar contents in infected coconut leaves due to fungal infection was also reported by various workers (CPCRI, 1981, 1982; Karthikeyan and Bhaskaran, 1997; Subramanyan, 2003).

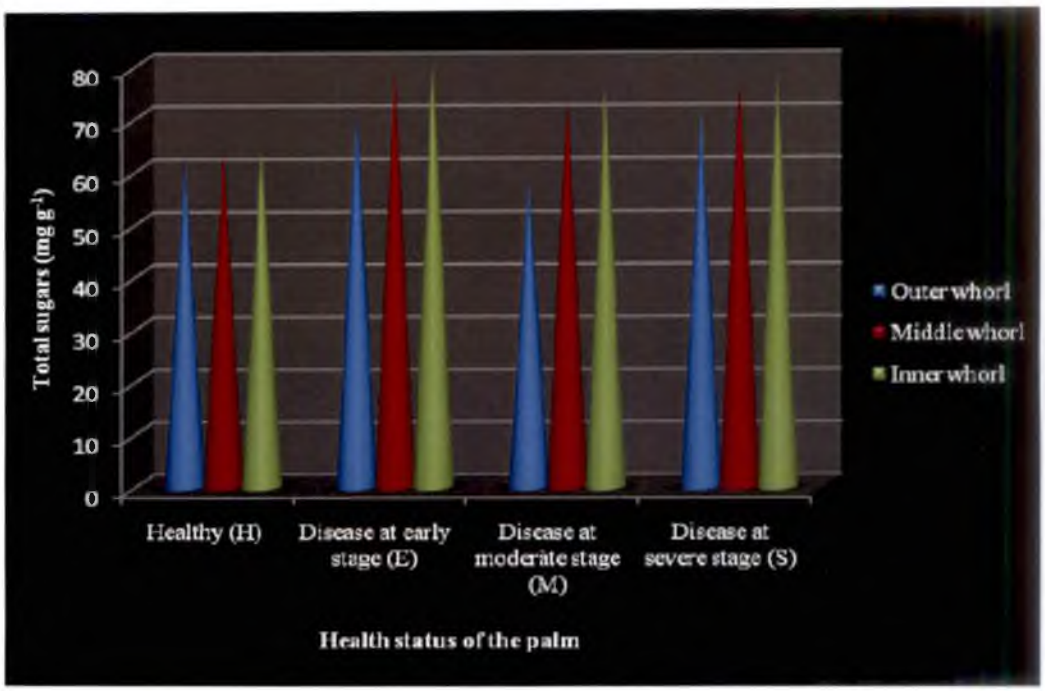


Fig. 10 Changes in total sugars of coconut leaves at different stages of infection

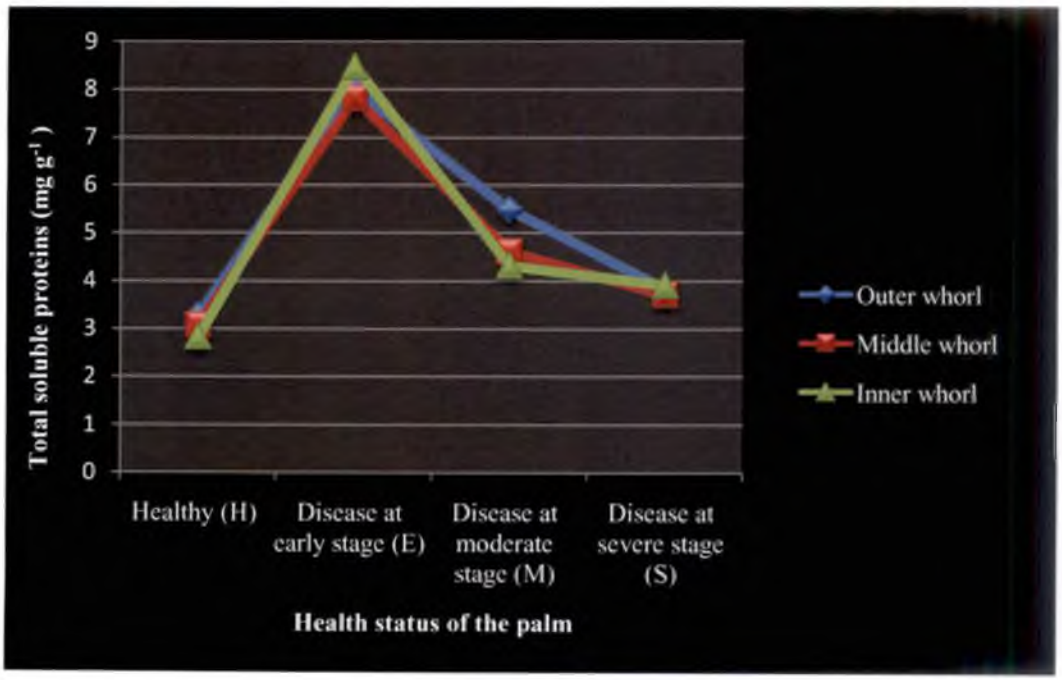


Fig. 11 Changes in total soluble proteins of coconut leaves at different stages of infection

The total soluble protein contents in the different whorls of the infected palms were found to be significantly higher than that of the healthy palms at all stages of infection (Fig. 11). Consequent to infection the total soluble proteins increased to the highest level at early stage of infection in all the whorls of the palm and thereafter it decreased to significantly lower levels at moderate stage and to further lower levels at the severe stage of infection. A decrease in protein fractions were observed in the leaf tissues of root (wilt) affected coconut palms (Pillai and Shanta, 1965). The leaf protein content showed no significant difference in the early stages of Lethal Yellowing affected palms and reduced from the foliar discolouration phase. At this stage the protein content dropped to 45 % of its original level (Leon *et al.*, 1996). The soluble protein content was adversely affected in Thanjavur wilt diseased coconut palms in the E. coast region of Tamil Nadu (Vijayaraghavan and Ramachandran, 1988). An increase in the total amount of proteins has been found in maize bushy stunt phytoplasma-infected maize plants (Junqueira *et al.*, 2004). Contradictory results have been obtained in different host plants infected with different Mollicutes. A possible explanation for this difference could be due to the fact that extremely susceptible plants were used in those experiments. In particular, resistant host plants accumulate higher protein content than susceptible ones, supporting the hypothesis that accumulation of PR-proteins contributes to the increase of total proteins in infected tissues (Musetti, 2010).

The result showed that the activity of PAL in the infected palms at all stages of infection in different whorls of the palm were significantly higher than that of the healthy palms and follows the similar trend as that of protein at different stages of infection. There was a very rapid increase in the PAL activity at early stages of infection in all whorls of the palm. Thereafter these values decreased significantly at moderate stage of infection and at severe stage of infection (Fig. 12). The levels of phenylalanine ammonia lyase was higher in root (wilt) affected coconut palms (Joseph and Jayasankar, 1980). The lowest PAL activity was recorded in palms having zero disease indexes. PAL activity

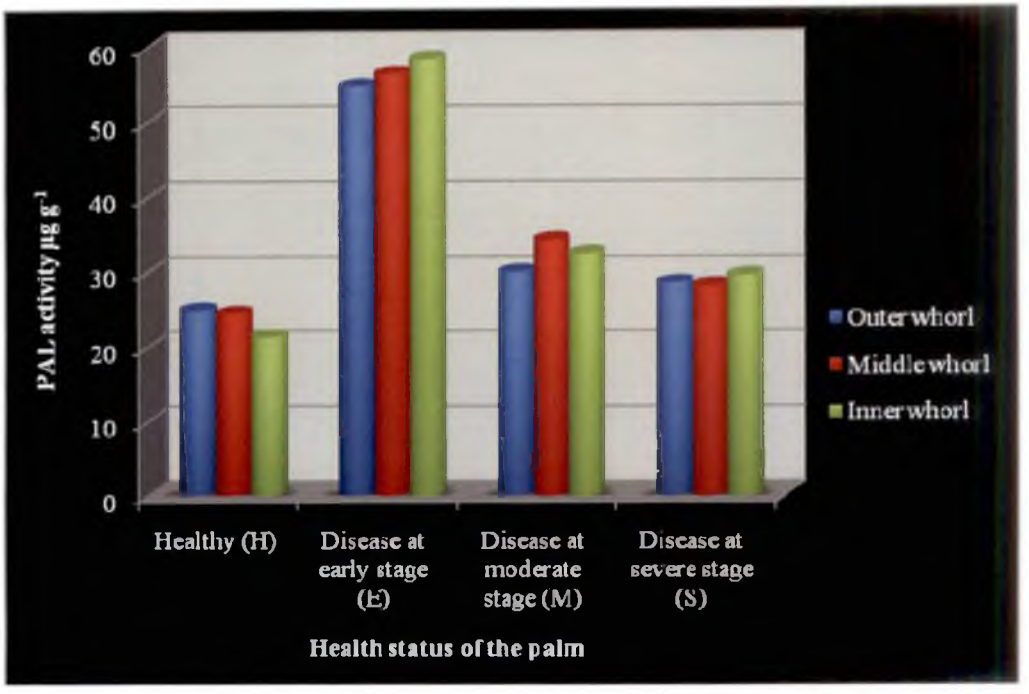


Fig. 12 Changes in PAL activity of coconut leaves at different stages of infection

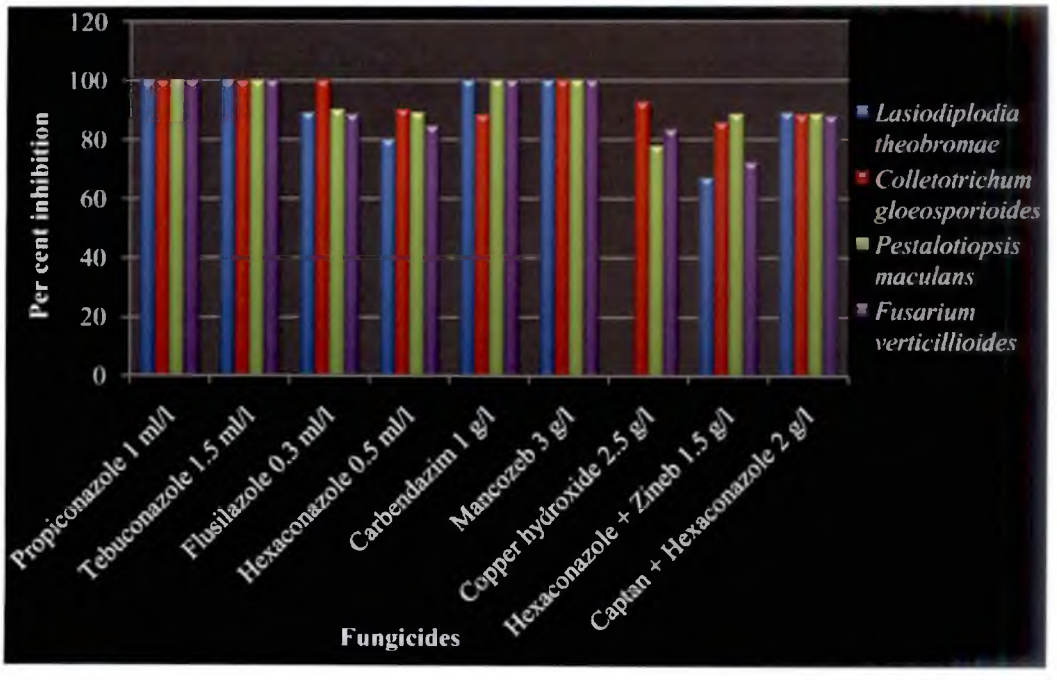


Fig. 13 Efficacy of fungicides against the predominant foliar pathogens in the Yellowing Disease affected coconut palms

increased from healthy to apparently healthy and then it decreased with increase in intensity of the disease. However in diseased palms the PAL activity was markedly higher than that of healthy palms (Joseph, 1983). An increase in enzyme activity was noticed immediately after contraction of disease and in the later stages of the disease the levels of enzyme activity were decreased but significantly higher than the levels found in healthy palms/ seedlings (Mayilvaganan and Jacob, 2008).

The bioassay of fungicides with *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Pestalotiopsis maculans* and *Fusarium verticillioides* at the recommended dose revealed that propiconazole (1ml l^{-1}), tebuconazole (1.5ml l^{-1}) and mancozeb (3 g l^{-1}) were highly effective in completely inhibiting the mycelial growth of all the pathogens tested (Fig. 13). Carbendazim (1 g l^{-1}) was also highly effective in complete inhibition of the growth of *L. theobromae, P. maculans* and *F. verticillioides* while flusilazole (0.3 ml l^{-1}) was highly effective in completely inhibiting the growth of *C. gloeosporioides* (Fig. 13). Propiconazole was found to be the best in the inhibition of mycelial growth of leaf rot pathogens as well as *L. theobromae* in the previous studies (Vrinda, 2002; Srinivasan *et al.*, 2006; Bhaskaran *et al.*, 2007a). Tebuconazole, which was effective against *L. theobromae* inciting leaf blight of coconut under field conditions previously (Warwick and Abakerli, 2001), exerted complete inhibition of mycelial growth of all the four pathogens tested in the present study. Mancozeb has been recommended as an effective fungicide in sequential spraying (Srinivasan and Gunasekaran, 1996d; KAU, 2007) and for alternating with hexaconazole (Koshy, 1999) against leaf rot disease of coconut and was found to have higher efficacy against *C. gloeosporioides* (Vrinda, 2002; Srinivasan *et al.*, 2006) and *L. theobromae* (Bhaskaran *et al.*, 2007a) under *in vitro* conditions. Carbendazim, which was reported to be effective under *in vitro* conditions against *C. gloeosporioides*, *Fusarium solani* (Srinivasan and Gunasekaran, 1998; Vrinda, 2002; Srinivasan *et al.*, 2006) and *L. theobromae* (Bhaskaran *et al.*, 2007a; Meena *et al.*, 2008) had shown complete inhibition of

mycelial growth of *L. theobromae, P. maculans* and *F. verticillioides* and 88% inhibition of *C. gloeosporioides* in the present study.

Previous studies highlighted the excellent fungicidal properties of hexaconazole against leaf rot pathogens under *in vitro* and *in vivo* conditions (Srinivasan and Gunasekaran, 1998; Srinivasan and Gunasekaran, 1999b; Koshy *et al.*, 2001, 2002; Vrinda, 2002; Srinivasan *et al.*, 2006; Srinivasan, 2008; Srinivasan, 2010). However it was found to be not much effective against any of the pathogens (80-90 % inhibition) tested *in vitro* in the present study. The combination products of hexaconazole also failed to produce more than 90 per cent inhibition of mycelial growth of any of the pathogens among which combination with zineb was found to be the least effective (68-88.5% inhibition). Hexaconazole was reported to exert varying levels of inhibition of mycelial growth ranging from 43.5% to 100% against *L. theobromae* causing leaf blight of coconut (Bhaskaran *et al.*, 2007a; Meena *et al.*, 2008). In the present study it inhibited 80% mycelial growth when it was tested alone and in combination with zineb and captan it inhibited 67% and 88.9% mycelial growth respectively.

From the fungicidal evaluation study it is concluded that propiconazole, tebuconazole and mancozeb were excellent in inhibiting the mycelial growth of foliar fungal pathogens associated with Yellowing disease of coconut. Hence more detailed efficacy evaluation study with these fungicides in the field are to be taken up for determining most effective fungicides for managing foliar pathogens associated with Yellowing disease of coconut.

Summary

6. SUMMARY

A detailed survey was carried out at the Instructional Farm, College of Agriculture, Vellayani and the incidence of Root (Wilt) Disease and Yellowing Disease of coconut were observed in all the six blocks of the Instructional Farm. Out of 6107 total palms in the farm 561 palms were affected by Root (Wilt) Disease (9.19 %) and 127 palms (2.08 %) were showing symptoms of Yellowing disease.

The disease intensity of the Root (Wilt) affected palms were calculated using the simplified method described by Nambiar and Pillai (1985) and it was found that out of the 561 Root (Wilt) affected palms 19.8 % (111 palms) were at the advanced stage of infection (above 50 % disease index), 70.8 % (397 palms) were at the moderate stage of infection (disease index between 20 and 50 %) and 9.4 % (53 palms) were at the mild infection stage (disease index below 20 %). Altogether 242 infected palms (43%) out of the total Root (Wilt) Disease infected palms were found to be unproductive (less than 10 nuts/ palm/ year) and hence recommended to cut and destroy.

A new evaluation method was designed to estimate the disease intensity of Yellowing Disease of coconut in Kerala based on three parameters *viz.*, foliar symptoms, inflorescence dieback and loss of productivity. Among the 127 Yellowing disease affected palms, 120 palms were yielding palms among which 37.5% had shown total loss of productivity (0 nuts), 23.3 % of the palms had less than 10 nuts/ palm/ year, 25.8% had 10-25 nuts/ palm/ year and 16 palms had more than 25 nuts/ palm/ year. Among these 87 palms (72.5%) had shown either above 50% foliar symptom severity or above 50 % severity in inflorescence dieback or yielded less than 10 nuts per palm either singly or in combination. All these palms along with the infected pre-bearing palms making a total of 94 palms (74%) out of the 127 Yellowing Disease affected palms are to be removed.

The characteristic symptoms of RWD were flaccidity, ribbing, yellowing and necrosis. In disease advanced palms, the crown size and spathes were greatly reduced and inflorescence production ceased. Fourty two per cent of the RWD

affected palms were 'super infected' with leaf rot disease also which started as minute water-soaked lesions on the margins and distal ends of leaflets of the unopened spindle leaf. These lesions were merged together, rotting extended into the interior of the spindle and were blown off by wind when the spindle unfurled. In Yellowing Disease affected palms sudden appearance of chlorosis/ yellowing/ bronzing of one or more leaves in the middle whorl coupled with flaccidity was the characteristic symptom. Rapid drying and necrosis of inflorescence and abnormal shedding of flowers, buttons, immature nuts and mature nuts occurred in succession either simultaneously or prior to yellowing and the palms became barren within a period of 4 to 8 months. On the chlorotic/ yellowed leaves intense brown to black leaf spots developed which enlarged, coalesced together and blighted the leaflets. As the disease advanced more number of leaves were turned yellow and gradually the spindle leaf withered and growing point rotted in severe cases. Incidence of leaf rot disease was 52.5% in the case of Yellowing Disease affected palms.

The extensive isolations conducted over a period of time (October 2009 to July 2010) from the symptomatic leaves of Yellowing Disease affected coconut palms and elaborate pathogenicity studies proved that a number of fungi were associated with leaf rots, leaf spots and leaf blights on these leaves. After the characterization and identification it was found that *Alternaria alternata, Aspergillus niger, Cephalosporium* spp., *Chalara fimbriata, Colletotrichum gloeosporioides, Curvularia* spp., *Fusarium verticillioides, F. semitectum, Lasiodiplodia theobromae, Pestalotiopsis maculans, P. palmarum, Phomopsis* spp., *Arthrinium* spp. and *Verticillium* spp. were the associated pathogens. Among these *C. fimbriata, P. maculans* and *Verticillium* spp. are new reports on coconut foliage. On artificial inoculation on detached leaflets, the pathogen isolates produced angular, oval or irregular brownish water soaked lesions on detached spindle and mature leaflets. *A. alternata, Curvularia* spp. and *Verticillium* spp. were non-pathogenic on mature leaves of the Yellowing disease affected coconut palms. The various pathogen isolates differed in their virulence

and it was found that *C. fimbriata* was the most virulent with respect to both the time taken for symptom expression and the size of the lesion produced in the spindle and mature leaflets. While *C. gloeosporioides* was highly virulent on the spindle leaflets, *F. verticillioides, P. maculans* and *L. theobromae* were the moderately virulent pathogens on mature leaflets.

The maximum number of 14 different pathogens were isolated from the symptomatic leaves during the summer season (March-May, 2010). *C. gloeosporioides, P.maculans, L. theobromae* and *P. palmarum* were the predominant pathogens during June-July while *F. verticillioides* was more during October-January seasons. Among the various pathogens, *L. theobromae* was the most frequently isolated pathogen followed by *C. gloeosporioides* and *P. maculans.*

Biochemical analysis of the coconut leaves with respect to total sugars, total soluble proteins and phenylalanine ammonia lyase activity showed that they were significantly higher in the leaves of the infected palms at all stages of infection. There was a very significant increase in the total sugars, total soluble proteins and PAL activity at early stages of infection which thereafter decreased significantly at moderate stage of infection. Total sugars increased at severe stage of infection while total soluble proteins and PAL activity decreased to further lower levels.

In vitro evaluation of fungicides against the predominant pathogens associated with Yellowing disease (*L. theobromae, C. gloeosporioides, P. maculans* and *F. verticillioides*) showed that propiconazole, tebuconazole and mancozeb were highly effective in completely inhibiting the mycelial growth of all the pathogens, while carbendazim was highly effective against *L. theobromae, P. maculans* and *F. verticillioides.* Flusilazole showed complete inhibition of growth of *C. gloeosporioides.*

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*Originals not seen.

FOLIAR FUNGAL PATHOGENS ASSOCIATED WITH YELLOWING DISEASE OF COCONUT

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ABSTRACT

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ABSTRACT

The study entitled 'Foliar fungal pathogens associated with yellowing disease of coconut' was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani.

A detailed survey was undertaken to study the incidence and intensity of Root (Wilt) Disease and Yellowing disease in the Instructional Farm, Vellayani. Both the diseases were observed in all the six blocks of the Farm. Out of 6107 palms in the farm 561 palms were affected by Root (Wilt) Disease (9.19 %) and 127 palms (2.08%) were showing symptoms of Yellowing disease. A total of 242 (43%) Root (Wilt) affected palms and 94 (74%) Yellowing disease affected palms are to be cut and removed since they are unproductive.

The characteristic symptoms of Root (Wilt) Disease were flaccidity, ribbing, yellowing and necrosis. The Root (Wilt) Disease affected palms (42%) were 'super infected' with leaf rot disease also. In Yellowing Disease affected palms sudden appearance of chlorosis/ yellowing/ bronzing of one or more leaves in the middle whorl coupled with flaccidity was the characteristic symptom. Rapid drying and necrosis of inflorescence, abnormal shedding of flowers, buttons, immature nuts and mature nuts occurred in succession either simultaneously or prior to yellowing and the palms became barren within a short period. On the chlorotic/ yellowed leaves intense brown to black leaf spots developed which enlarged, coalesced together and blighted the leaflets. The affected palms usually succumb within a short span of time.

Foliar fungal pathogens associated with leaf spots/ blights on the symptomatic leaves of Yellowing disease affected palms were isolated and the pathogenicity was proved. On artificial inoculation on detached spindle as well as mature leaflets, the pathogens produced tiny brown water soaked lesions of angular or oval or irregular shapes. Among the different pathogens *Chalara fimbriata* was the most virulent one. Based on the morphological and cultural

characteristics the following foliar fungal pathogens on Yellowing disease affected palms were identified: *Alternaria alternata, Aspergillus niger, Colletotrichum gloeosporioides, Cephalosporium* spp., *Chalara fimbriata, Curvularia* spp., *Fusarium verticillioides, F. semitectum, Lasiodiplodia theobromae, Pestalotiopsis maculans, Pestalotiopsis palmarum, Phomopsis* spp., *Arthrinium* spp. and *Verticillium* spp. Among them, *L. theobromae, C. gloeosporioides* and *P. maculans* were the most frequently isolated pathogens.

The biochemical analysis revealed that total sugars, total soluble proteins and phenylalanine ammonia lyase activity were higher in the leaves of diseased palms.

Among the nine fungicides tested *in vitro*, Propiconazole, Tebuconazole and Mancozeb were found to be equally effective against *L. theobromae, C. gloeosporioides, P. maculans* and *F. verticillioides.*

Appendices

APPENDIX - I

Composition of media

1. Carnation Leaf - piece Agar (CLA)

Sterile carnation leaf pieces	- 3-5 mm^2 (one piece per 2 ml medium)
Agar	- 20 g
Distilled water	- 1 l

2. Carrot Agar (CA)

Carrot	- 400 g
Agar	- 20 g
Distilled water	- 1 l

3. Czapek's Dox Agar (CDA)

$NaNO_3$	- 2 g
K_2HPO_4	- 1 g
$MgSO_4.7\ H_2O$	- 0.5 g
KCl	- 0.5 g
$FeSO_4$	- 0.01 g
Sucrose	- 30 g
Agar	- 20 g
Distilled water	- 1 l

4. Malt Extract Agar (MEA)

Malt extract	- 20 g
Agar	- 20 g
Distilled water	- 1 l

5. Potato Dextrose Agar (PDA)

Potato	- 200 g
Dextrose	- 20 g
Agar	- 20 g
Distilled water	- 1 l

6. Potato Sucrose Agar (PSA)

Potato extract	- 500 ml (1.8 kg potato in 4.5 l water)
Sucrose	- 20 g
Agar	- 20 g
Distilled water	- 500 ml

APPENDIX - II

Lactophenol - Cotton Blue

Lactophenol	- 100 ml
Cotton Blue	- 1 to 5 ml 1% aqueous solution
Glacial acetic acid	- 20 ml

APPENDIX - III

Anthrone reagent

Anthrone reagent made by dissolving 200 mg of Anthrone in 100 ml ice cold 95 per cent concentrated Sulphuric acid.

APPENDIX – IV

Buffers for biochemical analysis

1. Acetate buffer (0.1 M)

A: 0.2 M solution of acetic acid = 11.55 ml in 1000 ml

B: 0.2 M solution of sodium acetate = 16.4 g $C_2H_3O_2Na$ in 1000 ml

22.7 ml of A was mixed with 27 ml of B and the volume made upto 100 ml.

2. Borate buffer (0.1 M)

A: 0.2 M solution of boric acid = 12.4 g in 1000 ml

B: 0.05 M solution of borax = 19.06 g in 1000 ml

50 ml of A was mixed with 30 ml of B and diluted to a total of 200 ml. 0.05 g PVP was added.

APPENDIX – V

Stock dye solution for estimation of protein

100 mg of Coomassie brilliant blue G-250 was dissolved in 50 ml of 95 per cent ethanol and 100 ml of concentrated orthophosphoric acid was added. The volume was made upto 200 ml with water and kept at 4°C. The working dye was prepared just before use by diluting the stock solution to five times with water.

APPENDIX – VI

Weather data during the study period

Unit: AAS, Vellayani

Latitude: 8.5^0N

Height above MSL: 29 m

Hours of Observation: 07:22 hours, 14:22hours

Year	Month	Maximum Temperature (°C)	Minimum Temperature (°C)	Relative Humidity (%)		Rain (mm)
				I	II	
2009	October	29.7	24.5	89.1	85.00	102.7
2009	November	29.0	24.3	90.7	82.7	234.0
2009	December	30.7	23.7	90.2	85.7	51.6
2010	January	31.4	22.9	89.2	83.0	25.2
2010	March	34.0	24.4	88.6	72.2	0.0
2010	April	34.6	25.5	86.1	69.1	61.0
2010	May	32.8	25.0	86.8	78.2	278.9
2010	June	30.4	22.9	89.1	81.4	245.2
2010	July	30.6	22.8	87.5	77.3	199.3