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Preliminary studies on wilt disease complex in cocoa

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Abstract

The wilt disease complex of cocoa was first noticed in India in 1998, in Karnataka. The leaves became yellow or brown, branches wilted and finally the plant died. Pods of the affected trees did not show any external symptoms, but shrivelled slowly leading to the death of the trees. The affected plants were consistently associated with *Xylosandrus* beetles which inhabited short pin hole borings made by them. Several fungi were found associated with the disease of which *Ceratocystis* sp.? was found to be consistently present. Pathogenicity trials with inoculation of *Ceratocystis* sp.*?* by wound inoculation–cotton plugging method produced internal symptoms of wilt in the seedlings. Field trials with systemic chemicals showed that the diseased plants recovered when Propiconazole @ 0.2 %, and Imidacloprid @ 0.2 % were used simultaneously as whole plant drenching. The incidence of fresh infection was less when compared with the other treatments.

Key words : Cocoa, Wilt disease complex, *Ceratocystis sp?.*, *Xylosandrus* beetles, Propiconazole, Imidacloprid.

Introduction

Cocoa (*Theobroma cacao* L.) is an exotic crop, introduced in India in early 20th century with limited cultivation under the government owned farms. Systematic cultivation of cocoa in India started in 1970s. Both the varieties of "Criollo" and "Forastero" were introduced, however due to its high susceptibility to the different pests and diseases, Criollo variety was slowly replaced with Forastero. Cocoa is cultivated in India in an area of 16,200 ha producing 7000 MT of dry beans, with Karnataka contributing the major share (1700 MT from 8,900 ha) (Balasubramanian, 2002). It is grown mainly in the southern states of India, *viz.*, Kerala, Karnataka, Tamil Nadu and Andhra Pradesh and the cultivation is being extended to the non-conventional areas in other states, where coconut and arecanut are grown. Diseases caused by microorganisms are one of the major constraints in the production of cocoa resulting in great economic losses to the farmers. Detailed survey of cocoa gardens in Kerala, Karnataka and Tamil Nadu during 1980 indicated that the diseases caused by *Phytopthora* and *Colletotrichum*, vascular streak dieback and zinc deficiency were the major diseases in India causing significant economic losses (ChandraMohanan and Kaveriappa, 1981). *Ceratocystis* wilt disease complex (WDC) in cocoa caused by *Ceratocystis fimbriata* (Ell. and Hals) Walter., is one of the major production constraints in this crop which has been a major challenge to plant pathologists in the cocoa growing countries of the world (Zalasky, 1965; Thorold, 1975; Bezerra, 1997). Wilt diseases of cocoa is also known as *Ceratocystis-Xyleborus* complex and has been reported from major cocoa growing countries like Colombia, Costa Rica, Ecuador, Venezuela, Trinidad, Hawaii, and the Phillipines (Entwistle 1972). The diseased trees are always associated with the ambrosia beetles, whose young ones normally feeds on the fungs (Bakshi, 1950). The disease was first observed in India in 2000, in the Hunsur taluk of Mysore district, Karnataka (Chowdappa and Rohini Iyer, 2000; Chowdappa *et al.*,2000). However the outbreak of the disease in parts of Karnataka caused great concern among the farmers in that area. So a preliminary study was carried out involving the disease occurrence and its distribution in the Mysore district of Karnataka, pathogens involved in the disease and an adhoc field trial including a few chemicals and ***Pseudomonas fluorescens*** as a biocontrol agent.

Materials and methods

Mysore was selected for the survey since the wilt disease complex was first reported from Hunsur taluk in Mysore district. Further the disease was also confined to this taluk and a few gardens in Mysore district (Chandramohanan, 2002). Roving survey was conducted in the cocoa gardens in seven taluks of Mysore, in both the summer months (pre monsoon- March to May) and in the post monsoon season (September to November). Survey in post monsoon season was conducted for three years *viz.*, 2003, 2004 and 2005 and that of summer months in 2004, 2005 and 2006. Survey was conducted in the following areas *viz.*, Mysore taluk, K R Nagara, T N Pura, Hunsur, Nanjangud, H D Kote, and Periyapatna. The gardens were given a rating of 1 to 5, where 1 implies the best maintenance and 5 implies the worst maintenance. The maintenance factors were given on a total appraisal of the different factors in the garden (Table 1).

The ratings were then analyzed along with the percentage disease infection on cocoa using ANOVA test for significance. Infected plant samples were collected from the garden and standard tissue isolation procedure was followed using PDA and NA media (Dhingra and Sinclair, 1986). For pathogenicity tests, spore suspension containing 21 X 10^3 cfu per ml was used in cocoa seedlings using modified standard methods *viz.*, Pin prick method of inoculation, whole plant drenching and wound inoculation with cotton plugging method (Emmatty, 1974; Singh and Sarma, 1975; Dhingra and Sinclair, 1986). The plants were examined for infection till 60th day after inoculation. A farmer's garden in Yellal panchayat in Mysore district, Karnataka, with cocoa plants of age about 5 years, planted at a standard spacing of 2.7 X 5.4 M as an intercrop with coconut, was selected for conducting chemical trials along with a biocontrol agent *viz., P. fluorescens*. The plants were given a standard dose of recommended fertilizers and had disease incidence of 45.5%. The garden was divided into 4 plots of equal size with each plot having at least a minimum of ten diseased plants. Four replications were there for each treatment and five trees per replication were maintained. The pesticides used for the trials were selected based on the literature survey for the management of *C. fimbriata* in cocoa and other crops (Chee, 1970; Martin, 1971; Wood, 1975; Roux *et al.*, 2000; Yang et al 2000). Further among those pesticides, the products which were readily available locally were selected for the trials. Treatments were given at an interval of three months and observations on fresh infection and percentage recovery were made after thirty days of application.

Table 1. Criteria followed for giving ratings to the field maintenance

No	Irrigation (type and water availability per day per tree)	Frequency of field monitoring	Weeding	Pest management	Pruning	Overall appearance of the garden	Marks awarded	Rating
1	Drip irrigation, 20 litres	Daily	Regular, Hand weeding+ weedicide application	Prophylactic And therapeutic	Regular	Excellent	80-100	1
2	Sprinkler, 10-15 litres	Once in three days	Hand weeding once in at least three months	Prophylactic And therapeutic	Regular	Good	60-79	2
3	Sprinkler Less than 10 litres	Once in a week	Hand weeding once in three to six months	Only therapeutic	Once in Two years	Fair	40-59	3
4	Basin Irrigation Less than 10 litres	Once in 15 days	Not regular	Not regular	Not regular	Bad	20-39	4
5	Basin irrigation, less than 2 litres	Not regular	Not regular	Not regular	Not regular	Worst	0-19	5

Results and discussion

Survey

Roving survey conducted in the cocoa gardens of Mysore district showed that the wilt disease complex (WDC) was the highest in Hunsur taluk, where the disease was first observed. It was observed that in most of the infected gardens the farmers had lost interest and started neglecting the gardens. Thus the percentage

incidence was more in most of the gardens surveyed in 2005 and 2006 (Table 2) and many of the cocoa gardens had been cut down.

Hence it was decided to relate the maintenance of the garden and the percentage disease incidence. It was found that maintenance of the garden had a strong relation with the incidence of wilt disease complex

Table 2. Survey and current status of the disease in Mysore district-2005-2006

Location	Gardens surveyed			% of infection		
	03-04	04-05	05-06	03-04	04-05	05-06
Mysore taluk	24	15	10	22.0	25.0	27.0
K R Nagara	14	11	8	28.0	34.0	43.90
T N Pura	17	10	7	24.0	30.0	37.5
Hunsur	15	9	7	56.0	78.0	75.0
Nanjangud	21	10	8	30.0	35.0	40.4
H D Kote	23	13	7	34.0	46.0	49.0
Periyapatna	18	11	8	35.0	46.0	57.0

(Table 3 and Fig. 1). When the garden was well maintained (rating 1) the percent disease incidence was significantly less (24.15 and 32.11 in the summer and post monsoon observations respectively) when compared to the worst maintained (rating 5) gardens where it was very high (70.40 and 78.08). This showed that by proper hygiene and maintenance of the garden the disease could be managed in an effective manner. Also the meagre availability of soil moisture in the neglected gardens (ratings 3 to 5) caused severe stress in the plants and hence they became weakened. Iton (1959) observed that *Xyleborus* beetle which were important in the transmission of this disease were attracted to the stressed plants, due to emanation of ethylene from stressed plants. Further it was observed that the disease was always higher in the case of post monsoon observations. This may be because of the high availability of spores of the pathogen in the high humid atmosphere and also the insect vectors *viz.*, the *Xyleborus* beetles. Similar reports were made by Moller *et al.*,(1969) in the case of stone fruit trees, where they had reported that the infection by *Ceratocystis. sp.* had a strong correlation with the availability of atmospheric moisture and the injuries caused by the *Xyleborus* beetles in the post monsoon season. Iton (1961) studied the transmission of the disease by wind and the two forms of spores *viz.*, chlamydospores and endoconidia, which were liberated to the atmosphere through the frass from the bore holes made by *Xyleborus* beetles.

Table 3. Rating Vs % infection

Rating	Summer % infection	Post monsoon % infection
1	24.15	32.11
2	32.45	39.48
3	52.92	49.01
4	61.56	69.04
5	70.40	78.08
Cd at 5%	7.21	6.54

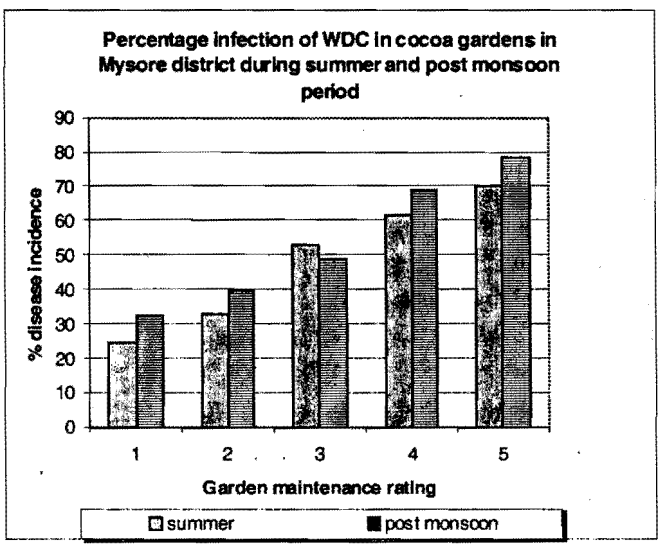

Fig. 1. Field survey for cocoa wilt complex in the Mysore district of Karnataka Rating (Garden Maintenance) : 1. Excellent 2. Good 3. Fair 4. Bad 5. Worst

Table 4. Pathogenicity trials

Method of inoculation	Days taken for appearance of symptoms	Symptoms noticed
Whole plant drenching	60	No symptoms
Pin prick method	42	Internal symptoms from the point of inoculation up to 3cms in both sides
Pin prick and cotton plugging method	30	-Do-

Pathogenicity studies

Several fungi were obtained from the WDC infected plant specimens i.e., *Ceratocystis sp.* (sent for identification to CABI, UK), *Fusarium* sp, *Botryodiplodia* sp, *Curvularia* sp, and *Colletotrichum* sp. However *Ceratocystis sp.* was found to be constantly associated with the infected plants and they were tested for Koch's postulates. The pathogenicity of *Ceratocystis sp.* in cocoa and other crops was earlier reported by many workers from other countries (Iton, 1959, in cocoa; DeVay *et al.*, 1968, in deciduous fruit trees; Somasekhara, 1999, in pomegranate). When they were inoculated through wound using cotton plugging method (Table 4), the symptoms of vascular discolouration up to a distance of 3 cm either way from the point of inoculation, was observed on the 30th day of inoculation. In the pin prick

method the symptoms were seen only on the 45th day as against no symptoms in the full drenching method. This clearly showed that the pathogen required a point of entry to infect the host plant, which may be either the wounds caused by cultural operations or the *Xyleborus* insects. Moller and DeVay (1968) proved conclusively that the pathogen *Ceratocystis sp.* was carried by several *Scolytid* beetles namely *Carphophilus freemani* and *Scolytus rugulosus* helping infection of deciduous fruit trees. In this study also the presence of insect pest *Xyleborus crassiculus* was found to be constantly associated with the disease in most of the WDC infected cocoa plants. It was observed that the disease was always associated with the *Xyleborus* beetles which bore shot holes in the stem, about 1mm diameter, with a small accumulation of its faecal matter at the mouth of the hole. The role of *Xyleborus corniculatus, X. hirtellus, X. trinidadensis* in the transmission of *Ceratocystis sp.* and in the process spreading the cocoa wilt disease from the infected trees to the healthy ones was reported by Iton and Conway,(1961). *Xylosandrus compactus* and *X. crassiusculus* were isolated from the infected trees in Hunsur, Mysore district, Karnataka (Chowdappa *et al.*,2002) The association of the fungus *Graphium* sp with the frass materials of the insects was also observed during this survey. Symptoms of WDC appeared as sudden wilting of a portion of the tree, starting from the tip of a chupon, followed by rapid wilting of the whole tree in a span of twenty days (Chandramohanan, 2002). Leaves hung in a pendulous fashion in the initial stages, with yellow to brown discoloration. The fungus produced large amount of spores in the galleries made by the *Xyleborus* beetles. Numerous chlamydospores were present in the discoloured wood and also frass present in the galleries. The fungus produced both asexual (thin walled/ thick walled endoconidium and very thick walled chlamydospores) and sexual spores (ascospores) (Fig.2a, 2b)

Table 5. Chemical control of WDC

	Treatment	% Recovery*	% Fresh infection*
T1	Propiconazole 0.2% +Imidacloprid 0.2%	**78.00**	**10.62**
T2	Propiconazole 0.2% + Carboryl 0.2%	71.25	19.75
T3	Tricyclazole 0.2%+Imidacloprid 0.2%	62.87	32.62
T4	Tricyclazole 0.2+ Carboryl 0.2%	58.87	15.50
T5	Calixin 0.2% +Imidacloprid 0.2%	34.25	28.87
T6	Calixin 0.2% + Carboryl 0.2%	39.37	31.62
T7	*Pseudomonas fluorescens* 10g per plant	24.12	33.12
T8	Control- no spray	12.50	64.62
	CD (± 0.05)	5.29	5.54

Fig 2a. Ascospore of *Ceratocystis* sp?.

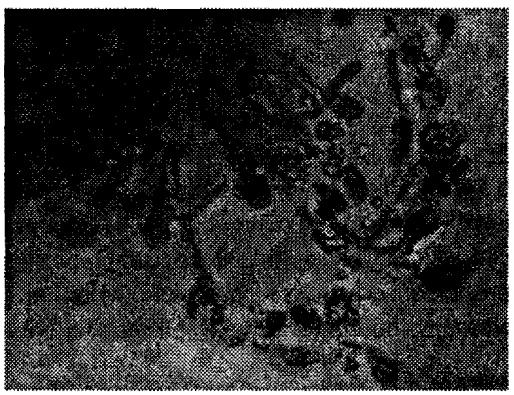

Fig 2a. Ascospore of *Ceratocystis* sp?.

Chemical trials

Since the disease was rampant in the cocoa gardens threatening the cocoa cultivation and also spreading an adhoc chemical recommendation was necessary for the management of the disease. Hence a field trial with pesticides based on their effectiveness studied by the earlier workers and their local availability, was laid in the farmer's garden at Mysore with 45.5 percentage disease incidence was selected and treatments were imposed (Table 5). The results indicated that Propiconazole @0.2% and Imidacloprid @0.2% when applied as foliar application simultaneously, gave 78.00 percentage recovery of the diseased plants when compared with 12.5% in the control (Table 5). The incidence of fresh infection in the same treatment was only 10.62% when compared with 64.62% in the control. Systemic fungicides of the azole groups were used with some success in controlling *Ceratocystis sp.* by post-harvest dips of *Ipomoea* roots (Yang *et al.*,2000). Use of systemic insecticides and azoles group of fungicides were tried by many workers with partial success. However resistance breeding was considered to be a better option in the management of this disease (Wood, 1975). Fungicides injected into the stems of *Platanus* species may provide some protection (Chee KH, 1970).

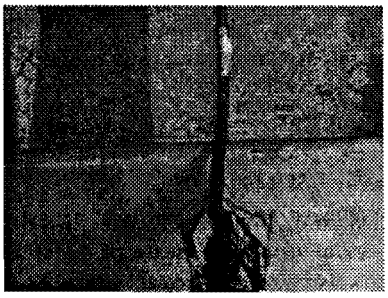

Fig 3a. Wound inoculation +cotton plugging method

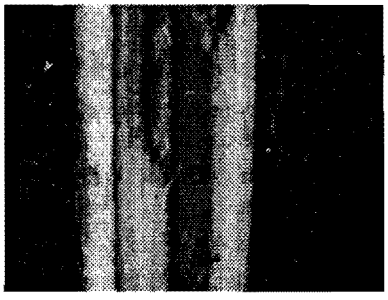

Fig 3b . Internal symptoms in the vascular bundles of the inoculated plants

Resistances to some diseases by the different clones of cocoa have been observed by Simmonds, 1994. Iton (1959) observed that cocoa clones ICS 38 and 45 were highly susceptible to the wilt complex, whereas ICS 1, 6, 40, 95, 98 and SCA 6 were highly resistant. He also observed that progenies of the cross ICS 95 and 98 were highly resistant to the disease.

Conclusion

From the above studies it was established that the WDC in cocoa was caused by a fungus resembling *Ceratocystis sp.*? and the Xyleborus beetles. *Xylosandrus compactus* and *X. crassiusculus* were found to be constantly associated with the disease. However further work has to be done to ascertain the nature of the disease complex and to find out whether the insect enters the host first or the pathogen enters. Proper maintenance of the gardens reduced the percentage of disease incidence.

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Fig 4a. Initial stages of WDC in cocoa

Fig 4b. Complete wilt of the tree

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