International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 2287-2291

Received: 07-07-2017
Accepted: 08-08-2017

Sangeetha Chinnusamy
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Beena Saralemma
Department of Plant Pathology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Correspondence
Sangeetha Chinnusamy
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Fungicidal studies on foliar fungi associated with die back disease of mango grafts in nursery

Sangeetha Chinnusamy and Beena Saralemma

Abstract
Two experiments were conducted to manage the die back disease of mango graft in nursery against two predominant fungal pathogens like *Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*. In the first experiment, all eight fungicides were tested under *in vitro* and *in planta* conditions with recommended dose. Among the eight fungicides only four fungicides were selected for second experiment (cuso4 + lime, copper hydroxide, copper oxychloride and carbendazim) to control the die back disease effectively. The highest per cent reduction over control of incidence (71.44%) and severity (80.02%) recorded by Bordeaux mixture.

Keywords: *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, Fungicides and Mango seedlings.

Introduction
Mango (*Mangifera indica* L.) is considered as the King of fruits and very useful tropical and subtropical countries in the world, which is cultivated in more than 87 countries but nowhere it is so greatly valued as in India. The main mango growing states are Uttar Pradesh (23.86), Andhra Pradesh (22.14), Karnataka (11.71%), Gujarat (6.00%), Tamil Nadu (5.09%) and the rest of the states have quite less production. Mango is not considered as a commercial crop of Kerala, but mango trees are inevitable components of homesteads of the state. The total estimated area under mango cultivation in this state is 75,911 ha with an annual production of 3, 23,517 tonnes.
Mango is affected by a number of diseases at all stages of its development *i.e*. from nursery to harvest. Young grafted mango plants also suffer from rapid necrosis and drying up of leaves. The die back symptom was initiated on leaf margin and leaf tip as light brown necrotic area with irregular dark brown margin. Later, it spread towards midrib as large blighted area was observed. The die back disease was considered as one of the major constraints in mango nurseries. The disease incidence may occur at any time of the year, but it is most conspicuous during October to November. In this context, the present study was undertaken to evaluate the efficacy of selected fungicides to manage the foliar pathogens associated with die back disease of six months old mango grafts.

Material and Methods
1. *In vitro E*valuation of Fungicides against Die Back Pathogens
The effectiveness of fungicides against dieback pathogens was tested under *in vitro* condition. Eight promising fungicides at the three different concentrations (Table 1) tested against the predominant foliar fungal pathogens associated with die back disease of mango graft in nursery *viz*., *C. gloeosporioides* and *B. theobromae* employing the poisoned food technique (Zentmeyer, 1955) [1]. The fungicides tested were $cuso_4$ + lime, copper oxychloride 50% WP (Fytran, Travancore copper fungicide private limited); copper hydroxide 77% WP (copper (II) hydroxide, Kocide 101, M/s E.I. DuPont India Ltd., Gurgaon); carbendazim 50% WP (methyl benzimidazol-2-ylcarbamate), (Bavistin, M/s BASF India Ltd., Mumbai); mancozeb 75% WP (manganese, ethylene-bis (dithiocarbamate) (polymeric) complex with zinc salt, (Dithane M-45, M/s Dow Agro Sciences India Pvt. Ltd., Mumbai); zineb 75% WP (zinc ethylene-bis (dithiocarbamate) (polymeric), (Avtar, M/s Indofil Industries Ltd., Mohali); captan 50% WP (N(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide) (Captaf) and Hexaconazole 5% SC ((RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol), (Contaf, M/s Rallis India Ltd., Bangalore).

The required quantity of fungicides was added in to 250 ml conical flask which containing 100ml of sterilized, cooled PDA medium. Three different concentrations of each fungicide was mixed separately with the medium and poured into sterilized Petri dishes @20 ml per plate. Mycelial disc of 10mm diameter were cut from actively growing seven day old culture of the pathogens and placed at the centre of each Petri dish containing poisoned medium. Three replications were maintained for each treatment and the medium without fungicide served as control. The Petri dishes 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 (PI) of growth over control was calculated by the formula (Vincent, 1927) [2]

PI=100(C-T)/C

Where

C = Growth of pathogen in control (mm)

T = Growth of pathogen in treatment (mm)

Table 1: Fungicides used for *in vitro* evaluation against dieback pathogens

Chemical name	Trade name	Dose (mL L^{-1})/ Concentrations (Per cent)
Cuso4+lime	Bordeaux mixture	5,10 and 15/ 0.5,1 and 1.5
Copper oxychloride	Fytran 50%WP	2,3 and 4 / 0.2,0.3 and 0.4
Copper hydroxide	Kocide 101 77%WP	0.5, 1.5 and 2.5/ 0.05,0.15 and 0.25
Mancozeb	Indofil M-45 75%WP	2, 3 and 4/ 0.2,0.3 and 0.4
Zineb	Indofil Z-78 75%WP	2, 3 and 4/ 0.2,0.3 and 0.4
Captan	Captaf 50%WP	2, 3 and 4/ 0.2,0.3 and 0.4
Hexaconazole	Contaf 5%SC	0.5, 1, and 1.5/ 0.05,0.1 and 0.15
Carbendazim	Bavistin 50%WP	0.5, 1 and 2/ 0.05,0.1 and 0.2

Evaluation of Different Fungicides against Die Back Diseases of mango graft under *In Planta* condition

Two experiments were laid out under *in planta* condition to study the efficiency of fungicides and one commonly used insecticide against dieback disease of mango grafts. The recommended dose of fungicides and insecticide was used for the experiments. The first experiment was conducted during the month of july to september, with all the eight fungicides, and insecticides quinalphos were included in this experiment. The foliar application of treatments was given on 15 day after establishment of grafts and four sprayings were given at 15 days interval. The details of the first experiment are as follows

Variety - Neelam
Design - CRD
Replications - 3
Number of treatments - 10
Number of plants/ treatment - 39

Based on the first experiment result, the most effective fungicide selected and second experiments were conducted during October – December. The details of the second experiment are as follows

Variety - Neelam
Design - CRD
Replications - 3
Number of treatments - 8
Number of plants/ treatment - 21

Assessment of Disease Incidence and Severity

Observation on disease incidence and severity were recorded on 10 days after each spraying. For assessing the disease incidence, total number of plants and plants infected by dieback disease in each treatment were recorded separately. The per cent disease incidence (PDI) of dieback disease was calculated separately using the formula.

PDI = Number of plants infected x100/Total number of plants observed

Disease severity of dieback disease from each treatment was calculated using 0-4 scale (Ambika and Abraham, 1979) [3] as given below

Grade : Description
0 : No necrotic lesions / streaks
1 : Up to 3 necrotic lesions/ streaks – general vigour of flushes affected
2 : 4-6 coalescing or non –coalescing lesions/ streaks – general vigour of flushes affected
3 : Above 6 coalescing or non –coalescing lesions/ streaks –general vigour of flushes affected
4 : Lesions /streaks confluent and drying of affected flushes

Statistical Analysis

Analysis of variance was performed on the data collected in various experiments using the statistical package (Freed, 1986) [4]. Multiple comparisons among treatment means were done using DMRT.

Result and Discussion

The main objective of the present study was the management of die back disease of mango graft in nursery. For this, at first an *in vitro* evaluation of different fungicides was carried out. Fungicides such as $Cuso_4$ + lime, copper oxychloride, copper hydroxide, mancozeb, captan, hexaconazole, carbendazim and zineb, each at three different concentrations were evaluated against die back pathogens. Among these fungicides, $Cuso_4$ + lime at all the three concentrations recorded hundred per cent inhibition on the growth of all the pathogens (Table 2 and 3). The effectiveness of $cuso_4$ + lime against die back pathogens of many crops was reported by many workers (Sharma and Kaul, 1990; Sharma and Badiyala, 1994; Sharma and Gupta, 1994; Ebenezer and Subramanian, 1995; Patil *et al*. 2007 and Sohi *et al*., 1973) [5-10] and hence the present result is in conformity with the earlier reports. It was followed by carbendazim which showed hundred per cent inhibition on the growth of *C. gloeosporioides* at all the three concentrations (Table 2). Carbendazim showed hundred per cent inhibition of *B. theobromae* at 0.1 and 0.2 per cent concentrations whereas at 0.05 per cent concentration, it recorded only 76.9 per cent inhibition over control. Earlier studies also reported the effectiveness of 0.1 per cent carbendazim for the management of *B. theobromae* causing stem end rot of mango (Singh *et al*., 1989) [11]. The fungicide hexaconazole was found to be very effectively inhibiting the growth of *C.gloeosporioides* causing anthracnose disease in guava (Sohi *et al*., 1973) [10]. Hexaconazole at 0.15 per cent concentration recorded the maximum inhibition (65.2 per cent) on growth of *B. theobromae* (Table 3).

After the *in vitro* evaluation of fungicides, an *in planta* experiment was conducted two times to know the effect of various treatments on the management of die back disease (Table 4 and 5). From the field observations and search of literatures, it was observed that the die back disease of mango

grafts was always associated with an insect attack. The injuries made by the insect predispose the plants for infection and also the die back pathogen could easily enter the plant through the wounds made by the insect. In this experiment, an insecticide like quinalphos was also included. So in the first *in planta* experiment the recommended dose of all fungicides were used for the *in vitro* evaluation and the insecticide, quinalphos @ 0.05 per cent were included (Table 4). The observations made on per cent disease incidence and severity of die back disease of mango revealed that all the treatments were superior to control. The incidence of dieback disease was observed only on control plants in the early stage of the experiment. Ten days after the last application of treatments, the highest reduction in disease incidence was observed in plants sprayed with $cuso_4$ + lime and copper oxychloride (91.67 per cent). It was followed by hexaconazole, carbendazim and copper hydroxide (80.56 per cent). The insecticide, quinalphos recorded 70.01 per cent reduction over control. The observation on per cent disease severity revealed that all treatments were superior to control. The highest reduction in disease severity (90.47 per cent) was observed in plants treated with one per cent $cuso_4$ + lime, 0.3 per cent copper oxychloride, 0.1 per cent carbendazim, 0.15 per cent copper hydroxide and all these treatments were found statistically superior than all other treatments. The efficiency of copper fungicides *viz*., $cuso_4$ + lime, copper oxychloride, copper hydroxide and the systemic fungicides hexaconazole and carbendazim for the management of plant diseases was reported earlier by many workers. The previous studies reported that $cuso_4$ + lime at one per cent concentration was the best to control *C. gloeosporioides* (Sharma and Kaul, 1990; Ebenezar and Subramanian 1995) [5, 8]. Similarly, Sharma and Gupta (1994) [7] showed the effectiveness of one per cent $cuso_4$ + lime against *B. theobromae*. The effectiveness of carbendazim against *C. gloeosporioides* and *B. theobromae* was reported earlier by many workers (Patil *et al*., 2007; Sohi *et al*. 1973; Singh *et al*., 1989; Deepthy, 2003; Sharma and Verma, 2007) [9-13]. Earlier studies reported the complete inhibition on the growth of *Colletotrichum* sp. by hexaconazole (Sharma and Badiyala 1994; Gupta *et al*., 2005) [6, 14]. The treatments T_9 (quinalphos) were also on par with the above treatments. From this, it was revealed that the insecticide quinalphos were equally efficient to the copper fungicides and systemic fungicides in the management of die back disease of mango. The fungicidal property of quinalphos was earlier reported and hence our observations confirm the earlier reports (Deepthy, 2003 and Bindu, 1996) [12-15].

A second experiment under *in planta* condition was carried out in which the best treatments from the *in vitro* evaluation and also from the first *in planta* experiment were used. Thus six treatments were selected *viz.* one per cent $cuso_4$ + lime, 0.15 per cent copper hydroxide, 0.3 per cent copper oxychloride, 0.1 per cent hexaconazole and 0.1 per cent carbendazim to confirm the result of first experiment. Observations on PDI and PDS of die back disease were taken separately and data did not show any significant difference among the treatments except in the die back incidence (Table 5). The highest per cent reduction of disease severity over control was exhibited by $cuso_4$ + limes (T_1) (80.02 per cent) and was on par with all other treatments. Similarly the highest reduction in PDI was also noticed in plants sprayed with $cuso_4$ + lime (T_1) (71.44 per cent). But it was on par with all other treatments except T_3 (copper oxychloride, 0.3 per cent) which recorded only 28.56 per cent reduction over control. It was concluded that all the five fungicides *viz*., Bordeaux mixture, copper hydroxide, hexaconazole and carbendazim and copper oxychloride were equally effective for the management of disease severity of die back of mango grafts. As discussed earlier, these observations are in conformity with the earlier reports. Many scientists observed that 0.1 per cent carbendazim was very effective to control die back of mango caused by *B. theobromae* (Sharma and Badiyala, 1994; Sohi *et al.*, 1973) [6and10]. Similarly early report said that the effectiveness of one per cent $cuso_4$ + lime to control *C. gloeosporioides* causing diseases in citrus, cashew and guava respectively (Ebenezar and Subramanian,1995; Patil *et al*., 2007 and Bindu,1996) [8,9 and 15].

Summing up the results of *in vitro* evaluation of fungicides against die back pathogens, it was found that $cuso_4$ + lime at 0.5, 1.0 and 1.5 per cent concentrations was most effective to control the pathogens since it recorded hundred per cent inhibition on growth of these pathogens. Similarly carbendazim also recorded hundred per cent inhibition of *C. gloeosporioides* and *B. theobromae* except at the lowest concentration of 0.05 per cent which was observed in case of *B. theobromae.* Hexaconazole at 0.1 and 0.15 per cent showed more than 80 per cent inhibition on the growth of *C. gloeosporioides*. The present study concluded that selected six fungicides of $cuso_4$ + lime, carbendazim, copper oxychloride, copper hydroxide and hexaconazole exhibited effective management of die back disease which not significantly differ each other.

Table 2: *In vitro* evaluation of fungicides against *C. gloeosporioides*

Fungicides	Concentrations (Per cent)	Colony diameter (mm)* DAI				Per cent inhibition over control
		2	4	6	8	
$Cuso_4$ + lime #	0.5	0	0	0	0	100
	1.0	0	0	0	0	100
	1.5	0	0	0	0	100
Copper oxychloride 50%WP	0.2	17.3^{bc}	40.0^{b}	52.3^{de}	52.0^{d}	42.2
	0.3	13.3^{efg}	36.2^{c}	50.5^{e}	52.0^{d}	42.2
	0.4	11.0^{hi}	31.3^{de}	48.3^{e}	49.0^{d}	45.6
Copper hydroxide 77WP	0.05	13.7^{ef}	22.7^{f}	31.7^{g}	33.3^{f}	63.0
	0.15	11.7^{ghi}	21.5^{f}	27.8^{gh}	29.3^{fg}	67.4
	0.25	11.2^{hi}	15.0^{ghi}	24.2^{h}	24.7^{h}	72.6
Mancozeb 75WP	0.2	14.0^{e}	32.8^{d}	55.3^{cd}	58.7^{c}	34.8
	0.3	12.2^{fgh}	29.7^{e}	50.8^{e}	53.2^{d}	40.9
	0.4	11.7^{ghi}	30.5^{de}	49.5^{e}	50.7^{d}	43.7

Captan 70WP	0.2	13.5^{efg}	20.8^{f}	31.7^{g}	38.3^{e}	68.5
	0.3	11.8^{ghi}	17.0^{g}	33.3^{g}	33.0^{f}	63.3
	0.4	11.7^{ghi}	16.0^{gh}	23.7^{h}	25.7^{g}h	71.4
Hexaconazole5%SC	0.05	10.3^{hi}	13.0^{hij}	17.8^{i}	20.7^{i}	77.0
	0.1	10.0^{i}	12.5^{ij}	15.7^{i}	17.8^{i}	80.2
	0.15	10.0^{i}	10.5^{j}	15.2^{i}	16.7^{i}	81.4
Carbendazim 50WP #	0.05	0	0	0	0	100
	0.1	0	0	0	0	100
	0.2	0	0	0	0	100
Zineb75WP	0.2	18.2^{b}	42.7^{b}	64.3^{b}	66.7^{b}	25.9
	0.3	16.0^{cd}	42.3^{b}	64.7^{b}	66.8^{b}	25.8
	0.4	15.0^{de}	35.8^{c}	58.5^{c}	60.3^{c}	33.0
Control	-	29.7^{a}	60.8^{a}	83.0^{a}	90.0^{a}	-

DAI – Days after inoculation, * Mean of three replications, # Not included in statistical analysis, In each column figures followed by same letter do not differ significantly according to DMRT

Table 3: *In vitro* evaluation of fungicides against *B. theobromae*

Fungicides	Concentrations (Per cent)	Colony diameter (mm)* DAI			Per cent inhibition over control
		2	4	6	
$Cuso_4$ + lime #	0.5	0	0	0	100
	1.0	0	0	0	100
	1.5	0	0	0	100
Copper oxychloride 50%WP	0.2	23.5^{hi}	37.7^{efg}	45.3^{ef}	49.7
	0.3	21.8^{hi}	34.0^{gh}	42.8^{fg}	52.4
	0.4	20.7^{ijk}	31.5^{hi}	38.3^{hi}	57.4
Copper hydroxide 77WP	0.05	23.7^{h}	37.5^{efg}	46.7^{def}	48.1
	0.15	21.3^{hij}	36.8^{efg}	45.7^{ef}	49.2
	0.25	18.3^{kl}	29.0^{ij}	40.7^{gh}	54.8
Mancozeb 75WP	0.2	34.7^{cd}	45.7^{bc}	58.0^{b}	35.6
	0.3	31.0^{ef}	42.8^{cd}	50.2^{cd}	44.2
	0.4	28.7^{fg}	37.7^{efg}	46.3^{ef}	48.6
Captan 70WP	0.2	30.2^{efg}	38.5^{ef}	46.8^{de}	48.0
	0.3	29.2^{fg}	36.5^{fg}	44.8^{ef}	50.2
	0.4	27.3^{b}	35.5^{fg}	43.3^{efg}	51.9
Hexaconazole 5%SC	0.05	20.8^{hijk}	28.8^{ij}	40.5^{gh}	55.0
	0.10	18.5^{jkl}	27.3^{j}	35.7^{i}	60.3
	0.15	16.3^{l}	23.3^{k}	31.3^{j}	65.2
Carbendazim 50WP #	0.05	10.7^{m}	14.8^{l}	20.8^{k}	76.9
	0.1	10.0^{m}	10.0^{m}	10.0^{l}	88.9
	0.2	10.0^{m}	10.0^{m}	10.0^{l}	88.9
Zineb75WP	0.2	38.8^{b}	49.0^{b}	58.0^{b}	35.6
	0.3	36.0^{c}	40.7^{de}	50.7^{c}	43.7
	0.4	32.5^{de}	40.7^{de}	47.0^{de}	47.8
Control	-	46.0^{a}	60.8^{a}	83.0^{a}	-

DAI – Days after inoculation, * Mean of three replications, # Not included in statistical analysis, In each column figures followed by same letter do not differ significantly according to DMRT

Table 4: Effect of various treatments on per cent disease incidence (PDI) and severity (PDS) of die back disease of mango grafts (First trial)

Treatments No.	Fungicides	Per cent reduction over control	
		PDI	PDS
T_1	$Cuso_4$ + lime (1%)	91.67^{a}	90.47^{a}
T_2	Hexaconazole (0.1%)	80.56^{ab}	84.94^{a}
T_3	Copper oxychloride 50%WP (0.3%)	91.67^{a}	90.47^{a}
T_4	Mancozeb 75WP (0.3%)	30.56^{c}	30.14^{c}
T_5	Zineb (0.3%)	40.02^{c}	35.69^{c}
T_6	Captan (0.3%)	50.01b^{c}	65.08^{b}
T_7	Carbendazim50WP (0.1%)	80.56^{ab}	90.47^{a}
T_8	Copper hydroxide 77WP (0.15%)	80.56^{ab}	90.47^{a}
T_9	Quinalphos (0.05%)	70.01^{abc}	79.35^{ab}
T_{10}	Control	0.00	0.00

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

Table 5: Effect of selected treatments on per cent disease incidence (PDI) and severity (PDS) of die back disease of mango grafts (second trial)

Treatments No.	Fungicides	Per cent reduction over control	
		PDI	PDS
T_1	$Cuso_4$ + lime (1%)	71.44[a]	80.02[a]
T_2	Hexaconazole (0.1%)	57.13[ab]	70.03[a]
T_3	Copper oxychloride 50%WP (0.3%)	28.56[b]	40.05[a]
T_4	Carbendazim50WP (0.1%)	57.13[ab]	70.03[a]
T_5	Copper hydroxide 77WP (0.15%)	57.13[ab]	60.03[a]
T_6	Control	0.00	0.00

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

Acknowledgement
The authors are thankful to Kerala Agricultural University for providing financial assistance and facilities for the study.

References

1. Zentmeyer GA. A laboratory method for testing soil fungicides with *Phytophthora cinnamomi* as test organism. Phytopath. 1955; 45:398-404.
2. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1927; 159:850.
3. Ambika B, Abraham CC. Bio-ecology of *Helopeltis antonii* Sign. (Miridae: Hemiptera) infesting cashew trees. Entomn. 1979; 4:335-342.
4. Freed R. MSTAT version 1.2. Department of crop and soil science. Michigan State University, 1986.
5. Sharma RL, Kaul JL. Field evaluation of fungicides for control of olive anthracnose. Indian J. Mycol. Pl. Path. 1990; 20(2):185-187.
6. Sharma IM, Badiyala SD. Effect of pre-harvest fungicidal sprays against stem end rot of mango fruits in storage caused by *Botryodiplodia theobromae* Pat. causal organism of canker and die back of mango. Indian J. Mycol. Pl. Path. 1994; 24(2):146-147.
7. Sharma IM, Gupta VK. Evaluation of different fungicides on *Botryodiplodia theobromae Pat*. Causal organism of canker and die back of mango. Indian J. Mycol. Pl. Path. 1994; 24(2):146-147.
8. Ebenezar EG, Subramanian KS. Chemical control of die back of acid lime caused by *C. gloeosporioides*. 1995; 48:112.
9. Patil KAS, Gaikwad AP, Sonawane RB, Nagrale DT. Efficacy of different fungicides against *C. gloeosporioides* causing anthracnose of guava. J. Mycol. Pl. Path. 2007; 37(3):592.
10. Sohi HS, Sokhi SS, Tiwari RP. Studies on the storage rot of mango caused by *Colletotrichum gloeosporioides* Penz. and its control. Phytopath Mediterranean. 1973; 12:114-116.
11. Singh A, Aulakh KS, Chahal SS. Effect of different fungicides on *B. theobromae*, the causal organism of canker and die back of pear. Indian J. Mycol. Pl. Path. 1989; 19(2):214-215.
12. Deepthy KB. Evalution and management of pest complex in cashew grafts. M.Sc. (Ag.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, 2003, 55.
13. Sharma A, Verma KS. *In vitro* cross pathogenicity and management of *Colletotrichum gloeosporioides* causing anthracnose of mango. Ann. Plant Prot. Science. 2007; 15:186-188.
14. Gupta S, Kalha CS, Vaid A, Rizvi SHE. Integrated management of anthracnose of french bean caused by *Colletotrichum lindemuthianum.* J. Mycol. Pl. Path. 2005; 35:432-434.
15. Bindu N. Die back in cashew incited by the tea mosquito bug, *Helopeltis antonii* Signorett as influenced by wound pathogens and plant biochemical. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 1996, 54.