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DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF FUNGICIDES AGAINST FUNGUS *FUSARIUM MANGIFERAE*

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Abstract

Eight fungicides were evaluated for their *In vitro* effect on the colony growth of *Fusarium mangiferae* after 3, 8 and 16 days of inoculation in pre-amended Potato dextrose agar (PDA) medium. The fungicides showed variable response in inhibiting the colony growth of the pathogen according to their nature and specificity at different minimum inhibitory concentrations (MICs). Benlate 50 WP and Carbendazim proved to be the best fungicides giving 100% suppression of the colony growth. When decrease of colony growth over control was examined, Benlate and Carbendazim showed 100% decrease over control after 3, 8 and 16 days of inoculation. The fungicides Score 250 EC, Daconil W 75 and Captan 50 WP proved to be comparatively less effective. The fungicides were classified into three types i.e., I, II and III in reference to the sensitivity of *F. mangiferae.* Fungus proved highly sensitive to type-I fungicides (Benlate 50 WP, Carbendazim, Topsin-M 70 WP and Copper oxychloride 50 WP) with 100% suppression at tested MICs. The studies will be helpful to devise suitable control strategy to curb malformation in mango orchards.

Introduction

Mango (*Mangifera indica* L.) is a prominent commercial fruit of Indo-Pakistan sub-continent. It is susceptible to various biotic and abiotic stresses (Shahbaz *et al*., 2009). The major obstacle in the establishment of economically profitable orchards in the world is mango malformation (MM). The term refers to deformation of vegetative apex in seedlings / saplings or inflorescences in mature trees which ultimately turns into abnormal appearances. Since its first record by Maries (Watt, 1891), little is known about nature of the disease and the pathogen. This disease has been reported from India, Bangladesh, Burma, UAE, Indonesia, Malaysia, USA, Australia, Egypt, South Africa, Sudan, Cuba, Central America, Brazil, Mexico, Israel, Philippines and Thailand (Kumar *et al*., 1993). During the last two decades or so, the problem of MM has assumed an alarming magnitude. It has a serious impact on the propagation of trees and commercial fruit production. Despite hectic efforts, it defies complete solution. Overall yield losses may be as high as 90% (Ploetz, 1999). In Pakistan, MM is a serious disease in the orchards of Punjab and Sindh provinces. Almost all the cultivars are susceptible and lack of management skills has aggravated the problem.

Most of the attempts to control malformation have met with little success in the world. Pruning of symptomatic tissues (Covarrubias, 1980; Pinkas & Gazit, 1992; Pernezny & Ploetz, 2000) and application of systemic fungicides like benomyl (Siddiqui *et al*., 1987) have shown some efficacy.

In order to maintain the economic status of mango, a solution oriented approach is required to combat the disease and to maximize production. Fungicides provide a useful potential tool to control a disease where other measures prove ineffective. Use of fungicides is an essential component of integrated management against mango malformation. Fungicides may act on or interrupt the metabolic system of the pathogen (Bilgrami & Dube, 1976). The effectiveness of a fungicide depends on its innate toxicity and permeation. Different protectants and systemic fungicides have been reported to be used *In vitro* against the fungus *F. mangiferae*.

Kumar (1983) found Carbendazim as highly effective fungicide in *In vitro* tests against *Fusarium* sp. isolated from malformed mango tissues. Pujol *et al*. (1997) determined the minimum inhibitory concentrations (MICs) of amphotericin B, micinazole, ketoconazole, flucytosine, itraconazole and fluconazole for isolates of different *Fusarium* species including *F. subglutinans* by a broth microdilution method. In general, *Fusarium* species strains showed resistance to all the antifungals tested. However, the most active agent was amphotericin B.

The isolates of *F. moniliforme* collected from various cities of Japan were examined for their sensitivity to Pefurazoate and Benomyl. The MICs of the compounds were in a range from 0.78 ppm to 12.5 ppm. The isolates were classified into four types on the basis of their sensitivity to both the fungicides (Wada *et al*., 1990). Hamamura *et al*. (1989) examined the sensitivity of *F. moniliforme* to triflumizole and clarified the existence of less sensitive isolates valued at 1,000 ppm or more of MIC. The MIC values of triflumizole against *F. moniliforme* were widely distributed in a range from 0.2 ppm to 400 ppm.

The conventional breeding has little impact on cultivar development in mango. Almost all the cultivars are derived from open pollinated/chance seedlings (Litz, 1994).The problem is intensified due to lack of resistance in available local and exotic cultivars. *In vitro* studies against *F. mangiferae* are barely sufficient and even that report application of old fungicides with only one or few test doses. There is no report on systematic splitting of doses (10, 25, 50 and 100 ppm) with precise information on retainment of efficacy after different study periods. Similarly, sensitivity types, taking into count different minimum inhibitory concentration (MIC) values, have not been characterized against *F. mangiferae*.

The objectives of the study were to evaluate different fungicides under lab conditions to find out the most effective one for final use in the field trial. The results of these studies will be helpful to the mango growers to adopt the most suitable control strategy.

Materials and Methods

In vitro evaluation of various fungicides to check the colony growth of the fungus *F. mangiferae* was done through poisoned food technique described by Borum & Sinclair (1968) on potato dextrose agar (PDA) medium. The experiment was conducted in Completely Randomized Design (CRD) with 9 treatments. Eight fungicides viz., Benlate 50 WP, Topsin-M 70 WP, Carbendazim, Score 250 EC, Copper oxychloride 50 WP, Trimiltox Forte, Daconil 75 WP and Captan 50 WP, were tested each with 4 doses/ MICs i.e., 10, 25, 50, and 100 ppm (Table 1).

After autoclaving, 25 ml of PDA medium amended with fungicides in four different concentrations in separate 100 ml flasks was poured in sterilized 90 mm Pyrex Petri plates. The PDA medium without fungicide was kept as control. The fungus *F. mangiferae* was picked from purified culture in the form of a 5 mm agar disc and inoculated in the center of each Petri plate. Four replicate plates were inoculated for each fungicidal concentration. The dishes were incubated at 25°C receiving fluorescent light with 12 hr cycling. Mean colony diameter was measured after 3, 8 and 16 days of inoculation.

Table 1. Nomenclature of fungicides used in the study.

Sr. No.	Trade name	Common name	Technical name	Chemical group
1.	Benlate 50 WP	Benomyl	Methyl 1-(butylcarbamoyl)benzimidazole-2-ylcarbamate	Benzimidazole
2.	Carbendazim	Carbendazim	Methyl benzimidazole-2-ylcarbamate	Benzimidazole
3.	Topsin-M 70 WP	Thiophanate methyl	Dimethyl 4,4'(ophenylene)bis(3-thioallophanate)	Benzimidazole
4.	Copper oxychloride 50 WP	Copper oxychloride	Dicopper chloride trihydroxide	Copper
5.	Trimeltox forte	Copper + Mancozeb	Tetra copper tricalcium sulphate	Carbamate
6.	Captan 50 WP	Captan	N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide	N-trihalomethylthio
7.	Score 250 EC	Difenoconazole	Cis,trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazole-1-ylmethyl)-=1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether	Azole
8.	Daconil W 75	Chlorothalonil	Tetrachloroisophthalonitrile	Phthalimide

Data analysis: Two readings of colony growth were taken for each Petri dish. Mean colony diameters (mm) of *F. mangiferae* were used to calculate percent decrease over the control for each treatment. Mean colony growth in different fungicidal treatments and percent decrease of growth in each treatment over control were analyzed by analysis of variance as three factorial combination of fungicide, concentration and days. Means were compared at *P*= 0.05 by Least significant difference (LSD) test.

Results

In this study, fungicides were evaluated for their effect on mycelial growth of *F. mangiferae* to identify best effective chemicals. Eight selected fungicides are known to be effective against fungi of the same class correlated to *Fusarium* spp. The fungicides showed variable response in inhibiting the colony growth of the pathogen according to their nature and specificity at different MIC values. Benlate and Carbendazim proved to be the best fungicides giving no growth of the fungus after 3, 8, and 16 days of inoculation. The effect was retained till the termination of the experiment after 16 days. Topsin-M followed Benlate and Carbendazim & showed 12 and 13.88 mm mean colony diameter at 10 ppm dose, after 8 and16 days, respectively, while no growth was observed after 3 days at the same dose. Least increase in growth (1.88 mm) was recorded even after interval of 8 days at 10 ppm dose (Table 2). Copper and Trimeltox followed the Benzimidazole group (Benlate, Carbendazim, Topsin-M). Copper showed 0.37 and 1.62 mm colony diameter at 10 and 25 ppm after 3 and 8 days while 4.12 and 12.63 mm after 16 days at the same doses, respectively. Comparatively improved efficacy was exhibited at 50 ppm where colony diameter was reduced to zero after 3 and 8 days but 1.12 after 16 days, respectively. The best Copper dose was 100 ppm which yielded no growth at all the three observations. Trimeltox displayed less effect at 10 ppm dose. The fungal growth of 1.37 mm after 3 days reached 5.5 and 25.0 mm, after 8 and 16 days, respectively. At 25 ppm, although growth was zero after 3 and 8 days but 10.75 mm was observed after 16 days showing its reduced efficacy; this difference was significant at $P < 0.05$. The best doses were 50 and 100 ppm which showed only 6.0 and 4.0 mm growth after 16 days, respectively.

Based on the results, fungicides were classified into three types i.e., I, II and III, in reference to the sensitivity of *F. mangiferae* (Table 3). Fungus proved highly sensitive to Benlate, Carbendazim, Topsin-M and Copper oxychloride which were assigned type I with 100% suppression at 10, 10, 25 and 100 ppm MICs, respectively. Copper oxychloride and Topsin-M joined the same type but they achieved complete control at 25 and 100 MICs, respectively. Trimeltox was placed under type II with 98% suppression. Fungus was moderately sensitive (MS) to this fungicide as compared to type I fungicides at a MIC of 50 ppm. Fungus showed comparatively less sensitivity (LS) to Captan, Score and Daconil and they were classified under type III with suppression level upto 91%. MIC for these fungicides was measured as 100 ppm.

Discussion

Overall suppressive effect was displayed by all the fungicides and the colony growth decreased with increase in fungicidal concentration. Keeping in view the efficacy and cumulative performance, tested fungicides may be placed into three groups. First group constitutes highly effective fungicides viz. Benlate, Carbendazim and Topsin-M. Second group includes Copper oxychloride and Trimeltox. This group appeared to be medium effective. Third group comprises of Score, Daconil and Captan and these fungicides were comparatively less effective.

Table 2. Mean values of colony growth of *Fusarium mangiferae* recorded after 3, 8 and 16 days of inoculation on medium pre-amended with 4 different fungicidal concentrations by food poison technique.

Sr. No.	Fungicide	MICs	Mean colony growth (mm)		
			3 days	8 days	16 days
1.	Benlate	10	0.00 a	0.00 a	0.00 a
		25	0.00 a	0.00 a	0.00 a
		50	0.00 a	0.00 a	0.00 a
		100	0.00 a	0.00 a	0.00 a
2.	Topsin-m	10	0.00 a	12.0 0 m-p	13.88 o-r
		25	0.00 a	0.00 a	0.00 a
		50	0.00 a	0.00 a	0.00 a
		100	0.00 a	0.00 a	0.00 a
3.	Carbendazim	10	0.00 a	0.00 a	0.00 a
		25	0.00 a	0.00 a	0.00 a
		50	0.00 a	0.00 a	0.00 a
		100	0.00 a	0.00 a	0.00 a
4.	Score	10	3.12 b-f	13.25 n-q	29.38 w
		25	1.87 a-d	10.38 k-m	21.63 t
		50	1.62 a-d	6.75 g-j	17.13 s
		100	0.00 a	4.75 e-h	12.63 mp
5.	Copper oxychloride	10	0.37 ab	1.62 a-d	12.63 m-p
		25	0.37 ab	1.62 a-d	4.12 c-g
		50	0.00 a	0.00 a	1.12 ab
		100	0.00 a	0.00 a	0.00 a
6.	Trimeltox	10	1.37 a-c	5.50 f-i	25.00 u
		25	0.00 a	0.00 a	10.75 kn
		50	0.00 a	0.75 ab	6.00 g-j
		100	0.00 a	0.00 a	4.00 c-g
7.	Daconil	10	2.37 a-e	8.75 j-l	28.25 v-w
		25	2.50 a-e	8.25 i-k	20.75 t
		50	0.00 a	7.00 g-h	16.25 r-s
		100	0.00 a	6.75 g-j	15.75 q-s
8.	Captan	10	5.75 f-i	25.75 uv	67.88 v
		25	4.37 dg	25.00 u	67.88 v
		50	1.50 ac	11.25 l-o	51.13 x
		100	0.00 a	5.12 e-h	10.63 k-n
9.	Control	-	14.99 p-s	51.20 x	76.00 z
		-	14.69 p-s	49.98 x	76.00 z
		-	14.50 p-s	51.40 x	75.98 z
		-	14.85 p-s	51.45 x	76.02 z
	LSD		**2.803**		

Means sharing the same letters are not significantly different at $p<0.05$ by LSD test.

Table 3. Sensitivity of *Fusarium mangiferae* to 8 fungicides.

Sr. No.	Fungicide	Sensitivity	MICs (ppm)	Sensitivity type
1.	Benlate 50 WP	S	10	I (Suppression of 100%)
2.	Carbendazim	S	10	
3.	Topsin-M 70 WP	S	25	
4.	Copper oxychloride 50 WP	S	100	
5.	Trimeltox forte	MS	50	II (98%)
6.	Captan 50 WP	LS	100	III (88-92%)
7.	Score 250 EC	LS	100	
8.	Daconil W 75	LS	100	

S = Sensitive, MS = Moderately sensitive, LS = Less sensitive

Benlate and Carbendazim were highly effective and retained their efficacy even at lowest concentration achieving 100% decrease over control. Topsin-M proved to be equally good except that it was less effective at low dose (10 ppm) but displayed equivalent effect at higher doses (25, 50 and 100 ppm). Our studies are consistent with the findings of Mamza *et al*., (2010) who tested six fungicides *In vitro* against fungus *F. pallidoroseum* and found benomyl to be the best fungicide completely inhibiting mycelial growth of the fungus at 1.5x, 1.0x and 0.5x mg a.i/ml. In a similar study, Pandey & Chakrabarti (2004) applied Carbendazim against *F. moniliforme*. Carbendazim inhibited the growth of the germ tubes produced by conidia and also reduced the infection rate. However, Gaur & Chakrabarti (2009) found Captan and carbendazim to be most effective in arresting the mycelial growth of *F. mangiferae*.

Benlate, Carbendazim and Topsin-M belong to the same Benzimidazole group. They are systemic and are readily absorbed to reach the target part. Benlate and Carbendazim have the same active ingredient Methyl 1-2 Benzimidazole carbamate (MBC). Great similarity in fungitoxic spectrum, mode of action and their chemical structures has been reported. They interfere with mitosis in cell in green plants and fungal hyphae and have good correlation between *In vitro* efficacy and fungal disease. Topsin-M although based on Thiourea, is classified under Benzimidazole group due to conversion to Benzimidazole ring for its activity and resemblance of fungicidal spectrum to that of Benlate (Singh, 1984). These fungicides on treatment with water produce MBC. They show broad spectrum of fungitoxic activity against members of class Fungi Imperfecti. They have been found much effective against *Fusarium* species in *In vitro* and *In vivo* studies.

Copper oxychloride and Trimeltox showed medium efficacy. Copper oxychloride completely suppressed the fungal growth when added to the medium at higher concentration (100 ppm). It is used to control diverse diseases and is reported to be effective against *Fusarium* spp., (Nene & Thapliyal, 1993). Although it is non systemic but copper kills fungal spores by combining with sulphhydral group of certain enzymes. Inactivation of fungal enzymes by copper ions gives good inhibition than other non systemic fungicides. Trimeltox also contains copper as an important ingredient.

When sensitivity values were measured, quite similar trend of Benlate and Carbendazim was found. They had similar effect on the sensitivity pattern of fungus *F. mangiferae*. Both the fungicides fell in type I and fungus showed sensitivity at 10 ppm MIC. Copper oxychloride and Topsin-M shared the same type but they achieved complete control at 25 and 100 MICs, respectively. This differentiates the minimum inhibitory concentration at which these fungicides proved effective and displayed their

lethal effects against the fungus. The present work is supported by the findings of Wada *et al*., (1990) who tested three chemicals against *F. moniliforme* isolates. Fungus proved sensitive to Benlate at 12.5 ppm MIC.

The MIC values of type I fungicides ranged from 10 to 100 ppm. Type II (Trimeltox) and III (Captan, Score, Daconil) were effective at 50 and 100 ppm, respectively. Type III fungicides required higher doses to manifest their fungitoxicity. Non systemic fungicides prevent infection largely by inhibition of spore germination and germ tube elongation. They required increased concentrations probably to give a better effect. Still at higher doses, the effect could not be retained for a longer period as observed in their interaction with days. In studies on *In vitro* evaluation of fungicides against *Sclerotinia minor*, Daconil was found less effective to check the vegetative stage mycelium at low concentrations. Sectors of rapidly growing colonies developed on the periphery of colonies (Porter & Lankow, 1981). Score, although systemic, but could not exhibit desired effect due to non-specificity against *Fusarium* spp.

The use of fungicides in the laboratory *vis-à-vis* field depends on their *In vitro* efficacy at minimal, economically acceptable dosages and their efficient and rapid transport to the infection site. Indiscriminate or inappropriate use can encourage the development of resistance in fungi. Evaluation of MICs of 8 fungicides helped to standardize the best fungicides viz. Benlate and Carbendazim against *F. mangiferae* in the present study. This study will be much helpful in future to devise fungicidal application schedule for commercial orchards.

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