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EFFECTIVENESS OF DIFFERENT FUNGICIDES AGAINST PREDOMINANT AND VIRULENT FUNGUS *FUSARIUM NIVALE* THE CAUSE OF MANGO MALFORMATION DISEASE

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ABSTRACT

Fusarium nivale (Fr.) Ces. a recently reported fungus of mango malformation disease (MMD) is a predominant and virulent fungus in mango orchards of Sindh, Pakistan. In the current study, *in vitro* and *in vivo* attempts were made with commercial fungicides to reduce the severity of *F. nivale*. Mycelial growth of *F. nivale* was significantly inhibited at low and high doses of Thiophanate methyl and Fosetyl- Aluminium. Metalaxyl+Mancozeb and Mancozeb also reduced growth of fungus at their high doses (6.830 and 11.900mm), respectively, as compared to Copper oxychloride (18.083 mm) and control (40.750 mm). Thiophanate methyl and Fosetyl- Aluminium significantly reduced infection in Desi, Almas and Dusheri to 16.60 and 19.00%; 17.60 and 19.80%; and 20.60 and 22.00% after first spray, with decreased percent of malformation 72.33 and 68.33%; 71.11 and 67.54%; and 67.81 and 65.62% and over untreated control. The second spray of Thiophanate methyl and Fosetyl- Aluminium fungicides completely inhibited infection of *F. nivale*, and 100.0% reduction in malformation disease in Desi, Almas, and Dusheri as compared to Metalaxyl+Mancozeb (78.73, 73.84, 72.64%) and Mancozeb (73.65, 73.69, 69.41%), Copper oxychloride and in control. The application of Thiophanate methyl and Fosetyl- Aluminium would be useful in integrated management of MMD.

Keywords: *Fusarium nivale*; Mango Malformation; Intensity; Fungicides

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the oldest and the most important fruit of the tropical world including Indo Pak subcontinent where it is known to be cultivated from ages. Pakistani mangoes have attained a good reputation due to their excellent flavour, delicious taste and nutritive values (Muhammad *et al.*, 2002). Mango is the second important fruit crop of the country to earn foreign exchange by exporting to Middle East and Europe (Tahir *et al.*, 2003).

The average yield, however, is not appreciable which is due to the negligence of growers and attacks of

several insect pests, abiotic factors and diseases, about 81 diseases have been recorded on mango (Pernezny and Simone, 2000). Mango malformation disease (MMD) is a serious threat to this crop and is of increasing significance because of great demand of mango in the international market and expansion of mango production worldwide for export (Ahmed *et al.*, 2002). Mango malformation is of two types, vegetative and floral, floral malformation is more prevalent in bearing mango tress, however, vegetative malformation mostly appears on seedlings (Khaskheli *et al.*, 2008a). MMD causes shortened inflorescence, sterility and aborted hermaphrodite flowers and the male flowers increase in number and size (Kumar *et al.*, 1993; Akhtar *et al.*, 1999; Ahmed *et al.*, 2002; Iqbal *et al.*, 2006). MMD was first reported in India in 1891

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(Ploetz, 2001). It is found elsewhere in Asia [Israel, Malaysia, and Pakistan (Ploetz, 2001)], Africa [Egypt, South Africa, Sudan, Swaziland, and Uganda (Ploetz *et al.*, 2002)] and Americas [Brazil, El Salvador, Mexico, Nicaragua, the United States and Venezuela (Noriega *et al.*, 1999 and Britz *et al.*, 2002)]. In Pakistan, Khaskheli *et al.*, (2008b) confirmed with pathogenicity *Fusarium nivale* (Fr.) Ces., as first record in Pakistan and also the first report of its association with mango malformation disease in Sindh, Pakistan. In another study, Iqbal *et al.*, (2006) isolated four fungi viz. *F. mangiferae*, *F. pallidoroseum*, *F. oxysporum* and *Alternaria alternata* from malformed mango parts. Akhtar *et al.*, (1999) verified the association *F. moniliforme* and *G. fujikuroi* with the disease.

Several attempts were made to manage the MMD, however, due to differences in the reported species some time difficult to control the extent of MMD. Some research lines indicate that broad spectrum systemic fungicides are beneficial for the control of the disease (Lonsdale and Kotze, 1993). Kumar *et al.*, (1995) found that mangiferin, the metabolites of mango induced changes in isolates of *Fusarium moniliforme* (*Gibberella fujikuroi*). Iqbal *et al.*, (2011) conducted study on management of mango malformation through physical alteration and chemical spray, in their study the treatment with clipping at 45 cm distance followed by spray of benomyl results 70.37% decrease over previous year's count.

For the last many years researchers have been involved to study various aspects of MMD but so far its management no systematic work is reported to have been done in Sindh province of Pakistan. The present studies were therefore carried out to investigate management with available fungicides to get the solution of MMD in Sindh province.

MATERIALS AND METHODS

Fungal culture: The fresh culture of *Fusarium nivale* (Fr.) Ces. obtained from the Department of Plant Pathology, Sindh Agriculture University, Tando Jam, Pakistan was used in the current experiment. Furthermore, *Fusarium nivale* (Fr.) Ces. was confirmed and proved with pathogenicity test as first recorded fungi from mango malformation disease in Sindh, Pakistan in our previous experiments.

In vitro fungicide test: Five different fungicide were tested *in vitro*; Fosetyl- Aluminium, Copper oxychloride, Mancozeb, Metalaxyl+Mancozeb, and Thiophanate methyl. The basal medium was amended with three different doses of each fungicide. The doses of each fungicide were prepared according to their active ingredient (Table 1). Petri dishes containing amended PDA medium were inoculated with 3 mm disk removed with sterilized cork-borer from the growing margin of a 7 days old culture of *Fusarium nivale*. Petri dishes containing PDA medium without fungicides were used as control. All the plates were incubated at $25 \pm 1^{\circ}\text{C}$ for about 10 days. Mycelial growth of the fungus was recorded in mm after 24 hours of inoculation and after 10 days of growth. The experiment was conducted in completely randomized design (CRD) with four replications for each treatment.

In vivo fungicide test: Effect of different fungicides on the disease development on inoculated mango nursery was carried to confirm the efficacy. 10 healthy seedlings of each 3 susceptible varieties i.e. Almas, Dusheri, and Desi were inoculated by injecting 3ml spore suspension (10^7 conidia ml^{-1}) of the fungus *Fusarium nivale* just below growing tip of the nursery. After 30 days of inoculation, nurseries were sprayed two times with five different fungicides at 10 days interval. Mango nurseries sprayed with distilled sterile water were treated as control. The experiment was arranged in randomized complete block design (RCBD) with three replications. The fungicides at their different doses used are mentioned in Table 1. To record the data, samples were collected 72 hours after each spray to find the reduction in extent of infection. The infection percentage was determined using 0-5 scale described by Townsend and Henberger (1973): where; 0 =No infection (0%), 1= Slightly infection (20%), 2=Moderate infection (40%), 3=Slightly severe infection (60%), 4=Severe infection (80%) and 5=Dead inflorescence (100%).

Statistical analysis: Data were analysed according to standard procedures for analysis of variance, ANOVA (linear model), and separation of means (least significant difference, LSD) of all parameters was determined using Statistix 8.1 (Analytical Software 2005). All differences described in the text were considered significant at the 5 % level of probability.

Table 1. List of different fungicides used for the management of *Fusarium nivale*

Fungicide	Dose used (under Laboratory) in 100 ⁻¹ ml Medium	Dose used (under Field) in 100 ⁻¹ ml Water
Fosetyl- Aluminium 80 WP	1. 250 mg 2. 300 mg 3. 350 mg	350 mg per
Copper oxychloride 50 WP	1. 200 mg 2. 250 mg 3. 300 mg	300 mg
Mancozeb 45 WP	1. 250 mg 2. 300 mg 3. 350 mg	350 mg
Metalaxyl+Mancozeb 72 WP	1. 200 mg 2. 250 mg 3. 300 mg	300 mg
Thiophanate methyl 70 WP	1. 100 mg 2. 150 mg 3. 200 mg	200 mg

RESULTS

***In vitro* efficacy of different fungicides against *Fusarium nivale*:**

The efficacy of five different fungicides (Thiophanate methyl, Fosetyl- Aluminium, Metalaxyl+Mancozeb, Copper oxychloride and Mancozeb) used against *F. nivale* at their different doses showed significant difference ($P < 0.05$) in the reduction of mycelial colony growth. However, Thiophanate methyl and Fosetyl- Aluminium at their lower and higher doses completely inhibited the colony growth of the fungus even after 10 day's growth (Figure 1). Metalaxyl+Mancozeb at its lower dose also reduced mycelium growth (8.880 mm) of fungus. However, there was no significant difference in colony growth of the fungus at medium and high doses of Metalaxyl+Mancozeb (Figure 1), respectively (Table 2). Mancozeb was found less effective against the fungus as compared to Metalaxyl+Mancozeb (Figure 1). There was no significant difference in the colony growth of the fungus recorded at low and medium dose of Mancozeb (Table 2). Copper oxychloride was the least effective at its respective doses, in retarding the colony growth of the fungus (Figure 1). All the fungicides at their respective doses significantly retarded the growth of fungus as compared to control (40.750 mm) (Table 2).

Efficacy different fungicides on disease development:

The efficacy of five different fungicides were tested on 10 healthy seedlings of three selective susceptible mango varieties inoculated with *F. nivale* showed significant difference in the diseases development. The infection percentage of tested seedlings after first and second sprays is revealed in Table 3 that depicted significant variation in the development of disease caused by *F. nivale*.

Effect on Desi variety: The data recorded after the first spray for Desi variety showed that Thiophanate methyl reduced the infection of *F. nivale* to 10.0% i.e. 72.33% percent decreased in malformation intensity over untreated control (Table 3). Fosetyl-Aluminium, Metalaxyl+Mancozeb and Mancozeb also reduced the infections as compared to Copper oxychloride i.e. 19.0, 20.60, 23.80 and 33.00% with reduction in disease intensity is 68.33, 65.66, 60.33 and 45.00% over untreated control (Table 3). The second spray of Thiophanate methyl and Fosetyl-Aluminium completely reduced the infection of fungus, with reduction in disease intensity by 100.0% as compared to Metalaxyl+Mancozeb, Mancozeb, and Copper oxychloride with reduction disease intensity by 78.73, 73.65 and 68.88% over control (Table 3).

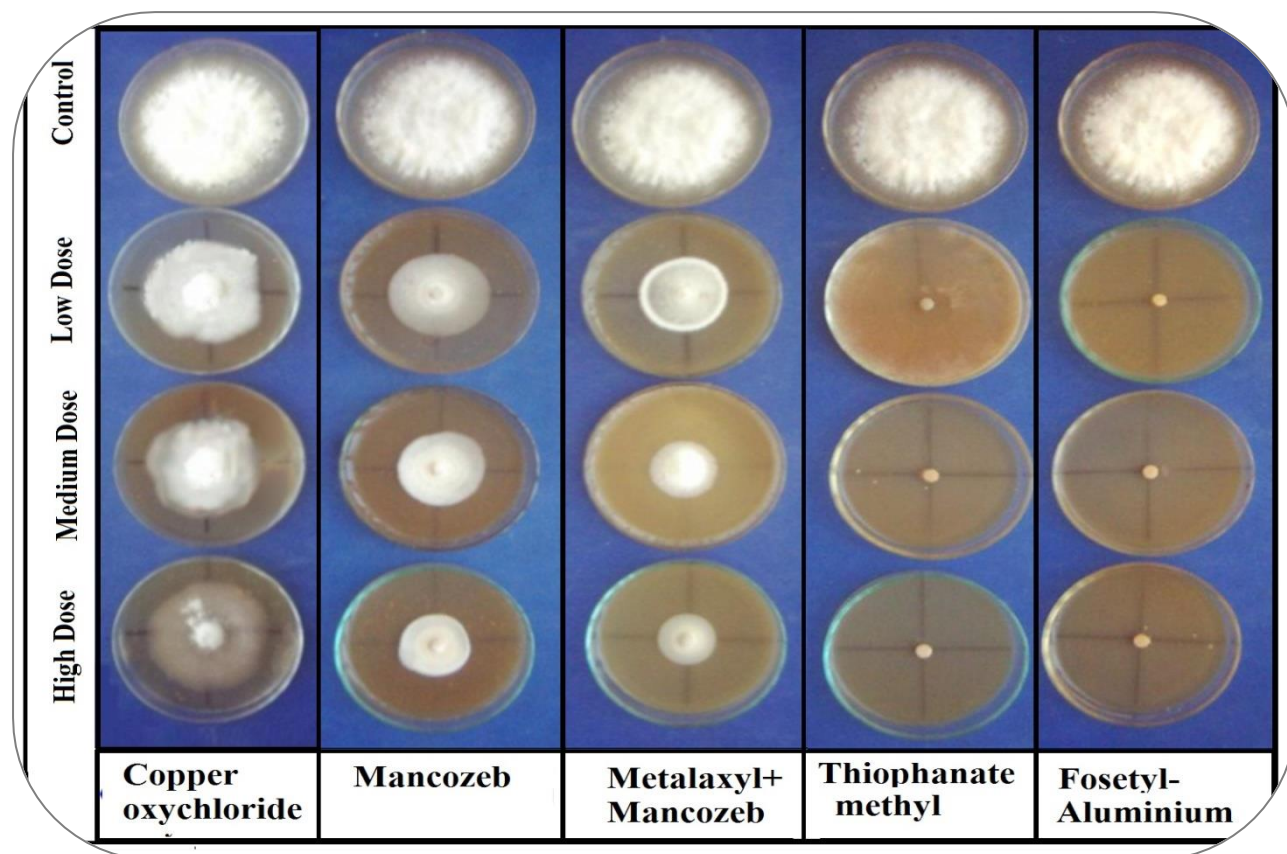


Figure 1. *In vitro* efficacy of different fungicide against mycelial growth of *Fusarium nivale*

Table 2. Effect of different fungicides on mycelial growth of *Fusarium nivale*

Fungicide	Dose (mg)/ 100 ml. medium		Radial colony growth (mm)
Thiophanate methyl70 WP	1	100.0	3.416 i
	2	150.0	3.375 i
	3	200.0	3.250 i
Fosetyl- Aluminium 80 WP	1	250.0	4.916 h
	2	300.0	4.750 h
	3	350.0	4.583 h
Metalaxyl+Mancozeb72 WP	1	200.0	8.880 f
	2	250.0	7.230 g
	3	300.0	6.830 g
Mancozeb45 WP	1	250.0	13.338 d
	2	300.0	13.090 d
	3	350.0	11.900 e
Copper oxychloride 50 WP	1	200.0	21.833 b
	2	250.0	21.500 b
	3	300.0	18.083 c
Control	-		40.750 a
LSD (P = 0.05)			1.144

Table 3. Effect of different fungicide on infection (percent) of on mango seedlings of Desi variety inoculated with *Fusarium nivale*

Fungicide	Dose (mg)/ 100ml distilled water	Infection (%) after			
		First spray	*Reduction (%) over control	Second spray	Reduction (%) over control
Thiophanate methyl70 WP	200.0	16.60 e	72.33	0.00 e	100.0
Fosetyl- Aluminium 80 WP	350.0	19.00 d	68.33	0.00 e	100.0
Metalaxyl+Mancozeb72 WP	300.0	20.60 d	65.66	13.40 d	78.73
Mancozeb45 WP	350.0	23.80 c	60.33	16.60 c	73.65
Copper oxychloride 50 WP	300.0	33.00 b	45.00	19.60 b	68.88
Control	(-)	60.00 a	-	63.00 a	-
LSD (P= 0.05)		2.064	-	2.797	-

*Reduction (%) = Untreated seedlings – Treated seedlings/ Untreated seedlings x 100

Note. Figures followed by the same letter within a column were not significantly different according to the LSD (least significant difference) test at P<0.05

Effect on Almas variety: Thiophanate methyl and Fosetyl- Aluminium after first spray also reduced the infection of fungus by 17.60 and 19.80% i.e. 71.11 and 67.54 percent decrease in malformation incidence over untreated control (61.0%), respectively on Almas variety (Table 4). Metalaxyl+Mancozeb, Mancozeb and Copper oxychloride also significantly reduced the infection of the fungus, with reduction percent in malformation intensity by 65.57, 60.98 and 44.26% respectively (Table 4). The data of second spray revealed that Thiophanate methyl and Fosetyl- Aluminium reduced the infection of fungus by 100.00%. Metalaxyl+Mancozeb, Mancozeb, and Copper oxychloride were also found effective in reducing infection of the fungus and percent decrease in disease intensity by 73.84, 73.69 and 69.53% over untreated control of Almas variety, respectively (Table 4).

Effect on Dusheri variety: The frequency of fungal infection on Dusheri variety was also significantly decreased by Thiophanate methyl and Fosetyl- Aluminium after first spray, 20.60 and 22.00%, respectively, with percent reduction in intensity of malformation by 67.81 and 65.62%, respectively (Table 5). Metalaxyl+Mancozeb, Mancozeb, and Copper oxychloride were also proved to be effective in reducing infection of *F. nivale* with percent decrease in disease intensity by 60.93, 59.37 and 42.18% (Table 5). In the second spray with Thiophanate methyl and Fosetyl- Aluminium, infection of the fungus was completely reduced, decreasing incidence of the disease by 100.0% as compared to Metalaxyl+Mancozeb (18.60%), Mancozeb (20.80%) and Copper oxychloride (21.00%), with percent decrease in malformation intensity by 72.64, 69.41, and 69.11% over untreated control of Dusheri variety, respectively (Table 5).

Table 4. Effect of different fungicide on infection (percent) of on mango seedlings of Almas variety inoculated with *Fusarium nivale*

Fungicide	Dose (mg)/ 100ml distilled water	Infection (%) after			
		First spray	*Reduction (%) over Control	Second spray	Reduction (%) over control
Thiophanate methyl70 WP	200.0	17.60 e	71.11	00.0 d	100.0
Fosetyl- Aluminium 80 WP	350.0	19.80 d	67.54	0.00 d	100.0
Metalaxyl+Mancozeb72 WP	300.0	21.00 d	65.57	17.00 c	73.84
Mancozeb45 WP	350.0	23.80 c	60.98	17.10 c	73.69
Copper oxychloride 50 WP	300.0	34.00 b	44.26	19.80 b	69.53
Control	(-)	61.00 a	-	65.00 a	-
LSD (P= 0.05)		2.160	-	2.700	-

*Reduction (%) = Untreated seedlings – Treated seedlings/ Untreated seedlings x 100

Note. Figures followed by the same letter within a column were not significantly different according to the LSD (least significant difference) test at P<0.05

Table 5. Effect of different fungicide on infection (percent) of on mango seedlings of Dusheri variety inoculated with *Fusarium nivale*

Fungicide	Dose (mg)/ 100ml distilled water	Infection (%) after			
		First spray	*Reduction (%) over Control	Second spray	Reduction (%) over control
Thiophanate methyl70 WP	200.0	20.60 d	67.81	00.0 d	100.0
Fosetyl- Aluminium 80 WP	350.0	22.00 cd	65.62	00.0 d	100.0
Metalaxyl+Mancozeb72 WP	300.0	25.00 cd	60.93	18.60 c	72.64
Mancozeb45 WP	350.0	26.00 c	59.37	20.80 b	69.41
Copper oxychloride 50 WP	300.0	37.00 b	42.18	21.00 b	69.11
Control	(-)	64.00 a	-	68.00 a	-
LSD (P= 0.05)		2.58	-	2.17	-

*Reduction (%) = Untreated seedlings – Treated seedlings/ Untreated seedlings x 100

Note. Figures followed by the same letter within a column were not significantly different according to the LSD (least significant difference) test at P<0.05

DISCUSSION

Mango (*Mangifera indica* L.) is one of the most important fruit crops grown in tropical and subtropical lowlands throughout the world. It is also a fact that Pakistani mangoes are considered far superior because of its excellent flavour and delicious taste. However, mango is attacked by a number of diseases (Pernenzky and Simone, 2000) and the malformation is amongst the important one. Currently, malformation is the threatening disease and occurs wherever mango grown in Pakistan (Ahmad *et al.*, 2002). For the last many years research workers have been involved to study various aspects of mango malformation disease (MMD). There are also several lines of reports available in the literature regarding the specific cause of this disease. Numerous fungi have been reported associated with MMD and possibly the causal agents of the disease. Freeman *et al.*, (2000) reported that *Fusarium subglutinans* (*Gibberella fujikuroi*) has been associated with mango floral and vegetative malformation. Iqbal *et al.*, (2006) isolated four fungi viz. *F. mangiferae*, *F. pallidoroseum*, *F. oxysporum* and *Alternaria alternata* from malformed mango parts. Khaskheli *et al.*, (2008b) isolated and identified *Fusarium nivale* (Fr.) Ces., as first record in Pakistan and also the first report of its association with mango malformation in Sindh, Pakistan.

However, the study regarding the management of MMD is not well documented. In current study, five different fungicides were tested *in vitro* and *in vivo* to see their effect on growth of *F. nivale* and disease development. Thiophanate methyl and Fosetyl-Aluminium at their low and high doses completely inhibited mycelial growth of *F. nivale*. Mycelial growth of the fungus was also reduced at low dose of

Metalaxyl+Mancozeb. Mancozeb was found to be less effective in reducing mycelial growth of the fungus as compared to Metalaxyl+Mancozeb. Similarly, Copper oxychloride was also found least effective against *F. nivale* at low dose and medium dose, respectively.

When these fungicides tested on *Fusarium nivale* inoculated seedlings for diseases development, varied responses of fungicides were observed. Thiophanate methyl and Fosetyl- Aluminium reduced the infection of *F. nivale* in Desi, Almas and Dusheri varieties after first spray, with highest reduction percent in malformation over untreated control. Metalaxyl+Mancozeb and Mancozeb also reduced the infection as compared to Copper oxychloride, with decrease in intensity. Thiophanate methyl and Fosetyl- Aluminium after second spray completely reduced infection of the fungus with 100.0% decrease in intensity of the disease as compared to Metalaxyl+Mancozeb, Mancozeb and Copper oxychloride over untreated control on all tested seedlings of Desi, Almas and Dusheri varieties. Some other reports are also inconsistency with the current study to reduce the intensity MMD through different chemicals as well as with physical measures; however, their reported causal agents are different from the current tested fungi. Hafeez-ur-Rehamn *et al.* (1989) used Benlate and certain biological antagonists separately and as well as in combination. Benlate in both cases gave better result in reducing malformation intensity. Kumar *et al.*, (1995) found that mangiferin, a metabolite of mango caused changes in various isolates of *Fusarium moniliforme* (*Gibberlea fujikuroi*).

It is suggested based on the current findings that Thiophanate methyl and Fosetyl- Aluminium are the most effective fungicides against *F. nivale*, the causal

agent of MMD in Sindh, Pakistan. These fungicides could be used for preventive as well as curative control of *F. nivale* and other species of *Fusarium*.

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