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CHEMICAL CONTROL OF *LASIODIPLODIA THEOBROMAE*, THE CAUSAL AGENT OF MANGO DECLINE IN SINDH

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Abstract

Mycelial growth of *Lasiodiplodia theobromae* was significantly inhibited by Carbendazim and Thiophanate-methyl when used @ 1 ppm a.i. or more. Alliete was effective at relatively high concentrations i.e., @ 1000 and 10000 ppm a.i., whereas, Copxykil, Cuprocaffaro and Thiovit failed to inhibit the mycelial growth of *L. theobromae*. In field experiment, Carbendazim was found to be more effective than Thiophanate-methyl and Alliete in reducing the fungal infection in mango plants, suppressing the gum exudation, dieback and wilting resulting in significant enhancement in vegetative growth of plants.

Introduction

Since the late nineties, mango decline or dieback disease has become one of the most severe problems in mango orchards of the Sindh province (Khanzada *et al*., 2004a, b). In most cases, the disease has been characterized by the exudation of gum, wilting, dieback, vascular browning and death of the whole tree (Narasimhudu & Reddy, 1992; Khanzada *et al*., 2004a). Our previous studies have established that *Lasiodiplodia theobromae* (Syn: *Botryodiplodia theobromae*) is a causative fungus of this disease (Khanzada *et al*., 2004b). In other parts of the world, similar association between *L. theobromae* and mango decline has been observed by many research workers (Das-Gupta & Zachariah, 1945; Narasimhudu & Reddy, 1992; Sharma, 1993; Simone, 1999; Savant & Raut, 2000, Al Adawi *et al*., 2003; Ramos *et al*., 1997; McSorley *et al*., 1981; Schaffer, 1994; Gonzalez *et al.*, 1999). *Lasiodiplodia theobromae* is a cosmopolitan soil-borne fungus causing both field and storage diseases on more than 280 plant species including crops, fruits and plantation trees (Khurana & Singh, 1972; Talukdar, 1974; Sing *et al.*, 1977; Ilag & Marfil, 1977; Domsch, *et al.*, 1980; Sutton, 1980). The fungus also produces similar symptoms in many other economic plants such as root rot of *Brachychiton poplneus* seedlings (Sandlin & Ferrin, 1992), leaf necrosis and stem cankers on Proteas (*Protea magnifica)* (Denman, 2002; Denman *et al*., 2003), Cashew gummosis in Brazil (Cardoso *et al.*, 2004), gummosis disease of Japanese apricot and peach trees (Li *et al*., 1995), canker in white cedar (Sandrock *et al*., 1999), collar rot of peanut (Phipps & Porter, 1998), die back of Kumquat (Ko *et al*., 2004), blueberry stem blight (Cline & Milholland, 1992), storage rot of Taro, black-band disease of jute, crown rot diseases of banana fruit, fruit rot of coconut, stem-end rot of mango fruit, soft rot of papaw, guava, litchi, sapodilla fruit and die-back in lemon plant fruits (Anthony *et al*., 2004; Mortuza & Ilag, 1999; Alam & Nahar, 1990; Alam *et al*., 2001; Wall & Cruz, 1991). In Pakistan, the fungus has been reported on more than 50 plant species (Ahmed, *et al.*, 1997). The objective of this study was to evaluate some of the available fungicides for the mango decline management *in vitro* and *in vivo* conditions.

Materials and Methods

Pathogen inoculum: Culture of *L. theobromae*, previously isolated from mango was obtained from Karachi University Culture Collection (KUCC0027) and maintained on Potato Sucrose agar (PSA) at room temperature.

***In vitro* screening of fungicides:** Both contact and systemic fungicides were used for *in vitro* evaluation by food poison method (Dhingra & Sinclair, 1985). The registration status, active ingredients, trade name and formulations of the fungicides are given in Table 1. The tested fungicides were suspended in sterile water and added to molten PSA medium to obtain a final concentrations of 1, 10, 100, 1000 and 10,000 ppm a.i. PSA without fungicides served as control. Approximately 20 ml of fungicides amended or control PSA medium was poured into each 90-mm diam., Petri dish. After solidification each dish was inoculated with a 5-mm diam., disc obtained from an actively growing margin of *L. theobromae* colony on PSA. There were 3 replicates of each treatment. The Petri dishes were incubated at room temperature and daily radial mycelial growth was recorded till the upper surface in control treatment was fully covered with the mycelial growth of the fungus. The daily mean colony diameters for each fungicide concentration were calculated.

Table 1. List of fungicides with their description.

Trade name	Active ingredient	Formulation	Mode of action	Distributor/ Manufacture
Thiophanate-methyl	Thiophanate-methyl	70 WP	Systemic	Kissan Supplies Services (KSS)
Carbendazim	Carbendazim	50 WP	Systemic	Kissan Supplies Services (KSS)
Alliete	Fosetyle almonium	80 WP	Systemic	Bayer (Pvt.) Ltd.
Thiovit	Sulphur	80 WP	Contact	Novartis (Pvt.) Ltd.
Copxykil	Copper oxychloride	50 WP	Contact	Stedec Technology commercialization corporation of Pakistan
Cuprocaffaro	Copper oxychloride	50 WP	Contact	Pan Pacific (Pvt.) Ltd.

Field experiment: The field experiments were carried out at a commercial mango orchard "Dehli Farm", situated at Tandojam, district Hyderabad of the Sindh province. The farm had a history of mango decline and plants were showing severe disease symptoms. Carbendazim, Thiophanate-methyl, Alliete that gave the best inhibition of *L. theobromae in vitro* were used in the field trials. Carbendazim @ 2 gL^{-1}, Thiophanate-methyl @ 1.43 gL^{-1} and Alliete @ 1.25 gL^{-1} were dissolved in water to get a final concentration of 1000 ppm a.i. There were ten plants per fungicide treatment. The plants were thoroughly sprayed three times with 15 days interval. Before the first spray the plants were tagged and all dead branches were removed by pruning. After the first spray treated and control plants were irrigated with canal water and provided necessary farmyard manure and urea fertilizer. Isolation from the branches of treated and control plants were made before each spray to determine the infection on each plant. The effect of fungicides on mango plants was also evaluated by assessing the diseases severity and disease incidence before and after treatment. Disease incidence and severity were evaluated with the help of a model proposed by Cardoso *et al.*, (2004) for evaluating disease incidence and severity of cashew gummosis caused by *L. theobromae*. Incidence

and severity were assessed at the same time after some modification. Incidence (*I*) was based on the presence of typical symptoms of the disease. Severity (*S*) was estimated by the equation $S = \sum (x_i n_i)/n$, in which *x* represented modified disease grade (Cardoso *et al*., 1998) (0, No symptoms; 1, Little wilting of upper tips; 2, Wilting and small exudates of gum, yellowing of leaves, little browning of vascular tissues; 3, Drying of branches, heavy gum exudation from branches, large scale browning of vascular tissues; or 4, Splitting of the bark, gum exudation from branches as well as from main trunk, drying of more than half of the tree), n_i represented the number of diseased plants on the *i*th grade of the disease scale and *n* was the total number of diseased plants evaluated. Disease incidence ($I = \sum x/N$) was the proportion of diseased plants, which consisted of the number of diseased plants (*x*) divided by the total number evaluated (*N*). Both disease severity and incidence were evaluated before the 1st, 2nd and 3rd spray and after two weeks of 3rd spray of each fungicide.

Results and Discussion

***In vitro* experiment**: On the basis of their efficacy, fungicides were grouped into three categories. The 1st category comprises of the most effective fungicides that inhibited fungal growth at very low concentrations i.e., Carbendazim and Thiophanate-methyl. The Alliete that showed moderately less efficacy was placed in the 2nd group. Remaining three fungicides *viz*., Copxykil, Cuprocaffaro and Thiovit that failed to inhibit the mycelial growth of *L. theobromae* made the 3rd group.

Banik *et al.,* (1998) also found that Carbendazim at 400 ppm completely inhibited the linear growth of *L. theobromae* followed by Thiophanate-methyl at 450 ppm. Sheler *et al*., (1997) reported that Thiophanate-methyl (0.1%) and Benlate (0.1%) could completely suppress *in vitro* growth of *L. theobromae*. Similarly, Mahmood *et al*., (2002) reported that Benlate @ 100 ppm and Topsin-M (Thiophanate-methyl) @ 50 ppm completely inhibited the colony growth of *L. theobromae*. Li *et al*., (1995) also found that Thiophanate-methyl suppressed mycelial growth and conidial germination of *L. theobromae*.

F**ield experiment:** In field experiment, Carbendazim proved to be the highly effective fungicide for the control of decline disease followed by Thiophanate-methyl and Alliete (Fig. 2). The fungal infection in treated mango plants gradually reduced with the number of fungicidal sprays. It was accompanied with a gradual reduction in the disease severity and disease incidence in treated trees as compared to untreated control trees (Fig. 3). It was also noted in the trees treated with fungicides that new vegetative growth comprising of new shoots and leaves appeared and increased with each fungicidal treatment. After the 3rd spray the trees sprayed with Carbendazim or Thiophanate-methyl showed promising results. They produced large number of new shoots and complete disappearance of typical symptoms of the disease. However, the plants sprayed with Thiophanate-methyl still exhibit little gum exudation. Trees treated with Alliete produced less vegetative growth than trees treated with Carbendazim or Thiophanate-methyl. In untreated control plants, disease severity was increased with increase in time. None of the fungicides elicited any phytotoxic response under the field conditions.

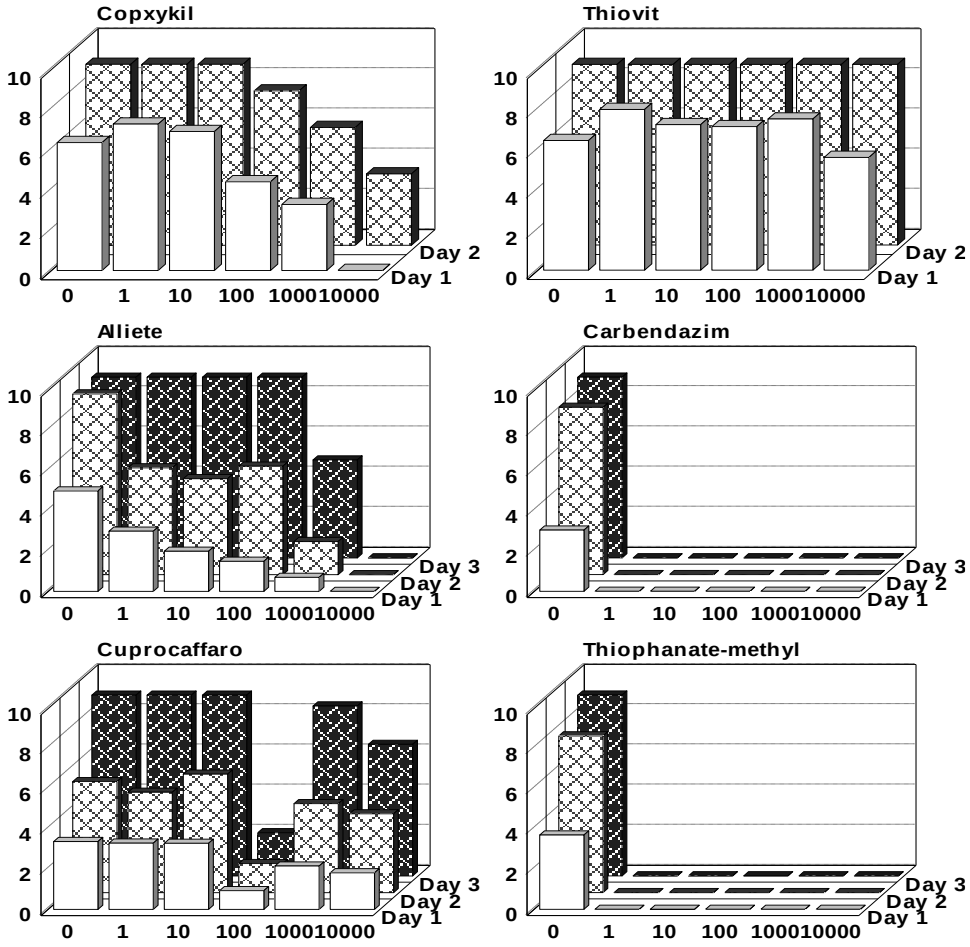

Fig. 1. Effect of fungicides on the colony growth *L. theobromae*.

Our results are in accordance with Mahmood *et al*., (2002) who recorded that 1st foliar spray of Topsin-M (Thiophanate-methyl) @1 gL^{-1} reduced the infestation of *L. theobromae* to 10% and 2nd spray of the same fungicide completely inhibited the fungus as no tissue yielded this fungus. Similarly, Rawal (1997) observed that die-back of mango caused by *L. theobromae* was controlled by spray of Carbendazim @ 0.1%, Methylethiophanate @ 0.1% or Chlorothalonil @ 0.2% at fortnightly interval. Lonsdale & Kotze (1993) reported that broad-spectrum systemic fungicides are beneficial for the control of mango die-back disease. In India this disease was effectively controlled by pruning the affected portions and spraying the wounded areas with 5:5:50 Boradeaux mixture (Parkash & Raoof, 1989). According to the Narasihudu & Reddy (1992) mango gummosis could be controlled by using a paste of Boradeaux mixture or Carbendazim. The gummosis disease in Japanees apricot and peach orchards caused by *L. theobromae* was controlled by Thiophanate-methyl (Li *et al*., 1995). Fungicides also provided effective control of the stem end rot of mango fruits caused by *L. theobromae* (Samadisi *et al*., 1990; Sangchote, 1988; Muller & Burt, 2000; Johnson *et al*., 1990).

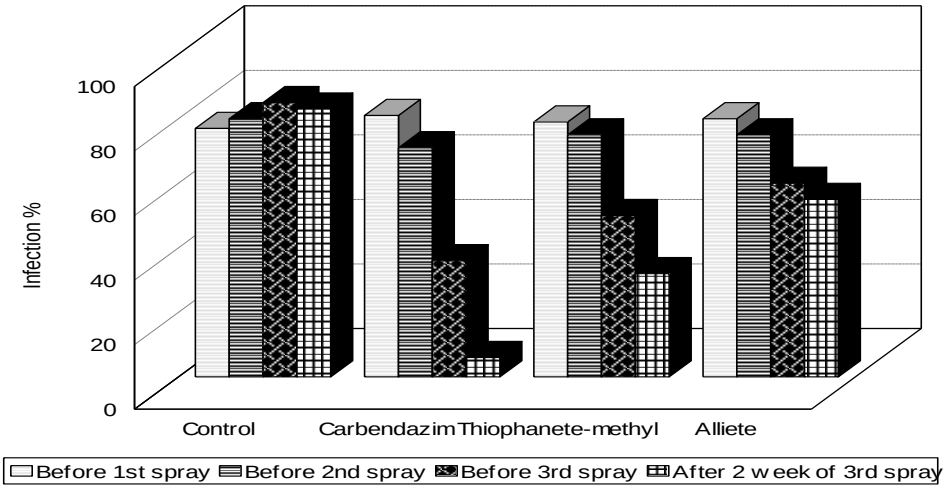

Fig. 2. Effect of Fungicidal sprays on infection% of *L. theobromae.*

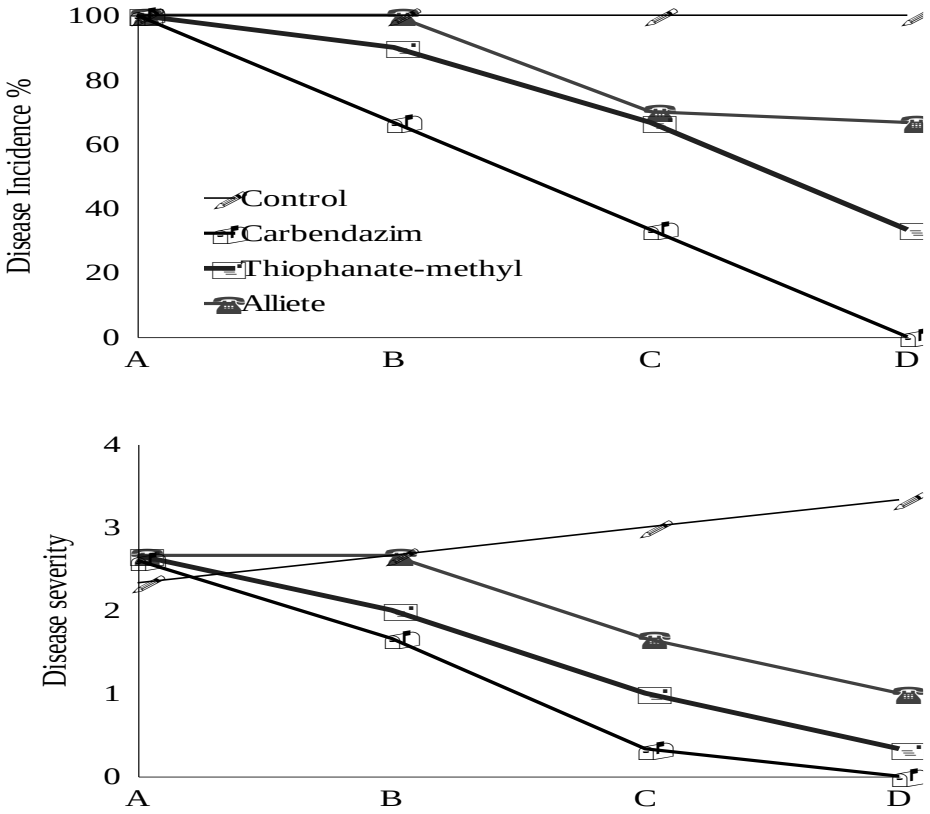

Fig. 3 Effect of fungicidal spray on mango decline

A= Before 1st spray, B= Before 2nd spray, C= Before 3rd spray, D= After 2 weeks of 3rd spray

The results of the present study would suggest that proper sanitation, irrigation and fertilization with at least three fortnightly sprays with Carbendazim would help in the management of mango decline under field condition.

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