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***Full Length Article***

# Efficacy of Systemic Fungicides against *Ceratocystis manginecans*; the Cause of Mango Quick Decline

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## Abstract

*Ceratocystis manginecans* Van Wyk, is a cause of mango quick decline, which is a disease that lead to losses of 74% in all mango growing areas of Punjab, Pakistan. Under these circumstances, a quick remedy to control the disease is necessary. Therefore, systemic fungicides, such as bavistin, thiophanate methyl, difenoconazole and benlate were evaluated against *C. manginecans* isolates from diseased mango plants of South Punjab, Pakistan. *In vitro* experiment was conducted with all the above-mentioned fungicides at active ingredient concentrations of 250 ppm, 500 ppm, and 750 ppm a.i. against the two fungal isolates CMK04 and CMR35. Results revealed that bavistin significantly inhibited the growth of two isolates CMK04 and CMR35 at concentrations of 500 ppm (78 and 70%) and 750 ppm (87 and 79%), after nine days of incubation. Followed by Bavistin, thiophanate methyl also showed significant inhibition at 750 ppm (65 and 68%), after nine days of incubation. Difenoconazole and benlate were the least effective in the inhibition of the fungal growth of the isolates. Green house experiment was carried out with the Bavistin, thiophanate methyl and their combination at 500 ppm and 750 ppm concentrations in one-year old artificially inoculated mango seedlings. Bavistin again alone and in combination with thiophanate methyl was significant to manage the fungal infection at concentration of 750 ppm (80%) and (78%), after twenty-one days of application, respectively. Under field conditions, Bavistin alone and in combination with thiophanate methyl, at 500 ppm and 750 ppm calibrated concentrations, were evaluated in diseased or infected 8 to 10 years old mango plants in three different years (2014, 2015 and 2016). Bavistin significantly inhibited the disease for three years 2014, 2015, 2016 at concentration of 750 ppm (51, 58 and 63%, respectively), after forty-two days of treatment. Thiophanate methyl & bavistin combination also reduced the fungal infection (40, 36 and 39%) at 750 ppm, respectively. Conclusively, Bavistin alone and in combination with thiophanate methyl has the potential to control mango quick decline, caused by *C. manginecans*. 

**Keywords:** *Ceratocystis manginecans;* Mango quick decline; Bavistin; Thiophanate methyl

## Introduction

Mango (*Mangifera indica* L.), Family Anacardiaceae, is originated in Southeast Asia, and is entitled as the ‘‘King of fruits’’ due to its excellent flavor and fragrance. It is a rich source of vitamins A, C and E, sugar (10–20%), minerals such as potassium and magnesium, and dietary fiber (Ajila and Parsada, 2008). It is also used in various food preparations at home and food industries for mango pulp, mango peel, mango jam, jellies and pickles etc. World mango production is 38.7 million tons, where Pakistan ranked 6$^{th}$ sharing 1.9 million tons of production on an area of 17.3 thousand hectares (FAO, 2018). Pakistan exports a quantity of 0.11 million tons of mango fruits in other mango importing countries (FAO, 2018). Mango as a perennial crop is exposed to nature that either made the trees to boost up or vulnerable to open challenges of pests and diseases at every stage of its growth. All over the world more than140 mango diseases are known (Parkash, 1998; Arshad, 2008). Despite mango production ranks the sixth place, production of mango is still low due to various biotic and abiotic constraints (Gupta, 1989; Akhtar *et al.*, 1999; Masood *et al.*, 2008). Among biotic problems, mango anthracnose, powdery mildew, bacterial blight, malformation disease, slow and quick decline are well known (Kazmi *et al.*, 2005; Asad *et al.*, 2010), but the major limiting factor is Mango Quick Decline (MQD) has become a major threat to production in recent years.

Mango Quick decline hammered the trees with losses up to 60% (Al-Subhi *et al.*, 2006; Saeed *et al.*, 2007; Al-Adawi *et al.*, 2014), in all mango growing countries of the World (Batista, 1960; Ploetz, 2003; Anjum *et al.*, 2016). In Pakistan, orchards have been suffering from its destructive and latent infection (Masood *et al.*, 2008; Chohan *et al.*, 2015; Naqvi and Perveen, 2015). If the disease remains unable to manage, it would lead to more devastation in

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future (Rajwana *et al.,* 2011; Saeed *et al.,* 2011). So, in this situation, it is the dire need to save the germplasm for future generation (Douthett, 2000). This decline is caused by a systemic devastating wilt causing soil-borne fungus, *Ceratocystis manginecans* (Rossetto and Rinero, 1990; Wyk *et al.,* 2007; Rashid *et al.,* 2016) that survives in the soil by producing chlamydospores (Khanzada *et al.,* 2004; Al-Adawi *et al.,* 2014). *C. manginecans* is responsible for the clogging of the vascular bundles of the mango trees which in turns leads to the death of the tree. Keeping in view the severity caused by this disease, management should be carried out, inhibiting the proliferation of this fungus in the vascular system of the trees (Amin *et al.,* 2011). Therefore, systemic fungicides can be a good source of easy, direct and quick disease management method in the present situation to prevent the farmers from heavy losses. Keeping this in view, the aim of this study was to screen appropriate systemic fungicides to check mango quick decline disease. Experiments were conducted at laboratory, green house and field level.

## Materials and Methods

**Isolates:** *C. manginecans* isolates were isolated from diseased plants of different mango growing districts of Southern Punjab, Pakistan. They were morphologically and molecularly identified, and two isolates, CMK04 (KX499537) and CMR35 (KX499538), were selected due to their previous history of virulence.

**Impact of systemic fungicides *in vitro*:** Efficacy of four systemic fungicides namely benlate (50% WP), bavistin (50% WP), difenoconazole (3% EC), thiophenate-methyl (70% WP) at 250 ppm, 500 ppm, and 750 ppm active ingredient, respectively, were evaluated on their ability to control the two fungal isolates CMK04 and CMR35 of MQD by Poison Food Technique (Dhingra and Sinclair, 1985). Each fungicidal ppm solutions based on their active ingredients were prepared and added in the molten MEA (50°C) and poured into the Petri plates with three replications of each fungicide. Control was maintained without adding any fungicide to the growth medium. Mycelial plugs of 5 mm from seven days old culture were placed on the poisoned and control MEA Petri plates. Plates were incubated at ± 25°C. Data for percent colony growth was conducted after three days, six days and nine days. The efficacy of fungicides was expressed as percent inhibition of mycelial growth over control (Vincet, 1947) using the following formula

$$\text{Inhibition (\%)} = \frac{\text{RG in control} - \text{R.G. in treatment}}{\text{R.G. in control}} \times 100$$

Where, R.G. = Radial colony growth.

**Impact of systemic fungicides in the greenhouse:** After the data interpretation and statistical analysis of *in vitro* results, the most effective fungicides alone and in combinations were used to manage the disease in the greenhouse trial. Mango plants were artificially inoculated for the efficacy of fungicidal treatments in response to the disease management. Three soil drenching of 1 L of total volume with 25, 50 and 75 g of fungicides for the final concentrations of 250, 500, 750 ppm, respectively, were tested; first = before inoculation, second = two weeks after inoculation and third = four weeks after inoculation. Sterilized water treated mango seedlings were considered as control. Disease progression data was recorded three times: seven, fourteen and twenty-one days after inoculation. Disease progression was measured by lesion length, wilting, discoloration and gummosis. Percent disease inhibition was measured by disease severity of treatments over control.

**Impact of systemic fungicides in the field:** Efficacy of fungicides was also tested in infected 08 to 10 years old mango plants at field conditions for three consecutive years, 2014, 2015 and 2016, based on the evaluation of the greenhouse results. Three soil drenching with fungicides calibrated concentrations of 500 and 750 ppm, respectively, were tested after two weeks interval. Untreated plants were considered as control. Data was recorded for the percentage reduction in disease incidence of field trees, in comparison to the control.

Data were statistically analyzed with SAS/STAT software (S.A.S. Institute, 1990). LSD test at 5% level of significance was used to determine post-hoc comparison among treatments (Steel *et al.,* 1996).

## Results

**Impact of systemic fungicides *in vitro:*** The systemic fungicides at different concentrations significantly inhibit the growth of two *C. manginecans* isolates: CMK04 and CMR35 (Fig. 1 and Fig. 2). Bavistin significantly inhibited the growth of both isolates, CMK04 and CMR35, at concentrations of 500 ppm (78 and 70%) and 750 ppm (87 and 79%) nine days after inoculation, respectively. Thiophanate methyl also showed significant inhibition of two isolates at 750 ppm (57%) and (68%) nine days after inoculation. While, difenoconazole and benlate displayed the least significant values, in comparison to bavistin and Thiophanate methyl (Fig. 1 and Fig. 2). Two isolates have non-significance differences of growth inhibition between each other.

**Impact of systemic fungicides in the greenhouse:** The effect of bavistin, thiophanate methyl, alone and thiophanate methyl + bavistin combination at concentrations of 250, 500 and 750 ppm were selected for the green house trial based on their responses in the *in vitro* experiment. These fungicides significantly reduced the growth of the fungus, *C. manginecans,* in inoculated mango seedlings on different concentrations. Bavistin significantly reduced the percentage of lesion length in the treated artificially inoculated mango seedlings, when compared to the control, at concentrations 500 ppm (70%) and 750 ppm (80%)

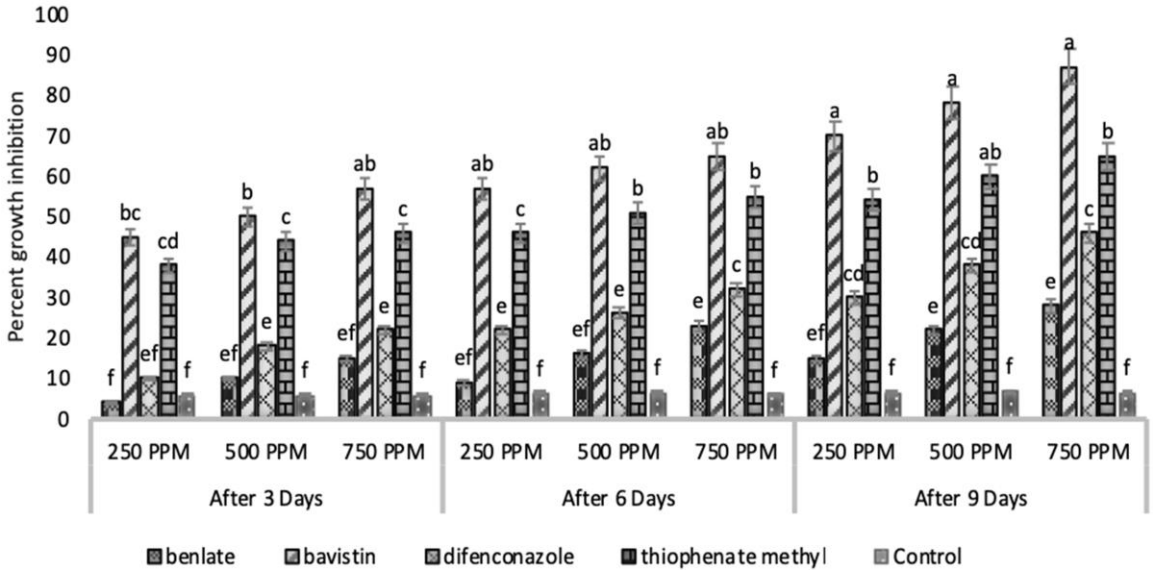


**Fig. 1:** Efficacy of systemic fungicides for *in vitro* percent inhibition of *Ceratocystis manginecans* isolate CMK04 Bars for each treatment denoted with different letters are significantly different from each other at $P < 0{\cdot}05$

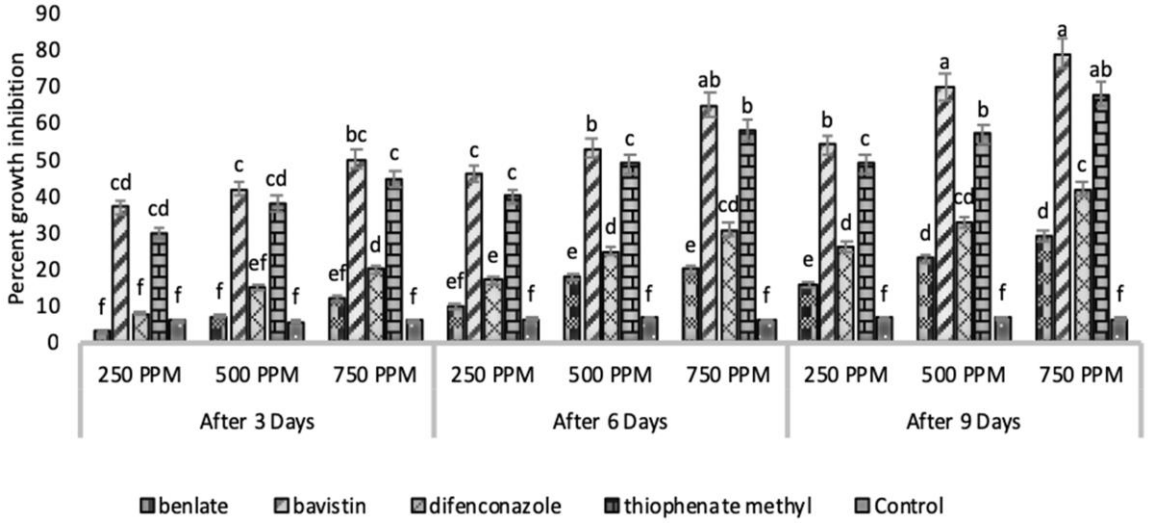


**Fig. 2:** Efficacy of systemic fungicides for *in vitro* percent inhibition of *Ceratocystis manginecans* isolate CMR35
Bars for each treatment denoted with different letters are significantly different from each other at $P < 0{\cdot}05$

twenty-one days after application. Thiophanate methyl + bavistin was also significant different from the control at 500 ppm (66%) and 750 ppm (78%) twenty-one days after application. Thiophanate methyl alone was the least effective at 500 ppm (40%) and 750 ppm (47%) twenty-one days after application. Bavistin alone and thiophanate methyl + Bavistin combination showed significant reduction in lesion length at 500 ppm & 750 ppm, in comparison to the control. Thiophanate methyl was less effective alone than when in combination with another fungicide (Fig. 3).

**Impact of systemic fungicides in the field:** Based on the efficacy of Bavistin alone and in combination with thiophanate methyl in the greenhouse experiment, they were selected with calibrated concentrations of 500 and 750 ppm in three different field trials during 2014, 2015 and 2016. In all years, bavistin significantly reduced the percentage of disease severity in affected mango plants at concentrations of 750 ppm in the respective years 51% (2014), 58% (2015) and 63% (2016) forty-two days after application. Thiophanate methyl + bavistin combination at concentrations of 750 ppm showed 40% (2014), 36% (2015) and 39% (2016) of disease inhibition, forty-two days after application, in comparison to the control (Fig. 4).

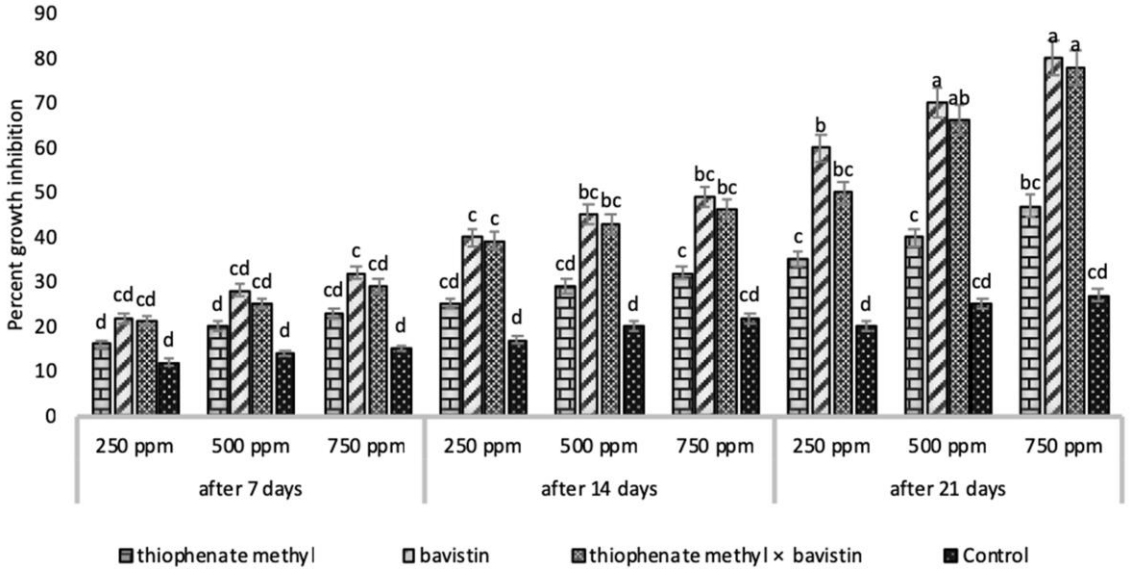


**Fig. 3:** Efficacy of fungicides against *Ceratocystis manginecans* in green house mango plants during September, 2014
Bars for each treatment denoted with different letters are significantly different from each other at $P < 0{\cdot}05$

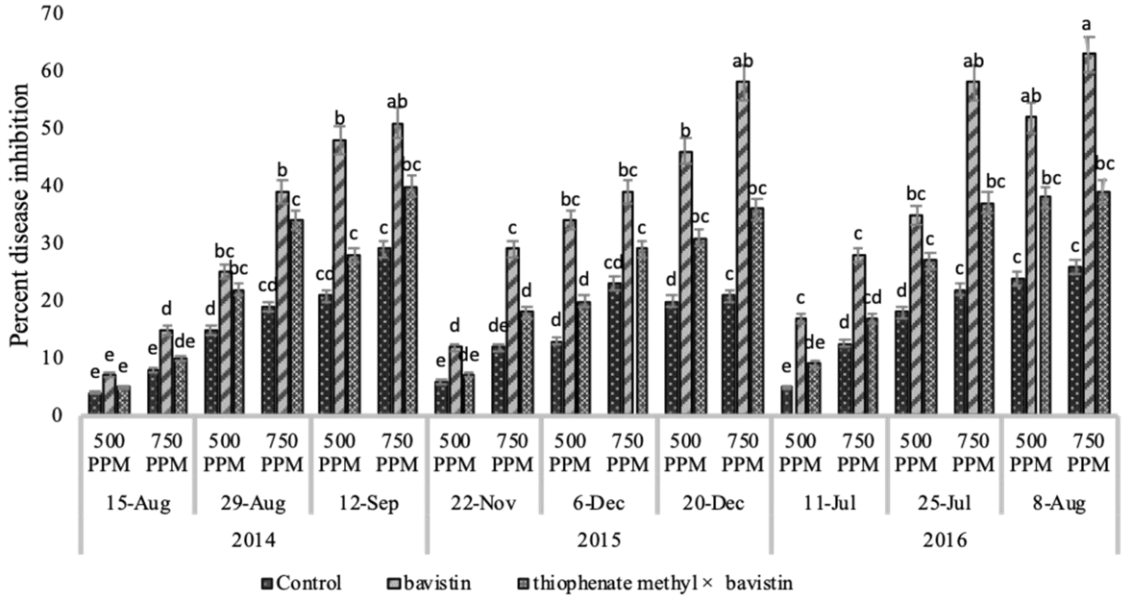


**Fig. 4:** Efficacy of fungicides against *Ceratocystis manginecans* in field during 2014, 2015 and 2016
Bars for each treatment denoted with different letters are significantly different from each other at $P < 0{\cdot}05$. a=significant to e=non-significant

## Discussion

Mango quick decline has a high impact in mango production due to its gradually increase in disease incidence every year. Results of this study provides clear evidences that the systemic chemical bavisitin alone or in combination with thiophanate methyl can control the systemic fungal pathogen, displaying great potential for the management of mango quick decline. *In vitro* experiments showed that bavistin has the potential to inhibit the fungal growth of two isolates CMK04 and CMR35 at 500 and 750 ppm concentration (78 and 70%) & (87 and 79%) nine days after incubation. Thiophanate methyl was significant in fungal growth inhibition, in comparison to difenoconazole and benlate, which were the least significant. The observations of present study are in confirmation with the results published by Rehman *et al.* (2011), who reported that carbendazim (bavistin) was significant in the inhibition of the fungal

growth of and *L. theobromae*, in comparison to Dithane M-45, Daconil, Alliete and Agrofit, which were the least significant at all concentrations. Mode of action of bavistin is not well elucidated in the management of the systemic fungi, *C. manginecans*, however, systemic fungicides acts by binding with b-tubulin polymers of fungi, which takes part in a key role in nuclear partition and result in reticence of polymerizing activity of microtubules of the hyphal cell wall. Systemic fungicide also cause barrier in diverse dictatorial cellular activities including mitosis, meiosis and cell form preservation (Nene and Thapliyal, 1982). So, being systemic, the fungicide bavistin may have same mechanism of action.

Based on the *in vitro* results, the greenhouse studies were devised. In the greenhouse experiments, bavistin alone and in combination with thiophanate methyl significantly reduced the lesion length of the *C. manginecans* infection in artificially inoculated mango seedlings at both concentrations, in comparison to the control. These results corroborate greenhouse studies conducted with *L. theobromae* inoculated mango plants with different fungicides by Arshad (2008), where Thiophanate methyl, Derosal and Pre-cure combi showed best performance.

In field trial studies, bavistin was effective alone and in combination, for three different years with no significant difference. Similarly, *L. theobromae* was effectively managed by thiophanate methyl, in comparison to score, which was the least effective in disease management of field affected mango plants (Arshad, 2008). With the passage of time the fungicides effect will be reduced. Presence of pathogen inoculum can make the pathogen again aggressive on the host plants, due to fungicide resistance developed by the pathogen over the years. Therefore, continuous and scheduled application of these fungicides in field conditions will reduce the inoculum and disease pressure, avoiding the emergence of fungicide resistance in the future.

## Conclusion

The present study concluded that bavistin alone and in combination with thiophanate methyl has a great potential for the maximum growth inhibition of the fungal pathogen *C. manginecans* in all three different experiments, *in vitro*, greenhouse and field studies, at the concentrations of 500 and 750 ppm. Additional data on efficacy of a large number of fungicides could provide a wide range of efficient products, allowing producers to have a diversity of substances that could be combined wisely for a better disease management and to prevent fungicide resistance.

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