



IN-VITRO ASSESSMENT OF SOME FUNGICIDES FOR THE MANAGEMENT OF SUDDEN DEATH IN MANGO

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ABSTRACT

Mango production in Pakistan has faced enormous losses due to the sudden death malady. It has reduced the farm income significantly. To devise an effective control strategy for this disease, present work was carried out to evaluate the *In-Vitro* efficacy of fungicides. In this experiment five fungicides including, thiophenate methyl, radomil, score, alliette, and carbendzim were tested at four concentrations i.e. 5, 10, 20 and 40 ppm with three replications under completely randomized design. The results of this study showed that all fungicides significantly inhibited the mycellial growth of *Ceratocystis fimbriata* as compared to control. Thiophenate methyl was the only fungicide that was effective at its lower concentrations 5 and 10 that showed overall 92.02% mean mycellial inhibition followed by carbendzim which produced 74.84% inhibition as a whole. All other fungicides, score alliette and radomil proved very effective at their higher doses i.e. 20 ppm and 40 ppm and showed significant reduction in mycellial growth. These results suggest that the use of thiophenate methyl and carbendzim can be very useful for the management of sudden death in mango.

Keywords: fungicides, mango, management, sudden death

INTRODUCTION

Sudden death of mango has gained attention in Pakistan in late nineties. A number of orchards in southern Punjab and Sindh were affected by this menace. The situation got worst due to its uncertain etiology for a considerable period of time (Kazmi *et al.*, 2005). As a result mango production in the country was impaired. Mango growers not only in Pakistan but also in Oman, Australia and Brazil faced huge economic losses due to this disease (Al Adwai *et al.*, 2006). The disease produced identical symptoms in all these areas where mango is affected and the diseased trees show the blackening of the collar region, oozing of dark colored material from the affected parts, gummosis, drying of the twigs and leaves (Prakash and Singh, 1976; Ploetz *et al.*, 1997; Fateh *et al.*, 2006). A number of predisposing factors including the presence of dead and dry trees in orchards, improper irrigation, intercropping and the lack of proper orchard management practices favored the spread of this problem. The severity of sudden death was more in less attended orchards (Mailk *et al.*, 2005; Khaskheli *et al.*, 2011). The association of bark beetle with the sudden

decline of plants is well documented. These beetles serve as wounding agent for the infection and transmission of the pathogens in healthy plants (Ploetz and Ploetz 2003; Maqsood *et al.*, 2008). A number of fungal flora especially *Ceratocystis fimbriata*, *Ophiostoma ulmi* and *Ceratocystis fagacearum* responsible for wilt syndrome in many plants are transmitted by these beetles (Graham, 1967; Lanier and Peacock, 1981; De Nooij, 1988, Paine *et al.*, 1997). Iqbal and Saeed (2012) have reported the association of *Ceratocystis fimbriata*, *Lasiodiplodia theobromae*, *Fusarium* sp., *Alternaria* sp. and *Aspergillus* sp. with *Hypocryphalus mangiferae*. *C. fimbriata* produces a typical aroma which attracts these insects and as a result sticky spores of fungus are transported to mango trees (Hansen, 1993; Christen *et al.*, 1997). The feeding of beetles on trees is an important source of fungal infection on xylem tissues. Such infection results in the blockage of vascular vessels which causes the plant to wilt (Khuhro *et al.*, 2005; Al-Adawe *et al.*, 2006). Similarly the deficiency of manganese and iron plays a key role in decline of mango plants. It also contributes in the establishment of fungal infection on mango trees. Due to such a complex nature of this problem there is a need

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to devise a proper program for its management. Currently the mango cultivars harbor no resistance against the malady, therefore application of fungicides is the best strategy to cope up this problem in mango orchards (Akem, 2006). Application of fungicides provides a rapid remedy for crop diseases. Keeping in view the intricate condition of sudden death in mango, the present study was designed to evaluate the efficacy of certain fungicides for *In-Vitro* management of this problem.

MATERIALS AND METHODS

Isolation of pathogen

Infected samples of mango were brought from mango orchards to plant pathology laboratory of Nuclear Institute of Agriculture (NIA), Tandojam. These samples were cut into small pieces, surface sterilized with 5% chlorox for two minutes and blotter dried. The pieces were placed on Potato Dextrose Agar medium (PDA). All the petri dishes were incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 10 days. Carrot disc method was also used to isolate *Ceratocystis fimbriata*. The initial growth of the fungus was purified on PDA medium.

Effect of fungicides on mycellial growth of *Ceratocystis fimbriata*

The present study was planned to test different fungicides (Table 1) for the management of sudden death in mango trees. The experiment was conducted *In Vitro* by using the food poison method (Dhingra and Sinclair, 1985). These fungicides were evaluated at different concentrations viz. 5 ppm, 10 ppm, 20 ppm and 40 ppm. Three replications of each concentration were used under randomized complete block design. The test fungicides were added to the liquefied PDA media. After the solidification of medium, five petri dishes for each replication were inoculated with a 5-mm fungal disc of actively growing *Ceratocystis fimbriata*. The media (PDA) plates without fungicides served as control. The petri dishes were incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and the mycellial growth was recorded on daily basis till the control plates were fully covered with the fungus. The percent inhibition of fungal growth by fungicides was calculated according to Sireesha and Venkateswarlu (2013). The data were

statistically analyzed with the help of Statistix 8.1 Software.

RESULTS AND DISCUSSION

The relative performance of the fungicides used in this study against *Ceratocystis fimbriata* has been presented in Table 2. It is evident from the results that Thiophenate methyl was the most effective fungicide among the other tested chemicals. It produced 83.27% and 84.80% reduction in fungal growth at 5 and 10 ppm, respectively, while the petri plates with its 20 and 40 ppm concentration exhibited 100% reduction in the colony growth of the tested fungus. This level of efficacy is evident from previous studies on role of chemicals for the management of mango decline (Li *et al.*, 1995; Sheler *et al.*, 1997; Banik *et al.*, 1998; Mahmood *et al.*, 2002; Diedhiou *et al.*, 2014). The results of the present work further revealed that carbendzim produced more inhibition of fungal growth as compared to alliette, radomill and score at 5 ppm and 10 ppm (Table 2). In this study the fungal growth in the petri plates containing radomill and score was more as compared to plates containing thiophenate methyl, carbendazim and alliette, however redomill and score produced significant reduction over control (Table 3). The petri plates containing 20 ppm and 40 ppm concentration of each fungicide showed maximum inhibition in fungal growth. The fungal growth in all treated petri plates was retarded as compared to the vigorous growth in un-treated petri plates. Our results are in concurrence with those of Vijaya *et al.* (2007) and Sonyal *et al.* (2015) who have shown the effect of systemic fungicides on *C. paradoxa* and *C. fimbriata*, respectively and observed complete inhibition of both fungi under *In-Vitro* conditions. Figure 1 shows the percent mean reduction in fungal growth by the tested chemical. Thiophenate methyl with 92.01% proved the best chemical fungicide, followed by carbendzim with 74.84% inhibition. Alliette and score were ranked third and fourth in reducing the fungal colonies on PDA while radomill showed least percent mean inhibition. The study indicates that Thiophenate methyl and carbendzim can be very useful against the sudden death of mango.

$$\text{Percent mycellial inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Sudden death of mango is a type of vascular wilts. This occurs due to the interaction of *Ceratocystis*, *Lasiodiplodia* and bark beetle (Al-Adawii, 2003). Apart from these biotic factors physical damage on plants, high soil pH, intercropping and nutritional deficiencies play a vital role in the decline of mango trees (Malik *et al.*, 2001; Al-Adawi 2003; Malik *et al.*, 2004; Saeed *et al.*, 2007). The progress of such diseases in mango can be limited by the use of suitable fungicides like Thiophenate methyl and carbendazim which is evident from our results. Similarly alliette, score and radomill can be very effective against sudden death. The application of fungicides against mango sudden death has also been suggested by Mehmood and Gill (2002); Malik *et al.* (2011) and Maqsood *et al.* (2014). To increase the effect of these

fungicides it is necessary to repeat the sprays of these chemicals on mango trees so that the susceptible portion must be covered to prevent the establishment of pathogens (Akem, 2006). However, their use will be more efficient and effective as the other disorders in mango orchards are managed properly. As mango sudden death has created huge economic losses. There is a need to devise an integrated approach for its management (Ismail *et al.*, 2012; Dahar *et al.*, 2017). New chemistry products must be evaluated against sudden death to have better alternates. Similarly, good orchard management and timely application of fungicides like Thiophenate methyl and Carbendazim will be very effective in reducing the proliferation of this problem.

Table 1. The source and types of fungicides tested for the control of the pathogen

Trade Name	Active Ingredient	Type	Source
Thiophenate Methyl	Thiophenate methyl (70WP)	Systemic	Local Market
Score	Difenaconazol (250EC)	Systemic	Local Market
Alliette	Fosetyly Aluminium (80%W/W)	Systemic	Local Market
Ridomill	Metalyxl+Carbendazim (68WG)	Systemic	Local Market
Carbendazim	Carbendazim (80WP)	Systemic	Local Market

Table 2. Effect of fungicides on mycellial growth (mm) of *Ceratocystis fimbriata*

Fungicides	5ppm	10ppm	20ppm	40ppm	Mean
Thiophenate Methyl	1.45±0.10E	1.33±0.06E	0.00±0.00E	0.00±0.00B	0.65±0.03E
Carbendzim	4.40±0.10D	3.30±0.10D	1.07±0.07D	0.00±0.00B	2.19±0.06D
Alliette	5.33±0.17C	4.17±0.31C	1.27D±0.07C	0.00±0.00B	2.69±0.04C
Score	6.23±0.15B	5.57±0.23B	1.92±0.08B	0.00±0.00B	3.43±0.11B
Radomill	6.33±0.10B	5.33±0.17B	1.47±0.17C	0.00±0.00B	3.27±0.05B
Control	8.67±0.17A	8.75± 0.14A	8.75±0.14A	8.85±0.07A	8.75± 0.03A
LSD (0.05)	0.41	0.57	0.33	0.09	0.18
SE	0.19	0.26	0.15	0.04	0.08

Table 3. Percent mycellial inhibition of *Ceratocystis fimbriata*

Fungicides	5ppm	10ppm	20ppm	40ppm	Mean
Thiophenate Methyl	83.27±0.42A	84.80±0.57A	100.00±0.00A	100.00±0.00A	92.02±0.22A
Carbendzim	49.08±0.04B	62.28±1.2B	87.88±0.76B	100.00±0.00A	74.81±0.12B
Alliette	38.47±0.35C	52.37±0.44C	85.52±1.70BC	100.00±0.00A	69.08±0.60C
Score	28.08±0.13D	39.08±0.34D	83.31±1.01C	100.00±0.00A	62.37±0.31D
Radomil	26.92±0.01E	36.37±0.57E	78.17± 1.43D	100.00±0.00A	60.65±0.47E
LSD (0.05)	0.79	2.26	3.60	0.00	1.22
SE	0.36	1.01	1.62	0.00	0.55

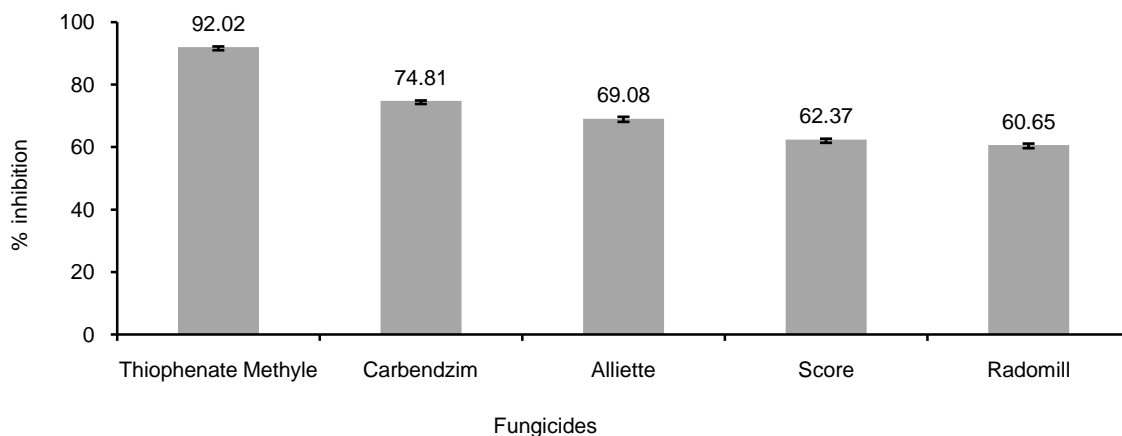


Figure 1. Mean mycelial inhibition (%) of *Ceratocystis fimbriata* by fungicides

CONCLUSION

It is evident from the present results that thiophenate methyl and carbendazim significantly inhibited the growth of *Ceratocystis fimbriata* under *In-Vitro* conditions. However all other fungicides were also found very effective against the tested fungus at their higher doses. Therefore the timely application of these chemicals will limit the problem of sudden death in mango. Due to the complex nature of this problem proactive approach must be adopted in mango orchards.

REFERENCES

- Al-Adawi, A. O., M. L. Deadman, A. K. Al-Rawahi, Y. M. Al-Maqbali, A. A. Al-Jahwari, B. A. Al-Saadi and I. S. Al-Amri, 2003. *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. *Plant Pathology*, 52: 419.
- Al-Adawi, A. O., M. L. Deadman, A. K. Al-Rawahi, Y. M. Al-Maqbali, A. A. Al-Jahwari, B. A. Al-Saadi., I. S. Al-Amri and M. J. Wingsfield. 2006. Etiology and causal agents of mango sudden decline disease in Sultanate of Oman. *European Journal of Plant Pathology*, 116 (4): 247-254.
- Akem, C. N. 2006. Mango anthracnose disease: present status and future research priorities. *Plant Pathology Journal*, 5: 266-273.
- Banik, A. K., S. A. K. M. Kaiser and R. S. Dhua. 1998. Evaluation of some systemic and non-systemic fungicides against *Botryodiplodia theobromae*, the cause of dieback disease of mango (*Mangifera indica* L.). *Journal of Soils and Crops*, 8: 199-222.
- Christen, P., J. C. Meza and S. Revah. 1997. Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata*: influence of the substrate type and the presence of precursors. *Mycological Research*, 101: 919-919.
- Dahar, G. Y., M. Ismail, M. A. Abro and R. M. Memon. 2017. *In-Vitro* evaluation of plant extracts for the management of sudden death disease in mango. *Journal of Biology Molecular Science*, 5 (1): 31-37.
- De Nooij, M. P. 1988. The role of weevils in the infection process of the fungus *Phomopsis subordinaria* in *Plantago lanceolata*. *Oikos*, 52 (1): 51-58.
- Dhingra, O. D. and J. B. Sinclair. 1985. Basic plant pathology methods. Boca Raton, FL, USA, CRS Press.
- Diedhiou, P. M., Y. Diallo, R. Faye, A. A. Mbengue and A. Sene. 2014. Efficacy of different fungicides against mango anthracnose in Senegalese Soudanian agroclimate. *American Journal of Plant Sciences*, 5: 2224-2229.
- Fateh, F. S., M. R. Kazmi, I. Ahmad, and M. Ashraf. 2006. *Ceratocystis fimbriata* isolated from vascular bundles of declining mango trees in Sindh, Pakistan. *Pakistan Journal of Botany*, 38 (4): 1257-59.
- Graham, K. 1967. Fungal-insect mutualism in trees and timber. *Annual Review of Entomology*, 12: 105-126.
- Hansen, H. P. 1993. Volatile metabolites produced by species of *Ophiostoma* and *Ceratocystis*. In: Wingfield M. J., K. A. Seifert and J. F. Webber (Eds.) *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity*, (pp. 117-126) APS Press, St Paul, USA.

- Iqbal, N. and S. Saeed. 2012. Isolation of mango quick decline fungi from mango bark beetle, *Hypocryphalus mangiferae* S. (Coleoptera: Scolytidae). *Journal of Animal and Plant Science*, 22 (3): 644-648.
- Ismail, M., S. A. Anwar, M. I. Haque, A. Iqbal and N. Ahmad and M. A. Arain. 2012. Seed borne fungi associated with cauliflower seeds and their role in seed germination. *Pakistan Journal of Phytopathology*, 24 (1): 26-31.
- Khaskheli, M. I., M. M. Jiskani, M. H. Soomro, M. A. Talpur and G. B. Poussio. 2011. Prevalence of Mango Sudden Decline/Syndrome (MSDS) on various varieties at the orchards of different age in the vicinity of Tando Qaiser, Hyderabad, Sindh. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences*, 27 (2): 160-167
- Kazmi, M. R., F. S. Fateh, K. Majeed, A. M. Kashkhely, I. Hussain, I. Ahmad and A. Jabeen. 2005. Incidence and etiology of mango sudden death phenomenon. *Pakistan Journal of Botany*, 17 (2): 154-158.
- Khuhro, R. D., S. M. Nizamani, Q. D. Abbasi, G. S. Solangi and M. M. Jiskani. 2005. Mango tree mortality due to Asian ambrosia beetle, *Xylosandrus crassiusculus* Mot. (Coleoptera: Scolytidae), *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences*, 21 (1): 39-42.
- Lanier, G. A. and J. W. Peacock. 1981. Vectors of pathogen. *In: Compendium of Elm diseases*. (Eds.): R. J. Stipes and R. J. Campana. American Phytopathology Society Minn. pp. 14-16.
- Li, H. Y., R. B. Cao and Y. T. Mu. 1995. *In-Vitro* inhibition of *Botryosphaeria dothidea* and *Lasioidiplodia theobromae* and chemical control of gummosis diseases of Japanese apricot and peach trees in Zhejiang province, China. *Crop Protection*, 14: 187-191.
- Mahmood, A., A. Saleem and K. M. Akhtar. 2002. Mango decline in Pakistan and its management. *Pakistan Journal of Phytopathology*, 14: 40-43.
- Mahmood, A. and M. A. Gill. 2002. Quick decline of mango and *In-Vitro* response of fungicides against the disease. *International Journal of Agriculture and Biology*, 4 (1): 39-40.
- Malik, M. T., C. A. Haq, A. G. Grewal, M. I. Khan and M. Nisar. 2001. An integrated approach to recover naturally infected mango plants with dieback (*Diplodia natalensis*). *Journal of Agriculture Research*, 39: 51-58.
- Malik, M. T., A. A. Dasti and S. M. Khan. 2005. June. Mango decline disorders prevailing in Pakistan. *In: Proceedings of International Conference on Mango and Date palm: Culture and Export*, University of Agriculture, Faisalabad, Pakistan, pp. 20-23.
- Malik, M. T., A. D. Altaf, and S. M. Khan. 2004. Some manageable predisposing factors for collar/stem rot of mango. *Pakistan Journal of Phytopathology*, 16 (2): 37-42.
- Malik, M. T., C. N. Akem, M. R. Kazmi and F. S. Fateh. 2011. Towards chemical management of mango sudden death disease in Pakistan. *In: International conference on "New Frontiers in Plant Pathology for Asia and Oceania*, Darwin-Australia, pp. 26-29.
- Maqsood, A., S. Saeed and A. Sajjad. 2008. Characterization and damage patterns of different bark beetle species associated with mango sudden death syndrome in Punjab, Pakistan. *Pakistan Entomologist*, 30 (2): 163-168.
- Maqsood, A., M. Salman and S. Saeed. 2014. Fungicide injection, an efficient management technique of mango sudden death disease in Punjab, Pakistan. *Pakistan Journal of Phytopathology*, 26 (2): 259-263.
- Paine, T. D., K. F. Raffa, T. C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual Review of Entomology*, 42:179-206.
- Ploetz, R. C., D. Benscher, A. Vázquez, A. Colls, J. Nagel and B. Schaffer. 1997. Mango decline research in Florida, an apparently wide-spread disease complex. *Acta Horticulture*, 455: 547-557.
- Ploetz, R. and R. Ploetz. 2003. Diseases of mango. *Diseases of tropical fruit crops*, 327-363.
- Prakash, O. M. and U. N. Singh. 1976. New disease of mango. *In: Proceedings of fruit research workshop*. May 24-28, 1976. Hyderabad, India. pp. 300-302.
- Saeed, S., N. Hussain, R. Attique and A. Masood. 2007. Etiology and management of sudden death phenomenon in mango. *Second Annual Report*. Dept. of Entomology, University of College Agriculture, Bahauddin Zakariya University, Multan. pp. 15-24.
- Sheler, S. A., D. N. Padule, D. M. Sawant and B. K. Konde. 1997. *In-Vitro* evaluation of fungicides against *Botryodiplodia*

- theobromae*, the cause of dieback disease of mango. Indian Journal of Plant Protection, 25: 118-120.
- Sireesha, O. and N. Venkateswarlu. 2013. *In-Vitro* evaluation of botanicals and panchagavya against leaf blast fungus *Pyricularia grisea*. Asian Journal Pharmacy Clinical Research, 6 (5): 84-86.
- Sonyal, S., M. S. Giri, H. S. Mahesha, K. B. Palanna, M. S. Hurakadli and A. Pappachan. 2015. Effect of fungicides on growth of *Ceratocystis fimbriata* ELL. and Halst. Causing wilt in Pomegranate. International Journal of Pure Applied Biosciences, 3 (4): 28-32.
- Vijaya, H. K., S. Kulkarni and Y. R. Hegde. 2007. Evaluation of plant extracts against *Ceratocystis paradoxa* causing sett rot of sugarcane. Karnataka Journal of Agricultural Sciences, 20 (1):168-169.

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