

SYMPTOM DEVELOPMENT AFTER ARTIFICIAL INOCULATION OF *BOTRYODIPLODIA THEOBROMAE*, A POSSIBLE CAUSAL ORGANISM TO QUICK DECLINE IN MANGO TREES

Shafqat Saeed*, Muhammad Ijaz Khan and Asad Masood

Department of Entomology, University College of Agriculture, Bahauddin Zakariya University, Multan, Pakistan

*Corresponding author e.mail: bumblebeepak@gmail.com

The fungus, *Botryodiplodia theobromae* is one of the causal agents of quick decline which was artificially inoculated to mango varieties to see quick decline symptoms. The most famous varieties, i.e. cv. Ratol-12, black chaonsa, white chaonsa, Fajri, Dosehri, Langra, Sindhri and Summer Bahisht were established in earthen pits under complete randomized design (CRD) and was observed up to three months for their establishment. Three months after transplanting, *B. theobromae* was artificially inoculated in the test plants which showed that Dosehri variety was comparatively tolerant to the disease as compared to others. Regarding the appearance of percent disease symptoms, Ratol-12 showed the highest disease symptoms followed by Langra, Fajri and then black Chounsa. So it is concluded that *B. theobromae* is not the major causal organism of this disease and other sources may also be involved in the development of disease.

Keywords: Mango, varietal resistance, fungi, *Botryodiplodia theobromae*, quick decline

INTRODUCTION

Mango (*Mangifera indica* L.) is the most important fruit of the tropical world. It is indigenous to India and Southeast Asia. Growing countries are India, Pakistan, China, Mexico, Bangladesh, Thailand, Philippine, Indonesia, Nigeria, Brazil, Florida and Oman. Mango is one of the most important foreign exchange earning fruit of Pakistan, through exports to many countries, mainly to England, Saudi Arab, Dubai, Germany, France, Holland, Thailand, Bangladesh, Singapore, Italy and Malaysia. In Pakistan mango is grown mainly in Punjab and Sindh provinces. There are many varieties of mango grown in Pakistan but most famous of these are Sindhri, Dosehri, Summer Bahisht, Chounsa, Anwer Ratol, Langra and Malda (Masood *et al.*, 2010).

According to FAO, Pakistan stood fourth during 1995 among mango growing countries of the world and come after India, Mexico and China (Khalid *et al.*, 2002). In 2004 Pakistan stood sixth instead of fourth among the mango growing countries of the world. The main reason of reduction in the ranking of Pakistan is due to the increased number of mango orchards being cutting away because of mango insect pests and disease problems and competition with other crops like cotton (Anonymous, 1996). The mango production is also vulnerable by the attack of number of diseases (Khalid *et al.*, 2002). Among all diseases infecting mango, the mango quick decline is the most severe threat to the Pakistan mango industry. Recently, incidence of this menace was found 20 and more than 60 percent in Punjab and Sindh Provinces of Pakistan, respectively and 60 percent in Al Batinah region of Oman (Al-Adawi *et al.*,

2006; Saeed *et al.*, 2006). Typical symptoms of mango decline include terminal and marginal necrosis of leaves, which ultimately lead to the death of leaf blade. The dieback gradually progresses to large branches with eventual reduction in the number of secondary roots (Ramos *et al.*, 1991). The bark of affected tree is discolored and darkened at a certain distance from the tip. Usually in young green twigs, the disease may lead to the browning of leaves and upward rolling of thin margins resulting in fall of leaves, ultimately in the drying and death of twigs (Anonymous, 1995). In India the disease is caused by *Botryodiplodia theobromae* (Rawal, 1998) while in Oman by *Botryodiplodia theobromae* and *Ceratocystis fimbriata* (Al-Adawi *et al.*, 2003; Al-Adawi *et al.*, 2005). In Brazil *Ceratocystis fimbriata* causes the disease in mango (Batista, 1960). Although, *B. theobromae* is frequently isolated from diseased mango tree and also give rise to mild symptoms especially gummosis in inoculated plants (Al Adawi *et al.*, 2006).

The main objective of the study is to observe resistance of different mango varieties against *Botryodiplodia theobroma* and to study the expression of disease symptoms especially gummosis.

MATERIALS AND METHODS

One year old healthy plants of eight different varieties of mango were collected from a commercial nursery and planted in pits at a depth of 3 feet at Agricultural Farm of University College of Agriculture, Bahauddin Zakariya University, Multan. Before transplanting the nursery plants,

earthen pits were made with tractor mounted digger and also filled with water to leach down the salts deep into the soil. After 15 days when salts were leached down into the soil, pits were filled with mixture of farm yard manure (FYM) and silt in which mango plants were planted under complete randomized design (CRD) with 5 plants for each variety. Finally, 3 plants of each of the eight varieties were treated with fungus and remaining 2 plants for each variety were treated as control. The most famous mango varieties, i.e. Ratol-12, black chaonsa, white chaonsa, Fajri, Dosehri, Langra, Sindhri and Summer Bahisht were selected due to presence of the symptoms of gummosis on large areas during the field surveys.

After transplanting the nursery in the field, establishment of plants was observed for a period of three months during which dead plants were replaced with healthy plants. After establishment of plants, these were inoculated with a fungus species *Botryodiplodia theobromae* arranged from Mango Laboratory, National Agriculture Research Center (NARC), Islamabad, Pakistan. Nursery was managed by proper agronomic practices, i.e. hoeing, timely irrigation, and NPK (Nitrogen, Phosphorous, Potash) application. Before inoculation all the equipments were sterilized in order to prevent the chances of infection by any other pathogen. Inoculation of fungus (*B. theobromae*) was made by insertion a piece of fungal colony (5 mm²) growth on Potato Dextrose agar (PDA) plate and inserted in slanting cuts (Fig. 1 and 2) in collar portion of each plant with sterilized scalpel and covered with a polythene sheet (Fig. 3) (Mullen *et al.*, 1991). In plants declared as control treatment, only agar slant without any fungal growth was inserted in slanting T-shaped cuts under the bark.

Disease evaluation: The presence or absence of disease (quick decline) in the plants was recorded in different mango varieties. The severity of disease symptoms in twigs, branches, leaves and stem of individual plant was rated using a scale from 1 to 5 (Ramos *et al.*, 1997) corresponding to percent disease severity from 0 to 100 % which has been described as under:

1. Plants free of disease = 0%
2. An early stage of infection characterized by browning of leaf petioles and mid-veins and presence of distal or marginal leaf blade necrosis in one or two branches = 25%
3. The presence of dead leaves, which may remain attached, in the tips of several branches, vascular browning, and evidence of pathogen invasion of vascular tissues = 50%
4. Dead leaves and progressive defoliation extending too many larger branches = 75%
5. Sever decline or dieback that extended to major portions of the plant = 100%

The disease development symptoms were observed in inoculated and control plants of each variety on monthly basis for a period of four months.

RESULTS

Bark splitting was the most frequent disease symptom of quick decline disorder. It was observed that 100% plants of Ratol-12 showed this symptom both in treated and control conditions followed by 33% in Sindhri in both cases (treated and control) while 33% plants in treated Summer Bahisht and Fajri but control was devoid of bark splitting symptom. Remaining varieties did not show the bark splitting symptoms. Gummosis produced in treated plants of Sindhri was 66% whereas there was no gummosis in control treatment. Similar disease incidence was recorded in treated and control plants of Langra. About 33% plants of Fajri showed gummosis symptoms in control and treated plants. Similar symptoms of gummosis were also observed on treated plants of Black Chaonsa. Gummosis symptoms were not observed on the plants of Summer Bahisht and white Chaonsa. Stem rotting (cankers and black streaks) was absolutely absent in all the plants of mango varieties. Symptoms of healthy plants were also recorded by observing new braches/inflorescences. It was found that 100% plants of Sindhri produced new leaves followed by 66% of Ratol-12 and Chaonsa treated plants whereas 33% of other varieties plants except treated Fajri and white Chaonsa plant and same were the case in control plants of Dosehri and Langra. As the disease progressed, leaves turned yellow because physiology of plants is affected when disease attack occurs. Among the observed varieties of mango, only 66% Fajri and 33% Ratol-12 treated plants leaves turned yellow while all other plants had no yellowing of leaves. All plants of Sindhri and Fajri while treated and control plants of Ratol-12 and Dosehri had no any defoliation, respectively. But 66% treated plants of black Chonsa and Langra exhibited defoliation while 0% and 33% plants showed curling correspondingly. Some of the varieties were found without any symptoms of disease like Sindhri, Dosehri, Summer Bahisht and white Chonsa. Marginal necrosis was recorded as 0% on untreated plants of all varieties except 33% of Fajri and other treated plants of all varieties excluding that of Ratol-12 with 100% plants (Table 1).

In case of observed symptoms of plants, 50% disease symptoms were found on treated plants of Sindhri and black Chaonsa compatible with untreated plants of Ratol-12. Results showed that Dosehri variety was comparatively tolerant to the disease as compared to other varieties of mango whereas Ratol-12 showed the highest disease symptoms followed by Langra, Fajri and black Chaonsa. No significant difference was observed in the treated and untreated plants of white Chonsa showing 38% disease symptoms of quick decline (Table 2).

Table 1. Evaluation of disease in *Botryodiplodia theobromae* inoculated varieties of mango

Sr. #	Varieties	Disease symptoms (%)	
		Treated plants	Plants as control
1	Sindhri	50	25
2	Ratol 12	75	50
3	Fajri	62	38
4	Black Chonsa	50	38
5	Dosehri	38	0
6	Langra	63	25
7	Summer Bahisht	63	25
8	White Chaunsa	38	38

DISCUSSION

In present study, the disease symptoms were not only produced by inoculated plants but also appeared in the untreated healthy plants which may be infested due to the spread of inoculums through different means of dissemination, i.e. air, water or insect. So it is possible that *Botryodiplodia theobromae* may not be the main causal organism of this disease. Although, *B. theobromae* is associated with quick decline mango tree and also give rise to one or more of the symptoms of quick decline in inoculated plants. But, it is an opportunistic pathogen and becomes more virulent in combination with others fungi, i.e. *Ceratocystis fimbriata* and *Fusarium aesculi* (Ploetz *et al.*, 1996; Al Adawi *et al.*, 2006). Recently, Shahbaz *et al.* (2009) investigated that *B. theobromae* (Pat.) Griffon and Maubl was relatively more frequently isolated from trees showing symptoms of decline in Pakistan. Therefore, mango quick decline is considered as a disease complex in which pathogenic fungi, bark beetle and cultivation method are involved (Malik *et al.*, 2005; Al Adawi *et al.*, 2006; Masood *et al.*, 2009). Early authors, (Westerdijk and Buisman, 1929) supposed MSDS might be transmitted and spread by various dissemination means, i.e. air, water, and insects. In spite of other means of disease dissemination, bark beetle species are

expected to be involved as putative vectors (Ribeiro, 1980; Al Adwai *et al.*, 2006; Masood *et al.*, 2008). The elm bark beetle, *Scolytus mutistriatus* and *S. scheveyrewi* (Coleoptera: Scolytidae) has been reported as the primary vector of Dutch elm disease caused by fungus, *Ophiostoma novoulmi* which can be transmitted into healthy elm trees and also re-isolated the same fungus from adult beetles as well as from infested tree (Jacobi *et al.*, 2007). Although, bark beetle, *Hypocryphalus mangiferae* was formerly reported as an indigenous wood borer in mango as secondary pest (Mohyuddin and Mahmood, 1993) but now due to its role in disease transmission as a vector it has gained the status of primary pest, i.e. due to transmission of *Ceratocystis fimbriata* and *Lasiodiplodia theobromae*, the causal organisms of mango sudden death syndrome (Al Adawi *et al.*, 2006; Masood *et al.*, 2009; Masood *et al.*, 2010). However, research is needed to further investigate other means of disease dissemination and other cultural practices in the etiology of this disease in Pakistan.

The evaluation of disease symptoms due to the inoculation of fungus *Botryodiplodia theobromae*, the possible casual organism of mango quick decline was described to assess the most tolerable available mango variety against the attack of quick decline. According to our studies, cv. Doshari and white Chaunsa was found more tolerant while cv. Ratol-12 followed by Summar Bahisat was observed more susceptible in the development of disease symptoms, i.e. gummosis, bark splitting, leaf yellowing and defoliation. These findings would be helpful in development of resistant mango varieties keeping in view disease scale based on the characteristic symptoms of mango quick decline. These symptoms of mango quick decline observed are also reported alone or in combination of more symptoms in different mango orchards in Oman, Brazil or Pakistan (Ploetz *et al.*, 1996; Al Adwai *et al.*, 2006; Iqbal *et al.*, 2007). Initial symptoms of this disease include gummosis from the bark, bark splitting, yellowing and withering of leaves. On scrapping of diseased tree trunk, usually vascular discoloration, cankers and in some cases oozing of bad smelled liquid were observed (Masood *et al.*, 2010). Ramos

Table 2. Evaluation of disease symptoms (%) in treated and untreated mango varieties

Disease Symptoms	Disease symptoms (%) in treated mango varieties (n=9)								Disease symptoms (%) in untreated healthy mango varieties (n=9)							
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈
Bark splitting	100	33	33	0	0	0	0	33	33	33	0	0	0	0	0	0
Gummosis	0	66	33	33	0	66	0	0	0	0	33	33	0	0	0	0
Stem rotting	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leaf appearance	66	100	0	66	33	33	33	0	66	100	33	33	0	0	33	33
Leaf yellowing	33	0	66	0	0	0	0	0	0	0	0	0	0	0	0	0
Defoliation	0	0	0	66	0	66	33	0	0	0	0	0	0	0	0	0
Marginal necrosis	0	100	100	100	100	100	100	100	0	0	33	0	0	0	0	0

T₁= Ratol-12; T₂= Sindhri; T₃= Fajiri; T₄= Black Chonsa; T₅= Doshari; T₆=Langra; T₇= Summar bahisat; T₈= White Chonsa



Figure 1. An inverted T-shape cut



Figure 2. Inoculation of fungus



Figure 3. Wrapped by polythene



Figure 4. Gummosis



Figure 5. Black streak in stem



Figure 6. Cankers/stem rots



Figure 7. Bark splitting



Figure 8. Yellowing and drying

Pictorial representation of inoculation and disease symptoms on mango plants

et al. (1997) determined the resistance of *Mangifera indica* to tip dieback caused by *Botryosphaeria ribis*, anamorphic state *Fusicoccum* sp., on 361 trees of 122 cultivars of mango. Three trees from each cultivar were selected and the disease was evaluated on a 1 to 5 scale ranging from those free of tip dieback to those with extensive branch necrosis. At the end they found that two *Mangifera* species (*M. odorata* and *M. zeylanica*) showed the least mean disease rating which shows that field resistance to tip dieback may be present in some mango cultivars. Khalid *et al.* (2002) gave the assessment keys for some important diseases of mango and demonstrated that these are important in any study relating disease severity to disease losses and subsequent management tactics.

Al-Adawi *et al.* (2003) studied that 60% of the trees of mango were infected in the parts of Al Batinah region of Oman. They observed that trees showed gummosis from the trunk, wilting and eventual browning of leaves on the single branch. Wood of the diseased tree is stained dark brown, spreading from point of infection. In the lesions produced on the bark of tree, pycnidia were observed and the pathogen *Diplodia theobromae* was isolated from the infected trees. Van Wyk *et al.* (2005) studied the DNA based characterization of *Ceratocystis fimbriata* which is associated with the mango decline in Oman. They obtained the sequence data for the internal transcribed spacer 1 and 2 regions and the 5.8 S rRNA gene regions and compared it with the *C. fimbriata* from several hosts and geographic areas. So the isolates from Oman were reported to represent *C. fimbriata sensu lato* that were most closely related to an isolate from mango in Brazil. They concluded that mango pathogen in Oman might have originated in Brazil. Rawal (1998) reviewed that dieback in mango is due to *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*. He told that during the attack of dieback pathogens epidermal and sub epidermal cells shriveled in the early stage. The areas of cambium and phloem show brown discoloration and yellow gum like exudates flows out of the cells. This concept is elaborated by Aslam (2005) that mango is indigenous to India and Southeast Asia and told that the causal organism of mango decline in India is *Botryodiplodia theobromae* Pat. and *Alternaria alternate*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis* spp. and other fungi caused mango decline in USA. He also described that the deficiencies of micronutrients may predispose the trees to infection by fungal pathogens. In this study, there were no differences observed in the treated and untreated plants of white Chonsa showing 38% disease symptoms of quick decline and some healthy plants showed disease symptoms without inoculation. So it is concluded that *Botryodiplodia theobromae* is not the major causal organism of this disease and other factors are also involved in the development of disease. Therefore, it is necessary to investigate the other

disease facilitating factors and means of disease dissemination so that a sustainable management strategy can be devised for suppression of mango quick decline.

ACKNOWLEDGEMENT

We acknowledge for the technical support of Mr. Munawar Raza Kazmi, (NARC) Mr. Faisal Sohail Fateh (NARC) and Mr. Muhammad Tariq Malik (MRS). This study was funded by PARC project "Etiology and Management of Sudden Death Phenomenon in Mango Orchards" and Mango ASLP project.

REFERENCES

- Al-Adawi, A.O., M.L. Deadman, A.K. Al Rawahi, A.J. Khan and Y.M. Al Maqbali. 2003. *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. Pl. Pathol. 52:419-425.
- Anonymous. 1995. Mango orchards survey and research prospects. Dept. Hort., Uni. Agri. Faisalabad, Pakistan, pp.148.
- Anonymous. 1996. National Coordinated research project on mango crisis. First Annual Report. Dept. Hort., Uni. Agri. Faisalabad, Pakistan, p.240.
- Batista, A.C. 1960. *Ceratocystis fimbriata* Ell. & Halst. Sobre *Mangifera indica* L. Publicacao 244, Instituto de Micologia da Universidade do Recife, p.1-46.
- Iqbal, Z., E.E. Valeem, M. Shahbaz, K. Ahmad, Z.I. Khan, M.T. Malik and M. Danish. 2007. Determination of different decline disorders in mango orchards of the Punjab, Pakistan. Pak. J. Bot. 39:1313-1318.
- Jacobi, W.R., R.D. Koski, T.C. Harrington and J.J. Witcosky. 2007. Association of *Ophiostoma novo-ulmi* with *Scolytus scolytus* (Scolytidae) in Colorado. Pl. Dis. 91:245-247.
- Khalid, P. S. Akhtar and S. Alam. 2002. Assessment keys for some important diseases of mango. Pak. J. Biol. Sci. 5:246-250.
- Malik, M.T., A.A. Dasti and S.M. Khan. 2005. Mango decline disorders prevailing in Pakistan. Proceedings of International Conference on Mango and Date palm: Culture and Export. University of Agriculture, Faisalabad, Pakistan, 20-23-June, p.25.
- Masood, 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. Pak. Entomol. 30:163-168.
- Masood, A., S. Saeed and N. Erbilgin. 2010. Role of stressful mango host conditions in attraction and colonization by the mango bark beetle, *Hypocryphalus mangiferae* Stebbing (Coleoptera: Curculionidae: Scolytinae) and in the symptom development of quick decline on mango trees in Pakistan. Entomological

- Research 40:317-327.
- Masood, A., S. Saeed, M.T. Malik, N. Iqbal and M.R. Kazmi. 2010. Methodology for the evaluation of symptoms severity of mango sudden death syndrome in Pakistan. Pak. J. Bot. 40(2): 1289-1299.
- Masood, A., S. Saeed, A. Sajjad and M. Ali. 2009. Life cycle and biology of mango bark beetle, *Hypocryphalus mangifera* Stebbing: as a possible vector of sudden death disease of mango in Pakistan. Pak. J. Zool. 14:281-288.
- Mohyuddin, A.I. and R. Mahmood. 1993. Integrated control of mango pests in Pakistan. Acta Hort. 341:467-483.
- Ploetz, R.C., D. Bensch, A. Vazquez, A. Colls, J. Nagel and B. Schaffer. 1996. A re-evaluation of mango decline in Florida. Pl. Dis. 80:664-668.
- Ramos, L., J., S.P. Lara, R.T. McMillan and J.R. Narayanan. 1991. Tip dieback of mango (*Mangifera indica*) caused by *Botryosphaeria ribis*. Pl. Dis. 75:315-318.
- Ramos, L.J., S.P. Lara, R.T. McMillan and J.R. Narayanan. 1997. The resistance of mango (*Mangifera indica*) cultivars to tip dieback disease in Florida. Pl. Dis. 81:509-514.
- Rawal, R.D. 1998. Management of fungal diseases in tropical fruits. In: *Tropical Fruits in Asia: Diversity, Maintenance, Conservation and Use*. (Eds.): R.K. Arora and V. Ramanathia
- Rao. Proceedings of the IPGRI-ICAR-UTFANET Regional training course on the conservation and use of germplasm of tropical fruits in Asia held at Indian Institute of Horticultural Research, 18-31 May 1997, Bangalore, India.
- Shahbaz, M., Z. Iqbal, A. Saleem and M.A. Anjum. 2009. Association of *Lasiodiplodia theobromae* with different decline disorders in mango (*Mangifera Indica* L.). Pak. J. Bot. 41:359-368.
- Van Wyk, M., A.O. Al Adawi, B.D. Wingfield, A.M. Al Subhi, M.L. Deadman and M.J. Wingfield. 2005. DNA based characterization of *Ceratocystis fimbriata* isolates associated with mango decline in Oman. Australasian Pl. Pathol. 34:587-590.
- Westerdijk, J. and C. Buisman. 1929. De iepenziekte: Rapport over het onderzoek verricht op verzoek van de Nederl. Heidemaatschappij. Nederl. Maatschappij, Arnham.