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

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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 gummiosis 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 gummiosis.

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, Sindhi and Summer Bahisht were selected due to the 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 Sindhi 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 Sindhi 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 branches/inflorescences. It was found that 100% plants of Sindhi 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 Sindhi and Fajri while treated and control plants of Ratol-12 and Dosehri had no any defoliation, respectively. But 66% treated plants of black Chaonsa and Langra exhibited defoliation while 0% and 33% plants showed curling correspondingly. Some of the varieties were found without any symptoms of disease like Sindhi, Dosehri, Summer Bahisht and white Chaonsa. 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 Sindhi 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 Chaonsa showing 38% disease symptoms of quick decline (Table 2).
The evaluation of disease symptoms due to inoculation of fungus *Botryodiplodia theobromae*, the possible causal 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. 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 Adawi et al., 2006; Masood et al., 2009). 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 novouulmi* 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.

**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, Shabbaz 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 Adawi 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 novouulmi* 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.

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

<table>
<thead>
<tr>
<th>Sr. #</th>
<th>Varieties</th>
<th>Disease symptoms (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treated plants</td>
</tr>
<tr>
<td></td>
<td>T1= Ratol-12</td>
<td>T2= Sindhri</td>
</tr>
<tr>
<td>1</td>
<td>Sindhri</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Ratol 12</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>Fajiri</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>Black Chonsa</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>Dosehri</td>
<td>38</td>
</tr>
<tr>
<td>6</td>
<td>Langra</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>Summer Bahisht</td>
<td>63</td>
</tr>
<tr>
<td>8</td>
<td>White Chaunsa</td>
<td>38</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Disease Symptoms</th>
<th>Disease symptoms (%) in treated mango varieties (n=9)</th>
<th>Disease symptoms (%) in untreated healthy mango varieties (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 T2 T3 T4 T5 T6 T7 T8</td>
<td>T1 T2 T3 T4 T5 T6 T7 T8</td>
<td>T1 T2 T3 T4 T5 T6 T7 T8</td>
</tr>
<tr>
<td>Bark splitting</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>Gummosis</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td>Stem rottning</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leaf appearance</td>
<td>66</td>
<td>100</td>
</tr>
<tr>
<td>Leaf yellowing</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Defoliation</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Marginal necrosis</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

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

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