Pak. J. Bot., 43(3): 1793-1798, 2011.

QUICK DECLINE OF MANGO IN PAKISTAN: SURVEY AND PATHOGENICITY OF FUNGI ISOLATED FROM MANGO TREE AND BARK BEETLE

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Abstract

Mango sudden death syndrome (MSDS) has become an increasing threat for mango production all around the world. The present study was conducted to identify the association of pathogenic fungi with mango quick decline tree and the bark beetle. During survey, the most evident symptoms of this disease were gummosis and rotting, cankers and vascular discoloration along with holes made by *Hypocryphalus mangiferae*. The mango varieties viz., Malda and Ratol were found to be more tolerant against this disease. From diseased tree as well as from *H. mangiferae*, the most frequently isolated fungi were *Lasiodiplodia theobromae*, *Ceratocystis fimbriata* and *Phomopsis* sp. The isolation of C. *fimbriata* from beetle on PDA was relatively at low frequency (2.0%) as compared to *L. theobromae* and *Phomopsis* (24% and 6.0%). By carrot disc technique, the isolation of *C. fimbriata* was significantly higher (7.33%) but *L. theobromae and Phomopsis* sp., were not isolated. The formers fungi were re-isolated from artificially inoculated and symptomatic mango plants. After six months of inoculations, disease symptoms i.e., wilting, oozing and black streaks were developed which showed significant differences among all treatments. Our findings suggested that *C. fimbriata* and *L. theobromae* are both pathogenic to mango causing mango quick decline in Pakistan. Both fungi were frequently isolated from diseased tree as well as *H. mangiferae* which may be involved in the dissemination and as a facilitating agent for the entry of the pathogens.

Introduction

Mango (*Mangifera indica* L.) is one of the most important fruit crops in tropical and subtropical areas of the world. Pakistan produces 3.95% of the world mangoes and is ranked 5th after big producers i.e., India, China, Thailand and Mexico. There are more than 250 cultivars of mango grown in Pakistan over an area of about 0.099 million hectares with annual production of about 1.037 million tones. Pakistan exports 7-10% of its total production valued at around US$20 million per year (Anon., 2007).

The mango yield in Pakistan has been decreased due to various biotic and abiotic factors. Mango quick decline is the most recent rigorous threat to the Pakistan mango industry, and the fungi *Ceratocystis fimbriata* Ellis & Halst, is the first plant pathogen associated with it in Brazil, Oman and Pakistan (Ribeiro 1980; Malik *et al*., 2005; Al Adawi *et al*., 2006; Saeed & Masood, 2008). Recently, Shahbaz *et al*., (2009) established that *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl was relatively more frequently isolated from trees showing symptoms of decline in Pakistan than the other fungi and isolation of *L. theobromae* from different regions ranged from 8 to 61%. However, research is needed to confirm the etiology of the disease in Pakistan and to evaluate the incidence of the disease in mango production regions and among most grown mango varieties. The trees die suddenly in their numbers and there is no end in sight. This phenomenon has also been reported from some other parts of the world i.e., Brazil and Oman (Al Adwai *et al*., 2006). Initial visible symptoms of this disease include gummosis from the bark, bark splitting, streaking and vascular discoloration beneath the gummosis. The leaves of the trees wither out but remain attached with the dying tree. On scrapping of severely infected trunk, usually produced rotted cankers and in some cases oozing of bad smelled liquid (Masood *et al*., 2010). These symptoms described above may be found alone or in combination of two or more symptoms in different mango orchards in Brazil or Pakistan (Ploetz *et al*., 1996; Iqbal *et al*., 2007). The mortality of the tree is usually by the blockage of xylem and phloem vascular bundles in the proper flow of nutrients (Khuhro *et al*., 2005).

The most frequently found bark beetle on diseased tree was identified as *Hypocryphalus mangiferae* Stebbing (Coleoptera: Scolytidae)**.** It preferred diseased or dried portions of wood and made tiny holes from which saw dust emitted that was the characteristic damage pattern of this beetle. When the bark of the woody stem peeled off, irregular galleries were observed showing black appearance (Masood *et al*., 2008). The beetle produces galleries in the cambium of affected trees, feeding primarily on fungi and also serving as a wounding agent that facilitates infection and transmission of the pathogens (Ploetz, 2003). The fungi, *Ceratocystis fimbriata* and *Phomopsis subordinaria* have both been shown to be transmitted through different bark beetles species and other insects (Graham, 1967; De Nooij, 1988). Dutch elm disease caused by *Ophiostoma ulmi* have spread throughout Europe, Central Asia and North America and disseminated by various elm bark beetles mainly of the genus *Scolytus* (Lanier & Peacock, 1981; Brasier, 1987). Transmission of *Ceratocystis fagacearum* (Brentz) Hunt, caused oak wilt was intensively studied in North America whereby nitidulid beetles attracted by exuding sap, carry *C. fagacearum* to wounded oak and such wounds provide ground as infection courts (Gibbs & French, 1980).

Focusing on the increasing threat of mango sudden death syndrome in Pakistan, the present study was conducted to investigate the etiology of the disease and the association between the bark beetle and the spread of the disease in perspective of developing appropriate management strategies. The objectives of this study were therefore to: a) conduct a survey of affected mango trees and varieties across production regions of Pakistan, b) to determine the relation between infected trees and beetle attack, by collecting associated beetles from infected trunks and identify the species directly associated with the observed disease symptoms c) make isolations of putative pathogenic fungi from infected tree trunks and from beetles collected from the disease symptomatic trees, to compare and d) conduct pathogenecity tests to establish direct associations of the fungi in development of mango sudden death under controlled conditions.

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Material and Methods

1. Study area: This study was conducted in the mango orchards of Multan, Pakistan during 2006-2008 production seasons. We selected three locations in Multan district; Bund Bosan (30.268 ºN and 71.495 ºE), Shujabad (30.266 ºN and 71.494 ºE) and Khanewal road (30.270 ºN and 71.250 ºE). On the whole, 30 mango orchards were visited i.e., 10 orchards in each location. During the surveys, 10 trees of each mango variety per orchard were randomly examined for sudden death disease severity.

2. Disease severity and intensity: The most evident symptoms of Mango Sudden Death Syndrome (MSDS) i.e., black steaks and cankers were observed by scratching the bark from collar portion of tree up to main stem. Disease severity scores were assigned based on visual observation scales using a formula "Disease Severity = (Area of diseased tissue/Total tissue area) X 100" (Cooke, 1998). For assessment of MSDS, we developed a scale on visual basis of disease severity symptoms i.e., oozing, cankers, rotting, blackening, bark splitting, drying of twigs or branches, curling or drying of leaves, shedding and attachment of leaves appeared in five different portions of tree. The infected portion of MSDS tree on each portion i.e., collar, roots, main stem, main limbs and leaves of mango tree corresponded to the scale in the following way: Scale-0= no sign, 1= 1-10%, 2= 11-20%, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60% and 7= more than 60% area infected with mango sudden death symptoms (Masood *et al*., 2010). Disease intensity was calculated by using the formula devised by McKinney (1923) which was further simplified according to MSDS symptoms (Masood *et al*., 2010). This was based on a disease intensity index estimated as:

$$\text{Disease intensity index} = \frac{0(n) +1(n) +2(n)+3(n)+4(n)+ 5(n) + 6(n) + 7(n)}{\text{Total No. of Plants observed}} \times \frac{100}{7}$$

where, n= number of trees falling in each category

3. Pathogen isolation from diseased tree: For isolation of fungal pathogens, we removed small wooden pieces beneath the bark, manifested with beetle holes. Two standard methods of fungal isolation were used. In first method, plates of potato dextrose agar amended with streptomycin (SPDA) were used as the standard medium of isolation (Al Adawi *et al*., 2006; Rivera-Vargas *et al*., 2006). The petri plates were incubated at 25± 2^{o}C and further sub-cultured on fresh media plates for further purification. In the second method, we used carrot discs for the isolations from the plant tissue (Moller & DeVay, 1968). Once the ascomata has been developed, ascospore masses were transferred to SPDA plates and then into new SPDA plates for further purification. After 7-10 days, the fungal growths were microscopically observed and identified on the basis of culture morphology, spores and conidia of the specific fungi (Domsch *et al*., 1980; Upadhyay, 1981).

4. Pathogens isolation from bark beetles: Bark beetle adults were caught from infected mango trees simply by peeling off the bark from collar or above ground stem portion. To isolate associated fungi, the beetles were immersed in 1% NaOCl for three minutes followed by four serial washing with sterilized distilled water and then blotted dry on sterile filter paper. The beetles were slightly crashed and then aseptically placed onto potato dextrose agar (PDA). The plates were then wrapped with parafilm in order to avoid any contamination chance. The plates were incubated at 25±2^{o}C for 7-10 days and observed for pathogen colony growth from the beetles. The observed pathogen colonies from the beetles were sub-cultured and identified microscopically (Al Adawi *et al*., 2006). Pathogens were also isolated from bark beetles using the carrot baiting technique by placing the beetles in a cavity made on the inner surface of a pair of carrot discs and incubated at same temperature for colony growth and isolation (Jacobi *et al*., 2007).

5. Pathogenecity and evaluation of disease symptoms

Plant materials: The experiment was conducted during April to November, 2008 at Bahauddin Zakariya University, Multan, Pakistan (30.26307^{o}N and 071.50584^{o}E, latitude 308 ft elevation from sea level). A pot media was prepared which consisted of gravelly clay-loam, rotten farm yard manure and sterilized wheat and rice straw. Mango plants (2-3 years old) cv. "Chounsa" was planted in 45x 60 cm earthen pots. The whole experiment was performed under lathe house conditions in an insect proof compartment, made up of fine mesh cloth (Leach, 1940).

Pathogenecity: As the fungi, *Ceratocystis fimbriata*, *Lasiodiplodia theobromi* were frequently isolated from diseased tree as well as from the bark beetles. Therefore, only these two fungal pathogens were used in inoculations into healthy plants. To confirm the plants (under investigation) as disease free, isolations were made from the stem portion before the start of the experiment. The sampling sites were then covered with parafilm after sterilizing with 1% Sodium hypochlorides (NaOCl).

Three isolates of each fungi, *C, fimbriata* and *L. theobromae* were inoculated into healthy plants. In addition to above treatment, *C. fimbriata* and *L. theobromae* were collectively inserted under the bark as a composite treatment. In the last treatment, healthy plants were used to serve as a control. Five replications for each treatment were made under completely randomized design (CRD) in the lathe house. Inoculation of fungi was made by placing a piece of fungal colony (5 mm², obtained from leading edges of actively growing fungal culture on PDA) in slating cuts under the bark with sterilized scalpel and then covered with parafilm (Mullen *et al*., 1991). In healthy control, only agar slant was placed without any fungal growth. For re-isolation of fungi, total 12 to 15 stem pieces were excised from the above and below the point of inoculation and surface sterilized before planting on PDA according to above mentioned procedure. Representative fungal isolates that have been confirmed to be pathogenic to mango plants cultures were submitted to Department of Mycology and Plant Pathology, Quaid-i-Azam Campus, University of Punjab, Lahore- Pakistan (accession number FCBP 1012).

6. Symptoms evaluation: Success of infection was estimated by the occurrence of MSDS symptoms. For measuring plant wilting, we counted the number of wilted leaves out of total leaves in each observation and calculated the wilting percentage. Appearance of wilting and number of oozing points on plants were assessed every fortnight between April and November 2008. Length of black streaks (cm) was

observed on dissected plants at the end of experiment. To describe differences of infection, symptoms on plants were analyzed in three specific periods i.e., four months post infection (pi), six months pi.

7. Isolation frequencies of fungi: For isolation of fungi from treated or diseased plants, the stem portion was removed from the point of infection and made small pieces (0.5mm) of wood stripes. Each strip was placed on PDA and the frequency of isolation was calculated. For statistical comparison re-isolation frequencies were calculated (no. of isolates with the fungi/ total no. of isolates x 100) (Jankowiak & Bilanski, 2007).

8. Data analysis: Data regarding disease severity, symptoms i.e., black streaks, oozing and wilting of all the treatments and isolation frequencies of fungi were subjected to statistical analysis using analysis of variance (ANOVA). The means were compared using least significant difference (LSD) test. The data was analyzed using computer software XLSTAT (2008).

Results and Discussion

1. Disease symptoms and its severity on mango varieties: During the survey, the mango varieties found in the orchards were Cvs. Langra, Dosehri, Chounsa, Chounsa late, Fajri, Ratol, Malda, Non-grafted, Almas and Sindhari. All these varieties showed disease symptoms of mango Sudden death. Initial disease symptoms were gummosis from the bark of the infected trees. These affected trees usually displayed other symptoms, including bark splitting, rotting signs and stem vascular discoloration beneath the gummosis. Most diseased trees also showed signs of damage caused by the bark beetle. When the infected portion was scratched, cankers became visible, in most cases producing a pungent odor. The leaves of diseased trees were withered and remained attached to the dying tree. All the ten varieties were evaluated at each orchard individually keeping in view the disease scale. A total of 30 orchards comprising 1800 mango trees were surveyed in Bund Bosan, Shujabad and Khanewal locations. The differences on the basis of disease intensity among the varieties in three locations were highly significant at probability level (p<0.01). The maximum disease severity was recorded on Almas (16.81) followed by Sindhari (15.91) and Chounsa (14.05). These three varieties were considered to be the most susceptible to the disease. Minimum disease intensity was found on Malda (9.605) followed by Ratol (10.33) and Langra (12.33), all regarded as more tolerant to the disease (Fig. 1). The mango variety, Chounsa, the susceptible one, was the predominant variety planted in most orchards surveyed, thus accounting for the overall high disease intensity values. In these districts, the overall maximum disease intensity on all the surveyed varieties was observed in Khanewal (18.32) followed by Shujabad (13.08). But the overall minimum severity was found in Bund Bosan area (10.74) (Fig. 2).

2. Pathogen Isolation from the diseased mango trees: In this study, a total of 900 isolations were made from main stem and collar portion of diseased trees. The predominant fungi that were isolated on PDA were *Ceratocystis fimbriata*, *Lasiodiplodia theobromae* and *Phomopsis* sp., *Aspergillus* spp., and *Fusarium* sp., from Band Bosan, Shujabad and Khanewal locations. From these locations, the comparative high frequency of *Aspergillus* (28%) was recorded followed by *L. theobromae* (16%) whereas; the minimum frequency was obtained in ascending order from *C. fimbriata* (3.1%), *Fusarium* (4.22%) and *Phomopsis* (7.21%) as Table 1. Most of the time, growth of *Aspergillus* and *L. theobromae* dominated the growth of other pathogenic fungi on PDA media i.e., *C. fimbriata* and *Fusarium*. Isolation of fungi from the growing margins of discolored tissues yielded *C. fimbriata* and *L. theobromae* while *Phomopsis* was isolated from collar region near the soil surface and the beetles were also collected for isolation of fungi.

3. Isolation from the bark beetle: The most frequently found bark beetle on diseased tree was identified as *Hypocryphalus mangiferae*. The fungal isolation studies from the bark beetles showed that *C. fimbriata*, *L. theobromae* and *Phomopsis* sp., were frequently isolated from the adult bark beetles. The fungi, C. *fimbriata* appeared at a relatively low frequency (2.0%) as compared with *L. theobromae* and *Phomopsis* (24 and 6.0%) respectively by using the SPDA isolation method. The isolation of *C. fimbriata* by the carrot disc technique, however, was significantly higher (7.33) as compared to SPDA method (2.0). The fungi, *L. theobromae* or *Phomopsis* sp., were not isolated from the bark beetle by the carrot technique (Table 2).

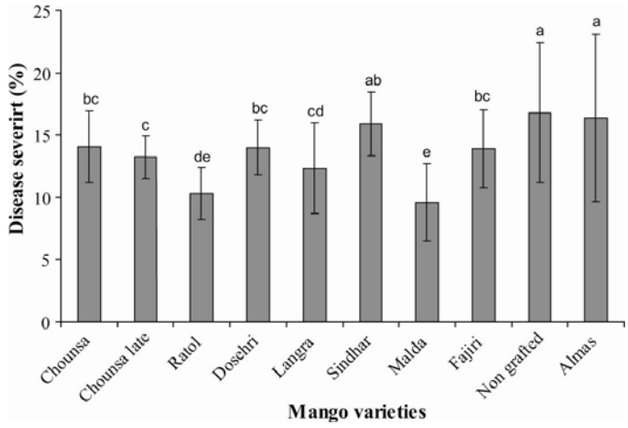

Fig. 1. Disease severity (%) of Sudden death of mango on different varieties at three locations of Multan region.

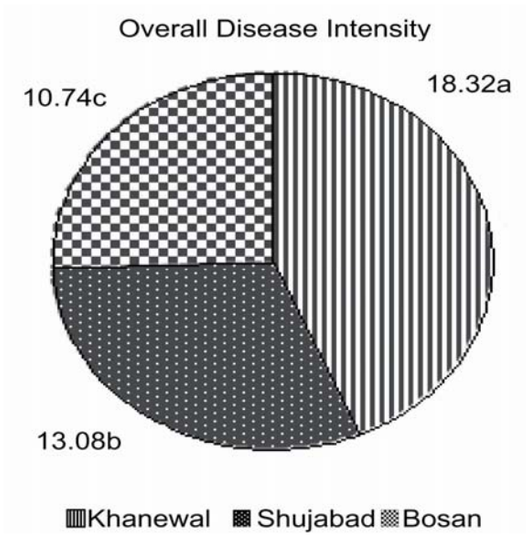

Fig. 2. Overall disease intensity on all surveyed varieties at Khanewal, Shujabad and Bosan area of Multan region.

Table 1. Isolation of fungi from diseased mango trees at three locations of Multan, region.

Locations	Isolation frequency of fungi (%)				
	Ceratocystis fambriata	***Lasiodiplodia theobromae***	***Phomopsis* sp.**	***Aspergillus* sp.**	***Fusarium* sp.**
Band Bosan	4.33	20	7.33	25.33	3.0
Shujabad	2.3	15	5.3	31.0	4.33
Khanewal	3.6	13.33	9.0	27.06	5.33
Average isolation frequency	3.1	16,03	7.21	28.0	4.22

N=300 Isolates at each location

Table 2. Isolation of fungi from bark beetle, *H. mangiferae* on PDA and Carrot disc Techniques.

Location	*Source of fungus	PDA			Carrot discs		
		Ceratocystis fambriata	***Phomopsis* sp.**	***Lasiodiplodia theobromae***	***Ceratocystis fambriata***	***Phomopsis* sp.**	***Lasiodiplodia theobromae***
Bund Bosan	Beetle	3/150 (2.00)	9/150 (6.00)	24/150 (16.0)	12/150 (8.0)	0	0
Shujabad	Beetle	0/150 (0.00)	6/150 (4.00)	39/150 (26.0)	6/150 (4.00)	0	0
Khanewal Road	Beetle	6/150 (4.00)	12/150 (8.00)	45/150 (30.0)	15/150 (10.0)	0	0
Total isolation frequency (%)		2.00	6.00	24	7.33	0	0

*N= 300 bark beetles collected from each location

Table 3. Reisolation of fungal isolates from healthy plants after inoculations in mango plants cv. Chounsa during April-November, 2008.

Isolate code	Location	Species (mango variety)	Fungal Pathogen	Reisolation frequency (%)
S1	Khanewal Road, Multan	Chaunsa	*Ceratocystis fambriata*	02.80 b
S2	Khanewal Road, ultan	Chaunsa late	*Ceratocystis fambriata*	03.00 b
S3	Bosan Road, Multan	Chaunsa late	*Ceratocystis fambriata*	05.30 b
S4	Kabirwala, Multan	Chaunsa	*Lasiodiplodia theobromae*	23.40 a
MRS1	MRS, Shujabad	Chounsa	*Lasiodiplodia theobromae*	32.50 a
MRS2	Khokar farm, Shujabad	Chounsa	*Lasiodiplodia theobromae*	21.00 a
S1 and S4	Khanewal&Kabirwala, Multan	Chounsa	*Ceratocystis fambriata* & *Lasiodiplodia theobromae*	13.0 a & 39.0 a

Table 4. Evaluation of disease symptoms after inoculation of fungal pathogens in mango plants cv. Chousa during April-November, 2008.

Plants inoculated	Host symptoms			
	Oozing (number)	**Wilting (after 4 months)**	**Wilting (after 6 months)**	**Streaks length (cm)**
Ceratocystis fambriata	1.80 b	51.0 a	89.0 a	12.00 a
Ceratocystis fambriata	02.0 b	26.80 bc	70.20 b	2.13 b
Ceratocystis fambriata	2.40 b	38.0 ab	91.0 a	1.42 b
Lasiodiplodia theobromae	1.40 bc	08.0 d	32.40 d	1.88 b
Lasiodiplodia theobromae	0.6 cd	16.60 cd	49.00 c	0.470 b
Lasiodiplodia theobromae	0.6 cd	11.00 d	32.00 d	0.30 b
Ceratocystis fambriata & *Lasiodiplodia theobromae*	4.40 a	48.00 a	92.00 a	15.10 a
Healthy plant as a control	0.0	6.0 d	16.00 e	0.00
ANOVA values: df; F; P	7; 11.33; <0.0001	7; 13.27; <0.0001	7; 30.64; <0.0001	7; 10.12; 0.0001

Mean values sharing similar letters show non-significant differences (P<0.05) by using LSD test.

4. Pathogenecity and evaluation of disease symptoms: After six months of inoculations, disease symptoms i.e., wilting, oozing and black streaks were developed which showed significant differences among all treatments (Table 4). Initial wilting (after four months) appeared high enough on plants inoculated with *Ceratocystis fimbriata* alone or in combination with *Lasiodiplodia theobromae* which became more drastic after six months. Whereas, wilting was non significant in plants inoculated with *L. theobromae* as compared with control. While, maximum oozing and black streaks were exhibited by all the plants inoculated with *C. fimbriata* (Table 4).

The plants artificially inoculated with the fungus, *C. fimbriata* and *L. theobromae* was reisolated in variable frequencies and non-significant differences were observed among treatments (F= 5.36; P= 0.009 and F= 1.09; P=0.379) (Table 3). Control plants did not exhibit any of the fungus growth and symptoms except some mild wilting.

The pathogenecity test demonstrated that the most frequently isolated fungi, *Ceratocystis fimbriata and Lasiodiplodia theobromae* developed symptoms of Sudden death of mango after inoculation in healthy plants. This test was conducted in the light of Koch's postulates; the fungi must be recovered from healthy plant after inoculation of disease; the same fungal pathogens must be associated with the diseased plants (Koch, 1883). *C. fimbriata* proved to be the more pathogenic in the development of disease symptoms singly or in combination with *L. theobromae*. Although, *L. theobromae* is associated with diseased tree and also give rise to mild symptoms in inoculated plants. Actually, *L. theobromae* is an opportunistic fungus and becomes more virulent in combination with others fungi especially *C. fimbriata* (Al Adawi *et al*., 2006). The fungus, *C. fimbriata*, is the more virulent in development of disease symptoms as it has also caused similar disease on mango in Brazil (Ribeiro,

1980) and Oman (Al Adawi *et al*., 2006) leading to ultimate tree death. This conclusion is also based on the consistent isolation of these entities from the stem of diseased trees as well as from the bark beetle, *Hypocryphalus mangiferae* on infected mango trees. These fungi might contribute to the symptoms development of Sudden death of mango which includes bark splitting, rotting of stems, vascular discoloration and gummosis. This disease symptom has also been reported from different mango growing areas of the world (Ploetz *et al*., 1996). The mango trees were badly affected by this disease up to 30-40% in India and 60% tree mortality in Oman (Prakash & Srivastava, 1987; Al Adawi *et al*., 2006).

During the study, different isolation techniques were used to isolate fungi from the bark beetles like Potato Dextrose Agar amended with the antibiotics streptomycin (SPDA) and carrot discs technique (Al Adawi *et al*., 2006). The fungi, *C. fimbriata*, *L. theobromae* and *Phomopsis* sp., were consistently isolated through the SPDA method which is also used in isolation of fungi from mango stem by Al Adwai *et al*., (2006). The carrot disc techniques has significantly higher frequency of *C. fimbriata* growth which is modified by soaking the carrot discs in streptomycin and wrapped with Parafilm to reduce contamination (Moller & DeVay, 1968). In Brazil, similar results were obtained by Ribeiro (1980) but the maximum isolation frequency of *C. fimbriata* from the *H. mangiferae* beetles by carrot method was of 1%. The species composition of fungi associated with the bark beetle has also been studied in the Raciborskie forests in Poland. The most important fungus isolated from overwintered adults, larvae, new adults and from galleries at various stages of development, was *Ceratocystis laricicola* (Jankowiak *et al*., 2007). Larvae of *H. mangiferae* could be grown on axenic cultures of *C. fimbriata* (Abrahão & Wegmuller, 1969). This study also shows for the first time the association of *Phomopsis sp.,* with the sudden death of mango in Pakistan, due to its consistent isolations from collar region of diseased trees near the soil surface. *Phomopsis* sp., has been isolated from necrotic lesions in infected mango trees in Florida (Rivera-Vargas *et al*., 2006). Along with other fungi such as *L. theobromae and Fusarium aesculi* they caused one or more of the symptoms of sudden death (Ploetz *et al*., 1996). The fungi, *C. fimbriata* and *Phomopsis* sp., were successfully isolated from the main trunk rather than from branches of mango tree and the beetles were mostly confined to that portion of the trees (Wood, 1982; Webber, 1990). However, is commonly attributed to *Phomopsis* spp., an endophytic existence on various vascular plants species (Suryanarayanan *et al*., 2006). Despite the high frequency of *Phomopsis* spp., isolations from disease plants and bark beetle *H. mangiferae*, the fungi cannot to be considered causal agent of sudden death of mango in Pakistan without any pathogenic proofs.

Bark beetles are often associated with infected and trees of low vigor. The incidence of bark beetle as a wounding agent for entry of fungal spores has also been observed in Brazil and Oman (Ribeiro, 1980; Al Adawi *et al*., 2006). The root pathogenic fungi are known to be important in predisposing trees to stresses and attack by bark beetle species (Goheen & Hansen, 1993). Beetle population increases in areas of high disease incidence when combined with short-term triggering events such as drought, nutrients or salts stress (Cobb, 1989). Wainhouse *et al.,* (1998) described the tunneling mechanism of bark beetle, *Cryphalus trypanus* on *Calophyllum inophyllum* var. takamaka and serve as vector of wilt pathogen in Seychelles. The elm bark beetle, *Scolytus mutistriatus* and *S. scheveyrewi* (Coleptera: Scolytidae) has been reported as the primary vector of Dutch elm disease caused by the fungus, *Ophiostoma novo- ulmi*. These bark beetle species transmit the fungus into healthy elm trees and also isolate the same fungus from adult beetles as well as from infested tree (Jacobi *et al.*, 2007). Similarly, the fungal mutual relationship with beetle, *Xyleborus glaratus* has also caused mortality to redbay (*Persea borbonica*) in Georgia and southeastern South Carolina which produced black discoloration disease symptoms in sapwood like mango tree (Fraedrich *et al.*, 2008). The phylogenetic relationships have revealed close association between fungi (*Ceratocystis* and O*phiostoma*) and the bark beetles due to preservation of fungal spores in special sac like structure of the beetle´s mycangia (Rollin *et al*., 2001). *H. mangiferae* has also been found as a wounding agent for the penetration of *C. fimbriata*. Therefore, it may be involved in the development of blight (Seca, in Portuguese) disease of mango in Brazil (Ribeiro, 1980). The symptoms of the blight disease are very similar to those observed on diseased mango trees in Pakistan. Wainhouse *et al*., (1998) indicated that the beetles collected from the main tree trunks may carry spores of fungal pathogens. Although, bark beetle prefers diseased trees but is also capable of attacking on healthy trees due to stresses (Wood, 1982). It is plausible that the attacks of beetles on the main stem, large limbs of healthy or temporarily stressed trees could be sufficient to introduce the pathogens (Flowers *et al*., 2001).

Based on conclusions, the association of *H. mangiferae* with the diseased tree also exhibited saw dust coming out from attack sites (Roberto *et al*., 2007; Masood *et al*., 2008), may possibly act as a vector of spores of *C. fimbriata*, *L. theobromae* and *Phomopsis* sp. on mango. The rapid spread of mango quick decline across all the mango growing areas of Pakistan suggests the involvement of the disease vector, *H. mangiferae* which necessitates that its role as putative vector should be addressed for future prospective. In spite of pathogens and vector, mango trees are increasingly vulnerable to the infection due to improper irrigation, root injuries either by termites or ploughing and lack of phytosanitary measures in the orchards (Malik *et al*., 2004). Therefore, it should be necessary to develop integrated management systems for mango production to minimize the risk of and the damage through the disease.

Acknowledgements

This research was funded by Higher Education Commission (HEC) Indigenous scholarship batch III and Agriculture Sector Linkage Program (ASLP) mango project. I am also grateful for the identification of bark beetle species by MEFEIS (Museum of Entomology of the Faculdade de Engenharia de Ilha Solteira), Ilha Solteira (city), state of São Paulo, Brazil (Carlos Flechtmann Ph.D.), also by Dr. Roger A. Beaver, Chiangmai 50180, Thailand and for fungi identification to the Plant Pathology and Mycology Department, Punjab University, Lahore, Pakistan. We also appreciate the technical assistance and cooperation of M. Tariq Malik, Plant Pathologist, Mango Research Station, Shujabad, Multan-Pakistan.

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(Received for publication 26 March 2010)