Pak. J. Bot., 42(1): 409-415, 2010.

POTENTIAL OF *FUSARIUM MANGIFERAE* AS AN ETIOLOGICAL AGENT OF MANGO MALFORMATION

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Abstract

Taxonomy of the causal fungus of mango malformation (MM) disease has passed through different phases. The fungus at first named as *F. moniliforme* was elevated to species level as *F. subglutinans.* Two new species viz. *Fusarium mangiferae* and *F. sterilihyphosum* Britz. found responsible for causing MM have been characterized in South Africa in the year 2002. Presence of *F. mangiferae* in Asian clade emphasized the need to confirm the specific species in the mango orchards of Pakistan. The assay of malformed parts of mango varieties obtained from five districts of the Punjab province of Pakistan revealed the association of four fungi viz., *F. mangiferae*, *F. pallidoroseum*, *F. equiseti* and *Alternaria alternata* while *F. mangiferae* proved to be the major infecting fungus. The colonies of *F. mangiferae* were tinged with purple and rosy buff color on Potato dextrose agar (PDA) medium. Macroconidia were four celled with dorsal and ventral surfaces almost parallel. Maximum within tissue infection (40.53%) in five districts was caused by *F. mangiferae.* The present studies reveal the infectivity and dominant association of *F. mangiferae* with malformed tissues of diverse origins.

Introduction

Mango (*Mangiferae indica* L.) is a unique species in fruit trees with respect to growth, specific nature and diversity. Like other crops, it is prone to various biotic and abiotic stresses being major obstacle to mango production (Iqbal *et al*., 2008; Shahbaz *et al*., 2009). Amongst biotic problems, MM has become a crux with yield losses ranging from 80% to 100% (Ginai, 1965; Ploetz *et al*., 2002). Two types of malformation viz., vegetative and floral have been characterized with similar etiology (Schlosser, 1971a; Kumar & Beniwal, 1987a). The disease affects vegetative shoots of juvenile plants causing severe damage in nurseries. It also affects floral panicles causing deformation and hypertrophy (Kumar & Beniwal, 1992; Ploetz, 1994).

Etiologies like viral (Kausar, 1959; Das *et al*., 1989), acarological (Singh *et al*., 1961) and physiological (Sattar, 1946) were claimed previously but rejected due to lack of etiological association. Examination of *Fusarium* strains isolated from malformed inflorescences of diverse international origins has explored new taxa. *F. mangiferae* Britz., relates to strains previously identified as *F. subglutinans* and regarded responsible for causing MM disease worldwide (Britz *et al*., 2002).

Isolations from malformed parts have ever displayed the dominance of *F. mangiferae*. The presence of this fungus in several orchards in Israel was determined by PCR analysis and the pathogen was detected in the infected samples towards the length of the branches. The majority of the pathogen was observed in the grafted scions with least

instance of fungal movement below the graft union (Lahav *et al.*, 2001). The fungus *F. mangiferae* was found widely distributed in symptomatic tissues of mango obtained from diverse origins showing upto 97.0% infection (Iqbal *et al*., 2003).

Freeman *et al*., (1999, 2004) confirmed *F. mangiferae* as the etiological agent of MM by artificial inoculations. Conidia of the pathogen were reduced quickly in soil under controlled laboratory and field conditions. Natural infections were also assessed on fruitlets, fruit and seeds in severely infected orchard, one month after fruit set and at fruit maturity. In infected trees, the skin of all the fruit was 100% infected while seed and seed coat remained disease free. The pathogen could not be isolated from healthy tissue or from flesh of both healthy and diseased fruit.

The objectives of the study were to glean an insight into association of different fungi with malformed tissues of different districts, compare their infection levels, track the dominant fungus infecting the mango tissues and prove its infectivity. The results could elucidate the frequent fungus hosting mango tissues in a range of cultivars of diverse regions which is the primary cause of this malady.

Materials and Methods

Sampling: The studies to ascertain the association of different fungi with malformed parts were conducted during the flowering cycle (March-April) in the Punjab province of Pakistan. Five mango growing districts were visited with three locations in each district. The sampled orchards showed moderate to high disease severity. Five commercial varieties viz. Dusehri, Chaunsa, Langra, Anwar ratole and Malda were selected to obtain the samples. From each location, five panicles alongwith 6-8 cm shoot portion of each variety, were collected. In this way 15 samples were collected per District with a total of 75 samples. The samples were tagged and transported in ice box to ensure maximum recovery of fungi as described by Iqbal *et al*., (2003).

Tissue processing: The experiment was arranged in completely randomized design (CRD) with three replications. Ten tissue pieces (5 mm long each) cut from peduncles and panicle-shoot juncture of each sample were disinfested for 2 min in 1% NaOCl solution. The tissues were processed and placed into glass Petri plates containing PDA medium as described by Ploetz & Gregory (1993). The plates were incubated at 25ºC with a 12 h light period followed by examination after 6-7 days of incubation (Iqbal *et al*., 2008). The colonizing fungi were identified based on characteristics specific for each fungus (Ellis, 1980; Nelson *et al*., 1983; Britz *et al*., 2002). The colonies of *F. mangiferae* were purified on Carnation leaf agar (CLA) medium, specific for *Fusarium* spp., and the identification was confirmed on the basis of typical micro and macroconidia.

Pathogenicity: Three isolates of *F. mangiferae* viz., FM-21, FM-23 and FM-25 were tested for their virulence by artificial inoculations on healthy mango seedlings. The inoculum was prepared by transfer of stored culture to CLA and incubation under cool-white fluorescent light for 14 days at 25ºC to promote sporulation (Viljoen *et al*., 1997). The experiment was conducted on nine months old seedlings raised from stones sown in June-July in CRD with six replications for each isolate. The temperature in the growth chamber was maintained at 25ºC with a 12 hour alternate cycling of light and dark. The plants were sprayed with Metasystox (Bayer) and Carbendazim (Agrevo) to prevent mite and fungal infection, respectively (Das *et al*., 1989; Iqbal *et al*., 2006).

The inoculum, containing 10^5 conidia per ml of H_2O (measured using a Haemacytometer), was applied by spore spray and wounding as described by Ploetz & Gregory (1993). Inoculum was sprayed over and below the buds of shoots arising from the main stem. In case of wound inoculation, a small flap was made at the base of the apical buds followed by insertion of a 3 mm piece of agar below the flap. In control seedlings, inoculation was done with insertion of sterile CLA or spray of deionized water. Determination of post inoculation colonization by *F. mangiferae* isolates in inoculated plants was done by re-isolation from tissues distal to the inoculation site. Disease rating was done by a 1-5 rating scale for characterization of pathotypes as described by Iqbal *et al*., (2006).

Rating 1: Bud swelling for both vegetative and floral malformation
Rating 2: Eruption of multiple buds (vegetative) or short thick rachis (floral)
Rating 3: Clustered buds / shortened internodes (vegetative) or thickened peduncles (floral)
Rating 4: Small scaly leaves (vegetative) or enlarged flowers (floral)
Rating 5: Bunchy apex (vegetative) or compact deformed panicle (floral)

Results and Discussion

Microscopic assay: The examination of malformed parts of common varieties of five districts of the Punjab revealed the association of four fungi viz., *F. mangiferae*, *F. pallidoroseum*, *F. equiseti* and *Alternaria alternata.* The major infecting fungus was found as *F. mangiferae*. The colonies of *F. mangiferae* were mostly dense and tinged with dark purple or rosy buff color on undersurface of Petri dishes on PDA medium. Top of the culture showed mixed coloration. Macroconidia were slender, three-septate with dorsal and ventral surfaces almost parallel. The size of macroconidia was in the range of 4-5 x 43-58 µm. The microconidia were abundant, fusiform and oval to allantoid.

Tissue infection: Maximum within tissue infection (60.66%) by *F. mangiferae* was recorded in Khanewal district followed by 46.00 and 41.0% in Multan and T.T. Singh, respectively (Table 1). Least infection of 19.33% was observed in Muzaffargarh district. District wise frequency of other fungi remained much low. Maximum overall infection of 12.13% was noted in Khanewal followed by 10.79% in Multan. T.T. Singh, Bahawalpur and Muzaffargarh descended with 9.73, 7.59 and 3.86% infection, respectively (Table 1).

Recovery of *F. mangiferae*: *F. mangiferae* proved to be the dominant fungus infecting 40.53% of the assayed tissues and showed statistically highly significant infection levels as compared to other fungi (Table 2). Fungi viz., *F. pallidoroseum*, *A. alternata* and *F. equiseti* caused infection in 1.99, 1.06 and 0.53% tissues, respectively. Frequency of their spores was also much less during microscopic observations.

Recovery of *F. mangiferae* from different mango varieties: A perusal of the Table 3 revealed that cv. Chaunsa exhibited maximum recovery (100%) of *F. mangiferae* in Khanewal, Dusehri (76.66%) in Khanewal, Langra (46.66%) in T.T. Singh, A. ratole (60%) in Multan and T.T. Singh and Malda (66.66%) in Khanewal and Multan, respectively. Dusehri showed maximum tissue infection of 50.66% (76 of 150 tissues) followed by Chaunsa (49.33%) (74 of 150 tissues), while Langra had minimum infection % age of 32.66 (49 of 150 tissues) among 5 tested cultivars. A. ratole and Malda followed with 35.33 and 34.66% infection in the same order.

Table 1. Fungi associated with malformed tissues of mango collected from five districts of the Punjab.

Sr. No.	District	% Tissue infection				Mean
		A. alternata	*F. equiseti*	*F. mangiferae*	*F. pallidoroseum*	
1.	Bahawal Pur	0.00i	1.33hi	35.33d	1.33ghi	7.59d
2.	Khanewal	0.00i	0.00i	60.66a	0.00i	12.13a
3.	Multan	3.33fg	0.00i	46.00b	4.66f	10.79 b
4.	Muzaffargarh	0.00i	0.00i	19.33e	0.00i	3.86 e
5.	T.T. Singh	2.00gh	1.33hi	41.33c	4.00f	9.73c

Table 2. Recovery of *F. mangiferae* from malformed tissues of five districts of the Punjab.

Sr. No.	Fungus	Tissue infection (%)
1.	*F. mangiferae*	40.53a
2.	*F. pallidoroseum*	1.99b
3.	*A. alternata*	1.06c
4.	*F. equiseti*	0.53c

Table 3. Recovery of *F. mangiferae* from malformed tissues of five local cultivars.

Sr. No.	District	% Recovery					Mean
		A. Ratole	Chaunsa	Dusehri	Langra	Malda	
1.	Bahawalpur	23.33k	76.66b	40.00b	33.33i	3.33n	35.33d
2.	Khanewal	23.33k	100.00a	76.66b	36.66h	66.66c	60.66a
3.	Multan	60.00d	26.66j	50.00e	26.66j	66.66c	46.00b
4.	Muzaffargarh	10.00m	20.00l	46.66f	20.00l	0.00o	19.33e
5.	T.T. Singh	60.00d	23.33k	40.00b	46.66f	36.66h	41.33c
	Mean	35.33b	49.33a	50.66a	32.66c	34.66b	40.53

A high percentage of infection frequency of *F. mangiferae* in malformed tissues of all the common cultivars in the present study confirms the possible role of this fungus in causing MM. The diseased sections yielded typical and abundant macroconidia of the fungus on selective growth media. Cumulative within tissue infection of 40.53% (Table 2) was exhibited by the same fungus while in other fungi it ranged from 0.53 to 1.99% only. Cultivar wise recovery of *F. mangiferae* differed from 32.66 to 50.66% in five cultivars. These findings are corroborated by the recent literature (Freeman *et al*., 2004). Ploetz & Gregory (1993) isolated *F. subglutinans* [*mangiferae*] as predominant fungus from malformed Keitt panicles in three orchards. An average of 85.4% of the pedicel and peduncle tissues from malformed panicles yielded the fungus. Other fungi including *F. oxysporum*, *Botryodiplodia* sp., and *F. roseum* were observed at much lower frequencies. Extensive recovery of *F. mangiferae* as a causal agent from malformed tissues of different cultivars grown in diverse agro climatic zones of the world has been proved (Britz *et al*., 2002; Ploetz *et al*., 2002).

Although *F. pallidoroseum* exceeded *A. alternata* and *F. equiseti* in tissue infection but it is worth mentioning that it is a saprophytic fungus on mango. *A. alternata* is often found as a common contaminant. *F. equiseti* is identified in rare cases from mango (Iqbal *et al*., 2003). In the present study, it was isolated from only 0.53% tissues.

Table 4. Induction of malformation symptoms on nine months old mango seedlings by artificial inoculations.

Treatment	Type of symptoms			Disease rating			Disease incidence (%)		
	FM-21	FM-23	FM-25	FM-21	FM-23	FM-25	FM-21	FM-23	FM-25
Wound inoculation	Scale like leaves	ni	Bunchy apex	4	ni	5	66.66	0.00	83.33
Spore spray	ni[a]	ni	ni	ni	ni	Ni	0.00	0.00	0.00
Control	ni	ni	ni	ni	ni	Ni	0.00	0.00	0.00

ni[a] =not infected

Pathogenicity studies: Isolate FM-25 proved to be highly pathogenic causing 83.33% disease incidence in inoculated seedlings with 5 disease rating followed by FM-21 which showed 66.66% incidence with 4 rating (Table 4). FM-23 could not cause infection in any of the seedlings and was classified as non pathogenic. Re-isolation of the isolates from malformed tissues confirmed that the infectivity was caused by inoculated isolates. Wounding method was successful in establishing a fungal infection in mango. Initial symptoms of infection, such as bud swelling, appeared approximately three months after inoculation. Full symptoms, consisting of bunchy apex with excessive proliferation, shortened internodes and scale like leafy structures developed five to six months post-inoculation. No disease symptoms developed in control plants till the termination of the experiment.

Although some information on etiological relationship of *F. mangiferae* was previously available in the world literature but confused citations caused ambiguity regarding the cause of the disease. A novel experiment by Freeman *et al*., (1999) unequivocally confirmed the causal relationship between *F. mangiferae* and MM, fulfilling Koch's postulates for both forms of the disease. They used GUS Gene stained mycelium for inoculation of mango seedlings and flowering plants and successfully manifested symptoms of malformation.

Previously many attempts to prove pathogenicity of mango malformation in various countries of the world proved futile due to dearth of knowledge on mechanism of action of the fungus *F. mangiferae* and physiology of pathogenesis. The normal practice of pathogenicity is to spray inoculum or make a slit into which a fungal disk is placed and kept under humid conditions (Summanwar *et al*., 1966). Following infection by slit inoculation, a large amount of mangiferin is accumulated at the site of wound and kills the pathogen. So symptoms of malformation are not manifested. This is because in the old literature, *Fusarium* sp., has been negated as the causal organism of MM due to absence of typical fusarial symptoms and repeated unsuccessful attempts to reproduce disease by artificial inoculation (Chakrabarti, 1996; Ibrahim & Foad, 1981).

Mangiferin (1, 3, 6, 7, tetrahydroxyxanthone-C_2-B-D glucoside, $C_{19}H_{18}O_{11}$) is a normal vegetative growth enhancer of mango and it also acts as the defensive chemical compound of the host (Ghosal *et al*., 1977; 1979). Mangiferin prevents the pathogen to go deeper into the host cells. It stimulates the growth at low concentration but becomes inhibitory at high concentration. In the present study, a small flap was made so there was least accumulation of mangiferin. The pathogen did not find chemical resistance from the host and easily established itself in the host cells (Chakrabarti & Kumar, 1998).

Buds are potential infection sites and finding a wound or avenue caused by mites, mechanical means or environmental factors, fungus enters the tissues. A single

macroconidium which attacks the buds can cause infection. Proliferation starts and the cells of the buds are turned to malformed condition. In the beginning symptoms remain latent but with the passage of time with the massive production of macroconidia, typical symptoms of MM are produced. Symptoms are only produced by wound inoculation (Ploetz & Gregory, 1993). Attempts to produce disease by spore spray proved futile.

Conclusion

The present studies were aimed to track the potential fungus hosting the malformed tissues of mango obtained from ecological proximity or distant origins. The determination of the etiological agent will be much helpful to devise inoculum specific management strategies in future to minimize the afflictions in mango orchards caused by mango malformation. The studies reveal the dominant association of *F. mangiferae* with malformed tissues of diverse origins.

Acknowledgments

We thank Pakistan Agricultural Research Council (PARC) for providing financial assistance under ALP to conduct this study.

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(Received for publication 15 July 2009)