

## Nematode and Fungal Communities Associated With Mango Decline of Southern Punjab

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**Abstract.-** Plant parasitic nematodes and fungal pathogens associated with decline of mango (*Mangifera indica* L Family-Anacardiaceae) were assessed within southern Punjab plantations. The fungi most often isolated from symptomatic terminal branches of decline mango trees were *Alternaria alternata*, *Ceratocystis fimbriata*, *Colletotrichum gloeosporioides*, *Fusarium* spp., *Botryodiplodia theobromae*, and *Natrassia mangiferae*. Three fungal pathogens viz., *B. theobromae*, *C. fimbriata*, and *N. mangiferae* were also identified from roots, barks and vessels collected from the stem and the collar region. Incidence of *B. theobromae* was 87% of sampled trees, whereas incidence of *C. fimbriata* and *N. mangiferae* was relatively low (ca 5.5%). Plant parasitic nematodes associated with declining trees included *Criconebella sphaerocephala*, *Helicotylenchus dihystera*, *Hemicriconeoides mangiferae*, *Hoplolaimus indicus*, *Meloidogyne* spp., *Pratylenchus brachyurus*, *Rotylenchulus reniformis*, *Trichodorus* spp., *Tylenchorhynchus claytoni*, *Tylenchus filiformis* and *Xiphinema* spp. Populations of *H. mangiferae*, *Macroposthonia* spp. and *Rotylenchulus reniformis* were very high. Possible factors contributing to mango decline must include *H. mangiferae* and *R. reniformis*. Association of fungi with tissues symptomatic of mango decline in the presence of none to high population levels of plant parasitic nematodes is an area in need of further study to understand the relationship between nematodes and fungal pathogens in inducing decline syndrome in mango.

**Key words:** Fungi, mango decline syndrome, nematodes.

### INTRODUCTION

Mango, *Mangifera indica* L. Family - Anacardiaceae, is grown primarily in tropical regions of Pakistan, India, USA, Oman, China, Indonesia, Brazil, Mexico and Thailand. Production from these orchards stood at 26,786,757 MTs in 2008 with Pakistan producing 5%, India 50%, China 15%, Indonesia 7%, Brazil 4%, China 5%, Mexico 9% and Thailand 5% (FAO, 2008). The world mango production area was about 3,500,753 ha in 2008 with 61% located in India and 5% in Pakistan (FAO 2008). In 2008 Pakistan orchards had climbed to 1, 66,223 ha over that of 88272 ha in 1995 but the production stood at the same level (FAO, 1995, 2008).

A major factor impacting vitality and yield of mango is a decline syndrome recognized in virtually all mango-producing regions of the world. *Botryosphaeria ribis* was the first pathogen to be

reported as a primary cause of mango decline (Ramos *et al.*, 1991). Although fungi are the implicated incitants in many locations, abiotic stresses including host nutritional deficiencies are thought to play a role (Schaffer *et al.*, 1988). It is further speculated that these deficiencies may predispose trees to infection by fungal pathogens including *B. ribis* and *Physalospora* sp. which attack shoots.

Many additional fungi have been associated with symptomatic tissues exhibiting bud necrosis, tip die-back, gummosis and vascular discoloration including: *Alternaria alternata*, *Cladosporium* sp., *Colletotrichum gloeosporioides*, *Dothiorella dominicana*, *Fusarium* spp., *Lasiodiplodia theobromae*, *Penicillium* sp., *Pestalotiopsis* sp. and *Phomopsis* spp. (Ploetz *et al.*, 1996). Jiskani (2002) reported that fungi such as *B. theobromae*, *Alternaria*, *Acremonium*, *Scytalidium*, *Fusarium* and *Ceratocystis* stained both the surface and the deeper wood of mango. Sharma (1993) established *B. theobromae* as a primary cause of die back of mango and *C. gloeosporioides* (*Glomerella cingulata*), *Pestalotia mangiferae*, *Phoma* sp.,

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*Sclerotium (Corticium) rolfsii*, *Rhizoctonia solani*, *Diplodia* sp. and *F. solani* were also reported pathogenic. One year later Narasimhudu and Reddy (1992) isolated *B. theobromae* from mango trees severely affected by gummosis and confirmed its pathogenicity. Recently *Ceratocystis fimbriata* has been isolated from vascular bundles of declining trees and is considered to be one of the contributing factors of mango decline (Fateh *et al.*, 2006; Wyk *et al.*, 2005).

Nematodes have also been associated with presence of mango decline. McSorley *et al.* (1981) consistently found high populations of the nematode *Hemicriconemoides mangiferae* in soils around old declining mango trees but few numbers of this nematode were counted on younger trees. Two nematode genera, *H. mangiferae* and *Xiphinema brevicolle* have been reported from declining mango trees in South Africa where these two nematodes caused decline of lychee, *Litchi chinensis* (Milne *et al.*, 1971, 1975). McSorley *et al.* (1980) also found *H. mangiferae* and *Rotylenchulus reniformis* on declining mango trees in southern Florida. Nematodes including *Criconebella sphaerocephala*, *Helicotylenchus dihystra*, *Hoplolaimus indicus*, *H. mangiferae*, *Meloidogyne* spp., *Pratylenchus brachyurus*, *R. reniformis*, *Trichodorus* spp, *Tylenchus filiformis* and *Tylenchorhynchus mashoodi* have also been found associated with mango decline trees (Siddiqui, 2007). Among these nematodes *H. mangiferae* was known to be pathogenic to mango trees and its infestation induced a slow decline of mango (McSorley *et al.*, 1980; McSorley and Parrado, 1982; Sauer, 1981).

On the basis of the work done by above researchers it is concluded that mango decline is a disease complex involving several different fungi (Jiskani, 2002; Ploetz *et al.*, 1996), nematodes (McSorley *et al.*, 1980; McSorley and Parrado, 1982; Sauer, 1981) and uptake of soil nutrients (Schaffer *et al.*, 1988). Mango decline is a prevalent disorder that has been affecting mango trees in southern Punjab, Pakistan. The association of different fungi with mango decline has been reported from Sind, Pakistan (Jiskani, 2002; Fateh *et al.*, 2006) but there are absolutely no data on fungal and nematode interactions associated with mango decline in southern Punjab, Pakistan. This study was

planned to simultaneously investigate fungi and nematodes associated with mango decline in the most important production areas of southern Punjab.

## MATERIALS AND METHODS

### *Disease assessment*

During 2008-10, a survey was conducted to assess the distribution of mango decline in 17-mango orchards located in four tehsils within the Rahim Yar Khan region of southern Punjab. The appearance or nonappearance of decline was recorded for 250 mango trees. The severity of decline foliage symptoms was sorted on a 1 to 5 scale, where 1 is healthy trees, free of disease; 2 is trees with marginal leaf necrosis; 3 is trees with bare dead terminal twigs retained for a long time; 4 is trees with bare decayed terminal twigs and bark gummosis; and 5 is trees entirely dead also exhibiting bark gummosis.

### *Isolation and identification of pathogens*

#### *Fungal pathogens*

Plant tissues include roots, stems, branches, and bark from trees displaying disease symptoms were collected, surface-sterilized with 1% sodium hypochlorite for 3 min and rinsed three times with sterile distilled water. Branches and roots exhibiting vascular discoloration were incubated in mist chambers. Bark was removed and sections from the vascular tissue were dissected with a sterile scalpel and placed on potato dextrose agar. Streptomycin sulfate was added to the medium to prevent bacterial growth. These mist chambers / Petri plates were incubated at 24±2°C for seven days for the growth of fungi. The fungi isolated were identified with the help of keys (Booth, 1977; Neergaard, 1979).

#### *Plant parasitic nematodes*

During 2008-10, 17-mango-orchards of varying ages (20 to 40 years) located in four tehsils of Rahim Yar Khan were sampled for plant-parasitic nematodes. Six mango cultivars evaluated were Longra, Dosari, Summer Beshat, Chonsa, Fajri and Anwar Retol. The surface 15 to 25 cm of these soils averaged 60% to 80.5% sand, 5.5% to 21.5% silt and 10.7% to 22.7% clay with 0.7% to 3.25% organic matter.

The sampling plan involved collection of nematodes from both soil and root samples for each mango decline severity category. The number of trees among categories varied from orchard to orchard. One composite sample of fifteen cores for each tree-category from an individual orchard was also collected. Each sample consisted of soil and roots collected with a hand trowel from three locations around each tree. A total of 85 samples were collected from 17 mango orchards during this investigation.

Roots were separated from soil by careful washing under tap water to remove adhering soil particles, then towel dried. Nematodes were extracted from 20g of smallest roots after placement in a mist-chamber for 3 days (McKenry and Roberts, 1985). A composite soil sample of 100 ml<sup>3</sup> was mixed with water in a bucket, hand stirred and nematodes sieved through #40 mesh over #325 mesh sieves. Debris collected on the 325 mesh sieve was then washed onto a modified Baermann funnel apparatus and allowed to settle for 3-days. During this period, nematodes that had migrated and settled were collected and examined in a dish under a stereo-binocular microscope at  $\times 40$ .

Plant parasitic nematodes were identified to genus and species using a compound microscope. Nematode identifications were based on the morphology of adult and second stage juveniles with the help of taxonomic keys (Handoo, 2000; Mai *et al.*, 1996; Sher, 1966).

## RESULTS

### *Severity of mango decline*

The number of mango cultivars and number of trees assigned for each mango production and disease rating are listed in Table I. The lowest mean disease rating was found in mango orchards located at Sadiqabad while highest ratings were found in mango orchards of Liaquatpur. Two other locations, Rahim Yar Khan and Khanpur, displayed an intermediate disease rating.

### *Fungi isolated*

A range of fungal pathogens was recovered from stringently disinfected symptomatic plant tissues collected from terminal branches of six

commercial mango cultivars (Table II). Isolated fungi included *A. alternata* (FR.: FR) Keissl.; *C. gloeosporides* (Penz) Penz. & Sacc. in Penz. (teleomirph; *Botryodiplodia theobromae* (Moug.:Fr.) Ces. & DeNot.); *Ceratocystis fimbriata*, Ellis & Halst; *Fusarium* sp. and *Nattrassia mangiferae* Syd. B. Syd.) B. Button & Dyko. No attempt was made to identify the species of *Fusarium*. Substantial variation existed among the mango cultivars for the various fungi found associated with diseased tissues (Table II). *Botryodiplodia theobromae*, *C. fimbriata*, and *N. mangiferae* have been reported to be involved in mango decline syndrome and in our survey were isolated from bark and vessels of symptomatic tissues from stem and collar regions as well as from roots (Table III). Propagules of *B. theobromae* were found highest on tissues taken from stem and collar regions where least propagules of *C. fimbriata* were found. Propagules of *N. mangiferae* were intermediate in abundance at this sampling site. Propagules of *B. theobromae* were also more abundant on roots compared to propagules of *N. mangiferae* and *C. fimbriata* (Table III).

**Table I-** Severity of decline in seventeen mango orchards from four production areas of southern Punjab

Locations	No. of trees	No. of cultivars	Mean disease rating*
Rahim Yar Khan	80	6	2.50±0.15
Sadiqabad	50	5	1.80±0.23
Khanpur	45	6	2.75±0.11
Liaquatpur	75	4	2.90±0.24

\*Disease rating was 1 to 5 scale, where 1 is healthy trees, free of disease; 2 is trees with marginal leaf necrosis; 3 is trees with bare dead terminal twigs retained for a long time; 4 is trees with bare decayed terminal twigs and bark gummosis; and 5 is trees entirely dead also exhibiting bark gummosis.

### *Nematodes isolated from mango decline trees*

Eleven genera of plant parasitic nematodes were isolated and identified during this investigation including: *C. sphaerocephala*, *H. dihystra*, *H. mangiferae*, *H. indicus*, *Meloidogyne* spp., *P. brachyurus*, *R. reniformis*, *Trichodorus* spp., *T. claytoni*, *T. filiformis* and *Xiphinema* spp. (Table IV). *Rotylenchulus reniformis* and *H. mangiferae*

**Table II.- Fungi recovered from symptomatic terminal branches of six mango cultivars sampled from seventeen orchards during a survey of southern Punjab.**

Cultivars	Trees sampled*	Samples per tree**	Incidence of recovery (%)***					
			Aa	Cg	F	Bt	Cf	Nm
Longra	12	4	0	23	15	12	6	2
Dosari	5	7	1	5	12	2	8	5
Summer Beshat	6	8	0	12	25	4	7	6
Chonsa	5	5	2	15	31	15	23	7
Fajri	5	9	3	27	42	17	13	12
Anwar Retol	7	3	2	25	18	11	17	14

\*Variable numbers of declined trees were sampled; \*\*Variable numbers of symptomatic tissues were collected.

\*\*\*Fungi were Aa, *Alternaria alternata*, Cg, *Colletotrichum gloeosporoides*, F, *Fusarium* sp., Bt, *Botryodiplodia theobromae*, Cf, *Ceratocystis fimbriata*; Nm, *Natrassia mangiferae*.

**Table III.- Frequency of fungal pathogens recovered from plant parts symptomatic of mango decline.**

Fungal pathogens recovered	Stem		Collar Region		Roots
	Bark	Vessels	Bark	Vessels	
<i>Botryodiplodia theobromae</i>	77.5	66.25	72.25	75.00	56.25
<i>Natrassia mangiferae</i>	20.00	30.00	18.75	18.75	20.00
<i>Ceratocystis fimbriata</i>	2.50	3.75	8.75	6.25	23.75

Frequency, number of infected samples / total number of samples  $\times$  100

**Table IV.- Frequency and density of plant parasitic nematodes associated with mango trees in southern Punjab.**

Nematodes	Nematode population levels								
	All trees*			Healthy trees**			Decline trees***		
	Frequency (%)	Roots 20 g	Soil 100 ml <sup>3</sup>	Frequency (%)	Roots 20 g	Soil 100 ml <sup>3</sup>	Frequency (%)	Roots 20 g	Soil 100 ml <sup>3</sup>
<i>Criconebella sphaerocephala</i>	49.2		22.0	3.0		5.0	5.0		7.0
<i>Helicotylenchus dihystra</i>	61.6		43.0	57.0		56.0	78.0		61.0
<i>Hemicriconemoides mangiferae</i>	81.5		91.7	51.0		91.0	87.0		89.0
<i>Hoplolaimus indicus</i>	1.5		2.0	4.1		2.0	0.0		0.0
<i>Meloidogyne</i> spp	8.0	5.0	35.0	12.0	7.0	21.0	15.0	9.6	33.7
<i>Pratylenchus brachyurus</i>	4.5	5.8	10.0	7.8	3.7	10.0	1.5	12.0	9.6
<i>Rotylenchulus reniformis</i>	67.2		121.0	98.0	19.0	101	84.2		151.9
<i>Trichodorus</i> spp.	9.0		5.0	17.0		11.0	13.0		9.0
<i>Tylenchorhynchus claytoni</i>	12.0		7.0	15.0		25.0	17.0		28.0
<i>Tylenchus filiformis</i>	34.0		23.0	34.0		43.0	12.0		22.0
<i>Xiphinema</i> spp.	17.8		2.0	7.9		1.5	23.4		4.9

\*mean of 250 samples collected from 17 mango orchards; \*\*mean of 85 samples; \*\*\*mean of 165 samples.

were found most frequent in samples and their population was the highest. *Hemicriconemoides mangiferae* was found more frequently in samples collected from declining rather than healthy mango trees. Occurrence of *H. dihystra*, *Meloidogyne* spp., *Trichodorus* spp., *T. claytoni* and *Xiphinema* spp. was more frequent and at higher numbers in samples collected from declining mango trees than from healthy trees. Both healthy and declining trees

contained *H. indicus*, *Meloidogyne* spp., *P. brachyurus*, *Trichodorus* spp., *T. claytoni* and *T. filiformis* less frequently and at population levels that were relatively low.

Frequencies of finding *C. sphaerocephala*, *H. dihystra*, *H. mangiferae*, and *R. reniformis* were also higher over that of *H. indicus*, *Meloidogyne* spp., *P. brachyurus*, *Trichodorus* spp., *T. claytoni*, *T. filiformis* and *Xiphinema* spp. on all the trees

regardless of tree health. *Hemicriconemoides mangiferae* was very abundant with 15% of the samples having a population greater than 175 / ml<sup>3</sup>.

## DISCUSSION

Mango decline syndrome displays diverse symptoms including dieback of terminal shoots with or without accompanying defoliation, gummosis on branches, and scaffold limbs, vascular discoloration, marginal chlorosis and necrosis of leaves, foliar deficiencies and root degeneration (Ploetz *et al.*, 1996; Schaffer, 1994). Several different organisms have been found contributing to mango decline syndrome. Various pathogenic fungi have been indicated as cause of decline symptoms (Alvarez-Garcfa and Lopeez-Garcfa, 1971; Ramos *et al.*, 1991). Ring nematode, *H. mangiferae* has been consistently associated with mango decline (McSorley *et al.*, 1980, 1981, 1982). An association of host nutrition with mango decline has been narrowed to manganese and iron as limiting elements in affected trees (Schaffer *et al.*, 1988). Association of different fungi with mango decline has been reported from Sind, Pakistan, Florida, USA and India (Fateh *et al.*, 2006; Ploetz *et al.*, 1996; Verma and Singh, 1970). Associations with nematodes have been reported from Florida and South Africa (McSorley *et al.*, 1980, 1981, 1982; Siddiqi, 2007) where they isolated either fungi or nematodes only from mango trees exhibiting mango decline symptoms (McSorley *et al.*, 1980, 1981, 1982; Ploetz *et al.*, 1996). Prior to this study no attempt had been made to investigate both fungi and nematodes simultaneously from the same declined trees. The primary objective of this study was to investigate the association of fungal pathogens with declining symptomatic terminal branches, roots, and stems, while also including nematode data from roots and soil.

Six fungi including *A. alternata*, *C. gloeosporides*, *Fusarium* sp. *B. theobromae*, *C. fimbriata* and *N. mangiferae* have been identified from terminal branches of mango trees exhibiting decline symptoms. These findings are in line with that of Ploetz *et al.* (1996) and Ramos *et al.* (1991). These fungal pathogens vary in virulence and have been found inducing symptoms of mango decline

thus contributing to mango decline syndrome (Ploetz *et al.*, 1996). Substantial variation existed among cultivars for the various fungi isolated (Table II). Ploetz (2004) observed frequent association of *A. alternata*, *C. fimbriata*, *C. gloeosporioides* and *Fusarium* spp. with mango trees showing sudden death disease symptoms. *Colletotrichum gloeosporioides* contributes to mango decline by inducing flower blight, fruit rot, and leaf spots (Arauz, 2000). Meanwhile, *B. theobromae* is an agent of die-back and bark canker (Shah *et al.*, 1991). *Fusarium* species particularly *F. mangiferae* is blamed for causing misshapen growth of both vegetative and productive parts of the mango trees leading to great yield losses (Kumar and Beniwal, 1992, 1993). Trees infected by *C. fimbriata* exhibit wilting symptoms as vascular tissues become discolored and has been isolated from decline trees in Oman, Pakistan and Florida (Al-Adawi *et al.*, 2006; Fateh, *et al.*, 2006; Ploetz *et al.*, 1996).

*Botryodiplodia theobromae* Pat., (synonyms: *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.) can be an important pathogen that induces decline symptoms on mango. It has induced a serious dieback disease of mango in India (Verma and Singh, 1970), in Salvador as well as in Egypt (Acuna and Waite, 1977). It was found associated with trunk canker disease of mango in Indonesia (Muller, 1940) and Malaysia (Lim and Khoo, 1985) and also caused gummosis and dieback of mango in Puerto Rico (Alvarez-Garcfa and Lopeez-Garcfa, 1971).

Mango decline in the production area of southern Punjab exhibited a wide variety of symptoms including foliage chlorosis and necrosis, bare dead terminals, bark gummosis and dead trees. Rating scales to assess disease severity have been proposed depending upon the condition of trees and the location of that damage (Asad *et al.*, 2010; Ramos *et al.*, 1997). We used a scale from 1 to 5 to assess the severity of decline symptoms in twigs and branches of individual trees. The primary root systems of these trees had been well-developed, but few secondary roots were present, which might be due to feeding of nematodes (McSorley *et al.*, 1980, 1981, 1982; Sauer, 1981).

Several different factors have been found to predispose mango to fungal diseases.

*Botryodiplodia theobromae*, usually a weak pathogen, was found damaging to mango trees after lengthy periods of drought exposure (Acuna and Waite, 1977). This pathogen also damaged mango trees that had been sun scorched in Puerto Rico (Alvarez-Garcfa and Lopeez-Garcfa, 1971) while in Indonesia sun scorch, tar, and tangle-foot all predisposed mango trunks to canker by *B. theobromae* (Muller, 1940).

Mango decline syndromes have been reported on other tree plantations including apple, citrus, and peach (Brown and Britton, 1986; Brown and Hendrix, 1981). The associated fungi are not aggressive pathogens, and the hosts only develop symptoms if they have been affected by cold temperatures, droughts, nutrient deficiencies (Schaffer *et al.*, 1988), or mechanical injury by nematodes (McSorley *et al.*, 1980, 1981, 1982; McKenry *et al.*, 1989). These stresses and other factors may be responsible for mango decline in the southern Punjab.

The simultaneous occurrence of fungal pathogens and high density of ring nematode, *H. mangiferae* with declined mango trees suggests that this nematode predisposed the plants to root week fungal pathogens by inducing physiological changes. For example, predisposition of *Prunus* spp. by ring nematodes in sandy soil can lead to bacterial canker disease caused by *Pseudomonas syringae* (Nyczepir, 1990) which has been related to lower carbohydrate concentrations in roots and scions of nematode infested trees (Olien *et al.*, 1995).

*Meloidogyne* spp. were rarely found and low in numbers although they occur widely on many vegetable crops grown in this area (Anwar *et al.*, 2007). Several other species associated with mango including *C. sphaerocephala*, *H. dihystra*, *H. indicus*, and *R. reniformis* have broad host ranges. *Rotylenchulus reniformis* has been reported in abundance on atemoya, *Myrciaria floribunda*, *Averrhoa carambola*, and *Macadamia integrifolia* in Florida (McSorley *et al.*, 1982). *Criconemella sphaerocephala* and *Xiphinema* spp. were not found on vegetable crops in southern Punjab, but they exhibit wide distribution on many tropical fruits in Pakistan and Florida (Anwar and Chaudhary, 1975; McSorley *et al.*, 1982).

Our frequent association of *H. mangiferae* at

high population levels among declining trees is of particular interest. Severity of mango decline has previously been found significantly correlated with density of *H. mangiferae* (McSorley *et al.*, 1981). It has also been reported to damage both lychee and mango in South Africa (Milne *et al.*, 1971, 1975) as well as mangoes in Florida (McSorley *et al.*, 1980, 1981). This potentially serious parasite has also been reported on longan, lychee, and soursop (McSorley *et al.*, 1982). Other Criconematidae are known to be involved with predisposition of *Prunus* spp. to highly damaging above-ground bacterial infections (McKenry *et al.*, 1989; Nyczepir, 1990) leading to short life of infected plants over that of non-infected ones (Nyczepir *et al.*, 1983).

Future studies are needed to determine the nematode population levels of *H. mangiferae* which are damaging to the mango cultivar alone or in combination with other biotic factors. Association of fungi with tissues symptomatic of mango decline in the presence of none to high population levels of plant parasitic nematodes is an area in need of further study to understand the relationship between nematode and fungal pathogens in inducing decline syndrome in mango.

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