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Mango sudden decline: An emerging threat in nursery and mango orchards of Andhra Pradesh

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Abstract
Mango sudden Decline (MSD) was observed with clear signs for the first time in the mango orchard and nurseries in the state of Andhra Pradesh. Symptoms were misdiagnosed to dieback and were different in peculiar with gum exudations, splitting of bark, beetle entry holes, vascular discolouration, wilting and rapid death. Morphological and microscopic characterization revealed the presence of *Ceratocystis fimbriata* and *Lasiodiplodia theobromae* from the infected bark and twigs. The isolates showed strong pathogenicity on mango cultivar, totapuri fulfilling Koch's postulates. In addition, an association of pathogenic fungi with mango sudden decline and the bark beetle, *Hypocryphalus mangiferae* was supported with the evidence of signs and their movement in mango seedlings at nurseries. Furthermore this is the first report of MSD caused by *Ceratocystis fimbriata* and *Lasiodiplodia theobromae* complex proved to be an emerging threat to mango production in the nursery for grafts production in India.

Keywords: Mango; ceratocystis; lasiodiplodia; dieback; gummosis; India; scolytid beetle

Introduction
Mango (*Mangifera indica* L.) is one of the choicest fruit crops of tropical and sub-tropical regions of the world. India is the largest producer and home of more than 1000 varieties of mangoes being cultivated in the states of Andhra Pradesh, Uttar Pradesh, Bihar, Karnataka, Tamil Nadu, West Bengal, Orissa and Maharashtra with a production of 187.79 lakh tones contributing to 46 percent of total fruit production [6]. There are nearly about 30 varieties grown commercially for export from South India especially in Andhra Pradesh which includes totapuri, Alphonso, Baneshan, Neelum, Rasalu and Swarnarekha amongst totapuri alone contribute 27% of the total's mango export value [4]. Despite of all this, mango production in Andhra Pradesh (AP) has been hit by a new disease called Mango Sudden Decline (MSD) which has been reported elsewhere in India named mango wilt but outside of Andhra Pradesh. The disease was reported but not described clearly. It is a complicated disease with peculiar symptoms characterized by tip die back, bark splitting, beetle entry holes, tunnels, amber coloured gum exudation, vascular discoloration of the woody tissues and wilting on one side of the tree followed by death of the entire tree. The principal pathogen in many of the countries has been identified as *Lasiodiplodia theobromae* [7, 16, 1]. Various authors have isolated and reported C*eratocystis fimbriata sensulato* as the first plant pathogen associated with mango sudden decline in Brazil, Oman, Pakistan and China [1, 27, 18, 31]. A mango bark beetle, *Hypocryphalus mangiferae* has also been implicated as a vector of the mango sudden decline in Pakistan [27, 18]. A single newsletter has been published from CISH, Lucknow but there is very less data and information of the pathogen complex. Moreover, no record of *Ceratocystis fimbriata* on mango in AP till date and only reported for its host range on *Hevea*, *Populus* and *Punica* [11, 28, 29]. Since lustrum, it has emerged as a new threat of extinction, as mango trees are becoming victim at an alarming rate especially in the orchards of India, Brazil, Oman and Pakistan. Nursery diseases are an important consideration as healthy grafts are prime need for establishment of good mango orchard. It has become a worrying threat since young and matured mango trees are dying at an alarming rate [20]. Mostly the *Ceratocystis* genera are weak pathogens vectored by scolytid beetles [22]. Recently transmission of *C. fimbriata* by bark beetles, *Hypocryphalus mangiferae* Stebbing, has been proved as a vector of mango sudden decline in mango orchards [1, 19]. Hence a study on record of vectors is also remained of prime importance at nursery level diagnosis. To the best of our knowledge, this is the first report of signs of MSD in AP and finding the source of infection caused by the complex of *Ceratocystis fimbriata* Ellis and Halst and *Lasiodiplodia theobromae* (Pat.)

Griffon & Maubl at mango orchards.

Materials and Methods

Unexpectedly during a diagnostic visit of mango orchard at Bangarupalem, Chittoor district in Andhra Pradesh, "Totapuri" cultivar of three year old plants cultivated in black calcareous soils showed signs of bark splitting, gum exudation, beetle tunnelling, galleries of beetle vector and vascular discoloration prior to complete dieback which resembled to the mango disease "Seca", in Brazil, Mango sudden decline and Mango Sudden death syndrome in Pakistan and Oman, respectively. Infected samples were collected by removing the bark and cutting strips longitudinal from the field and kept for pathogen isolation at Department of Horticultural Plant Pathology, College of Horticulture, Anantharajupeta, Dr YSR Horticultural University (Dr YSRHU), AP.

Fungal isolation and purification

Fungal isolation was carried by tissue isolation method. Longitudinal cross-sections were made from diseased plant twigs and the pathogen was isolated from affected tissues (Fig. 2). Tissues were cut into pieces of 2–5 mm, washed and surface-sterilized with 1% sodium hypochlorite for 1 min, followed by three consecutive washings in sterile distilled water. Tissues were then transferred onto PDA (Himedia Laboratories Pvt Ltd., Mumbai, India) plates of pH 6.0, supplemented with streptomycin (Himedia Laboratories Pvt Ltd., Mumbai, India) at a rate of 25 mg/L of the growth medium in order to inhibit the bacterial contaminants. Petri dishes were incubated at 28 ±2 ^{o}C for three days and the mycelia growing out of the plated tissue was aseptically sub-cultured on to fresh PDA and lastly purified by using hyphal-tip isolation technique [14].

Morphological and microscopic characterization

Cultural and microscopic characters were studied for the isolates retrieved from the infected mango trees. The microscopic observations were done under Olympus CX31, Binocular light microscope (Olympus Opto Systems India Pvt. Ltd., UP, India) to characterize different fungal structures. After the preliminary identification, cultures were sent to Indian Type Culture Collection, New Delhi for authoritative confirmation and deposition of pathogens.

Pathogenicity Test

An experiment was conducted to evaluate symptoms on 2 year old mango grafts and Koch postulates were followed to test the pathogenicity of isolates. Furthermore, stem inoculation was done by placing a 5mm disc of cultured isolates separately at cut end of the stem wrapped with para film in triplicates. Control is maintained by placing an uncultured 5mm PDA plug wrapped with para film. However to know the source of infection, "Railway Koduru" in YSR Kadapa District called as valley of fruit farms, surrounded by huge number of mango nurseries were also surveyed for the presence of MSD symptoms and the bark beetle vectors.

Results

A diagnostic visit to Bangarupalem village in Chittoor district showed clear symptoms of MSD in 'totapuri' cultivar of bark splitting, amber coloured gum exudation, beetle tunnelling, beetle entry holes and vascular discolouration prior to complete dieback of the tree (Fig 1A-G). The percent disease incidence (PDI) was severe by 30.0 per cent in the main field and pathogens were isolated from the infected samples by tissue isolation technique identified as *Ceratocystis fimbriata* Ellis and Halst and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl based on their morphological and microscopic characters (Fig. 2). Colonial and microscopic images of *Lasiodiplodia theobromae* demonstrated that the pathogen is a vigorous producer of septate mycelia (Fig. 2a, b, c) and immature hyaline conidia on PDA (Fig. 2g, h). Dark coloured sporulation was not prominent at early stages due to alteration in temperatures. But when the culture plates were incubated at 40^{o}C under dark conditions started to produce pycnidia with dark coloured, single septate two celled conidia with londitudinal striations confirming the pathogen *Lasiodiplodia theobromae* (Fig. 2j, k, i, l). Another destructive threat pathogen, *Ceratocystis fimbriata* was also isolated and the colonial and microscopical morphology revealed its existence and association with the bark beetle as vectors. The frass of beetles was observed under microscope for the association of fungus which made a remarkable presence of conidia, mycelium and perithecia of the fungus. Cultures identification was done based on the morphological and conidial characteristics and identified cultures were deposited at Indian Type Culture Collection, New Delhi. To prove the microbial pathogenicity of the disease by verifying the pathogen and its progression in plants, mango grafts were inoculated with the isolated pathogens (unshown/unpublished data). The results of inoculation on tissues were similar to the disease symptoms in the field and the re-isolation of the pathogen from the inoculated plants confirmed Koch's postulates. The control grafts remained symptomless totally over the incubation period. However to know the primary source of infection, Railway Koduru area was surveyed for the presence of MSD symptoms and bark beetles (Fig. 3a-e). Undoubtedly approved by the most evident symptoms of MSD i.e., black streaks, cankers observed by scratching the bark from collar portion of tree up to main stem, bark splitting, amber coloured gum exudations and beetle entry holes in the cultivars such as Baneshan, ImamPasand, Alphonso, Neelum and Totapuri with moving presence of bark beetles as vector with in the galleries forming tunnels (Fig 4a-f). This made us clear with the source of emerging threat from the mango nurseries producing grafts infected with MSD which is then carried over to the main fields. Moreover clear management trials needed to be standardised to control the emerging threat.

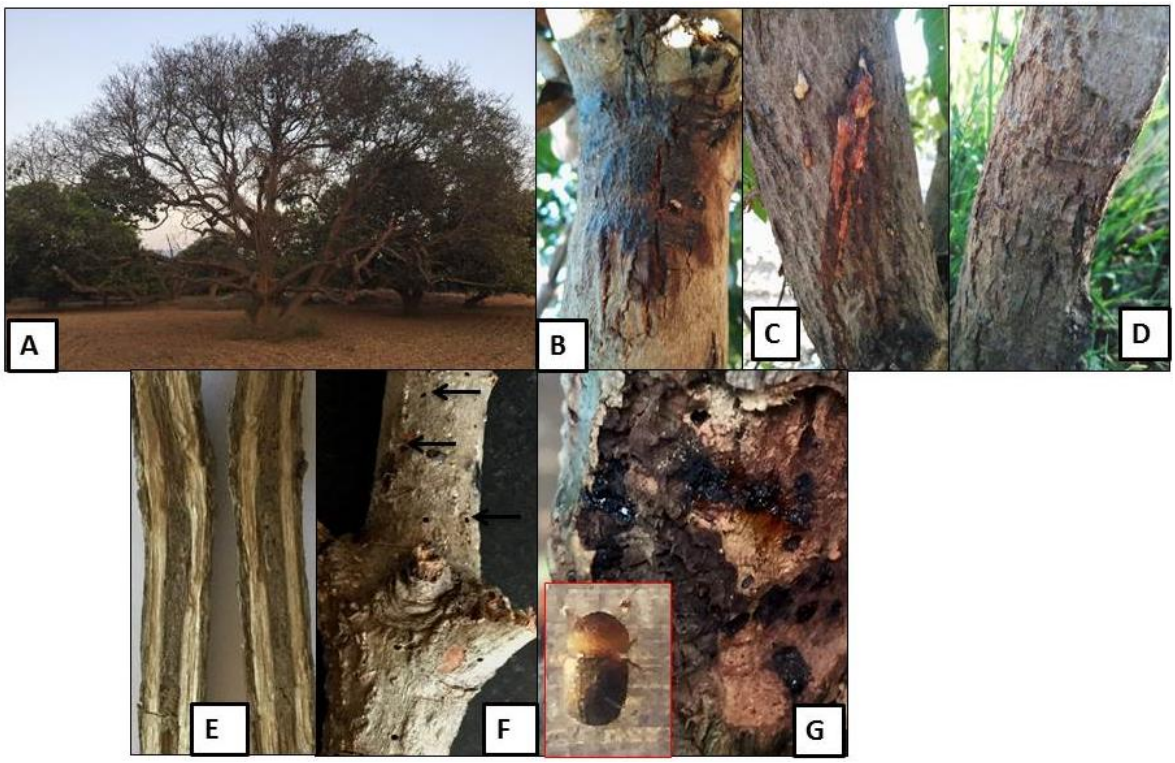

Fig 1: Symptoms of MSD at main field in mango variety, totapuri

A). Dieback of main tree B). Bark splitting C). Gum exudations D). Black Streaks E). Vascular Discoloration F). Beetle entry holes G). Beetle tunnels
Arrows indicate entry holes of beetles
Inset shows the Scolytid beetle, *Hypocryphalus mangiferae*

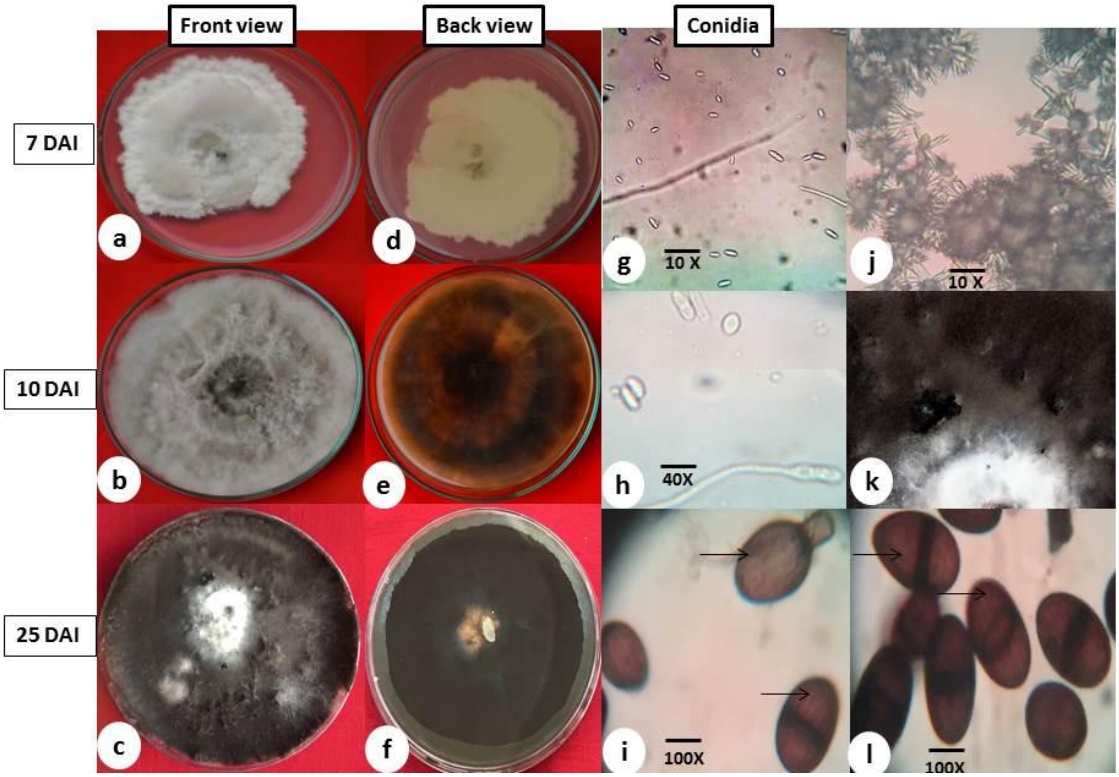

Fig 2: Morphological and microscopic characters of *Lasiodiplodia theobromae* isolates retrived from mango trees g, h Hyaline bicelled geminating conidia at 7, 10 DAI; i, l Coloured geminating bi celled conidia at 25 DAI; j prepycnidial stage; k pycnidia on culture dish; Arrows indicate striations on conidia of *Lasiodiplodia theobromae*

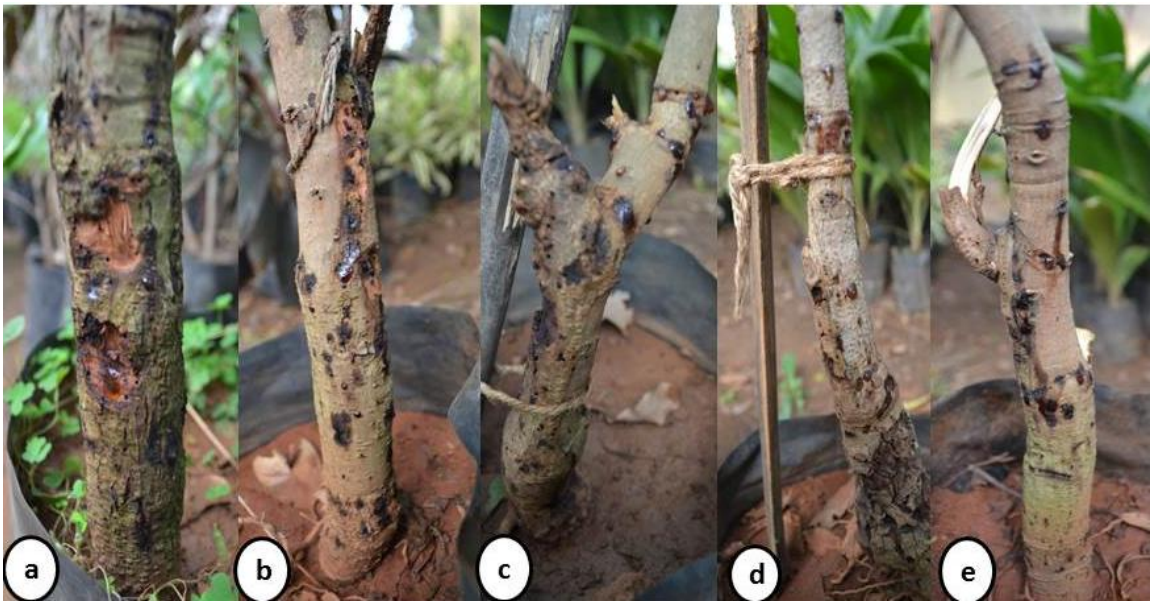

Fig 3: MSD symptoms at nursery in mango varieties

a). Baneshan b). Imam Pasand c). Alphonso d). Neelum e). Totapuri

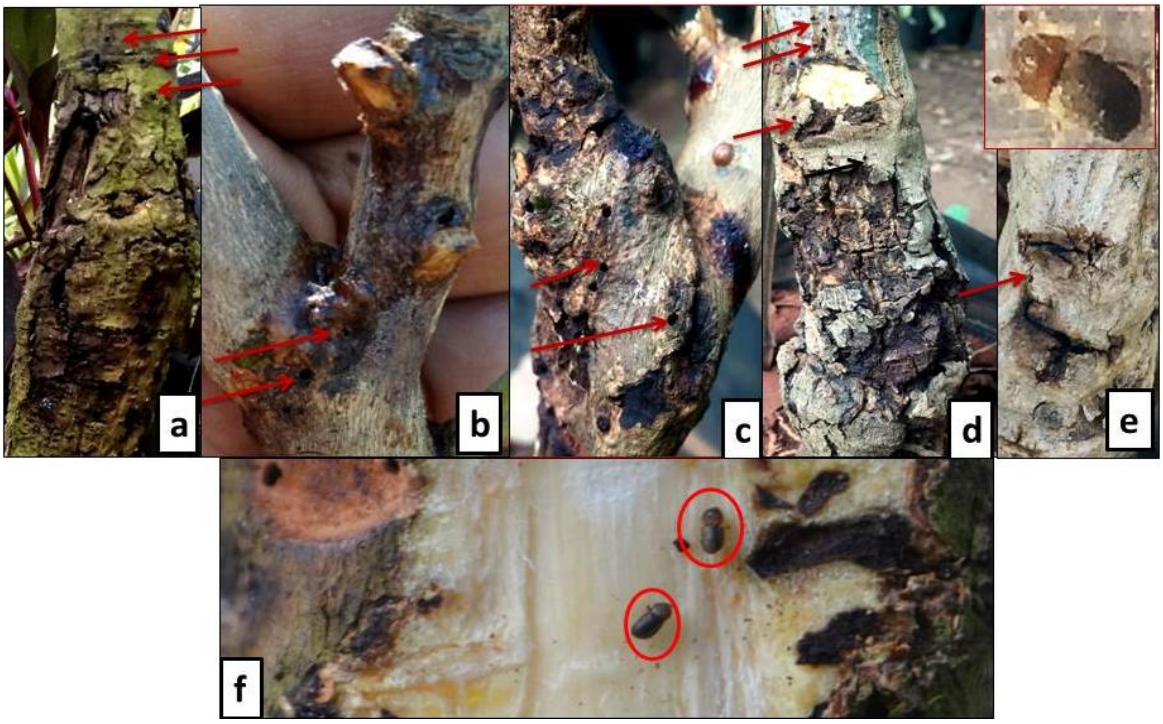

Fig 4: Clear Symptoms of bark splitting, beetle entry holes, tunnels and gum exudations from two year old rootstocks at nursery in mango varieties

a). Baneshan b). Imam Pasand c). Alphonso d). Neelum e). Totapuri f). Infected Neelum with beetles and galleries
Arrow indicates the entry holes of bark beetles Inset shows the bark beetle *H. mangiferae*

Discussion

Mango (*Mangifera indica* L.) is known as "the king of fruits" because of its popularity among the fruits of tropical regions [30]. Mango production is a profitable venture and can bring huge returns to the growers. It was however, realized through this study that mango sudden decline has a great economic impact on fruit yield. Recently, many mango trees or parts of trees began wilting and dying by an unknown emerging disease synonymous to dieback caused by *Lasiodiplodia theobromae*. This is characterized by sudden collapse of severely affected mango trees which initially appear as gummosis from the stem and branch decline followed by vascular discolouration [18]. Observed that mango sudden decline usually causes the death of affected trees within six months of first symptom appearance [1, 27]. further noted terminal and minimal necrosis of leaves which finally lead to the death of leaf blade of diseased mango trees. The above-mentioned symptoms may be found alone or in combination in different mango orchards [27, 8]. In Andhra Pradesh, typical symptoms of MSD has been observed and is yet to develop to an epidemic phase, causing fast-spreading death in mango orchards in a short period of time. Preferably accurate diagnostic measures can lead to effective management of the disease. In this report, we aimed to determine the causal

agent(s) of MSD on mango trees for the potential threat associated with this disease in Andhra Pradesh. The observations from diagnostic visit of main field symptoms provided clear destructive threat which is caused in combination of *Ceratocystis* and *Lasiodiplodia* and are confirmed with the findings of other workers [12, 1, 2, 18]. The colonial and microscopic images of *Lasiodiplodia theobromae* and *Ceratocystis fimbriata* demonstrated that the pathogens are vigorous producers of mycelia, conidia and perithecia on PDA. The mycelial and conidial characteristics of *L. theobromae* and *C. fimbriata* were in consistence with other researchers [25, 19, 5, 10, 15] reported maximum infection of *L. theobromae* in mangoes affected by decline symptoms [23]. isolated *L. theobromae* from mango trees affected by gummosis and successfully proved its pathogenicity. At present [21] mentioned that the MSD is caused by the combination of *L. theobromae* and *C. fimbriata* vectored by *H. mangiferae* in Pakistan which is in accordance with our new report in AP. *Hypocryphalus mangiferae* has been found to be consistently associated with MSD infected trees and considered as the vector of this disease [1, 27, 17]. The visual observations by splitting the bark revealed that *H. mangiferae* overwintered under the phloem portion of mango as an immature adult having light yellowish appearance. To prove the microbial pathogenicity of the disease by verifying the pathogen and its progression in plants of mango were inoculated with the isolated pathogen (unshown/unpublished data). The results of inoculation on tissues were similar to the disease symptoms in the field and the re-isolation of the pathogen from the inoculated plants confirmed Koch's postulates. Similarly our data corroborate with those of previous pathogenicity tests, which have been done on grapes, banana and mango [3, 13, 9, 26]. In general, seeds after germination are liable to attack by different soil borne organisms and for these reasons, healthy seedlings have to be reared to ensure quality mango grafts production in nurseries. Keeping the facts in mind, surveys were undertaken in and surrounding farms of Railway Koduru to know the prevalence of MSD in nurseries. Approvable evidences supported for the presence of exact symptoms of MSD similar to the main field along with the remarkable movement of bark beetles as vectors at nursery on mango grafts. Many researchers have assumed to be the cause, but no earlier reports as that of evidence. To the best of our knowledge, this is the first report of evidence of MSD at nursery level which carries over the source of infection to the mango orchards in India.

Conclusion

MSD is a sudden and rapid emerging threat in a wide area prevalent as an epidemic. This is the first report of MSD at mango nursery level and orchard where the scolytid beetles carry over the pathogen infection to the mango orchards in India. Hence accurate diagnostic and management trials are needed to be emphasised to prevent yield losses in mango. Further research should be focused on molecular characterization of pathogens, molecular diagnostic techniques and management of the disease at nursery and mango orchards.

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