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PATHOGENICITY OF *LASIODIPLODIA THEOBROMAE* AND *FUSARIUM SOLANI* ON MANGO

MUHAMMAD ALI KHANZADA, ABDUL MUBEEN LODHI AND SALEEM SHAHZAD

Pest & Disease Research Lab (PDRL),
Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract

Mango plantations in different areas of Sindh were found to suffer from a decline disease showing symptoms of drying of branches from the tip accompanied with a heavy exudation of yellowish-brown gum from stem and its branches and browning of vascular tissues. Under severe conditions, the disease results in death of the plant. There was an invariable association of *Lasiodiplodia theobromae* with aerial as well as underground parts. *Fusarium solani* was mostly isolated from root but very rarely from the stem. Inoculation of healthy plants with *L. theobromae* either alone or in combination with *F. solani* produced typical symptoms whereas *F. solani* failed to produce these symptoms.

Introduction

Mango (*Mangifera indica* L.) is an economically important fruit crop in the tropical and subtropical areas of the world. In Pakistan, mango is grown over an area of 94,100 ha with an annual production of 916,800 tons. In Sindh, more than 320, 700 ton of mango are produced from orchards spread over an area of 43,500 ha (Anon., 2001). Mango is subjected to a number of diseases at all stages of its development i.e., from nursery to the consumption of fruits and it is estimated that the production could be increased by 28% if the crop is protected against various diseases (Rawal, 1998).

In a survey of mango growing areas in Sindh, most of the mango orchards were found to suffer from a decline disease that is killing the plants and becoming epidemic in different areas. Mango decline has also been reported from different parts of the world. It is considered by some scientists as a disorder and by others as a disease. Schaffer *et al*., (1988) reported the deficiency of manganese, iron or both elements in the most of the declining trees. Rawal (1998) reported that decline is enhanced by beetle (*Xyleborus affinis*), relative humidity above 80%, temperature of 25-31.5^{o}C and rains.

Different workers have reported various fungi viz., *Lasiodiplodia theobromae* (Synonyms: *Botryodiplodia theobromae*, *Diplodia theobromae*) (Das-Gupta & Zachariah, 1945; Al Adawi *et al.,* 2003), *Botryodiplodia ribis*, *Fusarium equiseti*, *Diplodia* sp., *Colletotrichu*m sp., *Curvularia* sp., and *Oidium* sp., (Ramos *et al*., 1991, 1997), *Alternaria alternata, Dothiorella dominicana* (Darvas, 1993; Ploetz *et al*.,1996), *Phomopsis* sp., (Ploetz *et al*., 1996), *Hendersonula toruloidea* (Reckhaus & Adamou, 1987), *Physalospora rhodina* (Alveraze & Lopez, 1971), *Colletotrichum gloeosporioides*, (Rawal, 1998; Savant & Raut, 2000; Ploetz *et al*., 1996; Sharma, 1993), *Rhizoctonia solani*, *Pestalotia mangiferae*, *Phoma* sp., *Sclerotium rolfsii* and *F. solani* (Sharma, 1993) as cause of mango decline. Johnson *et al*., (1992) reported that several fungi cause this disease and that the host and environmental factors influence the prevalence of different species in different situations.

A preliminary survey of mango orchards in different districts of Sindh showed that the disease is becoming epidemic in the province. Studies were, therefore, carried out to study the symptomatology, severity and cause of the disease. Pathogenicity of the most frequently isolated organisms viz., *L. theobromae* and *F. solani* were also tested which is presented herein.

Materials and Methods

Survey of infected fields: A survey of mango growing areas of Sindh *viz.*, Hyderabad, Mirpurkhas, Sanghar, Nawabshah, Nausheroferoz, and mango plants growing in different parts of Karachi was carried out to study the symptoms, spectrum and severity of mango decline disease in these localities. Root and shoot samples were collected and isolations were made in Pest & Disease Research Lab (PDRL), Department of Botany, University of Karachi. Root and shoot samples were cut into 1-2 cm long pieces, surface sterilized with 5% sodium hypochlorite solution for two minutes and placed in Petri plates containing either potato sucrose agar (PSA) or two layers of moistened blotter paper and incubated at 30^{o}C with 12 hours alternate periods of light and darkness. Specimens were examined after one week for fungal growth. Baiting technique (Plaats-Niterink, 1981) was also used for the isolation of Oomycetous fungi where root pieces or one table spoon soil from the rhizosphere were placed in Petri dishes containing sterilized distilled water and a few sterilized sesame or hemp seeds along with 2-3 pieces of grass blade. The plates were incubated for 7 days with daily observation and baits colonized by fungi were transferred to corn meal agar (CMA) and potato carrot agar (PCA) for purification and identification. The organisms isolated were identified up to species level after reference to Booth (1971), Domsch *et al.*, (1980), Ellis (1971, 1976), Plaats-Niterink (1981) and Sutton (1980). The frequency of the fungi in the collected specimens was recorded using the following formula:

$$\text{Colonization \%} = \frac{\text{Number of pieces colonized by a pathogen}}{\text{Total number of pieces}} \times 100$$

Pathogenicity test: Pathogenicity tests of the most frequent fungi viz., *L. theobromae* and *F. solani* were carried out in screen-house of PDRL and at farmer's fields near Hyderabad. In each experiment, apparently healthy looking plants of var. *GulabKhasa* were selected and specimens were taken from their branches and roots to confirm the absence of the test pathogens. Plants found infected with the test pathogen(s) were not used in the study. Roots and stem of separate plants were inoculated with the test organisms either alone or in combination.

A cut in the stem or root was made using a sterilized knife. A 1x2 cm inoculum block from 5 days old culture of a test fungus on PSA was placed in the gap and the inoculated portion was wrapped with Para film. A 1x2 cm PSA block without fungus was placed in the control plants. Plants were irrigated after inoculation and the wrapping material was removed from the stems after 2 weeks of inoculation. Plants were monitored for the development of disease symptoms and isolations were made from roots, stem and branches of the test plants to confirm the pathogenicity. The experiments were carried out in randomized complete block design with four replications.

Results and Discussion

Survey of infected fields: Mango decline was frequent in almost all the areas visited during the present studies. In affected plants, twigs die from the tips back into old wood conforming a scorched appearance. Leaves on the affected branches turn brown with their margins rolled upwards, which fall leaving a dead branch (Fig. 1A). Brown streaks in vascular region were seen on splitting the twigs along the long axis (Fig. 1B). The outer wood cracks and exudes yellow to brown gum-like substance (Fig. 1C). Under severe conditions, trees show bark splitting (Fig. 1D), gummosis from the main trunk and its branches (Fig. 1E), wilting, and eventual browning of leaves on one or more branches leading to the death of the whole tree (Fig. 1F). The disease may occur at any time throughout the year. The symptoms were more severe in areas under water stress as compared to regularly watered areas.

A total of 11 fungi *viz., Aspergillus niger, Curvularia lunata, Drecshslera australiensis, Fusarium solani, F. semitectum, Lasiodiplodia theobromae, Pythium aphanidermatum, Rhizoctonia solani, Rhizopus oryzae, Trichoderma harzianum* and *T. longibrachiatum* were isolated from roots, stem and shoot of mango plants. Of these, *L. theobromae* was the most abundant fungus that was isolated from all the location as well as from all the plant parts. *F. solani* was the second most frequent fungus that was mostly isolated from roots. All other fungi were isolated occasionally with very low frequencies (Table 1). Maximum disease incidence was recorded at Tandojam followed by Mirpurkhas, whereas, minimum was observed in Hala, District Nawabshah (Fig. 2).

Decline disorder has been observed in nearly all mango growing regions of the world (Alveraze & Lopez, 1971; Rawal, 1998; Schaffer *et al*., 1988). In India, this disease has been recognized as a significant disease since 1940. It was reported as the serious most disease in Jaipur district (Verma & Singh, 1970) that also affected 30-40% of the plantations in the Moradabad region of Uttar Pradesh (Prakash & Srivastava, 1987). Narasimhudu & Reddy (1992) found that mango trees in Andhra Pradesh, India were severely affected by gummosis. Reckhaus & Adamou (1987) reported its occurrence from Niger since the early 1980s. Ploetz *et al.,* (1996) and Simone (1999) reported the same disease from Florida. In Oman, since 1999, up to 60% of trees were found affected in parts of the Al Batinah region (Al Adawi *et al.,* 2003). The incidence of fungi associated with diseased plants varied within the plant parts and locations.

Pathogenicity test: After one month of inoculation, plants inoculated with *L. theobromae* alone or with *F. solani* showed typical symptoms of the disease, whereas, no such symptoms were observed on plants inoculated with *F. solani* alone. Un-inoculated plants showed no gummosis (Fig. 3A). Plants inoculated with *L. theobromae* either alone or in combination with *F. solani* showed gummosis, whereas, no gummosis was observed in plants inoculated with *F. solani* alone (Fig. 3 B,C,D,G). In control plants, no vascular browning was observed whereas in plants inoculated with *L. theobromae* either alone or with *F. solani*, browning of vascular tissues was prominent (Fig. 3 E,F). Similarly, wilting and death of leaves at the branch apices was only observed where plants were inoculated with *L. theobromae* either alone or in combination with *F. solani* (Fig. 3H,I).

Fig. 1. Symptoms of mango decline and gummosis disease. A. An affected tree showing a dead branch. B. Browning of vascular tissues. C. Gum exudation. D. Bark splitting. E. Gummosis on main trunk and its branches. F. A tree killed by the disease.

Table 1. Fungi isolated from root and stem of mango plants growing in different locations of Sindh, Pakistan.

Fungi isolated	Colonization % at different locations													
	Hyderabad		Mirpurkhas		Sanghar		Nawabshah		Nausheroferoz		Karachi		Overall	
	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem
Aspergillus niger	4.5	6.2	2.1	7.7	4.8	5.6	2.3	2.1	0..0	10.5	0.0	0.0	3.9	5.8
Curvularia lunata	1.7	0.0	0.0	0.0	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0
Drecshslera australiensis	1.1	0.0	0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	1.1	0.0
Fusarium semitectum	1.1	1.2	2.1	0.0	9.5	2.8	2.3	2.1	6.3	0.0	0.0	0.0	2.1	1.1
F. solani	13.6	1.9	10.4	3.8	9.5	2.8	16.3	2.1	6.3	5.3	0.0	9.4	14.1	3.1
Lasiodiplodia theobromae	22.2	41.2	35.4	46.2	42.9	50.0	34.9	43.8	50.0	57.9	0.0	90.6	33.9	47.4
Pythium aphanidermatum	1.2	0.0	2.18	0.0	9.5	0.0	0.0	0.0	9.4	0.0	0.0	0.0	3.2	0.0
Rhizoctonia solani	1.1	0.0	2.18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0
Rhizopus oryzae	6.8	15.8	6.3	11.5	4.8	16.7	2.3	8.3	3.1	13.2	0.0	0.0	6.4	13.3
Trichoderma harzianum	1.1	1.9	4.2	0.0	0.0	5.6	2.3	6.3	0.0	0.0	0.0	0.0	1.8	2.2
T. longibrachiatum	2.8	5.8	4.2	3.8	14.3	11.1	7.0	6.3	6.3	5.3	0.0	0.0	4.9	5.6

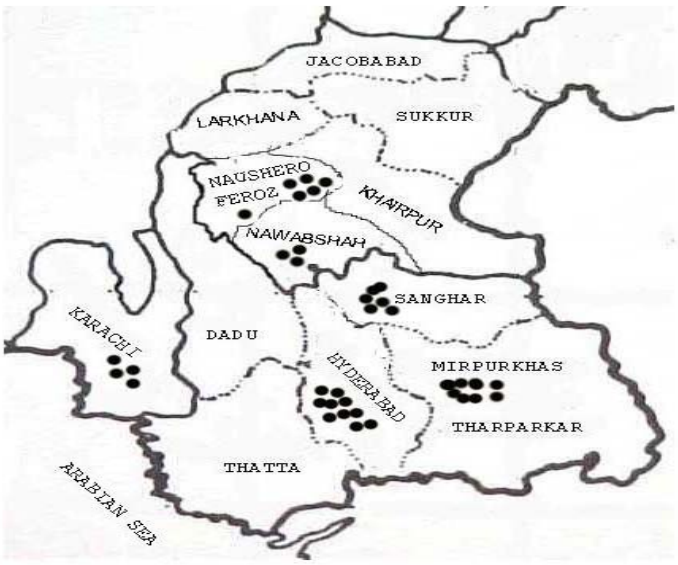

Fig. 2. Distribution and severity of mango decline and gummosis in Sindh.
*Number of dots indicates the relative intensity of the disease.

Severity of the symptoms was the same when *L. theobromae* was used either alone or in combination with *F. solani* (Table 2). It clearly indicated that the decline disease is caused by *L. theobromae* and *F. solani* plays no significant role in disease development. It was interesting to note that the symptoms appeared early and were more prominent in stem inoculated plants as compared to root inoculated plants (Table 2). This could be attributed to the short distance that would have been covered by the pathogen or its affects to reach the apical parts. Re-isolation from the dead and green branches of *L. theobromae* inoculated plants showed up to 95% recovery of the fungus**.**

The results of the present studies are in close confirmation to those of Das-Gupta & Zachariah (1945) who recorded similar symptoms of dieback and found that after artificial inoculations with *Lasiodiplodia theobromae*, *Phoma* sp., and two *Fusarium* spp., only the *L. theobromae* caused the disease. Similarly, Narasimhudu & Reddy (1992) reported *L. theobromae* associated with mango trees severely affected with gummosis. Sharma (1993) observed *L. theobromae, C. gloeosporioides, R. solani, Pestalotia mangiferae, Phoma* sp., *S. rolfsii* and *F. solani* as pathogenic. He also reported that a mixed infection was common and *L. theobromae* was the primary cause of the disease, and necrosis caused by other pathogens may facilitate invasion by *L. theobromae*. Simone (1999) also reported *L. theobromae* and *Botryospheria ribis* as the cause of dieback in Florida. Savant & Raut (2000) observed that dieback of mango stone grafts was incited by *C. gloeosporioides* and *L. theobromae* either alone or in combination. They believed that disease manifestation was enhanced in combination of pathogens. Al Adawi *et al.,* (2003) also reported *Diplodia theobromae* [*Lasiodiplodia theobromae*] as a cause of the mango decline in Oman.

Fig. 3. Pathogenicity test. A. Control plant showing no disease symptoms. B. Gummosis on a *L. theobromae* inoculated plant. C. No gummosis on *F. solani* inoculated plant. D. Gummosis on a plant inoculated with *L. theobromae* and *F. solani*. E. Vascular tissues in control plant. F. Browning of vascular tissues in a plant inoculated with *L. theobromae*. G. Drying and gum exudation in apical branch of a tree inoculated with *L. theobromae*. H. & I. Necrosis of leaves in the shoot apices of *L. theobromae* inoculated plants.

Table 2. Severity of symptoms on plants inoculated with *Lasiodiplodia theobromae* and *Fusarium solani*.

Pathogen	Inoculated in	Symptoms produced on mango plants		
		Drying of tips	Gum exudation	Internal browning
Lasiodiplodia theobromae				
	Stem	3*	3	3
	Roots	3	2	3
Fusarium solani				
	Stem	0	0	0
	Roots	0	0	0
L. theobromae + *F. solani*				
	Stem	3	3	3
	Roots	3	2	3
Control		0	0	0

* 0= No symptoms, 1= Very light, 2= Moderate, 3= Severe symptoms.

Association of *L. theobromae* with mango plants has already been reported from Pakistan (Mirza & Qureshi, 1978) but decline disease was not observed. Recently, Mahmood & Gill (2002) and Mahmood *et al.,* (2002) also isolated *L. theobromae* along with *Colletotrichum gloeosporioides, Fusarium oxysporum, F. semitectum* and *F. solani* from declining mango plants in Faisalabad, Punjab but were not able to determine the actual pathogen. Results of the present study would suggest that *L. theobromae* is the causative agent of mango decline and gummosis in Sindh.

References

Al Adawi, A.O., M.L. Deadman, A.K. Al Rawahi, A.J. Khan and Y.M. Al Maqbali. 2003. *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. *Plant Pathology,* 52: 419-419.

Alveraze, G.L.A. and J.G. Lopez. 1971. Gummosis, dieback and fruit rot disease of mango (*Mangifera indica* L.) caused by *Physalospora rhodina* (B&C) Cke. *Puerto Rico J. Agric*., 55(4): 435-450.

Anonymous. 2001. *Pakistan Statistics Year Book, 2001*. Ministry of Food, Live Stock and Agriculture, Govt. of Pakistan, Islamabad.

Booth, C. 1971. *The genus Fusarium.* Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.

Darvas, J.M. 1993. *Dothiorella dominicana*, an important mango pathogen in South Africa. *Acta Hort. (ISHS*), 341: 321-328.

Das-Gupta, S.N. and A.T. Zachariah. 1945. Dieback of mango. A new disease in India. *Ind. J. Bot. Sci*., 24(1): 101-108.

Domsch, K.H., W. Gams and T.H. Anderson. 1980. *Compendium of soil fungi*. Vol.1. Academic Press, N.Y. pp.

Ellis, M.B. 1971. *Dematiaceous hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 608 pp.

Ellis, M.B. 1976. *More Dematiacious Hyphomycetes.* CMI, Kew, Surrey, England. 507 pp.

Johnson, G.I., A.J. Mead, A.W. Cooke and J.R. Dean. 1992. Mango stem end rot pathogens - Fruit infection by endophytic colonization of the inflorescence and pedicle. *Annals Appl. Bot*., 120: 225-234.

Mahmood, A. and M.A. Gill. 2002. Quick decline of mango and *in vitro* response of fungicides against the disease. *Int. J. Agric. & Biol*., 4(1): 39-40.

Mahmood, A., A. Saleem and K.M. Akhtar. 2002. Mango decline in Pakistan and its management. *Pak. J. Phytopathol*., 14: 40-43.

Mirza, J.H. and M.S.A. Qureshi. 1978. *Fungi of Pakistan*. Dept. of Plant Pathology, Univ. of Faisalabad, Pakistan. 311 pp.

Narasimhudu, Y. and P.S.N. Reddy. 1992. A note on gummosis of mango. *Indian Phytopathol*., 45(2): 261-262.

Parkash, O. and K.C. Srivastava. 1987. *Diseases caused by Fungi: Mango Diseases and their Control*. Today & Tomarrow's Printers & Publishers, New Dehli.

Plaats-Niterink, Vander, A.J. 1981. *Monograph to the genus Pythium. Studies in Mycology No.21.* Centraalbureau voor Schimmelcultures, Baarn. 242 pp.

Ploetz, R.C., B. David and B. Schaffer. 1996. A re-examination of mango decline in Florida. *Plant Dis*., 80(6): 664-668.

Ramos, L.J., T.L. Davenport, R.T. McMillan and S.P. Lara. 1997. The resistance of mango (*Mangifera indica*) cultivars to tip dieback disease in Florida. *Pl. Dis*., 81: 509-514.

Ramos, L.J., S.P. Lara, R.T. McMillan and K.R. Narayanan. 1991. Tip dieback of mango (*Mangifera indica*) caused by *Botryodiplodia ribis*. *Pl. Dis*., 75: 315-318.

Rawal, R.D. 1998. Management of fungal diseases in tropical fruits. In: *Tropical Fruits in Asia: Diversity, Maintenance, Conservation and Use*. (Eds.): R.K. Arora and V. Ramanatha Rao. Proceedings of the IPGRI-ICAR-UTFANET Regional training course on the conservation and use of germplasm of tropical fruits in Asia held at Indian Institute of Horticultural Research, 18-31 May 1997, Bangalore, India.

Reckhaus, P. and I. Adamou. 1987. *Hendersonula* dieback of mango in Niger. *Plant Dis*., 71:1045.

Savant, N.V. and S.P. Raut. 2000. Studies on symptomatology of dieback of mango stone grafts. *Acta Hort. (ISHS)*, 509: 823-832.

Schaffer, B., K.D. Larson, G.H. Snyder and C.A. Sanchez. 1988. Identification of mineral deficiencies associated with mango decline by DRIS. *Hort. Sci*., 23: 617-619.

Sharma, I. 1993. A note on population dynamics and etiology of die back of mango in Himachal Pradesh. *New Agriculturist*, 2(2): 229-230.

Simone, G.W. 1999. *Disease Control in Mango* (*Mangifera indica*). *Plant Pathology Department Document PDMG-V3-22.* Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Sutton, B.C. 1980. *The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata.* CMI, Kew, Surrey, England. 696 pp.

Verma, O.P. and R.D. Singh. 1970. Epidemiology of mango dieback caused by *Botryodiplodia theobromae* Pat., *Ind. J. Agric. Sci*., 40: 813-818.

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