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ANATOMICAL STUDIES OF FUNGAL AFFECTED MANGO TREES OF BARNALA, DISTRICT BHIMBER, AZAD KASHMIR, PAKISTAN

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Abstract

Pathological studies were conducted by anatomical methodology to know the effect and severity of mycoflora on mango (*Mangifera indica* L.) trees of Barnala, District Bhimber, Azad Kashmir. In etiological studies, it was seen that stem blight or die back become evident by discoloration and darkening of the bark as well as exudation of gum from infected portion is major symptom of it. While on the leaves, angular water soaked spots lesions, surrounded by clear patches was noticed. A total of 5 populations each of healthy and affected specimens of same species were anatomically analyzed and 50 readings of various parameters were calculated. The size and diameter of anatomical tissues i.e., vessel diameter (25µm), vessel length (708µm), ray height (172µm), ray width (71µm), fiber length (32µm), and lumen diameter (45µm) were found to be increased in comparison to healthy mango trees. In other observations, there was considerable decrease in fiber length (36µm), fiber diameter (22µm), lumen diameter (55µm), number of vessel per square mm (41µm) and vessel diameter (50µm) in fungal infected trees which hampers yield of trees due to less photosynthetic activity. Several fungi including *Botryodiplodia theobromae, Ceratocystis fimbriata, Phoma* spp., *Aspergilus* spp., *Cladiosporium* spp., and *Nattrasia* spp., were found invading vascular tissue of mango trees (60-85% decline severity) which make tree declined with passage of time. This is illumined and crystal clear that mycoflora is impediment in yield of mango crops in the area and other parts of country.

Introduction

Pakistan is bestowed with diverse agro-ecological conditions which are appropriate and favourable for the production of a great variety of crops, vegetables and fruits. Among fruits, mango (*Mangifera indica* L.) has an important place in Pakistan export revenue ranking 2nd on the basis of country's production (Anon., 2001) and 4th in case of exports (Maqbool *et al.*, 2007; Amin *et al.*, 2010). Pakistan is at fifth rank in major producing countries of the globe after India, China, Thailand and Mexico (FAO, 2005). Currently, the world's mango production is 27960 thousand tons; 5.97% (1753.9 thousand tons) of which comes from Pakistan (MINFAL, 2006). Pakistan is one of the leading mango exporting countries along with other major exporters being Mexico, China, India and Philippines (Amin *et al*., 2008).

Mango (*Mangifera indica* L.) belongs to family Anacardiaceae and more than 250 cultivars of mango are grown in Pakistan covering an area of 0.099 million hectares with annual production of 1.037 million tones (Anon., 2004). Mango provides us not source of fruit and nutrition but also livelihood to their harvesters. Inter alia, its production remains hampered due to a number of factors and diseases impending its growth and fruit yield.

According to one estimate production of mango could be increased by 28% if the crop is protected against various diseases (Rawal, 1998). It is indubitable that various mango diseases are prevailing in different regions of country and anthracnose (AN), die back disease (DBD), root rot (RR) and others (wilts and cankers) are commonly infecting the crop (Jason *et al.*, 2005; Nafees *et al*., 2011). Tip die back disease occurs on the branches/ trunks of infected trees that start drying slowly and suddenly branch become completely dried/ killed resulting gummy substances oozes out or remain hanging on the tree. Khan & Bokhari (1970) carried out a survey in the irrigated plantations in Bhagat in Punjab. The results of the studies focused attention on the serious nature and vast extent of losses due to die-back: the number of trees after the third thinning declined by half as compared to uninfected areas. In previous studies, ace decrease demonstrated in yield of all infected mango trees (21%) was described. In financial terms, the loss was about 31% of the total expected income.

Different species of mycoflora infect mango trees hampering its growth and yield. Literature depicts that fungi *viz*., *Lasiodiplodia* (Gupta & Zachariah (1945; 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 anf Adamou, 1987), *Physalospora rhodina* (Alveraze & Lopez, 1971), *Colletotrichum gloeosporioides*, (Rawal, 1998; Savant and Raut, 2000; Ploetz *et al*., 1996; Sharma, 1993), *Rhizoctonia solani*, *Pestalotia mangiferae*, *Phoma* sp., *Sclerotium rolfsii* and *F. solani* (Sharma, 1993) are cause of mango die back disease (DBD) in the world. 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. DBD of mango was due to attack of mycoflora which infected vascular tissue of plant and ample anatomical medications were expected and ipso facto good yield is impeded (Jason *et al*., 2005). It is incumbent to the horticulturists and agronomy researchers to conduct research imperative to find pros and cons of DBD in mango species of (Azad Kashmir) Pakistan and formulate eradication mechanism by controlling invasion of different pathogens.

There is hitherto nothing in comprehensive in the literature for anatomical and pathological information on

mango trees from Barnala (Azad Kashmir) area. The main objective of the study was to find modifications in morphology and anatomy of healthy and decline affected mango trees. Secondly, find out causative agent of DBD and its subsequent impact on yield of mango. This inordinate and novel anatomy research on mango tree will provide base for horticulturists and agronomists to formulate different measures to eradicate this menace from the country which will boost mango yield boosting exports and generating better revenue.

Materials and Methods

Sample collection: Wood samples for this study were collected from the stems of mango (*Mangifera indica* L.) trees growing in different areas of Barnala, District Bhimber Azad Kashmir (Pakistan). Five samples of each healthy and affected tree were collected with saw and borer. These samples were brought to the laboratory at the National Agriculture Research Centre (NARC) Islamabad for further studies. Some herbarium specimens were also prepared and placed in Department of Botany, Mirpur University of Science & Technology (MUST) Bhimber Campus, Azad Kashmir for future reference study.

Anatomical studies: Thick discs (blocks) for each healthy and decline affected sample were cut with surgical blade and age of the sampled plants was determined by counting the annual rings with hand lens and naked eye. The small blocks were boiled in water until fully water logged and sections were trimmed at different angles (Khattak, 1984). For preparation of transverse and longitudinal section (12µm) slides, trimmed slices were immediately submerged in methanol/glacial: acetic acid fixative (3:1, v/v). Slides were soaked in two changes of distilled water and then stained for 5 min., in 0.01% (w/v) safranine. After rinsing twice with distilled water, the slides were dehydrated in an ascending ethanol series to 95% ethanol and counter–stained in 0.05% alanine blue (w/v). Slides were rinsed three times in absolute ethanol and were removed through three changes of xylene. Cover slip was placed on sections mounted in Canada balsam (Ahmed, 1992).

Microscopy: Sections were observed and examined under microscope (Carl Zeiss Inc., Thornwood, NY, USA), viewed using differential interference contrast (DIC) microscopy, and images were captured using a digital CCD camera (Hamamatsu Photonics, Hamamatsu, Japan). Photographs of sections were rotated and adjusted for brightness and image sharpness using Adobe Photoshop CS (Adobe Systems, San Jose, CA, USA). The backgrounds of all images were also converted to white using Photoshop.

A comparative anatomy microscopic (CAM) analysis was conducted by measuring differences in fiber length (FL), fiber diameter (FD), lumen diameter (LD), vessel diameter (VD), ray height (RH), ray width (RW) of healthy and decline affected samples. Fifty readings were taken at random for all the samples. For the measurement of FL, match stick sized splinters were cut down and were placed in test tube and were boiled in 20% HNO_3 with addition of small amount of $KCLO_4$ until the fibers were torn apart and fibers started separating from each other (Khattak & Ghazi, 2001). The boiled material was washed with dist. water. A small amount of material containing fibers was placed on glass slide and observed under microscope. In CAM five or more readings were taken for each parameter for all the samples.

Isolation of pathogens: Five sq. inch portion of vascular bundle of each healthy and decline affected tree (50-60% disease) were cut with a sharp axe until the vascular bundle were exposed. These were taken as small chips with grey to black strip like fungal lesions. These infected chips were cut into small pieces and after surface sterilization with ethanol were placed in 9cm diameter Petri dishes (PD) containing moist Whitman filter papers. PDs were incubated at 25^{o}C until sporulation occurred. When the woody tissues were showing a little growth, they were transferred to PDA plates. The plates were incubated till the growth was completed. After this immediately snaps were captured by microscopic digital camera. The data obtained was identified with help of literature and lab specimens.

Results and Discussion

Mango, *Mangifera indica* L., is a popular tropical fruit in Pakistan, India, Israel, Egypt, Africa and Central & South America. Decline disorders of trees are getting importance throughout the world (Johnson *et al.,* 2005). The declined affected hosts vary from forest to fruit trees. Inclusion of various pathogens makes this problem more complex. There is a dire need of a comprehensive study to know the deviation from the normal in the vascular bundles of affected trees.

A comparison of the diseased and healthy vascular tissues (from collar portion, roots and branches) anatomy was conducted. Mango production remains hampered due to a number of diseases at different stages of its development (Rawal, 1998). Mango decline was frequent in almost all observed sample trees. 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. The outer wood cracks and exudes yellow to brown gum-like substance. Under severe conditions, trees show bark splitting and vascular tissue is infected. 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. DBD or Decline disorder has been observed in nearly all mango growing regions of the world (Alveraze & Lopez, 1971; Rawal, 1998; Schaffer *et al*., 1988). In DBD and other fungal infections not plant morphology is altered but also vascular tissue is varied significantly which is major cause of inordinate loss in yield.

The analysis of vascular tissue by cross sectional comparative study was conducted for decline affected samples (DAS) and healthy sample (HS). A stark variation in annual rings, wood characteristics like porous, semi-porous and diffused porous were observed between the two samples. In DAS wood was comparatively more diffuse and porous than healthy sample (HS). Vessels were found having web-like

structure and discolored responsible for damaging vessel cells which resulted in blockage of up take of minerals nutrients and water (Atia *et al.*, 2003).

During this analysis several fungi including *Botryodiplodia theobromae, Ceratocystis fimbriata, Phoma* spp., *Aspergilus* spp., *Cladiosporium* spp., *and Nattrasia* spp., were found invading in vascular tissue of mango trees with 60-85% decline severity (as shown in Figs. 1 and 3).

It was noted that in DAS av. vessel length was 708µ (ranging from 500-1500 microns) and av. number of vessels per seq. was 10 (ranging from 8-15) (Fig. 1) and in HS av. vessel length was 91µ and av. number of vessel per sq.mm was 50 (Table 1). The pictorial of tangential sections revealed that ray features were uni-seriate, bi-seriate and multi-seriate storied and non-storied (Fig. 1). Its comparative analysis depicted that HS samples rays had non-storied, number of rays was 10, av. ray height was 41µ, av. ray width was 182µ and av. number of cells per ray along ray width was 50µ (Fig. 1) and av. number of cells along ray height was 42 , respectively (Table 1). While in DAS samples rays were non-storied, number of rays was 35, av. ray height was 172µ, av. ray width was 35µ and av. number of cells per ray along ray width was 35µ (Fig. 1) and av. number of cells along ray height was 55, respectively (Table 1). In observations some rays were discolored and in some rays cells were few or quite absent (Fig. 1). It was point of interest that almost all the rays were blocked with some reddish brown substance (Ploetz *et al*., 1997). Radial sections were prepared to study fibers which make up the appreciable volume of wood. In HS mostly, fibers were thick walled with narrow diameter and some were thin walled with large diameter. Av. fiber length was 36µ and average fiber diameter was 22µ and lumen diameter was found 55µ (Fig. 1). In DAS fibers had av. fiber length was 32µ and average fiber diameter was 10µ and lumen diameter was found 45µ (Fig. 1 & Table 1). A large gap was seen between vessels and gum-like substances were present in some fibers as well as in vessels which cause blockage (Fig. 1). Different decline disorders are identified by gummosis but in most of the orchards, combined forms of these disorders are manifested (Prakash & Singh (1976). In the present study, tip die back, twig blight and gummosis were evident in two or more combinations (Zafar *et al.*, 2007).

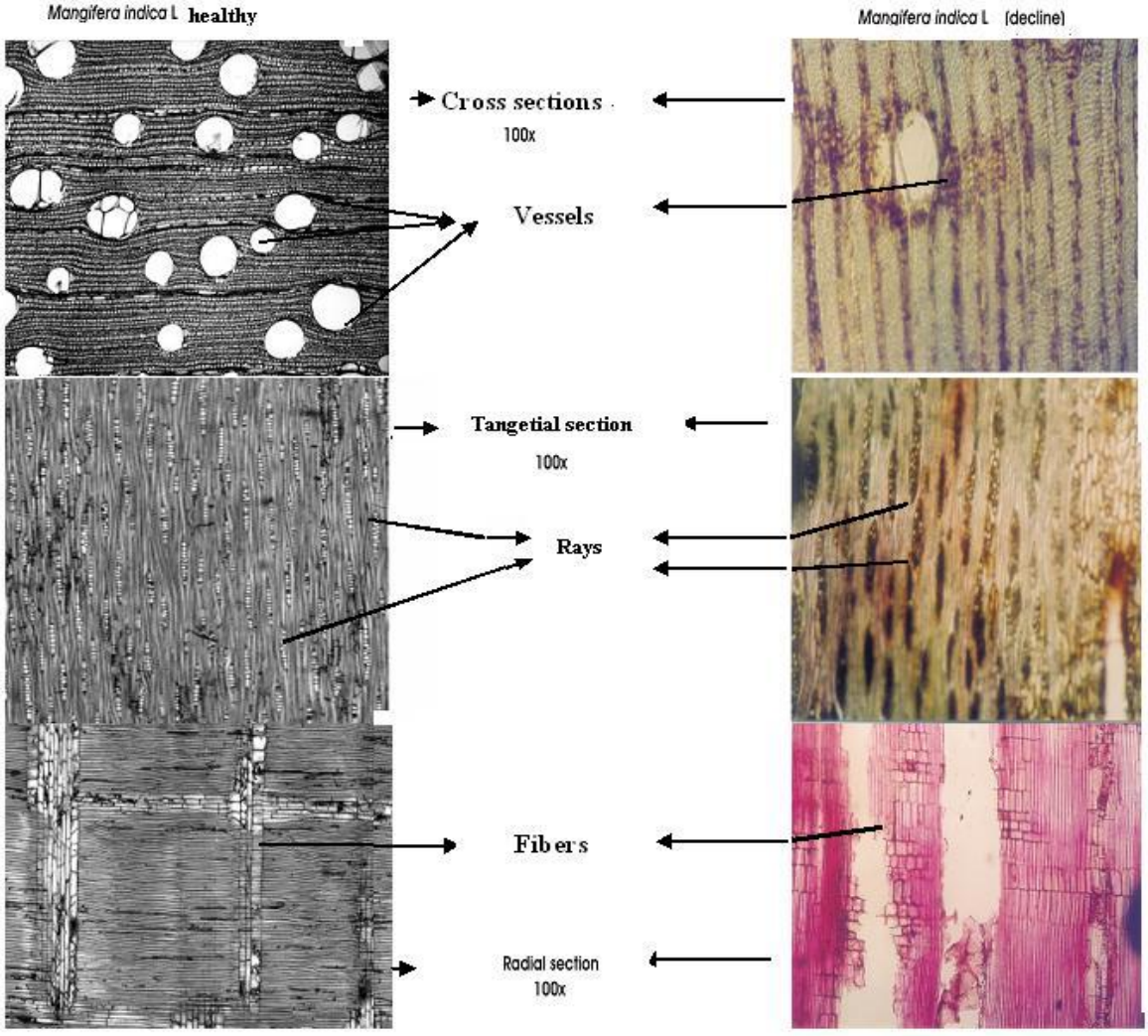

Fig. 1. Comparative anatomy of healthy and decline affected mango tree samples.

Fungi are aggressive and vigorous pathogens causing various type of disease symptoms like tip dieback (Fig. 2 & Fig. 3) and twig blight which do affect vascular tissue (Ragab *et al*., 1971). Eventually a complex mango decline form severely damaging the mango plant is produced. It was explored that conventional practices of farming, poor orchard and disease management, improper cultural, plant protection measures and non recommended intercropping predispose mango trees to decline complex.

It was discovered that disease affected trees which were properly disposed off after their dearth were becoming reservoir for inoculums of various fungi disseminating infection to nearby trees or neighbouring orchards (Zafar *et al*., 2007). Fuel to the fire on this attack of quick decline/ collar rot, which once established, kills the plants within days. Hence, detailed and comprehensive measures should be carried to find root cause of DBD and how it spreads and invades the vascular tissue of mango trees. This study will be useful to formulate an integrated strategy to combat decline complex of mango trees and subsequently increasing fruit yield and boosting revenue of the country.

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Fig. 2. Fungal spores growing in the Petri dishes.

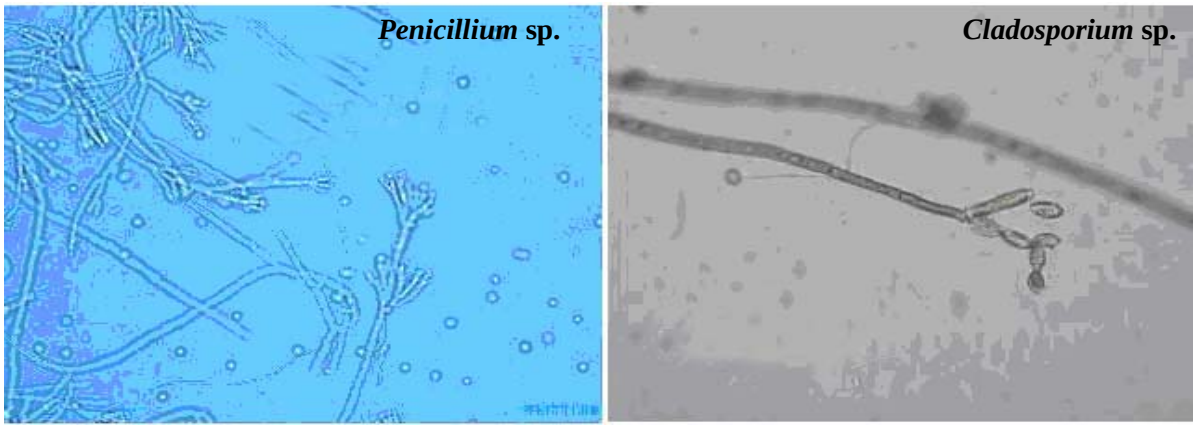

Fig. 3. Fungi isolated from decline affected mango tree samples.

Table 1. Comparative anatomical characteristics of healthy and decline mango sections.

S. No.	Anatomical characteristics	Healthy mango			Decline mango		
		Mean	Max	Min	Mean	Max	Min
1.	Fibers length: mm	36 ± 0.35	50	25	32 ± 0.45	42	20
2.	Fibers diameter: µm	22 ± 0.40	30	11	10 ± 0.65	22	10
3.	Lumen diameter: µm	55 ± 0.50	75	40	45 ± 0.80	70	35
4.	Vessels diameter: µm	360 ± 0.2	700	170	25 ± 0.44	65	11
5.	Number of vessels per.sq.mm	50 ± 0.55	60	30	10 ± 0.66	15	8
6.	Rays height; µm	41 ± 0.60	70	40	172 ± 0.15	730	422
7.	Rays width: µm	182 ± 0.65	812	502	71 ± 0.35	95	50
8.	Vessels length: mµ	91 ± 0.75	90	70	708 ± 0.64	1500	500
9.	No. of cells per ray along ray width	50 ± 0.45	95	70	35 ± 0.75	20	11
10.	No. of cells per ray along ray height	42 ± 0.66	27	15	55 ± 0.86	70	30
11.	No. of Ray per sq.mm	10 ± 0.025	7	5	35 ± 0.25	70	45

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