Available online at http://www.journalijdr.com

ISSN: 2230-9926

IJDR

International Journal of
DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 4, Issue, 8, pp. 1679-1683, August, 2014

Full Length Research Article

FREQUENCY DISTRIBUTION OF *FUSARIUM NIVALE* (FR.) CES. AND OTHER FUNGI ASSOCIATED WITH MANGO MALFORMATION DISEASE (MMD) IN SINDH, PAKISTAN

[1*]Ibrahim Khaskheli, M., [2]Mumtaz A. Pathan, [2]Mithal Jiskani, M., [2]Azeem Khaskheli, M., [3]Jurio Khaskheli, A. and [4]Fakhar I. Khaskheli

[1,2]Department of Plant Protection, Sindh Agriculture University, Tando Jam-70060, Pakistan
[3]Department of Biotechnology, Sindh Agriculture University, Tando Jam-70060, Pakistan
[4]Pakistan Central Cotton Research Institute, Sakrand, Sindh, Pakistan

ARTICLE INFO

Article History:
Received 12th May, 2014
Received in revised form
13th June, 2014
Accepted 30th July, 2014
Published online 31st August, 2014

Key words:
Mango,
Malformation,
Frequency,
Fusarium nivale

ABSTRACT

Mango Malformation Diseases (MMD) is one of the serious threats, causing tremendous economic losses every year to mango growers/contractors. In the current study, six fungi were isolated from primary and secondary tissues of malformed parts of the inflorescence. The fungi identified as *Fusarium nivale, F. oxysporum, F. moniliforme, F. semitectum, Alternaria alternata* and *Aspergillus niger*. *F. nivale* was isolated predominantly from the diseased samples collected from Mirpurkhas, Hyderabad, Tando Allah Yar and Sanghar districts. *F. nivale* was isolated with highest frequency from Mirpurkhas (55.00%) followed by Hyderabad (46.00%), Tando Allah Yar (35.00%) and Sanghar (30.00%) as compared to *F. oxysporum* (18.00-28.00%), *F. moniliforme* (13.00-25.00%), *F. semitectum* (7.00-15.00%) and other fungi. Desi, Almas and Dusheri showed maximum infection percent in Mirpurkhas ranging from 83.00-95.00%, while in Hyderabad (81.33-93.50%) as compared to Tando Allah Yar (55.33-75.33%) and Sanghar districts (40.60-45.66%). Comparatively less infection percent was observed in Fajri in Mirpurkhas and Hyderabad districts (35.25-36.30%) then the other mango varieties. The overall frequency of *F. nivale* on all the mango varieties at Mirpurkhas was 60.174% followed by Hyderabad (58.431%). Maximum infection frequency was obtained with *F. nivale* (82.08%) colonizing 985 tissues out of 1200 tissues, *F. oxysporum* (11.90%) colonizing 143 tissues, *F. moniliforme* (7.08%) with 85 tissues colonized as compared to other fungi.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the finest fruits and the most important in tropical and subtropical areas of the world. Mango is considered for superior in quality and is well established in international market to earn foreign exchange (Tahir *et al*., 2003). Mango malformation occurs in vegetative as well as in floral parts, floral malformation is more common in mango trees (Ahmed *et al.,* 2003). Mango malformation caused shortens inflorescence, sterility and aborted hermaphrodite flowers and male flowers increased in number and size (Kumar *et al*., 1993). Ploetz (1994) studied fifty five isolates of *Fusarium subglutinas* from malformed mango trees in Florida in single vegetative compatibility group (VCG).

Corresponding author: Ibrahim Khaskheli, M.,
Department of Plant Protection, Sindh Agriculture University, Tando Jam-70060, Pakistan

Numerous fungi have been reported associated with MMD and possibly the causal agents of the disease. However, according to Ploetz (1994), the confusion still remains about the actual cause of MMD, nevertheless, *F. moniliforme (Gibberella fujikuroi*) and *F. mangiferae* have been identified in recent past as the causal organisms of the disease (Chakrabarti & Kumar, 1998); (Nirengerg & O'Donell, 1998). Nirenberg and O'Donell (1998) showed small microscopic differences to distinguish *Fusarium mangiferae* from other taxa in the *Gibeberella fujikuroi* species complex. *F. mangiferae* can also be differentiated morphologically from two other species that are associated with mango malformation disease. Freeman *et al*. (2000) reported that *Fusarium subglutinas (Gibberella fujikuroi)* has been associated with mango floral and vegetative malformation. Britz *et al.* (2002) gave formal description for the two new texa that were named *F. mangiferae* and *F. sterilihyphosum.* Iqbal *et al.* (2006) isolated four fungi viz. *F. mangiferae, F. pallidoroseum, F. oxysporum*

and *Alternaria alternata* from malformed mango parts. Khaskheli *et al.* (2008) isolated and identified *Fusarium nivale* (Fr.) Ces., as first record in Pakistan and also the first report of its association with mango malformation in Sindh, Pakistan. However, Koch's postulates have only been completed for *Fusarium subglutinans* (Chakrabarti and Ghosal, 1989); (Kumar and Beniwal, 1992); (Ploetz and Gregory, 1993) and (Summanwar *et al*., 1966), *Fusarium oxysporum* (Bhatnagar and Beniwal, 1977) and *Fusarium nivale* (Fr.) Ces. (Khaskheli *et al.,* 2008). Yet there is some controversial regarding species identification (Ploetz, 1994) and the inoculation methods used (Kumar *et al.,* 1993). No information is available on frequency distribution of the fungi associated with mango malformation disease in Sindh. Hence the present study was taken first time to estimate the frequency distribution of the fungi associated with mango malformed tissues.

MATERIALS AND METHODS

Mango malformed samples were collected from 10 mango varieties: Desi, Dusheri, Almas, Chaunsa, Sindhri, Neelam, Langra, Swarnarika, Siroli and Fajri, while surveying from 4 mango growing districts of the Sindh, Pakistan i.e. Mirpurkhas, Hyderabad, Tando Allah Yar and Sanghar. The samples were taken from both floral and vegetative malformed types including malformed branches, malformed flowers with and without cover (layer).

Isolation and identification of disease causing fungi

Samples collected from different parts were passed through the process of isolation as described by Neergard (1979) and Pathak (1987); i.e. 100 pieces of infected primary and secondary tissues of each variety were treated with 0.1% Clorox (Sodium hypochlorite) for two minutes and rinsed twice in distilled sterile water for 2-3 minutes each. The treated tissues were dried thoroughly on sterilized blotter papers and five pieces were placed in glass petridishes containing sterilized potato-dextrose agar medium. All the petridishes were incubated at 25^0C for about seven days. The isolated fungi were identified with the help of keys (Booth, 1977); (Nelson *et al*., 1983) and (Barnett and Hunter, 1996). About 35 isolations were done throughout the experiment. The frequency of the isolated fungi in the collected samples from each locality was recorded using the following formula:

$$\text{Colonization (\%)} = \frac{\text{Number of pieces colonized with fungus}}{\text{Total number of pieces studied}} \times 100$$

The overall infection frequency (%) of all the isolated fungi was also determined on the basis of the tissues colonized by each fungus.

RESULTS AND DISCUSSION

Six fungi i.e., *Fusarium nivale, F. oxysporum, F. moniliforme, F. semitectum, Alternaria alternata* and *Aspergillus niger* were isolated from primary and secondary tissues of malformed parts of the inflorescence (Fig. 1). *F. nivale* was isolated with highest frequency from Mirpurkhas (55.00%) followed by Hyderabad (46.00%), Tando Allah Yar (35.00%) and Sanghar (30.00%), respectively (Fig. 2). Other fungi showed medium type of infection like *F. oxysporum* (18.00-28.00%), *F. moniliforme* (13.00-25.00%), and *F. semitectum* (7.00-15.00%). *Alternaria alternata* and *Aspergillius niger* responded with lowest frequency viz. 3.00-8.00% and 2.00-6.00%, respectively (Fig. 2).

It is also important to mention here that *Fusarium nivale* is the first recorded fungus from mango malformation diseases in Pakistan (Khaskheli *et al*., 2008). Kumar *et al*., (1993) reported that mango malformation was responsible for shorten inflorescence, sterility and aborted hermaphroditic flowers, and also increased male flowers in number and size. Nirenberg and O'Donell (1998) differentiated *Fusarium mangiferae* from two other species that are associated with the disease. Freeman *et al.,* (2000) showed the association of *F. subglutinas (Gibberella fijikuroi)* with floral and vegetative malformation. Ploetz *et al.* (2002) found that *Fusarium* spp. are common associated with diseases of tropical, perennial plants. Britz *et al*. (2002) described the two new taxa for *Fusarium* spp., were named *F. mangiferae* and *F. sterilihyphosum* associated with malformation disease.

Identification of *Fusarium nivale* (Fr.) Ces.

The fungus was identified as *F. nivale* on the basis of morphological characteristics like, colonies white, with little discoloration of the agar medium, conidia borne sparsely in aerial mycelium. Conidia curved, with a pointed apex, 1-3 septate and 10-30 X 2.5-5.0µ (Fig. 3).

Frequency distribution of *Fusarium nivale* (fr.) Ces.

Maximum frequency of *F. nivale* was recorded in Mirpurkhas (95.00%) and Hyderabad (93.00%) followed by Tando Allah Yar (75.35%), respectively (Table 1). Mango varieties: Desi, Dusheri and Almas showed highest infection in Mirpurkhas, Hyderabad and Tando Allah Yar ranging from 75.35-95.00%, 65.45-90.00% and 55.33-83.00%, respectively (Table 1). While medium type of infection percent was obtained in Chaunsa (50.45-65.00%), Sindhri (45.33-55.33%), Neelam (41.25-52.25%) and Langra (30.66-45.66%). The lowest colonization percent of the fungus was observed in Swarnarika (34.20-40.45%), Siroli (30.75-38.75%) and Fajri (30.66-36.30%), respectively (Table 1). The overall frequenscy of *F. nivale* on 10 mango varieties at Mirpurkhas was 60.174% followed by Hyderabad (58.431%), Tando Allah Yar (46.410%) and Sanghar (34.837%), respectively (Fig. 4).

Table 1. Frequency of *Fusarium nivale* from ten mango varieties of different localities

Variety	localities				Total	Mean
	Mirpurkhas	Hyderabad	Tando Allah Yar	Sanghar		
Desi	95.00	93.35	75.35	45.66	309.36	77.34
Dusheri	90.00	88.66	65.45	41.33	285.44	71.36
Almas	83.00	81.33	55.33	40.60	259.66	64.91
Chaunsa	65.00	64.25	50.45	38.33	218.03	54.50
Sindhri	55.33	52.33	45.33	35.30	188.29	47.07
Neelam	52.25	48.30	41.25	32.35	174.15	43.53
Langra	45.66	42.66	35.33	30.66	154.31	38.57
Swarnarika	40.45	40.33	34.20	30.15	145.13	36.28
Siroli	38.75	37.85	30.75	28.33	135.68	33.92
Fajri	36.30	35.25	30.66	25.66	127.87	31.96

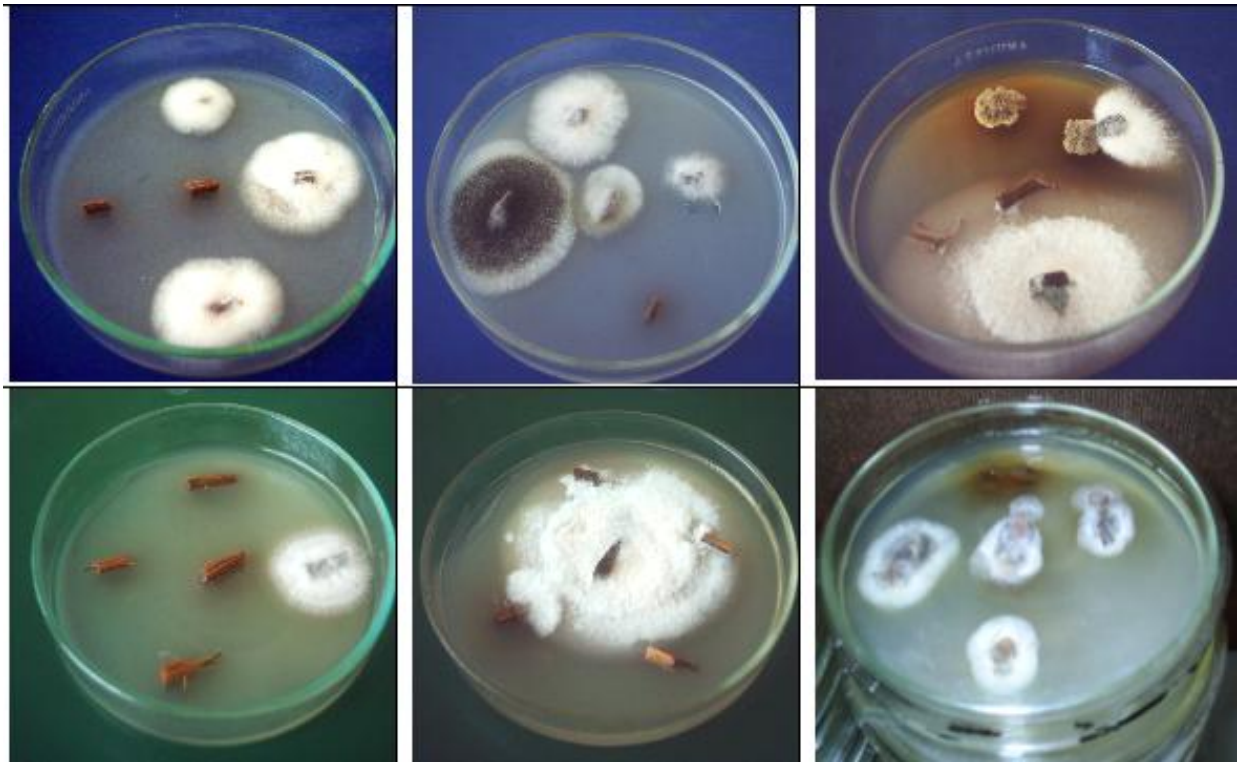

Figure 1. White mycelial growth of *Fusarium* spp. isolated from mango inflorescence tissues infected with malformation disease

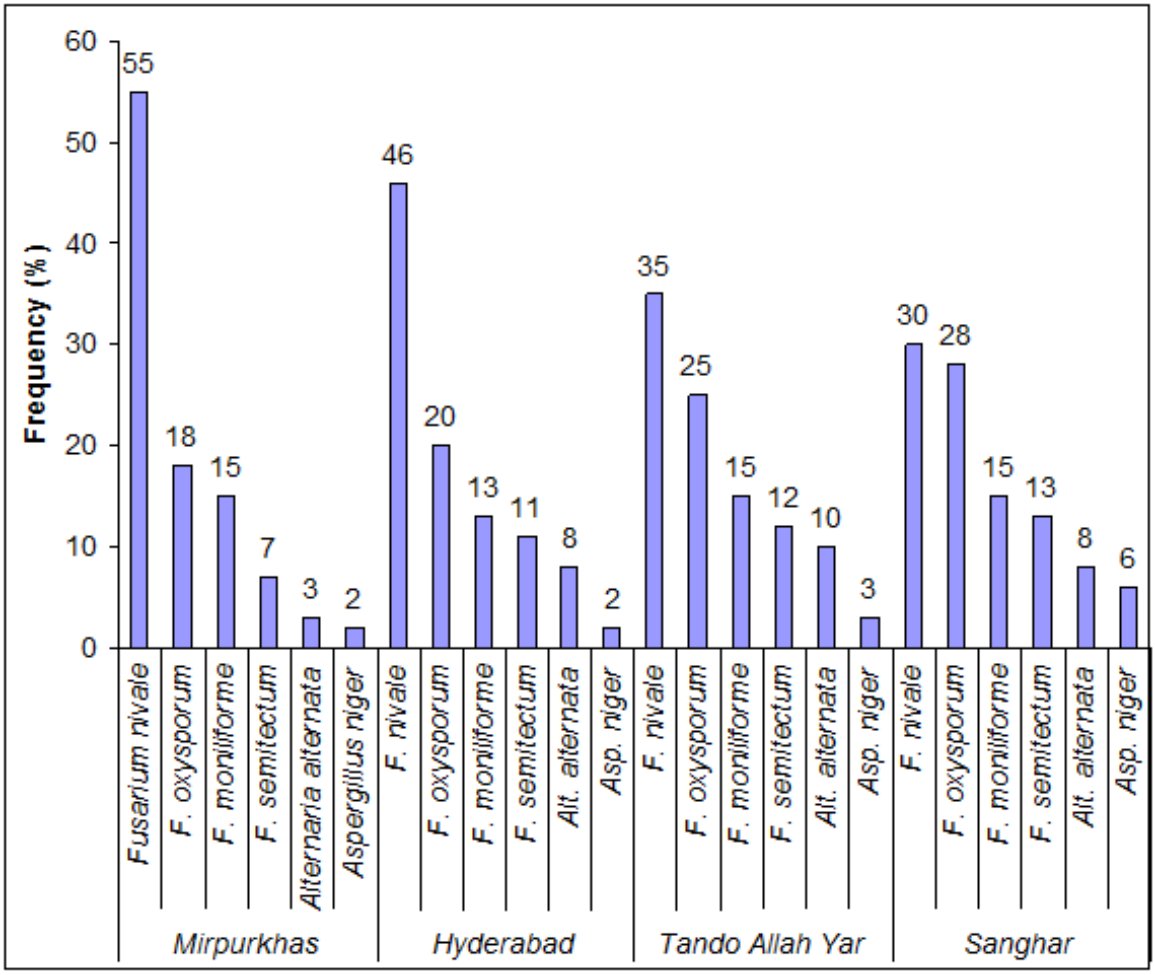

Figure 2. Fungi isolated from malformed parts of mango

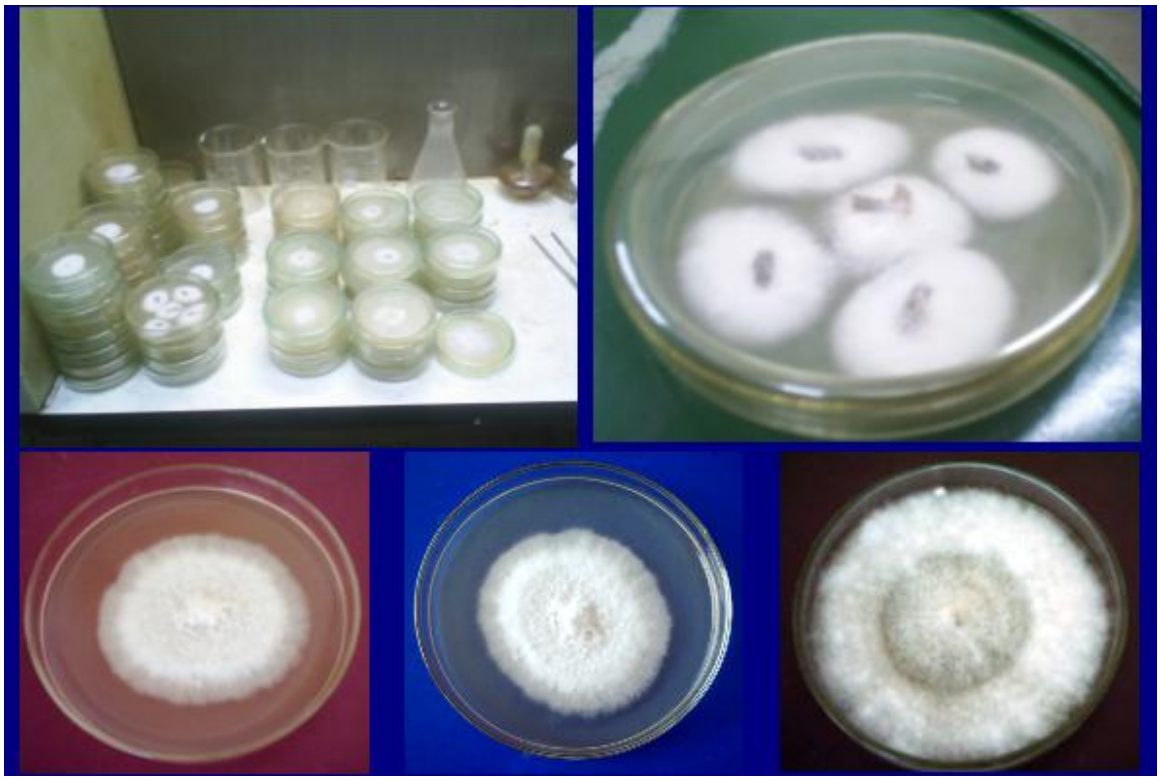

Figure 3. White mycelial colony growth of *Fusarium nivale* obtained from single spore isolation technique

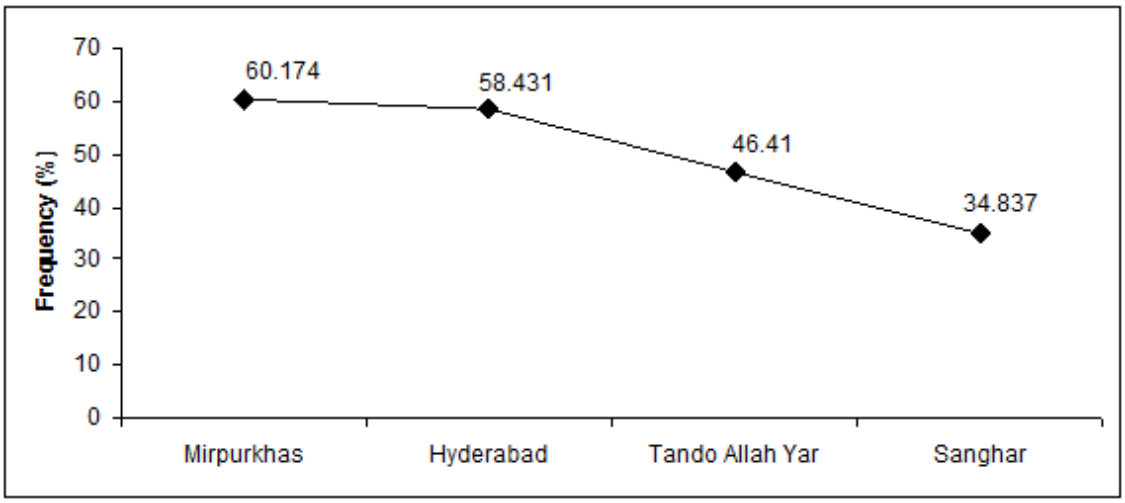

Figure 4. Mean frequency of *Fusarium nivale* at different localities from ten mango varieties

Table 2. Overall infection of different fungi isolated from malformed tissues often mango from different localities

Fungi isolated	Tissues colonized (out of 1200)	Infection frequency (%)
Fusarium nivale	985	82.08
F. oxysporum	143	11.90
F. moniliforme	85	7.08
F. semitectum	63	5.25
Alternaria alternata	45	3.75
Aspergillus niger	22	1.83

F. nivale colonized as the most frequent fungus of 82.08% colonizing 985 tissues out of 1200 (Table 2). *F. oxysporum* (11.90%), *F. moniliforme* (7.08%) and *F. semitectum* (5.25%) as compared to *Alternaria alternata* (3.75%) and *Aspergillus niger* (1.83%) respectively (Table 2). Ploetz (1994) found variation in the infection amongst the fifty five isolations of *Fusarium subglutinans*. Iqbal *et al.,* (2006) isolated *F. mangiferae, F. pallidoresum, F. oxysporum* and *Alternaria alternata* from malformed mango parts. Whereas Marasas *et al.,* (2006) found the association of *F. moniliforme* var. *subglutinans* from both vegetative and floral tissues. Lesile and Summerell (2006) found *Fusarium* spp. as commonly associated with higher plants in terrestrial ecosystem.

REFERENCES

Ahmad, F., I. A. Hafiz, A. A. Isi, S. Ahmed and M. Khan. 2002. Mango varietal susceptibility to malformation and its control. *Asion J. Plt. Sc*. 1 (2): 158-159

Barnett, H. L. and B. B. Hunter. 1996. Illustrated Genera of imperfect fungi. 4th Ed. A. P.S. Press, Sant Paul, Minnesota, USA.

Bhatnager, S.S. and S.P.S. Beniwal. 1977. Involvement of *Fusarium oxysporium* in causation of mango malformation. *Plant Dis. Rep*. 61:894-898.

Booth, C. 1977. The genus Fusarium. Commonwealth Mycological Institute. Kew, Surrey, England.

Britz, H., E. T. Steenkamp, T. A. Coutinho, B. D. Wingfield, W. F. Marasas and M. J. Wingfield. 2002. Two new species of *Fusarium* Section Liseola associated with mango malformation. *Mycologia*, 94 (4): 722-730.

Chakrabarti, D.K and S. Ghosal. 1989. The disease cycle of mango malformation induce by *Fusarium moniliforme* var. *subglutinans* and the curative effect of mangiferin-metal chelates. *J. Phytopathol*.125:218-246.

Chakrabarti, D.K and R. Kumar 1998. Mango malformation: role of *Fusarium moniliforme* and mangiferin. *Agriculture- Reviews Karnal* 19:126-136.

Freeman, S., M. Manimon and Y. Pinkas. 2000. Etiology of mango malformation disease using GUS transformants of *Fusarium subglutinans*. *Acta Hort*., 509: 751-758.

Iqbal Z, A. A. Dasti and A. Saleem. 2006. Role of *Fusarium mangiferae* in causation of mango malformation disease. *J. Res. Sc.* 17 (1): 9-14.

Khaskheli, M. I., M. A., Pathan, M. M. Jiskani, K. H. Wagan, M.H. Soomro and Gul Bahar Poussio. 2008. First Record of *Fusarium Nivale* (Fr.) Ces. associated with Mango Malformation Disease (MMD) in Pakistan. *Pak. J. Bot.* 40(5) 2641-2644.

Kumar, J. and S. P. S. Beniwal. 1992. Role of *Fusarium* species in the etiology of mango malformation. (Abstr.) Pages 17 in: Int. Mango Symp., 4th, Miami.

Kumar, J., U. S. Singh and S. P. Beniwal. 1993. Mango malformation: One hundred years of research. *Ann. Rev. Phytopathol*. 31: 217-232.

Leslie, J. F. and B. A. Summerell. 2006. The Fusarium Lab. Manual. Blackwell, Ames, IA.

Marasas, W. F. O., R. C. Ploetz, M. J. Wingfield and E. T. Steenkamp. 2006. Mango malformation disease and the associated *Fusarium* species. *Phytopathol*. 96: 667-672.

Neergaard, P. 1979. Seed Pathology. Vol. I. The MC-Millan Press Ltd., London. p.240.

Nelson, P. E., T. A. Tousson and W. F. O. Marasas. 1983. *"Fusarium species: An Illustrated Manual for Identification"*. The Penn. State Univ., Press, University Park, USA. p.123

Nirenberg, H. I. and K. O'Donnell. 1998. New *Fusarium* species and combination with the *Gibberella fujikuroi* complex. *Mycologia*. 90: 434-458.

Pathak, V. N. 1987. *Laboratory Manual of Plant Pathology*. 2nd Ed. pp:23-50, Oxford IBH Publ. Co., New Delhi.

Ploetz, R. C. and N.F. Gregory. 1993. Mango malformed in Florida: Distribution of *Fusarium subglutinans* in affected trees, and relationship among strains within and among different orchards. *Acta Hort.* 341: 388-394.

Ploetz, R. C. 1994. Distribution and prevention of *Fusarium subglutinans* in mango trees affected by malformation. *Can. J. Bot*. 72: 7-9.

Ploetz, R. C., Q. I. Zheng, A. Vazguez and M. A. Abdel Sattar. 2002. Current status and impact of mango malformation in Egypt. *Int. J. Pest Management*. 48: 279-285.

Ploetz, R. C. 2003. Diseases of tropical fruit crops. CAB Publ., Wallingford, UK.

Summanwar, A.S., S.P. Rayehandhuri and S.C. Pathak. 1966. Association of fungus *Fusarium moniliforme* sheld. With the malformation in mango. *Indian Phytopathol.* 19: 227-228.

Tahir, F. M., M. Ibrahim and K. Hamid. 2003. Effect of drought stress on vegetative and reproductive growth behavior of mango (*Mangifera indica* L.). *Asian J. Plt. Sci.* 2 (1): 116-118.

****