

**FIRST RECORD OF *FUSARIUM NIVALE* (FR.) CES.
ASSOCIATED WITH MANGO MALFORMATION DISEASE
(MMD) IN PAKISTAN**

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Abstract

During a survey of mango plantations in Sindh for investigating the association of fungi with mango malformation disease (MMD), six fungal species viz., *Fusarium nivale* (Fr.) Ces., *F. oxysporium*, *F. moniliforme*, *F. semitectum*, *Alternari alternata* and *Aspergillus niger* were isolated and identified on the basis of their colony characteristics and conidial morphology. *F. nivale* (Fr.) Ces., was predominantly isolated from malformed tissues of infected inflorescence; it produced white colonies and some discoloration of the agar medium was noticed around the growing mycelium. This is the first record of *Fusarium nivale* (Fr.) Ces., from Pakistan and also the first report of its association with mango malformation in Sindh, Pakistan.

Introduction

Mango (*Mangifera indica* L.) is one of the most important fruits of tropical and subtropical regions of the world, second major fruit crop of Pakistan and is well established in international market to earn foreign exchange. Its average yield is not appreciable due to many reasons including 81 pathogenic, physiogenic and miscellaneous diseases of mango (Pernezny & Simone, 2000). Mango malformation disease (MMD) is a serious threat to this crop (Akhtar *et al.*, 1999; Ahmed *et al.*, 2002). It occurs as vegetative as well as floral malformation; in vegetative malformation, it mostly appears on seedlings, while in floral malformation it is more prevalent in bearing mango trees. MMD causes shortened inflorescence, sterility and aborted hermaphrodite flowers and the male flowers increase in number and size (Hafiz, 1986; Kumar *et al.*, 1993; Akhtar *et al.*, 1999; Ahmed *et al.*, 2002; Iqbal *et al.*, 2006). For the last many years researchers have been studying various aspects of this disease but so far its specific cause has not been known (Kumar *et al.*, 1993). Some of the researches reported *Fusarium* spp., viz. *F. subglutinans* (*Gibberella fujikuroi* var. *subglutinans*) (Freeman *et al.*, 2000; Lahav *et al.*, 2001), *F. mangiferae* and *F. sterilihyphosum* (Britz *et al.*, 2002; Iqbal *et al.*, 2006), *F. moniliforme* var. *subglutinans* (Kumar *et al.* 1993; Akhtar *et al.* 1999; Mararsas *et al.*, 2006) as cause of mango malformation. However, Koch's postulates were only established for *F. subglutinans* (Mararsas *et al.*, 2006; Chakrabarti & Ghosal, 1989; Kumar & Beniwal, 1992; Ploetz & Gregory, 1993 and Summanwar *et al.*, 1966) and *Fusarium oxysporum* (Bhatnagar & Beniwal, 1977).

In Pakistan, Akhtar *et al.*, (1999) verified the association *F. moniliforme* and *G. fujikuroi* with the disease; yet, there are some controversies regarding species identification (Ploetz, 1994) and the inoculation methods used (Kumar *et al.* 1993). No systematic work on etiology of mango malformation is reported to have been done in Sindh province of Pakistan. The present studies were therefore carried out to investigate and ascertain the actual cause of MMD in Sindh province.

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Materials and Methods

Samples were taken from floral as well as vegetative malformed plant parts including malformed branches, malformed flowers with and without cover (layer) from Tando Qaiser, District Hyderabad. Samples collected from different parts were passed through the process of isolation as described by Neergard (1979) and Pathak (1987); wherein, 100 pieces of infected twigs were treated with 0.1% NaOCl for 2 minutes and rinsed twice in distilled sterilized water. Tissues were dried on sterilized blotter papers and 5 pieces were placed on 9cm glass Petri-dishes (Pyrex) containing sterilized potato dextrose agar medium (Saleem & Nasir, 1991). All the Petri dishes were incubated at $25\pm 1^{\circ}\text{C}$ for 7 days. The isolated fungi were identified on the basis of their colony characteristics and conidial morphology with the help of keys (Booth, 1977; Nelson *et al.*, 1983; Barnett & Hunter, 1972). For verification of the identity of the new fungus (other than already known fungi), culture obtained from a single spore of the most frequent fungus was sent in small culture tubes to Dr. J.H. Mirza, Chairman Culture Collection Laboratory, Department of Mycology and Plant Pathology, University of the Punjab, Lahore.

Results and Discussion

A total of 6 fungal species viz., *Fusarium oxysporium*, *F. moniliforme*, *F. semitectum*, *Alternaria alternata* and *Aspergillus niger* and *F. nivale* (Fr.) Ces were isolated from the malformed tissues of infected inflorescence. *Fusarium nivale* (Fr.) Ces is reported for the first time from Pakistan and this is also the first report of its association with mango malformation disease (MMD) in Sindh, Pakistan. The fungus (*F. nivale*) produces white colonies causing a little discoloration of the agar medium (Fig. 1A). Conidia are borne sparsely on aerial mycelium, they are curved with pointed apex, 1-3 septate and $10\text{-}30 \times 2.5\text{-}5.0\mu$ in size (Figs. 1B & 2).

A number of pathogens have been reported associated with MMD and possibly the causal agents of the disease but not *F. nivale*; thus it adds to the list of pathogens associated with 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; Nirenberg & O'Donnell, 1998). Akhtar *et al.*, (1999) verified the association *F. moniliforme* and *G. fujikuroi* with the disease in Pakistan. Freeman *et al.*, (2000) reported the association *F. subglutinans* (*Gibberella fujikuroi*) with floral and vegetative malformation. The presence of *F. sugglutinans* (*G. fujikuroi* var. *subglutinans*) was also confirmed as cause of mango malformation by Lahva *et al.*, (2001) while Iqbal *et al.*, (2006), isolated *F. mangiferae*, *F. pallidorseum*, *F. oxysporum* and *Alternaria alternata* from malformed mango parts. However, in all cases a confusion regarding the fungal species associated with MMD still remains (Ploetz, 1994). The present study conducted is so far the first evidence of *Fusarium nivale* (Fr.) Ces., association with MMD as the causal agent and is the first record in Sindh, Pakistan. The nature and status of MMD in different ecologies thus needs to be investigated in depth.

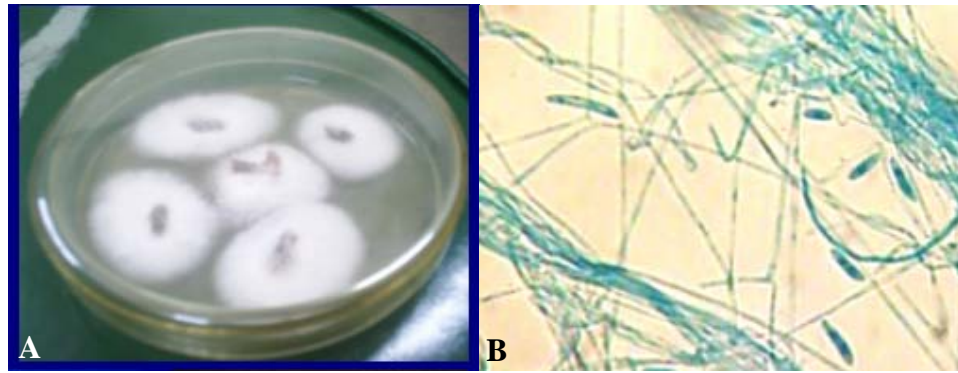


Fig. 1. Pure colonies (A) and Mycelial growth of *Fusarium nivale* (Fr.) Ces.

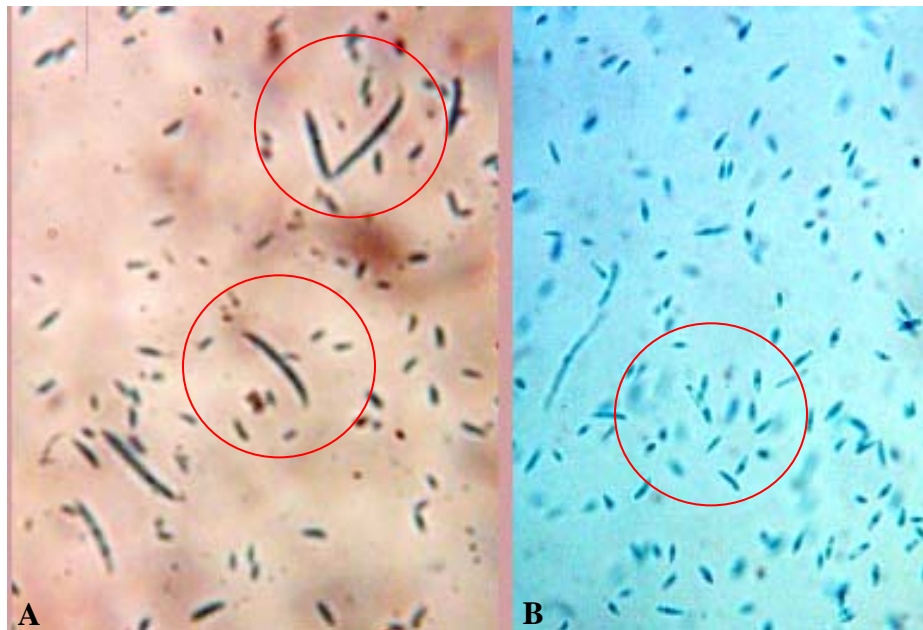


Fig. 2. Macro Conidia (A) and Micro conidia (B) of *Fusarium nivale* (Fr.) Ces.

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(Received for publication 22 January 2008)