

ISOLATION OF MANGO QUICK DECLINE FUNGI FROM MANGO BARK BEETLE, *HYPOCRYPHALUS MANGIFERAE* S. (COLEOPTERA: SCOLYTIDAE)

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ABSTRACT

The studies were carried out to isolate the mango quick decline fungi from mango bark beetle, *Hypocryphalus mangiferae* S. collected from mango orchards of Nawab pur, Lutfabad and Faiz pur bhatian, Multan, Pakistan. Different life stages of *H. mangiferae* were collected from diseased mango trees and fungal isolations were carried out from each stage as well as from different body parts (head, thorax and abdomen) of adult. Five types of fungi i.e. *Ceratocystis fimbriata*, *Lasiodiplodia theobromae*, *Fusarium* sp., *Alternaria* sp. and *Aspergillus* sp. were frequently isolated from life stages and body parts of *H. mangiferae*. However, their isolation frequency varied in different life stages and body parts. The consistent isolation of these fungi suggests their close association with *H. mangiferae* but the exact symbiotic relation between them need to be investigated.

Key words: *Hypocryphalus mangiferae*, life stages, isolation frequency, *Ceratocystis fimbriata*, *Lasiodiplodia theobromae*, *Fusarium* sp.

INTRODUCTION

Mango, *Mangifera indica* L. is the main fruit of tropical and subtropical parts of the world. In Pakistan, mango is cultivated on an area of 167.5 thousand hectares with 1,732 thousand tons average production (Balal *et al.* 2011). However, its production is decreasing due to various factors mainly insect pests and diseases (Ploetz *et al.* 1996; Talpur and Khuhro 2003).

Presently, mango industry is facing a serious threat of mango quick decline (MQD) resulting in huge losses all over the world. It has been reported from various districts of Pakistan with incidence of 10-28% in Punjab (Asif *et al.* 2011). Thousands of mango trees in Pakistan have been affected by this destructive disease which has lowered the mango production (Jiskani 2006). The tree infected with MQD shows the symptoms of gummosis, canker formation, bark splitting, drying of twigs, branches and curling of leaves (Masood *et al.* 2010). Al Adawi *et al.* (2006) and Al-Subhi *et al.* (2006) reported the association of three fungal pathogens, *Ceratocystis fimbriata*, *C. omanensis*, and *Lasiodiplodia theobromae*, with the MQD in Oman. Van Wyk *et al.* (2007) suggested *Ceratocystis manginecans* as the causal organism of MQD in Oman and Pakistan. *Ceratocystis manginecans* is not a new species and is closely related to *C. fimbriata* (VanWyk *et al.* 2007). Kazmi *et al.* (2005) reported the association of *Fusarium* sp. in addition to above three fungal pathogens with MQD.

Hypocryphalus mangiferae has been found to be consistently associated with MQD infected trees and considered as the vector of this disease (Al Adawi *et al.* 2006; Saeed and Masood 2008; Masood *et al.* 2009

Masood *et al.* 2010). This beetle usually prefers weakened and stressed trees (Wood 1982; Masood *et al.* 2010). The adults attack the mango trees and penetrate the trunk, limbs and branches. After making holes in the bark, the adult females make irregular galleries and lay about 42.3 ± 9.90 eggs in 4.2 ± 1.41 batches. *H. mangiferae* overwinter as adult under the bark from late November to late February. It resumes activity in January but maximum activity has been found during May-August (Masood *et al.* 2009) which is critical period for the dissemination of MQD in mango orchards.

As *H. mangiferae* has been proved previously as a vector of MQD in mango orchards (Saeed and Masood 2009) and various fungi have been isolated only from the adult stage of the *H. mangiferae*. However, in the present experiment we were interested to study different life stages of *H. mangiferae* with respect to fungi isolations. These measures allowed us to answer the following questions: (a) Do MQD causing fungi found in different life stages of *H. mangiferae*? (b) What are the relative proportions or frequencies of MQD fungi in different life stages and body parts of adult *H. mangiferae*?

MATERIALS AND METHODS

Collection of samples: The experiments were conducted in April-July 2008. Life stages of *H. mangiferae* and sapwood samples were collected from three severely infested orchards of Nawab pur, Lutfabad and Faiz pur bhatian, Multan, Pakistan. For this, twelve bark beetle infested logs (20×25cm) were taken from each orchard with surface sterilized axe and transported to mango laboratory, University College of Agriculture, Bahauddin

Zakariya University, Multan. Eggs, grubs, pupae and adults of *H. mangiferae* were removed from field collected logs by gently peeling the bark with a sharp knife. About 200 adults, 100 grubs, 100 pupae and five egg colonies were separated from logs of each orchard and kept individually in sterile micro tube with the help of camel hair brush. These micro tubes were stored at 5°C until isolation. We also took sapwood samples (10×15cm) from underneath of the galleries of *H. mangiferae* for fungal isolation.

2.3. Processing of samples: To isolate fungi, specimens were surface sterilized for 1 min. with 1% sodium hypochlorite solution (NaOCl) followed by three serial washings with distilled water for 1 min each. A total of 25 adults, 60 eggs, 30 grubs and 30 pupae from each orchard were used for fungal isolation. For isolation of fungi from different parts of adult *H. mangiferae*, 40 surface-disinfected specimens were aseptically cut into head, thorax and abdomen under stereomicroscope (Haanstad and Norris 1985). All these main samples were divided into sub samples i.e. 5 sub samples for adults, 12 sub samples for eggs and so on. Each sub sample (containing 5 specimens) was crushed in micro tube (1.5ml) with the help of glass rod and 0.5 ml homogenate was spread over potato dextrose agar (PDA) plate in 9cm Petri dish.

In order to isolate fungi from sapwood under galleries of *H. mangiferae*, 180 sub samples (each measuring 5mm) from the every orchard were cut with sterile scissor. These sub samples were placed on the surface of PDA after surface disinfection as describe earlier.

All isolations were carried out on 2% PDA supplemented with 1g streptomycin as an antibiotic. Petri dishes were incubated at 25±2°C for 30 days and periodically observed for fungal growth. A small piece of each fungal species was taken and placed on PDA under aseptic conditions for further purification and identification of fungi.

Identification of fungi: Isolated fungi were identified on the basis of their colony characteristics and conidial morphology. Slides were prepared by taking a minute portion of fungal colony from the growing margin with the help of inoculating needle and identified by viewing under the microscope at 40 and 100X lenses.

Data analysis for fungal isolation: The occurrence frequencies of each fungus isolated from life stages, body parts and sapwood under galleries of *H. mangiferae* were calculated by using the formula as described by Yamaoka *et al.* (1997).

$$F = (NF/NT) \times 100$$

Where F represents the frequency of occurrence (%) of a fungus, NT is the total number of samples from which isolations were carried out, and NF is the total

number of samples from which a particular fungus was isolated.

RESULTS

Isolation of fungi: Five types of fungi were isolated, *C. fimbriata* (fig. 4), *L. theobromae* (fig. 5), *Fusarium* sp. (fig. 6), *Alternaria* sp. (fig. 7) and *Aspergillus* sp. (fig. 8) fungi, from our different treatments (life stages, body parts and sapwood under galleries of *H. mangiferae*). Since three types of fungi (*C. fimbriata*, *L. theobromae*, *Fusarium* sp.) isolated in our study being already reported as causal agents of mango quick decline (Kazmi *et al.* 2005; Al Adawi *et al.* 2006; Al-Subhi *et al.* 2006; Massod *et al.* 2010) so we have only discussed these fungi. The isolation frequency of the remaining fungi is also given in the fig. (1, 2, 3).

Life stages of *H. mangiferae*: Among the MQD fungi isolated from life stages, the frequency of *Fusarium* sp. (3.33-13.33%) was higher compared to that of *C. fimbriata* (2.22-6.67%) and *L. theobromae* (0.55-1.33%). All the three MQD fungi were mainly detected from adults and grubs (1.33-13.33%, 3.33-4.44%) followed by eggs and pupae (0.55-3.33%, 0.00-2.22%) respectively (Fig 1).

Body parts of adult *H. mangiferae*: Data of isolation of fungi from body parts of adults revealed that *Fusarium* sp. (2.50-8.33%) is the main fungi as compared to *C. fimbriata* (1.67-4.76%) and *L. theobromae* (0.00-1.67%). The three MQD fungi were mostly obtained from abdomen (1.67-8.33) followed by head (1.67-2.50). None of these fungi were isolated from thorax (fig. 2).

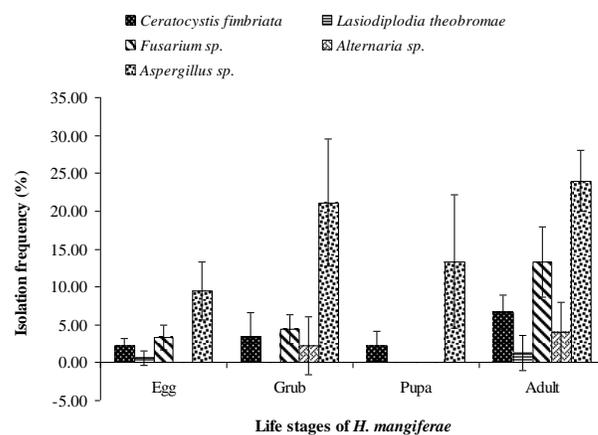


Fig. 1. Frequency of fungi isolated from life stages of *Hypocryphalus mangiferae*

Sapwood under galleries of *H. mangiferae*: The fungal isolation from sapwood under galleries revealed similar fungi as isolated from life stages and body parts of *H. mangiferae*. Frequency of *Fusarium* sp. (19.81%) was

higher in comparison with *C. fimbriata* (5.18%) and *L. theobromae* (2.77%) (fig. 3).

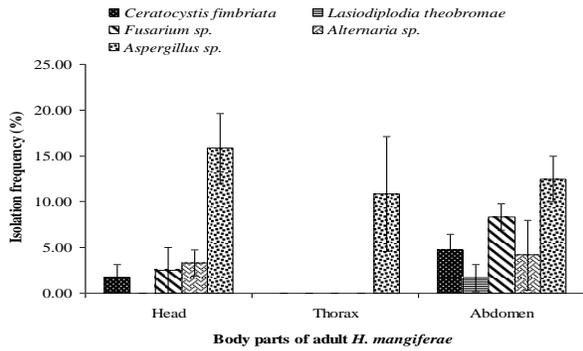


Fig. 2. Frequency of fungi isolated from body parts of adult *Hypocryphalus mangiferae*

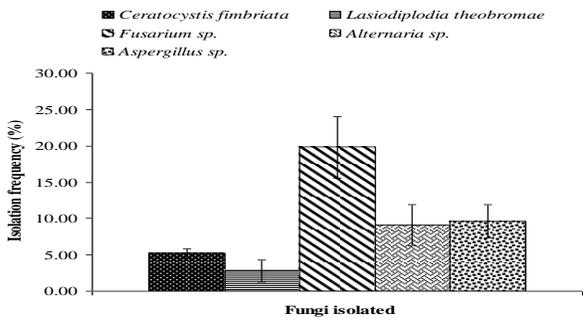


Fig. 3. Frequency of fungi isolated from sapwood under galleries of *Hypocryphalus mangifera*



Fig. 4. *C. fimbriata*

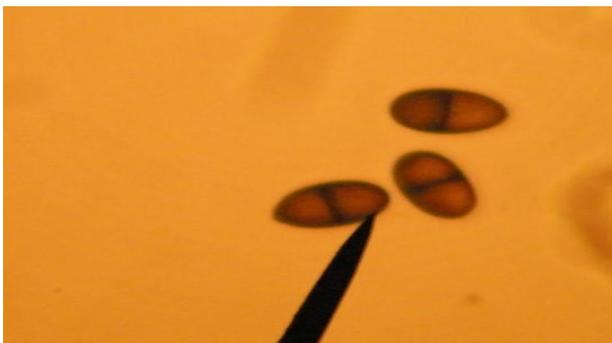


Fig. 5. *L. theobromae*



Fig. 6. *Fusarium sp.*



Fig. 7. *Alternaria sp.*

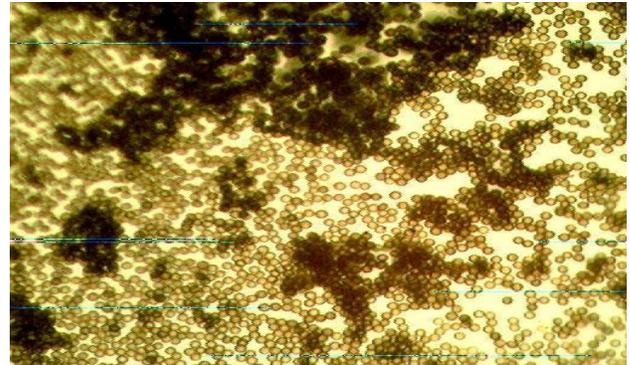


Fig. 8. *Aspergillus sp.*

DISCUSSION

Mango quick decline is a very destructive disease of mango orchards throughout the mango growing areas of the world. In this study, three reported MQD fungi (*C. fimbriata*, *L. theobromae* and *Fusarium sp.*) were found to be associated with eggs, grubs, pupae and adults of *H. mangiferae* (Fig.1). Consistent isolation of *Fusarium sp.* and *C. fimbriata* from different life stages of *H. mangiferae* in our study indicate a close association of these two fungi with *H. mangiferae*. In spite of less frequent isolation of these two fungi from *H. mangiferae* stages, there might be a symbiotic

relationship between them as found in other bark beetle species, *Dendroctonus frontalis* Z (Barras 1973; Goldhammer *et al.* 1990). Bark beetles transport the fungi to new host (Barras and Perry 1972; Harrington 1993) and in turn these fungi make new host more suitable for reproduction of the bark beetles (Leufven 1991; Paine *et al.* 1997; Reynolds 1992; Solheim and Safranyik 1997; Krokene and Solheim 1996, 1998).

Our results clearly showed the highest frequency of occurrence of MQD fungi in adults and grubs as compared to eggs and pupae. This difference may be related to the mobility and activity of the two stages. Adults and grubs are the damaging stages and they might utilize MQD fungi along with phloem sap as their food. This might be the reason of maximum isolation of MQD fungi from adults and the grubs.

However, when we compared the isolation frequency from adults and grubs, MQD fungi were more frequently isolated from adults. This frequent isolation of fungi from adults might be due to the fact of grazing on the fungal mycelia after emergence from the pupa. As these beetles emerge from diseased trees, they are loaded with spore of fungi on or in their bodies (Harrington 2005). When they bore in the bark of other healthy or stressed trees to lay eggs, they make an egg chamber in the gallery (Wood 1982). During this process, they also deposit spores of fungi in the egg chamber. The fungi deposited in the egg chamber start growing under the bark and as the grubs emerge, they start grazing on fungal mycelia along with wood and phloem sap (Harrington 2005). *C. fimbriata* was isolated with highest frequency from adults showing its capacity to transmit *C. fimbriata* to other mango plants as previously reported by Wood, 1982; Flower *et al.* 2001; Al- Adawi *et al.* 2006; Saeed and Masood 2008.

Consistent isolation of MSDS fungi from abdomen in our study (Fig. 2) might indicate that *H. mangiferae* either feeds on these fungi to improve its nutrition and reproduction or harbour them to survive harsh environmental conditions. These findings address the need to find out exact symbiotic or mutualistic relationship of MQD fungi with *H. mangiferae*.

Acknowledgements: This research was funded by Higher Education Commission (HEC) Indigenous Scholarship Batch IV and mango ASLP Australia project. Authors are thankful to Muhammad Tariq Malik (Assistant Plant Pathologist, Mango Research Station Shujaabad) and Faisal Sohail Fateh (Scientific Officer, NARC Islamabad) for their help in identification of fungi. Authors are also thankful to Hafiz Azhar Ali Khan Ph.D Scholar Agri-Entomology for his help in this research.

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