INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 2, p. 192-198, 2015

RESEARCH PAPER OPEN ACCESS

Mango Inflorescence Midge (*Erosomyia indica* Felt) as possible vector of *Fusarium mangiferae* Britz, the causal agent of Mango Malformation Disease

Usman Atif, Syed Atif Hasan Naqvi*, Rashida Perveen

Department of Plant Pathology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan, Pakistan

Key words: *Mangifera indica* L., MMD insect vector, MMD epidemiology, MMD dissemination.

http://dx.doi.org/10.12692/ijb/7.2.192-198 Article published on August 29, 2015

Abstract

Mango is a matchless species in fruit trees with respect to specific nature, growth and diversity. This current study was conducted to determine the potential role of mango inflorescence midge as a possible vector of the *Fusarium mangiferae* Britz. The pathogen was isolated under *in-vitro* on potato dextrose agar media from various samples of the symptomatic, asymptomatic inflorescence and mango inflorescence midge samples. The results of the isolation of *F. mangiferae* Britz from symptomatic inflorescence showed maximum recovery from treatment 04, with 83.77% infection frequency. *Alternaria alternata* ranked second with infection frequency 35.55%. Similarly, the isolation frequency of *F. mangiferae* Britz from asymptomatic samples was calculated 65.92, 65.92 and 62.25% respectively, from treatment 03, 05 and 04 and *A. alternata* with 35.55% infection frequency. Isolation frequency of *F. mangiferae* Britz from mango inflorescence midges (*Erosomyia indica* Felt) showed maximum recovery of the problematic fungi i.e., *F. mangiferae* Britz from treatment 04 and 03 and 05 with 96.55, 93.44 and 84.44% respectively. *F. mangiferae* Britz was the only isolated fungi from the mango midge samples which showed this insect to be the vectors of the fungi as a source of dispersal in the orchards of mango. The present study may be concluded with the fact that *E. indica* Felt is the possible vector of the *F. mangiferae* Britz which carry it towards the healthy inflorescence from the diseased ones and the infectivity and dominant association of *F. mangiferae* Britz with malformed tissues.

* **Corresponding Author:** Syed Atif Hasan Naqvi ✉ atifhasanshah@hotmail.com

Introduction

Mango (*Mangifera indica* L.) is unique specie with respect to growth, nature and peculiar characteristics. In Pakistan, total area under fruit cultivation is 853.4 thousand hectares with production of 7178.8 thousand tonnes while area under mango cultivation is 167.5 thousand hectares with production of 1.732 thousand tonnes being the second major fruit crop of Pakistan after citrus; ranked fourth in the world for its production (FAO, 2014). Like other crops, it is prone to numerous biotic and abiotic factors being major obstacle to mango production. In biotic problems, Mango Malformation Disease has become a crux with yield losses ranging from 80-to-100% (Ploetz and Gregory, 1993). This century's old problem inflicts enormous losses every year. According to Singh *et al.* (1961), trees between 4-to-8 years age suffer the most (90.9%) from vegetative malformation. Since first record by Maries from Bihar (Watt, 1891), it has been reported from Pakistan, India, Egypt, South Africa, Brazil, Israel, Central America, Mexico, U.S.A, Sudan, Cuba, Australia, Bangladesh and United Arab Emirates. As malformed inflorescence fails to produce fruits, the damage of individual tree may vary from 50-80% and in severe cases the loss may be almost total (Summanwar, 1967). Two types of malformation viz., vegetative and floral have been characterized with similar etiology (Kumar and Beniwal, 1987a). The disease affects vegetative shoots of juvenile plants causing severe damage in nurseries. It also affects floral panicles causing deformation and hypertrophy (Ploetz, 1994). Malformation is noticed on seedlings, saplings and floral organs. Floral malformation attacks inflorescences on mature plants. Cultivar susceptibility varies greatly depending upon the variety, age of the tree and prevailing environmental conditions. The flower malformation amounted to 54% with malformation severity of 17% (Sao-Jose *et al.*, 2000). Etiologies like viral (Das *et al.*, 1989), acarological (Singh *et al.*, 1961) and physiological (Sattar, 1946) were claimed previously but rejected due to lack of etiological association. Examination of *Fusarium* strains isolated from malformed inflorescences of diverse international origins has explored new taxa; *F. mangiferae* relates to strains previously identified as *F. subglutinans* and regarded responsible for causing MMD worldwide (Britz *et al.*, 2002). Isolations from malformed parts have ever displayed the dominance of *F. mangiferae* Britz. The fungus *F. mangiferae* Britz was found widely distributed in symptomatic tissues of mango obtained from diverse origins showing upto 97.0% infection (Iqbal *et al.*, 2003). Freeman *et al.* (2004) confirmed *F. mangiferae* Britz as the etiological agent of MMD by artificial inoculations. Conidia of the pathogen were reduced quickly in soil under controlled laboratory and field conditions. MMD is the major impediment to the establishment of economically profitable orchards. It is intriguing the scientists because of its destructive and widespread nature. Gradual and steady dissemination has made it an insuperable obstacle. It is also known as a century old problem of mango. The major objective of study was to glean an insight into association of different fungi with malformed tissues and to determine the possible role of Mango midges (*E. indica* Felt) as a carrier of *F. mangiferae* Britz through isolation of the fungi from the vector samples.

Materials and methods

Study site

The current research was conducted at experimental orchard and the Laboratory of Plant Pathology section, Mango Research Institute located at (30.1977° N, 71.4696° E, 710m elevated from sea level), Old Shujabad Road, Multan-Pakistan, from February to May 2014.

Experimental design

The study was carried out in a randomized complete block design in experimental orchard of the MRI, Multan with three blocks irrespective of the mango cultivar grown in that specific block. Each block contains ten mango plants and among them five were randomly selected for sampling of symptomatic and asymptomatic inflorescence and mango inflorescence midge and considered as a treatment. Hence there were five treatments with three replicates. All the samples were collected in the month of March and

preserved at 4°C for *in-vitro* studies.

In vitro experimentation

The studies to ascertain the association of different fungi with the malformation affected mango inflorescence were conducted under the controlled conditions with the collection of 15 samples of each symptomatic and asymptomatic inflorescence and mango inflorescence midges (*E. indica*) from each block. Immediately after collection of samples, they were kept into icebox to avoid heating during transit and ensure maximum recovery of fungus while the samples of collected mango inflorescence midges were kept into glass vials with caps and brought to Plant Pathology Laboratory of MRI, Multan. Five pieces, 3-5 mm long were excised from 45 samples making a total of 225 bits and the midges were crushed in the pestle and mortar for the isolation of fungi with the same number of pieces. Bits of both samples were surface disinfested in 1% Sodium hypochlorite (NaOCl) solution for two minutes, rinsed twice with sterilized distilled water, dried on sterilized blotting papers and placed in sterilized 9 cm Pyrex glass Petri dishes containing Potato Dextrose Agar Medium (Akhtar, 2000). The plates containing samples pieces were incubated at 25°C temperature receiving the illumination of 600 Lux. The plates were examined after 6-7 days of incubation. The tissue bits yielding different fungi were undergone identification based on characteristics specific for the fungus (Nelson *et al.*, 1983).

Infection frequency

The infection frequency (%) of the isolated fungi was calculated by the formula described as: Infection frequency % = (number of samples colonized / total number of samples incubated × 100.

Statistical analysis

The collected datasets of isolation frequency of fungus was subjected to analysis of variance (ANOVA) using (SAS® 2002). Treatments means were compared by using the Fisher's least significant differences (LSD) at ($P = 0.05$).

Results

The results of the study showed that maximum recovery of the problematic fungi i.e., *F. mangiferae* Britz observed from symptomatic inflorescence were recovered from treatment 04, with 83.77% followed by treatment 01 with 75.55% infection frequency. *A. alternata* ranked second with infection frequency 35.55% from treatment 01, 02 and 05, respectively (Table 1).

Table 1. Isolation frequency of *Fusarium mangiferae* Britz from symptomatic inflorescence samples.

Treatment	Nature of sample	Fungi isolated	Infection frequency (%)
01	Inflorescence	*Fusarium mangiferae*	75.77 ± 0.33 b
		Alternaria alternate	35.55 ± 1.11 d
02	Inflorescence	*Fusarium mangiferae*	35.55 ± 1.33 d
		Alternaria alternate	35.55 ± 1.63 d
03	Inflorescence	*Fusarium mangiferae*	72.81 ± 1.15 b
04	Inflorescence	*Fusarium mangiferae*	83.77 ± 1.55 a
05	Inflorescence	*Fusarium mangiferae*	67.77 ± 1.11 c
		Alternaria alternate	35.55 ± 1.13 d
LSD*	-------	-------	4.51
DF*	-------	-------	08
EMS*	-------	-------	1.01

Means followed by the same letter in infection frequency (%) column are not statistically different at ($P= 0.05$),

*LSD= Least significant difference

*DF= Degree of freedom

*EMS= Error mean square.

The results of the isolation frequency of *F. mangiferae* Britz, from asymptomatic samples showed that maximum recovery was made from the treatment 03 and 05 followed by 04 with 65.92, 65.92 and 62.25% respectively. *Alternaria alternata* ranked second with infection frequency 35.55% from treatment 01, 02 and 05, respectively (Table 2).

Table 2. Isolation frequency of *Fusarium mangiferae* Britz from asymptomatic inflorescence samples.

Treatment	Nature of sample	Fungi isolated	Infection frequency (%)
01	Inflorescence	*Fusarium mangiferae*	46.21 ± 0.54 c
		Alternaria alternata	35.55 ± 1.11 d
02	Inflorescence	*Fusarium mangiferae*	35.55 ± 1.00 d
		Alternaria alternata	35.55 ± 1.09 d
03	Inflorescence	*Fusarium mangiferae*	65.92 ± 0.74 a
04	Inflorescence	*Fusarium mangiferae*	62.25 ± 0.58 b
05	Inflorescence	*Fusarium mangiferae*	65.92 ± 1.74 a
		Alternaria alternata	35.55 ± 1.11 d
LSD*	-------	-------	2.53
DF*	-------	-------	08
EMS*	-------	-------	1.81

Means followed by the same letter in infection frequency (%) column are not statistically different at (*P= 0.05*)

*LSD= Least significant difference

*DF= Degree of freedom

*EMS= Error mean square.

The results of the isolation frequency of *F. mangiferae* Britz, from mango inflorescence midges (*E. indica* F.) samples showed only the maximum recovery of the problematic fungi i.e., *F. mangiferae* Britz was made from the treatment 04 and 03 followed by 05 with 96.55, 93.44 and 84.44% respectively. Besides the *F. mangiferae* Britz, no fungi was isolated from the midges samples which showed that this insect is the vectors of the *F. mangiferae* Britz, which not only carry the pathogen towards the healthy inflorescence from the diseased inflorescence but also a source of pathogen dispersal in the orchards of mango (Table 3).

Table 3. Isolation frequency of *Fusarium mangiferae* Britz from mango inflorescence midge samples.

Treatment	Nature of sample	Fungi isolated	Infection frequency (%)
01	*Erosomyia indica*	*Fusarium mangiferae*	77.43 ± 0.33 d
02	*Erosomyia indica*	*Fusarium mangiferae*	71.99 ± 0.33 e
03	*Erosomyia indica*	*Fusarium mangiferae*	93.44 ± 0.11 b
04	*Erosomyia indica*	*Fusarium mangiferae*	96.55 ± 0.11 a
05	*Erosomyia indica*	*Fusarium mangiferae*	84.44 ± 1.11 c
LSD*	-------	-------	1.55
DF*	-------	-------	08
EMS*	-------	-------	0.68

Means followed by the same letter in infection frequency (%) column are not statistically different at (*P= 0.05*),

*LSD= Least significant difference

*DF= Degree of freedom

*EMS= Error mean square.

Discussion

Malformation is a serious problem of mango industry and has become a limiting factor in the establishment of economically viable orchards. Despite hectic efforts, complete control has not been achieved yet. Floral malformation is most important because it directly hits the yield of the plants leaving unproductive inflorescences. The results of the study showed that maximum recovery of the problematic fungi i.e., *F. mangiferae* Britz observed from symptomatic inflorescence were recovered from treatment 04, with 83.77% followed by treatment 01 with 75.55% infection frequency. These findings are corroborated by the recent literature (Freeman *et al*., 2004). Ploetz and Gregory (1993) isolated *F. subglutinans mangiferae* as predominant fungus from malformed Keitt panicles in three orchards. Britz *et al*. (2002) reported various fungi including *F. oxysporum*, *Botryodiplodia* sp., and *F. roseum* at much lower frequencies from the symptomatic diseases inflorescences. Extensive recovery of *F. mangiferae* as a causal agent from malformed tissues of different cultivars grown in diverse agro climatic zones of the world has been proved (Ploetz *et al*., 2002). Although *F. pallidoroseum* exceeded *A. alternata* and *F. equiseti* in tissue infection but it is worth mentioning that it is a saprophytic fungus on mango. *A. alternata* is often found as a common contaminant. *F. equiseti* is identified in rare cases from mango (Iqbal *et al*., 2003). Although some information on etiological relationship of *F. mangiferae* was previously available in the world literature but confused citations caused ambiguity regarding the cause of the disease. A novel experiment by Freeman *et al*. (1999) unequivocally confirmed the causal relationship between *F. mangiferae* and MMD, fulfilling Koch's postulates for both forms of the disease. Previously many attempts to prove pathogenicity of MMD in various countries of the world proved futile due to dearth of knowledge on mechanism of action of the fungus *F. mangiferae* and physiology of pathogenesis. The normal practice of pathogenicity is to spray inoculum or make a slit into which a fungal disk is placed and kept under humid conditions (Summanwar *et al*. 1966). Following infection by slit inoculation, a large amount of mangiferin is accumulated at the site of wound and kills the pathogen. So symptoms of malformation are not manifested.

This is because in the old literature, *Fusarium* sp. has been negated as the causal organism of MMD due to absence of typical symptoms and repeated unsuccessful attempts to reproduce disease by artificial inoculation (Chakrabarti, 1996). Buds are potential infection sites and finding a wound or avenue caused by mites, mechanical means or environmental factors, fungus enters the tissues. A single macroconidium which attacks the buds can cause infection. Proliferation starts and the cells of the buds are turned to malformed condition. In the beginning symptoms remain latent but with the passage of time with the massive production of macroconidia, typical symptoms of mango malformation are produced. Symptoms are only produced by wound inoculation (Ploetz and Gregory, 1993).

Attempts to produce disease by spore spray proved futile. Good orchard management occupies paramount importance in this context and plays a vital role in checking the disorder (Noriega-Cantu *et al*., 1999). The results of these studies will be helpful for future statistics, management, forecasting and experimental designs.

Conclusion

F. mangiferae Britz is the possible vector of mango malformation diseases and mango inflorescence midges are the potential vector of the fungus *Fusarium mangiferae* in the mango orchards.

Acknowledgements

The authors gratefully acknowledge the provision of research facilities by Mango Research Institute, Multan.

Abbreviations

MMD= Mango malformation disease; MIM= Mango inflorescence midge.

References

Akhar KP. 2000. Fresh potato extract the best source for the growth of *Colletotrichum gloeosporioides* causing anthracnose of mango and *Fusarium subglutinans* isolated from malformed inflorescence of mango. Pakistan Journal of Phytopathology **12,** 134-136.

Food and Agricultural Organization of the United Nations. 2014. Crops. Primary & Domain= SUA. Rome. http://apps.fao.org./lim500/nphwrap.plFAOSTATDatabase/Production

Britz H, Steenkamp ET, Coutinho TA, Wingfield BD, Marasas WFO, Wingfield MJ. 2002. Two new species of *Fusarium* section Liseola associated with mango malformation. Mycologia **94,** 722-730.

Chakrabarti DK. 1996. Disease Scenario in Crop Protection, Vol.1, Delhi, India: International Books and Periodical Supply Service. **49**-59 p.

Das GC, Rao DP, Lenko PC. 1989. Studies on mango malformation in mango clone- Chiratpudi. Acta Horta **231,** 866-872.

Freeman S, Maimon M, Pinkas Y. 1999. Use of GUS transformants of *Fusarium subglutinans* for determining etiology of mango malformation disease. Phytopathology **89,** 456-461.

Freeman S, Gueta DK, Korolev N, Sztejnberg A. 2004. Epidemiology and survival of *Fusarium mangiferae*, the causal agent of mango malformation disease. Acta Horta **645,** 487- 491.

Iqbal Z, Saleem A, Dasti AA. 2003. Comparative distribution of *Fusarium subglutinans* in malformed and non-malformed tissues of mango. Pakistan Journal of Phytopathology **15,** 17-20.

Kumar J, Beniwal SPS. 1987a. A method of estimating cultivar susceptibility against mango malformation. Tropical Pest Management **33,** 208–11.

Kumar J, Singh US, Beniwal SPS. 1993. Mango malformation: One hundred years of research. Annual Reviews of Phytopathology **3,** 217–32.

Nelson PE, Tousson TA, Marasas WFO. 1983. *Fusarium* species: An illustrated Manual for identification, USA: The Penn. State University Press, University Park, P 123.

Noriega–Cantu DH, Teliz D, Aguilera GM. 1999. Epidemiology of Mango Malformation in Guerrero, Mexico, with traditional and integrated management. Plant Disease **83,** 223–8.

Ploetz RC, Gregory N. 1993. Mango malformation in Florida: Distribution of *Fusarium subglutinans* in affected trees, and relationships among strains within and among different orchards. Acta Horta **34,** 388-394.

Ploetz RC. 1994. Distribution and prevalence of *Fusarium subglutinans* in mango trees affected by malformation. Canadian Journal of Botany **72,** 7-9.

Ploetz RC, Zheng Q, Vazquez A, Sattar MAA. 2002. Current status and impact of mango malformation in Egypt. International Journal of Pest Management **48,** 279-285.

Sattar A. 1946. Diseases of mango in Punjab. Punjab Fruit Journal **10,** 56-58.

Singh K, Jawanda KJS. 1961. Malformation in mangoes. Punjab Horticulture Journal **1,** 18–22.

Singh LB, Singh SM, Nirvan RS. 1961. Studies on mango malformation. Review, symptoms, extent, intensity and cause. Horticulture Advances **5,** 197-217.

Summanwar AS, Raychaudhuri SP, Pathak SC. 1966. Association of fungus *Fusarium*

moniliforme with malformation in mango. Indian Phytopathology **19,** 227-228.

Summanwar AS. 1967. Mango malformation, serious economic consequences. Horticulture **1,** 12–6.

Sao JAR, Souza SE, Pina AV, Ataide EM. 2000. Incidence and severity of mango malformation in Bahia State, Brazil. Acta Horta **509,** 765–771.

SAS Institute. 2002. Version 8.00 SAS Institute Inc; Cary, North Carolina, USA.

Watt G. 1891. Dictionary of the economic products of India. The Mango Tree **5,** 149.