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Effect of temperature and relative humidity on growth and sporulation of *Fusarium mangiferae* under *in vitro* conditions

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ABSTRACT

An experiment was conducted during 2008-2009 under *in vitro* to find out the optimum conditions that are favorable for growth and sporulation of *Fusarium mangiferae*. with varying temperatures ranging from 10 to 40 ± 1°C with 5°C fraction at two levels of relative humidity (35 ± 2 and 65 ± 2 %). The growth of *F. mangiferae* was observed after 3, 5, 7, 9, and 12 days of incubation. The spore count was done on the 12th day using haemocytometer. The fungal growth was observed at 20-30°C.There was no growth of fungus at temperatures below 10 and above 40°C. The temperatures 27°C followed by 25°C with 65% relative humidity were found optimum for better growth and sporulation of *F. mangiferae*. Temperature, relatively humidity and the interaction effects are highly correlated. The result of the present studies indicates that *F. mangiferae* may be responsible for inducing the symptoms of malformation disease in mango at 25 to 27°C with 60-65% relative humidity.

Key words: *Fusarium mangiferae*, temperature, relative humidity, spore count.

INTRODUCTION

Mango malformation is a serious threat to mango cultivation and economic losses of up to 60% have been reported in different commercial varieties in India. It causes gross deformation of vegetative and floral tissues in mango (Ploetz, 8). Affected flowers are either sterile or abort shortly after fruit set; as a consequence fruit yield was significantly reduced (Zheng and Ploetz, 11). The etiology of malformation has been a contentious issue and a wide range of biotic and abiotic factors have been reported to cause the disease, including viruses, mites and nutritional deficiencies (Ploetz, 8). In 1966, it was shown that *Fusarium moniliforme* var. *subglutinans* (Wollenw and Reinking) Nelson, Tousson and Marasas was actually responsible for the disease. In India, researchers were first to report that *Fusarium moniliforme* (recognized later as *F. moniliforme* var. *subglutinans*) was the cause of the floral (Summonwar *et al*., 9) and vegetative (Varma *et al*., 10) forms of the disease. Recently, *Fusarium moniliforme* were described as members of a new species, *Fusarium mangiferae* Britz, Wingfield and Marasas sp. (Britz *et al*., 3). and shown to cause mango malformation disease by artificial inoculation (Britz *et al*., 3; Marasas *et al*., 6).

Atinsky *et al*. (2) reported based on *in vitro* studies that the *Fusarium mangiferae* was found to commence the germination when the temperature was above 5°C. They also reported that both conidial germination and colony growth increased with a corresponding elevation in temperature and reached a peak at optimal temperatures of 28 and 25°C respectively. The epidemiology of this disease is poorly understood (Kumar *et al*., 4). Keeping in view of these basic information available on *Fusarium* species infecting different crop plants the present study was undertaken to evaluate optimum conditions favourable for the growth of *Fusarium mangiferae* mycelia.

MATERIALS AND METHODS

The study was conducted at the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi. Pure culture of *Fusarium moniliforme* (currently named as *Fusarium mangiferae*) obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology. The culture was maintained in Potato Dextrose Agar (PDA) slant for further study. (Aneja, 1). The experiments were conducted by using fresh culture of the test pathogen. Pathogen was inoculated in Petri plates (90 mm) containing sterile PDA and incubated at 25±2°C. The mycelia growth of the pathogen was studied at different temperatures and relative humidity (RH). To test the effect of temperatures different fractions of 5 was chosen starting from 10-40±1°C and in addition the pathogen was also incubated at 27±1°C. Similarly, two levels of relative humidity 35±2 and 65±2% were tested in BOD incubator. Measurement of colony growth were determined by placing a fresh culture of *Fusarium moniliforme* (8 mm diameter disc) in the centre of Petri plates in solid state containing PDA medium. The radial growth of the fungus was measured at 3, 5, 7, 9 and 12th day of incubation. Total numbers of condia were counted on 12th day for all temperatures and relative

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humidity combinations. The mycelia material from a whole plate was thoroughly smeared with sterilized distilled water (5 ml) and again rinsed with 2 and 3 ml of water and collected in a vial. Each vial was shaken well for 5 min. and one ml of suspension was again diluted in 9 ml of water. The vial was vigorously shaken for 5 min. 1 µl was added to the improved Neubauer haemocytometer and covered with cover slip. Total numbers of conidia were counted by using compound microscope. The conidia count was calculated with the procedure followed by Mather and Roberts (7).

The average values observed from five replications were taken for analysis. The experiment was conducted using complete randomized design and the data were statistically analysed using SAS software.

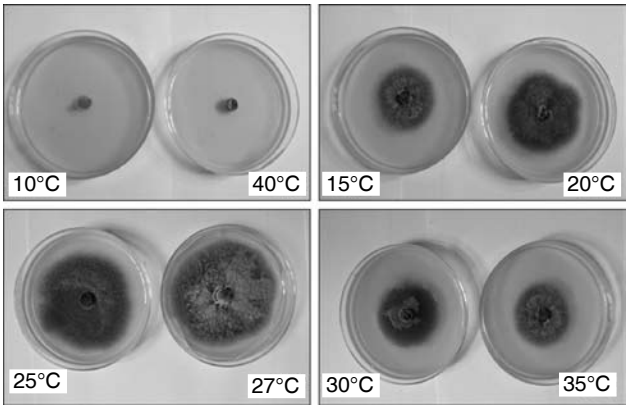

Fig. 1. Radial growth of *Fusarium mangiferae* at different temperatures with 65% RH.

RESULTS AND DISCUSSION

The radial growth of *Fusarium mangiferae* was observed in cultures maintained at temperatures from 15-35°C. No fungal growth was observed at both extremes, *i.e.*, low temperatures (10°C) and at high temperatures (40°C). Similar observations were recorded for both relative humidity conditions (Fig. 1). Maximum growth of *F. mangiferae* was recorded at 27°C followed by 25°C. These two temperature conditions differed significantly from all other temperature conditions (Fig. 2). The mycelia growth of *F. mangiferae* at 27° and 25°C was maximum at 65% relative humidity when compared with 35% relative humidity (Fig. 2) and differed significantly between the two relative humidity conditions. The mycelial growth increased steadily after three days of inoculation and the maximum growth was observed on the 12th day after inoculation. The two levels of temperatures, *i.e.* 27° and 25°C significantly influenced better growth of pathogen. The temperature and relative humidity levels are highly correlated and the fungus growth under controlled conditions was maximum under 27°C with 65% RH, whereas the growth was minimum in case of lower relative humidity. The radial growth of *F. mangiferae* observed under *in vitro* conditions was confirmed with the results reported by Atinsky *et al*. (2). However, the initial temperature required for fungal germination was found to vary. The initial temperature required for the *F. mangiferae* mycelial growth was above 10°C, while Atinsky *et al*. (2) reported 5°C. The optimum conditions favourable for the maximum mycelial growth of *F. mangiferae* were similar with the findings of Atinsky *et al*. (2). Liao (5) who also reported the growth of *F. circinatum* under similar conditions.

The number of spores varied significantly under different temperature and relative humidity regimes. Numbers of micro-conidia steadily increased from 15 to 27°C and followed the reversed trend up to 35°C. The maximum numbers of micro conidia were at 27°C followed by 25°C with 65% relative humidity. There was no formation of macro-conidia at very low and high temperatures under both the levels of relative humidity. The maximum numbers of macro-conidia were recorded at 25° followed by 27°C with 65% RH.

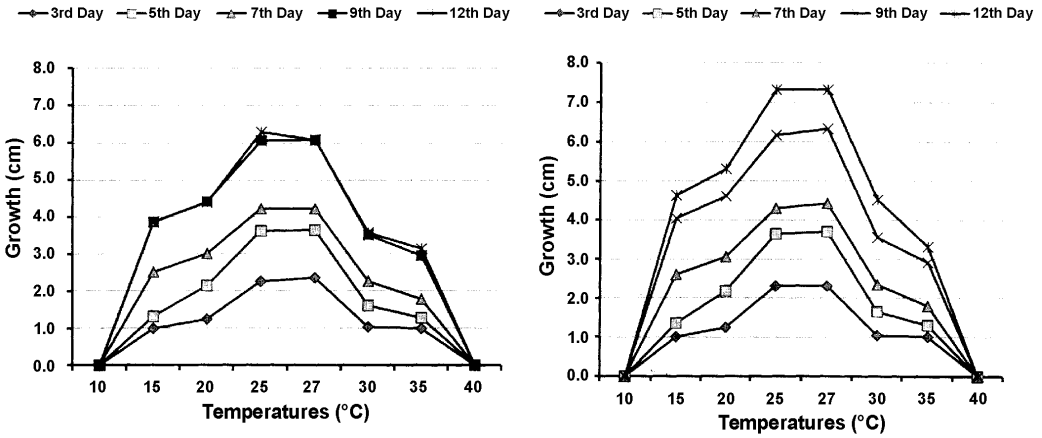

Fig. 2. Effect of temperature on growth of *Fusarium mangiferae* cultures at (a) 35% and (b) 65% relative humidity.

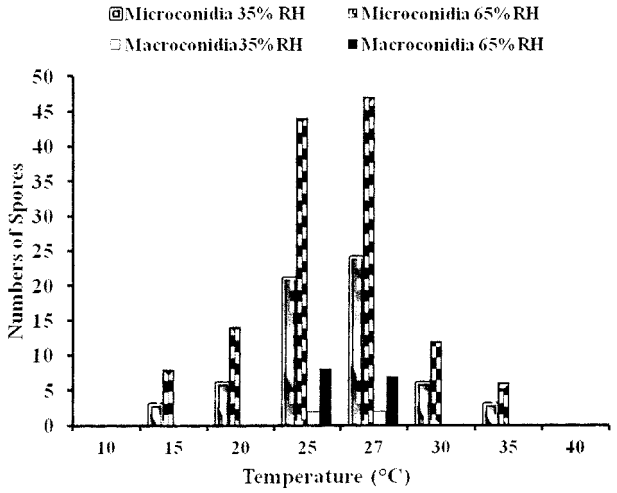

Fig. 4. Effect of temperature on micro and macro-conidia (10^{-5}/ml) of *Fusarium mangiferae* culture at 35 and 65% relative humidity.

However, low level of RH (35%) resulted less number of macro conidia at 27° and 25°C (Fig. 3).The relative humidity and temperature are highly significant for the growth and development of test pathogen. Britz *et al*. (3) reported that 26°C and 65%RH were favourable for increasing mango malformation. The fungus survived under soil at the above conditions for long period, which was similar to the present finding that the *F. mangiferae* culture had maximum growth at 27° and 25°C with 65% RH. Similarly, the number of conidia was maximum at 27° and 25°C with 65% RH (Fig. 3), which indicated that the relative humidity was an important for the pathogen growth and disease development.

The optimum temperatures for maximum growth of test pathogen (*F. mangiferae*) were 27°C followed by 25°C with 65% RH. The fungal growth was maximum under high relative humidity. The pathogen inoculum (micro- and macro-conidia) was also maximum at 27°C with 65% RH. Thus, the *F. mangiferae* culture can grow maximum under the temperature ranging from 23 to 28°C with a relative humidity of 60-65%. The experiment results concluded that the temperature and relative humidity are the critical factors for growth of pathogen, which might be the main reason for expression of mango malformation symptoms under field conditions in northern parts of India.

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