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Effect of Salicylic Acid on Mycelial Growth and Conidial Germination of Two Isolates of *Fusarium mangiferae*

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A B S T R A C T

Mango malformation, a century old malady of *Mangifera indica*, is considered as the major constraints for mango production worldwide. Several reports claimed that *Fusarium* species particularly *Fusarium mangiferae* is associated with mango malformation. Salicylic acid is an important signaling molecule that plays crucial role in plant microbial interactions. The present study was conducted to observe *in-vitro* application of different concentrations of salicylic acid on mycelial growth and conidial germination of two isolates of *Fusarium mangiferae.* The experimental findings showed that salicylic acid inhibits mycelial growth and conidial germination of two isolates of *Fusarium mangiferae* more effectively at moderate to high concentrations. The inhibition of mycelial growth of both isolates of *Fusarium mangiferae* was found to be pH dependent and was more in acidic condition as compared to alkaline condition.

Introduction

Mango malformation is a most destructive, century old malady of *Mangifera indica*, reported first time in 1891 from Darbhanga district of Bihar, India. Now apart from India, malformation has been confirmed in most of the mango growing countries: Australia, Bangladesh, Brazil, China, Cuba, Egypt, El Salvador, Israel, Malaysia, Mexico, Myanmar, Nicaragua, Oman, Pakistan, Senegal, South Africa, Spain, Sri Lanka, Sudan, Swaziland, USA, Uganda and the United Arab Emirates (Kumar *et al.,* 1993; Kumar *et al.,* 2011). Although malformation does not kill the tree, the vegetative phase of the disease impedes canopy development and the floral phase reduces fruit yield dramatically (Freeman *et al.,* 2014). In India, most of the commercially important cultivars such as Amrapali, Mallika, Neelum, Chausa, Dashehari, Bombay Green, and Langra are susceptible to this disease. It is undoubtedly a complex puzzle among scientific communities due to its annual recurrence widespread, catastrophic nature, mysterious etiology and no effective control methods (Kumar *et al.,* 1980; Sirohi *et al.,* 2006). Previous reports indicate that fungus species particularly *Fusarium mangiferae* is the cause or probable cause of mango malformation (Youssef *et al.,* 2007; Kvas *et al.,* 2008; Freeman *et al.,* 2014; Joshi *et al.,* 2014). Salicylic acid is a natural plant hormone that plays important role in plant

microbial interactions. Previous studies have also demonstrated that exogenous application of salicylic acid has wide range of effectiveness against fungal pathogen under both *in-vivo* and *in-vitro* conditions (Wu *et al.,* 2008; Mandal *et al.,* 2009; Makandar *et al.,* 2012; Qi *et al.,* 2012). The objective of this study was to investigate the *in vitro* effect of salicylic acid on mycelial growth and conidial germination of two isolates of *Fusarium mangiferae*.

Materials and Methods

Salicylic acid preparation

Salicylic acid (SA), (GRM1476-500G) used in this experiment was purchased from Himedia laboratories (Mumbai, MH, India). The 100 mM stock solution was prepared by dissolving 13.81 g of salicylic acid in minimum volume of ethanol and then autoclaved distilled water was added to make final volume 1 L. Different concentrations of salicylic acids solutions (0, 0.1, 0.25, 0.5, 1.5, 2.5, 5 and 10 mM) were prepared through serial dilution from stock solution. Distilled water without salicylic acid was used as the control.

Isolation and identification of *Fusarium mangiferae*

Ten isolates of *Fusarium mangiferae* were isolated from malformed tissues of different mango cultivars e.g. Amrapali, Langra, Dashehari, Bombay Green, Mallika and Chausa grown in the Experimental Mango Garden Department of Plant Physiology, GBPUA&T, Pantnagar. The single spore culture and hyphal tip isolation techniques were used to obtain pure culture of *Fusarium mangiferae* as previously described procedures (Nelson *et al.,* 1983, Britz *et al.,* 2002, Ansari *et al.,* 2013). Briefly, 5 mm long malformed tissues were sterilized for 2 min in 0.1% $HgCl_2$ solution and rinsed thrice with sterilized deionized water and kept on potato dextrose agar (PDA) slants/plates containing streptomycin sulfate. These slants/plates were incubated at 27^0C in BOD incubator for 3-4 days. The identification of *Fusarium* isolates were done on the basis of typical macro and micro-conidia on carnation leaf agar (CLA) and presence of purple orange color on PDA media. The pure culture was maintained at 4^0C on PDA medium for future use. Out of ten isolates only two isolates of *Fusarium mangiferae* were chosen for mycelial growth and conidial germination studies.

Effects of salicylic acid on mycelial growth

The effect of salicylic acid on the *in vitro* growth of *Fusarium mangiferae* was tested as previously described method (Qi *et al.,* 2012). Briefly, modified spezieller nahrstoffarmer agar (SNA) media (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$, 0.5 g KCl, 1 g glucose, 1 g sucrose, and 20 g agar per litre) amended with different concentrations of salicylic acid (0, 0.1, 0.25, 0.5, 1.5, 2.5, 5 and 10 mM) were prepared in sterile perti plates (90 mm diameter). Each petri dish was inoculated with a 5 mm agar plug cut from the edge of 3-5 days old cultures obtained from the same medium. Plates with sterile water were used as control. Mycelial growth was measured for consecutive one to six days at pH 5.6 and expressed as millimeter (mm).To observe effect of different pH (3.6, 4.6, 5.6 and 8.0) on the mycelial growth, the pH of modified SNA media was adjusted with HCl or KOH. All plates inoculated with isolates of *Fusarium mangiferae* were kept in a dark cabinet at 27^0C. The experiment was repeated two times with 3 replicates of each treatment.

Effects of salicylic acid on conidial germination

The effect of salicylic acid on conidial germination of *Fusarium mangiferae* was

tested using a slight modification of previously described procedures (Wu *et al.*, 2008; Qi *et al.*, 2012). Briefly, 5 mm agar plug was taken from 7-d-old PDA culture and inoculated in potato dextrose broth (PDB) aseptically. The liquid cultures were incubated at 27^0C with shaking at 120 rpm for seven days. The broths were filtered through four layers of sterile cheesecloth to collect conidial suspensions. The concentration of conidia was determined by using a haemocytometer and adjusted 1x 10^6 conidia/ml with sterile distilled water. To study the effect on conidial germination, 100 conidia were aseptically transferred on the surface of each modified SNA plate containing different concentrations of salicylic acid. The number of germinated conidia was counted after 60 h of incubation at 27^0C in the dark. Conidia were considered to have germinated if the germ tube length was equal to or greater than conidial diameter.

Statistical Analysis

The statistical analysis of data was carried out with analysis of variance for completely randomized design (CRD). Critical difference (CD) was evaluated at 5 % level of significance. The means were tested at P > 0.05 using STPR software designed at Department of Mathematics, Statistics and Computer Science, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar, India.

Results and Discussion

Metabolite colour and growth rate

The *Fusarium mangiferae* isolates exhibited different metabolite colour on potato dextrose agar (PDA). The Fm-1 isolate exhibited white-light orange colour from upper view and white-yellow colour from lower view. The Fm-2 showed white colour from upper view and white-orange colour from lower view. Both isolates produce macro and micro-conidia on carnation leaf agar. However, both isolates demonstrated different growth rate i.e. rapid and slow growth rate on PDA media. The Fm-1 exhibited rapid growth rate on PDA media whereas Fm-2 exhibited slow growth rate (Figure 1).

Mycelial growth of *Fusarium mangiferae* isolates

The mycelial growth of two isolates of *Fusarium mangiferae* was significantly influenced by different concentrations of salicylic acid on SNA media. It was observed that lower concentrations of salicylic acid (0.1 and 0.25 mM) promoted mycelial growth as compared to control at pH 5.6. In most cases, 0.5 mM concentration of salicylic acid also promoted mycelial growth. But concentration higher than 0.5 mM decreased mycelial growth of both the isolates of *Fusarium mangiferae*.

At higher concentrations of salicylic acid (5 and 10 mM), mycelial growth was observed very low as compared to control. Among different treatment, 0.5 mM salicylic acid had maximum mycelial growth in Fm-1 while Fm-2 exhibited highest growth in 0.25 mM salicylic acid concentrations at 6 day (Figure 2). Further, the growth of mycelia seems to be pH dependent. Both isolates of *Fusarium mangiferae* exhibited more growth of mycelial after 3 days at basic pH 8.0 as compared to acidic pH 5.6. However, it was observed that as the pH of the medium decreased the mycelial growth of both isolates also declined gradually (Figure 3).

Conidia germination of *Fusarium mangiferae* isolates

The percentage of conidia germination of two isolates of *Fusarium mangiferae* was tested at pH 5.6 after 60 h of incubation at 27 ^{0}C.

Fig.1 Colony morphology of two isolates of *Fusarium mangiferae* on potato dextrose agar. (A) Upper view, (B) Lower view, (C) Conidia, produced in carnation leaf agar visualized under light microscope (10X).

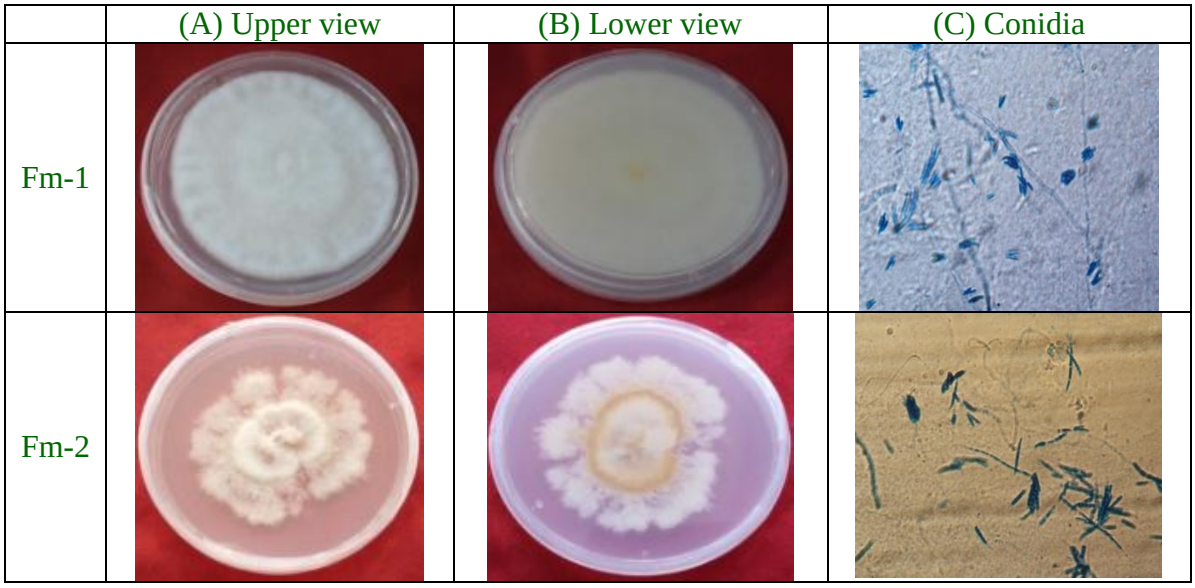


Fig.2 Effect of salicylic acid on mycelial growth of two isolates of *Fusarium mangiferae* (A) Fm-1, (B) Fm-2 on modified spezieller nahrstoffarmer agar (SNA) media at pH 5.6

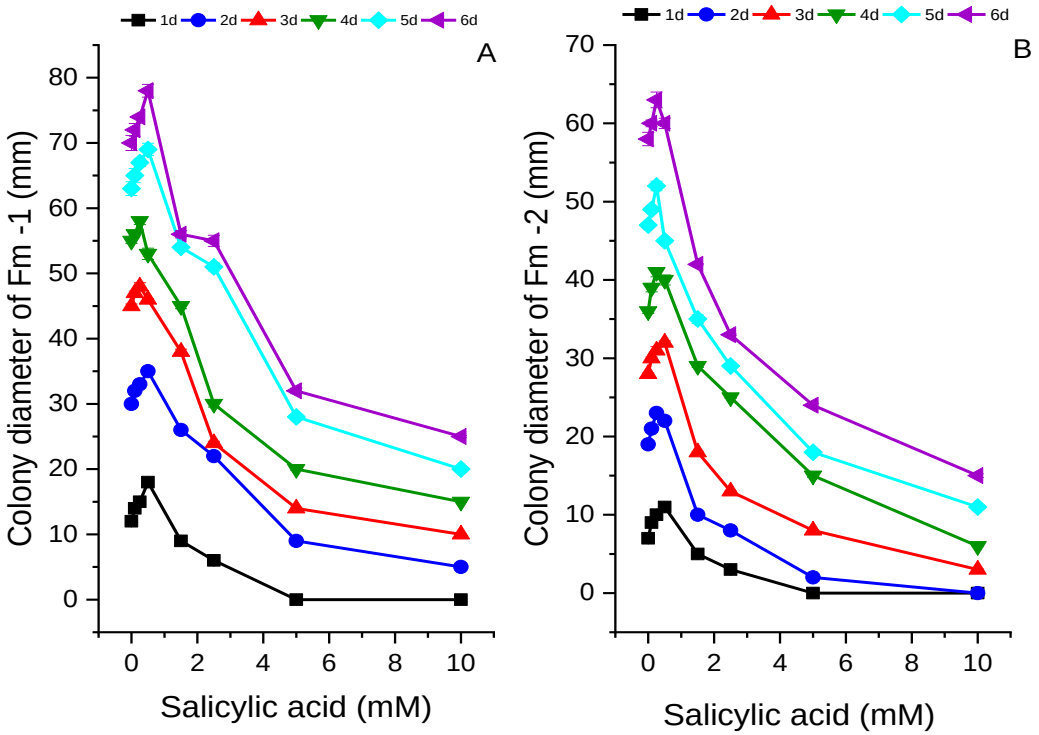


Fig.3 Effect of salicylic acid (SA) on mycelial growth of two isolates of *Fusarium mangiferae* (A) Fm-1, (B) Fm-2 at different pH on modified spezieller nahrstoffarmer agar (SNA) media

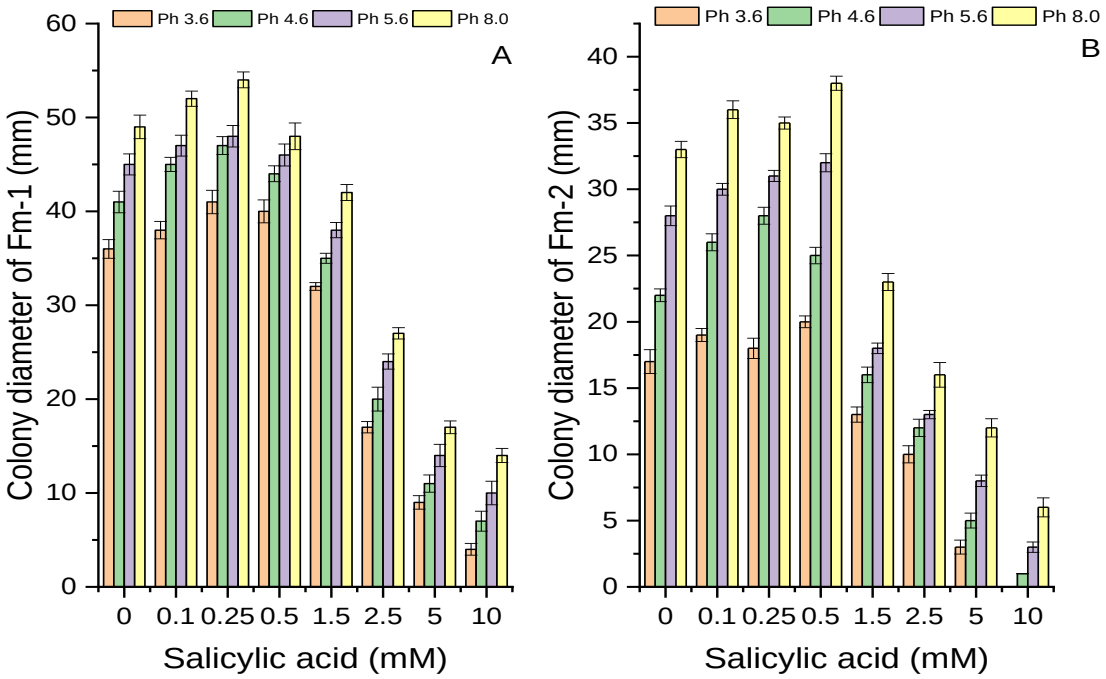
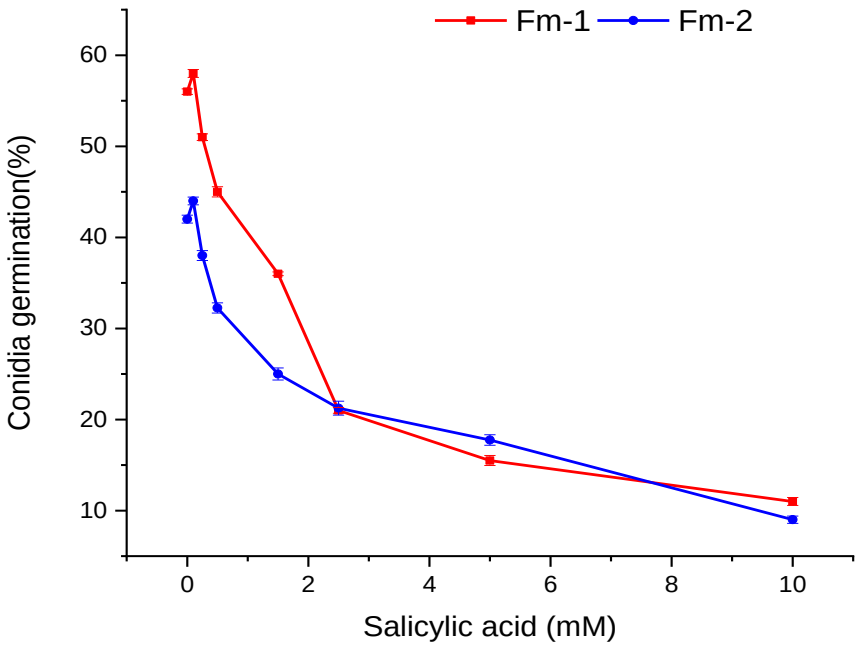


Fig.4 Effect of salicylic acid (SA) on conidia germination (%) of two isolates of *Fusarium mangiferae* (Fm-1 and Fm-2) at pH 5.6 on modified spezieller nahrstoffarmer agar (SNA) media



Generally, the percentage of conidia germination decreased gradually up to 0.5 mM salicylic acid concentration. Beyond this concentration increasing concentrations of salicylic acid causes a sharp decline in the percentage of conidia germination in both isolates of *Fusarium mangiferae*. However, the percentage of conidia germination varied between the isolates. The Fm-1 exhibited more percentage of conidia germination (56 %) than Fm-2 (42 %) under control condition (Figure 4).

Salicylic acid is a well-known signaling molecule that plays crucial role in plant microbial interactions (Hayat *et al.*, 2012). It is produced naturally inside plants and its level increases many fold during various biotic and abiotic stresses. Previous studies have reported that salicylic acid contributes in plant defense response either by altering the physiology of host plants or inhibiting the growth and infection of pathogens (Chen *et al.*, 2009; Fragnière *et al.*, 2011). The aim of this study was to investigate *in-vitro* application of salicylic acid on mycelial growth and conidia germination of two isolates of *Fusarium mangiferae*. Our findings suggest that mycelial growth of two isolates of *Fusarium mangiferae* varies with different concentrations of salicylic acid and seems to be pH dependent.

The salicylic acid mediated mycelial growth inhibition was more pronounced at acidic pH than basic pH in both the isolates. The percentage of conidia germination in both the isolates decreased with increasing the doses of salicylic acid. This suggested that salicylic acid affects conidia germination in a dose dependent manner. The findings of our results were in accordance with Wu *et al.*, 2008 and Qi *et al.*, 2012, who also reported that *in-vitro* conditions exogenously applied salicylic acid inhibited mycelial growth and conidial germination of other *Fusarium* pathogens e.g. *Fusarium oxysporum* f.sp. *niveum* and *Fusarium graminearum* respectively. Our *in-vitro* experimental finding suggests that exogenous application of salicylic acid could inhibit the infection and colonization of *Fusarium mangiferae* in host plant tissues and might be very helpful in controlling mango malformation.

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