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Behaviour of *Fusarium nivale* causal agent of mango malformation against different culture media and range of different temperatures and *in-vitro* control

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Abstract:

Mango malformation caused by Fusarium nivale is a serious threat to mango cultivation in various countries of the world including Pakistan. Present studies clearly showed that Fusarium nivalae was observed as the most dominant fungus with the maximum (78%) frequency followed by Alternaria (10%). There was a significant effect of fungicides on the overall growth of the fungus. The minimum mycelial colony growths was observed in Cabritop (07 mm) at 75 ppm dose, hence it was found to be the best fungicide, followed by Romeo, Acrobat, Melodiduo and Dragan as compared to control. In case of botanical extracts, the minimum mycelial colony growth was recorded by using Garlic extract, hence it was found to be the best followed by

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Datura, Neem, Safeda and Akk as compared to control. The most suited temperature for the maximum mycelia growth was recorded at 25 ^{0}C followed by 30 ^{0}C and 35 ^{0}C. However, the minimum colony growth was observed at 10 ^{0}C and 40 ^{0}C. The suitable medium for the maximum mycelia growth was recorded on PDA medium followed by V-8, PCA and mango branches. However, the minimum colony growth was recorded on mango leaves. This study proved that mango malformation can be successfully managed through the integration of all the possible control measures through IPM strategies. Further work is needed on the mechanism involved at cellular and molecular level.

Key words: mango malformation, fungicides, plant extracts, nutrient media.

Introduction:

Mango plant *Mangifera indica* L. is originated to the Asia is being cultivated in almost all tropical regions of the world (Ploetz *et al*., 2001). It is one of the fruits, which is extensively utilized for food, juice, flavor, fragrance color and also a common ingredient in new functional foods often called super fruits (Maqbool *et al*., 2007). The mango crop is of significant importance because of its demand in the international market and export value. Pakistan is 5th leading mango exporting country in the world and contributed 916.4 MT mangos that are 3.9% of the total world production (FAO, 2010; MINFAL, 2011). Major mango growing countries of the world includes India, Pakistan, Brazil, Australia, South Africa, Egypt, USA, Bangladesh and Philippines Many mango varieties are being cultivated in Pakistan, however, Sindhri, Langra, Chaunsa, Fajri, Samar Bahist, Anwar Ratole, Dasehri, Saroli, Tuta Pari, Neelam, Maldah, Collector, Began Palli are the famous varieties (Iqbal, 2004).

Besides its excellent nutritive, diuretic values and economical importance of the mango fruit, its production is

badly affected by some biotic and abiotic factors especially insects and diseases (Shahbaz *et al*., 2009). A number of diseases like powdery mildew, Anthracnose, Tip-die back and malformation have been reported on mango trees (Diczbalis, 1997). It has been reported that approximately 81 diseases have been found to attack the mango crop and reduces the overall yield and causes losses in mango production worldwide (Pernezny and Simone, 2000). Among all the diseases, mango malformation is one of the serious threats to mango production in the world where ever mango trees are grown. Malformation causes deformation of vegetative and floral tissues in mango (Chakrabarti, 2011). The yield losses due to malformation in mango may vary from 60-80% but if the disease may occurs in severity these losses may be increased up to 90% (Ploetz. *et al*., 2002).

Mango malformation being the major threat to the crop worldwide yet the etiology of the disease is still not conformed. The recent literatures and the studies regarding the disease have shown that it is a complex problem which is caused by the more than two species of *Fusarium*. Physiological studies regarding the etiology of the disease have proved that *Fusarium mangiferae* as the cause of the disease (Britz *et al*., 2002). . During a survey conducted in southern Pakistan on the different mango orchards for the identification of the causal agent, six fungal species including one new record of *Fusarium* specie (*Fusarium nivale)* (Fr.) Ces, *F. oxysporium, Fusarium moniliforme, Fusarium semitectum, Alternari alternata* and *Aspergillus niger* were isolated and identified on the basis of their colony characteristics and conidial morphology (Khaskheli *et al*., 2008).

The studies on the management of mango malformation revealed that the disease can be controlled with suitable fungicides like Benlate and Topsin-M (0.2%) in the month of July to august (Muhammad *et al*., 1999). Pandey and

Chakrabarti (2004) studied the effect of Bavistin on *Fusarium moniliforme* on disease development; the infection rate in treated plants was decreased, it was less effective during the first year but it showed better control in the second year of the treatment. Kumar and Beniwal (1992) reported that mango malformation can be managed up to satisfactory level by some broad spectrum systemic fungicides. Control of this disease can be difficult even with chemical treatments, and alternative methods are needed. Nowadays, the world is emphasizing on the development of alternative control strategies to reduce dependency on synthetic fungicides. In this way some botanical extracts are also effectively used as alternative strategy against some fungi causing disease in economical important crops and reduced yield of such crops. Plants have the ability to synthesize some important aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1999). Plant leaf extracts of *Datura stramonium* and *Calotropis procera* were found to be highly significant in reducing the mycelial growth of the pathogens. Leaf extracts of *Parthenium hysterophorus* and *Ricinus communis* and *Phyllanthus amarus* and *Tinospora cordifolia* showed the high inhibition of the growth of the pathogen. The other plant extracts like, *Azadirachta indica, Jatropha gossypifolia, Lawsonia inermis, Eichhornia crassipes, Verbesena enceloides* and *Morus alba* were also found effective in reducing the growth of pathogens. Mostly used plant extracts are, Neem (*Azadirachta indica*,), Garlic (*Allium sativum*, Linn), Eucalyptus (*Eucalyptus globulus*), Turmeric (*Curcuma Longa*, Linn), Tobacco (*Nicotiana tabacum*) and Ginger (*Zingiber officinale)* (Sharma *et al.,* 2002). Therefore keeping in view the losses caused by the disease worldwide, the objectives of our research work was to evaluate the most effective and environment friendly approach to manage the mango malformation disease. The results of the present study

will be utilized in improving the better control strategies against mango malformation.

Materials and Methods

Survey and sampling

The survey was conducted from four mango growing districts of southern Pakistan, including Sanghar, Hyderabad, Mirpurkhas and Tando Allahyar. Three orchards were surveyed from each district. Samples were taken from diseased trees, showing typical malformed symptoms (short, thickened and much enlarged or hypertrophied and highly branched) along with some healthy portion. The samples were kept in polythene bags and after proper labeling brought to the laboratory for further process.

Isolation and purification of causal fungus:

Samples of infected branches and inflorescence were washed with distilled water than cut into small pieces and surface sterilized with 5% mercuric chloride ($HgCl_2$). Then these pieces were rinsed twice with sterilized distilled water, plated on three layered tissue paper for drying and transferred to Petri plates containing potato dextrose agar medium. Isolation of fungi was done by standard techniques (Pathak, 1987). The Petri plates were kept under favorable conditions at temperature of 25 ± ^{0}C with 12 hours cycling of light and darkness. After 24 hours, growth of hyphal tips was observed. The growth was calculated with the help of formula given below;

$$\text{Colonization} = \frac{\text{Pieces colonized by the pathogen}}{\text{Total number of pieces}} \times 100$$

The distinct fungal growth colonized on tips was purified and identified with the help of hand book "The Isolation and Identification of Fungi" by Frank .M. Dugan.

Pathogenicity test:

Pathogenicity test of the fungus *Fusarium nivale* was conducted in the laboratory on apparently 12 healthy looking seedlings (17-20 healthy branches) of available mango variety Sindhri. The nursery was transplanted in sterilized earthen pots and then inoculated with fresh culture of the fungus *Fusarium nivale*. Inoculations were done with three different techniques (i) 5ml spore suspension of the fungus was injected below the growing tips of 3 seedlings. (ii) 3 seedlings were sprayed with 5ml spore suspension of the fungus; (iii) 3 seedling were drenched with 5ml spore suspension of fungus, whereas, the 3 seedlings were treated with 5ml sterilized distilled water and served as control. The inoculated portion was covered with muslin cloth. The muslin cloth was removed after 2 weeks of inoculation. Plants were monitored daily for disease development.

***In-vitro* evaluation of selected fungicides:**

The experiment was conducted in Randomized Complete Block Design (RCBD) with five treatments and three replicates (Steel *et al.,* 1997). The *in-vitro* sensitivity of the fungus *Fusarium nivale* to five fungicides viz. Cabriotop, Melodyduo, Acrobat, Dragon and Romeo were tested by food poison technique. Three concentrations (25, 50 and 75 ppm) of each fungicide were added to PDA medium at the time of pouring into 20 cm glass Petri plates. After solidification, 5 mm mycelia discs of seven days old culture of *Fusarium nivale* were placed in the center of plates and incubated at temperature of 25 ±1 ^{0}C. Data on mycelial colony growth of the fungus was recorded after 24 hours till 8th days of inoculation, while the petriplates

containing only PDA medium were treated as control (Steel *et al*, 1997).

In-vitro evaluation of botanical extracts:

The present studies were planned to find out most effective botanical extracts against *Fusarium nivale*. Sensitivity *of Fusarium nivale* was studied by using inhibition zone technique against five plants extracts viz., Safeda (*Euclyptus camaldulensis*), Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Akk (*Calotropisprocera*) and Datura (*Datura stramonium*). For the preparation of aqueous extract, 75 gm fresh leaves of each plant were macerated in 25 ml of sterilized water with the help of pestle and mortar. The macerated plant extract was first passed through four layered sterilized muslin cloth and then filtered through Whatman's filter paper.. The sterilized PDA medium was poured into the sterilized petri plates. These petri plates were allowed to solidify. Three concentrations (0.5, 1.0 and 1.5 ml) of each extract were added to PDA medium at the time of pouring into glass petri plates with the help of sterilized pipette.

Effect of different ranges of temperatures on the colony growth of *Fusarium nivale*

The fungus *Fusarium nivale* was grown on different ranges of temperatures to study the growth response of fungus against different ranges of temperatures i.e. (10 ^{0}C, 25 ^{0}C, 30^{0}C, 35 ^{0}C and 40 ^{0}C). For this purpose PDA medium was used. First the media was sterilized in autoclave at 121 ^{0} C for 20 minutes. After sterilization the media was poured into sterilized petriplates and allowed to solidify. After solidification 5 mm disk of 7 days old mycelia culture of *Fusarium nivale* was transferred into petridishes.

Effect of different solid media on the colony growth of *Fusarium nivale*

The fungus *Fusarium nivale* was grown on five different non-synthetic media viz., potato dextrose agar, V-8 agar, mango flower extract agar, mango branch extract agar and potato carrot agar. All the media after preparation were sterilized in autoclave at 15 lbs for 20 minutes. Petridishes containing about 20 ml of each medium were inoculated with 5 mm disk of 7 days old culture was taken from the growing margins of the petri plate. All the plates were incubated at 25 ±1 ^{0}C for about 7 days.

Data analysis

Statistical analyses were carried out using Statistica software (Statsoft Inc., Tulsa, OK). The ANOVA/MANOVA module was used for analyses of variance (ANOVAs) and for multiple comparisons of means (tests of Neuman and Keuls).

Results and Discussion

Disease incidence and most frequent fungi

The overall maximum (47%) disease incidence was recorded in cv. sindhri followed by chounsa (28%), langra (24%) and dusheri (20%). The orchards of Mirpurkhas showed maximum incidence (34%) in all assessed varieties, followed by Hyderabad (31%), Tando Allahyar (28%) and Matiari (26%) districts (Data not shown). *Fusarium nivalae* was observed as the most dominant fungus, with the maximum (78%) frequency followed by *Alternaria* (10%). The minimum (05%) frequency was recorded as *Nattrasia mangiferea* followed by *Aspergillus* sp., respectively (Fig. 1). Our results are in confirmation with the studies of Khaskheli *et al*., (2008), in which he isolated the *Fusarium nivalae* as most predominant fungus in all mango varieties.

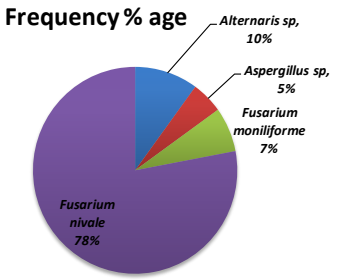

Figure.1 Frequency % age of most predominant fungi isolated from mango malformed tissues

Effect of different fungicides on colony growth of *Fusarium nivalae*

Effect of different fungicides on colony growth of *Fusarium nivalae* showed highly significant difference between treatments (Fig. 2). The minimum mycelial colony growth was observed in Cabritop (07 mm) at 75 ppm dose, hence it was found to be the best fungicide followed by Romeo, Acrobat, Melodi Duo and Dragan as compared to control. Overall, all tested fungicides had decreased the colony growth of the fungus at highest doses as compared to the lowest doses. The fungal growth was increased as incubation period was increased in all fungicides and control. As the fungus *Fusarium nivalae* is of slow growing nature, hence the data was recorded at 10, 15 and 25 days of incubation period. However, the minimum colony growth was recorded during first 10 days of incubation period as compared with control (Fig.2). So our results are in agreement with other studies on the management of mango malformation that reported that the disease can be controlled with Benlate and Topsin-M (0.2%) (Muhammad *et al*., 1999). Pandey and Chakrabarti (2004) also revealed that the Bavistin fungicide is more effective against *F. moniliforme* the disease development decreased in treated plants. Kumar and Beniwal.

(1992) reported that mango malformation can be controlled at least at satisfactory level by selected systemic fungicides.

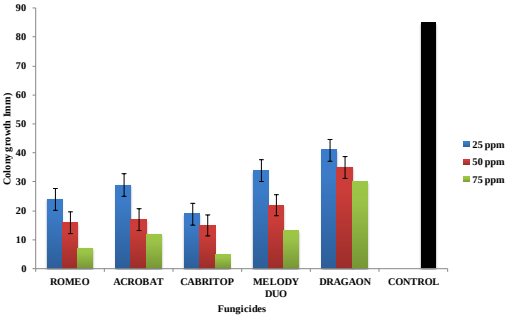

Figure.2 Effect of different selected fungicides on the colony growth of *Fusarium nivalae*

Effect of different plant extracts on colony growth of *Fusarium nivalae*

Effect of different plant extracts on colony growth of *Fusarium nivalae* showed highly significant difference between treatments (Fig. 3). The minimum mycelial colony growth was recorded in case of using Garlic extract, hence it was found to be the best followed by Datura, Neem, Safeda and Akk as compared to control. The gradually decreased colony growth of the fungus was recorded at highest dose as compare to the lower doses. The growth was increases as incubation period was increased in all extracts and control, however plant extracts could be considered as responding better as compare to control. The minimum colony growth was recorded during first 10 days of incubation period as compared to 15 and 25 days of incubation period that showed maximum colony growth, but not maximum as compared with control (Fig. 3). In previous studies botanical extracts have been reported to affect the disease development against diverse type of pathogens. For example, plant leaf extracts of Datura and Akk were found to be highly significant in reducing the mycelial growth of the *Parthenium*

hysterophorus, Ricinus communis and *Phyllanthus amarus*. The other extracts like, Neem, cotton leaf jatropha, *Hina,* Water lilly, Crown beard and White mulberry were also found effective in reducing the growth of pathogens (Sharma *et al.,* 2002).

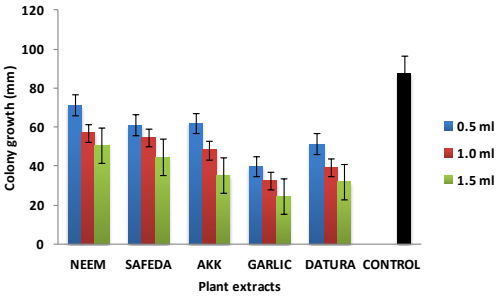

Figure.3 Effect of different plant extracts on colony growth of *Fusarium nivalae*

Effect of different ranges of temperatures on mycelia growth of *Fusarium nivale*

The fungus *Fusarium nivale* was grown on different ranges of temperatures to study the growth response of fungus against different ranges of temperatures. The overall results showed that there was a highly significant effect ($P<0.005$) of different ranges of temperatures on the mycelial growth of *F. nivalae*. The maximum mycelia growth was recorded at 25 ^{0}C followed by 30 ^{0}C and 35 ^{0}C. However, the minimum colony growth was observed at 10 ^{0}C and 40 ^{0}C (Fig.4). Our results are in confirmation with the earlier studies which showed that temperatures of 25 °C, 30 °C and 35 °C were found better suited for the growth of *F. mangiferae* than temperatures of 20 °C or 40 °C. Conidium germination of *F. mangiferae* was maximum at 30 °C and minimum at <15 °C, moreover, the mango malformation showed highest severity at 10–15 °C temperature range world-wide (Ansaria *et al.*, 2013).

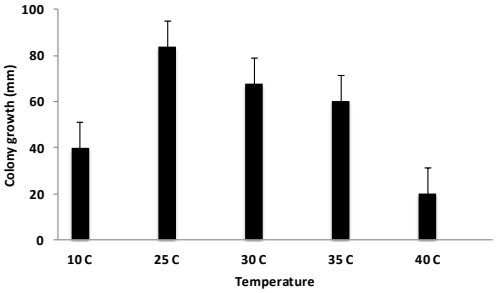

Figure 4. Effect of different ranges of temperatures on mycelial growth of *Fusarium nivale*

Effect of different media on mycelial growth of *Fusarium nivale*

The fungus *Fusarium nivale* was grown on different types of media to study the growth response of the fungus. The overall results showed that there was a highly significant effect ($P<0.005$) of different media on the mycelial growth of *F. nivalae*. The maximum mycelia growth was recorded on PDA medium followed by V-8, PCA and mango branches. However, the minimum colony growth was observed on mango leaves (Fig.5). These results are in confirmation with Farooq *et al*., (2005) who reported that high growth rate of *F. oxysporum* has also been observed in PDA and Czapek's Dox agar medium.

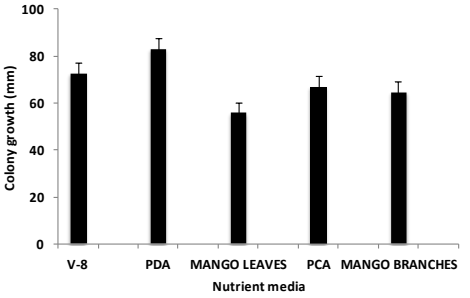

Figure 5. Effect of different nutrient media on the mycelial growth of *Fusarium nivale*

Conclusion:

We conclude that this study have support our hypothesis that mango malformation can be successfully controlled not only with chemical fungicides but it can also be managed through botanical extracts at least at satisfactory level. It will greatly help growers to find out other management strategies which are safe and environment friendly.

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