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Investigations on Fusarium wilt disease of mango nursery and Its *In-Vitro* control by applying different fungicides on the linear colony growth of *Fusarium oxysporum*

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Abstract

Fusarium wilt is one of the most serious and destructive diseases of mango nursery throughout Pakistan. This disease cause huge losses in mango nursery upto 22-25%. Keeping in view the losses caused by the Fusarium wilt in mango nursery, the present studies were planned to see the present position of the problem of Fusarium wilt disease by survey of disease incidence in different districts and to manage the disease by various methods of management like, use of chemicals and botanical extracts. The main objectives of the study were, (i) to record the disease incidence of Fusarium wilt in different mango nurseries of two districts of Sindh including, Hyderabad and Mirpurkhas (ii) to evaluate *in vitro* effect of different fungicides to manage this disease. For this purpose five fungicides were selected namely Nativo, Alliete, Cabriotop, Acrobat and Romeo. The concentrations of tested fungicides were kept (30, 60, 90 ppm) All the experiments were carried out according to the Randomized Completely Block Design (RCBD) with five treatments and three replications.

The results of our study showed that the disease is present in high frequency throughout all the districts surveyed. The maximum disease incidence of Fusarium wilt was recorded from Mirpurkhas (60.0%) followed by Hyderabad (55.0%). Five fungicides were tested for their efficacy against *Fusarium oxysporum*. Among all of them, Nativo was found more effective in reducing the linear colony growth of the fungus (3.33 mm) followed by Alliete (8.66 mm) and Cabriotop (19.00 mm), Whereas, the Acrobat was found moderately effective to reduce the linear colony growth of the fungus (24.70 mm) and Romeo was less effective (30 mm) as compared to control (82 mm). All the fungicides at their highest doses significantly reduced the linear colony growth of fungus as compared to control (82.67 mm). So, we can conclude that for the management of Fusarium wilt of mango nursery the more suitable fungicides are Nativo and Alliete, these chemical fungicides and can manage the disease as well. These studies will be proved fruitful for researchers and growers in future for management of the Fusarium wilt of mango nursery.

Keywords: Chemical fungicides, mango nursery, *Fusarium oxysporum*

Introduction

The Mango is technically known as (*Mangifera indica*, L) and is one of the members of family *Anacardiaceae*. Mango is called as “king of the fruits” due to its delicious taste. Mango is cultivated worldwide in tropical and sub tropical areas (Ploetz *et al.* 2001) [44]. Major mango growing countries of the world includes India, Pakistan, Brazil, Australia, South Africa, Egypt, USA, Bangladesh and Philippines. Mango trees as well as fruits have some religious importance in some countries of the world. Mango is known as nationalized fruit of India, Philippines and Pakistan while, in Bangladesh It is consider as national tree (FAO, 2013) [20]. The farming of *mangifera indica* started from south East Asia and Indo Pak sub continent. There are about 41 recognized species of mango originating as forest trees with fibrous and resinous fruits. Cultivation of mango was started 4000 years ago in the world from India. Cultivation of mango is as old as Indo Pak civilization. Its growth and culture in the sub continent was introduced by the Mughal Rulers especially Akbar who implanted a mango nursery in his residency and named as Lakh Bag (Bompard, 1993) [4]. Chemical control method is mostly used as traditional method of disease management in the world. The use of chemical substances as foliar application proved to be effective in reducing wilt disease

(Pandey and Chakrabarti, 2004) [45]. Gupta and Bansal, (2003) [24] tested Carbendazim, Mancozeb, Captan, Thiram, and Topsin- M at 0.2% concentration against *F. oxysporium* inducing fenugreek wilt under pot conditions. Carbendazim was found significantly effective followed by Mancozeb. Plants may have capability to manufacture secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan, 1999) [14]. The components are phenolic structures, for example carvacrol, eugenol and thymol, were extremely vigorous to fungus. The groups of fungi which may exhibit antifungal substances and attends as protection mechanisms of plants against pathogens (Shrivastava *et al.*, 1998) [56]. Harender and Kapoor, (1996) [28] assessed oil cakes from groundnut, mustard, sesame and cotton seed for their ability to reduce wilt of tomatoes caused by *Fusarium oxysporum* f *splycopersici* and found Groundnut and mustard at a 2% concentration were most effective in reducing the pathogen population more than 70%. However, groundnut was superior to mustard as it not only reduced a higher disease index (77.1%) but it also improved plant growth. Cotton seed cake was the least effective. generally used plant extracts are, Neem (*Azadirachta indica*), Garlic (*Allium sativum*) Eucalyptus (*Eucalyptus globulus*) Turmeric (*Curcuma Longa*) Tobacco (*Nicotiana tabacum*) Ginger (*Zingiber officinale*) (Sharma *et al.*, 2002).

Objectives of study

- To determine the incidence of the *Fusarium* wilt of mango nurseries at different orchards of Mirpurkhas and Hyderabad districts.
- Evaluate the *in-vitro* effect of different fungicides against linear colony growth of the fungus.

Materials and Methods

Survey of infected fields

A survey of mango nurseries of districts Hyderabad and Mirpurkhas was carried out to record the incidence of *Fusarium* wilt. Four nurseries were surveyed from each district.

Disease incidence

During the survey observations were recorded on the incidence of the *Fusarium* wilt of mango nursery. The incidence of the disease was calculated according to the disease incidence formula.

| | | |
|-----------------------|-------------------------|-------|
| Disease incidence (%) | Number of wilted plants | x 100 |
| | Total number of plants | |

Isolation and identification of the disease causing fungi

Samples were taken from infected branches, twigs and roots of infect nursery plants. Collected samples were then brought to the Plant Pathology laboratory for isolation and identification process as described by Pathak, (1987) [47], where, the samples were first surface sterilized twice with distilled sterilized water and then were treated with 0.5% NaOCl (Sodium hypochlorite) for 2 minutes. After surface sterilization the samples were dried on sterilized blotter papers and placed in petriplates containing sterilized potato dextrose agar medium. All the petridishes were incubated at 25 ± 1C for about seven days. After seven days of inoculation the fungi isolated, were then identified with the help of keys for identification of fungi by Nelson *et al.*, (1983) [43] and with the help of characteristics of fungi mentioned in the book “The Isolation and

Identification of Fungi” by Frank. M. Dugan.

Identification of *Fusarium* spp

Fusarium spp. isolated from rotted tissues of infected roots of mango nursery were then identified by studying their colony characteristics and conidial morphology using the keys described by Nelson *et al.* (1983) [43] and with the help of characteristics of fungi mentioned in the book “The Isolation and Identification of Fungi” by Frank. M. Dugan.

General characteristics of *Fusarium oxysporum*

Mycelium appeared yellow or reddish-brown or blue-black. Macroconidia straight, short or long 5-8 septate and 40-75 x 25.5-5 micron. Microconidia are 1-3 septate, with a short beak and 22-48 x 3.4 microns.

Effect of different fungicides on the linear colony growth of the *Fusarium oxysporum*

Five different fungicides were tested under *in-vitro* conditions for their efficacy against predominant fungus *Fusarium oxysporum* (Native, Cabriotop, Alliete, Acrobat and Romeo). The standard (aqueous) solution of these fungicides was prepared according to their active ingredients. The layout of the experimentation was conducted in complete randomized block design (RCBD) in 5 treatments and 3 replications briefly by Steel *et al.*, (1997) [54]. The doses of the fungicides were kept same (30, 60, 90 ppm) one of the each doses of the fungicide was mixed with 100 ml media and poured into petridishes 5 mm disk of the fungus was taken from the growing margin of 7 days old culture of *Fusarium oxysporum* with the help of sterilized cork borer. Petridishes containing only PDA medium without fungicides were used as control. All the petridishes were then transferred to incubator on 25 ± 1 C for about 8 days. Mycelial growth of the fungus was recorded in mm after 24 hours of inoculation till 8 days of inoculation.

| S. Number | Name of Fungicides | Dose in ppm |
|-----------|--------------------|------------------------------|
| 1. | Nativo | i. 30 ppm in 100 ml medium |
| | | ii. 60 ppm in 100 ml medium |
| | | iii. 90 ppm in 100 ml medium |
| 2. | Cabriotop | i. 30 ppm in 100 ml medium |
| | | ii. 60 ppm in 100 ml medium |
| | | iii. 90 ppm in 100 ml medium |
| 3. | Alliete | i. 30 ppm in 100 ml medium |
| | | ii. 60 ppm in 100 ml medium |
| | | iii. 90 ppm in 100 ml medium |
| 4. | Acrobat | i. 30 ppm in 100 ml medium |
| | | ii. 60 ppm in 100 ml medium |
| | | iii. 90 ppm in 100 ml medium |
| 5. | Romeo | i. 30 ppm in 100 ml medium |
| | | ii. 60 ppm in 100 ml medium |
| | | iii. 90 ppm in 100 ml medium |

Results

Survey of different mango nurseries

A Survey of different mango nurseries of district Hyderabad and Mirpurkhas was carried out to observe the incidence of *Fusarium* wilt in the mango nurseries of these districts. During the survey it was observed that almost all the nurseries were suffering from some severe diseases like, *Fusarium* wilt, Malformation, Anthracnose, Leaf spots and Blight. Among all of them, the *Fusarium* wilt was found most dominating disease throughout all the nurseries visited. The maximum disease incidence was recorded from Mirpurkhas (60.0%)

whereas, the disease incidence in district Hyderabad was bit low as compared to district Mirpurkhas, maximum incidence of *Fusarium* wilt recorded from district Hyderabad was (55.0%).

Isolation and identification of the fungi causing *Fusarium* wilt of mango

Nursery

The collected diseased specimens were then brought to laboratory for isolation and identification process of the associated fungi caused. The isolation and identification process reveals the association of different kinds of the pathogens with the rotted root parts of the mango nursery. Among all the isolated fungi, *Fusarium oxysporium* remains most frequent and pre-dominant fungus and was identified on the bases of their morphological characteristics mentioned in the book "identification of the fungi" written by Frank M. Dugan and with the help of electronic microscope, help from the senior Professors of the department was also taken in this regard (Fig. 2).

Effect of different fungicides on the mycelial colony growth of the fungus (*Fusarium oxysporium*)

During the study, five different fungicides were tested against *Fusarium oxysporium* the casual organism of *Fusarium* wilt of mango nurseries for their efficacy under *in-vitro* conditions at different doses. Data was recorded on the regular basis from 24 hours of inoculation till 8 days. Data was analysed by using the Statistics 8.1 application of the computer which shows that all the used fungicides significantly reduced the linear colony growth of *Fusarium oxysporium* ($p < 0.000$). Among them, Nativio was found more efficient in reducing the linear colony growth of the fungus at their highest dose (3.33 mm) as compared to its lowest dose (8.83 mm) respectively, followed by Alliete which reduce the radial colony growth of the test fungus at its highest dose (8.66 mm) and at lowest dose (20.17 mm) whereas, the Cabriotop was less effective in arresting the linear colony growth of the test fungus as compared to Nativio and Alliete. Cabriotop reduced the fungal growth at highest dose (19.00 mm) and at lowest dose (34.01 mm), while, Acrobat at its highest dose reduce the fungal growth (24.70 mm) and at the lowest dose (44.90 mm) and Romeo was found less effective as compared to other four fungicides which reduce the fungal growth at highest and lowest dose (30.50 and 44.90 mm) respectively. All the fungicides at their respective doses significantly retarded the growth of fungus as compared to control (82.67 mm).

Effect of selected fungicides on the linear colony growth of the *Fusarium oxysporium* causal agent of wilt in mango nursery

| S. NO | Fungicides tested | Dose (PPM) / 100 ml. medium | Radial colony growth (mm) |
|-------|-------------------|-----------------------------|---------------------------|
| 1 | Nativo | i. 30.0 | 8.833 j |
| | | ii. 60.0 | 6.500 j |
| | | iii. 90.0 | 3.333 k |
| 2 | Alliete | i. 30.0 | 20.17 h |
| | | ii. 60.0 | 14.33 i |
| | | iii. 90.0 | 8.667 j |
| 3 | Cabriotop | i. 30.0 | 34.00 e |
| | | ii. 60.0 | 26.00 g |
| | | iii. 90.0 | 19.00 h |
| 4 | Acrobat | i 30.0 | 44.90 c |
| | | ii 60.0 | 32.10 ef |

| | | | |
|---|----------------------|-----------|---------|
| | | iii 90.0 | 24.70 g |
| 5 | Romeo | i. 30.0 | 48.50 b |
| | | ii. 60.0 | 37.17 d |
| | | iii. 90.0 | 30.50 f |
| 6 | Control | - | 82.67 a |
| | LSD ($P < 0.0000$) | | |

Discussion

Fusarium wilt is most serious and devastating diseases of mango nursery throughout the world including Pakistan, which causes heavy losses to young nursery plants and may lead to their death. So, therefore keeping in view the incidence and the losses caused by the disease in mango nurseries, the survey of two districts of Sindh, Hyderabad and Mirpurkhas was carried out to record the incidence of the disease in different mango nurseries of these districts. During the survey it was observed that the *Fusarium* wilt was found frequently present in all the mango nurseries of the both districts with more or less incidence. The maximum incidence was recorded from Mirpurkhas district (60.0%) whereas, the incidence of the disease from the Hyderabad district was recorded up to (55.0%). These studies were investigated by Wrather and Phipps, (2002) [62] and Montgomery, (2009) [42], who found 50-70% and 9.4 % Infection on cotton due to *Fusarium* spp. Moreover Khanzada *et al.*, (2004) [33] reported 60% mango decline intensity due to soil borne microflora accompanied with *Fusarium oxysporium* also.

In our studies, isolation and identification of the collected diseased specimens showed the association of three fungi i-e., *Fusarium oxysporium*, *Allernaria alternata* and *Aspergillus niger* from rotted root tissues. Among them *Fusarium oxysporium* was found in highest frequency from all the associated fungi. The isolated fungi were then identified on the basis of morphological characteristics and color of the colonies of fungi. The identification was also done with help of taxonomical keys described by Nelson *et al.*, (1983) [43] and with help of a hand book "isolation and identification of fungi" written by Frank. M. Dugan. These results are in link with the studies of Hakro, (2006) [27] isolated 7 different species of fungal microbes from roots of deceased mango plant and found that the *Fusarium oxysporium* and *Rhizoctonia solani* were frequently associated with the tissues of the roots of mango.

Keeping in view the economic importance of mango, its export value and the losses caused by the *Fusarium* wilt disease in the nurseries of mango, management practices like chemical were carried out under *in-vitro* conditions against the *Fusarium oxysporium* to find out the most effective and eco friendly cost effective and easily available control measure to manage the *Fusarium* wilt disease of mango nursery. For this, reason our studies showed that, among all the tested fungicides, Nativio was found most efficient in arresting the mycelial colony growth of the fungus followed by Alliete and Cabriotop corresponding. Whereas, Acrobat and Romeo were sought less effective in reducing the mycelia colony growth of the fungus as compared to control. These studies are in connection with the previous studies of Sultana and Abdul, (2013) [52] used different microbial agents, oilcakes and fungicides *in-vitro* and *in-vivo* to control *Fusarium oxysporium* the cause of seed rot, seedling and root infection of bottle gourd and cucumber. Alliette, Benlate and Carbandazim totally inhibit the colony growth of *F. oxysporium* at 100 ppm. Whereas, Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the mycelial colony growth at 100 ppm. Fungicidal treatment of

bottle gourd and cucumber seeds artificially infested with *F. oxysporium* significantly reduced seedling dead and root infection. Benlate, Carbendazim and Topsin-M completely checked seedling mortality in bottle gourd. Gupta and Bansal, (2003) [24] tested Carbendazim, Mancozeb, Captan, Thiram, and Topsin M at 0.2% concentration against *F. oxysporium* inducing fenugreek wilt under pot conditions. Carbendazim was found significantly effective followed by Mancozeb. Buchvarova *et al.*, (1989) [7] found that Vitavax 200 HP showed best control *in-vivo* but under *in-vitro* conditions, the best were Mugibon, Vitavax 200-NP and Homai 80 WP against *Fusarium oxysporium*.

Summary, conclusions and recommendations

Summary

All the studies were carried out under *in-vitro* conditions at Plant Pathology Laboratory, Faculty of Crop Protection, Sindh Agriculture University Tandojam during the year 2014. The main objectives of the study were (i) to record the disease incidence of *Fusarium* wilt of mango nursery (ii) to evaluate certain chemical and botanical strategies to manage the disease. All the experiments were carried out according to the Randomized Completely Block Design (RCBD) layout system which comprised factors (Fungicides). The chemical fungicides used were, Nativo, Alliete, Cabriotop, Acrobat and Romeo at three different concentrations (30, 60, 90 ppm), three replications were made of each treatment. The results of the study are summarized as under

Results regarding the survey of different mango nurseries of district Hyderabad and Mirpurkhas showed that the *Fusarium* wilt disease is frequently found in all the districts surveyed with less or more incidence. The maximum disease incidence was recorded from Mirpurkhas followed by Hyderabad

The isolation and identification process reveals the *Fusarium oxysporium* as pre dominant fungus associated with the rotted parts of roots. The *Fusarium oxysporium* was identified on the basis of their morphological characteristics and color of the colonies with the help of taxonomical keys described by Nelson *et al.*, (1983) [43] and a hand book" isolation and identification of fungi" written by Frank. M. Dugan.

The results of different fungicides indicated that the *Fusarium* wilt can be managed through certain broad spectrum systematic fungicides. The fungicides used against the fungus significantly reduce the linear colony growth of the fungus. Among them, Nativo was more effective in reducing the colony growth of the fungus followed by Alliete and Cabriotop whereas; the Acrobat and Romeo were found very less effective in arresting the colony growth of the fungus as compared to control.

Conclusions

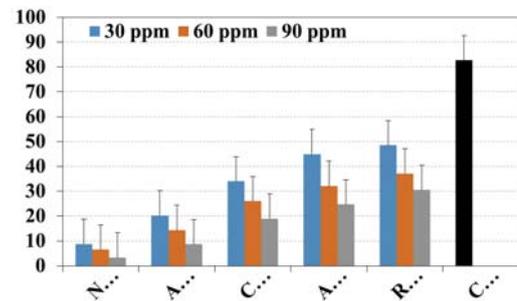
The present studies were conducted to report the disease incidence of *Fusarium* wilt disease of mango nurseries in two districts of Sindh namely Hyderabad and Mirpurkhas and to find out the more effective measures to manage the disease. Our studies showed that *Fusarium* wilt disease of mango nursery is one of the major serious threats to mango nursery. During the studies regarding the management it was found that the disease can be managed significantly through certain management strategies like by the use of chemical fungicides.

Suggestions

Keeping in view the results of present research work, it is suggested that certain fungicides such as Nativo and Alliete

should be recommended against this disease. Fungicides (Nativo and Alliete) should be evaluated at field conditions level at different nurseries of the mango at two different localities Hyderabad. And Mirpur khas district.

Effect of Selected Fungicides on the Mycelial Colony Growth of the *Fusarium Oxysporium*



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