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Comparative Effectiveness of Fungicides, Botanical Extracts and Bio-Control Agent against *Fusarium Nivale* Casual Agent of Mango Malformation

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Abstract:

Mango malformation disease, caused by Fusarium nivale, is a common threat for mango trees in field as well as nursery plants throughout the world. The management of the disease is very difficult; however it can be managed up to satisfactory level through certain chemical, botanical and biocontrol practices. Here, we studied the efficacy of some selected fungicides (Cabriotop, Romeo, Dragon, Melody due and Acrobat), botanical extracts (Neem, Garlic, Akk, Dattura and Safeda) at three different concentrations (25, 50, 75 ppm and 0.5, 1.0, 1.5 ml) respectively and biocontrol agent (Trichoderma harzianum) 5 mm mycelial disk to find out the suitable and eco- friendly approaches to manage the disease under in-vitro condition and to evaluate the alternative control measures to minimize the dependency and use of chemical fungicides. The results showed that the chemical fungicides like, Cabrioop and Acrobat can manage the disease well, whereas, the botanical extracts like, Garlic and Datura can also control the disease, however as alternative strategies to control the disease the biocontrol agent Trichoderma harzianum has also proved best to minimize the

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linear growth of the Fusarium nivale. Therefore different botanical extracts and biocontrol agents must be evaluated under in-vitro conditions and then applied to field conditions.

Key words: Management, Fusarium nivale, Mango malformation

INTRODUCTION

Fruits have become an integral part of human diet as they supply vitamins and minerals, the important constituents essential for human health [1]. Among these fruits mango (*Mangifera indica*), generally named as "King of the Fruits" and is one of the important exporting fruit of the world cultivated in almost 90 countries of the world. It is most relished fruit in the world and has attained a special place in the array of the delicious fruits and holds a typical nutritional and therapeutic value. Pakistan is the 5th leading mango exporting country of the world with annual production of around one million tons and shares of 7.6% of the mango export of the world [2]. The soil and climatic condition are suitable in Pakistan particularly in Punjab and Sindh, diseases are some of the significant causes leading to its low production. Its production is badly affected by a number of diseases at all stages of development i.e. from nursery to the consumption of fruits and cause heavy yield losses to the crop. If these threats managed at early stage of its development the production could be increased by 28% [3]. Among all the biotic threats of mango, mango malformation is one of the most severe and destructive diseases of mango throughout the world. It was first was first reported from India in 1891 [4] and then distributed worldwide, nowadays it is frequently found in all the mango growing countries of the world [5]. Generally two types of malformation are reported in the affected trees, Vegetative and Floral [6]. Normally, floral

malformation is more damaging for inflorescence than Vegetative malformation. Malformed inflorescence becomes much enlarged and crowded with hypertrophied axes of the panicles and do not produce fruits, or drop the fruit at early stages [7]. It has been reported that the malformation disease is more severe in early emerging flower buds as compared to lateral buds which remains free from disease this difference in the severity is due to increase in temperature because laterally the temperature increases which does not supports the pathogen to grow during panicle development. Lower temperature and high humidity supports the growth of the pathogen [8].

MATERIALS AND METHODS

Evaluation of selected Fungicides

The evaluation of the selected fungicides (Romeo, Acrobat, Cabriotop, Melody due and Dragon) was done according to the Completely Randomized Design (CRD) with five treatments and three replications against *Fusarium nivale*, through food poisoning technique [9]. The standard solution was prepared according to the active ingredients of the fungicides. Three concentrations (25, 50, 75ppm) of each fungicide were added to PDA medium at the time of pouring into 9 cm glass Petri plates. After solidification, 5 mm discs of seven days old culture of *Fusarium nivale* were placed in the centre of test plates. Petridishes containing PDA medium without fungicides were used as control. All the inoculated plates were incubated at 25 ± 1^{O}C for about 8 days. The linear colony growth of the fungus was recorded in mm after 24 hours of inoculation till 8 days of growth [10]. Percent decrease over control of fungicides was calculated by following formula:

$$\% \text{ Decrease} = \frac{\text{Control - Treatment} \times 100}{\text{Control}}$$

Evaluation of selected botanical extracts

Five different botanical extracts i.e. Neem (*Azadirachta indica*), Safeda (*Eucalyptus camaldulensis)*, Akk (*Calotropi sprocera*), Garlic (*Allium sativum*) and Datura (*Datura stramonium*) were evaluated under *in-vitro* conditions through food poisoning technique [9] against the *F.nivale*. The layout system for the experiment was selected as Completely Randomized Design (CRD) with five treatments and three replications. The basal medium was amended with three different doses of each extract (0.5, 1.0, 1.5ml). 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 extract obtained was considered standard and was stored in freezer for further studies in laboratory. The sterilized PDA medium was poured into the sterilized Petri plates. These Petri plates were allowed to solidify and different doses of plant extracts were poured into the PDA media with the help of sterilized pipette. Than inoculation of actively growing culture of *Fusarium nivale* (7 days old) were done in each Petri plate with the help of sterilized inoculating needle. All these Petri plates were then transferred to incubator at 25±1°C and data of mycelial growth of test fungus and inhibition zone by test plant extracts were recorded after 24 hours till 8 days of days of inoculation. Control was similarly carried out with only difference that plant extracts replaced by PDA media [10]. Percent decrease over control of fungicides was calculated by following formula:

$$\% \text{ Decrease} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

Evaluation of bio-control agent

The evaluation of the biocontrol agent was carried out to find the alternate control measure of fungicides under *in-vitro* conditions. The most prominent biocontrol agent *Trichoderma harzianum* was tested against *Fusarium nivale* the casual agent of mango malformation through dual culture method [11]. 5mm disk of seven days old mycelial culture of *Fusarium nivale* was obtained from the growing margins and placed on a fresh PDA plate (3 cm from the center) and then a 5mm mycelial disc of *Trichoderma harzanium* was obtained from 7days old mycelial culture and placed 3 cm away from the inoculum of the pathogen. The petriplates containing only the disc of *Fusarium nivale* were considered as control. The data of the pathogen in dual culture and control plates was measured after 24 hours till seven days. The petri plates were then incubated at 25 ± 1^0C. The growth inhibition percentage was calculated as described by Vincent and Budge [12]. The percentage inhibition growth of dual culture plates was calculated over control according to the formula:

$$(R1 - R2) \times 100$$

Where,
R1 = diameter of fungal colony in control
R2 = diameter of fungal colony in dual inoculation.

RESULTS AND DISCUSSION

Evaluation of Selected fungicides under *in-vitro* conditions against *Fusarium nivale*

All the tested fungicides significantly reduce the mycelial growth of fungus *Fusarium nivale* ($p<0.000$). Among all the tested fungicides, Cabriotop was found effective in suppressing the mycelial growth of the fungus *Fusarium nivale* up to (5.00 mm) followed by Romeo (7.33 mm) and Acrobat (11.67 mm) whereas, the Melody due reduced the colony growth of the test

fungus up to (13.23 mm) and Dragon (30.33 mm). All the fungicides at their respective doses significantly retarded the growth of fungus as compared to control (85.33 mm) (Fig.1, Tab. 1). These studies are in connection with the previous studies of [13], who used four fungicides against *F.oxysporium* and found that Benlate and Topsin-M at 100 and 50 ppm inhibited colony growth of *F.oxysporium*.

Evaluation of selected botanical extracts under *in-vitro* conditions against *Fusarium nivale*

The used botanical extracts significantly retarded the linear colony growth of test fungus ($p<0.000$). Among all the botanical extracts, Garlic was found the most effective in reducing the radial growth of the fungus at their highest dose (24.50 mm) followed by, Datura which suppress the fungal mycelilal growth up to (32.00 mm), whereas the Akk reduced the colony growth up to (35.33 mm) and the other two botanical extracts Safeda and Neem were found less effective in suppressing the linear colony growth of the *Fusarium nivale*. Safeda reduced the growth up to (44.67 mm), whereas Neem extract reduces the growth of the fungus up to (50.83 mm) (Tab. 2). These results are in association with studies of Rukhsana *et al.*, [14], who also tested different botanical extracts for their antifungal activity and reported that (*Eucalyptus camaldulensis)* exhibited highly pronounced antifungal activity. Jha *et al.,* [15] tested the efficacy of four botanicals such as Bel (*Aegle marmelos*), Neem (*Azadirachta indica*), Onion (*Allium cepa*.) and Garlic (*Allium sativum*) and evaluated separately or in integrations of different concentrations against spore germination of *Helminthosporium maydis*. Plant extracts proved inhibitory against the spore germination of *H. maydis* ranging from 50 to 100%.

Evaluation of bio-control agent *Trichoderma harzianum* under *in-vitro* conditions against *Fusarium nivale*

The bio-control agent *Trichoderma harzianum* was found significant in suppressing the mycelial growth of the *Fusarium nivale* ($p<0.000$). *Trichoderma harzanium* reduces the activity of the *F. nivale* up to (47.33 mm) as compared to control (87.67 mm) (Tab.3). These results are in line with Matroudi *et al*., [16] used the *Trichoderma harzanium, Trichoderma atroviride* and *Trichoderma longibrachiatum* on Canola stem rot caused by (*Sclerotinia sclerotiorum*) and Pea wilt caused by *Fusarium oxysporum* and found that *Trichoderma harzianum* have antagonistic effects on *Sclerotinia sclerotiorum.*

CONCLUSION

The present studies were carried out under *in-vitro* condition to find out most effective and eco- friendly approache to manage the mango malformation disease caused by *Fusarium nivale*. In this trial five fungicides *viz*, Cabriotop, Romeo, Acrobat, Melody duo and Dragon were applied against *Fusarium nivale* through food poison technique. Data was statistically analysed and revealed that Cabriotop and Romeo proved to be the best fungicides as compared to others (Acrobat, Melody duo And Dragon). This *in-vitro* sensitivity data clearly demonstrate sensitivity of *F. nivale* to fungicides. Studies on botanical extracts against *Fusarium nivale*, revealed that Garlic and Datura extracts proved to be the best plant extracts whereas Akk, Safeda and Neem extracts were least effective in suppressing the growth of *Fusarium nivale*, it revealed from analysis that *Trichoderma harzianum* can also restrict the mycelial growth of *Fusarium nivale* significantly.

Fig. 3: Effect of different fungicides against *Fusarium nivale*

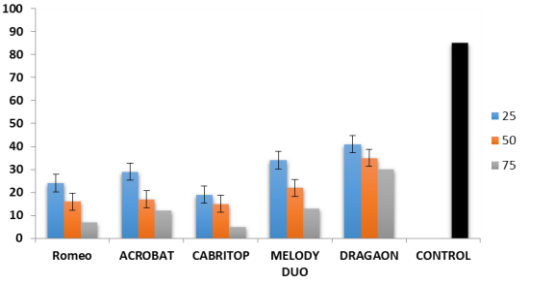

Table 1. Effect of different fungicides on radial growth of *Fusarium nivale*

Fungicides	**Dose (PPM) / 100 ml. medium**	**Radial colony growth (mm)**
CABRIOTOP	i. 25.0 ii. 50.0 iii. 75.0	19.50 h 15.67 j 5.000 n
ROMEO	i. 25.0 ii. 50.0 iii. 75.0	24.50 f 16.33 j 7.333 m
ACROBAT	i. 25.0 ii. 50.0 iii. 75.0	29.83 e 17.50 i 11.67 l
MELODY DUE	i. 25.0 ii. 50.0 iii. 75.0	34.23 d 21.70 g 13.23 k
DRAGON	i. 25.0 ii. 50.0 iii. 75.0	41.33 b 35.83 c 30.33 e
CONTROL	-	85.33 a
LSD ($p < 0.000$)		1.104

Fig: 2. Effect of different botanical extracts against *Fusarium nivale*

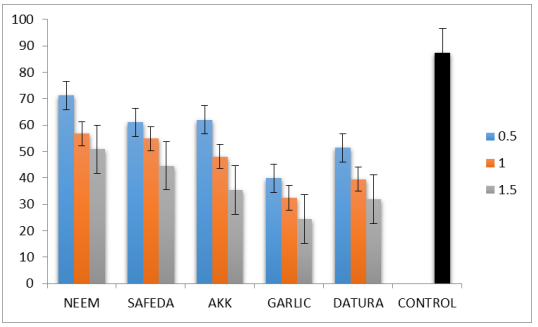

Table 8. Effect of different botanical extracts on mycelial growth of *F. nivale*

Botanical extracts	**Dose (mg)/ 100 ml. medium**	**Radial colony growth (mm)**
GARLIC	i. 0.5	40.00 j
	ii. 1.0	32.50 l
	iii. 1.5	24.50 m
DATURA	i. 0.5	51.50 g
	ii. 1.0	39.50 j
	iii. 1.5	32.00 l
AKK	i. 0.5	62.17 c
	ii. 1.0	48.33 h
	iii. 1.5	35.33 k
SAFEDA	i. 0.5	61.00 d
	ii. 1.0	54.67 f
	iii. 1.5	44.67 i
NEEM	i. 0.5	71.33 b
	ii. 1.0	56.67 e
	iii. 1.5	50.83 g
CONTROL	-	87.50 a
LSD (P < 0.0000)		1.271

Fig. 3. Effect of biocontrol agent (*Trichoderma harzianum*) against *Fusarium nivale*

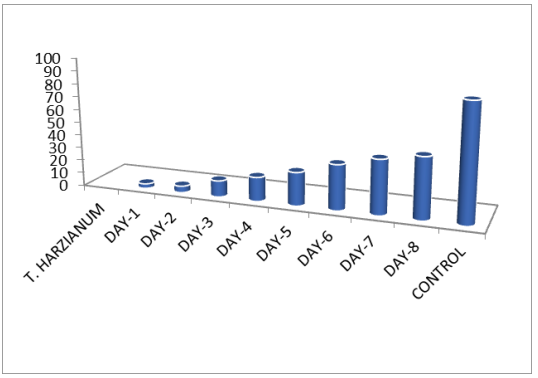

Table. 9. Effect of *Trichoderma harzianum* on the mycelial growth of *Fusarium nivale*.

Days after inoculation	Control (Radial colony growth (mm))	*Trichoderma harzianum* (Radial colony growth (mm))
DAY – 1	7.00	2.667 i
DAY – 2	18.0	5.667 h
DAY – 3	33.0	12.33 g
DAY – 4	47.0	19.17 f
DAY – 5	58.0	24.83 e
DAY – 6	69.0	35.00 d
DAY – 7	78.0	41.00 c
DAY – 8	87.67 a	47. 33 b
LSD (P < 0.0000)	1.810	

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