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## RESEARCH ARTICLE

### A Comprehensive Study On Ornamental Plants for Exploration of the Dynamics of Biotic Stresses

<sup>a</sup>Yasir Iftikhar\*, <sup>a</sup>Sana Ahmed, <sup>a</sup>Muhammad A. Zeshan\*, <sup>b,c</sup>Muhammad U. Ghani, <sup>d</sup>Muhammad N. Sajid, <sup>a</sup>Komal Ambreen, <sup>a</sup>Sonum Bashir, <sup>a</sup>Muhammad A. Shabbir

<sup>a</sup> Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha, 40100, Pakistan.

<sup>b</sup> Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, 38000, Pakistan.

<sup>c</sup> National-Regional Joint Research Center for Soil Pollution Control and Remediation in South China, Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Institute of Eco-Environmental and Soil Sciences, Guangdong Academy of Sciences, 808 Tianyuan Road, Tianhe District, Guangzhou, 510650, China.

<sup>d</sup> Potato Research Station Sahowali, Sialkot. 51060, Pakistan.

Corresponding Author:

Yasir Iftikhar, Email: yasir.iftikhar@uos.edu.pk, Muhammad.ahmad@uos.edu.pk

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#### ABSTRACT

Plants produced for aesthetic purposes, whether indoors or outdoors uses, are determined ornamentals, consisting of a variety of flowers, shrubs and trees. They can be grown in the ground, in potting compost, or without any soil at all. However, they are hugely admired and have high demand worldwide, many decorative plants are very important economically for a variety of sectors. Ornamental plants are affected by various diseases in the horticultural industry as a whole that deteriorate the economic and aesthetic value. Phyto-pathogens such nematodes, bacteria, fungi, and viruses can cause diseases like leaf spots, blights, cankers, galls, mosaic etc. This study is aimed to assess the prevalence, and severity of leaf spots, color variegation and leaf mosaics in ornamental plants followed by pathogenicity studies and in-vitro management. The survey for diseases assessment in ornamental plants i.e. conocarpus, crown of thorns, firebush and rose was carried out at 3 distinct locations such as the administrative block, the academic block, and the tissue culture laboratory area at College of Agriculture (COA), University of Sargodha (UOS) Pakistan. As a whole, maximum disease severity (68%) was recorded on conocarpus and minimum was on fire bush (5%) when summarized from all locations on all dates of data recording. A combination of Metalaxyl + Mancozeb, Topsin M, and Copper oxychloride were assessed against fungus at 3 different concentrations (50 ppm, 100 ppm, and 150 ppm) under laboratory conditions. The fungal colony diameter was reduced to 2.5 mm when treated with the combination of Metalaxyl + Mancozeb, whereas it was largest with Copper oxychloride. The study contributed for development of sustainable management options in ornamental plants and strengthened the knowledge of gardeners, landscapers for human wellbeing.

**Keywords:** Ornamental plants, Diseases, Landscape, Aesthetic.

#### INTRODUCTION

Ornamental plants are grown for aesthetic purposes indoors and outdoors which can be grown straight in the field, in potting mixture, or in a soilless culture medium i.e. peat moss, vermiculite (Li *et al.*, 2022a). Ornamental

plants comprised of potted plants, woody ornamentals, bulbs, corms, and cut flowers play a vital role in environmental beauty (Toscano *et al.*, 2019). In hotter climates, they offer shade and a cooling effect, whereas

in colder climates, they bulk out and help to prevent frost by holding onto soil heat (Capotorti *et al.*, 2019). Globally, ornaments are becoming a significant source of revenue as these offer attractions for a variety of birds, boosting natural beauty (Nawrath *et al.*, 2022).

Ornamental plants are subjected to abiotic and biotic factors that limit the potential of their production. Drought, salinity and temperature variation affect the growth of ornamental plants and predispose them for the attack of pathogens (Leotta *et al.*, 2024). Numerous microbiological species such as bacteria, fungi, nematodes and viruses, usually infect ornamental plants. These infections have detrimental effect on the growth and development of the plants affecting their commercial value (Ristaino *et al.*, 2021). The cultivation of ornamental plants is a significant part of the global horticultural business, and fungal infections i.e. *Alternaria*, *Myrothecium*, *Colletotrichum* and *Phytophthora* spp. can result in 30-40% yield losses (Mekapogu *et al.*, 2021). Pathogens can cause ornamental plants to lose their attractive and healthy appearance, which can result in an abrupt mass disease outbreak that is highly problematic for the market (Eike *et al.*, 2022). The soil-borne fungus *Fusarium oxysporum* is one of the most harmful pathogenic constraints for ornamental plants (Scott *et al.*, 2010). *Botrytis cinerea* is the most prevalent fungus that affects ornamental plants, especially cut flowers (Bika *et al.*, 2020). Bacterial symptoms mainly manifest as leaf spot and blights while chlorosis and mosaic mottling are typical signs of viral infection (Bonanomi *et al.*, 2018). Precise and accurate assessment of the severity of the disease is crucial for monitoring, forecasting, and estimating yield losses of ornamental plants. The majority of fungal diseases in ornamental plants are mostly controlled by chemical fungicides (Gullino *et al.*, 2021). Aqueous extract of neem and carbendazim inhibits the mycelial growth of fungus when mixed with culture medium (Pretty and Bharucha, 2015). Fungicides such as Ridomil Gold and Dithane M-45 provide excellent control against *Fusarium solani*, *Alternaria alternate* and other fungi under *in-vitro* and *in-vivo* conditions (Srivastava *et al.*, 2018). Proper diagnosis of ornamental diseases, understanding the host pathogen interaction and devising management strategies are the area of thirst in the field of ornamental plants. The objectives of the present study were to assess disease severity in ornamental plants, to conduct

pathogenicity test on ornamental plants affected by various pathogens and to evaluate different fungicides for the management of diseases.

#### MATERIALS AND METHODS

Research work was performed in Fungal Culture Laboratory, College of Agriculture, University of Sargodha, Pakistan.

**Survey and disease severity assessment:** A comprehensive survey of 3 locations i.e. Admin block (32°75'7"N, 72°41'11"E), Academic block (32°08'01"N, 72°41'14"E) and Tissue culture lab (32°08'09"N, 72°41'15"E) area at College of Agriculture (CoA), UOS (Pakistan) was conducted. The data of disease severity for color variegation, leaf spot and leaf mosaic was recorded based upon visual observations on the ornamental plants grown in these sites. Disease severity was recorded by using following formula

Disease severity = Number of symptomatic leaves/Total number of leaves in a plant×100

**Collection of diseased samples:** On the basis of symptoms; color variegation (yellowing, streaking, bronzing), leaf spots (black edges, fungal fruiting bodies, necrosis) and leaf mosaic (chlorosis, mottling, mosaic, leaf distortion) samples were collected from diseased plants. Diseased leaf samples were collected in a brown paper bag, then brought to laboratory and stored in a refrigerator (Dawlance) at 4°C for further procedure.

**Isolation of pathogens from diseased ornamental plants:** Leaf samples were collected from diseased plant and cut into small pieces, sterilized with sodium hypochlorite solution for 30 seconds wash 3 times with distilled water and dried on paper towel under aseptic conditions. Potato dextrose agar (PDA) media was used for isolation; that was prepared by mixing potato starch from 200g peeled potatoes, 15g each of dextrose and agar in 1L of distilled water (Usman *et al.*, 2023). The mixture was later on sterilized by using autoclave and poured on petri dishes for solidification. Samples were placed on PDA media plate; the procedure was completed in aseptic environment under laminar flow chamber and incubated at 25°C as this is the standard optimal temperature for most of the plant pathogenic fungi (Yaqoob *et al.*, 2024). The plates were observed for fungal growth, colony development and pigmentation after 4 to 5 days.

**Identification of isolated pathogens:** The fungi were identified by using morphological characteristics such as colony color, shape, hyphal morphology and size, spore

shape as these features are commonly considered for identification for most of the plant pathogenic fungi (Asif *et al.*, 2023).

**Pathogenicity tests for confirmation of Pathogens:**

Pathogenicity test was carried out in Plant Pathology Laboratory at COA, UOS (Pakistan). Healthy leaf samples were washed with tap water, surface sterilized followed by 3 washings in distilled water were dried and placed in petri plates lined with tissue papers. A sterilized needle was used to injure the leaf surface under aseptic

conditions and pathogen was inoculated. The petri plates were sealed to maintain humidity and symptoms were observed after few days of incubation. The symptoms were compared with original samples and fungi was re-isolated for fulfillment of Koch's postulates.

**Preparation of stock solution:** For all fungicides, stock solutions were prepared separately by dissolving 1g of fungicide in 999 mL of distilled water. For preparation of different concentrations this stock solution was used. The details of fungicides are given below:

Fungicide	Product Name	Company	Formulation
Metalyxl+Mancozeb	Ridomil Gold MZ	Syngenta	WP
Thiophanate Methyl	Topsin M	Arysta Life Science	WP
Copper Oxchloride	Oxicab	Agricides (Pvt.) Ltd.	WP

**Preparation of fungicidal concentration:** By using stock solution, 3 different concentrations were prepared for each fungicide. A 50 ml stock solution dissolved in 950 mL distilled water; 50 ppm concentration was prepared. For 100 ppm concentration; 100 ml of stock solution was taken and dissolved in 900 ml of distilled water. For preparation of 150 ppm concentration, 150 ml of stock solution was dissolved into 850 ml of distilled water.

**Poisoned Food Technique:** The poisoned food technique was employed to test the efficacy of 3 different fungicides (Mj *et al.*, 2017). *In-vitro* experiment was conducted by using 3 concentrations viz. 50 ppm, 100 ppm, and 150 ppm of each fungicide. Each treatment was used in 3 replicates were used and each concentration was applied in 3 plates. Fungicides in respective concentrations were added in culture medium during preparation. After solidification of culture medium (PDA), a 5mm mycelial plug of fungi was inserted in the center of the plates (Kafle *et al.*, 2022). The data was recorded on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> days after incubation of petri plates in incubator at 25°C which is optimum temperature for growth of plant pathogenic fungi. The percentage inhibition was calculated using the following formula:

$$\text{Percent inhibition} = \frac{C-T}{C} \times 100$$

C= Colony diameter in control

T= Colony diameter in treatments (Das *et al.*, 2022).

**Data recording and Statistical analysis:** The data of disease severity for color variegation, leaf spots and leaf mosaics were recorded in ornamental plants at 3 different sites and each data was recorded weekly for 3 times. The data was analyzed statistically by using Statistix 8.1 software; analysis of variance (ANOVA) was used for the comparison of means of disease severity.

**RESULTS**

**Temporal assessment of disease severity:** Disease severity was recorded in 4 different ornamental plants at CoA (UOS) Pakistan. There was variation in disease severity on the ornamental plants; as Conocarpus showed the maximum disease severity (52.21%), followed by Crown of Thorns, which exhibited (46.07%) color variegation and 44.61% leaf spot severity. A moderate range of leaf spot (25.66%) and 30.26% leaf mosaic severity was recorded in rose while minimum leaf spot severity (15.89%) was recorded in Fire Bush (Table 1). There was significant difference in temporal distribution of disease severity as a whole i.e. decreased with the passage of time. The fluctuations in disease severity on ornamental plants were further elaborated on the basis of standard deviation which indicated highest variability in disease severity in conocarpus and minimum for Fire Bush. It is also evident from the results that the rate of decrease in plant susceptibility with the passage of time was maximum in conocarpus.

The spatial distribution of disease severity was recorded on 4 ornamental plants. The maximum leaf disease severity (68.83%) was recorded at admin block area in conocarpus and minimum (38.61%) was in tissue culture area. The trend of color variegation disease severity was similar in crown of thorns at both locations i.e. maximum at admin block (45.16%) and minimum (24.35%) at tissue culture area (Table 2). There was a slight different trend in case of leaf mosaic disease severity of crown of thorns and leaf spot of rose in which maximum (51.73%) and (47.31%) was found, respectively in academic block. Leaf spot disease severity of Fire bush and leaf mosaic disease severity of rose was maximum in tissue culture

area (25.65% and 41.28%), respectively. There were significantly higher differences of disease severity for leaf spot of conocarpus and rose for locations which is evident from the higher values of standard deviations. Standard deviation values are indicating the greater difference of disease severity at 3 locations while the difference was almost uniform for crown of thorns. The significance of increasing or decreasing trend of disease severity for each location and plant was determined by trend analysis performed through linear regression. It is evident that trend for conocarpus leaf spot is significant; slope value

showing the decreasing trend for disease severity across locations and model is explaining 99% variability in disease. Moderately decreasing trend of disease severity was found in case of color variegation and leaf mosaic diseases of crown of thorns and model is explaining 76% and 57% variability. Lowest disease severity trend for location was observed in Fire bush i.e. location has only 3% influence on disease severity. There may be the role of plant resistance level, pathogen load or microclimate of the plant which are explaining the disease variability trend.

Table 1. Temporal distribution of ornamental plants diseases

Time Period	Plant	Disease Severity (%)	Standard deviation (SD)
1	Conocarpus (Leaf spot)	52.21 a	15.24
2	Conocarpus (Leaf spot)	45.33 b	
3	Conocarpus (Leaf spot)	23.05 c	
1	Crown of thorns (Color variegation)	46.07 a	12.01
2	Crown of thorns (Color variegation)	33.16 b	
3	Crown of thorns (Color variegation)	22.08 c	
1	Crown of thorns (Leaf Spot)	44.61 a	3.96
2	Crown of thorns (Leaf Spot)	36.71 b	
3	Crown of thorns (Leaf Spot)	41.11 c	
1	Fire Bush (Leaf spot)	12.47 a	2.13
2	Fire Bush (Leaf spot)	11.98 b	
3	Fire Bush (Leaf spot)	15.89 c	
1	Rose (Leaf Mosaic)	20.42 a	5.51
2	Rose (Leaf Mosaic)	21.06 b	
3	Rose (Leaf Mosaic)	30.26 c	
1	Rose (Leaf Spot)	25.66 a	7.35
2	Rose (Leaf Spot)	25.51 b	
3	Rose (Leaf Spot)	12.85 c	

\*Letters for each 3 rows are describing the statistical difference in values

Table 2. Spatial Distribution of ornamental plant diseases

Sites	Location	Plant	Disease Severity (%)	Standard deviation (SD)
1	Admin Block	Conocarpus (Leaf spot)	68.83 a	15.14
2	Academic Block	Conocarpus (Leaf spot)	52.13 b	
3	Tissue Culture	Conocarpus (Leaf spot)	38.61 c	
1	Admin Block	Crown of thorns (Color variegation)	45.16 a	9.78
2	Academic Block	Crown of thorns (Color variegation)	44.94 a	
3	Tissue Culture	Crown of thorns (Color variegation)	24.35 b	
1	Admin Block	Crown of thorns (Leaf Mosaic)	49.62 a	5.05
2	Academic Block	Crown of thorns (Leaf Mosaic)	51.73 a	
3	Tissue Culture	Crown of thorns (Leaf Mosaic)	41.39 b	
1	Admin Block	Fire Bush (Leaf spot)	23.81 a	6.05
2	Academic Block	Fire Bush (Leaf spot)	16.12 b	
3	Tissue Culture	Fire Bush (Leaf spot)	25.65 a	
1	Admin Block	Rose (Leaf Mosaic)	38.17 a	6.05
2	Academic Block	Rose (Leaf Mosaic)	34.53 b	
3	Tissue Culture	Rose (Leaf Mosaic)	41.28 c	
1	Admin Block	Rose (Leaf Spot)	33.12 b	6.05
2	Academic Block	Rose (Leaf Spot)	47.31 a	
3	Tissue Culture	Rose (Leaf Spot)	28.96 c	

\*Letters for each 3 rows are describing the statistical difference in values

Table 3. Trend analysis on spatial distribution of disease severity in ornamental plants

Plant	Disease	Slope	Intercept	P-value	R <sup>2</sup>
Conocarpus	Leaf spot	-15.04	83.41	0.03*	0.99
Crown of Thorns	Color variegation	-10.51	58.87	0.33	0.76
Crown of Thorns	Leaf Mosaic	-04.13	55.81	0.46	0.57
Fire Bush	Leaf spot	+0.91	20.02	0.88	0.33
Rose	Leaf Mosaic	+1.64	34.87	0.69	0.22
Rose	Leaf spot	-2.05	40.62	0.86	0.046

-value of slope = decreasing trend; + value of slope = increasing trend

\* indicates significance; R<sup>2</sup>=co-efficient of determination

**In-vitro assessment of various chemical fungicides against fungi:** The efficacy of fungicides was evaluated observing the growth inhibition of fungi from petri dishes where treatments were applied. The effect of all the fungicides was significant for growth inhibition in all

replications at all concentrations as compared to control. The maximum growth inhibition of fungi (*Cercospora personata*, *Colletotrichum gloeosporides*, *Alternaria alternate*) was recorded by Metalaxyl+Mancozeb followed by Topsin-M and Copper oxychloride (Figure 1).

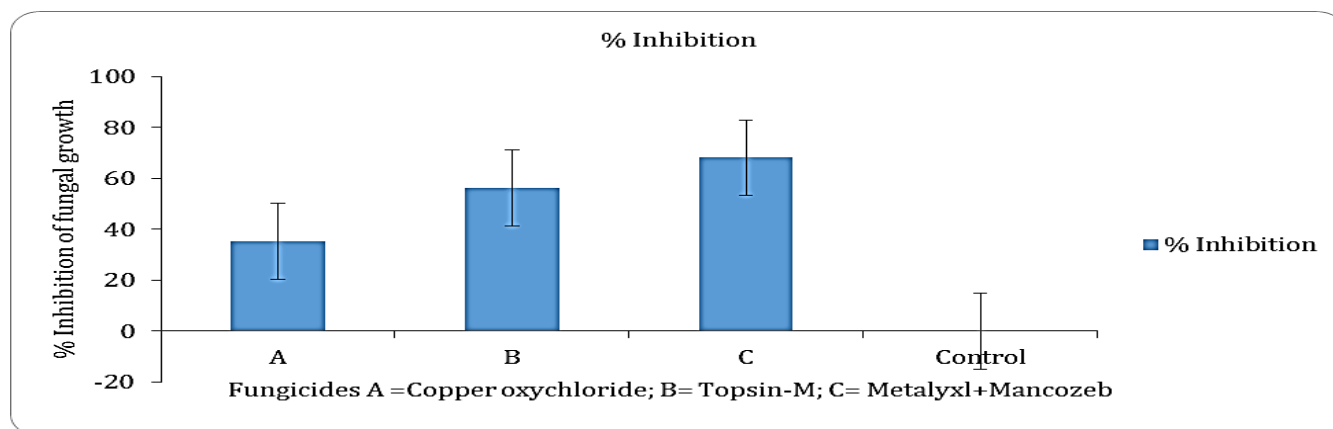


Figure 1. Effect of fungicides on % inhibition of fungi as a whole

The trend of %growth inhibition was recorded in *Alternaria alternata* by all fungicides followed by *Cercospora personata* and *Colletotrichum gloeosporides* (Fig 2). It is obvious from the results that *Alternaria* spp. are more susceptible to the fungicides particularly metalaxyl+mancozeb. The response of *Colletotrichum* was minimum indicating that it is least affected by the fungicides used.

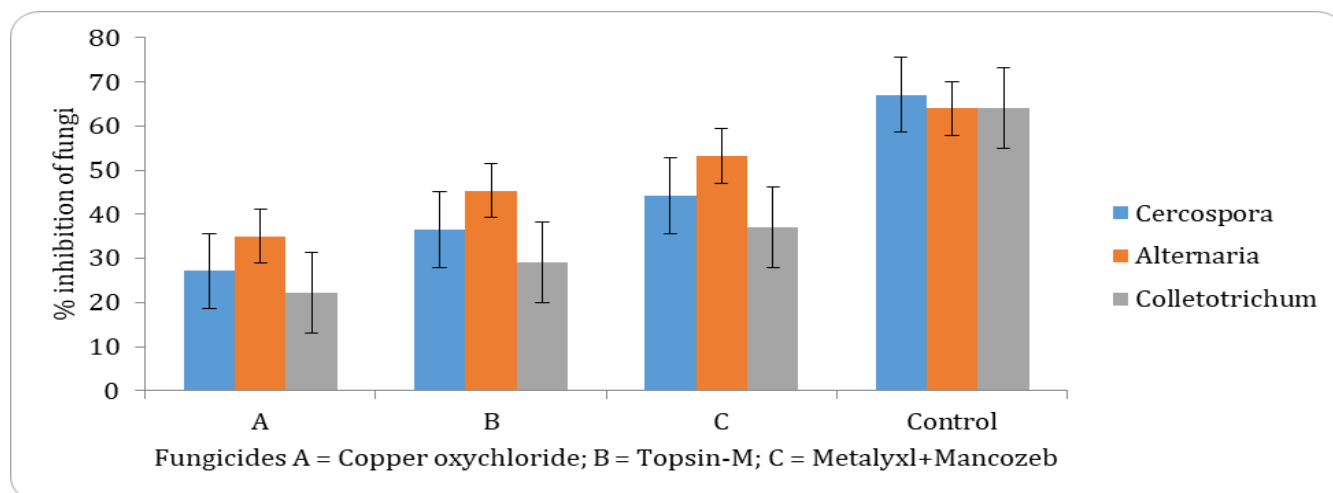


Figure 2. Effect of fungicides on % inhibition of different fungi

The mycelial development was least hampered by all fungicides at 50 ppm on the 3<sup>rd</sup>, 5<sup>th</sup>, and 7<sup>th</sup> days, respectively while it was maximum at 150 ppm

(Figure 3). There is significant increase in growth inhibition with the enhancement of fungicidal concentrations.

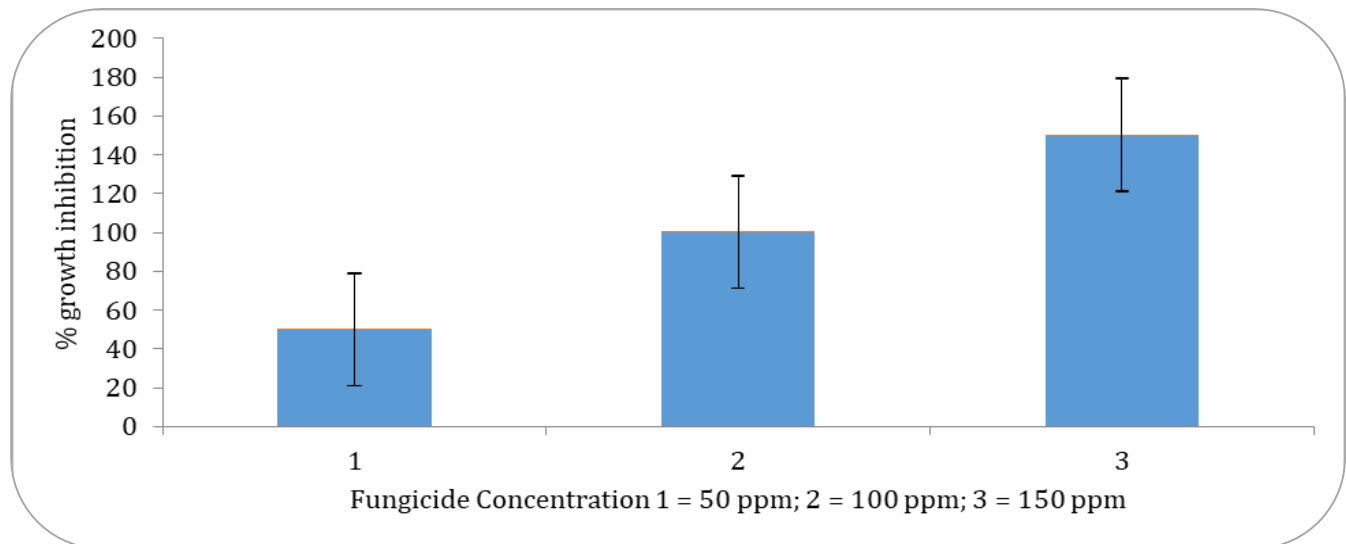


Figure 3. Comparison of different concentration on fungal growth inhibition

## DISCUSSION

Ornamentals are attacked by a wide range of microorganisms such as fungi, viruses, and bacteria that negatively impact the growth and appearance of the ornamental plants affecting their marketability. The assessment of plant disease incidence and severity determines the geographic distribution and state of the disease within a region (Shi *et al.*, 2023). Longer periods of illness prevalence are found in areas with cool, humid weather (Saba *et al.*, 2022). Koch's postulates were employed to determine the pathogenicity of microorganism (Atiq *et al.*, 2023). Pathogenicity was accomplished by using a uniform technique i.e. isolation, inoculation and re-isolation for testing and plant disease assessment that allow for inter-study comparison, which improves accuracy. Strategies for managing disease control include chemical control, biological control, and breeding for resistance and tolerance (Blanco *et al.*, 2023).

The pathological distribution of ornamental plants at 3 sites of COA, UOS (Pakistan) was very striking. Conocarpus was found as the most affected ornamental plant at all locations while Fire Bush was least affected. It was also noted there was a slight different response of all plants at all locations. The varied trend of disease response may be the consequence of resistance level, pathogen virulence at the particular site or microclimate of the plant (Lahlali *et al.*, 2024). Plants have natural defense responses against

pathogens which they use to restrict pathogen establishment particularly through production of reactive oxygen species (Rao *et al.*, 2020). The mechanism of resistance varies in different plants; some have only single resistance gene (R) while others have various resistance genes providing a moderate level resistance against many invading pathogens (Wiesner-Hanks and Nelson, 2016). In current study, temporal data of disease severity indicated a decreasing behavior with the passage of time. The decreasing trend of disease could be the result of resistance development in plants over time or may be the possibility of less favorable environment for pathogen activity (Laine *et al.*, 2023). Weather variables have a vital impact on plant disease development as these affect the virulence of pathogen. The mycelium of certain fungal pathogens is deteriorated at high temperature and humidity (Li *et al.*, 2022b). The conocarpus was the most affected ornamental plant in present study, but the extent of disease reduction with temporal changes was also maximum. The highest susceptibility of conocarpus towards fungal diseases was also confirmed by the findings of (Abbas *et al.*, 2021) who described more than 38% leaf spot disease intensity due to *Alternaria alternata*.

The results showed the greater variability in spatial distribution of diseases that may be the attribution of difference in inoculum level or microclimate. Inoculum load varies from field to field depending upon soil structure,

moisture and layout of plantations (Hussain *et al.*, 2019). Microclimates of a particular location has significant role in the pathogen progression and inhibition such as the more shady areas change the level of humidity that further influence the pathogen behavior accordingly (Fox *et al.*, 2024).

This experiment highlights the comparative effectiveness of 3 synthetic fungicides (Metaxy+Mancozeb, Topsin-M and Copper oxychloride) against 2 fungal pathogens isolated from ornamental plants. The fungicide Ridomil Gold MZ (Metaxy+Mancozeb) gave maximum growth inhibition of fungi under *in-vitro* conditions. The most effective role of Ridomil is due to the combined effect of 2 fungicides which it contains as its active ingredients, both have different mechanism of action. Metaxy and Mancozeb act synergistically as systemic and contact fungicides, respectively (Banaee *et al.*, 2023). Metaxy react with the RNA polymerase enzyme of the fungus resulting in inhibition of RNA synthesis leading to limited activity or death of pathogen (Matson *et al.*, 2015). Mancozeb has reportedly act on multiple sites of the fungus that disrupts the biochemical pathways and thus pathogen activity is reduced (Lyagin *et al.*, 2023). The efficacy of Mancozeb was also confirmed by the research of (Thesiyi *et al.*, 2020) who reported 94% inhibition of the fungus. The results of our

study also supported by the findings of Muhammad *et al.* (2019) who worked on the Mancozeb mode of action with the result that it disrupts the fungal enzymes.

In present experiment, Topsin-M was the second most effective fungicide against the isolated fungi from ornamental plants. The most promising mechanism of action of Topsin-M is the disruption of microtubule formation that is essential for intracellular transport (Requena *et al.*, 2008). The disturbance in microtubule formation affects the formation of processing bodies that regulates the mRNA metabolism of fungus due to which its reproduction is impaired (Sweet *et al.*, 2007). A similar efficacy of Topsin-M was described by (Neindow *et al.*, 2020) who achieved 89% growth inhibition of *Cercospora personata* under *in-vitro* conditions.

According to results of the current study, the least effective fungicide was copper oxychloride. Our findings were supported by the research outcome of (Manjunatha *et al.*, 2023) who found minimum growth inhibition while used against different fungi *in-vitro* as compared to other fungicides. Copper oxychloride produces the reactive oxygen species (ROS) that disrupts the fungal structures (Miner *et al.*, 2019). Ben-M'henni *et al.* (2022) described better efficacy of copper oxychloride for fungal management than flutriafol fungicide.

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**Contribution of Authors:**

Yasir Iftikhar	: Conceptualized and designed study
Sana Ahmed	: Conducted field surveys for disease assessment; collected data
Muhammad A. Zeshan	: Supervised the research work; contributed in editing of the manuscript
Muhammad U. Ghani	: Statistical analysis and interpretation of results
Muhammad N. Sajid	: Helped in pathogenicity and in-vitro testing of fungicides
Komal Ambreen	: Identified knowledge gaps and contributed in discussion improvement
Sonum Bashir	: Assisted in data collection and laboratory experiments
Muhammad A. Shabbir	: Provided technical expertise on ornamental plant biology; reviewed manuscript