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## EFFICACY OF DIFFERENT FUNGICIDES AGAINST CITRUS MELANOSE DISEASE IN SARGODHA, PAKISTAN

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### ABSTRACT

Citrus fruit is infected by various biotic factors including fungi, bacteria, viruses, nematode and spiroplasma. These biotic factors reduce the quality and quantity of citrus fruit. Among these biotic factors, fungi are playing a major role. Among the fungi, *Diaporthe* spp. cause significant disease of citrus crop known as citrus melanose. The disease is one of the emerging diseases of citrus trees in Pakistan. The objective of this study was *in-vivo* monitoring of citrus melanose disease and *in-vitro* evaluation of six different fungicides (Topsin-M, Copper oxychloride, Success, Aliette, Kumulus and Mancozeb) at different days of intervals. For this purpose, surveys of different citrus growing Tehsils of Sargodha were conducted for the collection of samples from infected citrus groves. Food poisoning technique was used to evaluate fungicides against *Diaporthe citri*. Three replicates of each fungicide and concentrations of 100, 200 and 300 ppm were used, and data were recorded after 3, 5 and 7-day's interval. Results revealed that all fungicides significantly inhibited mycelial growth of *Diaporthe citri*. The maximum percent inhibition was obtained with the application of Mancozeb, while minimum percent inhibition was obtained with the application of Success with values 36% and 8% respectively.

**Keywords:** Citrus, *Diaporthe* spp., Citrus melanose, Fungicides, Percent inhibition.

### INTRODUCTION

Citrus is one of the most significant crops in the world. It is grown all around the world from tropical to subtropical regions. Citrus is a reservoir of nutrients and among the nutrient's vitamin C is well known in the perspective of human health. Vitamin C helps in absorption of iron, zinc and other foods. Currently, annual worldwide production of citrus is 115 million tons. Brazil having 20 million tons of production is at the top, and Pakistan is ranked the 13<sup>th</sup> among the citrus-

producing countries (Mubeen *et al.*, 2015a, b). Pakistan is bestowed with fertile land and favorable climatic condition for citrus cultivation. Among citrus, Pakistan is the sixth-largest Kinnow producer of the world with a total plantation area of 8.6453 million hectares giving 128 million tones production during the crop year 2017-18 (FAO, 2018). Kinnow is famous due to its unique and delicious taste (Iftikhar *et al.*, 2020). Citrus is cultivated in all provinces of Pakistan, but most of the citrus is cultivated Punjab province due to adequate irrigation water and favorable climatic conditions. Punjab annually produced 98% of the total production of the country out of which 70% is Kinnow. Sargodha is major citrus producing areas of Punjab contributing 43% in citrus production (Knoema, 2019). Several abiotic and biotic agents currently threaten the citrus production. Among the biotic agents, fungi, bacteria, viruses, nematode and

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spiroplasma are responsible for causing major citrus diseases (Iftikhar *et al.*, 2020). Among these biotic agents, fungi contribution is high in causing severe diseases and consequently reducing citrus yield in Pakistan. Among the fungi, *Diaporthe* ssp. is one of the notorious citrus pathogens and causing billions of dollars losses annually. The pathogen is causing a severe disease known as citrus melanose. Apart from citrus melanose, the pathogen also causes diseases such as stem canker of soybean, canker disease of apple, pear and plum rootstocks, sunflower diseases and grapevine trunk diseases. Based on association with the host, initially, the *Diaporthe* was familiarized largely, however at this time it is recognized that species are not host-specific and even a single species can be present on more than one host (Rehner and Uecker, 1994). It is placed in the family *Diaporhaceae*, order *Diaporthales*, class *Sordariomycetes* (Mondal *et al.*, 2004). *Diaporthe citri* Wolf is sexual or teleomorph stage, while *Phomopsis citri* Fawc. is the anamorph stage. *Diaporthe citri* produces two-celled hyaline ascospores, and each cell consists of two oil droplets or guttulate. *Diaporthe citri* causes two fatal diseases on Citrus fruit. This fungus cause melanose disease in perfect stage is categorized as the occurrence of lesions on fruit and leaves. The imperfect stage, the fungus causes a postharvest disease, "stem-end rot" (Whiteside, 1980). Firstly, discovered the melanose near Florida in 1892 by Swingle and Webber (Wolf, 1926). It was not confirmed until 1928 after the discovery of Koch's postulates causal agent of melanose disease was confirmed as *Diaporthe citri* (Bach and Wolf, 1928). Almost all citrus cultivars are affected by *Diaporthe citri*, but very severe in lemons and grapefruit (Mondal *et al.*, 2007). *Diaporthe citri* is reported in seven citrus-producing countries out of top ten citrus-producing countries (FAO, 2018). pulp It is not only affecting the pulp of the fruit, but also reduces the market value (Gopal *et al.*, 2014). Melanose disease is prevalent in all citrus-producing areas in about 80 countries. The characteristic symptoms of *Diaporthe citri* are teardrop, blemishes and mudcake. The young tissues of leaves and green twigs are most susceptible (Dewdney and Timmer, 2011). Temperature between 24°C-28°C is optimum for the development of disease. The primary symptoms appeared after 4-7 days of post-infection. Similarly, when the temperature is low, the symptoms take a longer time to appear. Melanose is rarely severe on the spring growth flush. In summer melanose can be severe enough that it may cause defoliation, particularly

in years following freeze-induced twig die-back. There is a tendency of the disease to form tear-streak and water droplet patterns on the fruits. Initially, the lesions begin as tiny water-soaked spots with a translucent yellow halo which disappears over time. A gummy substance is exuded with the rupturing of the cuticle. This gummy substance hardens and becomes brown and made the texture of sandpaper on the surface of leaves and twigs. The lesions can be disseminated, bunched or streaks. The leaves remain pale green or turn yellow in the severe case and eventually fall. In maximum cases, it becomes challenging and not economical to overcome melanose disease on the fruit and foliage. Whiteside (1997) worked on the efficacy of fungicides to check action against citrus melanose. He used different fungicides including basic Copper sulphate, Chlorothalonil, Dithianon and Benomyl. A single spray of BCS, dithianon and captafol, was applied to post-bloom as long protecting action on fruit rind. Benomyl results in minute protection against melanose, but it wasn't like the other fungicides, it repressed the production of inoculum on dead twigs. Timmer *et al.* (1998) worked on copper fungicides improve control of melanose in Florida. They concluded that copper-based fungicides were helpful for the management of citrus melanose disease. In present research, this aspect is studied whether the control of citrus melanose disease can be improved by increasing the frequent application of copper fungicide. The fungus causes severe losses of tree and crop production to occur from nursery to fruit production. In Pakistan, no major work has been done for the management of this disease and no information is available on the management of this disease, so it is the need of time to manage disease to reduce quality losses as well as to maintain fruit production. In the present study, we monitored and calculated disease incidence in Sargodha District. Also evaluated the different marked-available fungicides against *Diaporthe citri*.

#### **MATERIALS AND METHODS**

The research was conducted in the Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan.

**Collection of samples:** Based on characteristic symptoms, diseased samples were collected from the aerial portion of trees including leaves and fruits during the surveys of citrus orchards.

**Data Recording:** To determine disease incidence and severity, five Tehsils (Silanwali, Shahpur, Kotmomin, Bhalwal and Sargodha) of Sargodha District were

selected. From each Tehsil, three citrus orchards were selected (Table 2). The disease severity of citrus melanose was recorded following 0-5 severity scale as proposed by Rossman *et al.* (2013) as shown in Table 1

and disease incidence was calculated using the following formula:

$$\text{Disease Incidence} = \frac{\text{Number of infected Plants}}{\text{Number of Total Plants}} \times 100$$

Table 1. Disease severity scale

Scale	Response
0	No melanose
1	Mild melanose
2	Suitable for fresh market
3	Moderate speck melanose, suitable only for processing
4	Severe speck melanose
5	Severe tear-stain or mudcake melanose

Table 2. Study area, samples and data collection based on symptomology

Sr. No.	Tehsil	Orchards	No. of Samples	Symptoms Observed
1	Silanwali	127 NB	3	Moderate speck melanose, suitable only for processing
		124 NB	3	Mild melanose
		121 NB	3	Suitable for fresh market
2	Shahpur	Wadhi	3	Severe speck melanose
		Hussain Shah	3	Suitable for fresh market
		Sultan Pur	3	Suitable for fresh market
3	Bhalwal	05 SB	3	Severe speck melanose
		10SB	3	Severe speck melanose
		Miani	3	Suitable for fresh market
4	Kotmomin	6 SB	3	Severe tear-stain or mudcake melanose
		Mateela	3	Severe speck melanose
		65 SB	3	Severe tear-stain or mudcake melanose
5	Sargodha	64 NB	3	Mild melanose
		86 NB	3	Severe tear-stain or mudcake melanose
		37 NB	3	Moderate speck melanose, suitable only for processing

**Isolation of Pathogen and Identification:** Potato dextrose agar (PDA medium) was prepared according to the standard composition (200 g peeled potatoes, 20 g dextrose and 15 g agar). The pathogen *Diaporthe citri* was isolated from the citrus melanose and preserved at 4°C. Working cultures are made regularly and stored at 25°C. Then the pathogen was identified based on cultural and morphological characters like colony colour, shape, size, hyphal, the morphology of sexual structure such as perithecia and pycnidia and conidia as well as using

molecular methods. These characters were compared with reported literature (Bach and Wolf, 1928; Kuhara, 1999).

**Evaluation of Fungicides:** Six fungicides were evaluated against the pathogen (Table. 3). The Stock solution for each fungicide was made separately by liquefying 1g of fungicide in 999 ml of distilled water, and this was called a stock solution. From the stock solution, three different concentrations (100 ppm, 200 ppm and 300 ppm) were prepared for each fungicide.

Table 3. Name of fungicides

Sr. No.	Fungicide	Active ingredient	Company name
1	Topsin-M	Thiophanate Methyl	Arysta
2	Copper oxychloride	Copper oxychloride	Capricorn
3	Success	Chlorothalonil & Metalaxyl	Arysta
4	Aliette	Fosetyl-Al 80%	Bayer
5	Kumulus	Sulphur 800g/Kg	FMC
6	Mancozeb	Cymoxanil 8% + Mancozeb 64%	Jaffer group

**Food Poisoning Technique:** To check the efficacy of the different fungicides food poison technique was used. Potato dextrose agar (PDA) was prepared and amended with fungicides concentrations before pouring. Three plates were poured for each concentration. Five mm mycelial plug of fungi was placed in the center of the plates. Plates were incubated at 27°C, and data were taken after 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup>-days. Percentage inhibition was measured by using the formula given by Fitsum *et al.* (2014); (C= Colony diameter in control and T= Colony diameter in treatment).

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

## RESULTS

**Monitoring and disease incidence:** Disease incidence were calculated during the surveys of different tehsils of Sargodha. Three hundred plants observed in each Tehsil. The highest disease incidence of 27% was recorded in Kotmomin. The 21% disease incidence was calculated in Bhalwal and Sargodha and 14% in Silanwali. The lowest disease incidence of 13% was recorded in Shahpur (Table 4).

Table 4. Disease incidences of citrus melanose in different Tehsils of Sargodha

S. No	Tehsils	Total Plants	Infected Plants	Disease Incidence%
1	Silanwali	300	42	14%
2	Shahpur	300	40	13%
3	Bhalwal	300	65	21%
4	Kotmomin	300	82	27%
5	Sargodha	300	65	21%

**Evaluation of fungicides against *Diaporthe citri*:** The fungicides showed significant results against the mycelial growth of *Diaporthe citri*. The data was recorded on 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup>-days intervals through the inhibition percentage technique. Mycelial growth of *Diaporthe citri* was also significantly inhibited by all fungicides on the 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup>-days at different concentrations. Maximum percent inhibition was given by Mancozeb 35% and 36%,

respectively at concentrations 100-300ppm, followed by Kumulus, 27%, Aliette 22%, Copper Oxychloride 18%, Success 21% and Topsin M 13%. Minimum percent inhibition was given by Success 8%, Topsin M 10%, Coper Oxychloride 9%, Aliette 17%, Kumulus 21% and Mancozeb 32% (Table 5; Fig. 1 A, B, C, D, F and E).

Table 5. Effect of different concentration of fungicides against the disease

Fungicides	Contraction	Inhibition rate		
		3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day
Mancozeb (A)	100ppm	32%	35%	36%
	200ppm	34%	36%	39%
	300ppm	39%	40%	44%
Kumulus (B)	100ppm	24%	27%	20%
	200ppm	18%	23%	24%
	300ppm	22%	25%	21%
Aliette (C)	100ppm	22%	20%	21%
	200ppm	18%	22%	19%
	300ppm	17%	19%	20%
Copper Oxychloride (D)	100ppm	15%	14%	16%
	200ppm	10%	9%	10%
	300ppm	17%	18%	17%
Success (E)	100ppm	15%	17%	17%
	200ppm	10%	8%	13%
	300ppm	17%	18%	21%
Topsin M (F)	100ppm	12%	10%	11.50%
	200ppm	16%	13%	13.50%
	300ppm	15%	12%	13.50%

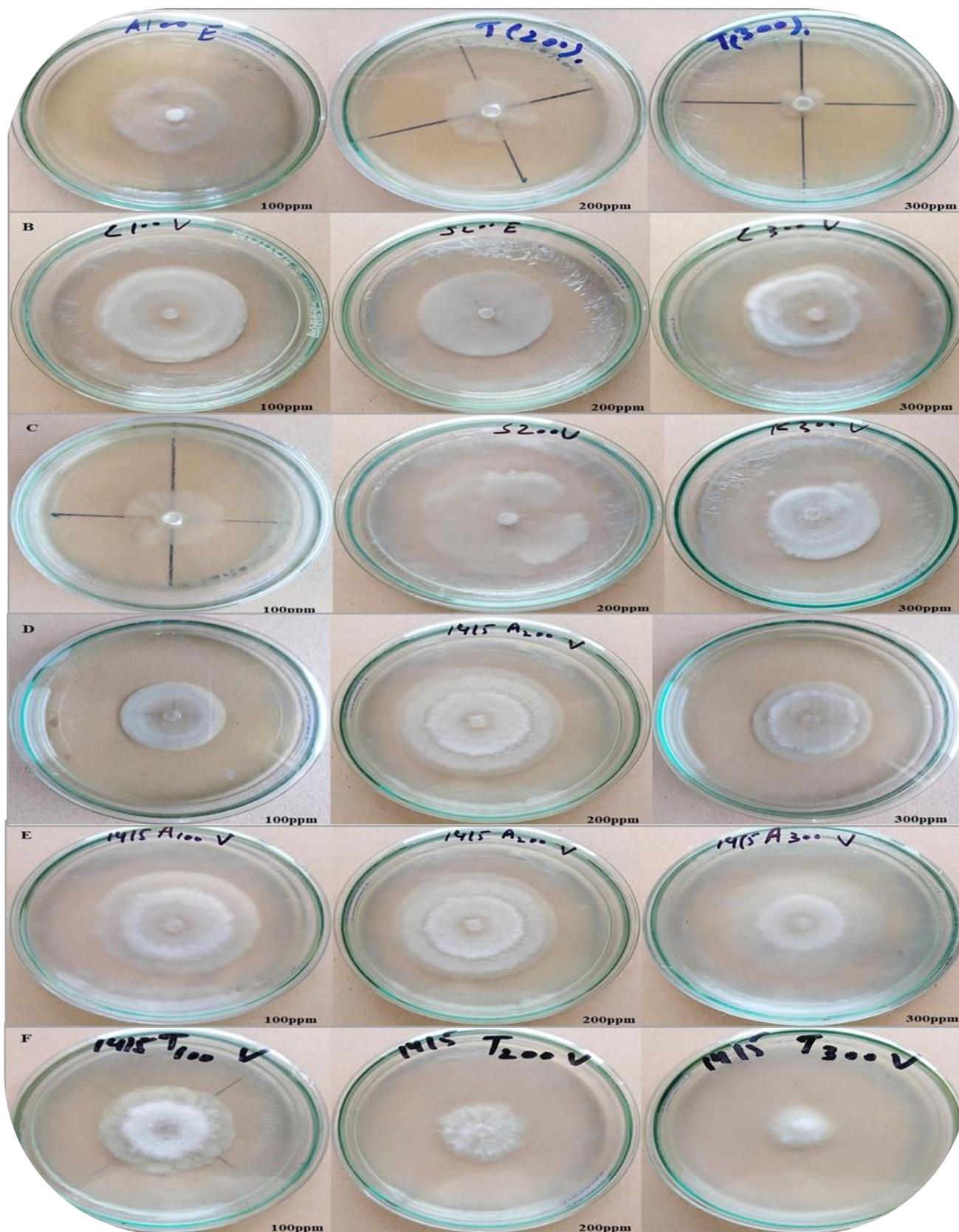


Figure 1. Effect of fungicides against the mycelial growth of *Diporthye citri*

## DISCUSSION

Citrus melanose disease is a severe threat to citrus groves. *Diaporthe citri* is most fatal casual organism for Citrus melanose disease. *Diaporthe citri* Wolf (the fungal pathogen) is the teleomorph or sexual stage while *Phomopsis citri* fawc. is the asexual or anamorph stage. The fungus affects typically the leaves and fruits but it also causes stem-end rot of citrus, and the fungus survives as saprophyte in dried branches. The symptoms vary from minute dark brown to black colour spots on fruits and leaves. The zones range 0.1-0.5 mm in diameter having tear-stained and mudcake patterns. The pathogen normally does not affect the pulp of the fruit, but it creates severe rind blemishes on fruit. In present research disease incidence of citrus melanose disease was calculated by an extensive survey of citrus groves in five Tehsils of Sargodha including Silanwali, Shahpur, Bhalwal, Kotmomin and Sargodha, further three different localities were selected in each Tehsil. The highest disease incidence was calculated as 27% in 66SB in Kotmomin flowed as 19% in 121NB Silanwali, 14% in Shahpur, 13% in Bhalwal 21% and 21% in 86NB in Sargodha. Our finding also resembles Whiteside (1980) who worked on the control of melanose disease by applying different fungicides at different intervals to check the efficacy of fungicides. The periods on which environmental conditions were suitable for infection of melanose were calculated regularly for fifteen years. The severity of melanose disease was related to the number of infection periods that occurred between early April and petal fall and the period late June for rind resistance. There was nil number of possible significant infection days in April in 11 years, 1 for each of 3 years and 2 for the remaining years, while the average of a number of these days was 3.7 for the month of May and 6.0 for the month of June. The recorded data was combined with the readings of spraying period experiments on the grapefruit orchards. This supports the argument that the optimum response for a single copper-based fungicide spray is generally obtained when the application is made in the late April and early May. The observations which were produced from 1966 to 1980, showed that the severity of *Diaporthe citri* on fruit was compared with the number of infection periods during petal fall (early April) and at the time of rind resistance (late June). Simultaneously the results of spray timing experiments

on grapefruit trees supported the contention that an optimum response to a single Cu based fungicide spray is typically obtained when an application is delayed until late April or early May. The pre-shoot growth treatment with a Cu fungicide, unlike one with Difolatan (captafol), failed to control. we also evaluated the *in-vitro* to the efficacy of six fungicides (three concentrations of each) through food poison technique against *Diaporthe citri* after 3<sup>rd</sup> day, 5<sup>th</sup> day and 7<sup>th</sup> day's intervals (Sultana and Ghaffar, 2013). Among these fungicides maximum percent inhibition was given by Mancozeb, 32%, 35% and 36%, at 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day, respectively at concentrations 100-300 ppm followed by Kumulus, 27%, Aliette 22%, Copper Oxychloride 18%, Success 21% and Topsin M 13%. Minimum percent inhibition was given by Success 8%, Topsin M 10%, Coper Oxychloride 9%, Aliette 17%, Kumulus 21% and Mancozeb 32%. Mycelial growth of Diporthe citri was also greatly repressed by all fungicides at 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day at different concentrations. Our result resembles to Ruehle and Kuntz (1940). They worked on the commercial control of citrus melanose disease. The spraying experiments were conducted in commercial orange and grapefruit orchards in central Florida, a single application of 6-6 (hydrated lime) 100 Bordeaux mixture results in the reliable control of melanose (*Diaporthe citri*). Similarly, a single application of a 3-3-100 mixture was sometimes equal to the stronger spray, but under conditions of severe infection, it couldn't prove useful, while applying two applications of the 3-3-100 spray, with the interval of three to four weeks produces better results as compare to a single treatment of the 6-6-100 formula. Pre-growth spraying experiments were also conducted that were proved less efficient. Chen *et al.* (2010) worked on *in-vitro* and *in-vivo* screening of fungicides for controlling citrus melanose. Four fungicides (tebuconazole, difenoconazole, prochloraz manganese chloride complex and mancozeb) were used in order to calculate their efficacy against the mycelial and conidial growth of *Diaporthe citri* and the inhibitory effects of these fungicides were determined in these experiments. Their results showed that the application of mancozeb is more effective than the other three fungicides, and they suggest that the mancozeb is the most effective one against the control of citrus melanose disease. Our observations are resembling with them.

## CONCLUSION

High disease incidence of citrus melanose was recorded

in Kotmomin while the lowest disease incidence was recorded in Shahpur. Moreover, all fungicides have significantly inhibited citrus melanose disease. However, the efficacy of the fungicides varies with the different concentrations and days. The most effective fungicides were found to be Mancozeb followed by Success.

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#### REFERENCE

- Bach, W.J. and F.A. Wolf. 1928. The isolation of the fungus that causes citrus melanose and the pathological anatomy of the host. *Journal of Agricultural Research*, 37:243-252.
- Chen, G., L. Jiang, F. Xu and H. Li. 2010. In vitro and in vivo screening of fungicides for controlling citrus melanose caused by *Diaporthe citri*. *Journal of Zhejiang University*, 36: 440-444.
- Dewdney, M. M and L.W. Timmer. 2011. *Citrus Pest Management Guide*. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. USA. 145.
- FAO. 2018. *FAO Production Year Book*, FAO, Rome, Italy.
- Fitsum, S., M. Amin, T. Selvaraj and A. Alemayehu. 2014. In vitro evaluation of some fungicides and bioagents against common bean anthracnose (*Colletotrichum lindemuthianum* Sacc. *Magnus*) Briosi Cavara. *African Journal of Microbiology Research*, 8: 2000-2005.
- Gopal, K., M. Lakshmi, S. Sarada, T. Nagalakshmi, T.G. Sankar, V. Gopi and K. Ramana. 2014. Citrus melanose (*Diaporthe citri* Wolf): A review. *International Journal of Current Microbiology and Applied Science*, 3:113-124.
- Iftikhar, Y., F. Bakhtawar, I. Hussain, A. Sajid, M. Mubeen, M.A. Zeshan, M.A. Sohail, N. Fatima, M. Umer, S. Iqbal. 2020. Detection of *Spiroplasma citri* causing citrus stubborn disease in Sargodha, Pakistan *International Journal of Botany Studies*, 5: 481-485.
- Knoema.com/atlas/Pakistan/topics/Agriculture/Crops-Production-Quantity-tonnes/Citrus-fruit-production/2019.
- Kuhara, S. 1999. The application of the epidemiologic simulation model "MELAN" to control citrus melanose caused by *Diaporthe citri* (Faw.) Wolf. *Food and Fertilizer Technology Center Extension Bulletins*, National Fruit Research Institute, Japan. 8: 1-8.
- Mondal, S., J.P. Agostini, L. Zhang and L.W. Timmer. 2004. Factors affecting pycnidium production of *Diaporthe citri* on detached citrus twigs. *Plant Disease*, 88: 379-382.
- Mondal, S., A. Vicent, R. Reis and L.W. Timmer. 2007. Efficacy of pre-and post-inoculation application of fungicides to expanding young citrus leaves for control of melanose, scab, and *Alternaria* brown spot. *Plant Disease*, 91: 1600-1606.
- Mubeen, M., H. M. I. Arshad, Y. Iftikhar, I. Bilqees, S. Arooj and H.M.A. Saeed. 2015a. In-vitro efficacy of antibiotics against *Xanthomonas axonopodis* pv. *citri* through inhabitation zone techniques. *International Journal of Agriculture and Applied Sciences*, 7: 67-71.
- Mubeen, M., H. M. I. Arshad, Y. Iftikhar, M.U. Irfan and I. Bilqees. 2015b. Bio-chemical characterization of *Xanthomonas axonopodis* pv. *citri*: a gram-negative bacterium causing citrus canker. *Journal of Science & Nature*, 6: 151-154.
- Rehner, S.A., and F.A. Uecker. 1994. Nuclear ribosomal internal transcribed spacer phylogeny and host diversity in the *Coelomycete phomopsis*. *Canadian Journal of Botany*, 72:1666-1674.
- Rossmann, A., D. Udayanga, A. Lisa, B. Castle and K. D. Hyde. 2013. Proposal to conserve the name *Phomopsis citri* H.S. Fawc. (*Diaporthe citri*), with a conserved type, against *Phomopsis citri* (Sacc.) Traverso & Spessa (Ascomycota: Diaporthales: Diaporthaceae). *Taxon*, 62: 627.
- Ruehle, G.D. and W.A. Kuntz. 1940. Melanose of Citrus and its commercial control. *Bulletin. Florida Agricultural Experiment Station*, 349: 54.
- Sultana, N. and A. Ghaffar. 2013. Effect of fungicides, microbial antagonists and oil cakes in the control of *Fusarium oxysporum*, the cause of seed rot and root infection of bottle gourd and cucumber. *Pakistan Journal of Botany*, 45: 2149-2156.
- Timmer, LW., S. Zitko and L. Albrigo. 1998. Split applications of copper fungicides improve control of melanose on grapefruit in Florida. *Plant Disease*, 82: 983-986.
- Whiteside, J. 1977. Sites of action of fungicides in the control of citrus melanose. *Phytopathology*, 67: 1067-1072.

Whiteside, J. 1980. Timing of fungicide spray treatments for citrus melanose control. Paper presented at the Proceedings of the Florida State Horticultural Society. USA.

Wolf, F.A. 1926. The perfect stage of the fungus which causes melanose of citrus. Journal of Agricultural Research, 33:621-625.

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Umair Anwar	:	Performed the experiment.
Mustansar Mubeen	:	Wrote the manuscript.
Yasir Iftikhar	:	Supervised, designed the experiment and wrote the manuscript.
Muhammad A. Zeshan	:	Performed the experiment.
Qaiser Shakeel	:	Edit the manuscript.
Ashara Sajid	:	Performed the experiment.
Muhammad Umer	:	Analysis the data.
Aqleem Abbas	:	Prepared tables.