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EFFICACY OF FUNGICIDES AGAINST *PENICILLIUM ITALICUM* CAUSING CITRUS BLUE MOLD

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ABSTRACT

Citrus is an economically important fruit crop in Pakistan; however, its productivity is affected by various insect pests and pathogens. Spores of *Penicillium italicum* Warmer are airborne and cause citrus blue mold disease in citrus plants. The main objective of this study was to assess the in-vitro efficacy of different concentrations of selected fungicides against *P. italicum* applied at different times. Before pouring, potato dextrose agar was made and modified with fungicides. For each concentration, three plates were poured. After the plates had solidified, a 5 mm mycelial plug of the fungus *P. italicum* was inserted in the center Five fungicides i.e., Nativo, Topsin-M, Metalaxyl+Mancozeb, Copper Oxychloride, Success were tested at three concentrations i.e., 100 ppm, 200 ppm, and 300 ppm. The findings of the study showed that Nativo was the best fungicide as the inhibition rate of *P. italicum* was 81.0-87.0%, after 7 days of application. The second most effective fungicide was Success, which showed 77.5-81.0% inhibition of *P. italicum* after 3 days and 78.0-80.0% after 7 days of application. Similarly, Topsin M showed 68.3-77.0% inhibition of *P. italicum* on 3 days and 77.0-81.0% on 7 days of application. Metalaxyl+Mancozeb and copper did not prove effective against *P. italicum*, even at higher concentrations. The higher concentration of each fungicide resulted in maximum inhibition of *P. italicum*. Thus, the current study suggests that Nativo, Success, and Topsin M could manage the citrus blue mold disease at the standardized concentration.

Keywords: *Penicillium italicum*, fungus, citrus blue mold, kinnow, fungicides, remedy.

INTRODUCTION

Citrus is the most widely produced and exported fruit crop in the World (Papoutsis *et al.,* 2019). It's eaten in the form of natural or derived products. Citrus fruits are necessary for humans since they are utilized in pharmaceuticals. However, in recent years, undesirable climatic conditions e.g., hot temperatures, below-average rainfall after the first two blooms and fruit set (Tayel *et al.,* 2015), and an increase in the frequency of pathogenic diseases have resulted in a significant decline in orange production (DaCunha *et al.,* 2018; Yang *et al.,* 2020). According to Singh *et al.* (2012), economic losses can range from 30 to 50% of total production (Vitoratos *et al.,* 2013; Yun *et al.,* 2013; Youssef & Hussien, 2020).

Pakistan's kinnow is well known for its distinct delicious flavor. With almost 2.5 million tons of citrus produced on 510,431 acres, Pakistan is ranked 12th in the world. Citrus is grown in all of Pakistan; however, the majority of citrus is grown in Punjab due to the abundant irrigation water, favorable weather conditions, and a larger population. Punjab produces 2,315,895 tons each year, accounting for 98% of the country's total production, with kinnow accounting for 70% of it. Sindh produces 31,259 tons annually, while annual production of KPK is 3,125 tons. Balochistan produces 7,350 tons annually (Usman *et al.,* 2018).

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Citrus fruit export is a significant source of forex for Pakistan with an annual value of 200 million US dollars (Malik 2014; Siddique & Garnevska, 2018). Pakistan has a citrus output per hectare of 10-12 tons, but other citrus-producing countries have yields of up to 26 tons per hectare (Fateh *et al.,* 2017).

The main reason for lower yield is citrus diseases caused by viruses, bacteria, fungi, nematodes, and *spiroplasma. Spiroplasma* causes stubborn citrus disease and reduces 25-32% of fruit yield than non-infected plants (Mello *et al.,* 2010). Citrus is infected with thirty different viruses, the most frequent of which are citrus ring spot virus and yellow vein clearing virus (Alshami *et al.,* 2003). Three important citrus diseases caused by bacteria have their effect on citrus yield; *Xanthomonas axonopodis* is the cause of citrus canker; the *Xyllelafastidiosa* variegated chlorosis is the cause of citrus variegation; and *Candidatus liberibacter* of greening disease.

There are over forty nematode species known to be pathogenic to citrus trees, including the most important cause of the slow decline of the citrus *Tylenchulus semi-penetrants* (Sharif *et al.,* 2018). Numerous fungal diseases are responsible for less citrus yield per hectare. The dry rot of *Fusarium* is an important pathological disease of the soil that is caused by *Fusarium solani*. It affects the plant root system, thereby reducing the plant's nutrition capability. This fungus also produces toxins that move in the plant's xylem system and plug vessels. *Diplodianatalensis*, *Armillaria mella*, *Pythium* spp., and *Thielaviopsis basicola* are other fungal pathogens involved in soil-borne diseases with negative effects on yield. Malsecco, which was first seen in Greece in 1880, is the cause of vascular disease by the fungus *Phomatracheiphila*. It enters the stomata systemically and occupies the xylem. Then symptoms of wilting start including discoloration of wood, venial chlorosis, leaf wilting, and di-back of twigs (Solel & Salnero, 2000). Brown citrus spot, stem red citrus, and black rot citrus are known to produce brown to black spots on fruit and leaves. Fruit is internally infected with black rot, caused by *Alternaria citri* (Mojerlou & Safaie, 2012). Several Colletotricum species are proven to cause important diseases and the most important one is citrus anthracnose (Guarnaccia *et al.,* 2018). *Diporthecitri*, tear falling, blemishes, and mud cake are major symptoms of citrus fruit. *Elsienofawcettii* and *Elsieno australis* are responsible for citrus scab, which affect citrus fruit, leaves, or shoots badly during warm and wet weather (Gopal *et al.,* 2014).

Pathogens attacking citrus fruit in pre- and post-harvest phases cause severe recurring economic losses. Production, cultivar, environment, damages, and post-harvest techniques influence the degree of losses caused by these illnesses. Blue mold is the most common postharvest disease in cold-stored citric fruit, while green mold can cause 60–80% degradation under ideal conditions. In the pre- and post-harvest phases, rind wounds are caused by necrotrophic pathogens (Ballester *et al.,* 2010). As a result, the treatment of citrus blue and green molds is required for long-term storage success. *P. italicum* can also induce small lesions (19.9 11.0 nm) at room temperature (33.9 4.5 percent relative humidity) in ambient storage circumstances. In the ambient and cold storage conditions, the lesions' growth rate is 4.8 and 1.4 mm/d. In addition, *P. italicum* led infections showed initial signs of cold lesion growth after 12-13 days, and after 2-3 days, first signs were observed under ambient conditions (Louw & Korsten, 2015).

Blue mold disease has symptoms such as watery, soft, and colorless fruit, loss of shine in affected peel tissue, and increased sensitivity owing to mechanical damage (Louw & Korsten, 2015; Papoutsis *et al.,* 2019). As white mycelium grows in the infected tissue and extends into the blue conidia, it sporulates (Louw & Korsten, 2015). The transmission of fungal spores in the air causes infection to spread, and the damaged healthy fruit can only be contaminated by direct touch before or after harvest (Kellerman *et al.,* 2016). The disease becomes more severe as the fruit matures. Temperatures between 20˚C and 25˚C, as well as high spore concentrations in skin lesions, exacerbate disease severity (Papoutsis *et al.,* 2019). Control of citrus diseases after harvest is essential to preserve quality and shelf life in the market which can take several weeks for transport from the producer to the consumer. To minimize post-harvest decay, fungicides such as thiabendazole and imazalil are important. The trade in citrus overseas would be considerably reduced without the use of fungicides. The sale of fresh Florida citrus was estimated to decrease by a minimum of 50 percent without using thiabendazole, with a decrease of $250 million in fresh fruit sales alone (Schirra *et al.,* 2008). Such reductions could also lead to lower prices

for grapefruit juice because more grapefruit can be reallocated to production. Based on their initial infections, the major postharvest conditions can be divided into two categories. *Penicillium digitatum*, *P. italicum*, *Geotrichum citriaurantii*, *Diplodianatalensis*, *Phomopsiscitri*, *Colletotrichum gloeosporioides*, *Phytophthora* species, and *Alternaria citri*cause pre- and post-harvest infections (Palou, 2014).

Citrus is the most widely produced and exported fruit crop in the World (Papoutsis *et al.,* 2019) It's eaten in the form of natural or derived products. Citrus fruits are necessary for humans since they are utilized in pharmaceuticals. However, in recent years, undesirable climatic conditions for example hot temperatures, below average rainfall after the first two blooms and fruit set (Tayel *et al.,* 2015), and an increase in the frequency of pathogenic diseases have resulted in a significant decline in orange production (DaCunha *et al.,* 2018; Yang *et al.,* 2020). According to Singh *et al.* (2012), economic losses can range from 30 to 50 percent of total production (Vitoratos *et al.,* 2013; Yun *et al.,* 2013; Youssef & Hussien, 2020).

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MATERIALS AND METHODS

The study was conducted under in vitro conditions in the Fungal Culture Bank and Plant Disease Diagnostic Laboratory at the College of Agriculture, University of Sargodha. A survey was carried out to obtain diseased samples from various citrus plantations in Sargodha. Based on characteristic symptoms, infected fruit samples were collected and stored in an incubator at 4˚C. *Penicillium itlaicum* was identified using an Olympus microscope in the fungal bank laboratory.

PDA Medium: For the evaluation of fungicides, the food poisoning technique was used. The medium of potato dextrose agar was prepared using the ingredients given below (Table 3.1).

Table 3.1 Ingredients used for the preparation of PDA

Sr.No	Ingredients	Quantity
1	Potato Starch	200 g
2	Agar	17 g/l
3	Dextrose	17g/l

Preparation of Medium: Unpeeled potatoes were boiled for 30 minutes in 1000 ml of water and then filtered through a cheese cloth to save effluent. The effluent was treated with dextrose, agar, and water. The media was sterilized by autoclaving at 121˚C for 15 minutes before being poured onto glass petri plates.

Storage of Media: Medium is sensitive to both light and temperature. Plates were kept at 2-8 ˚C, away from direct light. Plates could be used for a week if they were kept in a clean, sterile environment.

Preparation of stock solution: A stock solution was made for each fungicide by dissolving 1 g of fungicide in 999 ml of distilled water. This stock solution was used to make a variety of concentrations.

Preparation of fungicidal concentration: A stock solution was used to make three different concentrations of each fungicide. Using 100 mL of stock solution and 900 mL of distilled water, a 100 ppm concentration was achieved. 200 ml of the stock solution was dissolved in 800 ml of distilled water to achieve a 200-ppm concentration. For the production of the 300 ml concentration, 300 ml of stock solution was dissolved in 700 ml of distilled water (Table 3.2).

Table 3.2. List of fungicides used for control of *Penicillium italicum*

Sr. No.	Fungicide	Active ingredient	Manufacturer
1	Nativo	Trifloxystrobin+Tebuconazole	Bayer
2	Topsin-M	Thiophanate Methyl	Arysta
3	Metalaxyl+Mancozeb	Metalaxyl+Mancozeb	Green Zone
4	Copper Oxychloride	Copper Oxychloride	Capricorn
5	Success	Chlorothalonil & Metalaxyl	Arysta

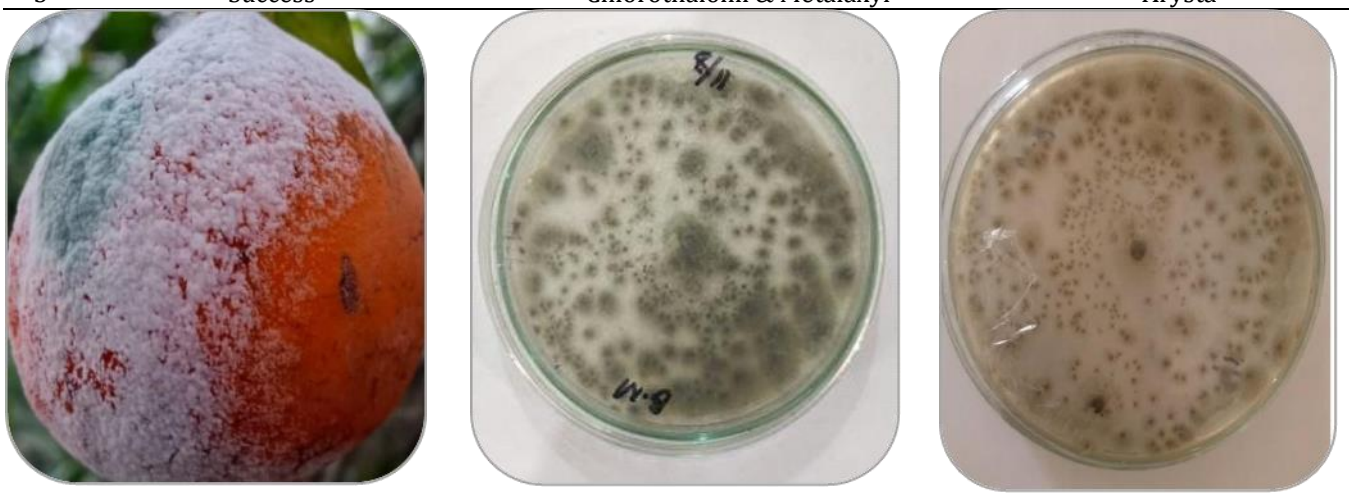


Figure 3.1. Infected citrus fruit &pure culture plate of Bluemold

Poisoned Food Technique: The efficacy of five different fungicides was tested using the food poisoning technique. In an in vitro experiment, three concentrations of each fungicide were prepared: 100 ppm, 200 ppm, and 300 ppm. Each therapy was repeated three times in this study. Before pouring, potato dextrose agar was made and modified with fungicides. For each concentration, three plates were poured. After the plates had solidified, a 5 mm mycelial plug of the fungus *P. italicum* was inserted in the center (Gautam *et al.,* 2017). Data was collected after 72, 120, and 168 hours of incubation at 25˚C, followed by pouring. The following formula was used to calculate percentage growth:

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

C= Colony diameter in control

T= Colony diameter in treatment

DATA ANALYSIS

ANOVA (one way) was performed to check the efficacy of fungicides at different concentrations on the inhibition rate of *P. italicum*. Means were separated by

LSD all-pairwise comparison test at α= 0.05. All the analyses were performed using Minitab 17.0 software.

RESULTS

Penicillium italicum was discovered based on morphological characteristics. On CYA, it generated colonies with a diameter of 30-40 mm, however on MEA, it produced colonies with a diameter of up to 55 mm. Colonies were dark green and flat, with medium and reverse brown coloring. Penicilli were triverticillate, with conidia that ranged from ellipsoidal to cylindrical (Figure 4.1).

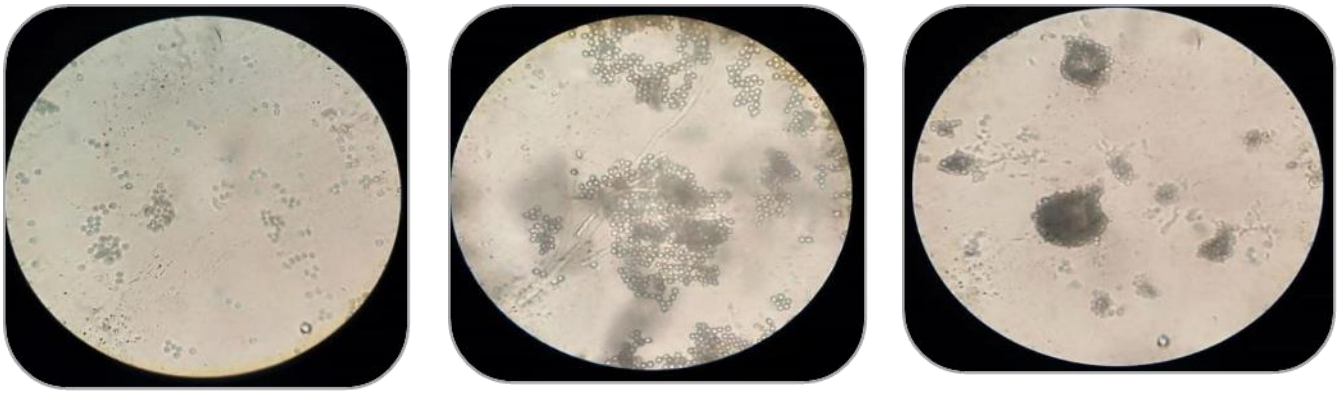


Figure 4.1. Microscopic Identification of blue mold

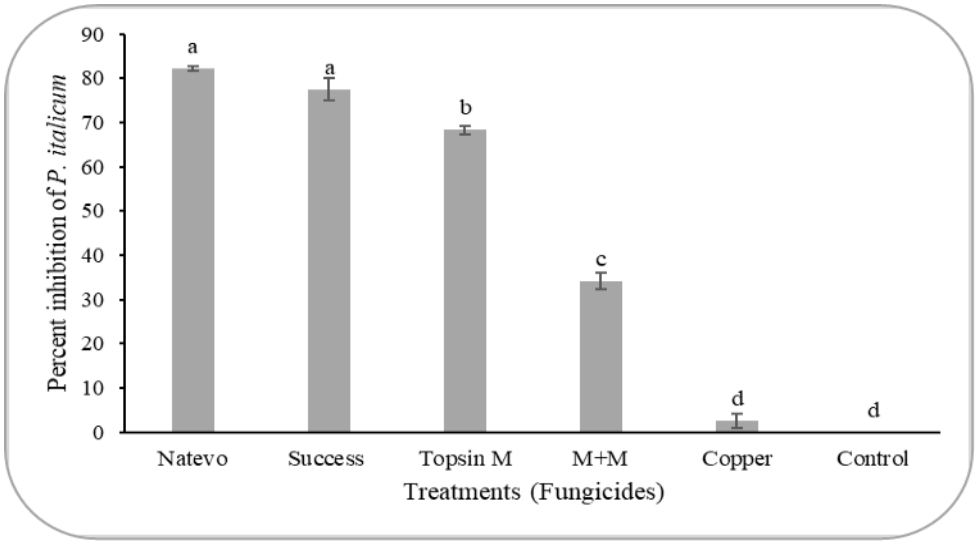
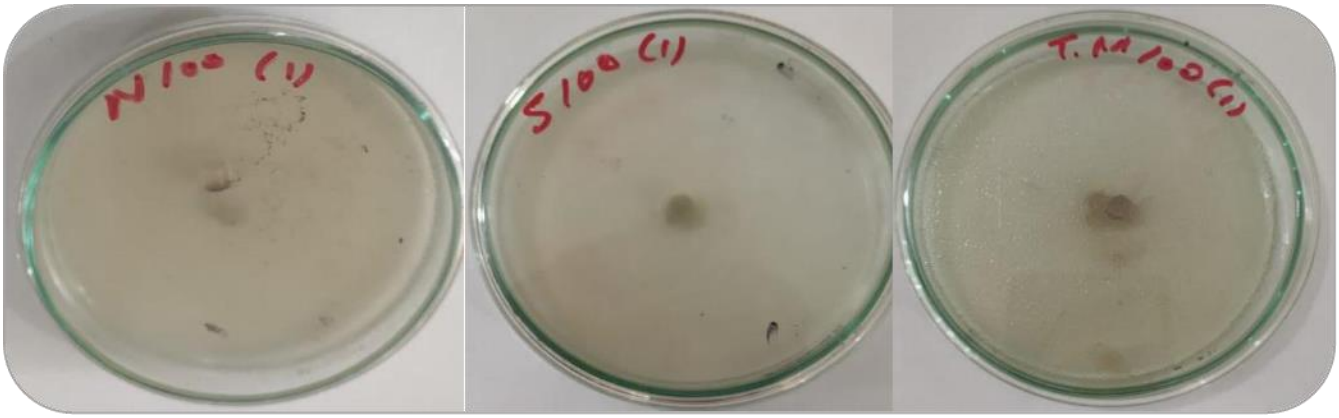
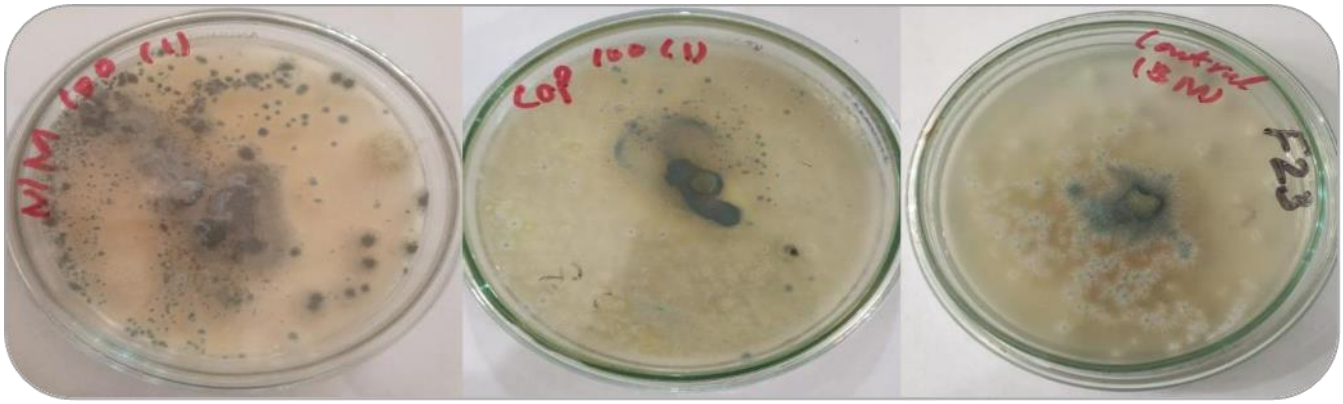


Figure 4.2.1 Percent inhibition of *Penicillium italicum* after 3 days of fungicides' application

After 3 days of fungicide application (100 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (82.2%) followed by Success (77.5%). Copper Oxychloride showed minimum inhibition (2.50%) of *P. italicum*. Metalaxyl+ Mancoze (MM) was not effective in this regard (Figure 4.2.1; Figure 4.2.2).



Nativo (100 ppm) Success (100 ppm) Topsin M (100 ppm)



Metalaxyl+ Mancoze Copper Oxychloride Control (100 ppm)

Figure 4.2.2. Pictorial view of inhibition of *Penicillium italicum* after 3 days of fungicides' application

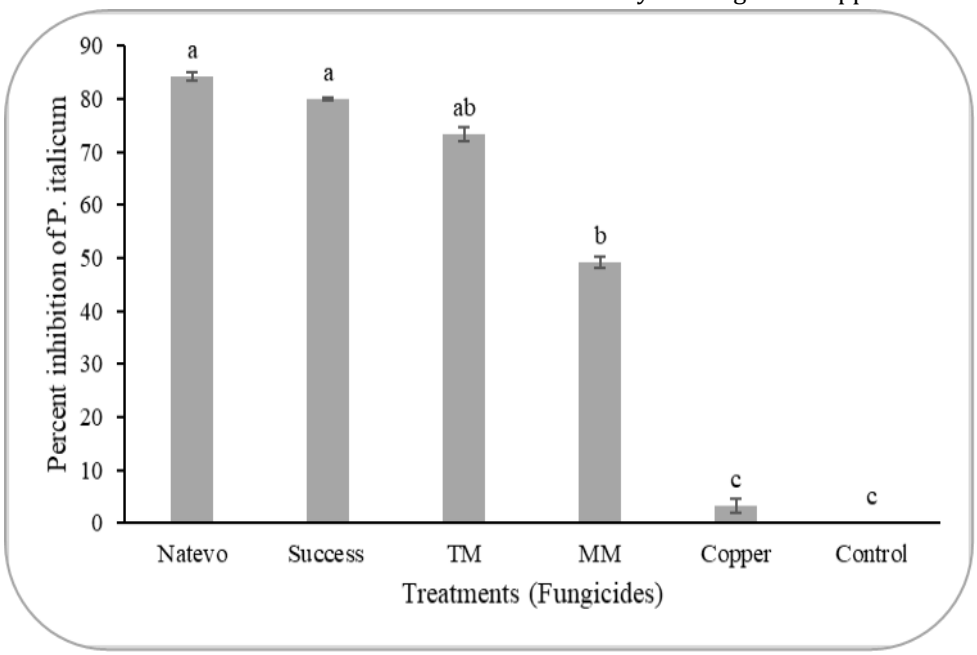
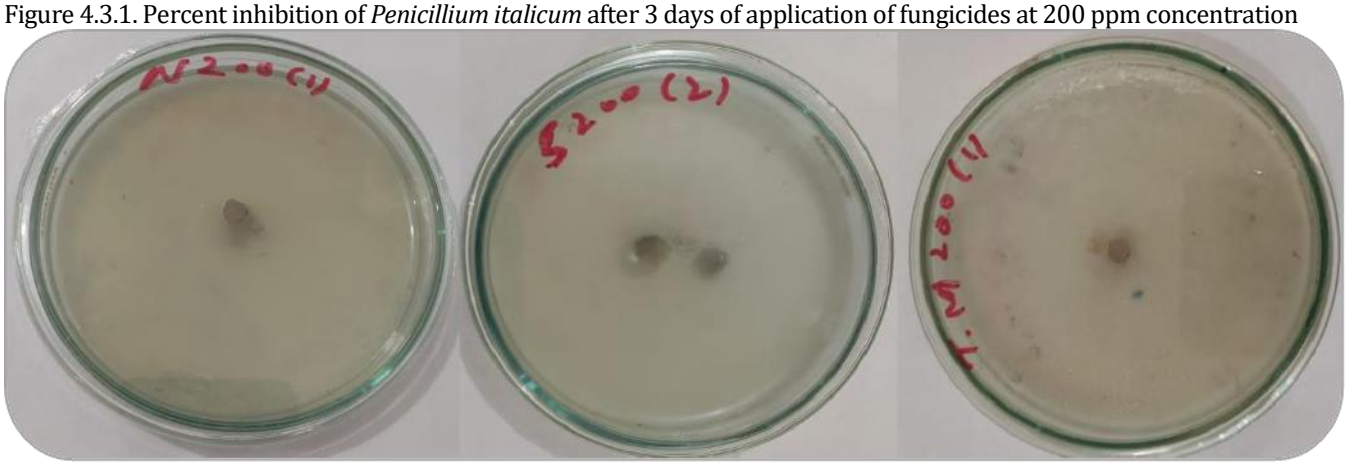
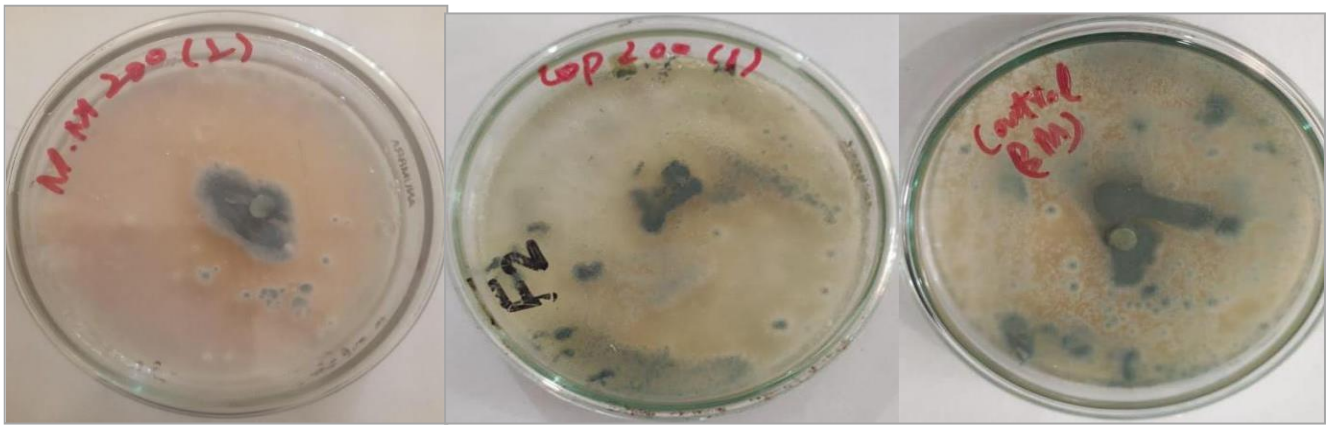


Figure 4.3.1. Percent inhibition of *Penicillium italicum* after 3 days of application of fungicides at 200 ppm concentration



Nativo (200 ppm) Success (200 ppm) Topsin M (200 ppm)

After 3 days of fungicidal application (200 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (84.33%) followed by Success (80.0%). Copper Oxychloride showed minimum inhibition (3.33%) of *P. italicum* (Figure 4.3.1; Figure 4.3.2).



Metalaxyl+Mancozeb Copper Oxychloride Control **(**200 ppm)

Figure 4.3.2. Pictorial view of inhibition of *Penicillium italicum* after 3 days of application of fungicides at 200 ppm concentration

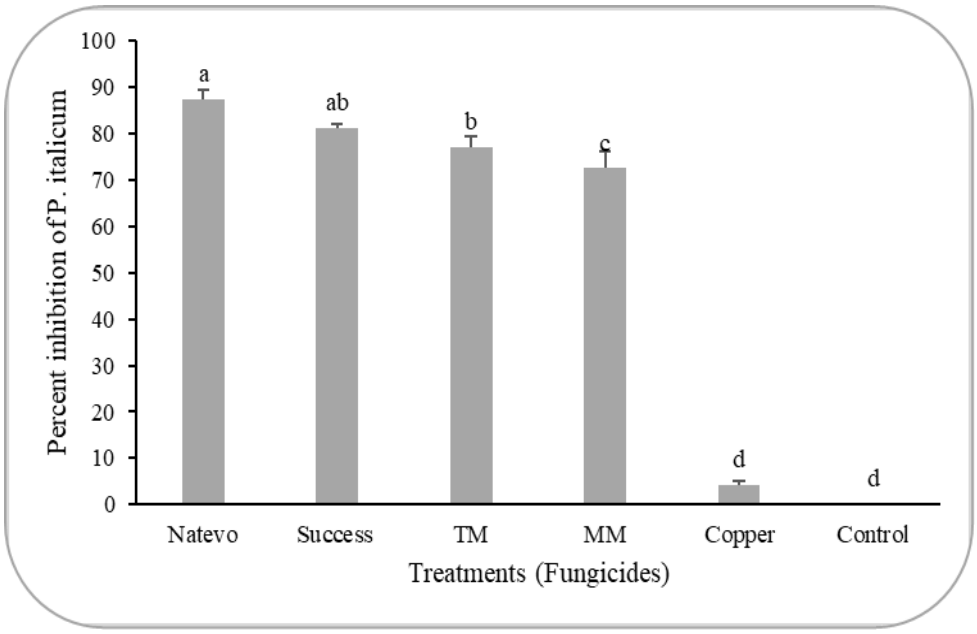
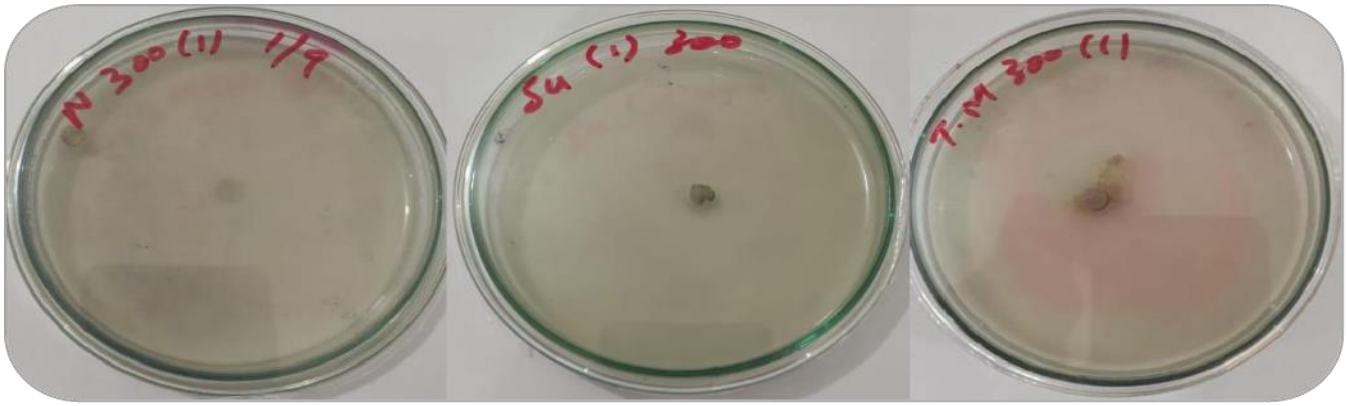
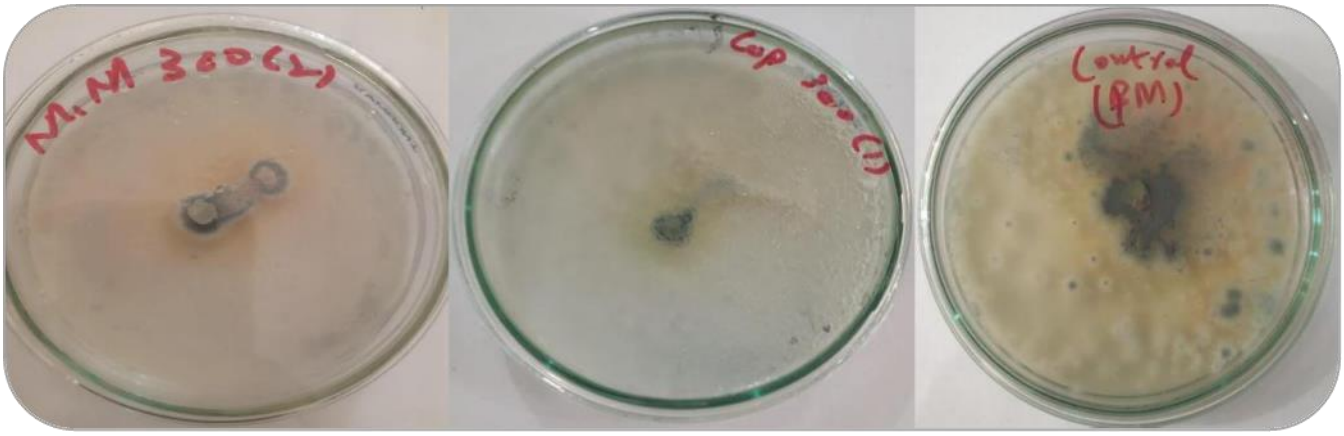


Figure 4.4.1. Percent inhibition of *Penicillium italicum* after 3 days of application of fungicides at 300 ppm concentration.

After 3 days of fungicidal application (300 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (87.5%) followed by Success (81.25%). Copper Oxychloride showed minimum inhibition (4.17%) of *P. italicum* (Figure 4.4.1; Figure 4.4.2).



Nativo (300 ppm) Success (300 ppm) Topsin M (300 ppm)



Metalaxyl+ Mancozeb Copper Oxychloride Control (300 ppm)

Figure 4.4.2 Percent inhibition of *Penicillium italicum* after 3 days of application of fungicides at 300 ppm concentration.

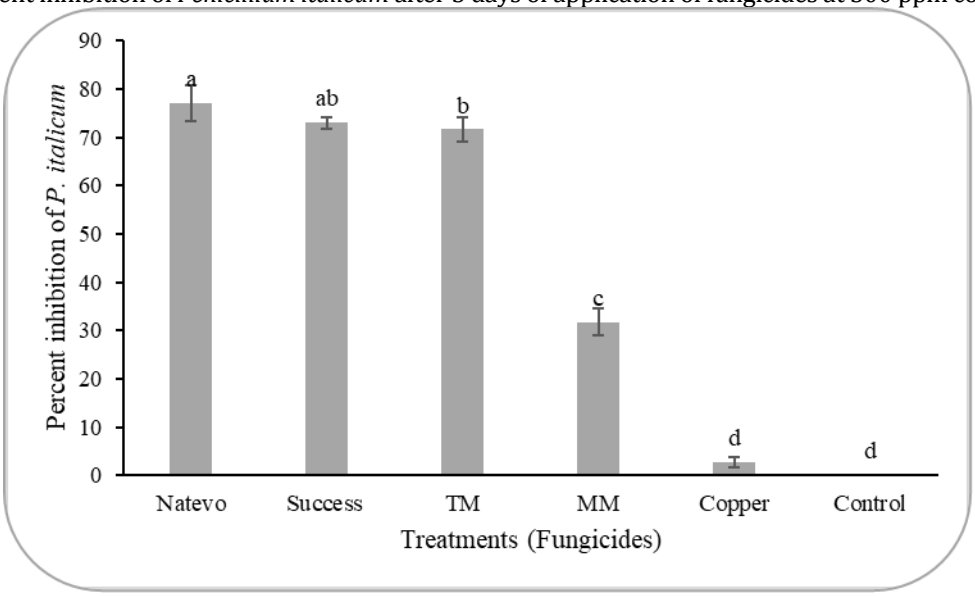
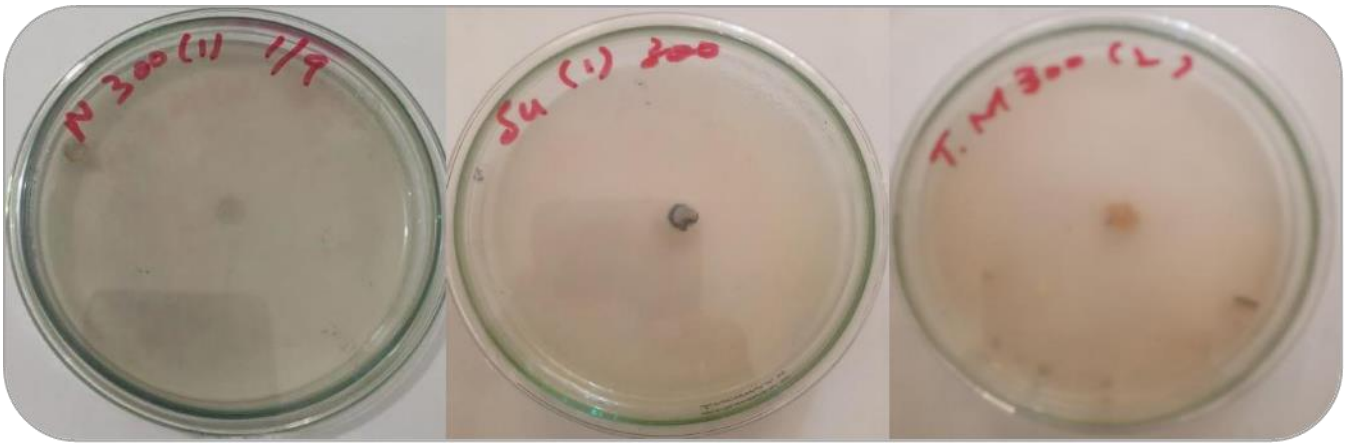


Figure 4.5.1 Percent inhibition of *Penicillium italicum* after 5 days of application of fungicides at 100 ppm concentration

After 5 days of fungicidal application (100 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (77.0%) followed by Success (72.9%). Copper Oxychloride showed minimum inhibition (2.7%) of *P. italicum* (Figure 4.5.1; Figure 4.5.2).



Nativo (300 ppm) Success (300 ppm) Topsin M (300 ppm)

Metalaxyl+ Mancozeb Copper Oxychloride Control (300 ppm)

Figure 4.6.2. Pictorial view of inhibition of *Penicillium italicum* after 7 days of application of fungicides at 300 ppm concentration

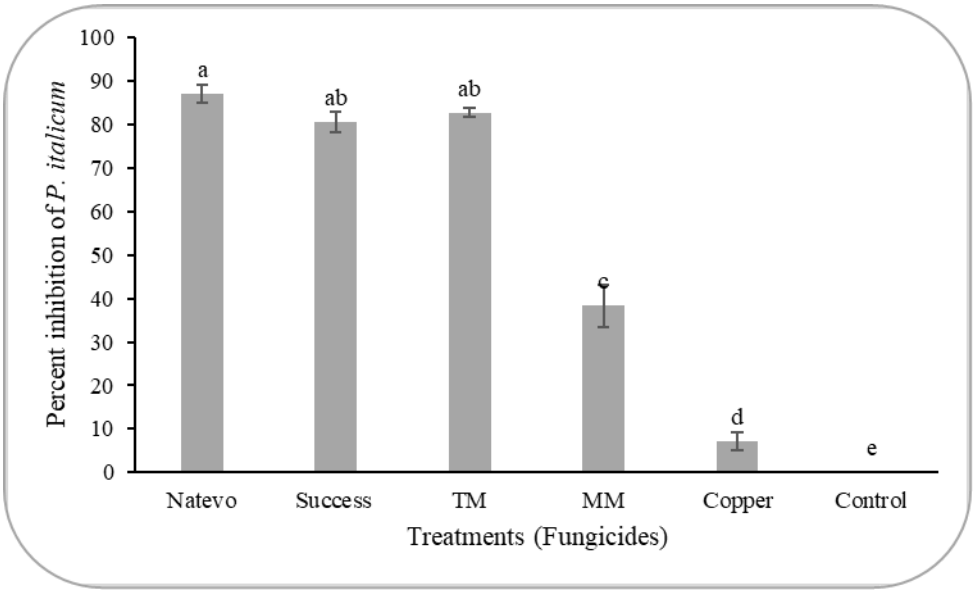
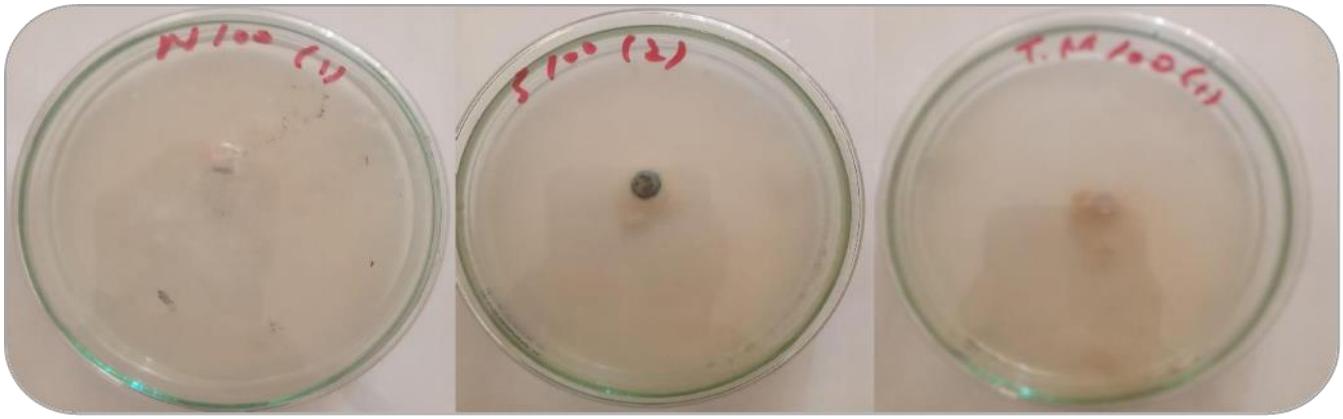


Figure 4.6.1 Percent inhibition of *Penicillium italicum* after 7 days of application of fungicides at 300 ppm concentration

After 7 days of fungicidal application (300 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (87.2%) followed by Topsin M (82.8%). Copper Oxychloride showed minimum inhibition (7.2%) of *P. italicum* (Figure 4.6.1; Figure 4.6.2).



Nativo (100 ppm) Success (100 ppm) Topsin M (100 ppm)

Metalaxyl+ Mancozeb Copper Oxychloride Control (100 ppm)

Figure 4.6.2. Pictorial view of inhibition of *Penicillium italicum* after 7 days of application of fungicides at 300 ppm concentration

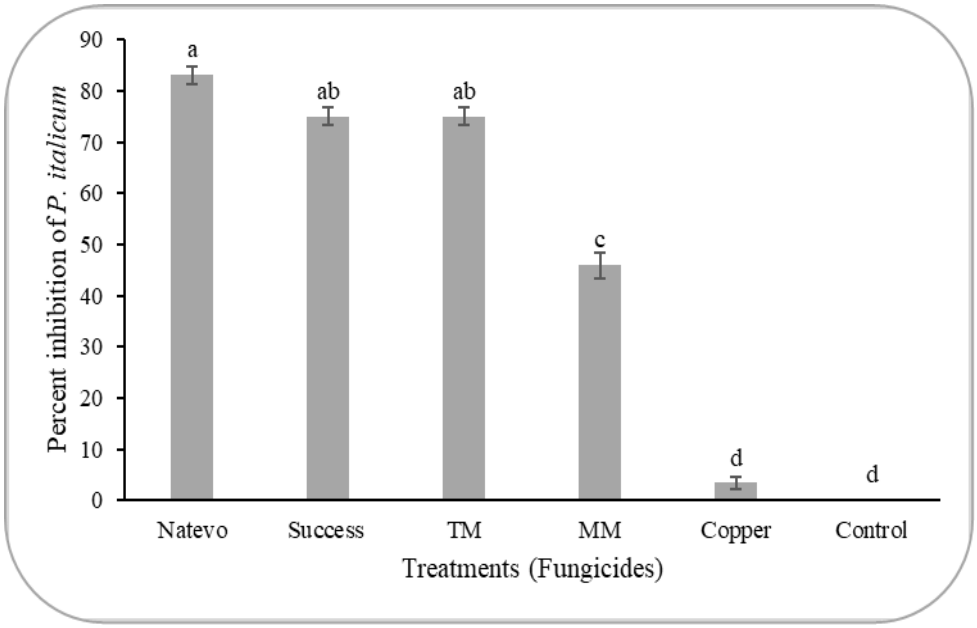
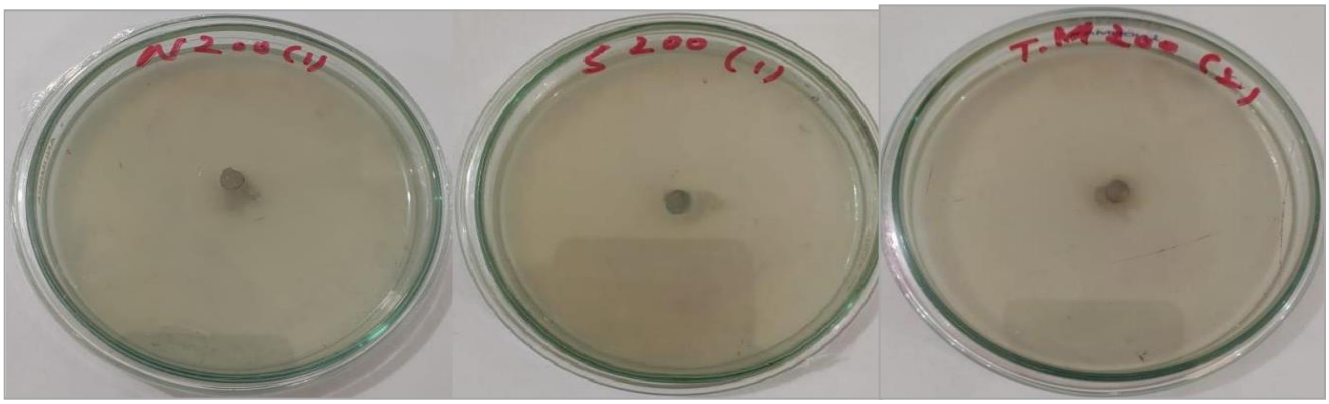


Figure 4.7.1 Percent inhibition of *Penicillium italicum* after 5 days of application of fungicides at 200 ppm concentration

After 5 days of fungicidal application (200 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (83.12%) followed by Success (75.0%). Copper Oxychloride showed minimum inhibition (3.38%) of *P. italicum* (Figure 4.7.1; Figure 4.7.2).



Nativo (200 ppm) Success (200 ppm) Topsin M (200 ppm)

Metalaxyl+ Mancozeb CopperOxychlorid Control (200 ppm)

Figure 4.7.2. Pictorial view of inhibition of *Penicillium italicum* after 5 days of application of fungicides at 200 ppm concentration

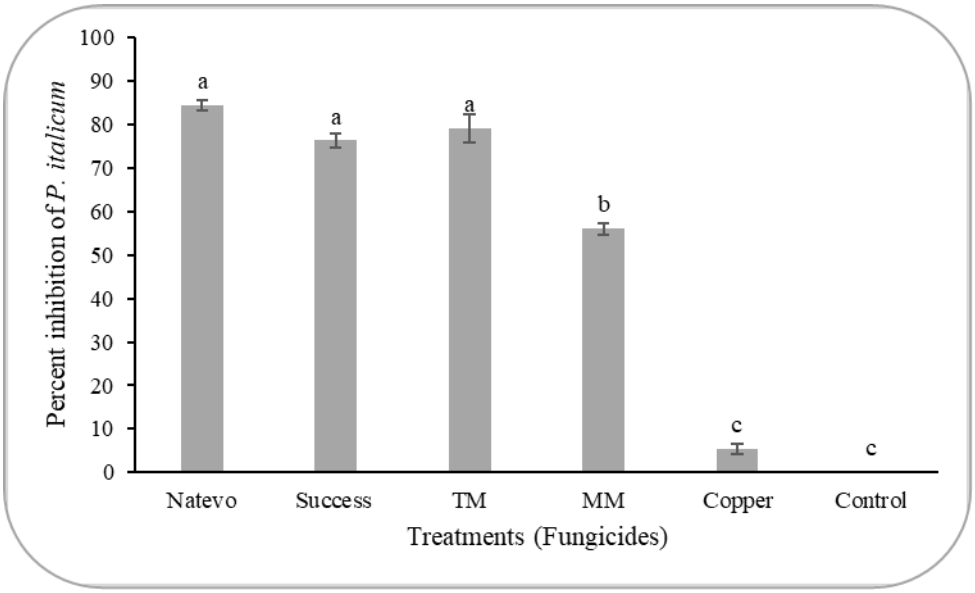
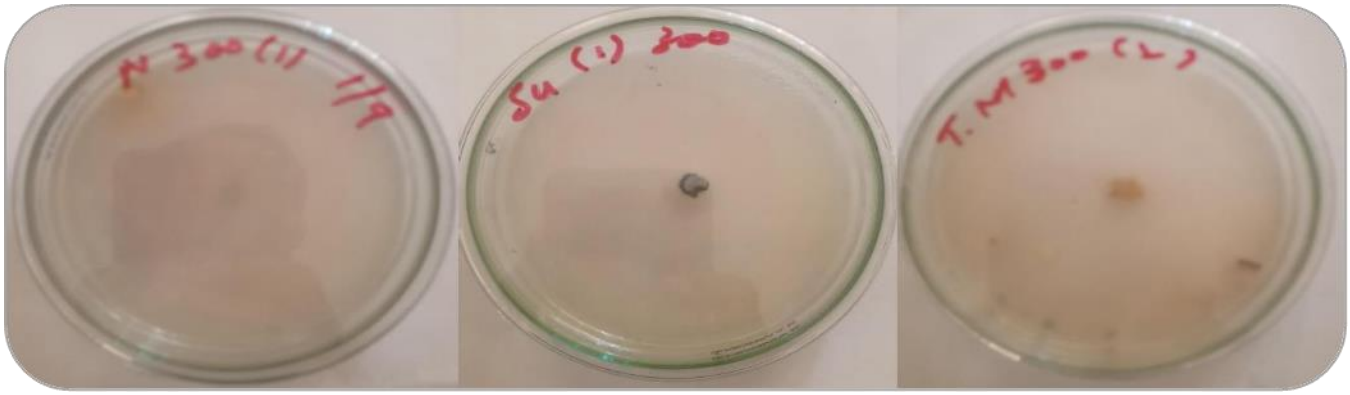
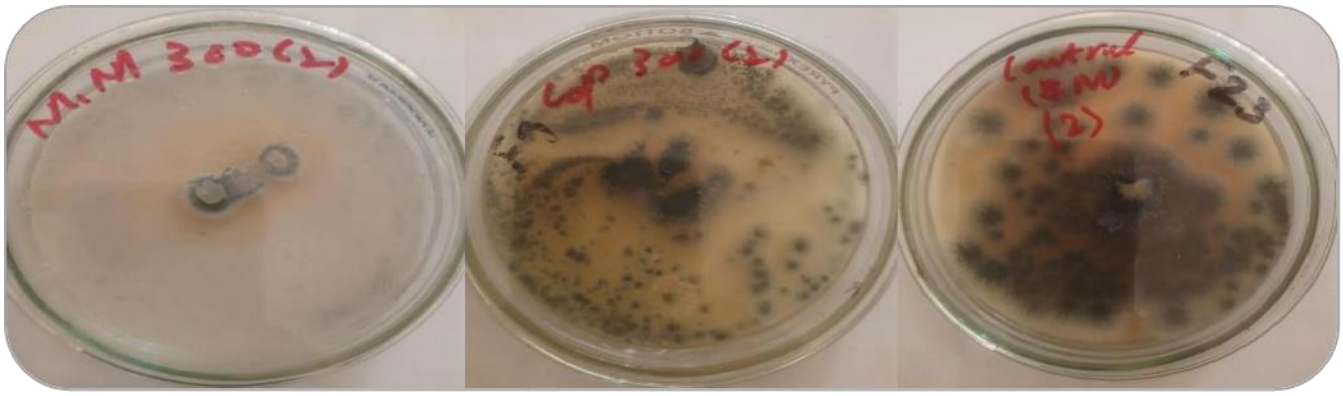


Figure 4.8.1 Percent Inhibition of *Penicillium italicum* after 5 days of application of fungicides at 300 ppm concentration

After 5 days of fungicide application (300 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (84.5%) followed by Success (76.4%). Copper Oxychloride showed minimum inhibition (5.4%) of *P. italicum* (Figure 4.8.1; Figure 4.8.2).



Nativo (300 ppm) Success (300 ppm) Topsin M (300 ppm)



Metalaxyl+ Mancozeb Copper Oxychloride Control (300 ppm)

Figure 4.8.2. Pictorial view of inhibition of *Penicillium italicum* after 5 days of application of fungicides at 300 ppm concentration

After 7 days of fungicidal application (100 ppm concentration), maximum inhibition of *P. italicum* was observed through application of Nativo (81.11%) followed by Success (77.78%). Copper Oxychloride showed minimum inhibition (3.33%) of *P. italicum* (Figure 4.8.1; Figure 4.8.2).

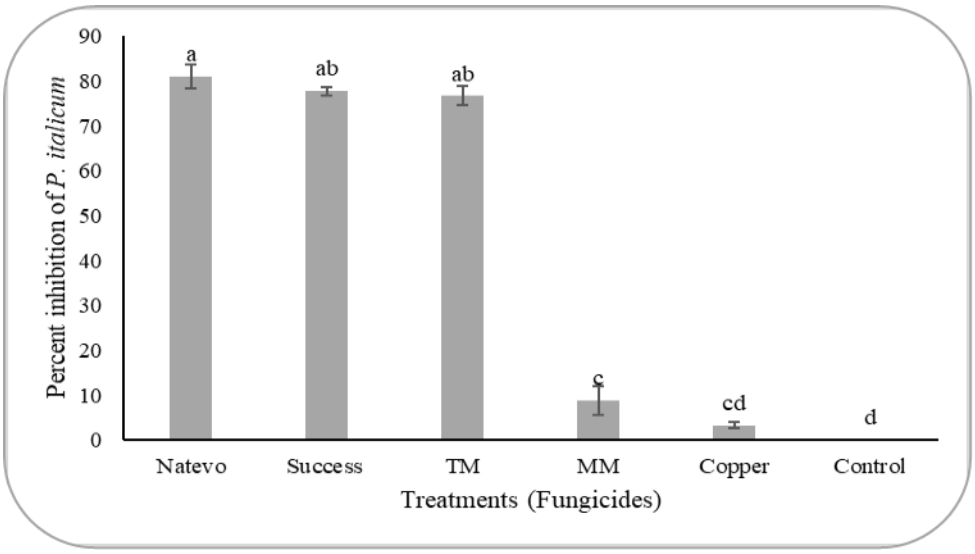
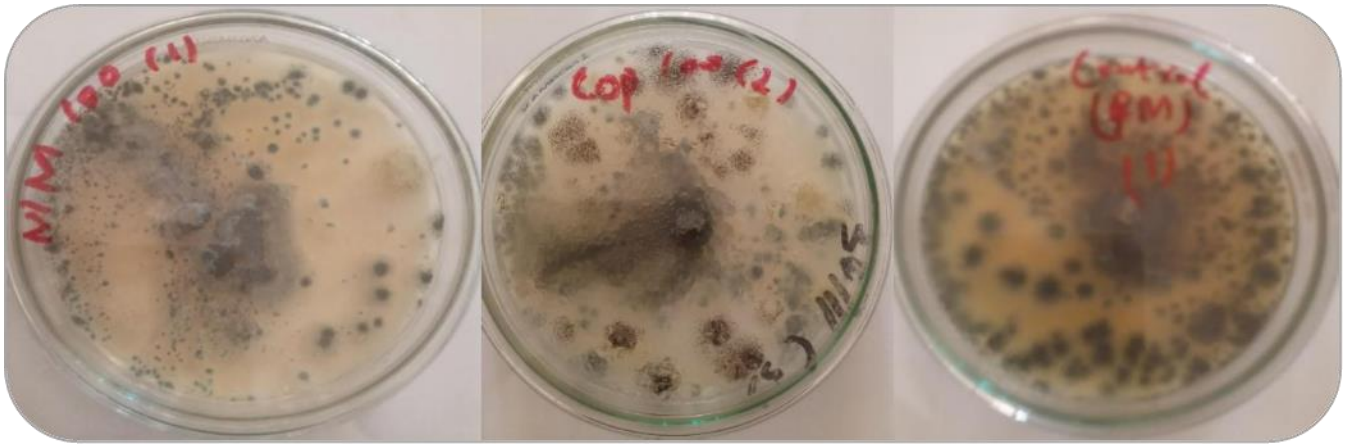


Figure 4.9.1. Percent Inhibition of *Penicillium italicum* after 7 days of application of fungicides at 300 ppm concentration

After 7 days of fungicidal application (300 ppm concentration), maximum inhibition of *P. italicum* was observed through the application of Nativo (87.22%) followed by Topsin M (82.78%). Copper showed minimum inhibition (7.22%) of *P. italicum* (Figure 4.9.1; Figure 4.9.2)

Nativo (100 ppm) Success (100 ppm) Topsin M (100 ppm)



Metalaxyl+ Mancozeb Copper Oxychloride Control (100 ppm)

Figure 4.9.2 Pictorial view of inhibition of *Penicillium italicum* after 7 days of application of fungicides at 300 ppm concentration

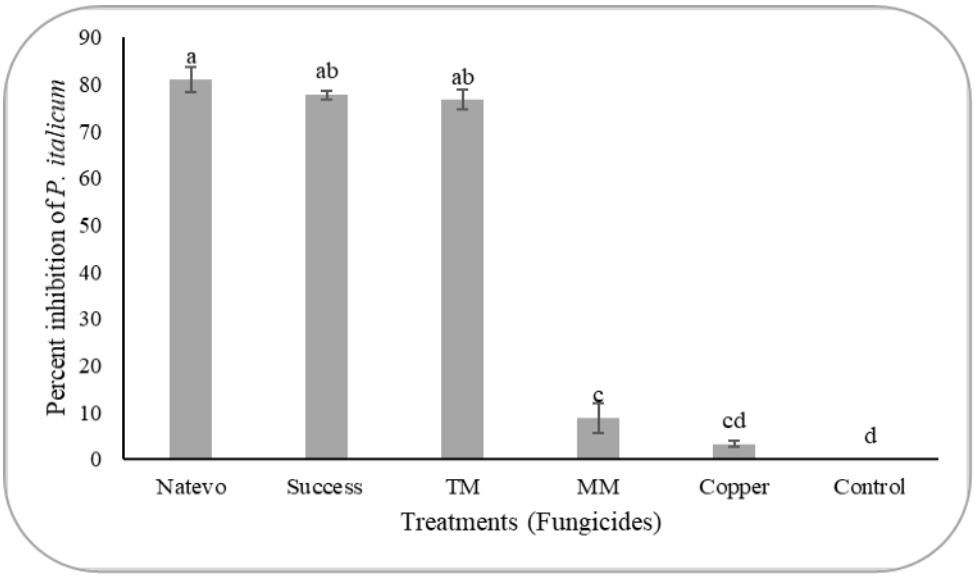
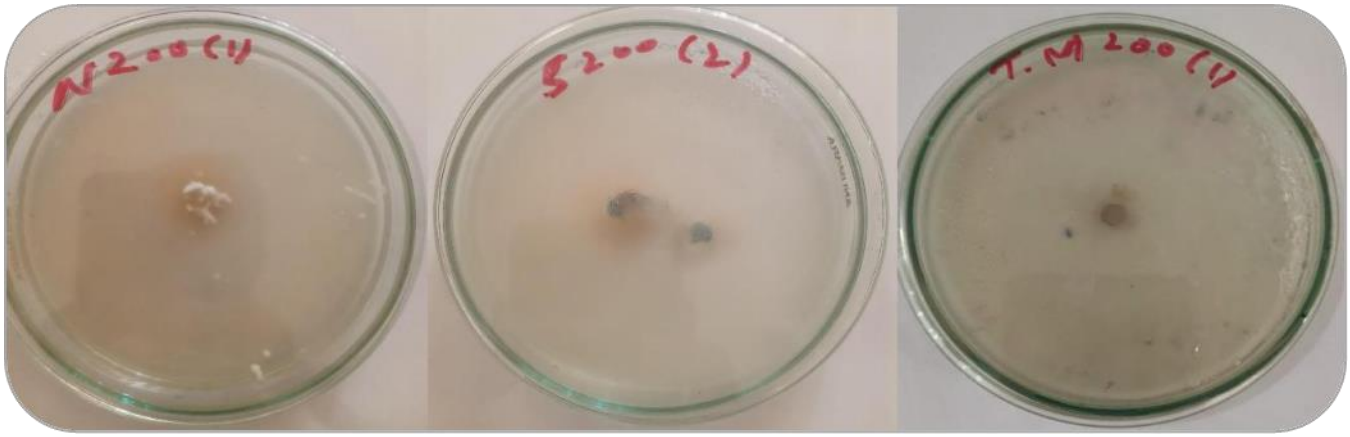
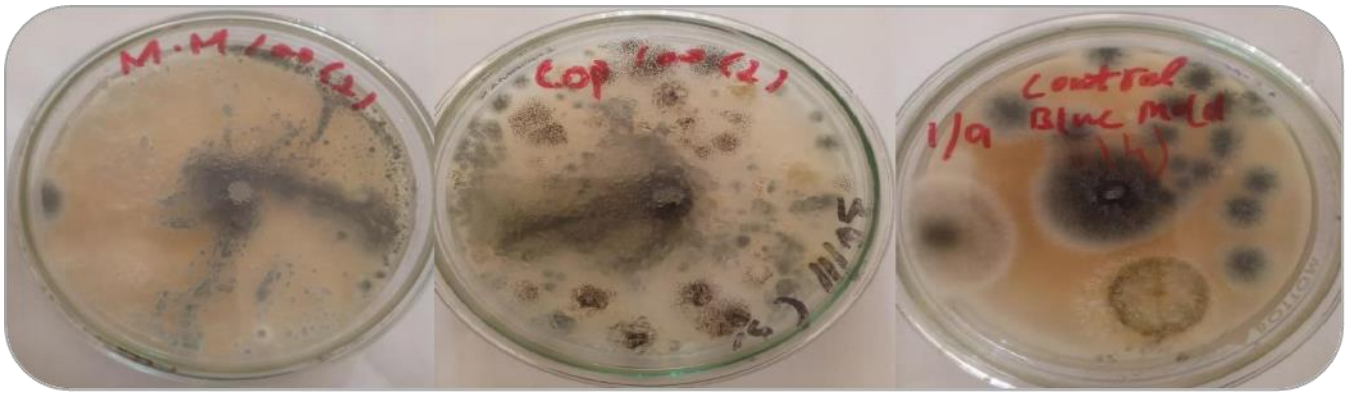


Figure 4.10 Percent inhibition of *Penicillium italicum* after 7 days of application of fungicides at 200 ppm concentration

After 7 days of fungicidal application (200 ppm concentration), Nativo (86%) exhibited maximum inhibition of *P. italicum* followed by Success (79%). Copper Oxychloride showed minimum inhibition (6%) of *P. italicum* (Figure 4.10.1; Figure 4.10.2)



Nativo (200 ppm) Success (200 ppm) Topsin M (200ppm)



Metalaxyl+ Mancozeb Copper Oxychloride Control (200 ppm)

Figure 4.10.2 Pictorial view of inhibition of *Penicillium italicum* after 7 days of application of fungicides at 200 ppm concentration

DISCUSSION

Citrus blue mold is caused by *Penicillium italicum* is destroying global and local production of citrus in Pakistan (Khan and Javed, 2022). *Penicillium italicum* Wehmer is the most destructive pathogen and has been widely reported as a postharvest pathogen in citrus. The major threat of *P. italicum* is due to its fine powdered spores, which are airborne. It is the postharvest phytopathogen that is responsible for blue mold disease in the citrus crop. This pathogen is being controlled currently by the application of various chemicals including Imazalil, Fludioxonil, Pyrimethanil, and Tiabendazole (Singh *et al.,* 2012; Papoutsis *et al.,* 2019), however, these chemicals are harmful to humans and the environment as well. In addition, the development of resistance to these chemicals is another threat due to widespread applications. To address all these problems, an alternate method or new product is being explored to control citrus postharvest phytopathogens (Arrebola *et al.,* 2010; Tayel *et al.,* 2015).

In the present study, the percent inhibition of *P. italicum* was recorded after the application of various fungicides under laboratory conditions. Nativo chemical proved to be very effective and showed the highest inhibition of *P. italicum* as compared to other fungicides. After 3 days, 82-87.5% inhibition of *P. italicum* was recorded at 100 to 300 ppm concentrations. After 7 days of application of Nativo, the inhibition rate was 81.0-87.0%, which means the fungicides had a long-term effect on the phytopathogen. The second most effective fungicide was Success which showed 77.5-81.0% inhibition of *P. italicum* after 3 days and 78.0-80.0% after 7 days of application. Similarly, Topsin M showed 68.3-77.0% inhibition of *P. italicum* at the 3 days and 77.0-81.0% on the 7 days of application. Metalaxyl+Mancozeb and copper were not effective against *P. italicum*, even at higher concentrations.

Nativo is the combination of Tebuconazole and Trifloxystrobin and is highly effective against *P. italicum* because it regulates antioxidant properties (Mohsin *et al.,* 2020). Our findings agree with Pathan *et al.* (2020) who reported that tebuconazole+trifloxystrobin could effectively control the pathogen. *Austropucciniapsidii* causal agent of Myrtle rust. Previously scientists have reported that a combination of tebuconazole and trifloxystrobin is the best strategy to control phytopathogens (Martins *et al.* 2014; Masson *et al.* 2011, 2013). A combination of fungicides may act as systemic demethylation inhibitors, e.g., tebuconazole and cyproconazole, having various properties to enhance efficacy (Zauza, 2008; Erincik *et al.,* 2016).

After Nativa, Success which is the combination of Chlorothalonil & Metalaxyl, was found more effective than others under consideration. On the other hand, fungicides that have a single mode of action may enhance the risk of resistance development in pathogens (Hollomon, 2015). As in the case of metalaxyl fungicides, pathogens have developed resistance. When metalaxyl was first introduced into the market, the mode of action of this fungicide was not completely understood, however, its systemic activity was only revealed at the time. Despite incomplete knowledge of the mode of action of this fungicide (Matson *et al.,* 2015), it is inarguable that this fungicide is involved in RNA Polymerase-I, a single mode of action (Randall *et al.* 2014). This might explain why this fungicide developed resistance so fast after introducing into the market. However, the product of the mixture of two fungicides

can enhance the efficacy reducing the chances of resistance development in pathogens. Alike, the fungicide Success is a mixture of Chlorothalonil and Matalaxyl and proved effective in the inhibition of *P. italicum*. Fungicides having different modes of action may result in synergistic action.

Sahi *et al.* (2012) reported that Topsin M is effective against Botryodiplodia theobromae, a causal agent of the quick decline of mango. In previous studies, Topsin-M showed the best inhibition of various pathogens such as Ceratocystis Manginecans and C. Fimbriata (Kumari *et al.,* 2021). Nativo, Succes, and Topsin M evaluated in this study exhibited significant inhibition of *P. italicum*. Therefore, these fungicides must be tested at the field level in combination to find out the most potential applications for integrated disease management programs for citrus blue mold disease.

CONCLUSION

Nativo chemical proved very effective and showed the highest inhibition of *P. italicum*as compared to other fungicides. The second most effective fungicide was Success which showed considerable inhibition of *P. italicum* after 3 days, 5, and 7 days of application. Similarly, Topsin M also showed significant inhibition of *P.italicum*. Metalaxyl+Mancozeb and Copper Oxychloride were not effective against *P. italicum*, even at higher concentrations. Nativo, Succes, and Topsin M evaluated in this study exhibited significant inhibition of *P. italicum*. Therefore, these fungicides must be tested at the field level in combination to find out the most potential applications for integrated disease management programs for citrus blue mold disease.

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Malik A. Rehman	:	Designed the treatment plan and facilitated the conduction of research
Asad Abbas	:	Conducted the research experiment and wrote the results
Zafar Iqbal	:	Supervised the research experiment
Mujahid Ali	:	Helped in statistical data analysis and designing of figures
Salman Ahmad	:	Wrote the introduction and discussion of the results
Muhammad Asim	:	Wrote the abstract and helped in results writing
Muhammad Nadeem	:	Wrote the conclusion and reference section and compiled the whole article according to journal style