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Research Article

In Vitro Evaluation of Fungicides Against Mango Anthracnose Caused by *Colletotrichum gloeosporioides*

Sana Inayat[1, *], Asad Ullah[2], Adeeba Anwar[3], Aneesa Kaleem[4]

[1]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan
[3]Department of Plant Pathology, University of Agriculture Sub-Campus, Burewala, Pakistan
[4]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

Abstract

Mango is a significant fruit crop in our country, but it is often attacked by anthracnose, a disease that causes considerable pre and post-harvest losses. There are different methods to control this disease but management through the use of various fungicides, often in combination is more suitablef, under field conditions. This study was conducted in vitro experiments using the poison food technique to induce anthracnose disease in mangoes. Efficacy of 10 different fungicides was investigated, including contact fungicides like Blitox (Copper oxychloride 50% WP) and Indofil M-45 (Mancozeb 75% WP), as well as systemic fungicides like Topsin-M 70 WP (Thiophanate-M methyl 70% WP), Amistar Top (Difenconazole @ 12.5%), Aliette (Fosetyl Aluminium 80%), Score (Difenconazole @ 25% EC), Meriman (Captan WP 50%), Tilt (Propiconazole 25% EC), mirador (Azoxystrobin @ 20%), and Native (Tebuconazole+ Trifloxystrobin 75% WG). These fungicides were tested at three concentrations (0.05, 0.1, and 0.15) to evaluate their effectiveness in inhibiting the growth of *Colletotrichum gloeosporioides*, the causal agent of anthracnose in mangoes. The results of this study showed that among the fungicides tested, Tilt (Propiconazole 25% WP), a systemic fungicide, exhibited superior inhibition, achieving complete 50 percent mycelial inhibition and preventing sporulation across all three concentrations. In contrast, Meriman (Captan WP 50%) showed the lowest inhibition, at 31.57 percent, compared to the other fungicides tested. These findings underscore the importance of selecting the appropriate fungicide for effective management of anthracnose in mango crops.

Keywords

Fungicides, *Colletotrichum gloeosporioides*, Mango Anthracnose, Concentrations

1. Introduction

Fruits are well-known for their nutritional value and economic significance. They are essential food products in around the worldwide. By providing vital components for growth, including vitamins, minerals, amino acids, carbs, lipids, and many other essential nutrients, they contribute significantly to human nutrition and help maintain good and

*Corresponding author: Sanainayat88@gmail.com (Sana Inayat)

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normal health [1]. Mangos are members of the family Anacardiaceae and genus *Mangifera*. The region between northeastern India, Bangladesh, and northwestern Myanmar is said to be origin of mangoes. The mango tree is a large fruit tree that can reach heights and widths of up to 30 meters (100 feet) and more, with a trunk circumference of more than 12 feet. Mangoes are mostly sweet, though different cultivars have different tastes and textures. Alphonso, for example, has a soft, pulpy, juicy texture that is similar to an overripe plum; Linnaeus originally characterized this species in 1753 [2]. The mango tree is the national tree of Bangladesh and the national fruit of Pakistan, India, and the Philippines [3]. The ideal pH range for mango trees is from 4.5 to 7.0, which is considered neutral to slightly acidic soil [4]. Over 50% humidity is preferred for mango trees. The ideal temperature range for a mango tree is between 80- and 100-degrees Fahrenheit. After the tree has blossomed, the mango fruit takes 3-5 months to ripen [5]. Most tropical and warmer subtropical regions without frost are suitable for mango cultivation [3]. Lipids (0.27) Fibers (1.8) Vitamin C (0.027) Carotene (Vit-A) (0.008) Thiamin (0.00056) Niacin (0.00058) Calcium (Ca) (0.01) Fe (iron) (0.00013) Potassium (K) (0.0156) phosphorus (P) (0.011) and Magnesium (Mg) 0.009. A raw mango comprises very little fat, 15% carbohydrates, 1% protein, and 84% water [2].

Mango production reached 56 million tons worldwide in 2019, with India accounting for 46% (or 26 million tons) of the total production. In 2019, 2.3 million tons of mangoes were produced in Pakistan [6]. According to the global production of mangoes in 2020, some other significant mango-producing nations in terms of total tons produced are Indonesia, Nigeria, Pakistan, Thailand, Brazil, Bangladesh, Mexico, and the Philippines. Pakistan is the sixth-largest mango-producing nation in the world, with 1717 thousand tons produced on 171 thousand hectares of mango cultivation in 2016 [7].

Mango anthracnose disease is caused by *Colletotrichum gloeosporioides*. On leaves, spots usually appear close to the edges. They have a tan to dark brown color, frequently with a darker edge. When immature leaf blossoms appear during a period of precipitation, they may become infected. These diseases typically show up along the margins of immature leaves, where lesions form and eventually turn a pale green or bronze color. In these cases, the damaged areas frequently have a semi-circular form. When the weather is really wet, young twigs may show signs of a darkened region that extends from the tip backward. Occasionally, when the new shoots become defoliated.

When anthracnose affects immature fruits, it manifests as huge, black, sunken lesions, and the infected fruits eventually fall off. Large to medium-sized, unripe green fruits with sunken, glossy, black lesions are indicative of pre-harvest anthracnose. Oozing and splits are common with these fruits. The majority of fruit anthracnose is caused by latent infections, which show up as gray-black, slightly depressed patches on the maturing fruit's skin. During that period, the characteristic acervuli, or pink to orange spore masses, appear on the tissue. Lesions caused by anthracnose are typically seen in ripening fruit, although they can also be seen in early, green fruits, with a higher prevalence in larger green fruits. This fungus produces more spores when it's rainy or humid weather. Rain and wind increase in the spores' dissemination. Anthracnose can destroy inflorescences, infect immature fruit, and cause fruit drops in regions where rain is common during blooming and fruit set. It may result in significant losses [8]. Many viruses infest mango trees from blossom to harvest and while they are in storage, which significantly reduces the quantity and quality of fruit produced. After fruits are harvested, microbes cause them to rot or spoil, which is a major problem for the mango business. In Asian countries, microbial degradation accounts for 17.0-26.9% of total postharvest losses. Postharvest losses of fresh mango fruits have been observed to vary from 25-40% in India and up to 69% in Pakistan [9]. Similarly, anthracnose, which can occur in the field or during storage, is a significant barrier to the production of mangoes in Bangladesh [10, 11].

2. Materials and Methods

2.1. Collection of Disease Sample

Disease samples of mango leave were collected from the experimental area of Ayub Agriculture research Institute, Faisalabad and used for the isolation of fungus and wrapped in paper envelop with great care. Care should be taken while collecting the sample it should not be insect /pest damage and broken. Each envelope was labeled with the date, name and place. These samples were carried to the lab and stored at 4 ℃ for further study.

2.2. Preparation of Culture Media (PDA)

Potato infusion (300g), Agar-agar (20g) Dextrose (20g) and water (1liter) these ingredients were taken. Firstly, 300g peeled potatoes were boiled in 2 liter of water for 30 minutes until the potato became soft. The water was filtered with muslin cloth and used for further media preparation. Then 20g of agar and 20g dextrose was weighed with the help of electric balance and were poured in a 2000 ml capacity media bottle. The volume was made up to 1000 ml by adding the potato starch extracted water. The ingredients were thoroughly mixed using an electric stirrer and then autoclaved at 121^{o} C for 20 minutes under 15 pounds per square inch (PSI) pressure. After autoclaving, the media was allowed to cool. The Luke-warm media was poured in sterilized petri dishes and allowed to solidify.

2.3. Isolation of the Pathogen

Diseased leaves were washed with tap water and then

air-dried. The infected leaves were cut along with healthy portion into small (6 mm) pieces and were surface sterilized with 1% NaOCl for 1 minute and then washed with sterile distilled water for three times to remove the traces of sodium hypochlorite and air-dried on the filter paper. The pieces of diseased leaves were transferred to sterilized Petri plates (3 leaf bits per Petri plates) containing PDA and the plates were wrapped with cling film under aseptic condition under laminar air flow and incubated at room temperature (25±1 ℃). After 72 hours, colonies that produced from the bits were transferred to fresh PDA medium. Colonies which developed from such culture was occasionally observed for mycelia growth and sporulation under microscope. Mycelial and spore character were means for identification of the pathogen.

2.4. Identification of the Fungus

There are two methods to identify the pathogen one method is macroscopic in which we can identify on the bases of colony color shape size and the other one is microscopic in which we can identify on the basis of mycelial spore size and color etc. Pathogen identification was done based on its mycelial and spore characteristics as described by Barnett and Hunter [10]. Following identification, the pathogens were transferred to new PDA slants and incubated at 25±1 ℃ for future use. The fungus was sub-cultured on PDA slants and allowed to grow at 25±1 ℃ for 7 days. Slants were preserved in a refrigerator at 5 ℃. Sub-culturing was performed in a month, and these cultures were utilized throughout the study.

2.5. In-Vitro Evolution of Different Fungicides

To evaluate the relative efficacy of different fungicides, two contact fungicides, six systemic fungicides one strobulin compound and one Combi product were taken and evaluated by poison food technique and used at 0.05%, 0.1% and 0.2% against pathogen. The mango pathogen *C. gloeosporioides* was cultured on PDA in Petri plates for seven days before initiating the experiment. To acquire the preferred concentration on the basis of whole product and active ingredients present in the chemical, by adding required quantity of fungicide, fungicide suspension was prepared in PDA. The media was poured into sterilized Petri plates. After the media solidified, 15 ml of poisoned medium was added to each plate, with each treatment replicated three times at three different concentrations. A 0.5 cm mycelial disc was taken from the margin from seven-day-old culture and placed in the center of each Petri plate containing poisoned media. The plates were then incubated at 25±1 ℃ until the fungus grew to the edges of the control plate. Controls without any fungicide were also maintained. The colony diameter was measured in three directions, and the average was calculated. The plates were also observed for the presence or absence of sporulation. Percent inhibition of growth was calculated using the formula provided by Vincent [12].

$$I = \frac{C - T}{c} \times 100$$

Where,
I = Per cent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Table 1. *Different fungicides with concentrations.*

Sr No.	Fungicide/ trade name	Active ingredient	Concentration
1	Indofil M- 45	Mancozeb 75% WP	0.05 0.1 0.15
2	Blitox	Copper oxychloride 50% WP	0.05 0.1 0.15
3	Topsin-M 70 WP	Thiophanate-M methyl 70 %	0.05 0.1 0.15
4	Amistar Top	Difenconazole @ 12.5%	0.05 0.1 0.15
5	Aliette	Fosetyl Aluminium 80%	0.05 0.1 0.15
6	Score	Difenconazole @ 25% EC	0.05 0.1 0.15
7	Meriman	Captan WP 50 %	0.05 0.1 0.15
8	Tilt	Propiconazole 25% EC	0.05 0.1 0.15
9	mirador	Azoxystrobin @ 20%	0.05 0.1 0.15
10	Native	Tebuconazole+ Trifloxystrobin 75% WG	0.05 0.1 0.15

2.6. Statistical Analysis

The data was subjected to statistical analysis using Analysis of Variance (ANOVA) at a significance level of 5%. The analysis was performed using the "R" software. Treatment means were compared using Tukey's HSD test and presented using "Microsoft Office version 2016" software.

3. Results

Table 2. *Different fungicides concentrations with inhibition percentage.*

Fungicide/ trade name	Active ingredient	Concentration Inhibition	Mean inhibition
		0.05 0.1 0.15	
Indofil M- 45	Mancozeb 75% WP	35.99 39.95 39.31	38.41
Blitox	Copper oxychloride 50% WP	26.15 50.00 50.00	42.05
Topsin-M 70 WP	Thiophanate-M methyl 70 %	29.05 44.00 42.00	38.50
Amistar Top	Difenconazole @ 12.5%	45.05 48.00 48.25	47.10
Aliette	Fosetyl Aluminium 80%	39.50 29.50 28.00	32.33
Score	Difenconazole @ 25% EC	41.51 43.63 43.30	42.81
Meriman	Captan WP 50 %	28.03 39.31 27.38	31.57
Tilt	Propiconazole 25% EC	50.00 50.00 50.00	50.00
mirador	Azoxystrobin @ 20%	31.78 37.66 37.66	35.70
Native	Tebuconazole+ Trifloxystrobin 75% WG	46.02 48.13 48.13	47.43

In this investigation 10 fungicides were evaluated for their antifungal activity out of 10 fungicides 6 fungicides were systemic fungicide 1 was strobulin compound 1 fungicide were combi compound and 2 were contact fungicides. Among the different fungicides tested, a Combi product Flurilazole + Carbendizim 37.5% WP and systemic fungicides Propiconazole 25% WP showed significantly superior inhibition over other chemicals with complete 50 per cent mycelial inhibition and absence of sporulation in all the three concentrations i.e., 0.05%, 0.1%, 0.15%. Tebuconazole + Trifloxystrobin 75% WG showed inhibition of 47.43%. Systemic fungicide Difenconazole 25% EC showed 42.81% inhibition. The least mycelial inhibition was shown by captan wp 50% with inhibition 31.57% followed by strobulin compound Azoxystrobin with 35.70 per cent inhibition. Among the systemic fungicides tested, Propiconazole 25% WP exhibited 50% inhibition of mycelial growth and absence of sporulation. This was followed by Difenconazole at 12.5%, which showed 47.10% inhibition, and Difenconazole at 25% EC, which showed 42.81% inhibition. In the contact fungicides tested, Copper oxychloride at 50% WP showed the highest inhibition at 42.05%, followed by Mancozeb at 75% WP with 38.41% inhibition. Whereas systemic fungicide Thiophanate-M methyl 70 % showed inhibition 38.50 followed by Fosetyl Aluminium 80% with inhibition 32.33% and Captan WP 50% with inhibition 31.57%. on the other hand, combi products i.e. Tebuconazole+ Trifloxystrobin 75% WG show maximum inhibition 47.43% while minimum inhibition and absence of sporulation was recorded by captan WP 50%. Systemic fungicides Propiconazole 25% EC efficacy was increase as compared to 0.05 and 0.1%. other systemic fungicide i.e. Copper oxychloride 50% WP Difenconazole @ 12.5% Azoxystrobin @ 20% Tebuconazole+ Trifloxystrobin 75% WG efficacy was remain constant. All fungicides were showed different inhibition on different concentrations but among contact fungicides Copper oxychloride 50% WP showed maximum 50% inhibitions at 0.1 and 0.15 concentrations in case of systemic fungicides Propiconazole 25% EC also showed maximum inhibition of 50% at 0.1 and 0.15 concentrations and combi product Tebuconazole+ Trifloxystrobin 75% WG showed maximum inhibition at 0.1 and 0.15 concentration inhibition was 48.13 in these two concentrations. Fungicides having maximum mycelial inhibition and absence of sporulation like systemic fungicide i.e. Propiconazole 25% EC and Difenconazole @ 12.5% combi products Flusilazole 12.5% + Carbendazim 37.5% WP and contact fungicide Tebuconazole + Trifloxystrobin 75% WG Copper oxychloride 50% WP can be used to control mango An-

thracnose caused by *Colletotrichum gloeosporioides.*

4. Discussion

In this study, we assessed the effectiveness of 10 fungicides in inhibiting the growth of the *Colletotrichum gloeosporioides.* pathogen causing anthracnose disease in mango. These fungicides were tested at 3 different concentrations i.e. 0.05, 0.1 and 0.15. to evaluated the mycelial inhibition and absence of sporulation of fungus *Colletotrichum gloeosporioides* which cause mango anthracnose disease. Among the various systemic fungicides tested, Propiconazole 25% WP exhibited 50% inhibition of mycelial growth and absence of sporulation. Patel [13] reported that carbendazim and propiconazole demonstrated 85% efficacy against C. *gloeosporioides*. Variations in results may be due to the chemical composition and different concentrations. Tebuconazole + Trifloxystrobin 75% WG showed 47.43% inhibition whereas, among contact fungicides, Copper oxychloride 50% WP showed maximum inhibition of 42.05%. Likewise, Tebuconazole 25.9% EC and Tebuconazole 50% + Trifloxystrobin 25% WG showed maximum percent inhibition. The current study is in line of Golakiya et al. [14] who investigated the efficacy of various fungicides at different concentrations (100 ppm, 250 ppm, and 500 ppm) against *C. gloeosporioides*. Tebuconazole + Trifloxystrobin was found to inhibit the formation of the fungal cell wall, thereby halting reproduction and further growth. Trifloxystrobin was observed to restrict respiration in plant pathogenic fungi. Additionally, Mancozeb 75% WP demonstrated 38.41% inhibition. Ekbote et al. [15] also noted significant inhibition by mancozeb at a 0.3% concentration. In another study Sushma et al., [16] reported that mancozeb 75% showed 87.96 inhibition at 0.1 concentration variations might be due to chemical compositions. The efficacy of triazole fungicides like Propiconazole may be attributed to their interference with fungal sterol biosynthesis, leading to the halt of ergosterol biosynthesis. Since ergosterol is crucial for fungal cell wall structure, its absence can result in severe damage and cell death. These results suggest that a single fungicide or a combination of these could effectively manage anthracnose disease in field conditions. A similar study by Nene and Thapliyal [17] reported on the efficacy of triazoles, which inhibit the sterol biosynthesis pathway in fungi. Most fungicides showed maximum inhibition of mycelial growth at higher concentrations but decreased in effectiveness with lower concentrations. These results are in in line with that of Sudhakar [18]. Azoxystrobin @20 showed 35.70% inhibition Sushma et al., [16] reported that Azoxystrobin showed 59.44 growth inhibition which is statically similar to our results.

5. Conclusions

This study evaluated the efficacy of 10 fungicides against mango anthracnose caused by *Colletotrichum gloeosporioides.* Among these, two were contact fungicides, six were systemic fungicides, one was a strobilurin compound, and one was a combination product. The fungicides were applied at three different concentrations (0.05, 0.1, and 0.15). Copper oxychloride exhibited the highest inhibition among contact fungicides, while propiconazole 25% EC showed the highest inhibition among systemic fungicides. The strobilurin compound and combination product also showed inhibition, lower than that of systemic and contact fungicides. This study underscores the potential of these fungicides in controlling anthracnose disease, highlighting the critical role of chemical composition and concentration in determining efficacy. Future research could focus on conducting field trials to validate these findings and optimize fungicide applications for practical use.

Abbreviations

PDA: Potato Dextrose Agar

Conflicts of Interest

The authors declare no conflicts of interest.

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