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Research Article

Novel Studies on the Prevalence of Leaf Spot Disease of Banana and its Chemical Control in Lower Sindh, Pakistan

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Abstract

In the current study, different chemical fungicides were used for effective management of *Fusarium oxysporum,* which is a causal agent of banana leaf spot disease. A survey of the banana-infected field was conducted in various regions of Sindh, Pakistan; Recorded disease incidence showed that Thatta was severely affected by leaf spot up to (46.66%), followed by Badin (28.88%) and minimum disease incidence was recorded in Hyderabad (24.44%). The pre-dominant pathogen was morphologically identified as *Fusarium oxysporum*; for confirmation of casual fungal, a pathogenicity test was done by two different methods i.e root dipping and drenching method. It was observed that the root dipping method of inoculation showed higher leaf spots (06) development on banana as compared to drenching method; leaf spot (03) of inoculation. To determine the efficacy of various chemical fungicides, such as Melodyduo, Aliette, Prevail, Topsin-M and Ridomil gold, were used at different doses, such as 100, 200, and 300 ppm, the findings of the study indicates that *F. oxysporum* was completely stopped to grew against the Prevail and Topsin-M (0.00 mm) at 100, 200 and 300 ppm, respectively, while the vegetative growth of the targeted fungi was slightly high against Ridomil Gold (18.16, 15.66 and 5.75 mm), as well Melody duo (24.33, 19.08 and 13.75 mm). Maximum mycelial growth of the pathogen was observed under Aliette (32.00, 27.83 and 26.08 mm). The *Fusarium oxysporum* vigorously developed under control (90mm). Present findings of the study suggested that Prevail and Topsin-M considerably retarded the mycelial growth and positively controlled the targeted pathogen.

Keywords: Novel studies; Leaf spot disease; *Fusarium oxysporum;* Survey

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Introduction

Considerable economic loss to plant products can occur when banana plants are attacked by the most important plant pathogens such as fungi, bacteria, phytoplasmas and viruses. Among such pathogens, fungi can cause severe damage to plant products and can cause various diseases in vegetable and fruit species. Over several decades, various attempts have been made to prevent and control the plants from such heavy loss (Inouye *et al.*, 2006). In this regard, scientists have developed synthetic fungicides to check or to stop the growth of such pathogens on host plants. These pesticides are able and highly effective in controlling a variety of postharvest diseases of fruits and vegetables. On other hand, the continuous and repeated use of various fungicides may disturb the equilibrium of ecosystems and pesticide residues in food produces high risks for health than insecticides and herbicides (Gomi *et al.*, 2003).

Chemical control is an integrated disease management that can be combined with biological control products (Elmer and McGovern, 2004). The severity of target disease was significantly reduced, when induced in the root system, indicating a disease reduction of up to 80.6% by demethylation and benomyl-inhibiting fungicides. The findings have also shown that other quaternary ammonium supplements are effective as *Foc* sterilants and should be substituted by currently inactive sterilants (Nel *et al.*, 2007). Benomyl and demethylation-inhibiting fungicides greatly decreased *F. oxysporum* disease incidence when used as root dip method, up to 80.6 percent reduction in disease is seen (Nel *et al.*, 2007). Fungicides are routinely implemented in most developing countries according to a fixed timetable (Marin *et al.*, 2003). Based on previous study it is very difficult to manage this disease, in our research study we identified the best fungicide to manage this disease.

Methodology

Survey and sampling of leaf spot disease in banana in lower Sindh

Different fields of Hyderabad, Thatta and Badin were surveyed for the collection of infected leaves. The disease incidence of leaf spot disease was calculated through the following formula;

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased tress leafs observed}}{\text{Total number of tress leafs observed}} \times 100$$

Isolation, identification and multiplication of the causal fungus

The infectious part of the banana leaf was cut into small 3-4 mm long pieces, and the surface was sterilized for 2 minutes with 5% commercial bleach. The sterilized parts were washed twice with sterilized water and placed over the surface of the sterilized blotting paper for drying, and then the sterilized portions were placed on petri dishes containing fresh Potato Dextrose Agar (PDA) medium. Usually, each plate was placed with 5 pieces of infected samples. In order to monitor the sporulation of the fungi, all Petri plates were placed in the incubator at a temperature of 25°C±2 for 7 days. In the meantime, complex fungal colony appeared which was extracted using the technique of single spore isolation and the method of hyphal tip. The pure culture of the fungus was made and preserved for potential use after diagnosis.

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Pathogenicity test

A pathogenicity test was carried out on healthy banana plants. Two methods were used in this study, root dip method and drenching method. Six healthy plants were selected and inoculated with *F. oxysporum* to check the severity of the disease. The inoculated plants were covered with plastic bags, and the progress of the disease was observed.

Efficacy of different fungicides

In-vitro experiments of certain selective chemical fungicides viz., Prevail, Ridomil Gold, Melody-Duo, Topsin-M., and Aliette were tested through food poisoning technique against the causal pathogen. For this research before poring, the PDA medium was incorporated with 3 separate concentrations (100, 200, 300 ppm). The fungicide-free PDA medium was kept as control. The 5 mm diameter agar disc of the test fungus was cut from 8-10 days old culture plate using a sterile cork borer and placed in the centre of the PDA plate after solidifying the media. The inoculated plates were incubated at 25°C. The growth of the radial colony of the test fungus was recorded by drawing two perpendicular lines in the center of the plate at the back of the petri plates. Data on colony growth were recorded in millimeters after every 24 hours along with these lines until the control plates were filled.

Statistical analysis

Experimental data collected for various parameters were compiled and tabulated in a manner suitable for statistical analysis. To analyze the data, statistical software (Statistics 8.1) was used to perform an analysis of variance (ANOVA). The purpose of the ANOVA was to determine significant differences among treatment groups. Additionally, the least significant difference (LSD) test, as suggested by Gomez and Gomez in 1993, was applied for mean comparison of the treatments.

Results and Discussion

This study was carried out to evaluate the effect of different fungicides against *Fusarium oxysporum*, the causal agent of leaf spot disease in banana. For this, survey and sampling were done and the pathogenicity test against target pathogen was performed using drenching and root dipping method of inoculations. The antifungal potential of different fungicides like Melodyduo, Aliette, Prevail, Topsin-M and Ridomil Gold was conducted at various doses, i.e. 100, 200 and 300 ppm, using food-poisoned methods for evaluating the successful and effective fungicide for *F. oxysporum* growth inhibition.

Disease incidence (%) at different locations of lower Sindh

The results in (Figure 1) shows that the disease incidence from different locations of Hyderabad, Thatta and Badin. Maximum disease incidence was recorded in Thatta 46.66% followed by Badin 28.88%. Minimum disease incidence was recorded in Hyderabad 24.44%.

Pathogenicity test

The pathogenicity test has been conducted on the banana seedling with two to three leaf stages by seedling root dip in spore suspension method and drenching with fungal culture method. The disease symptoms were observed in plants inoculated by both methods such as root dipping method and drenching method.

Results regarding pathogenicity test are presented in Figure 2. The data shows that

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maximum leaf spot 6 were observed in root dipping method followed by 3 leaf spots were oberved in drenching method. No leaf spot were observed in control treatment. On the basis of pathogenicity test, it was observed that root dipping method of inoculation showed higher percentage of spots as compared to drenching method of inoculation.

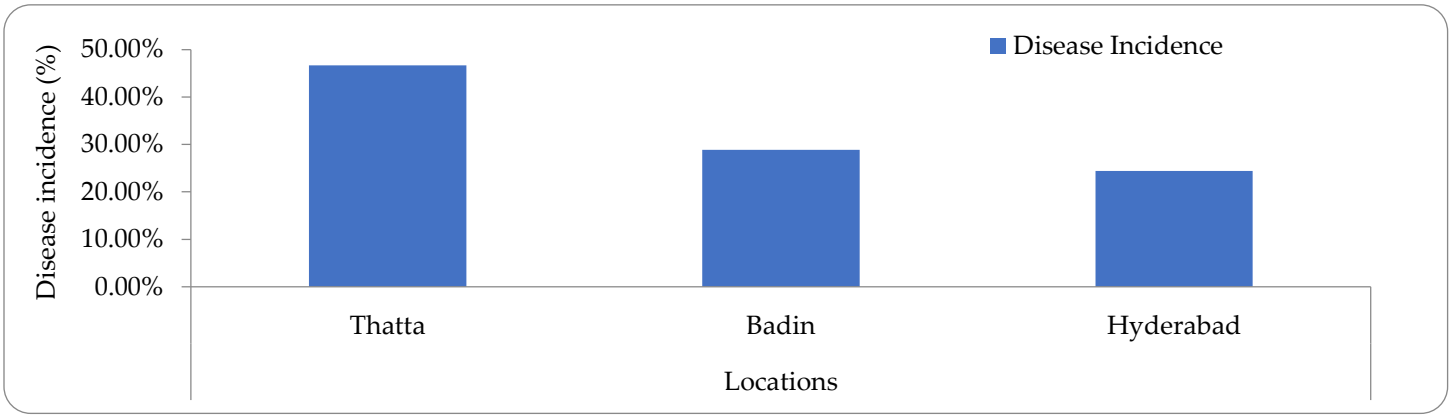

Figure 1. Disease incidence (%) of leaf spot disease of banana caused by *Fusarium oxysporum* at various locations of lower Sindh (Hyderabad, Badin and Thatta district).

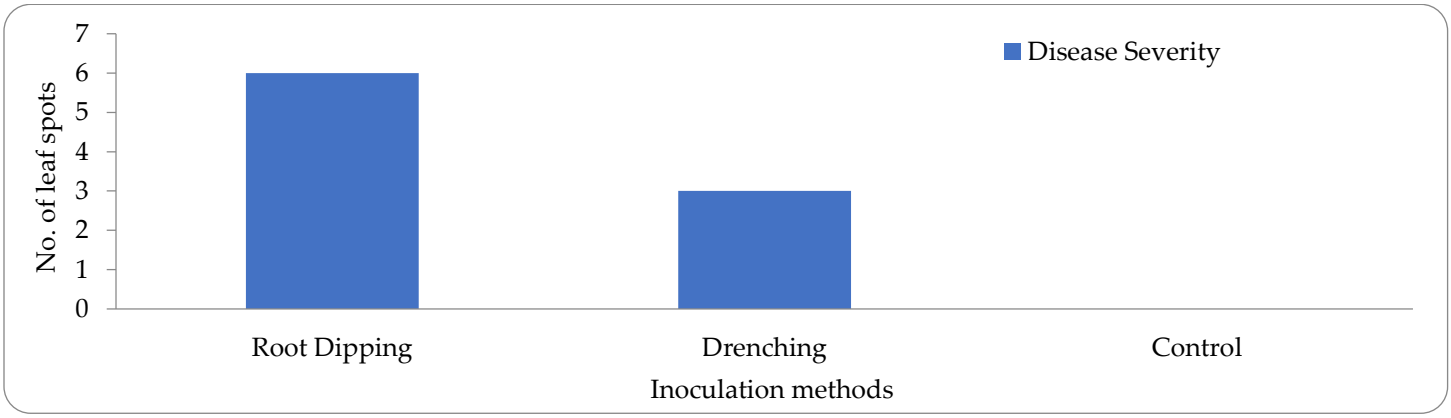

Figure 2. Leaf spots in root dipping and drenching inoculation methods.

Efficacy of various synthetic fungicides against targeted fungus *Fusarium oxysporum* under in-vitro conditions

The results (Figure 3) demonstrate that linear colony development of *Fusarium oxysporum* was not observed for prevail and Topsin-M (0.00 mm) at various concentrations i.e. 100, 200 and 300 ppm, respectively followed by Ridomil Gold (18.16, 15.66, and 05.75 mm), Melody duo (24.33, 19.08 and 13.75 mm), and maximum growth of fungus were determined under Aliette (32.00, 27.83 and 26.08 mm) (Figure 4). The fungus growth was observed upto 90 mm under control. Minimum growth of fungus was observed with Prevail and Topsin-M followed by Ridomil Gold, Melody duo, and maximum growth of fungus were determined under Aliette. Minimum linear colony growth of *F. oxysporum* was observed at 300 ppm and maximum linear colony growth of *F. oxysporum* was observed at 100 ppm for all fungicides except Prevail and Topsin-M. A statistic study of the results reveals that fungicides at various concentration levels

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for the linear colony growth of fungus differ significantly.

Figure 3. Pathogenicity test were performed through root dip method and drenching method.

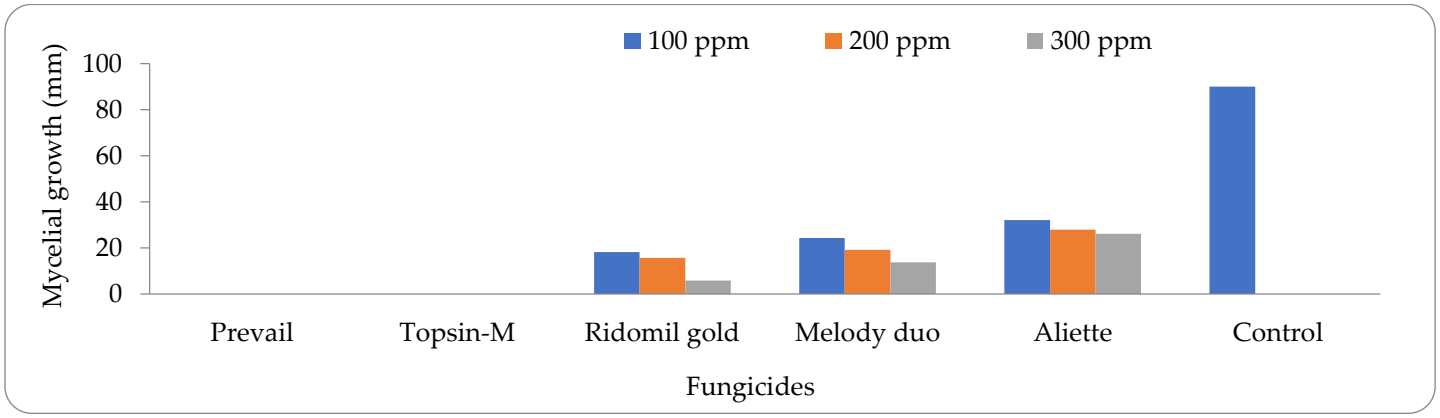

Figure 4. Efficacy of various synthetic fungicides against the mycelial colony growth of *Fusarium oxysporum*. Alphabetical letters denote statistically significant differences using student's t-test ($P<0.05$, $P<0.01$). Error bar represent the SD of the average from three different biological replicates.

Discussion

In the current study there was maximum disease incidence in Thatta 46.66% followed by Badin 28.88%. Minimum disease incidence was recorded in Hyderabad 24.44%. According to my knowledge I did not found any similar survey or disease incidence report of leaf spot disease in Sindh or in Pakistan.

It was suggested that chemical fungicide 'Prevail and Topsin-M' has been highly effective in regulating the growth of the target pathogen in mycelial colony at 100, 200, and 300 ppm. The findings of the current study are consistent with the (Sultana and Ghaffar, 2013) study where they found that fungicides like Prevail, Topsin-M, Benlate, and Carbendazim prevent *F. oxysporum* colony development entirely at 100 ppm while Ridomil, Vitavax, Aliette, and Mancozeb inhibited colony development at 1000 ppm

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altogether. Fareed *et al.* (2015) showed Ridomil as effective to control growth of cucumber Fusarium wilt causing fungus as compared to Score, Copper oxychloride, Cabriotop, and Antracol. This is indicating that *F. oxysporum* infecting different crops or in different regions may change its response to fungicides. In contemporary studies, six fungicides were tested against the growth of *F. oxysporum*, i.e., Nativo, Topsin-M, Carbendazim, Benomyl, Difenoconazole and Alliete, at varying concentrations. Among these fungicides, a major reduction in fungal growth was expressed by Carbendazim at 500 ppm. Carbendazim has disrupted metabolism, slowed pathogen growth and production (Iqbal *et al.*, 2010). It also limits the impact to pathogenic microtubules with chemical force and disturbs their ionic concentration (Magnucka *et al.*, 2007).

The results are similar with Parsa Sahar *et al.* (2013), where four fungicides were tested at 400, 600 and 800 ppm concentrations, Topsin-M, Alliete, Difenoconazole and Nativo against *F. oxysporum* by food poison method. Topsin-M recorded maximal pathogen inhibition at 800 ppm (76.66 percent) and decreased mycelial development, similar to my result where *F. oxysporum* no linear colony growth was observed on Topsin-M, followed by Difenoconazole, Alliete and Nativo at 67.50, 53.50 and 42.40 percent respectively, compared to control. In order to minimize disease occurrence of Fusarium wilt caused by *F. oxysporum*, the effectiveness of Topsin-M was evaluated. It expressed a decrease of 83.4 percent after 45 days at 800 mg / g soil concentration (Yucel *et al.*, 2007).

Conclusion

It was concluded on the basis of current research findings that maximum disease incidence was recorded in Thatta 46.66% and minimum disease incidence was recorded in Hyderabad 24.44%. Tested pathogen were failed to grow on petri plated treated with Prevail and Topsin-M fungicide at all evaluated concentrations.

Conflict of Interest

The authors have not declared any conflict of interest.

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