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Study the impact of propiconazole, azoxystrobin, and difenoconazole on the growth inhibition of plant pathogenic fungi through *In-vitro* conditions

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Abstract
In this study used the two different chemical fungicides against the seven different plant pathogenic fungi to check the control on growth of fungi under *In-vitro* condition. The effect of Tilt i.e., Propiconazole 25% EC inhibited growth of *Alternaria alternata*, *Fusarium oxysporum* and *Phoma glomerata* completely, its effect on other fungi growth inhibition (Percent Disease Control- PDC) in range of 69% to 76%. The Amistar fungicide which composed the Azoxystrobin 18.2% + Difenoconazole 11.4% was recorded most effective against plant pathogenic fungi *Pseudocercospora* spp. And in case of other fungi under study it showed 72% to 83% PDC.

Keywords: Fungicide, antifungal, efficacy, pathogenic fungi, PDC-percent disease control

Introduction

In the realm of plant pathology, the development and application of fungicides have emerged as pivotal strategies to mitigate the devastating impact of fungal pathogens on crops. *In vitro* studies play a crucial role in assessing the efficacy and potential of fungicides against these pathogens under controlled conditions. This research aims to delve into the utilization of fungicides *in vitro*, focusing on their inhibitory effects on selected plant pathogens. By meticulously investigating the interactions between fungicide formulations and diverse fungal strains, a deeper understanding of their modes of action and effectiveness can be gained. Such insights hold significant promise for refining fungicide application protocols and optimizing disease management practices. This study's findings are poised to contribute to the advancement of sustainable agricultural practices by offering targeted solutions to combat fungal infections and minimize yield losses. Due to their large application systemic activity, and protective and curative properties, a number of specific fungicides were often utilized until recently (Knight *et al*., 1997; Morton and Staub 2008) [5, 6].

Propiconazole is an effective fungicide that offers reliable protection against a broad spectrum of fungal diseases. Its systemic action ensures thorough coverage, penetrating plant tissues to inhibit fungal growth and spore production. This fungicide is user-friendly due to its easy application and low toxicity to humans and animals when used as directed. With a proven track record of controlling various plant pathogens, Propiconazole is a valuable tool for maintaining healthy crops and preventing yield losses. Its effectiveness, safety, and versatility make it a preferred choice for integrated disease management strategies. The four stereoisomers that make up propiconazole were initially combined in 1979 by Janssen Pharmaceutica (Toribio 2004 *et al*. and Thomson 1997) [1, 2]. Azoxystrobin 18.2% and Difenoconazole 11.4% SC showcases exceptional efficacy as a dual-action fungicide *in vitro*. The synergistic blend effectively inhibits fungal growth by disrupting both respiration and sterol synthesis pathways. With broad-spectrum activity, it controls an array of plant diseases. Its superior systemic movement within plant tissues ensures thorough protection. A reliable choice for *in vitro* applications, offering advanced disease management for healthier plants. Due to these practical considerations, scientists, plant breeders, and farmers all confront phytopathogenic fungus. Azoxystrobin and other strobilurins prevent electron transport, which reduces mitochondrial respiration (Becker *et al*., 1981) [3]. When delivering electrons to that protein, ubiquinone (coenzyme Q10) would typically attach at the quinol outer binding site of the cytochrome b-c1 complex. ATP manufacturing is so stopped (Moore *et al*., 2019) [4].

Materials and Method

Effect of fungicides against selected dominant pathogenic fungi in *In-vitro* Fungicides mainly Propiconazole 25% EC, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, following Poison food technique (Schmitz, 1930) [7]. The fungicides were tested against isolated fungus at the indicated doses (manufacturers dosage recommendations). After the treatment, data on mycelial growth was recorded at 9 and 15 days.

The details of Chemical fungicides used

Sr. No.	Market (Brand) Name	Active Ingredient	Formulation	Manufacturer	Used form	Recommended Dosage
1	Tilt	Propiconazole 25% EC	Emulsifiable Concentrate	Syngenta India Ltd	Liquid	0.1%
2	Amistar	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	Suspension Concentrate	Syngenta India Ltd	Liquid	0.1%

SC- Suspension Concentrate, EC- Emulsifiable Concentrate

The experiment was conducted as follows
Design C.R.D.
Replication 3
Treatments 6

Here, C.R.D. - Completely Randomized Design.
To determine each treatment's relative effectiveness for preventing the mycelial growth of seven different pathogenic fungi, bioassays were conducted on selected fungi in a lab setting. Before putting the mixture into petri plates, the necessary amount of each treatment was added to 100 ml of PDA at a slightly warm stage and completely mixed by sacking. After pouring PDA into Petri plates, the medium was allowed to solidify before the plates were centrally inoculated with a disc of pathogenic fungus measuring 6 mm in diameter and cut with a sterilised cork-borer taken from the edge of an actively growing culture that had been incubating for 10 days. Without any type of treatment, control was employed as such in the medium. For the pathogen to grow, three replications of each treatment were incubated at 26±2°C. The effectiveness of several compounds was evaluated by counting the millimetres (mm) of the fungal colony's radial growth. When compared to the control, the inhibition was measured in terms of the percentage of fungal growth that was inhibited. After 6 and 10 days of incubation, the radial development of the fungus was measured in order to evaluate the effectiveness of various treatments. The following formula was used to compute the percentage of mycelial growth inhibition (McKinney, 1923).
The following formula was also used to calculate the percent inhibition over control.

Percent Disease Control (PDC) PDC
$= growth\ in\ control - growth\ in\ treatment\ growth\ in\ control \times 100$

OR

$$(PDC) = C - T\ /C \times 100$$

Where
C = Growth in control (untreated). T = Growth in fungicide treated plate.
Fungicide effectiveness on mycelial growth and the percentage of isolated fungi that decreased were measured 5, 7, and 12 days after inoculation and noticed 6, 10, 15, and 20 days afterwards.
In these experiments tried to check the various fungicides against the surveyed dominant pathogenic fungi. Here the table indicating the values as control where no any kind of treatment given to the fungi while other three values are the treatment of given fungicide at three different concentrations. These numbers indicate the radial growth of colonies in petri-dish in millimetre units. To calculate Percent disease Control (PDC) taken lowest value from all three available values. For each fungicidal treatment here used three different concentrations, by keeping manufacturers recommended concentration should be an average. Two other fungicidal concentrations were lesser and the other one was higher than recommended by manufacturers. To calculate the Percent Disease Control (PDC) formula described in chapter three, by using that formula here we calculated the values of PDC. For PDC we considered only the lowest value of concentrations from the three concentrations we used.

Table 1: Effect of Tilt (Propiconazole 25% EC)

Sr. No.	Fungi	0.05% (mm)	0.1% (mm)	0.15% (mm)	Control (mm)	PDC
1	*Alternaria alternata*	08	00	00	55	100
2	*Phomopsis* spp.	32	21	12	48	75
3	*Colletotrichum capsici*	35	14	14	60	76.66
4	*Fusarium oxysporum*	20	00	00	62	100
5	*Curvularia lunata*	30	24	17	58	70.68
6	*Phoma glomerata*	22	00	00	50	100
7	*Pseudocercospora* spp.	25	18	16	52	69.23

Fungicide brand name Tilt composed the Propiconazole 25% EC (Emulsifiable Concentrate). These experiments used its three different concentrations in such a way that the recommended concentration (0.1%) comes from the mean value of all three. More or less in all fungi inhibited its growth due to this treatment but mostly growth inhibition in *Alternaria alternata*, *Fusarium oxysporum* and Phoma *glomerata.* The percent of disease control (PDC) here ranges between 69.23% to 100% for different fungi by this fungicide.

Table 2: Effect of Amistar (Azoxystrobin 18.2% + Difenoconazole 11.4% SC)

Sr. No.	Fungi	0.05% (mm)	0.1% (mm)	0.15% (mm)	Control (mm)	PDC
1	*Alternaria alternata*	34	26	15	55	72.72
2	*Phomopsis* spp.	22	18	13	48	72.91
3	*Colletotrichum capsici*	27	16	10	60	83.33
4	*Fusarium oxysporum*	25	14	13	52	75
5	*Curvularia lunata*	18	17	14	58	75.86
6	*Phoma glomerata*	25	18	12	50	76
7	*Pseudocercospora* spp.	20	12	00	52	100

The market name fungicide Amistar constituents are Azoxystrobin 18.2% with additional Difenconazole 11.4% SC (Suspension Concentrate). By this treatment mostly affected up to nil growth fungi was *Pseudocercospora* spp.

Other six fungi affected and the 0.1% as well as 0.15% influence more as increase the concentration of fungicide. Compared with control in all cases the fungi growth is reduced by too many levels. The percent of disease control

(PDC) here ranges between 72.72% to 100% for different fungi by this fungicide

Discussion

The presented data highlights the efficacy of the tested fungicide against a range of plant pathogenic fungi. At a concentration of 0.05%, the fungicide exhibited notable inhibition against Alternaria alternata, resulting in an 8 mm reduction in fungal growth as compared to the control. A similar trend was observed with Phoma glomerata, where the fungicide completely suppressed fungal growth at this concentration. Increasing the concentration to 0.1% led to enhanced inhibitory effects against Phomopsis spp., with a progressive decline in fungal growth from 32 mm to 12 mm. Moreover, Colletotrichum capsici and Pseudocercospora spp. also displayed sensitivity to this concentration, showcasing reductions of 21 mm and 16 mm, respectively, in comparison to the control.

At 0.15%, the fungicide continued to exhibit substantial antifungal activity. Notably, Curvularia lunata showed a significant reduction in growth from 30 mm to 17 mm. Similarly, *Colletotrichum capsici* and *Pseudocercospora* spp. maintained their sensitivity, further restricting growth to 14 mm and 14 mm, respectively. In contrast, *Fusarium oxysporum* displayed higher resistance, with no observable inhibition at both 0.05% and 0.1% concentrations. However, complete growth suppression was achieved at 0.15%, emphasizing the concentration-dependent nature of the fungicidal effect. The present study demonstrates the potential of the tested fungicide to effectively control a spectrum of plant pathogenic fungi. The variation in sensitivity among different fungal species suggests the importance of tailored fungicide concentrations for optimal disease management. These findings underscore the significance of continued research in refining fungicidal strategies and advancing plant protection measures in agriculture.

The study investigated the efficacy of Amistar, containing Azoxystrobin 18.2% + Difenoconazole 11.4% SC, against various plant pathogenic fungi at different concentrations. At 0.05%, the fungicide exhibited varied effectiveness, significantly reducing Alternaria alternata growth from 34 mm to 15 mm, and Phomopsis spp. growth from 22 mm to 13 mm. Colletotrichum capsici showed partial sensitivity, decreasing growth from 27 mm to 10 mm. Enhanced inhibition was observed at 0.1%, with Fusarium oxysporum growth reduced from 25 mm to 13 mm, and Curvularia lunata from 18 mm to 14 mm. Phoma glomerata exhibited a slight reduction from 25 mm to 12 mm. At 0.15%, Amistar displayed continued antifungal activity, causing complete growth suppression in *Pseudocercospora* spp., whereas Colletotrichum capsici displayed limited sensitivity. The study underscores Amistar's potential in controlling diverse plant pathogenic fungi. The concentration-dependent responses highlight the importance of tailoring fungicide application for optimal disease management. These findings contribute to the understanding of Amistar's role in integrated pest management strategies, promoting crop health and productivity.

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