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Study on Growth Inhibition of Plant Pathogenic Fungi in In-Vitro Conditions by Some Chemical Fungicides

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Abstract
In this study used the three different chemical fungicides against the seven different plant pathogenic fungi to check the control on growth of fungi. Hexaconazole, Mancozeb and combination of Tebuconazole and Trifloxystrobin were treated to fungi under in-vitro conditions against the *Alternaria alternata, Phomopsis* spp., *Colletotrichum capsica, Fusarium oxysporum, Curvularia lunata, Phoma glomerata* and *Pseudocercospora* spp. The effect of Hexaconazole 5% SC showed complete 100 percent inhibition of *Alternaria alternata and Fusarium oxysporum.* The results of Mancozeb 75% WP use were inhibited Phomopsis spp., Curvularia lunata and Phoma glomerata completely. The effect of Tebuconazole and Trifloxystrobin chemicals together completely inhibited growth of *Fusarium oxysporum.*

Keywords – Fungicide, Antifungal, Efficacy, Pathogenic fungi

Introduction-

This research article aims to present a comprehensive analysis of the in-vitro efficacy of select fungicides against a range of pathogenic fungi. By examining the inhibitory effects of these fungicides on key fungal isolates, we seek to enhance our understanding of their potential as control agents. Furthermore, this study will shed light on the broader implications of fungicide application, including the emergence of resistance and the ecological impact on non-target organisms. In the context of sustainable agriculture and integrated disease management, it is essential to assess fungicides' performance under laboratory conditions before their deployment in the field. Through this investigation, we aspire to contribute to the refinement of fungicide selection and application strategies, ultimately fostering more effective disease management and promoting the long-term resilience of agricultural systems.

In the subsequent sections of this research paper, we will detail the materials and methods employed in the study, present the results of our in-vitro experiments, and discuss the implications of our findings in the broader context of fungal disease control. By addressing these aspects, we aim to offer valuable insights that contribute to the ongoing advancement of effective strategies to combat pathogenic fungi and secure global food production. Numerous particular fungicides were widely used up until recently due to their wide application windows, systemic activity, and protective and curative characteristics (Knight *et al*., 1997; Morton and Staub 2008).

Unquestionably, these compounds have contributed greatly to the agriculture business and led to huge productivity increases. These most widely used chemical fungicides, meanwhile, have significant drawbacks. The development of infections with diverse fungicide resistances as a result of the careless use of these fungicides has made the management of the illnesses much more challenging (Talibi et al., 2014). Furthermore, due to their unfavourable effects on non-target species (carcinogenicity, high and acute residual toxicity), potential dangers to environmental pollution (long degradation time), and increasing restrictions on their repetitive and exclusive use, fungicides are no longer widely used (Bai et al., 2013). The current study focuses on the in vitro efficiency of fungicides against *Alternaria solani* because of the plant's economic significance, the yield losses caused by early blight, and its impact on yield.

The genus *Alternaria* contains a variety of deuteromycetes, which are harmful plant parasites for families like Solanaceae, Cucurbitaceae, and Brasicaceae. Worldwide species that coexist as saprophytes and weak parasites are included in the Alternaria genus. Tiny black spots are regularly produced in a variety of conditions on pods and delicate twigs (Valkonen and Koponen, 1990). The *Alternaria* genus is responsible for some of the most prevalent plant diseases in the world. On all of their hosts, the different Alternaria cause some of the highest absolute gross losses of any pathogen

(Agrios, 2005). Khalid et al. (2004) and Choulwar et al. (1994) present a thorough, comparative analysis of the morphological variations of the several forms of *Alternaria* found in cucurbitaceous, brassicaceous, and solanaceous crops.

Materials and Method

Effect of fungicides against selected dominant pathogenic fungi in in-vitro Fungicides mainly Hexaconazole 5% SC, Mancozeb 75% WP, Tebuconazole 50% + Trifloxystrobin 25% w/w (75 WG) following Poison food technique (Schmitz, 1930). 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	Contra Plus	Hexaconazole 5% EC	Emulsifiable Concentrate	Rallis India Ltd (Tata enterprises)	Liquid	0.1%
2	Dithane M-45	Mancozeb 75% WP	Wettable Powder	Bayer Crop Science Ltd	Powder	0.2%
3	Nativo	Tebuconazole + Trifloxystrobin 75%	Water Dipersible granules	Bayer Crop Science Ltd	Granules	0.05%

WP- Wettable Powder, 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.

1. **Effect of Contaf Plus (Hexaconazole 5% SC)**

Table No. 1.

Sr. No.	Fungi	0.05% (mm)	0.1% (mm)	0.15% (mm)	Control (mm)	PDC
1	*Alternaria alternata*	28	00	00	55	100
2	*Phomopsis* spp.	33	26	22	48	54.16
3	*Colletotrichum capsici*	42	24	13	60	78.33
4	*Fusarium oxysporum*	00	00	00	52	100
5	*Curvularia lunata*	32	20	16	58	72.41
6	*Phoma glomerata*	36	28	18	50	64
7	*Pseudocercospora* spp.	32	26	14	52	73.07

The Contaf Plus fungicide composing Hexaconazole 5% SC (Suspension Concentrate) its effect in-vitro was observed to inhibit the growth in case of *Alternaria alternata* and *Fusarium oxysporum* very well. *Fusarium oxysporum* was highly sensitive for this fungicide so it showed nil growth at 0.05% of hexaconazole. Comparatively with fusarium less sensitivity is shown by *Alternaria alternata* with hexaconazole because it shows some growth at 0.05%. All other fungi in this experiment got affected by its growth partially but not completely. The percent of disease control (PDC) here ranges between 54.16% to 100% for different fungi by this fungicide.

2. **Effect of Dithane M-45 (Mancozeb 75% WP)**

Table No. 2

Sr. No.	Fungi	0.1% (mm)	0.2% (mm)	0.3% (mm)	Control (mm)	PDC
1	*Alternaria alternata*	40	38	13	55	76.36
2	*Phomopsis* spp.	22	00	00	48	100
3	*Colletotrichum capsici*	40	26	12	60	80
4	*Fusarium oxysporum*	32	24	11	62	82.25
5	*Curvularia lunata*	16	00	00	58	100
6	*Phoma glomerata*	18	00	00	50	100
7	*Pseudocercospora* spp.	24	16	15	52	65.38

Dithane M-45 composed of the Mancozeb 75% WP (Wettable Powder) the manufacturer recommended effective concentration that is 0.2% observed more effective. At this recommended concentration got nil or no growth for 3 different pathogenic fungi *Phomopsis*, *Curvularia* and *Phoma glomerata.* For other fungi it positively interrupted the colony growth as well as rate of growth with time. The above 0.2% concentration gives their effect nearly the same. The percent of disease control (PDC) here ranges between 65.38% to 100% for different fungi by this fungicide

3. **Effect of Nativo (Tebuconazole 50%+ Trifloxystrobin 25% w/w (75 WG))**

Table No.3

Sr. No.	Fungi	0.01% (mm)	0.05% (mm)	0.1% (mm)	Control (mm)	PDC
1	*Alternaria alternata*	36	20	14	55	73.07
2	*Phomopsis* spp.	34	42	23	48	74.54
3	*Colletotrichum capsici*	24	06	00	60	52.08
4	*Fusarium oxysporum*	35	26	19	52	100
5	*Curvularia lunata*	24	20	13	58	63.46
6	*Phoma glomerata*	28	22	16	50	77.58
7	*Pseudocercospora* spp.	26	12	00	52	68

The Nativo contains the two different chemical constituents in its composition Tebuconazole 50% and Trifloxystrobin 25% by weight so this

proportion together showed 75 WG (Water granules). In this treatment observed more effect on *Colletotrichum capsici* and *Pseudocercospora* spp. The concentration recommended showed a good effect nearly on all fungi but our experimental conclusion recommends 0.1 % concentration better than 0.05%. The percent of disease control (PDC) here ranges between 52.08% to 100% for different fungi by this fungicide.

Discussion

In these tests, it was attempted to compare several fungicides to the dominant pathogenic fungus that had been studied. The values for controls show that the fungus received no treatment at all, while the other three values show that fungicide was applied at three different concentrations. These statistics represent the colony's radial growth on petri dishes measured in millimetres. In order to determine the percent disease control (PDC), the lowest value out of the three options was chosen. Here, three different concentrations of each fungicidal treatment were utilised, with the manufacturers' recommended concentration serving as the average. Two alternative fungicidal concentrations exist, but one of them is lower and the other is higher than what manufacturers advise. Some fungicides exhibited no signs of growth from all fungicidal treatments. It implies that fungicides were used successfully to halt the development of these fungus. Our study came to these results about fungi and fungicidal combinations, which are displayed in table number 31. Given the success of the combination of Hexaconazole 5% SC in treating *Alternaria alternata*, this combination should be used. In our study, these combinations demonstrated good control compared to other fungicidal chemical compositions.

Contaf Plus fungicide, composed of Hexaconazole 5% SC, effectively inhibited the growth of *Alternaria alternata* and *Fusarium oxysporum* in-vitro. Fusarium oxysporum was highly sensitive to hexaconazole, showing nil growth at 0.05%. *Alternaria alternata* showed less sensitivity, showing some growth at 0.05%. The fungicide had a partial effect on growth, with a percent of disease control (PDC) ranging from 54.16% to 100% for different fungi. Dithane M-45, containing Tebuconazole 50% and Trifloxystrobin 25%, showed more effect on Colletotrichum capsici and Pseudocercospora spp. The recommended concentration of 0.1% was better than 0.05%, with a PDC range of 52.08% to 100%, *Fusarium oxysporum*, and Phoma glomerata. Overall, the fungicide effectively inhibited fungi growth, with a PDC range of 72.72% to 100% for various fungi.

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