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Flusilazole and Penconazole Had Potent Effects Against *Fusarium oxysporum*, with no Compatibility with *Trichoderma harzianum*

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ABSTRACT

Fusarium oxysporum is a ubiquitous, widespread, soil-borne fungus with significant economic importance. More than 120 plant species, including some economically important crops like tomato, are susceptible to infection with this fungus. A series of laboratory experiments were conducted to assess the inhibitory effect of eight fungicides on *F. oxysporum*. These fungicides can be classified into a single mode of action fungicides (Flint, Previcur N, Punch and Topas) or multi-mode of action fungicides (Acrobat copper, Consent, Maxim XL and Roxyl Plus). Results revealed that, the single mode of action fungicides such as Punch (Flusilazole) and Topaz (Penconazole) were the most toxic to the *F. oxysporum* with IC_{50} = 0.152 and 0.989 ppm, respectively. While Maxim XL (Mefenoxam + Fludioxonil) was the most effective multi-mode of action fungicide with IC_{50} =1.66 ppm. The influence of the most effective fungicide on *F. oxysporum* on the fungal antagonist *Trichoderma harzianum* was investigated. All tested fungicides had a potent effect on *T. harzianum*.

1. INTRODUCTION

Fusarium oxysporum is an economically destructive species, which causes a lot of plant diseases, including wilt, rot, and damping-off. It is a phylogenetic diverse soil-borne fungus that had a broad host range counting horticultural and grain crops **(LeBlanc *et al.*, 2017; Xiong and Zhan, 2018)**. That pathogen came in fifth place among the top ten phyto pathogenic fungi **(Dean *et al.*, 2012)**. Fusarium wilt is a serious disease produced by *F. oxysporum* strains that poses a significant hazard to agriculture **(Fisher *et al.*, 2012)**.

There are diverse ways to control Fusarium wilt. It included the use of chemical control, which consisted of the use of fungicides and soil fumigants **(Rahman *et al.*, 2021)**, as well as the biological control of fusarium wilt, which included the use of non-pathogenic strains of *Fusarium* spp. **(Bahadur, 2022)**, the use of plant extracts as well as the addition of organic matter to the soil to control this disease **(Bubici *et al.*, 2019)**. Also, crop rotation contributes to the control of that disease **(Joshi, 2018)**. Soil-borne pathogens, such as *Fusarium* spp., are extremely difficult to manage with chemical fungicides **(Maurya *et al.*,**

2019). But fungicides have been widely used to reduce fungal infections and boost agricultural yield **(Bubici *et al.*, 2019)**. Several fungicides, including benzimidazole carbamate, dazomet, vancide, dimethyl dithiocarbamate, ethyl mercury phosphate, and mancozeb, have been used to control fusarium wilt disease **(Rahman *et al.*, 2021)**. But because of the unconscious and excessive use of fungicides, the problem of resistance of many fusarium strains to diverse groups of pesticides has emerged **(Lemanceau and Alabouvette, 1991; Rahman *et al.*, 2021)**.

Resistance is the ability of the strain to tolerate high concentrations of the fungicide without affecting its growth, which discourages the growth of susceptible strains. This resistance is due to a mutation at the fungicide target of action or the ability of the fungus to metabolize these fungicides **(Cools and Fraaije, 2008)**. Fungicides can be classified based on the site of action into single-site fungicides and multiple-site fungicides **(Hu and Chen, 2021)**. Also, a fungicide may consist of one active ingredient or a mixture of two or more active ingredients. Multi-site fungicides and mixtures of fungicide are used to overcome fungal resistance **(Netto *et al.*, 2020)**. Biological control appears to overcome the resistance of fungi to the action of fungicides **(Maurya *et al.*, 2019)**. The genus *Trichoderma* is among the most prominent and commonly used organisms for plant growth promotion and biological control of plant pathogens **(Maurya *et al.*, 2019)**. *T. harzianum* had been used effectively for control banana fusarium wilt disease **(Bubici *et al.*, 2019)**.

Therefore, the objective of this research is to evaluate the effectiveness of some fungicides against fusarium wilt, whether single-site fungicides or multiple-site fungicides. Additionally, to investigate the influence of effective fungicide on *F. oxysporum* f. sp. *lycopersici* pathogen on the growth of the fungal antagonist *T. harzianum*. Thus, we can use both chemical and biological control in parallel.

2. MATERIALS AND METHODS

2.1. The tested fungicides

Laboratory experiments were conducted to assess the effect of some fungicides on the growth of the *F. oxysporum* f. sp. *lycopersici* and to estimate the IC_{50} value for each. Also, the effect of the most effective fungicide on *F. oxysporum* on the growth of the fungal antagonist *T. harzianum*.

The fungicides that used in the experiments were classified into two groups: single-site fungicides (or fungicides containing one active ingredient that affects one site of action) and multiple-site fungicides (fungicide containing more than one active ingredient and therefore multisite effect). The detail of fungicides used in this experiment, including their trade name, the active ingredient, formulation, and field application rates are specified in Table 1.

2.2. Isolation, purification, and identification of the causal organism

Tomato plant samples (cv. Super Strain B) with the characteristic signs of vascular wilt disease were taken from various locations in EL-Gharbia Governorate. To detach the pathogen, tomato plant roots were clipped into little pieces. The infected parts were then surface sterilized by submerging them in a 0.5% sodium hypochlorite solution for two minutes, then three times with distilled water.

Samples were dried between two layers of sterilized filter papers to remove the excess water, before being placed on potato dextrose agar (PDA) medium on Petri dishes. Dishes that had been inoculated were incubated at 18–20 °C for 10–15 days while being checked daily to

see if the pathogen's mycelium was growing. The hyphal tip method was used to purify the cultures. Stock cultures were kept on PDA slants and kept at 5°C in the refrigerator. Cultural, microscopic, and phytopathological characteristics of *Fusarium oxysporum* f. sp. *lycopersici* were taken into consideration to identify the pathogenic isolates according to **Alexopoulos and Mims (1979)**.

2.3. Fungal antagonist

A pure isolate of the antagonist, namely *Trichoderma harzianum*, was obtained from the Department of Integrated Pest Management, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. The antagonist fungi were kept at 4°C on potato dextrose agar (PDA) medium until use.

2.4. In vitro evaluation of fungicides against F. oxysporum and T. harzianum

Eight fungicides shown in Table 1 were evaluated against *Fusarium oxysporum*. While the most effective fungicides against *F. oxysporum* were evaluated against the antagonist *T. harzianum*. Graduated concentrations of the tested pesticides were prepared i.e., 0.01, 0.1, 1, 10, 100, 1000, 2000 and 4000 mgL^{-1}.

Table 1: Details of fungicides used in the experiments

Trade Names	Active ingredient	Field application rate
Single active ingredient fungicide /single site of action		
Flint	Trifloxystrobin	20g/100L
Previcur N	Propamocarb	250 ml/100L
Punch	Flusilazole	3 ml/100L
Topas	Penconazole	150ml/100L
Multi-active ingredient fungicide/Multisite of action		
Acrobat copper	Dimethomorph/Cooper Oxychloride	150g/100L
Consento	Propamocarb/ Fenamidone	250 ml/100L
Maxim XL	Fludioxonil /Mefenoxam	300 g/100L
Roxyl Plus	Metalaxyl/Copper Hydroxide	150 g/100L

Based on the active ingredient, the obligatory quantity of each fungicide was considered and mixed thoroughly with autoclaved and cooled (45°C) PDA medium, while distilled sterilized water served as a negative control. The medium (fungicide modification PDA) was then aseptically placed into 7cm Petri plates and allowed to solidify. After solidifying the medium, all the plates were inculcated aseptically with a 5mm culture disc of a 7-day-old *F. oxysporum* culture and incubated at 18-20°C until pathogenic fungal mycelial growth covered the surface of the medium in the control treatment (after 7 days). The percent of growth inhibition of the test pathogen was calculated using the formula according to **Otadoh *et al.* (2011)** as follows:

$$I (\%) = [(C-T) /C] \times 100$$

Where:

I = Percent inhibition (Reduction %), C= Growth of the pathogen in control Petri plate, and T= Growth of the pathogen in the treatment.

2.5. L.D.P lines and statistical analysis

Growth inhibition percentages were plotted on probit graph paper against fungicides concentrations. Data were statistically analyzed according to the method of **Litchfield and Wilcoxon (1949)**. The obtained data including slopes of the regression lines and IC_{50} values with their 95% confidence limits were recorded.

3. RESULTS AND DISCUSSIONS

3.1. Fungicides efficacy against the pathogenic fungi F. oxysporum

3.1.1. Single-site fungicides

The effect of single-site fungicide on *F. oxysporum* radial growth was recorded in Table 2 and illustrated in Fig. 1. Data revealed that, the most effective fungicides against *F. oxysporum* were Punch and Topas with IC_{50}= 0.152 and 0.989 ppm, respectively with no significant difference between them. But Topas had IC_{99} over 10^4 ppm. This means that, this fungicide cannot be used to control *F. oxysporum*. Moreover, the Flint and Previcur N fungicides had a moderate effect against *F. oxysporum* with IC_{50}= 19.52 and 53.17 ppm, respectively with no significant difference between them. Like fungicide Topas, both fungicides (Flint and Previcur N) cannot be used to control the pathogen fungi (they had IC_{99} more than 10^4 ppm).

Table 2: Growth Inhibition (IC_{50}) of tested fungicides (single active ingredient/ single mode of action) on *F. oxysporum* pathogen

Fungicide	IC_{50}	Confidence intervals		IC_{99}	Value slop
		lower	upper		
Flint	19.519	0.208	1828.810	>10000	0.287
Previcur N	53.174	2.555	1106.532	>10000	0.495
Punch	0.152	0.010	2.204	14.882	0.973
Topas	0.989	0.018	53.830	>10000	0.412

Except Punch, all fungicides had a small value slop (ranging between 0.287 and 0.495). Thus, the fungus population is heterogeneous against the toxic effect of these fungicides. Moreover, the fungus population tends to be resistant to the toxic action of these tested fungicides. While Punch fungicide had a slightly high-value slope (0.973). Accordingly, the *F. oxysporum* population is homogeneous against the toxic effect of Punch fungicide. Moreover, *F. oxysporum* population is not going to be a resistant against the action of Punch fungicide. These results are consistent with the results obtained by **Ghante *et al.* (2019)** who found that Flusilazole (Punch) is one of the most effective fungicides in reducing the fungal growth of *F. oxysporum* f. sp. *udum*.

Flusilazole [1-{[Bis (4-fluorophenyl) (methyl) silyl] methyl}-1H-1, 2, 4-triazole] the active ingredient of Punch is an organ silicon fungicide, belongs to the group of systemic triazole fungicides. Its mode of action is inhibiting the cytochrome P_{450}-dependent 14α-demethylase activity. Which is necessary for converting lanosterol into ergosterol. Ergosterol is an essential component of cell membranes. This leads to the disintegration of cell membranes **(Henry and Sisler, 1984; Roberts and Hutson, 1999)**. That unique mode of action makes that fungicide more effective, and more selective. But, due to its site-specificity, flusilazole has a fairly high inherent risk of resistance advance in target pathogens fungi.

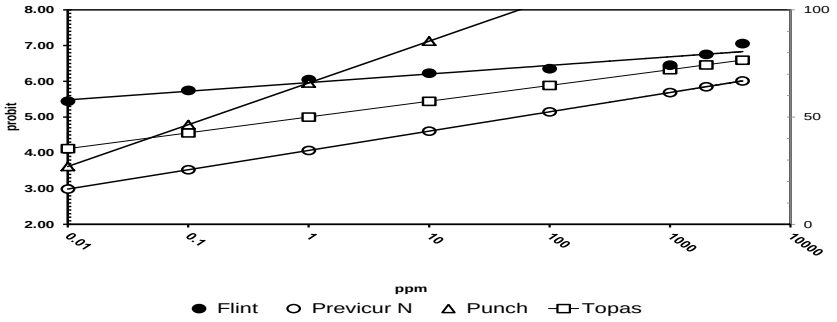

Fig. 1: Probit regression line for the growth inhibitory effect of single site fungicide against *F. oxysporum* pathogen

3.1.2. Multi-site fungicides

The effect of multi-site fungicide on *F. oxysporum* radial growth was recorded in the Table 3 and illustrated in Fig. 2. Data showed that the most effective fungicide against *F. oxysporum* was Maxim XL (IC_{50}= 0.166). Maxim XL had IC_{99} = 2140.26. This means that, this fungicide can be used to control *F. oxysporum*, economically. The Roxyl Plus fungicide had a moderate effect against *F. oxysporum* (IC_{50}= 1901.82 ppm). But, Acrobat copper, Consento, and Roxyl Plus fungicides cannot be used to control the pathogen fungi where they had IC_{99} more than 10^4 ppm. All fungicides had a small value slope (ranging between 0.23: and 0.66). Thus, the *F. oxysporum* population is heterogeneous against the toxic effect of these fungicides and tends to be resistant to these fungicides. This argument is consistent with the findings of **El-Morsi *et al.* (2012)** who found that Maxim XL completely inhibited the growth of *Fusarium* sp. Fungus *in vitro*.

Table 3: Growth Inhibition (IC_{50}) of tested fungicides (multi-active ingredient/multi-mode of action) on *F. oxysporum* pathogen

Fungicide	IC_{50}	Confidence intervals		IC_{99}	Value slop
		lower	upper		
Acrobat copper	1.95×10^5	153.00	2.48×10^8	>10000	0.30
Consento	1.94×10^6	154.70	2.43×10^{10}	>10000	0.23
Maxim XL	1.66	0.07	40.82	2140.26	0.66
Roxyl Plus	1901.82	35.72	101248.45	>10000	0.41

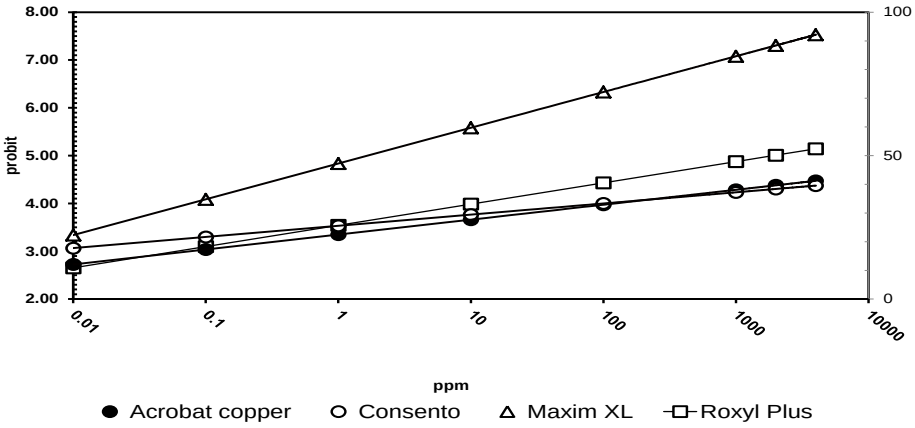

Fig. 2: Probit regression line for the growth inhibitory effect of multi-site fungicide against *F. oxysporum* pathogen

Maxim XL (Mefenoxam + Fludioxonil) is a seed treatment fungicide which is used to control certain seed-borne and soil borne diseases. Mefenoxam is a systemic fungicide, with a potent inhibition effect against mycelium growth and sporulation **(Staub and Young, 1980)**. Its mode of action is inhibiting the synthesis of ribosomal RNA by influencing the RNA polymerases activity **(Davidse *et al.*, 1983)**.While Fludioxonil is a non-systemic, long residual fungicide. The mode of action of Fludioxonil is inhibiting the transport-associated phosphorylation of glucose, which inhibits the mycelial growth rate **(Lee *et al.*, 2019)**. Fungicides are often mixed to overcome the development of the phenomenon of resistance, and when mixing these fungicides, they must be different in their mode of action. This is evident in the most

effective fungicide (Maxim XL) in our experiment.

3.2. The toxic effect of efficacious fungicides against F. oxysporum on T. harzianum

The toxic effect of efficacious fungicides against the pathogenic fungi *F. oxysporum* (Punch and Topas) on the antagonist fungal *T. harzianum*radial growth were recorded in the Table 4 and illustrated in Fig. 3. Results showed that, Punch (Flusilazole) and Topas (Penconazole) had a potent effect against *T. harzianum* (IC_{50} = 0.32 and 4.16 ppm, respectively) with no significant difference between them. Also, the two fungicides had large value slop (1.23 and 1.48, respectively). Thus, the fungus population is homogeneous against the toxic effect of these two fungicides. Similarly, the triazole fungicides (Hexaconazole, Propiconazole, and Penconazole) at different doses were found to be highly inhibitory against *T. harzianum* **(Bhat and Srivastava 2003; Khalko and Pan, 2009)**.Additionally, Benomyl, Penconazole, Propiconazole, and Prochloraz had a highly toxic effect on the mycelial growth of *T. harzianum* **(Sushir *et al.*, 2015)**.

Table 4: The inhibitory effect of tested fungicides on the fungal antagonist *T. harzianum*

Fungicide	IC_{50}	Confidence intervals		IC_{99}	Value slop
		lower	upper		
Punch	0.32	0.02	6.46	10.00	1.23
Topas	4.16	0.34	50.28	61.71	1.48

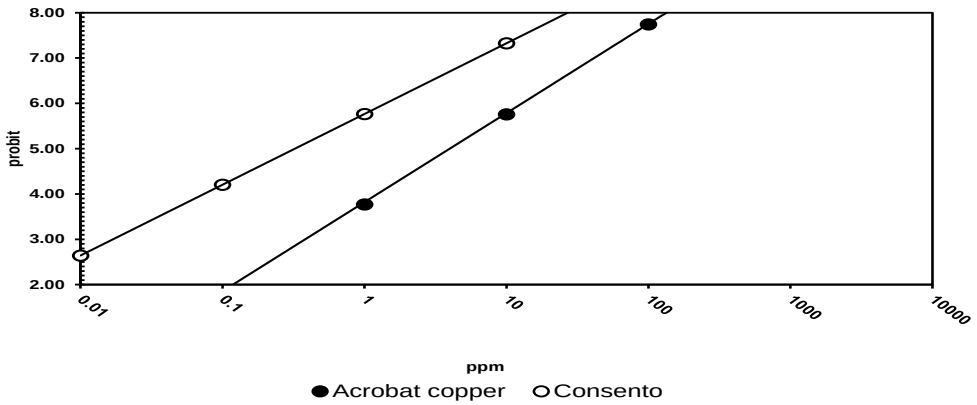

Fig. 3: Probit regression line for the growth inhibitory effect of the effective fungicide against *F. oxysporum* pathogen on fungal antagonist *Trichoderma harzianum*

Conclusion

The single site triazole fungicides Punch (Flusilazole) and Topas (Penconazole) had a potent effect against *F. oxysporum* but had no compatibility with *Trichoderma harzianum*.

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