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Sensitivity of *Fusarium oxysporum* Isolates Collected from Strawberry Roots to DMI Fungicides Difenoconazole, Tebuconazole and Prochloraz

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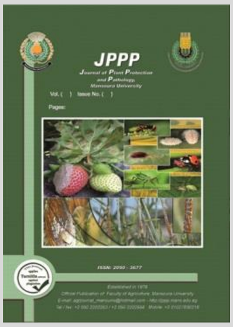

ABSTRACT

Soil borne fungi control is particularly difficult as they survive in soil and in host plant debris for long period. Strawberry plants infected heavily by *Fusarium oxysporum* causing *Fusarium* wilt leading to serious decrease in the crop production. The application of protective fungicides extensively is the essential strategy to control the disease. However, resistant populations to the common fungicides are widely detected recently.This study aimed to detect *F. oxysporum* resistant populations to difenoconazole, tebuconazole and prochloraz and to find a good strategy to control the disease. In the current study, 115 *F. oxysporum* isolates were collected from four main strawberry-producing governorates(Beheira, Ismailia, Dakahlia and Qalubiya)in Egypt and used. The results showed that among collected isolates, 71 isolates (61.73%) were resistant to difenoconazole while 85 isolates (73.92%)were resistant to tebuconazole and 2 isolates(1.74%)were resistant to prochloraz.The EC_{50} of difenoconazole, tebuconazole and prochloraz were determined for a set of sensitive and resistant isolates using the mycelial growth inhibition technique. The EC_{50} mean value for difenoconazole-sensitive isolates was 0.08 µg/ml while, the EC_{50} mean value for difenoconazole-resistant isolates was 1.27µg/ml. Tebuconazole's mean EC_{50} concentration for susceptible isolates was 0.04µg/ml, in contrast, the mean EC_{50} value for resistant isolates was 0.139µg/ml and likewise for prochloraz sensitive isolates had an average EC_{50} of 0.024µg/ml, while resistant isolates had an average EC_{50} of 6.97µg/ml. The combination of trifloxystrobin 25% and tebuconazole 50% tested with two concentrations 10 and 100 µg/ml showed high ability in the management of difenoconazole resistant isolates and exhibited 100% mycelial growth inhibition.

Keywords: *Fusarium oxysporum*, Strawberry, DMI fungicides, Fungicide Resistance, prochloraz

INTRODUCTION

Strawberry (Fragaria × ananassa) is non-traditional Egyptian export crop participating significantly in economic development. Northern parts of Egypt climate is similar to that of the Mediterranean climate, Egypt fertile soils, and its position all contribute to the country's high strawberry production and financial success. These elements can work together to provide early fruiting, a lengthy harvest season, high quality, affordable output, and proximity to export markets (Abd-Elgawad 2019). Egypt occupied fourth position among all nations, trailing only Spain, the United States, and Turkey by a little over 479 thousand tons in 2018 (Abozaid and Eldeeb 2019). In Egypt, strawberry is commonly linked to a serious fungus infection on the soil. *Fusarium oxysporum* f.sp. *fragariae* is a serious problem for strawberry sustainable production under continuous cropping. It infects host plants by penetrating plants through roots and is responsible for severe damage and economic losses, it also causes severe damage and yield losses and eventually causes strawberry plants to wilt and die (Essa 2015). Black root rot is a complicated disease brought on by one or more fungi, such as *Macrophomina phaseolina*, *Fusarium oxysporum* and *Rhizoctonia* species, Egypt has seen a significant increase in the prevalence of this root rot disease in recent years (Abd-El-Kareem et al., 2019). Four fungi with varying frequencies were found on the naturally infected strawberry plants grown in the Qalubiya Governorate. These fungi were identified as *Fusarium oxysporum*, *Verticillium dahliae*, *Rhizoctonia solani* and *Pythium sp*., according to a pathogenicity test, only *F. oxysporum* and *V. dahlia* were able to produce the characteristic wilt symptoms (Essa 2015).

The sterol demethylation inhibitors (DMIs) registered in Egypt and includes many chemical classes such as piperazines, pyridines, pyrimidines, imidazoles and triazoles, each group includes many of active ingredients. Triazoles include difenoconazole, penconazole, tebuconazole and many of others active ingredients. Recently, many fungicides failed to give adequate control for the disease due to emergence of resistant populations(Lin et al 2009) . Alternating fungicide usage is advised in Egypt to stop or postpone the establishment of resistant fungus populations. DMIs are a class of systemic fungicides that target the integrity of cell membranes by preventing C14 demethylation during sterol synthesis. Inhibitors of sterol biosynthesis are effective tools for controlling different types of fungi, including members of the ascomycetes, basidiomycetes, and oomycetes, three major genera of plant pathogens, and this has led to the DMI being an essential component of control programs against significant plant pathogens. Sterols and their derivatives promote and maintain growth and development in fungi by acting as membrane constituents and engaged in control of metabolism.

Numerous scientists have found that many phytopathogenic fungi were resistant to difenoconazole fungicide such as *Fusarium graminearum*, *Lasiodiplodia theobromae* and *Botrytis cinerea* (Rekanović et al., 2010, Li et al., 2020 and Zhang et al., 2020). Similarly, resistance to tebuconazole reported in different studies, according to (Chen

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et al., 2021), a total of 362 *F. graminearum* strains were found to be tebuconazole-resistant, and the prevalence of this resistance was 30.7%. The resistance of the generated *Fusarium oxysporum* f. sp. *fragariae* isolate to tebuconazole recorded 34.22 folds after 36 times selection in comparison to the sensitive isolate, according to (GU et al., 2010). Only seven *F. fujikuroi* strains were found to be prochloraz susceptible, while 82 strains were resistant, according to the findings of the study (Peng et al., 2022). The present investigation conducted to detect the resistance frequencies to DMI fungicides difenoconazole, tebuconazole and prochloraz among *Fusarium oxysporum* populations collected, to determine the half-maximal effective concentration (EC_{50}) values for the resistant and sensitive isolates, respectively, and in the end to find out the effectiveness of certain fungicide mixtures to management DMI resistant populations.

MATERIALS AND METHODS

Fungal isolates: -

Four governorates *i.e.,* Behera, Ismailia, Dakahlia, and Qalubiya were chosen for strawberry plants collection and fungal isolation. Strawberry plants (Festival cv.) with typical wilt symptoms were collected from these governorates. For each governorate, four commercial fields were visited. *Fusarium oxysporum* isolates were isolated from diseased strawberry roots, each root came from a different plant. The diseased strawberry roots were cleaned with tap water to get rid of any soil that was stuck to them, then they were surface sterilized in 1% sodium hypochlorite solution for three minutes, washed multiple times in sterilized distilled water, and finally dried with sterilized filter papers. The sterilized root fragments were transferred aseptically to wet blotter-coated plates, where they were cultured for 5-7 days at 25 °C. On the Potato Dextrose Agar medium, different kinds of fungal colonies were seen, using the hyphal tip technique, the fungal strains were purified (Hawker.1956). The Mycological Research and Plant Disease Survey Department, Plant Pathology Research Institute, A.R.C., Giza, Cairo, Egypt, identified the isolated fungi based on their physical and cultural characteristics as given by Leslie and Summerell (2008). The identified isolates were then kept at 4°C until use in 5-mL plastic tubes with PDA medium slants.

Fungicides and Fungicides mixtures used in the present study are given in table (1):

Table 1. Fungicides and Fungicides mixtures used

Fungicide or mixtures	Active ingredient	manufacturer
Difenoconazole	95% technical grade	Shanghai Heben-Eastsun Medicaments Co. Ltd. -china
Tebuconazole	97% technical grade	Hefei Yifeng Chemical Industry Co., Ltd. -china
Prochloraz	95% technical grade	Rosi chemical Co., Ltd
Nativo	75%WG (trifloxystrobin 25 % + teconazole 50 %)	Bayer AG German
Destruwwr	32.5 % SC (difenoconazole12.5% + azoxystrobin 20%)	Jiangsu Lanfeng Biochemical Co., Ltd. -china
Fenozon	50% W/V (Difenoconazole25%+ Propiconazole25%)	Jiangsu Greenscie Chemical Co.,Ltd. -china

Determination of fungicide resistance: -

In this experiment, discriminatory concentrations (concentrations that completely prevent the sensitive isolates' mycelial development) of tebuconazole, difenoconazole and prochloraz were determined. To create a stock solution containing 100μg/ml of each fungicide, the appropriate amount of each was dissolved in pure acetone. The discriminatory concentrations of tebuconazole, difenoconazole and prochloraz (0.1μg/ml, 1μg/ml, and 0.1μg/ml) respectively, were prepared and used to separate between resistant and sensitive isolates. A three-day-old colony of each isolate's mycelial plug (5mm in diameter) was cut with a cork borer and placed upside-down into the media that had been treated with the fungicide. The resistant isolate could grow on the modified media. While the sensitive isolates could not grow on the modified medium.

Sensitivity of *Fusarium oxysporum* to fungicides: -

By calculating the effective concentration (EC_{50}) values for the 15 Dif^S and 15 Dif^R isolates that were examined in the experiment, the sensitivity to difenoconazole was determined. A technical grade to create final concentrations, the active ingredient (a.i.) of difenoconazole 95% was dissolved in 100% acetone, adjusted to a concentration of 100μg/ml, and added to PDA at (0, 0.005, 0.01, 0.05, 0.1 ,1 and 2.5 μg/ml) and (0, 0.01, 0.05, 0.5,1 and 5μg/ml) to test sensitive isolates and resistant isolates, respectively.

At the same way the experiment evaluated 16$Tebu^S$ and 11$Tebu^R$ isolates, and the sensitivity to tebuconazole was assessed by calculating the effective concentrations (EC_{50}) that resulted in 50% growth inhibition. A technical grade the active ingredient (a.i.) of tebuconazole, 97%, was dissolved in 100% acetone, adjusted to a concentration of 100μg/ml, then added to PDA to achieve final concentrations at (0, 0.01, 0.05, 0.1, 0.5 ,1 and 5μg/ml) and (0, 0.005, 0.01, 0.05, 0.1,1 and 2.5μg/ml) to test resistant isolates and sensitive isolates, respectively.

Concerning the prochloraz, the sensitivity was calculated by calculating the effective concentrations (EC_{50}) for the 19 Pro^S and 2 Pro^R isolates that were tested in the experiment. Prochloraz 95% active ingredient [a.i.] of technical grade was dissolved in 100% acetone, adjusted to a concentration of 100μg/ml, then added to PDA to generate final concentrations at (0, 0.01, 0.05, 0.1 and 0.5μg/ml) and (0, 0.5, 1, 2.5, 5 and 10μg/ml) to test sensitive isolates and resistant isolates, respectively.

Statistical Analyses: -

The Data Processing System (DPS) program, created by Hangzhou Reifeng Information Technology Ltd., Hangzhou, China, was used to calculate the EC_{50} value for each isolate. Because there was no significant difference between the two studies ($P>0.05$), the EC_{50} values from the two trials for each isolate were averaged (Hamada *et al* 2011).

Assessment of fungicides mixtures efficacy in controlling resistant isolates: -

Using the approach of poisoned food, three different commercial fungicide mixes were evaluated for their effectiveness against *F.oxysporum*. The mixtures tested were Nativo mixture 75%WG (trifloxystrobin 25% +tebuconazole 50%), Fenozon50% W/V (Difenoconazole25%+ Propiconazole25%) and Destruwwr mixture 32.5% SC (difenoconazole 12.5% + azoxystrobin 20%). To create a stock solution with a concentration of 100μg/ml, the appropriate amounts of each mixture were dissolved in sterilized distilled water. Final concentrations 10 and 100µg/ml were prepared in PDA medium and added to sterilized petri dishes. Each dish had a mycelial disc (5 mm in diameter) of *F.oxysporum* injected in the center of it. The plates were incubated at (25°C) and the inculated plates untreated controls were used for comparison. The isolates that could grow on the modified PDA were labeled as resistant, whereas the ones that could not grow were labeled as sensitive.

RESULTS AND DISCUSSION

Resistance in *F. oxysporum* isolates to fungicides: -

The present sampling strategy resulted in 115 isolates from the four investigated governorates. The discriminatory concentration of 1µg/ml of difenoconazole, 0.1µg/ml of tebuconazole and 0.1µg/ml of prochloraz allowed differentiating the sensitive strains from resistant strains. From the screened isolates, due to their ability to grow on media modified with discriminating concentrations, 71 isolates (61.73%) were resistant to the fungicide difenoconazole, while 44 isolates (38.26%) were sensitive as they were unable to grow. The frequency distribution of resistant and sensitive isolates to the DMI fungicide difenoconazole (table2) showed the following: 64% (16 out of 25) resistant isolates were detected in population of Behera, 50% (6 out of 12) in Ismailia, 60.7% (17 out of 28) in Qalubiya and 64% (32 out of 50) in Dakahlia.

Resistance to difenoconazole was detected in many phytopathogenic fungi and reported by numerous authors, including (Li et al., 2021) stated that among 90 *Venturia inaequalis* isolates, 21.1% were resistant to difenoconazole. The prevalence of difenoconazole resistance was 11.7% in *B. cinerea* isolates obtained from five provinces in northern, central, and southern China in 2011 (Zhang et al., 2020). Furthermore, the above-mentioned results clearly showed that the highest percentages of difenoconazole-resistant isolates were detected in Dakahlia governorate, while the lowest was detected in Ismailia. This variation between governorates could be attributed to a high selection pressure that occurred as a result of usage the fungicide extensively in the Dakahlia control programme.

Table 2. Information about the sensitivity against difenoconazole of 115 Fusarium oxysporum isolates collected from strawberry fields in Egypt

Place	year of collection	number of isolates	number of	
			DIF^S	DIF^R
Behera	2021	25	9 (36%)	16 (64%)
Ismailia	2021	12	6 (50%)	6 (50%)
Qalubiya	2021	28	11 (39.28%)	17 (60.71%)
Dakahlia	2021	50	18 (36%)	32 (64%)
Total	/	115	44	71
Resistant percentage%			(38.26%)	(61.73%)

DIF^S = sensitive to difenoconazole DIF^R = resistant to difenoconazole

For tebuconazole, the findings indicated that 85 isolates (73.92 %) were resistant to tebuconazole whereas 30 isolates (26.08%) were sensitive to the fungicide. The frequency distribution of resistant and sensitive isolates (table 3) indicated that 56, 91.6, 64.3 and 84% of the isolates showed resistance in Bahira, Ismailia Qalubiya and Dakahlia governorates, respectively. According to the earlier findings, it was clear that Ismailia governorate had the highest percentage of tebuconazole-resistant isolates, whereas Bahira governorate had the lowest percentage. According to (Chen et al. 2021), out of the 362 *Fusarium graminearum* strains gathered, 30.7% of them exhibited tebuconazole resistance. While among 297 *Fusarium pseudograminearum* isolates collected in 2022 season from wheat (8.62%) was resistant to tebuconazole (Zhang et al 2023).

Table 3. Information about the sensitivity against tebuconazole of 115 Fusarium oxysporum isolates collected from strawberry fields in Egypt

Place	year of collection	number of isolates	number of	
			$Tebu^S$	$Tebu^R$
Behera	2021	25	11 (44%)	14 (56%)
Ismailia	2021	12	1 (8.3%)	11 (91.6)
Qalubiya	2021	28	10 (35.7%)	18 (64.3%)
Dakahlia	2021	50	8 (16%)	42 (84%)
Total Resistant percentage%	/	115	30 (26.08%)	85 (73.92%)

$Tebu^S$ = sensitive to tebuoconazole $Tebu^R$ = resistant to tebuconazole

Among 115 isolates collected from four tested governorates, the frequencies of prochloraz resistant isolates were 2, 0, 0, and 4% in Dakahlia, Qalubiya, Ismailia and Beheira, respectively. The other isolates were sensitive to prochloraz for all tested governorates. Table 4 displayed the frequency distribution of isolates' prochloraz sensitivity and resistance. According to (Qin et al. 2022), prochloraz resistance was present in 92.1% of the 89 *F. fujikuroi* strains that were isolated from the Heilongjiang Province. In a similar vein, prochloraz resistance in *F. fujikuroi* was reported to be severe in China by (Gao et al 2022) with resistance rates of 34.56%, 45.33%, and 48.45% from 2019 to 2021.

Table 4. Information about the sensitivity against prochloraz of 115 Fusarium oxysporum isolates collected from strawberry fields in Egypt:

Place	year of collection	number of isolates	number of	
			Pro^S	Pro^R
Beheira	2021	25	24 (96%)	1 (4%)
Ismailia	2021	12	12 (100%)	0 (0%)
Qalubiya	2021	28	28 (100%)	0 (0%)
Dakahlia	2021	50	49 (98%)	1 (2%)
Total	/	115	113	2
Resistant percentage%			(98.26%)	(1.74%)

Pro^S = sensitive to prochloraz pro^R = resistant to prochloraz

Determination of EC_{50} of *F.oxysporum* sensitive and resistant isolates to difenoconazole:

15 *F.oxysporum* susceptible isolates (Dif^S) and 15 *F.oxysporum* resistant isolates (Dif^R) were randomly chosen and analyzed using the mycelial growth inhibition assay to determine the EC_{50} of difenoconazole. The EC_{50} values for isolates that were difenoconazole sensitive ranged from 0.01 to 0.15µg/ml, and the highest value EC_{50} of isolates (40%) were > 0.1 µg/ml (Fig 1). The EC_{50} values for difenoconazole resistant isolates ranged between 0.846 to 2.55 µg/ml, and the isolates' greatest EC_{50} value (53.33%) ranged from 1 to 1.99µg/ml. RF (Resistance Factor) values ranged between 10.43 and 31.54 (Table 5). (Lin et al., 2009) examined *Fusarium oxysporum* f.sp. *fragariae* for susceptibility to the primary triazole fungicide difenoconazole, and the EC_{50} values ranged from 0.1307 to 9.0317.

Table 5.Sensitivity EC50's of Fusarium oxysporum isolates collected from strawberry rot roots to difenoconazole

Difenoconazole (EC_{50})				
Sensitive		Resistant		
Isolate	EC_{50}	Isolate	EC_{50}	RF
H4	0.0464±0.03	E3	0.9412±0.1	11.60
H24	0.0323±0.015	B8	0.9617±0.2	11.86
E8	0.0243±0.02	D52	0.9257±0.3	11.417
D24	0.016±0.015	D43	0.994±0.15	12.25
D29	0.0344±0.01	B24	0.8461±0.35	10.43
E4	0.0532±0.01	H1	1.2139±3.6	14.97
B3	0.0858±0.02	D15	1.0148±1	12.51
D21	0.0616±0.035	D40	1.934±0.577	23.85
B18	0.0579±0.15	D33	1.0002±0.57	12.33
H15	0.1002±0.1	B13	1.089±1	13.43
D39	0.1223±0.1	B16	1.061±0.57735	13.08
B1	0.115±0.003	E13	1.0611±1	13.08
D55	0.15±0.025	H18	1.2475±1	15.38
D1*	0.1301±0.1	H29	2.5574±2.08	31.54
B15	0.1267±0.1	B21	2.2545±1.52	27.8

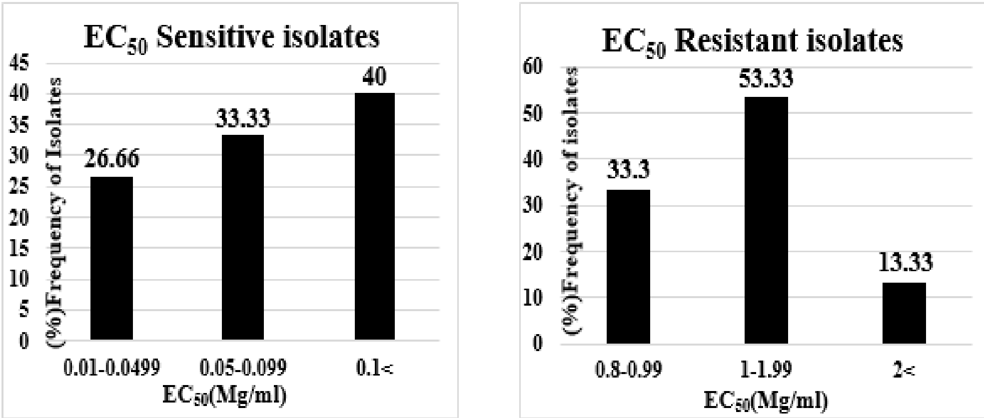

Fig. 1. Frequency distribution of effective concentration (EC_{50}) of difenoconazole to inhibit 50% of mycelial growth.

Determination of EC_{50} of *F.oxysporum* sensitive and resistant isolates to tebuconazole:

16 $Tebu^S$ and 11$Tebu^R$ isolates were chosen at random, examined using a mycelial growth inhibition experiment, and the EC_{50} values for tebuconazole-sensitive isolates were calculated. The EC_{50} values of tebuconazole-sensitive isolates ranged between 0.0137 to 0.08 µg/ml, and the highest value EC_{50} of isolates (50%) ranged between (0.01-0.299 µg/ml) while, the EC_{50} values for tebuconazole resistant isolates ranged from 0.119 to 0.221 µg/ml, and the highest value EC_{50} of isolates (54.54%) ranged from 1 to 0.1299µg/ml (Fig 2). RF (Resistance Factor) values ranged between 2.97 and 5.508(Table 6). (Lin et al., 2009) tested the sensitivity of *Fusarium oxysporum* f.sp. *fragariae* to the main triazole fungicide tebuconazole and the EC_{50} values ranged between 0.1107 to 1.1670 µg/ml.

Table 6. Sensitivity EC50's of Fusarium oxysporum isolates collected from strawberry rot roots to tebuconazole

Tebuconazole (EC_{50})				
Sensitive		Resistant		
Isolate	EC_{50}	Isolate	EC_{50}	RF
B13*	0.0254±0.015	D33	0.1194±0.1	2.97
B3	0.0194±0.01	D15	0.1226±0.05	3.054
B11	0.0137±0.0115	D9	0.1246±0.0577	3.104
B1	0.0253±0.01	D39*	0.1236±0.057	3.079
D35	0.0278±0.015	D18	0.2211±0.1	5.508
B9	0.0165±0.01	E6	0.1279±0.11	3.186
B6	0.0262±0.015	E14	0.1349±0.1	3.36
H29	0.0259±0.02	E2	0.1462±0.115	3.64
B13	0.0313±0.015	D24	0.1534±0.14	3.82
E12	0.0712±0.032	D40	0.1746±0.152	4.349
H4	0.0624±0.04	D43	0.1859±0.1527	4.63
H12	0.0798±0.04			
B8	0.061±0.035			
H16	0.08±0.03778			
B15	0.0523±0.04			
H15	0.054±0.03			

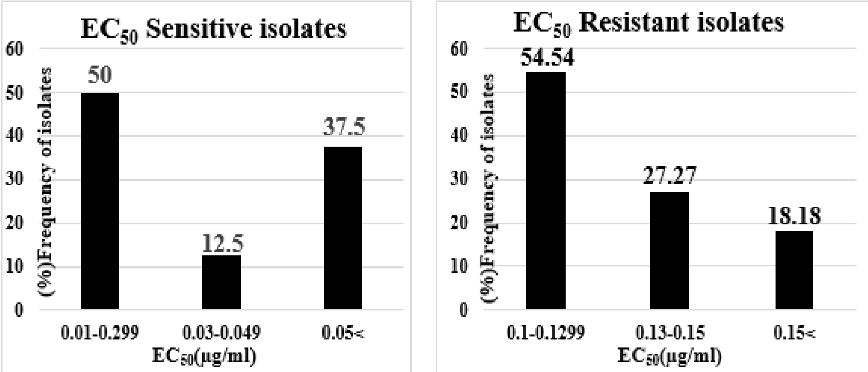

Fig.2. EC_{50} of *Fusarium oxysporum*-resistant and sensitive isolates which tested by using tebuconazole fungicide.

Determination of EC_{50} of *F.oxysporum* resistant and sensitive isolates to prochloraz:

For EC_{50} determination, 19 *F.oxysporum* sensitive isolates (Pro^S) and 2 *F.oxysporum* resistant isolates (Pro^R) employing the mycelial growth inhibition assay to select and test. The EC_{50} values for Prochloraz sensitive isolates ranged between 0.0019 to 0.194 µg/ml, and the highest value EC_{50} of isolates (73.68%) ranged between (0.01-0.0499 µg/ml) (Fig 3) while, the EC_{50} values for Prochloraz resistant isolates ranged between 5.2 to 8.6µg/ml . (Table 7)

Table 7. Sensitivity EC50's of Fusarium oxysporum isolates collected from strawberry rot roots to prochloraz

prochloraz (EC_{50})				
Sensitive		Resistant		
Isolate	EC_{50}	Isolate	EC_{50}	RF
D20	0.0054±0.0017	D7	8.6197±3.05	353.19
B11	0.0019±0.0015	B48	5.3222±2	218.078
B15	0.0041±0.0032			
B10	0.0081±0.0015			
E12	0.0295±0.026			
E15	0.0346±0.02			
D43	0.0343±0.03			
B13	0.0177±0.01			
D21	0.194±0.04			
D24	0.0202±0.0152			
E6	0.0108±0.01			
D35	0.0208±0.02			
B35	0.0334±0.032			
D39	0.0419±0.015			
B16	0.0201±0.0378			
E13	0.0114±0.01			
H29	0.0298±0.0173			
H21	0.0235±0.015			
D3	0.0968±0.03			

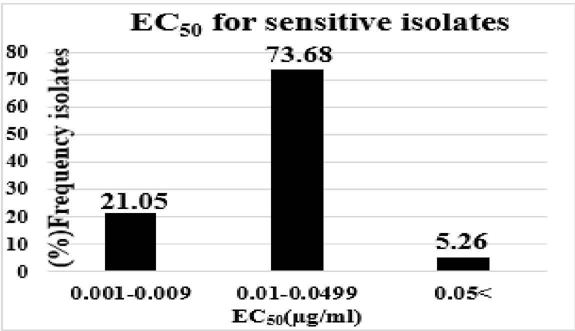

Fig. 3. EC_{50} of *Fusarium oxysporum*-sensitive isolates which tested by using prochloraz fungicide.

To assess the sensitivity of *F. oxysporum* isolates to prochloraz, (Mihajlović et al., 2021) performed *in vitro* experiments. The results revealed that the EC_{50} value was 0.07µg/ml.

The efficiency of some mixture of fungicides in controlling some resistant isolates: -

(Table 8) shows mycelia growth inhibition percentages resulted from using three commercial mixtures (difenoconazole + azoxystrobin, difenoconazole + propiconazole and tebuconazole + tryfloxystrobin) tested against five resistant isolates to difenoconazole in order to find successful strategy to control the resistant populations. The studied mixes all demonstrated strong abilities to manage the resistant isolates, the best mixture was tebuconazole + tryfloxystrobin with inhibition percentages ranged between 94.32 to 97.70 % when the mixture used at low concentration 10µg/ml. While, in case of usage the mixture at high concentration 100 µg/ml full inhibition was achieved for all the resistant isolates. The lowest efficient mixture was difenoconazole + azoxystrobin which give inhibitions ranged between 56.14 to 83.78 %. The previous results were in harmony with (Bhimani et al., 2018) reported that fungicide combinations tebuconazole 50% + trifloxystobin 25% WG were significantly inhibited the growth of *Fusarium oxysporum Schlecht*. In vitro. Similarly, (V Govardhan Rao et al., 2020) evaluated the combinations between tebuconazole +trifloxystrobin employing the approach of poisoned food to combat *Fusarium oxysporum* f.sp. *melongenae*. The results revealed that the tested mixture recorded no mycelial growth. Moreover, (Ponnusamy et al., 2021) stated that tebuconazole 50% + trifloxystrobin 25% WG completely inhibited (100% inhibition) the mycelial growth of the fungus in vitro so that the recommendation was made to use the mixture for controlling the wilt disease in carnation.

Table 8. Mycelial growth inhibition percentages resulted from using different mixtures to control resistant isolates.

Isolates name	Fungicides Mixtures					
	Difen	Difen+Azoxy	Difen+Propi		Tebu+Tryfloxy	
	Concentration µg/ml					
	1	10	10	100	10	100
D1	30.65±0.226	56.14±1.27	79.21±0.14	97.12±0.07	96.74±0.14	100
H22	35.8±0.42	66.38±0.21	95.1±0.28	100	97.7±0.35	100
D40	24.54±0.14	83.78±0.35	85.39±0.21	96.09±0.042	94.32±0.28	100
E6	38.99±0.23	83.78±0.28	82.29±0.28	96.09±0.049	94.32±0.28	100
H9	33.61±0.28	75.84±0.25	86.11±0.54	96.38±0.21	94.49±0.212	100

Difen:difenoconazole, Azoxy:azoxystrobin, Propi:propiconazole, Tebu:tebuconazole, Tryfloxy:tryfloxystrobin

CONCLUSION

Extensive usage of DMI fungicides to control fusarium root rot in strawberry caused by *Fusarium oxysporum* lead to development of resistant populations. High frequencies of resistance to DMI fungicides difenoconazole, tebuconazole and prochloraz were detected in the most of the governorates tested. 60.86% of the total isolates gathered were difenoconazole-resistant while, 73.92% of the total isolates were resistant to tebuconazole and 1.74% of the total isolates gathered were prochloraz-resistant. In the current study, the Nativo mixture 75%WG (trifloxystrobin 25 % + tebuconazole 50 %) was the most effective fungicide mixture tested against the resistant isolates of *F.oxysporum* to difenoconazole.

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حساسية عزلات الفيوزاريوم اوكسسبوريوم التي تم تجميعها من جذور الفراولة لمبيدات من مجموعه الDMI (الدايفينوكونازول ، التيبوكونازول و البروكلوراز)

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الملخص

تعد مكافحة الفطريات التي تنتقل عن طريق التربة أمرًا صعبًا بشكل خاص لأنها تعيش في التربة وفي حطام النباتات المضيفة لفترة طويلة. إصابة نباتات الفراولة بشدة بفطر فيوزاريوم أوكسيسبوريوم مما أدى إلى الذبول الفيوزاريومي حيث أدى ذلك إلى إنخفاض خطير في إنتاج المحصول. إن إستخدام المبيدات الفطرية الوقائية على نطاق واسع هو الإستراتيجية الأساسية للسيطرة على المرض ومع ذلك فقد تم إكتشاف مجموعات مقاومة للمبيدات الفطرية الشائعة مؤخرًا على نطاق واسع. هدفت هذه الدراسة إلى الكشف عن مجموعات فطر فيوزاريوم أوكسيسبوريوم المقاومة للدايفينوكونازول والتيبوكونازول والبروكلوراز وإيجاد استراتيجية جيدة للسيطرة على هذا المرض. في الدراسة الحالية، تم جمع 115 عزلة من فطر فيوزاريوم أوكسيسبوريوم من أربع محافظات رئيسية منتجة للفراولة (البحيرة، الإسماعيلية، الدقهلية والقليوبية) في مصر وتم إستخدامها فى الدراسة . أظهرت النتائج أن 71 عزلة (61.73%) من بين العزلات المجمعة كانت مقاومة للدايفينوكونازول بينما كانت 85 عزلة (73.92%) مقاومة للتيبوكونازول وعزلتان (1.74%) مقاومة للبروكلوراز. تم قياس التركيز المثبط 50 % من نمو الميسليوم للديفينوكونازول والتيبوكونازول والبروكلوراز لمجموعة من العزلات الحساسة والمقاومة. وكانت متوسط قيمه EC_{50} للعزلات الحساسة للدايفينوكونازول 0.08 ميكروغرام/مل، بينما كانت متوسط قيمة EC_{50} للعزلات المقاومة للدايفينوكونازول 1.27 ميكروغرام/مل وكان متوسط قيمة EC_{50} للعزلات الحساسة للتبوكونازول 0.04 ميكروجرام/مل، في المقابل، كان متوسط قيمة EC_{50} للعزلات المقاومة 0.139 ميكروجرام/مل وبالمثل بالنسبة للعزلات الحساسة للبروكلوراز حيث كان متوسط قيمة EC_{50} هو 0.024 ميكروجرام/مل، بينما كان متوسط قيمة EC_{50} للعزلات المقاومة 6.97 ميكروجرام/مل. أظهر مخلوط ترايفلوكسيستروبين 25% وتيبوكونازول 50% الذي تم إختباره بتركيزين 10 و100 ميكروغرام/مل قدرة عالية في علاج العزلات المقاومة للدايفينوكونازول وأظهر تثبيط نمو الفطريات بنسبة 100%.