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Sensitivity of *Botrytis Cinerea* Isolates Collected from Strawberry to Carbendazim, Diethofencarb and Iprodione

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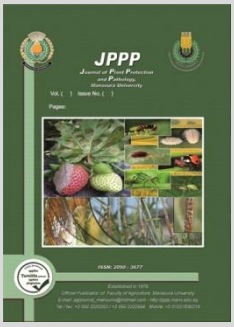

ABSTRACT

Currently strawberry is considered essential strategic crop in Egypt as it has great economic value. Grey mould disease, which caused by *Botrytis cinerea*, is a serious disease significantly reduces strawberry production globally. Protective fungicides considered essential tool in management strategies. Recently, fungicides ineffectiveness observed widely in many countries. Therefore, this investigation carried out to test the sensitivity of 311 isolates of *Botrytis cinerea* collected between 2019 and 2021 from strawberry open fields in Egypt's major strawberry-producing governorates (Beheira, Ismailia, Qalyubie, and Dakahlia) to diethofencarb, iprodione and carbendazim. The isolates were tested to distinguish resistant isolates and determine the EC_{50} values for sensitive and resistant isolates. The results showed that 7.4, 37.94 and 93.77% of the isolates found to be resistant to diethofencarb, iprodione and carbendazim, respectively. During the 2019 and 2021, resistance frequencies among *B. cinerea* isolates significantly increased, rising from 91.91% to 96% for carbendazim, and from 22.79% to 59% for iprodione while, resistance frequencies among *B. cinerea* isolates decreased gradually for diethofencarb from 10.29% to 4%. The mean EC_{50}values for the sensitive isolates were 0.021, 0.027 and 0.0548µg/ml, while the mean EC_{50}values for the resistant isolates were 141.06, 24.94 and 0.7161 µg/ml for carbendazim, diethofencarb and iprodione, respectively. There were little variations in osmotic sensitivity to NaCl between iprodione sensitive and resistant isolates. All carbendazim resistant isolates detected were sensitive to diethofencarb except three isolates showed dual resistance to diethofencarb and carbendazim.

***Keywords*:** Strawberry, Carbendazim, Diethofencarb, Iprodione, *Botrytis cinerea*

INTRODUCTION

In all temperate locations of the world, strawberry cultivation is widespread in both open fields and greenhouses. Strawberry plants infected with numbers of phytopathogenic fungi among them grey mold, caused by *Botrytis cinerea*, is considered one of the most severe diseases not just for strawberry but for many other crops. The disease leading to loses in crop production seriously as *B. cinerea* was discovered in the leftover plant components. High humidity promotes the growth of disease. The infection of strawberry flowers with *B. cinerea* lead to premature fruit drop and reducing crop production (Adnan *et al* 2019).

To stop the infection from developing, protective fungicides must be used widely in strawberry fields to control grey mold. Due to *B. cinerea* great genetic variability, polycyclic nature and abundant sporulation increased the number of fungicides sprays necessary for its effective control. The highly selective pressure resulted in rapid emergence of resistant populations leading to failure of various fungicides to control the fungus (Amiri *et al* 2013, Al-Zahraa *et al* 2022). *B. cinerea* is indicated as high-risk pathogen because of the rapid development of fungicide resistance (Brent *et al*. 1998; Leroux *et al*. 2002). Benzimidazoles (MBC), N-phenyl carbamates (NPC), and dicarboximides (DCs) used commonly for the control of grey mould. Following the introduction of the previous fungicides groups to prevent grey mould in recent decades, resistance to these fungicides has been reported in numerous countries (Baroffio *et al*. 2003; Bardas *et al*. 2010; Sun *et al*. 2010; Weber 2011). Carbendazim used in agriculture since1970 as MBC's first systemic and single-site fungicide (Walker *et al*. 2013). Ability to bind to -tubulin and stop microtubule assembly is the mechanism of this fungicides group to inhibit fungus in addition to its ability to inhibit mycelial growth and germ-tube elongation (Leroux *et al*. 1999). An additional antimicrotubule fungicide is the N-phenyl carbamates (NPC) fungicide diethofencarb, which usually exhibits negative cross-resistance with MBC fungicides it was typically used with carbendazim to control *B. cinerea* (Leroux *et al*. 1999; Zhang *et al*. 2009b). However, the intensive use of the mixture led to the emergence of new phenotype resistant to both carbendazim and diethofencarb in several countries (Elad *et al*. 1992; Sun *et al*. 2010).

Iprodione is a dicarboximide site-specific fungicide with a rather limited range of activity that only affects *Botrytis* species and the closely related genera *Monilinia* and *Sclerotinia* (Grabke *et al*., 2014). In filamentous fungi, the histidine kinase (HK), a key component in the two-component HK signal transduction pathway, found to be encoded by BcOS-1 gene. Cui *et al*., (2002) reported that DCs involves blocking the ability of HK signal transduction pathway to sense differences in osmolarity in *Botrytis spp*. The cross resistance between diethofencarb and

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carbendazim (Die) was also detected by Fan *et al* 2017. The present investigation was conducted to identify the frequency of carbendazim, diethofencarb, and iprodione resistance in *B. cinerea* isolates obtained from the main governorates in strawberry production in Egypt, monitoring the development of these fungicides resistance in *B.cinerea* over a period of three years from 2019 to 2021, to identify the effective concentrations EC_{50} of the resistant and sensitive isolates, and to detect cross resistance between diethofencarb and carbendazim

MATERIALS AND MSETHODS

Fungal isolates and culture conditions:-

From strawberry open fields 311 *B. cinerea* isolates collected to conduct this study between 2019 and 2021. Beheira, Ismailia, Qalyubie, and Dakahlia governorates were chosen for the isolates collection and four commercial yearly strawberry farms per governorate were selected. Strawberry fruit collected from commercial fields (one fruit showing typical grey mould symptoms taken from a different plant). *B. cinerea* isolates were grown on (PDA) medium at 20°C in the dark (200 ml of potato juice was made from 200 g of potatoes, 20 g of agar, 20 g of dextrose, and 1 L of distilled water). The single hyphal tip technique was used every year to isolate and purify the fungi strains. After isolation, the isolates were maintained in 15-mL plastic tubes filled PDA medium at 4°C.

Active ingredients: -

The following fungicides technical grades were used: carbendazim (98% a.i.; Shanghai Shennong Pesticide Co. Ltd., Shanghai, China), diethofencarb (95.4% a.i. jiansu Lanfeng Biochemical Co., Ltd.) and iprodione (96.5% a.i.; Heyi Agricultural Chemical Co. Ltd., Zhejiang, China).The fungicides dissolved in 100% acetone, while carbendazim dissolved in lactic acid to prepare a stock solution containing 100 mg/ml.

Determination of fungicide resistance in *B. cinerea*:-

Previous studies conducted by Lu *et al*. (2016), Yin *et al*. (2015), and Fan *et al*. (2016) used 10, 5, and 5 g/ml as discriminatory concentrations (a concentration that completely inhibits the sensitive isolates' mycelial growth) to distinguish sensitive isolates from resistant isolates for iprodione, carbendazim and diethofencarb, respectively. For each isolate, five 5 mm diameter mycelial plugs were separated from the 3-day-old colony margin and deposited upside-down onto the media that had already been treated with the fungicide. Colony diameter was measured after three days at 22°C. According to whether or not they can grow on media that has been treated with the fungicide, isolates are classified as resistant or sensitive for each fungicide.

Sensitivity of *B. cinerea* to carbendazim, diethofencarb and iprodione: -

11 carbendazim sensitive isolates (car^S) and 19 carbendazim resistant isolates (car^R) selected randomly to assess the effective concentration that inhibit 50% of mycelium growth (EC_{50}) values. Technical grade carbendazim (98% active ingredient [a.i.] was dissolved in100% lactic acid, adjusted to a concentration of 100 mg/ml, and added to PDA media to prepare final concentrations at (0, 0.005, 0.01, 0.05, 0.1 and 0.5 µg/ml) to test sensitive isolates and (0, 50, 100, 150, 200 and 250 µg/ml) to test resistant isolates. The sensitivity to diethofencarb was detected by evaluating (EC_{50}) values for 11 diethofencarb resistant (Die^R) and 17 diethofencarb sensitive (Die^S) isolates. Technical grade diethofencarb (95.4% active ingredient [a.i.] was dissolved in100% acetone, adjusted to a concentration of 100 mg/ml, and added to PDA to prepare final concentrations at (0, 0.005, 0.01, 0.05, 0.1 and 0.5 µg/ml) to test sensitive isolates (0, 5, 25, 50, 75 and 100 µg/ml) to test resistant isolates. The sensitivity to iprodione was determined by evaluating the (EC_{50}) values for 11 iprodione resistant (ipr^R) and 17 iprodione sensitive (ipr^S) isolates. Technical grade iprodione (96.5% active ingredient [a.i.] was dissolved in100% acetone, adjusted to a concentration of 100 mg/ml, and added to PDA to produce final concentrations at (0, 0.01, 0.05, 0.1, 0.25 and 0.5 µg/ml) to test sensitive isolates and (0, 0.1, 0.5, 1, 2.5, 5 and 7.5 µg/ml) to test resistant isolates. The experiment was repeated twice.

Data Processing System (DPS) program used to calculate the EC_{50} value for each isolate, (developed by Hangzhou Reifeng Information Technology Ltd., Hangzhou, China). For each isolate, the average of the EC_{50} values obtained from the two trials was used in the data analysis as there was no significant difference ($P>0.05$) between both experiments (Hamada *et al* 2011). RF (Resistance Factor) values for each fungicide assessed by dividing the EC_{50} value of each resistant isolate by the mean EC_{50} value of the sensitive isolates.

Cross resistance between carbendazim and diethofencarb

To figure out the resistance of *B. cinerea* isolates to diethofencarb and carbendazim, all carbendazim resistant isolates tested for sensitivity to diethofencarb. Discriminatory concentration used to detect diethofencarb resistant was 5µg/ml.

Determination of osmotic sensitivity to iprodione

To assess the osmotic sensitivity, 5-mm mycelia plug grown on 3-day-old PDA plates were removed from the edge of each isolate's actively growing colonies and put upside down at the center of PDA plates altered with 1, 2, 4 and 8% NaCl, or PDA plates as controls. The diameters of the colonies were measured in two perpendicular directions after 3 days of incubation at 22°C in the dark. A formula, (percent inhibition = (1-mean colony diameter on treated plates/mean colony diameter on non-treated plates) × 100%) was used to determine the percentage of mycelial growth inhibition. The experiment was repeated twice and four replicate plates were utilized for each isolate (Fan *et al* 2016 and Adnan *et al* 2019).

RESULTS AND DISCUSSION

Resistance in *B. cinerea* isolates to carbendazim

Among 311 isolates obtained, 291 (93.57%) were resistant to the carbendazim fungicide because of their ability to grow in the modified media adjusted with discriminatory concentration 10 µg/ml, whereas only 20 (6.43%) were sensitive which may be returned to significant selection pressure occurred. Additionally, the percentage of resistance to carbendazim increased gradually from 91.91% in 2019 to 93.33% in 2020 and again to 96% in 2021 (Table l).

In the same trend with our results, high carbendazim frequencies of resistance observed by numerous authors, such as Cai *et al* (2015), who reported that 84% of *B. cinerea* isolates were resistant to carbendazim. Also Rodrı´guez *et al* 2014 mentioned that 74.2 % from isolates of *B.cinerea* were resistant to carbendazim. Similarly, high carbendazim resistance frequency 81.6% also was noticed by Lu *et al* 2016 who studied

the sensitivity of *B. cinerea* isolates to carbendazim. While, moderate frequencies of resistance were reported by Leroch *et al* 2013who reported that the sensitivity of isolates of *B. cinerea* obtained from several locations in Germany (northern, central, and southern German) to carbendazim for four years (2008-2011) were48.0%, 43.3%, and44.7% in southern German, northern, and central, respectively.

Table 1. Resistance frequencies to carbendazim among Botrytis cinerea isolates collected during 3 years (2019-2021)

Location	Year of collection	No. of isolates	No. of		% of resistance
			Car^S	Car^R	
Beheira	2019	30	5	25	83.3
Ismailia	2019	44	4	40	90.9
Qalyubie	2019	33	1	32	96.9
Dakahlia	2019	29	1	28	96.6
Beheira	2020	26	Zero	26	100
Ismailia	2020	26	3	23	88.5
Qalyubie	2020	23	2	21	91.3
Beheira	2021	25	Zero	25	100
Ismailia	2021	25	Zero	25	100
Qalyubie	2021	25	1	24	96
Dakahlia	2021	25	3	22	88
Total	/	311	20	291	
Resistant percentage%			6.43	93.57	

Car^S carbendazim sensitive Car^R carbendazim resistant

Resistance of *Botrytis cinerea* isolates to diethofencarb

23 isolates (7.4%) were resistant to the fungicide diethofencarb they could grow on media that had been modified with discriminatory concentrations of 5 µg/ml, whereas 288 (92.6%) isolates were sensitive. Additionally, it was noticed that the percentage of diethofencarb resistance was 10.29% in 2019, which decreased to 6.67% in 2020 and decreased to 4% in 2021 (table 2).

Rodrı´guez *et al* 2014 stated that frequency of resistance to diethofencarb was 31.8% among *B. cinerea* strains tested during the sudy.

Table 2. Resistance frequencies to diethofencarb among Botrytis cinerea isolates collected during 3 years (2019-2021)

Location	Year of collection	No. of isolates	No. of		% of resistance
			Die^S	Die^R	
Beheira	2019	30	25	5	16.67
Ismailia	2019	44	38	6	13.64
Qalyubie	2019	33	32	1	3.03
Dakahlia	2019	29	27	2	6.89
Beheira	2020	26	26	Zero	Zero
Ismailia	2020	26	23	3	11.54
Qalyubie	2020	23	21	2	8.69
Beheira	2021	25	25	Zero	Zero
Ismailia	2021	25	25	Zero	Zero
Qalyubie	2021	25	24	1	4
Dakahlia	2021	25	22	3	12
Total	/	311	288	23	
Resistan percentage%			92.6	7.4	

Die^S diethofencarb sensitive Die^R diethofencarb resistant

Resistance in *Botrytis cinerea* strains to iprodione

In the same trend with our results, frequencies of resistance reported by Rodrı´guez *et al* 2014who mentioned that 31.8% of *B. cinerea* strains obtained were resistant to diethofencarb.

118 isolates (37.94%) found to be resistant to the dicarboximide fungicide iprodione since they could grow in media modified with discriminatory concentrations of 5 µg/ml, whereas 193 (62.06%) were sensitive. Additionally, it was reported that the iprodione resistance percentage increased from 22.79% in 2019 to 37.33% in 2020 and 59% in 2021(table 3).In the same trend with our results moderate frequencies of resistance detected by several authors such as Lopes *et al* 2017 who studied the sensitivity of *B. cinerea* to iprodione and observed that 25.7% from isolates were resistance to the fungicide. Also, Lu *et al* 2016 mentioned that 31.6% from *B. cinerea* strains were insensitive to iprodione. Monitoring the decreasing and increasing of iprodione resistance frequencies in *B. cinerea* populations were investigated by many authors such as Yin *et al* 2015 who found that the frequency of iprodione-resistant (IprR) isolates that collected from Zhejiang Province in China ranged from 46.0 to 94.2%. While, Pokorny *et al* 2016 reported that there were no *B. cinerea* isolates detected resistant to iprodione.

Table 3. Resistance frequencies to iprodione among Botrytis cinerea isolates collected during 3 years (2019-2021)

Location	Year of collection	No. of isolates	No. of		% of resistance
			Ipr^S	Ipr^R	
Beheira	2019	30	22	8	26.67
Ismailia	2019	44	36	8	18.18
Qalyubie	2019	33	25	8	24.24
Dakahlia	2019	29	22	7	24.14
Beheira	2020	26	16	10	38.46
Ismailia	2020	26	16	10	38.46
Qalyubie	2020	23	15	8	34.78
Beheira	2021	25	13	12	48
Ismailia	2021	25	7	18	72
Qalyubie	2021	25	8	17	68
Dakahlia	2021	25	13	12	48
Total	/	311	193	118	
Resistant percentage%			62.06	37.94	

Ipr^S iprodione sensitive Ipr^R iprodione resistant

Sensitivity of *Botrytis cinerea* strains to carbendazim

11 car^S and17 Car^R isolate chosen randomly and used to calculate the EC_{50} for carbendazim using mycelial growth inhibition technique. Carbendazim EC_{50} values ranged between 0.0059 to 0.0323 µg/ml, with a mean of 0.021 µg/ml and EC_{50}values for (54.54%) of the isolates exceeding 0.02 µg/ml. While the EC_{50} values for resistant isolates ranged between 12.98 to 265.05 µg/ml with a mean of 141.06 g/ml. the isolates with the highest EC_{50} values (63.1%) exceeded 100 µg/ml. Resistance Factor values ranged from648.79 to 13252.63fold (Fig.1and table 4).

In the same trend with our results, many authors such as Cai *et al* (2015) they tested *B.cinerea* sensitivity to carbendazim by evaluated EC_{50} values and the results illustrated that the value of EC_{50} ranged between 0.04 to 0.13 µg/ml for the sensitive isolates and >100µg/ml from resistant isolates. In addition, Sautua *et al* (2019) they tested *B. cinerea* sensitivity to carbendazim by evaluating the half-maximal effective concentration value and the results illustrated that the EC_{50} values for the sensitive isolates varied from 0.03 to 0.1 µg/ml. Also, Panebianco *et al* (2015) who assessed the (EC_{50}) half-maximal effective concentration (µg/mL) values of iprodione to *B. cinerea* and reported that 260 isolates (86.1%) were susceptible to carbendazim, and the values ofEC_{50} less than 1 µg/ml. while(13.9%)42 isolates considered resistant, EC_{50}were over than 100 µg/ml,

Table 4. EC50 values assessed for Carbendazim resistant and sensitive isolates

Carbendazim (EC_{50})				
Sensitive		Resistant		
Isolate	**EC_{50}**	**Isolate**	**EC_{50}**	**RF**
MB 9	0.0058 ± 0.0003	S 114	12.9718 ± 0.0178	648.79
MB 43	0.0146 ± 0.0002	MB 52	24.9314 ± 0.0260	1246.92
S 18	0.0158 ± 0.0002	S 37	43.0056 ± 0.0124	2150.015
S 29	0.0167 ± 0.0002	MB 154	43.2048 ± 0.0050	2160.145
S 230	0.0187 ± 0.0002	49	58.6832 ± 0.2215	2938.26
BN 310	0.0193 ± 0.0021	S 133	71.2942 ± 0.4871	3555.655
S 282	0.0207 ± 0.0003	BN 306	94.6126 ± 0.5539	4734.365
S 4	0.0214 ± 0.0002	33	124.3870 ± 1.2284	6241.945
BN 261	0.0296 ± 0.0003	BN 6	127.5974 ± 0.9502	6397.475
H4(34)	0.0327 ± 0.0021	41	156.1509 ± 1.3339	7821.625
MB 46	0.0324 ± 0.0002	137	159.6957 ± 1.4648	7996.79
		19 B	168.4307 ± 1.3561	8441.155
		S 23	174.1204 ± 1.0994	8714.56
		S 144	207.6985 ± 1.5624	10398.06
		S 216	217.2067 ± 0.9675	10874.785
		MB 172	236.8279 ± 0.7731	11844.62
		MB 117	241.0554 ± 0.7963	12053.55
		BN 317	250.1406 ± 1.5783	12535.045
		MB 136	265.0482 ± 0.9207	13252.63

RF Resistance Factor

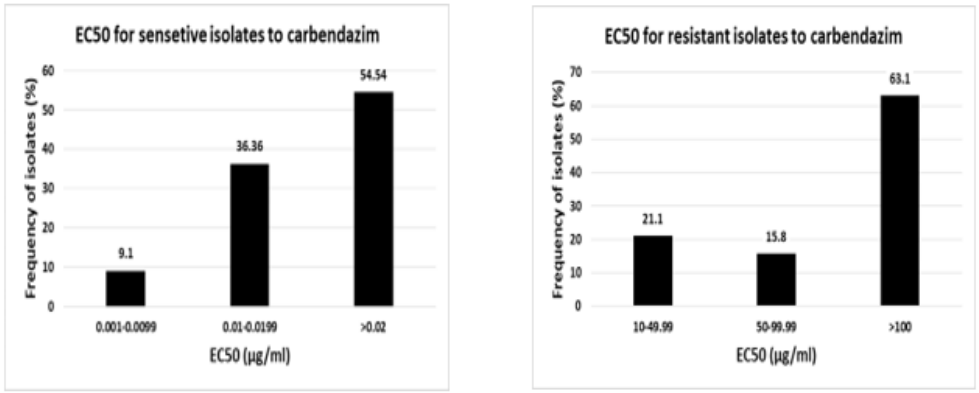

Fig. 1. determination of EC_{50} for carbendazim sensitive and resistant isolates.

Sensitivity of *Botrytis cinerea* isolates to diethofencarb: -

17 Die^S isolates and11 Die^R isolates selected randomly and used mycelial growth inhibition technique to determine the EC_{50} for diethofencarb. The values of EC_{50} ranged from 0.0084 to 0.052 µg/ml with a mean of 0.027 µg/ml and the majority of the isolates (64.71%) tested had EC_{50}values of isolates ranged between 0.01 to 0.0299 µg/ml. While, The EC_{50} values of resistant isolates ranged between 17.02 to 47.86 with a mean of 24.94 µg/ml. and the highest values EC50 of strains (45.46%) ranged between 20 to 29.99 µg/ml. Resistance Factor values ranged from 630.5 to 1772.6 fold. (Fig.2and table 5)

Table 5. EC50 values assessed for diethofencarb resistant and sensitive isolates

diethofencarb (EC_{50})				
Sensitive		Resistant		
Isolate	**EC_{50}**	**Isolate**	**EC_{50}**	**RF**
S 133	0.0084 ± 0.0002	S 4	17.1108 ± 0.1872	630.526
S 283	0.0160 ± 0.0002	MB 43	17.4734 ± 0.2806	643.996
136	0.0172 ± 0.0002	S 230	17.7345 ± 0.0742	657.56
S 286	0.0218 ± 0.0002	BN 261	18.6310 ± 0.3438	689.841
S 218	0.0222 ± 0.0002	S 206	20.3472 ± 0.3844	751.222
S 20	0.0232 ± 0.0002	H4(34)	22.6970 ± 0.2379	844.281
BN 296	0.0243 ± 0.0002	S 18	23.4168 ± 0.3667	869.359
153	0.0248 ± 0.0002	S 29	23.5270 ± 0.1167	870.63
BN 291	0.0263 ± 0.0003	BN 310	25.7316 ± 0.3468	959.063
BN 81	0.0273 ± 0.0002	S 282	39.6159 ± 0.1972	1469.937
BN 301	0.0275 ± 0.0003	MB 46	47.6132 ± 0.5741	1772.641
MB 112	0.0290 ± 0.0003			
BN 276	0.0321 ± 0.0003			
BN 55	0.0330 ± 0.0002			
H4(1)	0.0358 ± 0.0002			
BN 47	0.0368 ± 0.0002			
33	0.0523 ± 0.0015			

RF Resistance Factor

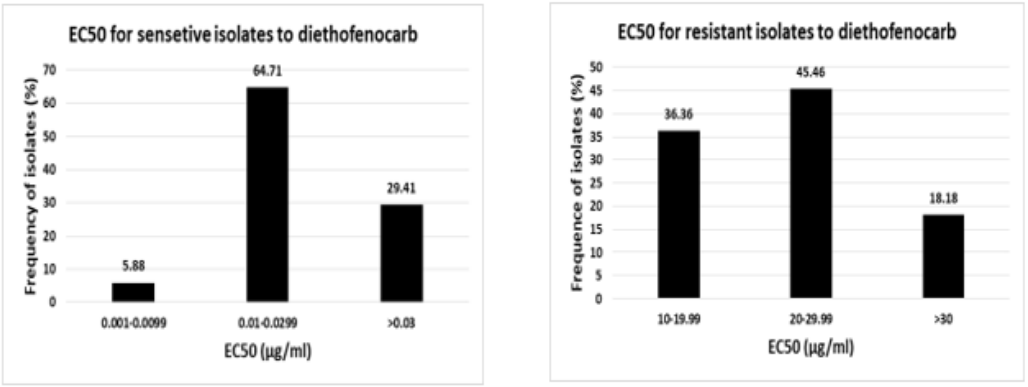

Fig 2.determination of EC_{50} for diethofencarb sensitive and resistant isolates.

Numerous authors studied *B.cinerea* sensitivity to diethofencarb. Our results were in harmony with Sun *et al* (2010) who determined (EC_{50}) the half-maximal effective concentration (µg/mL) values of diethofencarb to *B. cinerea* and the results showed that the values of EC_{50} were >50 µg/mL for diethofencarb resistant isolates. While, Zhang *et al* (2009) estimated the half-maximal effective concentration value for diethofencarb against *B.cinerea* and the results showed that the sensitivity of isolates to diethofencarb were two different levels sensitive (S) and resistant (R). Sensitive isolates were not able to grow on 25 µg/ml diethofencarb and the values of EC_{50} ranged between 0.01 to 16.5 µg/ml. R isolates were able to grow on 100 µg/ml diethofencarb.

Sensitivity of *Botrytis cinerea* strains to iprodione: -

18 Ipr^S isolates and14 Ipr^R isolates selected randomly and used mycelial growth inhibition technique to determine the EC_{50} for iprodione. The values of EC_{50} ranged between 0.0132 to 0.1338 µg/ml with a mean of 0.0548 µg/ml and (50 %) more than 0.05 µg/ml were the highest EC_{50}values. While, resistant isolates the values of EC_{50} ranged between 0.4517 to 1.0137 with a mean of 0.7161 µg/ml. and the highest EC_{50} values of isolates (78.57%) ranged between 0.5 to 0.999 µg/ml. Resistance Factor values ranged from8.21 to 18.43 fold. (Fig.3and table 6)

Table 6. EC50 values assessed for iprodione resistant and sensitive isolates

iprodione (EC_{50})				
Sensitive		Resistant		
Isolate	**EC_{50}**	**Isolate**	**EC_{50}**	**RF**
MB 46	0.0132 ± 0.0002	MB 48	0.4517 ± 0.0002	8.213
33	0.0156 ± 0.0002	S 283	0.4642 ± 0.0001	8.44
BN 310	0.0184 ± 0.0002	BN 312	0.5831 ± 0.0002	10.602
S 18	0.0260 ± 0.0002	S 218	0.5891 ± 0.0004	10.711
S 20	0.0345 ± 0.0003	MB 49	0.5930 ± 0.0002	10.782
S 4	0.0358 ± 0.0002	MB 133	0.6279 ± 0.0003	11.418
S 29	0.0373 ± 0.0002	MB 117	0.6919 ± 0.0002	12.58
S 282	0.0399 ± 0.0004	MB 118	0.7989 ± 0.0002	14.525
H4(34)	0.0405 ± 0.0002	S 258	0.8023 ± 0.0002	14.587
S 230	0.0509 ± 0.0003	BN 41	0.8073 ± 0.0003	14.676
S 133	0.0530 ± 0.0003	S 216	0.8083 ± 0.0002	14.696
BN 291	0.0649 ± 0.0002	BN 60	0.8740 ± 0.0003	15.891
MB 43	0.0781 ± 0.0002	S 167	0.9194 ± 0.0002	16.716
BN 55	0.0808 ± 0.0002	MB 172	1.0136 ± 0.0004	18.431
BN 276	0.0832 ± 0.0002			
BN 81	0.0857 ± 0.0002			
BN 301	0.0957 ± 0.0002			
136	0.1355 ± 0.0027			

RF Resistance Factor

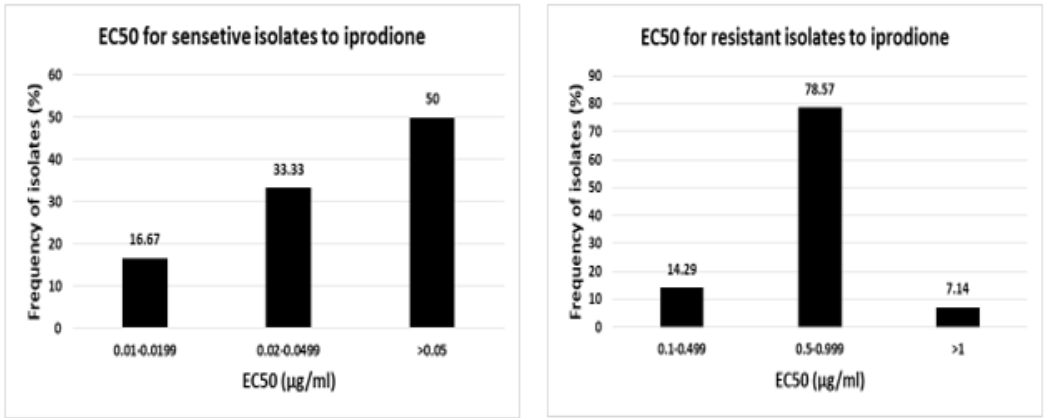

Fig 3. determination of EC_{50} for iprodione sensitive and resistant isolates

Pokorny *et al* (2016) determined (EC_{50}) the half-maximal effective concentration (µg/mL) values for iprodione using *B. Cinerea* isolates and the results showed that the values of EC_{50} ranged between 0.36 to 1.41 µg/ml for iprodione sensitive isolates. Also, Panebianco *et al* (2015) tested the sensitivity of *B. cinerea* to iprodione through determination of (EC_{50}) values and the results showed that most of *B. cinerea* strains tested (89.7%) were susceptible to iprodione. The values of EC_{50} ranged between 0.1 to 0.69 µg/ml and the most frequency of values ranged between 0.2–0.29 µg/ml. while, the percentages of resistance to iprodione were (10.3%) 31 isolates which could grow on modified media that amended with concentrations over than 1 µg/ml.

Resistance to carbendazim and diethofencarb

In the current study, the results illustrated that all carbendazim resistant isolates found to be sensitive to diethofencarb except three isolates showed dual resistance to diethofencarb and carbendazim. Based on the previous result usage of this mixture might led to success the control of *B. cinerea* populations. In the same trend with our results, Fan *et al* (2017) detected that dual resistance between diethofencarb and carbendazim.

Determination of osmotic sensitivity to iprodione

The osmotic sensitivity test showed that the radial mycelium growth of both Pro^R and Pro^S isolates reduced on Potato Dextrose Agar treated with 8% NaCl. According to the results there were no significant differences observed between sensitive and resistant isolates ($P > 0.05$). Similar to our results, Fan *et al* (2016) detected that no significant differences between sensitive and resistant isolates.

CONCLUSION

Due to the ability of *B. cinerea* to development resistant populations to many fungicides, alternation of a group of fungicides such as carbendazim, diethofencarb and iprodione could be successful strategy in the control process According to the results obtained in current study, resistant to carbendazim detected with very high frequencies in all the governorates tested, reducing the number of applications and alternate it with other fungicides such as diethofencarb in order to control this disease effectively.

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حساسية عزلات البوترايتس سناريا التى تم تجميعها من الفراولة لمبيد الكاربندازيم والديثوفينكارب والايبروديون.

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الملخص

تعتبر مصر واحدة من الدول الرئيسية المنتجة للفراولة. خلال هذه الدراسة تم تجميع 311 عزلة من فطر *Botrytis cinerea* المسبب لمرض العفن الرمادي الذى يؤدي إلى خسائر كبيرة في إنتاج الفراولة في جميع أنحاء العالم وتعتمد عملية مكافحته بشكل أساسي على إستخدام المبيدات الفطرية الوقائية. في الآونة الأخيرة فقدت العديد من هذه المبيدات كفائتها بسبب ظهور السلالات المقاومة ، تهدف الدراسة الحالية إلى الكشف عن العزلات المقاومة لمبيدات الكاربيندازيم ، والداي إيثوفينوكارب ، والأيبروديون في المحافظات الرئيسية فى إنتاج الفراولة في مصر (البحيرة ، الإسماعيلية القليوبية ، الدقهلية) بين 2019 و 2021 وتتبع تغيرها خلال سنوات الدراسة. أظهرت النتائج أن 93.77 و 7.4 و 37.94٪ من العزلات كانت مقاومة لمبيدات الكاربيندازيم ، الداي إيثوفينوكارب والأيبروديون على التوالي. بتتبع نسب المقاومة بين الأعوام المختلفة للدراسة وجد أن هناك زيادة ملحوظة في نسب المقاومة للكاربيندازيم ، والأيبروديون بين عزلات *B. cinerea* خلال موسمي 2019 و 2021 حيث كانت 91.91٪ في 2019 إرتفعت إلى 96٪ في 2021 للكاربندازيم وأيضا لمبيد الأيبروديون حيث كانت 22.79٪ في 2019 وإرتفعت إلى 59٪ في 2021 بينما إنخفضت تدريجيا لمبيد الداي إيثوفينوكارب حيث كانت 10.29٪ في 2019 و إنخفضت إلى 4 ٪ في 2021. كما وجد أن متوسط التركيز المثبط ل50% من نمو الميسيليوم للعزلات 0.021 ، 0.027 و 0.0548 ميكروجرام / مل بالنسبة للعزلات الحساسة بينما كانت 141.06 ، 24.94 و 0.7161 ميكروجرام / مل للعزلات المقاومة للكاربيندازيم ،الداي إيثوفينوكارب و الأيبروديون ، على التوالي. أظهرت جميع العزلات المقاومة للكاربيندازيم التي تم اكتشافها حساسيتها لمبيد الداي إيثوفينوكارب فيما عدا ثلاث عزلات أظهرت مقاومة مزدوجة لكلا المبيدين ، مما يشير إلى أن إستخدام خليط من كلاهما قد يؤدي إلى نجاح السيطرة على السلالات المقاومة لمبيد الكاربيندازيم.