J. Agric. Sci. Mansoura Univ., 29(7): 4203 - 4221, 2004

PHYSIOLOGICAL PROPERTIES AFFECTING THE RESISTANCE OF *BOTRYTIS CINEREA* ISOLATES TO FUNGICIDES

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ABSTRACT

Forty isolates of *Botrytis cinerea* were isolated from pepper (10 isolates), strawberry (15 isolates), and grape (15 isolates) collected from different governorates in Egypt. All tested isolates proved to be pathogenic to the hosts from which these isolates were isolated. The current acquired fungicidal resistance level in the natural population of *Botrytis cinerea* isolated from pepper, strawberry, and grape to the fungicides Sumisclex and Tecto was estimated. Resistance factors to the tested fungicides obviously differed from one isolate to the other. The fungus proved to be unspecific to a certain host which means all fungus isolates belong to only one population. The recalculated resistance factors ranged from 1.0 to 30.7 for Sumisclex but do not change for Tecto. Fungal acquired resistance to Sumisclex was negatively correlated with the catalase enzyme, while it was positively correlated with polyphenol oxidase, peroxidase and catalase enzymes for Tecto. The content of flavonoids (antioxidant substances) was higher in isolates resistant to Sumisclex than in sensitive ones. No clear correlation was observed between the sterol content of resistant isolates and the resistance to Sumisclex or Tecto.

INTRODUCTION

The genus *Botrytis* is widely known as a group of fungi causing destructive and economically important plant diseases. This is particularly true of those forms grouped together as the species *Botrytis cinerea*. The fungus *B. cinerea* infects a wide range of host plants either in greenhouse or in the field world wide, whereas other species are much more restricted in this respect (Smith *et al.* 1980).

In Egypt, *B. cinerea* attacks many different crops causing tremendous pre- and post-harvest losses and has been isolated from several crops (Hussein and Ali 1985, Hussein *et al.* 1985 and Abbas, 1995). In practice till now, fungicides are considered the most effective method to control *Botrytis* diseases. Many specific fungicides were developed especially to control *Botrytis* diseases and called Botrycides (Nakazawa and Yamada 1997). In Egypt, Sumisclex and Tecto are widely used to control these diseases (Anonymous 2001).

Recently, chemical control by using pesticides has faced criticism due to chemical residues and acquired pesticide resistance. *Botrytis cinerea*, which proved to be the causal organism of gray mold of strawberry, developed resistance to Benlate fungicide (Hussein and Ali 1985), and also to Sumisclex (Mansoor 1996). The frequency of fungicide resistance reports has accelerated since the mid-1960s, paralleling the introduction of new

compounds that attack specific biochemical targets in the pathogen. Many of these are systemic fungicides, but the systemic property is not a requirement for resistance development, since several protective fungicides such as Dodine, Fentin, and Ipodine have shared in the problem. Dekker (1983) stated that problems due to acquired resistance to fungicides significantly increased after the introduction of systemic fungicides in practice. This raises the question whether the phenomenon of acquired resistance is related in some way or another to systemic action. It seems, therefore, that the chance of development of resistance to systemic fungicides is greater than for conventional ones. Although, the resistance to conventional fungicides may arise by the change in the permeability of cell membrane or by increasing the ability to detoxify the fungicide. However, it doesn't imply that resistance will develop to all new systemic fungicides in the future. This will depend not only on the potential of fungi to mutate to resist a certain fungicide, but also on the probability that a resistant pathogen population will readily build up in the field.

The present investigation was carried out to spot light on the relationship between the fungicidal acquired resistance and the changes in some fungal physiological characters.

MATERIALS AND METHODS

1- Isolation and identification of the causal organism:

Infected samples of strawberries, grapes and pepper pods were collected from different fields and retail markets located in different governorates of Egypt as follows:

- Strawberry collected from Giza, Kalubeia, and Ismaelia Governorates and retail markets in Cairo.
- Grape collected from Giza, Gharbeia, and Monofeia Governorates and retail markets in Cairo.
- Pepper collected from Giza, Monofeia and Kalubeia Governorates and retail markets in Cairo.

The samples were cut into small pieces, sterilized using a 0.1% solution of mercuric chloride ($HgCl_2$) for one minute then washed in sterilized water many times and dried between sterilized filter papers.

The sterilized pieces were directly transferred onto PDA medium and incubated at 20°C.

Purification of the isolated fungus was carried out using the single spore technique; and pure isolates were preliminarily identified according to Menzinger (1966), Subromanian (1971) and Smith *et al.* (1980). The identification was confirmed by The Department of Survey and Identification of Fungi Researches, Plant Pathology Research Institute, ARC.

Table 1: The different isolates and the crops isolated from and locations.

Crop	Locations	Number of isolates
Pepper	Giza, Behera, Kalubeia and retail markets	10
Strawberry	Giza, Kalubeia, Ismaelia, and retail markets	15
Grape	Giza, Gharbeia, Behera and retail markets	15

2- Pathogenicity test:

In order to insure the pathogenicity of the different isolates of the fungus, a pathogenicity test was conducted under laboratory conditions. Spore suspensions of the different isolates (1×10^5 / ml) were prepared from two weeks old cultures grown on PDA medium.

Strawberries (Douglas var.), grapes bunches (Thompson seedless var.) and pepper pods (California wander var.) were surface sterilized using Ethyl alcohol, washed thoroughly with sterilized distilled water and dried in the laminar flow hood. The fruits were artificially inoculated by spraying them with the different spore suspensions using a manual atomizer (each isolate was tested on the crop from which it was isolated). The barriers and pods were kept in sterilized humidity chambers (covered plastic jars) for ten days at 20°C±3. The isolates which established and produced any colony on the fruits were considered pathogenic isolates regardless of the size of infection zones.

3- Estimation of the recent acquired fungicidal resistance level in natural population of *Botrytis cinerea*.

To estimate the resistance level for each isolate of *Botrytis cinerea*, the percentage of spore germination in different concentrations of fungicides suspensions was determined using the slide-germination fungicidal bioassay technique (Sharvelle, 1979) and adapted by Aschmawy (1997). Then the inhibition index, EC_{50}, and resistance factor were calculated as follows:

The inhibition index was calculated according to the Abbott formula (Fröhlich, 1979):

$$\text{Inhibition index (II)} = \frac{A - B}{A} \times 100$$

Where, A = percentage of germinated spores in control (sterilized distilled water).

B = percentage of germinated spores in treatment (fungicide suspension).

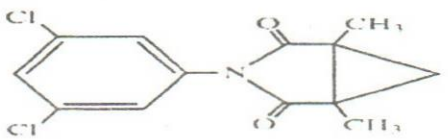
EC_{50} values were calculated using the Main trend sub-program of Excel computer program Microsoft office.

The resistance index was calculated according to Fröhlich (1979) as follows:

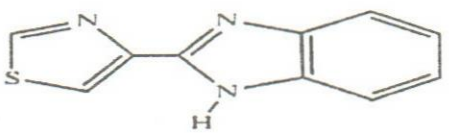
$$\text{Resistance factor (R F)} = \frac{EC_{50} \text{ for resistant isolate}}{EC_{50} \text{ for the most sensitive isolate}}$$

4. Tested fungicides:

Commercial name — Sumisclex 50% wp.
Common name — Procymidone
Chemical name — N-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane -1,2-dicaboximide.



Commercial name : Tecto 45% fl.
Common name : Thiabendazole
Chemical name : 2-(4-thiazolyl)-benzimidazole



Fungicide suspensions containing different concentrations (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 20.0 ppm active ingredient) of the aforementioned fungicides were used .The fungicide suspension (0.2 ml) was placed on a slide using a micro pipette and allowed to dry. A droplet (0.2 ml) of spore suspension (1 x 10^5 spore /ml.) was added on the residue of the fungicide exactly and the slides were mounted on two glass rods in a Petri-dish containing sterilized water and covered. The Petri-dishes were incubated at 20° C for 24 h. and germinated spores were microscopically counted. The inhibition index for each concentration of each fungicide, EC_{50} and resistance factor were calculated as mentioned before.

5. Relationship between acquired fungicidal resistance and the changes in some physiological fungal characters:

The effects of acquiring fungicidal resistance in different isolates of *B.cinerea* on the activity of some oxidative enzymes as well as some biochemical substances were studied. Some isolates with different resistance factors were selected to be involved in these studies. Correlation coefficients among the enzymatic activity and resistance factors of the different isolates were calculated.

5.1. Effect on activity of the oxidative enzymes:

The crude enzymes were prepared and the activities of polyphenoloxidase, catalase, peroxidase and ascorbic acid oxidase were determined as described by (Maxwell and Bateman, 1967). The isolates were grown separately on Czapek's liquid medium at 20°C for 21 days. After

incubation the cultures were filtèred. The filtrates were centrifuged at 3000 rpm for 20 minutes and the clear supernatants were used as the crude enzymes.

A portion of supernatants from culture filtrates and mycelium were boiled to inhibit the enzyme activity to serve as a control, then the following procedures were conducted to estimate the activity of each enzyme.

5.1.1 Polyphenoloxidase :

Reaction mixtures contained 0.5 ml enzyme extract, 0.5 ml (0.2 N) sodium phosphate buffer at pH 7.0 and 0.5 ml (10^{-3}N) catechol brought to a final volume of 3.0 ml with distilled water.

The activity of phenol oxidase was expressed as the change in absorbency of 1.0 ml of extract per min. at 495 nm, using a UV spectrophotometer.

5.1.2 Peroxidase :

Peroxidase activity was determined according to Allam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm. The sample cuvette contained 0.5 ml (0.1 *N*) sodium phosphate buffer pH 7.0, 0.3 ml enzyme extract, 0.3 ml (0.05*N*) pyrogallol, 0.1 ml (1.0%) H_2O_2, and distilled water to bring cuvette contents to 3.0 ml.

5.1.3 Catalase:

Catalase activity was assessed by spectrophotometeric methods (Maxwell and Bateman, 1967). The sample cuvette contained 0.5ml. 0.2 N. sodium phosphate puffer at pH 7.6, 0.3 ml 0.5 % H2O2, and 0.4 ml tissue extract, brought to a final volume of 3.0 ml. with distilled water. The data were expressed as the changes in absorbance by 0.1 ml. of extract per min at 240 nm.

5.1.4 Ascorbic acid oxidase:

Ascorbic acid oxidase activity was measured based on the disappearance of ascorbate at 265 nm. The sample cuvette containing 1.0 ml 0.2 *N* sodium phosphate buffer (pH 6.2), 0.2 ml 10^{-3} N ascorbic acid and 0.2 ml enzyme extract was brought to a final volume of 3.0 ml with distilled water. The results were expressed as the changes in UV absorbency for the first 2 min of the reaction per 0.1 ml extract.

5.2. Effect on some biochemical substances:

The effect of acquired fungicidal resistance on some biochemical substances in the mycelium mat of the *B. cinerea* with different resistance factors was carried out by assessment of the relative contents of flavonoids and the sterols.

5.2.1. Flavonoids:

Ten gr. of dry mycelium were macerated in 50 ml. 1% hydrochloric acid overnight, filtrated and filtrate was subjected to the following tests: 10 ml. of each filtrate were rendered alkaline with sodium hydroxide (15%). Appearance of a yellow color indicated the presence of flavonoid (Geissmann, 1962).

5.2.2 Sterols and Triterpenes:

1 gr. dry mycelium was extracted in 10 ml petroleum ether, then filtrated. The filtrate was evaporated to dryness. The residue was dissolved in 5ml. anhydrous chloroform, and filtrated. After that, 0.3ml of acetic anhydride were added to the filtrate, then a few drops of sulphuric acid down the side of

the tube; the formation of a reddish violet ring at the junction of the two layers indicated to the presence of unsaturated sterols and triterpenes. (Hanoson, 1972). Since the color density is proportional to the concentration of the substance, four degrees were visually distinguished, (-) = no color, (+) = light color, (++) = medium, (+++) = strong.

RESULTS AND DISCUSSION

1. Isolation and identification of the causal organism

In order to estimate the present situation of acquired fungicidal resistance in *B. cinerea,* samples of different crops showing gray mold were collected from different governorates. After isolation and purification 40 isolates were identified according to their cultural morphological and microscopic properties described by Menzinger (1966), Subermanian (1971) and Smith *et al.* (1980).

The different isolates and their origins are shown in table (1).

2- Pathogenicity

All the isolates were subjected to a preliminary pathogenicity test to determine which isolates were able to cause infection. The data obtained are tabulated below.

Table (2): Pathogenicity test of different *B. cinerea* isolates from different crops.

Tested crop					
pepper		strawberry		grape	
Isolate	Infection	Isolate	Infection	Isolate	Infection
P1	+	S1	+	G1	+
p2	+	S2	+	G2	+
p3	+	S3	+	G3	+
p4	+	S4	+	G4	+
p5	+	S5	+	G5	+
p6	+	S6	+	G6	+
p7	+	S7	+	G7	+
p8	+	S8	+	G8	+
p9	+	S9	+	G9	+
p10	+	S10	+	G10	+
		S11	+	G11	+
		S12	+	G12	+
		S13	+	G13	+
		S14	+	G14	+
		S15	+	G15	+

(+) means that the isolate could cause infection and gray mold, regardless of the disease severity.

The data indicate that regardless of the disease severity, all the isolates could infect the fruits of the crop from which each isolate was isolated and cause gray mold.

3- Estimation of the recent acquired fungicidal resistance level in the natural population of *Botrytis cinerea* .

To estimate the resistance level for each isolate of *Botrytis cinerea*, the percentage of spore germination in different concentrations of fungicides suspensions was determined using the slide-germination fungicidal bioassay technique and fungicide efficacy of different fungicide concentrations. EC_{50} and resistance factors (RF) were calculated. The data are set out in tables 3-8.

3.1. Estimation of the recent acquired fungicidal resistance level in the natural population of *Botrytis cinerea* on pepper.

Regarding the resistance level of the different isolates to Sumisclex fungicide (procymidone), the data obtained indicated a notable variation in the sensitivity of the different isolates to this fungicide (Table 3). However, this variation is divided into three groups; the first group contains 7 very sensitive isolates with EC_{50} ranging from 0.4 to 0.9 ppm and resistance factors (RF) ranging from 1.0 (P4 and P10) to 2.5 (P9). The second group contains two very convergent isolates P5 with EC_{50} of 6.4 ppm and resistance factor (RF) of 16.0 and P6 with an EC_{50} of 6.0 ppm and a RF value of 15. The third group contains only one isolate (P3) with EC_{50} of 12.3 ppm and an RF of 30.7. This isolate is very resistant.

Table 3: Fungicide efficacy, EC_{50} and resistance factors (RF) against the different *B. cinerea* isolates isolated from pepper grown on media amended with different Sumisclex (procymidone) concentrations.

Isolate No.	Fungicide concentrations in ppm.							EC_{50}	RF
	1	2	4	6	8	10	20		
P1	59.7	63.5	71.3	78.9	86.6	94.3	100	0.5	1.2
P2	72.0	74.9	80.8	86.6	92.5	98.4	100	0.5	1.2
P3	9.8	13.3	20.4	27.5	34.6	41.7	77.2	12.3	30.7
P4	53.4	59.3	71.1	82.8	94.6	100	100	0.4	1.0
P5	0.0	0.0	0.0	30.9	100	100	100	6.4	16.0
P6	9.2	17.3	33.7	50.1	66.5	82.9	100	6.0	15.0
P7	70.7	73.1	77.9	82.6	87.4	92.1	100	0.7	1.7
P8	69.0	72.3	79.0	85.7	92.3	99.0	100	0.7	1.7
P9	52.8	55.7	61.6	67.6	73.5	79.4	100	0.9	2.5
P10	51.5	54.3	59.8	56.2	70.7	76.2	100	0.4	1.0

On the other hand, no obvious variation was noted in the resistance level to Tecto fungicide (thiabendazole) of the different isolates of *B. cinerea* isolated from pepper. The EC_{50} ranged from 5.6 and 10.6 ppm with RFs of 1 to 1.8. No fungicidal resistance risk to this fungicide is expected (Table 4).

Table 4: Fungicide efficacy, EC_{50} and resistance factors (RF) against the different *B. cinerea* isolates isolated from pepper grown on media amended with different Tecto (thiabendazole) concentrations.

Isolate No.	Fungicide concentrations in ppm.							EC_{50}	RF
	1	2	4	6	8	10	20		
P1	12.6	18.2	29.6	40.9	52.2	63.5	100	7.6	1.3
P2	0.0	0.0	0.0	10.5	59.7	100	100	7.6	1.3
P3	0.0	0.0	0.0	0.0	6.4	40.3	100	10.6	1.8
P4	23.9	29.5	40.9	52.2	63.5	74.8	100	5.6	1.0
P5	0.0	0.0	11.8	25.7	39.6	53.4	100	9.5	1.6
P6	0.0	0.0	11.2	18.8	46.5	64.1	100	8.4	1.5
P7	0.0	0.0	1.8	18.0	34.3	50.6	100	9.92	1.7
P8	0.0	0.0	18.0	36.6	55.3	74.0	100	7.9	1.4
P9	13.0	17.8	27.8	37.8	47.5	57.3	100	8.5	1.5
P10	0.0	0.0	39.6	83.7	100	100	100	5.7	1.0

3.2- Estimation of the recent acquired fungicidal resistance level in natural population of *Botrytis cinerea* on strawberry.

The acquired fungicidal resistance level in the natural population of *Botrytis cinerea* on strawberry was estimated. The different isolates showed different reactions to Sumisclex as shown in table 5. It was observed that the isolate S3 was the most sensitive one where only 0.5 ppm were sufficient to cause 50% inhibition, compared with the isolate S10 which is considered the most resistant one (9.2 ppm). Other isolates such as S2, S6, and S8 required 4.1, 5.4 and 2.3 ppm to achieve the same effect, respectively. Therefore, three resistance categories could be distinguished. S3 was sensitive isolate; while S2, S4, S5, S8, S12, S13, S14 and S15 represented moderately resistant isolates. The resistant category includes S1, S6, S7, S9, S10 and S11 (Table 5).

Table 5: Fungicide efficacy, EC_{50} and resistance factors (RF) against the different *B. cinerea* isolates isolated from strawberry grown on media amended with different Sumisclex (procymidone) concentrations.

Isolate No.	Fungicide concentrations in ppm.							EC_{50}	RF
	1	2	4	6	8	10	20		
S1	0.0	0.0	0.0	11.3	45.5	79.6	100	8.2	16.4
S2	0.0	0.0	10.0	100	100	100	100	4.1	8.2
S3	72.8	76.6	84.2	91.7	99.3	100	100	0.5	1.0
S4	19.3	29.2	48.9	68.7	88.5	100	100	4.1	8.2
S5	30.9	37.4	50.4	63.4	76.5	89.5	100	3.9	7.8
S6	26.9	31.8	41.8	51.7	61.7	71.6	100	5.4	10.8
S7	0.0	0.0	0.0	17.5	42.8	68.0	100	8.5	17.0
S8	42.4	48.2	59.8	71.3	82.9	94.5	100	2.3	4.6
S9	0.0	0.0	0.0	28.3	58.1	87.8	100	7.4	14.8
S10	0.0	0.0	0.0	13.8	36.2	58.6	100	9.2	18.4
S11	0.0	0.0	0.0	0.0	81.7	100	100	7.3	14.6
S12	44.0	80.9	100	100	100	100	100	1.3	2.9
S13	48.7	51.9	58.3	64.8	71.2	77.7	100	1.3	2.6
S14	26.9	33.8	47.6	61.4	75.2	88.9	100	4.3	8.6
S15	0.0	0.0	10.0	100	100	100	100	4.1	8.2

Regarding the fungicide Tecto, EC_{50} and RF values ranged from 3.1 to 10.2 and from 1.0 to 4.8 without any obvious groping. Values of EC_{50} ranged from 1 ppm in case of S3 to 10.2 ppm in case of S7. This increase in EC_{50} was reflected in the resistant factors (RF) of the different isolates to this fungicide. A gradual increase in RF values was obviously obtained. The isolates can be arranged in ascending order according to their RF as follows: S3, S1, S13, S12, S8, S5, S2, S4, S14, S15, S6, S11, S9, S10, and S7; showing resistance factors of 1, 4, 5, 5.3, 5.7, 6.5, 6.6, 6.8, 6.9, 7.0, 7.6, 7.8, 8.0, 9.3 and 10.2, respectively (Table 6).

3.3. Estimation of the recent acquired fungicidal resistance level in the natural population of *Botrytis cinerea* on grape

Concerning Sumisclex, G13 was the only sensitive isolate. This isolate showed an EC_{50} value of 0.9 ppm. All other isolates showed higher EC_{50} values. These isolates showed two categories of resistance; the first one containing 11 isolates (G2, G4, G5, G6, G7, G8, G9, G11, G12, G14, and G15) and showing moderate resistance factors of 6.6, 9.5, 7.4, 7.6, 7.1, 4.7, 5.3, 5.7, 9.3, 7.8, and 6.8, respectively. The second category containing three highly resistant isolates G1, G3 and G 10 and showing resistance factors of 11.4, 14.1 and 11.7, respectively (Table 7).

Data also showed that there was a tight variation in the reaction of the different isolates to Tecto, with EC_{50} values ranging from 3.9 (G13), which is considered the most sensitive isolate, to 15.0 of the most resistant isolate G10 (Table 8). According to the resistance factor, the isolates can be divided into two groups. The first group contains sensitive isolates, G2 (RF = 1.8), G7 (RF = 1.9), G8 (RF = 1.2), G9 (RF = 1.5), G11 (RF = 1.5), G13 (RF =1) and G15 (RF = 1.8). The second group contains isolates that demonstrate a moderate resistance, G1 (RF = 2.9), G3 (RF = 3.8), G4 (RF = 2.5), G5 (RF = 2.0), G6 (RF = 2.0), G10 (RF = 3.8), G12 (RF = 2.3) and G14 (RF =2.3).

Table 6: Fungicide efficacy, EC_{50} and resistance factors (RF) against the different *B. cinerea* isolates isolated from strawberry grown on media amended with different Tecto (thiabendazole) concentrations.

Isolate No.	Fungicide concentrations in ppm.							EC_{50}	RF
	1	2	4	6	8	10	20		
S1	32.3	38.1	49.8	61.5	73.2	89.9	100	4.0	4.0
S2	0.0	0.0	0.0	0.0	100	100	100	6.6	6.6
S3	50.2	51.3	55.7	64.1	72.4	80.8	100	1.0	1.0
S4	0.0	4.1	23.2	42.2	61.3	80.3	100	6.8	6.8
S5	16.1	22.2	34.3	46.4	58.5	70.7	100	6.5	6.5
S6	0.0	0.0	0.0	0.0	78.4	100	100	7.6	7.6
S7	0.0	0.0	12.8	25.0	37.1	49.3	100	10.2	10.2
S8	8.2	17.1	34.9	52.7	70.6	88.4	100	5.7	5.7
S9	19.5	23.8	32.5	41.2	49.8	58.5	100	8.0	8.0
S10	0.0	0.0	0.0	28.5	32.0	59.3	100	9.3	9.3
S11	13.2	18.7	29.8	40.9	52.0	63.1	100	7.6	7.8
S12	0.0	0.03	21.6	67.8	100	100	100	5.3	5.3
S13	0.0	0.0	0.0	100	100	100	100	5.0	5.0
S14	0.0	0.0	8.1	36.4	64.7	93.0	100	6.9	6.9
S15	0.0	0.0	0.0	9.9	89.2	100	100	7.0	7.0

Table 7: Fungicide efficacy, EC_{50} and resistance factors (RF) against the different *B. cinerea* isolated from grape grown on media amended with different Sumisclex (procymidone) concentrations.

Isolate No.	Fungicide concentrations in ppm							EC_{50}	RF
	1	2	4	6	8	10	20		
G1	0.0	0.0	0.0	0.0	8.6	43.7	100	10.3	11.4
G2	0.0	0.0	23.7	49.3	74.8	100	100	6.0	6.6
G3	0.0	0.0	0.0	0.0	0.0	0.0	100	12.7	14.1
G4	0.0	0.0	0.0	0.0	35.8	80.2	100	8.6	9.5
G5	0.0	0.0	18.0	46.3	64.5	82.8	100	6.7	7.4
G6	0.0	0.0	13.7	38.8	64.0	89.1	100	6.9	7.6
G7	0.0	0.0	15.5	44.0	72.6	100	100	6.4	7.1
G8	26.2	33.3	47.4	61.4	75.5	89.6	100	4.3	4.7
G9	20.6	28.2	43.5	58.8	79.0	89.3	100	4.8	5.3
G10	0.0	0.0	0.0	0.0	4.7	39.1	100	10.6	11.7
G11	17.9	25.5	40.7	55.9	71.1	86.3	100	5.2	5.7
G12	0.0	0.0	0.0	0.0	0.0	100	100	8.4	9.3
G13	52.1	60.7	67.8	75.0	82.1	89.3	100	0.9	1.0
G14	0.0	0.0	0.0	0.0	87.5	100	100	7.1	7.8
G15	0.0	0.0	17.5	47.4	77.3	100	100	6.2	6.8

Table 8: Fungicide efficacy, EC_{50} and resistance factors (RF) against the different *B. cinerea* isolates isolated from grape and grown on media amended with different Tecto (thiabendazole) concentrations.

Isolate No.	Fungicide concentrations in ppm.							EC_{50}	RF
	1	2	4	6	8	10	20		
G1	19.0	21.9	27.6	33.4	39.2	44.9	73.7	11.6	2.9
G2	0.0	0.0	18.0	36.0	54.8	73.0	100	7.4	1.8
G3	0.0	0.0	0.0	0.0	0.0	0.0	100	15.0	3.8
G4	18.7	21.8	29.0	36.3	43.5	50.7	86.9	9.8	2.5
G5	17.0	22.1	32.4	42.6	52.9	63.2	100	7.9	2.0
G6	13.7	19.0	29.6	40.3	50.9	61.5	100	7.8	2.0
G7	0.0	0.0	0.0	0.0	10.0	100	100	7.5	1.9
G8	0.0	5.4	23.2	40.6	58.0	75.0	81.0	4.9	1.2
G9	12.9	20.2	34.8	49.3	63.9	78.4	100	6.1	1.5
G10	0.0	0.0	0.0	0.0	0.0	0.0	100	15.0	3.8
G11	0.0	16.7	33.8	51.0	68.1	85.2	93.8	5.9	1.5
G12	1.1	7.0	18.6	30.3	42.0	53.6	100	9.3	2.3
G13	30.9	37.4	50.2	63.1	76.0	88.8	100	3.9	1.0
G14	0.0	0.0	8.7	24.5	40.4	56.3	100	9.2	2.3
G15	0.0	0.0	0.0	0.0	100	100	100	7.4	1.8

Acquired resistance phenomenon is a world wide problem which faces all specialists working on the field of medicine and plant protection. *Botrytis cinerea* is one of the fungi that have been recorded as acquiring resistance to fungicides all over the world (Moustafa, 1980, Dieter, 1983; Beever *et al.* 1989; and Fabreges and Birchmore, 1998). This kind of resistance may be due to the wide host range of this fungus. In addition, *B. cinerea* is a heterokariotic fungus showing a great variability, which provides

a very good chance for the emergence of resistant types (Menzinger, 1966). These resistant types may become dominate under the selection processes by the intensive use of fungicides. Generally in Egypt, the problem of acquired resistance in *B. cinerea* has been studied earlier.

The data obtained in the present work indicate a noteworthy reduction of the resistance level in *B. cinerea* to the benzimidazole fungicide group since the lowest EC_{50} obtained was 0.1 ppm and the highest EC_{50} was 10 ppm. This fluctuation of the resistance level may due to the fact benzimidazole fungicides were introduced for plant disease control research in the 1960s and early 1970s. These fungicides were available in the Egyptian market late 1970s and early 1980s. From that time the benzimidazole fungicides, especially benomyl, were intensively used on strawberry and many other crops, which enhanced the development of different resistant types of the fungus to this fungicide group. In 1996, benomyl was one of the fungicides that were banned in Egypt (Anonymous, 1996). Accordingly, that led to an obvious decrease in the benzimidazole amount applied in the last few years, which may be the reason why the low resistance level for this fungicide was observed in the present work.
In 1996, Mansoor estimated the resistance level to Sumisclex in *B. cinerea* isolated from strawberry. He found that the EC_{50} ranged from 0.42 ppm to 4.3 ppm, representing RF values of 1 and 10.23, respectively. In our study the estimated EC_{50} for the same fungicide ranged from 0.5 to 9.2 ppm representing RF values of 1 and 18.4, which indicate continued increase of the risk.

4. Relationship between the fungicidal acquired resistance and the changes in some physiological fungal characters:

4.1. Effect on oxidative enzymes:

The effect of acquiring fungicidal resistance on the activity of different oxidative enzymes of *B.cinerea* was measured by assessment of the activity of 4 enzymes (polyphenoloxidase, catalase, peroxidase and ascorbic acid oxidase) in the culture filtrates of different isolates with different resistance levels. Correlation coefficients among the enzyme activity levels and resistance indexes of the different isolates were calculated.

4.1.1. Effect on activity of polyphenoloxidase enzyme

Concerning the effect of acquiring fungicidal resistance on the activity of polyphenoloxidase enzyme in *B.cinerea* (Table 9), the different isolates can be classified into three groups. The first group contains 4 isolates with low enzyme activity i.e.S3, S13, G4 and G6. The second grope contains 4 isolates which exhibited moderate enzyme activity i.e. G1, G8, P3 and P10. Two isolates, S7 and 3UV, represent the third group and manifest high enzyme activity. The calculated correlation coefficient between the enzyme activity and resistance index of the different isolates to Sumisclex showed low correlation in contrast to Tecto, where very high positive correlation was found.

4.1.2. Effect on activity of peroxidase enzyme

Regarding the effect of acquiring fungicidal resistance on the activity of peroxidase enzyme of *B.cinerea* (Table 10). The isolates could be also classified into three groups. The isolates G4, G6 and G8 composed the first group, which demonstrated low peroxidase activity. The most isolates showed moderate peroxidase activity, and could be classified into the second group. Two isolates, S7 and 3UV evince high peroxidase activity and constituted the third group. The calculated correlation coefficients between the enzyme activity and resistance factors of the different isolates to Sumisclex showed low negative correlations, in contrast to the corresponding coefficient for Tecto which exhibited a high positive correlation between the enzyme activity and resistance factor.

4.1.3. Effect on activity of catalase enzyme

In the case of catalase (Table 11), great variation in the enzyme activity among the different isolates was recorded, which ranged from 1.77 to 68.46. The lowest activity was recorded by G1, which showed 1.77. The most isolates showed similar rates of enzyme activity, which ranging from 11.34 to 21.96. The highest catalase enzyme activity was recorded by the 3UV isolate. In spite of these great catalase enzyme activity variations, a moderate negative correlation was found between the enzyme activity and the resistance to Sumisclex, while a high positive correlation was found between the enzyme activity and resistance to Tecto.

4.1.4. Effect on activity of ascorbic acid oxidase enzyme

The isolates were classified into two classes according to the ascorbic acid oxidase enzyme activity (Table 12). The first class containing two isolates G6 and G8 showed the highest ascorbic acid oxidase enzyme activity, 104.54 and 124.61 respectively. The rest of the isolates formed the second class with moderate enzyme activity ranging from 39.45 (S7) to 79.05 (G4). A low positive correlation was found to Sumisclex and a low negative correlation was found to Tecto.

The last data indicate positive correlation between resistance to Tecto and the activity of the polyphenoloxidase and peroxidase enzymes. In contrast resistance to Sumisclex showed only low correlations with those enzymes. It was also observed there is negative or low correlation between catalase and ascorbic acid enzyme activity and the resistance to Sumisclex was also observed. Resistance to Tecto correlated highly positively with catalase enzyme activity, but moderately negatively correlation with ascorbic acid oxidase enzyme activity. The last data confirm the data obtained by Mansoor (1996), who reported a remarkable increment in the oxidative enzyme activity correlated with the increased resistance to benzimidazole fungicides. It can be supposed that, the oxidative enzymes polyphenoloxidase and peroxidase play a role in the mode of resistance to benzimidazole fungicides. Concerning Sumisclex Edlich and Lyr (1987), reported that the content of intracellular lipid peroxidase in *B. cinerea* correlated well with the dicarboximide fungicide concentration. They added that catalase acts as protective enzyme by scavenging hydroxyl radicals and by degrading lipid hydroperoxidase, besides functioning in hydrogen

peroxidase cleavage. Superoxidase dismutase detoxifies mainly the highly reactive superoxide catalase radicals. The presence of all the protective components described above has been proven for *B. cinerea*. Since the mode of action of dicarboximide fungicides is based on the generation of active oxygen specimens, it can be speculated that changing the protective system toward higher efficacy would lead to resistance. Indeed, alternation of catalase, phenoloxidase, and superoxidase dismutase could be observed for several dicarboximide resistant isolates of *B. cinerea*. In particular, the increased levels of catalase in highly resistant isolates could account for resistance. However, a conclusive correlation between levels of such enzymes and the degree of resistance has not been found in resistant isolates of *B. cinerea* (Edlich and Lyr 1992). The lack of correlation for our data, since some dicarboximide resistant isolates in the present study showed high levels of catalase activity and other isolates showed negative reactions. The difference of catalase activity for different resistant isolates may due to, that most tested isolates were resistant to Sumisclex and at the same time to Tecto which lead to complicated interference in the different enzyme's activity. This data indicates that the mode of resistance to Tecto or Sumisclex depends not only on the oxidative and catalatic enzymes but other components may be involved too in the mode of resistance. Moustafa *et al.* (2002) found that resistance in *Botrytis fabae* to Antracol may beattributed to increasing oxidative enzyme activity, in addition to one or more substances , of non-enzymatic nature, produced by the resistant isolates.

Table 9: Effect of the acquisition fungicidal resistance on the activity of polyphynoloxidase enzyme.

solate	Enzyme activity									
	15s.	30s.	45s.	60s.	75s.	90s.	105s.	120s.	Equation for each 15s.	Calculated value/ min.
S7	0.119	0.120	0.121	0.121	0.122	0.123	0.123	0.123	y = 0.0006x + 0.1188	0.078
S3	0.005	0.006	0.007	0.007	0.007	0.007	0.008	0.008	y = 0.0008x + 0.0029	0.006
S13	0.003	0.004	0.006	0.007	0.007	0.008	0.008	0.009	y = 0.0008x + 0.0029	0.006
G1	0.012	0.012	0.012	0.013	0.013	0.013	0.013	0.013	y = 0.0002x + 0.0118	0.012
G4	0.007	0.009	0.009	0.009	0.010	0.010	0.011	0.013	y = 0.0007x + 0.0068	0.009
G6	0.008	0.009	0.009	0.009	0.009	0.009	0.011	0.014	y = 0.0006x + 0.007	0.009
G8	0.008	0.011	0.012	0.012	0.013	0.013	0.013	0.014	y = 0.0007x + 0.009	0.011
P3	0.015	0.016.	0.017	0.017	0.017	0.017	0.017	0.017	y = 0.0002x + 0.0156	0.016
P10	0.015	0.016	0.016	0.017	0.017	0.018	0.018	0.020	y = 0.0006x + 0.0144	0.016
S3UV	0.036	0.038	0.039	0.041	0.041	0.042	0.043	0.044	y = 0.0011x + 0.0357	0.040

N.B. S3UV= a new generated type obtained by exposing the mother isolate S3 to UV rays for different periods
Correlation coefficient
Sumisclex = 0.142849
Tecto = 0.434509

Table 10: Effect of the acquisition fungicidal resistance on the activity of peroxidase enzyme

Isolate	Enzyme activity									
	15s.	30s.	45s.	60s.	75s.	90s.	105s.	120s.	Equation for each 15s.	Calculated value/ min.
S7	0.06	0.066	0.072	0.08	0.086	0.088	0.095	0.101	y = 0.0058x + 0.055	0.078
S3	0.025	0.028	0.032	0.037	0.04	0.044	0.048	0.052	y = 0.0039x + 0.0207	0.036
S13	0.035	0.037	0.04	0.043	0.047	0.05	0.054	0.054	y = 0.003x + 0.0315	0.043
G1	0.026	0.031	0.037	0.037	0.044	0.045	0.048	0.05	y = 0.0034x + 0.0245	0.038
G4	0.01	0.017	0.024	0.029	0.035	0.039	0.045	0.048	y = 0.0054x + 0.0064	0.020
G6	0.017	0.021	0.025	0.028	0.032	0.035	0.038	0.042	y = 0.0035x + 0.014	0.028
G8	0.017	0.02	0.024	0.028	0.031	0.036	0.038	0.04	y = 0.0035x + 0.0137	0.027
P3	0.025	0.029	0.032	0.036	0.044	0.044	0.048	0.051	y = 0.0038x + 0.0214	0.036
P10	0.033	0.039	0.043	0.048	0.053	0.057	0.062	0.066	y = 0.0047x + 0.0291	0.047
S3UV	0.082	0.086	0.093	0.097	0.093	0.1	0.103	0.106	y = 0.0032x + 0.0805	0.093

Correlation coefficient
Sumisclex = - 0.24133
Tecto = 0.799082

Table 11 : Effect of the acquisition fungicidal resistance on the activity of catalaze enzyme.

Isolate	Enzyme activity									
	15s.	30s.	45s.	60s.	75s.	90s.	105s.	120s.	Equation for each 15s.	Calculated value/ min.
S7	20.92	21.61	21.96	22.4	22.7	22.24	22.27	22.28	y = 0.1662x + 21.3	21.96
S3	18.70	19.02	19.06	19.09	19.10	19.12	19.13	19.18	y = 0.2233x + 17.198	18.09
S13	19.79	19.88	19.91	20.07	20.09	20.12	20.13	20.13	y = 0.051x + 19.786	19.99
G1	1.74	1.75	1.76	1.78	1.78	1.78	1.82	1.82	y = 0.0115x + 1.7268	1.77
G4	9.91	9.95	10.05	10.23	11.04	14.82	15.78	16.05	y = 1.0387x + 7.5546	11.70
G6	11.14	11.17	11.24	11.45	11.52	11.54	11..62	11.63	y = 0.0792x + 11.058	11.34
G8	8.85	8.89	8.89	8.91	8.92	8.97	9.14	9.27	y = 0.0529x + 8.7421	8.95
P3	17.29	17.78	18.03	18.04	18.15	18.57	18.68	19.08	y = 0.2233x + 17.198	18.09
P10	18.22	18.59	18.75	18.82	19.11	19.59	21.74	22.06	y = 0.541x + 17.176	19.34
S3UV	68.01	68.08	68.28	68.50	68.58	68.90	68.94	69.08	y = 0.1635x + 67.811	68.46

Correlation coefficient
Sumisclex = - 0.4006
Tecto = 0.93862

Table 12 : Effect of the acquisition fungicidal resistance on the activity of ascorbic acid oxidase enzyme.

Isolate	Enzyme activity									
	15s.	30s.	45s.	60s.	75s.	90s.	105s.	120s.	Equation for each 15s.	Calculated value/ min.
S7	5.92	13.08	14.25	32.11	53.33	55.92	100	100	y = 14.755x - 19.57	39.45
S3	36.46	43.21	51.96	68.64	79.36	85.58	92.35	118.61	y = 11.099x + 22.075	66.47
S13	15.8	26.34	42.14	55.28	64.92	65.8	99.96	113.14	y = 13.454x - 0.1186	53.69
G1	29.96	40.28	48.27	55.21	74.75	82.74	99.97	179.21	y = 17.454x - 2.245	67.57
G4	42.59	50.6	67.81	70.12	90.18	97.67	130.96	139	y = 14.123x + 22.564	79.05
G6	65.64	87.99	95.82	107.24	111.45	118.71	130.18	166.43	y = 11.778x + 57.431	104.543
G8	97.61	105.92	114.3	122.59	130.92	133.31	136.86	200	y = 11.152x + 80.004	124.612
P3	25.3	51.68	69.24	91.21	95.59	95.59	96.69	121.95	y = 11.727x + 28.137	75.045
P10	1.19	4.78	15.47	49.97	61.89	65.47	79.72	119	y = 16.206x - 23.24	41.584
S3UV	29.78	40.53	60.8	62.15	64.86	71.61	79.68	89.16	y = 7.697x + 27.685	58.473

Correlation coefficient
Sumisclex = 0.214341
Tecto = - 0.22067

4.2. Effect on some biochemical substances:

The effect of acquired fungicidal resistance on some biochemical substances in the mycelium mat of the *B. cinerea* with different resistance factors was determined by assessment the relative contents of flavonoids, and sterols (Table 13). The gained data exhibited obvious differences among the different isolates concerning their flavonoid contents. The lowest concentration of flavonoids was found in mycelium mats of the isolates S3, S3UV and S13, in contrast to the S7, S10, G1 and G4 isolates, which demonstrated the highest concentrations of these components. S14, S15 and G6 showed moderate concentrations.

The last data manifests that the flavonoids content correlated positively with resistance to Sumisclex, unlike the sterol content, which didn't show certain trend.

Flavonoids are polyphenolic compounds. Over 4000 different flavonoids have been described. Flavonoids have a variety of biological effects on cell systems (Hollman, *et al.* 1996). They are very good antioxidants in their activity against iodophenol-derived phenoxyl radicals, superoxide anion radicals and lipid peroxidation (Zhang,and Shen 1997).

Although, all the available literature, deals with their biological effects on mammalian systems, in our work an obvious positive correlation was found between the resistance to Sumisclex and amount of flavonoids in the mycelium mat of the different isolates, which indicates, that flavonoids may play a role in the mode of resistance to Sumisclex side by side with the catalase enzyme as an antioxidant which protects the fungus against lipid peroxidase.

Table 13: Flavonoid and sterol content in mycelium of different isolates of *B. cinerea* with different resistance factors to Sumisclex and Tecto.

Isolates	Resistance factors		Substances	
	Sumisclex	Tecto	Flavonoids	Sterols
S3	1.2	1.0	+	+++
S3UV	1.2	24.1	+	+++
S7	20.4	10.2	+++	+++
S10	23.0	9.2	+++	+++
S13	3.2	2.5	+	++
S14	10.7	6.9	++	+++
S15	10.2	7.0	++	+++
G1	25.7	4.4	+++	+
G4	21.7	3.7	+++	+++
G6	17.2	3.0	++	++

(-) = no substance .
(±) = traces.
(+) = small amount.
(++) = moderate amount .
(+++)= great amount of substance .

REFERENCES

Abbas, I. E; 1995. Pathological studies on *Botrytis* spp. The causal organism of some vegetable diseases in greenhouses. Master of Science thesis, Agricultural Botany Department, Faculty of Agriculture , Al-Azhar University. 105 pp.

Allam, R. I.and J. P. Hollis, 1972. Sulfide inhibition of oxidases in rice root. Phytopathology 62 : 634 – 639.Ali. I.N.M. and M.S.H. Moustafa, 1991a. Prediction of acquired resistance in *Sphaerotheca macularis* the causal organism of powdery mildew Disease of strawberry in El-Sharkeia and Ismailia governorates. Egypt. J. Appl. Sci., 6 : 264 - 274.

Anonymous, 1996, Ministerial decree No. 874 / 1996, Egyptian Agricultural Ministry.

Anonymous, 2001. Pest Control Program. Ministry of Agriculture, Egypt. 247 pp.

Ashmawy, E. M. A; 1997. Studies on acquired resistance of *Alternaria solani* on tomato. Master of Science thesis, Economic Entomology and Pesticides Department, Faculty of Agriculture , Cairo University. 100 pp.

Beever, R. E; E. P Laracy and H. A. Pak, 1989. Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. Plant-Pathology. 1989, 38: 427-437.

Dekker, 1983. The fungicide-resistance problem. Neth. J. Pl. Path. 83 : 159 – 167.

Dieter, A; 1983. Development of resistance in *Botrytis* to dicarboximides in the Franken viticulture area. Bayerisches Landwirtschaftliches Jahrbuch. 1983, 60: 3, 346-349.

Edlich, W. and H. Lyr, 1987. Mechanism of action of dicarboximid fungicides, in Modern selective fungicides □ Properties, Application and Mechanism of action. Gustav Fischer Verlag. Jena.

Edlich, W. and H. Lyr, 1992. Target site of fungicides with primary effect on lipid peroxidation, In target sites of fungicide action. CRC press, 1992. 328 pp.

Fabreges, C; and R .Birchmore, 1998. Pyrimethanil: monitoring the sensitivity of *Botrytis cinerea* in the vineyard. Phytoma. 1998, No. 505, 38-41.

Fröhlich, G; 1979. Phytopathologie und Pflanzenschutz. VEB Gustav Fischer Verlag, Jena. 295pp.

Geismann, T. A; 1962. Chemistry of flavonoid compounds. Macmillan Company, New York.

Hanoson, J. R; 1972. Chemistry pf terpens and terpenoides. Academic Press New York, 155 – 206 pp.

Hollman, P.C.H.; M.G.L. Hertog,and M. B. Katan, 1996. Analysis and health effects of flavonoids. Food-chem. 1996. 57 : 43-46.

Hussein, M. S. and I. N. M. Ali, 1985. Development of Resistance to benomyl by *Botrytis cinerea* the Causal organism of gray mold of Strawberry Fruits under field conditions. The 1st. Nat. Conf. of Pests & Dis. of Veg. & Field Crops in Egypt. Ismailia, 1985, 2 : 589 – 901.

Hussein. M .S., I. N. Ali and H. Khalifa, 1985. Occurrence of gray mold disease on cucumber and melon cultivated in green-houses. The 1st. Nat. Conf. of Pests & Dis. of Veg. and Field Crops in Egypt. Ismailia, Oct. 1985. 2 : 1042 – 1053.

Mansoor, H. M. A. M; 1996. Studies on Acquired resistance in *Botrytis cinerea,* the causal organism of gray mold disease of strawberry to some systemic fungicides. Master of Science thesis, Plant Protection Department, Faculty of Agriculture , Al-Azhar University. 82 pp.

Maxwell, D.P. and D.F. Bateman. 1967. Changes in the activities of some oxidases in extracts of *Rhizoctonia* infected bean hypocotyls in relation to lesion maturation. Phytopathology. 57 : 132-136.

Menzinger, W; 1966. Zur Variabilität und Taxonomie von Arten und Formen der Gattung *Botrytis cinerea*. Zbl. f. Bakt. 11 : 141- 195.

Moustafa, 1980. Untersuchungen zur Fungizidresistenz von *Botrytis cinerea* unter Bruchsichtigung des Einsatzes von Benzimidazol-Fungiziden bei Lagerkohl (*Brassica oleracea* var. *Capitata*). Ph. D. Thesis, Biowissenschaftlichen Fakultät des Wissenschaftlichen Rates der Humboldt Universität zu Berlin. 141 pp.

Moustafa, M. S. H; El-Dakar, M. R. Helal and E. M. A. Ashmawy, 2002. Mode of fungicidal resistance in *Botrytis fabae* to Antracol. The first conference of the Central Agricultural Pesticide Laboratory, 3-5 September, 2002, 385 - 388.

Nakazawa, y. and M. Yamada, 1997. Chemical control of gray mold in Japa. – A history of combating fungicide resistance-. AJ. 71 : 2 – 5.

Sharvelle, E. G; 1979. Plant disease Control. AVI Publishing Company, I. N. C. Westport. 331 pp.

Smith, j. R. C; K. Verhoeff and W. R. Jarvis, 1980. The biology of *Botrytis*. Academic Press, London, New York, Toronto, Sydney and San Francisco. 317 pp.

Subromanian, C. V; 1971. Hyphomycetes. Indian Council of Agricultural research. New Delhi University of Madras. 930 pp.

Zhang, J; and X. Shen, 1997. Antioxidant activities of baicalin, green tea polyphenols and alizarin in vitro and in vivo. J. nutr environ -med. Abingdon, U.K. : Carfax Publishing Company. June 1997. 7 : 79-89.

تأثير إكتساب صفة المقاومة للمبيدات الفطرية سوميسكلكس وتكتو على بعض الصفات الفسيولوجية لبعض عزلات فطر البوترايتس سيناريا

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فطر البوترايتس سيناريا المسبب لمرض العفن الرمادي من الفطريات الشرسة ، فهو يهاجم عديد من المحاصيل المختلفة سواء قبل الحصاد أو بعده. ورغم أنه حتى الآن مازالت المبيدات تعتبر من أهم الوسائل لمقاومة هذا المرض، إلا أن ظهور بعض السلالات مقاومة لهذه المبيدات يؤدى لحدوث مشكلة خطيرة تحد من استخدام هذة المبيدات فى مقاومة هذه الأمراض. ونظرا لأن مستويات مقاومة هذه السلالات عملية ديناميكية غير ثابتة تختلف من وقت لآخر ومن مكان لآخر, لذلك فان هذا البحث يهدف الى :

١- تقييم الوضع الحالى لمستويات المقاومة فى فطر البوترايتس سناريا المعزولة من محاصيل مختلفة لمبيدى السوميسكليكس و التكتو.

٢- دراسة تأثير أكتساب صفة المقاومة للمبيدين موضع الدراسة على بعض الخصائص والصفات والفيسيولوجية .

ولتحقيق ذلك تم عزل ٤٠ عزلة من فطر البوترايتس سيناريا ؛ ١٥ عزلة من ثمار العنب و ١٥ عزلة من ثمار الفراولة و ١٠ عزلات من ثمار الفلفل والتى تم جمعها من محافظات مختلفة سواء من الحقل أو الصوب أو أسواق الخضر، ويمكن تلخيص النتائج المتحصل عليها فيما يلى:

أولا : تقييم الوضع الحالى لمستويات المقاومة فى فطر البوترايتس سيناريا المعزولة من محاصيل مختلفة.

١- تقييم الوضع الحالى لمستويات المقاومة فى فطر البوترايتس سيناريا المعزولة من الفلفل.

- بالنسبة لمستويات المقاومة لمبيد السوميسكلكس، يوجد اختلافات واضحة فى مستويات المقاومة لهذا لمبيد حيث تراوح معامل المقاومة مابين ١,٠ - ٣٠,٧ .
- بالنسبة لمستويات المقاومة لمبيد التكتو، لم تلاحظ اختلافات واضحة فى مستويات المقاومة لهذا المبيد حيث تراوح معامل المقاومة مابين ١,٠ - ١,٨ .

٢- تقييم الوضع الحالى لمستويات المقاومة فى فطر البوترايتس سيناريا المعزولة من الفراولة.

- بالنسبة لمستويات المقاومة لمبيد السوميسكلكس، يوجد اختلافات واضحة فى مستويات المقاومة لهذا المبيد حيث تراوح معامل المقاومة مابين ١,٠ - ١٨,٤ .
- بالنسبة لمستويات المقاومة لمبيد التكتو، تلاحظ اختلافات متدرجة واضحة فى مستويات المقاومة لهذا لمبيد حيث تراوح معامل المقاومة مابين ١,٠ - ١٠,٢

٣- تقييم الوضع الحالى لمستويات المقاومة فى فطر البوترايتس سيناريا المعزولة من العنب.

- بالنسبة لمستويات المقاومة لمبيد السوميسكلكس ، يوجد اختلافات واضحة فى مستويات المقاومة لهذاالمبيد حيث تراوح معامل المقاومة مابين ١,٠ – ١١,٧ .
- بالنسبة لمستويات المقاومة لمبيد التكتو، لم تلاحظ اختلافات كبيرة فى مستويات المقاومة لهذا المبيد حيث تراوح معامل المقاومة مابين ١,٠ – ٣,٨

ثانيا: تأثير اكتساب صفة المقاومة للمبيدين موضع الدراسةعلى بعض الخصائص الصفات الفيسيولوجية.

١- تأثير اكتساب صفة المقاومة للمبيداين موضع الدراسةعلى على إنزيمات الأكسدة

- المقاومة لمبيد التكتو كانت مرتبطة ايجابيا مع النشاط الانزيمى للبوليفينول أوكسيديز و البيرأوكسيديز والكتاليز.
- كانت المقاومة لمبيد السوميسكلكس مرتبطة سلبا مع نشاط انزيم الكاتاليز.
- تشير هذه النتائج الى أن النشاط الانزيمى لأنزيمات البوليفينول أوكسيديز و البيرأوكسيديز والكتاليز قد يكون لها دور فى ميكانيكية مقاومة الفطر لمبيد التكتو.

٢ – تأثير اكتساب صفة المقاومة للمبيدين موضع الدراسةعلى بعض المركبات الحيوية:

- وجدت زيادة ملحوظة فى محتوى ميسليوم العزلات المقاومة للسوميسكلكس من الفلافونيد.
- لم يلاحظ أرتباط بين محتوى الميسليوم من الأسترولات ومقاومة الفطر للمبيدات.