

STUDIES ON STRAWBERRY FRUIT ROT INCITED BY *Botrytis cinerea*

Mohamed M. El-Morsy; Ali M. Koriem; Amira M. Tawfik; M. I. Elian

Department of Plant Production, Faculty of Technology & Development, Zagazig University, Egypt.

e.mail: *Mohamedabdella8@gmail.com, Ali.koriem@yahoo.com, Amt2222@yahoo.com, drmohamedelian@gmail.com*

ABSTRACT:

Strawberry (Fragaria sp.) is among the most favourite and delicious on which the demand has been increased all over the world during the last decades. Strawberry fruit rots cause much losses to fruits both in quantity and quality in field and market. Numerous pathogenic fungi cause rots of fruits such as, leather rot caused by Phytophthora cactorm. grey mold caused by Botrytis cinerea, rhizopus leak caused by Rhizopus nigricans and hard brown rot caused by Rhizoctonia (corticium) solani. The presence of grey mould is the most common reason for fruit rejection by growers, shippers and consumers, leading to significant economic losses.

The traditional strategy of control of postharvest strawberry decay rely on the application of fungicides during the crop growing cycle. In this study Botrytis cinerea, Rhizopus nigricans and Rhizoctonia solani were isolated from the diseased strawberry fruits. Botrytis cinerea, was the most effective on inducing strawberry fruit rot. Pyrimethanil, Difenconazole and Azoxystrobin fungicides were used to control strawberry fruit rot under field conditions. Azoxystrobin was the best one in decreasing the cinerea infection and disease severity. The residues of the three fungicides were determined after 0, 3 and 6 days of application. Strawberry cultivars were differ in their reaction of B. cinerea infection. Strawberry fruit contents i.e. TSS, total acidity and vitamin C B. cinerea infection. Strawberry fruit contents i.e. TSS, total acidity and vitamin C content were affected due to B. cinerea infection.

***Conclusively,** strawberry fruit rot disease caused great losses in fruits in field, market and exportation. The caused fungus Botrytis cinerea was isolated and pathogenicity test was studied. The susceptibility of Strawberry cultivars for Botrytis cinerea infection as well as the residues of some used fungicides were determined in order to know the safety period for human consumption. The effect of infection on Strawberry fruits components i.e total acidity, vitamin C, and TSS were determined.*

Keywords: Strawberry fruit, rot incited *Botrytis cinerea*

INTRODUCTION:

Strawberry (*Fragaria sp.*) is among the most favourite and delicious on which the demand has increased all over the world during the last decades. It is grown in some governorate in Egypt, such as Kalubia, Tahrir, Ismaelia and Garbia. Fruits are not only consumed fresh, but also may be canned as processed in different forms. Also, fresh as well as canned strawberry fruit are exported in considerable quantities to Arab and Europe countries. Strawberry fruit rots cause much losses to fruits both in quantity and quality in the field and market.

Numerous pathogenic fungi cause rots of fruits such as, leather rot caused by *Phytophthora cactorm*, grey mold caused by *Botrytis cinerea*, rhizopus leak caused by *Rhizopus nigricans* and hard brown rot caused by *Rhizoctonia (corticium) solani*. All the above mentioned causals of fruit rots of strawberry were isolated and recorded a long time ago (**Stefan Petrash *et al.*, 2016**). *Botrytis cinerea* is an airborne plant pathogen, necrotrophic fungus that infects fruit in the field, storage, transport and market leading to significant economic losses. *B. cinerea* mainly enters the host via wounds or natural openings. *Botrytis cinerea* affects more than 200 plant species all parts of Plant organs include fruits, flowers, leaves, storage organs, and shoots. The traditional strategy of control of postharvest strawberry rely on the application of fungicides during the crop growing cycle. Conventional fungicides are applied around flowering, and treatments can be repeated up to harvest. Nowadays, there are many alternatives to conventional fungicides that are characterized by low impact on the environment and on human health. These include biological control agents, natural compounds, decontaminating agents, physical methods, and their combinations (**Iqbal *et al.*, 2020**). Despite the negative impact on human, animal and environmental health, synthetic fungicides are the most common agrochemicals used to control *Botrytis cinerea*, the causal agent of grey mold disease (**Elad *et al.*, 2007**). **Legard *et al.*, (2005)**, reported that crop phenology and epidemical donation were used to design a reduced use fungicide program for control of Botrytis fruit rot in winter annual strawberry. Fungicide spray programs during early and late periods of the season using high and low rates of captan were evaluated with or without second peak bloom applications of fenhexamid during the 1999-2000 and 2000-2001 seasons. Under Egyptian conditions, it was found that Rubigan, Topsin M. Benlate, Tecto 40 FI and Bayieion were the most effective in depressing strawberry mildew development, while Soril 80. Flawable sulphur and Vegil 7166 were the less effective ones (**Khafagi, 1989**). Many recent studies are concerned with the degradation and the residues of the fungicides used against strawberry diseases. For the Bayleton fungicide used against strawberry powdery mildew, the degradation of triadimefon and its metabolite triadimenol was investigated after 14 days from the last application of Bayleton. For Benlate and Daconil used against strawberry fruit rot diseases, the residues of both fungicides were

determined in washed and unwashed fruits at 3 days intervals up to 15 days after the last spray (**Koriem et al., 1991**).

Therefore, the objective of this study was aimed to isolate and identify the causal fungi of strawberry fruit rot and their pathogenicity test. Susceptibility of some strawberry cultivars for *B. cinerea* infection has conducted some fungicides were used to control strawberry fruit rot and their residues were determined in order to define the safety period for human consumption. Some fruit components i.e. TSS, vitamin C and total acidity were correlated to fruit infection.

MATERIALS AND METHODS

Isolation and identification of the causal organism:

Diseased strawberry (*Fragaria* sp.) fruits showing rots disease symptoms were collected from two Governorates, i.e., El-Qalubia and El-Behaira.

Collected samples were subjected to isolation trials. The infected tissues were small pieces and were surface sterilized with sodium hypochlorite (0.5%) for 3 minutes. Then washed several times with sterilized distilled water and dried between sterilized filter papers and transferred directly to the PDA medium in petri dishes 9cm. The plates were incubated for 7 days at $22 \pm 2^\circ\text{C}$. The fungi growing from the lesion pieces were transferred to potato dextrose agar (PDA) slants. The fungus was purified by the single spore technique and kept on the slant of PDA in test tubes at 5°C . Pure isolates were identified according to the morphological characteristics of mycelium, spores.

Pathogenicity test:

Pathogenicity tests for isolates of *B. cinerea* were carried out in the lab. The following technique was adopted in the pathogenicity study.

Healthy strawberry fruits of California cv. (El-Qalubia) were surface sterilized by immersing in 2.0% sodium hypochlorite solution for two minutes, then washed several times by sterilized tap water and immediately at the rate of three fruits per group. Three replicates for each treatment were used.

The number of fruits having specific rots disease symptoms was counted after 7 days of incubating and the percentage of infection was calculated according to **Balogum et al., (2005)**.

The purified isolated fungi were identified according to their morphological features using the description of **Barnett and Hunter (1998)**. The isolated fungi were maintained on a PDS slant, kept in the refrigerator at $5-8^\circ\text{C}$, and sub-cultured till used.

The identification was confirmed at the Disease Survey and Mycology Department, Plant Pathology Institute, Agriculture Research Center, Egypt. The identified are *Botrytis cinerea*.

Disease assessments:

Disease readings were determined for fruits according to disease index rating which was made to include the average diameter of the infected areas. The following numerical rates were suggested to facilitate visual determination and to give a satisfactory comparison:

0 = No rot

1 = Scattered small rots

2 = Rots coalescing and including about 0,25 to 0.50 of fruit area.

3 - More than 0.50 of the fruit area was infected.

Readings were converted to disease index according to the equation by **Townsend and Heuberger (1943)** as follows:

$$\text{Disease index \%} = \text{Sum} \frac{(n \times r_1) + (n \times r_2) + (n \times r_3)}{3N} \times 100$$

Where:

n: is the number of fruits in each numbered rates: r_1 , r_2 , and r_3 the ratings

N: is the total number of inoculated fruits multiplied by the maximum numerical rates 3

The percentage of infection was determined according to the following formula.

$$\% \text{ Infection} = \frac{\text{Number of rotted fruits}}{\text{Total number of tested fruits}} \times 100$$

Efficiency of some fungicides on strawberry fruit rot under field conditions:

The experiment was carried out at Qalubia Governorate during 2020 and 2021 seasons. A complete randomized block design four replicates was used in this study. Each plat was 1/100 of feddan. California strawberry cv. was used. The usual agriculture of strawberry cultivation i.e. fertilization and irrigation were followed. Plants were left for natural infection. Three, fungicides i.e. pyuimethonil, Difenocnazole and Azoxystibin were used to study their effect on controlling fruit rot. Plants were sprayed 4 times every two weeks intervals (Table 1). The percentage of disease severity and disease incidence were determined according to the following formula:

$$\% \text{ Disease incidence} = [\text{Number of rotted fruits} / \text{Total number of tested fruits}] \times 100$$

Disease severity was recorded according to an empirical scale with six degrees: 0 = healthy strawberry, 1 = 1% to 20% fruit surface infected, 2 = 21% to 40% fruit surface infected, 3 = 41% to 60% fruit surface infected, 4 = 61% to 80% fruit surface infected, 5 = more than 81% of fruit surface infected (**Romanzzi *et al.*, 2000**).

Table 1: Common name and trade name of the used fungicides:

Common name	Trade name
Pyrimethanil	Pyrys 40% SC
Difenoconazole	Eurozole 25% EC
Azoxystrobin	Azostar 25% EC

Residue determination:

After the fourth spray of fungicides the residues of pyrimethonil, Difwenocenazale and Azoxystrobin were determined as follow: strawberry fruits were harvested at the suitable stage of maturity. Four samples were collected at random from plats which were sprayed with the three used fungicides of the one hour of the fourth spray at the second season to determine the initial deposit of these compounds samples were taken of 3 days intervals.

Method of residues determination of the three used fungicides were determined by central laboratory of residue analysis of pesticides and heavy metals in food, Agric. Res. Cen. Ministry of Agriculture.

- Method Name: QuEchERs method
- Method Description: Quick and Easymethod (QUECH ERs) for determination of pesticides residues in foods using LC-MSMS GC-MSMS (European standard Method EN 15662-2018).

Effect of *B. cinerea* infection on some strawberry fruit quality parameters:**1. Total soluble solids (TSS)**

Juice of the treated fruits was used to estimate total soluble solids using a hand refractometer.

2. Vitamin C content

The vitamin C content of each sample of juice was measured using 2,6-dichloro-indophenol titration and 2% oxalic acid as a substrate as described by **Lucass (1944)**. The results were expressed in mg/100 m of the juice. The amount of ascorbic acid was calculated according to the following formula

$$\text{Ascorbic acid (mg)} = 100 \times V \times S \times D$$

Where;

V = volume of 2,6-dichloro-indophenol, S= concentration of 2.6 dichlorophenolendophenol, D= Dilution of sample,

3. Titratable acidity (TA)

Titrateable acidity was measured as citric acid by titration against NaOH (0.1 N) using phenolphthalein as an indicator. The percentage of acidity was calculated as mg/100 mL of the juice according to **A.O.A.C. (1980)**.

$$\% \text{ Titratable acidity} = \frac{\text{Volume NaOH} \times N \text{ of NaOH} \times 0.064}{\text{Sample volum of strawberry fruit (mL)}}$$

RESULTS AND DISCUSSION

1) Collected sample and number of isolates:

The collected samples from El-Qalubia governorate were 10 while the collected sample from El-Behiara were 25. The Fungal isolates were 14 from El-Qalubia while 33 from El-Behiara (Table 2) and fig. 1.

Table 2: Isolates Table

Governorate	Sample Number	Isolate Number
El-Qalubia	10	14
El-behiara	25	33
Total	35	47

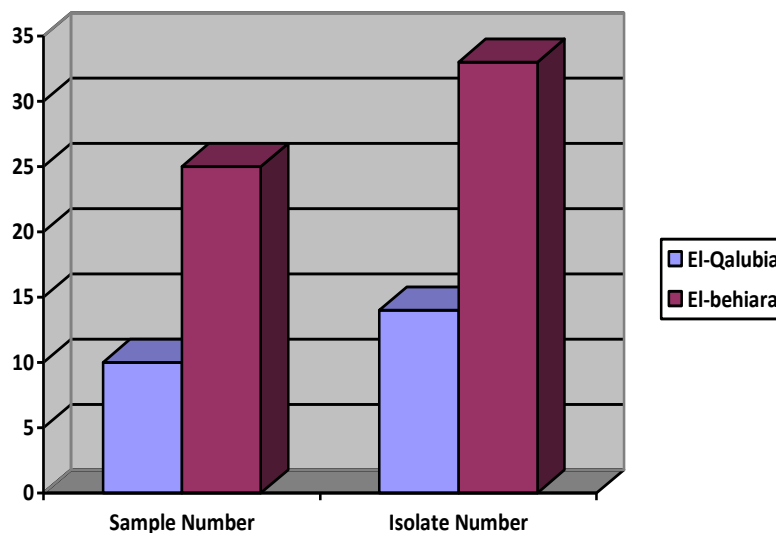


Fig 1. Sample and isolate numbers

Isolation of the causal organisms:

Botrytis cinerea, *Rhizopus nigricans* and *Rhizoctonia solani* were isolated from the diseased strawberry fruits.

Pathogenicity test of the isolated fungi:

Pathogenicity tests (% infection and % disease severity) were carried out for the isolated fungi *i.e.* *Botrytis cinerea*, *Rhizopus nigricans* and *Rhizotonia solani*. Data in Table 4 indicate that *B. cinerea* was the most effective on inducing rot strawberry fruits.

Table 3: Pathogenicity test of the isolated fungi :

Isolated Fungus	% infection	% disease severity
<i>Botrytis cinerea</i>	75	80
<i>Rhizopus nigricans</i>	50	60
<i>Rhizoctonia solani</i>	35	40

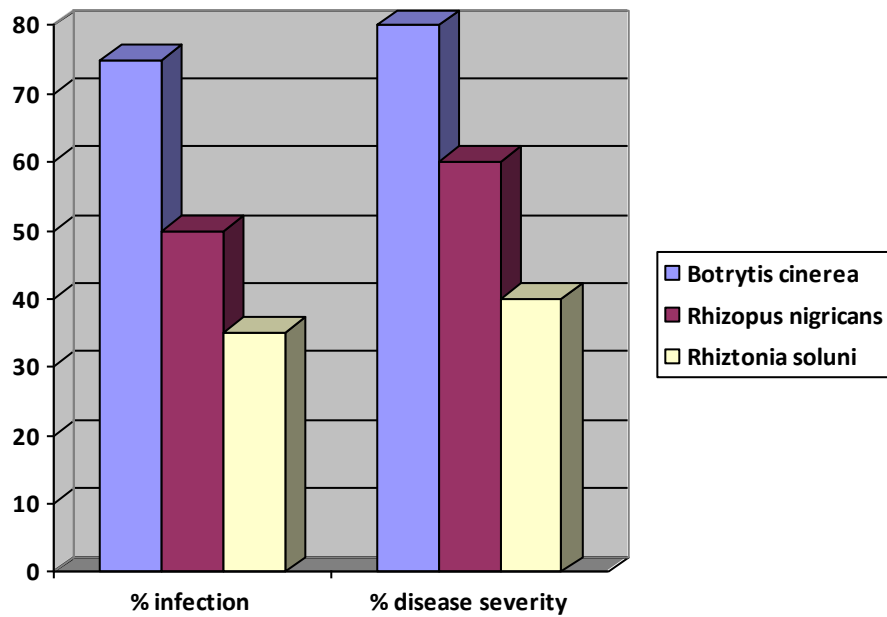


Fig. 2 . Pathogenicity test of the isolated fungi

Susceptibility of strawberry cultivars to Botrytis cinerea infection:

indicate that *B. cinerea* was pathogenic to strawberry cultivars, i.e. fortona, sunsation, festival, and florida. There are differences between the rotted fruits percentage caused by *B. cinerea*.

Festival cv., was superior in resistance of pathogenic to *B. cinerea* in fruits and the percentage of rotted fruits was recorded at the lowest pathogenic (5.2%). This cultivar was followed by fortona cv. while the percentage of rotted fruit was highest as a result of sunsation and Florida, respectively. These results may be due to the genotype characters in these cultivars and had the ability to resist the *B. cinerea* infection (Seuo *et al.*, 2008).

Table 4: Susceptibility of strawberry cultivars to *Botrytis cinerea* infection:

Tested Cultivar	% rotted fruits caused by <i>B. cinerea</i>
Fortona	6.5
Sensation	10.2
Festival	5.2
Florida	11.5
LSD (0.05)	2.64

**Fig. 3** Susceptibility of strawberry cultivars to *Botrytis cinerea* infection***Efficiency of some fungicides on strawberry fruit rot under field conditions:***

Data in Tables (5 – 1 & 5 – 2 and and Fig. 4 & 5) show in general, that all the tested fungicides (4) spray at 14 days intervals) gave sufficient control against strawberry fruit rot caused by the fungus *B. cinerea*, in comparison with control during the two successive seasons, The least percentages of both infection (18.90% and 12.91%) and disease severity (11.70% and 9.61%) and the highest yield (7.77 and 8.34 ton/feddan) were found with spraying of Azoxystrobin during 2020 and 2021 seasons respectively, while difenoconazole was less effective in this respect.

Regarding the percent reduction in injury Azoxystrobin during 2020 season and pyrimethanil during 2021 season were the test fungicides.

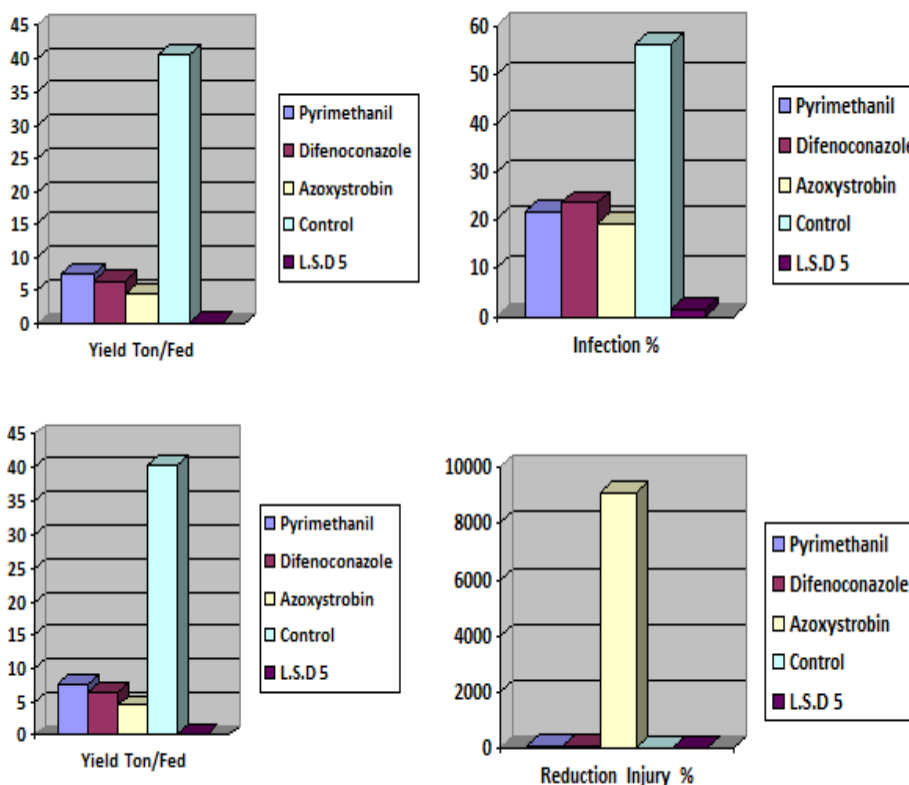
Data in Tables (5 – 1 & 5 –2 and Fig.4 & 5) also indicate that, in general, all the tested fungicides increased the yield in comparison with the control. The

percentage of the increases different from 39.25% to 70.39% during 2020 season and from 36.31% to 77.07% during 2021 seasons. Azoxystrobin and pyrimethanil were the best in reducing injury during the two seasons.

Table 5-1: Effect of some fungicides on strawberry fruit rot caused by *B. cinerea* under field conditions at qolubia governorate during 2020 season:

Treatment	2020				
	Infection %	Disease Severity %	Reduction Injury %	Yield Ton/Fed	% of yield Increase
Pyrimethanil	21.30	15.60	86.13	7.43	64.94
Difenoconazole	23.30	20.30	80.26	6.35	39.35
Azoxystrobin	18.90	11.70	9077	4.44	70.39
Control	56.30	40.40		4055	
L.S.D 5%	1.62	1.36		0.13	

2020



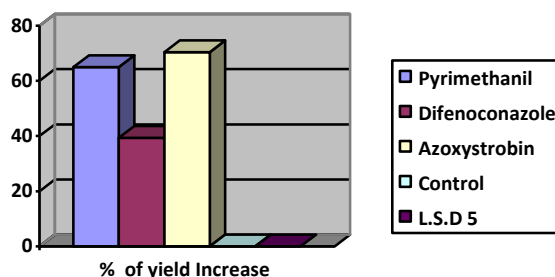
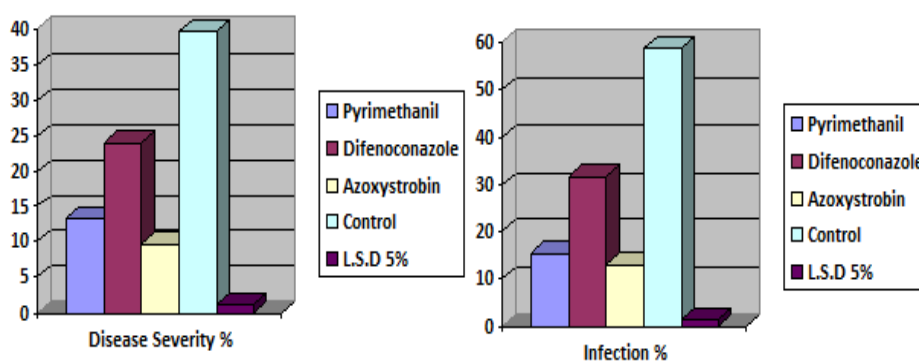


Fig. 4. Effect of some fungicides on strawberry fruit rot caused by *B. cinerea* under field conditions at qolubia governorate during 2020 season

Table 5-2: Effect of some fungicides on strawberry fruit rot caused by *B. cinerea* under field condition at Qalubia Governorate during 2021 season:

Treatment	2021				
	Infection %	Disease Severity %	Reduction Injury %	Yield Ton/Fed	% of yield Increase
Pyrimethanil	15.20	13.20	91.37	7.62	61.78
Difenonazole	31.81	24.11	67.03	6.42	36.31
Azoxystrobin	12.91	9.61	96.34	8.34	77.07
Control	58.72	39.60		4.71	
L.S.D 5%	1.21	1.13		0.11	



2021

On the other hand, difenoconazole was the last one for reducing injury (80.26 and 67.03) respectively during the two seasons. The best fungicide

tested that showed the highest reduction in fruit rot of strawberry as well as increasing the yield during the two seasons was Azoxystrobin.

It is quite clear that all the tested fungicides during the two successive seasons reduced the infection and increased the yield significantly. Similar results were obtained by many works (Koriem *et al.*, 1991; Jordan, 1973; Legard *et al.*, 2022 and Stefan Petrash *et al.*, 2016).

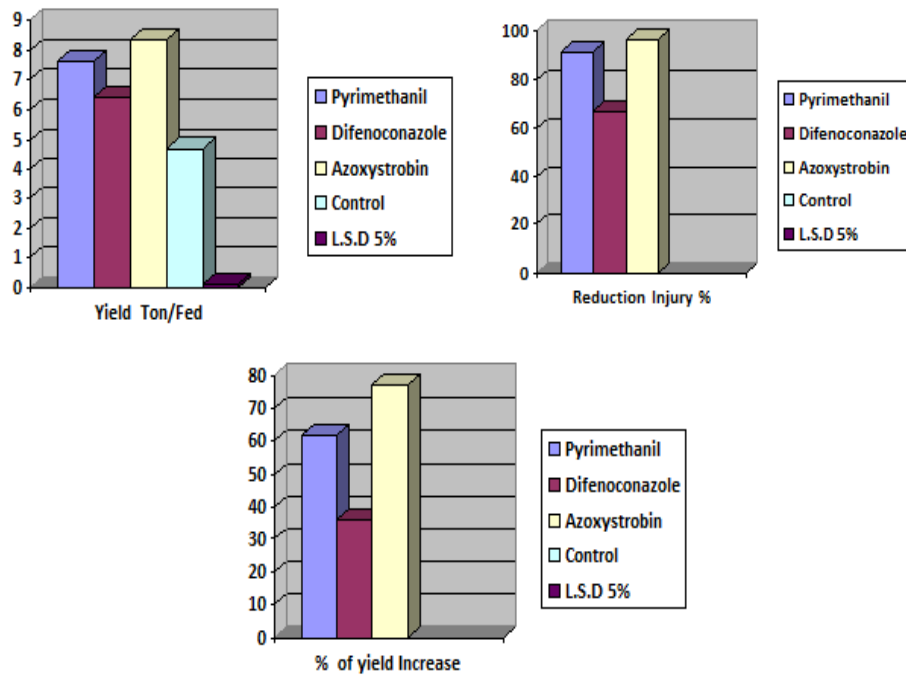


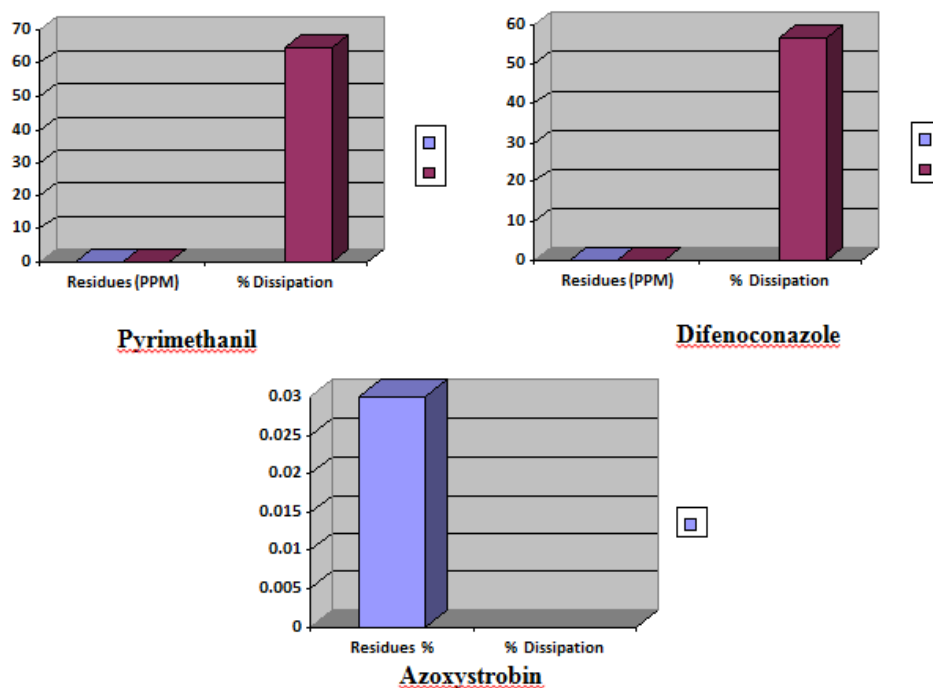
Fig. 5. Effect of some fungicides on strawberry fruit rot caused by *B. cinerea* under field conditions at Qalubia Governorate during 2020 season

Residue determination:

Data in Table 6 and Fig. 6 demonstrate that pyrimethanil degraded rapidly (from 0.017 to 0.011) than the other two fungicides. The experimental period varied for each fungicide. These levels depended on the initial deposits and the reaction between the treated surface and the chemical applied. The results indicated that the initial deposits of pyrimethanil, Difenoconazole and Azoxystrobin were 0.017, 0.023 and 0.03 ppm respectively. These figures decreased gradually after 3 days until reached 0.011 and 0.013 for pyrimethanil and Difenoconazole respectively while it was not detected for Azoxystrobin.

Table 6: Residues of pyrimethanil, Difenoconazole and Azoxystrobin in strawberry fruits :

Days after Spraying Residues	Residues (PPM)					
	Pyrimethanil		Difenoconazole		Azoxystrobin	
	Residues (mg/L)	% Dissipation	Residues (mg/L)	% Dissipation	Residues %	% Dissipation
Initial	0.017		0.023		0.03	
3	0.011	64.70	0.013	56.52	Not detected	—
6	Not detected		Not detected		—	—

Susceptibility test of strawberry cultivars to *Botrytis cinerea*:
Residues (PPM)**Fig. 6.** Residues of pyrimethanil, Difenoconazole and Azoxystrobin in strawberry fruits

Data in Table 6 also, indicate that the percentage of residues dissipation was (64.70, 56.52 and not detected) on pyrimethanil, Difenoconazole and Azoxystrobin respectively. The residue of Azoxystrobin was not detected after 3

days while pyrimethanil and Difenoconazol were not detected after 6 days after spraying. These results were arrived at by that obtained by **Ramadan, (1992); Gwan and Burgers, (1973); Koriem et al., (1991) and Adnan et al., (2019).**

To the best of author knowledge the allowable level of pyrimethanil, Difenoconazole and Azoxystribin in the strawberry fruits is unknown. So, one can say that strawberry fruits picked after spraying could not be marketed with apparent safety for human consumption until the strawberry fruits are free from any residues of these fungicides.

Effect of B. cinerea infection on strawberry fruit quality parameters:

a) TSS (Total Soluble Solids):

Data in Table 7 revealed the cultivars of strawberry had differences in infected by *B. cinerea*. Fortona cv, followed by festival cv, were the most effective in infection to *B. cinerea* and caused rotted fruits. While, the Florida and sensation cultivars improved high susceptibility infection to *B. cinerea* (10 and 12, respectively).

These results are in agreement with those reported by **Mertely et al., (2002) and Chandler et al., (2006).**

Table 7: Effect of *B. cinerea* infection on TSS content of the tested cultivars strawberry:

The tested cultivar	<i>Uninfected</i>	<i>Infected</i>
Fortona	12.5	10
Sunsation	10	12
Festival	8	10
Florida	10	11
LSD (0.05)	0.09	0.07

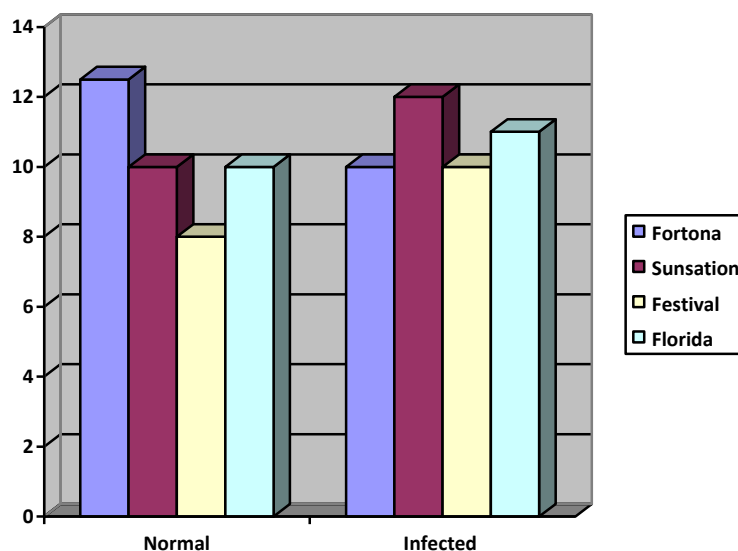
Total acidity (%):

Data in Table 8 and Fig. 8 illustrated that the tested cultivars of strawberry had a good taste and total acidity, when compared between them. Results indicated that the total acidity significantly increased by the infection of *B. cinerea*. All the tested cultivars had clear differences between them in increasing the total acidity caused by *B. cinerea*.

The cultivars of fortona and festival, are the most effective in the content of total acidity with the lowest increases compared with the other treatments, *i.e.* Florida and sunsation, respectively. These results are confirmed by **Chandler et al., (2006).**

Table 8: Effect of *B. cinerea* infection on total acidity (%) of tested strawberry cultivars :

The tested cultivar	<i>Uninfected</i>	<i>Infected</i>
Fortona	0.332	0.373
Sunsation	0.223	0.392
Festival	0.153	0.252
Florida	0.112	0.363
LSD (0.05)	0.007	0.009

**Fig. 8.** Effect of *B. cinerea* infection on TSS content of the tested cultivars strawberry**Ascorbic acid % (Vitamin C):**

Data in Table 9 and Fig. 9 confirm that the ascorbic acid concentration of the tested cultivar significantly affected by infection of *B. cinerea* and caused a decreases in the concentration of strawberry fruits. Festival and fortona cultivars improved a good variety to keeping with the concentration of vitamin C with small losses, while the cultivars of Florida and sunsation has affected by high decreasing in the concentration of vitamin C by the infection of *B. cinerea*. The results are similar to **Seuo *et al.*, (2008)**.

Table 9: The effect of *B. cinerea* infection on vitamin C content in the tested strawberry cultivars:

The tested cultivars	Uninfected	Infected
Fortona	45.1	41.2
Sunsation	39.1	35.3
Festival	42.4	40.2
Florida	40.9	38.7
LSD (0.05)	1.5	1.7

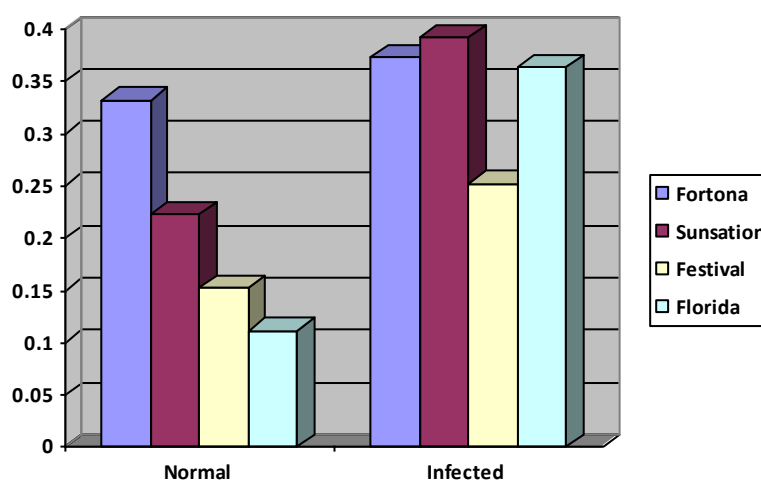


Fig. 9 . Effect of *B. cinerea* infection on vitamin C content in the tested strawberry cultivars

Conclusively, strawberry fruit rot disease caused great losses in fruits in field, market and exportation. The caused fungus *Botrytis cinerea* was isolated and pathogenicity test was studied. The susceptibility of Strawberry cultivars for *Botrytis cinerea* infection as well as the residues of some used fungicides were determined in order to know the safety period for human consumption. The effect of infection on Strawberry fruits components *i.e* total acidity, vitamin C, and TSS were determined.

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دراسات علي مرض عفن الثمار في الفراولة الذي يسببه الفطر بوترايتيس سينيريا

محمد مسعد المرسي، علي محمد كريم، أميرة مسعد توفيق، محمد إبراهيم عليان
قسم الانتاج النباتي - كلية التكنولوجيا والتنمية - جامعة الزقازيق - مصر

تزايد الطلب علي استهلاك الفراولة خلال الفترة الأخيرة، وتتعرض ثمار الفراولة
إلي خسائر كمية ونوعية في الحقل والأسواق نتيجة الإصابة بالعديد من الفطريات
الممرضة وأهمها العفن الرمادي المتسبب عن الفطر بوترايتيس سنيريا والذي يكون سبباً
في رفض الثمار من خلال المنتجين والمستهلكين مما يسبب خسائر اقتصادية كبيرة؛
الإستراتيجية التقليدية لمكافحة أعفان الثمار هو استخدام المبيدات الفطرية؛
في هذه الدراسة تم عزل فطريات ريزوبس وريزكتونيا وبوترايتيس سنيريا من
الثمار المصابة بالأعفان، واتضح أن الفطر بوترايتيس سنيريا كان الأهم في أحداث
الإصابة لثمار الفراولة؛ استخدمت ثلاث مبيدات فطرية لمكافحة عفن ثمار الفراولة تحت
ظروف الحقل وكان أكثرها كفاءة هو المبيد الفطري أزوس ستردين في التأثير علي نسبة
الإصابة وشدها، ثم تقدير متبقيات هذه المبيدات الثلاثة في الثمار يوم الرش وبعد ثلاثة ثم
سنة أيام من الرش؛ أصناف الفراولة المختلفة أظهرت درجات متفاوتة من حيث قابليتها
للإصابة بفطر بوترايتيس سنيريا.

تأثرت مكونات ثمار الفراولة مثل السكريات الذائبة والحموضة وفيتامين س بدرجات متفاوتة بعد الإصابة بمرض عفن الثمار المتسبب عن الفطر بوترياتيس سنيرا. **التوصية :** يسبب مرض عفن ثمار الفراولة خسائر فادحة للثمار كماً ونوعاً فى الحقل وفى الأسواق والتصدير.

وقد تم عزل وتعريف المسبب المرضى وهو فطر بوترياتيس سينيريا وكذلك إجراء القدرة المرضية لهذا المسبب. وتم دراسة قابلية أصناف الفراولة للإصابة بعفن الثمار وتقدير متبقيات المبيدات بعد فترات من الرش بغرض فترة الامان للاستخدام الادمى ومعرفة بعض مكونات الثمار وتأثرها بالإصابة مثل المواد الصلبة وفيتامين سى والحموضة الكلية.