

STUDIES ON STRAWBERRY LEAF SPOT DISEASE IN EGYPT

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ABSTRACT

Fungal diseases of strawberry are important worldwide and occur in all parts of the plant. Alternaria alternata and Botrytis cinerea are the major causal agents of the leaf spots.

Survey and distribution of strawberry leaf spot were carried out in EL-Sharkia and EL-Ismailia governorates. 12 and 20 samples were collected from two governorates with 25 and 35 average of disease incidence, respectively. Isolation and identification of the causal pathogen. Alternaria alternata and Botrytis cinerea were identified as the causal organisms of strawberry leaf spot disease.

Pathogenicity test show that A. alternata was the most effective on inducing strawberry leaf spot disease followed by B. cinerea. Susceptibility of different strawberry cultivars to A. alternata and B. cinerea showed that Fortuna and Winter star were the lowest infected cultivars whereas Festival and Sunsation were the most infected cultivars and also, Florida cultivar was the moderately infected.

All the tested fungicides for controlling leaf spot disease gave sufficient control against the causal organisms, in comparison with control in vitro. The results indicated that the fungicide Topsin M70 has the superior effect followed by Captan.

Conclusively, concerning phenolic compounds, it was found that total, free and conjugated phenols and oxidative enzymes peroxidase, polyphenol oxidase and catalase occurred at high levels in resistant cultivars in comparison with susceptible one. The accumulation of phenolic compounds occurred more rapidly in inoculated resistant leaves than in inoculated susceptible one.

Key words: Botrytis cinerea, Alternaria alternata , Strawberry cultivars, Phenolic compounds, Topsin, Captan

INTRODUCTION

Strawberry (*Fragaria x ananassa Duch.*) plant is a widely grown of family Rosaceae. Egypt produce 460245 tons from 11772 ha of the cultivated

area. Egypt, also exported 38543 tons with currency value estimated with 88million Dollars in the same year.

Cultivated area of Strawberry was 31606 Fed. and produce 539.482 tons (Agriculture Ministry) (**Anon, 2020**).

Observation of leaf spot symptoms on strawberry plants which was collected from EL- sharkia and EL- Ismailia governorates motivated us to find the causal agents of the disease.

Alternaria alternata and *Botrytis cinerea* are the most causal agents of the leaf spot. (**Fekri khan et al., 2021**). *Alternaria alternata* is common as parasite and worldwide distributed. (**Yan et al., 2022**).

Botrytis cinerea affects more than 200 plant species. All parts of plant organs include leaves and fruits. (**Guo et al., 2021**).

Therefore, the objective of this work aimed to survey and distribution of leaf spot disease, Isolation and Identification the causal pathogen. As well as effect some fungicides and susceptibility of strawberry cultivars for leaf spot disease. Also, the relationship between chemical compounds of strawberry plants such as phenolic compounds and oxidative enzymes associated with different disease reaction on strawberry cultivars were studied.

MATERIALS AND METHODS

1. Survey of strawberry leaf spot in EL- Sharkia and Ismailia governorates.

Strawberry (*Fragaria x ananassa Duch.*) has recently become an economically important crop in Egypt and is cultivated on approximately 31606 Fed. A survey and investigation for leaf spot of farmer's fields located across EL-sharkia and El-Ismailia governorates was carried out.

Survey studies on distribution and severity of leaf spot diseases was carried out at two growing seasons 2018 and 2019.

Observation and measurements of disease development were recorded as percentages of infection (number of infected plants), and as severity of infection by measuring the infected area (compared with the total area of each leaf as illustrated in Fig.1 of 100 randomly selected leaves with typical leaf spot symptoms.

2-Isolation and identification of the causal organisms:

Diseased strawberry plants showing leaf spot symptoms were collected from the selected fields.

Collected samples were subjected to isolation trails, *Botrytis sp.* and *Alternaria sp.* were isolated from the lesion appeared on diseased plants. The infected tissues were cut into small pieces and 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 9 cm. The plates were incubated for 7 days at 22 ± 2 °c. The fungal grown from the lesion pieces were transferred to potato dextrose agar PDA slants. The fungus was purified by single spore and or hyphal tip techniques. The isolates were kept on slants PDA medium in a refrigerator at 5 ° C. as stock culture for further studies.

Identification of the isolated organisms was don ten days post inocubation at 25 ° C and was made on the basis of culture characters and microscopical examination considered by **Clements and Shear (1957)**, **Gilman (1957)** and **Ramnath et.al. (1970)**.

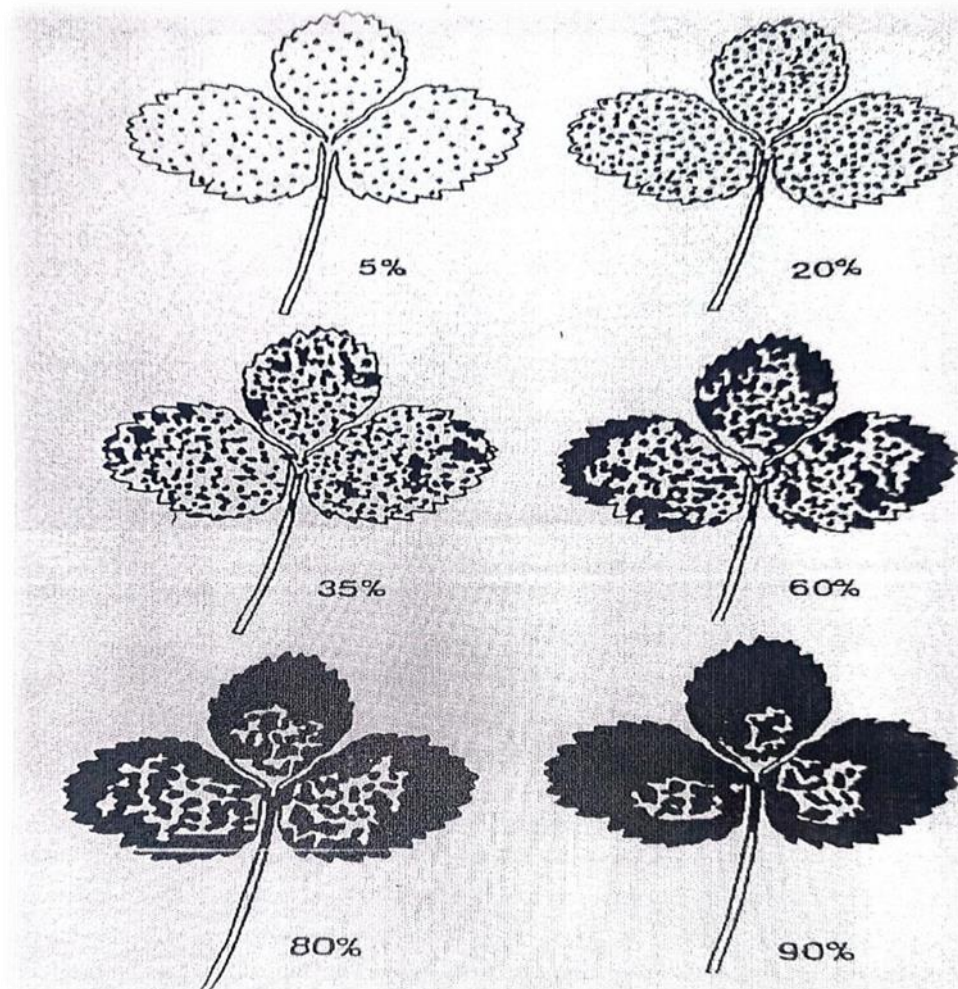


Figure.(1): Severity of infection to evaluate leaf area diseased.

3- Pathogenicity test :

Strawberry cultivars Fortuna, Festival and Winter star were used in the pathogenicity test of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium sp.* and *Rhizopus sp.* isolates.

Strawberry cvs. sown in pot (10 cm Diameter) in greenhouse to obtain 3 weeks old plants. Fungal inoculum for each isolate was prepared from 15 days old cultures which were thoroughly homogenized with 10 ml distilled water/each colony, for one minute in a blender. Then three pots each containing 5 strawberry plants, two weeks old, were inoculated by spraying with homogenized isolate inoculum at a rate of 15 ml/pot. This insured a uniformly heavy layer of inoculum on all leaves. Control plants were similarly treated with medium free from fungus. The inoculated plants were placed in a humid chamber at 100% relative humidity and at 20 °C to 25 °C for 24 hours post inoculation and then were transferred to open glass cages in the greenhouse.

The pathogenicity of these isolates, was determined according to **Cook and Timian (1962)** as follow :

- 1 (R) = resistant, small brown spots.
- 2 (MR) = moderately resistant, small heavy spots.
- 3 (MS) = moderately susceptible, mediate brown spots.
- 4 (S) = susceptible, heavy brown spots.

Resolution of the pathogenic isolates was don made as before and compared with original cultures.

4- Susceptibility of strawberry cultivars :

Two isolates of *A. alternata* and *B. cinerea* were used to inoculate the plants of five strawberry cultivars. Data were recorded post 10 days inoculation. Fortuna, Winter star, Festival, Sensation and Florida Strawberry cultivars were used. Inoculation, incubation and results were done as mentioned before.

5- Effect of different fungicides on the linear growth of tested pathogenic fungi in vitro:

This study was designed to investigate the inhibitory effect of two fungicides i.e. Topsin M70 and Captan, on the linear growth of *A. alternata* and *B. cinerea in vitro* Table 1. The used fungicides were tested at three concentrations as follow: 1%, 2%, 3%.

The amount required for obtaining a known concentration of any fungicide was calculated and added aseptically to known amount of warm sterilized PDA medium and poured before solidification into Petri dishes (9 ml/plate) then plates were inoculated at the center with equal discs (5 mm) obtained from the periphery of 10 days old cultures of *A. alternata* and *B. cinerea* . Plates contained media without any fungicide inoculated with *A. alternata* and *B. cinerea* was served as control treatment. Three plates were used for each

Table 1: The commercial name, chemical composition and active ingredients of the tested fungicides.

Fungicide	Chemical composition	Active ingredient, %
Topsin M70	1,2-Bis (3 methoxy carbonyl-1,2-thiourido) benzene.	70
Captan	Cis-N(trichloromethyle thio)-4-cyclo-hexan1,2dicarboximid	50

concentration. All plates were incubated at 25 ± 2 °C. The experiment was terminated when mycelial mats covered medium surface in control treatment. All plates were examined and growth reduction was calculated.

6- Determination of phenolic compounds:

Two resistant cultivars i.e. Fortuna and Winter star and one susceptible i.e. Festival were used to determine the activity of both phenolic and oxidative enzymes. Uninoculated and inoculated strawberry plants with *Alternaria alternate* or *Botrytis cinerea* were used.

Leaf samples were taken from each cultivar plants uninoculated and inoculated with either *A. alternata* or *B. cinerea* were used to determine the activity of both phenolic compounds and oxidative enzymes as follow:

Phenolic compounds were extracted and determined using the colorimetric methods as described by **Snell & Snell (1954)**.

Sample extraction:

A sample of 5 gm of leaf tissues was cut into small portions and immediately stored in ethanol solution (80%) in brown bottles and kept in dark at room temperature for one month until the tissues were colorless. The ethanolic extracts were filtered and evaporated to near dryness in a mild water bath at 60°C. Then the extracts were quantitatively transferred into 5 ml. of 50% isopropanol and stored in vials at 1 °C till the determination of phenolic compounds.

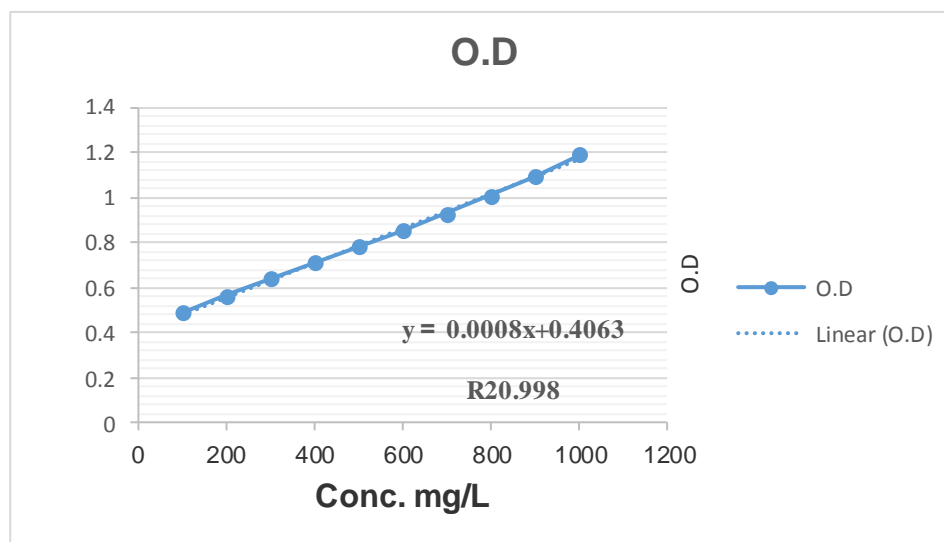
A) Determination of total phenolic compounds

One ml of extract was transferred into a measuring flask (10 ml) and treated with 0.25 ml HCL and boiled in a water bath for 10 min then cooled.

One ml of Folin – Denis reagent and 6 ml Na₂CO₃, were added. The mixture was completed to 10 ml with distilled water and the color density was measured at 520 nm using Spectronic 20. Total phenols were obtained from a standard calibration curve as mg Gallic acid equivalents/100g DW as shown in Figure. 2.

B) Determination of free phenolic compounds:

One ml of extract was transferred into a test tube, 1 ml distilled water, 1 ml folin-denis reagent, and 3 ml Na₂CO₃ 20% (w/v), were added. The mixture was completed to 10 ml with distilled water and the color density was measured using a spectrophotometer (Spectronic 20) at 520 nm. Free phenols were obtained from a standard calibration curve as a Gallic acid (ppm) as shown in Figure. 2. **El-Ghamry (Massarut) A. M. (1980).**



Figure(2): Standard curve of phenolic compounds

C) Determination of conjugated phenolic compounds:

The difference between total and free phenol concentration gave the concentration of bound phenol.

8. Determination of oxidative enzymes activity:

Determination of both peroxidase and polyphenol oxidase activities were measured in both inoculated and un inoculated strawberry leaves in adult plants. Crude enzyme extracts were prepared by triturating leaves in ordinary 10 ml 0.2 M. sodium phosphate buffer at pH 6.1 for 10 minutes at the rate of 5 ml per gram fresh weight. After straining through cheesecloth the triturates were centrifuged for 20 minutes at 3000 rpm. **Allam, and Hollis (1972).**

A) Determination of peroxidase enzyme activity:

Peroxidase activity was determined according to the method suggested, Which was measured at 430 nm. Reaction mixture of 0.1 ml of 0.2 M. pyrogallol solution, 1.0 ml of sodium phosphate buffer at pH 6.1 ,0.5 ml of 0.01 M hydrogen peroxide (H₂O₂). 0.05 ml of crude enzyme

preparation and distilled water to make a total volume of 3 ml. In the blank H₂O₂ was substituted by an equal volume of phosphate buffer at 5.1 pH. The increase in optical density at 430 nm was measured over a period of 5 minutes, at 30 seconds intervals. Peroxidase was expressed as change in absorbance at 430 nm per gram fresh weight of barley tissues per minute.

B) Determination of polyphenol-oxidase activity:

Polyphenol oxidase activity was determined according to **Esterbaner et al., (1977)**. The reaction mixture contained 2 ml enzyme extract, 1.0 ml of 10 M. catechol and 1.0 ml of 0.2 M. sodium phosphate buffer (at pH = 7) then the reaction mixture was brought to a final volume of 6.0 ml with distilled water. The activity of polyphenol oxidase was expressed according to the following equation:

$$\text{Enzyme activity [unit (mg. protein)]} = K \times (\Delta A/\text{min}).$$

Where: K (extension coefficient) is 0.272 mμ/cm at 490 nm for catechol. ΔA/min is the change in the absorbance of the mixture every 0.5 minute for 5 minutes period at 490 nm.

C) Determination of catalase activity:

Catalase enzyme was assayed according to the method of **Kato and Shimizu (1987)**. In the sample cuvette, 0.1 ml crude extract was mixed with 0.5 ml of 0.2 M. sodium phosphate buffer (at pH 7.6) and 0.3 ml of 0.5% H₂O₂. Then the mixture was brought to a final volume of 3 ml with distilled water. The breakdown of H₂O₂ was followed by measuring the absorbance at 240 nm. Moreover, the enzyme activity was calculated according to the following equation:

$$\text{Enzyme activity [unit (mg. protein)]} = K \times (\Delta A/\text{min}).$$

Where: K (extension coefficient) is 40 mμ/cm at 240 nm for H₂O₂. ΔA/min is the change in the absorbance per minute

RESULTS AND DISCUSSION

1. Survey and incidence of leaf spot disease:

Date in Table 2 show that the collected samples from EL-Sharkia governorate were 12 while the collected sample from Ismailia were 20 with 25 and 35 average of disease incidence respectively. The obtained fungal isolate were 19 from EL-Sharkia while 34 from EL- Ismailia.

Table 2: Survey of disease incidence in EL-Sharkia and EL- Ismailia Governorates

Governorates	Sample Number	Isolate Number	Average of disease incidence, %
EL-Sharkia	12	19	25
Ismailia EL-	20	34	35
Total	32	53	60

**Figure.(3):** Symptoms of leaf spot disease of naturally infected strawberry.

2. Isolation and identification of the causal organisms:

Samples of diseased leaves from strawberry plants showing typical leaf spot symptoms were collected during the two season (2018 -2019) from El Sharkia (Belbis and EL-salheyia) and Ismailia (EL-kassassin and Abo-swear) governorates of Egypt Fig.3.

The fungus can survive as mycelium or conidia for a long time and provide a source of infection (Fekrikohan *et.al.*, 2021). Pure culture of *Alternaria alternata*, *Botrytis cinerea*, *Fusarium sp.* and *Rhizopus sp.* were obtained from single spore or the hyphal tip techniques. (Riker and Riker, 1936), The isolates were used for pathogenicity test.

The identification of the isolated fungi was based on the description referring to (A key of Simmons 2007). Morphology of the isolates was examined, colonies were initially white, then turned light to dark brown produced abundant hyphae. Conidiophore straight or flexuous. Conidia were pale to brown, with beaks at tips. Conidia had 1-6 transept. Based on morphological characteristics of representative group, the fungus was tentively identified as *Alternaria alternata* (Simmons, 2007). To fulfill Koch's postulates, pathogenicity test were conducted by choosing one representative isolate from each regions on detached trifoliate strawberry leaves collected from healthy plants. Each of 10 disinfected leaves was inoculated with conidial suspension whereas to additional Leaves (controls) were inoculated with water.

3.Pathogenicity test:

Alternaria alternata, *Botrytis cinerea*, *Fusarium sp.* and *Rhizopus sp.* were isolated from strawberry leaf infected with leaf spots. Fourteen days old. isolates of the obtained fungi (grown at 25 °C and 12-12 photo period) were used to investigate the pathogenicity on the leaf of strawberry Festival cultivar (susceptible to leaf spots). Strawberry Festival cultivar plants were grown in pots under greenhouse conditions.

Table 3:- Pathogenicity test of the isolated fungi:

Fungus	Severity % of infected leaves after different periods.		
	5 days	10 days	15 days
<i>Alternaria alternata</i>	55.30	65.20	75.30
<i>Botrytis Cinerea</i>	35.20	40.50	45.20
<i>Fusarium sp.</i>	30.75	32.40	35.60
<i>Rhizopus sp.</i>	15.25	17.30	20.10

Pathogenicity test (% infection and % disease severity) were carried out for the isolated fungi i.e *Alternaria alternata*, *Botrytis cinerea*, *Fusarium sp.* and *Rhizopus sp.* Table 3 and Figure 4

Data in Table 3 indicate that *A. alternata* was the most pathogenic fungus on inducing strawberry leaf spot disease.

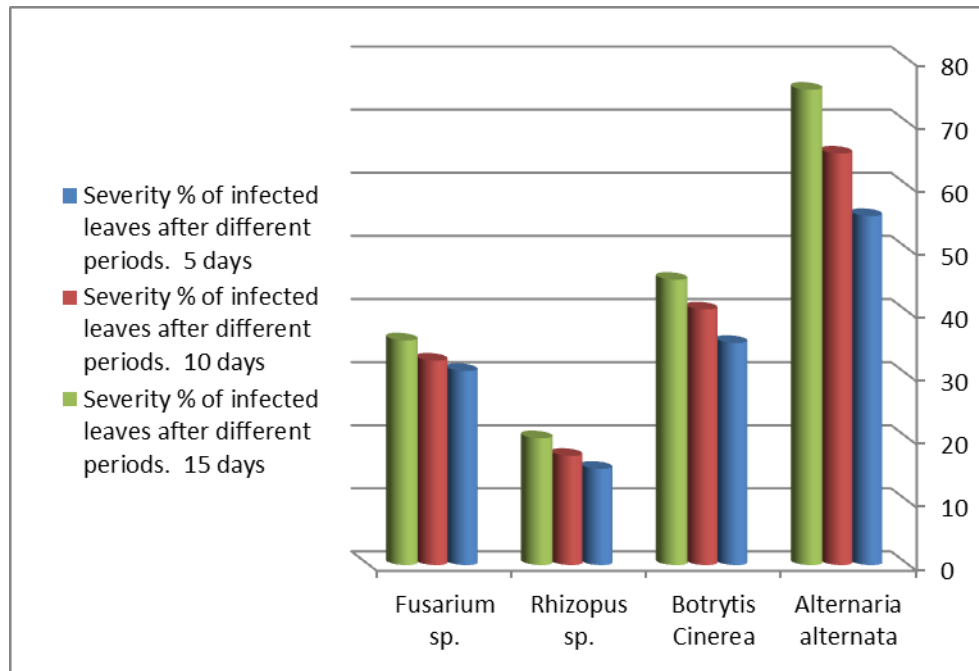


Figure.(4): Severity % of infection leaves after different periods, 5,10 and 15 days.

4. Susceptibility of strawberry cultivars to leaf spot:

One of the symptoms brown spot with the yellow margin. Many plant pathogens can cause this symptoms. *Botrytis* and *Alternaria* are the major causal agents of the leaf spots. Despite of the numerous reports about two fungi from different hosts.

Species of *Alternaria* are known as the serious plant pathogens, causing major losses in wide range of crops. The genus distributed worldwide as saprophytes, entophytes and plant pathogens (Peever *et al.*, 2004 & Woudenberg *et al.*, 2013). Infection by the *Alternaria* species typically cause the formation of necrotic lesions. One of the most Common species of the genus *Alternaria* is *A. alternata* that was reported as destructive plant pathogens and affect the wide range of the host plants causing leaf spot, blight and blossom rots. *Botrytis* is a fungus destroys the tissue of the living plants and can grow and continue to live on the host plant even after the tissue destruction (Wada, *et al.*, 1996).

Result in Table 4 and Figure 5 indicated that *A. alternata* and *B. cinerea* were pathogenic to strawberry causing leaf spot disease. Strawberry cultivars, *i.e.* Fortuna and Winter star were superior in resistance for pathogenic fungi,

and the percentage of leaf spot reaction were recorded at the lowest grade (1). The cultivars Festival and Sunstation were susceptible at the higher pathogenic (grade 4 and 4), respectively, while the cultivar Florida was moderately susceptible (grade 3) with pathogenic fungi. These results may be due to the genotype character in these characters and had ability to resistant the pathogenic fungi. Some chemical compounds of cultivars led to susceptibility for infection with *A. alternata* and *B. cinerea*.

Table 4: Susceptibility of different strawberry cultivars to *A.alternata* and *B. cinerea*

Cultivars	Type	Disease Reaction	
		<i>B. cinerea</i> Leaf spot %	<i>A. alternata</i> Leaf spot %
Fortuna	R.	5	5
Winter star	R.	10	10
Florida	MS.	60	50
Festival	S.	90	90
Sunsation	S.	90	80

Disease reaction are given on 1 to 4 basis.

1 = (R) = Being resistance.

3 = (MS) = Moderately Susceptible.

2 = (MR) = Moderately resistant.

4 = (S) = Susceptible.

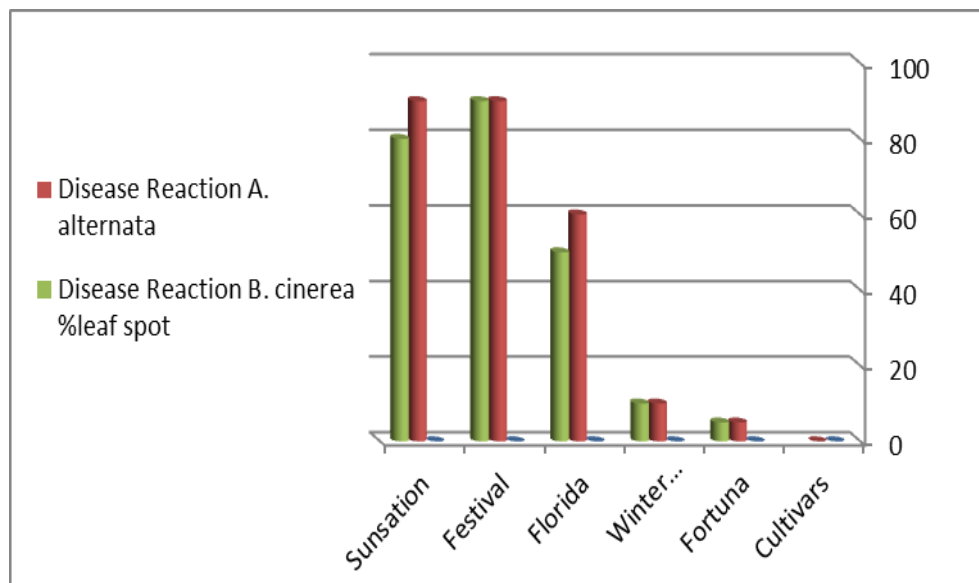


Figure.(5): Susceptibility of different strawberry cultivars to *A.alternata* and *B. cinerea*.

5. Effect of different fungicides on strawberry leaf spot disease:

Data in Table 5 and Figure 6 show in general, that all the tested fungicides gave sufficient reduction in linear growth of strawberry leaf spot pathogens *A. alternata* and *B. cinerea* in comparison with control. Topsin M70 was the most effective fungicide, at low concentration (1%). On the other hand, Captan was the least effective one for inhibition of growth (79.44 and 87.40), respectively. An conclusion Topsin M70 is the best fungicide tested that showed the highest inhibition of fungul growth, (Mass, 1970; Clark, 1976 and Yehia, *et al.*, 2011).

Table 5: Effect of some fungicides on the linear growth of *A. alternata* and *B. cinerea*.

Fungicide	<i>A. alternata</i>			<i>B. cinerea</i>	
	Conc. %	Growth measurement	% Inhibition of growth	Growth measurement	% Inhibition of growth
Topsin 70	1	0.00	100	0.00	100
	2	0.00	100	0.18	97.96
	3	0.00	100	0.00	100
Captan 50	1	2.10	76.66	1.92	78.70
	2	1.35	79.44	1.25	86.10
	3	0.58	66.52	1.13	87.40
Control		9.00		9.00	

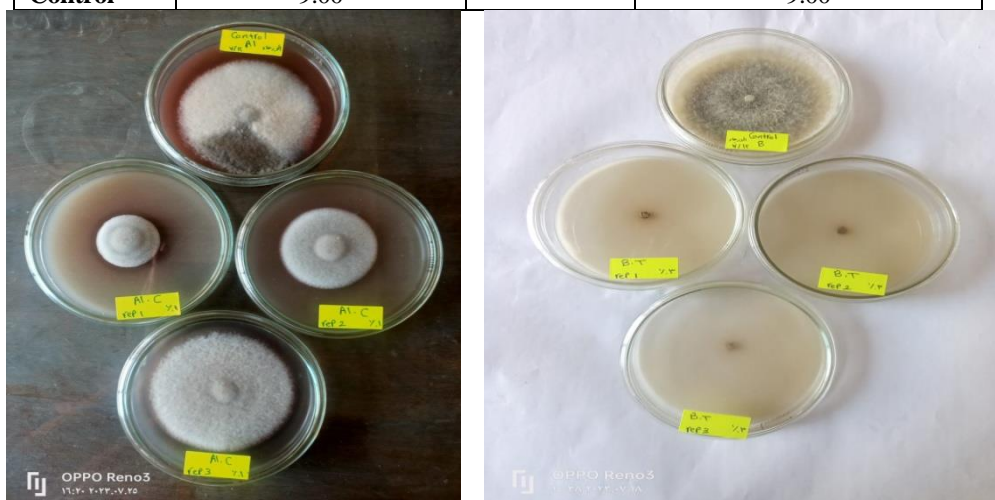


Figure 6: (A) (B)
 6- Effect of Captan on the linear growth of *A. alternata*
 B- Effect of Topsin M70 on the linear growth of *B. cinerea*.

6. Effect of phenol compounds and some enzyme activities on strawberry leaf spot:

a) Phenolic compounds:

Chemical analyses were carried out on three strawberry cultivars differ in their reaction to leaf spot diseases to focus the light on the dynamic nature of disease resistance. **El-Ghamry (Massarut) (1980)**. The importance of a biochemical study of defence reaction in the physiology of disease resistance is widely accepted. Phenolic compounds in plants as considered, by many authors, as major agents of both chemical resistance against disease incidence (**Maick and Singh 1980; Mahmoud, 2004 and Mahmoud et al., 2012**).

Union-collated strawberry plants (control) in Table 6 and Fig. 7 indicate that the levels of total phenol (7.99 and 7.80), Free (6.63 and 6.52) and conjugated Phenol (1.36 and 1.28) were higher in resistant strawberry cultivars Fortuna and Winter star as compared with susceptible one, Festival. These results are in agreement with those of many workers with host-pathogen combinations (**Maick and Singh, 1980**). Inoculation with leaf spot causal pathogens (*Alternaria alternata*, *Botrytis cinerea*) led to rapid increase in total, free and conjugated phenols in the resistant cultivars than in the susceptible one (**Mahmoud et al., 2012**). However, **Farkas and Kiraly (1962)**, were not able to establish a clean-cut relation between the phenolic content and resistance. The increase of phenolic compounds may result from either the synthesis of new aromatic compounds (**Biehn et al. 1968**) and / or the acceleration of accumulative phenol from neighboring cells (**Tomiyame, 1963**).

Data in Table 6 and Figure.7 indicate that the inoculation of Fortuna and Winter star (resistant strawberry cultivars) with either *Alternaria alternata* or *Botrytis cinerea* caused noticed increased on total phenol, free phenol and Conjugated phenolic compounds in comparison with the susceptible cultivar (Festival). On the other hand, inoculation with *Alternaria alternata* or *Botrytis cinerea* caused cleared increase on total, free and conjugated phenolic compounds in both resistant and susceptible cultivars in comparison with union-collated (control) plants. It was noticed that from Table 6 and Fig.7 inoculation with leaf spot pathogens led to rapid increase in total, free and conjugated phenol in the resistant cultivars i.e. Fortuna and winter star more than in the susceptible cultivar Festival.

However, data in Table 6 and Figure7 show also that the inoculation with leaf spot pathogens caused decrease of total, free and conjugated phenolic compounds in Festival susceptible cultivar in comparison with the resistant

ones. Similar results were found by **Tomiyama (1963)** in potato tubers infected with *Phytophthora infestans*. The decrease in phenolic compounds in the inoculated susceptible cultivar can be attributed to their utilization or splitting by the parasite. Another possibility is the decrease of metabolic activities of the susceptible host by pathogen products result in reduction of phenolic compounds.

b) Oxidative Enzymes:

The correlation between induced resistance and some biochemical changes in plant tissues, like the increased activity of enzymes and appearance of new polypeptide protein has become a model in study of plant disease resistance, this biochemical change becomes a marker to induce resistance. **Allam, and Hollis (1972)**.

Data in Table 7 and Figure 8 show the content of peroxidase, polyphenol oxidase and catalase enzymes on the three used strawberry cultivars. The three strawberry cultivars, two resistant (Fortuna and Winter star) and one susceptible (Festival) were used before and after inoculations with either *Alternaria alternata* or *Botrytis cinerea*.

Data presented the higher content of peroxidase, polyphenol oxidase and catalase enzymes in resistant cultivars (Fortuna and Winter star) in comparison with these in the susceptible cultivar (Festival).

Inoculated Fortuna cultivar with either *A. alternata* or *B. cinerea* showed decrease in the activities of the three enzymes in comparison with the control (without inoculation). The same trend was noticed for Winter star cultivar when inoculated with either *Alternaria alternata* or *Botrytis cinerea*.

Inoculation of Festival cultivar (susceptible one) with *A. alternata* or *B. Cinerea* caused clear increase in the activity of the three enzymes except in case of peroxidase enzyme when inoculated with *A. alternata* (0.005).

Phenolic compounds and oxidative enzymes in plants are considered, by many authors, as major agents of host chemical resistance against disease incidence.

Results indicated that total, free, conjugated and enzymes activities were found in higher amounts in the resistant cultivars than in susceptible ones. These results are in agreement with those of many workers with other host-pathogen combinations, *i.e.* **Farkas and Kiraly (1962)** ; **El-Gamry (1980)** ; **Abo-Shosha (1977)** and **Rizk (1982)**.

Table 6: Phenolic compounds expressed as mg Gallic acid equivalents/1g in inoculated and un inoculated three strawberry cultivars.

Strawberry cultivars	Fungus	Total phenolic compounds	Free phenolic compounds	Conjugated phenolic compounds
1- Fortun (Control)		7.99	6.63	1.36
2- Winter star (Control)	star	7.80	6.52	1.28
3- Festival (Control)		4.56	3.43	1.13
4- Fortuna + Al.		16.24	11.87	4.37
5- Fortuna + Bo.		12.97	10.87	2.10
6- Winter star + Al.		26.16	17.06	9.10
7- Winter star + Bo.		14.31	10.95	3.36
8- Festival + Al.		5.75	3.42	2.33
9- Festival + Bo.		12.87	8.77	4.10

L.S.D(0.05%)

0.16

0.53

0.01

1- Fortuna R

Al. *Alternaria alternata*

2- Winter star R

Bo. *Botrytis Cinerea*

3- Festival S

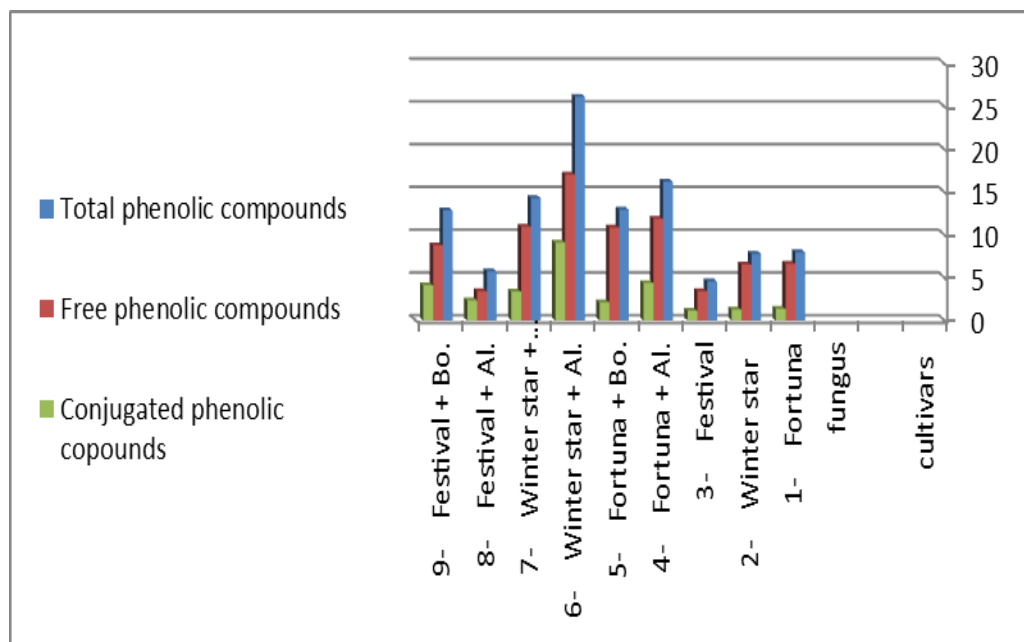


Figure.(7): Phenolic compounds expressed as mg gallic acid equivalents/1g in inoculated and un inoculated three strawberry cultivars.

Table 7:- Enzymes activities expressed as unit in mg. protein in inoculated and uninoculated three strawberry cultivars.

Strawberry cultivars	Peroxidase	Poly phenol Oxidase	Catalase
1- Fortuna (Cont.)	0.011	0.0009	0.544
2-Winter star (Cont.)	0.009	0.0009	0.416
3- Festival (Cont.)	0.006	0.0005	0.168
4- Fortuna + Al.	0.007	0.0016	0.328
5- Fortuna + Bo.	0.009	0.0005	0.232
6- Winter star + Al.	0.010	0.0004	0.152
7- Winter star + Bo.	0.008	0.0008	0.184
8- Festival + Al.	0.005	0.0006	0.208
9- Festival + Bo.	0.007	0.0020	0.384

L.S.D (0.05%)

0.065

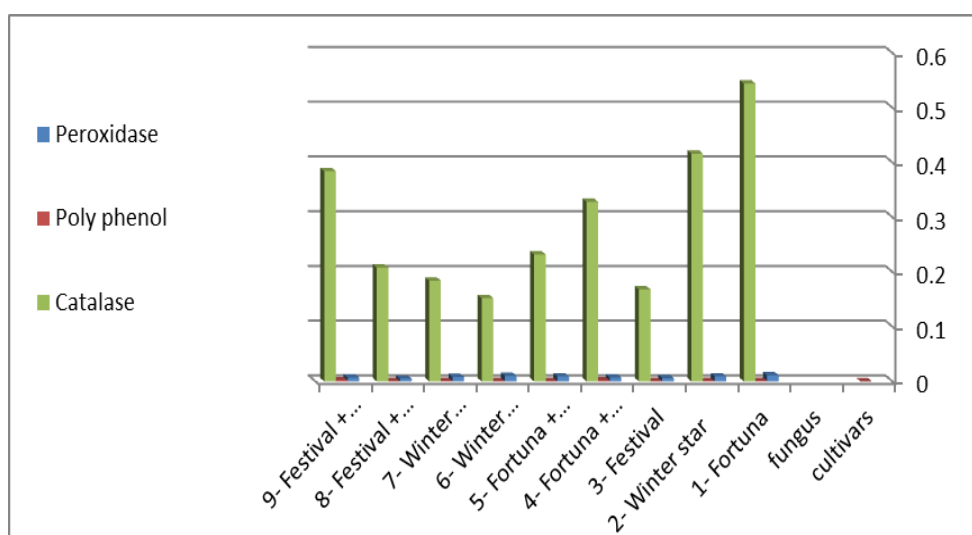
0.040

0.012

1- Fortuna R

2- Winter Star R

3- Festival S

Al. *Alternaria alternata*.Bo. *Botrytis cinerea*.**Fig.(8): Enzymes activities expressed as until in mg. protein in inoculated and uninoculated three strawberry cultivars.**

Conclusively, concerning phenolic compounds, it was found that total, free and conjugated phenols and oxidative enzymes peroxidase, polyphenol oxidase and catalase occurred at high levels in resistant cultivars in comparison with susceptible one. The accumulation of phenolic compounds occurred more rapidly in inoculated resistant leaves than in inoculated susceptible one.

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دراسات علي مرض تبقع الأوراق في الفراولة في مصر

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محصول الفراولة من أهم محاصيل الخضراوات الاقتصادية سواء لغرض الإستهلاك المحلي أو التصدير. ويتعرض محصول الفراولة لكثير من الأمراض الفطرية ومن أهمها مرض تبقع الأوراق الذي يسببه فطر ألترناريا ألترناتا و بوترايتس سينيريا مما يسبب خسائر فادحة في المحصول لتأثيره علي الأوراق الخضراء التي تقوم بعملية التمثيل الضوئي وقلة كفاءتها.

- وقد تم حصر لهذا المرض في محافظتي الشرقية (مركزي بلبس والصحية) والإسماعيلية (مركزي القصاصين وأبو صوير) وحسب إحصائية وزارة الزراعة لموسم ٢٠٢٠ وجدت مساحة الفراولة بالجمهورية ٣١٦٠٦ فدان وإنتاجية ٥٣٩٤٨٢ طن ووجد منها مساحة ٢٥٨١ فدان وإنتاجية ٤١١٦٢ طن في محافظة الشرقية ومساحة ٦٩٥٨ فدان بإنتاجية ١٢٧٦٨٨ طن في محافظة الإسماعيلية.

- تم دراسة قابلية أصناف الفراولة المختلفة للإصابة بمرض تبقع الأوراق واتضح أن صنف فور تانا و ونتر ستار أكثر مقاومة للمرض أما صنف فيستيفال فكان أكثرها قابلية للإصابة. كما تم دراسة تأثير المبيدات الفطرية علي النمو الميسليومي للمسببات المرضية وكان مبيد توبسين ٧٠ أكثرها تأثيرا علي النمو الميسليومي للفطر.

- اتضح من نتائج التحليل الكيماوي في تقدير المركبات الفينولية الكلية والحرية والمرتبطة في الأصناف المقاومة والأصناف القابلة للإصابة وكذلك تقدير النشاط الإنزيمي للإنزيمات الأكسدة من البيروكسيديز والبولي فينول اكسيديز والكاتاليز في الأصناف المقاومة والقابلة للإصابة.

التوصية: اتضح من النتائج أن محتوى الأصناف المقاومة من الفينولات الكلية والحرية والإنزيمات الأكسدة من البيروكسيديز والبولي فينول اكسيديز أعلى من محتوى الأصناف القابلة للإصابة كما وجد أن تراكم المواد الفينولية كان بصورة أسرع في أوراق النباتات المقاومة عن القابلة للإصابة.