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# Efficacy of Using Chemical Inducers and Biological Agents to Control Strawberry Leaf Spot Disease on Chemical Components and Enzyme Activity

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## Abstract

Strawberry plants are subjected to many pathogens. Fungal diseases of strawberries are important worldwide and occur in all parts of the plant, including leaves, crowns, and fruits. The results of the bio-agent experiment indicated that *Trichoderma harzianum* was the most effective in reducing the growth of *Alternaria alternata* and *Botrytis cinerea in vitro*, the main causes of leaf spots in strawberry plants. The inhibition zone was observed and mycelium of *T. harzianum* invaded the colony of the tested pathogens. The importance of the biochemical study of defense reaction in the physiology of disease resistance was accepted. The activity of total, free, and conjugated phenols as well as peroxidase, polyphenol oxidase, and catalase enzyme activities were determined in resistant and susceptible strawberry cultivars. All the total, free, and conjugated phenols increased in resistant cultivars (Fortuna and Winter Star) and decreased in susceptible cultivar (Festival). Oxidative enzymes, like the increased activity of enzymes that appear mince of new polypeptide protein, have become models in the study of plant disease resistance. The higher content of peroxidase, polyphenol oxidase, and catalase enzymes in resistant cultivars, were noticed compared with those in susceptible ones. It was found also that, the chemical inducers increased total phenols in resistant and susceptible cultivars i.e., potassium di-phosphate, ferrous sulphate, and oxalic acid. Inducer resistance also caused an increase of free phenols than the control. However, conjugated phenols accumulated faster after using chemical inducers in the leaves of the resistant cultivars, than the susceptible ones.

**Keywords:** Chemical inducers; Bio-agents; Phenols; Oxidative enzymes; Strawberry leaf spots; Fungicides.

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## 1. Introduction

The ability of a strawberry plant to respond efficiently to pathogens relies first on the physiological status of injured tissue (pre-formed mechanisms of defense) and secondly on the general ability to recognize and identify the invaders by surface plant receptors, followed by a broad range of induced mechanisms, which include cell wall reinforcement, production of reactive oxygen species, phytoalexin generation and pathogenesis-related protein accumulation.

Biological control, in recent years, made many advances against many pathogens of the crops. *Trichoderma* showed an interesting potential control against various pathogens. In this context, we consider it appropriate to make our contribution through the study *in vitro* of the power antagonist ***Trichoderma harzianum*** against ***Alternaria alternata*** and ***Botrytis cinerea***, the causing agents of leaf spot disease in strawberry plants [1]**.**

There has been much effort in the last few decades to introduce non-chemical methods for disease management, as alternatives to fungicide. These have included the application of bio-control agents such as ***Trichoderma harzianum*** Elad [2]**,** Cigdem and Merih [3] and Mokhtar and Aid [4].

Chemical analysis of phenolic compounds and oxidative enzyme activities are very important for supporting strawberry plants against leaf spot diseases.

The importance of the biochemical study of defense reaction in the physiology of disease resistance is widely accepted. Phenolic compounds in plants are considered, by many authors, as a major agent of host chemical resistance against disease incidence Rizk [5] and Mahdy and Eid [6].

The aim of this investigation is to study the effect of some induced chemical resistance and biological agents for controlling strawberry leaf spot disease and determine the activities of phenolic compounds and oxidative enzymes in resistant and susceptible (infected and uninfected) strawberry cultivars as affected with the using them.

# 2. Materials and Methods

## 2.1. Pathogenicity Test: 1

*Alternaria alternata, Botrytis cinerea, Fusarium* sp., and *Rhizopus* sp. were isolated from strawberry leaves infected with leaf spots, fourteen days old [7]. Isolates of the obtained fungi (grown at 25 $^0$C and 12-12 photoperiod) were used to investigate the pathogenicity on the leaf of the strawberry Festival cultivar (susceptible to leaf spots). Strawberry Festival cultivar plants were grown in pots under greenhouse conditions. Each pot were inoculated by spraying with homogenized spore suspension at a rate of 15 ml/pot, using hand sprayer. Inoculated plants were Inoculated plants were covered with a plastic bag, and kept under greenhouse conditions (20-27 °C, normal daylight). The inoculated plants were investigated for spot symptoms after 5,10 and 15 days from inoculation. The severity of infection leaves after the different periods was recorded for each isolate as follow:

Disease readings were determined for leaves 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 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.

Reading were converted to disease index according to the equation by Townsend and Heuberger [8] as follows:

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

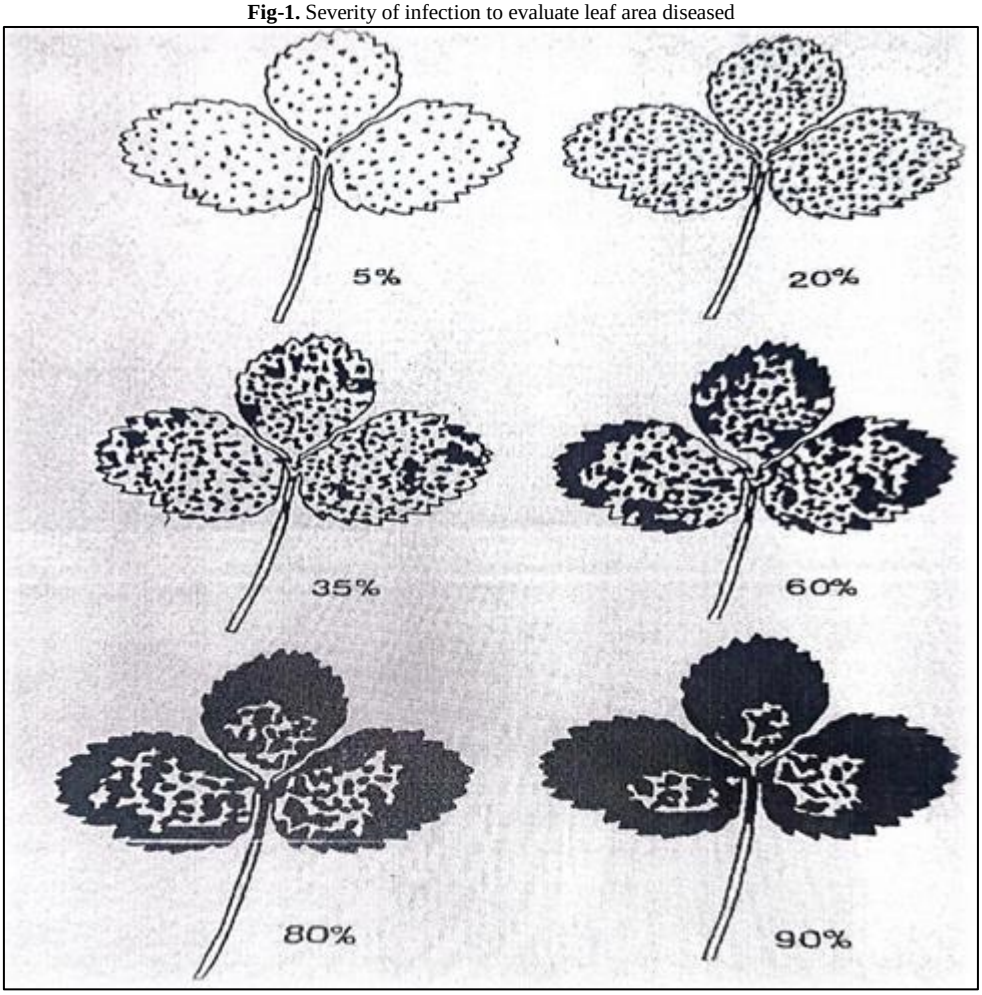
**Where:**

n: is the number of leaves in each numbered
rates: $r_1$ , $r_2$ and $r_3$ the ratings
N : is the total number of inoculated leaves multiplied by maximum numerical rates 3

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

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

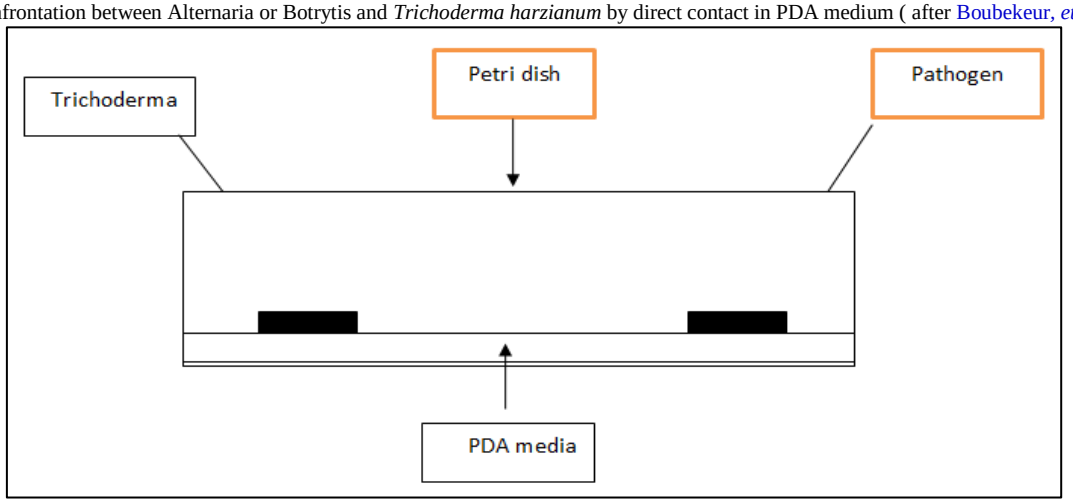
**Fig-1.** Severity of infection to evaluate leaf area diseased



## 2.2. Antagonism of *Trichoderma Harzianum* against *Alternaria Alternata* and *Botrytis Cinerea* the Causal Agents of Leaf Spot of Strawberry Plants

The amount required for obtaining a known concentration of any chemical was calculated and added aseptically to a known amount of warm sterilized Czapek's agar 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 containing media without any chemicals and inoculated with these fungi served as a control treatment. Three plates were used for each concentration. All plates were incubated at 22+2°C. The experiment was terminated when mycelial mats covered the medium surface in the control treatment, all plates were examined, and growth reduction was calculated as mentioned before [9].

**Fig-2.** Confrontation between Alternaria or Botrytis and *Trichoderma harzianum* by direct contact in PDA medium ( after Boubekeur, *et al.* [9])




## 2.3. Determination of Phenolic Compounds

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

Leaf samples were taken from each cultivar (uninoculated and inoculated) with either *A. alternata* or *Botrytis cinerea* and used to determine the activity of both phenolic compounds and oxidative enzymes as follows: Phenolic compounds were extracted and determined using the colorimetric methods as described by Snell and Snell [10].

### 2.3.1. Sample Extraction

A sample of 5 g of leaf tissues was cut into small portions and immediately stored in ethanol solution (80%) in brown bottles and kept in the 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$^{o}$C. Then the extracts were quantitatively transferred into 5 mL of 50% isopropanol and stored in vials at 1$^{o}$C till the determination of phenolic compounds.

#### 2.3.1.1. Determination of Total Phenolic Compounds

One mL of extract was transferred into a measuring flask (10 mL) 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 $Na_2CO_3$, 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 Fig. 2.

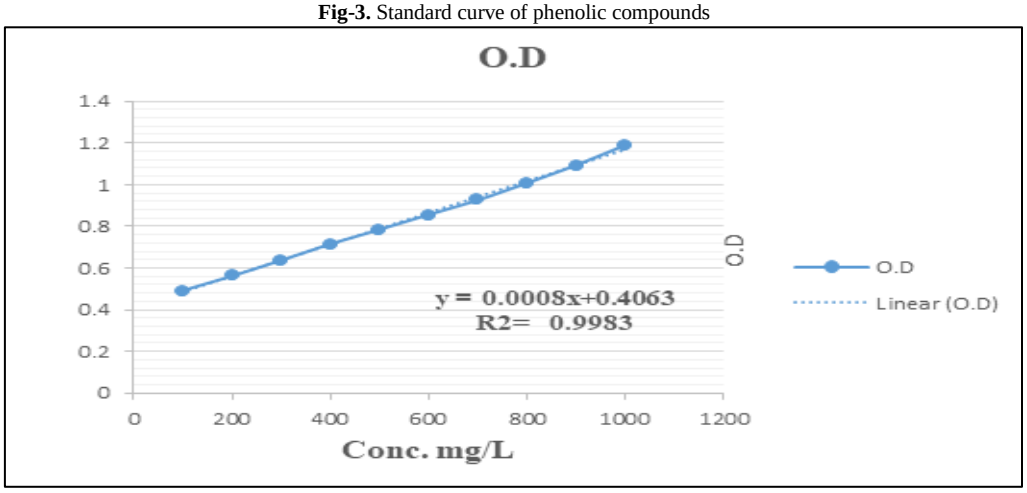
#### 2.3.1.2. Determination of Free Phenolic Compounds

One mL of extract was transferred into a test tube, 1 mL distilled water, 1 mL folin-denis reagent, and 3 mL $Na_2CO_3$ 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 (mg/L) as shown in Fig. 2.

#### 2.3.1.3. Determination of Conjugated Phenolic Compounds

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

**Fig-3.** Standard curve of phenolic compounds




## 2.4. Determination of Oxidative Enzyme Activity:- 4

Determination of both peroxidase and polyphenol oxidase activities was measured in both inoculated and uninoculated 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.

### 2.4.1. Determination of Peroxidase Enzyme Activity

Peroxidase activity was determined according to the method suggested by Colowick and Kaplan [11], 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 ($H_2o_2$). 0.05 mL of crude enzyme preparation and distilled water to make a total volume of 3 ml. In the blank $H_2o_2$ 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 a change in absorbance at 430 nm per gram of fresh weight of strawberry tissues per minute.

### 2.4.2. Determination of Polyphenol-Oxidase Activity

Polyphenol oxidase activity was determined according to Esterbaner, *et al.* [12]. 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:

Enzyme activity [unit (mg. protein) ] = K×( ΔA/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.

### 2.4.3. Determination of Catalase Activity

Catalase enzyme was assayed according to the method of Kato and Shimizu [13]. 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% $H_2O_2$. Then the mixture was brought to a final volume of 3 mL with distilled water. The breakdown of $H_2O_2$ was followed by measuring the absorbance at 240 nm. Moreover, the enzyme activity was calculated according to the following equation:

Enzyme activity [unit (mg. protein)] = K×(ΔA/min).

Where: K (extension coefficient) is 40 mμ/cm at 240 nm for $H_2O_2$. ΔA/min is the change in the absorbance per minute.

## 2.5. Effect of different Resistance Inducing Chemicals Activities of Phenol Compounds and Oxidative Enzymes

This study was designed to investigate the inhibitory effect of some chemicals, which were used later as resistance inducing compounds, on the *in vitro*. The used chemicals were as follows: Potassium di-phosphate ($K_2HPO_4$), Oxalic acid, (OX), and Ferrous sulfate ($FeSO_4$).

# 3. Results and Discussion

## 3.1. Pathogenicity Test

The isolated fungi i.e. *Alternaria alternata*, *Botrytis cinerea*, *Fusarium* sp., and *Rhizopus* sp. were tested for their pathogenicity on leaves of susceptible strawberry Festival cultivar.

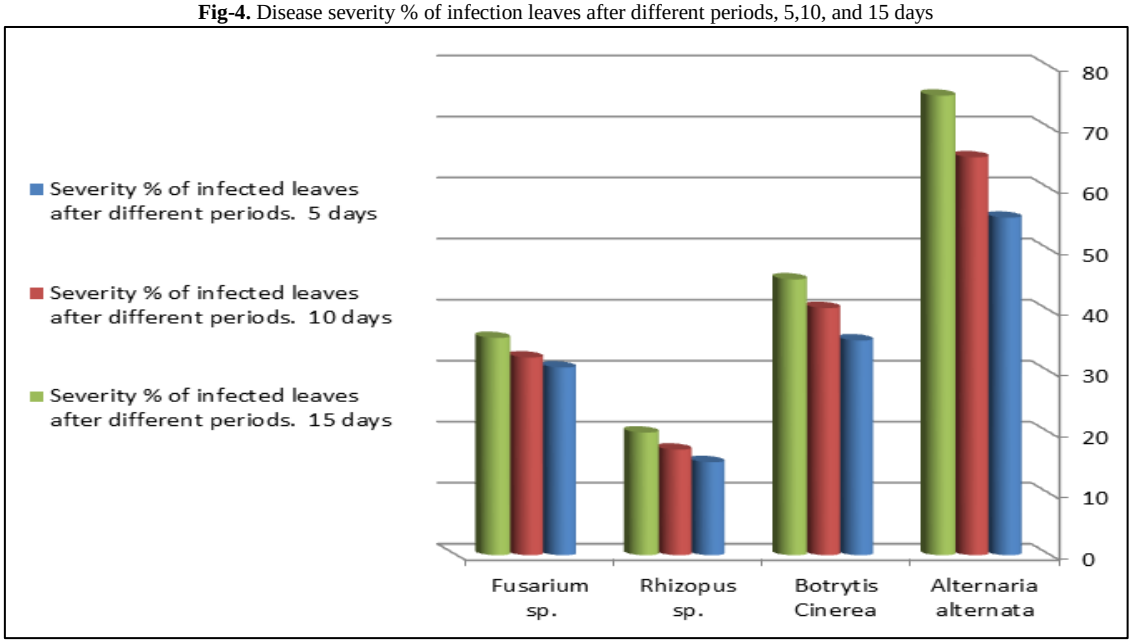
Data in Table 2: - indicate that the percentage of % disease severity was carried out for the isolated fungi i.e**.,** ***Alternaria alternata, Botrytis cinerea, Rhizopus*** **sp.,** and ***Fusarium*** **sp.**

Data show that the disease severity infection recorded that the most pathogenic fungus was *Alternaria alternata* (75.30 %) followed by *Botrytis cinerea* (45.20%) after 15 days of the inoculation, while the Fungus *Fusarium* sp. followed by *Rhizopus* sp. was the lowest pathogenic one (35.60 and 20.10 severity % of infection respectively) after 15 days from inoculation. Therefore, both *Alternaria alternata* and *Botrytis cinerea* were selected in the further studies [14].

**Table-1.** Pathogenicity test of the isolated fungi on strawberry festival leaves (susceptible cultivar) after 5,10 and 15 days from inoculation

| Fungus | Disease severity % of infected leaves after different periods. | | |
|---|---|---|---|
| | 5 days | 10 days | 15 days |
| *Alternaria alternata* | 55.30 | 65.20 | 75.30 |
| *Botrytis cinerea* | 35.20 | 40.50 | 45.20 |
| *Fusarium* sp. | 30.75 | 32.40 | 35.60 |
| *Rhizopus* sp. | 15.25 | 17.30 | 20.10 |

**Fig-4.** Disease severity % of infection leaves after different periods, 5,10, and 15 days




## 3.2. The Antagonistic Study

The PDA medium (Potato Dextrose Agar) which provides good growing conditions for both *A. alternata* and *B. cinerea* was used.

The result of direct confrontations (*in vitro*) of ***T. harzianum*** against ***A. alternata*** and ***B. cinerea*** in a PDA medium, showed a different inhibition in the mycelial growth of the tested fungi.

The inhibition was equal on the fourth day of the experiment to 65% and 58% in ***Alternaria* sp.** and ***Botrytis* sp.** respectively. The microscopic observations of mycelia showed that the mycelia of ***T. harzianum*** were capable of overgrowing and degrading mycelia and spores of ***Alternaria alternata*** and ***Botrytis cinerea*** coiled around the mycelia of pathogens.

Data in Table 2 show that using antagonistic fungus reduced the growth of ***A. alternata*** and ***B. cinerea***. ***Trichoderma harzianum*** was the best antagonistic fungus in inhibiting the mycelial growth by from 80% to 65% growth reduction in ***A. alternata*** and from 75% to 58% growth reduction in ***B. cinerea.*** However, the reduction of growth ***A. alternata*** was more than the reduction of ***B. cinerea*** after one and two days.

**Table-2.** Effect of antagonistic fungi on *A. alternata and B. cinerea*

| Day | *Trichoderma* + *A. alternata* | | Inhibition% | *Trichoderma* + *B. cinerea* | | Inhibition % |
|---|---|---|---|---|---|---|
| After 1 day | 2.2 | 43 | 80 | 2.2 | .53 | 75 |
| After 2 days | 4.4 | 1.4 | 68 | 4.03 | 1.5 | 62 |
| After 3 days | 5.7 | 1.9 | 65 | 5.1 | 2.03 | 60 |
| After 4 days | 6.7 | 2.3 | 65 | 6.2 | 2.5 | 58 |
| After 5 days | 7.4 | 2.1 | 70 | 6.9 | 2.3 | 65 |
| After 8 days | 8.00 | 2.3 | 70 | 8.1 | 2.5 | 68 |
| After 9 days | 8.2 | 2.2 | 72 | 7.3 | 2.4 | 66 |
| After 10 days | 8.7 | 2.5 | 70 | 7.7 | 2.6 | 65 |
| L.S.D 5% | | | 0.484 | | | 0.276 |

T = *Trichoderma harzianum*
A = *Alternaria alternata*
B = *Botrytis cinerea*

A highly significant effect was observed in the study a mycelial growth of ***Alternaria alternata*** and ***Botrytis cinerea*** colonies faced with ***Trichoderma harzianum*** which showed a reduction in growth compared with the control (Table 2). Transplanting simultaneous *Trichoderma* with one of *Alternaria* or *Botrytis* isolates showed faster growth of *Trichoderma* isolates than *Alternaria* or *Botrytis*. After 2 days of incubation, the dish is almost completely invaded by *Trichoderma.*

The growth of colonies was stopped on the fourth day of confrontation followed by the fifth day when one of the parasites came into direct contact with ***Trichoderma harzianum.*** After 8 days until 10 days of confrontation, the colonies of *Trichoderma* completely overlapped and covered the colonies of the parasite (*Alternaria* and *Botrytis*). *Trichoderma* is a fungus known for its mycoparasitism. Elad, *et al.* [15], Elad [2]**,** described the parasitic action of *T. harzianum* on *A. alternata* and *B. cinerea*. *Trichoderma* attacks its host by winding the mycelium around the host hyphae. Subsequently, mycoparasite penetrates the host cells and uses the cytoplasmic contents [7]

*In vitro*, trials through dual antagonist-pathogen culture could potentially indicate the potential of some microorganisms to produce antifungal chemicals to act as biocontrol agents [16]. Microorganisms acting through antibiosis, generally have a wide action spectrum, and thus pathogen inhibition by producing toxic substances is more effective than any other mechanism of action [17, 18].

**Fig-5.** Antagonism between *Trichoderma harzianum* and *A. alternata* after different periods

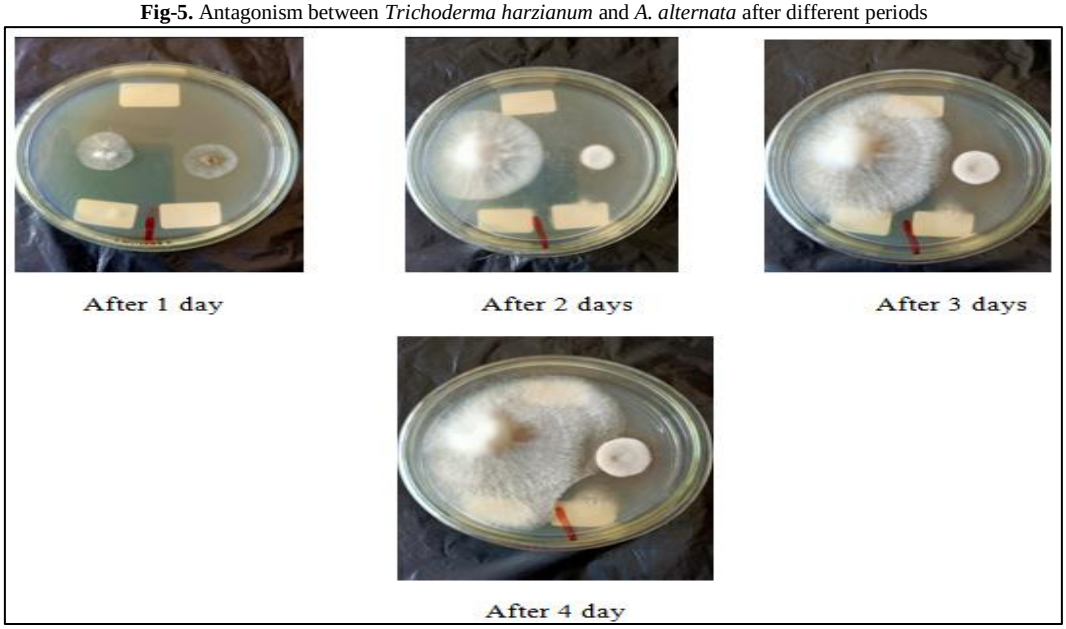
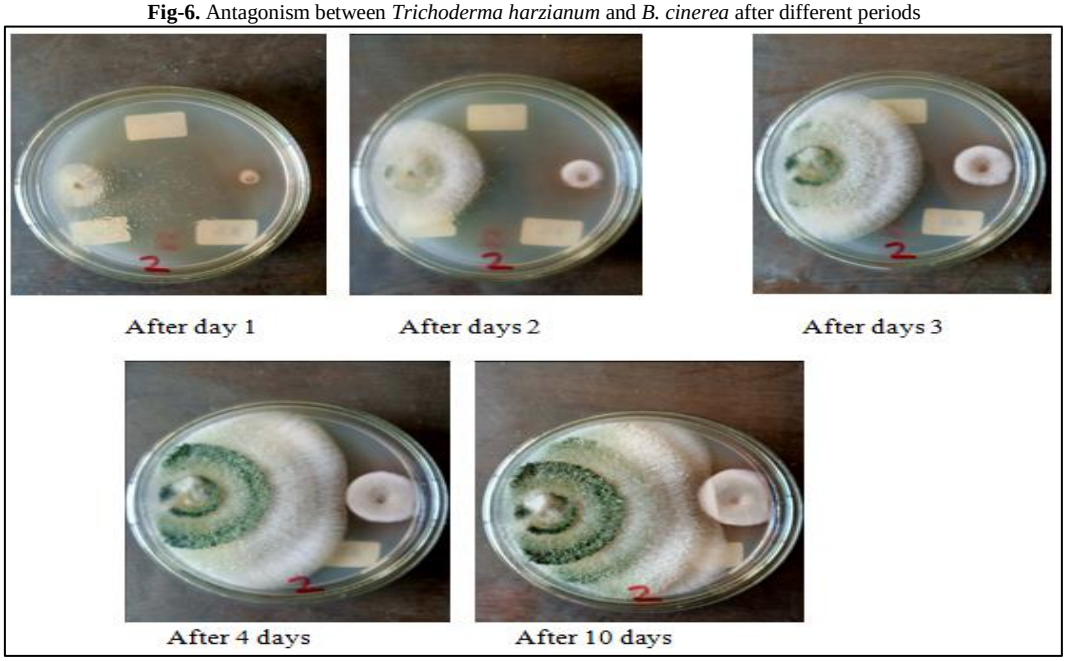



**Fig-6.** Antagonism between *Trichoderma harzianum* and *B. cinerea* after different periods




## 3.3. Phenolic Compounds

Chemical analyses were carried out on three strawberry cultivars that differ in their reaction to leaf spot diseases to focus the light on the dynamic nature of disease resistance. The importance of a biochemical study of defense reaction in the physiology of disease resistance is widely accepted. Phenolic compounds in plants as considered, by many authors, as major agents of chemical resistance against disease incidence [19-21]. Uninoculated strawberry

plants (control) in Table 3 indicate that the levels of total phenol (7.99 and 7.80), Free (6.63 and 6.52), and conjugated Phenols (1.36 and 1.28) were higher in resistant strawberry cultivars Fortuna and Winter star as compared with susceptible one, Festival. These results agree with those of many workers with host-pathogen combinations [20]. Inoculation with leaf spot causal pathogens (*Alternaria alternata*, *Botrytis cinerea*) led to a rapid increase in total, free, and conjugated phenols in the resistant cultivars than in the susceptible ones [21]. However, Farkas and kiraly [22] 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 [23] and/or the acceleration of accumulative phenols from neighboring cells [24]**.**

Data in Table 3 and Fig.7 indicate that the inoculation of Fortuna and Winter Star (resistant strawberry cultivars) with either ***Alternaria alternata*** or ***Botrytis cinerea*** caused a noticed increase in total phenols, free, and conjugated phenolic compounds in comparison with the susceptible cultivar (festival). On the other hand, inoculation with ***Alternaria alternata*** or ***Botrytis cinerea*** caused a clear increase in total, free, and conjugated phenolic compounds in both resistant and susceptible cultivars in comparison with uninoculated plants. It was noticed from Table 4 and Fig.6 that inoculation with leaf spot pathogens led to a rapid increase in total, free, and conjugated phenolic compounds in the resistant cultivars i.e., Fortuna and Winter Star more than in the susceptible cultivar Festival. However, data in Table 3 and Fig.7 show also that the inoculation with leaf spot pathogens caused a decrease of total, free, and conjugated phenolic compounds in Festival susceptible cultivar in comparison with the resistant ones. Similar results were found by Tomiyama [24] in potato tubers infected with ***Phytophthora infestans***. The decrease in phenolic compounds in the inoculated susceptible cultivar can be attributed to the utilization or splitting by the parasite. Another possibility is the decrease of metabolic activities of the susceptible host by pathogen products within obtained resulting in a reduction of phenolic compounds.

## 3.4. Oxidative Enzymes

The correlation between induced resistance and some biochemical changes in plant tissues like the increased activity of enzymes and the appearance of new polypeptides protein has become a model in the study of plant disease resistance, this biochemical, change become a morton to inducer resistance.

Data in Table 4 and Fig.8 show the content of peroxidase, polyphenol oxidase, and catalase Enzymes on the three used strawberry cultivars. Two resistant (Fortuna and Winter Star) and one susceptible (Festival) before and after inoculations with either *Alternaria alternata* or *Botrytis* cinerea

Data presented in Table 4 and Fig.8 show that higher content of peroxidase, polyphenol oxidase, and catalase enzymes in resistant cultivars (Fortuna and Winter star) in comparison with those in the susceptible cultivar (Festival).

Inoculated Fortuna cultivar with either *Alternaria alternata* or *Botrytis cinerea* showed a 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 *Alternaria alternata* or *Botrytis Cinerea* caused a clear increase in the activity of the three enzymes except in the case of peroxidase enzyme when inoculated with *Alternaria 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 [22]**;** El-Ghamry [25]**;** Abo-Shosha [26] and Rizk [5].

**Table-3.** Phenolic compounds expressed as mg gallic acid equivalents/1g in inoculated and uninoculated three strawberry cultivars.

| **strawberry cultivar / fungus** | **Total phenolic compounds** | **Free phenolic compounds** | **Conjugated phenolic compounds** |
|---|---|---|---|
| Fortuna (Control) | 7.99 | 6.63 | 1.36 |
| Winter star ( Control) | 7.80 | 6.52 | 1.28 |
| Festival (Control) | 4.56 | 3.43 | 1.13 |
| Fortuna + Al. | 16.24 | 11.87 | 4.37 |
| Fortuna + Bo. | 12.97 | 10.87 | 2.10 |
| Winter star + Al. | 26.16 | 17.06 | 9.10 |
| Winter Star + Bo. | 14.31 | 10.95 | 3.36 |
| Festival + Al. | 5.75 | 3.42 | 2.33 |
| Festival + Bo. | 12.87 | 8.77 | 4.10 |
| L.S.D(0.05%) | 0.16 | 0.53 | 0.01 |

Fortuna R
Winter Star R
Festival S

Al. *Alternaria alternata*
Bo. *Botrytis Cinerea*

**Fig-7.** Phenolic compounds expressed as mg gallic acid equivalents/1g in inoculated and uninoculated three strawberry cultivars

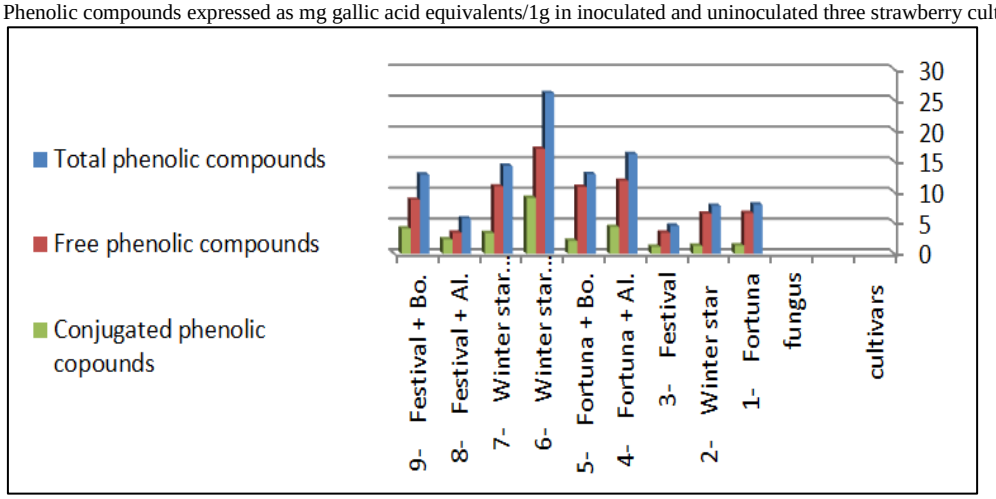


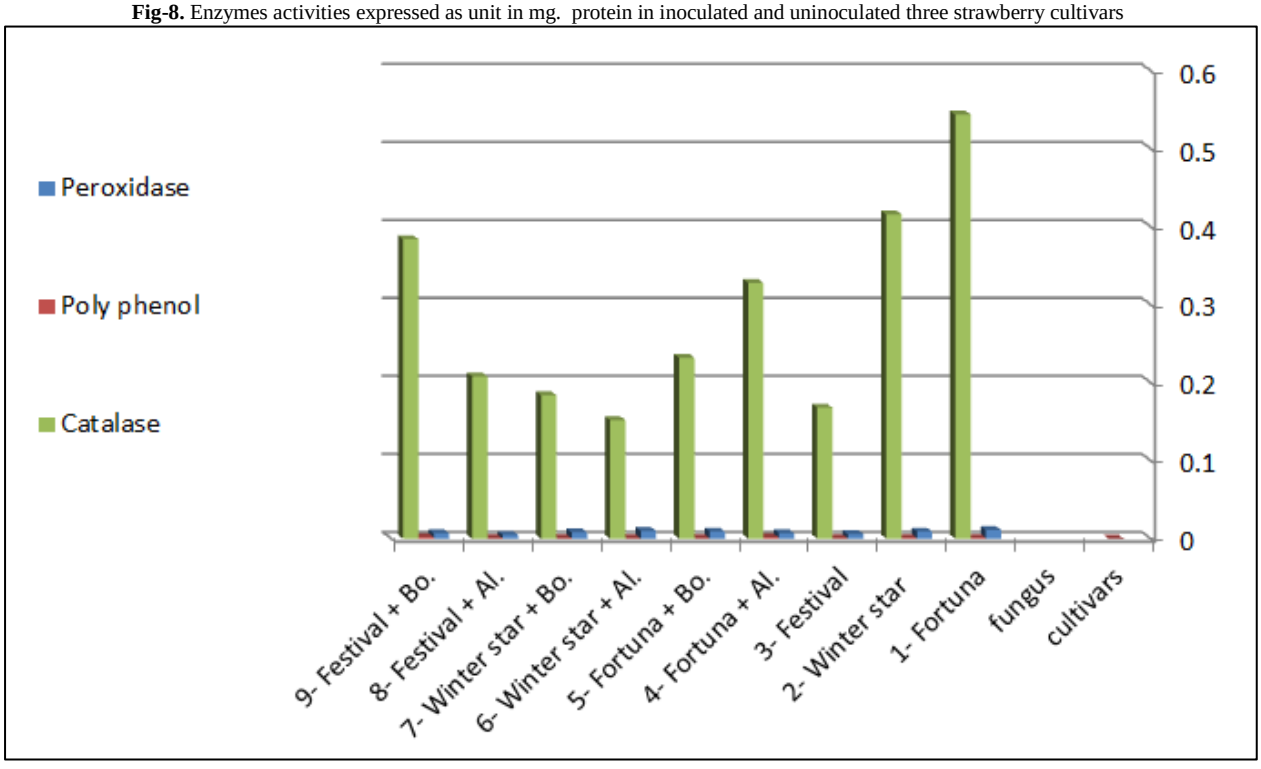

**Table-4.** Enzyme activities expressed as units in mg. protein in inoculated and uninoculated three strawberry cultivars.

| Strawberry cultivar / fungus | Peroxidase | Polyphenol oxidase | Catalase |
|---|---|---|---|
| Fortuna ( Cont.) | 0.011 | 0.0009 | 0.544 |
| Winter star ( Cont.) | 0.009 | 0.0009 | 0.416 |
| Festival ( Cont.) | 0.006 | 0.0005 | 0.168 |
| Fortuna + Al. | 0.007 | 0.0016 | 0.328 |
| Fortuna + Bo. | 0.009 | 0.0005 | 0.232 |
| Winter star + Al. | 0.010 | 0.0004 | 0.152 |
| Winter star + Bo. | 0.008 | 0.0008 | 0.184 |
| Festival + Al. | 0.005 | 0.0006 | 0.208 |
| Festival + Bo. | 0.007 | 0.0020 | 0.384 |
| L.S.D ( 0.05%) | 0.065 | 0.040 | 0.012 |

Fortuna R
Winter Star R
Festival S

Al. *Alternaria alternate*.
Bo. *Botrytis cinerea*.

**Fig-8.** Enzymes activities expressed as unit in mg. protein in inoculated and uninoculated three strawberry cultivars




## 3.5. Induced Resistance to Controlling Strawberry Leaf Spot Disease

Recently, because the food safety issues and environmental concerns, the use of synthetic chemicals in the technology of horticultural crops is highly restricted and much more research has been focused on generally regarded

as safe compounds and alternative strategy to fungicides. The antioxidant systems in plants included antioxidants such as Peroxidase, polyphenol oxidase, and catalase enzymes were studied [27].

**Table-5.** Effect of chemical inducers on phenolic compounds as mg. gallic acid equivalents/1g. DW

| No. | Treatment | Chemical inducers | Total phenols | Free phenols | Conjugated phenols |
|---|---|---|---|---|---|
| **1** | Control | Without | 7.99 | 6.63 | 1.36 |
| **2** | Control | Without | 7.80 | 6.52 | 1.28 |
| **3** | Control | Without | 4.56 | 3.43 | 1.13 |
| **4** | V1 Al. | Potassium di-phosphate ($K_2HPO_4$) | 18.28 | 12.28 | 8.00 |
| **5** | V1 Al. | Oxalic acid (OX) | 11.28 | 8.60 | 2.68 |
| **6** | V1 Al. | Ferrous Sulfate ( $Feso_4$) | 14.92 | 8.25 | 6.67 |
| **7** | V1 Bo. | Potassium di-phosphate ($K_2HPO_4$) | 13.09 | 10.61 | 2.48 |
| **8** | V1 Bo. | Oxalic acid (OX) | 12.85 | 8.50 | 4.35 |
| **9** | V1 Bo. | Ferrous Sulfate ( $Feso_4$) | 10.61 | 9.65 | 0.96 |
| **10** | V2 Al. | Potassium di-phosphate ($K_2HPO_4$) | 11.01 | 7.38 | 3.63 |
| **11** | V2 Al. | Oxalic acid (OX) | 13.94 | 9.25 | 4.69 |
| **12** | V2 Al. | Ferrous Sulfate ( $Feso_4$) | 20.29 | 12.03 | 8.26 |
| **13** | V2 Bo. | Potassium di-phosphate ($K_2HPO_4$) | 14.99 | 11.24 | 3.75 |
| **14** | V2 Bo. | Oxalic acid (OX) | 14.87 | 11.71 | 3.16 |
| **15** | V2 Bo. | Ferrous Sulfate ( $Feso_4$) | 9.06 | 3.86 | 5.20 |
| **16** | V3 Al. | Potassium di-phosphate ($K_2HPO_4$) | 6.20 | 4.56 | 1.64 |
| **17** | V3 Al. | Oxalic acid (OX) | 5.78 | 4.26 | 1.52 |
| **18** | V3 Al. | Ferrous Sulfate( $Feso_4$) | 5.99 | 3.94 | 2.05 |
| **19** | V3 Bo. | Potassium di-phosphate ($K_2HPO_4$) | 9.74 | 8.71 | 6.03 |
| **20** | V3 Bo. | Oxalic acid (OX) | 7.64 | 3.39 | 4.25 |
| **21** | V3 Bo. | Ferrous Sulfate ( $Feso_4$) | 7.29 | 5.40 | 1.89 |
| L.S.D (0.05%) | | | 0.16 | 0.53 | 0.01 |

V1= Fortuna Cultivar
V2= Winter Star Cultivar
V3= Festival Cultivar
Al.= *Alternaria alternata*
Bo.= *Botrytis cinerea*

**Fig-9.** Effect of chemical inducers on phenolic compounds as mg. gallic acid equivalents/1g. DW.

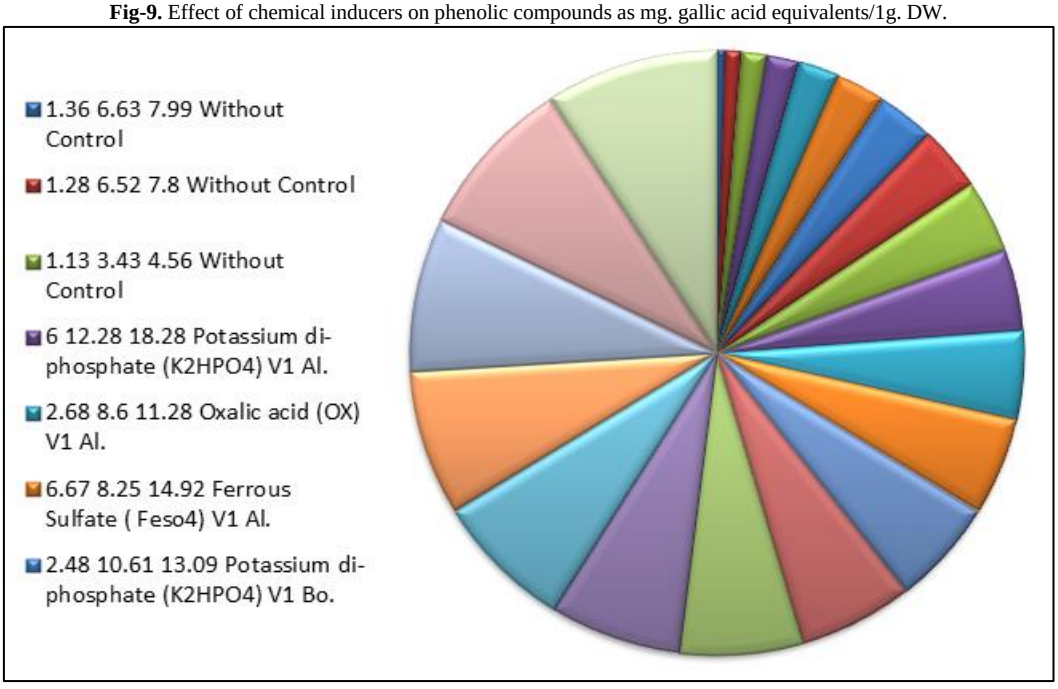


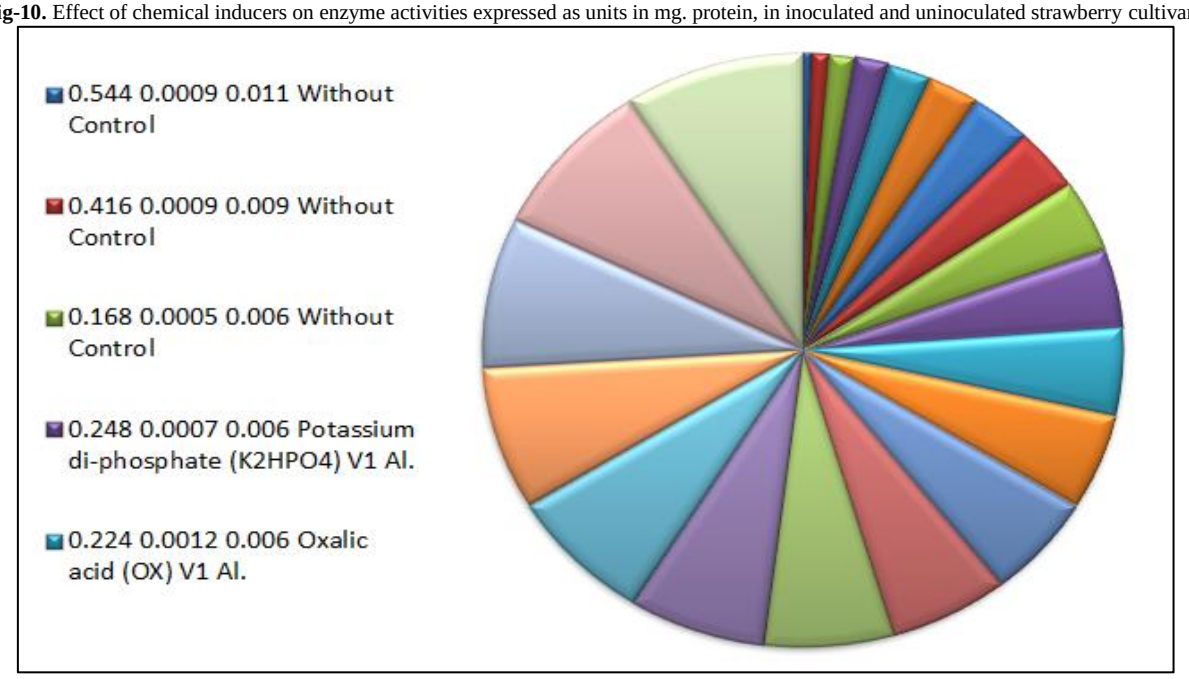
**Table-6.** Effect of chemical inducers on enzyme activities were expressed as units in mg. protein, in inoculated and uninoculated strawberry cultivars.

| No. | Sample | Chemical inducers | Peroxidase | Polyphenol Oxidase | Catalase |
|---|---|---|---|---|---|
| **1** | Control | Without | 0.011 | 0.0009 | 0.544 |
| **2** | Control | Without | 0.009 | 0.0009 | 0.416 |
| **3** | Control | Without | 0.006 | 0.0005 | 0.168 |
| **4** | V1 Al. | Potassium di-phosphate ($K_2HPO_4$)) | 0.006 | 0.0017 | 0.298 |
| **5** | V1 Al. | Oxalic acid (OX) | 0.006 | 0.0012 | 0.224 |
| **6** | V1 Al. | Ferrous Sulfate( $Feso_4$) | 0.012 | 0.0008 | 0.200 |
| **7** | V1 Bo. | Potassium di-phosphate ($K_2HPO_4$) | 0.021 | 0.0009 | 0.288 |
| **8** | V1 Bo. | Oxalic acid (OX) | 0.020 | 0.0006 | 0.016 |
| **9** | V1 Bo. | Ferrous Sulfate ( $Feso_4$) | 0.016 | 0.0013 | 0.160 |
| **10** | V2 Al. | Potassium di-phosphate ($K_2HPO_4$) | 0.005 | 0.0007 | 0.200 |
| **11** | V2 Al. | Oxalic acid (OX) | 0.006 | 0.0008 | 0.224 |
| **12** | V2 Al. | Ferrous Sulfate ( $Feso_4$) | 0.010 | 0.0009 | 0.232 |
| **13** | V2 Bo. | Potassium di-phosphate ($K_2HPO_4$) | 0.012 | 0.0008 | 0.280 |
| **14** | V2 Bo. | Oxalic acid (OX) | 0.004 | 0.0007 | 0.304 |
| **15** | V2 Bo. | Ferrous Sulfate ( $Feso_4$) | 0.005 | 0.0007 | 0.240 |
| **16** | V3 Al. | Potassium di-phosphate ($K_2HPO_4$) | 0.006 | 0.0012 | 0.144 |
| **17** | V3 Al. | Oxalic acid (OX) | 0.003 | 0.0011 | 0.200 |
| **18** | V3 Al. | Ferrous Sulfate ( $Feso_4$) | 0.011 | 0.0010 | 0.168 |
| **19** | V3 Bo. | Potassium di-phosphate ($K_2HPO_4$) | 0.005 | 0.0012 | 0.232 |
| **20** | V3 Bo. | Oxalic acid (OX) | 0.006 | 0.0011 | 0.212 |
| **21** | V3 Bo. | Ferrous Sulfate ( $Feso_4$) | 0.006 | 0.0009 | 0.248 |
| L.S.D (0.05%) | | | 0.067 | 0.038 | 0.015 |

V1= Fortuna Cultivar
V2= Winter Star Cultivar
V3= Festival Cultivar

Al.= Alternaria alternata
Bo .= *Botrytis cinerea*

**Fig-10.** Effect of chemical inducers on enzyme activities expressed as units in mg. protein, in inoculated and uninoculated strawberry cultivars.




Data in Table 5 and Fig. 9 show phenolic compounds, total, free, and conjugated phenols. The three cultivars differed greatly with respect to the amounts of phenols in the leaves of the plants.

Total phenols were found with higher amounts in leaves of the resistant cultivars Fortuna and Winter star (7.99 and 7.80) than the susceptible one (4.56). Generally, the resistant cultivars contained higher amounts of total phenols than the susceptible cultivars (Festival). It was found also that, the inducers resistance increased the number of total phenols in resistant as well as in susceptible ones (18.28, 20.29, and 9.74 by adding potassium di-phosphate, ferrous sulfate, and potassium di-phosphate) respectively. Data, also, show that infection with *B. cinerea* increased higher amounts of free phenols in resistant cultivars than in the susceptible ones, especially with the Winter Star cultivar.

Concerning free phenols, Table 5 and Fig. 9 exhibited that the leaves of resistant cultivars contained the highest number of free phenols compared with the other cultivars. Generally, free phenols were found to be higher inducers resistance than the control by adding (potassium di-phosphate, ferrous sulfate, and potassium di-phosphate) The accumulation of free phenols due to chemical inducers, was higher in the resistant cultivars than the susceptible ones (12.28,12.03 and 8.71) respectively.

Conjugated Phenols accumulated faster after using chemical Inducers in the leaves of the resistant cultivars than the susceptible ones. (8.00,8.26 and 6.03) by adding potassium di-phosphate, ferrous sulfate, and potassium di-phosphate) respectively. These results are in agreement with the finding of Ahmed, *et al.* [28] who found that trending cucumber seeds with *T. viride* or *B. subtilis* or citric acid increased the total phenols content in cucumber sown in soil inoculated with *Fusarium oxysporum* compared with untreated plants. Kessler, *et al.* [29]**,** mentioned that the antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals or chelating process.

Data in Table 6 and Fig.10 show that the chemical inducers significantly reduced the activity of peroxidase, polyphenol oxidase, and catalase compared with control. In most cases the resistant cult. i.e., Fortuna and Winter star exhibited higher activity of peroxidase (0.021) and (0.20) by using potassium di-phosphate and oxalic acid than the susceptible cultivar Festival (0.011) and (0.006) with Ferrous sulphate on ***A. alternata*** and ***B. cinerea.***

Results presented in Table 6 show that polyphenol oxidase activity was increased by adding the chemical inducers (potassium di-phosphate and Ferrous sulphate in a resistant cultivar (0.0017and 0.0013) and susceptible cultivars (0.012)

Data presented in Table 6 and Fig.10 show that catalase activity was higher in leaves of the tested cultivars and it is clear that enzyme activity was higher in the resistant cv., than susceptible one (0.298, 0.304, and 0.168) respectively. Data, also show that infection with *B. cinerea* exhibited higher Catalase, especially in the resistant cult. Fortuna and Winter Star (0.288 and 0.304) than in the susceptible cult. Festival. (0.248) by adding Ferrous sulphate Shoresh, *et al.* [18]**;** Gruenzweig, *et al.* [30]**.**

## 4. Conclusion

Oxidative enzymes, like the increased activity of enzymes that appear mince of new polypeptide protein, have become models in the study of plant disease resistance. The higher content of peroxidase, polyphenol oxidase, and catalase enzymes in resistant cultivars, were noticed compared with those in susceptible ones. It was found also that, the chemical inducers increased total phenols in resistant and susceptible cultivars i.e., potassium di-phosphate, ferrous sulphate, and oxalic acid. Inducer resistance also caused an increase of free phenols than the control. However, conjugated phenols accumulated faster after using chemical inducers in the leaves of the resistant cultivars, than the susceptible ones.

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## الملخص العربي

### فعالية استخدام المستحثات الكيماوية والعوامل البيولوجية في مكافحة مرض تبقع أوراق الفراولة على المكونات الكيمائية والنشاط الانزيمي في الأوراق

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يعتبر محصول الفراولة من أهم محاصيل الخضر الاقتصادية سواء لغرض الاستهلاك المحلي أو التصدير. ويتعرض محصول الفراولة لكثير من الأمراض الفطرية ومن اهمها مرض تبقع الأوراق الذي يسببه فطري ألترناريا ألترناتا و بوترايتس سينيريا مما يسبب خسائر فادحة في المحصول لتأثيره المدمر علي الأوراق الخضراء.

وقد تم عمل حصر لهذا المرض في محافظتي الشرقية ( مركزي بلبيس والصالحية ) والإسماعيلية ( مركزي القصاصين وأبو صوير ) وحسب إحصائية وزارة الزراعة لموسم 2020 وجدت مساحة الفراولة بالجمهورية 31606 فدان وإنتاجية 539482 طن ووجد منها مساحة 2581 فدان وإنتاجية 41162 طن في محافظة الشرقية ومساحة 6958 فدان بإنتاجية 127688 طن في محافظة الإسماعيلية.

تم إجراء اختبار القدرة الامراضية للطريات المعزولة من الأوراق المصابة ووجد أن الفطر ألترناريا ألترناتا أكثرها في شدة الإصابة يليه الفطر بوترايتس سينيريا وقد تم استخدام فطر ترايكودرما هارزيانم في تجارب التضاد مع الفطرين المذكورين واتضح من خلال النتائج التأثير المثبط من للترايكودرما على النمو الميسليومي لفطر الألترناريا وفطر البوترايتس .

تم عمل التحليل الكيماوي للمركبات الفينولية الكلية والحرة والمرتبطة للأصناف المقاومة والقابلة للإصابة من الفراولة وكذلك الإنزيمات المؤكسدة من البيروكسيديز والبولي فينول اكسيديز والكاتاليز واتضح من النتائج أن محتوي الأصناف المقاومة من المركبات الفينولية الكلية والحرة والإنزيمات المؤكسدة من البيروكسيديز والبولي فينول اكسيديز كانت أعلي من محتوي الأصناف القابلة للإصابة كما وجد أن تراكم المواد الفينولية كان بصورة أسرع في أوراق النباتات المقاومة عن القابلة للإصابة. تم استخدام المستحثات الكيماوية مثل ثيوكبريتات البوتاسيوم وسلفات الحديدوز واكساليك آسيد واتضح زيادة الفينولات الحرة في الأصناف المقاومة والقابلة للإصابة ووجدت زيادة الفينولات الحرة في الأصناف المقاومة عن الأصناف القابلة للإصابة كما وجد أن الفينولات المرتبطة تتراكم بطريقة أسرع في الأصناف المقاومة عن القابلة للإصابة .