Journal of Phytopathology and Pest Management 5(3): 55-66, 2018
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

# Potential resistance of certain sunflower cultivars and inbred lines against charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid

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**Abstract**

**In this study, fourteen sunflower cultivars and inbred lines (Giza 102, Sakha 53, Enosa, Bozoloke, $L_7$, $L_{16}$, $L_{22}$, $L_{26}$, $L_{35}$, $L_{36}$, $L_{46}$, $L_{49}$, $L_{60}$ and $L_{63}$) were evaluated for their resistance to charcoal rot disease. Obtained results indicated that, Giza 102 and Sakha 53 cultivars gave the lowest percentage of charcoal rot disease severity. While, inbred line $L_{46}$ showed the highest percentage of charcoal rot disease severity. The highest amount of total phenol contents was found in infected plants of the most resistant sunflower cultivars Giza 102 and Sakha 53. While, the most susceptible line $L_{46}$ showed the lowest amount of total phenol contents. Furthermore, the highest level of all determined enzymes activity Catalase, Peroxidase and polyphenoloxidase, were found in infected plants of the most resistant sunflower cultivars Giza 102 and Sakha 53. However, the lowest activity of enzymes was found in infected of the highest susceptible sunflower inbred line $L_{46}$. The results of this study indicated that cultivated resistant cultivars are effective and economical method for controlling charcoal rot disease of sunflower.**

**Keywords:** Enzymes, charcoal rot, *Macrophomina phaseolina*, phenol contents, sunflower.

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## Introduction

Sunflower (*Helianthus annus* L.) is one of the most important oil seed crops in Egypt and many other countries. Charcoal rot disease caused by the soil borne pathogenic fungus *Macrophomina phaseolina* is one of the serious diseases of sunflower growers in Egypt and all over the world (Jalil et al., 2013; Aboshosha et al., 2007; Khan, 2001; Mehdi et al., 1988). This fungal pathogen causes seedling blight, stem rot and charcoal rot. *M. phaseolina* is reports as the most fungal pathogens causing the charcoal rot disease on more than 500 plant species (Purkayastha et al., 2006). No chemical control currently exists for management of charcoal rot disease, and plant resistance for such disease has been hard to identify. Due to soil borne nature of the pathogen, control strategies other than host resistance are not much effective and economical. A yield loss claimed by charcoal-rot in many courtiers has been recorded up to 25% under favorable conditions for the growth and development of *M. phaseolina* (Jimenez et al., 1983; Tikhonov et al., 1976; Orellana, 1971). Planting resistant sunflower mixed cultivars reducing the effects of charcoal rot disease caused by *Macrophomina phaseolina*, compared to the same cultivars planted individually (Mahtab et al., 2013; Muhammad et al., 2011; Aboshosha et al., 2008). Development of resistant varieties is one of the most important methods for management of charcoal rot disease on sunflower (Aboutalebi et al., 2014). It is the cheapest source for management. Therefore, this work was planned for screening certain sunflower cultivars and inbred lines against charcoal rot disease.

## Materials and methods

**Collection of sunflower cultivars and inbreed lines:** Four cultivars and ten inbreed lines were used in this study, two Egyptian commercial sunflower cultivars (Giza 102 and Sakha 53) were obtained from (Agricultural Research and Experiments Center, Giza, Egypt) and two Russian sunflower varieties (Enosa and Bozolok) obtain from Russia as samples. Ten inbreed lines ($S_5$) obtained from Agronomy Department, Faculty of Agriculture, Assiut University, Egypt. Inbred lines were obtained from selfing of open pollinated cultivar "Maiak".

**Causal pathogen:** *Macrophomina phaseolina* (Tassi) Goid was isolated from naturally diseased sunflower plants, showing typical symptoms of charcoal rot disease during 2013 growing season from different fields in Assiut Governorate. Identification of the pathogen was carried out on 5-7 days old culture using the morphological and microscopic characteristics of mycelium and spores according to Booth (1977), Pitt (1979), Domsch et al. (1980) and Mahmoud and Budak (2011) and confirmed by Mycological Center, Faculty of Science, Assiut University, Egypt.

**Disease assessment:** Pre-emergence damping-off % was estimated by counting the number of the non-germinated seeds (2 weeks post-sowing). Post-emergence damping-off% was estimated by counting the number of damping-off seedlings (4 weeks post-sowing). Disease severity of charcoal rot was assessed through visual observation and symptoms on plants showing symptoms of charcoal rot at the end of the experiment (afusing numerical grades ranging from (1 to 10) according to

Smith and Carvil (1997) and Mahmoud et al. (2018) as follows:

(0)= No symptoms,
(1)= 0 ≤ 10 % stem infected area,
(2)= 10 ≤ 20 % stem infected area,
(3)= 20 ≤ 30 % stem infected area,
(4)= 30 ≤ 40 % stem infected area,
(5)= 40 ≤ 50 % stem infected area,
(6)= 50 ≤ 60 % stem infected area,
(7)= 60 ≤ 70 % stem infected area,
(8)= 70 ≤ 80 % stem infected area,
(9)= 80 ≤ 90 % stem infected area,
(10)= 90 ≤ 100 % stem infected area or dead plant.

$$\text{Disease severity} = \frac{\sum[(N \times 0) + (N \times 1) + \ \ldots\ldots\ (N \times 10)]}{\text{Maximum possible score x of stem evaluated}} \times 100$$

Where: (N) = number of plants in each group of diseased plants; and (0, 1, 2 ...10) = numerical grades of diseased plants.

**Greenhouse experiment:** Experiment was carried out in 2015 growing season at the greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University, Egypt. Sterilized pots (35 cm in diameter) were filled with sterilized sandy -loam soil which mixed thoroughly with the inoculums of *M. phaseolina* at the ratio of 2% of soil weight, then pots were irrigated. Soil infestation was carried out 7 days before seed sowing. Seed disinfestations were carried out by dipping the seeds in sodium hypochlorite solution (0.2%) for 2 minutes then rinsed several times with sterilized water. Inoculums of the pathogen was prepared by inoculating sterilized 1000 ml conical flasks containing barley medium (150g barley + 4g glucose + 200ml water) with *M. phaseolina* and incubated at 28 ±2°C for two weeks. Each pot was planted with 10 sunflower seeds for each cultivar. Four pots were used as replicates. Pots containing non infested soil mixed with 2% sterile barley medium were used as a control. The plants were irrigated when necessary and daily observed for infection progress. Percentages of survival plants were recorded after 2 and 4 weeks from sowing (as pre- and post-emergence damping-off, respectively). At the end of the experiment disease severity was assessed.

**Field experiment:** The experiment was carried out in Assiut University Farm, Assiut, Egypt during 2016 and 2017 growing seasons. The soil was divided into plots (plot = 3× 2.1 m). Each plot contained 4 rows, 60 cm a part. Each row contained 6 hills spaced 20 cm. Every hill was infested with the inoculum of *M. phaseolina* by adding 50 g/hill then covered with soil and irrigated at the same time. The inoculum was prepared as mentioned before in greenhouse experiment. After 7 days, every hill was sown with 5 sterilized seeds of each cultivar and inbred and covered with soil. After sowing all plots were irrigated. Plots containing non infested sterile barley medium (50g/hill) were used as control. Randomize complete block design with four replicates were used for each treatment. Percentages of survival plants were recorded after 2 and 4 weeks from sowing. At harvest time (12 weeks from planting) disease severity of charcoal rot was assessed.

**Determination of total phenol and salicylic acid contents in sunflower cultivars and inbreed lines, due to**

**infection by *M. phaseolina*:** Leaves of sunflower cultivars and inbred line, (infected and uninfected of two weeks old plants), were immersed in liquid N2, homogenized in 80% methanol (one gram plant material in 10 mL) and stored in the deep-freeze (-20°C) later, the homogenate were centrifuged at 1000 rpm for 30 min at 4°C, the pellet was discarded after addition of ascorbic acid (0.1 gm. for 5 mL) and the homogenates were evaporated in rotary evaporator at 65°C and repeated 3 times for 5 min. The residues were dissolved in 5 mL of 80% methanol. Four replicates were used each treatment (Rapp & Zeigler, 1973). Phenol contents were determined using the method described by Sahin et al. (2004) as follows: The reaction mixture was consisted of 0.02 methanol extract, 0.5 mL folin-ciocalteau reagent, 0.75 mL of $Na_2CO_3$ solution (20%) and 8 mL water. The mixture incubated for one hr. at 37°C in water bath. Methanol was used as blank. Total phenol contents were assayed spectrophotometrically at 767 nm as mg/g plant fresh weight. Gallic acid was used (0-5 mg) as a standard curve. Total phenol = mg Gallic acid / g plant material. Salicylic acid contents were determined using the method described by Dat et al. (1998) as follows: Sample 500 µL homogenate were mixed with 250 µL HCl (10N) and 1000 µL methanol. Sample were incubated in a water bath at 80°C for 2 h. neutralized with 4-5 drops 1 M $NaHCO_3$ and 1000 µL methanol were added. The OD was measured at 254 nm to calculate the content of salicylic acid and expressed as: Amount of total salicylic acid = µg/g plant material.

**Determination of oxidative enzymes:** For determination of oxidative enzymes (peroxidase, polyphenenoloxidase and catalase) leaves of sunflower cultivars and inbred line, (infected and uninfected of two weeks old plants), were treated with Liquid $N_2$ and homogenized with 0.1 M Na-acetate buffer (pH 5.2) (for one gram plant fresh weigh to10 mL buffer), centrifuged at 1000 rpm for 30 min at 4°C and the oxidative enzymes were determined in the supernatants. Four replicates were used for each treatment.

**Determination of peroxidase activity (PO):** Peroxidase activity was determined using the method descried by (Putter, 1974) as follows: Peroxidase activity was determined spectrophotometrically using quaiacol as common substrate for peroxidases. The reaction mixture was as follows: 0.2 mL supernatant, 1 ml 0.1 M Na-acetate-buffer PH 5.2, 0.2 mL 1% guaiacol and 0.2 mL 1% $H_2O_2$. The mixture was incubated at 25°C for 5 min and then measured at 436 nm. Extraction buffer was used as blank. Enzyme activity was calculated according to the change in absorbency and was expressed as enzyme in 1 mg protein.

**Determination of polyphenoloxidase activity (PPO):** Polyphenol oxidase activity was determined using the method described by Batra and Kuhn (1975). The reaction mixture was as follows: 0.5 mL supernatant, 2 mL 50 mM sorensen phosphate buffer pH 6.5 (preparation of Sorensen phosphate buffer, 6.8 gm. $KH_2PO_4$ with 8.99 gm. $Na_2HPO_42H_2O$ solved in 1000 ml water and 0.372 gm. EDTA was added the pH adjusted to 6.5) and 0.5 mL substrate

Brenzcatechol (sigma Aldrich) at 37°C for 2 hours and measured at 410 nm. PPO activity = OD 410 nm/mg protein.

**Determination of catalase activity:** Catalase activity was determined spectrophotometrically (Aedi, 1984). The homogenate of 0.5 mL for supernatant were mixed with 3 mL from Sorensen phosphate buffer pH 7.0 and 200 µL $H_2O_2$ 30%. Sorensen phosphate buffer was used as blank. Catalase activity was determined spectrophotometrically using spectrophotometer unicam UV calculated at $OD_{240}$ nm. The enzyme activity was expressed as changes in 240/mg protein/min.

**Statistical analysis:** The results were analyzed using ANOVA test and the means differences were regarded as significant using LSD test at 5% level of probability according to SAS software (SAS Institute, 1996).

## Results

**Reactions of certain sunflower cultivars and inbred lines to infection with charcoal rot disease caused by *Macrophomina phaseolina* under greenhouse conditions:** The results of this study are presented in Table (1). All tested sunflower cultivars and inbred lines were infected by *M. phaseolina*. The susceptibility of the tested cultivars to the disease is variable and varied according to the tested cultivars and inbred lines. There are a little difference in the rate of susceptibility between the tested sunflower cultivars and inbred lines. In both tested season, sunflower cultivars Giza 102 and Sakha 53, produce the highest percentages of survival plants (90% and 95%, respectively) in pre- and post- emergence damping-off as well as the lowest percentages of charcoal rot disease severity (10% for both cultivar). Whereas, inbred line $L_{46}$ was the most susceptible one and produced the lowest percentages of survival plants (40%) in pre- and post- emergence damping-off as well as the highest percentages of charcoal rot disease severity (70%). Other tested inbred lines ($L_{49}$, $L_{16}$, Bozolok, Enosa, $L_7$, $L_{63}$, $L_{60}$, $L_{35}$, $L_{36}$ and $L_{26}$) proved to be moderate resistant lines. Whereas, inbred line ($L_{22}$) showed a moderate susceptible reaction.

Table 1: Reactions of certain sunflower cultivars and inbred lines to infection with charcoal rot disease caused by *Macrophomina phaseolina* under greenhouse conditions.

| Cultivars/ Inbred lines | Survival plants relative to control after 2 week (%) | Survival plants relative to control after 4 week (%) | Charcoal rot disease severity (%) |
|---|---|---|---|
| Sakha 53 | 95 | 95 | 10 |
| Giza 102 | 90 | 90 | 10 |
| Bozolok | 70 | 70 | 30 |
| Enosa | 80 | 80 | 30 |
| $L_7$ | 85 | 85 | 35 |
| $L_{16}$ | 75 | 75 | 20 |
| $L_{22}$ | 55 | 55 | 60 |
| $L_{26}$ | 80 | 80 | 45 |
| $L_{35}$ | 70 | 70 | 45 |
| $L_{36}$ | 70 | 70 | 55 |
| $L_{46}$ | 40 | 40 | 70 |
| $L_{49}$ | 80 | 80 | 15 |
| $L_{60}$ | 75 | 75 | 40 |
| $L_{63}$ | 85 | 85 | 30 |
| L.S.D. $_{0.05}$ | 17.75 | 17.75 | 22.35 |

**Reactions of certain sunflower cultivars to infection with charcoal rot disease caused by *Macrophomina phaseolina* in the field:** Data presented in Table (2) showed that tested sunflower cultivars and inbred lines were varied in their resistance to infection with *Macrophomina phaseolina*. In both tested season sunflower cultivars Giza 102 and Sakha 53, showed the highest percentages of survival plants (90% for both cultivar) in pre- and post-emergence damping-off as well as the lowest percentages of charcoal rot disease severity (25% and 10% respectively). Whereas, inbred lines $L_{46}$ and $L_{22}$ produce the lowest percentages of survival plants (30% and 40%, respectively) in pre- and post- emergence damping-off as well as the highest percentages of charcoal rot disease severity (70% and 65% respectively). Inbred lines ($L_{49}$, $L_{16}$, Bozolok, Enosa, $L_7$, $L_{63}$ and $L_{60}$) proved to be moderate resistant lines. While, inbred lines ($L_{26}$, $L_{35}$ and$L_{36}$) showed an intermediate effect.

**Total phenol and salicylic acid contentsin sunflower cultivars and inbreed lines, due to infection by *M. phaseolina*:** Data in Table (3) indicate that each tested sunflower cultivars or inbred lines showed higher amount of total phenol contents in infected plants with *M. phaseolina* than uninfected plants. The highest amount of total phenol contents was found in infected plants of sunflower inbred line $L_{60}$ followed by Giza 102. While, $L_{46}$ inbred line gave lower amount of total phenol contents in infected plants than $L_{16}$ inbred line. Data in Table (4) showed that infected plants in each tested cultivars or inbred lines with *M. phaseolina* produced higher salicylic acid activity than uninfected plants (control). Infected plants of $L_{49}$ inbred line showed the highest amount of salicylic acid followed by inbred line $L_7$. The lowest salicylic acid activity was found in infected plants of inbred line $L_{46}$.

Table 2: Reactions of certain sunflower cultivars to infection with charcoal rot disease caused by *Macrophomina phaseolina* in the field during 2016 and 2017 growing.

| Cultivars/ Genotypes | Survival plants relative to control after 2 week (%) | | Survival plants relative to control after 2 week (%) | | Charcoal rot disease severity (%) | |
|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| Sakha 53 | 90 | 95 | 90 | 95 | 10 | 8.00 |
| Giza 102 | 90 | 95 | 90 | 95 | 25 | 8.00 |
| Bozolok | 70 | 65 | 70 | 65 | 30 | 37.50 |
| Enosa | 70 | 65 | 70 | 65 | 30 | 35.00 |
| $L_7$ | 65 | 65 | 65 | 65 | 35 | 36.25 |
| $L_{16}$ | 80 | 70 | 80 | 70 | 35 | 33.75 |
| $L_{22}$ | 40 | 45 | 40 | 45 | 65 | 58.75 |
| $L_{26}$ | 55 | 50 | 55 | 50 | 45 | 51.25 |
| $L_{35}$ | 55 | 50 | 55 | 50 | 45 | 52.50 |
| $L_{36}$ | 45 | 45 | 45 | 45 | 55 | 60.00 |
| $L_{46}$ | 30 | 30 | 30 | 30 | 70 | 73.75 |
| $L_{49}$ | 85 | 70 | 85 | 70 | 40 | 35.50 |
| $L_{60}$ | 60 | 40 | 60 | 40 | 40 | 63.50 |
| $L_{63}$ | 70 | 55 | 70 | 55 | 30 | 50.50 |
| L.S.D. 0.05 | 19.35 | 17.06 | 19.35 | 17.06 | 31.83 | 17.69 |

Table 3: Determination of total phenol contents in healthy and infected plants of sunflower cultivars and inbred lines growing under greenhouse conditions.

| Cultivars/ inbred lines | Total phenol contents (mg Gallic acid / g Fresh weight) | | |
|---|---|---|---|
| | Infected | Non infected | Mean |
| Sakha 53 | 5.280 | 5.240 | 3.760 |
| Giza 102 | 5.959 | 5.598 | 5.779 |
| Bozolok | 5.242 | 5.200 | 5.221 |
| Enosa | 5.179 | 5.141 | 5.160 |
| $L_7$ | 5.140 | 5.079 | 5.109 |
| $L_{16}$ | 2.610 | 2.515 | 2.563 |
| $L_{22}$ | 5.080 | 5.070 | 5.075 |
| $L_{26}$ | 5.180 | 5.178 | 5.179 |
| $L_{35}$ | 5.154 | 5.143 | 5.149 |
| $L_{36}$ | 5.082 | 2.688 | 3.885 |
| $L_{46}$ | 2.480 | 2.420 | 2.450 |
| $L_{49}$ | 5.820 | 5.058 | 5.439 |
| $L_{60}$ | 6.340 | 5.144 | 5.742 |
| $L_{63}$ | 5.482 | 5.260 | 5.371 |

L.S.D. at 5%: Cultivars (A) = 0.014, Infection (B) = 0.005, Interaction (A×B) = 0.019.

Table 4: Determination of salicylic acid in healthy and infected plants of sunflower cultivars and inbred lines growing under greenhouse conditions.

| Cultivars/ inbred lines | Salicylic contents (µg / g plant material) | | |
|---|---|---|---|
| | Infected | Non infected | Mean |
| Sakha 53 | 1.217 | 0.655 | 0.936 |
| Giza 102 | 0.949 | 0.212 | 0.581 |
| Bozolok | 0.541 | 0.459 | 0.500 |
| Enosa | 0.525 | 0.483 | 0.504 |
| $L_7$ | 1.912 | 0.533 | 1.223 |
| $L_{16}$ | 0.541 | 0.386 | 0.464 |
| $L_{22}$ | 0.769 | 0.533 | 0.651 |
| $L_{26}$ | 0.411 | 0.147 | 0.279 |
| $L_{35}$ | 1.380 | 0.586 | 0.983 |
| $L_{36}$ | 0.618 | 0.390 | 0.504 |
| $L_{46}$ | 0.161 | 0.106 | 0.134 |
| $L_{49}$ | 2.117 | 0.219 | 1.168 |
| $L_{60}$ | 0.181 | 0.121 | 0.151 |
| $L_{63}$ | 1.249 | 0.521 | 0.885 |

L.S.D. at 5%: Cultivars (A) = N.S., Infection (B) = N.S., Interaction (A×B) = N.S.

**Peroxidase activity (PO):** Data in Table (5) indicated that each tested sunflower cultivars or inbred lines showed higher amount of peroxidase activity in infected plants with the *M. phaseolina* than uninfected plants. The highest amount of peroxidase activity was found in infected plants of sunflower inbred line $L_{49}$ followed by Giza 102. Inbred line $L_{46}$ gave lower amount of peroxidase activity in infected plants than $L_{60}$ inbred line.

**Polyphenoloxidase activity (PPO):** Data in Table (6) showed that, infected plants in each tested cultivars or inbred lines with *M. phaseolina* produced higher polyphenoloxidase activity compared with uninfected plants. Infected plants of Enosa cultivar showed the highest amount of polyphenoloxidase followed by Sakha 53. The lowest polyphenoloxidase activity was found in infected plants of inbred line $L_{22}$.

Table 5: Determination of peroxidase activity in healthy and infected plants of sunflower cultivars and inbred lines growing under greenhouse conditions.

| Cultivars/ inbred lines | Peroxidase activity (unit / mg protein) | | |
|---|---|---|---|
| | Infected | Non infected | Mean |
| Sakha 53 | 2.935 | 2.164 | 2.888 |
| Giza 102 | 6.465 | 4.106 | 3.803 |
| Bozolok | 1.430 | 0.607 | 1.019 |
| Enosa | 4.328 | 1.922 | 2.798 |
| $L_7$ | 3.674 | 1.058 | 1.776 |
| $L_{16}$ | 3.57 | 1.524 | 2.547 |
| $L_{22}$ | 2.414 | 0.921 | 1.749 |
| $L_{26}$ | 2.282 | 1.448 | 1.865 |
| $L_{35}$ | 0.914 | 0.427 | 1.205 |
| $L_{36}$ | 1.264 | 0.601 | 0.933 |
| $L_{46}$ | 0.147 | 0.020 | 0.084 |
| $L_{49}$ | 8.554 | 1.892 | 4.481 |
| $L_{60}$ | 0.183 | 0.024 | 0.104 |
| $L_{63}$ | 0.888 | 1.892 | 1.832 |

L.S.D. at 5%: Cultivars (A) = 2.586, Infection (B) = 0.977, Interaction (A×B) = N.S.

Table 6: Determination of polyphenoloxidase activity in healthy and infected plants of sunflower cultivars and inbred lines growing under greenhouse conditions.

| Cultivars/ inbred lines | Polyphenoloxidase activity (unit / mg protein) | | |
|---|---|---|---|
| | Infected | Non infected | Mean |
| Sakha 53 | 3.933 | 0.686 | 2.310 |
| Giza 102 | 2.664 | 2.577 | 2.621 |
| Bozolok | 1.680 | 0.675 | 1.178 |
| Enosa | 4.228 | 2.367 | 3.298 |
| $L_7$ | 3.821 | 3.316 | 3.569 |
| $L_{16}$ | 3.428 | 0.831 | 2.130 |
| $L_{22}$ | 0.562 | 1.143 | 0.853 |
| $L_{26}$ | 1.461 | 0.866 | 1.164 |
| $L_{35}$ | 0.902 | 0.583 | 0.743 |
| $L_{36}$ | 1.298 | 0.679 | 0.989 |
| $L_{46}$ | 0.604 | 0.436 | 0.520 |
| $L_{49}$ | 2.553 | 0.279 | 1.416 |
| $L_{60}$ | 1.465 | 0.271 | 1.736 |
| $L_{63}$ | 0.730 | 0.666 | 0.698 |

L.S.D. at 5%: Cultivars (A) = 1.211, Infection (B) = 0.458, Interaction (A×B) = N.S.

Table 7: Determination of catalase activity in healthy and infected plants of sunflower cultivars and inbred lines growing under greenhouse conditions.

| Cultivars/ inbred lines | Catalase activity (unit / mg protein/ min) | | |
|---|---|---|---|
| | Infected | Non infected | Mean |
| Sakha 53 | 3.144 | 2.851 | 2.998 |
| Giza 102 | 3.232 | 3.143 | 3.188 |
| Bozolok | 3.049 | 3.044 | 3.047 |
| Enosa | 3.043 | 2.949 | 2.996 |
| $L_7$ | 3.091 | 3.017 | 3.054 |
| $L_{16}$ | 3.040 | 2.080 | 2.560 |
| $L_{22}$ | 3.146 | 3.145 | 3.146 |
| $L_{26}$ | 3.876 | 2.785 | 3.331 |
| $L_{35}$ | 3.147 | 3.145 | 3.146 |
| $L_{36}$ | 3.150 | 3.045 | 3.098 |
| $L_{46}$ | 2.925 | 2.076 | 2.501 |
| $L_{49}$ | 3.147 | 3.043 | 3.095 |
| $L_{60}$ | 3.144 | 3.037 | 3.091 |
| $L_{63}$ | 3.243 | 3.155 | 3.199 |

L.S.D. at 5%: Cultivars (A) = 0.011, Infection (B) = 0.004, Interaction (A×B) = 4.774.

**Catalase activity (CAT):** Data in Table (7) indicated that, each tested sunflower cultivars or inbred lines showed higher amount of catalase activity in infected plants with the *M. phaseolina* than uninfected plants. The highest amount of catalase activity was found on infected plants of inbred line $L_{26}$ followed by $L_{63}$. While, $L_{46}$ inbred line gave lower amount of catalase activity in infected plants than $L_{16}$ inbred line.

## Discussion

In the present study, we evaluated fourteen sunflower cultivars and inbred lines for infection by *M. phaseolina*. Most of the tested sunflower cultivars or inbred lines (Giza 102, Sakha 53, Enosa, Bozoloke, $L_7$, $L_{16}$, $L_{22}$, $L_{26}$, $L_{35}$, $L_{36}$, $L_{46}$, $L_{49}$, $L_{60}$ and $L_{63}$) were varied in their susceptibility to charcoal rot disease caused by *M. phaseolina*. Obtained results showed that sunflower cultivars Giza 102 and Sakha 53 produced the highest percentages of survival plants in pre- and post- emergence damping-off as well as the lowest percentages of charcoal rot disease severity and they proved to be resistant cultivars. Whereas, $L_{46}$ was the most susceptible inbred line. The resistance in sunflower cultivars can be attributed to increase the content of certain enzymes after infection (Papaiah & Narasimha, 2014; Roldan Serrano et al., 2007; Ramanathan et al., 2001; Kirstensen et al., 1999; Flott et al., 1989). In this study, salicylic acid (SA) concentration, phenolic compounds and the enzymatic activates or peroxidase (PO), polyphenoloxidas (PPO) and catalas (CAT) were determined in both infected and uninfected of sunflower cultivars and inbred lines. The results showed that the highest amount of total phenol contents were detected in the less susceptible sunflower cultivars (Giza 102 and Sakha 53). While, the lowest amount of total phenol contents was detected in inbred line $L_{46}$.Phenolics might play an important role in plant defense, phenols are essential for the biosynthesis of lignin, which consider an important structural component of plant cell walls and most notably phytoalexins. The oxidative enzymes played an important role in plant diseases resistance. Infected plants of sunflower cultivars Giza 102 and Sakha 53 cultivar showed a highest level in all determined enzymes activity (Catales, peroxidase and polyphenoloxidase). However, the lowest activity of enzymes found in susceptible sunflower $L_{46}$ line. The oxidative enzymes played an important role in plant diseases resistance (Saraswathi & Reddy, 2012; Cherif et al., 2007; Yang et al., 2002; Avdiushko et al., 1993). Such results are in line of that reported by Sulman et al. (2001), Mydlarz and Harvell (2007), Aboshosha et al. (2008) and Papaiah and Narasimha (2014). They mentioned that many plants enzymes are involved in defense reaction against plant pathogen. These include oxidative enzymes such as peroxidase, polyphenoloxidase, catales in the formation of lignin and other oxidative phenols that contribute to formation of defense barrier for reinforcing the cell structure**.** The less susceptible cultivars identified in the present study are expected to possess diverse resistance genes and could be

efficiently used as parents to improve resistance to charcoal-rot disease therefore, plant breeders should exert more efforts to improve and produce these cultivars to be used widely to overcome damping-off and charcoal-rot diseases (Mahmoud et al., 2015).

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