# Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
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http://ansinet.com

Plant Pathology Journal 8 (3): 79-89, 2009
ISSN 1812-5387


# The Use of Antioxidants and Microelements for Controlling Damping-Off Caused by *Rhizoctonia solani* and Charcoal Rot Caused by *Macrophomina phasoliana* on Sunflower

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**Abstract:** Seed soaking method or foliar spray of antioxidants (citric acid and salicylic acid at 10 mM) and microelements (manganese and zinc at 2 g $L^{-1}$) were tested to control of the damping-off and charcoal rot diseases of sunflower (varieties Sakha 53 and Giza 102). Field treatments in two different localities i.e., Tag El-Ezz, Dakahlia province and El-Serow, Damietta province were carried out. The high frequency isolated fungi (*M. phaseolina* and *R. solani*) presented in Tag El-Ezz location. On the other hand, *M. phaseolina* was isolated at a high frequency compared with *R. solani* in both locations. Sakha 53 was highly susceptible compered with Giza 102 when artificially infected with both *M. phaseolina* and *R. solani*. Laboratory results showed that salicylic acid alone or in combination with citric acid completely inhibited the linear growth of both pathogens i.e., *M. phasoliana* and *R. solani in vitro*. A positive correlation between the concentrations of Rizolex-T 50 and its effect on the fungal growth were recorded. The dose of 3 g $L^{-1}$ prevented the growth of *R. solani* linear growth *in vitro*. The greenhouse results revealed that Giza 102 variety was highly susceptible to the infection by *R. solani*. *M. phasoliana* showed severe symptoms in both sunflower varieties. On contrary, Giza 102 variety was tolerant to damping-off and charcoal rot diseases than Sakha 53 under field conditions. The application of Rizolex-T 50 followed by citric acid showed a highest percentage of healthy plants followed by the combination of citric acid and salicylic acid. The application of manganese combined with zinc was more effective than the microelements alone. All treatments of antioxidants and microelements significantly reduced the incidence of charcoal rot disease. On the other hand, no significant differences between Rizolex-T 50 and salicylic acid treatments was shown. Sakha 53 variety gave the highest values of plant height and number of leaves $plant^{-1}$ while Giza 102 recorded the highest values of stem diameter and flower head diameter. The application of citric acid combined with salicylic acid maximized the plant height followed by the mixture of manganese and zinc. Manganese treatment followed by the mixture of citric acid and salicylic acid then zinc were the most effective in increasing the number of leaves $plant^{-1}$. While, Rizolex-T 50 had no significant effect on plant height and number of leaves $plant^{-1}$. Microelements were more effective than antioxidants on enhancing the stem and flower head diameters. The combination between manganese and zinc followed by Rizolex-T 50 recorded the maximum values of the stem and flower head diameters. Giza 102 variety recorded the highest values of 100 seeds weight, total phenols, photosynthetic pigments and the percentage of seed oil when the above applications were carried out. Sakha 53 variety showed the highest plant yield under the above treatment. The mixtures of citric and salicylic acids were highly effective in increasing plant yield. The highest values of photosynthetic pigments were shown in salicylic acid treatment followed by Rizolex-T 50. Total phenols content was highest due to Rizolex-T 50 application followed by salicylic acid. Except zinc, seed oil concentration increased significantly in both antioxidants and microelements treatments on oil concentration.

**Key words:** Sunflower, damping-off, charcoal rot, *Rhizoctonia solani*, *Macrophomina phaseoliana*, antioxidants, microelements

## INTRODUCTION

Sunflower (*Helianthus annus* L.) is an agronomically and gastronomically interesting crop for its highly nutritional value of oil which goes up to 50% of seed contents (Gabriela and Castano, 2008). It is hygienically superior than other edible oil (Anonymous, 1994). Sunflower oil is quite palatable, free of all impurities, easy to refine and contains fat soluble vitamins A, D, E, K and good heart patients (Everrt *et al*., 1987; Gossal *et al*.,

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1988). Sunflower has diversifies uses in human food, livestock feed and other industrial products (Mahmood and Sadaqat, 2003).

A number of factors limit sunflower planting; growth and productivity include the partial interruption in the number of harvested heads as a result of broken plants caused by fungal infection (Conte *et al.*, 1989).

Charcoal rot of sunflower caused by *Sclerotium bataticola* (Taub.) [*Macrophomina phasoliana* (Tassi) Goid.] affect plants and shows grey to black discoloration at the base of the stem, which may extend upward thus hollowing the interior portion of the stem. Later the bith becomes shriveled and discolored (Ilyas *et al.*, 1982; Baumer and Hajdu, 1984). The disease also causes root and basal stem rot, premature ripening and drying of stalks (Mirza *et al.*, 1984).

Several investigators reported that the antioxidants may control seed and soil-borne fungal diseases (Shahda, 2001; Dmitrier *et al.*, 2003), as well as foliar fungal diseases (Hassan *et al.*, 2006). Moreover, the antioxidants enhance the level of plant phenols which play a major role in plant disease defense, growth and development (Hahlbrock and Scheel, 1989).

The resistance of plant may also induce when using chemical substances such as manganese and zinc (Marshner, 1986; Abd El-Hai *et al.*, 2007; El-Baz, 2007).

Elwakil and El-Metwally (2000) tested benzoic acid, citric acid, salicylic acid, sodium benzoate, sodium citrate and hydroquinone against seed-borne fungi of peanut (*Cephalosporium* sp., *F. moniliforme*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotium bataticola* and *Verticillium* sp.). The most effective antioxidant was hydroquinone, which showed a significant inhibitory effect on linear growth of these fungi. Low concentrations of hydroquinone (10 and 15 mM) prevented mycelial growth of *Cephalosporium* sp. and *S. bataticola.* No growth was observed in the other fungi at 20 mM concentration. Under greenhouse infested soil conditions, soaking seeds in a 20 mM hydroquinone solution for 12 h before planting in pots decreased pre- and post-emergence damping- off incited by all tested fungi.

The main trend in plant disease control today is avoid using toxic substances while a number of promising substances are available. So far, the aim of this research is to evaluate the effects of two safe control methods i.e. antioxidants (Citric acid and Salicylic acid) and microelements (Manganese and Zinc) as a seed treatment and foliar application for reducing sunflower damping-off and charcoal rot diseases as well as increase in seed productivity and quality.

## MATERIALS AND METHODS

**Isolation, purification and identification of the causal pathogens:** The causal pathogens were isolated from the two varieties of sunflower (Sakha 53 and Giza 102) showing typical symptoms of charcoal rot disease and collected from Tag El-Ezz, Dakahlia region and El-Serow, Damietta region. The infected plant parts were thoroughly washed in sterilized tap water then placed on PDA medium in Petri-dishes supplemented with streptomycin sulfate (0.035 g $L^{-1}$). The growing fungi were purified and identified according Ellis (1976) for *Macrophomina phasoliana* and Sneh *et al.* (1992) for *Rhizoctonia solani.*

**Preparation of the fungal inoculum:** The inocula of isolates of *Macrophomina phasoliana* and *Rhizoctonia solani* were prepared using sorghum: coarse sand: water (2: 1: 2 V/V) medium. The ingredients were mixed, bottled and autoclaved for 30 min. at 121°C and 1.5 air pressure. The sterilized medium was inoculated using agar discs, obtained from the periphery of 6 days old colony of each of the isolated fungi. The inoculated media were incubated at 28±1°C for 15 days, then used for soil infestation in a greenhouse experiment for studying the pathogenicity test.

### *In vitro* experiment

**Effect of tested antioxidants and microelements on the fungal growth:** The effect of different concentrations of citric acid, salicylic acid at 10 mM and their mixture and manganese, zinc at 2 g $L^{-1}$ and their mixture as well as Rizolex-T50 w.p. at 0.75, 1.5 and 3 g $L^{-1}$ were separately mixed in PDA medium before solidification, then poured in a sterile Petri dishes (9 cm) of each Petri dish. Five plates for each concentration were used as one replicate while each plate was inoculated with 5 mm fungal disc of each tested fungi. Treatments were kept at 27±1°C. The linear growth of the each fungus was measured when the full growth of tested fungi was observed in the check treatment. The average growth diameters were calculated.

### Greenhouse experiments

**Pots experiment (Pathogenicity tests):** The previously prepared fungal inocula were used to infest autoclaved soil at a rate of 4% soil weight (w/w) in plastic pots (30 cm diameter) filled with 5 kg autoclaved clay soil. The pots inoculated with *Macrophomina phasoliana* and *Rhizoctonia solani* watered and left for 3 days to ensure the distribution of inoculated fungi. Three pots were used for each treatment in all replicates. Seeds of sunflower cultivars (Sakha 53 and Giza 102) were surface sterilized in 1% sodium hypochlorite solution for 5 min followed by

3 successive rinses in sterilized tap water. The excess water was removed by placing the treated seeds between sterilized tissue paper until dryness. Seeds were sown at the rate of 5 seeds $pot^{-1}$. The percentage of pre- and post-emergence damping off were calculated on the basis of total number of seeds planted after 20 and 50 days from planting, respectively.

**Field experiments:** Filed experiment was carried out at two locations of Tag El-Ezz Agricultural Research Station Farm, Dakahlia Governorate and at El-Serow Agricultural Research Station Farm, Damietta Governorate, Egypt during the growing summer season of 2008. The experiment aimed to study the effects of 10 mM of citric acid, salicylic acid and their mixture and 2 g $L^{-1}$ of manganese, zinc (form of chelate) and their mixture as seed soaking. Rizolex-T50 w.p. at 3 g $kg^{-1}$ was used as seed coating. Germination, morphological characters, chemical composition and yield components as well as reduction in damage of charcoal rot disease (*Sclerotium bataticola* Taub.) were recorded. The above treatments were also used as a foliar application 35 days after sowing.

Sunflower seeds (c.v. Sakha 53 and Giza 102) were soaked in the tested chemicals for 3 hr., then air dried for 1 hr. before sowing. Seeds were planted on June 22, 2007 at both Tag El-Ezz and El-Serow Agricultural Research Station Farms then left to the natural infection which commonly occur in this regions.

A split plot design with three replicates was used in these experiments, the main plots represented varieties while sub-plots represented treatments. The area of each sub-plot was 10.5 $m^2$ (3.5×3.0 m). The sown seeds were applied at a rate of 375 seeds $plot^{-1}$.

After 20 days from sowing date, germination percentage and pre-emergence damping off were determined. In addition, at the age of 60 days from sowing, samples were taken to estimate the morphological characters (plant height and number of leaves $plant^{-1}$). The percentage of charcoal rot disease as well as photosynthetic pigments and total phenols were determined.

At harvest stage (84 days from sowing for Giza 102 and 91 days for Sakha 53), stem diameter (cm), flower head diameter (cm), seed plant yield (g) and weight of 100 seeds (g) were recorded. In addition, the percentage of seed oil was also determined.

**Determination of total phenolic compounds:** Total phenols were determined 60 days after sowing. Fresh shoot and the Folin-Ciocalteau reagent were used (Singleton and Rossi, 1965). Samples of (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10000 rpm for 15 min. under cooling and the supernatants were saved. The residues were extracted in 80% ethanol. The supernatants were taken and evaporated to dryness at room temperature. Residues were dissolved in 5 mL distilled water. One hundred microliters of each extract was water diluted to 3 mL. The 0.5 mL of Folin-Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was mixed thoroughly to the extract. The developed color was spectrophotomatrically measured at 650 nm. After 60 min, while catechol was used as a standard. The results were expressed as mg catechol/100 g fresh weight.

**Determination of photosynthetic pigments:** Photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted in 90% methanol for 24 h at room temperature after adding traces of sodium carbonate from the 2nd leaf from plant tip (Robinson and Britz, 2000), then photosynthetic pigments were determined sepctrophotometrically according to Mackinney (1941).

**Determination of oil in seeds:** The oil percentage in sunflower seeds was determined by using Soxhlet apparatus according to AOAC (1970) as follows:

Ten gram dried seed powder was extracted in a mixture of chloroform: methanol (2:1) for 6 h, while Soxhlet apparatus was used. Product oils were vacuum evaporated and the percentage of oil was calculated.

**Statistical analysis:** All data were statistically analyzed by the software CoStat (2005) in consultation with the analysis of variance (Gomez and Gomez, 1984). The means were compared using Least Significant Difference (LSD) at p = 0.05 as outlined by Duncan (1955).

## RESULTS

**Laboratory experiment**

**Isolation of fungi:** Results in Table 1 shows that the high frequency isolated fungi (*Macrophomina phaseolina* and *Rhizoctonia solani*) were found in Tag El-Ezz location. *Macrophomina phaseolina* was isolated also at high frequency number compared with *Rhizoctonia solani* in both locations. It was observed that Sakha 53 was less susceptible than Giza 102 when isolation of *Macrophomina phaseolina* and *Rhizoctonia solani* were carried out.

**Effect of antioxidants and microelements on fungal growth:** It is clear from Table 2 that, the tested antioxidants (citric acid, salicylic acid and their combination) significantly decreased the linear growth of *Macrophomina phasolaina* and *Rhizoctonia solani*. The application of salicylic acid at 10 mM and the combination between citric acid and salicylic acid showed a complete inhibition in the linear growth of both pathogens. Moreover, both microelements and their combination significantly decreased the linear growth of the tested pathogens. The treatment of Mn had no effect on the growth of *Macrophomina phasolaina*.

On the other hand, the effect of different doses of Rizolex-T 50 shows that all concentration of Rizolex-T 50 significantly decreased the linear growth of both fungi. The effect increases when increasing the doses from 0.75 to 3.00 g $L^{-1}$. Rizolex-T, 3 g $L^{-1}$ gave a complete inhibition in the linear growth of *Rhizoctonia solani*.

Table 1: Frequency of the isolated fungi from sunflower plant at Tag El-Ezz and El-Serow locations

| | | Frequency of fungi (%) | | |
|---|---|---|---|---|
| Locations | Varieties | *Macrophomina phaseolina* | *Rhizoctonia solani* | Total |
| Tag El-Ezz | Sakha 53 | 40.60 | 23.00 | 63.60 |
| | Giza 102 | 46.00 | 26.60 | 72.60 |
| Total | | 86.60 | 49.60 | 136.20 |
| El-Serow | Sakha 53 | 37.60 | 22.00 | 59.60 |
| | Giza 102 | 42.30 | 24.30 | 66.60 |
| Total | | 79.90 | 46.30 | 126.20 |

Table 2: Effect of antioxidants and microelements on the linear growth (cm) of the tested pathogenic fungi

| Treatments | *Macrophomina phaseolina* | *Rhizoctonia solani* |
|---|---|---|
| Citric acid 10 mm | 6.33b | 3.17c |
| Salcylic acid10 mM | 0.00g | 0.00f |
| Citric 10 mM+Salcylic 10 mM | 0.00g | 0.00f |
| Zinc 2 g $L^{-1}$ | 2.17f | 1.33e |
| Manganese 2 g $L^{-1}$ | 9.00a | 3.50bc |
| Manganese+Zinc 2 g $L^{-1}$ | 4.17d | 3.67b |
| Rizolex 3 g $L^{-1}$ | 1.67f | 0.00f |
| Rizolex 1.5 g $L^{-1}$ | 2.83e | 1.83d |
| Rizolex 0.75 g $L^{-1}$ | 4.83c | 3.83b |
| Check | 9.00a | 9.00a |
| LSD (5%) | 0.54 | 0.4398 |

Values with different letter(s) are not significnat

### Greenhouse experiments

**Pots experiment (Pathogenicity tests):** Results in Table 3 revealed that sunflower cultivar (Giza 102) was more sensitive to the infection by pre and post-emergence damping-off than Sakha 53 cultivar with no significant differences between the two varieties.

With regard to the effects of fungal isolates on the percentages of pre- and post-emergence damping-off as well as on the survival plants, data show that both tested fungi were pathogenic and causes typical symptoms of damping-off.

*Macrophomina phasoliana* showed to be highly virulent fungus. It gave the highest values of pre- and post-emergence damping-off (48.33 and 21.66%), respectively. The combination between both varieties and isolates significantly affected damping-off in turn survived plants. Generally, *Macrophomina phasoliana* showed highest percentage of pre- and post-emergence damping-off in both sunflower varieties than *Rhizoctonia solani*.

### Field experiment (Disease assessment)

**Damping-off disease:** The effect of antioxidants and microelements on germination and damping-off of sunflower plants under field conditions are shown in Table 4. Both sunflower varieties gave significant response to F-test. Giza 102 variety was the best in germination percentage within decreasing in damping-off.

Data also show that the highest values of germination percentage and decrease of damping-off occurred when citric acid treatment was followed by the combination treatment of citric acid and salicylic acid. On the other side, the combination between manganese and zinc showed a significant effect on the germination percentage than any microelements alone.

Concerning the effects of interaction between varieties and treatments, it was observed that the highest values of germination percentage in both sunflower

Table 3: Pathogenicity test of pre- and post-emergence damping-off and survival of sunflower plants infected with isolated fungi under greenhouse condition

| Treatments | Pre-emergence damping-off | Post-emergence damping-off | Survival plants |
|---|---|---|---|
| Sakha 53 | 25.00 | 12.00 | 63.00 |
| Giza 102 | 28.33 | 12.56 | 59.11 |
| F-test | NS | NS | NS |
| Check | 5.00 | 3.50 | 91.50 |
| *Macrophomina phaseolina* | 48.33 | 21.66 | 30.00 |
| *Rhizoctonia solani* | 26.66 | 11.67 | 61.67 |
| LSD (5%) | 4.30 | 3.25 | 5.59 |
| Sakha 53 | | | |
| Check | 3.33 | 2.67 | 94.00 |
| *Macrophomina phaseolina* | 46.67 | 21.67 | 31.67 |
| *Rhizoctonia solani* | 25.00 | 11.67 | 63.33 |
| Giza 102 | | | |
| Check | 6.67 | 4.33 | 89.00 |
| *Macrophomina phaseolina* | 50.00 | 21.67 | 28.33 |
| *Rhizoctonia solani* | 28.33 | 11.67 | 60.00 |
| LSD (5%) | 6.08 | 4.60 | 7.91 |

NS: Not significant

Table 4: Effect of antioxidants and microelements on germination percentage and damping-off disease of sunflower plant under field condition

| | Tag El-Ezz | | El-Serow | |
|---|---|---|---|---|
| Treatments | Germination (%) | Damping-off | Germination (%) | Damping-off |
| Sakha 53 | 82.57 | 17.43 | 84.54 | 15.46 |
| Giza 102 | 87.04 | 12.96 | 88.81 | 11.19 |
| F-test | * | * | * | * |
| Check | 73.66 | 26.34 | 75.19 | 24.81 |
| Rizolex-T 50 3 g $L^{-1}$ | 97.56 | 2.44 | 99.42 | 0.58 |
| Citric acid 10 mM | 93.04 | 6.96 | 95.96 | 4.04 |
| Salicylic acid 10 mM | 84.97 | 15.03 | 86.74 | 13.26 |
| Citric 10 mM+Salicylic 10 mM | 91.29 | 8.71 | 93.18 | 6.82 |
| Manganese 2 g $L^{-1}$ | 74.15 | 25.85 | 75.73 | 24.27 |
| Zinc 2 g $L^{-1}$ | 79.08 | 20.92 | 80.73 | 19.27 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 84.70 | 15.30 | 86.45 | 13.55 |
| LSD (5%) | 2.20 | 2.20 | 2.45 | 2.45 |
| Sakha 53 | | | | |
| Check | 69.26 | 30.74 | 70.68 | 29.32 |
| Rizolex-T 50 3 g $L^{-1}$ | 97.11 | 2.89 | 99.10 | 0.90 |
| Citric acid 10 mM | 90.80 | 9.20 | 94.66 | 5.34 |
| Salicylic acid 10 mM | 83.21 | 16.79 | 84.92 | 15.08 |
| Citric 10 mM+Salicylic 10 mM | 90.65 | 9.35 | 92.53 | 7.47 |
| Manganese 2 g $L^{-1}$ | 68.79 | 31.21 | 70.29 | 29.71 |
| Zinc 2 g $L^{-1}$ | 77.53 | 22.47 | 79.15 | 20.85 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 83.23 | 16.77 | 84.96 | 15.04 |
| Giza 102 | | | | |
| Check | 78.05 | 21.95 | 79.69 | 20.31 |
| Rizolex-T 50 3 g $L^{-1}$ | 98.01 | 1.99 | 99.73 | 0.27 |
| Citric acid 10 mM | 95.28 | 4.72 | 97.25 | 2.75 |
| Salicylic acid 10 mM | 86.73 | 13.27 | 88.55 | 11.45 |
| Citric 10 mM+Salicylic 10 mM | 91.93 | 8.07 | 93.82 | 6.18 |
| Manganese 2 g $L^{-1}$ | 79.52 | 20.48 | 81.17 | 18.83 |
| Zinc 2 g $L^{-1}$ | 80.63 | 19.37 | 82.31 | 17.69 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 86.16 | 13.84 | 87.95 | 12.05 |
| LSD (5%) | 3.11 | 3.11 | 3.47 | 3.47 |

*$p = 0.05$

varieties was recorded in Tag El-Ezz and El-Serow regions when the application of Rizolex-T 50 was followed by citric acid then with the combination of citric acid and salicylic acid. In addition, microelements significantly increased germination percentage and decreased damping-off. The mixture of manganese and zinc was more effect in this respect followed by the application of zinc alone.

**Charcoal rot disease:** Data presented in Table 5 show that there was a marked differences between treatments of different varieties regarding charcoal rot disease. Giza 102 variety was more tolerant than Sakha 53. All treatments of antioxidants and microelements on sunflower significantly reduced charcoal rot disease incidence compared with control treatment. Rizolex-T 50 was the most effective treatment followed by the combination between citric acid and salicylic acid then salicylic acid alone. It is worthy to mention that no significant differences between Rizolex-T 50 and salicylic acid treatments were found.

With respect to the application of microelements, data shown in Table 5 also indicate that all tested microelements significantly decreased charcoal rot disease. The combination between manganese and zinc was more effect in this respect followed by the treatment of zinc alone.

**Morphological characters:** The effect of citric acid and salicylic acid at 10 mM and their combination as well as manganese and zinc at 2 g $L^{-1}$ and their mixture on plant height and number of leaves $plant^{-1}$ after 60 days from sowing were determined. Stem diameter (cm) and flower head diameter (cm) at harvest stage of sunflower varieties were estimated and shown in Table 6. Data show that the variety Sakha 53 presented the highest values of plant height and number of leaves $plant^{-1}$. However, Giza 102 was the best variety in stem and flower head diameters.

Effect of tested treatments on the morphological characters showed that in all treatments, significantly increased plant height while the number of leaves $plant^{-1}$ were higher, Rizolex-T 50 did not show significant effect on the morphological characters. Citric acid in combination with salicylic acid followed by the mixture of manganese and zinc maximized plant height. Manganese treatment increased the number of leaves $plant^{-1}$ followed by the mixture of citric acid and salicylic acid then the treatment in which zinc was applied alone. The application of microelements were more effective on stem and flower head diameters than antioxidants alone. The combination between microelements recorded highest effects followed by zinc then manganese. Insignificant increase in flower head diameter was observed when citric acid alone or combined with salicylic acid were used.

Table 5: Effect of antioxidants and microelements on disease incidence of charcoal rot caused by *M. phaseoliana* (*Sclerotium bataticola*) after 60 days from sowing

| | Tag El-Ezz | | El-Serow | |
|---|---|---|---|---|
| Treatments | Disease incidence (%) | Reduction (%) | Disease incidence (%) | Reduction (%) |
| Sakha 53 | 5.64 | - | 6.88 | - |
| Giza 102 | 5.11 | - | 6.15 | - |
| F-test NS | - | NS | - | |
| Check | 13.53 | 0.00 | 16.28 | 0.00 |
| Rizolex-T 50 3 g $L^{-1}$ | 2.26 | 83.30 | 2.76 | 83.04 |
| Citric acid 10 mM | 5.38 | 60.52 | 6.67 | 59.03 |
| Salicylic acid 10 mM | 3.87 | 71.44 | 4.74 | 70.88 |
| Citric 10 mM+Salicylic 10 mM | 3.49 | 74.21 | 4.27 | 73.77 |
| Manganese 2 g $L^{-1}$ | 5.09 | 62.40 | 6.18 | 62.04 |
| Zinc 2 g $L^{-1}$ | 4.77 | 64.78 | 5.79 | 64.43 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 4.60 | 66.04 | 5.42 | 66.71 |
| LSD (5%) | 2.17 | - | 2.53 | - |
| Sakha 53 | | | | |
| Check | 12.02 | 0.00 | 14.44 | 0.00 |
| Rizolex-T 50 3 g $L^{-1}$ | 2.14 | 82.20 | 2.59 | 82.06 |
| Citric acid 10 mM | 5.90 | 50.94 | 7.33 | 49.24 |
| Salicylic acid 10 mM | 4.70 | 60.92 | 5.83 | 59.63 |
| Citric 10 mM+Salicylic 10 mM | 4.30 | 64.23 | 5.28 | 63.43 |
| Manganese 2 g $L^{-1}$ | 5.38 | 55.24 | 6.51 | 54.92 |
| Zinc 2 g $L^{-1}$ | 5.53 | 53.99 | 6.76 | 53.19 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 5.14 | 57.24 | 6.29 | 56.44 |
| Giza 102 | | | | |
| Check | 15.04 | 0.00 | 18.12 | 0.00 |
| Rizolex-T 50 3 g $L^{-1}$ | 2.38 | 84.18 | 2.93 | 83.83 |
| Citric acid 10 mM | 4.86 | 67.69 | 6.01 | 66.83 |
| Salicylic acid 10 mM | 3.03 | 79.85 | 3.65 | 79.86 |
| Citric 10 mM+Salicylic 10 mM | 2.68 | 82.18 | 3.26 | 82.01 |
| Manganese 2 g $L^{-1}$ | 4.79 | 68.15 | 5.84 | 67.77 |
| Zinc 2 g $L^{-1}$ | 4.00 | 73.40 | 4.83 | 73.34 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 4.05 | 73.07 | 4.55 | 74.89 |
| LSD (5%) | 3.07 | - | 3.58 | - |

NS: Not significant

The microelements and antioxidants significantly increased plant height in both sunflower varieties. Only salicylic acid did not show any effect in such parameters in Sakha 53 variety. Rizolex-T 50 had not any significant effect on plant height and number of leaves $plant^{-1}$ in both varieties. In contrast, the combination between manganese and zinc followed by Rizolex-T 50 recorded the maximum values of stem and flower head diameters especially on Sakha 53 variety. While, the highest values of Giza 102 flower head diameter occurred when zinc was applied followed by manganese combined with zinc then salicylic acid alone.

**Yield and its components:** Data in Table 7 show that Sakha 53 variety presented the best plant yield (83.86 g), while Giza 102 was the best for weight of 100 seeds (8.39 g).

Regarding the effects of both antioxidants and microelements on yield and its components, data show that plant yield and weight of 100 seeds were significantly increased. Manganese combined with zinc treatment was the most effective in increasing the plant yield followed by zinc alone then the combination between citric acid and salicylic acid. Meanwhile, all treatments did not show insignificant increase in 100 seeds weight except manganese which significantly increased this parameter.

**Total phenols, photosynthetic pigments and seed oil contents:** There were significant differences between sunflower varieties in photosynthetic pigments content (chlorophyll a and caroteinoids), total phenols and seed oil percentage which is shown in Table 8. The results presented a positive correlation between the photosynthetic pigments and seed oil percentage. Photosynthetic pigments and total phenols were increased significantly under application of antioxidants and fungicide treatments. The effect of salicylic acid treatment gave the highest values followed by Rizolex-T 50 in photosynthetic pigments. Rizolex-T 50 followed by salicylic acid to gave the highest values in total phenols. On the other side, these parameters were not affected when using microelements treatments except, with manganese which significantly increased chlorophyll b and carotenoids. Zinc treatment showed a significantly increase in chlorophyll a.

Table 6: Effect of antioxidants and microelements on sunflower morphological characters under field condition

| | Tag El-Ezz | | | | El-Serow | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Plant height (cm) | No. of leaves | Stem diameter (cm) | Flower head diameter (cm) | Plant height (cm) | No. of leaves | Stem diameter (cm) | Flower head diameter (cm) |
| Sakha 53 | 153.58 | 29.46 | 2.17 | 15.08 | 169.75 | 32.25 | 1.87 | 13.78 |
| Giza 102 | 142.54 | 26.00 | 2.50 | 17.08 | 157.96 | 28.38 | 2.18 | 15.73 |
| F-test | ** | ** | * | * | ** | ** | ** | * |
| Check | 135.00 | 25.17 | 1.73 | 13.00 | 149.67 | 27.67 | 1.43 | 11.75 |
| Rizolex-T 50 3 g $L^{-1}$ | 136.00 | 24.67 | 2.63 | 16.33 | 150.50 | 26.83 | 2.28 | 14.98 |
| Citric acid 10 mM | 147.83 | 27.50 | 2.22 | 14.83 | 163.00 | 30.00 | 1.92 | 13.53 |
| Salicylic acid 10 mM | 148.00 | 28.17 | 2.28 | 15.33 | 164.17 | 31.00 | 1.97 | 14.02 |
| Citric 10 mM+Salicylic 10 mM | 156.00 | 29.33 | 2.22 | 14.67 | 172.67 | 32.00 | 1.92 | 13.37 |
| Manganese 2 g $L^{-1}$ | 154.67 | 30.50 | 2.42 | 16.50 | 171.33 | 33.50 | 2.12 | 15.17 |
| Zinc 2 g $L^{-1}$ | 151.17 | 28.83 | 2.57 | 17.50 | 167.50 | 31.33 | 2.22 | 16.15 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 155.83 | 27.67 | 2.63 | 20.50 | 172.00 | 30.16 | 2.32 | 19.08 |
| LSD (5%) | 7.67 | 2.10 | 0.24 | 1.87 | 8.36 | 2.21 | 0.23 | 1.78 |
| Sakha 53 | | | | | | | | |
| Check | 141.33 | 29.00 | 1.87 | 12.67 | 156.67 | 32.00 | 1.57 | 11.43 |
| Rizolex-T 50 3 g $L^{-1}$ | 140.00 | 26.67 | 2.40 | 17.67 | 155.00 | 29.00 | 2.10 | 16.30 |
| Citric acid 10 mM | 152.00 | 31.67 | 2.27 | 13.33 | 166.67 | 34.67 | 1.97 | 12.03 |
| Salicylic acid 10 mM | 151.33 | 28.33 | 1.80 | 12.33 | 168.00 | 31.33 | 1.50 | 11.10 |
| Citric 10 mM+Salicylic 10 mM | 169.00 | 33.67 | 1.87 | 13.00 | 187.00 | 36.67 | 1.57 | 11.73 |
| Manganese 2 $gL^{-1}$ | 159.33 | 31.00 | 2.37 | 15.00 | 176.67 | 34.00 | 2.07 | 14.00 |
| Zinc 2 g $L^{-1}$ | 149.00 | 25.00 | 2.07 | 15.00 | 165.00 | 27.00 | 1.77 | 13.70 |
| Manganese 2 g $L^{-1}$ + Zinc 2 g $L^{-1}$ | 166.67 | 30.33 | 2.73 | 21.67 | 183.00 | 33.33 | 2.40 | 20.23 |
| Giza 102 | | | | | | | | |
| Check | 128.67 | 21.33 | 1.60 | 13.33 | 142.67 | 23.33 | 1.30 | 12.07 |
| Rizolex-T 50 3 g $L^{-1}$ | 132.00 | 22.67 | 2.87 | 15.00 | 146.00 | 24.67 | 2.47 | 13.67 |
| Citric acid 10 mM | 143.67 | 23.33 | 2.17 | 16.33 | 159.33 | 25.33 | 1.87 | 15.03 |
| Salicylic acid 10 mM | 144.67 | 28.00 | 2.77 | 18.33 | 160.33 | 30.67 | 2.43 | 16.93 |
| Citric 10 mM+Salicylic 10 mM | 143.00 | 25.00 | 2.57 | 16.33 | 158.33 | 27.33 | 2.27 | 15.00 |
| Manganese 2 g $L^{-1}$ | 150.00 | 30.00 | 2.47 | 18.00 | 166.00 | 33.00 | 2.17 | 16.63 |
| Zinc 2 g $L^{-1}$ | 153.33 | 32.67 | 3.07 | 20.00 | 170.00 | 35.67 | 2.67 | 18.60 |
| Manganese 2 g $L^{-1}$ + Zinc 2 g $L^{-1}$ | 145.00 | 25.00 | 2.53 | 19.33 | 161.00 | 27.00 | 2.23 | 17.93 |
| LSD (5%) | 10.85 | 2.97 | 0.33 | 2.64 | 2.64 | 3.13 | 0.32 | 2.52 |

*p = 0.05, **p = 0.01

Table 7: Effect of antioxidants and microelements on yield components at harvest stage of sunflower varieties

| | Tag El-Ezz | | El-Serow | |
|---|---|---|---|---|
| Treatments | Plant yield (g) | Weight of 100 seeds | Plant yield (g) | Weight of 100 seeds |
| Sakha 53 | 83.86 | 7.81 | 84.84 | 7.92 |
| Giza 102 | 76.42 | 8.39 | 77.54 | 7.26 |
| F-test | * | ** | NS | NS |
| Check 62.84 | 5.46 | 64.17 | 5.54 | |
| Rizolex-T 50 3 g $L^{-1}$ | 77.84 | 7.53 | 76.45 | 7.64 |
| Citric acid 10 mM | 71.54 | 6.48 | 73.02 | 6.57 |
| Salicylic acid 10 mM | 69.81 | 6.61 | 71.23 | 6.70 |
| Citric 10 mM+Salicylic 10 mM | 90.20 | 8.90 | 90.45 | 9.02 |
| Manganese 2 g $L^{-1}$ | 73.13 | 11.96 | 74.47 | 7.13 |
| Zinc 2 g $L^{-1}$ | 94.86 | 8.59 | 96.78 | 8.71 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 100.90 | 9.25 | 102.95 | 9.38 |
| LSD (5%) | 4.92 | 5.27 | 6.48 | 0.89 |
| Sakha 53 | | | | |
| Check | 66.04 | 5.99 | 67.50 | 6.10 |
| Rizolex-T 50 3 g $L^{-1}$ | 86.65 | 8.11 | 82.43 | 8.21 |
| Citric acid 10 mM | 73.95 | 6.15 | 75.47 | 6.24 |
| Salicylic acid 10 mM | 73.03 | 7.63 | 74.53 | 7.73 |
| Citric 10 mM+Salicylic 10 mM | 94.26 | 9.04 | 96.20 | 9.16 |
| Manganese 2 g $L^{-1}$ | 78.52 | 7.47 | 80.13 | 7.60 |
| Zinc 2 g $L^{-1}$ | 96.38 | 8.37 | 98.33 | 8.48 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 102.05 | 9.69 | 104.13 | 9.83 |
| Giza 102 | | | | |
| Check | 59.64 | 4.92 | 60.83 | 4.99 |
| Rizolex-T 50 3 g $L^{-1}$ | 69.03 | 6.96 | 70.47 | 7.07 |
| Citric acid 10 mM | 69.13 | 6.80 | 70.57 | 6.89 |
| Salicylic acid 10 mM | 66.58 | 5.59 | 67.93 | 5.68 |
| Citric 10 mM+Salicylic 10 mM | 86.14 | 8.76 | 84.70 | 8.88 |
| Manganese 2 g $L^{-1}$ | 67.73 | 16.45 | 68.80 | 6.66 |
| Zinc 2 g $L^{-1}$ | 93.35 | 8.81 | 95.23 | 8.94 |
| Manganese 2 g $L^{-1}$+Zinc 2 g $L^{-1}$ | 99.75 | 8.80 | 101.77 | 8.93 |
| LSD (5%) | 6.95 | 7.45 | 9.16 | 1.26 |

NS: Not significant, *p = 0.01, **p = 0.05

Table 8: Effect of antioxidants and some microelements on the photosynthetic pigments contents, total phenol and seed oil content of sunflower plant under field condition

| | Tag El-Ezz | | | | | El-Serow | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Total phenols mg/100 g fresh weight | Chlorophyll A mg $g^{-1}$ fresh weight | Chlorophyll B mg $g^{-1}$ fresh weight | Caroteinoids mg $g^{-1}$ fresh weight | Oil (%) | Total phenols mg/100 g fresh weight | Chlorophyll A mg $g^{-1}$ fresh weight | Chlorophyll B mg $g^{-1}$ fresh weight | Caroteinoids mg $g^{-1}$ fresh weight | Oil (%) |
| Sakha 53 | 653.52 | 1.45 | 0.91 | 0.65 | 43.53 | 666.85 | 1.49 | 0.93 | 0.66 | 44.42 |
| Giza 102 | 707.26 | 1.74 | 1.04 | 0.78 | 46.60 | 734.20 | 1.78 | 1.08 | 0.80 | 47.56 |
| F.test | ** | * | NS | * | ** | * | * | NS | * | * |
| Check | 521.92 | 1.39 | 0.89 | 0.67 | 42.91 | 532.57 | 1.41 | 0.91 | 0.69 | 43.80 |
| Rizolex-T 50 3 $g L^{-1}$ | 809.21 | 1.78 | 1.09 | 0.78 | 46.18 | 825.73 | 1.82 | 1.16 | 0.80 | 47.14 |
| Citric acid 10 mM | 753.64 | 1.57 | 0.98 | 0.69 | 46.72 | 769.02 | 1.61 | 1.00 | 0.71 | 47.68 |
| Salicylic acid 10 mM | 783.15 | 1.97 | 1.20 | 0.81 | 48.32 | 799.12 | 2.01 | 1.23 | 0.83 | 49.33 |
| Citric 10 mM+ Salicylic 10 mM | 732.35 | 1.68 | 0.97 | 0.75 | 47.44 | 747.32 | 1.72 | 0.99 | 0.77 | 48.42 |
| Manganese 2 $g L^{-1}$ | 634.67 | 1.45 | 1.01 | 0.65 | 43.09 | 647.58 | 1.48 | 1.04 | 0.66 | 43.97 |
| Zinc 2 $g L^{-1}$ | 619.16 | 1.50 | 0.83 | 0.67 | 42.37 | 631.78 | 1.53 | 0.85 | 0.69 | 43.25 |
| Manganese 2 $g L^{-1}$+ Zinc 2 $g L^{-1}$ | 589.04 | 1.44 | 0.81 | 0.68 | 43.45 | 651.07 | 1.47 | 0.83 | 0.69 | 44.36 |
| LSD (5%) | 48.62 | 0.07 | 0.19 | 0.04 | 1.1 | 48.43 | 0.07 | 0.20 | 0.04 | 1.10 |
| Sakha 53 | | | | | | | | | | |
| Check | 452.68 | 1.17 | 0.85 | 0.59 | 41.64 | 461.90 | 1.19 | 0.87 | 0.60 | 42.51 |
| Rizolex-T 50 3 $g L^{-1}$ | 790.85 | 1.69 | 1.04 | 0.73 | 44.45 | 807.00 | 1.73 | 1.06 | 0.75 | 45.36 |
| Citric acid 10 mM | 720.16 | 1.47 | 0.897 | 0.63 | 45.81 | 734.83 | 1.50 | 0.92 | 0.64 | 46.76 |
| Salicylic acid 10 mM | 763.41 | 1.94 | 1.15 | 0.76 | 46.09 | 778.97 | 1.98 | 1.18 | 0.78 | 47.04 |
| Citric 10 mM+ Salicylic 10 mM | 714.46 | 1.62 | 0.99 | 0.70 | 46.11 | 729.07 | 1.65 | 1.02 | 0.72 | 47.06 |
| Manganese 2 $g L^{-1}$ | 638.73 | 1.22 | 0.84 | 0.56 | 41.61 | 651.67 | 1.25 | 0.86 | 0.57 | 42.46 |
| Zinc 2 $g L^{-1}$ | 562.35 | 1.27 | 0.76 | 0.58 | 40.50 | 573.80 | 1.30 | 0.78 | 0.59 | 41.33 |
| Manganese 2 $g L^{-1}$ + Zinc 2 $g L^{-1}$ | 585.54 | 1.24 | 0.73 | 0.60 | 42.00 | 597.50 | 1.27 | 0.74 | 0.61 | 42.87 |
| Giza 102 | | | | | | | | | | |
| Check | 591.16 | 1.60 | 0.93 | 0.75 | 44.18 | 603.23 | 1.63 | 0.95 | 0.77 | 45.09 |
| Rizolex-T 50 3 $g L^{-1}$ | 827.58 | 1.87 | 1.14 | 0.82 | 47.91 | 844.47 | 1.91 | 1.26 | 0.84 | 48.92 |
| Citric acid 10 mM | 787.13 | 1.67 | 1.07 | 0.75 | 47.62 | 803.20 | 1.72 | 1.09 | 0.77 | 48.59 |
| Salicylic acid 10 mM | 802.89 | 1.99 | 1.25 | 0.86 | 50.56 | 819.27 | 2.03 | 1.28 | 0.88 | 51.61 |
| Citric 10 mM + Salicylic 10 mM | 750.24 | 1.75 | 0.95 | 0.81 | 48.77 | 765.57 | 1.79 | 0.97 | 0.83 | 49.78 |
| Manganese 2 $g L^{-1}$ | 630.60 | 1.67 | 1.18 | 0.73 | 44.56 | 643.50 | 1.70 | 1.21 | 0.75 | 45.48 |
| Zinc 2 $g L^{-1}$ | 675.96 | 1.72 | 0.90 | 0.75 | 44.24 | 689.73 | 1.76 | 0.92 | 0.78 | 45.17 |
| Manganese 2 $g L^{-1}$ + Zinc 2 $g L^{-1}$ | 592.54 | 1.63 | 0.90 | 0.75 | 44.90 | 704.63 | 1.66 | 0.92 | 0.77 | 45.84 |
| LSD (5%) | 68.53 | 0.10 | 0.27 | 0.05 | 1.56 | 68.50 | 0.11 | 0.28 | 0.05 | 1.55 |

NS: Not significant, *p = 0.05, **p = 0.01

The oil percentage in sunflower seeds was increased significantly in the treatment in which antioxidants and microelements were applied except, zinc treatment which did not show a significant effect on oil seed content.

In combination treatments, as shown in Table 8 the interaction between sunflower varieties and antioxidants or microelements had no significant effect on caroteinoids content and seed oil percentage. However, chlorophyll a and b as well as total phenols were increased significantly under interaction between varieties and antioxidants or microelements as well as Rizolex-T 50. The highest values occurred in salicylic acid treatment followed by Rizolex-T 50 then the mixture of citric acid and salicylic acid. The same trend was showed in chlorophyll b with the same percentage in all microelements treatment. Manganese treatment reveled a significant increase in oil contents of Giza 102.

## DISCUSSION

Plant resistance to pathogen requires the activation of complex metabolic pathways in the infected cells, aimed at recognizing pathogen presence and hindering its propagation within plant tissues (De Gara *et al*., 2003). In the present study, it was planed to investigate the possibility of using antioxidants and microelements on reducing the harmful effects of charcoal rot disease on sunflower productivity citric acid, salicylic acid, manganese and zinc were used in this study.

The results show that both antioxidants and zinc caused significant reductions in linear growth of both pathogenic fungi i.e., *Macrophomina phasolaina* and *Rhizoctonia solani* as well as damping-off and charcoal rot disease incidence.

The role of antioxidants on overcoming the injurious effects of both *Macrophomina phasolaina* and

*Rhizoctonia solani* may be attributed to the regulation of plant development and chilling of disease resistance (Dmitrier *et al*., 2003; Achuo *et al*., 2004). In addition, antioxidants may neutralize the harmful oxygen radicals released during then infections (Shahda, 2002). Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants (Sakhabutdinova *et al*., 2003).

It was recorded that salicylic acid level often increases after pathogen attack. It induces the expression of pathogenesis related proteins and initiate the development of systemic acquired resistance and hypersensitivity (Metwally *et al*., 2003). While, Klessing *et al.* (2000) stated that salicylic acid appears to regulate the delicate balance between pro- and anti death functions during hypersensitive response. The molecular events involved in salicylic acid signaling are not yet fully understood.

Salicylic acid accumulates at a high concentration in the immediate vicinity of incompatible infection sites and is considered a key endogenous regulator of defense responses, being involved both in localized defenses and in systemically acquired resistance (Chen *et al*., 1995). Moreover, White (1979) demonstrated that application of exogenous salicylic acid or its derivatives induces synthesis of pathogenesis related proteins and partial resistance to pathogens. On the other side, citric acid decrease growth of *Macrophomina phasolaina* and *Rhizoctonia solani* consequently decrease damping-off as well as charcoal rot disease percentage. These findings are in agreement with the finding of Elwakil and El-Metwally (2000), who shows that the most potent antioxidant on the linear growth of *Cephalosporium* sp., *F. moniliforme*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotium bataticola* and *Verticillium* sp. was hydroquinone. Galal *et al.* (2000) investigated sensitivity of *Alternaria radicina* and *A. tenuissima in vitro* against five antioxidants (ascorbic acid, benzoic acid, hydroquinone, salicylic acid and tannic acid), they found that the mycelial dry weights of both tested fungi were completely inhibited at 10 mM benzoic acid and salicylic acid. Hydroquinone at 10 mM caused a complete inhibition of the mycelial growth of *A. tenuissima*. At the lowest concentration, 0.1 mM, ascorbic acid and salicylic acid stimulated the growth of *A. tenuissima*, but inhibited the growth of *A. radicina*. Tannic acid at 0.1 mM stimulated mycelial growth of *A. radicina* and inhibited *A. tenuissima*. In addition, Glala *et al.* (2005) reported that treatment of tomato plants with salicylic acid, citric acid and mixture (SA + CA) recorded the highest value traits of tomato plants.

The role of microelements (manganese and zinc) in reducing the harmful effects of charcoal rot disease, hence increasing growth and productivity of sunflower plant may be discussed as follows:

Manganese plays a role in regulating the levels of auxin in plant tissues by activating photosynthesis especially photosystem two (Marschner, 1986). Auxin may induced the systemic resistance and encourage the meristemic activity of the plant which resulted in more cell division and cell enlargement (Devlin and Witham, 1983).

Manganese and zinc are co-factors of Super Oxide Dismutase (SOD), which considered enzymatic antioxidant, hence alleviate the harmful effect of Reactive Oxygen Species (ROS free radicals) caused by fungal stress. These findings are in agreement with Kostas and Christos (2006), they found that the foliar application of microelements (manganese and zinc) can be used to reduce the severity of tan spot disease on durum wheat, however the physiological basis of this pattern still unknown.

The positive effect of zinc on increasing the vegetative growth which lead to increase of plant tolerance and increase in yield components may be due to the role of zinc as essential constituent of three enzymes (Carbonic anhydrase, Alcohol dehydrogenase and superoxide dismutase). Also, zinc has marked effect on the level of auxin by it appears to be required in the synthesis of intermediates in the metabolic pathway, through tryptophan to auxin, which encourage the meristemic activity (Ohki, 1978; Devlin and Witham, 1983). Moreover, El-Samnoudi (1990) in faba bean, stated that the application of zinc and manganese accelerated flowering and thus increased flowering capacity and seed yield.

In this investigation, all tested antioxidants and manganese increased photosynthetic pigments in turn, it will increases carbohydrate content in plant tissues. Carbohydrates are the main repository of photosynthetic energy, they comprise structurally polysaccharides of plant cell walls, principally cellulose, hemicelluloses and pectin that consider a barrier against plant pathogens invasion and phenolic compounds are associated with structural carbohydrates, which play a major and important role in plant defense (Hahlbrock and Scheel, 1989). In addition, the enhancement in chlorophyll content is resulting from stimulating pigment formation and increasing the efficacy of photosynthetic apparatus with a better potential for resistance as well as decreasing phtotophosphorylation rate, which occurred after infection (Amaresh and Bhatt, 1988). In this connection, Rhodes and Wooltorton (1978) indicated that, the adaptation of plants to biotic and abiotic stress is due to the stimulation of protective biochemical systems and synthesis of secondary metabolites such as phenolics.

The increase in seed oil percentage due to antioxidants may be due to increase in photosynthetic pigments because there is a relationship between photosynthesis processes and biosynthesis during seed development (Smith *et al*., 1989). They added that induced sucrose translocated to the seeds which metabolized to precursor for oil.

It was found that all tested chemicals decreased damping-off and charcoal rot diseases and at the same time enhanced the vegetative growth and increased the total phenols and chlorophyll contents. So, in this research, it may be recommended to use a mixture of citric acid and salicylic acid at 10 mM plus manganese at 2 g $L^{-1}$ for seed soaking of sunflower to alleviate the harmful effect of charcoal rot disease and maximizing yield and its components. In addition, these are environmentally safe chemicals on public health.

## REFERENCES

AOAC., 1970. Official and Tentative Methods Analysis. 11th Edn., The Association of Official Agricultural Chemists, Washington, DC.

Abd El-Hai, K.M., M.A. El-Metwally and S.M. El-Baz, 2007. Alleviation of the damage of faba bean chocolate spot and rust diseases by some nutritional elements. J. Agric Sci., Mansoura Univ., 32: 8231-8243.

Achuo, E.A., K. Audenaert, H. Meziana and M. Hofte, 2004. The salicylic acid-dependent defense pathway is effective against different pathogens in tomato and tobacco. Plant Pathol., 53: 65-72.

Amaresh, C. and R.K. Bhatt, 1988. Biochemical and physiological response to salicylic acid in reaction to systemic acquired resistance Photosynthetica, 35: 255-258.

Anonymous, 1994. Tissue culture studies of sunflower. PARC Res. Digest, 5: 11-12.

Baumer, J.S. and F. Hajdu, 1984. Occurance of charcoal rot in Minnesota in 1983. Proceedings of the Sunflower Research Workshop, National Sunflower Assoc., 1984, Fargo Forth Dakota, USA., pp: 15-15.

Chen, Z., J. Malamy, J. Henning, U. Conrath and P. Sanchez-Casas *et al*., 1995. Induction, modification and transduction of the salicylic acid signal in plant defence responses. Proc. Natl. Acad. Sci. USA., 92: 4134-4137.

CoStat Pro., 2005. CoHort software. Version 6.311, 798 Lighthouse Ave. PMB 320, Monterey, CA, 93940, USA.

Conte, T., A. Zazzerini and L. Tosi, 1989. Changes in composition of sunflower oil extracted from a chenes of Sclerotium bataticola infected plants. J. Agric. Food Chem., 37: 36-38.

De Gara, L., M.C. De Pinto and F. Tommasi, 2003. The antioxidant systems via-a-vis reactive oxygen species during plant-pathogen interaction. Plant Physiol. Biochem., 41: 863-870.

Devlin, R.M. and F.H. Witham, 1983. Plant Physiology. 4th Edn., A Division of Wads Worth, Inc., Wadads Worth Publishing Co., Belmont, California.

Dmitrier, A., M. Tena and J. Jorrin, 2003. Systematic acquired resistance in sunflower (*Helianthus annus* L.). Tsitologiya-1-Genetika, 37: 9-15.

Duncan, D.B., 1955. Multiple ranges and multiple F-test. Biometrics, 11: 1-42.

El-Baz, S.M., 2007. Induction of resistance in some soybean varieties against root rot diseases by some chemical inducers. Egypt. J. Applied Sci., 22: 68-80.

El-Samnoudi, I.M., 1990. Influence of irrigation regime and microelements application (Zn, Mn and Fe) on faba bean yield and nutrient content of seeds. J. Agric. Sci. Mansoura Univ., 15: 1916-1921.

Ellis, M.B., 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycologyical Institute. Kew. Surrey, England.

Elwakil, M.A. and M.A. El-Metwally, 2000. Hydroquinone, a promising antioxidant for managing seed-borne pathogenic fungi of peanut. Pak. J. Biol. Sci., 3: 374-375.

Everrt, N.P., K.E. Robinson and D. Mascarenhas, 1987. Genetic engineering of sunflower (*Helianthus annus* L.). Biotechnol., 5: 1201-1204.

Gabriela, R. and F. Castano, 2008. Susceptibility of sunflower (*Helianthus annus* L.) hyprids to mid stem rot and broken stem caused by *Sclerotrinia sclerotiorum*. Ciencia E Investigacion Agraria, 35: 21-28.

Galal, A.A., M.N. Shaat and A.A. El-Bana, 2000. Sensitivity of *Alternaria radicina* and *Alternaria tenuissima* to some antioxidant compounds. J. Agric. Sci. Mansoura Univ., 25: 1553-1562.

Glala, A.A., A.M. Hoda and Z.F. Fawzi, 2005. Improving of tomato growth, health, earliness, productivity and fruit quality by chemically induced systemic resistance. J. Applied Sci. Res., 5: 362-372.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultreal Research. 2nd Edn., John Wiley and Sons, New York, pp: 229-308.

Gossal, S.S., L. Vasiljevic and D.S. Brar, 1988. Plant biotechnology and sunflower improvement. Proceddings of the 12th International Sunflower Conference, 1988, Novi Sad, Yugoslavia, pp: 599-599.

Hahlbrock, K. and D. Scheel, 1989. Phsiology and molecularbiology of phenyl propanoid metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol., 40: 347-369.

Hassan, M.E.M., S.S. Abd El-Rahman, I.H. El-Abbasi and M.S. Mikhail, 2006. Inducing resistance against faba bean chocolate spot disease. Egypt. J. Phytopathol., 34: 69-79.

Ilyas, M.B., M.A.R. Bahtti and M.A. Randhawa, 1982. Biology host parasite relationship and charcoal rot fungus (*Macrophomina phaseolina*). Tech. Bull. No. 1, Soc. Adv. Agric. Sci. Pak. Univ. Agric., Faisalabad, Pakistan. http://www.scialert.net/pdfs/ajps/2003/834-840.pdf.

Klessing, D.F., J. Dumer, R. Noad, D.A. Navarre and D. Wendenne *et al*., 2000. Nitric oxide and salicylic acid singaling in plant defense. Proc. Natl. Acad. Sci. USA., 97: 8849-8855.

Kostas, B.S. and D. Christos, 2006. Effect of foliar applied boron, manganese and zinc on tan spot in winter durum wheat. Crop Production, 25: 657-663.

Mackinney, G., 1941. Absorption of light by chlorophyll solution. J. Biol. Chem., 140: 315-322.

Mahmood, T. and S. Sadaqat, 2003. Evaluation of $S_1$ and $S_2$ progenies of sunflower (*Helianthus annuus* L.) for seed yield, its components and resistance to charcoal rot (*Macrophomina phaseolina*). Asian J. Plant Sci., 2: 834-840.

Marschner, H., 1986. Mineral Nutrition of Higher Plants. Acadmic Press, London.

Metwally, A., I. Finkemeier, M. Georgi and K.J. Dietz, 2003. Salicylic acid alleviates the cadmium toxicity in barly seedlings. Plant Physiology, 132: 272-281.

Mirza, M.S., W. Manzoor, M. Rashid and S.T.A. Khan, 1984. Screening sunflower cultivars for resistance to charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. Proceedings of the National Sunflower Workshop, Barani Agriculture Research Develpment Project, 1984, PARC, Isalmabad, Pakistan, pp: 36-38.

Ohki, K., 1978. Zinc concentration in soybean as related to growth, photosynthesis and carboni anhydrase activity. Crop Sci., 18: 79-82.

Rhodes, J.M. and L.S.C. Wooltorton, 1978. The Biosynthesis of Phenolic Compounds in Wounded Plant Storage Tissues. In: Biochemistry of Wounded Plant Tissues, Kahl, G. (Ed.). Gruyter, Berlin, pp: 243-286.

Robinson, J.M. and S.J. Britz, 2000. Tolerance of a field grown soybean cultivars to elvated ozon level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydrogenase redox status and long term photosynthetic productivity. Photosynthesis Res., 64: 77-87.

Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova, 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulg. J. Plant Physiol., Special Issue: 314-319.

Shahda, W.T., 2001. Antifungal activity of salicylic acid for controlling Alternaria alternata of tomato. J. Agric Sci., Mansoura Univ., 26: 6117-6129.

Shahda, W.T., 2002. Role of antioxidants in plant diseases control. Fac. of Agric., Alexandria Univ., September 2002, pp: 42.

Singleton, V.L. and J.A. Roosi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enology Viticuture, 16: 144-158.

Smith, A.J., R.W. Rinne and R.D. Seif, 1989. Phosphoenolpyruvate carboxylase and pyruvate kinase involvement in protein and oil biosynthesis during soybean seed development. Crop Sci., 29: 349-353.

Sneh, B., L. Burpee and A. Ogoshi, 1992. Identificatin of *Rhizoctonia* Species. APS Press, USA., pp: 133.

White, R.F., 1979. Acetyl salicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology, 99: 410-412.