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Resistance inducers for root and charcoal rots caused by *Macrophomina phaseolina* and their impact on sunflower (*Helianthus annuus* L.) growth parameters

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Abstract

The present work was carried out to study the effect of some safety compounds, e.g. plant guard & Rhizo-Nbiocide, Ocimum & Eucaliptus plant extracts, salicylic acid & ascorbic acid antioxidants compared to Rizolex-T in controlling root and charcoal rots of sunflower plants (Giza 102). Growth, and quality parameters in greenhouse and open field during 2016 and 2017 growing seasons were determined. Most treatments significantly protected sunflower plants from diseases and increased growth parameters compared to control plants. Under field conditions, naturally infected sunflower plants with *Macrophomina phaseolina* were significantly decreased compared to infected control. Vegetative growth parameters as plant height, head diameter, 1000 seed weight, percentage oil, protein of sunflower were increased significantly. The results provide an alternative and safe measures for control of sunflower root and charcoal rots.. Further trials with other safe compounds may be recommended

Keywords: root rot, charcoal rot, biocide, plant extracts, antioxidants, growth, yield, sunflower.

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops globally and is grown on over 22 million hectares worldwide it is belongs to the family Asteraceae. In sunflower, losses from charcoal rot can reach 60 to 90% if the conditions are favorable for infection**.** Charcoal rot of sunflower caused by *M. phaseolina* (Tassi) Goid was first time observed in USSR, but was not considered as an important disease of sunflower until 1957. Now, it has been as one of the most important and dangerous diseases threatening the crop throughout the world and reducing the yield around 20-30% (Sackston, 1981). The diseased plants are recognized by early maturing and showing black or charcoal type discoloration on stem from which the disease gets the name "charcoal root" (Khan, 2007). *Macrophomina phaseolina* is reported to be soil, seed and stubble born in the specific case of sunflower. The pathogen has been reported to cause seedling blight, damping off, basal stem rotand early maturity of sunflower (Khan, 2007). As a part in the increase of sunflower acreage, there is potential increase in several pathological problems also that deteriorate the seed quality and market value of the crop. Charcoal rot is considered one of the high challenges facing sunflower cultivation, thus it is necessary to control disease and improving seed yield by using safe compounds such as biological control agents and resistance inducers. Although, chemical control is the best for charcoal root disease but, it has high cost andharmful effect on human health and environment (Abd El- Hai et al, 2007). Accordingly, using safe compounds to control charcoal root disease and improve plant growth and yield is of a paramount important. The use of biocontrol agents, plant extract, salicylic acid, etc would build a reduction in the use of both fungicides and mineral fertilizers. Treating seeds of many crops with biocontrol agents was effective to suppress many pathogens. Ghanbarzadeh et al, (2016) concluded that applying *Trichoderma harzianum* was less effective for wilt control than the chemical fungicides, but it highly improved growth and yield of onion plants. Strains of *Bacillus* are known to show biological control activity against certain soil-borne phytopathogenic fungi and has the potential to produce known secondary metabolites which showed antagonistic activity against *Macrophomina phaseolina* in sunflower. (Leo Daniel et al., 2012). Several investigators reported that the antioxidants may control seed and soil borne fungal diseases (Dmitrier et al., 2003; Shahda, 2001). Moreover, the antioxidants enhance the level of plant phenols which play a major role in plant disease defense, growth and development (Halbrock & Scheel, 1989). Salicylic acid (SA) as resistance inducer plays an essential role in the defence response to pathogen attack, improved plant growth, photosynthesis and chlorophyll content of pea, hence decreased plant injuries (Popova et al., 2008). SA significantly increased seed germination, plant height, number of leaves, branches, root length and number of nodules as well as seed yield of ginger (Ghasemzadeh & Jaafar, 2013). Also, ascorbic acid is one of the abundantly occurring water-soluble antioxidant organic compounds and is mainly distributed in the cytosol of the plant. It is required in trace amount to maintain normal plant growth in higher plants. It is main source of vitamin C for humans and essential compound for plants with important roles as an antioxidant and as a modulator of plant development through hormone signaling

(Athar et al., 2008). Ascorbic acid is synthesized in higher plants and affects plant growth and development (El-Kobisy et al., 2005). The resistance of plant may be also induced when using plant extracts, Plant serves as renewable natural resources for a variety of biologically active chemicals. These chemicals bear a variety of properties viz, antibacterial, antifungal and antiviral (Parajuli et al., 1998). *Ocimum gratissimum* and *Eucalyptus globule* contains eugenol and shows some evidence of antifungal activity (Elaigwu et al., 2017). In view of the importance of this disease which cause considerable damage to sunflower plants and yield, this work was planned to control disease incidence and improve plant growth and yield by using Bio Safe Compounds (biocide, plant extracts, antioxidants) as resistance inducers.

Materials and methods

This work was carried out on pots in screenhouse and field of Arab-El-Awamer research station, Assiut Governorate, Egypt. Sunflower plants Giza 102 cultivar, seeds were sown in the first week of May in 2016 and 2017 seasons.

Isolation of the pathogens: Isolations were made from root samples of infected sunflower plants collected from different sunflower fields. The root portion of the plants was cut into small pieces with sterilized scissor. The root pieces were washed under running tap water then, sterilized by 1 % sodium hypochlorite for 2-3 minutes and washed with distilled sterile water. Treated root pieces were placed in each Petri dish containing potato-dextrose agar medium amended with 40 mg strepophenicol / 100 ml medium. The inoculated plates were incubated at temperature (25±1°C), for5-7 days for fungal growth. The fungal colonies were purified using hyphal tip isolation technique as described by (Brown, 1924). Identification of the isolated fungi was done at Mycology Center, Assiut University, Assiut, Egypt. Pure cultures were kept at 5°C on PDA slants in test tubes C for further studies .All isolates were tested against Sunflower for producing disease symptoms.

Pathogenicity test: Pathogenicity tests were carried out for ten isolates of *M. phaseolina* under greenhouse. Sunflower seeds Giza 102cultivar were surface sterilized by1 % sodium hypochlorite solution for 2 min, then washed with sterile water. Sterilized pots (30 cm diameter) containing sterilized sandy loam soil artificially infested with tested fungi. Soil inoculation was performed by mixing of 3% of the inocula with the soil in each pot (150g/5kg soil) and then irrigated directly.Pot was seeded with 5 seeds and three pots were used for each isolate as replicates. Percentage of infected plants with Pre-emergence-damping off, Post- emergence- damping off and charcoal rot were recorded after 15, 30 and 90 days from sowing , respectively The following formulae were used to determine the disease criteria.

Pre-emergence damping off %= Number of non-emerged plantlets/Number of sown seeds x100

Post-emergence damping off %= Number of diseased plantlets/Total number of plantletsx100

Charcoal rot %= Number of plant with charcoal rot/Total number of plants x100

Preparation of the fungal inoculum: Inoculum of ten pathogenic isolates of *Mcrophomina phaseolina* were prepared by inoculating sterilized milk bottles 0.5 L containing Barley medium (75g Barley + 25 pure sand + 2g sucrose + 0.1g yeast extract + 100ml water) as described by Abd-El-Moneem (1996), with the fungal isolates separately and incubated at 28℃ for two weeks.

Preparation of extracts: Fresh leaves of Eucaliptus and Ocimum plants used for extracting antifungal principles as per the method of (Shekawat & Prasada, 1971). Fresh plant leaves were washed with tap water followed by sterilized water, macerated separately in mortar by rat 1gm/1ml distilled water. The resulted crud extract were separately for each plant extract and filtered through Muslin cloth and sterilized in Seitz filter to give 100 % plant water extract solution. From this standard / stock solution(s), required concentrations (5%, 10% and 15%) were prepared by adding sterile distilled water.

Preparation of chemical inducers resistant: Salicylic acid and ascorbic acid were dissolved in ethanol as described by Galal and Abdou (1996). The flowing concentrations (4, 6 and 8ml) from such compound were tested in vitro on growth of *M. phaseolina* Aliquots (8ml) of the tested solutions were added separately to 990 ml PDA medium.

***In vitro* investigation of biocide, plant extracts, antioxidants and fungicide on growth of *Macrophomina phaseolin*:** Biocide (Plant guard & Rhizo-N), plant extracts (Ocimum & Eucaliptus), antioxidants (Salicylic acid & Ascorbic acid) and fungicide (Rizolex-T) were added separately to PDA medium. Petri-dishes (9cm diameter) containing PDA medium were inoculated in the center with discs (5mm) of *M. phaseolina* isolate (No.6) taken, from 5 days old culture and incubated at 27℃. Four replicates were used for each treatment. Discs which were immersed in sterile distilled water were used as control. Diameter of linear growth of funguswas measured in cm, when fungal growth filled up control Petri-dishes. Percentage of reduction in mycelial growth was calculated using the following formula:

$$MG_I\ (\%) = (D_C - D_T) / D_C \times 100$$

Where: $\%MG_I$ = % Inhibition of mycelial growth, D_C = diameter of control, D_T = diameter of test

Effect of biocide, plant extracts, antioxidants and fungicide on incidence of charcoal rot disease of sunflower as seed treatments: Sunflower seeds were rinsed several times with tap water and soaked for 3hours, in the solution or suspension of treatments and left to one hour in air before sowing under screenhouse and/or field (Abd El- Hai et al., 2009) of the following tested treatments:

1- Control (untreated),
2- Biofungicide Plant guard: 1ml contains $30x10^6$ spores of *Trichoderma harzianum*,
3- Biofungicide Rhizo-N: 1ml contains $30x10^6$ spores of *Bacillus subtilis*
4- Sweet basil (*Ocimum basilicum*, L) 15%
5- Eucaliptus (*Eucalyptus globules*, Labill) 15%

6- Salicylic acid ((8 mM)
7- Ascorbic acid (8 mM)
8- Fungicide treatment with Rizolex-T 50 WP at 3g/l.

Effect of biocide, plant extracts, antioxidants and fungicide on incidence of charcoal rot disease of sunflower under screenhoue conditions: Eight treatments above mentioned were used to study their effects on root-rot and charcol rot diseases of sunflower under greenhouse conditions. In pots (30 cmdiameter) filled with 5kg sterilized soil. Six seeds were sown in infested soil with inocula of the most aggressive *M. phaseolina.* Control was seeds soaked in water. Four pots were used for each treatment as replicates. Root-rot and charcol rotincidence were estimated at 30 and 90 days from planting date, respectively. The following formulae were used to determine the disease criteria.

Root-rot (%) = Number of diseased plantlets/Total number of plantlets x100

Charcoal rot (%) = Number of plant with charcoal rot/ Total number of plants x100

Effect of biocide, plant extracts, antioxidants and fungicide on incidence of charcoal rot disease of sunflower under field conditions: A field experiment was carried out in the experimental farm of Arab-El-Awamer research station, Assiut Governorate, Egypt in 2016 and 2017 growing seasons. Treated seeds were sown in rows 4 in each plot (3 m x 5.2 m) at 60 cm spacing between rows, and 25 cm between seedbeds. Each row contained 20 hills. Every hill was sown with 3 seeds. After 20 days from sowing, plants were thinned to one plant per hill. A complete randomized block design with four replicates was adopted. The recommended cultural practices for production were followed throughout the growth season. Irrigation and fertilization were applied according to good agricultural practices of growers in the region. Percentage of naturally infected plants in every plot was recorded at premature stage (30 days old) and mature stage(90 days old).At harvest time, plant samples (10 healthy plants each) were taken at random from each plot to determine the following were recorded:

1- Heightof plant (cm)
2- Head diameter (cm)
3- 1000Seed weight (g)
4- Oil percentage in the seeds was determined according to A.O.A.C., (1995) using soxhletapparatus using petroleum ether as a solvent.
5- Protein percentage was calculated by multiplying the Nby the converting factor 6.25 (Hymowitz et al., 1972).

Statistical Analysis: The experimental design was established as a randomized complete blocks design with four replicates. All data were statistically analysed according to Gomez and Gomez (1984).

Results and Discussions

Pathogenicity test of *M. phaseolina*: Ten isolates were tested for pathogenicity on seedlings of sunflower cultivar Giza 102 under greenhouse conditions. Isolates were recovered from infected plants of sunflower that showed Root and charcoal rots symptoms. Isolates were identified as *M. phaseolin*. All the isolates were able to cause Pre and post-emergence damping-off, and charcoal rot associated with discoloration of infected tissues at harvest time. Testing the pathogenic capability of the isolated fungi (Table 1) indicated that all tested isolates were pathogenic to sunflower plants with different rates. Such results are in accordance with that reported by Balamurugan et al. (2012), Muhammed et al. (2014) and Rafi and Dawar (2016). Our results showed that the percentage of infection reported by *M. Phaseolin* No.6 was (73.33%) followed by isolates No. 1 and 2 (66.66%, and 60.00% respectively) while isolates No. 4 and 9 caused the intermediate percentage of infection (53.33%) followed by the rest of the isolates.

Table 1: Pathogenicity of *M. phaseolina* on sunflower plants.

Isolates No.	Percentage of infected plants		
	Pre-emergence damping-off	Post-emergence damping-off	Charcoal rot
1	6.66	13.33	66.66
2	0.00	13.33	60.00
3	6.66	0.00	40.00
4	20.00	6.66	53.33
5	6.66	0.00	60.00
6	13.33	6.66	73.33
7	20.00	26.66	46.66
8	20.00	33.33	26.66
9	6.66	20.00	53.33
10	13.33	13.33	33.33
Control	0.00	0.00	0.00
L.S.D. at 5%	n.s	15.14	16.37

Evaluation of biocide, plant extracts,antioxidants, and fungicide on *M. phaseolin in vitro*: Linear growth of *M. phaseolina* was significantly decreased at all of the tested treatments (Table 2). The highest decreased in linear growth was pronounced by fungicide (Rizolex), and Plant guard by (58.7% and 57.3%) respectively. The ascorbic acid and thiamine gave the least decreased by 37.2%. The, mycelial growth *M. phaseolina* was influenced and much reduced by other treatments. These results agree with Vey et al. (2001) whom found that tested *T. harzianum* isolates T7 and T 14for their capability to produce volatile and non-volatile compounds that inhibit the pathogen growth *in vitro*. From our results, it is evident that volatile compounds of both *T. harzianum* isolates decreased the mycelial growth of *M. phaseolina*. A great variety of volatile secondary metabolites could be produced by *Trichoderma* spp. Such as ethylene, hydrogen cyanide, aldehydes, and ketones,which play an important role in controlling various plant pathogens. While Leo Daniel et al. (2102) confirmed that *B. subtilis* was strong antagonist against *M.phaseolina* of in sunflower. Abd El- Hai et al. (2009) found that antioxidants (salicylic acid and ascorbic acid) and Rizolex inhibition in the linear growth of *M. Phaseolina* in sunflower. Also, agree with Elaigwu et al. (2017)

that showed *O. basilicum* leaves extract and *E. globules* caused significant reduction of the mycelia growth of *M. Phaseolina* in sesame to because of an array of biologically active plant chemicals including triterpenes, proteins and steroids. Plant extracts (*Ocimum basilicum* and *Eucalyptus globules*) and antioxidants (Salicylic acid and Ascorbic acid) tested at three concentrations against *M. phaseolin*. According to the obtained results, 8 mM concentration of salicylic acid and ascorbic acid were chosen for testing the possibility of induced resistance in sunflower diseases.

Table 2: Effect of biocide, plant extracts, antioxidants and fungicide on *M. phaseolin in vitro*.

Treatments	Inhibition growth (%)
Control	0.00
Plant guard (30x10^6)	57.3
Rhizo-N (30x10^6)	51.8
Ocimum (15%)	46.9
Eucaliptus (15%)	42.4
Salicylic acid (8 mM)	48.9
Ascorbic acid (8 mM)	37.2
Fungicide (Rizolex-3g/l)	58.7
L.S.D. at 5%=	16.01

Effect of biocide, plant extracts antioxidants and fungicide on root rot and charcoal rot of Sunflower plants under screenhouse conditions: Figure (1) shows the effect of two of bio safe compounds, two plant extract and two antioxidants, and the commercial systemic fungicide Rizolex was also considered. All treatments have decreased the root rot and charcoal rot percentage of infected sun flower plants compared to control. Both Plant guard and fungicide had greater decreased in diseases caused by *Macrophomin phaseolin* by (16.66%) and (20.82%) respectively, followed by Rhizo-N and SA treatments by (24.99%) followed by the rest of the treatments. These results are similar to those of Abd El- Hai et al., (2009). Biological control by *Trichoderma* spp. against pathogens might be due to mycoparasitism and competition for nutrients (Karmel Reetha et al., 2104). Also, Leo Daniel et al. (2102) reported that *Bacillus subtilis* AF1 has antifungal properties through the secretion of b-1, 4-N-acetyl glucosaminidase and a b-1,3glucanase .It also exhibited moderate production of siderophore and chitinase enzyme as chitinase production is reported to act as good antagonistic agent. Elaigwu et al. (2017) have found that the crude extracts of *E. globules* and *O. gratissimum* decreased charcoal rot in sesame as well as they considered several potential the rapeutic activities like antiviral, antibiotic efficacies. In addition, antioxidants may neutralize the harmful oxygen radicals released during then infections. Sahar Zayan (2016) reported that Salicylic acid is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants via molecules that maintains resistance to biotic stress in plants. More over ascorbic acid was the most effective chemical inducers as they greatly retarded charcoal rot caused by *M. phaseolina*. It is found that Soaking of okra seeds in the Ascorbic acid and salicylic acid for 6 hr. before planting decreased charcoal rot infection. Whereas application of salicylic and ascorbic acids resulted in accumulation of pathogens is related proteins (PRs), which have been defined as plant proteins that are induced in pathological and related situations as Salicylic and ascorbic acids . Application of HA enhances the activity of antioxidants such as α- tocopherol, α-carotene, superoxide dismutase, and ascorbic acid concentrations in turf grass species. These antioxidants may play a role in the regulation of plant development, flowering, and chilling of disease resistance.

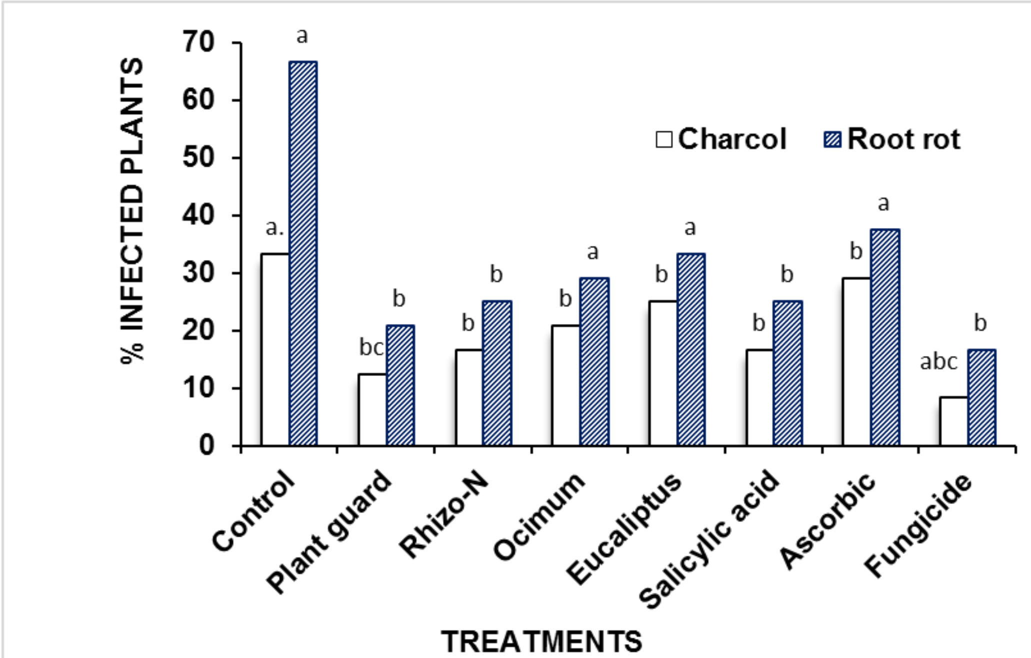

Figure 1: Effect of biocide, plant extracts, antioxidants and fungicide on root rot and charcol rot of sunflower plants under sreenhouse conditions.

Effect of biocide, plant extracts, antioxidants and fungicide on root rot and charcoal rot of Sunflower plants under field conditions: Table (3) shows the Effect of bio safe compounds, antioxidants, plant extracts and fungicide on root rot and charcoal rot of sunflower plants under field conditions. Treatments significantly reduced aforementioned both diseases. Data also show that the highest values of decrease occurred when fungicide (Rizolex) and Plant guard treatments, followed by the treatments of Rhizo-Nand salicylic acid. This finding is in agreement with those on the antagonistic activity of *T. harzianum* isolates T7 and T 14 against *M. phaseolina*. Khaledi and Taheri (2016) found that *T. harzianum* isolates reduced charcoal rot disease and promoted plant growth in the greenhouse. This antagonistic nature might be due to antibiotic nutrient competition and/or cell wall degrading enzymes (Kumar, 2013). The use of *B. subtilis* may also improve plant growth by suppressing non-parasitic root pathogen or by the production of biologically active substances or by unavailable minerai and organic compounds into forms available to plants. Zaki and Mahmood (1993) reported that *B. subtilis* can be used against *M. phaseolina* because it will be better for the control of this root-rot disease complex. Elaigwu et al. (2017) found that in sesame plant the antifungal activities of the extracts *E. globules* and *Ocimum gratissimum* include, antioxidant antifungal, antimicrobial, and antiviral, the antifungal activities of the extracts increased with increased as the concentration at field experiment conducted. Silva et al. (2010) reported that the essential oil of *O. gratissimum* contains eugenol and shows some evidence of antibacterial and antifungal activity. Also, the antifungal activity *E. globulus* extract may be attributed to the presence of some compounds. The major component was 1, 8-cineole (85.8%), B-pinine (7.2%) and B-myrcene (1.5%). *E. globules* could be a good antifungal efficacy, which may be used for formulating new, safer and eco-friendly fungicides (Cherkupally et al., 2107). Okra seeds soaked with chemical inducers (Salicylic acid and ascorbic acid) caused significant reduction charcoal rot disease either in pot or field experiments compared with the control treatment. Generally, ascorbic acid was the most effective chemical inducers as they greatly retarded charcoal rot caused by *M. phaseolina*, especially at the higher concentration (200 ppm). While SA recorded the lowest reduction compared with untreated plants (control). Several mechanisms that mediate the disease protection induced by different chemicals have been demonstrated, including blocking of disease cycle, the direct inhibition of pathogen growth and the induction of resistance to plant against pathogen infection. Also, demonstrated that application of exogenous chemical inducers or its derivatives induces synthesis of pathogenesis related proteins and partial resistance to pathogens (Ahn et al., 2005).

Table 3: Effect of biocide, plant extracts, antioxidants, and fungicide on root rot and charcoal rot of Sunflower plants under field conditions.

Treatments	Root-rot (%)		Charcoal rot (%)	
	2016	2017	2016	2017
Control	39.50	37.37	65.78	66.78
Plant guard (30x10^6)	4.75	4.75	10.10	10.50
Rhizo-N (30x10^6)	6.00	5.92	14.26	13.89
Ocimum (15%)	7.67	7.21	16.56	16.11
Eucaliptus (15%)	11.46	10.65	18.45	18.33
Salicylic acid (8 mM))	5.53	5.11	11.00	12.11
Ascorbic acid (8 mM)	12.89	11.98	20.55	19.57
Fungicide(Rizolex-3g/l)	0.00	0.00	6.35	7.51
LSD at 5%	3.02	1.93	1.95	1.37

Effect of bio biocide, plant extracts, antioxidants and fungicide on Some measurements during 2016, 2017 growing seasons: A perusal of the results showed (Table 4) that accessions have differed significantly in all treatments considered as plant height, Head Diameter, 1000-seeds weight, oil and protein contents being promoted as a sequence of the treatments compared to untreated plants. All treatments effect on different parameter of sunflower crops. For plant height the fungicide (Rizolex) recorded the highest increase followed in sequence Plant guard, *Ocimum*, Rhizo-N, Salicylic acid, Ascorbic acid and Eucaliptus, compared treatments to control.As for the rest of the parameters was fungicide (Rizolex) gave the best record of them followed by Plant guard, Rhizo-N, Salicylic acid, Eucaliptus and Ascorbic acid respectively compared to control. 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 concluded that induced sucrose located trans to the seeds which is metabolized to produse oil. The results agree with Abd El- Hai et al. (2009) whom found that seed soaking of sunflower in chemicals and fungicide (Rizolex) enhanced the vegetative growth and increased plant height and head diameter. Elaigwu et al. (2017) resulted extracts (*Ocimum* and Eucaliptus) have especially chloroform and antifungal constituents can effectively be used for the management of *M. phaseolina* and increased yield parameters. Reznikov et al. (2106) reported the highest crop yield values in soyabeen with *T. viride* and *B. subtilis*. The effect that *T. harzianum* isolates had an enhancing growth parameters in the plants inoculated by the pathogen, and reduced of disease severity, being were high in autoclaved soil. This result might be due to the better ability of *T. harzianum* in colonizing rhizosphere with better effect on plant growth and disease control in autoclaved soil along with absence of other competitive microorganisms (Khaledi & Taheri, 2016). Sahar Zayan (2016) tested chemical inducers (Salicylic acid and ascorbic acid) that significantly increased the tested growth parameters as plant height and branches number per plant compared with control treatment in both growing seasons.

Table 4: Effect of sunflower seed treatments with the tested biocide, plant extracts, antioxidants and fungicide on some measurements during 2016, 2017 growing seasons.

Treatments	Height of plant (cm)		Head diameter (cm)		1000-Seed weight (g)		Oil content (%)		Protein content (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Control	135.30	137.50	11.40	13.40	35.58	32.58	26.74	25.96	11.1	11.3
Plant guard ($30x10^6$)	160.30	161.00	17.40	17.81	61.50	60.68	42.66	41.07	16.7	15.4
Rhizo-N($30x10^6$)	159.30	159.90	16.80	16.50	58.62	57.38	40.08	40.42	16.1	16.1
Ocimum(15%)	161.00	161.00	15.96	16.00	55.98	55.50	39.37	39.22	14.4	14.6
Eucaliptus(15%)	146.00	147.90	15.50	15.30	55.13	54.13	38.19	37.80	14.6	14.9
Salicylic acid(8 mM)	158.50	157.60	15.80	15.00	56.65	57.20	40.98	40.64	15.8	15.9
Ascorbic acid(8 mM)	153.60	152.30	14.20	14.20	50.14	49.50	33.29	31.53	13.5	13.9
Fungicide(Rizolex-3g/l)	166.00	164.80	18.20	18.00	63.72	63.60	43.49	44.27	17.8	17.00
L.S.D. at 5%	5.40	1.92	1.62	1.96	1.00	1.44	2.50	1.75	1.52	1.06

Results from this study of chemical and biological seed treatments of sunflower to control charcoal rot will be useful for farmers to develop more efficient management strategies for this important disease. Additional research should be extended to further assess this plant–pathogen interaction under different environmental conditions. In addition, these are environmentally safe chemicals on public health.

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