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ORIGNAL PAPER

Management of the most Destructive Diseases of Chia plant and its Impact on the Yield

El-Kaed, S.A. [1]; **Mergawy, M.M.** [1] **and Hassanin, M.M.H.** [2]*

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ABSTRACT

Chia (*Salvia hispanica* L.) is one of the most important medicinal plants recently introduced to Egyptian cultivation and has significant multiusing in human curing. *Fusarium oxysporum*, *F. roseum*, *F. semitectum*, *F. solani*, *Macrophomina phaseolina*, *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were isolated from infected plants, showing root and stem rot symptoms collected from different localities of Giza and Fayoum governorates. The isolated fungi significantly realized different (%) of damping- off and root and stem rot. *Rhizoctonia solani*, *M. phaseolina* and *F. oxysporum* were the most aggressive fungi in the pathogenicity test. *Bacillus* spp. and *Trichoderma* spp. gave different degrees of antagonistic effects against the tested pathogens, *i.e.*, *F. oxysporum*, *M. phaseolina* and *R. solani in vitro*. Ascorbic and citric acids at 500 ppm recorded 100% seed germination *in vitro*. Under greenhouse conditions, Bio-Zeid, *T. harzianum*, *B. subtilis* and ascorbic acid gave the highest decreasing percentages of pre, post- emergence damping- off, root and stem rot and maximized healthy survivals. Under field conditions, Bio-Zeid, *T. harzianum* and ascorbic acid gave the highest decrease percentages of damping- off and root and stem rot and maximized plant survivals in the two experimental seasons, as well as increased plant growth parameters compared with the control (without treatment). Thus, it could be suggested that any of Bio-Zeid as formulated compound, also, ascorbic acid and *T. harzianum* can be formulated to be used for controlling chia damping-off and root and stem rot.

Keywords: Chia, *Salvia hispanica*, Bio-Zeid, ascorbic acid, citric acid, *Bacillus* spp. and *Trichoderma* spp.

*Correspondence: Hassanin M.M.H.
dr.hassanin.1978@gmail.com

Sarah A. El-Kaed
https://orcid.org/0000-0002-9529-6275

Madian M. Mergawy
https://orcid.org/0000-0002-4555-6219

1- Central Laboratory of Organic Agriculture, Agricultural Research Center, 12619, Giza, Egypt.

Mahmoud M.H. Hassanin
https://orcid.org/0000-0002-3127-0551

2- Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

INTRODUCTION

Chia (*Salvia hispanica* L.) is one of the most important autumn and winter medicinal plant crops in Egypt (Moghith, 2019), which belongs to the Family Lamiaceae. It was introduced, especially in the market of food products. The seeds have been subject to investigation, and they recognized beneficial effects on health due to its high protein content, antioxidants and dietary fiber (Ixtaina *et al.*, 2011). Chia seed contains up to 39% of an oil which has the highest known content of α-linolenic acid, up to 68% (Ayerza, 1995). Chia is one of the most efficient omega-3 (n-3) sources for enriching foods (Ayerza and Coates, 2001).

Damping-off, root and stem rot symptoms have been observed in chia plantations in Egypt which resulted in reduction of plant stand, vegetative growth. The causal pathogens of damping-off, root and stem rot diseases are mainly soil-borne. Percentages of frequency and occurrence of these diseases were always increased in the absence of the control measures in the field. Soilborne diseases, especially root and stem rot, caused by various pathogens, are amongst the most hazardous and destructive disorders on chia in Egypt. Chia was only recently documented to be susceptible to damping-off and root and stem rot. It is a destructive diseases limiting chia productivity (Nada, 2016). The primary pathogen responsible was identified as *Macrophomina phaseolina*, a widespread soilborne pathogen which has multiple commercial hosts (Su *et al.*, 2001). *Trichoderma* spp. and *Bacillus* spp. are now the most common biological control agents against plant diseases due to their capabilities to inhibit phytopathogens and increase growth promotion in plants (Kumar *et al.*, 2012 and Avşar *et al.*, 2017). Also, organic acids are being considered because conventional fungicides can result in accumulation of harmful residues which may lead to serious ecological and health problems. The use of organic acids (antioxidants) to control the fungal diseases was reported by several researchers (Prusky *et al.*, 1995, Khan *et al.*, 2001 and Zaky and Mohamed, 2009). Therefore, the objective of this study was to assess the effect of biocides and organic acids (antioxidants) on the incidence of damping-off and root and stem rot and crop parameters of chia plants in Egypt to develop management strategy of damping-off and root and stem rot chia plants.

MATERIALS AND METHODS

Isolation and identification of the associated fungi:

Infected chia plants showing root rot, stem rot and/or wilt diseases symptoms were collected from plantations of Giza and Fayoum governorates during January 2019 (Fig. 1). These were one or more of yellowing, stunting, stem blackening and dried shoots and wilt. Infected roots and stems were thoroughly washed several times with tap water, then cut into small pieces and surface sterilized by immersing in 1% sodium hypochlorite for 2 minutes, washed several times with sterilized distilled water and dried between folds of sterilized filter papers. The surface sterilized pieces were then plated onto potato dextrose agar (PDA) medium in Petri-dishes. The plates were incubated at 27±1°C for 7 days. The growing fungi were purified using single spore or hyphal tip techniques (Brown, 1924 and Hansen, 1926). Fungi were identified depending on their morphological features according to the descriptions of Booth (1971), Domsch *et al*. (1980), Niternik and Vandler (1981) and Nelson *et al*. (1983) and the identification was confirmed by the staff member of Department of Mycology, Plant Disease Survey and Plant Pathology Research Institute, ARC, Giza, Egypt. Pure cultures on PDA slants were kept at low temperature (5°C) for further studies.

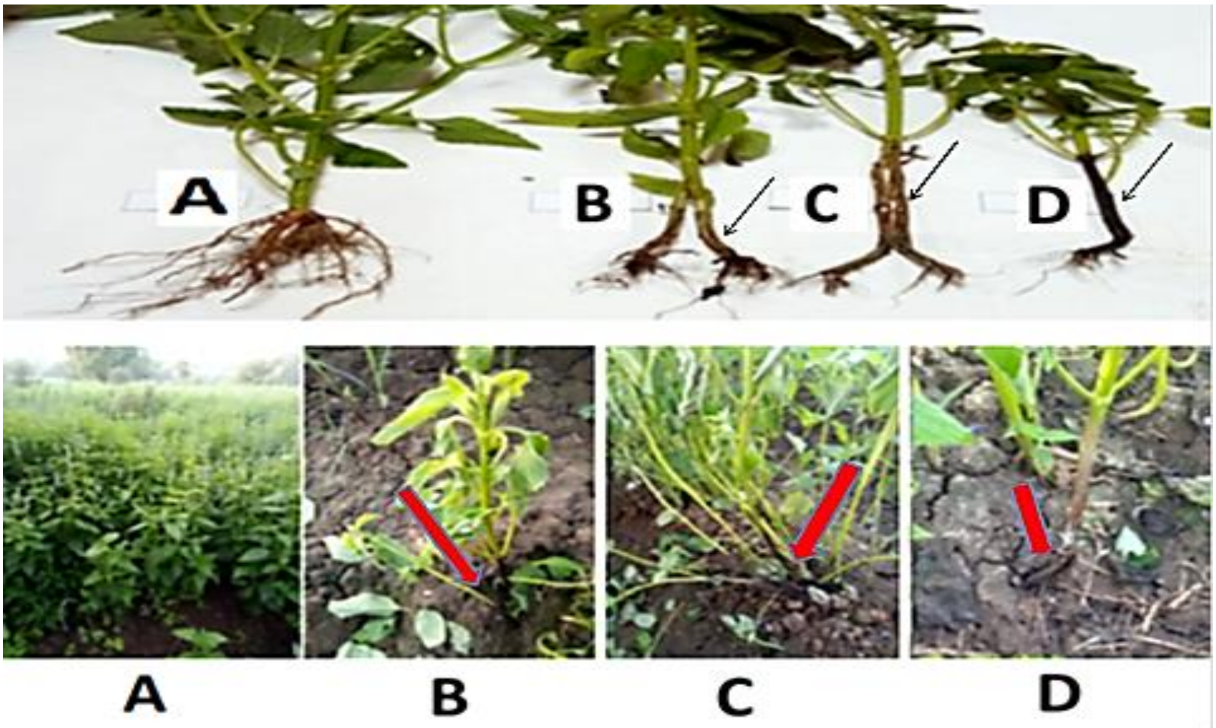

Fig. (1): Chia plants naturally infected by root and stem rot disease. (A: Healthy control; B, C, D: Diseased plants)

Pathogenicity test

Pathogenicity test was conducted to all the isolated fungi, *i.e., Fusarium oxysporum, F. roseum, F. semitectum, F. solani, Macrophomina phaseolina, Pythium ultimum, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Sandy clay soil (1 sand: 1 clay) was sterilized by 5 % formalin solution for two weeks, then left to dry for two weeks before use. Tested fungi were separately cultured on autoclaved sorghum grains medium (100 g sorghum + 50 g washed sand + 100 ml water) and incubated at 27±1°C for 15 days, except *S. sclerotiorum* was incubated at 18±2 ˚C. Soil infestation with the isolated fungi was applied at the rate of 1 % w/w, which was thoroughly mixed with the upper soil surface in plastic pots (20 cm diameter). Pots were watered till saturation one week before sowing to enhance colonization of the fungi. Pots were sown with 20 chia seeds for each. All treatments were replicated three times. Disease incidence was recorded as percentages of pre-, post- emergence damping- off and root and stem rot 15, 30 and 60 days after the sowing date, respectively using the following formula (Waller *et al*., 2002).

Disease incidence % =

$$\frac{\textbf{Number of infected plants}}{\textbf{Total number of planted seeds}} \times 100$$

Fungi were re-isolated from the infected plants and compared with the original isolates.

Antagonistic effect of some bioagents against mycelial growth of *F. oxysporum, M. phaseolina* and *R. solani in vitro*:

Isolates of *Bacillus* spp. and *Trichoderma* spp. used in this study were kindly obtained from the Central Lab of Organic Agriculture (CLOA), ARC, Giza, Egypt. The first isolate of *B. subtilis* (B1) was isolated from Beheira governorate and the second isolate (B2) was isolated from Fayoum governorate. The sequences of the isolates were submitted to NCBI Gen Bank and gave an accession number (MT110640) for (B1) and (MT110633) for (B2). The first isolate of *T. harzianum* (T1) was isolated from Ismailia governorate and the second isolate *T. asperellum* (T2) was isolated from Beheira governorate. The sequences of the isolates were submitted to NCBI Gen Bank and gave an accession number (MT111894) for (T1) and (MT110638) for (T2). Screening of antagonistic isolates for their capacities against *F. oxysporum, M. phaseolina* and *R. solani* was carried out using the dual culture plate method (Skidmore and Dickinson, 1976 and Rahman *et al.,* 2009). A loopful of 48 h old culture of any *B. subtilis* isolates grown on nutrient glucose broth was streaked on one side of Petri plate containing PDA medium. The other side of each plate was inoculated with disk (0.5 cm in diameter) of any tested pathogen taken from 7 days-old cultures grown on PDA medium.

Each plate was inoculated with *T. harzianum* at one side with a disk (0.5 cm in diameter) taken from the periphery of 7 days old culture. The opposite side of each plate was inoculated with a disk (0.5 cm in diameter) one of any pathogen (*F. oxysporum, M. phaseolina and R. solani*). Other plates only inoculated with the pathogen were used as a control treatment. Five replicates were used for each treatment. The plates were incubated at 25 ºC and the inhibition zone (if any) was recorded when the mycelial mats of the three pathogens covered the medium surface of the control plates. Only those isolates that produced a clear zone of inhibition or caused lyses of fungal mycelium were considered and used for further studies. The percentage of reduction in the mycelial growth of the pathogenic fungus was calculated as follows**:**

$$\textbf{\% Reduction} = \frac{\textbf{A – B}}{\textbf{B}} \times 100$$

A- Linear growth of the pathogenic fungus in the check plates (mm).

B- Linear growth of the pathogenic fungus in treated plates (mm).

Germination test:

Three organic acids (ascorbic acid, citric acid and oxalic acid) and six concentrations were prepared at the rates of 500, 1000, 2000, 3000, 4000 and 5000 ppm. Sets of 300 chia seeds were placed in Petri-dishes on layers of moistened blotters at the rate of 100 seeds/dish. Ten ml/dish from each concentration were added. Dishes were then incubated in controlled environment (27 °C) under alternating cycles of 12 hrs. light and 12 hrs. dark for 10 days. Percentage of germination was finally measured for each treatment.

Greenhouse experiments:

Effect of various control treatments on damping-off and root and stem rot diseases:

Under greenhouse experiment, 20 cm plastic pots filled with formalin sterilized mixture sand, peatmoss and clay soil (1: 1: 1 w: w: w) were used for planting. A set of four replicates was used for each treatment. The bioagents, *Trichoderma harzianum* (30×10^6 cfu/ml) and *Bacillus subtilis* (30×10^6 cfu/ml) were used at 5 ml/L. The biocides, Bio-Arc (*Bacillus megaterium*; 25 x 10^6 cell/g) and Bio-Zeid (*Trichoderma album*; 10 x 10^6 cell/g) were used at 5 g/L, also, the chemical elicitors, ascorbic acid and citric acid were used at 0.5 g/L water, compared to control without treatment.

Each treatment was applied as soil drench at sowing directly, as well as 15 days after sowing date. A set of 4 pots was used for each particular treatment and control. Eighty chia seeds were planted for each treatment (20 seeds / pot) in soil infested with *F. oxysporum*, *M. phaseolina* and *R. solani* (each alone). Percentages of disease incidence were determined as pre-, post- emergence damping-off and root and stem rot as well as survived plants after 15, 30, 60 and 60 days from sowing date, respectively.

Field experiments:

Field experiments were carried out at farm located in Aboxa village, Abshway county, Fayoum governorate, Egypt whereas, the disease of root and stem rot was found during three successive seasons, to evaluate the effect of each fungicide-alternative material, *i.e.* The bioagents *T. harzianum* and *B. subtilis* at 5 ml/L water, biocides, *i.e.* Bio- Arc and Bio-Zeid at 4 g/L water, the chemical elicitors, ascorbic acid at 0.5 g/L water and citric acid at 0.5 g/L water compared with control without treatment. Each treatment was applied as soil drench using pressurized backpack sprayer and spray wand equipped with one nozzle calibrated to deliver 100 ml/hill after seeding directly, as well as 15 days from sowing. The layout of the experiment was a split plot design with three replicates for each treatment. The plots were assigned to the soil drench treatment. The soil of the experimental site is classified as silty clay. The fields were prepared and divided into plots. The plot size was 4.2 m^2 (2.8 m x 1.5 m). Each plot consisted of four rows with 70 cm wide and 2.8 m long. Chia seeds were planted in hills 25 cm apart at the rate 5 seeds/hill. Seeds were planted on 1st September in both seasons in a naturally infested field. The recommended dose of nitrogen was applied at a rate of 200 kg/fed using ammonium nitrate (33.5% N). Phosphorus was applied and mixed with the soil before sowing at a rate of 250 kg/fed using calcium super phosphate (15.5% P_2O_5). Potassium was applied at a rate of 100 kg/fed using potassium sulphate (48% K_2O). Percentages of disease incidence were determined as pre-, post- emergence damping-off and root and stem rot as well as survived plants after 15, 30, 60 and 60 days from planting, respectively. Also, growth parameters were recorded as (Plant height (cm), Number of branches, Spike length (cm), Root length (cm), Seed yield/ plant (gm), Fresh weight (g/plant), Dry weight (g/plant), Number of spikes/ plant and Yield /feddan (Kg).

Statistical analysis:

The layout of this experiment was designed as factorial experiment in a complete randomized design with three replicates (Snedecor and Cochran, 1980). Statistical analysis was done by using the computer program MS-TATEC software version (4) using the L.S.D. test at 0.05.

RESULTS AND DISCUSSION

1- Isolation, purification and identification of the causal organisms and their frequency (%):

Eight fungal species belonging to five genera, *Fusarium, Macrophomina, Pythium, Rhizoctonia* and *Sclerotinia* were isolated from infected samples of chia plants. Samples showed root and stem rot symptoms were collected from different fields located in Giza and Fayoum governorates. Isolated fungi were identified as: *Fusarium oxysporum* Schlect., *F. roseum* Lk. Emend. Snyd. & Hans., *F. semitectum* Berk. & Rav., *F. solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Pythium ultimum* Trow, *Rhizoctonia solani* Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary. It is noticeable that *Fusarium solani, Pythium ultimum* and *Sclerotinia sclerotiorum* were not isolated from Giza governorate, in addition *Fusarium roseum* was not isolated from Fayoum governorate (Table, 1). Concerning mean percentages of frequency of the isolated fungi, data in (Table, 1) demonstrate that *F. oxysporum* (26.70%), *R. solani* (23.34%) and *M. phaseolina* (19.85%) recorded the highest means of frequency (%) for the isolation trials of the two governorates, while *S. sclerotiorum* (01.45%), *F. solani* (03.63%), *Pythium ultimum* (06.52%), *F. semitectum* (08.53%) and *F. roseum* (10.00%) recorded the lowest mean percentages of frequency. Such finding goes in accordance with the data obtained by Su *et al.* (2001) and Nada (2016)

2- Pathogenicity tests:

Pathogenicity test of the isolated fungi was conducted in the greenhouse. *Fusarium* spp., *Macrophomina phaseolina*, *Pythium ultimum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* were able to cause pre-, post-emergence damping- off and root and stem rot, as well as seedling survival was recorded (Table 2). All tested fungi significantly realized different percentages of pre- and post- emergence damping- off compared with the control treatment. However, *M. phaseolina*, *P. ultimum* and *R. solani* were the most pathogenic fungi as they recorded the highest percentages of pre - emergence damping- off (33.3, 31.7 & 26.7 %, respectively), while *F. oxysporum*, *R. solani* and *S. sclerotiorum* gave the highest percentages of post- emergence damping- off (36.7, 30.0 and 28.3%), respectively. On the other hand, symptoms of root and stem rot by all tested fungi appeared at 60 days after planting. However, *R. solani*, *M. phaseolina* and *F. oxysporum* were the most pathogenic fungi as they recorded the highest percentages of root and stem rot (10.0, 8.3 and 6.7%, respectively). Whereas *R. solani*, *M. phaseolina* and *F. oxysporum* recorded the lowest percentages of survivals (33.3, 43.4 and 44.9 %, respectively). In contrast, *F. roseum* recorded the lowest percentage of pre - emergence damping-off and root and stem rot (5 & 1.7 %, respectively), while *F. solani* and *P. ultimum* recorded the lowest percentage of post-emergence damping-off (6.7 and 3.8%, respectively). Whereas *F. roseum* and *F. solani* recorded the highest percentage of survivals (76.6 and 75.0 %). The aggressiveness of *M. phaseolina* and *R. solani* may be due to the presence of melanin pigment. Polak (1990) mentioned that melanin pigment plays a decisive role in the determination of virulence. Also, these results are, to somewhat, similar to those reported by Nada (2016) who found that *R. solani* followed by *F. oxysporum*, *F. solani* and *M. phaseolina* were the most pathogenic fungi on chia plants.

Table (1): Frequency percentages of fungi isolated from chia plants showing root and stem rot symptoms, in Giza and Fayoum governorates.

Isolated fungi	Giza		Fayoum		Mean
	No. of isolates	Frequency (%)*	No. of isolates	Frequency (%)*	
Fusarium oxysporum Schlect.	23	28.75	17	24.64	26.70
Fusarium roseum Lk. Emend. Snyd. & Hans.	16	20.00	0	00.00	10.00
Fusarium semitectum Berk. & Rav.	9	11.25	4	05.80	08.53
Fusarium solani (Mart) Sacc.	0	00.00	5	07.25	03.63
Macrophomina phaseolina (Tassi) Goid.	19	23.75	11	15.94	19.85
Pythium ultimum Trow	0	00.00	9	13.04	06.52
Rhizoctonia solani Kühn	13	16.25	21	30.43	23.34
Sclerotinia sclerotiorum (Lib.) de Bary	0	00.00	2	02.90	01.45
Total No.	80	100	69	100	-

*Frequency (%) = (Number of isolates of each fungus / Total number of isolates of all the tested fungi) x100.

Table (2): Percentages of pre- and post- emergence damping-off as well as root and stem rot infection caused by the tested fungi, under greenhouse conditions.

Tested fungi	% Damping-off		% Root and stem rot	% Survived plants
	Pre- emergence	Post- emergence		
Fusarium oxysporum	11.7	36.7	6.7	44.9
F. roseum	5.0	16.7	1.7	76.6
F. semitectum	23.3	23.3	3.3	50.1
F. solani	13.3	6.7	5.0	75.0
Macrophomina phaseolina	33.3	15.0	8.3	43.4
Pythium ultimum	31.7	8.3	3.3	56.7
Rhizoctonia solani	26. 7	30.0	10.0	33.3
Sclerotinia sclerotiorum	11.7	28.3	5.0	55.0
Control (without fungus)	0.0	0.0	0.0	100.0
L.S.D. at 5% for:	1.5	1.06	1.15	1.20

3- Antagonistic effect of some biocides against the growth of *F. oxysporum, M. phaseolina* and *R. solani in vitro*.

Data in Table (3) and Fig. (2) reveal that all the tested bioagents showed antagonistic effect against all tested pathogens, *i.e., F. oxysporum, M. phaseolina* and *R. solani*. The different bioagents also showed different degrees of antagonism. The highest antagonistic effect, compared with control, and other antagonists, was noticed when *T. harzianum* (T1) was used where percentages of reduction in linear growth of *R. solani*, *M. phaseolina* and *F. oxysporum* were 43.60, 37.33 and 25.00, respectively. *B. subtilis* (B2) occupied the second rank as a cause of inhibition against all tested pathogens. *T. asperellum* (T2) showed the least activity against *R. solani* and *F. oxysporum* and *M. phaseolina***.** It was found that the different tested antagonists either bacterial or fungal isolates were varied in their antagonistic effect against the three tested fungi under this study. This variation may be due to the amount and number of antifungal substances that are produced by the bioagent (Harman *et al.*, 2004 and Barakat *et al.*, 2006). *Trichoderma* spp. work through different mechanisms, such as mycoparasitism, also production of antifungal substances gliotoxin and trichodermin (Abd-El-Moity, 1976; Benítez *et al.*, 2004 and Harman *et al.*, 2004). According to the capacity of *Trichoderma* sp. in these regards, the effect of inhibition can be noticed.

Fig. (2): Antagonistic effects of *Trichoderma harzianum* and *B. subtilis* (B2) on growth of *F. oxysporum, M. phaseolina* and *R. solani in vitro*.

Table (3): Antagonistic effect of some bio controlling agents against the mycelial growth of *F. oxysporum, M. phaseolina* and *R. solani in vitro.*

Isolates	(%) Reduction in the linear growth of:		
	F. oxysporum	*M. phaseolina*	*R. solani*
Bacillus subtilis (B1)	24.00	21.66	20.80
B. subtilis (B2)	24.10	29.16	33.16
Trichoderma harzianum(T1)	25.00	37.33	43.60
T. asperellum (T2)	17.50	18.33	16.66
Control	0.00	0.00	0.00
L.S.D. at 5% for:	1.65	0.58	1.59

4- Effect of organic acids at different concentrations on germination of chia seeds:

The effect of organic acids at different concentrations on germination of chia seeds is presented in Table (4). Generally, there was a significant decrease in germination percentage when any of ascorbic and citric acids was used at concentrations above 500 ppm. Oxalic acid recorded 66 % germination percentage at 500 ppm, while ascorbic and citric acids gave maximum germination percentage (100 %) at 500 ppm. This showed that the use of oxalic acid at concentrations above 500 ppm had an inhibitory effect on seed germination. These results are, to somewhat, similar to those reported by Raza *et al.* (2013) who found that ascorbic acid is a natural antioxidant that plays an important role in plant metabolism as it is involved in embryogenesis during the process of seed formation. Its exogenous application may allow an endogenous increase in this antioxidant; but when applied at high concentrations, ascorbic acid can become detrimental, by nullifying (Takemura *et al.*, 2010) or simply reducing germination, as reported by Ishibashi and Iwaya-Inoue (2006) in wheat seeds treated with 50 and 100 mM ascorbic acid.

Table (4): Effect of three organic acids at different concentrations on seed germination percentage of chia seeds.

Treatments	% seed germination						Mean
	500 ppm	1000 ppm	2000 ppm	3000 ppm	4000 ppm	5000 ppm	
Ascorbic acid	100	93.0	90.0	84.3	62.3	35.3	77.5
Citric acid	100	89.0	73.6	56.0	49.3	33.0	66.8
Oxalic acid	66.0	0.0	0.0	0.0	0.0	0.0	11.0
*Control	100	100	100	100	100	100	100
Mean	91.5	70.5	65.9	60.1	52.9	42.1	63.8
L.S.D. at 5%	Treatments (T) =0.94		Concentrations (C) =1.08		(T) × (C) = 1.21		

* Germination of chia seed without treatment was done and recorded 100%.

5- Greenhouse experiments:

Effect of various control treatments on damping-off and root and stem rot diseases of chia plants under greenhouse conditions:

Data in Table (5) indicate that all the tested treatments significantly minimized the percentages of pre-, post- emergence damping-off, root and stem rot and maximized plant survivals (%) than the controls under greenhouse conditions. Biocide (Bio-Zeid), *T. harzianum*, *B. subtilis* and ascorbic acid gave the highest decrease percentages of pre-, post-emergence damping- off and root and stem rot. On the other hand, biocide (Bio-Arc) was the least effective treatment in percentage of pre-emergence damping- off, while citric acid was the least effective treatment in percentages of post-emergence damping-off and root and stem rot. The highest percentages of survivals, however, were recorded in case of using Bio-Zeid, *T. harzianum*, *B. subtilis* and ascorbic acid, respectively. On contrast, citric acid was the least effective treatment in increasing plant survivals. The positive efficacy of these treatments may be due to provided protection to chia plants against damping-off and wilt pathogens after planting of seeds as a result of their antifungal substances, which partially or completely prevent or delay the fungal infection processes and disease development. On the other hand, results of Nada, 2016, Kumar *et al.*, 2012, Avşar *et al.*, 2017, Prusky *et al.*, 1995, Khan *et al.*, 2001 and Zaky and Mohamed, 2009 might explain and support the present positive results of the tested treatments against pathogenic fungi to chia.

6- Field experiments:

Effect of various control treatments on damping-off and root and stem rot disease incidence of chia plants under field conditions:

Data in Table (6) indicate that all tested treatments significantly minimized the percentages of pre-, post- emergence damping-off, root and stem rot and maximized plant survivals (%) than the controls in Fayoum governorate during 2019/2020 and 2020/2021 seasons. Bio-Zeid *T. harzianum* and ascorbic acid gave the highest decrease percentages of pre- and post- emergence damping- off in both seasons. While Bio-Zeid, *B. subtilis* and *T. harzianum* gave the highest decrease percentages of root and stem rot in both seasons. On the other hand, Bio-Arc was the least effective treatment in both seasons, except for post-emergence damping-off (%) during 2019/2020 season.

The highest percentages of plant survivals, however, were recorded in case of Bio-zeid, *T. harzianum* and ascorbic acid, respectively during 2019/2020 and 2020/2021 seasons. On contrast, Bio-Arc was the least effective treatment in increasing survivals in the two seasons. Efficiency of bioagents (*Trichoderma* spp.) against pathogenic fungi may be due to production of inhibitory substances (Turner, 1971) and mycoparasitism (Ordentlich and Chet, 1989) or colonizing the court infection and preventing the pathogenic fungi for reaching susceptible plant tissues (Cook and Backer, 1983). On the other hand, these biocides were effective treatments against soil-borne fungi, and their diseases as reported by Abd-El-Moity

(1992), Brannen and Kenney, 1997, Hilal and Helmy (1998), Halawa (2004) and Hassanin (2013). Also, the antioxidant of organic acids mode of action was reported in many host-pathogen interactions, *i.e.*, many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase and polyphenoloxidase were detected as a result of infection with many pathogens (Clark *et al.*, 2002) or as a result of treatments with various antioxidents (Takahama and Oniki, 1994, El-Khallal, 2007 and Abdel-Monaim, 2008). Similar results were reported by Galal and Abdou (1996) who found that application of ascorbic acid as a soil drenching was better than foliar application to control fusarial diseases of cowpea. Aso, Mostafa (2006) reported that soaking cumin seeds or soil drenching with antioxidant solutions (salisylic, ascorbic, coumaric and benzoic acids) before planting resulted in resistant cumin seedlings against infection with *F. oxysporum* and *Acremonium egypticum*.

Table (5): Effect of different treatments on controlling damping-off and root and stem rot diseases of chia plants under greenhouse conditions.

Treatments (T)	Fungi (F)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Root and stem rot (%)	Plant survival (%)
B. subtilis	*F. oxysporum*	2.50	1.25	2.50	93.75
	M. phaseolina	5.00	2.50	1.25	91.25
	R. solani	6.25	2.50	2.50	88.75
	Mean	4.58	2.08	2.08	91.25
T. harzianum	*F. oxysporum*	0.00	1.25	1.25	97.50
	M. phaseolina	1.25	2.50	0.00	96.25
	R. solani	2.50	1.25	0.00	96.25
	Mean	1.25	1.67	0.42	96.67
Bio-Arc	*F. oxysporum*	5.00	6.25	3.75	85.00
	M. phaseolina	11.25	6.25	2.50	80.00
	R. solani	11.25	5.00	5.00	78.75
	Mean	9.17	5.83	3.75	81.25
Bio-Zeid	*F. oxysporum*	0.00	0.00	0.00	100.00
	M. phaseolina	1.25	0.00	0.00	98.75
	R. solani	2.50	0.00	0.00	97.50
	Mean	1.25	0.00	0.00	98.75
Ascorbic acid	*F. oxysporum*	2.50	2.50	5.00	90.00
	M. phaseolina	6.25	5.00	0.00	88.75
	R. solani	7.50	6.25	0.00	86.25
	Mean	5.42	4.58	1.67	88.33
Citric acid	*F. oxysporum*	3.75	8.75	6.25	81.25
	M. phaseolina	8.75	8.75	2.50	80.00
	R. solani	10.00	8.75	3.75	77.50
	Mean	7.50	8.75	4.16	79.58
Control	*F. oxysporum*	18.75	30.00	16.25	35.00
	M. phaseolina	31.25	27.50	10.00	31.25
	R. solani	23.75	17.50	18.75	40.00
	Mean	24.58	25.00	15.00	35.41
L.S.D. at 5 % for:					
Treatments (T) =		0.77	0.66	0.60	0.77
Fungi (F) =		0.50	0.43	0.39	0.50
(T) × (F) =		1.34	1.34	1.04	1.34

Table (6): Effect of different treatments on controlling damping-off and root and stem rot diseases of chia plants under field conditions.

Treatments	Pre-emergence damping-off (%)		Post-emergence damping-off (%)		Root and stem rot (%)		Plant survival (%)	
	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
B. subtilis	13.33	14.67	3.33	10.00	0.00	0.00	83.34	75.33
T. harzianum	6.67	10.00	0.67	1.00	0.00	0.00	92.66	89.00
Bio-Arc	26.67	36.67	7.67	20.00	2.33	3.00	63.33	40.33
Bio-Zeid	4.00	6.00	1.00	0.00	0.00	0.00	95.00	94.0
Ascorbic acid	10.00	11.67	3.33	7.67	0. 00	0.33	86.67	80.33
Citric acid	23.33	20.00	10.00	10.00	1.67	2.67	65.00	67.33
Control	60.00	63.00	17.67	15.67	5.67	6.66	16.66	14.67
L.S.D. at 5% for:	0.85	0.90	0.46	0.63	0.49	0.53	1.67	1.63

Plant growth parameters:

The obtained data (Tables 7, 8, 9 and 10) show that all treatments tested increased plant growth parameters, *i.e.*, Plant height (cm), number of branches, spike length (cm), root length (cm), seed yield/ plant (g), fresh weight (g/plant), dry weight (g/plant), number of spikes/ plant and yield/feddan (kg) than the control (without treatment). Differences between these treatments and the controls, however, were significant in Fayoum governorate during 2019/2020 and 2020/2021 seasons. Bio-Zeid, *T. harzianum* and ascorbic acid, respectively gave the highest increasing in all plant growth parameters in both two seasons. In contrast, the biocide (Bio-Arc) was the least effective treatments in plant height (cm), root length (cm), seed yield/ plant (g), fresh weight (g/plant) and dry weight (g/plant) during 2019/2020 season. While the bioagent (*B. subtilis*) was the least effective treatment in number of branches, whereas citric acid was the least effective treatment in spike length (cm), number of spikes/plant and yield/feddan (kg) during 2019/2020 season. On the other hand, the biocide (Bio-Arc) was the least effective treatment in plant height (cm), root length (cm), seed yield/ plant(g), fresh weight (g/plant) and dry weight (g/plant) during 2020/2021 season, while the bioagent (*B. subtilis*) was the least effective treatments in number of branches and number of spikes/ plant. Citric acid was the least effective treatments in spike length (cm) and yield/feddan (kg) during 2020/2021 season. The improvements in plant growth parameters due to soil treatment may be attributed to biochemical changing in the stem base tissues. This change includes increasing the activity of peroxidase enzyme, growth hormones and phenol compounds. Similar results on various crops, under naturally infested soil were reported by Hassanin (2013), Zhao *et al.*, 2014 and Halawa *et al.* (2018).

Table (7): Effect of different treatments on plant height, No. of branch/plant, spike length (cm), root length (cm) and seed yield/plant (g) of chia plants, grown in naturally infested soil, during of 2019/2020 season in Fayoum governorate.

Treatments	Plant height (cm)	Number of branches	Spike length (cm)	Root length (cm)	Seed yield/ plant (g)
B. subtilis	84.7	9.5	21.8	9.6	7.3
T. harzianum	88.1	13.7	25.9	10.7	12.6
Bio-Arc	79.3	10.9	18.2	7.6	6.6
Bio-Zeid	96.0	17.0	28.3	13.5	15.3
Ascorbic acid	87.6	13.8	23.7	10.1	9.0
Citric acid	82.9	11.3	13.4	8.9	6.9
Control	65.5	4.2	9.0	5.4	2.3
L.S.D. at 5% for:	0.82	0.74	0.59	0.49	0.56

Table (8): Effect of different treatments on fresh weight (g/plant), dry weight (g/plant), No. of spikes/plant and yield/ feddan (kg) of chia plants, grown in naturally infested soil, during of 2019/2020 season in Fayoum governorate.

Treatments	Fresh weight (g /plant)		Dry weight (g /plant)		No. of spikes/ plant	Yield/ feddan (kg)
	Roots	Shoots	Roots	Shoots		
B. subtilis	6.7	45.6	3.7	25.6	17	422.5
T. harzianum	7.6	50.1	4.1	30.7	23	615.0
Bio-Arc	6.1	40.1	3.2	22.8	19	401.1
Bio-Zeid	8.3	53.0	4.8	35.6	28	623.7
Ascorbic acid	7.1	48.2	4.0	30.1	22	493.3
Citric acid	6.9	42.3	3.8	27.3	18	335.6
Control	3.1	22.3	1.6	13.7	8	176.4
L.S.D. at 5% for:	0.44	0.99	0.36	0.98	1.32	2.45

Table (9): Effect of different treatments on plant height, No. of branches/plant, spike length (cm), root length (cm) and seed yield/plant (g) of chia plants, grown in naturally infested soil, during of 2020/2021 season in Fayoum governorate.

Treatments	Plant height (cm)	Number of branches	Spike length (cm)	Root length (cm)	Seed yield/ plant (g)
B. subtilis	81.3	8	19.1	7.5	5.1
T. harzianum	86.5	12	23.3	8.5	10.0
Bio-Arc	77.0	9	16.3	6.0	4.1
Bio-Zeid	90.0	15	26	11.6	13.7
Ascorbic acid	85.8	12	21	8.4	7.4
Citric acid	80.3	10	12.5	6.8	4.7
Control	61.0	3	8	4.1	1.8
L.S.D. at 5% for:	0.93	1.32	1.03	0.60	0.41

Table (10): Effect of different treatments on fresh weight (g/plant), dry weight (g/plant), No. of spikes/plant and yield/ feddan (kg)of chia plants, grown in naturally infested soil, during of 2020/2021 season in Fayoum governorate.

Treatments	Fresh weight (g/plant)		Dry weight (g/plant)		No. of spikes/ plant	Yield/ feddan (kg)
	Roots	Shoots	Roots	Shoots		
B. subtilis	6.1	40.4	3.3	23.3	15	401.6
T. harzianum	7.1	48.0	3.9	28.4	22	601.0
Bio-Arc	5.4	36.5	3.0	19.6	18	394.3
Bio-Zeid	7.6	50.9	4.2	33.1	26	617.0
Ascorbic acid	6.5	43.5	3.8	28.3	20	480.0
Citric acid	5.8	38.6	3.2	25.4	16	319.0
Control	2.3	21.1	1.2	11.2	7	161.6
L.S.D. at 5% for:	0.47	1.28	0.66	1.47	1.32	1.93

CONCLUSION

biological control and organic acids have had an effect in management of damping-off and root and stem rot of chia plant, improving various plant growth standards.

REFERENCES

Abd-El-Moity, T. H. 1976. Studies on the Biological Control of White Rot of Onion. M. Sc. Thesis, Fac. Agric, Minufiya Univ., pp.121.

Abd-El-Moity, T.H. 1992. The use of *Trichoderma* spp. to control soilborne plant pathogens in Egypt. pp. 255-258. In: Biological control of plant diseases: Progress and Challenges for the Future. E.C. Tjamos, G.C. Papavizas and R. J. Cook, eds. Plenum press, New york, USA.

Abdel-Monaim, M.F. 2008. Pathological Studies of foliar and root diseases of Lupine with special reference to induced resistance. Ph.D. Thesis, Fac. Agric., Minia University. pp.175.

Avşar, C.; Koyuncu, H. and Aras, E.S. 2017. Isolation and molecular characterization of *Bacillus* spp. isolated from soil for production of industrial enzymes. Biological and Chemical Research. 3(9):72-86**.**

Ayerza, R. 1995. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. Journal of the American Oil Chemists' Society, 72: 1079-1081.

Ayerza, R. and Coates, W. 2001. The omega-3 enriched eggs: the influence of dietary linolenic fatty acid source combination on egg production and composition. Canadian Journal of Animal Science, 81: 355-362.

Barakat, R.M.; Al-Mahareeq, F. and Al-Masri, M.I. 2006. Biological control of *Sclerotium rolfsii* by using indigenous *Trichoderma* spp. isolates from Palestine. Hebron Univ. Res. J., 2(2): 27-47.

Benítez T.; Rincón, A.M.; Limón, M.C. and Codón, A.C. 2004. Biocontrol mechanisms of Trichoderma strains. Int. Microbiol., 7(4):249-260.

Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycol. Inst., Kew, Surrey, England.

Brannen, P.M. and Kenney, D.S. 1997. Kodiak-A successful biological control product for suppression of soil-borne plant pathogens of cotton. J. Industrial Microbiol. & Biotechnol., (USA), 19(3): 169-171.

Brown, W. 1924. A method of isolating single strains of fungi by cutting out a hyphal tip. Annals of Botany, 38(150): 402-404.

Clark, F.S.; Guy, P.L.; Burritt, D.J. and Jameson, P.E. 2002. Changes in the activities of antioxidant enzymes in response to virus infection and hormone treatment. Physiol. Plantarum, 114: 157-164.

Cook, R.J. and Backer, K.F. 1983. The Nature and practice of biological control of Plant Pathogens. St. Paul, Minn., Am. Phytopathol. Soc., 359 pp.

Domsch, K.H.; Gams, W. and Anderson, T.H. 1980. Compendium of soil fungi, Vol. 1&2, Academic Press, London.

El-Khallal, M.S. 2007. Induction and modulation of resistance in tomato plants against Fusarium wilt disease by bioagent fungi (*A. mycorrhiza*) and/or hormonal elicitors (jasmonic acid and salicylic acid): 2- Changes in the antioxidant enzymes, phenolic compounds and pathogen related-proteins. Aust. J. of Basic and App. Sci., 1(4): 717-732.

Galal, A.A. and Abdou, E.S. 1996. Antioxidants for the control of Fusarial diseases in cowpes. Egypt J. Phytopathol., 24: 201-211.

Halawa, A.E.A. 2004. Pathological studies on some soilborne fungi attacking some of ornamental trees in Egypt. M.Sc. Thesis, Fac. Agric., Zagazig Univ. (Benha Branch), 186 pp.

Halawa, A.E.A.; Ali, A.A.M. and Hassanin, M.M.H. 2018. Efficiency of some organic acids as safe control mean against root and stem rot disease of *Coleus forskohlii*. J. of Phytopathol. and Pest Management, 5(2): 48-62.

Hansen, H.N. 1926. A simple method of obtaining single spore cultures. Science, 64: 384.

Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I. and Lorito, M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Reviews Microbiol., 2: 43-56.

Hassanin, M.M.H. 2013. Pathological Studies on Root Rot and Wilt of Black Cumin (*Nigella sativa*) and Their Management in Egypt. Ph.D. Thesis, Fac. Agric., Al-Azhar Univ. (Egypt), 137pp.

Hilal, A.A. and Helmy, A.A. 1998. Crown and root rot of turfgrasses in Egypt: Identification of the causal pathogens, pathogenicity and biological control. Egypt. J. Appl. sci., 13(1): 1-18.

Ishibashi, Y. and Iwaya-Inoue, M. 2006. Ascorbic acid suppresses germination and dynamic states of water in wheat seeds. Plant Prod. Sci., 9: 172–175.

Ixtaina, V.Y.; Martínez, M.L.; Spotorno, V.; Mateo, C.M.; Maestri, D.M.; Diehl, B.W.K.; Nolasco, S.M. and Tomás, M.C. 2011. Characterization of chia seed oils obtained by pressing and solvent extraction. J. Food Compos. Anal., 24: 166-174.

Khan, S. H.; Aked, J. and Magan, N. 2001. Control of the anthracnose pathogen of banana (*Colletotrichum musae*) using antioxidants alone and in combination with thiabendazole or imazalil. Plant Pathol., 50: 601-608.

Kumar, K.; Amaresan, N.; Bhagat, S.; Madhuri, K. and Srivastava, R.C. 2012. Isolation and characterization of *Trichoderma* spp. for antagonistic activity against root rot and foliar pathogens. Indian Journal of Microbiology, 52(2):137-144**.**

Moghith, W. 2019. Studies on growth and productivity of chia plant (*Salvia hispanica* L.) under Egyptian conditions. Ph.D. Thesis, Fac. Agric., Benha Univ. (Egypt), 169pp.

Mostafa, W.E.B. 2006. Studies on some cumin diseases. M.Sc. Thesis, Fac. Agric., Minia Univ. Pp.165Pp

Nada, M.G.A. 2016. Soilborne fungi causing disease problems on the medicinal plant chia (*Salvia hispanica* L.) in Egypt and the possibility of controlling damping-off. J. Biol. Chem. Environ. Sci., 11(3): 483-510.

Nelson, P.E.; Toussoum, T.A. and Morasas, W.F.O. 1983. *Fusarium* Species, An Illustrated Manual for Identification. The Pennsylvania State Univ. Press, 193pp.

Niternik, P. and Vandler, A.J. 1981. Monograph of the Genus *Pythium*. Studies in Mycology, No. 21, Central Burea Voor Schimmel Cultures, Baarn, Netherland. pp.242.

Ordentlich, A. and Chet, I. 1989. Biological control of soilborne plant pathogenic fungi by antagonistic Trichoderma. Israel Agresearch, 3 (1-2) XII: 137-152 (c.f. Rev. Pl. Pathol., 71 (12): 553, 1992).

Polak, A. 1990. Melanin as a virulence factor in pathogenic fungi. Mycoses, 33(5): 215-224.

Prusky, D.; Ohr, H.D.; Grech, N.; Campbell, S.; Kobiler, I.; Zauberman, G. and Fuchs, Y. 1995. Evaluation of antioxidant butylated hydroxyanisole and fungicide prochloraz for control of post-harvest anthracnose of avocado fruit during storage. Plant Disease, 79: 797-800.

Rahman, M.A.; Begum, M.F. and Alam, M.F. 2009. Screening of Trichoderma Isolates as a Biological Control Agent against *Ceratocystis paradoxa* Causing Pineapple Disease of Sugarcane. Mycobiology 37 (4): 277-285.

Raza, S.H.; Shafiq, F.; Chaudhary, M. and Khan, I. 2013. Seed invigoration with water, ascorbic and salicylic acid stimulates development and biochemical characters of okra (*Ablemoschus esculentus*) under normal and saline conditions. International Journal of Agriculture and Biology, 15(3): 486-492.

Skidmore, A.M. and Dickinson, C.H. 1976. Colony interactions and hyphal interference between *Septoria Nodorum* and phylloplane fungi. Trans. Brit. Mycol. Soc. 66: 57-64

Snedecor, G.W. and Cochran, G.W. 1980. “Statistical Methods”. 7th Ed, Iowa State Univ. Press, Ames, USA. 570 pp.

Su, G.; Suh, S.O.; Schneider, R.W. and Russin, J. S. 2001. Host specialization in the charcoal rot fungus, *Macrophomina phaseolina*. Phytopathology, 91(2): 120-126.

Takahama, U. and Oniki, T. 1994. Effects of ascorbate on the oxidation of derivatives of hydroxycinnamic acid and the mechanism of oxidantion of sinapic acid by cell wall- bond peroxidases. Plant Cell Physiol., 35: 593-600.

Takemura, Y.; Satoh, M.; Satoh, K.; Hamada, H.; Sekido, Y. and Kubota, S. 2010. High dose of ascorbic acid induces cell death in mesothelioma cells. *Biochemical and Biophysical Research Communications*, 394(2): 249-253.

Turner, W.B. 1971. Fungal Metabolites. Academic Press, London, N.Y., 446 pp.

Waller, J.M.; Lenne, J.M. and Waller, S.J. 2002. Plant Pathologist’s Pocketbook. 3rd ed. CABI Publishing, New York. pp. 27.

Zaky, W. H. and Mohamed, N.T. 2009. Efficiency of some important organic acids as safe control mean against root and stem rot of sweet basil. Egypt J. of Appl. Sci., 24(6A): 92-105.

Zhao, Y.; Selvaraj, J.N.; Xing, F.; Zhou, L.; Wang, Y.; Song, H.; Tan, X.; Sun, L.; Sangare, L.; Folly, Y.M.E. and Liu, Y. 2014. Antagonistic Action of *Bacillus subtilis* Strain SG6 on *Fusarium graminearum*. PLoS ONE 9(3): e92486.