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Controlling Alternaria Leaf Spot in Chia Plant Using Biological Agents with Biofertilizers and Their Effects on Quality, Productivity and Fixed Oil of Seeds

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Abstract: *Alternaria alternate* YZM1 (OQ569158) causes Chia (*Salvia hispanica* L.) leaf spot was isolated and molecular identified from Fayoum governorate, Egypt. This study was conducted in Abshaway county, Fayoum governorate during the 2019/2020 and 2020/2021 seasons. Our goal was to control Alternaria leaf spot, also improve plant growth, seed quality and fixed oil productivity using organic inputs as alternatives to toxic chemicals used in fertilization and fungicides. Compost and biofertilizer species including *Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum* and *Bacillus circulans*, were applied in both greenhouse and field experiments. Additionally, foliar spraying with biological control agents, including *Trichoderma harzianum*, *Bacillus subtilis* and *Streptomyces rochei*. Results indicated that, treatment with *Trichoderma harzianum* + *Streptomyces rochei* with biofertilizer led to the most significant reduction in disease incidence and severity (22.5% and 12.05% respectively). In addition, it significantly increased the activities of defense-related enzymes (peroxidase, polyphenoloxidase, and chitinase), total phenols, sugars and carbohydrates under greenhouse conditions. Furthermore, compost with biofertilizer increased plant height, spike number per plant, chlorophyll, plant NPK contents, seed yield per fed, fixed oil yield and seed quality under field conditions. According to our knowledge, this is the first report of *Alternaria alternata* causing Alternaria leaf spot on Chia in Egypt.

Key words: *Salvia hispanica, Alternaria alternata,* Bioagents, Biofertilization, fixed oil, yield.

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1. Introduction

Salvia hispanica L. plant is a new crop insert into the Egypt's agricultural system to add new varieties of therapeutic and aromatic plants (**Salman *et al.*, 2019**). Chia (family: *Lamiaceae*) is an herbaceous plant used for commercially and medically. Although it was previously grown in tropical and subtropical regions, today it is grown throughout the world as a valuable human food supplement and premium animal feed **(Abdel-Aty *et al.*, 2021).** Dry chia seeds have a high fixed oil yield with significant economic value that is utilized in the supplements

producing, medications, processed foods and dietary fibers. They are also rich in minerals, proteins, carbohydrates, phenolic compounds and essential fatty acids (**El-Serafy *et al*., 2020**).

The pathogen *Alternaria alternata* affects a wide variety of hosts. It creates small, round, brown spots on leaves, which are typical disease symptoms. As the disease progresses, the spots take on an uneven shape and eventually cause the leaves to wilt, dry out and shed. It lower seed quality and causes infection before and after harvest. **(Shoaib *et al*., 2021).** Chemical fungicides are generally used to control *Alternaria* disease, which have negative effects on both soil microorganisms and human health. **(Kayim *et al*., 2018)**.

Organic fertilizer promotes the growth of soil microorganisms, increases soil fertility and plant quality and productivity by improving biological activities, organic matter content, nitrogen and carbon content, soil structure and quality **(Akanmu *et al*., 2023).**

Bacteria and fungi that can promote plant growth, health, and disease control are known as biofertilizers and biocontrol agents. Biofertilizers such as *Azospirillum* spp., *Azotobacter* spp. and *Bacillus* spp. have the ability to fix nitrogen, solubilize potassium and phosphate in the soil, produce phytohormones (such as auxin and gibberellin), boost plant enzymatic activity and decomposition of organic materials. They cycle nutrients and ensure significant growth and development of the plant **(Hassouna *et al*., 2020; Kumar and Sharma, 2022).** while, Microbes with the ability to produce one or more antibiotics, antifungal enzymes such as glucanases, lipases, proteases and chitinases or enzymes capable of lysing the fungal cell wall, exhaust iron from the rhizosphere and causing systemic resistance known as biocontrol agents. **(Pirttilä *et al*., 2021)**.

Bacillus spp. plays an important role as plant growth promoter and biocontrol agent by synthesizing phytohormones (auxin, cytokinin and gibberellins), siderophores, antibiotics, volatile compounds, dissolving phosphorus, competition and inducing systemic resistance **(Ahmad *et al.*, 2019)**. *Trichoderma* spp. acts as biocides through its ability to inhibit plant pathogens, competition for nutrients and produce antibiotic compounds that inhibit the growth of pathogens **(Rahman *et al*., 2020)**. Streptomyces spp. plays an important role by secreting a wide range of antibiotics that can lysis fungal cell walls, so they have been shown to be effective biocontrol agents. **(Devi *et al*., 2022)**.

Copper compounds have the ability to decay fungal spores and conidia and inhibit spore germination, thus reducing the incidence of disease. This may be due to $CuSO_4$ providing the plant with two essential elements (Cu and S). Copper plays an important role in lignin formation, which affects the growth of fungi and has a toxic effect on them. Additionally, sulfur plays an important role in protein formation and the bonding of some amino acids **(Ghazy *et al*., 2020)**. Copper-based fungicides are commonly used to control lemon MSD in multiple applications to be effective. However, these treatments raise concerns regarding prolonged copper persistence in the environment, which can accumulate in soil and groundwater leading to toxic effects on plants, animals, soil microbiota and the contamination of food **(Abbate *et al.*, 2019).** According to **La Torre *et al*. (2018) and El boumlasy *et al*. (2022)**, the European Union has limited the use of copper-based fungicides. As a result, the goal of this study was to treat Alternaria leaf spot in chia plants using biological control agents with compost and biofertilizers. In addition to create a safe, chemical-free, healthy and exportable plant.

2. Materials and Methods

2.1. Used materials

Chia seeds cultivar: Misr 1 cultivar obtained from Crop Intensification Research Department, field Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt.

Biofertilizers inoculum: *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans* were kindly obtained from the Central Laboratory of Organic Agriculture (CLOA), ARC, Giza, Egypt.

The strains were activated in their specific medias as semi-solid malate medium **(Dobereiner *et al*., 1976)**, Pikosvskey's medium (**Pikovskaya, 1948)** and Aleksandrov's agar medium **(Parmar and Sindhu, 2013)** respectively. Then incubated at 30^{o} C on a rotary shaker at 120 rpm for 48 hours and adjusted to 10^{8} cfu /ml, they mixed in equal volumes to prepare the inoculum mixture of biofertilizers.

Chia seeds were soaked in inoculum for 30 minutes immediately before sowing. The inoculum is added as a first addition after sowing and repeated every 30 day (soil drench) after watering, it is add to all treatments in both greenhouse (5 ml/pot) and field (5 L/fed.) experiments **(Afifi *et al*., 2014)**.

Compost: Compost obtained from the same farm where the experiment was conducted out in Fayoum governorate, Egypt. It is add to all treatments in both greenhouse and field experiments, mixed with the soil during plowing at a rate of 12 m^3/fed**. (Moghith *et al*., 2021).**

The physical and chemical properties have been determined **(Page *et al*., 1982)**, as shown in Table (A).

Table (A). Physical and chemical properties of the compost

Physical and chemical properties	Value
Bulk density (%)	720
Moisture (%)	21.5
pH (1:10)	7.89
EC (1:10) dS/m^3.	4.88
Organic carbon (%)	22.12
Organic matter (%)	37.67
Ash (%)	69.70
C /N ratio	18: 1
Ammonia (ppm)	203
Nitrate (ppm))	58
Total nitrogen (%)	1.22
Total phosphorus (%)	1.08
Total potassium (%)	1.80
Fungus spores	Not detected

Bioagents: *Trichoderma harzianum, Bacillus subtilis* and *Streptomycis rochei* strains were kindly obtained from CLOA, ARC, Giza, Egypt. Molecular identification of the three bioagents has been performed previously and deposited in GenBank with accession numbers: MT110634, MT110633 and OP164572.1, respectively. All bioagents were sprayed at a rate of 1 L/50 L water (20 ml/L.).

Copperal max (fungicide): The active ingredient is copper sulfate ($CuSO_4$ 10%), which is allowed in organic agriculture, with a recommended concentration of 1L/200L water, produced by (CLOA), ARC. **(Mergawy, 2016)**. Copperal max (10%) was used at a rate of 5 ml/L.

2.2. Isolation and identification of the fungal pathogen

Twenty naturally infected Chia plant samples exhibiting typical leaf spot disease signs were gathered from various fields within the Fayoum Governorate, encompassing the counties of Abshway, Sinnoures, Etsa and El Fayoum. The infected leaves were chopped into tiny pieces (5mm), immersed in 70% ethanol for 0.5 min., rinsed three times with sterile water, then dried and put on water agar medium supplemented with streptomycin 100 µg/ml. The plates were incubated at 25 °C for 3- 7 days **(Park, *et al*., 2024)**.

Fungi isolates were placed onto potato dextrose agar medium (PDA) once their hyphae had emerged from the tissues. Fungal colonies were purified using a single spore isolation technique suggested by **Choi *et al.* (1999).** Identification was confirmed with PCR identification. Colony morphology was examined in terms of color, margin and texture. Conidia morphological characteristics and sporulation patterns were observed and photographed using a fixed camera light microscope at 40 x magnification.

2.3. Pathogenicity test

Four purified fungal pathogen isolates were tested and proven by Koch's postulates on 30-day-old pot grown Chia plants. Spore suspensions (5×10^4 cfu/ml) of fungal pathogen were prepared from a two-week old culture grown on potato dextrose broth media. Suspensions were suspend in deionized water and 0.2% malt extract. Three replicates were used; each replicate contained three pots (25 cm in diameter) and four seeds/ pot. Chia plants were sprayed with the spore suspension then covered with polyethylene covers to maintain high humidity. Uninoculated pots served as a control. Polyethylene covers were removed after 24 hrs, plants were watered regularly and disease development was monitored. Symptoms were observed 7 to 10-days after inoculation and compared with the original symptoms. The fungal isolates were re-isolated from artificially inoculated leaves and compared with the original isolates, and they were found to be identical **(Stammler *et al*., 2014 and Sharma *et al*., 2013)**. The percentages of disease incidence (DI%) and disease severity (DS%) of *Alternaria* spot were determined as the following equation:

DI% = (Number of plants with *Alternaria* leaf spot /Total number of plants) X 100

$DS\% = \Sigma d / (d\ max \times n) \times 100$

Where: (d) is the disease rating of each plant, (d max) is the maximum disease rating and (n) is the total number of plants tested in each replicate, as **Liu *et al*. (1995).** Ten plants were selected randomly from each treatment 14 days after inoculation and were used to estimate disease severity using the modified 0-5 rating scale in Table (B) and Fig (1).

Table (B). **Scale of disease severity for Chia leaf spot**

Grade	Disease severity
0	No infection
1	10- 20% leaf area infection
2	21- 40% leaf area infection
3	41- 60% leaf area infection
4	61- 80% leaf area infection
5	81- 100% leaf area infection

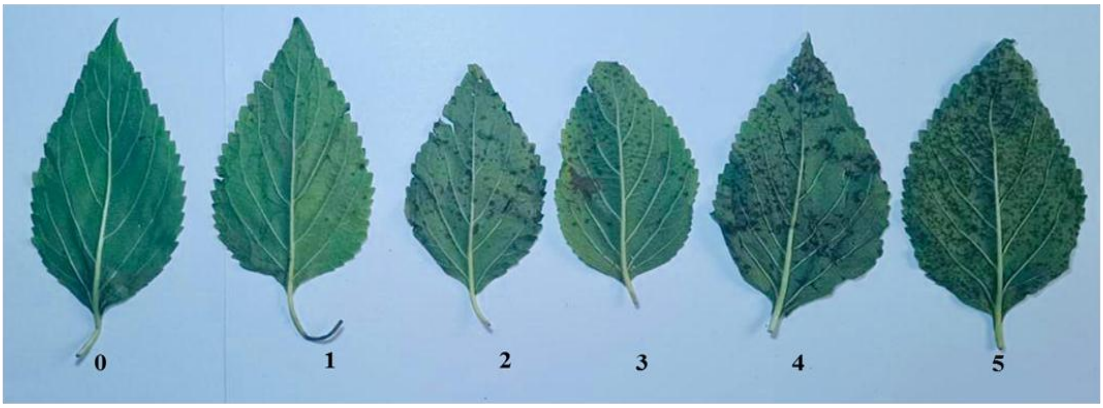


Fig. (1). *A. Alternaria* leaf spot disease index on Chia plant leaves, 0 = no symptom, 1 = 10-20% area covered by disease, 2 = 21- 40% area covered, 3 = 41-60% area covered, 4= 61-80% area covered, 5 = 81-100% area covered.

2.4. PCR Identification

The most aggressive fungal pathogen isolate was selected for PCR identification.

2.4.1. DNA Extraction

DNA was extracted from *Alternaria* sp. mycelium grown in 50 ml of potato sucrose broth (PSB) in 150 ml Erlenmeyer flasks at 25°C for 7 days at 120 rpm on orbital shaker. Total DNA extracted from mycelia was carried out using cetyl trimethyl ammonium bromide (CTAB) method as described by **Van Burik *et al.* (1998)** with a few changes. Briefly, approximately 0.5 g of the crushed powder was collected and resuspended in 800 µl of CTAB buffer and placed for 45 min. at 65°C, then centrifuged at 10000× g for 10 min. subsequently. About 650 µl of the supernatant was added to an equivalent volume of chloroform /Isoamyl alcohol (24:1) and centrifuged at 10000× g for 10 min. at room temperature. The supernatant was collected and 700 µl of ice-cold isopropyl alcohol was added then stored for 20 min at -20°C, then centrifuged at 10000× g for 5min. The precipitated pellet containing DNA was then washed twice with 70% ethanol and centrifuged for 5 min at 10000× g. Finally, the precipitated DNA was air-dried and then resuspended in 50 µl of TE buffer. DNA concentration and quality were measured using a spectrophotometery (NanoDrop One^{c}). Thermo Fisher Scientific, USA) then DNA was stored at -20°C and diluted to 100 ng/µL as the template for polymerase chain reaction (PCR) amplification.

2.4.2. Polymerase chain reaction (PCR) and Sequencing

Identification of *Alternaria* sp. was done based on amplification of the internal transcribed spacer (ITS) region of rDNA using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (TCCTCCGCTTATTGATATGC-3') primers (**White *et al.*, 1990**). The PCR Mixture was performed in a final volume of 25 µl containing 2µl (20 ng/ul) templet DNA, 12.5µl *amaR* OnePCR master mix (Gene DireX, Inc. USA), and 10 pmol of each primer. PCR reaction was performed in Applied Biosystems Proflex PCR System (Thermo Fisher Scientific. USA) as follows: an Initial denaturation step at 94°C for 3 min followed by 40 cycles of 94°C for 1 min, at 53°C for 45 sec, and an extension at 72°C for 90 sec and a final extension at 72ºC for 10 min. The PCR product was electrophoresed for 35 min at 100 V using 1.5% agarose gel in 1X TAE buffer (Tris-acetate-EDTA buffer at pH of 8). The gel was stained with EZ-View Stain (5 µl/100 ml) and observed under UV illumination using a gel documentation system. The PCR product was purified from the gel using a QI Aquick PCR Purification Kit according to the manufacturer's protocol and stored at -20 ºC for Sanger sequencing.

2.4.3. Phylogenetic analysis

The obtained sequences were compared with similar sequences in the NCBI Gen Bank using local Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned with Clustal W. and a Phylogenetic tree and molecular evolutionary analysis were conducted with the Maximum Likelihood with 1000 bootstrap replicates method using DNASTAR Lasergene MegAlign software ver. 7.1.0 (**Burland, 2000**).

2.5. Preparing of the biological agents

Trichoderma harzianum was grown in flasks (250 ml), each containing 200 ml of liquid Gliotoxin fermentation medium (GFM) **(Brain and Hemming, 1945**). Flasks were inoculated with 5 mm fungal discs from four-days-old culture. The inoculated flasks were then incubated in a shaking incubator at 170 rpm at 25° C for 12 days to stimulate toxin production **(Ali, 2021).** The suspension was adjusted to 15×10^{7} cfu/ml.

Bacillus subtilis was activated on fresh slants. Then, after 24 h, transferred to flasks containing 50 ml of Nutrient Glucose broth medium (NG). Inoculated flasks were incubated in a rotary shaker at 120 rpm for 3-days at 28±1°C. Bacterial concentration was adjusted to 15×10^{7}cfu/ml **(Dhingra and Sinclair, 1995).**

Streptomyces rochei was cultured on Starch Nitrate broth (SN) medium at 28° C for 7 days on a rotary shaker at 180 rpm. The suspension was adjusted to a concentration 15x 10^{7} cfu/ml.

2.6. Antagonistic effect of the bioagents against the pathogenic fungi

Petri dishes (9 cm diameter) were prepared, each containing 15 ml of (GFM) medium. Each plate was inoculated on one side with a disc (0.5 cm diameter) of the fungal pathogen from a 5-day-old culture grown on PDA medium. The opposite side of the plate was inoculated with a disc (0.5 cm in diameter) of a 4-day-old culture *T. harzianum*. Screening of *B. subtilis* against the fungal pathogen was

performed using the dual culture plate method **(Siddiqui *et al.*, 2001)**. A loopful of 48 hours-old culture of *B. subtilis* grown on nutrient glucose agar (NGA) was streaked on one side of the Petri plate containing NGA medium. The other side of each plate was inoculated with a disc (0.5cm in diameter) of the pathogen. The antifungal activity of *S. rochei* (7 days old culture) was also tested using the dual inoculation method **(Njoroge *et al*., 2018)**. Three plates were used as replicates; Plates inoculated only with the fungal pathogen were served as a control. The inoculated plates were incubated at 25 ± 2 ºC. When mycelial growth covered all medium surfaces in control plates, all plates were then examined and the percentage reduction in radial growth of the fungal pathogen was calculated by the following formula **(Abdel-Moity, 1985).**

Reduction of linear growth% = 100 - [G1/ G2 x 100]

Where, G1 = the growth diameter of the pathogenic fungus in treated plates (mm), and G2 = the growth diameter of the pathogenic fungus in the control plates (mm).

2.7. Interaction between the bioagents and the fungal pathogen using dual culture slides

This technique provided a clear view of any malformation in the mycelial growth of the pathogen. A sterilized microscopic glass slide was covered by a thin film of diluted PDA medium (1:10) under sterilized conditions. A diluted medium was used to decrease the fungal growth to facilitate its microscopic examination for any malformation in the fungal pathogen mycelia. The antagonist was inoculated at one side of the slide whereas the fungal pathogen was inoculated on the other side of the slide. Inoculated slides were placed in sterilized Petri dishes containing two filter papers saturated with 10 ml of sterilized distilled water. All plates were incubated at 25°C for 6-7 days. A fixed camera light microscope was used to examine and photograph any malformation (40x).

2.8. Greenhouse Experiment

A greenhouse experiment was conducted at CLOA, ARC, Giza, Egypt, to manage Alternaria leaf spot of chia. Pots (25 × 55 cm) with bottom holes were sterilized using a 5% formalin solution for 15 minutes and filled with disinfected sandy clay soil (1:2). Seven treatments were applied, each with three replicates, four pots/ replicate and 10 seeds/ pot (120 seeds/ treatment). All chia plants were artificially sprayed with the fungal pathogen. The bioagents (20 ml/L.) and the fungicide (Copperal max 5 ml/L.) were sprayed 30 days after sowing and repeated every 15 days. The artificial infection with the fungal pathogen was sprayed on plants 24 hours after the first application.

Seven treatments were used as follows:

(T_1) Biofertilizer + sprayed with water (control)

(T_2) Biofertilizer + sprayed with *Trichoderma harzianum*

(T_3) Biofertilizer + sprayed with *Bacillus subtilius*

(T_4) Biofertilizer + sprayed with *Streptomycis rochei*

(T_5) Biofertilizer + sprayed with *Trichoderma harzianum* + *Bacillus subtilius*

(T_6 Biofertilizer + sprayed with *Trichoderma harzianum* + *streptomycis* rochei

(T_7) Biofertilizer + sprayed with copperal max

We did not use a mixture of *Bacillus subtilis* and *Streptomyces rochei* due to incompatibility between the two isolates.

Percentages of diseases incidence (DI %) and diseases severity (DS %) of *Alternaria* spot were determined. In addition, total phenolic contents %**,** total carbohydrates % and sugars % in dried leaves were determined according to **Demirkol and Tarakci (2018); Chaplin and Kennedy (1994),** respectively.

Plant defense enzymes as Peroxidase activity (PO) nm/g fresh weight**,** Polyphenoloxidase activity (PPO) nm/g fresh weight and Chitinase activity mm N-acetylglucose amine equivalent released/g fresh weight were analyzed accordingly **Kar and Mishra (1976), Matta and Dimond (1963)** and **Boller *et al*., (1983)**, respectively.

2.9. Field Experiment

The field experiment was carried out on a private farm in Abshway County, Fayoum governorate, Egypt, during the winter growing seasons 2019/20- 2020/21. The experimental area was 12 m^2 with five rows (4 m in length, 60 cm between rows and 20 cm between plants) with a total of 100 plants. Seven treatments with three replicates for each treatment were applied.

Biofertilizers inoculum was added with irrigation, once a month at a rate of 5 L/fed. The bioagents 20 ml/L and the fungicide 5 ml/L were sprayed 30 days after sowing date, then repeated every 15 days.

Before sowing seeds, Soil physical and chemical analysis was estimated **(Chapman and Pratt, 1978)**, and microbiological counts were enumerated in the rhizosphere as total counts of fungi, bacteria and actinomyces **(Allen, 1959; Difco, 1985** and **Jensen, 1930)** respectively**.** In addition, N_2-Fixing bacteria, phosphate solubilizes bacteria and potassium release bacteria using semi-solid malate medium **(Dobereiner *et al*., 1976)**, Pikosvkey's medium (**Pikovskaya, 1948)** and Aleksandrov's agar medium **(Parmar and Sindhu, 2013)** respectively as shown in Table (C).

Table (C). Soil and irrigation water analysis in Fayoum governorate

Mechanical analysis (%)		Anions and Cations (mmq/ L.)			Total counts (CFU/ ml.)	
			Soil used	Irrigation water		
Sand	20.4	**pH (1:2.5)**	7.80	7.40	**Total Fungi**	1.00×10^4
Silt	27.3	**EC (ds/m^3)**	0.90	0.42	**Total Bacteria**	5.00×10^6
Clay	52.3	**Ca++:**	5.1	2.13	**Total Actinomycetes**	4.00×10^5
Texture class	**Clay**	**Mg++**	2.5	1.02	***Bacillus megaterium***	2.00×10^6
		Na+	1.38	1.01	***Bacillus circulans***	2.00×10^6
Available nutrients (ppm)		**K+**	0.03	0.04	**MPN in the rhizosphere/ ml.**	
		CO^{--}_3	--	--		
N	50	**HCO^{-}_3**	1.85	0.03		
P	4.67	**Cl^{--}**	1.56	1.09	***Azospirillum brasilense***	3.00×10^6
K	420	**SO_4^{--}**	5.60	3.08		
		$CaCO_3$	2%			

Disease assessment: Ten plants was taken randomly 60, 75 and 90 days after sowing in both seasons to determine disease incidence and disease severity

Evaluation of the biological activities of biofertilizer strains (Hebber *et al*., 1992)

Estimation of Soil enzyme activities: Nitrogenase (µ mole C_2H_4/g dry soil/h), dehydrogenases (µg TPF/ soil) and total phosphatase at 45, 90 and 130 days after sowing as (**Somasegaran and Hoben, 1994),** (**Skujins, 1976)** and (**Gerritse and van Dijk, 1978)** respectively**.**

Vegetative growth: Plant height (cm), branches number per plant, spike numbers per plant, 1000 seed weigh (g), seed yield per fed (kg) and oil yield per fed (L).

Chemical constituents: Total chlorophyll a, b and carotenoids were determined in fresh leaves (**Inskeep and Bloom, 1985)**. Total nitrogen, phosphorus and potassium % were determined in dried leaves and seeds **(Piper, 1950; Chapman and Pratt, 1961).** Total carbohydrates and sugars were determined in the seeds (**Chaplin and Kennedy, 1994).**

Evaluation of fixed oil productivity: Fixed oil % was calculated by the following equation:

Fixed oil % = Extracted fixed oil weight/seeds weight ×100.

2.10. Statistical analysis

The results were analyzed statistically using a completely randomized block design ($LSD_{0.05}$). **(Snedecor and Cochran, 1980).**

3. Results

3.1. Morphological identification of the pathogen

Based on the morphological characteristics of conidia and sporulation pattern, the fungal isolate was examined and photographed by fixed camera light microscope at 40x magnification. The isolate was initially identified as *Alternaria* spp. Symptoms of *Alternaria* leaf spot were observed on Chia leaves in Fayoum governorate (Abshaway county- Aboxa village) as small brown spots, which grow into irregular dark brown spots and on occasion progress to be a target-like pattern of rings Fig. (2).

Fig. (2). Naturally infected Chia plants with Leaf spot compared to healthy plants. (A) Healthy control plants. (B), (C) infected plants.

3.2. Pathogenicity test under greenhouse conditions

A pathogenicity test was conducted on 10 Chia plants, and Koch's postulates were applied. Small brown spots were observed 15 days after infection. Table (1) presents the results of the pathogenicity test for the four isolates of *Alternaria spp.* that were obtained from naturally infected chia plants in Ebshaway, Sinnoures, Etsa and El-Fayoum. All the tested isolates caused similar symptoms, but with significant variations. The Ebshaway isolate had the highest disease incidence and severity, with percentages of 45% and 26% respectively. On the other hand, the El-Fayoum isolate had the lowest percentages, with disease incidence and severity of 21.66% and 7.33% respectively, compared to the control. The most aggressive isolate was molecularly identified.

Table (1). Pathogenicity test of chia plant under greenhouse conditions against *Alternaria alternate*.

Location	Disease incidence%	Disease severity%
Ebshaway	45.00	26.00
Sinnoures	36.66	16.66
Etsa	31.66	12.66
El-Fayoum	21.66	7.33
Control	0.0	0.0
LSD $_{0.05}$	1.11	0.72

3.3. Molecular identification of *Alternaria* spp. isolate

Alternaria alternata was identified using molecular methods based on the sequencing of the internal transcribed spacer (ITS) region of rDNA using PCR the ITS-rDNA fragment (534 bp) was amplified compared with those of closely related isolates in the NCBI website using the BLAST software. The results revealed that the isolate has the highest similarity of 99.8, 99.8, 99.6 and 99.6 with *A. alternata* Turkey (MN826219.1), Iran (OQ455729.1), China (MZ350148.1) and Iraq (OP090358.1) isolates, respectively. In addition to 98.9 % identity with the Egyptian isolate (mic21) GenBank accession No. (MW850355.1). *Pythium aphanidermatum* is identified as an outgroup strain on the phylogenetic tree. Therefore, the sequence of the extracted isolate named *Alternaria alternata* YZM1 and granted the accession number OQ569158.1 on the Genbank. Fig. (3).

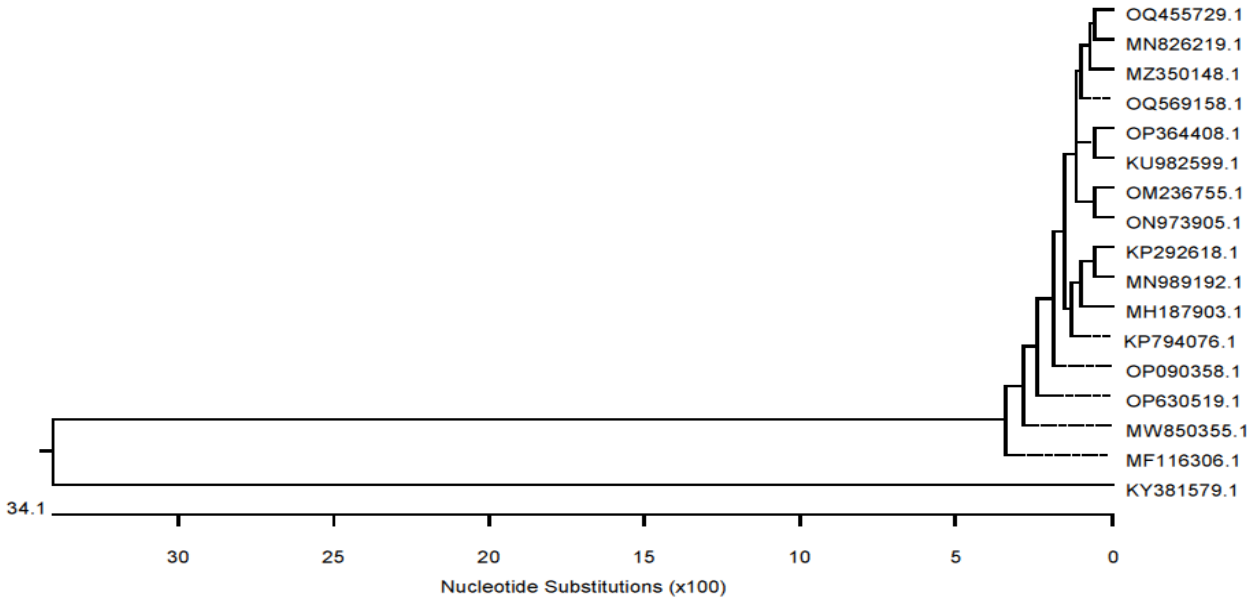


Fig. (3). The Maximum likelihood phylogenetic-tree-based ITS sequences and bootstrap support values. ITS sequences of rDNA of the *Alternaria alternata* isolate (YZM1) GenBank accession No. (OQ569158.1) in the present study which was aligned with closely related sequences accessed from the GenBank.

3.4. Antagonistic activity of the bioagents against *A. alternata* (YZM1)

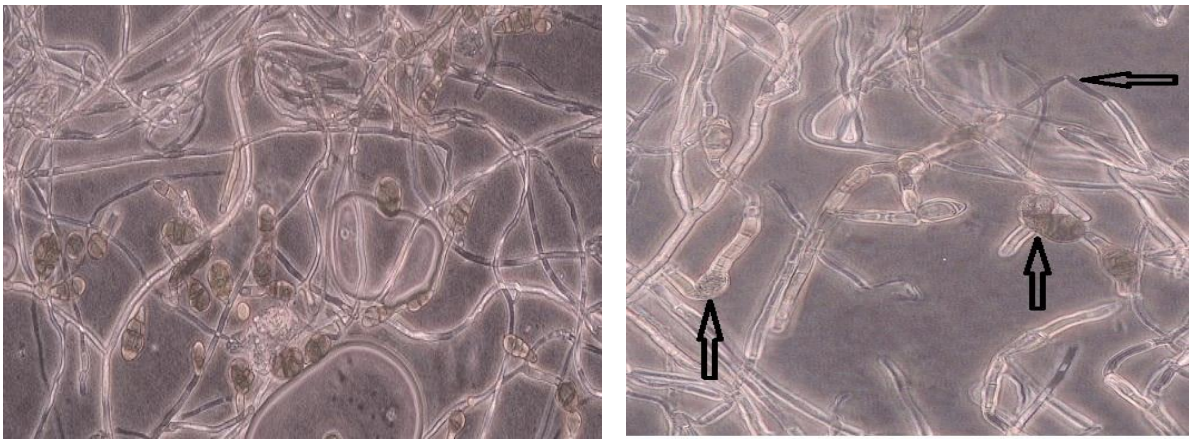
The antagonistic activity of *T. harzianum, B. subtilis* and *S. rochei* against *Alternaria alternata* (YZM1) was evaluated, using a dual culture method. All bioagents (*S. rochei, T. harzianum* and *B. subtilis,*) effectively inhibited the mycelia of *Alternaria alternata* on the PDA (60**,** 50 and 43.8% respectively). *S. rochei* showed the highest significant percentage of inhibition of mycelia growth (60%) compared to *T. harzianum* and *B. subtilis.* As shown in Table (2).

Table (2). Antagonistic effect of tested bioagents against *Alternaria alternata* (YZM1)

Treatments	Percentage of reduction in mycelial growth of *A. alternate* (%)
Streptomyces rochei	60.0
Trichoderma harzianum	50.0
Bacillus subtilis	43.8
Control	0
LSD $_{0.05}$	1.29

3.5. Interaction between the bioagents and *A. alternata* using dual culture slide technique

Through the microscopic examination, a malformation or lysis appeared in hyphae and spores of *A. alternata*, which was subjected to the bioagents compared with normal mycelium, which appeared with no deformities or lysis Fig. (4).



(A) **(B)**

Fig. (4). Normal mycelium of *A. alternata* (A); A malformation appeared in hyphae and spores which were subjected to the effect of *Streptomyces rochei* metabolites (B).

3.6. Greenhouse experiment

3.6.1. Effect of biofertilizers and bioagents on disease incidence and severity of Alternaria leaf spot of Chia plants under greenhouse conditions

Data in Table (3) indicate that all the bioagents *T. harzianum*, *B. subtilis* and *S. rochei* as well as copperal max significantly reduced the Alternaria leaf spot incidence and severity. T_6 using biofertilizers + foliar spraying with *T. harzianum* + *S. rochei* was the best effective treatment that significantly reduced DI to 22.5%, which caused a 73.52% reduction. The same treatment recorded 12.08% DS with a 78.36% reduction. Also applying biofertilizers + *T. harzianum*+ *B. subtilis* significantly reduced DI % and DS% giving 25% and 15.41, respectively. Plants treated with Copperal Max recorded the highest values than the bioagents (20 and 9.58%) in both DI and DS, respectively, compared to the control, which gave the highest values of DI (85%) and DS (55.83%), causing 0% reduction in both of them under greenhouse conditions.

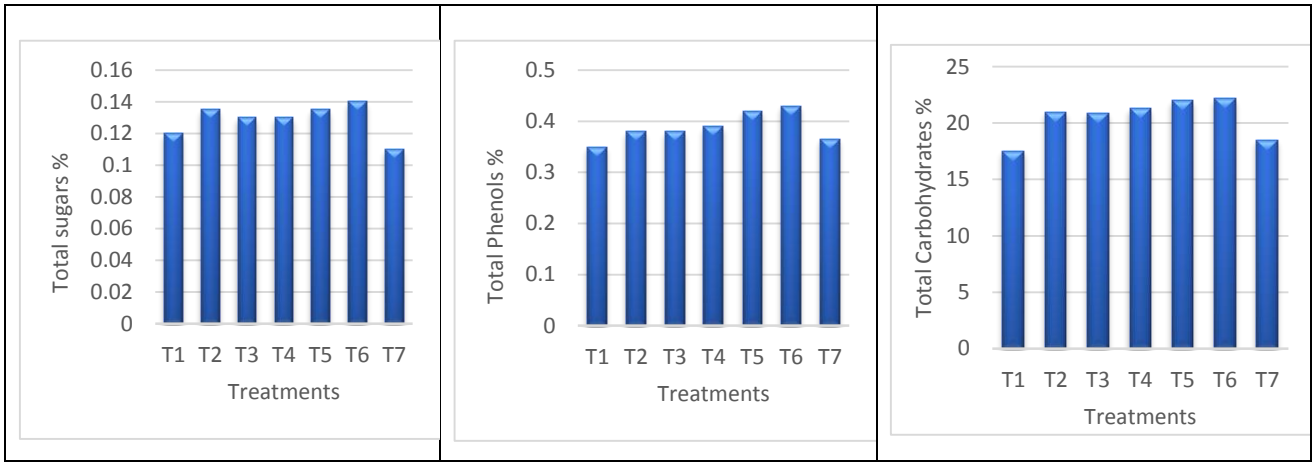
Table (3). Effect of biofertilizers and bioagents on disease incidence and severity of Alternaria leaf spot of chia plants under greenhouse conditions

Treatments	greenhouse conditions			
	Disease incidence %	Reduction %*	Disease severity %	Reduction %*
Biofertilizer + foliar spray with water (control)	85.0	0.00	55.83	0.00
Biofertilizer + *Trichoderma harzianum*	27.5	67.64	17.08	69.40
Biofertilizer + *Bacillus subtilis*	30.0	64.70	18.75	70.11
Biofertilizer + *Streptomyces rochei*	27.5	67.64	16.25	70.89
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	25.0	70.58	15.41	72.88
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	22.5	73.52	12.08	78.36
Biofertilizer + Copperal max	20.0	76.47	9.58	82.84
LSD at 0.05	0.61	0.08	0.05	4.19

*Reduction according to the control treatment.

3.6.2. Effect of biofertilizers and bioagents on total sugars, phenols and carbohydrates of Chia plants under greenhouse conditions

Data in Fig. (5) Showed that, treatments of compost and biofertilizer + foliar spray with *T. harzianum* and *S. rochei*, exhausted the highest values being 0.14, 0.48 and 22.18 % in total sugars, total phenols and total carbohydrates, respectively, followed by T_5, but T_7 (biofertilizer + copperal max) was the lowest values being 0.11, 0.32 and 19.0 % respectively.

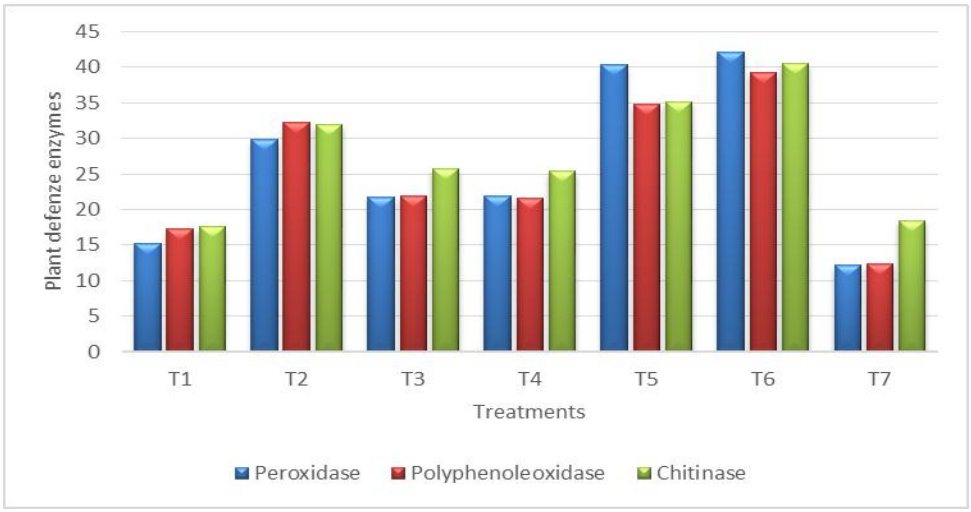


T_1 (biofertilizers), T_2 (biofertilizers + *T. harzianum*), T_3 (biofertilizers + *B. subtilis*), T_4 (biofertilizers + *S. rochei*), T_5 (biofertilizers + *T. harzianum*+ *B. subtilis*), T_6 (biofertilizers + *T. harzianum*+ *S. rochei*), T_7 (biofertilizers + Copperal max).

Fig. (5). Effect of biofertilizers and bioagents on total sugars, phenols and carbohydrates of Chia plants under greenhouse conditions.

3.6.3. Effect of biofertilizers and bioagents on Chia plant defense enzymes under greenhouse conditions

Data in Fig (6) indicate that peroxidase, polyphenoloxidase and chitinase have been affected by the PGPRs (bioagents and biofertilizers), Whereas, T_6 (biofertilizers + *T. harzianum* + *S. rochei*) was the best effective treatment, which increased the three enzymes in chia leaves that recorded 42.14, 39.22 and 40.55 respectively, followed by T_5 (biofertilizers + *T. harzianum* + *B. subtilius*). While T_7 (biofertilizers + Copperal max) recorded the lowest values in the three enzymes.



T_1(biofertilizers), T_2 (biofertilizers + *T. harzianum*), T_3 (biofertilizers + *B. subtilis*), T_4 (biofertilizers + *S. rochei*), T_5 (biofertilizers + *T. harzianum*+ *B. subtilis*), T_6 (biofertilizers + *T. harzianum*+ *S. rochei*), T_7 (biofertilizers + Copperal max).

Fig. (6). Effect of biofertilizers and bioagents on Chia plant defense enzymes under greenhouse conditions.

3.7. Field experiment

3.7.1. Effect of biofertilizers and bioagents on disease incidence and severity of *A. alternate* leaf spot of Chia plants under field conditions

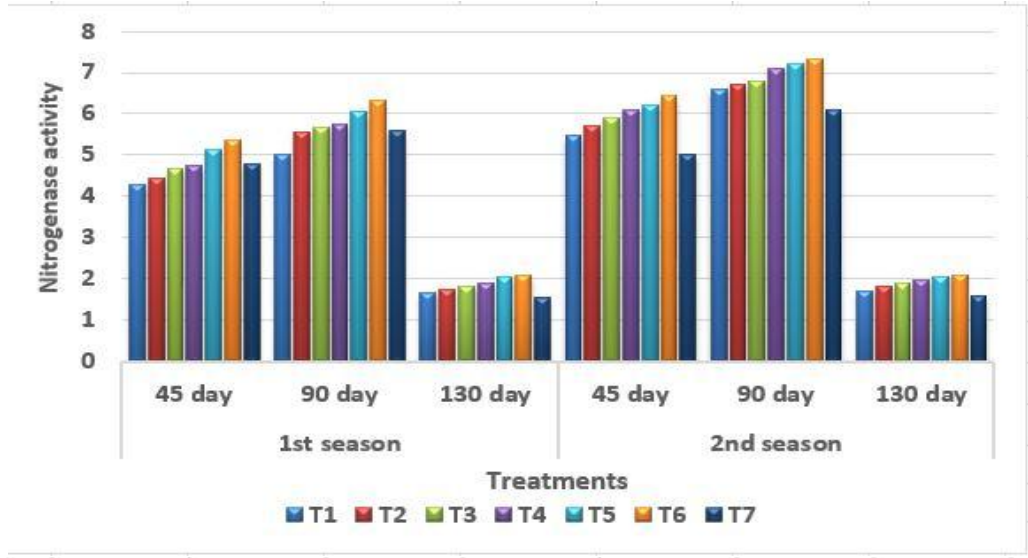
Data in Table (4) indicate that all the tested isolates *T. harzianum*, *B. subtilis* and *S. rochei* as well as copperal max significantly reduced the leaf spot incidence and severity. Plants that fertilized with compost and biofertilizers and sprayed with *T. harzianum*+ *S. rochei* reduced DI to 28- 25% causing 68.88- 72.82% reduction, as well as, 10.33- 7.83% in DS with 80.92- 85.79% reduction in first and second season respectively, compared to the control treatment.

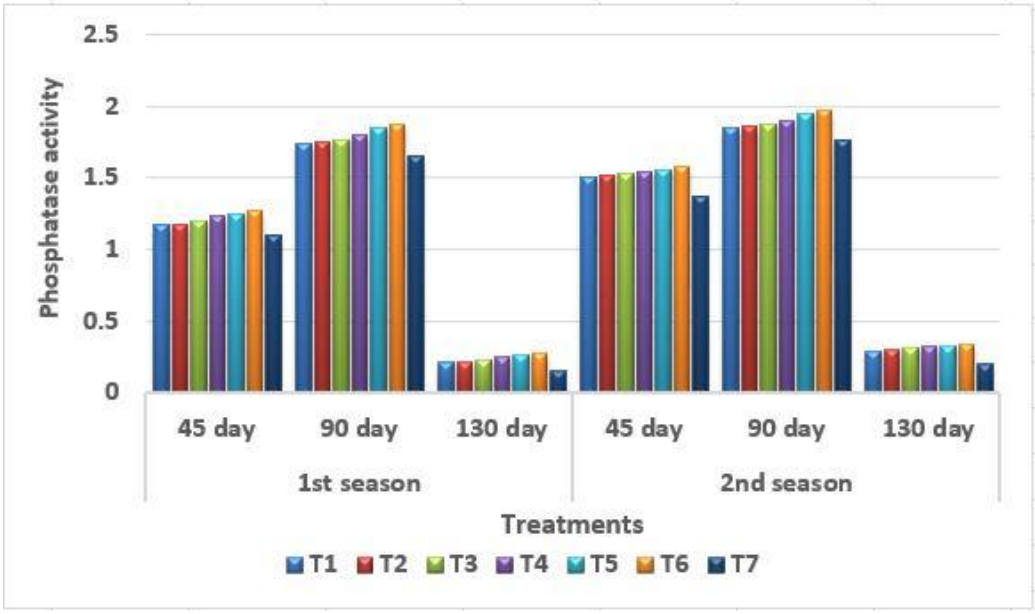
Table (4). Effect of biofertilizers and bioagents on disease incidence and severity of *A. alternate* leaf spot of Chia plants under field conditions.

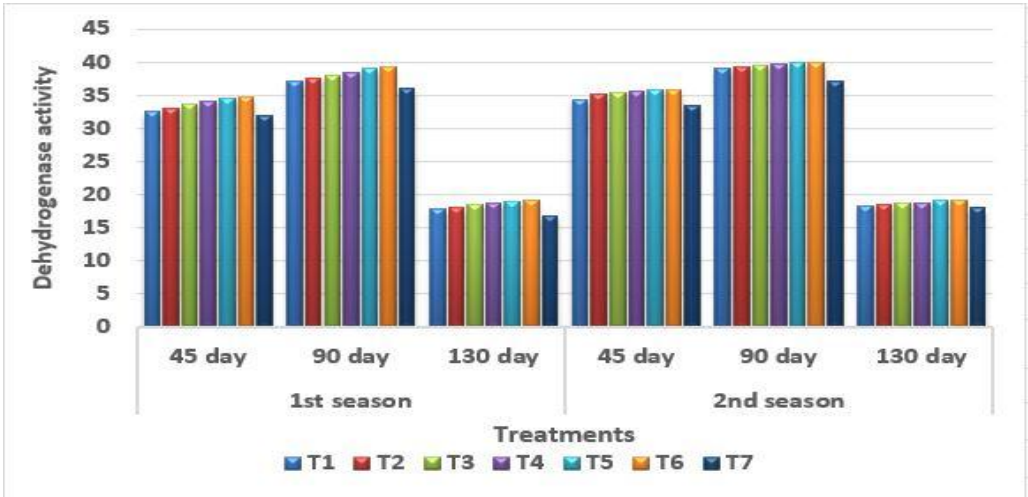
Filed conditions								
Treatments	**Disease incidence**		**Reduction %**		**Disease severity**		**Reduction %**	
	1st Season	**2nd Season**	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**
Biofertilizer + foliar spray with water (control)	90.0	92	0	0	54.16	55.16	0	0
Biofertilizer + *Trichoderma harzianum*	32.0	31	62.22	66.30	11.16	10.50	79.39	80.96
Biofertilizer + *Bacillus subtilis*	41.0	38	54.44	58.69	17.00	14.66	68.61	73.42
Biofertilizer + *Streptomyces rochei*	38.0	35	57.77	61.95	14.00	12.00	74.15	78.24
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	30.0	30	66.66	67.39	12.00	8.83	77.84	83.99
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	28.0	25	68.88	72.82	10.33	7.83	80.92	85.79
Biofertilizer + Copperal max	30.0	27	66.66	70.65	10.00	7.33	81.53	86.71
LSD at 0.05	**0.96**	**0.64**	**0.04**	**0.04**	**0.31**	**0.4**	**0.05**	**0.05**

3.7.2. Effect of biofertilizers and bioagents on the soil enzymes activities under field conditions

Data in Fig. (7) Indicate that the biological activity in the chia rhizosphere is expressed based on soil enzymes such as nitrogenase, phosphatase and dehydrogenase activities. The highest values of the three enzyme activities were recorded after 45-90 and 130 days of sowing in T_6 (biofertilizer + *T. harzianum* + *S. rochei*) was 5.36, 6.33 and 2.07 (n mole C_2H_4/g dry soil/h) in the first season, and 6.43, 7.34 and 2.09 in the second season, respectively. While phosphatase recorded 1.27, 1.88, and 0.28 µg/g soil/day in the first season and 1.58, 1.97, and 0.34 µg/g soil/day in the second season. Also, dehydrogenase recorded 34.71, 39.19, and 19.02 µg TPF/g dry soil/day in the first season and 35.8, 40.02, and 19.1 µg TPF/g dry soil/day in the second season compared to the control. Followed by T_5 and then other treatments. T_7 (biofertilizer + Copperal max) gave the lowest values.







T_1 (biofertilizers), T_2 (biofertilizers + *T. harzianum*), T_3 (biofertilizers + *B. subtilis*), T_4 (biofertilizers + *S. rochei*), T_5 (biofertilizers + *T. harzianum*+ *B. subtilis*), T_6 (biofertilizers + *T. harzianum*+ *S. rochei*), T_7 (biofertilizers + Copperal max).

Fig. (7). Effect of biofertilizers with bioagents on the soil enzymes activities (Nitrogenase, Phosphatase and Dehydrogenase) under field conditions.

3.7.3. Biological activities of the biofertilizer strains

Data in Table (5) show that, *Azospirillum brasilense, Bacillus megaterium* and *Bacillus circulans*, which were used as biofertilizers in this study, have the ability to excrete phytohormones and exopolysaccharides thereby improving soil health and fertility.

Table (5). Biological activities of the biofertilizer strains

Strain	Exopolysaccharide	Abscisic acid	Gibbereilic acid (GA)	Auxin (IAA)
Azospirillum brasilense	++	++	++	++
Bacillus megaterium	++	++	++	++
Bacillus circulans	++	++	++	++

3.7.4. Effect of biofertilizers and bioagents on the vegetative growth parameters of Chia plant under field conditions

The use of PGPR had a positive impact on the vegetative growth parameters. Table (6) shows that T_6 (biofertilizer + *T. harzianum* + *S. rochei*) gave the highest values for the vegetative growth parameters as plant height, number of branches/ plant, number of spikes/ plant, 1000 seed weight, total yield kg/ feddan and fixed oil content in both seasons, with values (118 and 120 cm.), (14 and 15), (30 and 32), (1.40 and 1.47 g.), (404 and 410 kg/ fed.) and (29.35 and 32.69 %), respectively. Followed by T_5 (biofertilizer + *T. harzianum* + *B. subtilius*) also showed relatively high values, while T_7 (biofertilizer + copperal max) recorded the lowest values in the first and second seasons.

Table (6). Effect of biofertilizers and bioagents on the vegetative growth parameters of Chia plant under field conditions

Treatments	Plant height (cm)	Number of branches/ plants	Number of spikes/ plants	1000 seed weight (g)	Total yield (kg/fed)	Fixed oil content
	First Season					
Biofertilizer + foliar spray with water (control)	95	6	12	1.21	304	24.23
Biofertilizer + *Trichoderma harzianum*	106	9	15	1.30	382	27.61
Biofertilizer + *Bacillus subtilis*	108	8	17	1.21	385	27.75
Biofertilizer + *Streptomyces rochei*	103	7	14	1.24	367	25.69
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	112	12	21	1.32	395	28.84
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	118	14	30	1.40	404	29.35
Biofertilizer + Copperal max	90	7	13	1.22	370	23.78
LSD at 0.05	**0.65**	**0.56**	**0.52**	**0.04**	**16.64**	**0.24**
	Second Season					
Biofertilizer + foliar spray with water (control)	103	8	20	1.27	381	26.00
Biofertilizer + *Trichoderma harzianum*	110	10	22	1.29	395	28.03
Biofertilizer + *Bacillus subtilis*	111	9	22	1.25	398	26.38
Biofertilizer + *Streptomyces rochei*	108	10	16	1.30	390	27.35
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	116	12	25	1.38	403	32.11
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	120	15	32	1.47	410	32.69
Biofertilizer + Copperal max	100	8	23	1.26	385	25.35
LSD at 0.05	**0.91**	**0.30**	**0.68**	**0.07**	**17.92**	**0.38**

3.7.5. Effect of biofertilizers and bioagents on the chemical components of Chia plant under field conditions

Table (7) shows that T_6 gave the highest values for chlorophyll a, b, carotenoid, total nitrogen, phosphorus and potassium % in the first and second seasons with the values of (14.53 and 15.26), (6.66 and 4.67), (4.67 and 4.83), (2.22 and 2.42), (0.35 and 0.37) and (4.83 and 2.32), respectively. This was followed by T_5, compared to T_7 that gave the lowest values.

Table (7). Effect of biofertilizers and bioagents on the chemical components of Chia plant under field conditions

Treatment	Chl.a	Chl.b	Carotenoid	Total nitrogen %	Total phosphorus %	Total potassium %
	(mg/100 fw)					
	First Season					
Biofertilizer + foliar spray with water (control)	11.75	3.61	3.68	1.93	0.20	4.60
Biofertilizer + *Trichoderma harzianum*	12.74	4.05	3.73	2.002	0.21	4.04
Biofertilizer + *Bacillus subtilis*	11.67	4.54	4.01	1.96	0.23	4.11
Biofertilizer + *Streptomyces rochei*	12.88	4.21	4.20	1.95	0.20	3.83
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	12.92	4.56	4.30	2.2	0.31	4.65
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	14.53	6.66	4.67	2.22	0.35	4.83
Biofertilizer + Copperal max	11.55	3.72	3.88	1.17	0.21	3.42
LSD at 0.05	**0.33**	**0.17**	**0.23**	**0.05**	**0.03**	**0.25**
	Second Season					
Biofertilizer + foliar spray with water (control)	12.33	3.89	4.60	1.97	0.23	0.98
Biofertilizer + *Trichoderma harzianum*	13.98	4.02	4.04	2.06	0.25	1.11
Biofertilizer + *Bacillus subtilis*	13.00	3.83	4.11	2.07	0.26	1.12
Biofertilizer + *Streptomyces rochei*	14.70	3.65	3.83	2.00	0.22	1.20
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	14.88	4.41	4.65	2.35	0.36	2.11
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	15.26	4.67	4.83	2.42	0.37	2.32
Biofertilizer + Copperal max	12.27	7.30	3.42	1.28	0.28	1.47
LSD at 0.05	**0.33**	**0.17**	**0.25**	**0.06**	**0.03**	**0.45**

3.7.6. Effect of biofertilizers and bioagents on seed quality (total sugars %, phenols %, carbohydrates % and antioxidants % in chia seeds

Table (8) indicates that, T_6 had the highest values in total nitrogen, protein, phosphorus, potassium, sugars, phenols and antioxidant content % in the chia seed in the first and second seasons. T_6 recorded the highest values (2.32 and 2.41), (14.50 and 15.06), (0.29 and 0.35), (1.18 and 1.23), (0.11 and 0.12), (0.077 and 0.085), (43.56 and 45.87) and (74.97 and 80.88) respectively, followed by T_5, while T_7 recorded the lowest values in both seasons.

Table (8). Effect of biofertilizers and bioagents on total sugars, phenols, carbohydrates and antioxidants in chia seeds

Treatments	Total nitrogen %		Protein %		Total phosphorus %		Total potassium %	
	1s Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Biofertilizer + foliar spray with water (control)	0.81	0.86	5.06	5.37	0.12	0.13	0.77	0.78
Biofertilizer + *Trichoderma harzianum*	1.09	1.12	6.81	7.00	0.19	0.27	1.00	1.05
Biofertilizer + *Bacillus subtilis*	1.06	1.11	6.62	6.94	0.20	0.25	0.94	1.00
Biofertilizer + *Streptomyces rochei*	1.14	1.23	7.13	7.69	0.20	0.25	1.01	1.06
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	2.24	2.36	14.00	14.75	0.26	0.30	1.11	1.13
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	2.32	2.41	14.50	15.06	0.29	0.35	1.18	1.23
Biofertilizer + Copperal max	1.17	1.28	7.31	8.00	0.20	0.26	0.93	0.99
LSD at 0.05	**0.07**	**0.07**	**0.1**	**0.11**	**0.02**	**0.04**	**0.03**	**0.03**
Treatments	**Total Sugars %**		**Total Phenols %**		**Total Carbohydrates %**		**Antioxidants DPPH %**	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Biofertilizer + foliar spray with water (control)	0.09	0.09	0.011	0.016	36.89	38.56	73.11	78.64
Biofertilizer + *Trichoderma harzianum*	0.08	0.08	0.052	0.061	41.75	43.48	73.18	78.88
Biofertilizer + *Bacillus subtilis*	0.08	0.08	0.043	0.047	37.78	39.85	73.14	78.77
Biofertilizer + *Streptomyces rochei*	0.08	0.08	0.031	0.037	40.99	43.00	72.55	77.71
Biofertilizer + *Trichoderma harzianum* + *Bacillus subtilis*	0.1	0.1	0.074	0.084	42.95	44.68	74.19	79.45
Biofertilizer + *Trichoderma harzianum* + *Streptomyces rochei*	0.11	0.12	0.077	0.085	43.56	45.87	74.97	80.88
Biofertilizer + Copperal max	0.07	0.08	0.031	0.039	25.56	26.88	70.72	74.00
LSD at 0.05	**0.01**	**0.006**	**0.002**	**0.002**	**0.27**	**0.23**	**0.21**	**0.20**

5. Discussion

Alternaria alternata is an important fungal pathogen causing losses in crop productivity. This study demonstrated the effectiveness of *Trichoderma harzianum* MT110634, *Bacillus subtilis* MT110633 and *Streptomyces rochei* OP164572.1, which used as a foliar spray to control *Alternaria* leaf spot and fertilized with both compost and biofertilizers including (*Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum,* and *Bacillus circulans*) under greenhouse and field conditions. The antagonistic activities of *T. harzianum* with *S. rochei* caused a pronounced decrease in the mycelia growth; *S. rochei* can effectively suppress *A. alternate* by several mechanisms including production of antibiotics such as streptomycin, hyperparasitism on pathogenic organisms, cell wall-degrading enzymes and induces of systemic resistance in the host plant **(Le *et al.*, 2022)**.

Rahman *et al*. (2020) reported that, Ashwagandha plants applied with *Trichoderma harzianum* as foliar spraying gave the lowest percentages of disease severity index to *A. alternata*. Additionaly, **Promwee and Intana (2022)** found that maximum linear growth inhibition on *A. alternata* was recorded with *T. harzianum*. This can be explained as *T. harzianum* had a different mechanism, such as mycoparasitism, production of antifungal substances as gliotoxin and trichodermin, and can compete for nutrients and space with the pathogens. In addition, **Johnson *et al*. (2021)** and **Ebrahimi- Zarandi *et al*. (2022)** explained that *T. harzianum* increases its mycoparasitic capacity through co-inoculating with *S. rochei.* That can release several active enzymes against phytopathogens, including chitinases,

amylases, cellulases, glucanases and lipases. Also volatile antifungal compounds such as alcohols, aldehydes and carboxylic acids that have a role against many fungal pathogens including *A. alternate***.**

Therefore, *T. harzianum* and *S. rochei* are able to produce specialized metabolites that develop systemic resistance through the accumulation of defense-related compounds such as total phenols, total sugars, carbohydrates and various plant defense enzymes against Alternaria leaf spot **(Kaari *et al*., 2022).** Also, **Torres-Rodriguez *et al*. (2022)** explained that, *Streptomyces* spp. able to activate both induced and systemic resistance that stimulate defense responses in plants through the overproduction of plants defense-related enzymes as peroxidase, polyphenoloxidase and chitinase. Also, enhancing the accumulation of total free phenolics compounds in the leaves and antioxidant enzymes, which strengthen the cell wall structure to prevent the entry of the phytopathogenic fungi and catalyzing the oxidation of phenolic compounds to mycotoxic quinones. In addition, **Abo-Elyousr and Almasaudi (2022)** explained that Co-inoculation of *T. harzianum* and *S. rochei* increased the activities of different defensive enzymes, such as polyphenoloxidase and peroxidase that leads to a reduction in diseases incidence, also phenol contents increased in plants treated with *T. harzianum* and *S. rochei*. This may have a direct relationship with the process by which *T. harzianum* and *S. rochei* induce resistance against the pathogen**.** In addition, *B. subtilis* induced an increase in the activity of antioxidant enzymes and its ability to produce biologically active substances as antimicrobial components (**Arias-Padró *et al.*, 2021).**

Multiple research has revealed that adding compost with biofertilizers significantly enhances soil fertility, crop quality and yield characteristics. Plant production and soil health are greatly affected by a variety of interactions between microorganisms, plants and soil. According to **Harman *et al*. (2020) and Baldi *et al*. (2021)** Said hat, the use of biofertilizer improves the degradation process and soil nutrient cycling by altering the microbial composition and increasing soil microbial diversity. Furthermore, there are many mechanisms that biofertilizers enhance plant growth, including N_2 fixation, mineralization of soil macro-micronutrients (such as Zn, P, Fe, K, etc.) and excretion of exopolysaccharides, phytohormone synthesis, siderophore, vitamins, enzymes and antibiotics. In addition, **Lopes *et al*. (2021)** referred that, Biofertilizers act as phytostimulants or plant growth regulators, allowing plants to produce phytohormones such as cytokinin, gibberellins, abscisic acid and indole-3-acetic acids (IAA) under biotic and abiotic stress situations. This protects plants by altering the level of phytohormones in them and encouraging root length, growth and the creation of root hairs, all of them work to improve soil water absorption.

Compost with biofertilizers increased the dehydrogenase, phosphatase and nitrogenase activities. According to **(Elayaraja and Senthilvalavan, 2019)** said that enriching the soil with organic matter and biofertilizers, increases nitrogenous compounds which activate nitrogenase enzymes and increased dehydrogenase activity. Furthermore, the addition of organic matter with phosphorus dissolving bacteria enhances phosphatase activity and enriches the soil.

Using compost with biofertilizers improved the indicators of vegetative growth. These findings were consistent with the findings of **Salman *et al*. (2019),** who reported that the growth-promoting properties of biofertilizers on chia plants included stimulating the production of phytohormones as gibberellins and indole acetic acid (IAA), which encourage the plant cell to divide and growth. In addition, organic and biofertilizers have a positive impact on plant growth parameters because they improve the physical, chemical and biological characteristics of the soil.

Chemical components of chia plant were investigated by **Mohamed and Ghatas (2020); Khater (2022),** which indicated that, Biofertilizers can fix atmospheric nitrogen and dissolve P and K, providing plants with elements used in the production of the chlorophyll molecule, nucleic acids, amino acid forms and proteins. As a result, contribute to increased plant height, branch number, root system expansion, and production of certain growth regulators such as auxins (indole-3-acetic acid or IAA). Also, improve the plant's ability to collect water and nutrients from the soil surrounding its roots. Also, **Abdul-Halim, *et al*. (2022)** found that, when Chia plants were fertilized with both compost and biofertilizer and sprayed with both *T. harzianum* and *S. rochei*, significantly enhanced chlorophyll content and improved plants photosynthesis processes. *T. harzianum* has the potential as a biostimulator that results in higher photosynthetic activity.

Regarding seed productivity, **Gururaj *et al*. (2022)** explained that Biofertilizers have a good effect on chia plants by peomoting nitrogen fixation, phosphorous solubilization, potassium mobilization and nutrient absorption. They also facilitate the transfer of nutrients to shoots and seeds, resulting in increased growth and productivity. Moreover, **Jyolsna *et al*. (2021); Kaymak *et al*. (2023)** stated that, Biofertilizer treatment on tomatoes may promote plant development and high yields due to phytohormonal effects such as indole acetic acid and gibberellins, lowering ethylene production and enriching soil components with nutrients provided by both compost and biofertilizers. **Dasgan *et al*., (2022)** reported that inoculation of basil with a mixture of *Azotobacter chroococcum* and *Azospirillum lipoferum* strains, recorded the highest levels of antioxidant activity, phenols and flavonoids compared to control. **El Sayed *et al*. (2022)** said that, plants grown with organic and biofertilizers had a significant increase in total chlorophylls, antioxidant compounds (total phenol and DPPH), carbohydrates, protein and oil in seeds, as well as mineral contents such as N, P, K, Zn, Fe, Mn, and Cu, and IAA content, which resulted in the best seed quality.

6. Conclusion

According to the results obtained, PGPR can be recommended as biological control agents and biofertilization against Alternaria leaf spot, promoting growth of chia (*Salvia hispanica* L.), seeds yield and fixed oil productivity. Therefore, organic fertilizer can be used with biofertilizers (*Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans*), in addition to bioagents (*Trichoderma harzianum* and *Streptomyces rochei*) as foliar spray, as they are considered a safe, natural and alternative applications to chemical fungicides and chemical fertilizers.

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