Egyptian Journal of Phytopathology, Vol. 50, No. 1, pp 31-50 (2022)
DOI 10.21608/ejp.2022.133129.1059

ORIGINAL PAPER

Evaluation of the Efficacy of some Bioagents Accompanied with Bio- and Mineral Fertilizers in Controlling Early Blight of Tomato and Improvement Yield

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Received: 13 April 2022 / **Accepted**: 29 May 2022 / **Published online**: 30 May 2022.

ABSTRACT

Tomato (*Lycopersicon esculentum* Miller) early blight caused by *Alternaria solani* is one of the most destructive diseases that reduce tomato production worldwide. The effect of organic manure, biofertilizer (N_2-fixer's bacteria) as *Azotobacter chroococcum* and *Azospirillum lipoferum* and the bioagents *Trichoderma harzianum* and *Bacillus subtilis* in controlling early blight of tomato cultivar 935 was evaluated under greenhouse and field conditions during the two successive growing seasons 2018/2019 and 2019/2020 at Abshway county, Fayoum governorate, Egypt. Adding biofertiizer and spraying tomato plants with bioagents or the fungicide Oxyplus (copper oxychloride) reduced the disease incidence and disease severity better than using the mineral fertilizer and spraying with bioagents or Oxyplus in both greenhouse and field experiments.
T. harzianum + *B. subtilis* with biofertilizer significantly increased the activities of defense-related enzymes i.e., catalase, polyphenoloxidase, peroxidase and the total content of phenols under greenhouse conditions. As well as, this treatment increased plant height, number of branches, chlorophyll content, plants NPK content, number of fruits/ plant and total yield under the field conditions compared to chemical fertilizer.

Keywords: Tomato, Early blight, *Alternaria solani*, PGPR, Biofertilizer, Biological control.

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INTRODUCTION

Tomato belongs to the family Solanaceae and is one of the most widely grown vegetable crops in the world, particularly in Egypt. In 2014, Egypt was the fifth all over the world in tomato production (FAO, 2017). Universally, tomatoes are considered as protective food. Tomato is a rich source of minerals, vitamins and organic acids. (Tiyagi *et al.*, 2015). Early blight is the common disease in tomato caused by *Alternaria solani* Ellis and Martin Jones and Grout. The disease is difficult to control because of its capacity to produce huge amounts of secondary inoculum (Atia and Ahmed 2011; Woudenberg *et al.*, 2014). Biological control is an important alternative method to avoid using the application of chemical pesticides in controlling plant diseases and encouraging the organic production of the crops (Atia, 2005; Atia and Ahmed 2011; Sarhan *et al.*, 2018 and Imran *et al.*, 2022). *T. harzianum* is the most common fungus used as biocontol agent against plant pathogenic fungi, also suppresses *A. solani* (Atia and Ahmed 2011& Camlica and Tozlu, 2019). Basamma and Shripad (2017) reported that *Bacillus* species are able to inhibit pathogens by antibiosis that efficiently inhibits the growth of *A. solani*. Isolates of *B. subtilis* and *T. harzianum* successfully suppressed early blight on tomato plants individually or in mixture. (Chowdappa *et al.*, 2013 and Imran *et al.*, 2022). *Trichoderma* spp. and *B. subtilis* are ecofriendly biocontrol agents and have been commercially marketed as biopesticides, biofertilizers and soil improvements (El-Fiki, 2017). Plant growth-promoting rhizobacteria (PGPR) have many beneficial effects on the soil by using organic manure and biofertilizer which include beneficial microorganisms, play a role in enriching all the micro and macronutrients as nitrogen fixation and phosphate and potassium solubilization or their mineralization in the soil, which leads to promotion the plant growth, improve crop yield and indirect effect on plant by removing of pathogens by producing secondary metabolites and ecology (Brar *et al*, 2015; Sarhan *et al.*, 2018 and Le *et al.*, 2018). *Azotobactor* spp. and *Azospirillum* spp. are the two most important N_2-fixing bacteria in crops. *Azotobactor* spp. secrete great amounts of bioactive substances that promote plant root growth and *Azospirillum* spp. have the ability to

produce plant growth regulatory substances along with N_2- fixation, stimulate plant growth and productivity (Meena *et al*., 2017).

The current study aimed to evaluate the effectiveness of some biological control agents and Oxyplus fungicide (copper oxychloride) with biofertilizers in controlling early blight of tomato plants compared to mineral fertilizer under greenhouse and field conditions. Moreover, the activity of defense- related enzymes, accumulation of phenolic compounds, growth parameters and yield of tomato plants were also studied.

MATERIALS AND METHODES

1-Tomato plant:

Tomato seeds 935 variety were obtained from the Dutch Company Enza Zeden. Seedlings were grown and prepared for use in the greenhouse and field during the progress of the present work.

2- Oxyplus 47.89 % WP (Copper oxychloride):

This fungicide was obtained from Delta for chemical company. And the rate of application is 250 g/ 100L water

3-Mineral or Chemical fertilizers:

Full-recommended dose of NPK was added according to the recommended dose by Ministry of Agriculture and Land reclamation. Egypt.

4-Organic fertilizer:

This fertilizer consisted of farmyard manure (FYM) and chicken manure (2:1) collected from local farm located at Abshway county, Fayoum governorate, Egypt. Chemical analyses (Table, 1) were carried out according to Trivedy and Goel (1986). Organic fertilizer was added to the soil before planting at the rate of 7 ton. /fed.

5-Biofertilizers:

The nitrogen fixer's bacteria (*Azotobacter chroococcum* and *Azospirillum lipoferum*) were isolated locally from the cultivated soil and used in biofertilizer treatments, The N_2- fixer's cultures containing 6×10^6 cfu/ ml. were used, 50 ml. of each bacterial strain suspension were mixed with each other's before inoculation and diluted as 1:10 ml. and used as soil drenching for each plot. These were added six times, the first one was after transplanting the seedlings then added monthly with each irrigation.

6-Treatments:

Treatments were divided into two groups, The first group received the biofertilizers, while the second group received the recommended dose of mineral fertilizer.

Group 1

T1: *B. subtilis* + biofertilizer

T2: *T. harzianum* + biofertilizer

T3: *B. subtilis* +*T. harzianum* + biofertilizer

T4: Copper oxychloride + biofertilizer

T5: Control (1) biofertilizer

Group 2

T6: *B. subtilis* + mineral fertilizer

T7: *T. harzianum* + mineral fertilizer

T8: *B. subtilis* + *T. harzianum* + mineral fertilizer

T9. Copper oxychloride + mineral fertilizer

T10: Control (2) mineral fertilizer

Table (1): Chemical properties of the organic fertilizer (FYM+ Chicken manure)

Chemical properties	Value
Bulk density (%)	720
Moisture (%)	19
pH (1:10)	7.80
EC (1:10) dS/m^3.	3.95
Organic carbon (%)	11.76
Organic matter (%)	28.00
Ash (%)	79.70
C /N ratio	18:1
Ammonia (ppm)	418
Nitrate (ppm)	220
T. N (%)	1.70
T. P (%)	1.00
T. K (%)	1.20

7- Laboratory experiments:

7-1-Isolation and Identification of the causal pathogen:

Tomato leaves with typical symptoms of early blight were collected from Abshway county, Fayoum governorate. The infected leaves were cut into small pieces; surface sterilized with sodium hypochlorite (0.5%) for 2-3 minutes, washed several times with sterilized distilled water. Small pieces from the edges of the sterilized pieces were dried between two folds of sterilized filter papers and transferred directly to PDA medium in Petri dishes 9 cm and incubated under 12 h light and 12h dark at 25±1°C as described by Naik *et al*. (2010). The plates were examined daily, and the emerged fungi were transferred to PDA slants. The isolated fungi were purified using the hyphal tip and/ or the single spore techniques according to Hawker (1960). Pure cultures were maintained on PDA slants and stored till used. Pure cultures were identified according to their morphological characteristics by the staff of the

Mycology and Plant Disease Survey Research Dept., Plant Pathol. Res. Inst., ARC as described by Barnett and Hunter (2003).

7-2- Preparation of bioagents:

T. harzianum and *B. subtilis* isolates used in this study were kindly obtained from the Central Lab of Organic Agriculture (CLOA), ARC, Giza, Egypt. Molecular identification was previously performed of the internal transcribed spacer (ITS) rDNA region for *T. harzianum*, as well molecular identification for *B. subtilis* was performed of 16S ribosomal RNA gene, the obtained sequences were deposited in GenBank with accession number MT110634 and MT110633, respectively.

7.2.1. *Bacillus subtilis*:

The culture of *B. subtilis* was activated on fresh slants. After 24 h, it was transferred to flasks with 50 ml of nutrient yeast dextrose broth (NYDB) medium. Inoculated flasks were incubated under a rotary shaker at 120 rpm for 3 days at 24±1°C. Bacterial concentration in the suspension was adjusted to approximately 5×10^6 cfu/ml by measuring absorbance at 600 nm (A600) in a spectrophotometer and using standard curves for *B. subtilis* (Dhingra and Sinclair, 1995).

7.2.2. *Trichoderma harzianum*:

Trichoderma isolates were grown in flasks (250 ml.), each contained 200 ml liquid gliotoxin fermentation medium (GFM) according to Brain and Hemming (1945). Tested flasks were inoculated with 5 mm fungal discs obtained from the periphery of four days old culture of the isolate. Inoculated flasks were incubated in shaking incubator with 170 rpm at 25°C for 12 days to stimulate toxin production (Ali, 2021).

The two bioagents were prepared as suspensions at concentration of 30×10^6 cfu /ml. and used each alone. The bioagents were also mixed together for other treatments at the rate of (1:1 v/v) and added to formula consisted of adjusted culture + 5 % Arabic gum + 0.5 % potassium soap to increase the adhesive capacity and improve distribution of bioagents on the surface of treated plants, then diluted as suspension (1:100 Litter water). All foliar treatments were sprayed at 45 days after transplanting then, repeated each 15 days.

7-3- Effect of antagonistic bioagents and Oxyplus fungicide on growth of *A. solani in vitro*:

Antagonistic assay was performed according to the dual culture method (Fahmi *et al.*, 2012) on potato dextrose agar medium (PDA). A disk (5 mm) of 5 days old culture of *A. solani* was placed at one side of 9 cm Petri dish. A loop of *B. subtilis* was streaked at the opposite side of the pathogenic agar disk. The antagonistic activity of *B. subtilis* was estimated by the inhibition of the fungal growth in comparison to a solely cultivated fungus (control). The decrease in fungal growth was determined by measuring the diameter in centimeter of the colony (Elkahoui *et al*., 2012).

The antagonistic potential of *T. harzianum* was evaluated against *A. solani* using the dual culture technique (Dhingra and Sinclair, 1995). Five mm. mycelial disc of the antagonistic fungus was taken from 5-days old culture and placed against the same sized mycelial disc of *A. solani* at the opposite end on 9 cm diameter PDA Petri plates. The pathogen and antagonist discs were placed at equal distances of the Petri plate. PDA plates inoculated only with the pathogen served as control. The plates were incubated at 28 – 30 ºC. Three replicates were used. When the plates of control were covered with the mycelial growth of *A. solani* (7-10 days later) the growth of the pathogen in both tests and the control was recorded according to the following equation descript by (Mayo *et al*., 2015) as follows.

The percent inhibition of radial growth = [(R1–R2)/R1] × 100.

Where:

R1 = radial growth of the pathogen in control.

R2 = radial growth of the pathogen in dual culture with antagonists.

As well as the fungicide Oxyplus was assayed by using the concentrations 1, 5, 10, 25 and 50 ppm. The radial growth of the fungal colony was recorded on the 10^{th} day when untreated control plates were observed to have maximum growth. The percent inhibition was calculated using the formula of (Vincent, 1947).

$$\mathbf{I = C – T/ C \times 100}$$

Where:

I = percent inhibition of mycelial growth.

C = radial growth of fungus in control.

T = radial growth of fungus in treatment

7-4- The Enumeration of total count of soil microorganisms:

Soil samples were taken from the tested soil before planting for microbiological analysis. The populations of fungi, bacteria and actinomycetes in the soil samples were estimated by soil serial dilution using appropriate dilution and media (Pramer and Schmidt, 1965). Total bacteria (Difco, 1985), fungi (Allen, 1957), and actinomycetes (Jensen, 1930) counts were estimated in the rhizosphere

zone of the plant. The colonies were counted and expressed per gram of dry soil.

7-5- Nitrogen fixing bacteria enumeration:

The most probable number technique (MPN) described by Reinhold *et al*. (1985) was used for the enumeration of *Azospirillum* spp. using the liquid N-deficient semi- solid malate medium (Doberiner *et al*., 1976) and *Azotobacter* spp. was counted on Ashby's medium (Abd-El-Malek and Ishac, 1968). Samples of soil rhizosphere were collected from the tested soil before planting, 10 g soil were shaken for 1 h in 90 ml sterilized water and serial dilutions were made. Tubes contained serial dilutions were incubated at 30° C for 15-21 days as described by Eid (1978), then identified according to cultural, morphological and physiological characteristics according to Bergy's Manual (George *et al*., 2005) as *Azotobacter chroococcum* and *Azospirillum lipoferum*.

7-5-1- Nitrogenase activity:

Acetylene reduction assays were performed to measure nitrogenase activity, which is responsible for the nitrogen fixation as described by Dart *et al*. (1972).

8- Green house experiment:

This experiment was carried out in the greenhouse of the Central Lab. of Organic Agriculture (CLOA), Agricultural Research Center, Giza, Egypt, to evaluate the efficiency of *B. subtilis*, *T. harzianum* and Oxyplus in controlling early blight caused by *A. solani* in tomato. Pots (25 cm. in diameter) with a bottom drainage hole were sterilized by dipping in a 5% formalin solution for 15 minutes and left for one week until complete disappearance of formalin odour. Pots were filled with steam disinfested sandy clay soil 1:2 (V/V). Pots were divided into two groups of treatments: organic and biofertilizer (First group), mineral fertilizer (Second group). Treatments consisted of five replicates, three tomato seedlings were transplanted in each pot. Mineral fertilizer was added according to the recommended dose by the Ministry of Agriculture and Land Reclamation. The biofertilizer as N_2- fixer's (*A. chroococcum* and *A. lipoferum*) cultures ($6X10^6$ cfu/ ml.) were used as soil drench with the irrigation water at concentration of 20 ml/L. added after transplanting the seedlings, then repeated every 15 days.

The bioagents, *T. harzianum* (30×10^6 cfu/ml) and *B. subtilis* (30×10^6 cfu/ml) were used at 10 ml/ L., the Fungicides Oxyplus was used at 2.5 gm./ L. Each treatment was applied as foliar application every 15 days post transplanting date. After two days from the second spray tomato plants were inoculated by spraying 20 ml of *A. solani* suspension containing 5×10^6 spore/ml. then, plants were kept in a climate chamber at a daily temperature of 28°C and 85% relative humidity.

8-1- Pathogenicity of fungal isolates:

a. Laboratory experiment:

Healthy young leaves of greenhouse-grown tomato plants (cv. 935) 45 days old were detached, washed with sterilized water and left between folds of sterilized filter papers for excess of water to dry, then placed on plastic trays, each contains wet filter papers. A volume of 25 ml of sterilized water was added to each tray, to provide a water source for the leaflets and to maintain high R.H. inside the trays. 50µl droplet of tested spore suspension ($5X10^4$ conidia/ml) were deposited on the upper surface of each leaflet (Badawy and Dib, 2012). Three replicates were used. A set of leaflets were inoculated by sterilized water only and kept as control. All trays were incubated at room temperature (25± 3°C) for 7 days then disease severity (%) was calculated (Badawy and Dib, 2012).

b. Greenhouse experiment:

Tomato seeds (cv. 935) were sown in ordinary cultivation trays filled with peat moss for 30 days under greenhouse condition. Growing seedlings were transplanted in pots (20-cm-diam.) filled with sterilized sandy-clay soil (1:1 w/w) at the rate of 3 seedlings/pot. Growing plants (4-weeks-old) were sprayed, using a fine atomizer, with the tested spore suspension ($5X10^4$ conidia/ml) of *A. solani*, then incubated under greenhouse conditions (25±5°C and 75-90% R.H.). Tomato plants sprayed with water only were kept as check. Three replicates were used for each isolate. Disease severity percentage was determined after 14 days post inoculation (El- Tanany *et al*., 2018).

8-2- Disease assessment:

A random sample of ten plants collected from each treatment was used for measuring disease incidence and disease severity and evaluate the effect of the tested preparations on controlling the disease.

A-Disease incidence (DI %) was calculated according to the following formula:

$$\textbf{DI \%} = (n / N) \times 100$$

Where:

n = Number of diseased plants sampled.

N= Total number of plants.

B-Disease severity: Ten plants were selected randomly from each treatment 14 days post inoculation and were used to estimate disease

severity using the modified 0-5 rating scale as described by (Pandey *et al.*, 2003).

Grade	Disease severity %
0	No symptoms
1	10 % of leaf surface area.
2	More than 10-25 % of leaf surface area.
3	More than 25-50 % of leaf surface area.
4	More than 50-75 % of leaf surface area.
5	More than 75-100 % of leaf surface area.

Disease severity % was calculated according to the following formula:

$$\mathbf{DS\ \% = \Sigma\ (n \times v) / 5\ N) \times 100}$$

Where:

n = number of leaves in each symptom's category.

V = numerical value of each category.

N = total number of leaves in sample.

8-3- Effect of the biofertilizer and the bioagents treatments on the activity of oxidative enzymes and total phenolic compounds in treated tomato plants:

Peroxidase activity was determined according to Kochba *et al.* (1977), polyphenoloxidase activity was determined according to Lisker *et al*. (1983), catalase activity was determined according to Aebi (1974), and the total phenols content was determined according to Snell and Snell (1953).

9- Field experiment:

The field experiment was carried out in a private farm located at Abshway county, Fayoum governorate, Egypt during the winter growing seasons of 2018/19 - 2019/20 to control early blight disease of tomato caused by *A. solani*. Three replicates (5×4 m^2) belonged to each treatment; each replicate consist of 4 rows (4 m.), contain 60 plants. The agricultural practices were conducted according to the recommendation of the Ministry of Agriculture and Land Reclamation, Egypt.

9-1- Soil and water analysis:

Physical and chemical analyses of soil and irrigation water were carried out before planting as described by Jackson (1973). The obtained results are illustrated in Table (2).

9-2- Disease assessment:

A random sample of ten plants representing each experimental unit was taken at 70 and 90 days post transplanting in both seasons to determine the disease incidence and the disease severity and to evaluate the effect of different preparations on controlling the disease as described under greenhouse experiment.

At the end of the experiment, the following vegetative characteristics i.e., plant height (cm), number of branches/plants, number of fruits/plant and fruit weight/plant (kg/plant) and total yield (kg/plot) were determined.

Table (2): Analysis of the tested soil and the irrigation water at Fayoum governorate.

Mechanical analysis (%)		Anions and Cations (mmq/ L.)	Soil used	Irrigation water
Sand	20.4	pH (1:2.5)	7.80	7.40
Silt	27.3	EC (ds//m3)	0.90	0.42
Clay	52.3	Ca^{++}:	5.1	2.13
Texture class	Clay	Mg^{++}	2.5	1.02
Available nutrients (ppm)		Na^{+}	1.38	1.01
N	50	K^{+}	0.03	0.04
P	4.67	CO^{-}_{3}	--	--
K	420	HCO^{-}_{3}	1.85	0.03
		Cl^{--}	1.56	1.09
		$SO4^{--}$	5.60	3.08
		$CaCO_3$	2 %	

9-3- Chemical analysis:

Photosynthetic pigments (mg/ g fresh weight of leaf) were determined according to Askar and Treptow (1993), total phenolic compounds (mg /100g fw) were estimated as described by Shahidi and Naczk (1995), total nitrogen % was determined as described by Piper (1950), total phosphorus and potassium % were determined by using the atomic absorption spectrophotometer (Barkin Elmer 3300) according to Chapman and Pratt (1961).

10- Statistical analysis:

The experiment was designed in complete randomized blocks (CRB). Data were analyzed using the MSTAT-C (1991) program version 2.10. Means of treatments were differentiated using the Least significant difference (LSD) at P=0.05 (Gomez and Gomez, 1984).

RESULTS

Lab experiment:

1-Effect of antagonistic bioagents on the growth of *A. solani*:

All tested bioagents were effective on reducing growth of *A. solani*, the causal of tomato early blight. Results in Fig. (1) indicate that T. *harzianum* + *B. subtilis* treatment was the most effective one that decreased the linear growth of the pathogen, being 0.9 cm. followed by *T. harzianum* which, reduced the growth, being 2.7 cm. Meanwhile, *B. subtilis* recorded 4.9 cm. compared to the control.

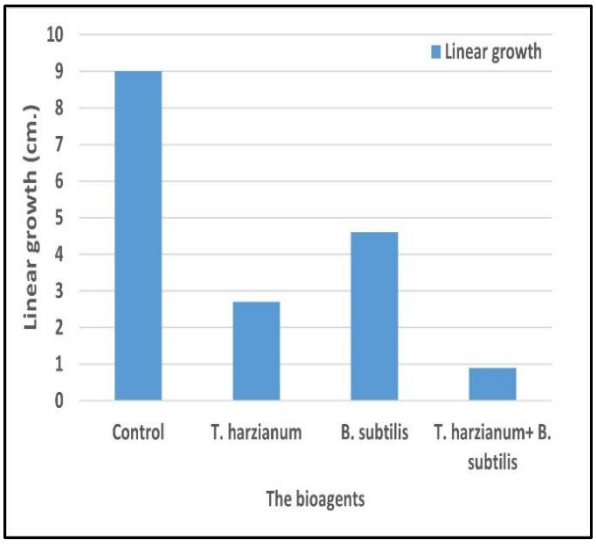

Fig (1): Effect of the tested bioagents on the linear growth of *Alternaria solani*.

2-Effect of Oxyplus fungicide on the growth of *A. solani*.

As shown in Fig. (2) all the tested f concentrations (1, 5, 10, 25 and 50 ppm) of Oxyplus fungicide inhibited the linear growth of *A. solani*. There was an inverse relation between the fungicide concentration and the linear growth. The maximum decrease of the linear growth was due to the fungicide concentration 25 ppm that entirely inhibited the growth being zero cm.

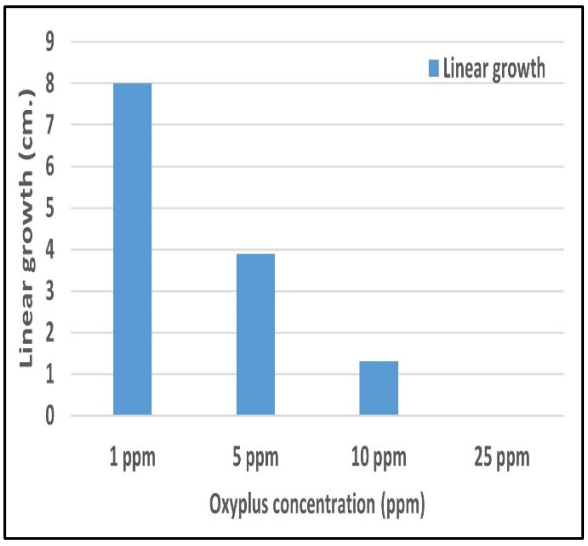

Fig (2): Effect of different fungicide concentrations on the linear growth of *Alternaria solani*.

3- The enumeration of total count of soil microorganisms and the MPN of N_2 fixer´s bacteria:

The obtained data (Table 4) reveal that bacteria in the rhizosphere of tomato plants recorded the highest population density, being 11×10^6 CFU/g soil followed by the fungi and the actinomycetes, being 3×10^4 and 2×10^5 CFU /g soil, respectively. The most probable numbers (MPN) showed that *Azotobacter* spp. recorded the highest populations being 8×10^4 CFU/ ml. of culture media followed by *Azospirillum* spp, being 5×10^4 CFU/ml of culture media.

The bacterial isolates were identified as *Azotobacter chroococcum* and *Azospirillum lipoferum*. The values of nitrogenase activities were higher with *A. lipoferum* than *A. chroococcum*, being 21.7 and 19.3 nmole C_2H_4 /g dry matter / hr. after transplanting, respectively.

Table (4): The microbial groups and N_2-Fixer´s isolated from the rhizosphere of tomato plants.

Microorganisms	Microbial counts (CFU/ml.)	
Bacteria	11×10^6CFU	
Fungi	3×10^4CFU	
Actinomycetes	2×10^5CFU	
Most probable number of N_2- fixer´s (MPN $\times 10^5$ ml^{-1} on specific cultural media)		
The isolates	Zero time	Nitrogenase Enzyme
A. lipoferum	5×10^4/ml	21.7
A. chroococcum	8×10^4/ml	19.3

Greenhouse experiment:

1-Effect of biofertilizers and bioagents on the incidence and severity of early blight in tomatoes under artificial inoculation under greenhouse conditions:

Data presented in Table (5) show that tomato plants (cv. 935) treated with two groups, *i.e.*, the first group included the bioagents *B. subtilis* and *T. harzianum* with biofertilizer N_2- fixers bacteria, *i.e., A. lipoferum* and *A. chroococcum* and the second group included the bioagents with mineral fertilizer. Tomato plants were sprayed with these biological agents two weeks before artificial infection with *A. solani*. *B. subtilis* and *T. harzianum* significantly reduced the disease incidence and disease severity in the two groups. Whereas the biofertilizer with the bioagents recorded the highest reduction in disease incidence and disease severity than the mineral fertilizer.

Combination of *B. subtilis* + *T. harzianum* with the biofertilizer significantly reduced the disease incidence and disease severity, being 62.33 and 5.1 %, respectively, followed by the fungicide Oxyplus with the biofertilizer treatment that recorded 76.10 and 5.33 %, respectively compared to the mineral fertilizer.

Table (5): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) under bio- and mineral fertilizers on early blight incidence and severity in tomato plants grown under greenhouse conditions.

Treatments	Disease incidence (%)			Disease severity (%)		
	Biofertilizer	Mineral fertilizer	Mean	Biofertilizer	Mineral fertilizer	Mean
B. subtilis	78.32	81.00	79.66	6.70	9.33	8.02
T. harzianum	78.33	82.77	80.55	6.67	9.00	7.84
B. subtilis + *T. harzianum*	62.33	75.35	68.84	5.10	8.33	6.72
Oxyplus fungicide	76.10	85.00	80.55	5.33	7.67	6.50
Control	78.10	75.00	76.55	8.33	12.23	10.28
Mean	74.64	79.82		6.42	9.31	
L.S.D at 0.05						
Fertilizers (F)		0.64			0.58	
Treatments (T)		0.46			0.41	
F × T		0.91			n.s.	

n.s.: no significant

2- Effect of biofertilizers and bioagents on the total phenolic compounds in tomato leaves under greenhouse conditions:

The tested bioagents gave higher content of phenol components in both seasons followed by Oxyplus fungicide compared to the control. Data in Table (6) also show that biofertilizer revealed the highest content of phenol compared to mineral fertilizer during the two seasons tested. T_3 (*B. subtilis* + *T. harzianum* + biofertilizer) was the most effective treatment, which recorded 2.84- 2.70 of total phenols, conjugated phenols (2.36- 2.31) and free phenols (0.47- 0.50) in both seasons, respectively followed by T_4 (Oxyplus + biofertilizer) then other treatments.

3- Effect of biofertilizers and bioagents on plant defense related enzymes in tomato leaves under greenhouse conditions:

The activity of plant defense related enzymes was measured in tomato leaves. The enzymatic activities of Peroxidase (PO), Polyphenoloxidase (PPO) and Catalase (Ca) were varied between treatments. Data in Table (7) show that the activity of resistance related enzymes was increased significantly elevated under combined effect of biocontrol agents with biofertilizer than with the mineral fertilizer. Therefore, *B. subtilis* + *T. harzianum* + biofertilizer significantly showed the highest values of peroxidase, catalase and polyphenoloxidase in both seasons, Peroxidase activity was 2.98- 2.99, catalase activity was 1.09- 2.88, and polyphenoloxidase recorded 0.07- 0.64 in the first and the second season, respectively compared with the other treatments.

Field experiment:

1-Effect of biofertilizers and bioagents on the incidence and severity of early blight in tomatoes under field conditions:

Biofertilizer and bioagents had a significant effect in reducing tomato early blight disease incidence and severity percentages during the two growing seasons, 2018/19 and 2019/20 compared with plants received mineral fertilizer and bioagents (Table 8). In the biofertilizer group, data indicated that all treatments reduced the disease incidence and disease severity percentage with different degrees compared to the control. The combined bioagents treatments *B. subtilis* +*T. harzianum* + biofertilizer recorded the lowest infection in comparison with the biocontrol agents, being 20.00- 10.67 % disease incidence and 11.61- 5.33 % disease severity in the first and the second season, respectively. Followed by copper oxychloride which showed the lowest percentage of disease incidence (20- 7.67%), and the lowest percentage in disease severity (11.6- 5.33%) during the two seasons, respectively

2- Effect of biofertilizers and bioagents on some plant growth parameters:

Tomato plants received biofertilizers recorded the highest values of all growth parameters compared to those received the mineral fertilizers. Plants treated with *B. subtilis* + *T. harzianum* + biofertilizer were significantly the higher plants (76.67- 107.33 cm) and showed the number of branches (6.33- 7.33) and produced the highest number of fruits/ plant (66.67- 72.00) compared to all other treatments in the two seasons, respectively (Table, 9).

Table (6): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio- and/ or mineral fertilizers on the phenol components (mg /100g fw) in tomato leaves infected with early blight under greenhouse conditions.

Treatments	Total phenols						Conjugated phenols						Free phenol					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	0.93	1.03	0.98	0.85	0.45	0.65	0.42	0.30	0.36	0.42	0.11	0.27	0.49	0.66	0.58	0.4	0.6	0.5
T. harzianum	0.43	1.01	0.72	0.65	0.83	0.74	0.43	0.17	0.30	0.50	0.14	0.32	0.42	0.65	0.54	0.37	0.6	0.49
B. subtilis +T. harzianum	2.84	2.80	2.82	2.70	1.75	2.22	2.36	1.16	1.76	2.31	0.37	1.43	0.47	0.48	0.48	0.5	0.5	0.5
Oxyplus fungicide	1.07	1.51	1.29	0.93	1.23	1.08	0.39	0.57	0.48	0.35	0.45	0.40	0.65	0.78	0.72	0.6	0.67	0.64
Control	0.76	0.55	0.66	0.76	0.11	1.15	0.28	0.10	0.19	0.34	0.10	0.22	0.31	0.04	0.18	0.3	0.02	0.16
Mean	1.21	1.38		1.18	0.87		0.78	0.46		0.8	0.23		0.47	0.52		0.43	0.48	
L.S.D. at 0.05																		
Fertilizers (F)		0.09			0.10			0.07			0.11			n.s.			n.s.	
Treatments (T)		0.07			0.07			0.05			0.08			0.06			0.06	
F × T		0.13			0.15			0.10			0.15			n.s.			n.s.	

BF: Biofertilizer & MF: Mineral fertilizer & n.s.: no significant

Table (7): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio- and/ or mineral fertilizers on the activities of oxidative enzymes in tomato leaves infected with *A. solani*

Treatments	Peroxidase						Catalase						Polyphenoloxidase					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	2.49	1.49	1.99	2.83	2.31	2.57	0.99	0.69	0.84	2.80	2.27	2.535	0.07	0.1	0.085	0.45	0.46	0.455
T. harzianum	2.06	1.51	1.785	2.79	2.68	2.735	0.54	0.49	0.515	2.79	2.80	2.795	0.04	0.07	0.055	0.46	0.45	0.455
B. subtilis + T. harzianum	2.98	2.37	2.675	2.99	2.83	2.91	1.09	1.11	1.10	2.88	2.81	2.845	0.07	0.11	0.090	0.64	0.50	0.570
Oxyplus fungicide	2.11	1.75	1.93	2.93	2.93	2.93	1.03	0.78	0.905	2.89	2.88	2.885	0.14	0.21	0.175	0.74	0.51	0.625
Control	1.34	1.3	1.32	2.57	2.17	2.37	0.55	0.46	0.51	2.39	2.09	2.24	0.07	0.03	0.05	0.30	0.33	0.32
Mean	2.20	1.68		2.82	2.58		0.84	0.71		2.75	2.57		0.079	0.10		0.52	0.45	
L.S.D. at 0.05																		
Fertilizers (F)		0.08			0.13			0.05			n.s.			0.03			n.s.	
Treatments (T)		0.05			0.09			0.03			n.s.			0.02			0.07	
F × T		0.11			0.18			0.06			n.s.			0.04			n.s.	

BF: Biofertilizer & MF: Mineral fertilizer & n.s.: no significant

Table (8): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio- and mineral fertilizers on tomato early blight disease incidence and severity in tomato plants grown under field conditions.

Treatments	Disease incidence (%)						Disease severity (%)					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	23.32	30.00	26.66	22.33	30.01	26.17	16.33	21.73	19.03	7.67	12.33	10.00
T. harzianum	25.00	31.76	28.38	16.33	20.67	18.50	16.20	24.32	20.26	17.00	19.00	18.00
B. subtilis+ *T. harzianum*	20.00	26.68	23.34	10.67	16.01	13.34	12.94	19.56	16.25	6.33	8.33	7.33
Oxyplus fungicide	20.00	25.00	22.50	7.67	12.33	10.00	11.61	16.05	13.83	5.33	6.67	6.00
Control	38.33	45.00	41.67	32.67	41.67	37.17	24.02	37.87	30.95	22.00	32.33	27.17
Mean	25.33	31.69		17.93	24.14		16.22	23.91		11.67	15.73	
L.S.D. at 0.05												
Fertilizers (F)		0.42			1.20			0.15			0.57	
Treatments (T)		0.30			0.85			0.11			0.40	
F × T		0.59			1.70			0.21			0.81	

BF: Biofertilizer & MF: Mineral fertilizer

Table (9): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio-and mineral fertilizers on some growth parameters of tomato plants grown under field conditions.

Treatments	Plant height (cm.)						No. of branches/plant						No. of fruit /plant					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	66.67	63.33	65.00	96.33	76.67	86.5	8.00	5.67	6.84	5.33	6.00	5.67	52.33	62.67	57.5	67.67	53.33	60.5
T. harzianum	74.33	65.33	69.83	109.0	90.67	99.84	6.00	6.33	6.17	7.00	5.67	6.34	63.00	60.00	61.5	62.00	42.34	52.17
B. subtilis + T. harzianum	76.67	65.40	71.00	107.33	90.00	98.67	6.33	7.76	7.05	7.33	6.33	6.83	66.67	66.33	66.5	72.00	56.67	64.34
Oxyplus fungicide	75.00	67.00	71.00	96.67	92.33	94.50	5.00	7.00	6.00	8.67	6.00	7.34	74.00	63.33	68.67	75.67	50.00	62.84
Control	60.33	50.01	55.17	96.33	63.33	79.83	6.00	4.67	5.34	5.33	4.67	5.00	51.67	46.33	49.00	49.33	34.33	41.83
Mean	70.6	62.21		101.13	82.6		6.27	6.29		6.73	5.74		61.53	59.73		65.33	47.33	
L.S.D. at 0.05																		
Fertilizers (F)		1.29			0.97			n.s.			0.94			0.38			1.00	
Treatments (T)		0.91			0.69			0.32			0.67			0.27			0.71	
F × T		1.82			1.37			0.64			1.33			0.54			1.42	

BF: Biofertilizer & MF: Mineral fertilizer & n.s.: no significant

3- Effect of biofertilizers and bioagents on photosynthetic pigments:

Data presented in Table (10) reveal that in both seasons, all biofertilizer treatments significantly increased leaf pigments content (chlorophyll a, chlorophyll b and carotenoids) compared to mineral fertilizer (NPK) in both seasons. T_4 (Oxyplus + biofertilizer) resulted significantly the highest chlorophyll a value (25.33 - 25.82 mg/ g fresh weight of leaves) in tomato leaves, respectively compared to the values recorded from other treatments, followed by T_3 (*B. subtilis* + *T. harzianum* + biofertilizer) which recorded 25.06- 23.64. On the other hand, chlorophyll b, T_4 plants gave higher values in the first season (47.94), but in the second season, the biofertilizer plus the bioagent treatment recorded the highest value. Concerning the carotenoids, the T_3 Treatment significantly caused the highest values in both experimental seasons.

4- Effect of biofertilizers and bioagents on macronutrients contents in tomato leaves:

The obtained results in Table (11) show that application of biofertilizer on tomato significantly increased the nutrient absorption of the macronutrients as compared to the control. The highest contents of N, P and K % were found in tomato plants treated with *B. subtilis* + *T. harzianum* + biofertilizer at the two experimental seasons, followed by T_4 plants (Oxyplus + biofertilizer).

5- Effect of biofertilizers and bioagents on tomato yield:

Data presented in Table (12) indicate that the highest weight of fruits/ plant was collected from tomato plants treated with *B. subtilis* + *T. harzianum* + biofertilizer which received bioagents and biofertilizer (3.77 – 4.18 kg/plant) in the first and the second season, respectively, followed by Oxyplus + biofertilizer which recorded 3.78 – 3.80 kg/plant. While mineral fertilizers recoded 3.36 kg/plant with the bioagents. Moreover, plants received the Oxyplus fungicide in the first and the second season yielded 3.21 – 3.16 kg/ plants.

In the same trend, the highest total yield was recorded due to using T_3 (*B. subtilis* + *T. harzianum* + biofertilizer) followed by T_4 (Oxyplus + biofertilizer) compared to the mineral fertilizers

DISCUSSION

Early blight is one of the most common diseases of tomato, caused by *A. solani*, causing the necrotic spots in concentric rings with a yellow halo reducing the photosynthetic area. It causes significant damage at all stages of growth and in all aerial parts of tomato, leading to a 35-78% loss in fruit yield (Mohamed *et al*., 2021).

Agricultural fungicides combat plant pathogens: but they have negative effects on the beneficial natural organisms. Therefore, it is necessary to develop environmentally friendly and biodegradable agricultural alternatives in order to control plant pathogens (Khalil and Adbelghany, 2021). So, the use of bioagents has become important as an alternative to fungicides to prevent postharvest losses in recent years. Plant growth-promoting rhizobacteria (PGPR) include a wide range of nonpathogenic soil microorganisms and beneficial soil rhizobacteria that play a major role in plant health and nutrition. These also prevent attack from pathogenic microorganisms (Morsy *et al*., 2009; Camlica and Toziu, 2019). In this study, the effect of the bioagents *Bacillus subtilis* and *Trichoderma harzianum* as well as the N_2 – fixer's biofertilizers *Azotobacter chroococcum*, *Azospirillum lipoferum* on *A. solani* was evaluated under laboratory, greenhouse and field conditions.

Results obtained in this study showed that the two bioagents reduced the linear growth of *A. solani* in laboratory experiments. This result is in agreement with the findings of Atia and Ahmed (2011); El-Farnawany (2006) and Imran *et al*. (2022) referred that *T. harzianum* inhibited growth of *A. solani*, the interaction between *T. harzianum* and conidia of *A. solani* resulted in deformations; connect spores, growing in the contact zone between spores and formation of a node -like structure between two successive conidia. In addition, *B. subtilis* has a vital role in reducing the growth of *A. solani*. Morsy *et al*. (2009) and Elkahoui *et al*. (2012) reported that *B. subtilis* produces antifungal substances or colonizing microsites faster than the surface fungi as lipopeptides belonging to the iturin and surfactin in the late phase of growth that inhibit *A. solani* growth. Also, Mazrou *et al*. (2020) cleared that the effectiveness of *Trichoderma* spp. may lies in its ability to produce toxic hydrophilic metabolites or lytic enzymes as glucanase and chitinase, which released by *Trichoderma* spp. at low levels, so it can act against pathogenic fungi before interacting with fungi, thus increasing the antagonistic ability of *Trichoderma* spp. In addition to, *Trichoderma* spp. act as mycoparasities produce lytic enzymes towards the host or prey then attach to it, wrapping around the organism's hyphae and penetrating them.

Table (10): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio-and mineral fertilizers on photosynthetic pigments (mg/ g fresh weight of leaf) in tomato leaves grown under field conditions.

Treatments	Chlorophyll A						Chlorophyll B						Carotenoids					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	24.07	24.07	24.07	22.75	18.01	20.38	42.49	28.27	35.38	18.38	14.22	16.30	4.23	4.23	4.23	3.07	3.39	3.23
T. harzianum	24.18	24.40	24.29	24.64	25.72	25.18	44.15	47.92	46.04	18.17	14.23	16.20	5.55	4.49	5.02	2.35	5.13	3.74
B. subtilis + *T. harzianum*	25.06	24.60	24.83	23.64	25.98	24.81	45.08	48.12	46.6	26.34	18.10	22.22	7.00	4.80	5.90	4.00	4.20	4.10
Oxyplus fungicide	25.33	24.32	24.83	25.82	15.56	20.69	47.94	45.95	46.95	17.01	18.09	17.55	5.55	3.75	4.65	2.91	4.99	3.95
Control	24.02	14.74	19.38	15.99	15.69	15.84	38.62	17.64	28.13	17.45	18.55	18.00	6.33	3.50	4.92	0.25	2.39	1.32
Mean	20.53	22.43		22.57	20.19		43.66	37.58		19.40	16.63		5.73	4.15		2.52	4.02	
L.S.D. at 0.05																		
Fertilizers (F)		0.11			0.06			0.12			0.06			0.26			0.11	
Treatments (T)		0.08			0.04			0.08			0.04			0.18			0.08	
F × T		0.16			0.09			0.17			0.08			0.37			0.14	

BF: Biofertilizer & MF: Mineral fertilizer

Table (11): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio- and mineral fertilizers on the NPK % in tomato leaves grown under field conditions.

Treatments	Total nitrogen %						Total phosphorus %						Total potassium %					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	2.30	1.67	1.985	2.245	2.10	2.168	0.02	0.018	0.018	0.02	0.02	0.02	1.63	1.79	1.71	1.63	2.15	1.89
T. harzianum	2.33	1.66	1.995	2.245	2.44	2.338	0.02	0.018	0.018	0.015	0.021	0.018	1.70	1.74	1.72	1.63	2.28	1.955
B. subtilis + *T. harzianum*	2.90	1.15	2.025	2.290	1.73	2.010	0.03	0.012	0.021	0.03	0.02	0.025	1.95	2.08	2.015	2.46	2.12	2.29
Oxyplus fungicide	1.78	2.61	2.195	1.725	1.01	1.363	0.015	0.015	0.015	0.02	0.02	0.02	2.56	1.53	2.045	0.90	2.23	1.565
Control	1.60	1.35	1.48	1.61	0.5	1.06	0.01	0.11	0.06	0.06	0.010	0.035	1.63	1.35	1.49	1.07	2.37	1.72
Mean	2.16	1.69		2.02	1.56		0.019	0.034		0.03	0.018		1.89	1.70		1.54	2.23	
L.S.D. at 0.05																		
Fertilizers (F)		0.05			0.22			0.01			0.02			0.07			0.11	
Treatments (T)		0.04			0.15			0.01			0.01			0.05			0.08	
F × T		0.08			0.30			0.01			0.03			0.09			0.15	

BF: Biofertilizer & MF: Mineral fertilizer

Table (12): Effect of different bioagents compared to Oxyplus fungicide (Copper oxychloride) with bio-and mineral fertilizers on the yield of tomato plants grown under field conditions.

Treatments	Weight of fruit (Kg/ plant)						Total yield (kg/fed.)					
	Season 2018/2019			Season 2019/2020			Season 2018/2019			Season 2019/2020		
	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean	BF	MF	Mean
B. subtilis	3.72	3.28	3.50	3.80	3.20	3.50	29787.33	26212.67	28000.00	30366.00	25573.34	27969.67
T. harzianum	3.56	3.09	3.33	3.61	2.93	3.27	28583.33	24712.67	26648.00	28872.67	23473.33	26173.00
B. subtilis + *T. harzianum*	3.77	3.36	3.57	4.18	3.36	3.77	30179.33	27192.67	28686.00	33446.00	26861.34	30153.67
Oxyplus fungicide	3.78	3.21	3.50	3.80	3.16	3.48	30240.00	25680.68	27960.34	30426.67	25214.01	27820.34
Control	2.76	2.40	2.58	2.98	2.16	2.57	22040.67	19194.00	20617.34	23846.67	17299.33	20573.00
Mean	3.52	3.07		3.67	4.94		28166.00	24598.54		29391.60	23684.27	
L.S.D. at 0.05												
Fertilizers (F)		0.05			0.04			394.10			351.43	
Treatments (T)		0.04			0.03			278.67			248.50	
F × T		0.07			0.06			557.34			496.99	

BF: Biofertilizer & MF: Mineral fertilizer

The results of enumeration of total counts of soil microorganisms and the MPN of N_2- fixer´s bacteria came in agreement with several researchers, who revealed that there are vast types of microbial species live into the soil and they are found in all types of soils (Kumar *et al*., 2015). These microbes may interact with plants, some with beneficial effect and others have harmful consequences as fungi, bacteria and actinomycetes that play a major role in plant health and productivity or disease (Kumar *et al*., 2015). In addition, Zhang *et al*. (2020) explained that soil microorganisms are the most active part of the soil and the main component of the soil decomposition system. Soil microorganisms play an important role in soil formation and development, organic matter decomposition, material transformation, energy transfer, the geochemical cycle and bioremediation. Yagmur and Gunes (2021) found that several PGPRs can act as very good biological control agents. These bacteria can provide great success against plant diseases, increase the solubility of plant nutrients in the soil and have an important role in increasing plant nutrition efficiency in various ways including biological nitrogen fixation, phosphorous dissolution and/or production of plant hormones, amino acids and organic acids and significantly increasing plant growth. Therefore, it has increasingly used in agricultural systems as a microbial fertilizer.

Under greenhouse conditions, results obtained showed that spraying tomato plants with the bioagents before inoculation with *A. solani* reduced the disease incidence and disease severity of tomato early blight. These results are in agreement with the findings of El-Tanany *et al*. (2018) who evaluated the effect of different isolates of *Trichoderma* on the linear growth of *A. solani* on PDA medium. In addition, Khalil and Adbelghany (2021) indicated that *T. harzianum* was the best bioagent, which inhibited the growth of *A. solani*,

Data of this study showed that phenol components were increased with using the biofertilizer and the bioagents more than with mineral fertilizer during the two seasons. These results are in agreement with those reported by Khalil and Adbelghany (2021) who found that treated tomato plants with the bioagents led to an increase in the total dissolved phenols in tomato leaves compared to the control. The highest significant increase in the total dissolved phenols in tomato leaves was obtained from tomato plants treated with *T. harzianum*.

The activity of plant defense related enzymes Peroxidase (PO), Polyphenoloxidase (PPO) and Catalase (Ca) was measured in leaves of tomato. Data showed that activity of the enzymes was increased significantly under the combined effect of biocontrol agents with biofertilizer than with the mineral fertilizer. These results are in line with those obtained by Adhikari *et al*. (2017) who reported that the plant growth-promoting rhizobacteria *B. subtilis* increased systemic resistance in tomato by inducing growth hormones and defense-related enzymes such as peroxidase, polyphenoloxidase and superoxide dismutase. Also, Abou-Zeid *et al*. (2018) found that the use of *Trichoderma* spp., *Bacillus* spp. as bioagents for induction of defense enzymes such as chitinase, peroxidase and polyphenoloxidase, play an important role in plant defense mechanisms against pathogens infection in treated bean plants. Morsy *et al*. (2009) and Sarhan *et al*. (2018) mentioned that using PGPR is an important method for plant diseases suppression due to its ability to stimulate plant defense in response to microbial infection including defense-related enzymes and pathogenesis related proteins such as β-1,3-glucanase, peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, indole acetic acid (IAA), lignin synthesis, accumulation of phenolic compounds and specific flavonoids.

Under field conditions, plants that received biofertilizer and bioagents (PGPR) exhibited a significant effect in reducing early blight disease incidence and disease severity percentage comparing with the plants that received mineral fertilizer and bioagents. This result is in agreement with Adhikari *et al*., 2017 and Imran *et al*., 2022 who reported that *Trichoderma* spp. have beneficial effect on plant growth and development. They are able to colonize the soil and/or parts of the plant, avoiding the multiplication of pathogens, producing cell wall degrading enzymes against the pathogens, producing antibiotics that can kill the pathogens, promoting the plant development and inducing the defense mechanisms of the plant. While Basamma and Shripad (2017) mentioned that *B. subtilis* is very important bioagent used for management of plant diseases. In this respect, Ali *et al*. (2017) indicated that a combination of antagonistic bacteria as *Bacillus* spp. with antagonistic fungi especially *Trichoderma* sp. increased plant protection than when being used individually.

Biofertilizers help in improving biological activities of beneficial microorganisms in the soil to improve the crop yield and production quality. Moreover, organic manures release the nutrients slowly; their effect is shown on the

current crop and reflected on the performance of other crops. The use of organic manures and biofertilizers is the only reason to producing good quality fruits without any harmful effect on soil health and the environment (Aly *et al*., 2003 and Brar *et al*., 2015).

Tomato plants in the biofertilizer group achieved the highest results in all growth parameters compared to plants in the mineral fertilizer group. The increases in most vegetative growth parameters may be refer to the ability of biofertilizers to release some compounds that may improving the plant growth characters, in addition that, organic fertilizer contributes to nutrients release through the decomposition of organic matter, production humate that can exchange for absorbed anions such as phosphorus. Some researchers had been indicated that organic fertilizers may increase soil fertility which is reflected on the potential of the crop production, changes in soil properties including nutrient bio availability, soil structure, water holding capacity, cation exchange capacity, soil pH, microbial community and activity (Ayeni *et al*., 2010 and Hafez *et al*., 2016). Similar results were reported by (Abd El All, 2019) who reported that the good growth (vegetative, physiological and chemical) of tomato plants resulting from growth in soil fertilized by bioorganic fertilizers.

In both seasons, all biofertilizer treatments significantly increased leaf total chlorophylls and carotenoids contents (Ali *et al*., 2019). N, P and K % in tomato leaves (Altuhaish *et al*., 2014). *Azotobacter* spp. and *Azospirillum* spp. can enhance plant growth development and yield of several crops in different soils. *Azotobacter* with other biofertilizers can fix 15-20 kg nitrogen/ha/crop and give about 10-15 % increase in yield. (Gothandapani *et al*., 2017). Baba *et al*. (2018) reported that the beneficial effects of *Azotobacter* and *Azospirillum* increase the rate of water and mineral uptake by roots, displacement of fungi and plant pathogenic bacteria and biological nitrogen fixation. *Azotobacter* synthesizes and secretes large amounts of vitamins, nicotinic acid, pantothenic acid, biotin, heteroxins, gibberelins etc., which enhance root growth of plants, improving the secretion of ammonia in the rhizosphere that helps in modification of nutrient uptake by the plants and produce plant growth regulatory substances along with N_2 fixation stimulate plant growth and productivity. Nosheen *et al*. (2021) reported that mixing two different types of biofertilizers increased the yield of crops significantly more than the single biofertilizer or chemical fertilizers.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFRENCES

Abd El All, A.M. 2019. Effect of some Bio-organic fertilizers on morphological and physiological traits of tomato plants. Int. J. Adv. Res. Biol. Sci. 6(2): 204-210.

Abd El-Malek, Y. and Ishac, Y.Z. 1968. Evaluation of methods used in counting *Azotobacter* sp. J. Appl. Bact., 331: 269-275.

Abou-Zeid, N.M.; Mahmoud, N.A.; Abbas, M.S.; Soliman, A. Sh. and Eid, H.T. 2018. Induction of systemic resistance against Tomato wilt disease under greenhouse conditions in Egypt. Bioscience Research, 15(4): 4559-4570.

Adhikari, P.; Oh, Y. and Panthee, D.R. 2017. Current status of early blight resistance in tomato: An Update. Int. J. Mol. Sci., 18.

Aebi, H. 1974. Methods of Enzymatic Analysis, H.U. Bergmeyer (Ed.), Verlag Chemie/ Academic Press Inc, Weinheim/ New York, USA, pp. 673–677.

Ali, A.A.; Abd El- kader, A.E.S. and Ghoneem, KH.M. 2017. Two *Trichoderma* species and *Bacillus subtilis* as biocontrol agents against Rhizoctonia disease and their influence on potato productivity. Egypt. J. Agric. Res., 95 (2): 527-541.

Ali, M.A.M.; El- Tokhy, A.I.A.; El- Sherbini, M.A.A.; Abdel- Dayem, S.M. and Khpalwak, W. 2019. Response of growth, yield and fruit quality of tomato (*Solanum lycopersicum* L.) to different organic fertilizer treatments under two pest control programs against *Tuta absoluta* in New Valley-Egypt. J. Sus. Agric. Sci. 45 (2): 37- 57.

Ali, A.M. 2021. The Competitive potential of different *Trichoderma* spp. to control Rhizoctonia root rot disease of pepper (*Capsicum annuum* L.). Egyptian Journal of Phytopathology, 49(1): 136-150.

Allen, O.N. 1957. Experiments in Soil Bacteriology. 3rd ed. Burgess Pablo. Co. USA, pp.117.

Altuhaish, A., Hamim, H. and Tjahjoleksono, A. 2014. Biofertilizer effects in combination with different drying system and storage period on growth and production of tomato plant under field conditions. Emir. J. Food Agric. 26 (8): 716-722.

Aly, A.Z.; Tohamy, M.R.A; Atia, M.M.M.; Abd-El-Moity, T.H. and Abed-El-Moneim, M.L. 2003. Role of organic matter in controlling some soil-borne and foliage diseases of cucumber. Proceedings of the International Symposium: on the Horizons Using Organic Matter and Substrates in Horticulture. Acta Hortic. 608: 209-217.

Askar, A. and Treptow, H. 1993. Quality assurance in tropical fruit processing. Berlin, Springer Verlag; pp.10–13.

Atia, M.M.M. 2005. Biological and chemical control of potato late blight. Annals Agric. Sci. Moshtohor, 43 (4): 1401-1421

Atia, M. M.M. and Ahmed, A.M. 2011. Controlling of tomato early blight disease using some of biotic and a biotic agent. J. Plant Prot. and Pathology, Mansoura Univ., 2 (4): 481-500.

Ayeni, L.S.; Omole, T.O.; Adeleye, E.O. and Ojeniyi, S.O. 2010. Integrated application of poultry manure and NPK fertilizer on performance of tomato in derived savannah transition zone of South West Nigeria. Nat. and Sci., 8 (2): 50 - 54.

Baba, Z.A.; Tahir, S.; Wani, F.S.; Hamidi, B.; Nazir, M. and Hamid, B. 2018. Impact of *Azotobacter* and inorganic fertilizers on yield attributes of tomato. Int. J. Curr. Microbiol. App. Sci., 7(2): 3803-3809.

Badawy, H.M.A. and Dib, N.D. 2012. Chemical control of tomato early blight disease. Egypt. J. Phytopathol., 40(2): 149-162.

Barnett, H.L. and Hunter, B.B. 2003. Illustrated Genera of Imperfect Fungi. American Phytopathological Society Press, St. Paul, Minnesota, USA, pp. 241.

Basamma, R.H. and Shripad, K. 2017. Bio-efficacy of *Bacillus subtilis* against foliar fungal disease of tomato. International Journal of Applied and Pure Science and Agriculture (IJAPSA), 2(2): 220-227.

Brar, N.S.; Thakur, K.S.; Kumar, R.; Mehta, D.K.; Sharma, N.; Kumar, D. and Kumar, S. 2015. Effect of organic manures and biofertilizers on fruit yield and its contributing trials of tomato (*Solanum lycopersicum* L.) Eco. Env. & Cons. 21(4): 1783-1787.

Brain, P.W. and Hemming, H.G. 1945. Gliotoxin a fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol., 32: 214-220.

Camlica, E. and Tozlu, E. 2019. Biological control of *Alternaria solani* in tomato. Fresenius Environmental Bulletin, 28(10): 7092-7100.

Chapman, H.D. and Pratt, P.F. 1961. Methods of Analysis of Soil, Plant and Water. Calif. Univ. Division of Agric. Sci., pp. 172-173.

Chowdappa, P.; Kumar, S.P.M.; Lakshmi, M.J. and Upreti, K.K. 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biological Control, 65(1): 109–117.

Dart, P.J.; Day, J.M. and Harris, D. 1972. Assay of nitrogenase by acetylene reduction. From Technical Report No. 149 from FAO / IAEA. Use of isotopes for study of fertilizer utilization by legume crops, pp. 85-100.

Dhingra, O.D. and Sinclair, J.B. 1995. Basic Plant Pathology Methods, 2nd ed., CRC Press Inc., pp.134-139.

Difco Manual 1985. Dehydrated Culture Media and Reagents for Microbiology, Tenth Edition Hardcover – USA, pp. 487- 623.

Dobereiner, J.; Marria, l.L.E. and Nery, M. 1976. Ecological distribution of *Spirillum lipoferum* Beijerink. Can. J. Microbiol., 22: 1464-1473.

Eid, M.A. 1978 Studies on the free N_2 – fixing bacteria in the rhizosphere of sugarcane. M. Sc. Thesis Fac. Agric., Cairo Univ.

El-Farnawany, M.A. 2006. Effect of seed treatment with *Trichoderma harzianum* on reducing tomato early blight incidence. Assiut J. Agric. Scie., 37(1): 201-216.

El-Fiki, I.A.L. 2017. Evaluation of some fungi toxicants for controlling tomato early blight disease. Journal of Phytopathology and Pest Management, 4(2):38-52.

Elkahoui, S.; Djébali, N.; Tabbene, O.; Hadjbrahim, A.; Mnasri, B.; Mhamdi, R.; Shaaban, M. and Limam, F. 2012. Evaluation of antifungal activity from *Bacillus* strains against *Rhizoctonia solani*. African Journal of Biotechnology, 11(18): 4196- 4201.

El-Tanany, M.M.; Hafez, M.A.; Ahmed, G.A. and Abd El-Mageed, M.H. 2018. Efficiency of biotic and abiotic inducers for controlling tomato early blight disease. Middle East J. Agric. Res., 7(2): 650-670.

Fahmi, A.I.; Al-Talhi, A.D. and Hassan, M.M. 2012. Protoplast fusion enhances antagonistic activity in *Trichoderma* spp. Nat. Sci., 10(5): 100–106.

FAO STAT (2017). Global Tomato Production in 2014. FAO, Rome.

George, G.; Brenner, D.J.; Krieg, N.R. and James, R.S. 2005. Staley Phylum XXVIII, 1106. Williams and Wilkins, 1984. In: Bergey's Manual of Systematic

Bacteriology 2nd. Vol. 2. Eds. George, Garrity, Don, J. Brenner; Noel, R. Krieg and James, R. Staley. The Proteo-bacteria (Part B 384-402. and C7-82.). (Springer-Verlag) Dordrecht Heidelberg, London, New York. pp. 1203.

Gomez, K.A. and Gomez, A.A 1984. Statistical Procedures for Agricultura Research. 2nd Ed. John Willey and sons. New York. pp. 680.

Gothandapani, S.; Sekar, S. and Padaria J.C. 2017. *Azotobacter chroococcum:* Utilization and potential use for agricultural crop production: An overview. Int. J. Adv. Res. Biol. Sci., 4(3):35-42.

Hafez, S.K.; El-Beik, A.K.; El-Tantawy, E.M. and El-Kassas, A.I. 2016. Effect of organic and bio- fertilizer source on summer squash under El- Arish conditions. Sinai Journal of Applied Sciences, 5(2): 155- 172.

Hawker, L.E. 1960. Physiology of Fungi. University of London Press, LTD. Warwick Square, London, pp. 1950.

Imran, M.; Abo-Elyousr, K.A.M.; Mousa, M.A. and Saad, M.M. 2022. Screening and biocontrol evaluation of indigenous native *Trichoderma* spp. against early blight disease and their field assessment to alleviate natural infection. Egypt. J. Biol. Pest Control, 32:40

Jackson, M.L. 1973. Soil Chemical Analysis (2nd Edition). Prentice Hall of India, New Delhi, chapt, 8:183- 204.

Jensen, H.L. 1930. The genus *Micromonospora orskov*, a little- known group of soil microorganisms. Proc. Linne on Soc. News. Wales. 40: 231- 248.

Khalil, M.E. and Adbelghany, R.E.A. 2021. Effectiveness of some biotic and abiotic agents to control tomato early blight disease caused by *Alternaria solani*. Egypt. J. Phytopathol., 49(1): 114-128.

Kochba, J.; Lavee, S. and Spiege- Roy, P. 1977. Difference in peroxidase activity and isozymes in embryogenic and non-embryogenic 'sharrouti' orange ovular callus lines. Plant and Cell Physiology, 18(46): 3-7.

Kumar, P.K.R.; Hemanth, G.; Niharika, P.S. and kolli, S.k. 2015. Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam District. IJAPBC, 4(2): 484- 490.

Le, T.A.; Pek, Z.; Takacs, S.; Nemenyi, A.; Daood, H.G. and Helyes, L. 2018. The effect of plant growth promoting rhizobacteria on the water-yield relationship and carotenoid production of processing tomatoes. Hort. Science, 53(6): 816–822.

Lisker, N.; Cohen, L.; Chaluz, E. and Fuchs, Y. 1983. Fungi infection suppress ethylene-induced phenylalanine ammonia-lyase activity in grapefruit. Physiological Plant Pathology. 22(3):331-338.

Mayo, S.; Gutiérrez, S.; Malmierca, M.G.; Lorenzana, A.; Campelo, M.P.; Hermosa, R. and Casquero, A. 2015. Influence of *Rhizoctonia solani* and *Trichoderma* spp. in growth of bean (*Phaseolus vulgaris* L.) and in the induction of plant defense-related genes. Front. Plant Sci., 6 (685):1-11.

Mazrou, Y.S.A.; Makhlouf, A.H.; Elseehy, M.M.; Awad, M.F. and Hassan, M.M. 2020. Antagonistic activity and molecular characterization of biological control agent *Trichoderma harzianum* from Saudi Arabia. Egypt. J. Biol. Pest Control, 30(4): 1-8.

Morsy, E.M.; Abdel-Kawi, KA. and Khalil, M.N.A. 2009. Efficiency of *Trichoderma viride* and *Bacillus subtilis* as biocontrol agents against *Fusarium solani* on tomato plants. Egypt. J. Phytopathol., 47(1): 47-57.

Meena, M.L.; Gehlot, V.S.; Meena, D.C.; Kishor, S.; Kishor, S.; Kumar, S. and Meena, J.K. 2017. Impact of biofertilizers on growth, yield and quality of tomato (*Lycopersicon esculentum* Mill.) cv. Pusa Sheetal. J. Pharmacogn. Phytochem. 6(4): 1579-1583.

Mohamed. A.A.; Salah, M.M.; Zen El- Dein, M.M.; El- Hefny, M.; Ali, H.M.; Al Farraj, D.A.; Hatamleh, A.A.; Salem, M.Z.M. and Ashmawy, N.A. 2021. Ecofriendly Bioagents, *Parthenocissus quinquefolia*, and *Plectranthus neochilus* Extracts to Control the Early Blight Pathogen (*Alternaria solani*) in Tomato. Agronomy, 11: 911.

MSTAT-C. 1991. A software program for the design, management and analysis of agronomic research experiments. Michigan State University, East Lansing.

Naik, M.K.; Prasad, Y.; Bhat, K.V. and Rani, G.S. 2010. Morphological, physiological, pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. Indian Phytopathol., 63(2): 168-173.

Nosheen, S.; Ajmal, I. and Song, Y. 2021. Microbes as biofertilizers, a potential approach for sustainable crop production. Sustainability, 13, 1868.

Pandey, K.K.; Pandey, P.K; Kalloo, G. and Banerjee, M.K. 2003. Resistance to early blight of tomato with respect to various parameters of disease epidemics. J. Gen. Plant Pathol., 69: 364–371.

Piper, C.S. 1950. Soil and Plant Analysis, The University of Adelaide Press, Adelaide, Australia, pp. 368.

Pramer, D. and Schmidt, E.L. 1965. Experimental Soil Microbiology. Minneapolis: Burgess Publ. Co., pp. 107.

Reinhold, B.; Hurek, T. and Fendrik, I. 1985 Strain- specific chemotaxis of *Azospirollum* spp. J. Bacteriol., 162: 190- 195.

Sarhan, E.A.D. 2018. Induction of induced systemic resistance in fodder beet (*Beta vulgaris* L.) to cercospora leaf spot caused by (*Cercospora beticola* Sacc.). Egypt. J. Phytopathol., 46(2): 39-59.

Shahidi, F. and Naczk, M. 1995. Food Phenolics: Sources, Chemistry, Effects and Applications. Technomic Publishing Co., Lancaster, pp. 331.

Snell, F.D. and Snell, C.T. 1953. Colorimetric Methods of Analysis, including Some Turbidimetric and Nephelometric Methods. Volume III, D. Van Nostrand Co., Inc., Princeton, Jersey, Toronto, New York and London, pp. 606.

Tiyagi, S.A.; Ansari, S.; Rizvi, R.; Mahmood, I. and Khan, Z. 2015. Evaluation of organic matter, bio-inoculants and inorganic fertilizers on growth and yield attributes of tomato with respect to the management of plant-parasitic nematodes. Emir. J. Food Agric., 27(8): 602-609.

Trivedy, R. K. and Goel, P. K. 1986. Chemical and biological method for water pollution studies. Environmental Publication Karad, India, 6: 10-12.

Vincent, J. M. 1947. Distortion of fungal hyphae in presence of certain inhibitors. Nature, 159: 850.

Woudenberg, J.H.C.; Truter, M.; Groenewal, d.J.Z. and Crous, P.W. 2014. Large spared *Alternaria* pathogens in section Porri disentangled. Stud. Mycol., 79:1-47.

Yagmur, B. and Gunes, A. 2021. Evaluation of the effects of plant growth promoting rhizobacteria (PGPR) on yield and quality parameters of tomato plants in organic agriculture by principal component analysis (PCA). Gesunde Pflanzen 73: 219-228.

Zhang, H.; Gao, Z.; Shi, M. and Fang, S. 2020. Soil bacterial diversity and its relationship with soil CO_2 and mineral composition: A case study of the Ihaiwu Experimental Site. Int. J. Environ. Res. Public Health, 17(16): 5699.