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Protective Action of Some Bio-Pesticides against Early Blight Disease Caused By *Alternaria Solani* In Tomato Plant

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Abstract

A field experiment was conducted in the experimental farm station of Botany and Microbiology Department, Faculty of Science, Al-Azhar University to investigate the efficient of some bio pesticides (cinnamon extract, cyanobacteria and plant growth promoting rhizobacteria) against early blight disease of tomato plant (Solanum lycopersicum) caused by the necrotrophic fungus *Alternaria solani*. Disease symptoms, disease index percent, morphological parameters, anatomical changes, photosynthetic pigments and phytochemicals indicators as response to induction of systemic resistance (SR) in tomato plants were recorded. The results demonstrated that *Alternaria solani* challenged plants treated with PGPR as well as cyanobacteria which showed the highest significant reduction in percent disease index (PDI) with 32.5 and 35 %, followed by treatment with cinnamon water extract showed (42.5 %). Significant improvements in all tested morphological parameters as well as histological changes of tomato plants were obtained due to use of the tested elicitors especially PGPR and cyanobacteria, respectively than control infected plants. The upper and lower epidermis were very compacted and smaller cells as well as appearance abnormal growth in lower epidermis cells, compared to healthy ones. Application of applied elicitors alleviated these challenging abnormalities in varied degrees, where the most potent treatment was PGPR followed by *cyanobacteria* and *Cinnamon extract* respectively, Inoculation of PGPR (mixture of two bacterial strains namely *Bacillus subtilis*, *Serratia marcescens*) caused highly significant increases in chlorophyll A, chlorophyll B and total chlorophyll A + B as well as carotenoids. The beneficial effects of the used treatments were extended to increase not only total phenol, total soluble protein content and carbohydrates but also the activities of Superoxide dismutase, peroxidase and polyphenoloxidase enzymes in comparison with control.

Key words: Tomato- – *Alternaria solani* – Bio-pesticides- Plant growth promoting rhizobacteria - Cyanobacteria- *Cinnamon extract*, *S. marcescens*, systemic resistance.

Introduction:

Tomato is the most important vegetable crop in Egypt. Some 186.000 ha is cultivated with this crop and annual production amounts to seven million tons, which are consumed either fresh or processed (**Ramadan, *et al*.,2008** and **Abd-El Kareem *et al*., 2006**). There are many pests and diseases damaging both the quality and quantity of tomato production. Several investigators recorded that diseases found on

tomato plants are considered of the most destructive and causes considerable losses in its yield which estimated at over 50%. Early blight of tomato caused by the necrotrophic fungus *Alternaria solani* is one of the most common diseases of tomatoes. The early blight disease causes a severe reduction in yield and high economic losses every growing season (**Waqas *et al*., 2016 , Abada *et al* 2008 and Abd-El-Khair and Haggag . 2007**). Soil borne diseases are controlled by chemical fungicides, long crop rotation, pasteurization of seedbeds with steam or fumigants and by breeding resistant tomato cultivars (**Spletzer and Enyedi, 1999**). The biocontrol of plant pathogens is currently regarded as a key practice in sustainable agriculture be-cause it exploits a natural resource (Yu **and Zheng 2006**). Bio-Pesticides can be classified as either microbial or biochemical, based on the active ingredient. Microbial pesticides include live organisms (e.g., beneficial bacteria, fungi, nematodes, and viruses) and/or their fermentation products as the active ingredient. Biochemical pes-ticides include plant extracts, pheromones, plant hormones, natural plant-derived regulators, clay, potassium bicarbonate, and enzymes as the active ingredient. **(Prasad and Naik, 2003).** A number of plant species have been reported to possess natural substances that are toxic to several plant pathogenic fungi (**Goussous *et al.* 2010**). The induction of plant resistance using non-pathogenic or incompatible microorganisms is also a form of biological control (**Schouten *et al.*, 2004**). Plant treatments with various biotic and abiotic agents can induce resistance against subsequent pathogen attack (**Walters *et al*., 2005**). **Adesemoye and Kloepper (2009)** compiled the benefits derivable from plant–PGPR interactions to include the following: improvements in seed germination rate, root development, shoot and root weights, yield, leaf area, chlorophyll content, hydraulic activity, protein content, and nutrient uptake—including phosphorus and nitrogen. Nature is bestowed with many biocontrol agents including plant growth promoting microorganisms (PGPM) could regulate plant growth by inducing defense responses in plants via a systemic resistance (ISR) and/or a systemic acquired resistance(SAR) (**Akkopru & Demir 2005 and Siddiqui 2006**).

This investigation aimed to study the positive performance of biological agents against early blight of tomato caused by the necrotrophic fungus *Alternaria solani*, which considered among the most difficult crop diseases to control.

Furthermore, to evaluate the effect of tested bio Pesticides as alternative and safety method in Integrated Management programs to management the early blight disease.

Materials and Methods:

1. Plant material: For the present investigation, Uniform Four Weeks-Tomato seedlings (*Solanum Lycopersicon* L. cv. Castle rock II PVP) were obtained from agricultural research center (ARC), ministry of agriculture, Giza, Egypt.

2. Isolation and maintenance of pathogen (*A.solani*): Alternaria solani was isolated according to (**Katan *et al*., 1991**). Tomato infected plant samples such as leaves, fruits and stems with typical disease symptoms were collected from different localities 2016 cropping season. For fungus isolation, small segments of diseased tissue along with some healthy portion (5 × 5 mm2) were cut by sterilized razor and surface sterilized in 2% Sodium hypochlorite (NaOCl) for 2 minutes. Surface sterilized plant tissue were rinsed by sterilized distilled water for removing the last trace of Sodium hypochlorite solution, dried on filter paper and placed Petri plates containing 15 ml of potato dextrose agar medium (PDA). Three to four pieces of sterilized tissue were placed in each Petri plate and incubated for 7 days at 25±2°C in incubator. The composition of potato dextrose agar medium used was as described **in Samson *et al*. (2002)**. And identified Morphological macroscopic and microscopic according to (Ellis 1976), the isolated fungus was maintained on PDA at 20°C for 7 days. To induce sporulation, cultures were transferred on 23-25°C for 6 days on PDA. Conidial suspensions were prepared as described in **Boedo *et al*. (2012)**. Spore density was counted by a hemocytometer and adjusted to 10^6 spores per mL.

3. Source and application methods of bio-pesticides:

For preparing plant Cinnamon water extract, dried leaves of plant obtained from Agriculture Research Centre (ARC) Giza Egypt. The dry leaves of plant crushed into powder. 0.5 gm of the powder was put in 50ºC boiled water and left for 1 hr then filtered into a conical flask. The aqueous infusion was sterilized by bacterial filter. An equivalent of 10 mg dried material per ml of aqueous infusion was obtained (**Adebolu and Oladimeji, 2007**). **Cyanobacteria** which contain mixture two strains of ***(****Nostoc muscorum, Anabaena oryzae****)*** were kindly provided by Microbiology Department;

Soils, Water and Environment Research Institute, Agricultural Research, Center (ARC). The inocula suspensions were approximately adjusted to 10^9 CFU/ml culture (colony formation unit). **Plant growth promoting rhizobacteria (*PGPR*) mixture of two bacterial strains namely** *Bacillus subtilis*, *Serratia marcescens*. Bacterial inocula were kindly provided by Biofertilizer production unit, Soil, Water and Environment Research institute, Agricultural Research Center (ARC), Giza, Egypt. The concentration of *B. subtilis* in suspension was counted by most probable number (MPN). *S. marcescens* strain isolated from Egyptian soils. The inocula suspensions were approximately adjusted to 10^9 CFU/ml culture (colony forming unit).

4. Field experiment:

The field trials were conducted at the Experimental garden of Faculty of Science, Al Azhar University Egypt in 2016 growing season. seedlings were planted in 4 groups each group were planted in 4 lines. After one week of planting, two lines of each treatment were infected with *A. solani* (4 mL/plant containing spore 10^6 per mL) consequently we have 8 treatments as following; :(1) plants without any treatments were referred as healthy control, (2) plants infected with *A. solani* as infected control, (3) plants treated with cinnamon plant extract (foliar treatment every 15 days), (4) plants treated with cinnamon plant extract (foliar treatment every 15 days), and infected with *A. solani* ,(5) plants treated with cyanobacteria,(6) plants treated with cyanobacteria and infected with *A. solani* (7) plants treated with PGPR (4 mL/plant containing 10^9 cfu/mL),(8) plants treated with PGPR (4 mL/plant containing 10^9 cfu/mL) and infected with *A. solani* . Disease development was recorded 15 days after inoculation. Disease index was recorded. The plant samples were collected for morphological, histological and biochemical indicators for resistance analysis when the plants were 32 days old (Stage I) and 47 days old (Stage II).

5-Disease symptoms and disease index: Disease symptoms were assessed 15 days after inoculation and the disease index was evaluated according to (**leath *et al.*, 1989**) with slight modifications using score consisting of five classes: 0(no symptoms), 1(slight yellow of lower leaves), 2(moderate yellow plant), 3(yellow halo around Browne spots), 4(concentric rings of raised and depressed dead tissue). `Disease index (DI) was calculated using the five-grade scale according to the formula: DI= (1n1+ 2n2 + 3n3 + 4n4)100/4nt. Where n1-n4 the number of plants in the indicated classes, and Nt total number of plants tested. Percent protection were calculated using

following formula: Protection % = A–B/A × 100% Where, A = PDI in non-treated control infected plants B = PDI in -treated plants.

6-Determination of growth parameters: Samples of tomato plants from each healthy and infected with *A.solani* treated with tested bio-pesticide were collected at the vegetative stages (32 and 47 days old plants) to determine growth characteristics (shoot and root length (cm) and number of leaflets per plant.

7- Histological changes*:* The effect of ***A.solani*** and bio-pesticides on anatomical structure of tomato leaves were studied according to **Corgan and Widmoyer (1971).**

8- Determination of pigments: The method used for the quantitative determination of pigments was that of **Vernon and Selly (1966)**.. **Total soluble proteins (**mg/100g of dry wt) were determined **according** to the method of **(Lowery *et al.*, 1951)** using casein as a standard protein. Contents of total soluble carbohydrates were determined using anthrone technique according to **(Umbriet *et al.*, 1969)**. **Determination of phenolic compounds** (mg/100g of dry wt) was carried out according to that method described by Daniel and **George (1972) Superoxide dismutase (SOD)** activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by **Marklund and Marklund (1974). Peroxidase activity** determined according to the method adopted by (**Srivastava 1987**). **The activity of polyphenol oxidase** enzyme was determined according to the method adopted by **Matta and Dimond (1963**).

9- Statistical analyses.

Experimental data were subjected to one-way analysis of variance (ANOVA) and the differences between means were separated using Duncans multiple rang test and the (L.S.D) at 5% level of probability using Co-state software **(Snedecor and Cochran, 1982)**.

3-Results:

Identification of causal pathogen:

fungus isolate was obtained from infected tomato leaves and fruits showing blight symptoms and identified as *A. solani*, a based on the morphological characteristics. Conidiophore as short irregularly branched. Conidia showing as spectating in both laterally and longitudinally, with up to six transverse and two to three longitudinal or oblique septa, clavate shape overall, tapering towards the

apices, forming a short beak, in culture usually 20–40 µm, with walls smooth to conspicuously rough.

percent disease incidence (PDI) and Protection%: Data presented in table (1) showed that application of all tested elicitors reduced significantly percent disease incidence (PDI) caused by Alternaria solani compared to untreated infected control plants. However, data showed that infection percent reached 70 % in untreated infected control plants. PGPR and Cyanobacteria were the best treatments and reduced percent disease indexes by 32.5 and 35 % respectively and came next treatment with cinnamon extract which recorded 42.5.

Table (1) Effect of tested Biopesticides on Percent disease incidence (PDI) caused by Alternaria solani .

Treatment	Classes					PDI %	Protection (%)
	0	1	2	3			
A. solani	0	2	2	2	4	70	0
Cinnamon + A. solani	2	3	2	2	1	42.5	39.28
Cyanobacteria + A. solani	3	3	2	1	1	35	50
PGPR + A. solani	3	4	1	1	1	32.5	53.5

Disease index (DI) was calculated using the five-grade scale according to the formula: DI= (1n1+ 2n2 + 3n3 + 4n4)100/4nt. Where n1-n4 the number of plants in the indicated classes, and Nt total number of plants tested. Percent protection were calculated using following formula: Protection % = A–B/A × 100% Where, A = PDI in non-treated control infected plants B = PDI in -treated plants.

Figure 2. Early blight symptoms on tomato leaf and fruits showed slight yellow of lower leaves, moderate yellow plant, Browne spots, angular spots, displaying the typical concentric rings and a yellow halo. concentric rings of raised and depressed dead tissue.

Growth characters:

Results in table (2) revealed that all growth characters in fungal -infected tomato plants were significantly decreased than that of non-infected ones (healthy plants) On the other hand, treatment with tested bio-pesticides resulted in different responses as regards the lengths of shoot and root as well as number of leaf of fungal-infected plants. These responses were varied according to the type of used bio-pesticides as follows: Application of Cinnamon extract resulted in increase in the shoot lengths of infected and non-infected plants. These observed increased were found to be statically significant especially at the second stages of growth. Treatment with cyanobacteria significantly increased the lengths of shoots of tomato plants. This was the case in infected and non-infected plants. Also, treatment with PGPR significantly increased the lengths of shoots of tomato plants. This was the case in infected and non-infected plants. Regarding the treatment with cyanobacteria, insignificant changes were recorded in root lengths throughout the two stages of growth. This was the case in fungal-infected and non-infected plants. On the other hand, different responses were observed as regards the root lengths due to the treatment with PGPR. Moreover, foliar treatment with cinnamon extract significantly increased root lengths of infected plants. This was the case throughout the two stages of growth. Concerning the effect foliar application of Cinnamon

extract and inoculation cyanobacteria or PGPR on the challenged plants with **Alternaria solani**, it was noticed that PGPR gave significant increase in tomato plants root lengths in comparison with cyanobacteria, followed by cinnamon-Alternaria infected tomato plants. Regarding the effect of different used Bio-pesticide, the obtained results revealed that the numbers of leaflets/plant were significantly increased in tomato-infected plants due to the treatment with different elicitors (PGPR, Cyanobacteria and cinnamon extract) respectively.

Table (2) Effect of tested Bio-pesticides on Shoot, root lengths and number of leaflets (per plant) in healthy and infected Tomato plants with *Alternaria solani* under field conditions.

Treatments	Shoot length (cm/plant)		Root length (cm/plant)		Number of leaflets (per plant)	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control	41.6 d	70.6 bc	19.6 bc	27.6 bc	125 d	244.3 c
A. solani	29.3 e	40 e	16.3 c	15.3 d	70 f	91.3 f
Cinnamon	48.3 b	67.6 c	18.6 c	28 bc	132 d	198.3 e
Cinnamon + A. solani	40 d	58 d	24 a	26 c	87 e	205 de
Cyanobacteria	57.6 a	76.3 b	27.3 a	31 b	165 b	216.6 de
Cyanobacteria + A. solani	54 a	66 c	23.3 ab	27 bc	151 c	206.6 de
PGPR	47 bc	90 a	25 a	36.6 a	211.3 a	283 a
PGPR + A. solani	44 cd	66.3 c	25.6 a	31 b	207 a	260 b
LSD at %5	4.25	6.2	4.15	4.4	125 d	244.3 c

Control(H.): untreated tomato plant **A. solani**: *Alternaria Solani.* **Cinnamon**: cinnamon plant extract**. PGPR**: mixture *Bacillus subtilis* &*Serratia marcescens.* **Cyanobacteria**: mixture of ***(****Nostoc muscorum & Anabaena oryzae****)***. **LSD**: Least Significant Difference

Histopathological changes:

Histopathological changes in tomato leaves tissues as evidence of the systemic acquired resistant reaction were elicited after 32 days of biotic inducers (bio-pesticide). **A. solani** -infected tomato leaf was revealed the destroyed so mesophyll, parenchyma, epidermis and vascular bundles (fig 2-B). The upper and lower epidermis were very compacted and smaller cells as well as appearance abnormal growth in lower epidermis cells, compared to healthy ones (fig 2- A). abnormal spongy tissue as well as they were not tubular parenchyma cells. The mesophyll cells showed relatively small or without intracellular spaces (fig 2- B). The mesophyll and Palisade tissues are large and have a lower number of cells and lacking chlorenchyma

with thin cell walls (fig 2- B) compared with healthy ones. Excessive growth (hyperplasia) or enlargement (hypertrophy) demonstrated as enlarged parts of lower epidermis, compared with healthy ones.

Application of applied elicitors alleviated these challenging abnormalities in varied degrees, where the most potent treatment was PGPR followed by *cyanobacteria* and *Cinnamon extract* respectively, where the upper epidermis is composed of tubular parenchyma cells. The mesophyll cells (palisade parenchyma) were cylindrical and tightly packed into two or these layers. The spongy parenchyma contains a large into two or three layers. The spongy parenchyma with intercellular spaces (fig 3 A, B & C). Also, Progressive increasing in lignin accumulation in epidermal cells, number of hairs, thickness of blade, number of xylem arms and phloem layers. The thickness and toughness of the outer wall of epidermal cells of infected plants and treated with applied elicitors. also forming necrotic lesions (spots) that are remarkably uniform in size and shape.

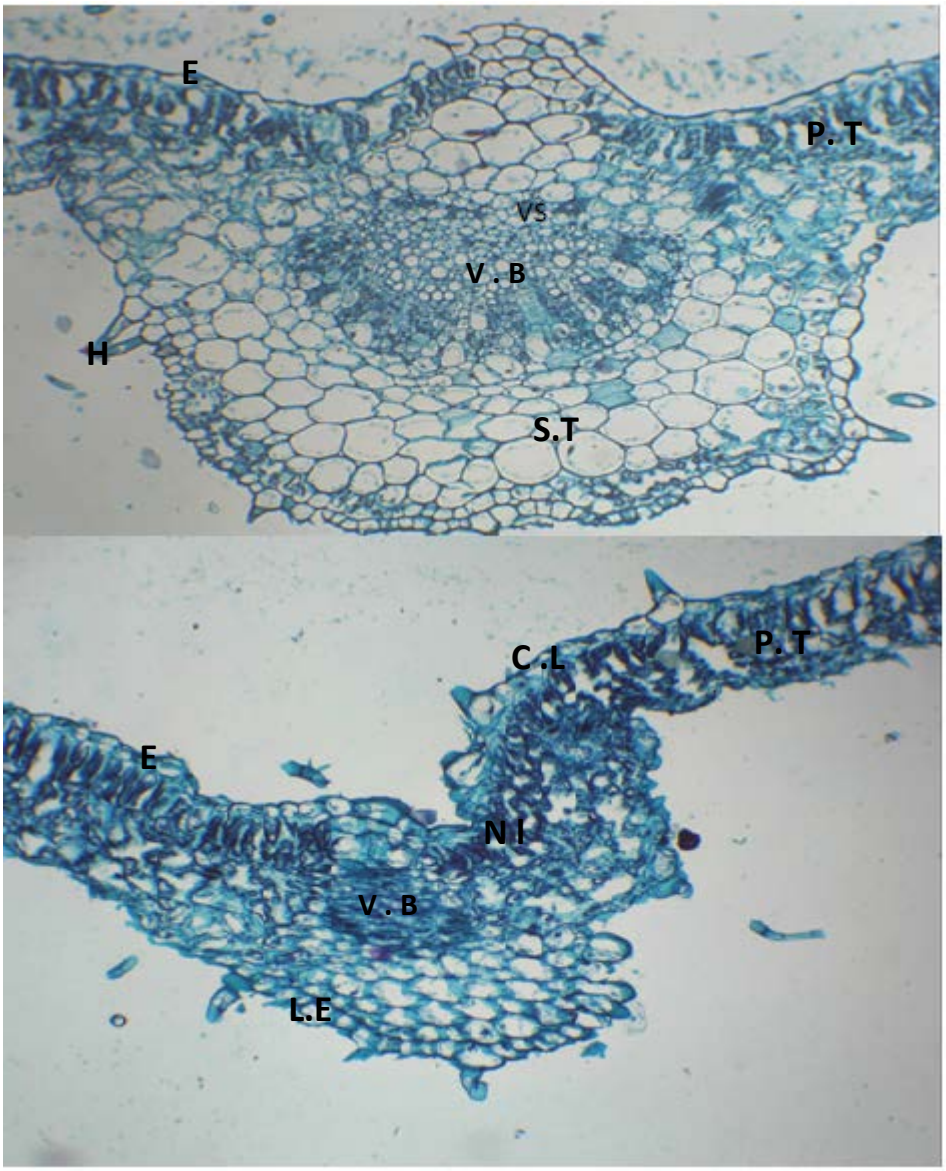


Fig (2). Light micrograph of tomato leaves cross section, (A) healthy Leaf and (B) infected leaf showing different changes in cells and tissues of 32 days post infection. H: hairs; Vp: vascular bundle; Up: upper epidermis; S.T: spongy tissue; Lp: lower epidermis; M: mesophyll; Nl : Necrotic lesions : HT; hypertrophy .

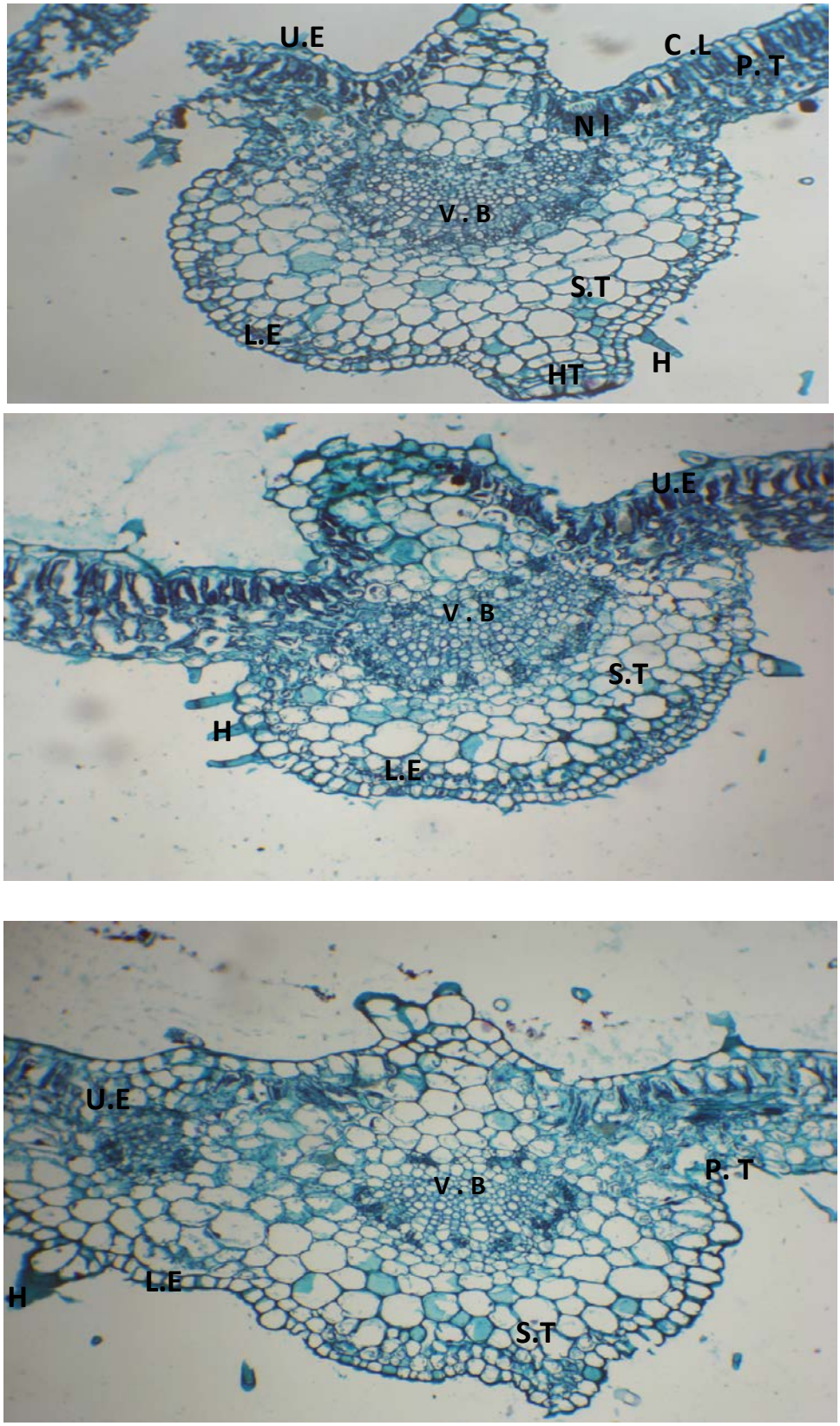


Fig (3). Light micrograph of infected tomato leaves treated with applied elicitors, cross section, (A) Cinnamon extract (B) cyanobacteria and (C) PGPR showing different changes in cells and tissues o 32 days post infection . H: hairs; Vp: vascular bundle; Up: upper epidermis; S: spongy; PT; palisade tissue; Le: lower epidermis; M: mesophyll. Nl : Necrotic lesions : HT; hypertrophy

Photosynthetic pigments:

Results in (table 3) Cleary revealed that contents of chlorophyll a, b as well as total chlorophyll a + b were highly significantly decreased in *Alternaria solani* - infected plants. This was the case throughout the two stages of growth. The plants treated with PGPR (healthy) showed significant increases in the contents of chl. a, b and total a+ b. Also, Alternaria - infected plants treated with PGPR cause significant increase in the contents of chl. a, b and total a+ b. compared with non-treated Alternaria -infected plants ones. Regarding the treatment with cyanobacteria, data revealed that the contents of chl.a, b as well as total a + b all were increased Alternaria -infected plants due to the treatment with cyanobacteria compared with those of control infected ones. The statically analysis of the obtained results showed that most of the aforementioned increases were highly significant. Generally, application of cyanobacteria enhanced the photosynthetic process in Alternaria - infected plants. Also, data obtained in (table 3) Cleary revealed that the contents of chl.a, b as well as total a + b all were increased Alternaria -infected plants due to the treatment with cinnamon extract (foliar treatment) compared with those of control infected ones. Generally, cinnamon treatment enhanced the photosynthetic process in Alternaria - infected plants. Results in table (3) Cleary revealed that, contents of carotenoids were significantly increased in tomato plants in response to Alternaria infection. In tomato, infected plants and pretreated with PGPR contents of carotenoids were decreased when being compared with that in Alternaria infected plants but not pretreated with PGPR. This was the case throughout the duration of the experiment. The statically analysis of the obtained results showed that the aforementioned decreases were highly significant. Also, the obtained results illustrated that in both healthy and Alternaria -infected plants, contents of carotenoids were decreased throughout the two stages of growth in response to the treatment with cyanobacteria as well as cinnamon extract. The observed decreases were found to be statistically, mostly, insignificant.

Table (3) Effect of tested Bio-pesticides on chlorophyll (a), chlorophyll (b), total chlorophyll (a+b) and carotenoids of tomato plants, infected with **Alternaria solani** under field conditions.

Treatments	chlorophyll(a) mg/g F.wt		chlorophyll(b) mg/g F.wt		Total chlorophyll (a+b) mg/g F.wt		Carotenoids mg/g F.wt	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control (H)	**11 b**	**16.4 b**	**5.2 ab**	**6.2 ab**	**16.28 b**	**22.75 b**	3.45 bc	3.89bcd
A. solani	**2.5 d**	**3.8 d**	**1.19 d**	**1.4 d**	**3.78 e**	**5.2 e**	4ab	5.1ab
Cinnamon	**7.6 c**	**11.4 c**	**4.3 b**	**5.1 b**	**11.97 c**	**16.59.c**	5 a	6.1 a
Cinnamon + A. solani	**3.3 d**	**5 d**	**2.4 cd**	**2.9 cd**	**5.77de**	**7.9de**	3.5 b	4.3 abc
Cyanobacteria	**9.9 b**	**14.8 b**	**3.9 bc**	**4.7 bc**	**13.89 bc**	**19.5 bc**	3.5 b	4.1 bc
Cyanobacteria + A. solani	**4.2 d**	**6.3 d**	**2.5 cd**	**3 cd**	**6.79d**	**9.4d**	3.3bc	4.1 bc
PGPR	**13.5 a**	**20.1 a**	**6.78 a**	**8.1 a**	**20.3a**	**28.3a**	3 bc	3.1 cd
PGPR + A. solani	**9.7 b**	**14.5 b**	**5.17 ab**	**6.2 ab**	**14.9 b**	**20.7b**	2 c	2.1 d
LSD at %5	**2.04**	**3.04**	**1.64**	**1.97**	**2.4**	**3.34**	**1.38**	**1.9**

Control(H.): untreated tomato plant **A. solani**: *Alternaria Solani.* **Cinnamon**: cinnamon plant extract**. PGPR**: mixture *Bacillus subtilis &Serratia marcescens.* **Cyanobacteria**: mixture of ***(****Nostoc muscorum & Anabaena oryzae****)***. **LSD**: Least Significant Difference

Total phenols:

Data generated in table (4) showed that, total phenols of shoot A. **solani** -infected tomato plants were significantly increased than that of non-infected ones (healthy plants). On the other hand, treatment with tested bio-pesticides resulted in different responses. This was the case throughout the different stages of growth. Application of cinnamon extract on infected plants showed significant increase in total phenols shoots during two stages of growth. contents of total phenols in shoots of A. **solani** -infected plants were increased due to the cyanobacteria treatment. Also, the obtained results illustrated that in both healthy and A. **solani** -infected plants, contents of phenols were increased throughout the two stages of growth in response to the treatment with PGPR. The observed increases were found to be statistically, mostly, significant. Concerning the effect foliar application of Cinnamon extract and inoculation cyanobacteria or PGPR on the challenged plants with ***Alternaria solani***, it

was noticed that it was found that PGPR & cyanobacteria show significant increase in total phenols of tomato shoots related to cinnamon extract, respectively during two stages of growth.

Table (4) Effect of Alternaria solani infection and induced resistance elicitors on Phenolic, Total soluble protein content and total soluble carbohydrate Roots in Tomato plants.

Treatments	Phenolic mg/100g d.wt		Protein mg/g d.wt		Carbohydrate mg/g d.wt	
	Stage I	Stage II	Stage I	Stage II	Stage I	Stage II
Control (H)	0.54 d	1.1 c	24.01 de	31.08 de	34.5 c	38.68 c
A. solani	0.98 b	1.12 c	11.9 f	23.56 ef	19.3 d	21.74 e
Cinnamon	0.79 c	0.91 d	21.22 e	20.38 f	22.68 d	43.3 b
Cinnamon + *A. solani*	0.85 c	1.34 a	12.81 f	22.37 f	21 d	22.9 de
Cyanobacteria	0.95 b	1.10 c	35.42 a	76.8 a	43.54 b	56.9 a
Cyanobacteria + *A. solani*	1.22 a	1.39 a	28.92 bc	41.06 b	36.86 c	25.24 de
PGPR	1.26 a	1.21 bc	31.96 ab	59.03 b	64.4 a	22.9 de
PGPR + *A. solani*	1.27 a	1.28 ab	26.99 cd	37.8 cd	35.93 c	46.76 b
LSD at %5	0.092	0.116	4.1	8.6	6.27	4.03

Control(H.): untreated tomato plant **A. solani**: *Alternaria Solani.* **Cinnamon**: cinnamon plant extract**. PGPR**: mixture *Bacillus subtilis &Serratia marcescens.* **Cyanobacteria**: mixture of ***(****Nostoc muscorum & Anabaena oryzae****)***. **LSD**: Least Significant Difference.

Total soluble protein contents:

The results in Table (4) showed that, total soluble protein contents in tomato shoots highly significantly decreased due to ***Alternaria solani*** infection through various stages of growth. Application of cyanobacteria and PGPR showed significant increase in total soluble protein contents of shoots, in both healthy and *A.* ***solani*** -infected plants in comparison with ***A. solani*** - infected plants ones. Regarding the effect of foliar treatment by cinnamon extract, it was found that insignificantly increased the total soluble protein contents in shoots of ***A.solani*** infected plants related to ***A.solani*** - infected plants but not treated with cinnamon extract through various stages of growth.

Total soluble carbohydrates:

Data obtained in table (4) reveled that, total soluble carbohydrate contents in tomato plant shoots were significantly decreased due to ***A.solani*** infection during stages of growth. It was found significantly increased in shoots of PGPR plants (either healthy or infected plants). Regarding the treatment with cyanobacteria, data revealed that ***A.solani*** - infected plants, were increased due to the treatment with cyanobacteria. Also, ***A.solani*** - infected plants, were increased due to the treatment with cinnamon extract . These increases were found to be statistically significant. Concerning the effect Cinnamon extract, cyanobacteria and PGPR on the challenged plants with ***A.solani***, it was found that (PGPR) show considerable increase in total carbohydrate contents related to (cyanobacteria and cinnamon), respectively during two stages of growth.

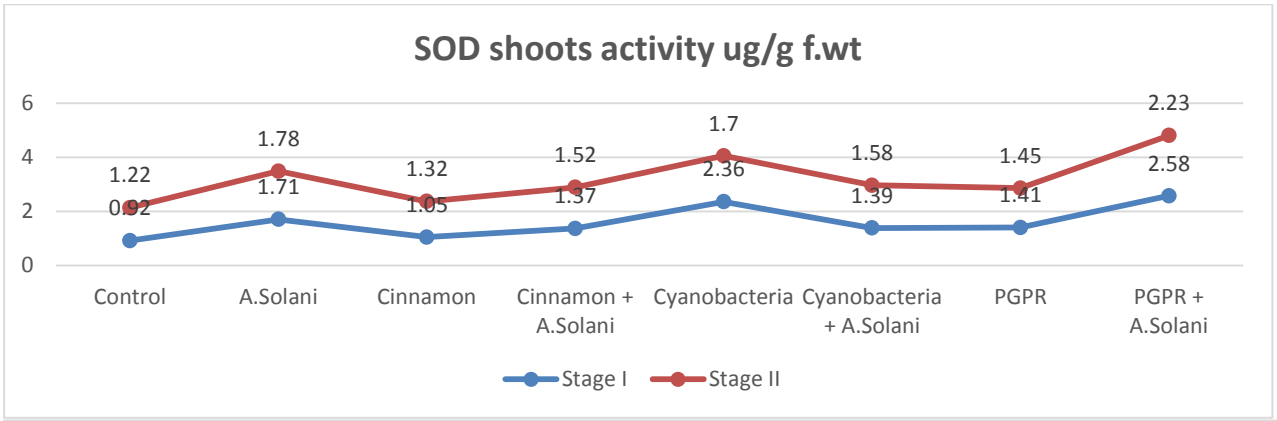
Oxidative enzymes activity.

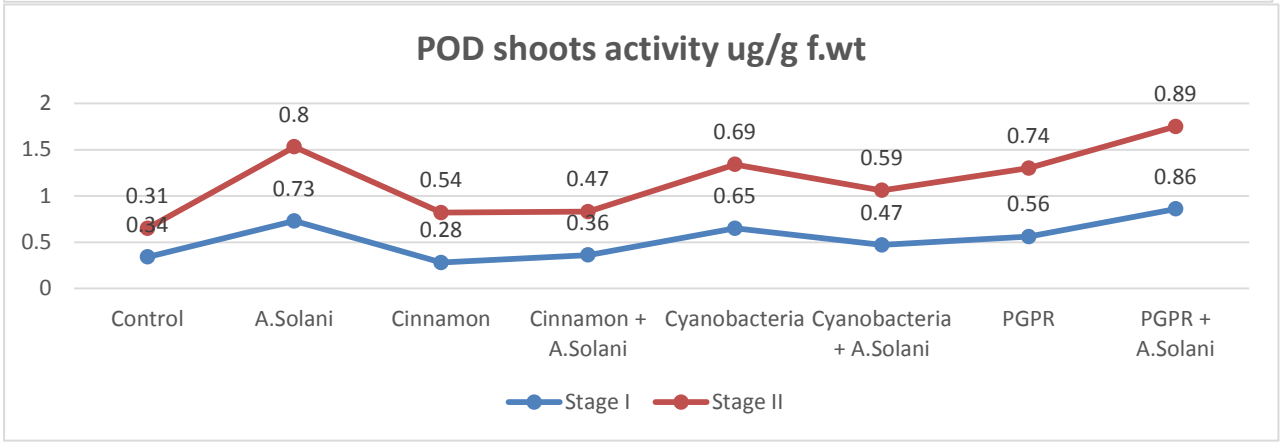
Data generated in fig. (4) Showed the changes in the activities of SOD, POD and PPO enzyme in tomato shoots in response to ***A.solani***, cinnamon extract, cyanobacteria and PGPR at different growth intervals.

The highest activity of **Superoxide dismutase** (SOD) was obtained in tomato plants infected with ***A.solani*** related to healthy untreated tomato plants. Treatment tomato plant with all tested bio pesticide significantly increased the activities of (SOD) enzyme in ***A.solani*** infected plants. It was found that infected tomato plants treated with PGPR gave the highest SOD activity followed by, Cyanobacteria. Also, infected plants and treated with cinnamon plant extract were the least effective and increased SOD activity. This was throughout different growth intervals.

For peroxidase activity, all treatments stimulated POD activity before challenge. This stimulation was observed throughout the two stages of growth, and when compared with that of healthy (non-infected) plants. After challenge with ***A.solani***, all tested bio- pesticide (cinnamon extract, cyanobacteria and PGPR), POD activities were found to be increased than that induced by ***A.solani*** alone. These increases were statistically significant and stilled throughout the duration of the experiment. Also results of the present work (fig 4) revealed that, tomato plants infected with ***A.solani*** gave highly significant increases in PPO activity related to healthy tomato plant. In

A.solani -infected plants, it was found that all inducers showed, mostly, insignificant changes in PPO activity throughout the two stages of growth.





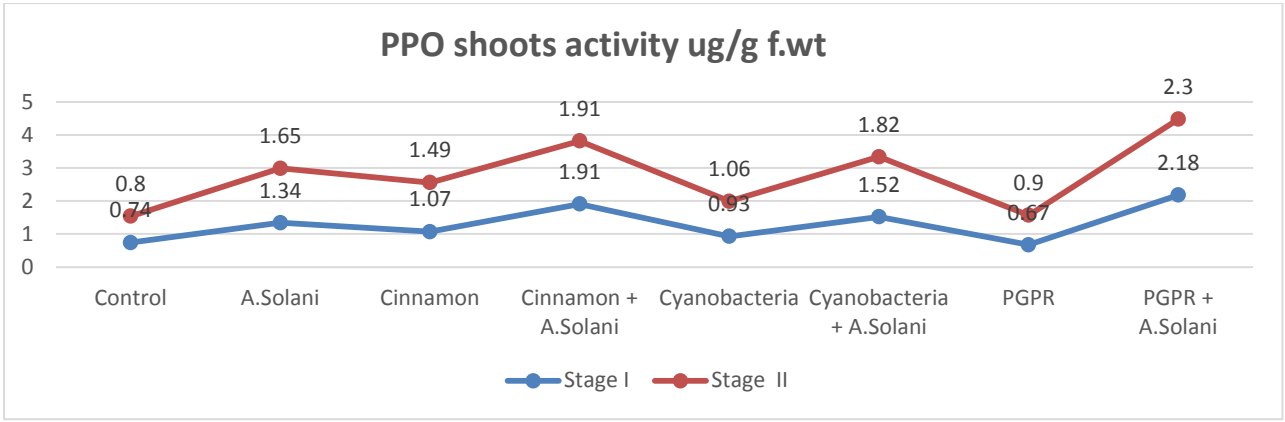


Fig (4): Effect of cinnamon , PGPR and cyanobacteria treatment on (SOD,PO and PPO) activities of tomato shoots at two stages of growth.

Control(H.): untreated tomato plant **A. solani**: *Alternaria Solani*. **Cinnamon**: cinnamon plant extract**. PGPR**: mixture *Bacillus subtilis &Serratia marcescens*. **Cyanobacteria**: mixture of ***(****Nostoc muscorum & Anabaena oryzae****)***. **LSD**: Least Significant Difference

4- Discussion:

It is well known that, resistance to pathogen infection can be enhanced within plants through bio-Pesticides. Bio Pesticides can be classified as either microbial or biochemical, based on the active ingredient. Microbial pesticides include live organisms (e.g., beneficial bacteria, fungi, nematodes, and viruses) and/or their fermentation products as the active ingredient. Biochemical pesticides include plant extracts, pheromones, plant hormones, natural plant-derived regulators, clay, potassium bicarbonate, and enzymes as the active ingredient. **(Prasad and Naik, 2003 &** Siddiqui, 2006**).** The objectives of this study were induction of systemic resistance in Tomato plants against ***A.solani*** infection. The first standard to govern the occurrence of systemic resistance in tomato plants, treatment with tested Bio-pesticides was reduced percentage of disease index; our results similar to **Yeole *et al* 2014** showed application of *Cinnamomum zeylanicum* possessed marginal to excellent antifungal activity. Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly **(Amadioha., 2000**) or induce systemic resistance in host plants resulting in a reduction of the disease development (**Kagale *et al.* 2004**). Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly (**Amadioha 2000**) or induce systemic resistance in host plants resulting in a reduction of the disease development (**Kagale *et al.* 2004**). Our results showed application of PGPR (*B. subtilis* and *S. marcescens)* recorded highly increasing suppress plant disease index as was reported by **Son *et al*., 2014 and Farrag *et al* 2017.** In addition to some species of Bacillus reported to induce systemic resistance in plants against pathogens and antagonists to *Alternaria solani* (**Atia and Amal 2011)**. PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere which indirectly boosts plant growth rate or through production of phytohormones (**Bhardwaj *et al*., 2014**). In this study results Cleary that best treatment recorded highly suppress plant disease index were cyanobacteria (*N. muscorum* and *A. enaoryzae*). Our results similar also to (**Farrag *et al*., 2017 and Hend *et al*., 2012).**These results could demonstrate by **Kiviranta *et al*., 2006** who proved that Cyanobacteria produce biologically active antifungal compounds for controlling plant pathogens.

The results of the present study showed a retarded growth in *Alternaria solani* -infected plants. Plant height, and the number of leaflets /plant were significantly decreased due to *Alternaria solani* infection. In this regards the reduction in all growth parameters development may be correlated with the disturbances in the supply or distribution of the growth regulating hormones **(Bos, 1978).** On the contrary, results of the present work showed that treatment of tomato plants with applied bio-pesticide (cinnamon extract, cyanobacteria and PGPR) respectively, then infected with *Alternaria solani* significantly improved plant growth, as shown by an increase of plant height, and the number of leaflets /plant, as well as increased the yield of tomato compared to the infected control under field conditions. Our results are in accordance with those reported by (**Farrag, *et al* 2017, Sharaf *et al* 2016 and Hend *et al*., 2012)** they reported that application of cyanobacteria on Tomato plant infected with different soil born disease, the plant heights, fresh and dry weight of plants were found to be improved significantly**.** These results could demonstrate by **Kiviranta *et al*., 2006** who proved that Cyanobacteria produce biologically active antifungal compounds for controlling plant pathogens. The observed increase growth parameters of tomato plant (either healthy or infected) might result from the capacity of these bacteria as well as cyanobacteria to form nitrogen-fixing as well as of releasing secondary metabolites, including plant growth regulators; as well as from solubilizing phosphate, so facilitating the uptake of nutrients from the root environment The improvement in the growth parameters of tomato plants following treatment with plant extract was explained by **Avenimelech (1986)** as due to changes in the physical, chemical and biological characteristics of the soil, which in turn increased plant growth and improved productivity.

Anatomical studies in the present work showed that, the upper and lower epidermis of infected leaves were very compacted and smaller cells as well as appearance abnormal growth in lower epidermis cells, than these in healthy ones. Abnormal spongy tissue as well as they were not tubular parenchyma cells. The mesophyll cells showed relatively small or without intracellular spaces. The mesophyll and Palisade tissues are large and have a lower number of cells and lacking chlorenchyma with thin cell walls compared with healthy ones. These results agree with **(Gómez-Rodríguez *et al*., 2003 and Khalil *et al* 2014**). Excessive growth (hyperplasia) or enlargement (hypertrophy) demonstrated as enlarged parts of lower

epidermis, compared with healthy ones. Which explain harmful of Alternaria on plant tissue. infected cells in most diseases are weakened or die, in some diseases, infected cells are induced to divide much faster (hyperplasia) or to enlarge a great deal more (hypertrophy) than normal cells and to produce abnormal amorphous overgrowths (tumors) or abnormal organs (Goethals *et al.,* 2001). The obtained results show many signs of resistance. where the upper epidermis is composed of tubular parenchyma cells. Also, Progressive increasing in lignin accumulation in epidermal cells, number of hairs, thickness of blade, number of xylem arms and phloem layers. **Vance *et al* (1980)** explained that A Lignified cell wall provide effective barrier to hyphal penetration. They also act as impermeable barrier for free movement of nutrient causing starvation of pathogen. The thickness and toughness of the outer wall of epidermal cells of infected plants and treated with applied elicitors. also forming necrotic lesions (spots) that are remarkably uniform in size and shape. These results may be explained by **Riedle-Bauer (2000**) which reported that thickness and toughness of the outer wall of epidermal cells are apparently important factors in the resistance of some plants to certain pathogens. The dead tissues, including the pathogen, are thus delimited by the cork layers and may remain in place, forming necrotic lesions (spots) that are remarkably uniform in size and shape for a host–pathogen combination (Goethals *et al.,* 2001).

Photosynthetic pigments content was positive affected as result to using the tested Bio pesticide before infection. In this study, chlorophyll degradation was produced in *Alternaria solani* infected plants than healthy ones. The decrease in chlorophyll is explained by **Kyselakova *et al.* 2011 and Ali *et al.*, 2006** this decrease after infection might be due to the generation of reactive oxygen species (ROS) causing damage to chlorophyll a that is mean the plant failed to capture the light and so photosynthesis will decrease or stopped. At the same time, marked increases in the contents of carotenoids were observed in infected plants as being compared with healthy ones. But showed different responses as regards the Photosynthetic pigments due to the application of different Bio pesticide used. Results obtained indicate that the harmful effect of *Alternaria solani* infection on photosynthetic pigments could be reduced via using of *Cinnamon extract, cyanobacteria and PGPR* that can enrich the plant and soil with N2 element these findings are supported by (**Farrag *et al* 2017, Sharaf *et al* 2016 and Abd El-Baky *et al.* 2010)**. It appeared from our results that

tested Bio pesticide induced tomato plants for increasing total chlorophyll pigments and carotenoids contents as an indication of systemic resistance and help infected tomato plants to tolerant against Alternaria *solani* infection (as bio inducer agents).

Total phenols play a significant role in the regulation of plant metabolic process and over all plant growth as well as lignin synthesis **(Lewis and Yamamoto, 1990)**. In addition, phenols act as free radical scavengers as well as substrates for many antioxidant enzymes **(Martin- Tanguy, 2001)**. It is quite evidence that, the greatest value of total phenols was achieved by using PGPR or Cyanobacteria on the Alternaria - infected plants more than on the healthy plants, indicating induction of systemic acquire resistant (SAR). These are in accordance with (**Attia ,2014, Farrag., *et al* 2017 and Sudhakar *et al.*, 2007)** they stated that phenolic acids are involved in phytoalexin accumulation, biosynthesis of lignin and formation of structural barriers, which play a major role in resistance against the pathogen. In this regard Phenol metabolism and cell wall lignification are thus involved in, and have consequences for, a number of cellular, whole plant and ecological processes, that might even provide plants, the immunity against destructive agents **(Sudhakar *et al.*, 2007)**. Total protein was determined as response to induction treatments. Our results showed that the total soluble protein increased significantly in shoots in plants due *Alternaria solami* infection. In the present work, Bio pesticide (Cinnamon extract, Cyanobacteria &PGPR) showed significant increase in total soluble protein contents of shoots in comparison with *Alternaria solami* - infected plants. Also, in challenged treatments, it was noticed that, significantly higher total protein content was observed in shoots of tomato plants treated with PGPR and treatment with cyanobacteria followed by Cinnamon extract, respectively. The indirect effects of PGPR in disease suppression are the activation of plant defense mechanisms when challenged with pathogens through production of proteins **(Rakib and Mustafa 2013)**. Addition of cyanobacteria increased total soluble protein content in the infected tomato plant. This increase may be due to the increase of the nitrogen fixation and nitrate reductase activity of cyanobacteria (**Haroun and Hussein, 2003; Osman *et al.*, 2016**). One of the most abundant groups of organic compounds in the plant kingdom is the carbohydrates. total soluble carbohydrate contents in tomato plant shoots were significantly decreased due to ***A.solani*** infection during stages of growth. In the present work, addition of (PGPR) show considerable increase in total carbohydrate

contents related to (cyanobacteria and cinnamon), respectively during two stages of growth. Soluble sugars are involved in the responses to a number of stresses, and act as nutrient and metabolite signaling molecules that activate specific or hormonal-crosstalk transduction pathways, resulting in important modifications of gene expression (**Couee *et al.*, 2006**). A large number of defense enzymes that have been associated with ISR include, peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), and proteinase inhibitors (**Van Loon, 1997).** These enzymes also bring about liberation of molecules that elicit the initial steps in induction of resistance, phytoalexins and phenolic compounds **(Van Loon *et al.*, 1998).** Our results showed that antioxidant enzymes activity increased significantly in plants infected with *Alternaria solami*. To obtain clearer indication on some defense-responsible enzymes, mean activities of Superoxide dismutase, peroxidase and polyphenol oxidase of the tested tomato plants were determined in this study. SOD, PO and PPO activities were greater in the plants treated with cinnamon extract, cyanobacteria & PGPR and challenged with *A. solami*., compared to control plants. In this respect, enhanced SOD, POD and PPO activities against disease and insect pests have been reported in several beneficial plant–microbe interactions **(Attia, 2014 , Farrag,*et al* 2017 and Harish *et al.*, 2009).** The signal molecules JA and SA are involved in some signal transduction system, which induce enzymes catalyzing biosynthetic reactions to form defense compounds such as polyphenols, alkaloids and pathogenesis- related (PR) proteins (**Vijayan *et al.*, 1998; Metraux 2001**).

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