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INFLUENCE OF SOME NATURAL PLANT EXTRACTS AND CERTAIN ANTIOXIDANTS ON INCIDENCE, PGPR INOCULATION AND DEFENSIVE SYSTEM AGAINST ROOT ROT DISEASES IN TOMATO

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ABSTRACT

In this work, effects of PGPR, some natural plant extracts and certain antioxidants on incidence of root rot diseases and defensive enzymes of tomato under greenhouse conditions were studied. Results showed that disease severity of tomato in soil infected with F. solani and R. solani were reduced in S1 and S2in the soil incubated with PGPR compared with the soil not incubated

Total phenolic compounds (TPCs) levels in untreated samples are always lower than those reported in treated ones.

The sane trend was also recorded in the case of TFs. Chlorophylls concentration fluctuated as a result of infection and treatments. Effectiveness of studied plant extracts could be arranged in the following order: WE> OBE> MOE> CCE.

Three different defensive enzymes i.e. catalase, peroxidase and polyphenol oxidase showed that the treatments led to decreases in the levels of PPO activities compared with the untreated sample except propylgallate (PG).

The five organic substances have effective antioxidant and could be arranging it in the following order: PG > Na-Tannate >L-AA > SA >Na-Benzaoate. All these five substance are phenolic compounds and have antioxidant potentials

Key words: Antioxidant, Catalase, defensive enzymes, *Fusarium solani*, incidence, peroxidase, PPO, *R. solani*, severity, TPCs, TFs

INTRODUCTION

Several soil-borne fungal infections, including *Fusarium* species, *Rhizoctonia solani*, and *Sclerotium rolfsii*, attack tomato plants, resulting in catastrophic illnesses such root rots and wilt as well as decreased crop yield and quality. (**Abdel-Monaim, 2010; Saad, 2006).**

A viable strategy for managing plant diseases is the induction of resistance in plants to combat pathogen infection. In plants that typically express vulnerability to pathogen infection, exogenous or endogenous stimuli have the potential to significantly alter host physiology and cause rapid and coordinated activation of defense-genes (**Mandal *et al*., 2009; Metwally, 2004)**. Salicylic acid, potassium salts, and sorbic acid are just a few examples of the different abiotic agents (chemical inducers) that can be used to create this induced resistance to infections (**Abdel-Monaim, 2010, and El-Mohamedy *et al.*, 2013**). On the other hand, applying these chemical inducers in the field has improved numerous vegetable plant growth characteristics, yield components, and fruit quality (**El-Mougy *et al*., 2004, Karlidag *et al*., 2009**).

The most significant plant pigments are flavonoids, which also function as chemical messengers, physiological regulators, and inhibitors against pathogens like *Fusarium oxysporum*. Flavonoids are also involved in UV filtration and symbiotic nitrogen fixation (**Zhou *et al*., 2014, 2017**). The deadly foliar fungal disease known as tomato early blight is caused by *Alternaria solani*. The protective effects of benzoic acid (BA) and two of its hydroxylated derivatives, protocatechuic acid (PCA) and r-hydroxybenzoic acid (HBA), against *A. solani* were examined by (**Nehela *et al*. 2021)**. All of the compounds under test displayed potent, dose-dependent fungistatic action against *A. solani* and significantly inhibited the disease's spread.

One of the main plant diseases that have an impact on tomato yield is fusarium wilt. **Farag *et al.* (2011)** examined the effects of neem (*Azadirachta indica*) and willow (*Salix babylonica*) aqueous extracts on *Fusarium* wilt disease in tomato seedlings. Tomato seedlings that were four weeks old were given a 10% dose of either neem or willow aqueous extracts before being exposed to *Fusarium oxysporum* four days later. Neem and willow aqueous extract treatments on tomato plants decreased the percentage of disease incidence to levels of 25.5% and 27.8% after 6 weeks of infection, respectively. The findings demonstrate that *Fusarium oxysporum* infection of tomato seedlings resulted in numerous morphological and biochemical changes, including a reduction in tomato shoot and root growth, an increase in lipid peroxidation, and a notable increase in the activities of antioxidant defensive enzymes such as POX, CAT, and SOD. Infected or non-infected seedlings treated with neem and willow aqueous extracts significantly showed a growth boost of tomato shoot and root. Additionally, neem and willow aqueous extract application with *Fusarium* considerably decreased the degree of lipid peroxidation and elicited high activities of antioxidant defensive enzymes after 3 and 7 days of infection. *Fusarium oxysporum* triggered the upregulation of numerous POX isoenzymes, as seen by the electro *Sclerotium rolfsii* can infect tomato (*Solanum lycopersicum* L.) plants, leading to crown rot in

adult plants as well as damping-off in nursery seedlings. Onion and garlic extracts, neem oil, salicylic, ascorbic, citric, and hydrogen peroxide, as well as various bioagents like *Bacillus subtilis*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, and *Trichoderma harzianum*, were all evaluated by **Farag *et al.* (2011**) for their effects on the pathogen's linear growth. The top three factors for reducing disease incidence and severity were neem oil, salicylic acid, and *P. fluorescens*, followed by *B. subtilis* and a decrease in the linear development of *Sclerotium rolfsii*. The results showed that ascorbic, citric, and onion extract had the lowest values. phoretic pattern of POX. It is possible to deduce that willow and neem

The present investigation was undertaken to study effects of (**a**)-some natural plant extracts and (**b**)-certain antioxidants on incidence and defensive enzymes against root rot diseases in tomato under greenhouse conditions to more elucidate their impacts on growth parameters,

MATERIALS AND METHODS

The soil used:

The soil used in this study was collected from the top layer (25 cm depth) of the soil at the farm of Faculty of Agriculture, Minia University

Seedlings:

Tomato transplants varieties 086 that have been used in this study were obtained from Horticulture Department, Faculty of Agriculture, Minia University.

Microbial strains:

The biofertilizing-PGPR strains used in the present work were reported in **Abdullah *et al*., (2023)**.

Disease Severity:

Tomato root rot was assessed as root rot severity by the method described by **Liu, *et al.*, (1995)** using arbitrary scale 0-5 where 0 =No infection plant, 1= 20-25%; 2= 26-50%; 3= 51--75% and 5= >75% were completely dead plants. Damping-off seedlings were considered dead plants and graded at maximum disease grade. After that root rot severity % were recorded.

Root rot disease severity % = [∑(n x v) x N] x 100

Where: n=number of plant within each infection categories, v=numerical values of infection categories, N= total number of plant examined and 50 constant, highest numerical value.

The percent of wilt disease was recorder using the following.

Disease severity of wilted plant was recorded by adopting the same scale based on percent disease index (PDI), According to (**Paz-Lago *et al*., 2000**) using the following scale of 0–5 degrees, diseased plants showed stunting, dark brown vacuolar discolouration, and mortality. The symptoms of wilting in the aerial part were noted, and the invasion score of roots was measured..

0 = Foliage or not damaged root
1 = Wilting or damaged root (20%).
2 = Wilting or damaged root (40%).
3 = Wilting or damaged root (60%).
4 = Wilting or damaged root (80%).
5 = Wilting or damaged root (100%).

Disease severity (%) = [∑(n x V) /5xN)] x 100. Where:

N= is the number of plants in each type of infection
V =stands for the classifications of infection values
N= stands for the entire sample of plants
The largest number, 5, is a constant**.**

Effect of certain plant extracts on the occurrence of root rot infections when grown in greenhouses tomato

Four plants' leaves, namely basil (*Ocimum basilicum*), camphor *(Eucalyptus globulus*), WE willow *Salix alba*. extracts, and MOE *Moringa oleifera*, were used to make aqueous plant extracts. The plant samples were cleaned with sterilised distilled water several times, cut into small pieces, and then macerated in 100 ml of sterilised distilled water using a mortar. The resulting extract was then twice squeezed through four layers of cheese cloth, centrifuged at 4000 rpm for 15 minutes, and sterilised with Seitz filtrate (**Hassan, 2006**).

Aqueous plant extracts' sterilised filtrates were stored in the fridge until they were needed. On the occurrence of root rot infections induced by F. solani and R. solani. tomato plants (086) under greenhouse conditions throughout the 2022 season, these plant extracts were sprayed onto all plant samples. With autoclaved sand clay soil, sterilised plastic pots (30 cm in diameter) were filled. As previously noted, infected soil was used to evaluate each pathogen. For each treatment, three replicates were employed. Each copy has three pots with four seeds each. As a control, no treatment with aqueous plant extracts was utilised. Following 60 days, total phenolic compounds (TPCs), total flavonoids (ITFs), and chlorophylls were measured in leaf samples.

4- Effect of certain anti-oxidants on the occurrence of root rot infections in greenhouse environments tomato

Salicylic acid, L-ascorbic acid, sodium benzoates, propylgallate, and Na-Tannate were five antioxidant compounds that were synthesised and sprayed on both damaged (*R. solani* and *F. solani*) and healthy plants in order to examine their effects. Under greenhouse circumstances and throughout the growing seasons of 2022, the impact of antioxidant compounds was examined on the prevalence of root rot on tomato plants (086). The final concentration (10 mM) of each chemical inducer was sprayed onto tomato samples. As previously noted, R. and F. solani were introduced to the soil. Three pots were utilised as replicates for each treatment, and sterilised samples were seeded in each pot. Infested soil was seeded with compound and untreated plants as a control. Leaf samples were collected after 60 days in order to measure the protective enzymes PPO, Catalase, and peroxidase.

Polyphenol oxidase activity:

According to **Matta and Dimond's (1963)** description, the activity of polyphenol oxidase was determined by monitoring the oxidation of catechol using a spectrophotometer at 546 nm.

Catalase activity:

According to **Maxwell and Bateman (1967)**, the activity of catalase (CAT) was determined by monitoring the oxidation of catechol with a spectrophotometer at 420 nm.

Determination of phenolic compounds:

Four tomato seedlings, both infected and uninoculated, were evaluated for phenolic compound concentration. To remove the tissues,

two grams of tomato seedlings were chopped into little pieces, instantly submerged in 95% ethanol for 10 minutes, and the percolate was extracted for 8–10 hours in soxhlet units using 70% ethanol until it was colourless. The mixed ethanol extracts were filtered and dried almost completely by evaporation on a water path at 70°C. Redissolved in a known volume of 50% isopropanol alcohol was the dried residues. According to Snell and Snell (1953), the latter isopropanol extracts were used to calculate total phenols using the Folin and Ciocatalteus reagent. Ten drops of conc. HCI (70%) were added to the samples, which were then quickly heated to boiling point and submerged in boiling water for ten minutes to measure the total phenols. 5 ml of 20% Na2CO3 and 1 ml of the reagent were added after cooling. After 30 minutes, the mixture was diluted to 10ml, and the determination was made using a spectrophotometer at 520 nm. Phenolic compounds were calculated as milligrams equivalent of catechol/1gm fresh weight of tomato samples with using the following formula:

$$\text{Sample conc. (mg)} = \frac{\text{Reading of sample X (Total volume of sample (cm) Sample volume (ml) X 100}}{\text{Standard curve of catechol X Fresh weight (gm)}}$$

Extraction of total flavonoids (TFs):-

The extract was filtered after being extracted for an hour in a Soxhlet extractor with 100 ml of distilled water or ethanol from powdered air-dried or air-dried samples (10 g).

Determination of total flavonoids (TFs): -

10 ml volumetric flasks were filled with a known amount of extract. 5 ml of distilled water and 0.3 ml of NaNO2 (1:20) were added. 5 minutes later, 3 ml of AlCl3 (1:10) were added. 2 ml of 1 mol litre-1 sodium hydroxide was added after 6 minutes, bringing the total to 10 ml with distilled water. Using a Spectrophotometer (Taizhou Radio Factory) and a blank at 510 nm, the solution was thoroughly mixed once again (**Zhuang *et al*., 1992**).

4.2. Determination of oxidative enzymes/

In four inoculated and noninoculate d tested peanut cultivars with tested path ogenic isolates, the activities of oxidative enzymes were measured.

According to **Goldschmidt *et al*, (1968)** instructions, 4 ml of 0.1M sodium phosp hate buffer pH 7.1 and 2 grammes of pur ified sand were added to the two gramme s of tomato seedlings that had been chop ped into small pieces and pulverised in a mortar.

Four layers of cheesecloth were used to f ilter the homogenate, and the filtrates we re then centrifuged at 3000 rpm for 20 mi n. at 6 °C.

For testing the oxidative enzymes catalas e (CAT), peroxidase (PO), and polyphen ol oxidase (PPO), supernatant fluids (enz yme extracts) were collected.

Pigment content determination

Ten gram of air-dried tomato plants were powdered to get finally powder, cold acetone was added and used for chlorophylls extraction. The clear supernatant was measured according to the method described by **Seely and Jensen (1965).**

Statistical analysis:

All obtained data are subjected to specific programme to calculate the changes according to the method described by **SAS (1985)**

RESULTS AND DISCUSSION

(1)-Effect of PGPR inoculation on disease severity in soil infected with *F. solani* and *R. solani*.

Results given in Table (1) showed that disease severity of tomato in soil infected with *F. solani* reached to be 89.25 and 88.32 whereas *R. solani* were 94.2 and 87.99 for S1 and S respectively. The lowest value of disease severity was recorded (5.44) in the case of non-infected soil with *R. solani* (S2). According to the findings presented by **Abdel-Fattah *et al.* (2011)**, the negative impacts on these parameters, as well as the severity and incidence of disease, were dramatically diminished when bean plants were colonised with AM fungus. According to the findings reported by **Xue *et al.* (2017**), *Trichoderma* stain TrichC70 may be utilised as an alternative to fungicide seed treatments to lessen the negative effects of the wheat *Fusarium* head blight's seed-borne phase..

(2)-Effect of PGPR inoculation on disease incidence of tomato in soil infected with *F. solani* and *R. solani*.

Disease incidence of tomato in soil infected with *F. solani* and *R. solani* was affected by PGPR inoculation and the result are given in Table (2). Soil infected with *F. solani* reduced the disease incidence to be 22 and 17% for S1 and S2, respectively. Soil infected with *R. solani* recorded disease incidence 11 and 17 for S1 and S2, respectively. Under greenhouse conditions, this chitinase preparation effectively decreased the incidence of disease caused by S. rolfsii in beans and R. solani in cotton. When an active E. coli cell carrying the chitinase gene (pLCHIA) was used, a similar result was attained. It indicates that lytic enzymes, such chitinase, which are crucial for controlling plant diseases, could be genetically engineered to increase the effectiveness of biocontrol agents. (**Chet *et al.*, 1990)**.

Effect of five antioxidants on TPCs, TFs and chlorophylls in tomato leaves of root rot diseases under greenhouse conditions

Levels of three groups of bioactive secondary metabolites having defensive roles are illustrated in Table (3). TPCs in untreated samples are always lower than those reported in treated ones. The highest concentrations (39±3.2) of TPCs were recorded in tomato plant infected with *Rhizoctonia solani* treated with Na-Tannate followed by *Fusarium solani* (35±3.3). The TPCs are bioactive secondary metabolites paly in important roles in defensive process against pathogenic fungi and bacteria. Total flavonoids (TFs μg/g) concentrations ranged from 425 for untreated plants treated with L-AA to 975 μg/g for infected plants with *R. solani* and treated Na-Tannate.

Photosynthetic pigments (chlorophylls) are also determined in the present study and the results are given in Table (3). The highest levels of chlorophylls are 1.32±0.3 for sample infected with *Fusarium solani* and treated by Salicylic acid (SA).

According to **Dar *et al.* (2017**) and **Zhou *et al.* (2014)**, phenolic compounds (PC) are a collection of secondary metabolites that contain at least one

phenol unit and one or more hydroxyl groups..

When plant tissues are exposed to various abiotic and biotic stressors, phenolic compounds frequently form and accumulate in the sub-epidermal layers (**Clé *et al.*, 2008**). According to **Srinivasan *et al*. (2007)**, PC are also renowned as potent antioxidants that may scavenge free radicals.

Among the most prevalent phenolic substances are benzoic acid and its hydroxylated counterparts, r-hydroxybenzoic acid and protocatechuic acid. In addition to having potential antibacterial and antifungal properties (**Li *et al.*, 2020**), benzoic acid also serves as a protective agent against a variety of environmental stresses, including heat, drought, and chilling stress (**Senaratna *et al.*, 2003; Williams *et al.*, 2003)**. For instance, benzoic acid and and 57 of its derivatives demonstrated potential antifungal effects against Eutypa lata, the phytopathogenic fungus that causes dieback disease, and they established a probable structure-activity link (**Amborabé *et al.*, 2002**). Furthermore, under laboratory conditions, BA decreased the growth rate of A. citri and A. alternata, the causative agents of stem-end rot and internal core rot (black rot) in citrus fruits (**Embaby *et al.*, 2013**).

Functions of bioactive secondary metabolites are defensive compound against pathogenic fungi and harmful bacteria. The higher the TPCs content, the stronger the antioxidative activity of the extract. Antioxidant activity of methanolic extracts of tomato containing PAs was high and safe compared to the common commercial antioxidant BHA (**Allam and Bassiuny, 2002, Zhou *et al.*, 2014, Zhou *et al.*, 2017**).

Effect of four plant extracts on TPCs, TFs and chlorophylls in tomato leaves of root rot diseases under greenhouse conditions

Results of TPCs, TFs and chlorophylls concentrations in tomato leave samples are given in Table (4). TPCs concentration ranged from 40±3.2 mg/g for infected plant with *Rhizoctonia solani* and treated with willow extracts (*Salix alba*) to 11±0.1 mg/g for untreated plant in camphor group. The sane trend was also recorded in the case of TFs. The camphor extract (CCE) recorded the lowest levels of TPCs, TFs and chlorophylls while willow extracts (*Salix alba*) reported the highest levels. Willow extracts extracted from *Salix alba* recorded the highest concentrations (1100±6) of TFs with infected plant with *Rhizoctonia solani* followed by *Fusarium solani*. Chlorophylls concentration fluctuated as a result of infection and treatments. Effectiveness of studied plant extracts could be arranged in the following order: WE> OBE> MOE> CCE. These results are consistent with those from **Abdurrahman and Hayriye (2016**), who discovered that methanol extracts of various plant parts (leaf, flower, root, fruit, and shoots) of *Trachystemon orientalis*, *Smilax excelsa*, *Rhododendron ponticum*, *Phytolacca americana*, and *Prunus laurocerasus* demonstrated significantly distinct antifungal activities against *Alternaria solani*, *Botrytis cinerea*, and *Rhizoctonia solani*.

Moringa leaf and seed extracts had antifungal characteristics that prevented *R. solani* and *F. solani* from growing. The antifungal effectiveness of the extracts was regulated by the

concentrations of moringa extract (**Goss *et al.* 2017**).

antibacterial drugs are required due to the prevalence of microbial infections; *M. oleifera* has been shown to be an effective antibacterial agent (**Chen and Verdes 2009**). The extracts of *M. oleifera* can act against germs like *Bacillus subtilis*, *Staphylococcus aureus*, and *Vibrio cholera*, according to a study by **Viera *et al.* (2010)**. *Pterygospermin*, *moringine*, and benzyl isothiocyanate were found to be responsible for the seeds' antibacterial properties **(Jahn *et al.*, 1986)**.

Moyo *et al.* (2012) examined the antioxidant capacity of leaves from Moringa oleifera in several in vitro settings. They claimed that goats treated with *M. oleifera* (MOL) or sunflower seed cake (SC) had an antioxidative effect on the activities of superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO), and reduced glutathione (GSH).

Effect of certain antioxidants on activities of some defensive enzymes of root rot diseases on under greenhouse conditions tomato

Results given in Table (5) showed enzyme activities of three different defensive enzymes i.e. catalase, peroxidase and polyphenol oxidase. Data illustrated in Table (5) showed that the treatments of the present work led to decreases in the levels of PPO activities compared with the untreated sample except propylgallate (PG). Treatment with L-AA inhibited the PPO activities. On the other hand, results showed that spraying SA promoted PPO activities with different extents. The highest values for PPO activities were observed to be 11-folds and 10-folds when PG in the case of plants treated with *Fusarium solani*, and *Rhizoctonia solani* respectively. The same trend was also recorded when Na-Tannate was used as antioxidant. The reasons for high PPO levels may be due to the accumulation of these compounds in tomato tissues that considered as PPO substrates and precursors for biosynthesis of many complicated polyphenolic acids. When L-Ascorbic acid (L-AA) and Na-Benzoate were present, the lowest PPO activity values were observed. In conjunction with ascorbic acid or citric acid, erythorbic acid, an isomer of ascorbic acid, has been utilised as an inhibitor of enzymatic browning (**Dennis, 1993**).,

Additionally, other researchers discovered that the antifungal properties of some natural plant products decreased plant pathogens while increasing oxidative enzymes in plants. Oxidative enzymes can play a significant role in a plant's ability to resist disease infection, which in turn increases growth parameters and seed yield (**Abdel-Monaim *et al.*, 2011**).

The activities of peroxidase in all treatment are always higher than those reported for untreated plants (control) and the highest level (4.325) was assayed in the extracts of PG-*Fusarium solani* followed by *Rhizoctonia solani (*4.125**).** Peroxidase is the second enzyme in the superoxide radical's conversion pathway from H_2O_2 to $H_2O + O_2$ and prevents harmful H_2O_2 accumulation in the inside of cells. For these reasons it consider defensive enzyme **(De la Rosa *et al.*, 2018**).

The findings demonstrated that Fusarium oxysporum infection of tomato seedlings resulted in numerous morphological and biochemical changes, including a reduction in tomato shoot

and root growth, an increase in lipid peroxidation, and a notable increase in the activities of antioxidant defensive enzymes such as POX, CAT, and SOD. (**Farag *et al.*, 2011)**.

Catalase activities in treated plants with Na-Tannate with *Fusarium solani* recorded the highest levels (1.810) followed by propylgallate with *Fusarium solani*.

Results existed in Table (5) also, showed that the five organic substances have effective antioxidant and could be arranging it in the following order: PG > Na-Tannate >L-AA > SA >Na-Benzaoate. All five of these substances are phenolic compounds with potential antioxidative effects. Through the phenylpropanoid and shikimic acid pathways, phenolics are produced from L-phenylalanine (De la Rosa et al., 2018). They are widely dispersed phytochemicals that are present in the majority of plant tissues and play a crucial function in how plants react to biotic and abiotic stressors, notably pathogen infection (**De la Rosa *et al.* 2018, Bennett and Wallsgrove 1994).**

Nehela *et al.* (2021), who studied tomato early blight, a damaging foliar fungal disease brought on by *Alternaria solani*, looked into the potential defensive effects of benzoic acid and two of its hydroxylated derivatives, r-hydroxybenzoic acid and protocatechuic acid, against *A. solani*.

The reducing abilities of both solvent extracts revealed strong antioxidant activity in a concentration-dependent manner. The acetone extract inhibited a larger proportion of DPPH, ABTS, and nitric oxide radicals than the reference standard antioxidants (vitamin C and BHT). MOL increased the antioxidant capacities of GSH (186%), SOD (97.8%), and catalase (0.175%). MOL significantly reduced the oxidation of lipids. The present investigation suggests that *M. oleifera* may be a source of compounds with potent antioxidant effects. **(2012) Moyo *et al***. According to **Yousef et al. (2013),** soaking *R. Solani sclerotia* in antioxidant-rich potassium tartrate and a combination of micronutrients for 48 hours caused a significant reduction in colony diameter of up to 52%, with complete *sclerotia* production inhibition occurring after 24 days of incubation. This knowledge provides fresh insight into the environmental elements that affect plant-pathogen interactions and may be used to create a management approach for the prevention of *R. solani* based on host nutrition.

Table (1). Effect of PGPR inoculation on disease severity of tomato in soil infected with *F. solani* and *R. solani*.

	Disease severity (%)	
	S1	S2
Non-infected soil with *F. solani*	7.50	8.20
Soil infected with *F. solani*	89.25	88.32
F. solani* + *T. viride	5.64	7.58
F. solani* + *B. megaterium	6.10	8.43
F. solani* + *A. chroococcum	16.21	22.17
F. solani* + *B. circulans	9.83	10.40
F. solani* + *T. viride* + *B. megaterium	11.23	16.20
F. solani* + *T. viride* + *A. chroococcum	19.32	18.44
F. solani* + *T. viride* + *B. circulans	12.33	13.65
Mix	22.40	21.31
Non-infected soil with *R. solani*	6.23	5.44
Soil infected with *R. solani*	94.2	87.99
R. solani* + *T. viride	7.11	9.3
R. solani* + *B. megaterium	10.55	9.21
R. solani* + *A. chroococcum	16.88	20.43
R. solani* + *B. circulans	10.5	11.26
R. solani* + *T. viride* + *B. megaterium	11.26	13.54
R. solani* + *T. viride* + *A. chroococcum	23.48	26.15
R. solani* + *T. viride* + *B. circulans	19.55	17.22
Mix	29.10	27.16

S_1:season one **S_2=season two**

Table (2). Effect of PGPR inoculation on disease incidence of tomato in soil infected with *F. solani* and *R. solani*.

	Disease incidence (%)	
	S1	**S2**
Non-infected soil with *F. solani*	22	17
Soil infected with *F. solani*	70.08	65.82
F. solani* + *T. viride	19.97	15.08
F. solani* + *B. megaterium	16.10	12.60
F. solani* + *A. chroococcum	22.05	26.34
F. solani* + *B. circulans	15.67	20.04
F. solani* + *T. viride* + *B. megaterium	17.06	20.37
F. solani* + *T. viride* + *A. chroococcum	25.99	20.94
F. solani* + *T. viride* + *B. circulans	18.17	17.82
Mix	26.57	27.15
Non-infected soil with *R. solani*	11	17
Soil infected with *R. solani*	76.70	70.82
R. solani* + *T. viride	16.28	22.63
R. solani* + *B. megaterium	16.39	22.92
R. solani* + *A. chroococcum	26.06	35.43
R. solani* + *B. circulans	15.05	17.93
R. solani* + *T. viride* + *B. megaterium	18.76	21.80
R. solani* + *T. viride* + *A. chroococcum	38.82	35.15
R. solani* + *T. viride* + *B. circulans	28.55	24.72
Mix	37.44	33.83

S_1:season one **S_2=season two**

Table (3): Levels of total phenolic compounds (TPCs mg/g), total flavonoids (TFs µg/g) and chlorophylls (mg/g) in tomato leaves treated with antioxidants in diseased and healthy plants *in vitro*

Antioxidants	Treatments	Bioactive secondary metabolites		
		TPCs	TFs	Chlorophylls
L-Ascorbic acid (L-AA)	Untreated plants (control)	16±1.6	425±4	0.652±**0.16**
	Treated plants with *Fusarium. solani*	21±1.9	505±4	0.787±0.17
	Treated plants with *Rhizoctonia. solani*	23±1.9	575±6	0.948±0.3
Propylgallate (PG)	Untreated plants (control)	19±1.5	475±5	0.875±0.2
	Treated plants with *Fusarium solani*	32±2.1	768±8	0.887±0.2
	Treated plants with *Rhizoctonia solani*	28±2.0	678±7	1.13±0.3
Salicylic acid (SA)	Untreated plants (control)	19±1.9	487±5	1.03±0.2
	Treated plants with *Fusarium solani*	32±2.2	600±6	1.32±0.3
	Treated plants with *Rhizoctonia solani*	31±3.2	587±6	1.20±0.3
Na-Tannate	Untreated plants (control)	18±1.7	450±4	0.657±0.16
	Treated plants with *Fusarium solani*	35±3.3	875±9	0.985±0.3
	Treated plants with *Rhizoctonia solani*	39±3.2	975±10	0.997±0.3
Na-Benzaoate	Untreated plants (control)	18±1.6	449±5	0.655±0.16
	Treated plants with *Fusarium solani*	24±1.2	599±**6**	1.05±0.31
	Treated plants with *Rhizoctonia solani*	23±2.1	654±7	1.02±0.21

Activity of PPO was determined colourimatrically at 485 nm and then expressed as enzyme unit. One unit of PPO activity was expressed as a change of absorbance of per min. per g.

Table (4): Levels of total phenolic compounds (TPCs mg/g), total flavonoids (TFs µg/g) and chlorophylls (mg/g) in tomato leaves treated with some plant extracts in diseased and healthy plants *in vitro*

Plant extracts	Treatments	Bioactive secondary metabolites		
		TPCs	**TFs**	**Chlorophylls**
CCE *Cinnamomum camphora* extract Camphor	Untreated plants (control)	11±0.1	268±3.0	0.589±0.09
	Treated plants with *Fusarium. solani*	16±0.3	300±3.0	0.624±0.09
	Treated plants with *Rhizoctonia. solani*	14±0.2	287±3.0	0.564±0.9
OBE *Ocimum basilicum*	Untreated plants (control)	15±1.9	447±5	0.73±0.2
	Treated plants with *Fusarium solani*	27±2.2	5600±6	0.92±0.3
	Treated plants with *Rhizoctonia solani*	26±3.2	597±6	1.26±0.3
WE willow extracts *Salix alba*	Untreated plants (control)	25±1.9	587±5	0.53±0.20
	Treated plants with *Fusarium solani*	38±2.2	987±6	1.62±0.30
	Treated plants with *Rhizoctonia solani*	40±3.2	1100±6	1.67±0.30
MOE *Moringa oleifera*	Untreated plants (control)	13±0.72	295±2	0.687±0.12
	Treated plants with *Fusarium solani*	15±0.85	310±2	0.852±0.12
	Treated plants with *Rhizoctonia solani*	17±0.92	325±3	0.922±0.24

Table (5): Activities of Catalase, Peroxidase and Polyphenol oxidase in tomato leaves treated with antioxidants in diseased and healthy plants *in vitro*

Antioxidants	Treatments/pathogen	Enzyme activity Δ_{Abs} per min. per g fw		
		Catalase	Peroxidase	Polyphenol oxidase
L-Ascorbic acid (L-AA)	Untreated plants (control)	0.489 ±0.01	0.551 ±0.02	0.958 ±0.05
	Treated plants with *Fusarium solani*	0.772 ±0.04	1.671 ±0.09	0.845 ±0.06
	Treated plants with *Rhizoctonia solani*	0.911 ±0.09	2.015 ±0.101	0.850 ±0.06
Propylgallate (PG)	Untreated plants (control)	0.821 ±0.05	1.024 ±0.15	0.671 ±0.06
	Treated plants with *Fusarium solani*	1.752 ±0.11	4.325 ±1.01	7.544 ±1.52
	Treated plants with *Rhizoctonia solani*	1.554 ±0.22	4.125 ±0.23	7.312 ±1.54
Salicylic acid (SA)	Untreated plants (control)	0.544 ±0.02	0.884 ±0.07	0.890 ±0.06
	Treated plants with *Fusarium solani*	0.750 ±0.07	1.649 ±0.14	1.281 ±0.14
	Treated plants with *Rhizoctonia solani*	1.190 ±0.04	2.450 ±0.11	4.950 ±0.51
Na-Tannate	Untreated plants (control)	0.781 ±0.07	0.929 ±0.09	0.798 ±0.07
	Treated plants with *Fusarium solani*	1.810 ±0.12	4.001 ±0.35	7.415 ±0.54
	Treated plants with *Rhizoctonia solani*	1.323 ±0.12	3.664 ±0.38	7.250 ±1.55
Na-Benzaoate	Untreated plants (control)	0.333 ±0.01	1.625 ±0.25	0.984 ±0.11
	Treated plants with *Fusarium solani*	0.651 ±0.01	2.184 ±0.12	0.974 ±0.09
	Treated plants with *Rhizoctonia solani*	1.09 ±0.08	3.041 ±0.34	0.954 ±0.45

The catalase activity was assayed as the change in absorbance/ ml of extract/min. at 420 nm. Activity of PPO was determined colourimatrically at 485 nm and then expressed as enzyme unit. One unit of PPO activity was expressed as a change of absorbance of per min. per g.fw

REFERENCES

Abdel-Fattah, G.M., El-Haddad, S.A., Hafez E.E. and Rashad, Y.M. (2011): Induction of defense responses in common bean plants by arbuscular mycorrhizal fungi. Microbiological Research Volume 166, Issue 4, 20 May 2011, Pages 268-281

Abdel-Monaim, M.F., Abo-Elyousr K.A.M. and Morsey K.M. (2011). Effectiveness of plant extracts on suppression of damping-off and wilt disease of lupine (*Lupina termis* Forsik). Crop prot., 30: 185-191.

Abdel-Monaim, M.F. (2010). Induced systemic resistance in tomato plants against *Fusarium* wilt disease. Pages 253-263. In Proceedings of the 2nd Minia Conference for Agriculture and Environmental Science, 22-25 March, 2010, Minia, Egypt.

Abdallah, A.R.; Saad' A.O.S., Hassan, A.A. Wafy, A.S.M.A. (2023). Impact of plant growth promoting microorganisms as a biofertilizer on enzymes activity and vegetative criteria of tomato crop. Minia Agric. Journal, Vol. pp

Abdurrahman, O. and D.S. Hayriye (2016). Antifungal Activity of Some Plant Extracts against Different Plant Pathogenic Fungi. Journal of Advances in Agricultural & Environmental Eng., 3(2): 1523-1531.

Allam, S.S.M. and Bassiuny, A.M.M. (2002). Chlorogenic acid, efficiency and safety aspects as antioxidant. *Rivista-Italiana-delle-Sostanze-Grasse.* 2002, 79: 7-8, 257-265.

Amborabé, B.E.; Fleurat-Lessard, P.; Chollet, J.F.; and Roblin, G. (2002): Antifungal effects of salicylic acid and other benzoic acid derivatives towards *Eutypa lata*: Structure-activity relationship. Plant Physiol. Biochem. **2002**, 40, 1051–1060.

Bennett, R.N.; Wallsgrove, R.M. (1994). Secondary metabolites in plant defence mechanisms. New Phytol. **1994**, 127, 617–633.

Chen, M. and Verdes R.P. (2009). Elucidation of bactericidal effects incurred by *Moringa oleifera* and Chitosan J US SJWP, 4 (2009), pp. 65-79

Chet, A. Ordentlich, Shapira, R. and Oppenheim, A. (1990): Mechanisms of biocontrol of soil-borne plant pathogens by Rhizobacteria. *Plant and Soil* volume 129, pages85–92(1990)

Clé, C.; Hill, L.M.; Niggeweg, R.; Martin, C.R.; Guisez, Y.; Prinsen, E.; Jansen, M.A.K. (2008). Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicum*; consequences for phenolic accumulation and UV-tolerance. Phytochemistry, 69, 2149–2156

Dar, S.A.; Rather, B.A.;Wani, A.R.; and Ganie, M.A. (2017). Chemical Science Review and Letters Resistance against Insect Pests by Plant Phenolics and their Derivative Compounds. Chem. Sci. Rev. Lett. **2017**, 6, 1941–1949.

De la Rosa, L.A.; Moreno-Escamilla, J.O.; Rodrigo-García, J.; Alvarez-Parrilla, E. **(2018).** Phenolic compounds. In Postharvest Physiology and Biochemistry of Fruits and Vegetables; Yahia, E., Carrillo-Lopez, A., Eds.;Woodhead Publishing, Elsevier: Sawston, UK, 2018; pp. 253–271. ISBN 9780128132784.

Dennis, J.A.B. (1993). The effects of selected antibrowning agents, selected packaging methods, and storage times on some characteristics of sliced raw potatoes. Dissertation, Oklahoma State University, Stillwater, USA

El-Mohamedy, S.R., Abdel-Kader, M.M., AbdEl-Kareem, F. and El-Mougy, N.S. (2013). Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens *in vitro*. Journal of Agricultural Technology 9: 1507-1520.

El-Mougy, N.S., Abd-El-Karem, F., El-Gamal, N.G., and Fotouh Y.O. (2004). Application of fungicides alternatives for controlling cowpea root rot diseases under greenhouse and field conditions. Egypt. J. Phytopathol. 32: 23-35.

Embaby, E.M.; Hazaa, M.; Hagag, L.F.; Ibrahim, T.E. and El-Azem, F.S.A. (2013). Decay of some citrus fruit quality caused by fungi and their control: II-control *Alternaria* rot or core rot decay by using some alternative fungicides. J. Appl. Sci. Res., 9, 5671–5678.

Farag, R.M.H., Abdou, Z.A., Salama, D.A., Ibrahim, M.A.R. and Sror, H.A.M. (2011). Effect of neem and willow aqueous extracts on fusarium wilt disease in tomato seedlings: Induction of antioxidant defensive enzymes. Annals of Agricultural Science (2011) 56, 1–7

Goldschmidt, E.E.; Goren, R. and Monselise, S.P. (1968). The IAA oxidase system of citrus roots. Planta , 72: 213-222.

Goss, M.,P. Mafongoya, 1. and Gubba, A. (2017). *Moringa oleifera* Extracts Effect on *Fusarium solani* and *Rhizoctonia solani* Growth. Asian Research Journal of Agriculture, 6(1): 1-10.

Hassan, T.I. (2006). The biological control via biofertilizers. M.Sc. Thesis, Fac. Of Agric., Minia Univer. 135 pp

Jahn, S.A. Musnad H.A. and Burgstaller, H (1986) The tree that purifies water: cultivating multipurpose Moringaceae in the Sudan Unasylva, 38 (1986), pp. 23-28 View in ScopusGoogle Scholar

Karlidag, H., Yildirim, E., and Turan, M. (2009). Exogenous applications of salicylic acid affect quality and yield of strawberry grown under anti-frost heated greenhouse conditions. J. Plant Nutr. Soil Sci. 172: 270-276.

Liu H.Y.; Lu, Y.F. and Chen W.J. (1995). Predictive equations for basal metabolic rate in Chinese adults: A cross-validation study. J. Am. Diet. Assoc., 95 (12): 1403-1408.

Mahmoud, A.Y. (2004). Integrated control of pod rots diseases of peanut. Ph.D. Thesis, Fac. Of Agric. Ain-Shams University, 154 pp.

Mandal, S., Mallick, N., and Mitra, A. (2009). Salicylic acid- induced resistance to *Fusarium oxysporum f. sp. lycopersici* in tomato. Plant Physiol Biochem. 47: 642-649

Matta, A and Dimond, A.E. (1963). Symptoms of *Fusarium* wilt in relation to quantity of Fungus and enzyme activity in tomato stems. Phytophathology, 53: 574-587.

Maxwell, D.P. and Bateman D.F. (1967). Change in the activities of

lesion maturation. Phytopathology, 57: 132-136 some oxidases in extracts of Rhizoctonia infected bean hypocotyls in relation.

Metwally, M.M. (2004). Resistance induction against disease of faba bean crop. Ph.D Thesis, Fac. of Agric., Cairo University, Egypt, 94 pp. 102-106.

Moyo, S., Oyedemi, P. Masika, .J. and Muchenje, V. (2012). Polyphenolic content and antioxidant properties of *Moringa oleifera* leaf extracts and enzymatic activity of liver from goats supplemented with *Moringa oleifera* leaves/sunflower seed cake. Meat Science, Volume 91, Issue 4, August 2012, Pages 441-447

Nehela, Y., Taha, N.A., Elzaawely, A.A., Xuan, T.D., Amin, M.A., Ahmed M.E. and El-Nagar, A. (2021). Benzoic acid and its hydroxylated derivatives suppress early blight of tomato (*Alternaria solani*) via the induction of salicylic acid biosynthesis and enzymatic and non-enzymatic antioxidant defense machinery**.** Journal Fungi **2021**, 7, 663. https:// doi.org/ 10.3390/jof 7080663

Paz-lago, D.; Gutierrez; A.B.Jr; Borges; A. Cabrera; G. Ramirez M.A. and Falcon, A. (2000). Tomato- *Fusarium oxysporum* interactions: Ii-Chitosan and msb induced resistance against FOL in

Saad, M.M. (2006). Destruction of *Rhizoctonia solani* and *Phytophthora capsici* causing tomato root-rot by *Pseudomonas fluorescens* lytic enzymes. Res. J. Agric. Biol. Sci. 2: 274-281.

SAS, (1985): SAS User s Guide: Basics, Version, 5 Edition SAS Institute, Inc., Cary, NC.

Seely G.R, and Jensen R.G. (1965): Effect of solvent on the spectrum of chlorophyll. Spectrochim Acta 21: 1835-1845

Senaratna, T.; Merritt, D.; Dixon, K.; Bunn, E.; Touchell, D.; and Sivasithamparam, K. (2003). Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. Plant Growth Regul. **2003**, 39, 77–81.

Snell, F.D. and Snell, CT (1953). Colorimetric methods. Pp.:66 Volume 111. Organi, D. Van Nostrand Company, Inc. Toronto, New York, London

Srinivasan, M.; Sudheer, A.R.; Menon, V.P. (2007). Ferulic acid: Therapeutic potential through its antioxidant property. J. Clin. Biochem. Nutr. **2007**, 40, 92–100.

Viera, G.H.F. Mourão, J.A. Ângelo, Â.M. Costa, R.A. and Vieira R.H.S.D.F (2010). Antibacterial effect (*in vitro*) of *Moringa oleifera* and *Annona muricata* against Gram positive and Gram negative bacteria Rev. Inst. Med. Trop. Sao Paulo, 52 (2010), pp. 129-132

Williams, M.; Senaratna, T.; Dixon, K.; and Sivasithamparam, K. (2003). Benzoic acid induces tolerance to biotic stress caused by Phytophthora cinnamomi in Banksia attenuata. Plant Growth Regul. **2003**, 41, 89–91.

Xue, A.G.; Guo, W.; Chen, Y.; Siddiqi, I., Marchand, I. and Ren, J.Ch. (2017): Effect of seed treatment with novel strains of *Trichoderma* spp. on establishment and yield of spring

wheat. Crop Protection Volume 96, June 2017, Pp 97-102.

Yousef, S.A, El-Metwally, M.M., Gabr, S.A and Al-Ghadir, A.H. (2013). New strategy for managing damping-off and root rot disease of cucumber caused by *Rhizoctonia solani* by seed soaking in formula of antioxidant with micronutrients. J Plant Pathol Microb 2013, 4:9 http://dx. doi.org/10.4172/2157-7471.1000196

Zhou Z., Chen X., Zhang M., and Blanchard C. (2014). Phenolics, flavonoids, proanthocyanidin and antioxidant activity of brown rice with different pericarp colors following storage. *J. Stored Prod. Res.* 59 120–125.

Zhou Z., Schenke D., Miao Y., and Cai D. (2017). Investigation of the crosstalk between the flg22 and the UV-B-induced flavonol pathway in .

Arabidopsis thaliana seedlings. *Plant Cell Environ.* 40 453–458.

Zhu, J.J., Li, Y.R. and Liao J. X. (2013). Involvement of anthocyanins in the resistance to chilling-induced oxidative stress in *Saccharum officinarum* L. leaves. *Plant Physiol. Biochem.* 73 427–433.

Zhuang, X.P.; Lu, Y.Y. and Yang, G.F. (1992): Extraction and determination of flavonoid in ginkgo. Chinese Herbal Medicine, 2: 122-124.

الملخص العربي

تأثير بعض المستخلصات النباتية الطبيعية وبعض مضادات الأكسدة على الإصابة ، وتلقيح بـ PGPR وأنظمة الدفاعي الانزيمية ضد أمراض تعفن الجذور في الطماطم

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في هذا البحث تمت دراسة تأثيرات الكائنات الحية الدقيقة المشجعة (PGPR) لنمو النبات وبعض المستخلصات النباتية الطبيعية وبعض مضادات الأكسدة على الإصابة بأمراض تعفن الجذور والإنزيمات الدفاعية للطماطم تحت ظروف الصوبة. أظهرت النتائج أن شدة مرض الطماطم في التربة المصابة بالفطر الفيوزاريوم سولانى F. solani وكذلك المصابة بالريزوكتونيا سولانى R.solani فى الموسم الاول الثانى انخفضت باستخدام PGPR مقارنة بالتربة غير الملقحة

تكون مستويات المركبات الفينولية الكلية (TPCs) في العينات غير المعاملة دائمًا أقل من تلك العبنات المعاملة.

تم تسجيل نفس الاتجاه أيضًا في حالة مجموع الفلافينويدات (TFs) كما تذبذب تركيز الكلوروفيل نتيجة العدوى و المعاملات ويمكن ترتيب فعالية المستخلصات النباتية تحت الدراسة بالترتيب التالي:الصفصاف ثم الريحان ثم المورينجا ثم الكافور.

أظهرت انشطة ثلاثة إنزيمات دفاعية مختلفة مثل الكتاليز والبيروكسيديز والبوليفينول أوكسيديز أن المعاملات أدت إلى انخفاض في مستويات أنشطة البوليفينول اوكسيديز (PPO) مقارنة بالعينة غير المعاملة باستثناء البيروجيلات (PG).

سجلت أنشطة الكتاليز في النباتات المعاملة بـتنات الصوديوم مع الفيوزاريوم سولانى أعلى مستويات لهذا الانزيم (1.810) يليها البيروجيلات مع الريزوكتنيا سولانى.

المركبات العضوية الخمسة المختبرة في هذه الدراسة لها نشاط عالي كمضادات اكسدة ويمكن ترتيبها كالتالي:البروجيلات ثم تنات الصوديوم ثم حامض الاسكوربيك ثم حامض السلسليك ثم بنزوات الصوديوم وكل هذه المواد الخمسة عبارة عن مركبات فينولية ولها جهد كمضادة للأكسدة.