Plant Archives Vol. 21, Supplement 1, 2021 pp. 1274-1280 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
doi link : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.201

INDUCTION OF SYSTEMIC RESISTANCE IN SOYBEAN USING SOME ECO-FRIENDLY MATERIALS AGAINST INFECTION OF *F. SOLANI* AND ITS EFFECT ON GERMINATION AND BIOCHEMICAL CHARACTERISTICS

Sawsan H. A. AL-Mayahi and Aalaa K. Hassan*
Department of plant protection, College of Agricultural engineering sciences, University of Baghdad, Iraq.
*Email: aalaammh@gmail.com

ABSTRACT

This study was conducted to evaluate the efficacy of some chemical and biological substances, individually or in combination in reducing fusarium root rot disease in soybeans caused by the fungus *Fusarium solani.* The results of laboratory evaluation showed that *A. chroococcum* bacteria achieved 58.90% inhibition by using dilution 10^{-7}. Whereas, the biochar scored 100% inhibition rate at a concentration 4%, while the potassium phosphate (K_2HPO_4) scored a complete and 100% inhibition of the growth of the fungus at of 200 mM concentration. At greenhouse conditions, the mixing treatment *A .chroococcum* with biochar was performed in the presence of pathogenic fungi, and treatment of K_2HPO_4 with *A.chroococcum* and biochar with pathogenic fungi, was the most efficient in reducing the incidence and severity of infection with *F. solan*i which was 0.00 and 0.00% respectively, which did not differ significantly from the treatment of K_2HPO_4 with *A.chroococcum* with pathogenic fungi, which was 6.67 and 3.33% respectively, compared to the percentage and severity of infection in the control treatment with, 76.67 and 75.00% respectively, as well as their cause of The fresh and dry weight of the plant was significantly increased among their treatments. The factors also showed an efficiency in inducing systemic resistance by increasing the peroxidase enzyme activity 7 and 14 days of adding the pathogen, compared to the treatment (without pathogenic fungi. The two mixing treatments between K_2HPO_4 with *A .chroococcum* and biochar with pathogenic fungi and the addition treatment of mixing *A .chroococcum* with biochar in the presence of the pathogen were 64.00, 70.00, 63.17 and 68.37 respectively the rate of change in photosynthesis / min / g fresh weight of plant leaves. The rate of change of plant leaves was successively, while the rate of change of the enzyme was achieved by the comparison treatment (without pathogenic fungi), which was 22.27 and 23.33 rate of change by photosynthesis / min / gm fresh weight of plant leaves respectively, followed by the remaining treatments.

Keywords **:** Soybean, *A. chroococcum*, Biochar, K_2HPO_4, *F.solani* , Induced systemic resistance.

Introduction

Soybean *Glycine max* (L.) Merrill, is one of the most important multi-use food crops for humans and animals (Kim *et al*., 2016). Soybean seed consists of 14–24% oil and 30 to 50% protein, It has a high nutrition value because it contains unsaturated fatty acids and most of the essential amino acids and some vitamins (Vahedi, 2011) Due to its multiple uses in many food industries for human consumption and it's use as a fodder crop, it ranked first in supplying animal feed with protein, and thus considered a food, industrial, fodder and fertilizer crop at the same time, Therefore it is called “the Miracle Crop" or "the gold that grows". (FAO, 2007; Al-Odeh *et al.*, 2009). The soybean crop is infected by many plant diseases that damage the shoot and root systems. The soil fungi are the most common pathogens that infect plant at various growth stages causing root rot and seedlings death (Navi & Yang, 2016; Abdelmajid *et al*., 2012 & Roth & Chilivers, 2019). *Fusarium solani* is the most widely spread and dangerous species in recent years (Diaz, 2012) This pathogen has a severe effect on plant parts under soil surface resulting in to a general weakness of the shoot system (Zheng *et al*., 2018). Numerous researches have demonstrated confirmed the negative effects of excessive use of chemical pesticides on human health and the environment, Therefore, the researchers focused on developing alternative methods of disease control that would reduce environmental pollution, such as using biological control (Adesemoye & Kloepper, 2009; Aboutorabi, 2018). Recently, attention has turned to a new type of biological control, which is known as induced resistance, which means stimulating the plant to resist the disease, whether by biotic or abiotic stimulating (Liorens *et al*., 2016). Matloob and Kim (2016) found that *A. chroococcum* bacteria could inhibit the pathogenic fungus *R. solan*i growth by 100% on PSA medium.On the other hand Jaiswal *et al*. (2018) indicated that the use of biochar at a concentration 3% resulted in improving cucumber plant resistance against root rot disease caused by *Pythium aphanidermatum*. El-Fawy and El-Said (2018) also found that foliar spraying of sesame plants with K_2HPO_4 at a concentration 100 mM reduced the severity of leaf spot disease caused by *Helminthosporium sesami*in in addition to increasing plant productivity .Therefore, this study aimed to evaluate the efficiency of some resistance induction agents in protecting soybean seedlings from infection by the pathogenic fungi and their effect on the growth of soybean plants.

Materials and Methods

Isolation, diagnosis and testing of pathogenicity of *F. solani:*

F. solani was isolated from the roots of soybean plants that showed root rot symptoms *F. solani* was diagnosed using taxonomic keys described by (Booth, 1977; Rezaee *et al*., 2018). The pathogenic fungus inoculum was prepared according to Dewan method (1989) using the millet seeds *Panicum miliaceum* The pathogenicity of the pathogenic fungus was tested using a sterile mixture consisting of agricultural soil and pitmos at (1: 1) ratio. Pathogenic fungus inoculum grown on millet seeds, was added to sterile soil at 1% (w / w).

Antagonistic ability test of *A. chroococcum* against *F. solani* under Laboratory conditions

A local isolation of *A. chroococcum* bacteria was obtained from the Central Health Laboratory/Department of Soil and Water Resources/College of Agricultural Engineering Sciences/University of Baghdad. The antagonistic ability of bacteria was tested against pathogenic fungi by taking a swab of bacteria grown on nutrient agar (NA) at the age of 48 hours with sterilized inoculating needle and streaking the bacteria about 2 cm from one edge of the dish. Then, 5mm diameter discs from the margin of the pathogenic fungus colony grown on PDA were sliced and placed about 3.5 cm from the bio-agent line and 3.5 cm from the other edge of 9 cm diam Petri dish. Each treatment was triplicated. The dishes were incubated for 5 days at 2 ± 25 °C than the percentage of the biological control effectiveness was calculated using the following formula:

Biological control effectiveness = A / A + B x 100

A = the distance between the bacteria line and the end of the fungal growth.

B= the fungal expansion towards the bacteria line

The inhibitory and competitive efficacy of the bacteria against the pathogenic fungus was tested by growing bacteria on the culture medium NA at 25±2 °C for 24 hours. Sequential decimal dilutions were performed to mitigate with 10^{-9}. One ml of each dilution was taken and placed in a sterile petri dish, then about 15-20 ml of the PDA culture medium was added in each dish with three repetitions for each dilution, distilled water was added as a control treatment. The Petri dishes were moved in a rotational movement, and after the hardening of the culture medium, each dish was inoculated with 5mm diameter disc taken from the edges of the a 5 days colony of the fungus *F. solani* grown on the culture medium PDA. The dishes were incubated at a temperature of 2 ± 25 °C. Each colony in dishes was measured every 24 hours. When the control dishes were filled with the fungal growth, the percentage of inhibition was calculated according to the following formula:

% inhibition = (mean of fungal growth diameter in control-mean of fungal growth diameter in treatment/ mean of fungal growth diameter in control) × 100

Effect of biochar on the growth of *F. solani*

The effect of biochar on the growth of the pathogenic fungus was tested using 5 concentrations 1, 2, 3, 4 and 5%. In addition to the control treatment, the concentration was prepared by adding the biochar to culture medium with a good shaking of flasks before the hardening of medium. The center of each dish was inoculated with 0.5 cm disk taken from the edge of the *F. solani* pathogen colony. The control treatment included was inoculated pathogenic fungi without adding biochar to the medium. The dishes were incubated at a 2± 25 °C for 5 days. The experiment was conducted in a completely randomized design with four, replicates the percentage of inhibition was calculated according to the previously mentioned formula.

Effect of K_2HPO_4 on the growth of *F. solani*

The effect of K_2HPO_4 on the growth of pathogenic fungi and biotic stimulating agent (*A .chroococcum*) was tested using 4 concentrations (0 , 50. 100 ,200) mM The chemical compound was sterilized by passing through 0.45µm sterile filters.K_2HPO_4 concentrations were added to the PDA medium with a good mixing separately homogenize them, than the medium poured into sterile 9cm diameter Petri dishes. Then dishes were inoculated with *F. solani* grown on the PDA medium. The dishes were incubated at 2± 25 ±°C for 5 days. The experiment was conducted in a completely randomized design with four replicates, the percentage of inhibition was calculated according to the previously mentioned formula.

Field evaluation of systemic inducing resistance agents against *F. solani* and the effect on some growth traits

The experiment was carried out in a plastic green house at Pesticide Laboratory / Agricultural Protection Department / Ministry of Agriculture, a mixture of loamy soil and pitmos (2: 1) ratio was autoclaved for 20 minutes for two consecutive times at an interval of 24 hours for each sterilization .Then the soil was distributed in plastic pots of 30 cm diameter at a rate of 4 kg / pot. The experiment included the following treatments:

1. only Sterile soil
2. Sterile soil + *F. solani*
3. Sterile soil + K_2HPO4
4. Sterile soil + Biochar
5. Sterile soil + *A .chroococcum*
6. Sterile soil + K_2HPO_4 + *F. solani*
7. Sterile soil + Biochar + *F. solani*
8. Sterile soil + *A .chroococcum* + *F. solani*
9. Sterile soil + K_2HPO_4 + Biochar + *F. solani*
10. Sterile soil + *A .chroococcum* + K_2HPO_4 + *F. solani*
11. Sterile soil + Biochar+ *A .chroococcum*+ *F. solani*
12. Sterile soil + K_2HPO_4 + Biochar+ *A. chroococcum* + *F. solani*

The soil was contaminated with 40 g per pot of the pathogen inoculum growing on millet seeds. Soybean Lee variety seeds were sterilized with sodium hypochlorite solution (2.0 % of free residual chlorine) for two minutes, then washed with sterile water Planting was done by placing 5 seeds/ pot. The fungal inoculum was added 15 days of planting. As for the control treatment (only sterile soil), it included adding millet seeds that were not contaminated with pathogenic fungi.

Inducing resistance agents against pathogenic fungi were added to soil at planting. K_2HPO_4 was applied at a concentration 200 mM at a rate of 50 ml / pot. While biochar was added at a concentration 4% at a rate of 160 g / pot by mixing it with the soil before planting. Whereas, the bacterial inoculum *A. chroococcum* was added at a rate of 100 ml / pot

of concentration 5×10^8 (CFU/mL) taken from a 48- hour old bacterial culture. The experiment was conducted using a completely randomized design with 12 treatments and three replicates per treatment. Seedlings death was measured after 4 days of contamination with fungal inoculum. The percentage of seedlings showing root rot symptoms was calculated according to Stanghellini and Phillips, (1975).

$$\% \text{ of seedlings death} = \frac{\textit{No. of dead seedlings}}{\textit{Totale number}} \times 100$$

Disease severity of infection was also expressed as a disease index rated on a 0–4 scale for root rots disease according to Alwan, (2014)where:

Healthy plants= 0

1- 25% of the root rot = 1

25 – 50% of the root rot = 2

50 - 75% of the root rot= 3

75 – 100% of the root rot = 4

The final disease severity index (DSI) for each pot was calculated according to McKinney (1923), by the following equation:

$$\%\text{disease severity} = \frac{No.of\ plants\ \ of\ 0\ degree\ \ X0+\cdots.+No.of\ plants\ \ of\ 5\ degree\ \ x\ 5}{No.of\ \ tested\ \ plants\ \ x\ 5} \times 100$$

The percentage of seed germination was calculated by using the following equation:

Fresh and dry weights of soybean plants were measured directly.

Samples were taken from plant leaves treated with systemic resistance stimulating agents at 7, 14 and 21 days after adding the pathogenic fungi in order to estimate peroxides enzyme activity according to Hammershildt *et al.* (1982) method

All data were subjected to analysis of variance, and the treatment means were compared by least significant difference (LSD) at 0.05 level of probability.

Results and Discussion

Pathogenicity test of *F. solani*

The results in (table (1)and Fig (1)) showed that the isolation of the fungus caused a significant decrease in the seed germination percentage of soybean under greenhouse conditions, when germination percentage was33% in the presence of pathogenic fungi compared to 100% germination percentage in control treatment (without pathogenic fungi)The pathogen's ability to reduce the germination percentage may be attributed to enzymes that digest pectin substances (Lozovaya *et al*., 2006) as well as metabolic compounds produced by the fungus (Zheng *et al*., 2018; Toghueo, 2019).

Table 1 : Pathogenicity test for isolation of *F. solani* fungi inpots

% germination	Treatments
100	Control
3.3	*F.solani*
9.25	L.S.D $_{0.05}$

• Each number represents the average of 3 replicates

Fig. 1 : Pathogenesis of *F.solani* fungus in middle WaterAgar on soybean seeds

Antagonistic ability test of *A. chroococcum* against *F. solani*

The results (Table (2) and Fig (2)) showed the efficiency of bacteria *A. chroococcum* when inhibited *F. solani* by 58.90% compared to 0% for control treatment. The effect of using these bacteria on inhibiting the growth of pathogenic fungi is due to the ability of these bacteria to produce metabolites and organic compounds and the production of indole acetic acid and some enzymes and antibiotics and the production of HCN and others (Zarrin *et al*., 2009; Herter *et al*., 2011 and Paul *et al*., 2014). In addition to its high ability to compete with pathogens for place and food (Hillel, 2005). Hence this result is consistent with what be found (Mali & Bodhankar, 2009; Mali *et al*., 2011) who demonstrated the ability of these bacteria to inhibit the growth of pathogens, especially *F. solani*.

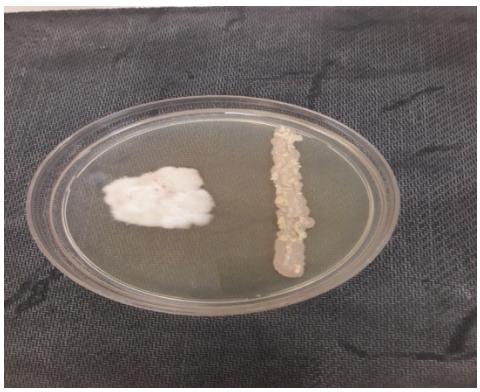

Fig. 2 : Effect of *A. chroococcum* on the growth of the pathogen on the PDA culture medium

Table 2: Test of antibacterial *A. chroococcum* against pathogenic fungus *F. solani*

% Inhibition	Treatments
58.90	*A. chroococcum*
00.00	control
17.73	L.S.D$_{0.05}$

• Each number represents the average of 3 replicates

The inhibitory effect of plant biochar concentrations on radial growth of the pathogenic fungus

Biochar prepared from *Eucalyptus* inhabited fungal growth significantly when fungal growth was 0.00 at 4% , 5% concentration compared to 9 cm for control treatment (Fig. 3). In this study, application of biochar could reduce the aver-age of fungal growth in all treatments. The biochar mode of action against the pathogenic fungus in PDA medium could be through its high adsorption ability. Biochar

can adsorb nutrients and elements which affects the permeability of mycelium cell membranes and inhabits the mycelium growth (Elad *et al*., 2010; 2011; Elmer & Pignatello 2011; Jaiswal, 2014; Hassan, 2017; Al-Luhaiby, 2020).

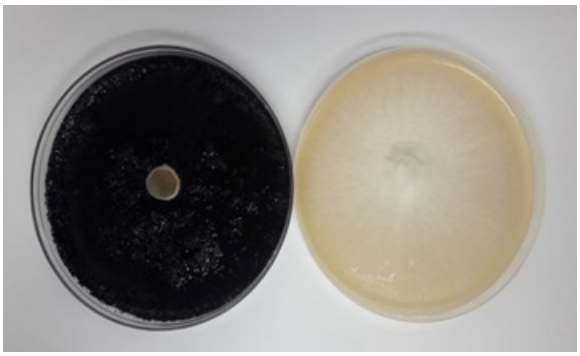

Fig. 3 : The effect of biochar on the growth of pathogenic fungi on PDA

Table 3 : The effect of biochar on the average of fungal mycelium growth on PDA medium

%(Inhibition)	Colony diameter (cm)	% (Concentrations)
0.00	9.00	0
37.77	5.60	1
68.61	2.82	2
87.22	1.15	3
100.00	0.00	4
100.00	0.00	5
4.43	0.39	L.S.D0.05

• Each number represents the average of 3 replicates

The effect of K_2HPO_4 against *F. solani* growth in laboratory

K_2HPO_4 concentration at 200 mM was the best when inhibited *F. solani by* 100% and reduced fungal grown on PDA up to 0.00 cm (Table 4, Fig 4). The ability of potassium phosphate to control the pathogen directly is due to its possession of anti-substances that inhibit the growth of fungal colonies, as it destroys fungal hyphae and new spores which preventing their spread (Reuveni *et al.*, 1998; Arslan, 2015). Several studies have indicated the efficiency of potassium phosphate in growth inhibition of pathogens, including *Fusarium solani* and, *Rhizoctonia solani*, and *Sclerotinia rolfsii* (Abdel-Ghany, 2008; Abdel-Kader *et al.*, 2012b; El-Mohamedy *et al.*, 2014; Jabnoun-Khiareddine *et al*., 2016).

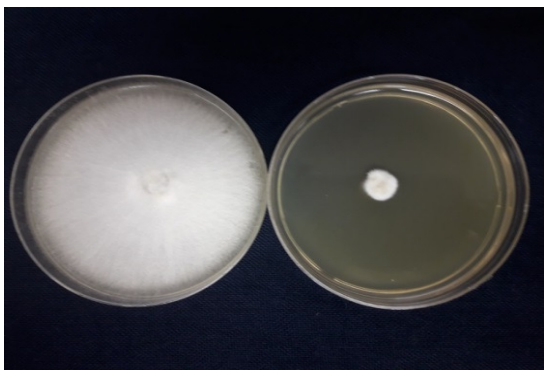

Fig. 4 : The effect of K_2HPO_4 on the growth of the pathogen *F. solani* on the PDA culture medium

Table 4 : Effect of K_2HPO_4 mycelial growth of *F. solani* in laboratory

Inhibition (%)	Colony diameter (cm)	Concentrations (mM)
0.0	9.00	0
46.1	4.58	50
85.8	1.27	100
100.0	0.00	200
6.21	0.55	$L.S.D_{0.05}$

• Each number represents the average of 3 replicates

Evaluation of the efficiency of some biological and chemical stimuli in reducing germination of soybean plants treated with *F. solani* under greenhouse conditions

The results in Table (5) showed that the two treatment of mixing *A .chroococcum* with charcoal in the presence of pathogenic fungi and treatment of K_2HPO_4 with *A. chroococcum* and biochar with pathogenic fungi were the most efficient in reducing the infection incidence and severity of *F.solani* when incidence and severity were 0.00 0.00%, respectively, followed by treatment of K_2HPO_4 with *A. chroococcum* with pathogen, which were 6.67 and 3.33% respectively, compared to the infection rate and severity of infection in the comparison treatment, which were 76.67 and 75.00% respectively.

Numerous studies have indicated that these compounds have high efficacy in controlling many plant pathogens (Kareem, 2014; Abdel-Monaim *et al*., 2015; Jabnoun-Khiareddine *et al.*, 2016; Juber *et al.,* 2016; Hashem *et al.*, 2017; Hasaan, 2017; Jaiswal *et al.*, 2019).

The two treatments of *A. chroococcum* with biochar added were to the best against the presence of the pathogen by scoring the highest increase in the average fresh and dry weight, which was 22.30 and 5.50 g / plant, respectively, whereases the treatment of K_2HPO_4 with *A. chroococcum* with the pathogen, which was 21.70 and 5.03 g/plant, respectively. The sequence, which was not significantly differentiated from the treatment of mixing K_2HPO_4 with *A. chroococcum* and biochar with pathogenic fungi, was 20.26 and 5.00 g / plant respectively, followed by the remaining other treatments (Figure 1).

Table 5 : The effect of some chemical and biological compounds on the percentage of severity of infection with *F.solani* under greenhouse conditions.

% Disease severity	infection %	Treatment
0.00	**0.00**	Control
75.00	76.67	(FS) *F. solani*
0.00	0.00	K_2HPO_4
0.00	0.00	(AC) *A .chroococcum*
0.00	0.00	(E) Eucalptase Biochar
28.30	36.67	K_2HPO_4 +FS
16.67	26.67	AC + FS
18.33	33.33	E + FS
3.33	6.67	K_2HPO_4 +AC + FS
10.00	10.00	K_2HPO_4 +E +FS
0.00	0.00	AC + E + FS
0.00	0.00	K_2HPO_4 + AC +E+ FS
8.88	**6.28**	$L.S.D_{0.05}$

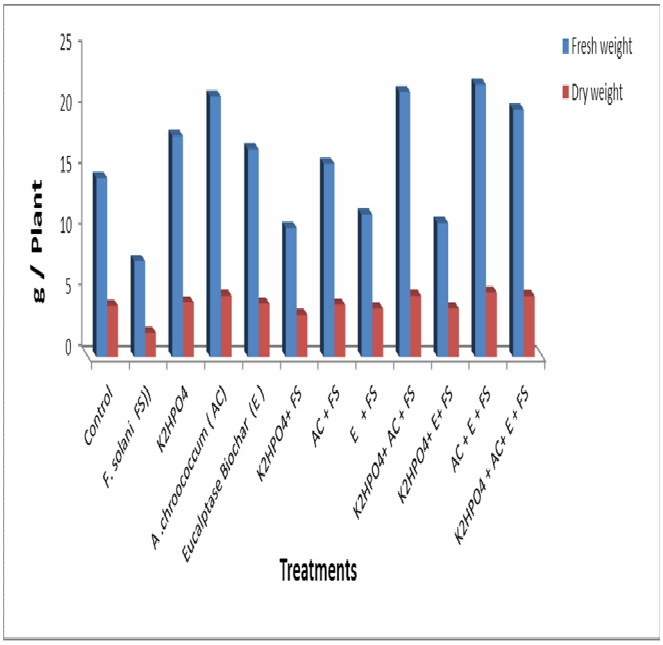

Fig. 1 : The effect of some chemical and biological materials on the fresh and dry weight of soybean plants under potting conditions

*Each number in the table is a rate of 3 replicates

1.49 = Fresh weight **L.S.D** $_{0.05}$

0.50 = Dry weight L.S.D $_{0.05}$

The reason for the efficiency of *A. chroococcum* bacteria in reducing the percentage of infection and severity of the pathogen may be due to its direct effect on plants by fixing atmospheric nitrogen and increasing the availability of phosphorous as well as the production of hydrogen cyanide (HCN) and vitamins (Gurikar *et al*., 2016).

Besides the production of plant hormones that either have an inhibitory effect or stimulate the phycological processes in the plant and microorganisms, including Gibberellins, Cytokinins and Auxins like Indole Acetic Acid (IAA), which stimulate plant growth and increase its efficiency in absorbing nutrients (Wani, *et al*, 2016). Moreover, it is due to its ability to produce some other microorganism-degrading enzymes (Catalase, Amylase, Esterase, Phosphatase, Phenol Oxidase, Peroxidase and Nitrogenase and the accumulation of phenolic compounds (Herter *et al*., 2011; Alwan and Hussein, 2019).

Kareem (2014) indicated that *A. chroococcum* has a high efficiency in controlling the pathogen *R. solani* that causes soybean root rot, and it also reduced the incidence and severity of disease under potting conditions as well as increased plant growth parameters such as plant height and. fresh and dry weights. While the efficiency of phytophthora is attributed to reducing the percentage and severity of infection through its direct effect as an inhibitory substance against plant pathogens and works to absorb allelopathic compounds toxic to plants or indirectly by improving the properties and quality of the soil in terms of the availability of nutrients that are easy to be absorbed by the plant as well as strengthening and abundance of beneficial microbes in soil (Elad *et al*. 2010; Bonanom *et al*. 2015; Akhter *et al*., 2015). Jaiswal *et al*. (2019) confirmed that the use of biochar reduced the severity of *P. aphanidermatum* infection that causes root rot and death of cucumber seedlings by increasing the microbial activity in the soil, absorbing toxins and metabolites and inhibiting the enzymes secreted by the fungi. While Abdel-Monaim *et al* (2015) showed that the use of K_2HPO_4 potassium phosphate reduced the percentage and severity of infection with the fungi that cause suger beet root rot, including *F. solani* and *F. oxysporum.*

Matloob and Alkim (2016) demonstrated the effectiveness of bacteria *A. chroococcum* against cotton seedling diphtheria caused by the fungus *R. soloni*, which increased the average root and shoot height and the fresh and dry weights of the plant. In a study conducted by Jaiswal and others (2017), it was shown that the use of biochar contributed to control *Fusarium* root rot disease on tomatoes, which resulted in an increase in growth indicators such as fresh and dry weight and plant high. These results are consistent with what was reported by El-Fawy and El-Said (2018) that spraying sesame plants with potassium phosphate K_2HPO_4 at a 100 mM concentration resulted in the control of sesame leaf spot disease caused by the fungus *Helminthosporium sesami* as well as increased plant productivity and some growth indicators such as increased plant height, fresh and dry weight, number of pods, and seed yield.

The results also indicated the presence of significant differences in the rate of activity of the peroxidase enzyme, estimated based on the rate of change in photosynthesis / minute / gram, fresh weight in soybean plants, Figure (2). As all the treatments outperformed the rate of enzyme activity on the treatment of *F. solani* after 7 and 14 days of contamination with the pathogen, which scored 35.00 and 38.10 respectively, while the highest enzyme rate was scored in the two K_2HPO_4 with *A. chroococcum* and biochar combination treatments with the pathogenic fungus included. The addition of *A. chroococcum* with biochar combination in the presence of the pathogen, scored 64.00, 70.00, 63.17 and 68.37, respectively, followed by other treatments. After that, the activity of the enzyme began to decrease gradually on day 21, but it remained significantly effective compared to the control treatment.

It is noticed from the above results the close relationship between the increase of th enzyme's effectiveness and the induced resistance. This was obvious in reducing the infection rate of the pathogen *F. solani* Then inhibiting the process of breaking down the cell wall, and the products that break down. The enzymes of pectinase act as stimulatory signals in the plant in response to the biological stresses represented by the presence of pathogens. They were triggering the sequential induction of many chemical defense means, including the building of Phytoalexins as well as the structural defenses by interacting the enzyme peroxidase with some proteins the cell wall to form cross-linkages and multiple compounds, which increases the rigidity of the cell wall, as peroxidase is a defense-related protein called PR-q (Almagro *et al*. 2009; Thakker *et al*., 2013; Siqueira *et al*., 2019)

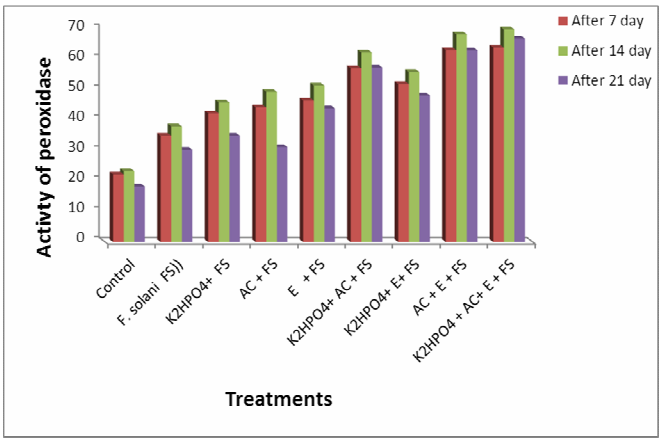

Fig. 2: The effect of different treatments on peroxidase activity (absorption value/min./g fresh weight)

• Each number represents the average of 3 replicates

L.S.D $_{0.05}$ After7 day =2.70

L.S.D $_{0.05}$ After 14 days = 2.22

L.S.D $_{0.05}$ After 21days = 2.56

References

Abdel-Ghany, R.E.A. (2008). Induced resistance for controlling root-rot disease of strawberry and their side effects on biological activities in soil (Doctoral dissertation, Ph. D. Thesis, Bot. Agric. Dept., Fac. Agric., Moshtohor, Benha Univ., Egypt.

Abdel-Kader, M.M.; El-Mougy, N.S.; Aly, M.D.E. and Lashin, S.M. (2012). Integration of biological and fungicidal alternatives for controlling foliar diseases of vegetables under greenhouse conditions. International Journal of Agriculture and Forestry. 2(2): 38-48.

Abdelmajid, K.M.; Ramos, L.; Leandro, L.; Mbofung, G.; Hyten, D.L.; Kantartzi, S.K. and Meksem, K. (2012). The 'PI 438489B'by 'Hamilton'SNP-based genetic linkage map of soybean [*Glycine max* (L.) Merr.] identified quantitative trait loci that underlie seedling SDS resistance.

Abdel-Monaim, M.F.; Atwa, M.A.M. and Morsy, K.M. (2015). Induce Systemic Resistance against Root Rot and Wilt Diseases in Fodder Beet (*Beta vulgaris* L. var. rapacea Koch.) by Using Potassium Salts. J Plant Pathol Microbiol, 6 (315): 2.

Aboutorabi, M. (2018). A Review on the Biological Control of Plant Diseases using Various Microorganisms. Journal of Research in Medical and Dental Science, 6(4): 30-35.

Adesemoye, A.O. and Kloepper, J.W. (2009). Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied microbiology and biotechnology, 85(1): 1-12.

Akhter, A.; Hage-Ahmed, K.; Soja, G. and Steinkellner, S. (2015). Compost and biochar alter mycorrhization, tomato root exudation, and development of *Fusarium oxysporum* f. sp. *lycopersici*. Frontiers in plant science, 6: 529.

Al-Luhaby, A.K. and Hassan, A.K. (2020). Evaluation the ability of some organic compounds is protecting bean seedling against infection with *Rhizoctonia solani*. Journal of Plant Archives, 20(1): 320-331.

Almagro, L.; Ros, L.G.; Belchi-Navarro, S.; Bru, R.; Barceló, A.R. and Pedreño, M. (2009). Class III peroxidases in plant defence reactions. Journal of Experimental Botany. 60(2): 377-390.

Al-Odeh, A.A.; Hadid, M.L. and Nimer, Y. (2009). Oil and sugar crops and their technology. Faculty of Agricultural Engineering / University of Damascus 225-310.

Alwan, K.F. (2014). Activity of some biocontrol agents against *Fusarium solani* and *Macrophomina phaseolina* caused cucumber root rot disease in Babylon Province. AL–Muthanna Journal of Agricultural Science, 2(2): 1-22.

Alwan, S.L. and Hussein, H.N. (2019). Efficacy of ecofriendly biocontrol *Azotobacter chroococcum* and *Lac-tobacillus rhamnosus* for enhancing plant growth and reducing infection by Neoscytalidium spp. in fig (*Ficus carica* L.) saplings. Journal of Kerbala for Agricultural Sciences, 6(1): 16-25.

Atef, M.N. and Haikel, N. (2008). Efficacy of Seed Treatment with Microbial Agents And/or Waste Products for the control of Cucumber Damping – off Botany Department, Faculty of Science, Cairo University, Egypt.

Bonanomi, G.; Ippolito, F. and Scala, F. (2015). A " black" future for plant pathology Biochar as a new soil amendment for controlling plant diseases. Journal of Plant Pathology, 97:(2).

Booth, C. (1977). Fusarium laboratory guide to the identification of the major species. Commonwealth Mycological Institute Kew, Surrey, England. 58 pp.

Dewan, M.M. (1989). Identify and frequency of occurrence of fungi in root of wheat and ryegrass and their effect on take – all and hostgrowth. Ph.D. Thesis. Univ. West Australia. 210pp.

Diaz Arias, M.M. (2012). *Fusarium* species infecting soybean roots: Frequency, aggressiveness, yield impact and interaction with the soybean cyst nematode.

Elad, Y., David, D.R.; Meller Harel, Y.; Lew, B. and Graber, E.R. (2010). The biochar effect: plant resistance to biotic stresses. Phytopathol. Mediator. 50: 335-349.

El-Fawy, M.M. and El-Said, M.A.A. (2018). Effect of Foliar Application of some Zinc and Phosphorus Sources on Controlling Helminthosporium Leaf Spot Disease and Production of Sesame. Journal of Plant Protection and Pathology, 9(3): 201-207.

El-Mohamedy, R.S.R.; Jabnonn-Khiareddine, H. and Daami-Remadi, M. (2014). Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers .Tunisian Journal of Plant Protection, 9: 45-55.

FAO, World Food and Agriculture Organization. 2007. The development of soybeans in Africa as a weapon to combat malnutrition and improve food security. Bulletin No. 401

Gurikar, C.; Naik, M.K. and, Sreenivasa, M.Y. (2016). *Azotobacter*: PGPR activities with special reference to effect of pesticides and biodegradation. In Microbial inoculants in sustainable agricultural productivity, 229-244.

Hammerschmidt, R.; Nuckles, F. and Kuc, J. (1982). Association of enhance activity with induced systematic resistance of cucumber to Colletotricum lagenrium. Physiol. plant pathol. 20:73-82.

Hashem, M.Z.; Samir, S.H. and Hassan, A.K. (2017). Detect activity of some biological factors to induce resistance in cantaloupe plant through peroxidase enzyme, phenqls and chlorophyll contents. The Iraqi Journal of Agricultural Science, 48(5): 1239-1246.

Hassan, A.K. (2017). Induction of Systemic Resistance of Eggplant against *Sclerotinia Sclerotiorum* infection using Bio char and bio – health. 14(4): 653 – 661.

Herter, S.; Schmidt, M.; Thompson, M.L.; Mikolasch, A. and Schauer, F. (2011). Study of enzymatic properties of phenol oxidase from nitrogen- fixing *Azotobacter chroococcum*. ABM Express. 1-14.

Hillel, D. (2005). Plant Growth Promoting Bacteria. Elsevier, Oxford, U. K., 103-115.

Jabnoun-Khiareddine, H.; Abdallah, R.; El-Mohamedy, R.; Abdel-Kareem, F.; Gueddes-Chahed, M.; Hajlaoui, A. and Daami-Remadi, M. (2016). Comparative efficacy of potassium salts against soil-borne and air-borne fungi and their ability to suppress tomato wilt and fruit rots. Journal of Microbial and Biochemical Technology, 8(2): 45-55.

Jaiswal, A.K.; Graber, E.R.; Elad, Y. and Frenkel, O. (2019). Biochar as a management tool for soilborne diseases affecting early stage nursery seedling production. Crop Protection. 120: 34-42.

Jaiswal, A.K.; Elad, Y.; Paudel, I.; Graber, E.R.; Cytryn, E. and Frenkel, O. (2017). Linking the belowground microbial composition, diversity and activity to soilborne disease suppression and growth promotion of tomato amended with biochar. Scientific reports. 7: 44382.

Juber, K.S.; Al-Juboory, H.H. and Al-Juboory, S.B. (2016). Identification and control of strawberry root and stalk rot in Iraq. International Journal of Environmental and Agriculture Research, 2(2): 54-63.

Kareem, F.H. (2014). Efficiency of some bioilogical factors and plant extractas against the fungus rhizoctonia solani kuhn reasoned root rot disease of soybean. Euphrates Journal of Agriculture Science. 6(4): 183-197.

Kim, E.; Hwang, S. and Lee, I. (2016). SoyNet: a database of co-functional networks for soybean (*Glycine max* L.). Nucleic Acids Res. (1): 1-13.

Liorens, E., Agustín, P.G. and Lapena, L. (2016). Advances in induced resistance by natural compounds: towards new options for woody crop protection. Scientia Agricola. 74: 90-100.

Lozovaya, V.V.; Lygin, A.V.; Zernova, O.V.; Li, S.; Wind Holm., J.M. and Hartman, G.L. (2006). Lignin degradation by *Fusarium solani*. Plant Dis. 9:77-82.

Mali, G.V. and Bodhankar, M.G. (2009). Antifungal and phytohormone production potential of *Azotobacter chroococcum* isolates from groundnut (*Arachis hypogea* L.). Asian J. Exp. Sci. 23: 293-297.

Mali, G.V.; Patil, R.C. and Bodhankar, M.G. (2011). Antifungal and Phytohormone Production Potential of *Azotobacter Chroococcum* Isolates From Groundnut (*Arachis Hypogea* L.) Rhizosphere and Their Effect on Nodulation and Dry Mass, Alongwith Native Rhizobia in Pot Culture Experiment. Research Journal of Chemistry and Environment Vol: 15, 2.

Matloob, A.A. and Al-Kim, F.A.A. (2016). Evaluation of the efficacy of *Azotobacter chroococcum* and *Trichoderma* spp. In the control of cotton seed diphtheria, caused by the fungus Kühn *Rhizoctonia solani*. Babylon University Journal / Pure and Applied Sciences: Issue 4 (24): 1002-1024.

Mckinney, H.H. (1923). Biological control of nematode pests by natural enemies. Ann. Rev. Pytopathol. 18: 415-440.

Navi, S.S. and, Yang, X.B. (2016). Impact of crop residue and corn-soybean rotation on the survival of *Fusarium virguliforme* a causal agent of sudden death syndrome of soybean. J. Plant Pathol. Microbiol, 7: 330-336.

Paul, S.; Bandeppa Aggarwal, C.; Thakur, J.K.; Rathi, M.S. and Khan, M.A. (2014). Effect of salt on growth and plant growth promoting activities of *Azotobacter chroococcum* isolated from saline soils. Environ Ecol. 32(4): 1255-1259.

Rezaee, S.; Gharanjik, S. and Mojerlou, S. (2018). Identification of *Fusarium solani* f. sp. *cucurbitae* races using morphological and molecular approaches. Journal of Crop Protection, 7(2): 161-170.

Rogovska, N.; Laird, D.; Leandro, L. and Aller, D. (2017). Biochar effect on severity of soybean root disease caused by *Fusarium virguliforme*. Plant and soil, 413.1-2: 111-126.

Roth, M.G. and, Chilvers, M.I. (2019). A protoplast generation and transformation method for soybean sudden death syndrome causal agents *Fusarium virguliforme* and *F. brasiliense*. Fungal biology and biotechnology, 6(1): 7.

Siqueira, I.T.D.; Cruz, L.R.; Souza-Motta; C.M.; Medeiros, E.V.; Moreira, K.A. (2019). Induction of acibenzolar-S-methyl resistance in cowpea to control anthracnose. Summa Phytopathologica, 45(1): 76-82.

Stanghellini, M.E. and Phillips, J.M. (1975). *Pythium apanidermatum*: It's Occurrence and control with pyroxychlor in the arabian desert at abu-dhabi. Plant Dis. Repor., 59: 559-563.

Thakker, J.N., S. Patel, P.C. Dhandhukia . 2013. Induction of defense-related enzymes inbanana plants: effect of live and dead pathogenic strain of *Fusarium oxysporum* f.sp. *cubense*. ISRN Biotech. 13:1

Toghueo, R.M.K. (2019). Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. Mycology, 1-21.

Vahedi, A. (2011). The effects of micronutrient application on soybean seed yield and on seed oil and protein content. J. Amer. Sci. 7(6): 44-49.

Wani, S.A., Chand, S.; Wani, M.A.; Ramzan, M. and Hakeem, K.R. (2016). *Azotobacter chroococcum*–a potential biofertilizer in agriculture: an overview. In Soil Science: Agricultural and Environmental Prospectives. pp:333-348. Springer, Cham.

Zarrin, F.; Saleemi, M.; Zia, M.; Sultan, T.; Aslam, M.; Rehman, R. and Chandhary, M.F. (2009). Anti Fungal activity of plant growth promoting Rhizobactera isolates against *Rhizoctonia solani* in wheat. African J. of Biotechnol. 8(2): 219-225.

Zheng, N.; Zheng, L.P.; Ge, F.Y.; Huang, W.K.; Kong, L.A.; Peng, D.L. and Liu, S.M. (2018). Conidia of one *Fusarium solani* isolate from a soybean-production field enable to be virulent to soybean and make soybean seedlings wilted. Journal of integrative agriculture. 17(9): 2042-2053.