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

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**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$_2$HPO$_4$) 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$_2$HPO$_4$ with *A.chroococcum* and biochar with pathogenic fungi, was the most efficient in reducing the incidence and severity of infection with *F. solani* which was 0.00 and 0.00% respectively, which did not differ significantly from the treatment of K$_2$HPO$_4$ 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$_2$HPO$_4$ 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$_2$HPO$_4$, *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 & Chilvers, 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 & Kloeper, 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 (Liores *et al*., 2016). Matloob and Kim (2016) found that *A. chroococcum* bacteria could inhibit the pathogenic fungus *R. solani* 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$_2$HPO$_4$ at a concentration 100 mM reduced the severity of leaf spot disease caused by *Helminthosporium sesami* 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 diameter Petri dish. Each treatment was replicated. 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:

\[
\text{Biological control effectiveness} = \frac{A}{A+B} \times 100
\]

\[A=\text{the distance between the bacteria line and the end of the fungal growth.}\]

\[B=\text{the fungal expansion towards the bacteria line}\]

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 10⁻⁴. 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:

\[
\% \text{ inhibition} = (\text{mean of fungal growth diameter in control-mean of fungal growth diameter in treatment/ mean of fungal growth diameter in control}) \times 100
\]

**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 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 K₂HPO₄ on the growth of *F. solani***

The effect of K₂HPO₄ 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. K₂HPO₄ 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 greenhouse 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 + K₂HPO₄
4. Sterile soil + Biochar
5. Sterile soil + *A. chroococcum*
6. Sterile soil + K₂HPO₄ + *F. solani*
7. Sterile soil + Biochar + *F. solani*
8. Sterile soil + *A. chroococcum + F. solani*
9. Sterile soil + K₂HPO₄ + Biochar + *F. solani*
10. Sterile soil + *A. chroococcum + K₂HPO₄ + F. solani*
11. Sterile soil + Biochar + *A. chroococcum + F. solani*
12. Sterile soil + K₂HPO₄ + 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. K₂HPO₄ 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 × 10⁸ (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).

\[
\% \text{ of seedlings death} = \frac{\text{No. of dead seedlings}}{\text{Total number}} \times 100
\]

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:

\[
\% \text{disease severity} = \frac{\text{No. of plants of 0 degree} - \text{No. of plants of 5 degree} \times 5}{\text{No. of treated plants} \times 5} \times 100
\]

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 was 93% 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 in pots**

<table>
<thead>
<tr>
<th>% germination</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Control</td>
</tr>
<tr>
<td>3.3</td>
<td>F. solani</td>
</tr>
<tr>
<td>9.25</td>
<td>L.S.D 0.05</td>
</tr>
</tbody>
</table>

*Each number represents the average of 3 replicates*

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

**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**

<table>
<thead>
<tr>
<th>% Inhibition</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>58.90</td>
<td>A. chroococcum</td>
</tr>
<tr>
<td>00.00</td>
<td>control</td>
</tr>
<tr>
<td>17.73</td>
<td>L.S.D 0.05</td>
</tr>
</tbody>
</table>

*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 average-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 inhibits the mycelium growth (Elad et al., 2010; 2011; Elmer & Pignatello 2011; Jaiswal, 2014; Hassan, 2017; Al-Luhaiby, 2020).

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

<table>
<thead>
<tr>
<th>% (Inhibition)</th>
<th>Colony diameter (cm)</th>
<th>% (Concentrations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>9.00</td>
<td>0</td>
</tr>
<tr>
<td>37.77</td>
<td>5.60</td>
<td>1</td>
</tr>
<tr>
<td>68.61</td>
<td>2.82</td>
<td>2</td>
</tr>
<tr>
<td>87.22</td>
<td>1.15</td>
<td>3</td>
</tr>
<tr>
<td>100.00</td>
<td>0.00</td>
<td>4</td>
</tr>
<tr>
<td>100.00</td>
<td>0.00</td>
<td>5</td>
</tr>
<tr>
<td>4.43</td>
<td>0.39</td>
<td>L.S.D0.05</td>
</tr>
</tbody>
</table>

*Each number represents the average of 3 replicates*

**The effect of K$_2$HPO$_4$ against F. solani growth in laboratory**

K$_2$HPO$_4$ concentration at 200 mM was the best when inhibited F. solani by 100% and reduced fungal growth 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-Mohameda et al., 2014; Jabnoun-Khiareddine et al., 2016).

**Table 4:** Effect of K$_2$HPO$_4$ mycelial growth of F. solani in laboratory

<table>
<thead>
<tr>
<th>Inhibition (%)</th>
<th>Colony diameter (cm)</th>
<th>Concentrations (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>9.00</td>
<td>0</td>
</tr>
<tr>
<td>46.1</td>
<td>4.58</td>
<td>50</td>
</tr>
<tr>
<td>85.8</td>
<td>1.27</td>
<td>100</td>
</tr>
<tr>
<td>100.00</td>
<td>0.00</td>
<td>200</td>
</tr>
<tr>
<td>6.21</td>
<td>0.55</td>
<td>L.S.D0.05</td>
</tr>
</tbody>
</table>

*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$_2$HPO$_4$ 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$_2$HPO$_4$ 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 be 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, whereas the treatment of K$_2$HPO$_4$ 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$_2$HPO$_4$ 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.

<table>
<thead>
<tr>
<th>% Disease severity</th>
<th>infection %</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>Control</td>
</tr>
<tr>
<td>75.00</td>
<td>76.67</td>
<td>(FS) F. solani</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>K$_2$HPO$_4$</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>(AC) A. chroococcum</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>(E) Eucalptase Biochar</td>
</tr>
<tr>
<td>28.30</td>
<td>36.67</td>
<td>K$_2$HPO$_4$ +FS</td>
</tr>
<tr>
<td>16.67</td>
<td>26.67</td>
<td>AC + FS</td>
</tr>
<tr>
<td>18.33</td>
<td>33.33</td>
<td>E + FS</td>
</tr>
<tr>
<td>3.33</td>
<td>6.67</td>
<td>K$_2$HPO$_4$ +AC + FS</td>
</tr>
<tr>
<td>10.00</td>
<td>10.00</td>
<td>K$_2$HPO$_4$ +E + FS</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>AC + E + FS</td>
</tr>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>K$_2$HPO$_4$ + AC + E + FS</td>
</tr>
<tr>
<td>8.88</td>
<td>6.28</td>
<td>L.S.D0.05</td>
</tr>
</tbody>
</table>

*Each number represents the average of 3 replicates*
Matloob and Alkim (2016) demonstrated the effectiveness of bacteria A. chroococcum against cotton seedling diphtheria caused by the fungus R. solani, 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 K2HPO4 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 K2HPO4 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 After 7 days = 2.70
  - L.S.D. 0.05 After 14 days = 2.22
  - L.S.D. 0.05 After 21 days = 2.56

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