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Article

Seed Treatment of Okra with Humic Acid to Control Pathogen of Damping-Off *Fusarium Solani*.

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ABSTRACT
The study was conducted in the fields of the College of Agricultural Engineering Sciences for the 2022 season, University of Baghdad / Al-Jadriya. The study aimed to evaluate the efficiency of soaking the okra seeds of the Hissainawya and Batra varieties with 0.05% Humic acid for 12 hours in controlling the disease of rot, damping off, and root rot caused by the fungus Fusarium solani. The results showed the effectiveness of soaking treatment with Humic acid in reducing the disease incidence with damping off and root rot of okra seeds of the Hissainawya variety when planted in soil contaminated with the pathogen. In comparison, it reached 25% compared to the pathogen treatment only, which amounted to 75% in field conditions after 30 days of sowing. The average infection ratio and severity in okra plants after 70 days of sowing the Hissainawya variety in treating soaking with Humic acid. Besides, the soil spraying after 40 days of sowing in soil contaminated with the pathogen was 26.67 and 41.67%, respectively, with a significant difference from the treatment of the pathogen F. solani, which amounted to 55.67 and 58.33%, respectively. Finally, treating okra seeds of Hissainawya and Batra varieties with Humic acid had a significant effect on increasing plant height and dry weight, in general. Moreover, the Hissainawya variety responded more to the treatment of soaking with Humic acid compared to the treatment of soaking seeds for the Batra variety in field conditions.
Key words: Fusarium solani, Humic acid, Soaking seeds, okra.

INTRODUCTION

Abelmoschus esculentus L. Moench is one of the most important plants of the Malvaceae family. It is believed that its original home is in Africa, specifically Eritrea and Sudan, from which it was transferred to other countries through North Africa. It is one of the desirable summer crops in Iraq, as it is cultivated for its green pods, and it is eaten either fresh or dried [1]. Okra fruits have a high nutritional value and are rich in calcium, phosphorous, and magnesium. It, likewise, contains some vitamins such as riboflavin, thymine, and vitamins A and C, as well as its ripe seeds contain about 20% edible oil [2]. The cultivated area of the okra crop in Iraq for the year 2020 amounted to about 64,146 dunums, with a productivity rate of 93,719 tons Central Statistics Agency, 2020 and [3]. The okra plant is affected by many plant diseases, including fungal diseases, which have increased in prevalence in recent periods.

The most important of these diseases is the disease of seedlings, damping off and rotting of the roots and stem. These are common diseases on the okra plant, and the fungus F. solani is one of the most important pathogens of root rot disease and the seedlings damping off [4, 5]. It can stay in the soil for about four years (Cook, 1983), or it can remain as a mycelium in grain residues [6]. Several methods have been used in controlling plant pathogens; chemical control is one of the most easily and rapidly influencing pathogens. However, repeated use has led to problems, including the emergence of resistance, its pollution of the environment and its danger to human and animal health [7]. Because of the aggravation of plant diseases on the one hand and the negative effects of chemical herbicides on the other hand, farmers are forced to graft or re-cultivate, and this leads to lost time and increased costs. In addition to the heterogeneity of growth, which causes delay and deterioration in production in many crops, such as vascular wilt diseases. [8] was found that soaking the seeds before planting them with some solutions accelerates the germination process, and this is known as seed priming, a technique that is easy to use, has low costs, and shortens time and effort. The principle or basis for these technologies is the treatment of seeds before planting. Accordingly, it has become necessary to use materials capable of activating okra seeds to improve the functional performance within the seed, which is reflected in the seedling characteristics and germination vigor. [9, 10]. Thus, the study aimed to evaluate the response of treating the seed of okra Hissainawya and Batra varieties with Humic acid in controlling the pathogen F. solani.

MATERIAL AND METHODS

Two varieties of okra seeds were used in this study (the first variety is Hissainawya, which is one of the common local varieties, and the second variety is Batra, which is a common variety in central Iraq, and its pods do not harden quickly. The crop is collected 65-70 days after sowing [11], obtained from some farmers from Al-Diwaniyah and Babylon governorates.

Isolation and morphological identification of fungi associated with infected okra roots

Several okra seeds were planted in pots containing soils brought from selected fields planted with okra in the governorates of Baghdad and Al-Diwaniyah, in which the disease of seedling damping off and root rot was previously recorded. After germination, examinations were conducted for the seedlings that clearly showed symptoms of seedling damping off and wilt. Samples were taken from the roots of the infected seedlings and were placed in bags with the sample number, place, and date of bringing the sample and brought to the laboratory and kept at a temperature of 4°C to isolate the fungi. The stem bases and the root were washed with tap water to remove dust and other plankton. It was cut into small pieces with a length of 0.5-1cm, sterilized with 10% local sodium hypochlorite solution for 2-3 minutes, then washed with sterile distilled water three times. The pieces were placed on sterile filter paper to remove excess water. Then, the roots were planted with three explants in each 9 cm petri dish containing P.D.A (Potato Dextrose Agar). The plates were placed in the incubator at 25 ± 2 °C for five days. Fungi isolates were purified by transferring parts from the edges of the colonies using a sterile needle to Petri dishes containing Potato Dextrose Agar (PDA) medium. The plates were incubated in the incubator at a temperature of 25 ± 2 ºC for five days, then the fungus was identified based on the taxonomic and morphological characteristics based on the approved taxonomic keys developed by [12, 13].

Effect of seed soaking treatment with Humic acid on the disease incidence of seedling damping off and seed rot

The field experiment was carried out in the Scientific Experiments Complex B near the apiary of the College of Agricultural Engineering Sciences / University of Baghdad. The land was prepared by plowing and harrowing with a disc plow, and it was divided into furrows, the distance between one furrow and another 75 cm, by eight furrows for both varieties. A concentration of 0.05% of Humic acid was prepared, and okra seeds were soaked for 12 hours. Then, the holes were made, and the distance between one hole to another was 20-25 cm. According to the Randomized Complete Block Design (RCBD), each block of the experiment included the following treatments:

The control treatment is symbolized by symbol C.

Treatment with Humic acid, symbolized by the symbol H.

Treatment with the fungus F. solani, symbolized by F

Treatment with the fungus F. solani and Humic, symbolized by H.F., as the seeds are soaked with Humic acid for 12 hours and planted in the hole to which the inoculum of the pathogen F. solani is added.

Isolated fungi borne on millet seeds

The method of [14] was followed to bear the pathogen F. solani isolated in this study from the roots and infected seeds of okra plants using seeds of local millet (Panicum miliacem). Millet seeds were washed to remove plankton, dirt, and dust, then soaked in distilled water for 3 hours and dried on filter paper to remove excess water. The soaked seeds were distributed in 300 ml glass flasks containing about 200 g of seeds and blocked with cotton plugs and aluminum foil. Then, the flasks were sterilized with an autoclave at a temperature of 121 °C and a pressure of 1.5 bar for half an hour, with re-sterilization the next day. The flasks were left to cool, and each flask was inoculated with five tablets (0.5 cm/tablet) taken from the culture medium (PDA) growing on a seven-day-old pure isolate of F. solani. All flasks were incubated at a temperature of +1 25 ° C for 14 days, taking into account the shaking of the flasks every two or three days to ensure the distribution of fungal growth on all seeds until all the seeds are covered with spores of the fungus F. solani, which are completely white to gray. The seed-borne pathogen inoculum was added at a rate of 5 g per hole, and two days before planting, okra seeds were planted for both varieties on March 8, 2022, after soaking them with Humic acid at a rate of 3 seeds per hole. Furthermore, the number of healthy seedlings in each hole was recorded from the beginning of planting and for a month after planting to calculate the disease incidence with seed rot and seedling damping off.

Disease incidence

The disease incidence was calculated according to the following equation: -

$$\text{The disease incidence} = \frac{\text{infected seedlings number}}{\text{total seedlings number}} \times 100$$

Subsequently, the process of thinning into one plant / a hole and the distance between a hole and another was 40 cm. All agricultural operations were carried out from fertilizing with DAP fertilizer and hoeing according to the crop need and according to the recommendations for cultivating okra [11]. Humic acid was added to the soil of plants in the field as a strengthening treatment after 40 days of planting. After the plant reached the production stage after 90 days of planting, the infection ratio of root rot disease was recorded according to the following equation:

$$\text{The infection ratio} = \frac{\text{infested plants number}}{\text{total plants number}} \times 100$$

The plants were removed from each treatment, and the roots were examined to assess the disease severity according to the pathological index followed [5] as listed in Table 1.

Degree	Index
0	The plant is healthy, and the root is white
1	Coloring 1-25 of the root in a light brown color
2	Coloring of more than 25-50 of the root in a dark brown color
3	Coloring of more than 50-75 of the roots in a dark brown color with yellowing of the leaves
4	Coloring more than 75-100 of the roots in the dark with the plant death,

Table 1. Pathological index

In the same role, the [15] equation mentioned in [16] was used to calculate the disease severity as follows:

$$\text{Disease Severity Index (\%)} = \frac{[\text{sum (class frequency} \times \text{ score of rating class)}]}{[(\text{total number of plants}) \times (\text{maximal disease index})]} \times 100$$

4: plant death	3: %75 of the root is infected	2: %50 of the root is infected	1: %25 of the root is infected	0: healthy roots

Figure 1 Pathological index

Plant Height (cm):-

The plant height was measured from the surface of the soil to the top of the plant using the tape measure, and the length of three replicates (plants) of the same treatment was recorded, as shown in Figure 2.

Figure 2. Pathological index of okra root rot on Fusarium solani

The shoot dry weight (g):

To calculate the dry weight, the plants were placed in the oven at a temperature of 65 ºC for 72 hours until the weight was constant. A sensitive scale was used to measure the dry weight.

RESULTS

Isolation and identification of fungi associated with infected roots and seedlings of okra

The results of the morphological identification, depending on the nature of the mycelium growth and the distinctive microscopic characteristics, showed that the pathogen was due to the fungus. solani F, as shown in Figure 3.

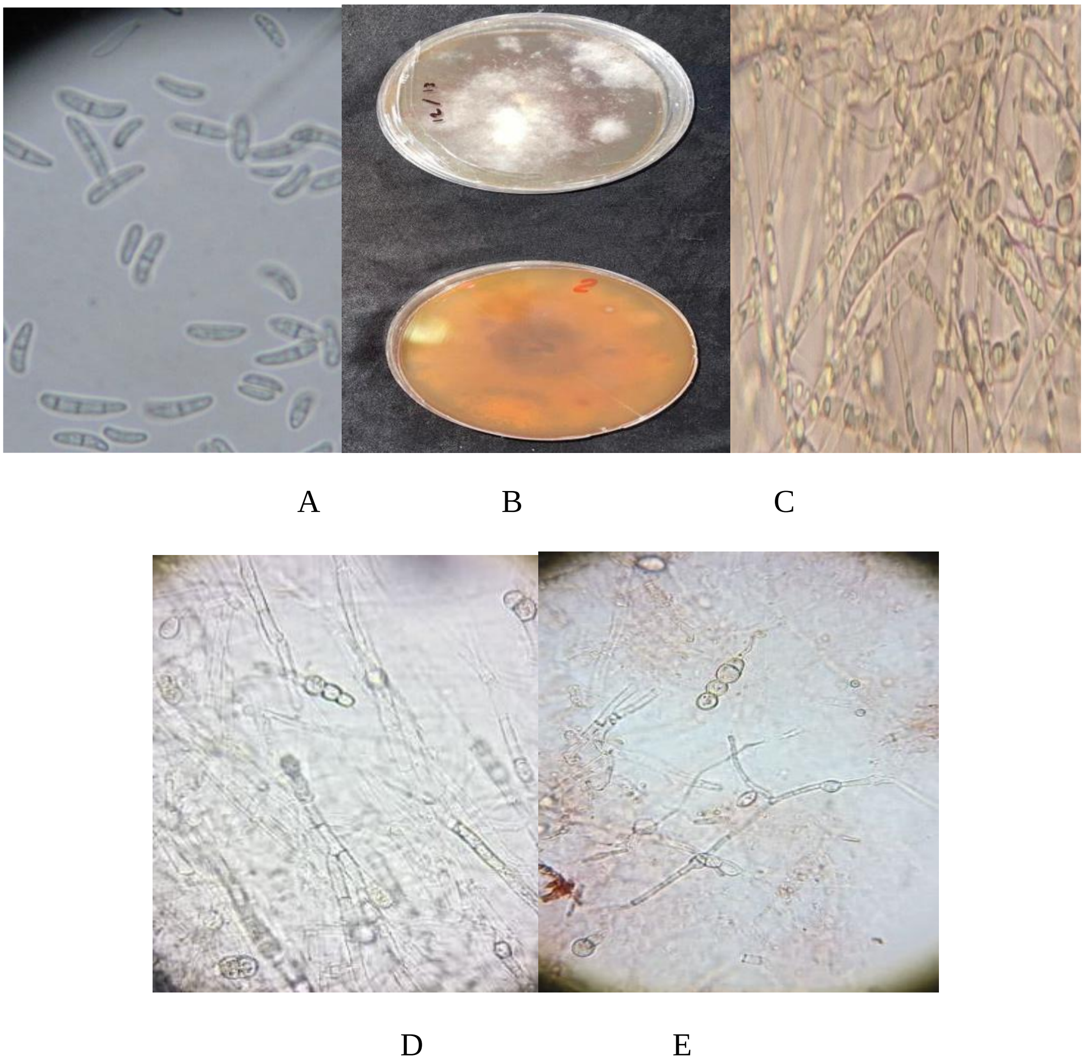

Figure 3. Some morphological and microscopic characteristics of *F. solani* fungus. (A) Macroconidia and Microconidia of the fungus with a magnification of X40. (B) The fungus colony on the PDA medium. (C) Macroconidia; (D) Mycelium; (E) Chlamydospores.

Disease incidence with seed rot and seedling damping off in field conditions

The results of Table (2) show the disease incidence of okra seeds of Hissainawya and Batra varieties, treated with Humic acid and planted in soils contaminated with the pathogen F. solani. The average disease incidence of okra seeds with the presence of the pathogenic fungus F. solani, in which the infection ratio was 70.83%, with a significant difference from the control treatment in which the percentage of germination reached 50%. The lowest disease incidence of okra seeds was achieved by soaking the seeds with Humic acid, as it reached 25%.

There are no significant differences in the disease incidence for both varieties (Hissainawya and Batra). Also, the lowest disease incidence of seedlings when treating seeds with Humic acid for each of the two okra varieties, Hissainawya and Batra, and the treatment of seeds planted in soil contaminated with pathogenic fungus (interaction treatment) was 25, 25, 25, and 41.67% for each of them, respectively.

In comparison, a significant difference was recorded from the control treatment in which the disease incidence of seedlings for the two cultivars of Hissainawya and Batra reached 58.33 and 41.67%, respectively. From Table (2), it can be observed that the germination percentage of the seeds of both varieties decreased in the control treatment (soaking with water only), which amounted to 58.33 and 41.67%, respectively. Besides that, the treatment of soaking them with Humic acid reduced the infection ratio to about half, as the infection ratio for both varieties reached 25 %.

Varieties / Treatments	Hissainawya	Batra	Average
Control	58.33	41.67	50
Fusarium solani	75	66.67	70.83
Humic acid	25	25	25
Humic Acid + F.s.	25	41.67	33.33
Average	45.83	43.75	
LSD 0.05	Varieties = NS	Treatments = 22.57	Interaction = 31.92

Table 1. Effect of seed treatment with Humic acid for okra cultivars Hissainawya and Batra on the average disease incidence with seed rot disease and seedling damping off after 30 days of sowing in field conditionsEach number represents the average reading for four plants.

Effect of Humic acid on the percentage of okra root rot as a result of infection with the fungus F. solani after 90 days of sowing:

Table (3) shows the results of the infection ratio of okra plants of Hissainawya and Batra varieties with Fusarium solani and treated with Humic acid. Significant differences exist in the infection ratio for the treatments of soaking seeds with Humic acid and the control treatment. In comparison, the lowest infection ratio reached 15.33 and 17.67, respectively, with a significant difference from the treatment of the fungus F. solani, in which the highest infection ratio was 50.00. From the same Table, it is evident that there are no significant differences between the two okra varieties, Hissainawya and Batra, where the infection ratio reached 28.92 and 26.83%, respectively, as for the interaction results between the two varieties of okra, Hissainawya, Batra, F. solani, and Humic acid. The highest disease incidence was recorded in the treatment of F. solani for the Hissainawya variety, which amounted to 55.67% compared to the control treatment, and for the same two varieties, where the infection ratio reached 18.33 and 17.00%, respectively.

Varieties / Treatments	Hissainawya	Batra	Average
Control	18.33	17.00	17.67
Fusarium solani	55.67	44.33	50.00
Humic acid	15.00	15.67	15.33
Humic acid + F.s.	26.67	30.33	28.50
Average	28.92	26.83	
LSD 0.05	Varieties = NS 6.83	Treatments = 9.65	Interaction = 13.65

Table 2. Effect of seed treatment with Humic acid for okra varieties Hissainawya and Batra on the disease incidence with *Fusarium solani* in field conditions

Disease severity

The results shown in Table (4) indicate that the highest disease severity of plants with the fungus F. solani when the seeds were treated with Humic acid against the pathogenic fungus amounted to 53.33% with a significant difference from the control treatment in which the severity reached 20%. However, when the seeds were treated with Humic acid, the severity decreased, reaching 8.50%, where there is no significant difference in the severity between the Hissainawya and Batra varieties. From the results of the same Table, it can be found that the interaction between the two Hissainawya and Batra, and the seeds treatment with Humic acid and the fungus F. solani. That the highest severity was recorded, significantly, for the fungus F. solani. For variety Hissainawya, it reached 58.33%. The lowest significant severity was when seeds were treated with Humic acid and planted in soil contaminated with the pathogen F. solani. For both varieties of study, in which the severity was 16.67 and 13.67%, respectively. These results are agreed with [20 21]. Those who confirmed that pathogenic fungi, including F. solani, are pathogens with high disease severity. The pathogens vary in severity on the plant host itself, which is clear evidence of the presence of special factors that control these abilities and are related to the genotypes of these fungi. There are also physiological aspects of the pathogenicity that lie in the biochemical effects of microorganisms, which is of fundamental importance in revealing the pathogenicity during the interaction between the pathogen and the plant host, such as the mechanism of the pathogen's effect on the host's defense means.

Plant height cm

The results of Table (4) show the height of okra plants for the study factors and their combinations, as the highest height was in plants treated with Humic acid. The average plant height reached 117.50 cm, superior to all experimental treatments with highly significant differences, as the height of okra plants in the control treatment reached 89.50 cm.

Varieties / Treatments	Hissainawya	Batra	Average
Control	21.67	18.33	20.00
Fusarium solani	58.33	48.33	53.33
Humic acid	16.67	13.67	15.17
Humic acid + F.s.	41.67	43.33	42.50
Average	34.58	30.92	
LSD 0.05	Varieties = NS 3.65	Treatments = 5.17	Interaction = 7.31

Table 4. Effect of seed treatment with Humic acid for the two okra varieties Hissainawya and Batra on the disease severity of root rot caused by Fusarium solani after 70 days of sowing in field conditions

As for the treatment of the pathogen F. solani. The average height of okra plants was as low as possible, recorded at 73.17 cm. From the same Table, it was observed that there are no significant differences between the average height of the two okra varieties, Hissainawya and Batra, as the average length of one plant reached 95.42 and 92.50 cm, respectively. There were also significant differences between the treatments of the two study factors (interaction treatment) in the mean length of okra plants. Thus, the highest height was in plants of the two humic acid treatments for both varieties, 127.67 and 107.33 cm, respectively, and the lowest height was in okra plants of Hissainawya and Batra varieties and treated with F. solani fungus, which reached 70.00, 76.33 cm respectively. In comparison, the height of the two varieties was 89.67 and 89.33 cm, respectively, for the control treatment.

Varieties / Treatments	Hissainawya	Batra	Average
Control	89.67	89.33	89.50
Fusarium solani	70.00	76.33	73.17
Humic acid	127.67	107.33	117.50
Humic Acid + F.s.	94.33	97.00	95.67
Average	95.42	92.50	
LSD 0.05	Varieties = NS	Treatments = 15.72	Interaction = 22.22

Table 4. Effect of seed treatment with Humic acid for okra varieties Hissainawya and Batra on average plant height (cm) in field conditions

Plant dry weight

It was found that the seeds treatment with Humic acid against the fungus F. solani affected the average dry weight of okra plants of the two varieties, Hissainawya and Batra, in the field. A significant difference was recorded between the Humic acid treatment, which gave the highest plant dry weight, which amounted to 233.75 g, with significant differences from the control treatment, which amounted to 66.25 g. However, the fungus F. solani treatment recorded the lowest plant dry weight, which amounted to 40.63 g compared to the control treatment. It was also observed that there are no significant differences between the two okra varieties, Hissainawya and Batra, where the average dry weight of plants reached 101.81 and 106.81 g, respectively, as shown in Table

(5). Moreover, it can be noticed an increase in the dry weight of okra plants of the two varieties, Hissainawya and Batra, as the treatment of plants with Humic acid recorded the highest plants' dry weight, which amounted to 225.00 and 242.50 g, respectively. Significant differences were recorded compared to the control treatment and for the two varieties, where the average dry weight was 65.00 and 67.50 g, respectively.

In contrast, the lowest dry weight of okra plants of Hissainawya and Batra in treating pathogenic fungus F. solani were 40.00 and 41.25 g, respectively. The reason for the plant's dry weight gain may be because organic matter contains Humic matter, as this substance has an important role in improving cell division and elongation. Plus, it affects the processes of photosynthesis, respiration, and protein synthesis and causes an increase in the rate of plant growth [26]. These results are similar to the (Al-Jabri, 2011)findings, where the addition of organic matter had a role in increasing the weight of the Alfalfa plant.

Varieties / Treatments	Hissainawya	Batra	Average
Control	65.00	67.50	66.25
Fusarium solani	40.00	41.25	40.63
Humic acid	225.00	242.50	233.75
Humic Acid + F.s.	77.25	76.00	76.63
Average	101.81	106.81	
LSD 0.05	Varieties = NS 9.70	Treatments = 13.72	Interaction = 17.40

Table 5. Effect of seed treatment with Humic acid for okra varieties Hissainawya and Batra on the plants' dry weight (g) in field conditions

DISCUSSION

The colony was white to gray, Microconida were spherical or oval, Macroconida were in large numbers and were spindle-shaped, and Chlamydospores had a rough wall similar to what was described by [12, 13]. The reason for activating the seeds treated with organic compounds is due to the effectiveness of Humic acid in raising the rate of enzymatic activity, which stimulates the food metabolism process. It likewise provided a quick energy source and materials for the biological construction of the developing embryonic axis [17]. These results are similar to [18] when using Humic acid to control fusarium wilt on tomato plants caused by the fungus Fusarium oxysporum f.sp lycopersici.

Humic acid leads to a change in the pattern of carbohydrate metabolism, and this leads to the accumulation of soluble sugars, which increases the osmotic pressure inside the cell walls and makes the plant more resistant to unfavorable conditions, as well as increases the ability of the plant's immune system [27]. The seeds are stimulated by soaking them with natural or manufactured solutions that increase the seed's vigor to resist stresses and increase the speed and percentage of germination and the homogeneity of germination [19].

There are three metabolic activities of microorganisms of importance in the pathogenicity. They are the secretion of decomposing enzymes, toxins, and growth regulators; pathogenicity may vary for pathogens according to the mechanism of action of these metabolites, either collectively or individually [22].

The reason for the increase in plant height may be due to the role played by Humic acid in increasing the development of chlorophyll and the assembly of sugars, enzymes, and amino acids, as the effect of Humic acid is similar to the effect of Auxins in increasing cell division and increasing vegetative growth rates, which is reflected in an increase in stem height [23].

This result agrees with the results of [24] when spraying Humic acid led to an increase in the height of the okra plant. These results are consistent with [25] who found that when Humic acid was used, it increased maize plant height.

CONCLUSIONS

The treatment of okra seeds of the two varieties, Hissainawya and Batra, with Humic acid had a significant effect on improving the different growth characteristics. It caused a decrease in their infection with seed rot, along with seedling damping off and root rot when planted in soils contaminated with the pathogen F. solani, as well as an increase in the dry weight of plants treated with Humic acid and an increase in the plant's height. Moreover, the response of the Hissainawya variety was more than that of the Batra variety in all the studied traits.

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