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Sensitivity test of Six Backcross Generations of Eggplant *(Solanummelongena)*for(*Rhizoctoniasolani*)kühn

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Abstract:*In the sensitivity of *eggplant for *Rhizoctaniasolani, samples were collected from greenhouses in the district of Abu Ghraib and other of the districts of Dujail, which showed symptoms of wilt, yellowing and canker and brown coloration in the infected area. The results of the *pathoginicity of R.solani*isolates in *eggplant seeds and seedlings of two weeks old showed that isolating R1 achieved 86.7% seed germination failure and seedling rate 88% compared to R2 *isolates which failed to germinate seeds and death of *eggplant seedlings by 20% and 13.3% respectively. The results of the plastic house experiment showed that *R.solani isolates had 66% of infected parent P1 (Father) and 44% parent P2 (Mother) respectively. The infection of F1 was reach to 57% and gradually began to decrease in the *genotypes BC1, BC2, BC3, BC4, BC5 and BC6, reaching 49.5, 47.8, 46.3, 46, 45, 44.6 %respectizvely*

Keyword: *Rhizoctoniasolani , Pathoginicity , eggplant , genotypes , isolates.*

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I. Introduction

Eggplant *Solaniummelongena* is one of the main vegetable crops, which has become particularly important in recent years, as well as the crops of the same Solanaceae family such as tomatoes, potatoes and peppers (Gramazio and others, 2017), with a global production of 49.9 million tons, 57% of production from China alone India, Iran, Egypt and Turkey. More than 1600,000 hectares have been allocated for the cultivation of eggplant in the world (FAO, 2013). Eggplant in Iraq is one of the economically important and food crops. *Rhizoctoniasolani* is one of the main causes of seed rot and seedling diseases Attack the plant during its various stages of growth, it affects the Seeds in the soil before and after emergence and affects the roots and has the ability to infect the plant over the surface of the soil, which affects the leaves, stems, fruits and horns, as well as fruits (Anne et al., 2002; Rivera et al., 2004).the early infection by *R.solani* Which leads to the damping off and death of seedling lead to areas free of healthy plants and late infection lead to the rot of the roots and

does not cause the killing of the plant, resulting to loss of production the plants become more resistant to the fungus of aging and survival healthy, although with the presence of fungus (Anees and others, 2009) *R.solani* is one of the most important soil endemic disease that are penetrate of plant (Mayo et al., 2015). The fungus causes seedling death and rotting of seeds, roots before and after emergence in many specieses of plants (Gajera et al., 2016). It also has a wide family range, causing leaf blight in some crops (Zhou et al., 2016). The fungus also cause the rotting of the crown area of tomato under the conditions of the plastic house (Hamza et al., 2016) and also causes significant annual damage to thousands of species of crops and horticulture (Chen et al., 2016).

The study aimed at:

1. Test the sensitivity of generations of backcross hybridization, parents and first generation hybrids to *Rhizoctoniasolani* under the conditions of the plastic house.
2. Test sensitivity of different ages for each of these genotypes (parents and hybrids and six generations of backcross hybridization) for the fungal infection *R.solani*.

II. Materials And Methods

Isolations of *Rhizoctoniasolani* which isolate from Samples of eggplant were collected from greenhouses in Abu Ghraib district and other isolate from same eggplant from the districts of Dujail district, which showed symptoms of wilt, yellowing, canker and prowning in the infection area. The samples were placed in polythene bags and transferred to the laboratory and placed in the refrigerator at 4° . Infected samples was washed with tap water for 30 minutes and cut into small pieces 0.3 cm from the area of infection and sterilized surface for 2 minutes by immersing in sodium hypochlorite solution (1% free chlorine) and then

transferred to sterilized water for 2-3 minutes, On sterile filter paper for drying from water And then transfer 4 pieces using sterile forceps in each 9 cm Petri dish containing 15 ml of the PDA medium (Potato Dextrose Agar) The media were sterilized with the auto clave at 121 ° C and pressure 1.5 kg / cm² for 20 minutes (tetracycline) 200 mg / kg before transfer 4 pieses and 10 samples per sample which incubated dishes at 25 ± 1 ° C for two days. Then, the developing colonies of the fungus were examined and pieces of fungus filaments were transferred to new dishes containing a PDA medium and incubated for 5 days at 25 ° C ± 1 º m, *R.solani* was diagnosed based on the phenotypic and microscopic characteristics mentioned in Parmeter), Whitney, 1970 and Alexopoulos and others, 1996 and Blazier and Conway, 2004). Parts of the colony of each growing colony were taken in sterile needle from dishes and transferred to test tubes containing the PDA. They were incubated for 5 days and then placed in the refrigerator until the end of the tests.

1- Preparation of *R. Solani* inoculum

The inoculation was prepared by growing the fungus on the local millet seeds *Panicummiliaceum*. Put 100 g of seeds in a 500 ml flask. Add 300 ml of water, soak for six hours, pour excess water and sterilize by auto clave at 121 ° C and pressure 1.5 kg / cm² for 20 minutes and twice for two days .The seeds of millet were inoculate by five 5 mm diameter tablets from the PDA medium containing the *R.solani* at the age of 7 days and incubated in 1 ± 25 ° C for 10 days. To ensure ventilation and distribution of fungus in all seeds (Pannecoucque and Hofte, 2009).

2- Pathogenecity of two isolate *R.solani*:

Sterile peatmoss was used for pathogenicity , peatmoss was sterile by autoclave at 121 ° C and pressure 1.5 kg / cm² for 1 hour and 2 times between the first and second hours 24 hours and put in sterile plastic bottles of half a kilo and 13 cm diameter, add the fungus inoculum loaded on the local millet seeds sterile by 3 g / The mixture was mixed with peat, with a comparative treatment without the addition of fungus. The peatmoss was rinsed and covered with polyethylene bags for three days. The seeds of the eggplant were then sown with 10 seeds/pot , sterile seed with sodium hypochlorite solution. Concentrate 1% chlorine for 2 minutes. The seeds in the comparison treatment calculated the percentage of germination.

3- Effect of Insulation in Eggplant Seedlings

The mixture of peat:loamy soil, 1:3 vol/vol which sterile by autoclave as same method was distributed in plastic containers of 1 kg in diameter and 15 cm in size. The fungus inoculum was added by 5 g / pot (Dewan, 1989) by mixing it .The soil (pot)was rinsed and covering with polyethylene bags for 3 days before the seedlings were transferred to them.

4- Effect of *R.solani* isolates on seedlings of different genotypes

- Producing seedlings

The seeds of the eggplant genotypes representing the parents and the hybrid and six generations of backcross hybridization were obtained from Dr. EnadDhaherAbood / Department of Plant Protection and sterilized surface using sodium hypochlorite concentration of 1% free chlorine for half an hour and then washed by sterile water and with water and planted seeds in poly dishes Stearin with 209 eyes, containing sterile peatmoss which sterilized by autoclave as mentioned in the inoculum preparation section. A week later, each seedling was transferred individually to other dishes containing sterilized peatmoss and left until tests were carried out, taking into account the irrigation process as needed.

Eggplant Sensitivity Test for *R.solani*

Seedlings were prepared (according to the previous paragraph), and a group of 1 kg and 13 cm diamter filled with mixed soil and peatmoss at 1: 3 loamy soil and digested twice with the autoclave at 121 ° C and 1.5 kg / cm² for 1 hour at a time and added *R.solani* inoculum loaded on millet seeds (as mentioned in the preparation of the inoculum) by 5 g / pot (Dewan, 1989) and mixed well with the soil and irtigated and covered by polyethylene bags perforated for 3 days and then moved the seedlings two weeks old in the original 3 seedlings / pot and 3 replicates for each genetic varieties with the treatment of comparison, added to the sterile millet only, conducted this experiment in Plastic House /Plant Protection Department of plant protection / University of Baghdad. Distributed to the design according to CRD design. A month later, the percentage of infection was calculated according to the following equation: Percentage of infection = (infected plants number) / (total number of plants) x 100 The disease severity index for plants was estimated using the pathological index of 4 degrees: 0 = healthy plant with no symptoms, 1 = slight discoloration at the bottom of the stemextending for a short distance, and 2 = canker around the stem extending of a long distance with plant wilting and 3 = plant death. The percentage of severity of the disease was calculated according to the Mckinney equation (1923) (0 × 0 in plant number) + (3 × 0) + (3 × 3) The experiment was carried out according to CRD design.

5- Effect of *R. solani* in seedlings of different ages of genotypes

Eggplant seedlings for genotypes (parents, hybrids F1 and six backcross generations) were produced according to the terms of the seedlings production section, taking into consideration the age of 30, 45, 60 days. The seeds were planted on 10/1/2017, 25/1, 10/2 A month after the last date, the seedlings were transferred to a pot 1 kg weight and 13 cm diameter. The same steps were followed as mentioned in the previous paragraph. One month after the seedlings were transferred, the percentage of infection and severity of infection was calculated on the plants as mentioned in the above paragraph.

III. Results And Conclusion

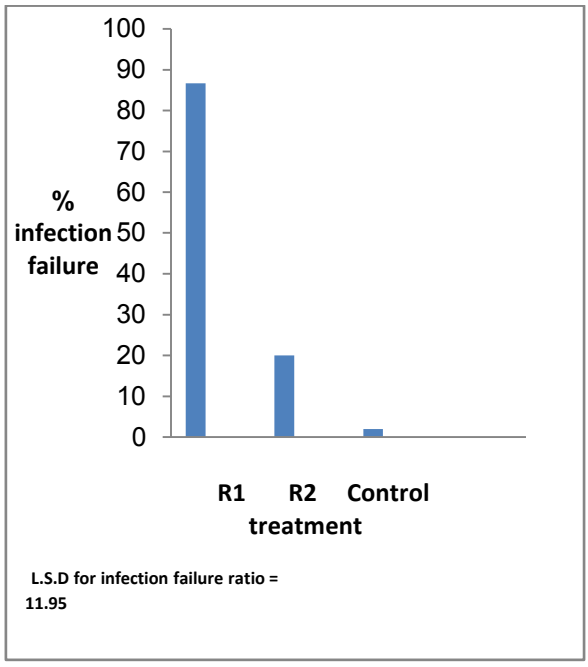
1- Isolation and Diagnosis of *R.solani*

A microscopic examination of a 72-hour fungus showed the presence of a hypha was divided slight brown to brown color, with short and many fungal cells divided by transverse barriers. The fungal mycelium is observed at almost vertical angles with a clear narrowing in the branches and the emergence of the branch, and it was noted that non-sexual or conidia were consist . All of these traits that can be observed under the microscope are applied to the properties of *R. solani*, which are found in scientific sources (Stalpers et al., 1996). Solana Rhizoctonia was identified using taxonomic characteristics (Parmeter, Whitney, 1970, Blazier and Conway, 2004) and using taxonomic keys (Whitney, Parmeter, 1970 and Alexopoulos et al., 1996). The diagnosis was confirmed by Dr. Hadi Mahdi Abood, Ministry of Science and Technology.

2- Pathoginicity of *R.solani*

- Germination of eggplant seeds (local variaty)

The results showed that Figure(1), eggplant seeds were highly sensitive to the isolation of the fungus R1 and the failure rate of germination was 86.7%. The fungus reduced seed germination to 13.3% while the localy eggplant seeds showed no sensitivity to isolation(R2). The failure of germination rate to 20% and did not affect the germination rate as much as 80%. The reason for the difference of the ability of isolates to cause such proportions is due to its various mechanisms known as secretion of enzymes which decay cell wall of hostor the secretion of toxic substances that cause the failure of germination (Inoue et al., 2002 and Rival, 2004 and Mohamad et al., 2006), and may be the difference between isolate is due to different isolates at the level of secretion of the enzymes for the analysis of pectin and cellulose, which are responsible for rotting in the seeds and thus preventing them from germination. Some researchers said that proteinase has a major role in determining the pathogenicity of the fungi *R. Solani* (Weinhold and Sinclair, 1996) , Or may be due to genetic differences between isolates of fungi (Hassan, 2002).

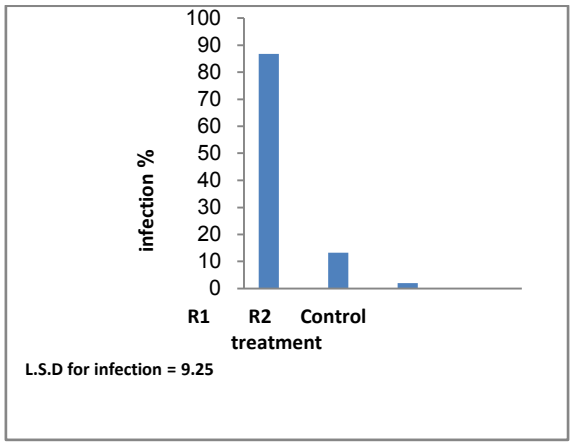


R1, R2 = *Rhizoctoniasolani*isolates

Figure (1) Effect of *R.solani* isolates in the germination of local eggplant seeds

- Effect of *R.solani* isolates in eggplant seedlings (local variety)

The results showed that Figure(2) the sensitivity of the local variety to isolates of the fungus R1 was 88%, which differed significantly with the R2 treatment and the comparison treatment, the infection rate was 13.3% and 0% respectively. It was developed into a general wilt of the plant several days after the fungal infection and eventually led to the death of most of the infected plants. Some studies have indicated that *R. solani* is the most frequent cause of seedling damping off disease in fields and nurseries (Jabr, 1996 and Hodges, 2003 , Jayed et al., 2004) . Jensen et al. (2002) reported that *R.solani* attacks the host's seeds, causes them to be rotted and prevents them from germinating, and attacks seedlings before they emergency, causing highly reducing of percentage seed germination the results are consistent with the damping off and root rot and canker of stem which nearly of soil serface and then die. Pannecoucque et al. (2008) mentioned that some isolates of *R.solani* caused severe lesions on the stems in most plants and got her full wilt after 10 days of inoculation with fungus, they mentioned Pannecoucque and Hofte (2009) The pathogenicity of *R.solani* on the production of enzymes for the analysis of pectin and cellulose, which causes the decay of cell walls, which leads to the rotting and decay of seeds. The cause of the difference between the two isolates may be due to the secretion of pectin and cellulose enzymes (Weinhold et al., 1996). The cause may be due to genetic differences between isolates (Hassan, 2002).

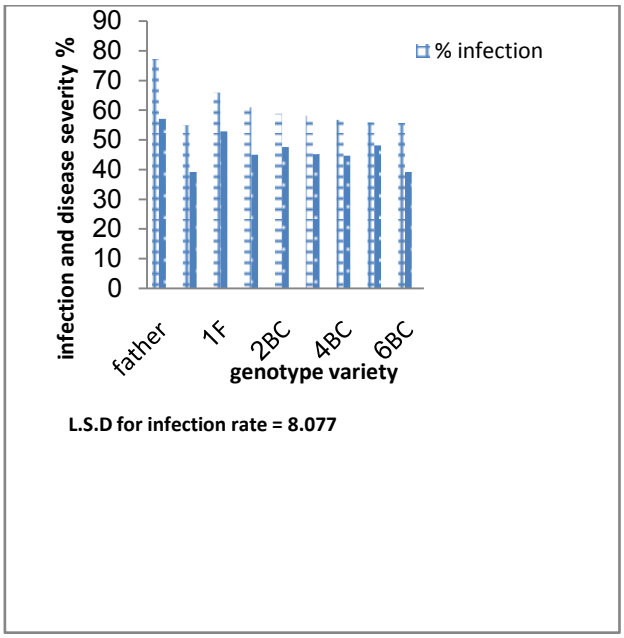


R1, R2 = *R. solani*isolates

Fig. (2) The percentage of infection with the isolates of *R.solani* for local eggplant veriety

- Effect of *R.solani* isolate (R1) on eggplant varieties

The results showed that the percentage of infection and disease severity of P1 was 77.3%, 57.2% respectively, and P2 55.3% and 39.3% respectively. The percentage of infection in F1 was between P1 and P2 66% and disease severity 52.9%, and gradually began to dicrease in the genotypes BC1, BC2, BC3, BC4, BC5 and BC6 (61, 58.9, 58.2, 56.8, 56, 55.7%) respectively and disease severity (45.1, 47.7, 45.3, 44.8 , 48.2, 39.3%) respectively. These results are accepted with what Liu and others found (2015), noting that the backcross of eggplant generations showed resistance to the wiltdisease caused by *Verticillumdahliae*, gradually increased continuously as forword in backcross generations.

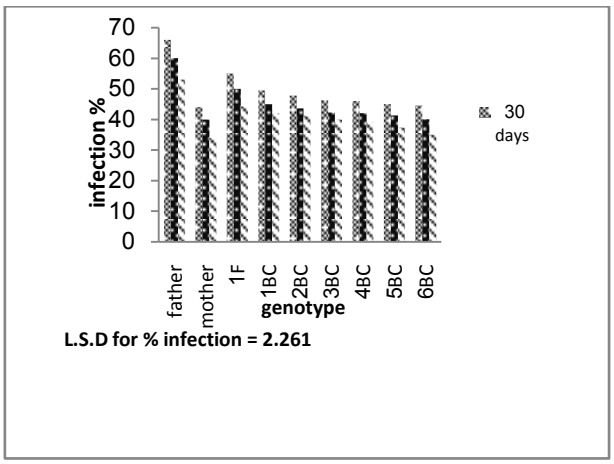


F1= hybrid first generation, BC = backcross

Figure (3): infection and disease severity of *R.solani* isolates on parents, hybrid and backcross generations of eggplant

- Effect of isolate *R.solani* on eggplant seedlings at different ages (30-45-60) daysof different genotypes

The results showed that the genotypes used in the experiment showed a different reaction to *R.solani* infection. In both genotypes P1 and P2, significant differences were found with the highest incidence of seedlings in the age of 30 days 66 and 44% on the relay and at the age of 45 days 60% and 40% respectively. The age of 60 days was 53% and 34% respectively, followed by genotype F1, which achieved 30 days 55%, 45 days, 50% and 44.2% 60 days, and no significant difference Between genotypes BC1, BC2, and BC3, with 30 days of age 49.5, 47.8 and 46.3% respectively, 45, 43.6 and 42.25% at 45 days respectively, 42, 41.2 and 40% at 60 days. The infection was concentrated in the lower stem area, ranging from mild to severe of canker with wilt and therefore plant death. The incidence of BC4, BC5 and BC6 There were no significant differences between the age of 30, 46, 45, 44.6%, 42, 41.3 and 40.1% at 45 days. The 60-day age was 39, 37.3 and 35%.

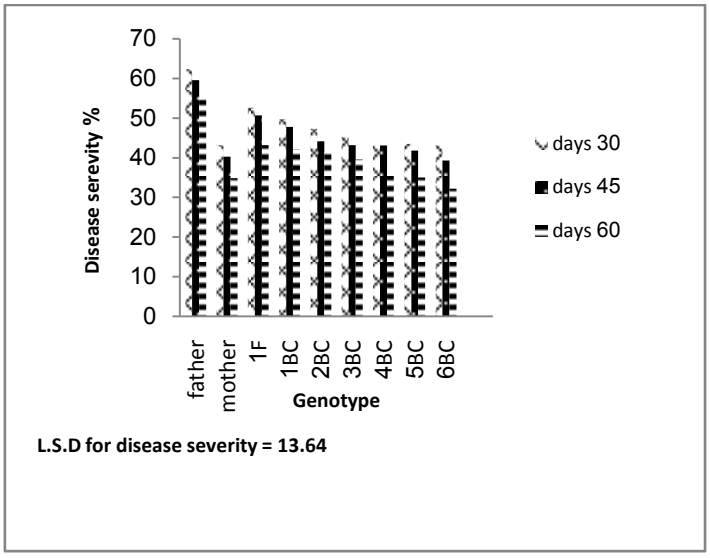


F1 hybrid first generation, BC = backcross generations

Figure (4) *R.solani* infection rate of eggplant genotypes with different ages (30-45-60 days)

The severity of the infection (Fig. 5) was found in the father (P1) and mother (P2) genotypes and F1 at the age of 30 days 62.3, 43.2 and 52.6% respectively and at the age of 45 days 59.6, 40.3 and 50.7% respectively. The age of the 60 days : 54.7, 34.9 and 43.2% and the disease severity began to decrease in the other of the genotypes as they reached the genotypes BC1 and BC2 less compared with other genotype reach at

the age of 30 days : 49.7 and 47.3% respectivily, and the age of 45 days : 47.8 and 44.2%, and the age of 60 days : 42.2 and 41.3% respectivily and no differences significantly between the four genotypes BC3, BC4, BC5 and BC6 as disease severity gradually decreased in the three ages, reaching 45.3 , 44.1, 43.5 and 43.2% at the age of 30 and 43.2, 43.1 and 41.8 and 39.3 at the age of 45 while the age of 60 days was reach 39.6 , 36.4 , 35.4 and 32.2%. These results are consistent with Pannecoucque et al. (2008) that some isolates of *R.solani* caused severe lesions on the stems in most plants and were completely wilt at 10 days after the fungal infection.



F1= hybrid first generation, BC =generation of backcross

Figure (5) Disease serevity*R.solani* for different genotypes at different times(30-45-60 days)

These results are consistent with Chauhan et al. (2000)was found that the severity of *R.solani* on plants varies with the age of plants. Plants aged 20 days are severely affected by the fungus when compared at 35 days of plant ages, and the sensitivity of the plants decreases as they forword of age. The results of this study are useful in strategic management of the disease. The cause of the decline in the genotype of backcross may be due to the restoration of resistance status after gradually decreasing.

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