THE USE OF *Aspergillus Niger* TO RESIST THE DISEASE OF SEED ROT AND DAMPING OFF CUCUMBER CAUSED BY *Rhizoctonia Solani* AND SOME PARAMETERS OF PLANT GROWTH

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ABSTRACT

The study was conducted to control the disease of seed rot and damping off cucumber caused by *Rhizoctonia solani* using *Aspergillus niger*, The results of the pathogenicity capacity test of fungi (*R. solani*) on the seeds of cucumber plant that the fungus negatively affected the germination of seeds, the germination percentage reached of 00.00%, while the fungi (*A.niger*) has worked to raise germination rate by 100.00% compared to the control treatment, which reached to 73.33%. *A.niger* fungus showed a high resistance against *R.solani* with rate of inhibition reached 91.58%. The effect of *A.niger* was found in some growth characteristics of cucumber plant on percentage of germination, stem diameter, Dry weight of leaves, leaf content of growth hormone Cytokinin (CKS), leaf content of phosphorus and Peroxidase with the presence or absence of pathogenic fungus (*Rhizoctonia solani*), these traits were found to have increased significantly which reached of (73.70, 7.8 mm, 1.97 mg, 2.98 mg / kg wet weight, 0.037 mg / kg dry weight, 0.14 units / micromole) respectively, compared with the lowest rates of control (40.00 mm, 5.2 mm and 0.65 mg, 1.19 mg / kg wet weight and, 0.009 $mg.g^{-1}$ dry weight, 0.04 unit /micromol), respectively.

Keywords: *Aspergillus niger, Rhizoctonia solani,* seed rot, cucumber plant.

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أستعمال *Aspergillus niger* في مقاومة مرض تعفن بذور وموت بادرات الخيار المتسبب عن *Rhizoctonia solani* وبعض موشرات نمو النبات

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الملخص

أجريت دراسة للسيطرة على مرض تعفن بذور وموت بادرات الخيار المتسبب عن الفطر *Rhizoctonia solani* باستخدام الفطر*Aspergillus niger* , أظهرت نتائج اختبار القدرة الأمراضية للفطر *R. solani* على بذور نبات الخيار ان الفطر اثر سلباً على انبات البذور اذ بلغت نسبة الانبات 00.00% اما الفطر *A. niger* فقد عمل على رفع نسبة الانبات اذ بلغت 100.00% قياس بمعاملة السيطرة التي بلغت 73.33%. كما اظهر الفطر *A. niger* قدرة تضادية عالية ضد الفطر الممرض *R. solani* وبنسبة تثبيط بلغت 91.58% , وجد من دراسة تأثير الفطر*A.niger* في بعض صفات النمو لنبات الخيار على النسبة المئوية للإنبات و قطر الساق والوزن الجاف للأوراق و محتوى الأوراق من هرمون النمو السايتوكاينين (CKS)ومحتوى الأوراق من الفسفور وانزيم البيروكسديز وبوجود او غياب الفطر الممرض *Rhizoctonia solani* وجد ان هذه الصفات قد زادت معنويا اذ بلغت (73.70 و7.8(ملم) و1.97 (ملغم)و2.98 (ملغم/كغم وزن رطب)و 0.037 (ملغم.غم $^{-1}$ وزن جاف)و 0.14 (وحدة /مايكرومول)على التوالي مقارنة بأقل المعدلات عند معاملة السيطرة التي كانت (40.00 و5.2(ملم) و0.65 (ملغم) و1.19 (ملغم/كغم وزن رطب) و0.009 (ملغم.غم $^{-1}$ وزن جاف)و0.04 (وحدة /مايكرومول).على التوالي .

1. INTRODUCTION

The cucumber plant *(Cucumis sativucs* L.), which is a summer crop belongs to the Cucurbitaceae family, it's an important vegetable crops. It is cultivated in all parts of Iraq and is cultivated in open and protected fields throughout the year. The cultivated area in Iraq is (82160 dunums) (Central Bureau of Statistics, 2015). The cucumber plant is exposed to many pathogens and is one of the main problems that cause a decrease in production. The most important

of which is the fungus *Rhizoctonia.solani*, which is a highly virulent pathogen infecting the plant at various stages of development (Agrios, 2007). Many objects have been used to resist pathogens, but their effect is weak, especially in *R.solani* resistance. The stone objects which are resistant to harsh conditions because They have the ability to still for several years alive in the soil or on plant manures It also has a large family and can be reconstructive (Howard et al. , 2007). As a result of the emergence of environmental pollution and the negative effects of the irresponsible use of chemicals in agriculture, recent studies have resorted to the use of some biocontrol agents to limit or reduce the incidence of pathogens found in soils (Yassin et al., 2013). These factors include *A.niger*, which has a high ability to grow and compete with pathological factors affecting plants, so it is used in bio-resistance as well as high antagonistic capacity against many pathogens. In addition, a fungus (*A.niger*) works to increase plant growth (Rikabi, 2008 and Gold, 2006). The research aims at:

The use of *A.niger* fungus as a vital agent against the rot disease and death of seedling that caused by R.solani fungus.

2. MATERIALS AND METHODS

Fungi used in the study:

1. *Aspergillus niger* fungus was obtained from Prof. Dr. Majeed Meteb Diwan, which was propagation and conservation done in the refrigerator under temperature of 4°C for conducted a laboratory and field studies (Characterized by non-production of toxins).

2. Get the fungus Rhizoctonia solani

The *R. solani* fungus were isolated from 10-day cucumber seedlings, taken from the greenhouses of the Abbasiya region in Najaf. Seedlings were brought to the laboratory, washed for several hours under running water, then washed with sterilized distilled water several times and placed on sterile filter paper to remove excess water, the vegetation for seedling was removed and the remaining part and the stem were cut into parts of about 0.5-1 cm long. These pieces were cultivated in the PDA-prepared dishes with 5 pieces per dish. The cultivating PDA was incubated in incubator at 25 ± 2 ° C and after 2- 3 days, The dishes were examined and the fungal colonies developed in the dishes of the infected seedlings were removed by transferring them several times to dishes containing the prepared nutrition media until the colonies were obtained pure, the fungus according to its own taxonomic keys (Tsuneo watanabe, 2002 and Domsch, 1980). The selected isolates were kept in the refrigerator on the PDA nutrition media in a test tube for subsequent studies.

3. Testing the pathogenicity of fungi *A.niger* and *R.solani*

The vaccine of two fungi *A. niger* and *R.solani* were added independently to sterile soil taken from the field of experiment, with a petri dish per 1500 g of soil placed in nylon bags. Mix the soil and vaccine well to ensure uniformity of the pollen with soil and distribute contaminated soil to 3 pots as replicates with diameter of (20 cm) and depth of (25 cm), taking into consideration the work compared to (3) replicates of sterile soil. Each pot was cultivated with 5 cucumber seeds of hybrid type after sterilization with sodium hypochlorite at 2% concentration for 2 minutes, washed with sterilizer distilled water twice and then put on sterile filter papers to dry it. The pots was irrigated with sterile water, covered with transparent nylon (polyethylene), placed in the greenhouse. When seedlings appeared, the percentage of seeds grown in different treatments was calculated. The percentage of seed germination was calculated according to the following equation:

$$\text{Percentage of germination of seeds} = \frac{\text{Number of seeds germinating}}{\text{Total number of seeds}} \times 100\%$$

4. Test the antagonistic capacity of the pathogenic fungi (*Rhizoctonia solani* and *Aspergillus niger*)

In this test, the double implant technique was used, where a 9 cm diameter petri dish divided which contains of the potato sucrose media, the sterilizer was divided into two

equal parts, then the center of each section was Vaccinated with a 0.5 cm disc from the colony edge of both two fungus *A. niger* and *R. solani* at age of 7 days, With a comparative treatment in which the tablet was vaccinated in the center of one half of the dish with fungi *A. niger* and *R.solani*, separately. The experiment was performed with three replicates per treatment and incubated at 25 ± 2 ° C for 5 days. The percentage of inhibition of pathogenic fungi was calculated according to the equation:

To inhibit pathogenic fungi% = $\frac{\text{Radial growth rate in control treatment} - \text{Radial growth rate of treatment}}{\text{Radial growth rate in control treatment}} \times 100\%$

5. Field experiment:

The experiment was conducted in mid-January 2017 in one of the greenhouses of Al-Abbasiya in Najaf Province, using the seeds of cucumber (hybrid) from the Department of Horticulture - Ministry of Agriculture, cultivated in clay trays in sterile soil until it reached the stage 3-4 leaves, was transferred to the soil of the plastic house with 500 m2 area, soil was prepared by tillage, smoothing, settling and covering the plastic house. After that, the land was divided in the form of a terraces with width of 1 m and the distance between the terrace and another 50 cm with a distance of 50 cm between the sides of the house, Drip irrigation pipes were laid along the length of terrace and at the rate of two lines on both sides of the substrate. The farm fertilization program was used. It is a comparative program in terms of the costs incurred when using chemical pesticides and mineral fertilizers and is used by the farmer for the crop management and obtained from the agricultural extension department of the Directorate of Agriculture.

Mix the *A. niger* mushrooms with the soil and repeat the same treatments with the addition of fungus *R.solani*

A. niger fungi were added to the soil with moisturizing. The pathogenic fungus *R.solani* was added to the soil before 48 hours of the addition of *A.niger* fungus. All the treatments were regularly fed until the stage of harvesting .The indicators measured the percentage of germination after 10 days and the diameter of the stem using the (Vernier) and dry weight by using a Sensitive Balance after weight stability for the fifth leaf (g) and estimated the following traits in the analysis laboratory / soil department / college of Agriculture / University of Kufa.

1. Estimate the percentage of phosphorus:
It was estimated using ammonium molybdate and ascorbic acid (Page et al., 1982).

2. Determination of the hormone concentration (Cytokinin Cks.) (Micromol.kg-1.dry weight)
The plant hormone (Cytokinin) was estimated according to the method used by Nuray et al. (2002).

3. Determination of the effectiveness of ascorbate peroxidase (unit / micromol)
The efficacy of peroxidase was estimated by the method (Nakana and Asada, 1981).

4. Statistical analysis:
All experiments were conducted factorial according to the complete random design (CRD) for laboratory experiments. As for the experiments of the pots and the field, they were conducted according to Randomized Complete Blocks Design (R.C.B.D). The average of the treatments was calculated according to the least significant difference method (LSD and 0.01 and 0.05) (Al-Rawi and Khalaf Allah, 2000). The results were analyzed using the Genstat12th Edition 2009 statistical analysis program. The results were analyzed using the Genstat12th Edition 2009 statistical analysis program.

3. RESULTS AND DISCUSSION

1. Effect of *Aspergillus niger* and *Rhizoctoni solani* fungi on the percentage of cucumber seed germination in plastic pots.

Table (1) shows that the pathogenic capacity of *A. niger* and *R. solani* in plastic pot. The *A.niger* fungus has increased the germination

rate by 100.00%, while the percentage of seed germination under the *R. solani* fungus is 00.00% compared to the control treatment of 73.33%.

Table 1: Test the effect of isolates *Aspergillus niger* and *T.harzianum* on the percentage of cucumber seed germination

fungi	Germination %
Aspergillus niger	100.00
Rhizoctoni solani	0.000
Control	73.33
L.S.D=0.05	19.46

It was found that the fungus *A.niger* worked to raise the germination rate because of its ability to secrete biochemical that stimulate germination and thus help to increase the ratio (Hasan, 2002). As for *R. solani*, it reduced the percentage of germination to 00.00 It is probably due to its high pathogenicity to produce some of the bacteriostatic enzymes (Bartz and others, 2013).

2. Effect of the *Rhizoctoni solani* and *Aspergillus niger*:

The results of the antagonistic experiment showed that *A. niger* had a high antagonistic capacity *R. solani* fungus, The percentage of inhibition of pathogenic fungi was 91.58% in P.S.A. This may be due to its high ability to produce toxic metabolites such as *Griseofulvan Flavicin, Aspergillin, Jawaheren, Funagalin* (Siddiqui et al., 2004 and Trabelsi, 2007), which may affect the pathogenic activity of pathogenic fungi and thus affect the pathogenic growth of pathogenic fungi as well as its production of pathogenic cells of Chitinase, Cellulase, lipase and pectinase cells (Nehwani et al., 2010, El-Ghany et al., 2010).

3. **Effect of *Aspergillus niger* on the percentage of cucumber seed germination after 10 days of cultivating in the plastic house and with the presence and absence of fungi *Rhizoctoni solani***

The results of Table (2) showed that the use of *A.niger* fungus in the ratio of seed germination option to the treatment of plant fertilization program and the treatment of fungus *Aspergillus niger* and the presence of fungus *R.solani* as well as the treatment of the program of fertilizing farms in the absence of fungus *R.solani*, where the percentage of seed germination 73.30 and 73 70 and 70.00% respectively, which was significantly excelled on other treatments.

Table 2: Effect of *Aspergillus niger* fungus in the percentage of cucumber seed germination after 10 days of cultivating in the plastic house, with the presence and absence of fungi *Rhizoctoni solani*

Treatments	**With the presence of *R.solani* fungus %**	**With the absence of *R..solani* fungus %**
Only soil	30.00	40.00
Farm fertilization program	73.30	70.00
***Aspergillus niger* fungus**	73.70	66.30
L.S.D=0.05	14.92	

The reason for the increase in seed germination is that *A. niger* has a positive effect on the improvement of most growth indicators such as germination percentage, plant height, fresh and dry weight of vegetative and root system (Al-hamdani, 2006). As well as the ability of *A.niger* mushroom on the production of compounds such as: hexyl maleic acid 3-n-2-Carboxymethyl, which has an important effect in accelerating the process of seed germination and increase the growth of the total vegetative (Mondal et al., 2000).

4. Effect of *Aspergillus niger* fungus on the diameter of cucumber stem

diameter (mm) in the plastic house and with presence and absence of *Rhizoctoni solani* after 40 days of cultivating.

The results of Table (3) showed the effect of the use of *A.niger* fungus in the stem diameter of the cucumber (mm). The treatment of the plant fertilization program with the presence of the fungus *R.solani*, in which the stem diameter of the 8.8 mm was significantly excelled than all the treatments except the same treatment in absence fungus *R.solani* which reached 8.1 mm

Table 3: Effect of *Aspergillus niger* fungus use on the cucumber stem diameter (mm) in the plastic house and with the presence and absence of *Rhizoctoni solani* after 40 days of cultivating.

Treatments	With the presence of *R.solani* fungus (mm)	With the absence of *R..solani* fungus (mm)
Only soil	5.5	5.2
Farm fertilization program	8.8	8.1
***Aspergillus niger* fungus**	7.8	7.5
L.S.D=0.05	0.7074	

5. Effect of *Aspergillus niger* on the dry weight of cucumber leaves (g) in the plastic house and with presence and absence of *Rhizoctoni solani* after 40 days of cultivating.

The results of Table (4) showed the effect of the use of *A. niger* in dry weight of cucumber leaves (g). *Aspergillus niger* fungus treatment excelled with presence of *R.solani* fungus, which reached 1.97 g significantly compared to all treatments**.**

Table 4: Effect of *Aspergillus niger* fungus use on the dry weight of cucumber leaves (g) in the plastic house and with the presence and absence of *Rhizoctoni solani* after 40 days of cultivating

Treatments	With the presence of *R.solani* fungus (gm)	With the absence of *R.solani* fungus (gm)
Only soil	0.96	0.65
Farm fertilization program	1.73	1.66
***Aspergillus niger* fungus**	1.97	1.87
L.S.D=0.05	0.027	

From Tables(3, 4) The increase in these traits may be due to the ability of the fungi *A.niger* to secrete many secondary compounds, such as the growth hormone AAI, which works to increase with the rate of cell division. This leads to an increase in the root system (Yadav et al., 2011), then positively reflected on the studied traits And perhaps due to the ability of some isolates of fungi in the release of nutrients from the soil and increase the readiness and transfer to the plant tissues and these elements such as phosphorus when the phosphorus enters the structure of lateral roots and Side root whiskers. This leads to an increase in the total root and vegetable (El-Ghany and others, 2010).

6. Effect of *Aspergillus niger* fungus on Percentage of phosphorus in leaves of cucumber in the plastic house and with presence and absence of *Rhizoctoni solani* after 40 days of planting.

The results of Table (5) showed the effect of the use of *A. niger* fungus on the percentage of phosphorus in leaves of cucumber to the superiority of the treatment of the addition of *A. niger* to the soil with the presence of fungus *R.solani* significantly excelled in the highest percentage of phosphorus, which amounted to 0.037% compared to all the treatments.

Table 5: Effect of *Aspergillus niger* fungus use on the Percentage of phosphorus in leaves of cucumber (g) in the plastic house and with the presence and absence of *Rhizoctoni solani* after 40 days of cultivating

Treatments	With the presence of *R.solani* fungus (gm)	With the absence of *R.solani* fungus (gm)
Only soil	0.013	0.009
Farm fertilization program	0.018	0.011
***Aspergillus niger* fungus**	0.037	0.025
L.S.D=0.05	0.01188	

The results of Table (5) showed an increase in the percentage of element P in leaves of cucumber plant when treating the soil with *A. niger*. It is possible that *A. niger* has a role in the secretion of certain compounds such as enzymes and organic acids that are linked to calcium phosphate and lead to liberation or release some nutrients and then increase their availability for plants (Al-Ghany et al., 2010). *A. niger* is one of the best fungi which dissolves phosphate in basal soils, which increase the root system, which helps the plant absorb nutrients from different places of soil (Achal) , 2005 and Richa et al., 2007). Phosphorus is an essential element in increasing the activity, growth and development of the plant's root system. This increases the plant's ability to absorb water and other nutrients. (Al-Sahaf, 1989). *A. niger* has the ability to dissolve and increase the availability of many important nutrients such as phosphorus and to increase leaf content of this element (Nehwani et al., 2010 and Azzawi et al., 2013). This increase is due to the increased availability of phosphorus in soil added to the *A. niger* and to the role of fungi in the secretion of organic acids and the secretion of phosphatase enzyme, which increases the level of dissolved phosphorus in phosphorus-poor soils (Grover, 2003 and Guang-Hua et al., 2005). The plant needs the phosphorus element because it enters with the proteins in the formation of cellular membranes such as plasma membrane, mitochondria, green plastids and Vacuole membrane by forming phospholipids such as Lecithin. It enters the construction of storage and energy transfer compounds and enzymatic accompaniments as well as entering into the installation of bio-membranes, Which form the basic components of building and plant permanence, as well as its role in increasing the occurrence and improve the quality and early maturity of fruits. It also helps in the formation of lateral roots of some plants and root whiskers (Tisdale et al., 1997).

7. Effect of use *Aspergillus niger* on leaf content of Cytokinin (mg / kg fresh weight) in plastic house and with the presence and absence of *Rhizoctoni solani* after 40 days of cultivating

The effect of the use of *A. niger* in leaf content of Cytokinin was observed in the results of the treatment of *A.niger* fungi with the presence and absence of *R.solani* fungus, in which the content of Cytokinin in leaves was 2.98 and 2.95 mg / kg fresh weight, respectively, and was significantly excelled than all other treatments.

Table 6: Effect of *Aspergillus niger* fungus use on the leaf content of Cytokinin (mg / kg fresh weight) in the plastic house and with the presence and absence of *Rhizoctoni solani* after 40 days of cultivating

Treatments	With the presence of *R.solani* fungus (mg/kg fresh weight)	With the absence of *R.solani* fungus (mg/kg fresh weight)
Only soil	1.23	1.19
Farm fertilization program	2.76	2.72
***Aspergillus niger* fungus**	2.98	2.95
L.S.D=0.05	0.02257	

The results of Table (6) showed an increase in the content of the Cytokinin hormone in cucumber leaves when treated with *Aspergillus niger*. El-Ghany et al., (2010) attributed this to the ability of *A. niger* to produce Cytokinin , Which increases the absorption of the roots of the growth hormone and then increase its concentration in the plant, or as a result of high content of leaves of nutrients, proteins, vitamins and carbohydrates (Yadav and others, 2011).

8. Effect of use *Aspergillus niger* on leaf content from pyroxidase concentration (unit / micromol) in the plastic house and with presence and absence of *Rhizoctoni solani* after 40 days of cultivating

The results of the effect of the use of *A. niger* in the leaf content of peroxidase showed that the highest concentration of peroxidase was 0.14 (unit / micromol) in the treatment of the addition of *Aspergillus niger* to the soil of cucumber plants after 40 days of cultivation with the presence of fungus *R. solani*, and that this treatment was significantly excelled than other treatments.

Table 7: Effect of *Aspergillus niger* fungus use on the leaf content from peroxidase concentration (unit/micromol) in the plastic house and with the presence and absence of *Rhizoctoni solani* after 40 days of cultivating

Treatments	With the presence of *R.solani* fungus (unit/micromol)	With the absence of *R.solani* fungus (unit/micromol)
Only soil	0.06	0.04
Farm fertilization program	0.08	0.05
***Aspergillus niger* fungus**	0.14	0.11
L.S.D=0.05	0.064	

The reason may be due to the role of *A.niger* in increasing nutrients in plant tissues, stimulating systemic resistance in plants and producing pathogens-related proteins, which included many enzymes produced by *A. niger*, Or perhaps due to the fact that *A.niger* fungus has increased the effectiveness of the enzyme peroxidase, Biological agents, such as fungi, have stimulated plant resistance against pathogens by stimulating the gene expression of the enzyme peroxidase, leading to plant resistance to pathogenic fungi (Al Murat, 2011). The induction of resistance against pathogens is due to the effect of the enzyme of peroxidase. This enzyme works with hydrogen peroxide in the break of pathogenic enzymes, including Pectinase, And activating the process of breaking the cell wall and the induction of Phytoalexines as well as structural payments to strengthen the walls such as the construction of Lignin and interact with the enzyme cell wall proteins to form cross-links and multiple compounds, increasing the hardness of the cell wall and the inability of the pathogen to penetrate the walls of the cell and impede the introduction (Hiber

et al., 2007) The increase in the enzyme and increase its effectiveness in the plant with the presence of fungus as a reaction to the plant because the plant infected with pathogens stimulates resistance against the causes of resistance to plants in the possession of their already antimicrobial disease.

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