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Effect of Different Concentrations of Neem oil on Damping off ond Root Rot Disease of okra And Tomato Caused by Fusarium Solani.

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Abstract: The Results of this study showed that the biopesticide Fytomax had high ability in inhibition the growth of the pathogenic fungus Fusarium solani when it used in concentration of 2ml/L as inhibition percentage reached 87.6%. On the other hand biopesticide increased seed germination of both tomato and okra seeds were soaked with biopesticide suspension at concentration of 2ml /L Where the percentage of seed germination reached 70% with okra seeds, Results also showed that biopesticide decrease damping - off and root rot diseases of both plants.

Key word: Fusarium solani, neem oil, tomato, okra, damping off, root rot

1. INTRODUCTION

The fungus Fusarim solani Mart is one of the soil-endemic fungi, and it is one of the economically important fungi where it causes several plant diseases such as root and stem rot disease,dampping off seedling and seed rot for several plants such as tomato, potatoes, peppers, eggplant, okra and several plants belonging to other families such as the cucurbit family and causes them Serious losses (Nelson, 2004, Boughalleb and El-Mahjoub, 2006, Roberti et al., 2012, Baker et al., 2013), Infection with this fungus is usually accompanied by the production of mycotoxins, phytotoxin, and this fungus is characterized by its high competitiveness (Gray et al., 1999 and Agrios, 2005).The bio control to plant diseases has minimal adverse effects on physiological processes in plants .It also has few environmental risks because the final products are easily converted into common organic materials that are less dangerous to humans and animals compared to commercial pesticides (Isman, 1989). The Meliaceae family, which is famous for its many uses in the control of many diseases (Abbasi et al., 2003 and Dubey et al., 2009), and infection caused by insect pests and nematodes (Schmutterer, 1995)The toxic effect of neem oil and its products is due to the fact that they contain compounds such as Azdiractin, Salannin, and Nimbin, which have an inhibitory, toxic, reducing or anti-toxin effect by some toxin-producing fungi (Geraldo et al., 2010 and Anand et al., 2010). Effectiveness in controlling fungi and their germs (Simone et al., 2009). In line with this trend, this research came to evaluate the effectiveness of Fytomax, extracted from neem seed oil, against the fungus F. solani.

Materials and methods:

Isolation and purification of pathogenic fungi

The fungus F. solani was isolated from the roots of tomato seedlings with signs of root rot, where the roots were cut into small pieces 0.5-1 cm, then sterilized with sodium hypochlorite solution NaOCl (10% of the commercial preparation) for 2 minutes, then washed with sterile distilled water, and after drying, transferred 3 -4 pieces were cut into Petri dishes containing sterile PDA culture medium and the antibiotic Chloramphenicol 250 mg/L was added to it, then the Petri dishes were incubated in the incubator for 7 days at a temperature of 25 ± 2 °C. 25 ± 2 °C for a week.

Testing the pathogenicity of the fungus F. solani

A soil mixture consisting of agricultural soil and peat moss in a ratio of (1:2) was used. Sterilize the mixture with an autoclave device at a temperature of 121 °C and a pressure of 15 pounds / inch² for an hour, and re-sterilization after 24 hours, the inoculum of the pathogenic fungus F. solani grown on millet seeds was added to the soil Sterile 0.5% (w/w)The soil and the inoculum were placed inside a cellophane bag and agitated well in order to distribute the vaccine homogeneously in the soil. The soil contaminated with fungi inoculum was distributed among six pots, with 1 kg of soil per pot. As for the control treatment, only sterilized millet seeds were added at an average of 0.5% (weight / weight), 10 seeds of tomato and okra were planted in each pot respectively.The pots were watered very carefully, then placed in the greenhouse, and after two weeks of planting, they were re-isolated from dead seedlings and plants that showed symptoms of infection for the purpose of verifying the pathogenic fungus.

Studying the effect of different concentrations of Fytomax on the growth of the fungus F. solani

Phytomax pesticide (produced by the English company Russell IPM UK) was obtained from A. Dr.. Ibrahim Jadoua Al-Jubouri A series of concentrations were prepared (0.5, 1.0, 2.0, 2.5) ml/liter of the culture medium by adding the withdrawn quantities of the pesticide to the medium before it solidified, then the medium containing the pesticide was poured into sterilized Petri dishes and after the solidification of the medium, the center of each dish was inoculated with a drop disc. 0.5 cm was taken from the edge of the fungi farm F. solani. The experiment was conducted in three replicates for each concentration, and the control treatment included growing the fungus on a pesticide-free culture medium.Then the dishes were incubated at a temperature of 25 ± 2 °C, and after the fungi colony in the control treatment reached the edge of the dish according to the growth rate of the fungi by taking the average of two perpendicular diameters passing through the center of the dish, then according to the diagonal growth rate of the fungi and the percentage of inhibition through the following equation:

$$\textbf{inhibition percentage \%} = \frac{\textit{The fungi diameter in control} - \textit{the fungi diameter in the treatment}}{\textit{The fungi diameter in control}} \times 100$$

Studying the effect of Fytomax on the infection of tomato seedlings with the fungus F. solani

A- Preparing the soil for cultivation:

This experiment was conducted in plastic pots with a capacity of 1 kg, the soil consisting of agricultural soil was sterilized with peat moss in a ratio of (1:2) with a formalin solution

consisting of 20 ml of commercial formalin / literThe solution was used in the amount of 3 liters / 1 m3 and the soil was left for 3 days covered with bags, then the bags were lifted and left exposed to the sun for 3 days for the purpose of volatilization of formalin (Tawajen, 1975).

Preparing the inoculum for the fungus F. solani:
fungi inoculum was prepared by growing it on millet seeds. The seeds were washed and then soaked for 6 hours, then placed in glass flasks of 500 ml capacity at a rate of 50 g / beaker, and then sterilized by an Autoclave at a temperature of 121 ° C and a pressure of 15 pounds / inch for 20 minutes. After taking it out, it was left to reduce the temperature of the beakers, and after it was lowered, each beaker was inoculated with tablets (2 tablets) taken from the edge of a recent farm for the fungus F. solani. The flasks were incubated at a temperature of 28 °C for 14 days, taking into account the stirring and stirring of seeds every 2-3 days to ensure the distribution of the fungal inoculum, then the potted soil was inoculated with mushrooms at an average of 0.5 g/kg soil, and the experiment included the following treatments and three replicates for each treatment.

1- Tomato seeds covered with 1% Phytomax pesticide for 10 minutes and then planted in soil contaminated with the fungus F. solani.
2- Tomato seeds covered with 2% Phytomax pesticide for 10 minutes and then planted in soil contaminated with the fungus F. solani.
3- Tomato seeds were planted in soil contaminated with the fungus F. solani, and after a week, the potted soil was irrigated with 1% Phytomax pesticide.
4- Tomato seeds were planted in soil contaminated with the fungus F. solani, and after a week, the potted soil was irrigated with Phytomax 2% pesticide.
5- The control treatment containing seeds planted in soil contaminated with the fungus F. solani.
6- The control treatment containing seeds planted in uncontaminated soil with the fungus F. solani.
The pots were planted at an average of 10 seeds / pot, and after germination, the percentage of germination was calculated, then the damping off percentage of seedlings was calculated through the following equations:

$$\text{Seedling damping off percentage\%}=\frac{Number\ of\ dead\ seedlings}{Total\ number\ of\ seedlings}\times 100$$

The same experiment was repeated using okra instead of tomato
2-4 Statistical Analysis
All laboratory experiments were coundected by a C.R.D. The averages were compared using the least significant difference method, R.L.S.D. and at a probability level of 0.01 for laboratory experiments (Al-Rawi and Khalafallah, 1980).

2. RESULTS

Diagnosis of pathogenic fungi
The fungus and its species, Fusarium solani Mart, were diagnosed based on approved taxonomic keys, and microscopic tests showed the formation of three types of spores, including Macroconidia in most cases straight or slightly curved, containing 3-7 transverse divisions with a small end As for the second type, it is Microconidia that is kidney-shaped or oval without septa or branches containing a single spore formed on a round false head and on

long monophialids. The colony color on the medium of the PDA is usually white to cream with sometimes brown pigments, while chlamydial spores form at intervals of 1-4 weeks terminal or interfacial (Booth, 1977 and Leslie and Summerell, 2006).

News of the pathogenicity of the fungus F. solani

The results showed the ability of the fungus F. solani to reduce seed germination and increase the percentage of seedling death on both plants, where the percentage of seed germination in tomato and okra reached 46.6 and 36.6, respectively, while in the comparison treatment it reached 76.6 and 70, respectively, for both plants.While the rate of seedling damping off in tomato and okra was 43.3 and 36.6, respectively, while the control treatment was 0% for both plants.

Studying the effect of different concentrations of Fytomax on the growth of the fungus F. solani

The results (Figure 1) showed the efficiency of the pesticide Fytomax in inhibiting the growth of the diameter of the pathogenic fungusF. solani, as the concentration of 2 ml / liter gave the best inhibition rate of 87.6% with statistically significant differences compared to the concentration of 0.5 ml / liter which gave the lowest inhibition rate of 42.4%. This is consistent with what was found in a previous study that the percentage of fungal growth inhibition increases directly with the increase in the concentration of Phytomax pesticide (Ashfaq Ahmed et al., 2016).

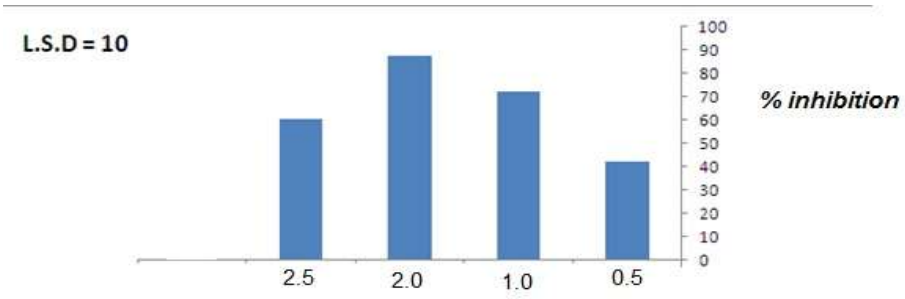

Figure (1) Studying the effect of different concentrations of Fytomax on the radial growth of F. solani.

Studying the effect of Fytomax on infecting tomato and okra seedlings with the fungus F. solani

The results in Table (1) showed that the fungus F.solani had a significant effect on reducing the percentage of seed germination with statistically significant differences from the control treatment (without fungus), where the percentage of seed germination in the fungus treatment was 20% compared to 86.7% for the control treatment. The results in Table (1) also showed that the use of the pesticide Phytomax had a significant effect on the percentage of tomato seedlings damping off, and in both cases when it was used as a preventive or therapeutic pesticide, the pesticide had a significant effect when used with a concentration of 1% as a preventive pesticide on the germination rate, which amounted to 66.7% And also a significant effect as a therapeutic pesticide when used at a concentration of 2%, where the percentage reached 60%, compared to the treatment of fungi only, when the percentage of germination was 20%.In the case of the percentage of seedling damping off, the preventive pesticide

treatment had a significant effect, where the peracentage of seedling damping off was 0% at a concentration of 2% compared to the fungus treatment only, as it reached 50%This is consistent with what Locke (1995) found that using neem oil at a concentration of 2- 10% in the field leads to a reduction in diseases caused by Fusarium oxysporum, Aspergillus niger and Alternaria alternata, and Niaz et al. (2008) also found that neem seed oil has an inhibitory activity against a number of Of the plant pathogenic fungi such as Fusarium moniliforme, Rhizoctonia solani and Macrophomina phasiolina.

Table (1) Effect of Fytomax on the percentage of germination and damping off of tomato seedlings

%**damping off seedlings**	**germination** %	**Treatments**
8.3	66.7	Phytomax pesticide (soaking the seeds) 1ml/L
0	60	Phytomax pesticide (soaking the seeds) 2 ml / L
27.8	53.3	Phytomax pesticide(Surface Irrigation) 1ml/L
9.4	60	Phytomax pesticide (Surface Irrigation) 2ml/L
50	20	F.solani fungi Only
0	86.7	Control
17.6	57.8	average
L.S.D for germination = 18.76 L.S.D for seedling damping off = 32.49 L.S.D for interaction between factors = 45.95		

The results in Table (2) also showed the effect of the pesticide Phytomax on the percentage of germination and seedling death of okra plant. The results showed the ability of the pesticide to raise the germination rate when it was used as a preventive pesticide with a concentration of 2%, where the germination rate reached 70% compared to the treatment of pathogenic fungi only, which amounted to 20%.The results also showed the moral effect of the pesticide on the percentage of okra seedlings damping off when it was used as a preventive pesticide with a concentration of 2% and a curative pesticide with a concentration of 1% and 2%, where the percentage of seedling damping off was 0% in all of them compared to the treatment of the fungus only, which amounted to 21.1%.This is consistent with what was found by Ramjegathesh et al. (2011). The use of neem oil at a concentration of 3% led to a reduction in the appearance of the fungus A.alternata that causes leaf blight on onions when spraying Allium cepa with oil at the onset of the disease and after 15 days. It was also noted that there was an increase in the amount of yield. ,The effect of neem in inhibiting the growth of fungi is also due to the chemical compounds it contains, such as Azdiractin, Salanin and Nimbin (Anand et al., 2010), and its effectiveness in controlling and sprouting fungi (Simane et al., 2009).

Table (2) Effect of Fytomax on the percentage of germination and damping off of okra seedlings

Treatments	% **germination**	%**damping off seedlings**
Phytomax pesticide (soaking the seeds) 1ml/L	50	8.9
Phytomax pesticide (soaking the seeds) 2 ml / L	70	0
Phytomax pesticide(Surface Irrigation) 1ml/L	40	0
Phytomax pesticide (Surface Irrigation) 2ml/L	60	0
F.solani fungi Only	20	21.1
Control	81.7	
average	53.6	
L.S.D for germination = 7.20 L.S.D for seedling damping off = 12.47 L.S.D for interaction between factores = 17.64		

The results of the experiment showed that the use of Fytomax, whether in seed soaking (preventive effect) or soil treatment (curative effect), reduced the negative effect of pathogenic fungi and may be due to the compounds it contains such as Azdirechtin, Salanin and Nimbin, which have an inhibitory effect on fungi (Anand et al., 2010).The effectiveness of the compound as a therapeutic pesticide is due to its direct effect on the mycelium by inhibiting the growth and sporulation of the fungus (Simone et al., 2009), and it stimulates systemic resistance in the plant (Singh and Prithiviraj, 1997). It may also be due to the use of neem extracts In the soil for a long time leads to an increase in the number of microorganisms in both the soil and the rhizosphere (Sarawaneeyaruk1 et al., 2015),Previous studies have also demonstrated that plant growth varies with the spread of beneficial microorganisms in the soil and rhizosphere and that a change in the number of beneficial microorganisms may enhance plant root growth and increase the decomposition of organic matter and the turnover of major plant nutrients (Madigan et al., 2011),The reason is also that neem oil works on the development of ammonia production in the soil, making it alkaline and this leads to an increase in antimicrobial activity (Dubey et al., 2009).

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