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STUDY THE COMPLEX DISEASE BETWEEN THE *FUSARIUM OXYSPORUM* AND NEMATODE *MELOIDOGYNE JAVANICA* ON CUCUMBER AND THE POSSIBILITY OF BIOCONTROL IT BY BIO-FUNGUS *TRICHODERMA HARZIANUM*

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Abstract

Agricultural pests are one of identified factors of crops as many botany scientists have shown, plant nematodes are one of the most important and dangerous five economic pathogens in the world due to their interaction with other organisms like fungi and bacteria. This study aimed to isolate pathogenic fungi associated with nematodes from cucumber roots and the ability to control them by bio-fungus Trichoderma harzianum and chemical fungicide topsin in Basra city in Iraq. Three types of fungi were isolated from the roots of cucumber plant infected with the nematode root pathogenic test of these fungi was carried out on the cucumber plant and the lowest proportion of seed germination was in F.oxysporum and M.phaseolina which reached to 67.60% and 75.00% respectively follow by R.solani which reached to 82.50%. The highest ratio for seedling death in F.oxysporum and M.phaseolina which reached to 18.51% and 16.66% respectively followed by R.solani which reached to 12.12% . The results showed the ability of bio-fungus T.harzianum to inhibit the growth of pathogenic fungus F.oxysporum by dual culture process, the antagonistic ratio reached to 1 in F.oxysporum according to the Bell scale. The using of bio-fungus T.harzianum in concentration 1.7 x 10^6 in vitro lead to reduce the eggs hatching ratio and death of nematode to 23.44% and 21.46% respectively. While the use of fungicide topsin lead to reduce the eggs hatching ratio and death of the nematode to 16.66% and 15.68% respectively. F.oxysporum is completely inhibited by fungicide topsin. Also the results showed the effect of different treatments of soil contaminated with nematode root knot M.javanica and non-contaminated soil with nematode root knot M.javanica in the laboratory on seeds germination and seedling death in T.harzianum + topsin treatment, T.harzianum treatment and control which reached to 100% . While the lowest ratio of seedling death and infection severity in non-contaminated soil with nematode root knot M.javanica which reached to 0.00%. Whereas the lowest number of root knot in the same treatments in contaminated soil reached to 8, 6 and 14 knots / plant respectively. The highest plant length was in T.harzianum + topsin treatment, T.harzianum treatment and control non-contaminated soil with nematode root knot M.javanica which reached to 143.00, 135.00 and 136.00 cm / plant respectively, while roots length was 15.00, 11.00 and 13.00 cm /plant.

Keywords: Fusarium oxysporum, Meloidogyne javanica, Trichoderma harzianum.

1. INTRODUCTION

There are many fungal diseases attacked cucumber plant in different growth stages which can results in plant yeild losses, among these diseases root rot and Fusarium wilt. the diseases of soil borne are very substantial and in charge of the lossing in plant yield. *Fusarium oxysporum f. sp. cucumerinum* considered a main pathogenic fungus caused fusarium wilt in cucumber plant which decreased the yield of this plant (Martinez et al. 2003). Also there are several soil borne pathogenic

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fungi such as; *Pythium spp*., *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Phytophthora spp.* can contaminate cucumber plant and caused root rot and damping-off (Bedlan,1986). Among many plant pathogens, nematoda are one of the most important and dangerous five pathogens in the world which are difficult to control in addition to their ability to break plant resistance to some other diseases (Alhazmi, 2009). Plant damage is increased when there is an interation between nematoda *Meloidogyne spp.* and fungal pathogens. There are many studies that clearly showed the role of nematoda in affecting the proportion and severity of infection of various pathogens, which result in a complex disease in which the symptoms are complex and more harmful from injury to any of these causes individually (Jarjis et al., 1992). *Trichoderma spp.* due to its mycolytic enzyme secretions has high potential to combat with the destructive plant pathogens. It fights with competitive pathogen for space and food by stopping its activities and keeling the pathogen thus play significant role in enhancing the growth of the crops of economic importance (Sajjad et al., 2017). This study was designed to study the complex disease between the *F.oxysporum* and nematode *M.javanica* on cucumber and the possibility of biocontrol it by bio-fungus *T.harzianum*.

2. MATERIALS AND METHODS

The fungi associated with nematoda were isolated from the roots of cucumber plants which showing symptoms of root nodules. The fungi were kept on PDA (slant) in the refrigerator at 2-8 ^{0}C until use and the percentage of colonies was calculated according to the following equation;

$$\%\text{Appearance of colonies} = \frac{\text{Number of samples showing species or genus}}{\text{Total number of samples}} \text{ X 100}$$

The mixture soil was mixed with a 1: 3 peat moss was sterilized at 121 ^{0}C and atmospheric pressure of 15 lbs. / Ang² for an hour and for two consecutive days and then placed in sterilized pots with capacity of 500 grams of dry soil. The vaccine was added by 1/3 dish / pot, and mix well with three pots for each fungus, leaving three pots without vaccination as a control treatment and then planted in each pot of ten seeds of cucumber plant carefully sown the seeds and then placed in the growth room at 31 ± 2 ^{0}C and lighting 16 hours a day with watering when needed (Al-waily, 2004). The percentage of germination after 10 days and the ratio of seedling death calculated after three weeks of germination according to the following equations (Mickenny, 1923; Al-waily, 1988).

$$\%\ \text{germination} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} x\ 100$$

$$\%\ \text{seedlings death} = \frac{\text{Number of dead seedlings}}{\text{Number of germinated seedlings}}\ x\ 100$$

Antagonism tests antithesis between strains pathogenic fungus *F.oxysporium* and bio fungus *T.harzianum* according to the method of Bell et al. (1982). Appropriate amounts of cucumber roots were collected from the root nodes of the Shatt al-Arab region in Basra and the infected roots were carefully washed for the purpose of disposing of the suspended soil without the egg bags. The roots were cut into small pieces 2-4 cm and then placed in an electric blender mixer and placed an appropriate amount of distilled water with 1% sodium hydroxide. Mixer function for 2 minutes followed by shaking manually 2 min, contents mixture were received in sieves ranging from 5 mm to 38 μm (400 mesh), then the concentration of eggs in the latter and washed for several minutes with tap water and distilled water. The eggs were collected in a 250 mL flask with distilled water

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and then incubated eggs at 28 ^{0}C and for 3-5 days to hatch into the larvae of the second stage, which is sufficient to hatch most of the eggs to the larvae of the second phase of the root nodules (Hussey and Barker, 1973). While the effect of *T.harzianum* and topsin fungicide on hatching eggs and larvae of the second phase of the root nodules *M.javanica* was investigated by adding 1 ml of freshly extracted eggs and 32 mL egg/ml and second larvae containing 22 larvae to a 5 cm diameter petri dish and add 3 ml of fungus with 1.7x10^6 concentrations and topsin fungicide by instructions of the company, the addition was individually and with three replicates with the treatment of control, which added distilled water only and incubated the dishes at a temperature of 28 ^{0}C and the rate of hatching was calculated after 72 hours. The efficacy of the funginicide topsin on *F.oxysporum* in PDA by addition of topsin to PDA before the it become solid with 1 mL / L and pour in sterile 9 cm diameter petri dishes and then vaccinated the center of each dish with a diameter of 0.5 cm with *F.oxysporum* colony, the treatments were in three replicates for both treatment and control. The effect of *T.harzianum* and topsin in controlling the disease complex; the laboratory experiment was carried out under the conditions of the green house where the mixture soil was mixed with the peat moss at 3: 1 and sterilized at 121 ^{0}C and atmospheric pressure of 15 lbs. / Ang² for an hour and for two consecutive days then packed in 2 kg of dry soil. The following treatments for contaminted and un-contaminted soil with nematoda were included;

Table 1. The treatments in pots in laboratory

Treatments	
1. *F.oxysporum*	**5. *T.harzianum***
2. *F.oxysporum* + *T.harzianum*	**6. *T.harzianum* + topsin**
3. *F.oxysporum* + topsin	**7. Topsin**
4. *F.oxysporum* + *T.harzianum* + topsin	**8. control**

3. RESULTS AND DISCUSSIONS

The results of isolation showed an accompaniment of three types of fungi with cucumber plant infected with *M. Javanica* which were *M.phaseolina*, *R.solani* and *F.oxysporum*. The most common species in all samples were *F.oxysporum*, with an appearance rate of 34.69% followed by *M.phaseolina* by 20.06% and the lowest appearance rate was in *R. solani*, amounting to 18.83%.

The results of the pathogenic capacity of fungi isolated from the seeds of cucumber contaminated with nemtoda, Table 2, showed that the less germination rate was in *F.oxysporum* which reached to 67.60%, followed by *M.phaseolina*, where the germination rate was 75.00% and *R.solani* 82.50%. The percentage of seedling death, Table 2, was highest in *F.oxysporum*, 18.51%, followed by *M.phaseolina* 12.12% and *R.solani* 16.66%.

Table 2. The pathogenic capacity of F.oxysporum, R.solani and M.phaseolina

Pathogenic fungi	**Appearance rate**	**Germination rate%**	**Seedling death%**
F.oxysporum	34.69	67.60	18.51
R.solani	18.83	82.50	12.12
M.phaseolina	20.06	75.00	16.66
L.S.D $_{0.05}$	**9.46**	**6.25**	**4.01**

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The results of the of pathogenic fungus *F.oxysporum* ,topsin fungicide and *T.harzianum* in cucumber plant indictors are shown in Table 3, which the lowest germination rate was in *F.oxysporum* treatment in un-contaminated soil which reached to 73.3%, while the highest seedling death ratio was in *F.oxysporum* treatment in contaminated soil which was 61.66 %. The severity of infection was in highest ratio in *F.oxysporum* treatment in contaminated soil which reached to 69.33%. the highest plant length and highest root length were *T.harzianum* + topsin fungucide treatment in un-contaminated soil which reached to 143.0 cm and 15.0 cm respectively. The highest number of nodes was in topsin fungucide treatment in contaminated soil which reached to 15.0.

Table 3. The effect of pathogenic fungus F.oxysporum , topsin fungicide and T.harzianum in cucumber plant indictors

	Uncontaminated soil					**Contaminated soil**					
Treatments*	**GER**	**SED**	**INF**	**LP**	**LR**	**GER**	**SED**	**INF**	**LP**	**LR**	**NN**
F.oxy	73.3	55.53	46.73	85.0	7.0	80.00	61.66	69.33	73.0	4.0	12.0
F.oxy+ T.h.	86.6	28.33	27.23	97.0	9.0	86.60	45.00	49.23	83.0	7.0	9.0
F.oxy+ Top.	86.6	48.33	35.16	94.0	7.0	93.30	55.53	53.90	80.0	4.0	13.0
F.oxy+T.h.+Top.	93.3	21.66	21.46	113.0	9.0	86.60	33.33	37.23	101.0	9.0	10.0
T.h.	100.0	0.00	0.00	135.0	11.0	100.0	8.33	5.50	107.0	10.0	8.0
T.h. + Top.	100.0	0.00	0.00	143.0	15.0	100.0	8.33	6.66	115.0	9.0	6.0
Top.	100.0	0.00	0.00	131.0	10.0	100.0	15.00	8.33	97.0	6.0	15.0
Control	100.0	0.00	0.00	136.0	13.0	96.00	21.66	6.60	102.0	6.0	14.0
Avarege	**92.47**	**19.23**	**16.32**	**16.75**	**10.12**	**92.81**	**31.10**	**29.59**	**94.75**	**6.87**	**10.87**
$L.S.D.0.05_{soil}$	**4.35**	**5.96**	**4.95**	**2.45**	**0.63**	**4.35**	**5.96**	**4.95**	**2.45**	**0.63**	**----**
$L.S.D.0.05_{treatments}$	**6.55**	**7.13**	**6.74**	**3.41**	**0.81**	**6.55**	**7.13**	**6.74**	**3.41**	**0.81**	**2.32**
$L.S.D.0.05_{intractions}$	**8.62**	**12.35**	**7.93**	**4.13**	**1.03**	**8.62**	**12.35**	**7.93**	**4.13**	**1.03**	**----**

*** GER= germination ratio, SED = seedlings death, INF = severity of infection, LP = plant length, LR = roots length, NN = nodes number, F.oxy = *F.oxysporum*, T.h. = *T.harzianum*, Top. = topsin fungicide.**

The results of antagonism tests show that the fungus *T.harzianum* have a high antagonism ability against the pathogen fungus *F.oxysporum*. the antagonistic ratio reached to 1 according to the scale of (Bell et al., 1982). *T.harzianum* in concentration 1.7 x 10^6 lead to reduce the eggs hatching ratio and death of nematode to 23.44% and 21.46% respectively. While the use of fungicide topsin lead to reduce the eggs hatching ratio and death of the nematode to 16.66% and 15.68% respectively. Also fungicide topsin inhibited the pathogenic fungus *F.oxysporum* with a ratio reached to 100% in PDA. One of the mechanisms of bio-fungi in combating pathogenic fungi in plants is antagonism, among various types of species being used as biocontrol agents, fungal genus *Trichoderma* produces different kinds of enzymes which play a major role in biocontrol activity like degradation of cell wall, tolerance to biotic or abiotic stresses, hyphal growth etc., (Waghunde et al., 2016). The effect of the pathogenic fungus on the germination percentage is due to the fact that *F. oxysporum* produces numerous toxic compounds that inhibit germination of seeds, including fusaric acid (Inoue et al., 2002). *T.harzianum* works to reduce the proportion and severity of diseases caused by *Fusarium spp.* in plant roots such as wheat, rice, tomatoes, eggplants, potatoes, field peas (Harman, 2000). Also the effectiveness of *T.harzianum* around the root area where Lo et al. (1994) reported the role of *T.harzianum* in the plants and the increase in production growth standards. Also, Yadav et al. (2011) showed that *T.harzianum* has the possibility to promote of plant hormone production, such as IAA. *T.harzianum* strains possess the strongest egg-parasitic ability and the best

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compatibility with nematoda. Because *Trichoderma* chitinolytic enzyme systems play an important role in egg-parasitism (Szabó et al., 2012). *T.harzianum* prefers both immature eggs and the egg containing juveniles in parasitum process, which can make a full morphological modification of juvenile of the egg interior and prevented the mature eggs from hatching (Uday et al., 2019). Also (Saifullah and Thomas, 1996) observed that *T. harzianum* have the ablility in growing on the surface of the egg and broke through the shell of the egg. The *Trichoderma* species conidia and hyphae were strongly connected to the egg surface and the germination of *Trichoderma* species hyphae is not only parasitised to the eggs and permeated the masses of the egg it is also parasitised to the second phase of egg juveniles (Sharon et al., 2007).

4. CONCLUSIONS

Our results showed that the infection of cucumber caused by fungus *F.oxysporum* increases in soils infected with nematoda. Also the using *T.harzianum* decreased the rate of egg hatching and larval death.

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6. REFERENCES

Alhazmi, A. S. (2009). *Introduction to plant nematology*. Scientific publishing and printing. King Saud University**,** p 431.

Al-waily, D. S. (1988).Studies of early blight of tomato caused by Alternaria solani. Master thesis. Agriculture College, University of Baghdad**,** p.76.

Al-waily, D. S. (2004). Studies on the incidence and control of the disease in the desert plantations. PhD thesis. College of Science, University of Basra.

Bedlan, G. (1986). The most important fungal diseases of cucumbers. *Pflanzenschutz*, 9**,** 8 –11.

Bell, D. K., Wells, C. D., Mirkham, C. R. (1982). In vitro antagonism of *Trichoderma* species against six fungal plant pathogens . *Phytopathology*, ***72,*** 379–382.

Harman, G. E. (2000). Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis, 84 (4),* 377–393.

Hussey, R.S., Barker, K. R. (1973). Acomparison of methods of collecting inocula of *Meloidogyne spp* including new techniques. *Plant Disease Report*, ***57,*** 1025–1028.

Inoue, I., Namiki, F., Tsuge, T. (2002). Plant colonization by the vascular wilt fungus *Fusarium oxysporum* requires FOW1, a gene encoding a mitochondrial protein. *American Society of Plant Biologists, 14,* 1869–1883.

Jarjis, M. M., al-Ani, R. A., al-Hiti, A. A. (1992). *Plant Diseases*. Baghdad University**,** p569.

Lo, C.T., Lin, C.Y. (2002). Screening strains of *Trichoderma spp* for plant growth enhancement in Taiwan. *Plant Cell, 11,* 215–220.

Martinez, R., Aguilar, M. I., Guirado, M. L., Alvarez, A., Gomez, J. (2003). *First report of Fusarium oxysporum in Spain*. Plant pathology. Black well science, Oxford, UK, 52 (3)**,** 410.

Mickenny, H. H. (1923). Influence of soil temperature and moisture on infection of wheat seedling by Helminthosporium sativum. *Agri. Research, 26,* 195 – 217.

Saifullah, Thomas, B. J. (1996). Studies on the parasitism of Globodera rostochiensis by *Trichoderma harzianum* using low temperature scanning electron microscopy. *Afro-Asian Journal of Nematology, 6,* 117–122.

Sajjad, H., Muhammad, I., Shagufta, B., Aamir, H. M., Salman, G., Shomaila I. (2017). Novel potential of *Trichoderma spp*. As biocontrol agent. *Journal of Entomology and Zoology Studies, 5(4),* 214–222.

Sharon, E., Chet, I., Viterbo, A., Bar-Eyal, M., Nagan, H., Samuels, G. J., Spiegel, Y. (2007). Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix. *European Journal of Plant Pathology, 118,* 247–258.

Szabó, M., Csepregi, K., Gálber, M., Virányi, F., Fekete, C. (2012). Control plantparasitic nematodes with *Trichoderma* species and nematode-trapping fungi: The role of chi18-5 and chi18-12 genes in nematode egg-parasitism. *Biological Control, 63,* 121–128.

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Uday, K., Bhagawati, B., Neog, P. P. (2019). Biological control of *Meloidogyne incognita* by *Trichoderma harzianum*. *Int.J.Curr.Microbiol.App.Sci, 8(02)***,** 2176–2188.

Waghunde, R. R., Rahul, M. S., Ambalal, N. S. (2016). *Trichoderma*: A significant fungus for agriculture and environment. *African Journal of Agricultural Research, 11(22)***,** 1952–1965.

Yadav, J., Verma, J. P., Tiwari, K. N. (2011). Plant growth promoting activities of fungi and their effect on chickpea plant growth. *Asian J. Biol. Sci., 4***,** 291–299.

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