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STUDY THE EFFECT OF *TRICHODERMA* SPORES LOADED ON ALGINIC AND 2% CMC WITH CHEMICAL FERTILIZER AND ORGANIC COMPOST AGAINST FUNGI *FUSARIUM OXYSPORUM* ON THE PLANT OF TOMATO IN THE FIELD

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Abstract
The study was conducted to determine the effect of the fungi Trichoderma harzianum and Trichoderma koningii loaded on alginic acid and 2% CMC in sterile and non-sterilized soil, treated with mineral fertilizer and organic fertilizer against pathogenic fungus Fusarium oxysporum on the plant of tomato in the field. The best results of the fungus T.harzianum and T.koningii loaded with alginic acid in sterile soil with organic compost against pathogenic fungus and improved plant growth. Where the growth indicators for the length of the plant were 186.66, 186.33, fruits weight were 3231.50, 3592.00 and number of fruits were 25.00, 21.00 for the two fungi T.harzianum and T.koningii respectively. While chlorophyll 69.33 and 69.66, nitrogen 6.57 and 6.54 and phosphor 0.591 and 0.585 for T.harzianum and T.koningii respectively, also the fungus T.harzianum and T.koningii loaded with alginic acid in sterile soil with organic compost improve photosynthetic process the results of photosynthesis16.75 and 16.29 and respiration 6.53 and 7.25espectively. This study was designed to determine the effect of bio fungus T.harzianum and T.koningii and their filters against the pathogen F.oxysporum in the laboratory and investigate the effect of T.harzianum and T. koningii loaded on alginic acid and 2% CMC on growth indicators.

Keywords: Fusarium oxysporum, Trichoderma harzianum, Trichoderma koningii.

1. INTRODUCTION

Seedling death, vascular degeneration and root rot are among the most important diseases that cause large losses in different crops and *Fusarium* wilt diseases, caused by pathogenic formae speciales of the soilinhabiting fungus *F.oxysporum* can cause severe losses in a wide variety of crop plants. (Alabouvette et al., 1993; Larkin and Fravel,1998). *Fusarium oxysporium*, is the main cause of fusarium wilt disease, it was first recorded in 1895 in the English Channel Islands (Walker, 1961), and fusarium wilt disease is a serious disease affecting plants in various conditions and in all stages of growth. (Walker, 1969 ; Decal et al., 2000). In addition, this fungus can also affect the fruits and caused infection, decomposition and fall (Agrios, 2005). Al-Bahadli et al. (1980) reported that the fungus spent two seasons in the form of chlamydia spores found in the soil or conidial spores in the affected host's residues and were a source of primary infection or in the form of mycelium or macroconidia. Several methods have been used to combat fusarium wilt disease perhaps the most important of these methods is the use of resistant varieties and t healthy seeds in addition to sterilizing the field soil with solar pasteurization by covering the soil with plastic sheets during the

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summer to reduce the incidence and severity of the disease. Several studies have been conducted over the past few years regarding the potential for biocompatibility of fusarium wilt using antifungal such as *Trichoderma* and *Pseudomonas* bacteria which produced iron chelate (Rose et al., 2003). The bio-fungus has many mechanisms that distinguish in inhibiting the work of plant pathogens such as competition on the site, food and bio-antagonism, the secretion of many enzymes to the walls of the pathogen host cells, and the stimulation of the host against the pathogen (Mukerji and Garg, 1987) and to wrap around the mycelium of the pathogen and penetrated it and then feeding on its contents and killing it. As well as the killing of other structures such as the fruit and stone bodies of these pathogens (Trutman and Keane, 1990). It has been revealed recently that the *T. harzianum* works to form yeast-like cells found among the tissues of the plant to protect and increase production (Mariola et al., 2007). The aim of the study is known the effect of *T.harzianum*, strain ICCF 417 and *T.koningii* strain ICCF 418 against pathogenic fungus *F.oxysporum* strain ZUM 2407 on tomato plant Buzau 47 .

2. MATERIALS AND METHODS

Antagonism tests antithesis between *F.oxysporum* fungus and bio fungus *T.harzianum* and *T.koningii*. in PDA medium according to the method of (Bell et al.,1982). Biogenic fungui *T.harzianum* and *T.Koningii* were grown in the nutrient medium liquid Potato Dextrose sterile and distributor in flasks conical 250 ml and a rate of 150 ml / flask in the shaker incubator at a temperature of 28^0C for 14 days. Then filtrated by filter paper No.1 Whatman after that filtrated by micro filter (Mllipore 0.22µm) and after was added filtrates biogenic fungi to the nutrient medium at 10, 20, and 30 ml / liter, taking into account adjustable rate agar before sterilization medium and three replications. The comparison treatment has included the addition of distilled water to sterile the PDA and the same ratios of filtrates. Mediums container and non-container on filtrates poured in a sterile petri diameter dishes 9 cm and then vaccinated circles after hardening tablets diameter, each 0.5 cm from the grown nutrient medium the pathogenic fungus *F.oxysporum* for five days in each dish center and then incubated dishes in the incubator at a temperature of 28^0C and the growth rate has been taking the measure diagonals rate perpendicular passing through the dish center after the reaching of the growth of fungus in the treatment of the comparison to the edge of the dish and applied damping equation to calculate the percentage of inhibition of fungus: (Matrood, 2015).The vaccine of *F.oxysporum* prepared depending on (Dewan, 1989). Loading *T.harzianum* and *T.koningii* on alginic was done according to (Minaxi, 2011) while the loading with 2% CMC according to (Vivek et al., 2016), *Trichoderma* spores which were 3.4×10^7 spore/mL for *T.koningii* and 3.8×10^7 spore/mL. The field experiment was conducted at the Baneasa Institute in Bucharest on June in a 140 m^2 area, after the soil was plowed, settled and installed a distillation system, it is divided into two halves. The first half was sterilized with pesticide Raisan .51, and covered with polyethylene for 14 days, the cover was then removed for a week for ventilation. Each half has two types of compost, a mineral fertilizer (NevaTec classic 12-8-16+3) from COMPO GmbH /Germany with ratio 0.525 kg/ 35 m^2 and organic fertilizer (Dox 10 N) from ITALPOLLINA/ Italy with ratio 2.625 kg/ 35 m^2. Then 5 lines were done the distance between one line to other is 1 m, in each line were holes with 17 depth x 24 diameter, the weight of soil with peat moss were 3 kg with ratio 1:1 and the distance between one hole to other is 70 cm, After that, every four holes were treated with special treatment as shown in Table 1.

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Table 1: The treatments shortcut

1. T.h. beads (alginic acid)	**3. T.k. beads (alginic acid)**	**5. T.h. + CMC**	**7. T.k. + CMC**	**9.Fungicide topsin**	**11. F.oxy**
2. T.h. beads (alginic acid). +F.oxy	**4. T.k. beads (alginic acid)+F.oxy**	**6. T.h.+ CMC +F.oxy**	**8. T.k.+ CMC +F.oxy**	**10. Fungicide topsin+ F.oxy**	**12.Control**

The soil was sprayed with topsin 70 WDG after planting process, after four months of planting, measurements were taken of plant height, chlorophyll which measured by OPTI-SCIENCES CCM-200 plus device, phosphorus was estimated depending on (Murphy and Riley, 1962 ; Cresser and Parsons,1979). nitrogen which measured according to (Bremner and Edwards,1965), phytosynthesis and respiration determined by *ADC BioScientific Ltd*. in (31-36) ^{0}C and light intensity (1200-1500), weight of the fruit and its number.

3. RESULTS AND DISCUSSIONS

The results of antagonism tests show that, Figure 1, that the fungi *T.harzianum* and *T.Koningii* 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). These results were similar to previous studies (Tran, 1998 ; Ngo et al., 2006) and the reason of this inhibition because that the fungus *Trichoderma spp.* of the most microbiology which widely used in the biological control field (Whipps, 1997; Hunt, 1999), the reason for this is due to the ease of isolation and the speed of its growth and it does not need to special dietary requirements and the variety of his work mechanisms (Paulitz, 1997; Howell et al., 2000). Also its ability to reduce the fungal growth from the pathogenic fungi such as *F. oxysporum* and *F.culmorum* and *F.moniliforme* and *R.solani* and *Sclerotinia Sclerotiorum* attributed these effects to the fungus's ability to produce antibiotics such as Trichodermol and Trichodermin and Gliotoxin and Pachybas and Emodin Chrysophancol (Domsch and Gams, 1970 ; Kuguk and Kivang, 2002)

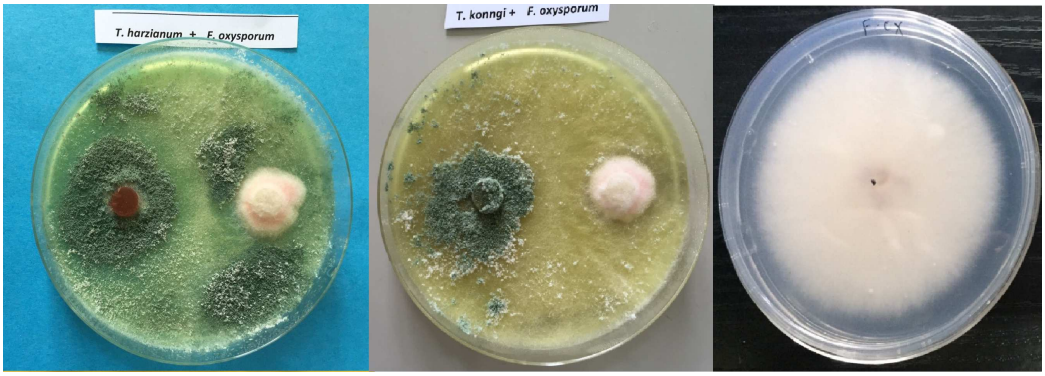

Figure 1. Antagonistic effect of fungi T.harzianum and T.Koningii on F.oxysporum in PDA medium

Results in Figure 2, show the ability of filtrates types of fungus *T.harzianum* and *T.Koningii* in a different concentrations to inhibit the pathogenic fungi. (Odebode, 2006) confirmed that the filtrates fungal biological control, including the fungus *T. harzianum* and *T. pseudo-koningii* have the ability in inhibiting the growth of many causes pathogenic to plants such as fungus *M. Phaseolina*, *Fusarium solani*, *Alternaria sp* and *Aspergillus niger* (Kredics et al., 2003).

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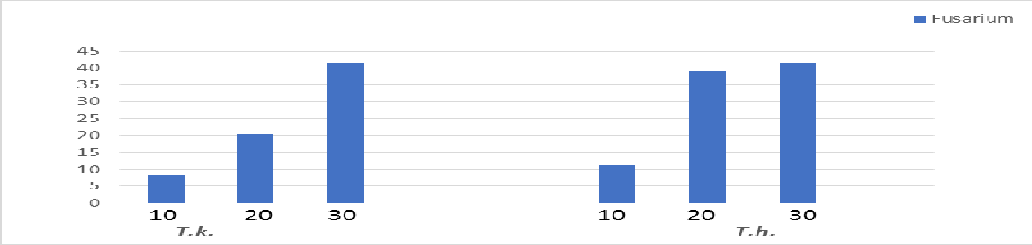

Figure 2. The inhibition of the pathogenic fungi in a different concentrations of filtrates types of fungus T.harzianum and T.Koningii

The results of the experiment showed a significant increase in the treatments containing *T.harzianum* and *T.Koningii* loaded with alginic acid for the length, number and weight of the fruits of tomato plant, where the length of the plant in the sterile soil and treated with organic fertilizer 186.66 and 166.33 cm respectively wtile in the soil contaminated with fungus *F.oxysporum*109.00 cm

Table 2: The effect of T.harzianum and T.Koningii loading on alginic acid and 2% CMC on F.oxysporum in plant growth indictors in tomato plant.

T	Non-sterile soil						Sterile soil					
	Chemical fertilizers			Organic fertilizers			Chemical fertilizers			Organic fertilizers		
	L(cm)	FW (g)	Nr	L(cm)	FW (g)	Nr	L(cm)	FW (g)	Nr	L(cm)	FW (g)	Nr
1	132.66	2086.50	23.66	141.66	2286.50	21.66	177.00	3290.00	25.66	186.66	3231.50	25.00
2	112.33	1386.00	18.00	120.33	1433.00	15.00	125.00	2604.75	23.00	137.00	2709.50	21.00
3	136.33	2165.50	21.66	146.66	2447.00	18.00	173.66	3317.50	25.00	186.33	3592.00	21.00
4	108.66	1310.00	18.00	122.66	1364.75	14.33	127.66	2590.25	25.33	133.33	2816.50	26.33
5	128.66	1775.25	20.00	132.00	1713.25	20.33	156.66	3011.25	24.00	166.33	3195.75	20.00
6	109.66	1152.00	15.33	115.00	1240.00	14.00	123.66	2168.25	22.66	128.66	2265.00	17.66
7	126.00	1875.25	18.66	135.66	1795.50	17.33	166.00	3115.50	25.33	169.00	3062.00	19.33
8	106.33	1090.25	16.66	114.00	1162.50	14.00	155.66	2377.00	21.00	164.33	2396.25	23.00
9	123.00	1687.75	20.00	126.00	1687.00	20.00	156.66	3079.50	28.00	155.33	3160.50	23.00
10	82.66	691.50	14.33	88.66	651.25	15.00	113.00	1254.25	17.33	115.00	1475.75	17.33
11	80.66	492.50	12.66	85.66	481.75	15.66	107.00	1153.00	18.00	109.00	1218.00	15.00
12	122.33	1653.25	19.66	126.00	1543.00	17.33	154.66	3032.50	25.33	158.00	3135.00	22.66
Av.	**114.10**	**1447.14**	**18.21**	**121.19**	**1483.79**	**16.88**	**144.71**	**2582.81**	**23.38**	**150.74**	**2688.14**	**20.94**
L.S.D 0.05	**1.75**	**47.22**	**1.69**	**5.41**	**34.43**	**1.68**	**5.49**	**67.81**	**1.41**	**5.62**	**62.22**	**1.37**

L = Length of plant, FW= fruits weight and Nr. = Number of fruit

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Some studies have confirmed that the fungus *T.harzianum* has a high potential to control (Poornima, 2011) .This superiority is due to the ability of fungi *T.harzianum* and *T.Koningii* to protect the roots of the plant from the pathogen *F.oxysporum* , through competition for the place and parasitism on the fungus *F.oxysporum* or the secretion of many antibiotics that inhibit the fungus *F.oxysporum* . and it work on reducing the proportion and the severity diseases that caused by a fungus *Fusarium spp*. in the roots of plants like wheat, rice, tomato, eggplant, potatoes, split peas (Michalikova and Michrina, 1997; Harman, 2000).

The results of Table 3 showed a significante increase in the chlorophyll 69.33 and 69.66, nitrogen 6.57 and 6.54 and phosphor 0.591 and 0.585 levels in the leaves of tomato plant with the treatment of the *T.harzianum* and *T.Koningii* loaded on the alginic acid respectively in sterile soil with organic fertilizers.Where the increase in the treatments containing the *T.harzianum* and *T.Koningii* to the role of these fungi in increasing the readiness of nutrients in the soil and make it easy absorption by the roots. Also results in Table 4 demonstrated the ability of biofungi *T.harzianum* and *T.Koningii* to improve the vital processes of photosynthesis 16.75 and 16.29 was higher with the treatment of the *T.harzianum* and *T.Koningii* loaded on the alginic acid in sterile soil with organic fertilizers.

Table 3: The effect of T.harzianum and T.Koningii loading on alginic acid and 2% CMC on F.oxysporum in chlorophyll, Nitrogen and phosphor in tomato plant.

T	Non-sterile soil						Sterile soil					
	Chemical fertilizers			Organic fertilizers			Chemical fertilizers			Organic fertilizers		
	CHLO	N%	P%	CHLO	N%	P%	CHLO	N%	P%	CHLO	N%	P%
1	56	5.31	0.51	58.33	5.42	0.531	66.33	6.32	0.590	69.33	6.57	0.591
2	47.33	3.04	0.411	45.33	3.11	0.406	52	3.65	0.415	54.33	4.73	0.433
3	57.66	5.44	0.515	60.66	5.54	0.523	67.66	6.43	0.553	69.66	6.54	0.585
4	43.33	3.08	0.406	45	3.14	0.409	54.33	3.74	0.416	54.66	3.83	0.432
5	52.33	5.09	0.442	54.33	5.14	0.433	58	5.97	0.463	62	6.01	0.482
6	42.33	2.86	0.317	41.66	2.95	0.324	49.66	3.52	0.342	49.33	3.56	0.357
7	53	5.12	0.452	55	5.18	0.457	59.33	6.04	0.474	63	6.22	0.477
8	40	2.90	0.321	38.66	3.12	0.332	48	3.61	0.353	48.33	3.67	0.363
9	52.33	4.52	0.421	47.33	4.60	0.452	57	5.54	0.456	56.33	5.62	0.436
10	32.66	2.63	0.265	33.33	2.73	0.268	41	3.04	0.293	34.66	3.22	0.293
11	33.66	2.54	0.264	29.66	2.86	0.264	35.33	3.04	0.285	36	3.62	0.285
12	52.33	4.40	0.422	51	4.47	0.445	60.66	5.48	0.473	66.33	5.63	0.484
Av.	**46.91**	**3.910**	**0.39**	**46.69**	**4.02**	**0.40**	**54.10**	**4.69**	**0.42**	**55.33**	**4.93**	**0.43**
L.S.D 0.05	**2.31**	**0.028**	**0.027**	**1.95**	**0.020**	**0.024**	**7.54**	**0.034**	**0.037**	**9.21**	**0.025**	**0.034**

****CHLO= chlorophyll, N = Nitrogen and P = Phosphor***

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Table 4: The effect of T.harzianum and T.Koningii loading on alginic acid and 2% CMC on F.oxysporum in photosynthesis and respiration in tomato plant.

T	Non-sterile soil				Sterile soil			
	Chemical fertilizers		Organic fertilizers		Chemical fertilizers		Organic fertilizers	
	PHYTO	RES	PHYTO	RES	PHYTO	RES	PHYTO	RES
1	12.00	6.19	12.50	6.25	15.70	6.74	16.75	6.53
2	7.26	4.53	7.85	4.34	8.28	4.64	8.25	4.52
3	12.42	6.98	13.05	6.36	14.33	6.25	16.29	7.25
4	7.57	4.52	7.71	4.34	7.54	4.73	8.35	4.83
5	10.45	5.92	9.9	5.73	11.85	6.62	14.03	6.86
6	6.61	3.32	6.65	3.54	7.17	3.93	7.36	4.14
7	10.18	6.16	9.32	5.82	11.30	6.23	10.46	6.25
8	6.78	3.45	7.40	4.13	6.89	3.85	7.82	4.23
9	9.32	5.78	10.12	5.83	11.27	6.96	15.62	6.47
10	5.34	2.15	5.70	1.72	4.92	2.34	5.82	1.79
11	3.96	4.42	4.91	3.56	6.47	2.2	4.16	1.91
12	9.43	5.22	9.24	5.45	14.54	6.94	15.79	7.02
Average	**8.44**	**4.88**	**8.69**	**4.75**	**10.02**	**5.11**	**10.89**	**5.15**
L.S.D 0.05	**0.33**	**0.024**	**0. 68**	**0.036**	**0. 62**	0.047	**0.81**	**0.049**

**** PHYTO = photosynthsis and RES = respiration***

Biological resistance fungi *Trichoderma spp.* in addition to their role in the release of nutrients such as nitrogen, sulfur and phosphorus through its analysis of protein, chitin and other substances by the enzymes they produce, and the fungi increase the efficiency of absorption of micro nutrients such as Mn, Fe, Cu and Zn by plants that became more ready to grow and thus increase the growth and activity of root and fruit buds (Altomare et al., 1999). Recently a new mechanism discovered showed that *T. harzianum* produces yeast-like cells found among plant tissues that protect and increase its production (Mariola et al., 2007), and it reflex positively on vital process like photosynthesis. While in *F.oxysporum* the growth indictors and photosynthesis decreased due to that this fungus is the main cause of fusarium wilt in tomato plant and the mechanism of wilt caused by this fungus occurs in one or more of the following ways: either because of the presence of the fungus and its reproductive units in the vessels of the root or stem causing the blockage, which prevents the transfer of raw materials and water to the top of the plant or wilt occurs due to the secretion of fungi of some toxins and acids and may cause the death of the cells of the wood tissues , and may wilt occurs when the fungus above secretes some of the enzymes that decompose the walls of the cell, through its effect on the substance of pectin, and thus disrupt the function of wood texture and the wilt is occur (Jarjis et al., 1992). The activity of the pathogen in woody tissue vascular plant usually continues to spread internally through the vessels of wood on hypha form or candida spores until the whole plant dies and the pathogenic fungi remain confined in tissues and some surrounding cells (Agrios, 1995).

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4. CONCLUSIONS

1. The pathogenic fungus *F.oxysporum* negatively affect on the growth iindictors and the vital processes of the tomato plant**.**
2. Using the *T.harzianum* and *T.Koningii* to reduce the negative effect of the fungus *F.oxysporum.*
3. *T.harzianum* and *T.Koningii* have the ability to increase nutrient availability such as phosphorus, nitrogen and chlorophyll, also stimulate plant growth and photosynthesis.

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