USING OF TRICHODERMA SPP. (*TRICHODERMA HARZIANUM* AND *TRICHODERMA KONINGII*) AND ITS EXTRACT TO CONTROL PATHOGENIC FUNGI IN THE SOIL IN VITRO

UTILIZAREA TRIPODERMA SPP. (*TRICHODERMA HARZIANUM* ŞI *TRICHODERMA KONINGII*) ŞI EXTRACTUL ACESTEIA PENTRU INHIBAREA CIUPERCILOR PATOGENICE DIN SOLUL IN VITRO

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Abstract. *The present study was designed to investigate the effect of Trichoderma harzianum and Trichoderma Koningii on pathogenic fungi such as Fusarium oxysporum, Rhizoctonia solani and Pythium sp. The results of the study showed the ability of fungi Trichoderma harzianum and Trichoderma koningii on the inhibition of pathogenic fungi Fusarium oxysporum, Rhizoctonia solani and Pythium sp. in medium PDA as well as the viability of bio-fungi on production of the enzymes cellulase, chitinase and amylase and dissolve phosphorus in the medium, also these fungi have effect on the growth indictors on tomato plant.*
Key words: *Trichoderma harzianum, Trichoderma koningii*, enzymes

Rezumat: *Prezentul studiu a fost conceput pentru a investiga efectul Trichoderma harzianum şi Trichoderma koningii asupra ciupercilor patogene cum ar fi Fusarium oxysporum, Rhizoctonia solani şi Pythium sp. Rezultatele studiului au arătat capacitatea fungiilor Trichoderma harzianum şi Trichoderma Koningii asupra inhibării fungilor patogeni Fusarium oxysporum, Rhizoctonia solani şi Pythium sp. în mediu PDA, precum şi viabilitatea bio-fungilor la producerea enzimelor cellulază, chitinază şi amilază şi dizolvarea fosforului în mediu, de asemenea, aceste ciuperci au efect asupra indicatorilor de creştere pe planta de tomate.*
Cuvinte cheie: *Trichoderma harzianum, Trichoderma koningii*, enzime

INTRODUCTION

In the context of specialization and high concentration of protective measures plant in order to stabilize or increase agricultural production, a key factor in obtaining high yields and guaranteed crop, keeping their quality seems high efficiency and versatility chemical methods of plant protection rapid return to traditional farming, organic seems to be the dominant system of safeguards that will be applied. *Trichoderma* species are the most common species of fungal used as biological control agents and are as commercial as biofungicide, biofertilizers

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and soil, to activate the system resistance induced in plants by treatments with preparations fungal (Jin *et al.* 1991; Inbar and Chet, 1992; Paulitz, 1997). It is considered that involves several mechanisms (Micoparazitismul; antibiosis, competition for space and nutrients, stress tolerance caused by increased development of roots and plant; Leaching and retention of inorganic nutrients, resistance induced; inactivation of enzymes patogenuluicare make species of *Trichoderma* agents biocontrol very efficient. It has an important activity fitostimulare nutrition. Most separates of the sort *Trichoderma* that were found to go about as mycoparasites of numerous financially essential ethereal and soil-borne plant pathogens, have been delegated *Trichoderma harzianum Rifai* (Gams and Meyer, 1998). The aim of this study to know the effect of *Trichoderma* spp and its filtrates on controlling pathogenic fungi in the soil.

MATERIAL AND METHOD

Antagonism tests antithesis between strains pathogenic fungus and bio fungus *T.harzianum* and *T.Koningii*. According to the method of Bell et al. (1982).Cellular enzymatic activity in solid cultural mediums cellulase enzyme adopted the method described by (Reese and Mandels, 1963) and (Yeoh *et al*., 1985). While amylase enzyme activity was detected by the method of (Hankin and Anagnostakis, 1975) and Chitinase detection was measured according to (Agrawal and Kotasthane, 2012). Screening of *T.harzianum* and *T.koningii* isolates for phosphate solubilization two fungal isolates were screened for their in vitro phosphate solubilizing potential in solid medium according to (Nautiyal, 1999). Fermentation assay method, the fermentation of *T.harzianum* and *T.Koningii* adopted by the method of (Tayung *et al*., 2011). Effect of *T.harzianum* and *T.Koningii* filtrates sterilized in the growth of pathogenic fungi adopted according to (Matrood, 2015). Prepare a vaccine of fungi, the vaccine of fungus *Trichoderma* was prepared depending on (Papavizas et al., 1982), *Pythium* was prepared according to (Pratt and Janke, 1980), *F.oxysporum* and *R.solani*. prepared depending on (Dewan, 1989).The effect of fungi *T.harzianum* and *T.Koningii* and their filtrates on pathogenic fungi on tomato plant in pots in the laboratory, take the peat moss then sterilized with $Ca_3(PO_4)_2$ (1g per 1Kg), then placed in sterilized pots. Add the fungal vaccine of the millet seeds, yellow corn flour into the soil by 1% w/w was mix well with the soil. Add the vaccine fungal bran into the soil after 5 days of fungi from the addition to vaccine pathogenic with ratio 1% w / w and mixing well, also irrigated pots daily for three days after that planted the seeds of tomato by five seeds per pot and put in a growth chamber at 28 ^{0}C after 10 days calculated the percentage of germination and after 20 days calculated the proportion of seedling death and after 50 days were measured growth indicators and estimate the proportion of phosphorus and chlorophyll in the leaves, where the treatments are as following; T.h. = *Trichoderma harzianum*, T.K. = *Trichoderma koningii*, ex T.h. = extract of *Trichoderma harzianum*, ex T.k. = extract of *Trichoderma koningii*, F.oxy = *Fusarium oxysporum*, R.solani = *Rhizoctonia solani*. The studied growth indictors, the ratio of germination and seedling death calculated by using (Mickenny, 1923) equation contained in AL-Waily (1988).The severity of the infection were calculated according to the following scale: 0=no infection,1= slight infection,2= mild infection 3= severe infection,4=very severe infection. The growth indicators were calculated after 50 days after planting. Determination of Phosphor and Chlorophyll, phosphorus was estimated depending on

Cresser and Parsons (1979) and Murphy and Riley (1962). To determine the individual levels of both chlorophyll a (Ca) and chlorophyll b(Cb) and the total amounts of carotenoids (C x+c) and chlorophylls (Ca+ Cb) [in pg.(ml of plant extract)-1] the measured absorbance values (A) at different wavelengths: (Smith and Benitez, 1955).

RESULTS AND DISCUSSIONS

The results of antagonism tests show that (fig. 1), that the fungi *T.harzianum* and *T.koningii* have a high antagonism ability against the pathogen fungi *F.oxysporum* and *Pythium spp.* the antagonistic ratio reached to 1, while against *R.solani* the antagonistic ratio reached to 2 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 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).

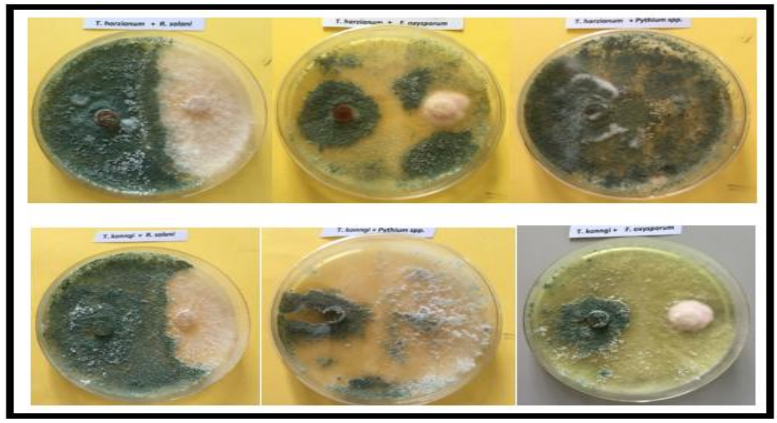

Fig.1 Antagonistic effect of fungi *T.harzianum* and *T.Koningii* on pathogenic fungi *F.oxysporum, Pythium* and *R.solani* in PDA medium

The results of enzymatic activity (fig. 2), show that the fungi *T.harzianum* and *T.Koningii* have the ability to production the cellulase enzyme (halo diameter) which reached to 5.75 cm and 6.5cm respectively, while the chitinase 5.25 cm and 6.25 cm respectively, and the amylase production was very low which reached to 1 cm and 0.75 cm respectively. *Trichoderma* species are capable of producing cell wall degrading enzymes such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease (Strakowska *et al*. 2014). These enzymes play an important role in cell wall degarding of pathogenic fungi because they contain chitin ,cellulose , clogan and proteins (Lorito *et al.,* 1994; Carsolio *et al.,* 1999). (Sonika Pandey *et al.*, 2015) found that a different types of fungus *Trichoderma* can produce cellulase in a different medium. Figure 1 shows the levels of enzymes in *T.harzianum* and *T.koningii*.

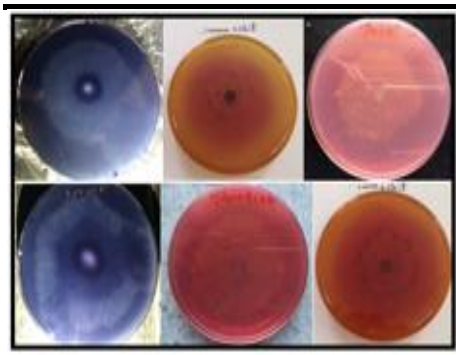

Fig. 2 Production of amylase, cellulase and chitinase in T.h and T.k

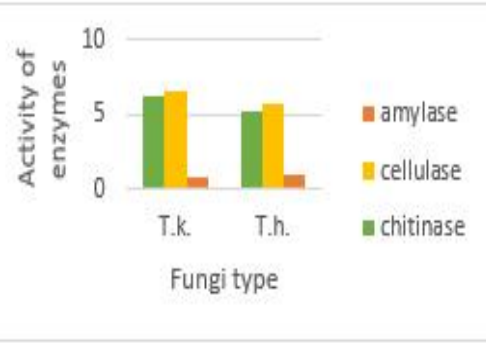

Fig. 3 Levels of amylase, cellulase and chitinase in T.h and T.k

Results in figure 4, 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* and *Fusarium solani* and *Alternaria sp* and *Aspergillus niger* and efficiently may outweigh the sometimes the efficiency of chemical pesticides (Kredics *et al.*, 2003).

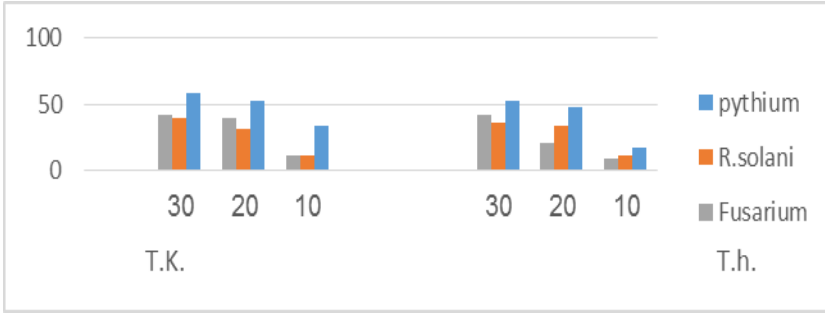

Fig. 4 The inhibition of the pathogenic fungi in a different concentrations of filtrates types of fungus *T.harzianum* and *T.Koningii*

Figure 5 show the results the ability of fungus *T.harzianum* and *T.koningii* in solid medium to dissolve the phosphorus, where the halo diameter was 1.8 cm and 2.5 for fungus *T.harzianum* and *T.Koningii* respectively. (Whitelaw, 2000) noted that the precipitation of phosphorus technology in the solid medium useful in isolating microorganisms are considered one of the successful methods of excellence fungi that have the ability to dissolve phosphorus and tested on the plant, are consistent with a study by (Saravana kumar *et al*, 2013), which demonstrated the ability of sorts fungus *Trichoderma spp* to form a transparent zone around the colonies, this indicates its ability to dissolve phosphorus.

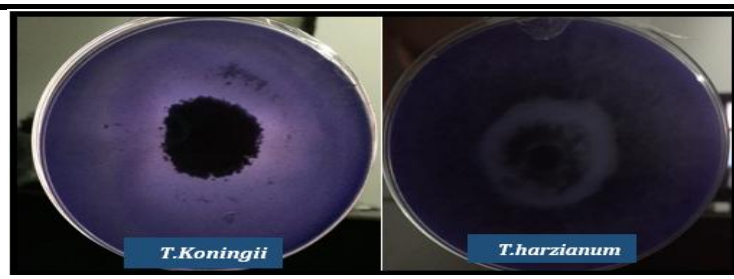

Fig. 5 Formation a halo by *T.harzianum* and *T.Koningii* in solid medium

Results in the table 1, the effect of *T.harzianum* and *T.Koningii* on pathogenic fungi *F.oxysporum*, *Pythium* and *R.solani* in germination, dead seedlings and the severity of infection in tomato plant. Some studies have confirmed that the fungus *T.harzianum* has a high potential to control a wide range of pathogens, Fungus showed effectiveness in resisting *R.solani* fungus that causes seed rot and seedling death and wilting wheat (Salih and Bidn, 1999) 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).

Table 1

The effect of *T.harzianum* and *T.koningii* on pathogenic fungi *F.oxysporum*, *Pythium* and *R.solani* in germination, dead seedlings and the severity of infection in tomato plant

Treatment	% germination*	% dead seedlings*	% infection severity*
T.h.	93.33	0	0
T.h. +F.oxy	73.33	18.18	15
T.h. + R.solani	66.66	20	25
T.h. + Pythium	66.66	20	12
T.k.	100	0	0
T.k. +F.oxy	80	16.66	17
T.k. + R.solani	66.66	27.27	28
T.k. + Pythium	73.33	20	10
ex T.h.	93.33	0	0
T.h. +F.oxy	73.33	27.27	35
T.h. + R.solani	60	33.33	42
T.h. + Pythium	66.66	30	37
ex T.k.	86.66	0	0
T.k. +F.oxy	73.33	20	34
T.k. + R.solani	66.66	30	40
T.k. + Pythium	66.66	22.22	35
F.oxysporum	66.66	27.27	50
R.solani	53.33	37.5	60
Pythium	60	30	45
control	93.33	0	0
$L.S.D_{0.05}$	0.35	11.02	4.21

Table 2 has shown the results of plant growth. The role of the fungus *T. harzianum* in the studied plants and increase production growth standards.Also its possibility to production plant hormones such as IAA (Malgorzata *et al*., 1997; Yadav *et al*., 2011).

Table 2

The effect of *T.harzianum* and *T.koningii* on pathogenic fungi *F.oxysporum, Pythium* and *R.solani* in plant growth indictors, phosphor and chlorophyll in tomato plant.

Treatment	Length of leaf (cm)	width of leaf (cm)	Length of stem (cm)	Length of root (cm)	No. of leaf	No. of leaflets	Dry weight (g)	Soft weight (g)	plant steam's diagram (cm)	Phosphor (mg/Kg)	Chlorophyll*		
	The average										C a	C b	C x+c
T.h.	16.66	3.33	24.33	13.5	5.33	9.33	2	7.083	0.85	0.46	5.53	2.67	384.07
T.h. +F.oxy	12.66	3.08	19.5	9	4.33	7.33	1.033	5.166	0.506	0.41	5.36	2.05	348.08
T.h. + R.solani	10.33	1.83	17	10	4.33	6	0.916	4.916	0.613	0.39	5.11	1.91	351.70
T.h. + Pythium	12	2.83	17.66	11	5.33	6.66	1.233	5.166	0.556	0.34	5.14	2.09	369.56
T.k.	14.33	3.16	27.66	14	6.33	8.33	2.466	11.473	0.79	0.45	5.25	2.84	407.67
T.k. +F.oxy	12.5	2.08	19.66	10	5	7.33	1.4	5.056	0.4	0.39	6.31	2.53	423.75
T.k. + R.solani	12	2.66	16.16	11	4	6.33	0.916	6.143	0.5	0.39	5.48	2.26	410.73
T.k. + Pythium	9.66	2.08	15.33	10.5	4.66	5.66	1.123	6.653	0.5	0.29	6.00	2.36	435.52
ex T.h.	12.83	2.83	23.66	11.5	5.66	6.66	0.086	4.066	0.506	0.24	8.87	3.42	585.63
T.h. +F.oxy	10.16	2.33	17.66	10	4	5	0.083	3.5	0.496	0.2	5.34	2.09	368.10
T.h. + R.solani	10	2	14	6	3.66	4.33	0.073	2.4	0.313	0.22	6.22	2.53	445.95
T.h. + Pythium	9.66	1.58	16.33	10.5	4.33	6	0.093	5.556	0.55	0.23	5.53	2.23	446.63
ex T.k.	12.16	2.16	20.66	11	5.33	6.66	0.096	4.633	0.706	0.26	8.04	3.00	532.65
T.k. +F.oxy	9.33	2	18.66	8	4	7.33	0.073	3.033	0.43	0.21	5.68	2.12	434.33
T.k. + R.solani	7	1.16	10.66	5.5	3	4.66	0.063	2.85	0.33	0.19	5.74	2.25	469.15
T.k. + Pythium	11.33	1.83	16	11	5	8	0.09	5.233	0.626	0.19	4.07	1.78	342.11
F.oxysporum	9	1.83	17	8	4	6.33	0.073	3	0.41	0.11	3.19	1.50	423.20
R.solani	7.66	1.33	11	5.5	2.66	4.33	0.046	2.8	0.203	0.18	6.12	2.51	463.18
Pythium	10.66	2	14.33	9	4	6.66	0.063	3.1	0.513	0.17	2.99	1.34	259.72
Control	14	2.83	23.33	12	4.66	6.333	1.233	4.366	0.716	0.23	6.04	2.57	448.36
$L.S.D_{0.05}$	2.76	1.22	9.36	3.22	1.00	1.21	0.68	1.65	**0.24**	0.019	0.95	0.73	9.67

Results in the table 2 also shown the percentage of phosphor. Some fungi such as *T.harzianum* and *Aspergillus higen* and *Penicillium* variable susceptibility to dissolve phosphorus component of organic and mineral sources as this fungus recorded an important role in the inhibition of the growth of pathogens through secreted compounds Siderapheras and a number of organic acids (Vassilev *et al*, 2006). Table 2 also the results of chlorophyll, the fungus biogenic effect in improving growth and biochemical components including total chlorophyll. (Dubova, 2012) reported that the increasing of chlorophyll due to the using of *Trichoderma* on cucumbers and lettuce, also the low level of chlorophyll has been observed in lettuce leaves as a result of live microorganisms preparation use and it causes changes in the chlorophyll *a* and *b* ratio.

CONCLUSIONS

1. This study showed that bio-fungi have the ability to produce enzymes such as cellulase, amylase and chitinase, also they have the ability to dissolve phosphor.

2. The ability of *T.harzianum* and *T.koningii* to inhibit the pathogen fungi through antagonism

3. Protection and improvement of tomato plant pathogenic fungi.

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