J. Agric. Sci. Mansoura Univ., 26 (4): 1989 - 2000, 2001.

INCREASING THE EFFICACY OF *Trichoderma harzianum* AS BIOCONTROL AGENT BY SLOW RELEASE NITROGEN FERTILIZERS FOR WILT CONTROL OF SUNFLOWER.

Nawar, Lobna, S.*; E.H. Ziedan and A.F. Sahab****

*** Biology Dept., Fac., of Science, King Abd El-Aziz Univ., Saudi Arabia**

**** Plant Pathology Dept., NRC , Cairo , Egypt**

ABSTRACT

Efficacy of *Trichoderma harzianum* Rifai isolates as a biocontrol agent against soil borne pathogenic fungi is depending on physiological, chemical and biological factors in plant and rhizosphere.

Applying a combination of slow release N fertilizer with *T. harzianum* to soil before sowing reduced the incidence of wilt disease of sunflower caused by *Fusarium oxysporum* and improved the growth parameters of the plant (plant height, fresh and dry weight).

Soil infested with *T. harzianum* isolates (T_2 or T_3) alone or in combination with the slow release N fertilizers namely, thiourea or urea rock phosphate (NP) reduced the percentages of pre and post emergence damping - off of sunflower plants as well as root colonization of the pathogen. The total count of the pathogen in the rhizosphere was also reduced

Keywords: Sunflower (*Helianthus annuus*) , *Fusarium oxysporum* , *Trichoderma harzianum* , slow release , nitrogen ,fertilizers , wilt diseases , rhizosphere, root colonization

INTRODUCTION

Wilt of sunflower (*Helianthus annuus* L.) caused by *Fusarium oxysporum* Schlect is considered to be the most destructive disease in many countries (Shaarhwy,1980 ; Gamal El-Din *et al.*, 1984 and Abd Allah, 1993)

Trichoderma spp. isolates have a substantial ability to suppress a wide range of plant pathogenic fungi by various mechanisms, including the production of cell wall degrading enzymes, i.e, polysaccharide layases, proteases and lipases (Mahadevamurthy *et al.*, 1990, Canullo. *et al.*, 1992; Harman *et al.*, 1993 and Haran *et al.*, 1996).

Some nitrogen fertilizers are well known for their suppressive effects on plant diseases. Soil amended with urea suppressed *F.oxysporum* f. sp. *cubense* (Sequeira, 1963), *Sclerotium rolfsii* (Henis and Chet, 1968). Woltz and Engelhard (1973) reported that nitrate nitrogen decreased the severity of *Fusarium* wilt of yellow delawar chrysanthemum. Zaher *et al.* (1979) reported that ammonium nitrate decreased the percentage of diseased senna plant by *Rhizoctonia solani*, *F.oxysporum* and *F.solani*. Gamal El-Din (1984) and Abd Allah (1993) reported that N.fertilizer alone significantly reduced the percentages of pre-and post emergence phase in sunflower.

Nofal and Sahab (1980) studied the effect of ammonium sulphate fertilizer on microorganisms in the rhizosphere of some soybean cultivars. They found that the application of ammonium sulfate greatly reduced the

densities of *Aspergillus ochraceus* , *A. terreus* ,*Rhizoctonia* and *Rhizopus* spp., while the count of *Fusarium* and *Trichoderma* spp.were increased.

Lyaskovskii and Pidolplichko (1990) reported that the slow release N fertilizers to barley increase the number of stems/pot by 26-31%, root/pot by 45-55% and root volume by 27-48% compared with mineral NPK. The slow release fertilizers increased the adsorbing surface area of the roots, their ion exchange capacity and the duration of their functional activity and tended to suppress the pathoganic activity of soil microorganisms.

Mahadevamurthy *et al.* (1990) reported that soil amended with urea, diammonium phosphate and potassium sulphate and treated with *T. harzianum* completely controlled the fungus causing ergot of *Pennistem americanum.*

Canullo *et al.* (1992) mentioned that slow release N fertilizers, *i.e.,* guanidine thiocyanate, guanylurea sulfate and thiourea suppress diseases caused by *Sclerotium rolfsii* and affected quantitative and/or qualitative changes, in composition of the soil microflora. *T. harzianum* pollution was significantly higher in soil treated with thiourea.

The purpose of this study was undertaken to evaluate the potential of two chitinolytic isolates of *T. harzianum* to control wilt disease of sunflower in combination with slow release N fertilizers. The effect of slow release N fertilizers in combination with *T. harzianum* on rhizosphere fungi of diseased sunflower root was also studied.

MATERIALS AND METHODS

Isolation and identification of the causal organisms:

Isolation of organisms associated with sunflower plants showing wilt and root-rot diseases collected from El-Giza Governorate, 1999 was performed. The specimens of infected root tissue were surface disinfested in 1% active chlorine for 2 minutes, washed several changes with sterile water, then plated on PDA medium and incubated on 27± 2^{o}C for 5 days. The isolated fungi were purified and identified of Booth (1971) and Nelson *et al.* (1983).

Pathogenicity test.

Plastic pots of 25 cm-diameter were filled with sterilized clay soil infested with the inoculum of each tested isolates. The isolates grown were individually for 15 days on sand-barely medium (1:1/w.w) and er ough water was added before sterilization . Three pots were used as replicates three were served as a ccntrol. Ten seeds were sown per pot. At the mature stage (75 day old) plants were carefully pulled out from the pots. Roots with discolor vessels in the longitudinal sections were counted as described by Ibrahim and Abd El-Rehim (1965). The percentage of infection of sunflower plant was determined as pre and post emergence damping-off 10 and 75 days after sowing.

The isolated fungi were used to infest soil in the current research.

Isolates of *T.harzianum:*

Four isolates of *T.harzianum* with capablity to parasitize on *F. oxysporum* were obtained from Plant Pathology Dept., of National Research Centre, Egypt and used in this investigation.

Effect of nitrogenous sources on the *in-vitro* growth and sporulation of *T.harzianum*:

The effect of substitution of different nitrogen sources were added in an equal proportion with respect to nitrogen content of $NH_4 NO_3$ of the chitin basal agar medium according to Monreal and Reese (1969). This medium contained colloidal chitin (1w/v dry weight), Yeast extract (0.05%), $(NH_4)_2 SO_4$ (0.1%), Mg SO_4 $7H_20$ (0.03%), KH_2 PO_4 (0.136%) and agar 2% in distilled water . The pH value of the medium was adjusted at 6.5. Three petri - dishes were used for each treatment and inoculated at the center with a disc (5- mm) of different isolates of *T.harzianum* and kept at 27±2°C. The diameters of the developed colonies were measured daily and the average was calculated. Spore production was also determined at the end of experiment using scale from - (no spores) to ++++ (abundant spores .)

Pot experiment:

This experiment was conducted in pots (25 cm-diameter) infested with a highly virulent isolate of *F. oxysporum* at the National Research Centre greenhouse during the summer season, 2000. Sunflower seeds cv.Miak were sown in soil infested with *T. harzianum* isolate T_2 or T_3 (10 ml / pot each ml contain $1x10^6$ CFU). Slow release nitrogen fertilizers i.e, thiourea or urea rock phosphate (NP) were amended per pot before sowing at the rate 0.7 g (140 kg/ faddan) and 1.5 g (280 kg/ faddan) respectively. A set of three pots was used as replicate for every treatment, while three was served as a control. Soil treatments were categorized into the following:

1- Un- infested soil (control)
2- Soil infested with *F.oxysporum*
3- *F.oxysporum* + *T. harzianum* (T_2)
4- " " + *T.harz.* (T_2) + thiourea
5- " " + *T.harz.* (T_2) + urea rock phosphate(NP)
6- " " + *T.harzianum* (T_3)
7- " " + *T.harz.* (T_3) + thiourea
8- " " + *T.harz.* (T_3) + NP

Preparation of *Trichoderma* inoculium :

A mycelial disc (1.0 cm diam.) from one week- old culture of the isolates T_2 and T_3 of *T.harzianum* were inoculated into duplicate of 500 ml flasks containing potato dextrose liquid medium. The cultures were grown at 25°C and shaken at 150 rpm for 5 days. Conidia were counted using a hemacytometer and the volumes of the suspensions were adjusted to obtain the numbers of conidia as indicated before in the experiment.

The percentage of post emergence damping-off, the length of shoot, fresh and dry weights were determined after 75 days.

Rhizosphere samples as adopted by Louw and weblely (1959) were taken 15,30,45,60 and 75 days after sowing. Sunflower plants were up-rooted with great care to obtain most of the root system intact. Root was then gently shaken to get ride of most of the adhering soil particles in order to obtain the rhizospheric soil. The root system was transferred to a wide-mouth reagent bottle containing 99 ml sterilized water. The bottle was shaken using a mechanical shaker for 15 min. Serial dilutions were made up by using test tubes containing 9 ml sterilized water as a diluent. The total fungal counts were carried out on Martin's medium (Allen, 1961) and peptone PCNB agar medium for counts *Fusarium spp.* (Nash and Snyder, 1962). Fungal plates were incubated at 27±2°C for 5 days.

Endophytic fungi

Endophytic fungi were isolated essentially as described by (Carver *et al.*, 1996). The main root of sunflower plants of the above experiment was cut into small segments (1cm) which were surface disinfected in 0.1% sodium hypochloride and plated onto Martin's agar medium supplemented by 1% Triton X- 100 for isolating *Trichoderma* spp. and peptone PCNB agar medium for isolating *Fusarium* spp. The plates were
incubated at 25± 2°C for 7 days , while the isolated fungi were identified.

Data were statistically analyzed according to (Steal and Torrie, 1960).

RESULTS

1-Isolation, identification and pathogenicity test.

Isolation trials from wilted sunflower plants revealed the incidence of *F. oxysporum* were in infected roots.

The pathogenicity test showed that the 3 isolates obtained were able to attack sunflower (Table ,1). However, isolate of (No,1) showed to be the most aggressive one causing post - emergence damping – off without any significant differences between the other isolates . On the average , the isolate No 1 caused more post - emergence damping off (42.2%) than the pre - emergence one (13.0%) . Re-isolation from diseased roots yielded only the same fungus used for the artificial infestation of the soil.

Table (1): pathogenicity levels of *F. oxysporum* isolates on sunflower

F. oxysporum isolates Number	Wilt disease incidence %	
	Pre-emergency	Post-emergency
1	13.0	42.2
2	22.0	31.1
3	13.0	24.3
Check plants (control)	00.0	7.7
L.S.D at 5%.	20.7	28.9

2- Effect of nitrogenous sources on growth of *T. harzianum* isolates:

The *in-vitro* effect of nine nitrogenous compounds and slow release N fertilizers on the mycelial linear growth and sporulation of the four isolates of *T.harzianum* (T_2, T_3, T_5 and T_9) were studied.

Table (2) shows that the linear growth and sporulation of the four tested isolates of *T.harzianum* were affected drastically when NH_4 Cl, $NaNO_3$ and peptone were applied to the medium instead of $(NH_4)NO_3$. Meanwhile, Amm. thiosulphate, thiourea, urea granulated coated and NP (urea rock phasphate) had a stimulatory effect on the growth and sporulation . Concerning isolates T_2 and T_3 of *T.harzianum* application of thiourea and NP significantly stimulated greatly their growth and sporulation and were used in the current research.

Table (2): Effect of different nitrogenous compounds and slow release N fertilizers on the linear growth and sporulation of some isolates of *T.harzianum* on solid medium.

	Trichoderma harzianum							
	T_2		T_3		T_5		T_9	
Nitrogen source	growth * (cm)	Sporulation **	*	**	*	**	*	**
$NH_4 No_3$ (control)	5.2	+ + +	5.0	+ +	6.0	++	6.5	++
$(NH_4)2 So_4$	2.5	+ + +	6.0	+ +	8.4	+ + +	5.2	+
NH_4 Cl	4.5	+	4.0	+	5.2	++	4.9	+
Na $N0_3$	4.0	+	3.3	-	5.9	++	3.1	-
Peptone	5.3	+	4.2	+	4.2	+	3.0	-
Amm.thiosulfate	8.6	+ + + +	8.3	+ + +	8.7	+ + + +	7.0	+ + +
thiourea	9.5	+ + + +	8.8	+ + + +	8.5	+ + + +	8.3	+ + +
Urea granula coated	8.4	+ + +	8.1	+ + +	8.2	+ + +	9.8	+ + + +
NP (urea rock phosphate)	8.7	+ + +	9.5	+ + + +	7.0	+ + +	9.8	+ + +
L.S.D. at 5%	0.89		1.39		4.1		1.38	

3- Effect of *T.harzianum* isolates and slow release N fertilizers on:

a- wilt disease incidence of sunflower

Table (3) shows clearly that soil treatment with *T. harzianum* isolates (T_2 and T_3) as single or in combination with slow release N fertilizers significantly reduced the pre and post emergence damping- off in sunflower plant than those plants cultivated in infested soil with *F. oxysporum alone.*

The disease severity was reduced to the maximum value of 11.7 and 16.7% for pre and post emergence respectively by using *T. harzianum* (T_3) + thiourea followed by *T.hazianum* (T_3) + urea rock phosphate. On the other hand, the least effective treatment on wilt disease incidence occurred with *T. harzianum* (T_3) and (T_2) as singl treatment respectively. Records on plant growth (Table,3) indicate that plants cultivated in infested soil with *T.harzianum* isolate (T_3) and applied with thiourea or urea rock phosphate fertilizers grow better than untreated ones. Its evident that the above treatments resulted in a significant increase in plant height, fresh and dry weight/plant than the control (check).

Table (3): Effect of slow release N fertilizers and *T.harzianum* isolates (T_2 and T_3) on occurrence of wilt and morphological characters of sunflower plant.

Infestation treatments	Wilt disease incidence		Shoot morphological characters		
	Emergence		Plant height (cm)	Fresh weight (g)	Dry weight (g)
	Pre	Post			
Un-infested (control)	18.3	50.0	59.93	7.77	2.12
Fusarium oxysporum(F.oxy.)	45.0	100.	48.10	15.54	3.24
F.oxy + T.harzianum (T_2)	28.0	60.	79.67	17.26	2.64
+ T.harz (T_2) + thiourea	25.0	56.7	64.80	25.98	9.88
+ T.harz (T_2) + urea rock	15.0	50.0	74.80	29.99	5.16
+ T.harzianum (T_3)	33.0	56.7	55.00	24.23	3.24
+ T.harz (T_3) + thiourea	11.7	16.7	86.37	46.99	8.66
+ T.harz (T_3) + urea rock	16.7	30.0	105.33	39.85	8.66
L.S.D. at 5%	7.27	18.84	22.58	15.01	3.60

b- Root colonization

Data illustrated in Fig. (1) show that infestation of soil with any of the tested antagonists *T.harzianum* (T_2 or T_3) individually or in combination with slow release N fertilizers reduce the percentage of root colonization by *F. oxysporum* than the corresponding figures of the control. The opposite was true concerning the root colonization with *T.harzianum* isolates. The most treatments resulted in high colonization by *T.harzianum* (100%) and zero infection with *Fusarium* oxysporum were showed in roots of plants cultivated in soil infested with *T.harzianum* (T_2 or T_3).

4- Total Fungal count in the rhizosphere of sunflower plants:

Data presented in Table (4) indicate that total fungal count in the rhizosphere of plants cultivated in soil infested with *F.oxysporum* of different treatments was increased as the plant grow up. The maximum count was found 75 days after sowing. Total fungal count in soil artificially infested with *F. oxysporum* was higher than in control during all stages of plant growth.

Generally, application of slow release N fertilizers to the infested soil with *F.oxysporum* and *T.harzianum* (T_2 and T_3) increased the total fungal count comparing the soil infested with pathogen and *T.harzianum* T_2 or T_3.

Table (4): Total fungal counts in the rhizosphere of sunflower plants cultivated in infested soil with *F.oxysporum* and treated with *T.harzianum* and slow release N fertilizers (1x10^4/g dry soil)

Infestation treatments	Time of sampling after sowing (days)				
	15	30	45	60	75
Un - infested soil (control)	2.68	2.97	46.6	129.0	69.5
F.oxysporum	167.8	692.2	481.8	962.3	3354.8
+ *T.harzianum* (T_2)	32.3	254.5	488.4	351.1	625.0
+ *T.harz* (T_2)+ thiourea	42.9	380.9	285.71	688.1	1410.2
+ *T.harz* (T_2)+ urea rock(NP)^x	49.5	266.6	400.6	446.1	1769.4
+ *T.harzianum* (T_3)	57.1	248.8	358.5	417.1	1250.0
+ *T.harz.*(T_3)+ thiourea	38.1	290.7	315.8	778.9	2097.9
+ *T.harz* (T_3)+ urea rock (NP)^x	26.3	285.7	547.9	572.0	2883.3

X = Urea rock phosphate.

5- *Fusarium* spp. count :

Data in Table (5) indicate that *Fusarium* spp. count in plant rhizosphere of control as well as *F. oxysporum.* infested soil were increased as the plant grow up to 75 days. Meanwhile, the maximum count of *Fusarium* spp in the plants rhizosphere of the other soil were observed 30 days after sowing. Data also showed that counts of *Fusarium* spp. in the rhizosphere soil infested with *F.oxysporum,* as expected were always greater than of other treatments. *T.harzianum* (T_2 or T_3) inoculation greatly reduced *Fusarium spp.* counts in the rhizosphere of sunflower plants cultivated in soil infested with *F.oxysporum* only.

Table (5) : *Fusarium* spp. count in the rhizosphere of sunflower plants cultivated in soil infested with *Fusarium oxysporum* and treated with *T.harzianum* and slow release N fertilizers (x10^4/g dry soil)

Infestation treatments	Time of sampling after sowing (days)				
	15	30	45	60	75
Un infested soil (control)	0.3	0.4	4.9	6.5	6.6
Infested with *F.oxysporum*	16.3	251.3	251.7	284.5	387.5
+ *T.horzianum* (T_2)	1.8	155.2	46.3	42.1	44.6
+ *T.harz.* (T_2)+ thiourea	1.4	166.7	47.6	45.9	51.3
+ *T.harz.* (T_2)+ urea rock *(NP)	1.8	146.7	71.5	74.4	59.9
+ *T.harzianum* (T_3)	5.0	124.4	79.6	50.1	104.7
+ T.harz.(T_3+ thiourea	9.0	116.3	87.7	150.8	104.9
+ *T.harz.* (T_3)+ urea rock (NP)	8.4	95.2	86.5	63.6	117.9

*(NP)= Urea rock phosphate

With regard to the effect of slow release N fertilizers on *Furarium* spp. count, the same trend observed in the total fungal count was obtained. As application of slow release N fertilizers in combination with *T.harzianum* (T_2 or T_3), it was observed that *Fusarium* spp. count increased than count of infested soil with *Trichoderma* isolatesT_2 or T_3 alone.

5- Frequency occurrence of different fungi in the rhizosphere:

Considering fungi in the rhizosphere of sunflower, as a whole, more than 5 genera were isolated from infested and uninfested soil with *F.oxysporum* (Fig.2). Four species of *Aspergillus*, i.e., *A.flavus* , *A .niger* , *A. sydowi* and *Asperigllus* sp. were common on both conditions. High frequencey occurrence of all fungal genera or species except, *Trichodrma* spp. was observed in the rhizosphere plant sowing in soil infested with *F.oxysporum*. In other word, inoculation with *Trichoderma* spp. (T_2 or T_3) as single or in combination with thiourea or urea rock phosphate (NP) reduced most of fungal or species than in the infected plants with *F. oxysporum*. As expected, the occurrence of *Fusarium* spp. showed higher frequency in soil infested with *F.oxysporum* than in the control. Applying thiourea or urea rock phosphate in soil inoculated with the isolate of *Trichoderma* spp. (T_2 or T_3) increased its density and reduced *F.oxysporum* frequency than in the other treatments.

DISCUSSION

Since , a great deal of current interest is being shown in the biological control of soil-borne pathogens, it was decided to follow the same trend to test the potential biocontrol of *T.harzianum* against *Fusaruim* wilt of sunflower *in-vivo*.

Isolation trials from wilted sunflower roots revealed the presence of *F.oxysporum* only. This fungus was previously reported to be associated with wilt disease of sunflower in Egypt (Gamal El-Din *at al.*, 1984 and Abd Allah., 1993). *F.oxysporum* was found in the present studies, not only to eleminate most sunflower plants but also to retard the morphogenesis of the ones that survived. Shoot length, fresh and dry weights of the surviving infected plants were significantly reduced. Similar results were also mentioned by (Abd Allah, 1993) in Egypt. Several investigators reported that application of *Trichoderma* spp. significantly reduced the disease incidence caused by *F.oxysporum* (Yousef *el al.* 1987 , Sivan and Chet, 1989 ; Harman *et al.* 1989 and 1993., Ziedan, 1998 and El-Gamal, (Nadia), 2000)

The present data showed that the addition of *T.harzianum* to soil infested with *F.oxysporum* reduced disease infection. Accumulated evidence in the literature suggest that antifungal metabolites may well have a part to play in antagonism (Dennis and Webster, 1971). The possible antagonistic effect on disease could be attributed to the production of hydrolytic enzymes such as chitinase and B 1-3 glucanase (Hadar *et al.*, 1979 ; Elad, *et al.*,1982 ; Canullo *et al.*, 1992 ; El-Gamal (Nadia) ,2000 and Ziedan and Elewa ,2000)

The present study indicated that soil treated with slow release N fertilizers, i.e., thiourea or urea rock phosphate + *T.harzianum* as combined treatments significantly reduced the pre-and post emergency damping - off of sunflower plant attacked by *F. oxysporum*. Also, the treatment significantly suppressed *Fusarium* spp. colonization in the root of sunflower and enhanced root colonization by *T. harzianum*. The results agree with those mentioned by Henis and Chet (1968) Mahadevamurthy *et al.*(1990) and Canullo *et al.*(.1992) .

Fig(1) Frequency occurrence of fungi in the rhizosphere of sunflower cultivated in soil infested with *Fusarium oxysporum* and treated with *T.harzianum* and some slow release N.fertilizers.

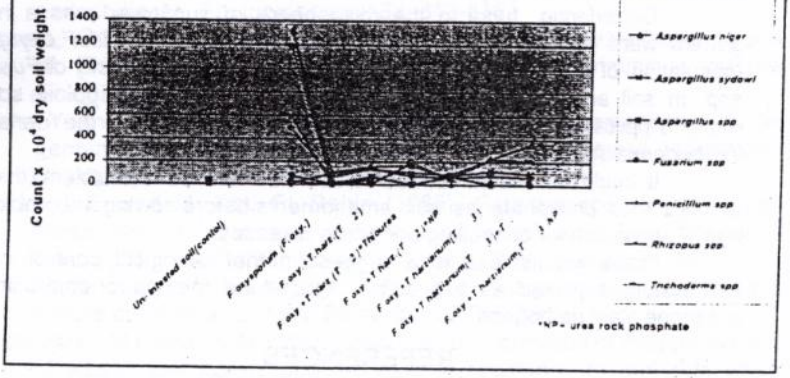
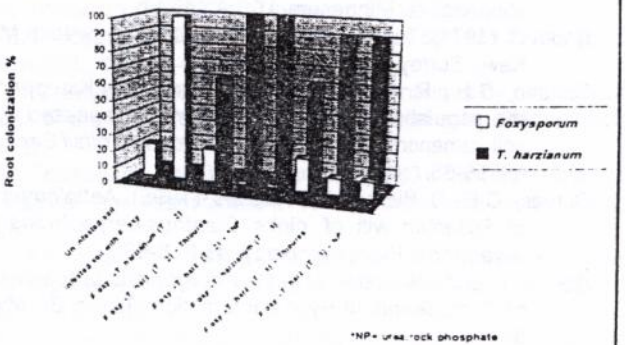


Fig. (1) Effect of slow release N fertilizers on the root colonization of sunflwer plant by *F.oxysporum* and *T. harzianum*



The data show that *T. harzianum* (T_3) + thiourea or urea rock phosphate proved to be the best treatment in reducing wilt disease incidence and increase the morphological parameter, i.e, plant height, fresh and dry weights of shoot system. The primary beneficial effect may be due to tolerance resulted from the improved phosphorus and nitrogen nutrition and allowing increased root growth. This would allow the plant to replace roots damaged and destroyed by the pathogenic microorganisms. *Fusarium* spp. count in sunflower rhizosphere was highered than in other treatments and in control as well as in highly colonized roots by *T.harzianum*.

Considering fungi in the rhizosphere of sunflower , as a hole 5 genera were isolated from infested and uninfested soil with *F.oxyspoum*. Inoculation of soil with *T.harzianum* in general reduced density of *Fusarium* spp. in soil and roots, reversibly stimulated *Trichoderma* spp. in soil and roots. Application of slow release N fertilizers increased the density of *Trichoderma* spp. in soil and roots of sunflower plants.

It could be recommended to use slow release N fertilizers (thiourea or urea rock phosphate as soil amendments before sowing in combination with *T.harzianum* for controlling soil borne diseases.

These results lead to a suggestion that biological control can be successfully exploited as a possible agricultural method for controlling the soil brone plant pathogens .

REFERENCES

Abd Alla, M.A. (1993). Studies on wilt and root-rot diseases of sunflower (*Helianthus annuus* L.) M.Sc.Thesis , Faculty of Agric., Moshtohor, Zagazig Univ.

Allen, O.N. (1961) Experiments on Soil Bacteriology. Burgess Publishing Co. Minneapolis, Minnesota, U.S.A.147 pp.

Booth,C. (1971). The Genus *Fusarium*. Commonwealth Mycological Inst., Kew,. Surrey, England., 237pp.

Canullo, G.H., R. Rodriguez-kabana and JW Kloepper (1992). Changes in the populations of microorganisms associated with the application of soil amendments to control *Sclerotium rolfsii* Sacc. Plant and Soil, 144 (c): 59-66.

Carver, C.E.; D. Pitt and D.J Rhodes. (1996). Aetiology and biological control of *Fusarium* wilt of pinks (*Dianthus caryophyllus*) using *Trichoderma aureoviride*. Plant Pathology, 45:618-630.

Dennis, L. and J.Webster (1971) . Antagonistic properties of species groups of *Trichoderma*. III Hyphal interaction .Trans. Br. Mycol. Soc., 57:363-369.

Elad, y.; I. Chet and y Henis (1982). Degradation of plant pathogenic fungi by *Trichaderma harzianum*. Can J. Microbiol., 28:719-725.

El Gamal, (Nadia), G.S. (2000). Biological and chemical control for root diseases of tomoto plant. Ph.D.Thesis Faculty of Agric.,Cairo Univ.

Gamal El.Din ; A. Eisa (Nawal) and A.A. shaarhwy (1984). Resistance of sunflower to damping off and charcoal rot disease caused by *Fusarium oxysporym* and *Macrophomina phasealina* in Egypt. Egypt. J. Phytopathol., 16 (1-2): 43-51.

Hadar, Y.; I. Chet and Y. Henis (1979). Biological control of *Rhizoctonia solani* damping- off with wheat bran culture of *Trichoderma harzianum*. Phytopathology, 69:64-68.

Haran,S.; H.Schickler and I. Chet (1996) . Mollecullar mechanisms of lytic enzymes involved in the biocontrol activity of *T.harzianum* Microbiol., 142(9): 231-233.

Harman, G.E; C.K. Hayes; M. Lorito; R.M. Broadway, R.M; Di Pietro, A.;Peterbaur, C.and A Tronsmo (1993). Chitinoloytic enzymes of *Trichoderma harzianum*. Purification of chitobiosidase and endochitinase. Phytopathology, 83: 313-318.

Harman, G.E.; A.G. Taylor and T.E. Stasz (1989). Combines effective strains of *Trichoderma harzianum* and solid matrix priming to improve biological seed treatment. Plant Dis., 73:631-637

Henis, Y and I. Chet (1968). The effect of nitrogenous amendment on the germinability of *S. ralfsii* and on accompanying microflora. Phylopathol., 58: 209-211

Ibrahim D.E. and M. Abd EL-Rehim.(1965). *Fusarium* root-rot and wilt on horse bean *(Vicia faba var. equina)* in UAR. Ann. Agric. Sci., Alex. Univ. ,13 : 412-426 Louw, H.A. and D.W. Webely (1959). The bacteriology of root region of the oat plant grow under collected pot culture conditions. J. Appl. Bacteriol., 22:216-226.

Lyaskovskii, M.I and V.N Pidolplichko (1990). Formation of the root system of wheat and barely and increase in resistance to root-.rots when compound slow- acting fertilizers is used . Agrokhimya, 3: 31-37 (C.F. Soil and Fert., 56 (2) :249.

Mahadevamurthy, S.; H. Prakash and H. Shetty (1990). Effect of fertilizer amendment of soil and antagonist treatment on sclerotial germination on *Claviceps fusiformis*. Plant Dis. Research, 5:212-215.

Monreal, J. and E.T. Reese (1969) The chitinase of *Serratia marescens*. Can.J. of Microbiol., 15:689-696

Nash, S.M. and W.C Snyder.(1962). Ouantitative estimatians by plate count of prcpagules of the bean root- rot *Fusarium* in field soils. Phytopathol, 52(6): 567-572.

Nelson, P.E.; T.A. Toussoum and W.F Marasas (1983). *Fusarium* spp. An illustrated Manual for Identification. The pennsylvania Univ., Park, USA. 189 pp.

Nofal, M.A. and A.F. Sahab (1980). Influence of Ammonium sulphate fertilizers on the rhizosphere microflora of some soyabean cvs.. Egypt. J. Agron., 5 (2) : 161-169.

Sequeira, L. (1963). Effect of urea applications on survival of *Fusarium oxysporum* of. cubense in soil. Phytopathology: 53:332-336

Shaarhwy, M.A. (1980). Studies on some diseases that attack the root of (*Helianthus annuus* L.) plant. M.Sc. Thesis Fac. of Agric, Cairo Univ,. Egypt.

Sivan, A.and I. Chet (1989). Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum*. J. Gen. Microbiol., 135:675-682

Steel, P.G. and J.H Torrie (1960). Principles and Procedures of Statistics. Mc Grow-Hill Book Company Inc., New York.

Yousef, S.A., M.S. Khalil; H.A. Eisa; Amr; M. Afaf and S.M El-Fangary (1987). Varietal reaction and control of flax wilt caused by *F.oxysporum* f. sp. *lini*. 6th Congr. Egypt, phytopath . Soc., Giza. pp. 359-371.

Zaher, E.A.; H.R. Abdelal; A.N. Ibrahim and A.Y. Ez El-Din (1979). Studies on root - rot disease. of senna (*Cassia acutifolia*). Egypt. J. Phylopathal., 11 (1-2): 1-11.

Ziedan E.H.E. (1998). Integrated control of wilt and root- rot diseases of sesame in ARE. Ph.D.Thesis Fac. Agric., Ain shams Univ.

Ziedan,E.H. and I..S Elewa, (2000). Treatment of sesame transplants with *Trichoderma* spp. and chitosan as control measures against wilt disease *Fusarium oxysporum* f.sp. *sesami*. 9th congress of phytopathology. Giza, Egypt.

Woltz, S.S. and A.W.Engelhard (1973). *Fusarium* wilt of chrysanthemum: I Effect of nitrogen source and lime live on disease develop. Phytopathol., 63: 155-157.

استخدام الاسمده النتروجينيه بطيئة التحلل لزيادة فاعلية الترتايكودرما هيرزيانم كعامل حيوى فى مقاومة مرض ذبول عباد الشمس

لبنى صادق نوار* و السيد حسين زيدان ** و احمد فرحات سحاب**

* قسم البيولوجى ، كلية العلوم جامعة الملك عبد العزيز ، جدة، المملكة العربية السعودية .

** قسم أمراض النبات ، المركز القومى للبحوث، القاهرة ، مصر .

تعتمد قدرة عزلات الفطر تريكودرما ها رزيانم فى المقاومة البيولوجية ضد العديد من الفطريات النباتيه الممرضه على العديد من العوامل الفسيولوجيه والكيماويه والبيولوجيه سواء فى النبات او التربة المحيطه بها ولزيادة قدرة كفاءة هذه العزلات تم استخدام بعض الاسمده الازوتيه بطيئة الانسياب لزيادة فعاليتها كعامل حيوى فى مقاومة مرض الذبول فى عباد الشمس

وقد تبين الاتى من الدراسة :

١- وجد أن معاملة التربه قبل الزراعة بأحد الاسمدة النيتروجينيه بطيئة التحلل وفطر الترايكودرما هيرزينام. أدت الى تثبيط مرض ذبول عباد الشمس المتسبب عن الفطر فيوزاريوم اوكسيسبورم كما أدت الى زيادة أطوال النبات وزيادة الوزن الطازج والجاف للمجموع الخضرى وخفض أعداد الفطريات الكليه فى ريزوسفير النبات مقارنة بغير معامله وتحت نفس ظروف الحقن بالمسبب للمرض.

كما أدت هذه المعامله الى خفض أعداد المسبب الممرض فى رايزوسفير النبات خلال التجربه وعلى العكس من ذلك لوحظ زيادة عالية لاستيطان الفطر ترايكودرما بالمجموع الجذرى للنبات والمنطقة المحيطه بالمجموع الجذرى (رايزوسفير).

٢- كانت معاملة التربة بالفطر تريكودرما هيرزايانم رقم (٢) ومادة الثيوريا أو يوريا روك فوسفات افضل المعاملات على وجه الاطلاق لخفض معدل حدوث الاصابه بمرض ذبول عباد الشمس وأعداد الفطريات الكليه والمسبب الممرض برايزوسفير النبات مقارنة بالتربه المحتويه بالمسبب الممرض غير المعامله إضافة لزيادة فعالية استيطان جذور النبات والرايزوسفير بالفطر ترايكودرما هارزيانم واكثرها زيادة لنمو النبات الخضرى.

لذا فان استخدام مثل تلك المواد من شأنها زيادة فعالية الترايكودرما هارزيانم كعامل حيوى فى مقاومة الامراض المحموله بالتربه .