Trichoderma spp. are known to be efficient producers of polysaccharide-hydrolyzing enzymes , proteases, and lipases all of which being involved in cell wall degradation (Lorito *et .al.*, 1993).

Recently, the antifungal properties of purified chitinolytic and glucanolytic enzymes from the biocontrol fungus *T. harzianum* have been described and evidence has been provided that these cell wall- degrading enzymes may act synergistically with nitrogen fertilizers. Jayarag, and Ramabadran,(1998) studied the effect of certain nitrogenous sources on the *in-vitro*, and *in -vivo* of antifungal ability of *T.harzianum*. They reported that urea,ammonium sulphate and ammonium chloride favoured the growth and survival of *T. harzianum* in soil. Ammonium sulphate enhanced the growth and survival of *T. harzianum* most , followed by urea and amm. chloride .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* . Harman, *et. al.*, (1993) reported that *T.harzianum* strain P1 produces a variety of chitinolytic enzymes including N-acetyl-ß-glucosaminidases, chitin 1,4-ß chitobiosidase and an endochitinase. The larger glycosylated chitobiosidase had a range between 4.0 -7.0 pH optimum. The endo chitinase optimum activity was at pH 4.0 and gradually declined as pH increased .

The purpose of this study was to evaluate *in - vitro* the ability of *T. harzianum* to inhibit the growth of *F. oxysporum* the wilt agent of sunflower. The effects of some physiological and environmental factors including the use of slow release N fertilizers, as nitrogen sources on the production of chitinolytic enzymes were also tested .

MATERIALS AND METHODS

Isolates of *Trichoderma* spp.:

Twelve cultures of *Trichoderma* spp. isolated from Egyptian soils were obtained from Plant Pathology Department of NRC (Cairo , Egypt) and were grown on potato dextrose agar medium(PDA) at 25°C .Identification of these cultures were carried out in the same department .

A preliminary test for the antifungal activity of the previous isolates of *Trichoderma* spp. against *Fusarium oxysporum* (the wilt agent of sunflower) was carried out on petri- dishes according to method described by Hamed, *et. al.* ,(1996).

Media:

1- Chitin-basal agar medium was used according to Monreal and Reese (1969), for growth and for chitinase enzyme production . This medium contained colloidal chitin (1% w/v dry weight); Yeast extract (0.05%) ., $(NH_4)_2 SO_4$ (o.1%) ; Mg SO4 . $7H_2O$ (0.03%) ; $KH_2 PO_4$ (o.136%) and agar, 2%, in distilled water and the pH value was adjusted at 6.5. Plates were prepared in double agar layers, the bottom layer of which consisted of chitin-free basal agar medium.

2- Chitin-mineral medium was used according to Okon *et. al.*, (1973), as a medium for chitinase production containing colloidal chitin (10g dry

weight) ; K_2 HPO_4.(0.9g) ; Mg SO_4. $7H_2O$ (0.2g) ; KCl (0.2g) ., NH_4NO_3(1.0g); Fe SO_4, $7H_2O$ (0.003g)., $MnCl_2$ (0.002g) and Zn SO_4. $7H_2O$(0.002g) in 1000 ml distilled water and the pH value adjusted at 6.3.

Screening of *Trichoderma* isolates for the production of chitinolytic activities :

This was carried out to determine their chitinolytic activities on chitin basal agar medium of Monreal and Reese (1969), by measuring growth diameter (mm), visual growth density { using rang from 0 (no growth) to + 4 (vigourous growth)} and visually chitin degradation (clear zone formation around growth) .

Factors affecting the chitinase production :

Each isolate of *T. harzianum* was grown in Erlenmeyer flasks (250ml) containing 50 ml of chitin mineral medium (Okon *et.al.*, 1973) on a rotary shaker at 150 r.p.m. and incubated at 28±2 °C. Experiments were carried out in shaken flasks to study the effect of various carbon sources, pH values, different nitrogen compounds and some slow release N fertilizers as nitrogen sources on chitinase enzymes production by the four selected isolates of *T. harzianum* .

Enzyme assay procedures:

Chitinase enzyme activity was determined according to procedure described by Clark (1964).One ml of the enzyme was incubated with 1 ml (1%) colloidal chitin in 0.05M citrate- phosphate buffer (pH 6.5). The reaction mixture was incubated at 50 °C for 60 min. A unit of enzyme activity is defined as the amount of enzyme required to produce 0.5µ mole per ml of N-acetyl glucosamine per hour at 50°C .

Protein detremination :

Protein concentration was determined by measuring the absorbance at 750n.m by the method of Lowery *et. al.* , (1951).
Specific activity is expressed as units / milligram protein /ml .

Partial purification of chitinase:

Cells were removed from the culture liquor (250 ml) by centrifugation at 4°C (4000 r.p.m.). The supernatant was freez-dried by lyophilizaticn to brownish residue . The residue obtained was dissolved in 10 ml of phosphate buffer , (0.05 M pH 6.6) .
The crude enzyme solution was dialyzed for 48 hr.against the same buffer at 4°C.

In the preceding part of this work, fractional percipitation of the the crude lyophilized chitinase was performed with ethanol , acetone and ammonium sulphate . Amm. Sulphate was the most suitable for the fractionation and it gave the highest protein recovery with active chitinolytic enzyme . To the dialyzate, solid ammonium sulphate was added to the supernatant with different concentrations (0-15%) , (15-30%) , (30-60 %) and (60-100%) , whereas, the enzyme precipitated in (0-30%) saturation . The precipitated enzyme was centrifuged at 4000 r.p.m. for 20 min. The supernatant which had no enzyme activity was discarded and the precipitate was dialyzed and used to study the properties of the enzyme.

RESULTS AND DISCUSSION

The preliminary test revealed that the 12 isolates of *T. harzianum* differed in their antifungal activity against *F. oxysporum* (Fig.1) The isolates no. T2, T3, T5, and T9 were the most promising and were selected for further studies .

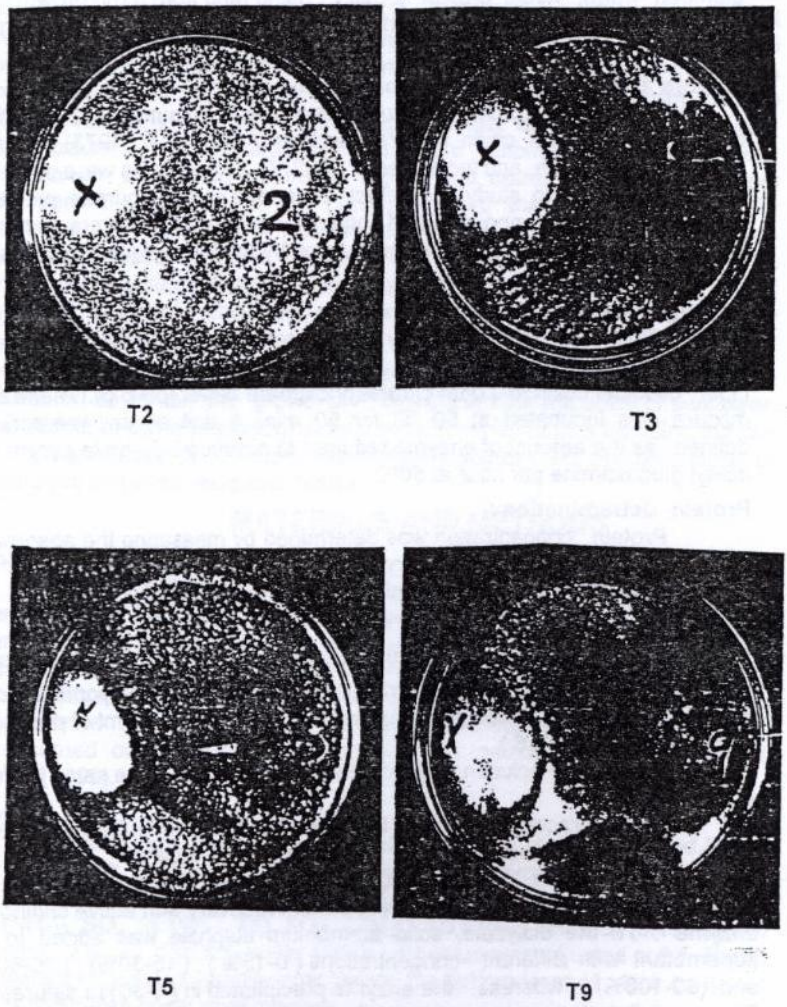

Fig.(1): The antifungal activity of *T. harzianum* isolates against *F. oxysporum*, the wilt agent of sunflower

Results in Table (1) indicated that all of the twelve's *Trichoderma* isolates have chitinolytic activity and can utilize chitin as an only carbon source for their growth.. Out of the twelve *Trichoderma* isolates T2, T3, T5 and T9 were surrounded by clear zones denoting a larger chitinolytic activity in comparison with other *Trichoderma* isolates which produced variable amounts of chitinase enzymes along the fermentation period allowed. It is clear also that the greatest linear growth , chitin degradation and chitinase enzyme production (1200,471.1,620,and 525.2 units / mg protein) were observed using the isolate T2, T3,T5,and T9 of *T. harzianum* respectively after 5 days incubation .These results have been confirmed by many authors (Hadar *et. al.*, 1979 ; Tronsmo and Harman,1992 ; Lorito *et. al.*,1993 and El-Barougy, 1997).

Factors affecting chitinase production

1-Effect of initial pH values :

Results in Table (2) showed that maximum chitinase production was obtained upon using chitin containing medium at a initial pH of 6.5 for the four *Trichoderma* isolates . Shifting towards more acidic or alkaline sides led to a corresponding decrease of chitinase production .These results are not similar to that obtained by Reid and Ogrydziak (1981), who found that optimal pH for chitinase production by *Serratia marcescens* is7.5 .Moreover ,chitinase production is sensitive to initial medium pH and to changes in pH during cell growth .

2-Effect of various carbon sources and chitin concentrations :

Untabulated data concerning the effect of different carbon sources revealed that the chitin monomer was the most favorable when used as a sole carbon source . Growing the four isolates of *T . harzianum* on the basal medium supplemented separately with N- acetylglucosamine , galactose , sucrose , and cellobiose resulted in a complete inhibition of chitinase production . Consequently, the chitinolytic activity was not detected when growing on various carbohydrates other than chitin .

Therefore, different concentrations of chitin were tested in the same medium within at the range of 0.25 to 2% . Data in Table (3) showed that chitinase production induced by the four isolates of *T. harzianum* was maximum at the concentration of 1% chitin beyond which a decrease in the enzyme production was evident .The same conclusion has been drawn by other workers (Monreal and Reese, 1969 for *S. marcescens* ; Haran *et al.*,1995 and Schickler *et.al.*,1998) for *Trichoderma harzianum*.They also found that addition of readily utilizable metabolites to cultures involved in the degradation of chitin caused catabolite repression. The repression was overcome as the additive is consumed. However, Abdel- Fatah (1995) for *Streptomyces cellulosae* found that chitinase production was increased as the amount of chitin in the medium increased and the maximal value of chitinase activity was attained at 1.5 % chitin.

3-Effect of nitrogen sources and some slow release N fertilizers.

The effect of substitution of different nitrogen sources and some slow release N fertilizers added in an equal proportion with respect to nitrogen

content of (NH_4NO_3) of the basal medium is presented in Table (4). Data indicated that isolates T2 and T5 of *T. harzianum* were stimulated by the use of slow release N fertilizers ammonium thiosulphate, urea, urea granula coated and urea + rock phosphate showing highest chitinase activity with % production of (129.3% and 115.9%) , (107.0% and 101.8%) , (103.0% and 101.3%) and (102.0% and 99.4%) in descending order respectively as compared to the control (NH_4 $N0_3$). On the other hand , the enzyme activity of T2,T3 and T5 gave slight increase when NH_4NO_3 was replaced by $(NH_4)_2$ $S0_4$.While,T3 isolate showed highest chitinolytic activity when urea + rock phosphate , urea , urea granule coated and or amm. thiosulphate were used compared with the control in descending order . Other nitrogen sources (NH_4Cl , $NaNO_3$ and peptone) decreased the enzyme activity of the four tested isolates compared with the control. On the other hand, the T9 isolate gave highest chitinolytic activity when replaced NH_4N0_3 (control) by urea granule coated , urea + rock phosphate and urea in descending order. These results are in agreement with that for chitinase from *Aeromonas* sp.(Hamed,1986) and Zhang *et al.* (1998) for *T. harzianum* . However , the present results differed from that reported by Abdel- Fatah (1995) using *St. cellulosae.*

In a subsequent experement , the effect of different concentrations of the most favorable N source for each isolate were added to the basal medium in an equivalent amount of nitrogen located in the control(NH_4N0_3) were investigated. Data in Table (5) clearly indicate that the increase in ammonium thiosulphate concentration in media used for T2 stimulated the production of chitinase enzyme, reaching its maximum value at concentration of 0.08% N , while isolate T5 showed a maximum enzyme activity at 0.04% N. Furthermore , the chitinase activity of the T3 and T9 isolates reached maximum at the concentration of 0 .08% N of urea+ rock phosphate and urea granula coated fertilizers respectively .

The use of higher levels of slow release N fertilizers (0.16 % and 0.3 2% N) was accompanied with drop in the chitinolytic activity of the four isolates *of T. harzianum* . These results agree with those obtained by Canullo *et. al.*, (1992)and Jayarag and Ramabadran (1998) for *T. harzianum.*

Partial purification of chitinase of *Trichoderma harzianum* isolates :

Several workers have used $(NH_4)_2SO_4$ for the precipitation of chitinase as an important step of their purification scheme. Jeuniaux (1963) collected 30-50 % saturated fraction at pH 5.2 . This was followed by adsorption and desorption on chitin . Monreal and Reese (1969) obtained a high yield with a 40- 80 % saturated fraction for the chitinase of *S. marcescens* , Morrissey *et. al.* , (1976) and Hamed *et.al.* , (1998) precipitated chitinase at 30 % saturation . In our work the precipitate obtained at 30 % saturation (Table 6) resulted in a 1.7, 1.6 , 1.1 and 1.24 fold for T2, T3 , T5, and T9 respectively. The resulting precipitates were dissolved in phosphate buffer (0.05 M,pH 6.6) and dialyzed against the same buffer,the enzyme samples (0.2 mg protein) were used to study the properties of the enzyme.

Some properties of crude chitinase enzymes

1-Effect of pH values: The optimum pH of enzyme activity was determined in different buffers : (0.05 M) citrate buffer, pH 3-6 ; phosphate buffer, ; carbonate bicarbonate buffer, pH 9-10, under standard assays conditions at 50 °C. The enzyme activities was optimally active at pH 6.6 in the four isolates of *T. harzianum* (Fig.2). These results are not similar to the optimal pH of *Arthrobacter* reported by Morrissey *et.al. (1976),Aspergillus carneus (Abdel Naby et.al.,1992) ,and Streptomyces venezulae DSM 4027 chitinase (Hamed et. al.,1998).*

2- Effect of temperature : The effect of temperature on chitinase activity showed that the optimum temperature of the chitinase activity for the four isolates was 50°C, however the activity decreased rapidly at 60°C (Fig.2) .This results is in agreement with that for chitinase from *Aeromonas sp.* (Hamed ,1986), *St. erythraeus* (Hara *et. al.*,1989), *A. carneus* chitinase Abdel Naby *et. al.* (1992),and Hamed *et. al.* (1998).

3-Effect of time reaction : The progress of enzymatic reaction with time was studied under optimum temperature and pH. The rate of colloidal chitin hydrolysis was linear with time up to 60 min , then it decreased slightly for the four isolates (Fig.2) .These are similar to the optimum time reaction of *St. erytharus* chitinase (Hara *et. al.*, 1989),and *St. venezulae* chitinase (Hamed *et. al.*, 1998).

4-Thermal stability of chitinase from *Trichoderma harzianum* isolates : Thermal stability of the enzymes were examined after incubating the enzyme solutions in phosphate buffer (0.05 M , pH 6.6) at different time intervals in a water bath set at 50°C . The results showed that T2,and T5 chitinase were stable for 15- 55 min., on the other hand T3 and T9 were stable for 15- 40 min. but were inactive when heated for 70°C (68.2 , 70.1 , 85.3 , and 90.3 %) loss of activity for T2, T3, T5 and T9, respectively. Similar results obtained from *St. venezulae* (Hamed *et. al.* 1998).

5-Metal ions and chelating agents : The effect of some metal ions and some reagents on the activity of T2 , T3 , T5 and T9 chitinase are summarized in table (7) Data showed that the metal ions Mn^{+2},Fe^{+2},Ca^{+2} and Co^{+2} slightly activated the enzyme, while Cu^{+2}, Zn^{+2},Hg^{+2} and EDTA showed relatively strong inhibitory effects .In general, chitinase of T2, T3, T5 and T9 reacts to these reagents in a manner very similar to other chitinases from *Streptomyces venezulae* (Hamed *et. al.* , 1998), *Bacillus* R.4 (Tominaga and Tsujisuka 1976), *Vibrio* sp. (Ohtakara *et. al.*, (1979), and *Verticillium alboatrum* (Pegg and Young, (1982). However , the inhibition pattern of metal ions and reagents clearly differed from that reported for chitinase from *Aeromonas* sp. (Ueda and Arai,1992).

These results suggest that *Trichoderma harzianum* chitinase and that its mycolytic activity may provide a possible biological activity against wilt diseases caused by *F. oxysporum*, such a suggestion requires further studies on viable mycelia and spores of the pathogen.

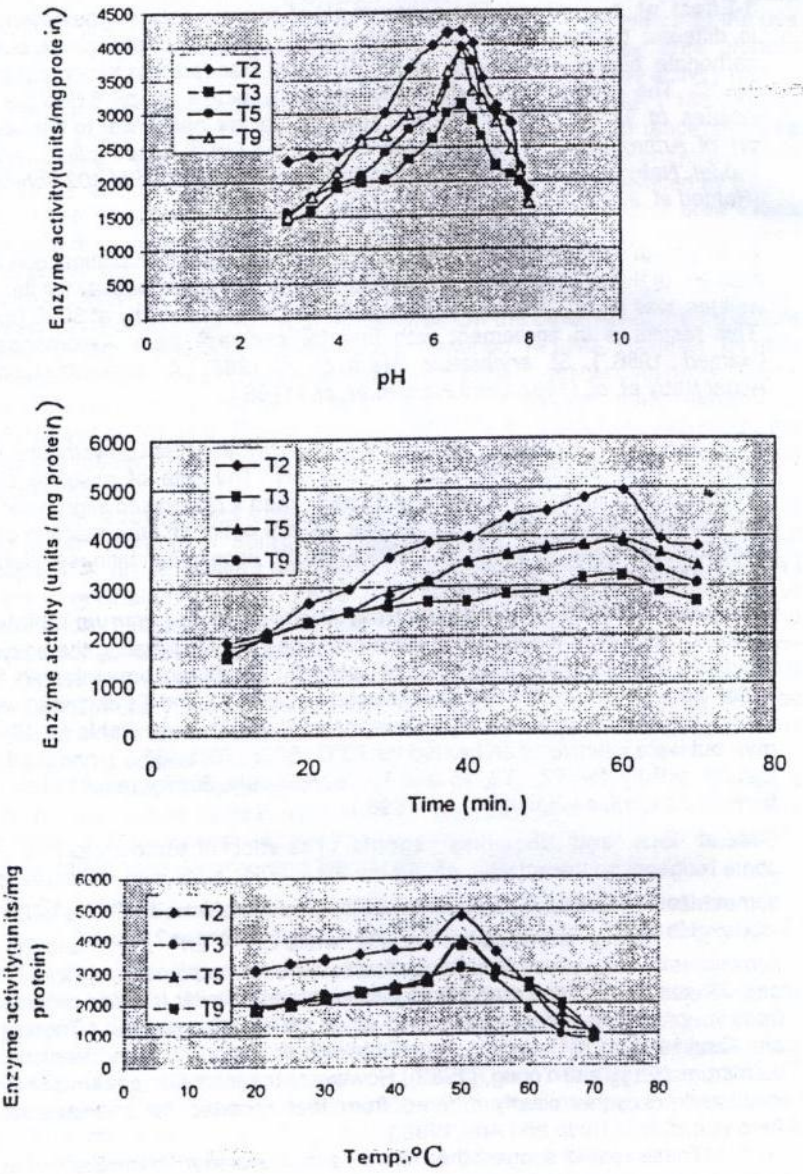

Fig.(2): Effect of some factors chitinase activity of the four *Trichoderma harzianum* isolates

Table (1): *In-vitro* growth and chitinolytic abilities of different *Trichoderma* isolates on chitin media

Isolates NO.	Growth diameter (mm)	*Growth	Chitin degradation	Chitinase activity (units /mg protein)
T. viride (T1)	4.0	+	-	**
T.harzianum (T2)	9.5	++++	++++	1200
T. harzianum(T3)	9.3	++++	+++	471.1
.T.harzianum (T4)	7.2	+++	+	**
T. harzianum (T5)	9.0	++++	++++	620
T.harzianum (T6)	7.0	+++	++	**
T.harzianum (T7)	7.2	+++	++	**
T.viride (T8)	6.0	++	-	**
T. harzianum (T9)	9.3	++++	+++	525.2
T. harzianum (T10)	6.5	++	+	**
T. harzianum (T11)	5.8	++	+	**
T. harzianum (T12)	6.6	++	+	**

*Vigorus growth ++++
Heavy growth +++
Moderate growth ++
weak growth +
No growth –
** Enzyme activity was not determined .
Tests were run in quadrable replicates and incubation was at 28±2 °C.

Table(2): Effect of different initial pH of the medium on the chitinase production by four *Trichoderma harzianum* isolates .

Initial PH	Chitinase activity (units / mg protein) No. of isolates			
	T2	T3	T5	T9
5.0	653.3	388.9	330	244.2
5.5	703.3	442	400.6	416.5
6.0	1166.6	486.4	556	584.1
6.5	1236.7	511.1	684	676.9
7.0	1196.3	432.9	625.6	552.5
7.5	1036	405.7	591.3	381.7
8.0	696.7	302.7	473.8	295
8.5	333.2	220	211.6	172.9

Each figure is an average of 4 replicated shaken cultures incubated at 28 ± 2 ^{O}C days.

Table (3):Effect of different concentrations of chitin on chitinase production of *Trichoderma harzianum* isolates

Chitin conc. %	Chitinase activity (units / mg protein) No. of isolates			
	T2	T3	T5	T9
0.25	590	131.1	147.8	130.8
0.5	1126.7	293.3	326.9	286.5
1.0	1236.9	511.1	684	567.9
1.5	1009	398.6	430.3	371.6
2.0	985.7	276.1	360.7	290.3

Each figure is an average of 4 replicated shaken cultures incubated at 28 ± 2 ^{O}C for 5 days.

Table (4): Effect of different nitrogenous compounds and slow release N fertilizers as a nitrogen sources on chitinase production of *Trichoderma harzianum* isolates.

Nitrogen source and slow release N fertilizers	Chitinase activity (units / mg protein) No.of isolates							
	T2	% production	T3	% production	T5	% production	T9	% production
Control	1236.7	100	511.1	100	684	100	576.9	100
$(NH_4)_2SO_4$	1250	101	528.8	103	692.4	101.2	574.6	99.6
NH_4Cl	1033.3	83.5	470.6	92.1	662.6	95.8	531.7	92.1
$NaNO_3$	986.6	79.7	445.8	87.2	605	88.5	490.5	85.0
peptone	766.7	61.9	441.3	86.3	652.4	95.4	499.8	86.6
Slow release N fertilizers								
Amm. thio –sulphate	1600	129.3	575.5	112.6	793	115.9	572.3	99.2
urea (SRF)*	1333.3	107	691.8	135.3	696	101.8	616.3	106.8
Urea granule coated	1276.6	103	671.1	131.3	693.2	101.3	742.5	128.7
urea +Rock phos.(NP)	1263.3	102	714.4	139.7	680.4	99.4	675.6	117.1

- *SRF = Slow release fertilizers

Each figure is an average of 4 replicated shaken cultures incubated at 28 ± 2 °C for 5 days.

Table (5): Effect of different concentrations of the most efficient slow release N fertilizers on the chitinase production of *T. harzianum* isolates.

N% of fertilizers	Chitinase activity(units / mg protein). No. of isolates			
	T2	T3	T5	T9
	Amm. thiosulphate	NP urea + Rock phosphate	Amm. thiosulphate	Urea granula cotated
0.04	766.7	666.9	793	688.7
0.08	1600	691.8	742.2	742.5
0.16	1390	658.9	640	663.5
0.32	1180	540.2	590.2	600

- Each figure is an average of 4 replicated shaken cultures incubated at 28 ±2 °C 2 for 5 days.

Table (6): Partial purification of chitinase

Purification Step	Total units	Protein (mg)	Specific activity	Recovery	Purification fold.
T. harzianum (T2)					
Supernatant	4879	221.3	22.04	100	1
$(NH_4)_2SO_4$ (0-30)	4593	119.1	38.5	94.1	1.7
T. harzianum (T3)					
Supernatant	3003	200.5	14.98	100	1
$(NH_4)_2SO_4$ (0-30)	2886	117.3	24.6	96.1	1.6
T. harzianum (T5)					
Supernatant	3900	225.1	17.33	100	1
$(NH_4)_2SO_4$ (0-30)	3790	200.0	18.95	97.2	1.1
T. harzianum (T9)					
Supernatant	3806	236.1	16.12	100	1
$(NH_4)_2SO_4$ (0-30)	3529	176.3	20.01	92.7	1.24

Table (7): Effect of some metals and some chelating agents on chitinase activity

Metal ion 10 M/mol	Strains No. Relative activity %			
	T2	T3	T5	T9
None	100	100	100	100
Mn^{+2}	140	133	137	131
Fe^{+2}	132	133	135	124
Ca^{+2}	125	119	121.2	118.5
Co^{+2}	110	108.3	113	119.1
Cu^{+2}	85.5	80.2	75.9	79.4
Zn^{+2}	26.2	22.2	28.3	23.1
Hg^{+2}	30.0	32.3	29.6	27.3
EDTA	18.2	15.1	16.7	13.3

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فسيولوجيا تكوين انزيم الكيتينيز لفطر تريكودرما هارزيانم

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وجد أن الترايكودرما النشطه فى تحليل فيوزاريوم اوكسى سبورم تقوم بإنتاج انزيم الكيتينيز وهو الاتريم المحلل للكيتين (المركب الرئيسى لجدار هيفات الفيوزاريوم) كذلك وجد أن ٤ عزلات من ١٢ عزله ترايكودرما لها نشاط تحليلى عالىعلى الكيتين وذلك بعد فترة تحضين ٥ أيام كذلك تم بحث العوامل المؤثره على انتاج الإنزيم بتجويد الظروف المزرعية وتقدير النشاط الانزيمى . لم يظهر النشاط الانزيمى عند نمو العزلات على بعض الكربوهيدرات مما يدل على ان الانزيم لا ينتج الا فى وجود الكيتين كذلك تم دراسة تأثير بعض المصادر النتروجين والاسمده النتروجينيه البطيئة الانسياب على انتاج الكيتينيز وجد أن الاسمدة النتروجينيه البطيئة الانسياب لها تأثير عالى على زيادة انتاج الانزيم مما يتيح الفرصه لاستخدام الترايكودرما لمقاومة الفطريات الممرضه فى التربه . كذلك تمم عمل تنقية جزئية للانزيم بواسطة كبريتات الامونيوم ثم تم دراسة اهم العوامل المؤثره على النشاط الانزيمى حيث وجد ان افضل أ س هيدروجينى كان ٦,٦ وافضل درجة حرارة كانت ٥٠درجه منويه وأفضل زمن للتحضين كان لمدة ساعة.

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CHITINASE FORMATION BY *TRICHODERMA HARZIANUM* ISOLATES.

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ABSTRACT

Isolates of *Trichoderma harzianum*, a powerful biocontrol agent against plant pathogenic fungi, whose activity lysed *Fusarium oxysporum* were found to liberate chitinase (E.C. 3.2.1.14, , chitin glucanohydrolase) , an enzyme essential for the hydrolysis of chitin, which is a major component of hyphal walls of fungi. Out of twelve isolates, only four showed high chitinolytic activity when grown on chitin containing medium. Factors involved in the production of chitinase were investigated. The enzyme was not produced in the presence of carbohydrates other than chitin, suggesting that chitinase is inducible and that chitin breakdown is regulated by a repressor-inducer mechanism. Maximum enzyme production was obtained upon using 1% chitin (w/v) in the growth medium . The chitinolytic activity was produced optimaly in the medium at pH 6.5 for the four *T. harzianum* isolates and gradually declined as pH shifting toward acidic or alkaline side . Different nitrogen sources were tested including pure inorganic salts , organic sources as well as some slow release nitrogen fertilizers in chitin containing media . The enzyme levels produced by the isolates T2,T3 and T5 of *T. harzianum* were stimulated upon using slow release nitrogen fertilizers as nitrogen sources such as ammonium thiosulfate ,urea granule coated and urea + rock phosphate (NP) yielding the highest chitinase activity production .Partial purification was performed on chitinase from *Trichoderma harzianum* isolates by (NH_4)$_2$ SO_4 preciptation achieving 1.7,1.6,1.1 and 1.24 fold from T2,T3,T5 and T9 respectively.Studies on partially purified enzyme preparations have shown that optimal activities were achieved at pH 6.6 for 60 min at 50°C incubation temperature .

Keywords :*Trichoderma harzianum* , Biological control, fungal chitinase, production parameters, Slow release N fertilizers, Wilt disease, Antagonism.

INTRODUCTION

The fungal antagonist *Trichoderma* spp. is an effective biocontrol agent against a range of important aerial and soil borne plant pathogens (Papavizas, 1985). The antagonistic ability of *Trichoderma harzianum* against several soil borne plant pathogens was reported by Dennis and Webster (1971). Application of the biocontrol agent to fields infested with *Rhizoctonia solani* and *Sclerotium rolfsii* successfully reduced the disease (Elad *et.al.* ,1981) . This fungal species produced extracellular chitinase when grown on the cell walls of *R.solani* (Hadar *et.al.*, 1979). It appears that the main mechanism involved in the antagonism of *Trichoderma* and pathogenic fungi is the release of lytic enzymes. One of such enzymes is chitinase which hydrolyses chitin, the β-1,4 linked polymer of n-acetyl glucosamine, which is an important structural constituent of many fungal cell walls .To obtain a reliable effect of the organism under different environmental conditions, we need to know more about the ecology of this antagonist. Canullo *et. al.* (1992) reported that chitinclytic fungi increased synergistically when guanidine thiocyanate and guanylurea sulfate were applied in combination.