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**PRODUCTION AND EVALUATION OF CELLULASE ENZYMES FROM
TRICHODERMA HAMATUM AND *RHIZOPUS NIGRICANS*.
BY**

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ABSTRACT

Two different fungi strains were used for production of cellulase enzymes. *Trichoderma Hamatum* was found to be the best cellulase producer. The optimum conditions for cellulase production using two different fungi was found to be by mandel's media (No.1) with (1 % w/v) cellulose powder and (1 % w/v) carboxymethyl cellulose as carbon source, at pH 5.8 and incubation for 9 days at 30° C. The final activity of carboxy methyl cellulase (CMC ase) and filter paper activity (FP ase) were 13.1 U/ml and 1.7 U/ml for *Trichoderma Hamatum*, 6.4 U/ml and 1.1 U/ml for *Rhizopus nigricans* by using CMC in Mandel's media after 9 days. However, the CMCase and FPase were 14.3 U/ml and 3.3 U/ml for *Trichoderma Hamatum*, 8.9 U/ml and 2.7 U/ml for *Rhizopus nigricans* by using cellulose powder in Mandel's media at the same incubation time. The relative activity of cellulase enzymes were 93.8 % and 91.4 % for *Trichoderma Hamatum* and *Rhizopus nigricans*, respectively after 63 days incubation time at 4° C. The optimum pH values were 5.0 for the first and 5.5 for the second and the optimum temperature was 55° C and 50° C for two fungi, respectively. The optimum enzyme concentrations for both were 0.5 ml (0.07-0.1 mg protein/mL) cultural filtrate. The K_m values were 2 mg/ml and 0.18 mg/ml and maximum reaction velocity (V_{max}) were 163.7 mM/L and 115.3 mM/L for *Trichoderma Hamatum* and *Rhizopus nigricans*, respectively.

INTRODUCTION

Lignocelluloses are complex polymers of cellulose, hemicellulose and lignin, and represent the most abundant renewable organic matter on earth, McCarthy, (1987).

The utilization of the enormous amounts of cellulosic materials, which are available in the form of plants waste, can be greatly simplified if the cellulosic materials are first hydrolyzed into its monomer glucose, Woodward, (1987). Biodegradation of lignocellulose is one of the nature's most important

biological processes. Through photosynthetic processes about 70 Kg of lignocellulose is produced per person per day, Lutzen,*et.al.*, (1983).

Although the enzymatic hydrolysis of cellulose is well established, but the high cost of enzyme production has hindered its industrial application. To reduce the cost of enzyme production, cheaper and more efficient production methods must be sought.

Fungi are considered as the most favourable micro - organisms as producers of a complex of cellulase enzymes, Kvesitadze, *et.al.*, (1986).

Anaam and Saad, (1987) studied the production of cellulase enzymes from fifty six micromycetes strains. They found that *Aspergillus terreus* was the best cellulase producer among 15 strains. Also, they found that the optimum conditions for cellulase production was pH 4.0 and incubation at 30° C for 3 days using selected strain by Mandel's medium with 8 % soyabean straw treated with NaOH.

Sanyal, *et.al.*, (1988) reported that the production of cellulolytic enzyme of *Aspergillus japonicus* was highly favoured on carboxymethylcellulose, jut powder, wheat bran and barley husk in comparison with microcrystalline cellulose and absorbent cotton. Carboxymethylcellulase and β -glucosidase were simultaneously induced by the addition of some carbon sources such as glucose in the growth medium, furthermore, utilization of carboxymethylcellulose (CMC) was prevented and CMC-ase synthesis was inhibited.

Gbikeloluwa and Murray, (1991) studied the different carbon growth sources i.e., solka floc and other sources of cellulase enzymes, they concluded that the pure cellulose powder sources was the best source producing cellulase by *Neurospora stophila* ATCC 36935. Also, they mentioned that the optimum pH and temperature for activity were 5.0 to 5.4 and 55°C, respectively.

Sharma, *et. al.*, (1991) found that the partial purification of extra cellular cellulase of *T. Viride* isolated from a forest soil was carried out by its precipitation of culture supernatant with ammonium sulfate, centrifugation at high speed, solubilization of protein in sodium acetate buffer and dialysis. The enzyme activit

Bastawde, (1992) studied the production of cellulolytic enzymes with fungi strain *Aspergillus terreus* on semisolid media. He found that high activities of cellulolytic enzymes were produced by shaking flasks for 8 days at 40°C or 14 days at 28° C in medium containing 2.5 % (w/v) cellulose powder and 1 % (w/v) wheat bran. The final activities of carboxymethyl cellulase (CMCase) and filter paper (FP ase) were 14.4 U/ml and 1.3 U/ml, respectively. Also, found that the obtained cellulase production was clearly and high activity and used for the hydrolysis of some agricultural wastes i.e., cotton; filter paper; bagasse and rice straw.

Cheorl and Dong, (1995) purified the specific endo- 1,4-D-glucanase, and exo-cellobiohydrolase (CBH) from *Bacillus circulans* F.2 by employing the fractionation process on the enzyme. The concentrated enzyme was applied on hydrophobic interaction column of TSK Gel (Toyopearl H w-50 S phenyl 6 pw) previously equilibrated with 1 M ammonium sulfate in 0.1 M phosphate buffer (pH 7.5) then the enzyme was eluted with a decreasing (NH₄)₂SO₄ gradient in 0.1 M phosphate buffer. The optimum pH of the cellulase enzyme was 4.5, while the optimum temperature was 45°C.

Okeke and Obi, (1995) Saccharified an agro-waste materials by cellulase from two fungi isolates, *Sporotrchum Pruinosum* and *Arthrographis* Sp.. They found that the highest degree of hydrolysis was 15.1 % for *Sporotrchum Pruinosum* and 7.5 % for *Arthrographis* Sp.

Sheldon and Willian, (1996) mentioned that the optimum temperature for *T. Reesei* growth was 30°C. However, the optimum temperature for cellulase production was 25-28 °C.

Therefore, the present study was undertaken with a view to determine the ability of two strains of fungi (*Trichoderma Hamatum* and *Rhizopus nigricans*) to production of cellulase enzemes, also studing some of their kinitic properties.

MAETRIALS AND METHODS

Microorganisms:-

Trichoderma Hamatum and *Rhizopus nigricans* were isolated from the rhizosphere of local bagasse plants and cotton bols. Identification of these organisms was carried in plant protection laboratory, Agric., Res. Center at Giza. Cellulose powder, Whatmann filter paper and Carboxymethyl cellulose were obtained from Merck Co. These materials were used as cellulosic sources.

Media for fungi:-

The standard cultures were maintained on potato/dextrose agar (PDA) slants containing in (g/L) : agar, 20 gm, potato 200 gm and glucose 20 gm.

Basal medium for cellulolytic enzyme production:

Media No.	Media composition G/L	References
Med. 1.	KH ₂ PO ₄ ; (NH ₄) ₂ SO ₄ , 1.4; CaCl ₂ .2H ₂ O 0.3; Urea, 0.3; MgSO ₄ .7H ₂ O, 0.3; Peptone 0.25; yeast extract, 0.1; Tween 80, 1.0; FeSO ₄ .7H ₂ O, 0.005; MnSO ₄ .H ₂ O, 0.0016, ZnSO ₄ . 7H ₂ O, 0.0014 and CoCl ₂ . 6H ₂ O, 0.002 and 1 % cellulose sole Carbon source.	Mandels and Weber, (1969).
Med. 2	Glucose 40.0; NaNO ₃ ; yeast extract, 0.1; KH ₂ PO ₄ , 2.0; CoCl ₂ . H ₂ O; and MgSO ₄ . 7H ₂ O, 0.3.	Aditya, <i>et al.</i> , (1990).
Med. 3.	Sucros, 150.0; ammonium - oxalate -monohydrate, 5.0; KH ₂ PO ₄ , 5.0; MgSO ₄ . 7H ₂ O 5.0; Kcl, 5.0; FeSO ₄ . 7H ₂ O 0.01 and yeast extract, 5.0.	Lee, <i>et al.</i> , (1992)
	The pH was adjusted to 5.8	

Enzyme assay:-

Enzyme assays were carried out on culture filtrates at pH 4.8 (0.05 M acetate buffer) and 50° C for 30 min. Filter paper activity (FPase) was performed according to the method of Mandels and Sternberg, (1976). Carboxymethylcellulose activity (CMC ase) was achieved according to the method described by Mandels and Weber, (1969). Released reducing sugar was determined colorimetrically as described by Nelson's (1944) and which was modified by Somgyi, (1952). The activity was expressed as micromoles of reducing sugar per/ml/min.

Determination of protein :-

Protein of enzyme production was determined by the method described by Bardford, (1976).

Effect of ammonium sulfate concentration on precipitation of enzyme produced from different fungi:-

Different concentrations of ammonium sulfate i.e 2, 4, 6, 8 and 10 % were used for precipitation of cultural filtrate solution which containing the cellulase produced by *Trichoderma Hamatum* and *Rhizopus Nigricans*. The precipitate was redissolved in 0.05 M acetate buffer (pH 4.5) , then it was reprecipitated by cold acetone many times to dryness as reported by Sharma, *et al.*, (1991). The enzyme activity and protein content were determined by the methods described before.

Evaluation of the produced enzymes:-

The different parameters which affect the enzyme activity were determined in order to evaluate enzyme activity and also to obtain the optimal conditions e.g. pH, temperature, and concentration of substrate and enzyme. The enzyme activity of produced cellulase was tested at different pH values, i.e 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 in 0.05 M acetate buffer , different temperatures , i.e 30, 35, 40, 45, 50, 55, and 60°C and different concentrations of enzyme i.e.

0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml/100 ml buffer solution at 1 % (w/v) carboxymethyl cellulose as substrate. The effect of substrate concentration on the reaction activity and velocity of produced cellulase enzyme was tested at different concentrations of carboxymethyl cellulose solutions i.e. 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 % .

Stability of cellulase enzymes produced from different fungi were evaluated according to the method described by Nicole and Anh,(1985).

RESULTS AND DISCUSSION

Effect of carbon source and media on the growth of different fungi:-

The influence of carbon source and media on the growth of *T. Hamatum* and *R. nigricans* are shown in Tables (1, 2). These results indicate that carboxymethyl cellulose and cellulose powder yielded a highly growth with *Trichoderma Hamatum* . While, the other fungi, *Rhizopus nigricans* growth was lowest value on carboxymethyl cellulose. But with cellulose powder as a carbon source gave slightly high value of growth compared with CMC. On the other hand, media (No. 1) was found to be the best media for growth of *Trichoderma Hamatum* and *Rhizopus nigricans*.

Table (1): Effect of carbon source on the growth of different fungi.

Source of fungi	Carbon source (1% w/v)	
	Carboxymethyl cellulose growth/cm	Cellulose Powder growth/cm
<i>Trichoderma Hamatum</i>	9.0	9.0
<i>Rhizopus nigricans</i>	2.3	4.4

Table (2): Effect of different media on fungi growth.

Fungi	Growth /cm		
	Media (1)	Media (2)	Media (3)
<i>Trichoderma Hamatum</i>	8.0	7.5	6.6
<i>Rhizopus nigricans</i>	5.0	4.8	2.0

Effect of fermentation time on the production of cellulase enzymes from *T. Hamatum* and *R. nigricans*:-

The activities of cellulase enzymes production by two fungi strains which growth on different carbon sources i.e carboxymethyl cellulose and cellulose powder at different fermentation times (3, 6, 9, 12 days) are shown in Tables (3, 4) . The results showed that the two fungi strains grown on cellulose powder gave better cellulase productivity than on the other cellulosic sources (carboxymethyl cellulose). This may be due to the presence of growth factors

which accelerate the fungi productivity, the fungi started using CMC as its substrate. Maximum activities of both enzymes in the culture filtrate with carboxymethyl cellulose were 13.1 μmol glucose/ml (CMCase) on the ninth day of fermentation, 1.7 μmol glucose/ml (FPase) on the sixth day of fermentation with *Trichoderma Hamatum* and 6.4 μmol glucose/ml (CMCase), 1.1 μmol glucose/ml (FPase) on the ninth day of fermentation with *Rhizopus nigricans*. On the other hand, the maximum activities of abovementioned fungi with cellulose powder as a source of carbon were 14.3 μmole glucose/ml (CMCase), 3.3 μmole glucose/ml (FPase), for *T. Hamatum* and 9.8 μmole glucose/ml (CMCase), 2.7 μmole glucose/ml (FPase), for *R. nigricans* on the ninth day of fermentation at 30° C and pH 5.8. These values of enzyme activities are in agreement with that reported by Bastawde, (1992). He found that enzyme activities (CMCase and FPase) for *Aspergillus terreus* strain were 14.2 U/ml and 1.4 U/ml, respectively, at incubation time of 14 days, 28° C and pH between 4.7 and 5.1.

Table (3): Activities of cellulase enzymes production by different fungi growth on Carboxymethyl cellulose.

Fermentation time (days)	Activities * μmol glucose/ml			
	<i>Trichoderma Hamatum</i>		<i>Rhizopus nigricans</i>	
	CMCase	FPase	CMCase	FPase
3	2.3	1.3	1.3	0.7
6	6.6	1.7	4.0	1.0
9	13.1	1.5	6.4	1.1
12	9.5	1.3	4.2	0.8

* The enzyme activities were the average of three independent experiments on pH 5.8 at 30° C.

Table (4): Activities of cellulase enzymes production by different fungi growth on cellulose powder.

Fermentation time (days)	Activities * μmol glucose/ml			
	<i>Trichoderma Hamatum</i>		<i>Rhizopus nigricans</i>	
	CMCase	FPase	CMCase	FPase
3	3.7	1.7	2.4	1.5
6	7.2	2.4	5.9	1.9
9	14.9	3.3	8.9	2.7
12	10.1	2.4	3.2	2.2

* The enzyme activities were the average of three independent experiments on pH 5.8 at 30° C.

Effect of ammonium sulfate concentration on the precipitation of cellulase enzymes production by *T. Hamatum* and *R. nigricans*:-

Different concentrations of ammonium sulfate i.e. 2 , 4, 6, 8 and 10 % were used to precipitate cellulase enzyme from culture filtrates of the two fungi strains. The results are shown in Table (5). From these results the enzyme activity was reached it a maximum at 8 % (w/v) ammonium sulfate for both two culture filtrates. The enzyme activities equalled 19.2 units/ ml (CMC ase) and 2.2 units/ml (FP ase) for *Trichoderma Hamatum*. The amount of protein enzyme was 0.137 mg/ml. But, the enzyme activities were 7.2 units/ml (CMC ase) at 10 % ammonium sulfate and 1.4 units/ml (FP ase) at 8 % and protein enzyme equalled 0.190 mg/ml for *Rhizopus nigricans*. These results are coincide to those reported by Bastawde,(1992).

Table (5): Effect of ammonium sulfate concentration on the precipitation of cellulase enzymes production from different fungi.

Ammonium sulfate concentration (% , w/v)	Enzyme Activities Units/ ml			
	<i>Trichoderma Hamatum</i>		<i>Rhizopus nigricans</i>	
	CMC _{ase}	Fp _{ase}	CMC _{ase}	FP _{ase}
2	0.4	0.2	-	-
4	2.0	1.8	0.8	1.0
6	6.9	2.0	3.0	1.2
8	19.2	2.2	4.5	1.4
10	15.4	1.0	7.2	-
Enzyme protein content mg/L	0.137		0.190	

Evaluation of cellulase enzymes produced from different fungi:-

Stability of cellulase enzymes:-

The effect of incubation time at optimum temperature of each produced enzyme and the reaction activities of both culture filtrate from *Trichoderma Hamatum* and *Rhizopus nigricans* are shown in Fig. (1). The relative activities were 94 % and 91 % after 63 days with *T. Hamatum* and *R. nigricans*, respectively. The obtained results are in agreement with that obtained by Nicole and Anh,(1985) and Okeke and Obi,(1995).

Effect of pH on the activity of cellulase enzyme:-

The effect of pH on the reaction activity of enzyme produced from different sources were tested at different pH values. Eight solutions of carboxymethyl cellulose (1 % w/v) with (50 mM) acetate buffer were adjusted to pH values of 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5. Table (6) and Fig. (2) show that the maximum reaction activities of cellulase enzyme production from *Trichoderma Hamatum* and *Rhizopus nigricans* amounted to 46.4 μmM/L/min at pH 5.0 for the first and 31.5 μmM/L/min at pH 5.5 for the second, respectively.

Such relative low value of optimum pH clearly indicate the importance of such acid media to fit the nature of the catalytic activity of the groups in the active site of enzyme, Wiseman, (1985). These results are in line with those obtained by Okeke and Obi, (1993).

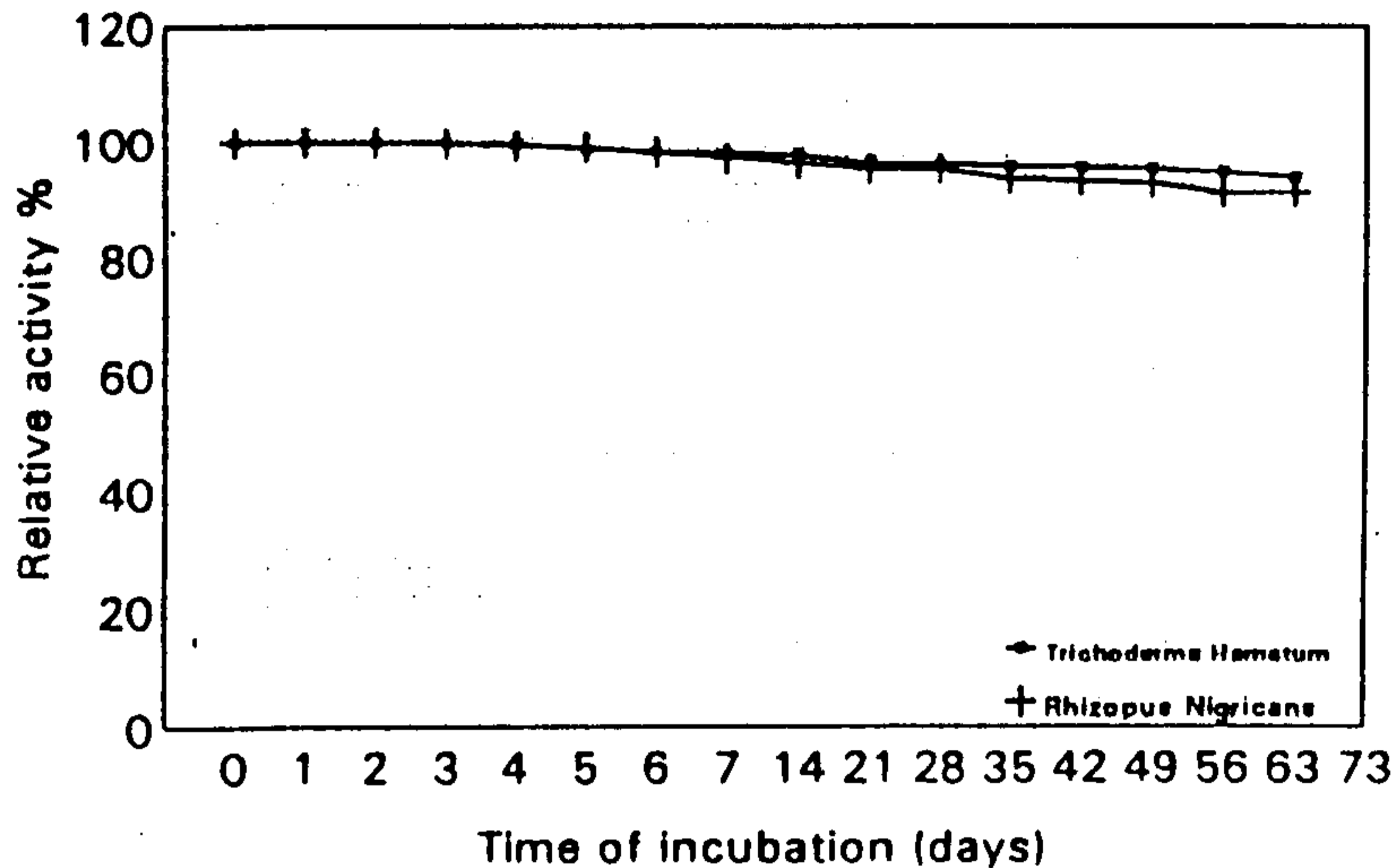


Fig. (1): Stability of cellulase enzyme produced by *Trichoderma Hamatum* and *Rhizopus Nigricans* at 4°C

Table (6): Effect of pH on the activity of produced cellulase enzyme from different fungi.

pH ^{ns}	Reaction activity (μ mM G/L/min)	
	<i>Trichoderma Hamatum</i>	<i>Rhizopus nigricans</i>
3.0	24.2	6.7
3.5	28.4	8.9
4.0	32.3	11.0
4.5	38.2	19.4
5.0	46.4	24.8
5.5	40.1	31.5
6.0	34.2	15.2
6.5	18.6	13.7

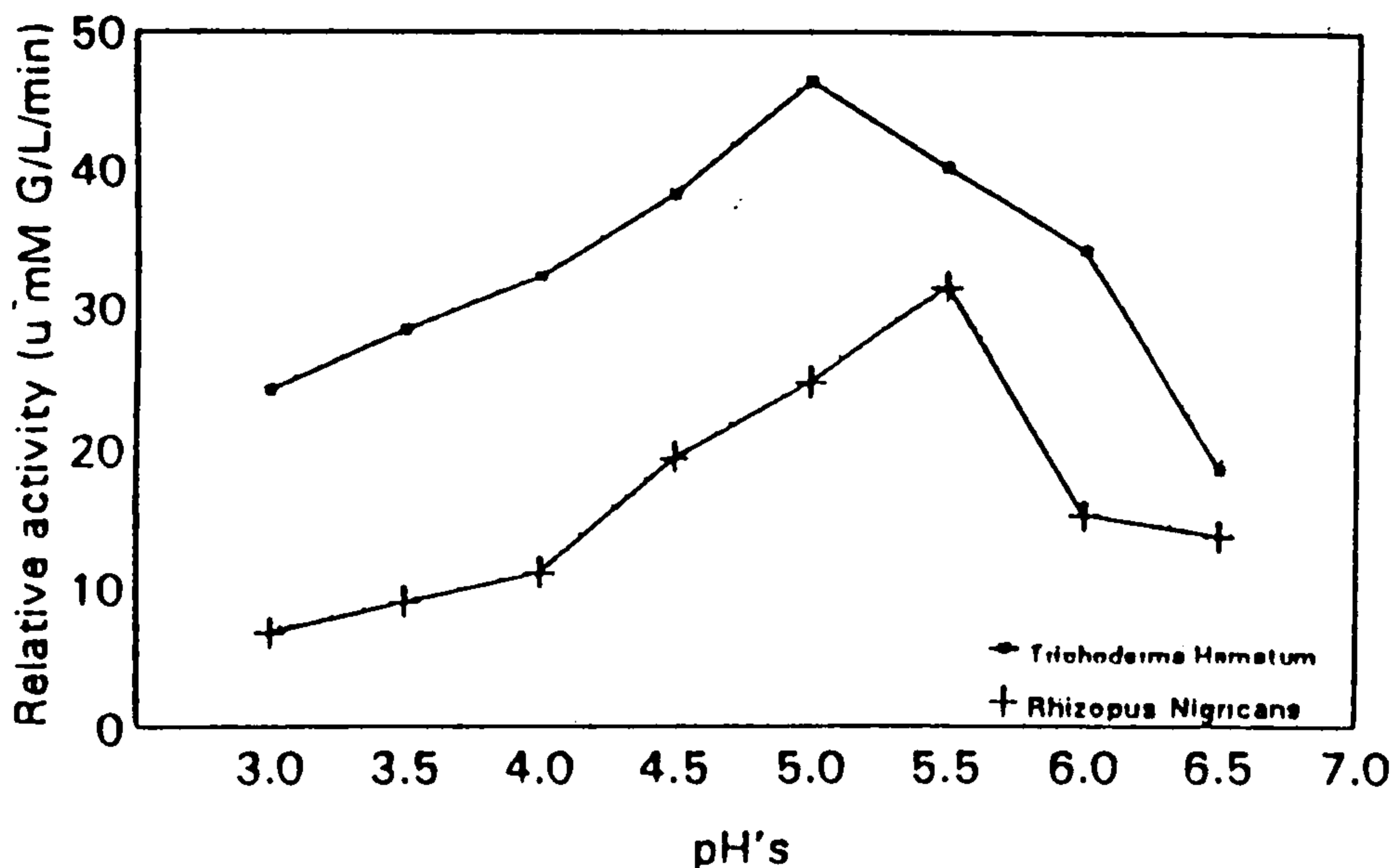


Fig. (2): Effect of pH on the reaction activity of cellulase enzyme produced from different fungi.

Effect of temperature on the activity of produced cellulase enzyme:-

Seven different temperatures, i.e 30, 35, 40, 45, 50, 55, and 60° C were chosen to estimate the optimum temperature of produce cellulase enzyme from different sources. The experiment was carried out at pH 5.0 for *Trichoderma Hamatum* and pH 5.5 for *Rhizopus nigricans* with (1 % w/v) carboxymethyl cellulose and duration of each experiment was 30 min.

Table (7) and Fig. (3) show that the reaction reached its maximum at temperature 55°C and equalled to 43.9 µm M glucose/L/min. for *Trichoderma Hamatum*. While, the maximum reaction activity reached to 23.3µmM glucose/L/min for *Rhizopus nigricans* at 50°C. These temperatures could be considered a balance between the increase of initial activity and destruction of the enzyme at high temperature, Plummer, (1972). The slight change in temperature of *Trichoderma Hamatum* and *Rhizopus nigricans* can be attributed to the change in physical characteristics of abovementioned fungi. However, such values for optimum temperatures are in good agreement with that obtained by Okeke and Obi, (1993) and (1995).

Table (7): Effect of Temperature on the reaction activity of produced cellulase enzyme from different fungi.

Temperature's C°	Reaction activity (μ mM G/L/min)	
	<i>Trichoderma Hamatum</i>	<i>Rhizopus nigricans</i>
30	18.6	9.3
35	19.2	12.6
40	20.5	15.7
45	31.6	18.4
50	39.9	23.3
55	43.9	21.4
60	40.2	18.1

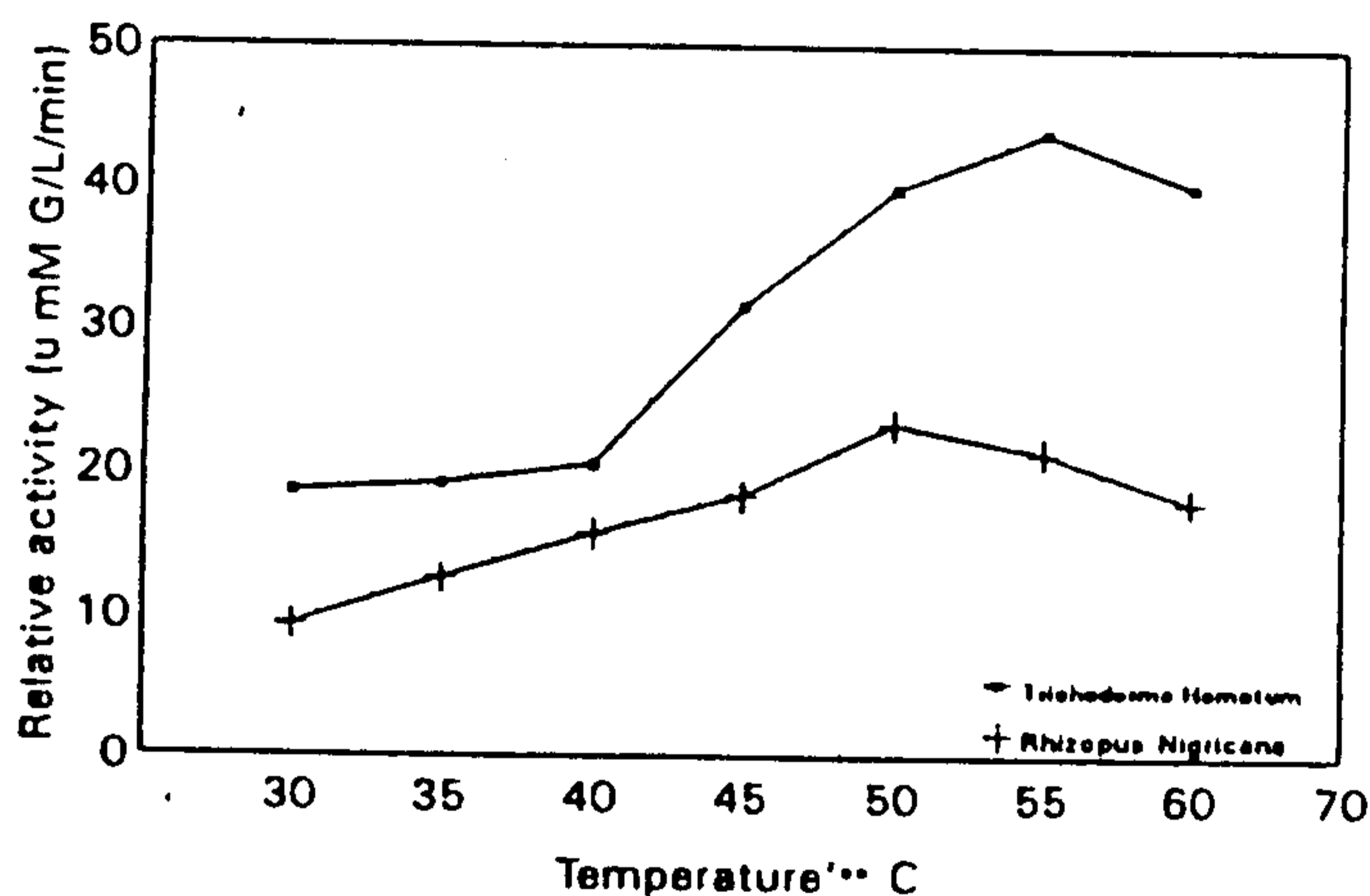


Fig. (3): Effect of temperature on the activity of cellulase enzyme produced from different fungi.

The results showed that cellulase enzyme produced from *Trichoderma Hamatum* reached its maximum activity ($67.5 \mu\text{mM}$ glucose/L /min) with CMC and activity ($63.7 \mu\text{mM}$ glucose/L /min) with filter paper at enzyme concentration equalled to $0.5 \text{ mg}/100 \text{ ml}$. On the other hand, the maximum activity of cellulase produced from *Rhizopus nigricans* at the same enzyme concentration, which gave reaction activity equal 30.2 and $20.5 \mu\text{mM}$ glucose/L/min for carboxymethyl cellulose (CMCase) and filter paper (FPase), respectively. The above results indicated that increasing enzyme concentration beyond these values lead to a decrease in the overall reaction activity.

Effect of substrate concentration on the reaction activity and velocity of cellulase enzymes production by *Trichoderma Hamatum* and *Rhizopus nigricans*:-

The obtained results are recorded in Table (9). These results showed that the rate of the enzyme reaction was increased up to a certain point with increasing concentration of carboxymethyl cellulose (CMC) as substrate until a

maximum activity reached and further increase in substrate concentration does not show any positive effect. The maximum activity (V_{max}) were 163.7 mM/L for *Trichoderma Hamatum* and 115.3 mM/L for *Rhizopus nigricans* at 1.8 % (w/v) of carboxymethyl cellulose. The lower value of V_{max} for *Rhizopus nigricans* can be due to the lower amounts and activities of enzyme production from this fungi. The K_m apparent values for different fungi, calculated by the Lineweaver - Burk method (1954), Fig. (4) were 2 mg/ml and 1.8 mg/ml for *Trichoderma Hamatum* and *Rhizopus nigricans*, respectively. These values of K_m are slight lower than that reported by Nicole and Anh,(1985) who found that K_m was 2.6 mg/ml with carboxymethyl cellulose (CMC) as substrate and acidophilic fungi strain NC - II (deposited as ATCC - 20677).

Table (8): Effect of enzyme concentration on the reaction activity of produced cellulase enzyme from different fungi.

Enzyme concentration mg/100 ml buffer	Reaction activity (μ mM G/L/min)			
	<i>Trichoderma Hamatum</i>		<i>Rhizopus nigricans</i>	
	CMC _{ase}	FP _{ase}	CMC _{ase}	FP _{ase}
0.1	22.0	26.6	10.7	4.9
0.2	36.4	32.4	13.3	10.6
0.3	43.3	42.5	17.2	13.3
0.4	56.9	54.5	20.5	16.6
0.5	67.5	63.7	30.2	20.5
0.6	66.5	61.2	28.4	20.1

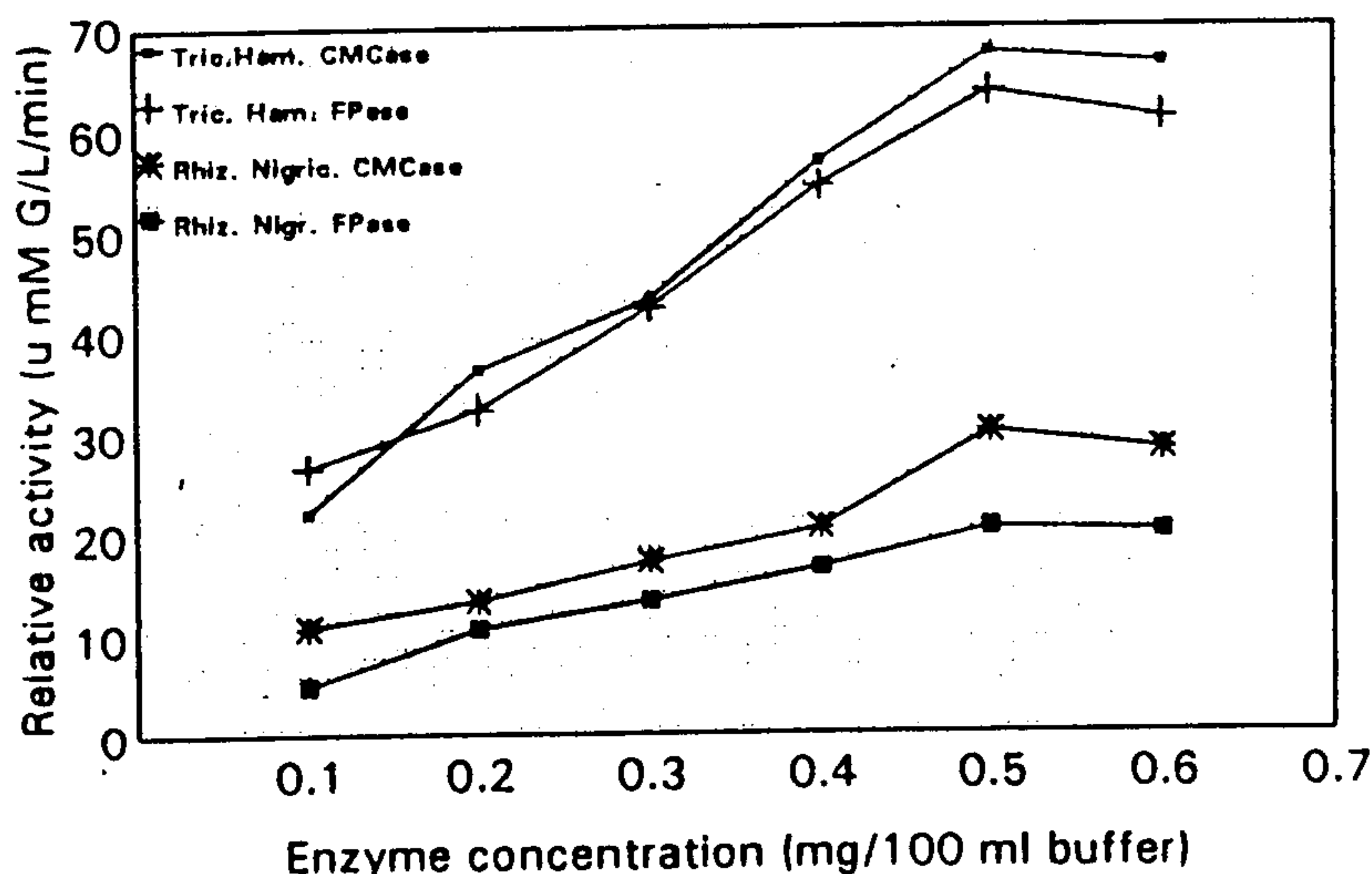


Fig. (4): Effect of enzyme concentration on the activity of cellulase enzyme produced from different fungi.

Table (9): Effect of substrate concentration on the reaction activity and velocity of produced cellulase enzyme from *T. Hamatum* and *R. Nigricans*.

Substrate concentration [S] (%W/V)	$\frac{1}{S}$	<i>Trichoderma Hamatum</i>			<i>Rhizopus nigricans</i>		
		Obtained reducing sugar mM/L	Reaction velocity (v)	$\frac{1}{V} \times 10^{-3}$	Obtained reducing sugar mM/L	Reaction velocity (v)	$\frac{1}{V} \times 10^{-3}$
0.2	5.00	75.6	81.9	12.21	62.1	60.7	16.47
0.4	2.50	88.2	109.1	9.17	67.3	79.5	12.58
0.6	1.67	90.5	122.8	8.14	72.8	88.7	11.27
0.8	1.25	97.5	131.0	7.63	78.2	94.1	10.63
1.0	1.00	110.4	136.4	7.33	82.4	97.7	10.24
1.2	0.83	118.5	140.3	7.13	86.9	100.3	9.97
1.4	0.71	129.5	143.2	6.98	92.2	102.2	9.78
1.6	0.63	145.2	145.5	6.87	102.2	103.6	9.65
1.8	0.56	163.7	147.3	6.79	115.3	104.8	9.54
2.0	0.50	162.8	148.8	6.77	112.4	105.8	9.45

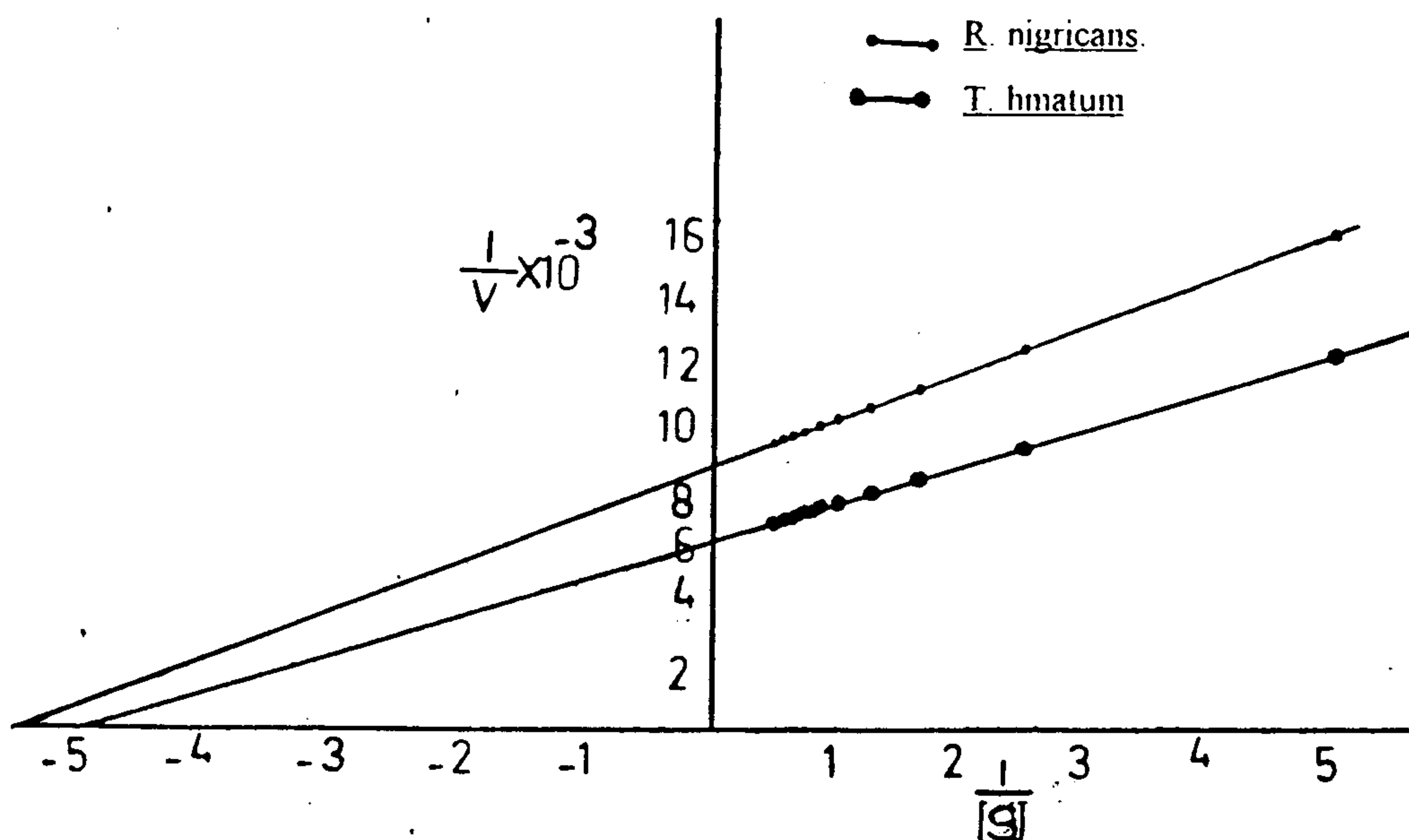


Fig. (5): Lineweaver Burk plots of cellulase enzyme produced from *T. hamatum* and *R. nigricans*.

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إنتاج وتقييم إنزيمات السليوليز من فطريات تراى كودرماهاماتيم
والريزوباس نيجريكانس

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تهدف هذه الدراسة إلى استخدام الفطريات فى إنتاج إنزيمات السليوليز حيث تم استخدام فطريات *Trichoderma Hamatum* (تراى كودرما هاماتيم) و *Rhizopus nigricans* (الريزوباس نيجريكانس) لقياس مقدرتهم على الإنتاج وقد تبين أن تراى كودرما هاماتيم لها مقدرة للإنتاج أعلى من الريزوباس نيجريكانس . بعد ذلك تم دراسة أنسب الظروف لإنتاج هذا الإنزيم حيث وجد أن بيئة Mandel المحتوية على كل من السليلوز بودرة والكربوكسى ميثيل سليلوز كمصدر للكربون ، مع درجة حموضة pH 5,8 ثم التحضين على 30 °م لمدة 9 أيام هى أفضل ظروف للإنتاج بواسطة كل من التراى كودرما هاماتيم والريزوباس نيجريكانس. كما أوضحت الإنزيمات المنتجة من التراى كودرما هاماتيم والريزوباس نيجريكانس أعلى درجة نشاط وتساوى 14,3 وحدة/ملى ، 8,9 وحدة/ملى على التوالى مع استخدام السليولوز بودرة فى بيئة Mandel بعد 9 أيام تحضين.

كما أوضحت الإنزيمات المنتجة درجة ثبات عالية لمدة 63 يوم على درجة 4م . وقد وجد أن رقم الـ pH الأمثل يساوى 5,0 للفطر الأول ، 5,5 للفطر الثانى بينما كانت درجة الحرارة المثلى لنشاط إنزيم السليوليز المنتج من التراى كودرما هاماتيم 55 °م ، 50 °م من الريزوباس نيجريكانس . كما أوضحت الدراسة أن السرعة القصوى وثابت ميكاليس لإنزيم السليوليز المنتج تساوى 163,7 ملليمول/لتر ، 2 ملليجرام / مللى لفطر تراى كودرما هاماتيم ، 110,3 ملليمول /لتر ، 0,18 ملليجرام / مللى لفطر الريزوباس نيجريكانس على التوالى.

من هذه الدراسة يتضح أن هذه الفطريات لها المقدرة على إنتاج إنزيمات السليوليز الذى يمكن استخدامه فى عمليات تكسير المخلفات السليولوزية وتقليل التلوث البيئى.