Ethanol and Xylitol Production from Xylanase Broth of *Thermomyces Lanuginosus* Grown on Some Lignocellulosic Wastes using *Candida tropicalis* EMCC2

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Abstract: Four different strains of *Thermomyces lanuginosus* were screened for cellulase-free xylanase enzyme. These were: *Thermomyces lanuginosus* A72, *T. lanuginosus* H72, *T. lanuginosus* U72 and *T. lanuginosus* YMN72.The potentiality of these strains to produce cellulase-free xylanase was screened on four different natural lignocellulosic substrates. Incubation period lasted for 7 days at 45 ºC. *Thermomyces lanuginosus* A72 and *T. lanuginosus* YMN72 generally exhibit a relatively higher xylanase activity as compared with other fungal strains when grown on cane bagasse and corn cobs; respectively. *T. lanuginosus* A72 showed xylanase activity (411 U/ml) on cane bagasse (1.0%) as carbon source, while *T. lanuginosus* YMN72 produced highest xylanase activity (428 U/ml) when grown on corn cobs (1.5%) as carbon source when incubated at 45ºC for seven days of growth. Both of the experimental fungal strains reached a maximum value of xylanase activity at slightly neutral pH (6.6) giving (442 U/ml & 723 U/ml respectively). Sodium nitrate (0.3 % w/v) was the best nitrogen source for *T. lanuginosus* A72 where xylanase activity reached 563U/mg., while ammonium nitrate (0.1% w/v) was the best nitrogen source for *T. lanuginosus* YMN72 giving xylanase activity 946U/ml. Gamma radiation affected xylanase produced by the two experimental strains. Thus radiation dose (1.0KGy) was the best for the production of xylanase by *T. lanuginosus* A72 giving activity (1082U/ml) with increasing 179% as compared with control value. On the other hand results showed that radiation dose (0.9KGy) was the best for the production of xylanase by *T. lanuginosus* YMN72 giving activity (1173U/ml) with increasing 121% as compared with control value. Maximum saccharification was obtained from treatment of cane bagasse by partially purified xylanase from *T. lanuginosus* A72 and *T. lanuginosus* YMN72 after 24 hrs of incubation. The maximum production of ethanol and xylitol were obtained after fermentation time 48 and 24 hrs giving (22.48 and 13.54 g/l) using enzyme broth of *T. lanuginosus* YMN72 using *Candida tropicalis* EMCC2.

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1. Introduction

In the present study, Four different strains of *Thermomyces lanuginosus* were screened for cellulase-free xylanase enzyme. These were: *Thermomyces lanuginosus* A72, *T. lanuginosus* H72, *T. lanuginosus* U72 and *T. lanuginosus* YMN72.The potentiality of these strains to produce cellulase-free xylanase was screened on four different natural lignocellulosic substrates, followed by irradiation with Gamma radiation then determination of saccharification and finally estimation of ethanol and xylitol produced by using *Candida tropicalis* EMCC2 in the xylanase broth of *T. lanuginosus* YMN72.

Fermentable sugars have high market value. The utilization of enzymatic hydrolysis to obtain sugars from agricultural residues is of great interest in modern biotechnology particularly for bioethanol production. Lignocellulosic biomass is composed of cellulose (35–50%), hemicellulose (20–35%) and lignin (10–25%) (Saha, 2003). Enzymatic hydrolysis of the hemicellulose is essential to facilitate complete cellulose degradation. As xylan is the major hemicellulose, xylanase action leading to production of xylose would make the production of bioethanol from lignocellulosic materials more profitable.

The utilization of hemicellulosic sugars is essential for efficient and cost-effective conversion of lignocellulosic material to fuel ethanol. The demand for fuel ethanol is expected to rise very sharply as a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion. MTBE has been found to contaminate groundwater. (Saha, 2003).

In addition, Xylitol; a five-carbon sugar alcohol has attracted much attention because of its potential use as a natural food sweetener. A dental caries reducer and a sugar substitute for diabetics (Saha and Bothast, 1997). Production of xylitol by fermentation is becoming more attractive because of the problems associated with its chemical production so the production rate of ethanol and xylitol from enzyme broth of *T. lanuginosus* YMN72 using *Candida tropicalis* EMCC2 was studied.

Hemicellulose is the second most common natural polysaccharide on earth; represents about 20-30% of lignocellulosic biomass (Saha, 2003; Collins *et al*., 2005). It is a storage polymer in seeds (Taiz and Honigman, 1976), and it forms the structural component in cell walls of woody plants and in the middle of plant cells (Eriksson, 1990). Classes of hemicelluloses are named according to the main sugar unit in the backbone chain of the polymer. The principal monomers present in hemicelluloses are D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, D-glucuronic acid and D-galacturonic acid.

Xylan is the most abundant of the hemicelluloses (Estebam *et al*., 1982; Saha, 2003). Xylan polysaccharides comprise 15-35% of hard wood and annual herbaceous plants, and accounts for 20-35% of the total dry weight in tropical plant biomass. In soft woods, xylan is less abundant and may comprise about 8% of the total dry weight. Xylan is found mainly in the secondary cell wall of plants and is considered to be forming an interphase between lignin and other polysaccharides (Srinvasan and Meenafshi, 1999).

Many organisms are known to produce different types of xylanases; nature of these enzymes varies between different organisms (Hann and zyl, 2003). Production of xylanases has been reported in a number of microorganisms, including bacteria (Rani and Nand, 1996); fungi (Sunna and antranikian, 1997; Jorgensen *et al*., 2005); yeasts (Leather, 1986) and actinomycetes (Nascimento *et al*., 2003).

Many studies have been done on the production of xylanases from thermophilic fungi by many investigators as *Thielevia terrestris* and *Thermoascus crustaceus* (Gilbert *et al*., 1993), *Melanocarpus albomyces* (Prabhu and Maheshwari., 1999; Saraswat and Bisaria, 2000; Gupta *et al*., 2002); *Ceriporipsis subvermispora* (Ramos *et al*., 2001; Milagres *et al*., 2005); *Humicola grisea* var. thermoidea (Salles *et al*., 2005; Medeiros *et al*., 2007), *Chaetomium thermophilum* (Katapodis *et al*., 2007), *Paecilomyces thermophila* (Yang *et al*., 2006; Yan *et al*., 2008; Zhang *et al*., 2010), *Sporotrichum thermophile* (Katapodis *et al*., 2006; Vafiadi *et al*., 2010), *Talaromyces stipitatus* (Mandalari *et al*., 2008), *Talaromyces thermophilus* (Maalej *et al*., 2009), *Thermoascus aurantiacus* (Katapodis *et al*., 2002; Katapodis and Christakopoulos, 2008; *Myceliophthora* sp. (Luo *et al*., 2005; Badhan *et al*., 2007; 2008), and *Thermomyces lanuginosus* [formerly *Humicola lanuginose*] (Katopodis *et al*., 2006; Puchart and Biely, 2008; Manimaran *et al*., 2009).

2.Materials and Methods

Microorganisms

Thermomyces lanuginosus A72 and *T. lanuginosus YMN72* were isolated from soil samples collected from Egypt and Yemen. They were isolated by direct soil plate method (Warcup, 1950) and identified according to Moubasher (1993). The two isolates were chosen as potent producers for cellulase-free xylanase.

Candida tropicalis EMCC2 was obtained from Microbiological Resources Center (Cairo, MIRCEN), Faculty of Agriculture, Ain Shams University and maintained on YPG agar slants [(g/l): glucose 20; yeast extract 10; peptone 20; agar 20] at 4 ºC, and subcultured every four weeks.

Medium

Czapek- Dox agar (CDA) medium was used to culture the experimental fungi and composed of the following ingredients per liter: Sucrose, 30 g; $NaNO_3$, 3.0 g ; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; KCl, 0.5 g and $FeSO_4.7H_2O$, 0.01 g.

Carbon Sources

Dry milled substrates of some agro-industrial by-products including cane bagasse, corn cobs, wheat bran, wheat straw and sugar beet pulp were used in the screening experiment to choose the most potent isolates for xylanase production.

Fermentation conditions

Optimized studies were carried out in 250 ml Erlenmeyer conical flasks containing 50 ml of fermentation medium. 50 ml *T. lanuginosus* YMN72 enzyme broth was added. Tween-80 (0.1 ml) was added to each flask. Concentrated solutions of yeast extract and peptone (5 ml each) were added to the enzyme broth to a final concentration of 2% and 3% (v/v), respectively (Latif and Rajoka, 2001). Finally, 2.5 ml yeast suspension of one day old culture was added. The SSF was carried out at 30°C. The whole flasks were harvested after every 24 hrs up to 96 hrs of incubation.

Analysis

Ethanol and xylitol released were determined and quantified by high performance liquid chromatography (HPLC) by the authorities of National Research center, Dokki, Cairo, Egypt; to whom the author is greatly indebted. Samples were filtered through a 0.45 µm membrane. Analysis of the samples were performed by using HPLC, Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with refractive index RID-10A Shimadzu detector, LC-16ADVP binary pump, and PL Hi-Plex Pb column, heater set at 80 °C. The mobile phase was 0.01% reagent grade calcium chloride prepared with deionized water, and the flow rate was 0.6 ml / min.

Standard of ethanol and xylitol with analytical grades were prepared Injection volume of each standard and samples was 20 µL.

Methods

Xylanase assay

Xylanase activity was determined according to (Bailey *et al*. 1992) by determination of the amount of reducing sugars liberated from oat spelt xylan (Sigma Chemical co., USA), as substrate due to the effect of *T. lanuginosus* enzyme preparation by the dinitrosalicylic acid (DNS) method (Miller, 1959). The xylanase assay was carried out in 50 mM acetate buffer (pH 5.0) at 50 ºC for 30min. The substrate was prepared by dissolving oat spelt xylan in acetate buffer (1.0%, w/v). The reaction mixture which contained 1ml of substrate solution and 1ml of enzyme solution (original filtrate or suitably diluted) incubated for 30 min at 50 ºC, then the reaction was stopped by adding 3ml of DNS reagent and the samples were heating in a boiling water bath for 5min, cooled and the developed color was measured spectophotometerally at 540 nm. The amount of reducing sugars liberated was quantified using xylose standard. One unit of xylanase activity was defined as the amount of enzyme required to release to 1µmol of xylose equivalents per minute.

Protein determination

The protein content of the purified enzyme was measured by UV absorbance at 280 nm (Markwell *et al*., 1978) using bovine serum albumin as a standard.

3. Results and Discussion

In the present study, we attempted the saccharification of dry milled agroresidues including cane bagasse, corn cobs, wheat bran, wheat straw and sugar beet pulp by partially purified xylanase produced from *T. lanuginosus* A72 and YMN72 using cane bagasse and corn cobs, respectively under solid state fermentation.

A- Optimization of the conditions for xylanase production by the experimental fungi

The potentiality of four fungal strains of *T. lanuginosus* namely; *T. lanuginosus* A72, *T. lanuginosus* H72, *T. lanuginosus* U72 and *T. lanuginosus* YMN72 to produce cellulase-free xylanase was tested using four natural lignocellulosic substrates (1.0% w/v) namely; cane bagasse, corn cobs, sugar beet pulp and wheat straw (Table 1) . *T. lanuginosus* A72 and *T. lanuginosus* YMN72 which were the most potent xylanase producers were chosen as the experimental fungal strains in this study. The results obtained showed that cane bagasse was the best substrate for *T. lanuginosus* A72 and this agrees with the results of Jain *et al*. (1998); Lemos *et al*. (2002); khalil *et al*. (2002); Milagres *et al*. (2004); Aboellil and Geweely (2005); Malabadi *et al*. (2007); Meshram *et al*. (2008). On the other hand, corn cobs was the best substrate for the production of xylanase by *T. lanuginosus* YMN72 and this result agrees with the results of Oliveira *et al*. (2006); Katapodis and Christakopoulos (2008).

Based on the data of Wang *et al*. (1994), Gaspar *et al*. (1997) and Kalogeris *et al*. (1998); the production of fungal xylanase is almost proportional to the initial substrate concentration of the culture medium. Thus, it became of interest to optimize this factor in our study. Different concentrations 0.5, 1.0, 1.5, 2.0% of the most suitable lignocelluloses were tested for xylanase activity. The results obtained (Table 2) showed that 1.0% cane bagasse gave the optimum production by *T. lanuginosus* A72 (411U/ml). This was in agreement with Khalil (2002) who reported that the optimum xylanase production by *Phanerochaete chrysosporium* was obtained in liquid medium containing 1.0% cane bagasse, but disagrees with the results of Meshram *et al*. (2008) who stated that maximum activity of xylanase was obtained on Mandel-Waber medium containing 1.63% cane bagasse as a carbon source by *Penicillium janthinellum* under surface culture conditions. On the other hand, 1.5% corn cobs gave the optimum production by *T. lanuginosus* YMN72 (428U/ml) (Table 3). This disagreed with the results with recorded by Katapodis *et al*. (2006) who reported that the optimum production by the thermophilic fungus *Sporotrichum thermophile* was obtained on fermentation medium containing 2.7% corn cobs as a carbon source.

The result revealed that the hydrolysis rate (total reducing sugars and saccharification percentage) of hemicelluloses of cane bagasse was much higher than other lignocellulosic substrates after 24 hrs of incubation with xylanase enzyme from *T. lanuginosus* A72 and *T. lanuginosus* YMN72. Other lignocelluloses revealed higher hydrolysis rate after 12 hrs of incubation with xylanase enzyme from *T. lanuginosus* A72 and YMN72 on wheat bran, corn cobs, sugar beet pulp and wheat straw, respectively. After an initial phase of rapid sugar formation, the rate of hydrolysis decreased. This could be due to enzyme inactivation or depletion of an easily hydrolysable fraction of hemicellulose (Chapla *et al*., 2010). Prasertsan and Oi, 2001) studied the hydrolysis rate of hemicelluloses extracted from palm cake wastes using the crude xylanase from *A. niger* after incubation time 4, 8, 16 and 24 hrs and found that maximum saccharification percentage was obtained after 8 hrs of incubation time giving 13.04%.

The initial pH of the medium has a great effect on the growth of the organism, on the permeability membrane, as well as on the biosynthesis and stability of the enzymes (Schoichi *et al.*, 1985; Prescott *et al*., 1999; Deacon, 2006).

Using citrate phosphate buffer, a range of pH from 5.0 – 7.8 were prepared and tested for production of cellulose-free xylanase. The results (Fig. 1) showed that the optimum pH value for both of the experimental fungi was 6.6 when incubated at 45°C for seven days of incubation. The maximum activity of xylanase was almost obtained with different microorganisms within narrow initial pH range around neutrality pH (5.0-7.0). These findings were confirmed by many authors e.g. Hoq *et al*. (1994); Gomes *et al*. (2000); El-Gindy (2002); Sonia *et al*. (2005); Katapodis and Christakopoulos (2008); and Khucharoenphaisan *et al*. (2009).

These results were similar with those reported by several investigators as the optimum pH for xylanase production at initial 6.5 for *T. lanuginosus* (Hoq *et al*., 1994; Puchart *et al*., 1999; Singh *et al*., 2000) and *Penicillium sclerotiorum* (Knob and Carmona, 2010). However, these results disagreed with the results of some investigators which recorded that optimum pH was 8.0 for *Aspergillus nidulans*. (Taneja *et al*., 2002) and 10.0 for *Aspergillus terreus* (Geweely *et al*., 2006).

Nitrogen sources are primary ingredients in growth media used for enzyme production by microorganisms (El-Shafei *et al*., 1990; and Bansod *et al*., 1993). The effect of different nitrogen sources was investigated under the best conditions for the production of xylanase (50ºC). Sodium nitrate (0.3%) was shown to be the best nitrogen source for *T. lanuginosus* A72 while ammonium nitrate (0.1 %) was the best nitrogen source for *T. lanuginosus* YMN72 (Figs.3 & 4). This was in agreement with the results recorded by Kalogeris *et al*. (1998) who studied the effect of different nitrogen sources on xylanase production by *Thermoascus aurantiacus* and reported that inorganic nitrogen sources showed maximum activities than the organic one. But this disagrees with Ali (2001) who reported that organic source (Asparagine) showed optimal xylanase production than the organic sources. Sodium nitrate (3.0%) was the best concentration of nitrogen source for *T. lanuginosus* A72. This agreed with Okafor *et al*. (2007) who found that a fermentation medium containing 0.3% $NaNO_3$ enhanced xylanase production by *Penicillium chrysogenum.* However, El-Gindy (2002) recorded that highest cellulase-free xylanase was obtained from cultures grown on medium containing 0.2% $NaNO_3$ by *Penicillium wortmannii.* On the other hand; ammonium nitrate (0.1%) was the best concentration of nitrogen source for *T. lanuginosus* YMN72. This disagrees with Tony *et al*. (2010) who recorded that optimum fermentation medium of xylanase production by *Aspergillus carneus* containing 3.0% NH_4NO_3 as nitrogen source. Many fungi utilize nitrates as nitrogen source forming ammonium salts through the action of nitrate and nitrite reductases (Deacon, 2006).

The effect of gamma radiation was investigated. Results (Figs 5-8) revealed that, the low gamma radiation doses of 1.0 KGy and 0.9 KGy for *T. lanuginosus* A72 and *T. lanuginosus* YMN72, respectively under surface culture conditions and by radiated fungal slants increased xylanase production, than the control value. Under surface culture conditions; the maximum xylanase production was obtained after exposing the fungal slants of selected strains of *T. lanuginosus* to dose levels 1.0 and 0.9 KGy giving 1082 and 1173 U/ml with increasing 179 and 121% as compared with the control value of *T. lanuginosus* A72 and *T. lanuginosus* YMN72, respectively. This could be attributed to the presence of an effective mutant due to the irradiation process (Ito and Nessa, 1996). In this connection, other workers have reported that the production of various fungal extracellular enzymes increased by mutating the tested fungi using the low ionizing gamma irradiation doses (Macris, 1983; Gunde-Cimerman *et al.,* 1985; El-Zawahry and Mostafa, 1991; Kumakura, 1993). Friedrich *et al*. (1982) found that, mutants results from gamma irradiated of *A. niger* produced much cellulolytic enzymes than wild type and the bioconversion of cellulosic waste substance was estimated. Macris (1983) concluded that, gamma irradiation induced mutants of *A. ustus* and *Trichoderma harzianum* could be grown faster than their wild types on cellulosic waste and produced high yield of cellulases as well as β-glucosidase. Also Shimokawa *et al*. (2007) studied the effect of gamma-ray irradiation on enzymatic hydrolysis of spent enokitake mushroom *Flammulina velutipe*s substrate containing corn cobs and rice bran as major components and found that the saccharifications rate of the spent substrate doubled with irradiation at a dose of 500 KGy.

The results of the present work showed that the maximum production of ethanol and xylitol were obtained after fermentation time 48 and 24 hrs of fermentation giving (22.48 and 13.54 g/l), respectively. Different results were obtained by different authors according to the yeast species, the type of lignocellulosic materials and the conditions of fermentation. (Latif and Rajoka, 2001) studied the production of ethanol and xylitol from enzyme broth of Chaetomium thermophile using *Saccharomyces cerevisiae* and *Candida tropicalis* by simultaneous saccharification and fermentation (SSF) using pretreated and dry corn cobs and found that a maximal ethanol concentration 23g/l from 200 g/l (w/v) dry corn cobs was obtained by *C. tropicalis* after 96 hrs of fermentation. While maximal xylitol

concentration of 21 g/l from 200 g/l (w/v) dry corn cobs was obtained by *C. tropicalis*.

B-Effect of partially purified xylanase on the saccharification of lignocellulosic substrates

This experiment was carried out to determine the hydrolysis rate of hemicelluloses extracted from lignocellulosic substrates including cane bagasse, corn cobs, wheat bran, wheat straw and sugar beet pulp using the xylanase enzyme from *T. lanuginosus* A72 and *T. lanuginosus* YMN72. Samples were taken at 12, 24, 36, 48, 60, 72, 84 and 96 hrs. of incubations at 50 ºC. Their filtrates were used for reducing sugars determination and saccharification percentage . The results (Tables 1 and 2) revealed that the hydrolysis rate of hemicelluloses from cane bagasse was much higher than other lignocellulosic substrates after 24 hrs of incubation with xylanase enzyme from *T. lanuginosus* A72 and *T. lanuginosus* YMN72 giving (7.380 mg/ml, 13.10% and 9.031mg/ml and 16.26%) of total reducing sugars and saccharification percentage, respectively.

Other lignocelluloses revealed higher hydrolysis rate after 12 hrs of incubation with xylanase enzyme from *T. lanuginosus* A72 on wheat bran, corn cobs, sugar beet pulp and wheat straw giving (3.824, 3.209, 2.376, 2.136 mg/ml) and (6.88, 5.78, 4.28, 3.84%) of total reducing sugars and saccharification percentage, respectively.

C-Production of ethanol and xylitol from xylanase broth of *Thermomyces lanuginosus* YMN72 by *Candida tropicalis* EMCC2

This experiment was carried out to determine the production rate of ethanol and xylitol from xylanase broth of *T. lanuginosus* YMN72 using *Candida tropicalis* EMCC2.

The results (Table 6) showed that the maximum production of ethanol and xylitol were obtained after fermentation time 48 and 24 hrs giving (22.48 and 13.54 g/l), respectively followed by decrease in ethanol and xylitol production with increase of fermentation time.

For treatment with xylanase enzyme from *T. lanuginosus* YMN72; higher hydrolysis rate was obtained after 12 h of incubation on wheat bran, corn cobs, sugar beet pulp and wheat straw giving (3.025, 2.809, 1.994, 1.702 mg/ml) and (5.45, 5.10, 3.59, 3.06%) of total reducing sugars and saccharification percentage, respectively.

Table (1): Effect of different natural lignocelluloses on the production of cellulase-free xylanase by the isolated fungi

Fungi / Substrates**	*Thermomyces lanuginosus* A72		*Thermomyces lanuginosus* H72		*Thermomyces lanuginosus* U72		*Thermomyces lanuginosus* YMN72	
	Protein mg/ml	**Xylanase activity U/ml**	**Protein mg/ml**	**Xylanase activity U/ml**	**Protein mg/ml**	**Xylanase activity U/ml**	**Protein mg/ml**	**Xylanase activity U/ml**
Cane bagasse *	**0.648**	**411**	0.607	211	0.588	102	0.686	113
Corn cobs*	0.499	152	0.495	111	0.461	316	**0.486**	**337**
Sugar beet pulp*	**1.084**	**309**	1.009	395	1.032	61	1.094	188
Wheat straw*	0.785	304	0.813	263	0.709	125	**0.834**	**279**

* Control (Modified Czapek's medium without substrate): 0.136 mg/ml protein and 125 U/ml Xylanase activity**.** **1.0% (W/V) of substrate.

Table (2): Effect of different concentrations of the best natural lignocelluloses on the production of Cellulase-free Xylanase by *Thermomyces lanuginosus* A72.

Substrate	Concentration of the substrate (%)							
	0.5		**1.0**		**1.5**		**2.0**	
	Protein mg/ml	**Xylanase activity U/ml**	**Protein mg/ ml**	**Xylanase activity U/ml**	**Protein mg/ml**	**Xylanase activity U/ml**	**Protein mg/ml**	**Xylanase activity U/ml**
Cane bagasse	0.298	194	**0.648**	**411**	0.678	314	0.809	230
Sugar beet pulp	0.430	25	1.084	309	1.140	362	1.319	215

* Control (Modified Czapek's medium without substrate): 0.136 mg/ml protein and 125 U/ml Xylanase activity.

Table (3): Effect of different concentrations of the best natural lignocelluloses on the production of Cellulase-free Xylanase by *Thermomyces lanuginosus* YMN72.

Substrate	Concentration of the substrate (%)							
	0.5		1.0		1.5		2.0	
	Protein mg/ml	Xylanase activity U/ml	Protein mg/ ml	Xylanase activity U/ml	Protein mg/ml	Xylanase activity U/ml	Protein mg/ml	Xylanase activity U/ml
Corn cobs	0.208	344	0.486	337	**0.460**	**428**	0.550	239
Wheat straw	0.313	168	0.834	279	0.862	381	1.132	159

* Control (Modified Czapek's medium without substrate): 0.142 mg/ml protein and 285 U/ml Xylanase activity**.** **1.0% (W/V) of substrate.

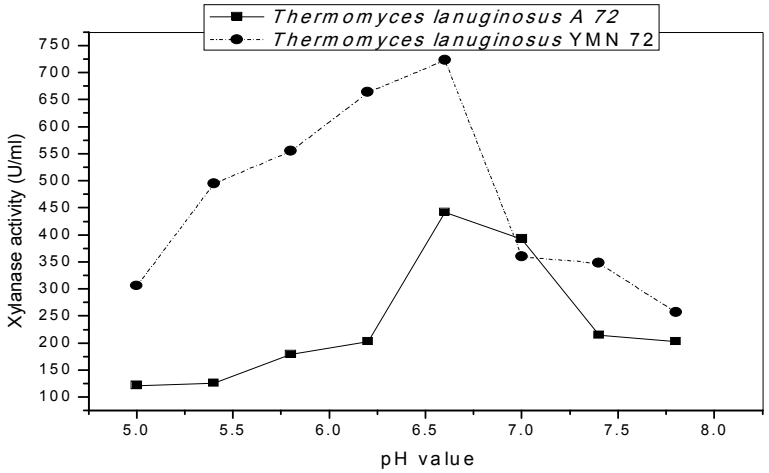

Fig. 1: Effect of pH values on the production of Cellulase-free Xylanase by *Thermomyces lanuginosus* A 72 and *Thermomyces lanuginosus* YMN72.

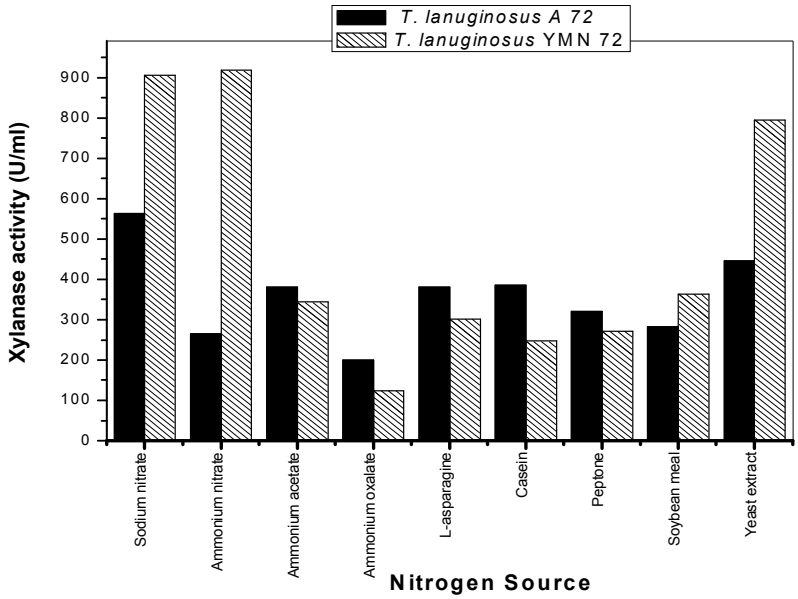

Fig. 2 : Effect of different nitrogen sources on the production of Cellulase-free Xylanase by *Thermomyces lanuginosus* A72 and *T. lanuginosus* YMN 72

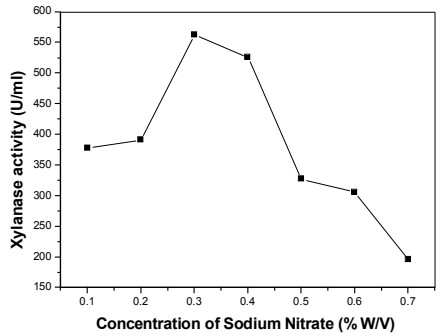

Fig. (3): Effect of different concentrations of sodium nitrate on the production of Cellulase-free Xylanase by *Thermomyces lanuginosus* A72.

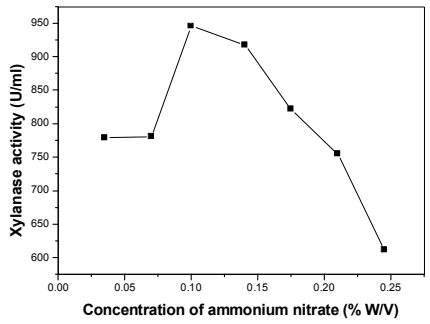

Fig. (4): Effect of different concentrations of Ammonium nitrate on the production of Cellulase-free Xylanase by *Thermomyces lanuginosus* YMN72.

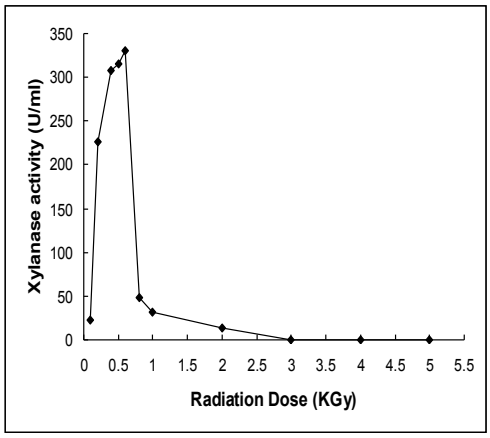

Fig. (5): Effect of gamma radiation on the production of Cellulase-free Xylanase by radiated spore suspension of *Thermomyces lanuginosus* A72.

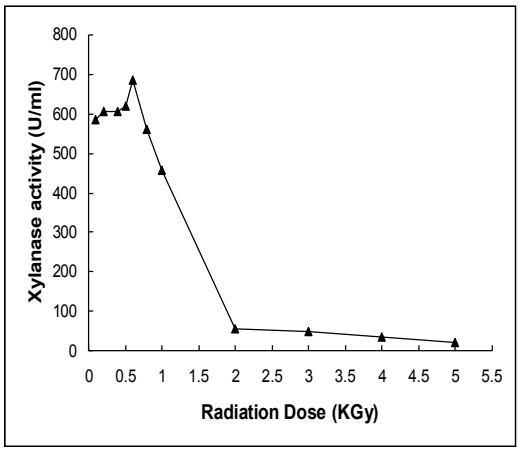

Fig. (6): Effect of gamma radiation on the Cellulase-free Xylanase by radiated spore suspension of *Thermomyces lanuginosus* YMN72.

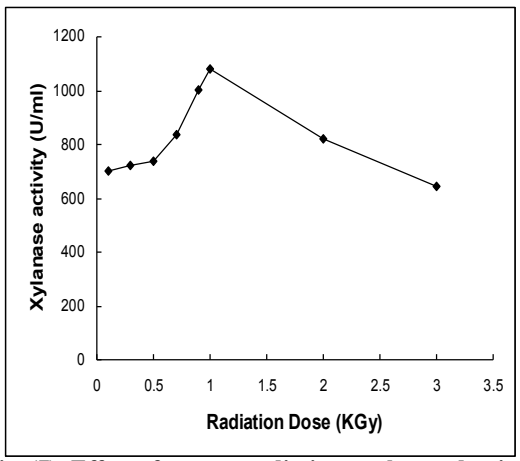

Fig. (7): Effect of gamma radiation on the production of Cellulase-free Xylanase by radiated fungal slants of *Thermomyces lanuginosus* A72.

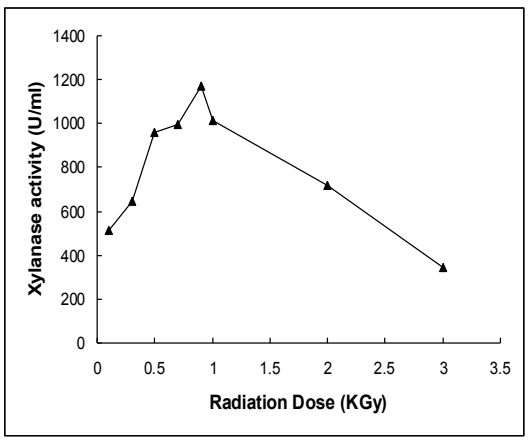

Fig. (8): Effect of gamma radiation on the production of Cellulase-free Xylanase by radiated fungal slants of *Thermomyces lanuginosus* YMN72.

Table (4): Determination of total reducing sugars extracted from enzymatic saccharification of lignocellulosic substrate by cellulase-free xylanase of *T. lanuginosus* A72(1) and *T. lanuginosus* YMN72(2)

Incubation Time (h)	Total Reducing Sugars (mg/ml)									
	CB		CC		WB		WS		SBP	
	1	2	1	2	1	2	1	2	1	2
12	3.956	3.406	3.209	2.809	3.824	3.025	2.136	1.702	2.376	1.994
24	7.380	9.031	2.272	2.154	1.809	2.689	1.751	1.651	2.180	1.634
36	4.982	2.994	0.976	1.171	1.400	2.000	1.589	1.351	1.791	1.580
48	4.918	2.920	0.951	1.143	1.304	1.917	1.071	1.137	1.320	1.497
60	4.666	2.686	0.940	1.131	0.900	1.637	1.036	1.069	1.040	1.480
72	4.456	1.934	0.904	1.126	0.829	1.471	1.031	0.997	0.909	1.466
84	4.312	1.891	0.880	1.072	0.624	1.214	0.929	0.914	0.869	1.429
96	4.060	1.451	0.869	0.977	0.491	1.171	0.896	0.894	0.869	1.286

1 = *Thermomyces lanuginosus* A72 2 = *T. lanuginosus* YMN72 CB: Cane bagasse
CC: Corn Cobs WB: Wheat Bran WS: Wheat Straw SBP: Sugar Beet Pulp

Table (5): Enzymatic saccharification percentage of lignocellulosic substrates by cellulase-free xylanase of *T. lanuginosus* A72 (1) and *T. lanuginosus* YMN72(2)

Incubation Time (h)	Saccharification percentage (%)									
	CB		CC		WB		WS		SBP	
	1	2	1	2	1	2	1	2	1	2
12	7.12	6.13	5.78	5.10	6.88	5.45	3.84	3.06	4.28	3.59
24	13.10	16.26	4.10	3.88	3.26	4.84	3.15	2.97	3.92	2.94
36	8.97	5.39	1.76	2.11	2.52	3.60	2.86	2.43	3.22	2.84
48	8.85	5.26	1.71	2.10	2.35	3.45	1.93	2.05	2.38	2.69
60	8.40	4.83	1.69	2.03	1.62	2.95	1.86	1.92	1.87	2.66
72	8.02	3.48	1.63	2.02	1.49	2.65	1.85	1.79	1.64	2.64
84	7.76	3.40	1.58	1.93	1.12	2.19	1.67	1.65	1.56	2.57
96	7.31	2.61	1.56	1.76	0.88	2.10	1.61	1.61	1.56	2.31

1 = *Thermomyces lanuginosus* A72 2 = *T. lanuginosus* YMN72 CB: Cane bagasse
CC: Corn Cobs WB: Wheat Bran WS: Wheat Straw SBP: Sugar Beet Pulp

Table (6): Ethanol and xylitol productivity from the fermentation of xylanase broth of *T. lanuginosus* YMN72 using *Candida tropicalis* EMCC2.

Fermentation Time (h)	Ethanol productivity (g/l)	Xylitol Productivity (g/l)
24	4.45	**22.48**
48	**13.54**	16.04
96	4.97	9.021

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