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Influence of Gamma-rays and Some Cultural Conditions on the Enhancement of Cellulase Production by Some Fungal Strains Isolated from Cellulosic Wastes

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ABSTRACT

In the present study, out of 51 fungal strains isolated from the cellulosic wastes, only 19 were CMCase-producers. *Aspergillus, Fusarium* and *Penicillium* were the most common fungal genera isolated from the cellulosic wastes. *Fusarium neoceras, Aspergillus fumigatus* and *Fusarium oxysporium* produced CMCase activity than *Trichoderma viride.* Out of 23 gamma-irradiated survivors from *A.fumigatus* and *F. neoceras* showing CMCase production, only two mutant strains *A.fumigatus* 8G-2 and *F. neoceras* 4G-2 produced the highest levels of CMCase than the parent strains. The results indicated that the maximum level of of CMCase activity was produced by *A.fumigatus* and *F. neoceras* strains under optiminizing conditions

Key words: Cellulosic wastes, Cellulase, Enzymes, Biodegradation, Fermentation, Moulds, Carboxymethyl cellulose, Carboxy- methyl cellulase and Mutation .

INTRODUCTION

Cellulose is the most abundant and renewable biopolymer. Cellulase is an inducible enzyme system [(1)]. All microorganisms studied so far have produced cellulase when grown on cellulose [(2-4)]. Cellulolytic enzymes are produced by a wide variety of bacteria and fungi, aerobes and anaerobes, mesophiles and thermophiles [(5)].

However, relatively few fungi and bacteria produce high levels of exteracellular cellulase capable of solubilizing crystalline cellulose extensively. So, for most of the studies have been on cellulase system of aerobic fungi as *Trichoderma viride, T. reesei, Penicillium pinophilum, Sporotrichum pulverulentum, Fusarium spp., Aspergillus spp., Talaromyces emersonii* and *T. koningii* [(6-11)].

Cellulase is a multicomponent enzyme complex containing three types of enzymes, the synergistic actions of which bring about the degradation of cellulose to glucose [(12, 13)].

The rate of cellulase production is influenced by environmental conditions, components of nutrient medium which might act as inducers or repressors cell density and growth rate [(4, 7)]. The effect of gamma irradiation on the sensitivity and production of cellulase by different fungal isolates was investigated [(14)].

The present study aim to isolate and identify fungal cellulase producers from different

cellulosic wastes and to study the effect of gamma irradiaton on the production of mutants higher than parent strains for the production of CMCase.

MATERIALS AND METHODS

Microorganisms

The fungal strains used in the present study were isolated from cellulosic wastes (bagasse, rice straw, wheat straw, and potato peels). Samples were collected from the upper Egypt governorates.

A standard strain of *Trichoderma viride* known to produce cellulase was kindly provided by MERCIN, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Isolation and identification of fungal isolates from cellulosic wastes

The cellulosic wastes, of 10 g were transferred to aliquots of 90 ml sterile saline solution (0.85 % NaCl) in 250 ml flasks then were shaken vigorously at constant speed (300 rpm). The cellulosic waste suspension was then subjected to serial dilutions (10 fold dilution) and 0.1 ml of the dilution from 10^4 to 10^7 was spread on the surface of Potato Dextrose Agar (PDA) plates. The plates were incubated for 5 days at 25^{o}C. All plates were examined daily and the developing colonies were picked up and subcultured on five PDA slants. Fungal isolates were identified according to [15].

Screening of the cellulolytic fungal isolates on synthetic medium

The minimal medium (100 ml) [16] supplemented with 1% commercial cellulose, pure cellulose, strips of filter paper Whatman No.1 and carboxymethyl cellulose (CMC) was dispensed in 250 ml conical flasks. The flasks were autoclaved at 120^{o}C for 15 min and inoculated with 4 ml spore suspension (5 x 10^6 spores / ml medium) from the selected fungal isolates and the references standard *T.viride.* The inoculated flasks were incubated at 28^{o}C as stationary cultures for 7 to 10 days.

Mycelial dry weight and CMCase were determined for each fungal isolate and *T. viride.* The best grown fungal isolates were identified as described by [15].

Preparation of spore suspension

The cultures of the selected fungal strains were inoculated on plates of PDA medium and incubated for 7 days at 28^{o}C.

Discs of 9mm diameter, from 7 day old cultures were used to inoculate five flasks (250 ml) for each organism containing 100 ml Sabouraud-dextrose agar medium. The flasks were incubated for 7 days at 28^{o}C. The spores were collected by adding 30 ml saline solution supplemented with 0.1 %. Tween 80 and the flasks were shaken slightly for 15 min, then the spores were collected by centrifugation at 500 rpm for 15 min. The pellet of spores after washing three times with saline solution, was resuspended in 5 ml saline solution and 4 ml of the spore suspension (10^6 spores / ml) were used for further investigation.

Irradiation conditions.

The Indian chamber of Cobalt-60 located at National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt was used for the irradiation treatments. The dose rate was 198 Gy/min at the time of experiment at room temperature. Heavy spore suspensions concentration (5 ml) from the selected fungal isolates were distributed into clean, dry and sterile screw capped tubes. The tubes were exposed to increasing doses of 0, 1, 2, 3, 4, 5 and 6 kGy. The un-

irradiated and irradiated samples of spore suspension (3 tubes/dose) were subjected to serial dilutions (10- fold) and the appropriate dilutions were plated (0.1 ml) on the surface of Sabouraud agar plates in duplicate. The plates were incubated for 7 days at 28°C and the count of the C.F.U were recorded. The selected colonies from different doses were subcultures on PDA slants. Spore suspension of all purified irradiated isolales were prepared as described before. Each spore suspension was used (4 ml) to inoculate 100 ml of the minimal medium [16] supplemented with 1% carboxymethyl cellulose.

Factors affecting CMCase production

Mycelial dry weight and CMCase activity were determined. The most producing CMCase of the tested strains (mutants) were recorded and used for further investigation.

Influence of initial pH of medium

The minimal medium (100 ml) supplemented with 1% CMC (Sigma) was dispensed in 250 ml Erlenmeyer flasks. The initial pH was adjusted with 0.1 N-NaOH or 0.1N-HCl to different values ranging from 2 to 8. The flasks were autoclaved at 121°C for 15 min. Each flask was inoculated with a spore suspension to give a final concentration of $5x10^6$ml of the medium and incubated at 25°C for 7 days.

Influence of shake or stationary cultures

The minimal medium was inoculated with the selected strains and incubated at 28°C as stationary or shake cultures. The flasks were analysed daily for 7 days.

Effect of incubation cultures

The minimal medium supplemented with 1% CMC, was inoculated with selected strains, then incubated at 28°C for 1, 2, 3, 5, 7 and 9 days. At the end of each incubation period, mycelial dry weight and CMCase were determined.

Influence of nitrogen sources

The minimal medium (100 ml) supplemented with 1% CMC and with 0.1% of nitrogen sources of ammonium sulphate, sodium nitrate, lead nitrate, magnesium nitrate, ammonium chloride, potassium nitrate and ammonium nitrate, was dispensed in 250 ml conical flasks, sterilized by autoclaving. Each flask was inoculated with 4 ml of the spore suspension of each strain and incubated at 28°C for 7 days as stationary cultures. Three replicates were used for each of the seven nitrogen sources of the tested fungal mutants and their parents.

Effect of carbon sources

Carboxymethyl cellulose (CMC) in the minimal medium was replaced by 1% glucose, maltose mannitol or sucrose as a sole source of carbon. Sugars were sterilized by Seitz filtration before adding to the autoclaved medium. The inoculated flasks were incubated at 28°C for 7 days as stationary cultures.

Dry weight determination

The mycelial growth were collected by porcelin funnel then were transferred to dried and preweighed filter paper Whatman No. 1 and washed several times with distilled water, and were dried at 60°C to a constant weight.

Enzyme determination

Carboxylmethyl cellulase (CMCase) was estimated as described by [17] using CMC as substrate. In a clean dry tube an aliquot of 1 ml of the supernatant was mixed with 1 ml of 0.5% CMC in acetate buffer and incubated at 63°C for 30 min and the absorbance was measured at 450 nm. CMCase in the culture filtrate was determined by using dinitrosalysilic acid reagent [18].

Protein determination

According to [19], 10ml of the filtrated culture was centrifuged at 5000 rpm for 10 min. One ml of the supernatant was used to determine extracellular protein, and 5ml of reaction mixture in a clean dry tube was added, then kept at room temperature for 10 min. 0.5 ml of folin reagent (Fluka), was added to the previous mixture. The tubes were leaved for 20min. at room temperature, the absorbance was measured at 750 nm by using Unicam.

RESULTS AND DISCUSSION

Isolation and identification of cellulase-fungal producers from the cellulosic wastes

Table 1 shows that twenty fungal isolates were recovered from baggase samples, twelve isolates were collected from each of rice straw and wheat straw samples and only seven isolates were selected from potato peel samples.

Also, from the table it is clear that *Aspergillus* (23 isolates), *Fusarium* (17 isolates) and *Penicillium* (11 isolates) were the most common fungal genera isolated from the different cellulosic wastes. The data recorded in Table-1 revealed that out of the 51 fungal isolates, 19 isolates were able to grow on the commercial and pure cellulose and CMC. The data showed that these fungal isolates were belonging to the three genera namely: *Aspergillus* (10 isolates), *Fusarium* (6 isolates) and *Penicillium* (3 isolates). Cellulase is a complex mixture of enzyme proteins with different specificities hydrolyze glycosidic bonds [20]. The major single protein is an exocellulase which amounts to 40-70% of the total cellulase protein and the enzyme is necessary for the hydrolysis of crystalline cellulose to glucose [21]. This enzyme activity is therefore called carboxymethyl cellulase activity [22].

From the results, it is obvious that media supplemented with CMC as a sole carbon source gave higher growth for the tested fungal strains than that observed when pure cellulose used.

In the present investigation (Table-2) the highest breakdown of the CMC in the medium was reported by the fungal isolates No. 2, 10 and 34 as compared with other fungal isolates. [23] revealed that in high crystalline substrate, the closely packed hydrogen bonded cellulose may be less accessible to cellulase attack than the loosly organized amorphous cellulose.

The data recorded in Table-3 revealed that the fungal isolates No. 2, 10 and 34 had the highest CMCase activity of 690, 185 and 280 µg/ml, respectively which were higher than that estimated for *T.viride* (35 µg/ml). These fungal isolates were identified as *Fusarium neoceras* (isolate No. 2), *Aspergillus fumigatus* (isolate No. 10) and *Fusarium oxysporium* (isolate No. 34). **El-Zawahry *et al.* (1987)** [24] isolated 31 different cellulolytic strains from cellulosic wastes of corn grains, peeled peanut seeds, cotton seed cakes, wheat grains and pea seeds and the authors cleared that all fungal isolates were belonging to the genera *Aspergillus* (20 isolates) *Paecilomyces* (6 isolates), *Fusarium* (3 isolates) *Penicillium* (one isolate) and *Trichoderma* (one isolate). The production of cellulase have been also reported for members of the genus *Aspergillus* as *A. niger, A. terreus* and *A. chevalieri* [25, 26], *Fusarium oxysporium* [27] and *Penicillium steckii* [28].

Effect of some cultural conditions on CMCase activity by *T.viride* and the selected fungal species.

In the present study, (Fig-1), it is clear that *A. fumigatus*, *F. oxysporum* and *F. neoceras* produced the highest values of CMCase activity as compared with *T. viride* after 7 days of incubation. Also, in the present investigation (Fig-2), the maximum CMCase activity in the culture filtrates of *A. fumigatus* (410 µg/ml) and *F. oxysporum* (680 µg/ml) was recorded at pH 4.0 whereas for *F. neoceras* (850 µg/ml) was at pH 5.0. It is clear that all the selected fungal isolates had the highest CMCase activity than *T. viride* (62 µg/ml, pH 5.0). Among carbon sources tested in this study (Fig-3), the highest CMCase activity was recorded with CMC (185 µg/ml) followed by glucose (130 µg/ml) and mannitol (65 µg/ml) for *A. fumigatus.* On the other hand, the highest CMCase activities were recorded with sucrose (950 µg/ml) followed by CMC (380 µg/ml) for *F. oxysporum*, whereas for *F. neoceras,* the highest CMCase activity was recorded with CMC (690 µg/ml) followed by glucose (260 µg/ml) and sucrose (170 µg/ml). From Fig-3, it was noticed that the highest CMCase activity was recorded with sucrose (375 µg/ml) followed by glucose (190 µg/ml) for *T. viride.* Fig-4, shows that the extracellular cellulase accumulated at the highest rates in the media containing the different nitrogen sources for *A. fumigatus*, *F. oxysporum* and *F. neoceras* as compared with *T. viride*. The present data are in agreement with that recorded by [7] who revealed that the rate of production of cellulase, an inducible extracellular enzyme, is greatly influenced by the nutrient medium composition and by several factors such as : type and concentration of cellulosic substrates, organic and inorganic nitrogen sources macro and trace elements and fermentation conditions e.g. age, types and amount of inoculum, pH, temperature aeration and stirring. Also, **(Steiner *et al.*, 1987 and Kvachadze and Yashvili, 1996)** [29, 30] who proved that carbon, nitrogen and phosphorus sources and other cultivation conditions influenced greatly the biosynthesis of cellulases by different fungal isolates.

Effect of gamma irradiation on the sensitivity and cellulase production by the fungal isolates

In the present work the selected fungal isolates were exposed to different dose levels of gamma irradiation for the enhancement of the enzyme activity.

Twenty three selected colonies of *A. fumigatus* and *F. neoceras* had been selected depending on the morphological changes i.e: growth diameter, colour, pigmentation and margin of the colonies as compared with parent strain.

As shown from Table-4, among 11selected mutant strains of *A. fumigatus* producing CMCase, one strain 8G-2, which recovered after the exposure of the parent strain to dose level 5.0 kGy, produced significant CMCase activity 925 µg/ml with mycelial dry weight of 1.51 g/L as compared with the parent strain (465 µg /ml and 1.73 g/1, respectively). In addition, the data in Table-4 show that out of 12 selected *F.neoceras* isolates, 7 strains produced significant CMCase activity than the parent strain. The data decleared that strain 4G-2, which selected after the exposure of the parent isolate to dose level 2.0 kGy, produced the highest level of C'MCase 1150 µg/ml as compared with the other mutants. It is clear from Table-4, that all the mutant had lower mycelial dry weight which ranged from 0.60 to 0.74 g/L as compared with the parent strain (1.97 g/l).

Furthermore, from Table-5, it is obvious that the selected mutant after exposure of *F. oxysporium*to gamma radiation showed lower CMCase activity than the parent one.

Sadana *et al.* (1979 and 1980) [31, 32] succeeded to select a mutant of *Solerotion rolfsii,* using UV irradiation secreated about two times more FPase activity and high levels of cellulase production

than parent strain. Further more, **EI-Zawahry and Mostafa (1983)**[33] recorded that the cellulase produced by irradiated *T. viride* isolates at 0.2, 0.4 and 0.8 kGy was about 1.3 to 1.9 fold of that produced by the parent strain. In the present investigation (Table-3), it is clear that the mutant strains *A. fumigatus* 8G-2 and *F. neoceras* 4G-2 produced CMCase by about 2.0 and 1.6 fold, respectively of that produced by the parent strain.

Sandhu and Kalra (1985) [30] indicated that there was no correlation between the myeclial dry weight and enzyme activity, that production of maximum cell mass may not accompanied by maximum cellulase yield. The effect of ionizing radiation as mutagenic agent on living cells are described by the summation of direct effect of radiation on the vital centers in the cells plus the indirect effects caused by chemical changes outside there centers [35].

The lethal effect of gamma radiation may be explained on the bases that gamma radiation induces DNA-damage single or double strand breaks, affects protein fingerprinting and enzymes and disrupter of protein-DNA complex so affecting gene expression [36]. Use of mutagens in the improvement of organic acids and cellulase production by microorganism from raw agro-industrial wastes was investigated [8, 10, 14, 37, 38 and 39]

4. Effect of cultural conditions on production of CMCase by *A. fumigatus* 8G-2 and *F.* neoceras 4G-2.

Effect of initial pH values

Fig.5 shows that CMCase reached its highest level for the mutant strain *F. neoceras* 4G-2 (1100 µg/ml) at pH 4.0 and at pH 5.0 for the mutant *A. fumigatus* G80-2 (450 µg/ml). From the present investigation (Fig. 5), it is clear that CMCase produced by the mutant strain *A. fumigatus* 8G-2 at pH 5.0 was about 4.0 fold of that produced by the parent strain, whereas the CMCase produced by the umlaut strain *F. neoceras* 4G-2 at pH 4.0 was about 2.2 fold of that produced by the parent strain. In the present study, it is clear that CMCase activity (Fig. 5) and growth rate (Fig-6) for the fungal isolates sharply decreased especially above pH 6.0 for both the parent and the mutant strains. The data show that the maximum mycelial dry weight for the parent and the mutant strain *A. fumigatus* 8G-2 was at pH 6.0, whereas for the parent and the mutant strain *F. neoceras* 4G-2 was at pH 5.0 and by increasing the pH values there was a significant decrease of the mycelial dry weight up to pH 8.0. **Sanahu and Kalra (1985)** [34], revealed that growth and cellulase production by *T. longibrachiatum* was markedly inhibited at pH lower than 4.0 and higher than 6.0, and the authors concluded that, this pH range has also been reported as the most favorable hydrogen ion concentration for cellulolytic enzyme synthesis in several fungal isolates. In a previous study, **EI-Zawahry *et al.* (1987)** [24] investigated that cellulase enzymes and mycelial dry weight produced by *T. knoningii* and *A. niger* were obtained at pH 5.0 and the cellulase production sharply decreased below pH 4.0 or about pH 5.0. The present data was found to be agreement with those obtained for CMCase production at pH from 4.0 to 6.0 by *T. viride, T. pseudokoningii, T. harinaum, A. terreus* and *A. fumigatus* by **Gomaa (1993),** [25] **and Fadel and Abd-El-Kader (1997)** [26] who investigated that the optimum pH for maximum cellulase activity varied with substrate type and also depending on the source of the enzyme.

Effect of shake and stationary culture

Table-6 shows that under stationary culture, the CMCase produced by the mutant strain *A.*

fumigatus 8G-2 was about 4.8 fold of that produced by the parent strain, wherease, the CMCase produced by the mutant strain *F. neoceras* 4G-2 was about 1.2 fold of that produced by the parent strain. In addition the data recorded in Table-6 revealed that the CMCase produced by the mutant strains *A. fumigatus* 8G-2 and *F. neoceras* 4G-2 was about 4.5 and 1.9 fold, respectively under stationary culture than in shake culture. In the present investigation (Table-6), it is clear that CMCase activity of the parent and the mutant fungal isolates produced by shaked flask culture was low compared with stationary culture, this may be due to inactivation of the specific enzymes as reported by [34]. In a previous study, **Wase *et al.* (1985)** [36] revealed that agitation, had a negative effect on fungal growth and cellulase production and the authors concluded that agitation may involve some rupturing of the mycelium liberating intercellular content and preventing cellulase production. Also, the present data are in agreement with that recorded by **LeJeune and Baron (1995)** [10] who investigated that the cellulase production was strongly affect by agitation where at the higher agitation rate (400 rpm) almost no enzymes are produced at this highest rate.

Effect of incubation period

Fig-7 shows that all the selected parent and the mutant strains showed the highest level of CMCase after 7 days of incubation at 28^{o}C. In addition, the data show that the CMCase produced by the mutant strain *A. fumigatus* 8G-2 (225 µg/ml) was 1.2 fold greater than that of the parent strain (185 µg/ml) and *F. neoceras* 4G-2 (920 µg/ml) was 1.3 fold than that produced by the parent (690 µg/ml). Also, the present data (Fig-7) shows that the CMCase produced by the mutant strain *F. neoceras* 4G-2 was about 4.1 fold of that produced by the mutant strain *A. fumigatus* 8G-2 after 7 days of incubation. In a previous study, **Fadel and Abd EL-Kader (1994)** [26] investigated the highest levels of both Fpase and β-glucosidase produced by *A. niger* F92 after 9 days incubation, while it is was after 8 days incubation for CMCasc. It was reported by, **Desai *et al.* (1982)** [41] that Fpase and CMCase were obtained after 10 days, while β-glucosidase was produced after 18 days incubation by *Scytalidium lignicola*, also cellulase was obtained after 10 days incubation by *T. reesei* [42]. In The present study (Fig-8), it is clear that there was a significant increase in the mycelial dry weight by increasing the incubation period for the parent and mutant strains and the maximum growth rate was at 9 days of incubation. **El-Zawahry *et al*, (1987)** [24] revealed that the highest cellulases and growth of *T. koningii* and *A. niger* were obtained after 15 days of incubation, while **Mandles *et al*, (1971)** [43] **and Gallo *et al.* (1978)** [44] reported that 14 days gave maximum production of cellulases by*T. birdie* and *T. reesei.*

Effect of nitrogen sources

In the present investigation (Table-7), it is clear that all nitrogen sources stimulated the growth of the mutant strains than parent strains, the maximum value for the mycelial dry weight was obtained when sodium nitrate and lead nitrate was used as nitrogen sources for the mutant *A. fumigatus* 8G-2 and ammonium nitrate, sodium nitrate, ammonium chloride and lead nitrate were used as nitrogen sources for *F. neoceras* 4G-2. **El-Zawahry *et al.* (1987)** [24] investigated that the addition of peptone, NH_4NO_3, $(KH_4)_2$ SO_4, NH_4Cl and $NaNO_3$ as a source of nitrogen to the basal growth medium gave the best results in the production of cellulases in addition to dry weight for *A. niger* and *T. koningii*. On the other hand, **Fadel and Abd El-Kader (1994)** [26] revealed that cellulase was affected greatly by nitrogen source in the culture medium of *A .niger* F-92, as CMCase, Fpase and β-glucosidase were stimulated by some organic or inorganic sources, i.e.: $(NH_4)_2$ SO_4, $HN_4H_2PO_4$, NH_4CL and soybean flour. These observations are in good agreement with the data of the present study (Table-7) that the nature of the nitrogen source markedly affected cellulase production by

microorganisms. Our present data were also discussed by several investigators [7, 25, 45] who revealed that the types and the initial concentrations of nitrogen sources had a great effect on the production of cellulases.

Effect of carbon sources

Table-8 shows the effect of different carbon sources on both the growth and CMCase activity. It is clear that the CMCase produced by the mutant strain *A. fumigatus* 8G-2 was about l.4, 1.2 and 2.4 fold compared with that produced by the parent strain in the presence of glucose, maltose, and mannitol, as a carbon source, respectively Also, from this table, it is clear that maltose stimulated the production of CMCase by the mutant strain *F. neoceras* 4G-2 by about 1.2 fold of that produced by the parent strain. In addition Table-8 shows that sucrose gave markedly low CMCase for the mutant strain *A. fumigatus* 8G-2 (45 µg/ml), whereas mannitol gave low CMCasc for the mutant strain *F. neoceras* 4G-2 (55 µg/ml). Also, Table-8 indicates that glucose and sucrose stimulated the production of CMCase by the mutant strain *F.neiceras* 4G-2 by about 1.4 and 4.2 fold, respectively of that produced by the mutant strain *A. fumigatus* 8G-2, whereas mannitol stimulated the production of CMCase by the mutant strain *A. fumigatus* 8G-2 by about 2.8 fold than that produced by *F. neoceras* 4G-2. In a previous study, **EL-Zawahry *et al.* (1987)** [24] reported that the highest cellulase produced by *T.koningii* and *A. niger* were obtained in the presence of CMC-NA salt in the basal medium as a source of carbon, whereas starch, cellubioase, sucrose, fructose and glucose gave markedly low yield. The present data are in agreement with that recorded by several investigators [14, 46] who indicated the great role of the carbon sources as inducer for CMCase and the other cellulases by a wid varity of fungal isolates as*A. niger, A. terreus, F. oxysporium, T. longibrachiatum, T. reesei, T. pseudokoningii, A. chevelieri* and *P. steckii.*

Table (1): Fungal genera isolated from the different cellulosic wastes.

Fungal genera	*Baggase*		*Rice Straw*		*Wheat Straw*		*Potato Peel*	
	No. of isolates tested	*No. of +ve isolates*	*No. of isolates tested*	*No. of +ve isolates*	*No. of isolates tested*	*No. of +ve isolates*	*No. of isolates tested*	*No. of +ve isolates*
Aspergillus	***8***	***6***	***6***	***2***	***5***	***1***	***4***	***1***
Fusarium	***7***	***3***	***4***	***2***	***5***	***1***	***1***	***0***
Penicillium	***5***	***2***	***2***	***1***	***2***	***0***	***2***	***0***
Total	***20***	***11 (55%)***	***12***	***5 (41.7%)***	***12***	***2 (16.7%)***	***7***	***1 (14.3%)***

Positive fungal genera were able to grow on pure and commercial cellulose and CMC for 7 days at 28^{o}C
-51 total fungal isolates testes.
-No. of total +ve fungal isolates 19 (37.25%) isolates.

Table (2): Total protein, extracellular protein, reducing sugars and CMCase activity of *T. viride* and the other selected fungal isolates.

Isolates No.	Fungal isolates identified	Total protein mg/ml	Extra- cellular protein ug/ml	Reducing sugars ug/ml	CMCase activity ug/ml
Standard	***Trichoderma viride***	41.3	132.0	300.0	35.0
10	*Aspergillus sp.*	43.2	185.0	550.0	185.0
11	*Aspergillus sp.*	38.4	166.0	200.0	120.0
15	*Aspergillus sp.*	45.6	187.0	270.0	100.0
16	*Aspergillus sp.*	32.6	185.0	370.0	170.0
24	*Aspergillus sp.*	39.2	188.0	340.0	140.0
30	*Aspergillus sp.*	38.5	166.0	474.0	170.0
37	*Aspergillus sp.*	35.0	184.0	300.0	120.0
39	*Aspergillus sp.*	33.2	130.0	375.0	140.0
45	*Aspergillus sp.*	32.0	184.0	350.0	150.0
51	*Aspergillus sp.*	48.0	120.0	320.0	120.0
2	*Fusarium sp.*	38.4	240.0	480.0	690.0
3	*Fusarium sp.*	40.8	181.0	520.0	120.0
25	*Fusarium sp.*	44.8	140.0	450.0	150.0
32	*Fusarium sp.*	44.6	210.0	380.0	130.0
34	*Fusarium sp.*	45.6	170.0	470.0	280.0
38	*Fusarium sp.*	34.0	180.0	330.0	130.0
31	*Penicillium sp.*	45.4	186.0	525.0	125.0
33	*Penicillium sp.*	45.6	135.0	270.0	140.0
43	*Penicillium sp.*	31.8	215.0	290.0	90.0

* Values are mean of three replicates.

Table (3): Growth of *T. viride* and the identified fungal species on filter paper, commercial cellulose, pure cellulose and CMC as well as CMCase activity.

Isolates No.	Fungal species identified	Growth on filter paper	Mycelial dry weight (g/l) *			CMCase activity (µg/ml)
			Commercial cellulose	Pure cellulose	CMC	
Stan.	*T.viride*	+++	2.94	0.98	1.29	35.0
2	*F.neoceras*	++++	4.31	1.30	1.47	690.0
10	*A.fumigatus*	+++++	5.61	1.17	1.36	185.0
34	*F.oxysporum*	+++++	4.47	1.49	1.89	280

* Values are means of three replicates
+++ : well growth
++++: Very well growth
+++++: excellent growth

*Table (4)**: Growth and CMCase production by irradiated isolates of* **Aspergillus fumigatus** *and* **Fusarium neoceras** *grown on 1% CMC for 7 days at 28°C.*

A.fumigatus				F. neoceras			
Mutant isolates	*Dose (kGy)*	*Mycelial dry weight (g/l)*	*CMCase activity (µg/ml)*	*Mutant isolates*	*Dose (kGy)*	*Mycelial dry weight (g/l)*	*CMCase activity (µg/ml)*
Parent	***0.0***	***1.73***	***465***	*Parent*	***0.0***	***1.97***	***700***
G-1	***0.50***	***1.87***	***455***	*G-1*	***0.50***	***0.63***	***1062***
G-2	***0.50***	***1.38***	***325***	*G-2*	***0.50***	***0.63***	***745***
G-3	***0.50***	***1.98***	***335***	*G-3*	***0.50***	***1.12***	***430***
2G-1	***1.00***	***1.93***	***455***	*2G-1*	***1.00***	***0.66***	***950***
2G-2	***1.00***	***1.81***	***435***	*2G-2*	***1.00***	***0.81***	***650***
2G-3	***1.00***	***1.37***	***280***	*3G-2*	***1.50***	***0.74***	***1015***
4G-1	***2.00***	***1.81***	***440***	*4G-1*	***2.00***	***0.40***	***895***
4G-2	***2.00***	***1.44***	***175***	*4G-2*	***2.00***	***1.74***	***1150***
4G-3	***2.00***	***2.00***	***210***	*5G-1*	***2.50***	***1.49***	***430***
6G-1	***3.00***	***1.26***	***403***	*5G-2*	***2.50***	***1.29***	***610***
8G-2	***5.00***	***1.51***	***925***	*6G-1*	***3.00***	***0.60***	***738***
				7G-1	***4.00***	***1.01***	***465***

Table (5): Growth and CMCase production by irradiated isolates of *F. oxysporun* grown on 1% CMC for 7 days at 28°C.

Mutant isolates	**Dose (kGy)**	**Mycelial dry weight (g/l)**	**CMCase activity**
Parent (control)	0.0	1.90	990
G-1	0.50	1.45	890
2G-1	1.00	0.57	623
3G-1	1.50	1.23	340
3G-2	1.50	1.26	685
4G-1	2.00	1.94	416
5G-1	2.50	1.38	370
5G-2	2.50	2.08	720
7G-1	2.50	0.807	615

Table (6): Effect of shake and stationary culture on CMCase activity by *A. fumigatus* and *F. neonceras* and gamma irradiation mutants grown on 1% CMC for 7 days at 28C°.

Fungal strains and mutants	**CMCase activity ug/ml***	
	Stationary culture	**Shake culture**
A. fumigatus (parent)	40.0	35.0
A. fumigatues 8G-2 (mutant)	190.0	42.0
F. neoceras (parent)	800.0	620.0
F. neoceras 4G-2 (mutant)	920.0	480.0

* Values are mean of three replicates.

Table (7): Effect of different nitrogen sources (1g/l) on growth and CMCase of *A. fumigatus* and *F. neoceras* and their gamma-irradiation mutants grown on 1% CMC for 7 days at 28°C.

Nitrogen sources	*A.fumigatus (Parent)*		A. fumigatus 8G-2		*F. neoceras (Parent)*		*F. neoceras 4G-2*	
	Mycelial dry weight (mg/l)	**CMCase activity (µ)g/ml**	**Mycelial dry weight (mg/ml)**	**CMCase activity (µg/ml)**	**Mycelial dry weight (mg/l)**	**CMCase activity (µg/ml)**	**Mycelial dry weight (mg/ml)**	**CMCase activity (µg/ml)**
Ammonium nitrate	110.0	350.0	270.0	600.0	380.0	1100.0	906.0	885.0
Sodium nitrate	316.0	260.0	820.0	450.0	1330.0	1000.0	1400.0	950.0
Potassium nitrate	150.0	310.0	210.0	500.0	672.0	750.0	1420.0	940.0
Ammmonium sulphate	300.0	320.0	680.0	500.0	800.0	1060.0	980.0	970.0
Magnesium nitrate	150.0	375.0	250.0	600.0	620.0	1125.0	1492.0	900.0
Ammonium chloride	300.0	260.0	488.0	625.0	790.0	920.0	974.0	1050.0
Lead nitrate	280.0	210.0	922.0	250.0	522.0	875.0	680.0	1000.0

* : Values are mean of three replicates.

Table (8): Effect of different carbon sources on growth and CMCase activity of *A. fumigatus* and *F. neoceras* and their gamma irradiation mutants grown on 1% CMC for 7 days at 28°C.

Carbon source	*A. fumigatus* (parent)		*A. fumigatus* 8G-2 (mutant)		*F. neoceras* (parent)		*F. neoceras* 4G-2 (mutant)	
	Mycelial dry weight (g/l)	CMCase activity µg/ml	Mycelial dry weight (g/l)	CMCase activity µg/ml	Mycelial dry weight (g/l)	CMCase activity µg/ml	Mycelial dry weight (g/l)	CMCase activity µg/ml
Glucose	2.64	130.0	3.39	180.0	2.52	260.0	2.65	260.0
Sucrose	3.75	36.0	3.96	45.0	3.26	170.0	3.39	190.0
Maltose	2.90	310.0	3.49	375.0	2.75	290.0	3.88	345.0
Mannitol	1.94	65.0	2.56	155.0	4.31	90.0	2.65	55.0

Values are mean of three replicates.

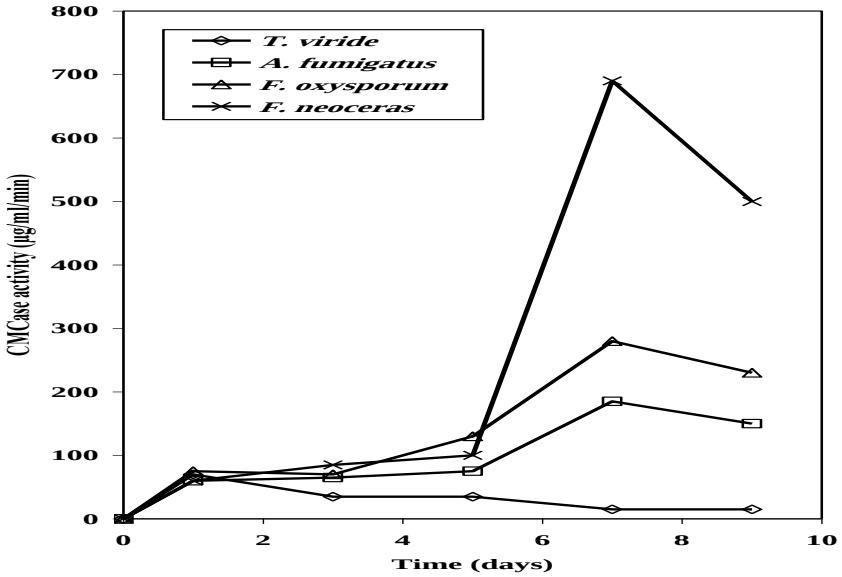

Fig (1): CMCase activity of *T. viride* and the selected fungal isolates

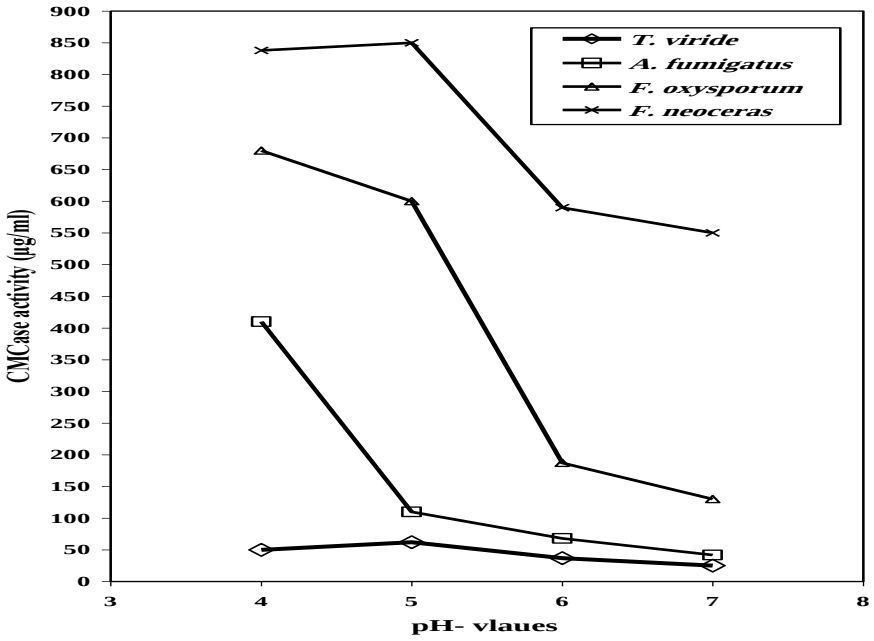

Fig. (2): Effect of pH value on the CMCase activity by *T. viride* and the selected fungal isolates.

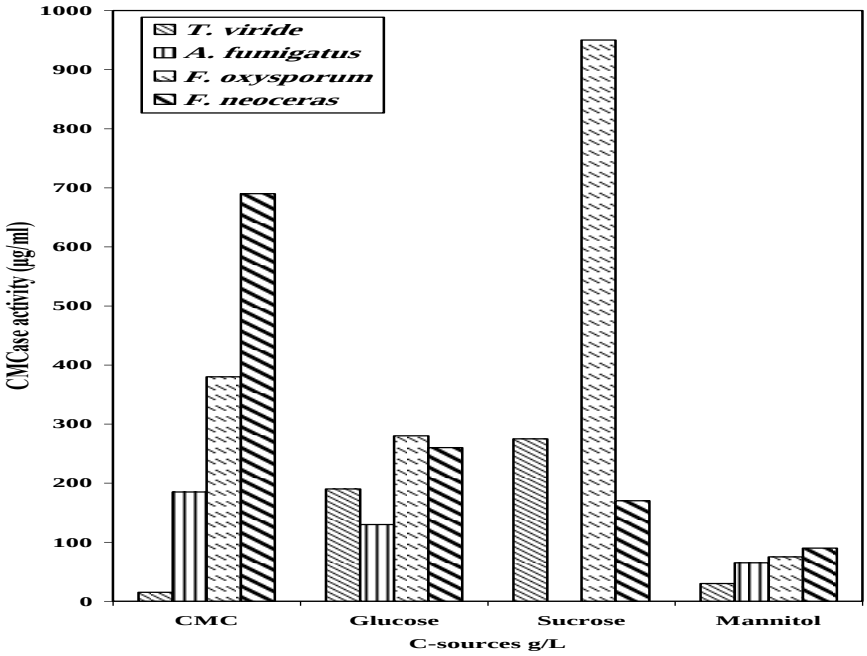

Fig. (3): Effect of different carbon sources on CMCase activity of *T. viride* and the selected fungal isolates.

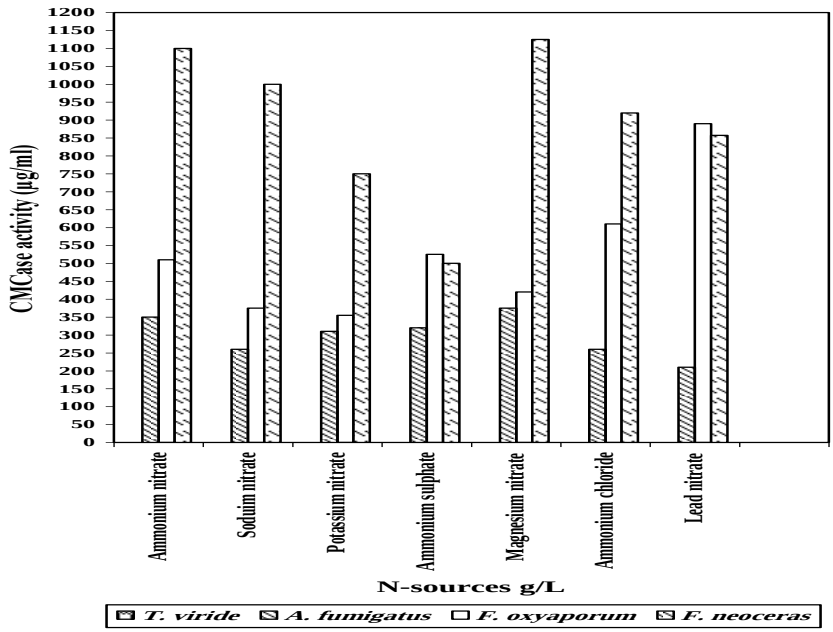

Fig. (4): Effect of different nitrogen sources on CMCase activity of *Trichoderma viride* and the selected fungal isolates.

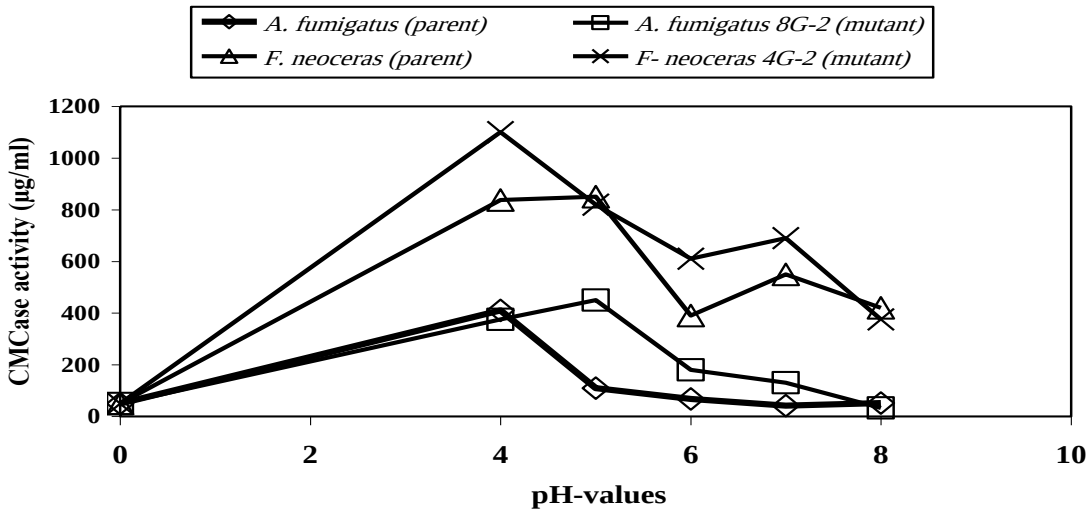

Fig. (5): Effect of different pH values on CMCase activity by *A. fumigatus* and *F. neoceras* and gamma irradiated mutants grown on 1% CMC for 7 days at 28°C.

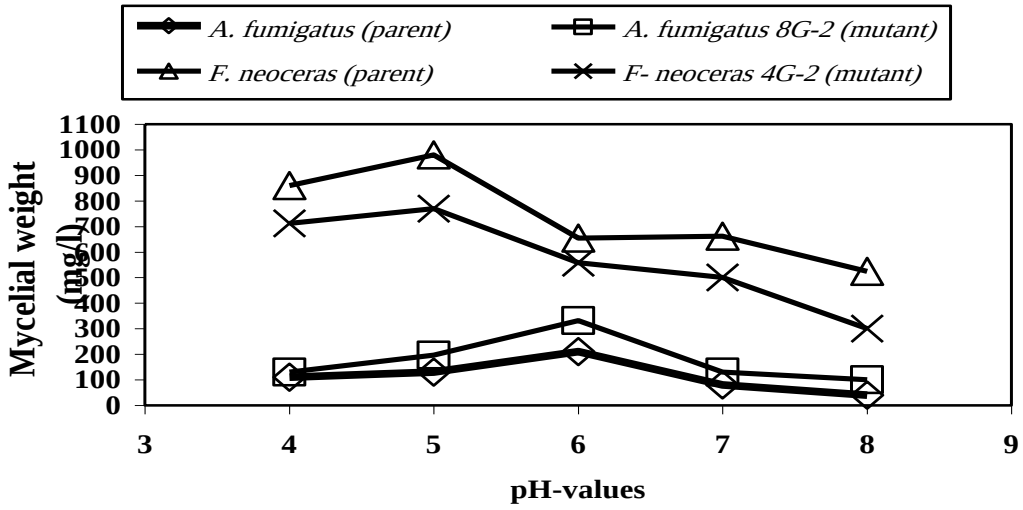

Fig. (6): Effect of different pH values on mycelial dry weight of *A.fumigatus* and *F. neoceras*and gamma irradiated mutants grown on 1% CMC for 7 days at 28°C

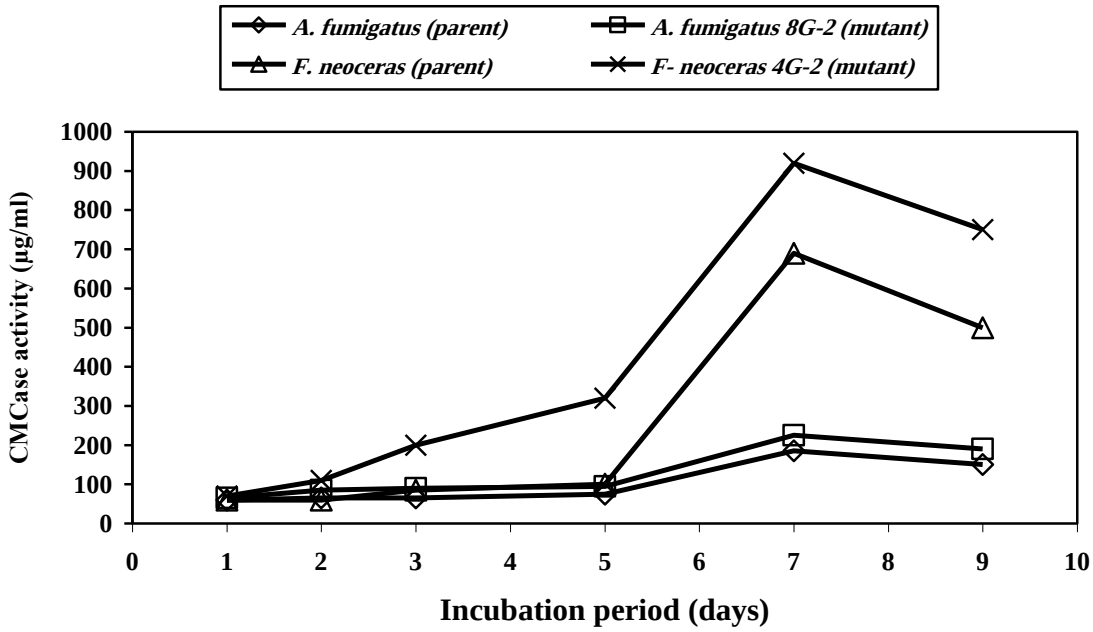

Fig. (7): Effect of incubation periods on the production of CMCase by the selected fungal isolates and gamma irradiated mutants grown on 1% CMC for 9 days at 28°C.

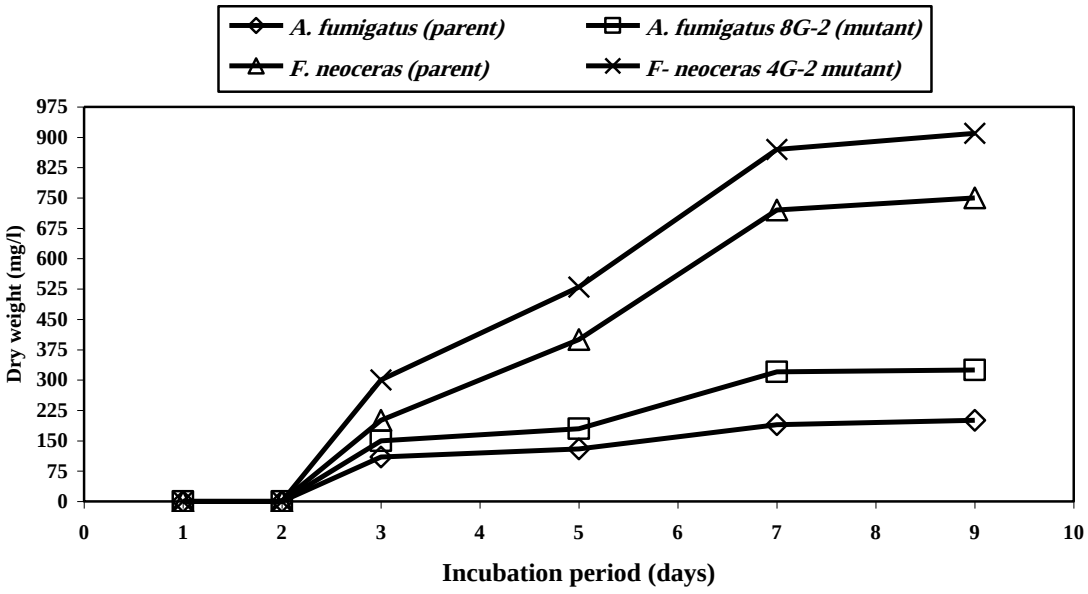

Fig. (8): Effect of the incubation periods on the mycelial dry weight of the selected fungal isolates and gamma irradiated mutants grown on 1% CMC for 9 days at 28°C.

CONCLUSION

Gamma irradiated fungal mutants were able to produce the highest levels of CMCase as compared to parent strain.

REFERENCES

1-Kubicek, C. P., Messner, R., Gruber, F., Mach, R. L. and Kubicek-Praz, E. M. (1993). The Trichoderma reesei cellulase regulatory puzzle-from the interior life of a secretory fungus. Enzyme Microbiol. Technol., 15, 90-99.

2-Ramos, L.P.; Nazhad, M.M.; and Saddler, J.N. (1993). Effect of enzymatic hydrolysis on the morphology and five structure of pretreated cellulosic residues. Enzyme Microbiol. Technol. 15: 821-831.

3-Peitersen, N. (1997). Continuous cultivation of Trichoderma viride on cellulose. Biotechnol. Bioengin XIX: 337-348

4- Arifoglu, N. and Ögel, Z. B. (2000). Avicel-adsorbable endoglucanase production by the thermophilic fungus Scytalidium thermophilum type culture Torula thermophila. Enzyme Microbiol. Technol. 27: 560-569.

5-Bhat, K. M. and Bhat, S. (1997). Cellulase degrading enzymes and their potential industrial applications. Biotechnol. Adv. 15: 583-620.

6-Le Jeune, R. and Baron, G.V. (1995): Effect of agitation on growth and enzyme production of Trichoderma reesei in batch fermentation. Appl. Microbiol. Biotechnol. 43: 249-258.

7-Umikalsom, M. S.; Ariff, A. B.; Shamsuddin, Z. H.; Tong, C. C.; Hassan, M.A. and Karim, M. I. A. (1997). Production of cellulase by a wild strain of Chaetomium globosum using delignified oil palm empty–fruit–bunch fiber as substrate. Appl. Microbiol. Biotechnol. 47: 590-595.

8-Lati fian, M.; Hamifi-Esfahani, Z. and Barzegar, M. (2007). Evaluation of culture conditions for cellulose production by two Trichoderma reesei mutants under solide-state fermentation conditions. Bioresource Technol. 98: 3634-3637.

9- Alam, Z.; Muyibi, S. A. and Wahid, R. (2008). Statistical optimization of process conditions for cellulose production by liquid state bioconversion of domestic wastewater sludge. Bioresource Technol. 99: 4709-4717.

10- Kovacs, K.; Kegyeri, L.; Szakacs, G.; Kubicek, C. P.; Galbe, M. and Zacchi, G. (2008). Trichoderma atroviride mutants with enhanced production of cellulose and □-glucosidase on pretreated willow. Enzyme Microb. Technol. 43: 48-55.

11- Ahamed, A. and Vermette, P. (2008). Culture-based strategies to enhance cellulose enzyme production from Trichoderma resei. RUT-C30 in bioreactor culture conditions. Biochem. Engineer. J. 40: 399-407.

12-Bravo, V.; Paez, M. P.; Aoulad, M.; Reyes, A. (2000). The influence of temperature upon hydrolysis of cellubiose by □-1,4- glucosidase from Aspergillus niger. Enzyme Microbiol. Technol. 26: 614-620.

13-Zorov, I. N.; Gusakov, A. V.; Baraznenok, V. A.; Bekkarevich, A. O.; Okunev, O. N.; Sinitsyn, A. P. and Kondrat'eva, E. G. (2001). Isolation and properties of cellobiase from Penicillium verruculosum. Appl. Biochem. Microbiol. 37: 588-592.

14- Abo-State, M. A. M. (2003). Production of carboxymethyl-cellulase by Fusarium oxysporium and F. neoceras-from gamma pretreatment lignocellulose wastes. Egypt. J. Biotechnol., 151-168.

15-Pitt, J. I. And Hocking, D. A. (1997): Fungi and Food Spoilage 2nd edition. Blackie Academic Press, London, U K.

16-Bahkali, A. H. (1995). Production of cellulase, xylanase and polygalacturonase by Verticillium tricorpus on different substrates. Bioresource Techno. 51: 171-174.

17-Wang, C. H.; Hseu, T.H. and Huang, C. M. (1988). Induction of cellulase by cello–oligosaccharides in Trichoderma koningii G- 39. J. Biotechnol. 9: 47-60.

18-Miller G. L. (1959): Use of dinitrosalysilic acid reagent for the determination of reducing sugars. Anal Chem, 31: 426-28.

19-Lowry, O. H. ; Rosebrough, N. J. ; Farr, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-276.

20-Klyosov, A. A. (1990): Trends in biochemistry and enzymology of cellulose degradation. Biochem, 29, 10577-10585.

21-Gomes, J.; Gomes, I.; Esterbauer, H.; Kreiner, W. and Steiner, W. (1989). Production of cellulases by a wild strain of Gliocladium virens: optimization of the fermentation medium and partial characterization of the enzymes. Appl. Microbiol. Biotechnol. 31: 601-608.

22-Romero, M. D.; Aguado, J.; González, L. and Ladero, M. (1999).Cellulose production by Neurospora crassa on wheat straw. Enzyme Microbiol. Technol. 25: 244-250.

23-Gregg, D. J. and Saddler, J. N. (1996). Factors affecting cellulose hydrolysis and the potential of enzyme recycle to enhance the efficiency of an integrated wood to ethanol process. Biotechnol. Bioengin. 51: 375-383.

24-El-Zawahry, Y. A., El-Fovly, M. Z., Helal, G. A. and A. A. Shindis (1987). Effect of nutrional requirement and environmental condition on the production of cellulase enzymes and microbial protein by some local fungal isolates. Egypt. J. Appl. Sci. 1, 471-488.

25-Gomaa, E. G. (1993): Induction of Volvoriella volvaceae cellulases on mixtures of xylose and cellulose. Fifth Arab Conf. Of Food Sci. and Technol. Dec (1993), pp. 326-342. Cairo, Egypt.

26-Fadel, M. and Abd El-Kader, M. M. (1994). Production of cellulases and □-glucosidase by new isolate of Aspergillus niger F. 92. Egypt. J. Microbiol. 29, 2, 175-182.

27-Christakopoulos, P.; Macris, B. J. and Kekos, D. (1989). Direct fermentation of cellulose to ethanol by Fusarium oxysporum. Enzyme Microbiol. Technol. 11: 236-239.

28-Famurewa, O. and Olutiola, P. O. (1991). Comparison of growth and cellulolytic enzyme production in Aspergillus chevalieri and Penicillium steckii from mouldy cacao beans. Folia Microbiol. 36: 347-352.

29-Steiner, W.; Lafferty, R. M.; Gomes, I and Esterbauer, H. (1987). Studies on a wild strains of Schizophyllum commune cellulase and xylanase production and formation of the extracellular polysaccharide Schizophyllan. Biotechnol. Bioengin. 30: 169-178.

30-Kvachadza, L. L. and Yashvili, T.S. (1996). Influence of cultivation conditions on the synthesis of extracellular cellulases by Chaetomium thermophile T-1. Appl. Biochem. Microbiol. 32: 557-560.

31-Sadana, J. C.; Shewale, J. G. and Desphande M. V. (1979). Enhanced cellulase production by a mutant of Sclerotium rolfsii. Appl. Environ. Microbiol. 38: 730-733.

32-Sadana, J. C.; Shewale, J .G. and Desphande, M. V. (1980). High cellobiase and xylanase production by Sclerotium rolfsii UV-B mutant in submerged culture. Appl. Environ. Microbiol. 39: 935-936.

33-El-Zawahry, Y. A. and Mostafa, I. Y. (1983). Study on the production of cellulase enzyme by non-irradiated and irradiated isolates of Trichoderma viride. Isotope and Rad. Res., 15, 103-110.

34-Sandhu, D. K. and Kalra, M. K. (1985). Effect of cultural conditions on production of cellulases in Trichoderma longibrachiatum. Trans. Br. Mycol. Soc. 84: 251-258.

35-Tuttle, S.; Stamato, T.; Perez, M. L. and Biaglow, J. (2000).Glucose–6-phosphate dehydrogenase and oxidative pentose phosphate cycle protect cells against apoptosis induced by low doses of ionizing radiation. Rad. Res. 153: 781-787.

36-Trumnore C. N.; Ehrlich R. C. and Mayers, Y. N. (2001).Changes in DNA confermation induced by gamma irradiation in the presence of copper. Rad Res., 155: 453-65.

37-Aziz H. N.; El-Zawahry Y, El-Fouly M Z .; El-Essawy A. and Khalaf A. (2000). Effect of gamma irradiation and some environmental factors on citric acid production from potato processing waste water by fungal strains. J. Environ. Research, 2: 77-89.

37-Haq Ikram U, Silander A, Qadeer M A and Javed I. (2004). Citric acid production by selected mutants of Aspergillus niger from cane molasses. Biores Technol. 93: 125-30.

39-Khalaf, S. A. (2004). Use of mutangenens in the improved of itaconic acid production by Aspergillus terreus RBF (local isolate) from raw starch material and refused banana fruits. Isot. Rad. Res.36: 355- 363.

40-Wase, D. A.; McManamey, W. J.; Raymahasay, S. and Vaid, A. K. (1985): Comparisons between cellulase production by Aspergillus fumigatus in agitated vessels and in an air–lift fermentor. Biotechnol. Bioengin XXVII : 1166-1172.

41-Desai, J. D., Desai, A. J. and Patel, N. P. (1982).Production of cellulases and □-glucosidease by shake cuture of Scytalidium lignicola. J. Ferment Technol, 6, 117-123.

42-Ogawa, K., Toyama, H. and Toyama, N. (1982). Native cellulose hydrolyzing cellulase of T. reesei. J. Ferment. Technol. 60, 349-356.

43-Mandels M, Weber J, Parizek M. (1971). Enhanced cellulose production by mutant of Trichoderma viride. Appl Microbiol. 21: 152-54.

44-Gallo, B. J., Anderotti, R., Roche, C., Ryu, D. and Mandels, M. (1978). Cellulase production by new mutant strain of Trichoderma reesei MCG77. Biotechnol. Bioengin. Symp. 8, 99-101.

45-Ellouz-Chaabouni, S.; Belguith, H. and Hassairi, I., M,Tad, K. and Ellouz, R. (1995). Optimization of cellulase production by Penicillium occitanis. Appl. Microbiol. Biotechnol. 43, 267-269.

46-Walter, S. and Schrempf, H. (1996). Physiological studies of cellulase (Avicelase) synthesis in Streptomyces reticuli. Appl. Environ. Microbiol. 62: 1065-1069.

تأثير أشعة جاما وبعض الظروف البيئية على زيادة إنتاج إنزيم السليولايز بواسطة بعض السلالات الفطرية المعزولة من مخلفات سيليولوزية

ناجى حليم عزيز[1] ، خيرية عبد الغنى يوسف [2] ، مرفت على أبو ستيت [1] ، عادل أحمد المحلاوى [2] و عبير معوض برتلا جرجس [1]

1 *المركز القومى لبحوث وتكنولوجيا الإشعاع*

2 *كلية العلوم ، جامعة عين شمس*

فى هذه الدراسة تم عزل وتعريف 51 عزلة من الفطريات من المخلفات السليلوزية ولقد إستطاعت 19 عزلة فطرية أن تفرز إنزيم السليلولايز وهذه العزلات تنتمى إلى أجناس Penicillium and Aspergillus تم تقدير إنزيم السيليوليز لمعظم الفطريات المعزولة ولقد لوحظ أن الفطريات F. neoceras A. fumigatus and F. oxysporium أنتجت أعلى مستويات لإنزيم السليوليز مقارنة بفطر T. viride ولقد تبين من هذه الدراسة أنه من بين 23 عزلة فطرية تم عزلها من الجرعات الإشعاعية المختلفة كان أفضل إنتاج الإنزيم بواسطة سلالتين فقط وهما A. fumigatus 8G-2 and F. neoceras 4G-2مقارنة بالسلالات الأم الغير المعرضة للجرعات الإشعاعية .

لوحظ فى هذه الدراسة أنه تحت الظروف البيئية الثابتة الرقم الهيدروجينى ، وكذلك إضافة بعض المصادر الكربونية والنيتروجينية أدت إلى زيادة مقدرة هذه السلالتين على إنتاج إنزيم السيليوليز. ولقد أثبتت هذه الدراسة أن إستخدام السلالات الفطرية المطفرة إشعاعيا تلعب دورا هاما فى إنتاج إنزيم السيليوليز وكذلك التخلص من المخلفات السيليولوزية.