# Effect of gamma irradiation on *Aspergillus niger* DNA and production of cellulases enzymes

Azza A. Mostafa

Department of Biological and Environmental, Faculty of Home Economics, Al- Azhar **Univ., Tanta, Egypt**
Email :azza_1965_1965@yahoo.com

**Abstract:** *Aspergillus niger* isolated from wheat straw was subjected to various doses of gamma irradiation (1KGy and 2KGy) to enhance the production of enzyme carboxymethyl cellulase (CMCase) and filter paper cellulase (FPA).A wild *Aspergillus niger* and *A. niger* subjected to various doses of gamma irradiation were screened for the production of cellulases by submerged cultivation in liquid mineral salt medium in which carboxymethylcellulose (CMC), wheat straw and corn cobs had been added as the sole carbon source. Cultivation conditions investigated include variation of the carbon source, pH, temperature and time of incubation. *Aspergillus niger*,which subjected through 2 KGy irradiation showed highest extracellular CMCase and FPA production which is higher than that of the wild type. Optimum conditions for the production of CMCase and FPA by the wild *A. niger* and *A. niger* subjected to 2 KGy. The optimized initial pH and temperature was 5.0 and 30$^{o}$C respectively and the use of corn cobs as the carbon source gave the highest CMCase and FPA by *A. niger* exposed to 2KGy after 2 day. The polymerase chain reaction (PCR) that includes random amplified polymorphic DNA (RAPD) was employed to investigate the influence of gamma radiation in inducing DNA-Polymorphisms. Number of amplified DNA fragments were 12 and 20 in irradiated *A. niger* (1KGy) and irradiated *A. niger* (2KGY) respectively
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## 1. Introduction

Lignocellulosic wastes are the largest group of wastes present on this plant causing environmental pollution (Rani, and Nand, 2000). It is estimated that the photosynthetic process produced 1.5 x 10 ton (150 billion tons) of dry material annually with respect to carbon of which about 50% is cellulose (Persson *et al., 1991)*. Cellulase(s) are industrially important enzymes that are sold in large volumes for use in industrial applications, for example in starch processing, animal feed production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp, paper and textile industries (Ögel *et al*. 2001, Abo-State *et al*. 2010). Moreover, there are growing markets for produced cellulases in the field of detergent industry and saccharification of agriculture wastes for bioethanol technology (Camassola and Dillon 2009, Vu *et al*. 2011).The cellulase complex secreted by filamentous fungi consists of three major enzyme components, an endo-1,4-β-glucanase [Carboxymethyl cellulase (EC 3.2.1.4)], a 1,4-β-cellobiohydrolase [Exoglucanase (EC 3.2.1.91) and a 1,4-β-glucosidase [Cellobiase (EC3.2.1.21)], which act synergistically during the conversion of cellulose to glucose (Bucht and Ericksson 1969, Almin *et al*. 1975). The cost of production and low yield of cellulases are the major problems for industrial applications. It has been reported that solid state fermentation (SSF) as an attractive process to produce cellulases economically is mainly due to its lower capital investment and lower operating expenses (Singhania *et al*. 2009). Production of cellulases by fungi in SSF using agricultural wastes has been reported (Fawzi 2009, Abo-State *et al*. 2010). Therefore, investigation on the ability of fungal strains to utilize inexpensive substrates and improvement of enzyme productivity are important. The lignocellulosic biomass, especially agricultural wastes, is known to be an excellent carbon sources for microbial enzyme production (Gao *et al*. 2007). The utilization of cheaper and indigenous substrate for cellulose production has contributed somewhat to economical recovery (Pandey *et al*. 2000), Various agricultural substrates, by products and microbial cultures have been used successfully in solid state fermentation for cellulase production (Madamwar *et al*. 1989 and Deunas *et al*. 1995). *Aspergillus genus* is known to be a good producer of cellulases (Oxenboll, 1994). Overproduction of the enzyme as the quantities produced by wild strains are usually too low (Pradeep and Narasimha 2011). The spectacular successful examples of strain improvement in industry are mostly attributed to the extensive application of mutation and selection (Vu *et al*. 2011). Such improved strains can reduce the cost of the processes with increased productivity and may also possess some specialized desirable characteristics (Karanam *et al.* 2008). Ionizing radiations like gamma irradiation have many advantages over chemical mutagens. Such ionizing radiations penetrate into tissues and generate the production of numerous compounds depending on the

dose rate of radiation. By the reaction of gamma rays and water, some influential reducing and oxidizing species and molecular products like OH and H2O2 are produced respectively (Parker & Darby, 1995). Mutagenic effectiveness of gamma rays has been reported to be higher than the chemical like EMS (Dhulgande *et al*., 2011). Irradiation by gamma ray may cause some mutations to the genes of cells through the DNA repair mechanisms within cells. Gamma radiations are short wave high energy electromagnetic radiations emitted from certain radioactive isotopes such as Cobalt60. The effect of gamma radiation doses on fungal enzyme production was studied by many workers (Shimokawa *et al*. 2007, Yousef *et al*. 2010). The aim of this study was to investigate high level production of extracellular carboxymethyl cellulase (CMCase) and filter paper activity( FPA) through the exposed of *A.niger* to gamma radiation method, and optimizing some parameters .

## 2. Material and Methods

**Sources of agricultural waste and pretreatment**

The substrates used for this work were wheat straw and maize cobs. The wheat straw and maize cobs were obtained from El – Mahla El- Kubra, Tanta government, Egypt. The substrates were washed thoroughly with water to remove surface dirt, and oven dried at $70^0$ C for 2 hours.

The dried samples were broken into pieces form with the aid of mortar and pestle in preparation for alkaline and steam treatment (Ali *et al*., 1991). The broken samples was measure into separate conical flasks containing 5% NaOH solution (1:20 w/v). This was autoclaved at 121$^o$C for 1 hour to free cellulose of lignin hold. The NaOH solution was drained off by sieving through a muslin sieve. Samples were rinsed several times with distilled water, neutralized with 0.1 M HCL and finally washed with distilled water. The pre-treatment samples were dried in oven at 70$^o$C for 24 hours and further broken to powder form in an electric blender.

**Organism :**

*A niger* was isolated from wheat straw and maintained on Potato Dextrose Agar slants at 4$^o$ C. The isolated *A. niger* were carefully identified by morphological characteristics include color of the colony and growth pattern studies, as well as their vegetative and reproductive structures observed under the microscope.

**Effect of gamma radiation on *Aspergillus niger* :**

*A. niger* grown on PDA plates for 6 days were exposed to two doses of gamma in (Atomic Energy Commission, El-Katameya, Cairo, Egypt). The doses were 1.0 KGy and 2 KGy. Three plates were used for each dose. One disc (10 mm diameter) from each plate was placed on center of PDA plates. Three replicates were used for each dose. The plates were incubated at 30$^o$ C for 6 days.

**Media preparation for enzyme production**

The basal medium comprised of (gram per litre of distilled water); $KH_2P0_4$, 10.0g; $(NH_4)_2$ $SO_4$, 10.5g; $MgSO_4$. $7H_20$, 0.3g; $CaCl_2$, 0.5g; $FeSO_4.7H_2O$, 0.013g; $MnS0_4.7H_20$, 0.04g; $ZnSO_4$. $7H_20$, 0.004g; $CoCl_2$. $6H_20$, 0.0067g; yeast extracts, 0.5; into separate 250 ml conical flask containing 100 ml of the basal medium was added 40g each of the treated carbon source (wheat straw and corn cobs) and 20g of Carboxymethylcellulose (for control). The pH of the media was then adjusted to 5. Media were sterilized in an autoclave at 121$^o$C for 15 minutes (Milala *et al.,* 2005).

**Fermentation**

Media were inoculated by scooping mycelia from plate culture (*A. niger* exposed to gamma radiation and non exposed to gamma radiation) with the aid of a wire loop and incubated at $30^0$C in an incubator. Samples of culture fluids obtained with aid of sterile pipette every 48 hours for 240 hours. These were centrifuged at 3,000 rpm for 15minute and the supernatant analysed as the crude enzyme.

**Assay of cellulases activity**

Endoglucanase activity, (carboxymethyl-cellulase; CMCase) activity was determined using the method recommended by Acharya *et al.* (2008). The reaction mixture contained 0.5 mL of 0.5% of CMC as substrate prepared in 0.5 M sodium acetate buffer pH 5.5 and 0.5 mL of enzyme extract. The control sample contained the same amount of substrate and 0.5 mL of the enzyme solution heated at 100$^o$C for 15 min. Both the experimental and control samples were incubated at 50$^o$C for 30 min. At the end of the incubation period, tubes were removed from the water bath, and the reaction was terminated by addition of 3 mL of 3, 5-dinitrosalicylic acid (DNSA) reagent per tube (Shazia *et al.,* 2010). The tubes were incubated for 5 min in a boiling water bath for color development and then were cooled rapidly. The activity of reaction mixture was measured against a blank sample at wavelength of 540 nm. The concentration of glucose released by enzymes was determined by comparing against a standard curve constructed similarly with known concentration of glucose. Unit enzyme activity was defined as the amount of enzyme required for liberating 1μM of glucose per millilitre per minute, in analysed conditions of reaction and was expressed as μM/mL/min.

For filter paper activity (cellulase; FPA) measurement , it is a combined assay for endo and exo β – 1,4 glucanase, according to Stephen *et al.* (2003) . 1 ml of culture supernatant as a enzyme source was added to WhatmanNo. 1 filter paper 50 mg immersed in 1 ml of 0.05 M sodium acetate buffer of pH 4.8. After incubation at 50$^o$ C for 10min, the reducing sugar

released was estimated by DNS method. One unit of filter paper activity was defined as the amount of enzyme released 1 mg of reducing sugar from filter paper per milliliter per minute of the reaction.

**Determination of time course for enzyme production**

The optimum time management for enzyme production was determined by assaying samples collected at 48 hours interval for cellulase activity on enzyme production.

**Effect of pH**

A wild type of *Aspergillus niger* and *A. niger* exposed to gamma ray (2KGy) were inoculated into a series basal media supplemented with cellulose, wheat straw or corn cobs separately with the pH varied from 3 to 7. The inoculated media were incubated at 30$^{o}$C .

**Effect of varying temperature**

The optimal temperature for enzyme production was determined by growing the a wild type of *Aspergillus niger* and *A. niger* exposed to gamma ray (2KGy in sterile media incubated at temperature of 30, 35, 40 and 45$^{o}$C. Cellulase activity of the enzyme were then determined.

**RAPD-PCR analysis.**

DNA was extracted from fungal samples by Cetyltrimethyl Ammonium Bromide (CTAB) according to Doyle and Doyle (1990). RAPD was performed using 10 random 13 mer primers. Polymerase Chain Reaction (PCR) was carried out in presence of 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2), 100 µM dNTPs, 5 picomole single random primers, 25 ng template DNA, and 0.5 unit of Taq DNA polymerase in a total volume of 25 µl. PCR amplification was performed in automated thermal cycler (MJ-Mini, Bio Rad) programmed as follow, 95°C for 4 min followed by 40 cycles of 1 min for denaturation at 94°C, 30 sec for annealing at 37°C and 1.30 min for polymerization at 72C°, followed by a final extension step at 72C° for 7 min. The amplification products were resolved by electrophoresis in 1.5 % agarose gels in 0.5 X TBE buffer and documented on Gel Documentation UVITEC, UK.

Table 1. PCR reactions were conducted using 13 mer primers (Metabion, Germany) with the following sequences:

| Primers | Sequences |
|---|---|
| P1 | 5′- CCGACTCTGGCGA-3′ |
| P2 | 5′- GTAAGCCGAGACA-3′ |
| P4 | 5′- ACCTGCCAACATA-3′ |
| P5 | 5′- GTAGGTCGCAGGT-3′ |
| P6 | 5′- TCGTGGCACATAC-3′ |
| P7 | 5′- TGTACGGCACACG-3′ |
| P8 | 5′- ACGGAGGCAGAGA-3′ |
| P9 | 5′- GTCTTCCGTCGTC-3′ |
| P10 | 5′- GTGTGCCTGGTGC-3′ |

**Statistical analysis:**

The data were presented as means ± standard deviation (SD). Analysis of variance was conducted using General Linear Model(univariate) a one – way ANOVA test followed by Duncan test using SPSS computer program (version 9.0) .

## 3. Results and Discussions

The wild type of *A. niger* when exposed to two gamma radiation doses ( 1 and 2 KGy ) gave different abilities to produce CMCase and FPA cellulase activity on the basal media supplemented with carboxymethel cellulose(CMC) as the carbon source, wheat straw and corn cobs as natural substrate that might be useful for production of enzymes in a commercial scale. *Aspergillus niger* is a potent producer of many industrially important enzymes and may genotypically be improved by exposure to gamma rays. The mutants recovered after treatment by gamma rays were found to be effective producers of enzymes (Awan *et al.*, 2011). Mutagenesis of *Aspergillus niger* by using chemicals has been reported earlier to improve many industrially important enzymes and other products.

Table 2 shows the effect of unirradiated and irradiated *A. niger* for production of CMCase and FPA cellulase on basal media supplemented with CMC. The highest CMCase and FPA cellulase production were obtained by *A. niger* when exposed to 2 KGy dose (62.9344 and 44.29 µMol/min/ml respectively) after 4 days for both. On the other hand, there is not significant effect between irradiated and non irradiated *A. niger* for production of CMCase while there are significant effect on FPA cellulase Abo- State *et al.* (2010) found enhanced productivity in CMCase by gamma-irradiation at dose 0.5 KGy with 21% increase as compared with un-irradiated control. CMCase activity of a mutant of *Aspergillus terrus* showed two-fold improved activity than that of the wild type (Vu *et al*. 2011).

Table 3 shows the effect of unirradiated and irradiated *A. niger* for production of CMCase and FPA cellulase on basal media supplemented with wheat straw as carbon source . The highest CMCase and FPA cellulase production were obtained by *A. niger* when exposed to 2 and 1 KGy dose (101.56 and 101.88 µMol/min/ml after 2 and 4 days respectively). On the other hand, there is significant effect between irradiated and non irradiated *A. niger* for production of CMCase and FPA cellulase . Abo- State *et al.* (2010) found that the best substrate for FPA cellulose activity by *A. niger* was wheat straw. Enhanced production of glucose oxidase was performed by mutagenesis of *Aspergillus niger* by gamma irradiation at dose of 80krad (Muhammad *et al*. 2012).

**Table.2. Effect of gamma radiation on CMCase and FPA(cellulase) production by irradiated and non irradiated *A. niger* on basal media supplemented with carboxymethyl-cellulose as carbon source .**

| Time (day) | CMCase | | | FPA(cellulase) | | |
|---|---|---|---|---|---|---|
| | Wild type of *A. niger* | *A. niger* exposed to (1KGy) | *A. niger* exposed to (2KGy) | Wild type of *A. niger* | *A. niger* (1KGy) | *A. niger* (2KGy) |
| 2 | 29.26±0.85 | 34.94±0.85 | 29.26±0.85 | 9.17±0.59 | 29.28±0.85 | 24.17±0042 |
| 4 | 43.97±0.67 | 30.62±1.68 | 62.68±1.54 | 36.78±0.15 | 32.59±1.12 | 44.29±0.56 |
| 6 | 57.45±1.14 | 30.96±0.85 | 42.04±0.98 | 16.93±0.41 | 23.49±0.32 | 30.70±0.57 |
| 8 | 38.35±0.85 | 55.11±1.7 | 44.31±1.13 | 15.66±0.29 | 27.80±0.95 | 18.19±0.14 |
| 10 | 31.25±0.82 | 50.54±1.7 | 31.26±1.14 | 14.13±0.12 | 9.39±0.42 | 7.36±0.56 |
| Mean | 40.06 ±11.2[a] | 40.44±10.95[a] | 41.91±12.5[a] | 18.534±9.8[a] | 24.514±8.5[b] | 24.944±12.8[b] |

Values are presented as mean ± standard deviation (n=3)
All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different.

**Table.3. Effect of gamma radiation on CMCase and FPA (cellulase) production by irradiated and non irradiated *A. niger* on basal media supplemented with wheat straw as carbon source**

| Time (day) | CMCase | | | FPA(cellulase) | | |
|---|---|---|---|---|---|---|
| | Wild type of *A. niger* | *A. niger* exposed to (1KGy) | *A. niger* exposed to (2KGy) | Wild type of *A. niger* | *A. niger* (1KGy) | *A. niger* (2KGy) |
| 2 | 27.55±0.29 | 37.66±0.87 | 101.56±1.2 | 11.09±0.54 | 22.12±0.57 | 96.71±0.39 |
| 4 | 30.75±0.58 | 97.70±3.68 | 78.012±2.2 | 13.60±0.97 | 101.88±0.31 | 35.51±0.28 |
| 6 | 76.29±3.16 | 81.39±1.26 | 73.39±2.7 | 39.44±0.29 | 37.77±0.51 | 35.45±0.29 |
| 8 | 63.04±3.62 | 73.17±2.52 | 58.23±1.12 | 30.33±0.83 | 30.98±0.29 | 38.35±0.29 |
| 10 | 57.38±3.41 | 45.81±1.46 | 57.60±1.87 | 34.63±0.49 | 27.55±0.29 | 40.04±0.28 |
| Mean | 51.003±26.3[a] | 67.156±23.3[b] | 73.76±16.8[b] | 25.817±12.08[a] | 44.058±33.3[b] | 49.211±24.6[c] |

Values are presented as mean ± standard deviation (n=3)
All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different.

Table 4 shows CMCase and FPA cellulase enzyme activities for a period of 10 days on basal media supplemented with corn cobs as carbon source. CMCase andFPA cellulase activities were maximal after 2 days for corn cobs by irradiated *A. niger* (2KGy), it was 109.48 and 106.63 µmol/min/ml respectively after two days for both. These results agreement with Abo- State *et al*. (2010) who found the best Avicelase producing mutant of *Aspergillus* was exposed to 2.0 kGy, which was the best also for protein production. Mutant No. "4" produced 31% Avicelase, 21% CMCase and 34% FPase more than the parent strain (control). El-Batal, and Abo-State, (2006), they found enhanced productivity in CMCase, FPase, Avicelase, xylanase, pectinase,α- amylase and protease by gamma-irradiation at dose 1.0 kGy.

**Table.4. Effect of gamma radiation on CMCase and FPA cellulase production by irradiated and non irradiated *A. niger* on basal media supplemented corn cobs as carbon source**

| Time (day) | CMCase | | | FPA(cellulase) | | |
|---|---|---|---|---|---|---|
| | Wild type of *A. niger* | *A. niger* exposed to (1KGy) | *A. niger* exposed to (2KGy) | Wild type of *A. niger* | *A. niger* exposed to (1KGy) | *A. niger* exposed to (2KGy) |
| 2 | 35.30±0.27 | 19.99±0.28 | 109.48±0.52 | 17.16±0.89 | 8.64±0.40 | 106.63±0.63 |
| 4 | 46.06±0.57 | 28.34±0.52 | 108.80±0.60 | 20.17±0.28 | 9.36±0.42 | 104.42±0.31 |
| 6 | 56.99±0.86 | 46.19±0.46 | 108.86±0.32 | 27.26±0.57 | 26.42±0.99 | 101.57±0.62 |
| 8 | 99.73±1.26 | 90.51±0.95 | 100.57±0.66 | 91.62±1.9 | 31.25±0.98 | 65.92±0.31 |
| 10 | 91.93±1.22 | 106.37±0.33 | 79.86±1.36 | 54.38±0.63 | 83.59±0.94 | 48.58±1.1 |
| Mean | 66.003±26.3[b] | 58.28±35.4[a] | 101.51±11.8[c] | 42.11± 29.01[b] | 31.85±28.4[a] | 85.42±24.6[c] |

Values are presented as mean ± standard deviation (n=3)
All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different.

**Effect of initial pH:**

Effect of initial pH on CMCase and FPA cellulase activity was analyzed at different pH ranging from 3-7. *Aspergillus niger* which exposed to 2KGy gamma ray and gave the highest enzyme activity compared with a wild *A. niger* were used. Tables 5 and 6 show the activity of CMCase and FPA cellulase produced by a wild *A. niger*. There are a significant effect on CMCase and FPA cellulase activity at different pH values. A wild *A. niger* grow on basal medium supplemented by carboxymethyl-cellulose produced maximum CMcase and FPA celluase at pH 4 (40.95 an46.66 µmo/min/ml respectively) while produced maximum CMcase and FPA celluase at pH 5 and 4 respectively on basal medium supplemented by wheat straw. On the other hand, a wild *A. niger* produced the highest CMcase and FPA celluase at pH 5 on basal medium supplemented by corn cobs.

**Table 5: Effect of initial pH on CMCase activity by non irradiated *A. niger* on basal media supplemented by different carbon source at 30°±2 C**

| Carbon source | pH3 | pH4 | pH5 | pH6 | pH7 |
|---|---|---|---|---|---|
| Carboxymethyl-cellulose | 32.38±.28$^{c}$ | 40.95±0.4$^{e}$ | 38.35±0.85$^{d}$ | 25.71±0.5$^{b}$ | 22±0.35$^{a}$ |
| Wheat straw | 29.52±1.12$^{b}$ | 41.91±0.51$^{c}$ | 63.04±3.62$^{e}$ | 20±0.51$^{a}$ | 52.38±0.18$^{d}$ |
| Corn cobs | 26.67±0.39$^{a}$ | 50.4±1.2$^{d}$ | 99.73±1.26$^{e}$ | 43.33±0.13$^{c}$ | 34.29±0.29$^{b}$ |

Values are presented as mean + standard deviation (n=3); All groups are compared to each other at p<0.05.
Values with different super scripts along a raw are statistically different.

**Table 6: Effect of initial pH on FPA cellulase activity by non irradiated *A. niger* on basal media supplemented by different carbon source**

| Carbon source | pH3 | pH4 | pH5 | pH6 | pH7 |
|---|---|---|---|---|---|
| Carboxymethyl-cellulose | 34.28±1.08$^{d}$ | 46.66±0.8$^{e}$ | 15.66±0.29$^{c}$ | 11.43±0.23$^{b}$ | 7.14±0.15$^{a}$ |
| Wheat straw | 20.95±0.25$^{c}$ | 38.15±0.2$^{e}$ | 30.33±0.83$^{d}$ | 17.62±0.5$^{b}$ | 13.81±0.19$^{a}$ |
| Corn cobs | 20.95±0.4$^{a}$ | 30.47±0.3$^{b}$ | 91.62±1.9$^{e}$ | 58.15±0.15$^{d}$ | 34.29±0.25$^{c}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different.

Tables 7 and 8 show the activity of CMCase and FPA cellulase produced by irradiated *A. niger* by gamma ray (2KGy). There are a significant effect on CMAase and FPA cellulase at different pH. Irradiated *A. niger* grew on basal medium supplemented with carboxymethyl-cellulose, wheat straw and corn cobs produced maximum CMcase and FPA celluase at pH 5. Same results have been reported by Umbrin *et al*. (2011) who found the optimum pH rang from 4 to 5 for cellulase activity of *A. niger*, Ghada (2011) found that the cellulase complex enzyme produced by *Aspergillus oryzae* has abroad pH range between 3.8 to 8 while Juliet and Oladiti (2013) found the optimum pH for cellulases activity by *A. niger* was 4.5.

**Table 7: Effect of initial pH on CMCase activity by irradiated *A. niger* (2KGy) on basal media supplemented by different carbon source**

| Carbon source | pH3 | pH4 | pH5 | pH6 | pH7 |
|---|---|---|---|---|---|
| Carboxymethyl-cellulose | 26.66±0.3$^{b}$ | 40.95±0.48$^{d}$ | 44.31±1.13$^{e}$ | 27.62±0.2$^{c}$ | 23.81±0.11$^{a}$ |
| Wheat straw | 31.69±0.49$^{c}$ | 37.61±0.31$^{d}$ | 58.23±1.12$^{e}$ | 26.66±0.26$^{b}$ | 18.15±0.3$^{a}$ |
| Corn cobs | 24.76±0.47$^{b}$ | 38.15±0.2$^{c}$ | 100.57±1.57$^{d}$ | 39.05±0.15$^{c}$ | 20.03±0.97$^{a}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different.

**Table 8: Effect of initial pH on FPA cellulase activity by irradiated *A. niger* on basal media supplementedby different carbon source**

| Carbon source | pH3 | pH4 | pH5 | pH6 | pH7 |
|---|---|---|---|---|---|
| Carboxymethyl-cellulose | 28.57±0.5$^{b}$ | 28.57±0.52b | 44.29±0.56$^{d}$ | 37.14±0.14$^{c}$ | 20±0.55$^{a}$ |
| Wheat straw | 26.66±0.3c | 29.91±0.41$^{d}$ | 38.35±0.29$^{e}$ | 25.71±0.5$^{b}$ | 13.33±0.13$^{a}$ |
| Corn cobs | 20.95±0.55$^{a}$ | 40±0.8$^{d}$ | 65.92±0.31$^{e}$ | 26.19±0.18$^{c}$ | 23.81±0.7$^{b}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different.

**Effect of incubation temperature :**

Influence of different incubation temperatures on the production of cellulolytic enzymes was examined . The inoculated flasks were incubated at different temperatures covering range from 30 to 45°C .The highest CMCase and FPAcellulase activity were at 30°C produced by a wild *A. niger* and the basal medium supplemented with corn cobs as carbon source produced the highest CMCase and FPA cellulase (99.73±1.26 and 91.62±1.9µmol/min/ml respectively) as presented in Tables 9 and 10.

**Table 9: Effect of incubation temperature on CMCase activity by wild *A. niger* on basal media supplemented by different carbon source**

| Carbon source | 30°C | 35°C | 40°C | 45°C |
|---|---|---|---|---|
| Carboxymethyl-cellulose | $38.35\pm0.85^{d}$ | $30.48\pm0.5^{c}$ | $24.371\pm0.30^{b}$ | $14.51\pm0.20^{a}$ |
| Wheat straw | $63.04\pm3.62^{c}$ | $21.43\pm0.3^{b}$ | $16.56\pm0.5^{a}$ | $15.31\pm20^{a}$ |
| Corn cobs | $99.73\pm1.26^{d}$ | $44.29\pm0.3^{c}$ | $16.58\pm0.4^{b}$ | $12.54\pm0.15^{a}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different

**Table 10: Effect of incubation temperature on FPA cellulase activity by wild *A. niger* on basal media supplemented by different carbon source**

| Carbon source | 30°C | 35°C | 40°C | 45°C |
|---|---|---|---|---|
| Carboxymethyl-cellulose | $15.66\pm0.29^{d}$ | $28.57\pm0.27^{c}$ | $17.21\pm0.15^{b}$ | $12.5\pm0.18^{a}$ |
| Wheat straw | $30.33\pm0.83^{c}$ | $25.5\pm0.30^{b}$ | $11.72\pm0.20^{a}$ | $11.23\pm0.12^{a}$ |
| Corn cobs | $91.62\pm1.^{9c}$ | $41.91\pm0.3^{b}$ | $15.5\pm0.15^{a}$ | $15.1\pm0.20^{a}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different

Table 11 and 12show the effect of different incubation temperature on CMCase and FPA cellulase activity in basal media supplemented with cellulose, wheat straw and corn cobs by irradiated *A. niger* (2KGy) by gamma ray. The maximum CMCase and FPA cellulase activity were achieved at 30°C (100.57±0.66 and 65.92±0.31µmo/min/ml respectively) on media supplemented with corn cobs.

**Table 11: Effect of incubation temperature on CMCase activity by irradiated *A. niger* (2KGy) on basal media supplemented by different carbon source**

| Carbon source | 30°C | 35°C | 40°C | 45°C |
|---|---|---|---|---|
| Carboxymethyl-cellulose | $44.31\pm1.13^{d}$ | $30.47\pm0.30^{c}$ | $19.5\pm0.25^{b}$ | $15.5\pm0.15^{a}$ |
| Wheat straw | $58.23\pm1.12^{c}$ | $23.81\pm0.30^{b}$ | $13.5\pm0.17^{a}$ | $12.5\pm0.11^{a}$ |
| Corn cobs | $100.57\pm0.66^{d}$ | $81.91\pm1.1^{c}$ | $15.5\pm0.20^{b}$ | $13.5\pm0.14^{a}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different

**Table 12: Effect of incubation temperature on FPA cellulase activity by irradiated *A. niger* (2KGy) on basal media supplemented by different carbon source**

| Carbon source | 30°C | 35°C | 40°C | 45°C |
|---|---|---|---|---|
| Carboxymethyl-cellulose | $18.19\pm0.14^{c}$ | $30.48\pm0.47^{d}$ | $14.5\pm0.13^{b}$ | $12.35\pm0.25^{a}$ |
| Wheat straw | $38.35\pm0.29^{d}$ | $26.66\pm0.57^{c}$ | $13.5\pm0.40^{b}$ | $11.89\pm29^{a}$ |
| Corn cobs | $65.92\pm0.31^{d}$ | $36.19\pm0.18^{c}$ | $21.13\pm11^{b}$ | $19.8\pm7^{a}$ |

Values are presented as mean ± standard deviation (n=3); All groups are compared to each other at $p<0.05$.
Values with different super scripts along a raw are statistically different

The temperature of fermentation medium is one of vital factors that have the deep influence on the yield and quality of the biosynthesis products (Ahmed *et al*., 2009). Juliet and Oladipo (2013) reported that the maximum cellulase activity at 32° C temperature. As the temperature was increased there was a gradual reduction in enzyme production. This may be due to the fact that higher temperature denatures the enzymes. High temperature may also lead to inhibition of microbial growth (Shazia *et al.,* 2010). Many workers have reported different optimal temperatures for cellulases production either in shake or bioreactor studies using *Aspergillus spp*. Suggesting that the optimum temperature for cellulases production also

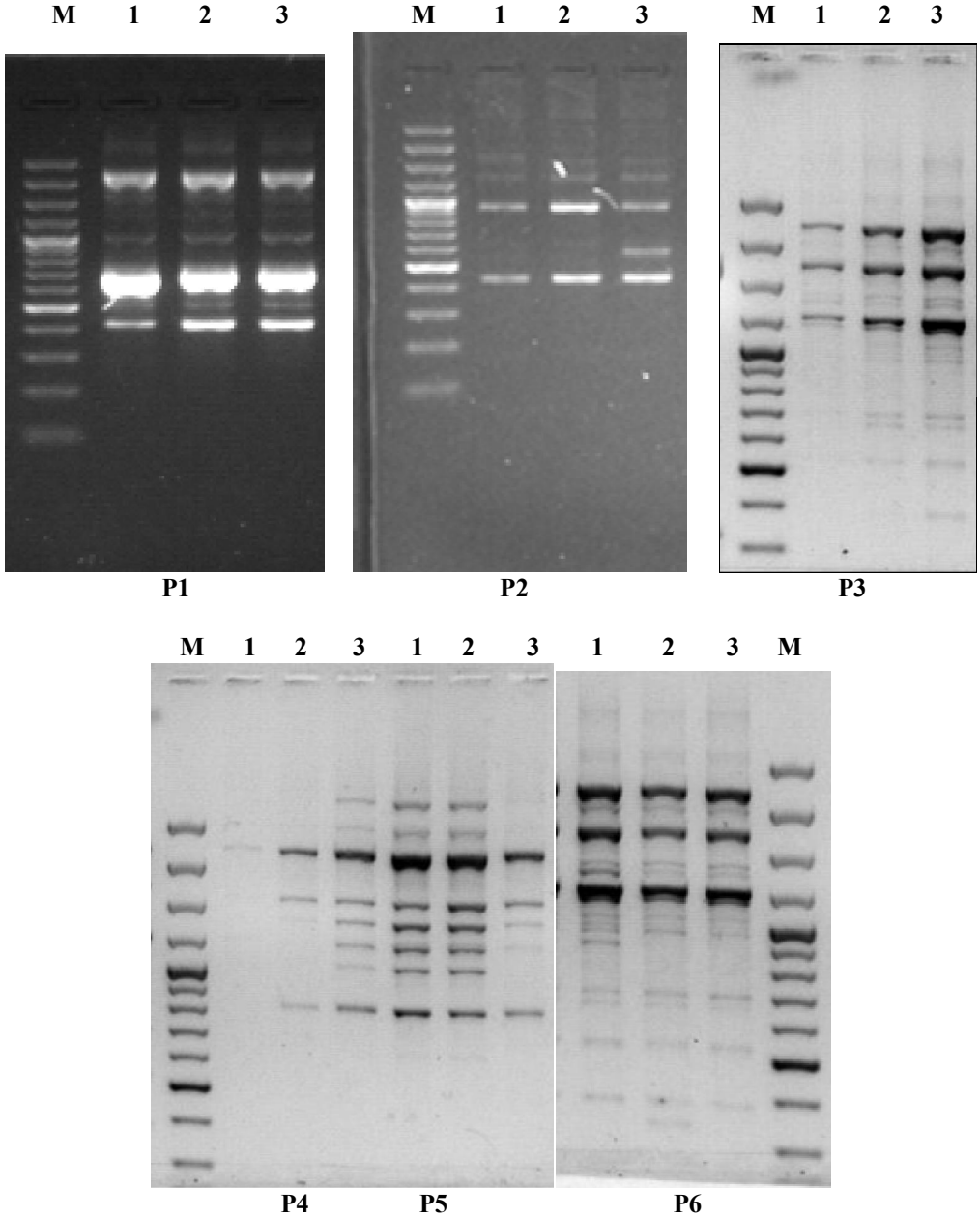
depends on the differences within the same genus of the same fungus (Akinyele *et al.,* 2013a).

**DNA analysis of *Aspergillus niger* exposed and non exposed to gamma irradiation by RAPD- PCR analysis**

Awild *A. niger* and *A. niger* exposed to gamma ray (1KGy and 2 KG) were used to study the effect of two doses of gamma rays on DNA, using RAPD analysis. Analysis of 10 RAPD primers [nucleotide sequences for the 10 primers are shown in (Table 1) leading to a band profiles with a number of amplified DNA fragments shown in (Table 13).

RAPD profiles for a wild *A. niger* and exposed to the gamma radiation (1KGy and 2KG are shown in (Fig. 1). The number of amplified DNA fragments that are generated by gamma rays are also varied depending on the doses of gamma radiation (Table 13).

**Fig. 1. RAPD Profiles of *non* irradiated and irradiated *A. niger* with 1KGy and 2KGy of gamma rays**




Lane M, marker. Lane 1, a wild-type *A. niger* Lane 2, *A. niger* exposed to gamma irradiation 1KGy, Lane 3, *A. niger* exposed to gamma irradiation 2KGy ( P1: primer 1, P2: primer 2, P3: primer 3, P4: primer 4, P5: primer 5, and P6: primer 6 )

**Table 13: DNA polymorphism induced by 1 and 2 KG Gamma rays in *A. niger***

| Primer | *A. niger (1KGy)* | | *A. niger (2KGy)* | |
|---|---|---|---|---|
| | Total bands | % polymorphism | Total bands | % polymorphism |
| P1 | 0 | 0 | 0 | 0 |
| P2 | 2 | 50 | 2 | 50 |
| P3 | 5 | 125 | 8 | 200 |
| P4 | 3 | 150 | 6 | 300 |
| P5 | 0 | 0 | 3 | 37.5 |
| P6 | 2 | 13.3 | 1 | 6.66 |

**Table 14. Number of Amplified DNA fragments produced by gamma ray**

| | |
|---|---|
| *Aspergillus niger* (1KGy) | 12 |
| *Aspergillus niger* (2KGy) | 20 |

Polymorphism may appear as shifts in band migration or missing bands, or differences in band intensities. The number of amplified DNA fragments that are generated by gamma radiation are also varied depending on the doses of gamma ray. The number of bands were 12 and 20 amplified fragments in A. *niger* exposed to 1KGy and 2 KGy gamma ray respectively (Table 14). Finally, these results revealed that RAPD technique can be easily used to demonstrate DNA polymorphism.

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