GENETICAL AND PHYSIOLOGICAL EFFECTS OF ULTRAVIOLET RADIATION ON *Cylindrocarpon heteronemum*

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Cylindrocarpon is one of the most common aquatic fungi that found on decaying vegetation in freshwater habitats (Booth, 1966). Production of chlamydospores allows the fungus to survive in the soil for extent length of time in the absence of a suitable host plant (Insutsa and Merwin, 2000; Dumroese *et al*., 2000). Over the past decade, the emphasis has been on the enzymatic hydrolysis of cellulose to glucose, the efficiency of which depends on many factors governed by the source of cellulose and its decomposition and structure, and of the cellulosic substrate. Both *Cylindrocarpon* and *Lichenicola* which causing corns rot of taro produce carboxymethyl cellulase (Cx) but not filter paper cellulase (C1) (Usharani *et al.*, 1987). Ashadi *et al.* (1996) and Harper *et al*. (1996) observed the optimal treatment conditions for increasing cellulase activity by ultraviolet (UV) mutagenesis by exposure of *Aspergillus terreus* and *Aspergillus niger* spores to ultraviolet germicidal lamp, respectively, at wavelength 250 nm at a distance of 30 cm, while strains of *Trichoderma harzianum,* with either elevated or reduced production of cellulase-complex enzymes and endoglucanse were constructed using UV-light mutagenesis (Gadgil *et al*.,1995; Melo *et al*., 1997). Desphpand *et al*. (1989) and Ashadi *et al*. (1996) reported that *Sclerotium rolfsii* UV-8 mutant produced a constituent cellulase free endoxylanase in synthetic medium containing cellulose 123 that used as a sole carbon source. *Trichodarma veridie* increased production of cellulases where prepared by UV radiation conidia with the greatest width and different reactions to near-UV black light-blue light. Denward *et al*. (1999) indicated that both photosynthetic active radiation (PAR) and UV-A affect the microorganisms involved in the decomposition of leaf litter. The ∝/β-D-glycosidase activity ratio was less than one in shaded microcosms, suggesting a change in the substrate dissolved organic matter composition towards more than β-glucosidic linkages as a result of solar radiation.

From genetic point of view, Prokaryotic and Eukaryotic cells developed a network of DNA repair systems that restore genomic integrity following DNA damage from endogenous and exogenous genotoxic stress (Friedberg *et al*., 1995). The system was able to cope with different kinds of DNA lesions induced by various physical and chemical agents

including environmental sunlight or UV light, ionizing radiation, medical drugs and oxidative metabolism. Defects in DNA repair may cause accumulation of mutations, genetic instability and cell death and, in higher organisms, tumorigenesis and life shortening. With regard to the multiple biological effects encountered after the induction of DNA damage, repair systems play an important role. Holbrook and Forance (1991) and Bender *et al.* (1997) suggested that repair systems play two roles; providing pathways for DNA metabolism, assuring cell survival with or without genetic changes, and a second, not yet much considered role providing signal(s) for the activation of DNA damage- inducible genes and modulation of gene expression. The latter effects are important for intra- and extra cellular signalling as well as for immunological responses (Krutmann *et al*., 1996; Cooper, 1996).

Several reports have been studied using the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique for screening the genetic variation within several species of fungi, *Uncinula nectar* (Delye *et al.,* 1995 and 1997); *Plasmopara viticola* (Seidel *et al.*, 1999); the genus *Alternaria* (Cooke *et al*., 1998); *Verticillium lecanii* and *Beauveria bassiana* (Mitina and Mattila, 2002). Also, there are some reports have been proved that the RAPD pattern which exposed to UV irradiation differed in all cases from control, like within *Ohiostoma ulmi* (Bernier and Hubbes, 1994); *Paecilomyes lilacinus* (Gunasekera *et al.*, 2000) and *Candida albicans* (Pesti, 2001).

In this study the effect of UV radiation on cellulose production of *C. heteronemum* as well as the fungus itself was evaluated associated with some factors such as temperature degrees, pH degrees and incubation periods.

MATERIALS AND METHODS

Fungal isolate and culture media

C. heteronemum was isolated from the River Nile (Elquanater Elkhayria) by El-Shafy (2000). The basic medium of potato dextrose agar (PDA) medium according to Fisher *et al.* (1982) was used for culturing *C. heteronemum.* The basic medium of cellulase medium according to Bland and Douglas (1977) and Data *et al.* (1989) was used for cellulase production.

Source of UV irradiation

Two germicidal UV lamp of 30 watt of the wave length 250 nm and manufactured by Philips in Holland were used for irradiation of samples. The samples of *C. heteronemum* were exposed to the following periods, ½, 1, 1½, 2, 2½, 3, 3½ and 4 hr and then incubated for a week. All irradiated samples were used for studying the growth, the enzymatic activities and genetically variation.

Cellulolytic activity

The basal medium was supplemented with different cellulose sources,

i.e., carboxymethyl cellulose (CMC), salicine, and filter paper (Watman No.1). *C. heteronemum* mycelia discs were inoculated on the surface of the cellulase medium and incubated at 20°C for 72 hr. The culture was further incubated at 50°C for 8 hr to detect celluloytic activity by adding the rosebengal to the medium as described by Bland and Douglas (1997).

Detection of cellulase activity on liquid medium

The culture was prepared by dispersing 49 ml Czapeck-Dox cellulase liquid medium in 250 ml Erlenmeyer flasks. The flasks were sterilized by autoclaving at 1.5 atmospheres and 121°C for 20 min. The medium was then inoculated in the presence of filter paper (Watman No. 1), and then the flasks were incubated at 20°C for 9 days to detect the cellulolytic activity. Cellulase was measured based on the method described by Mandels *et al.* (1976). At the end of incubation period, the culture media were centrifuged at 3000 rpm for 20 min then filtered through filter paper and the filtrates were taken and the cellulase activity and the protein content were detected by Lowery *et al.* (1951).

Factors affecting cellulase activity

In this experiment *C. heteronemum* was subjected to UV radiation. Then, the cellulolytic activity of UV-irradiated fungus was investigated under environmental factors such as temperature degrees, pH degrees and incubation periods, to reach to the optimum conditions for cellulase production. Cellulase activity of *C. heteronemum* was determined at different pH degrees: 3, 5, 7, 9 and 11. The pH was adjusted at different values by using 0.1 N HCl or 0.1 N NaOH. After autoclaving and cooling the flasks which contain 49 ml basal medium for each were inoculated with 1 ml of spore suspension of 7 days old culture. The inoculated flasks were incubated at 20°C, filtered and the clear filtrate was kept for determination of cellulase activity, protein content, and specific activity. Cellulase activity of isolate was determined at the following temperatures: 20, 30, 40 and 50°C respectively for 9 days. A Basel medium was inoculated with 1 ml spore suspension of *C. heteronemum* and incubated at 20°C at different incubation periods, .i.e., 5, 7, 9, 12 and 15 days. At the end of the incubation period, mycelia were separated by filtration and filtrates were kept for further use. Cellulase activity was measured in terms of C1, Cx and β -glucosidase (β-G) enzymes as described by Mandel *et al.* (1976).

Determination of C1 enzyme

To 5 mg filter paper (Watman No.1), 1 ml of sodium acetate buffer (pH 5.0) and 0.5 ml enzyme filtrate were added. The mixture was incubated at 50°C for 30 min (Sternberg, 1976; Nisizawa *et al.*, 1978). The liberated reducing sugars were measured as glucose using 1 ml of 3, 5 dinitrosalcylic acid (DNSA) reagent.

Determination of Cx enzyme

A volume of 0.5 ml of 1% CMC was mixed with 0.1 M sodium acetate buffer (pH 5.0) and 0.5 ml filtrate enzyme in a test tube and incubated 50°C for 30 min. The sugar released expressed, as activity of CX enzyme on CMC was determined as glucose using 1 ml of 3, 5 DNSA reagents. One activity unit was expressed as mg of glucose per ml of filtrate per unit, under the standard assay conditions (Su, 1976).

Determination of β-G enzyme

To 0.5 ml of filtrate enzyme culture, 0.5 ml 1% salicine was added and incubated for 30 min at 50°C the liberated reducing sugars were measured as glucose using 3, 5 DNSA reagent.

Determination of protein

Protein was estimated by the method of (Lowery *et al.*, 1951).

Extraction of genomic DNA extraction

The mycelium of each isolate exposed to UV was harvested and filtered from a colony growing on a PDA liquid medium; mycelia were ground in liquid nitrogen mixed with buffer. DNA was extracted and purified according to the CTAB method described by Nicholson *et al.* (1996). UV spectrophotometer and following gel electrophoresis estimated DNA concentration, by comparison with DNA standard marker.

RAPD-PCR analysis

Amplification reaction was carried out in a total volume of 5o µl as described by Bagheri *et al.* (1995). Each reaction mixture contained 50 ng genomic DNA as a template, 0.4 µM decamer oligonucleotide primer (Operon technologies, Alameda, CA) kit B, 20 primers as shown in Table (1). Two units of *Taq* DNA polymerase (Promega Crop, Madison, WI, USA), 5 µl of 10X buffer (500 Mm KCl, 100 mM Tris-HCl (pH=9.0) and 1% Triton-100), 1.5 mM $MgCl_2$, 0.2 mM dATP, dCTP, dTTP, dGTP) and deionizer dd.H_2O up to 50 µl. The reaction was overlaid with a drop of mineral oil to avoid evaporation. PCR amplification was performed in a Perkin-Elmer Cycler 480 (Norwalk, CT) for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94 for 1 min, annealing at 36°C for 1min, extension at 72°C for 2 min. The primer extension segment was extended to 5 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1% agrose gel at 60 volts for 3.5 hr with 1X TBE buffer. PCR products were visualized and photographed under UV light using a Polaroid camera.

DNA electrophoresis

PCR products were visualized by electrophoresis using 1.2% agarose gel. Gels were viewed under UV light on a 'Gel Doc 1000' System and analyzed using Molecular Analyst Software (Bio-Rad, Hercules, CA).

RESULTS AND DISCUSSION

Effect of UV radiation on enzymatic activity

The effect of different exposure times of UV irradiation on the cellulolytic ability were investigated on a solid medium. The results showed the ability of *C. heteronemum* to produce cellulase enzymes, which were variable as detected by the information of the clear zone around the fungal growth on the solid medium depend on the period of exposure of UV radiation. The least activity in the tested samples was after 4 hr exposure to UV radiation, while the most activity was the control with gradual decreasing by increasing period of UV irradiation (Table 2).

Ashadi *et al.* (1996) and Harper *et al*. (1996) observed the optimal treatment conditions for increasing cellulase activity by UV mutagenesis by exposure of *Aspergillus terries* and *A. niger* spores to UV germicidal lamp respectively, at wavelength 250 nm at a distance of 30 cm. They reported cellulase from a mutant (cellulase higher producer) obtained by UV irradiation, *A. niger* were fractionated into Acicel hydrolyzing cellulase (Avicalase), Cx, and β-G by DFAE-Sepharose CL-6B column chromatography and all purified enzymes were homogenous. They found a strong synergistic action was shown by the combination Avicalase alone with Cx and β-G.

Effect of temperature on cellulases production

Results in Table (3) illustrated the effect of temperature degrees on Cx, C1 and β-G enzymes as well as biomass, protein content and specific activity after exposure of *C. heteronemum* to different exposure periods of UV irradiation on liquid media. The results considering biomass production showed that biomass decreased with increase exposure period of UV irradiation at all different temperatures, while the maximum biomass (25 g/L) was obtained at 20°C. Biomass markedly decreased by rise in temperature and minimum value of biomass (0.2 g/L) obtained at 50°C after irradiation for 4 hr (Table 3). On the other hand, the results recorded that the exposure time of ½ hr to 1 hr of UV irradiation stimulate production Cx, C1, and β-G enzymes at all different temperatures, then the activity decreased to reach its lowest with increased temperatures and irradiation periods (Table 3). C1 and β-G enzymes showed high differences among the studied range of temperatures (20-50°C). These activities of both C1, and β-G enzymes increased and reached maximum activity (129 and 195 μ/L), respectively, at 20°C compared with control and rise in the incubation temperature up to 50°C decreased the activity.

In general, the 20°C was the most convenient for the production of total cellulases activity (498 μ/L), and 1 hr for the exposure period of UV radiation and the minimum production was 151 μ/L at

50°C and after 3 ½ hr of exposed period of UV radiation. Kvesitadze *et al.* (1999) found that fungi, comprising about 4000 cultures, were collected from different climatic zones of the southern Caucasus (Georgia) during 1967-1989. Almost all cultures in the collection showed a high potential for degrading basic different plant biopolymers such as cellulose and hemicellulose. Cultures of *Penicillium canescens* possessing comparatively low cellulase activity in their wild type were treated with UV light, which produced genetically stable mutant. Few of them had high xylanase and no cellulase activities. More than 6% of all cultures in the collection were thermophiles and, from these, 56 cultures with the highest cellulase and xylanase activity were selected. It was shown that under two thermophilis growth conditions, 40 and 48°C.

Effect of pH on cellulases production

Result in Table (4) illustrated UV irradiation on celluloytic activities on dry weight and protein content of *C. heteronemum* at different pH. Biomass production, Cx, C1, and β-G were markedly inhibited under conditions of high acidity pH 3.0 or high alkalinity pH 11.0, while pH 5.0 was the best for maximum dry weight (2.5 g/L) obtained at the control, Cx (152 μ/L), C1 (141μ/L) β-G (205 μ/L), and was also maximum for the production of total cellulases activity (498 μ/L) at 1 hr exposure of UV irradiation (Table 4). According to effect of exposure period of UV irradiation at 1 hr exposure, biomass production, Cx, C1, and β-G were stimulated at pH 5.0 and pH 7.0, and then activity decreased to reach its lowest with increased irradiation periods. Ashadi *et al.* (1996) reported that *Sclerotium rolfssi* UV-8 mutant produced a constituent cellulose free endoxylanase in synthetic medium containing cellulose 123 used as a sole carbon source. They added that the endoxylanase having specific activity (340 IU mg super (-1) has been purified from the culture filtrate and its biochemical properties have been studied and endoxylanase shows no action on cellulose and has a maximum activity on larchwood xylan at pH 4.5 and 65°C).

Effect of incubation period on cellulases production

The results of Table (5) showed that the growth of *C. heteronemum* reached its maximum growth after 9 days (2.5 g/L). The results showed clearly that cellulases especially Cx, C1, and β-G enzymes increased by increasing in incubation period and also reached their maximum activity (145 μ/L, 129 μ/L, 195 μ/L), respectively at 9 days incubation periods. Total cellulases activity showed the same trend and producing maximum (469 μ/L), after the same incubation period (Table 5).

RAPD-PCR analysis

To elucidate genetic markers for of *C. heteronemum* and the same one exposed to different periods of UV radiation (½, 1, 1½, 2, 2½, 3, 3½ and 4 hr), six primers (OPA-10, OPB-10, OPB-

11, OPC-06, OPZ-04 and OPZ-18) were used for the RAPD-PCR analysis of genomic DNA. Result of RAPD-PCR analysis are shown in Figs (1, 2 and 3) and Table (6), it elucidated clearly differences among the mycelium of control and others exposed to different periods of UV irradiation. DNA isolated from control which was characterized by the presence of 7 unique fragments that disappeared in the DNA extracted from mycelia exposed to different periods of UV radiation. These fragments have approximate sizes of 2186, 1742 and 553 (bp) with the primer OPC-06; 1604, 415 and 315 bp with the primer OPB-10, and 415bp with the primer OPB-11. However, genomic DNA analysis mycelium exposed to ½ hr period of UV radiation revealed the occurrence of 3 distinct DNA fragments were with the following sizes; 2014 and 695 (bp) with OPB-10; 2014 (bp) with OPB-11 which disappeared with control and the other treatments. DNA analysis of mycelium those exposed to 1 hr exposure period of UV irradiation was characterized by the presence of 7 unique fragments that disappeared in the DNA of control and other treatments as following: 2422 and 695 (bp) with OPB-10; 801 and 316 (bp) with POB-11; 3192 and 2299 (bp) with OPC-06 and 3554 (bp) with OPZ-04. In generally, exposure periods ½ and 1 hr of UV radiation made stimulation of growth and cellulose activities, so interesting fragments 2014, 695, 2422 and 695 (bp) with OPB-10; 2014, 801 and 316 (bp) with POB-11; 3192 and 2299 (bp) with OPC-06 and 3554 (bp) with OPZ-04 could be considered as stimulation a specific fragments causing stimulation of growth and activity because they have not been shown in all other treatments.

Seven DNA fragments were detected in mycelium of *C. heteronemum* exposed to 1½ hr of UV radiation as following: 3714 and 2026 bp (OPA-10), 963, 812, 395 and 310 (bp) with OPB-11 and 1141 (bp) with OPZ-04 which appeared only with this exposure time. A set of 10 DNA fragments with size, 872 bp with (OPA-10); 2388, 1537, 480, 395 and 310 bp with (OPB-10); 3656 and 429 (bp) with (OPB-11); 755 (bp) with OPC-06 and 3514 (bp) with (OPZ-04), were shown in the DNA isolated from mycelium exposed to 2 hr of UV irradiation. Another set of 7 DNA fragments were detected with mycelium treated at 2 ½ hr of UV irradiation as following: 2186 bp (OPA-10); 1297 and 735 bp (OPB-10), 37O8, 408 and 325 bp (OPB-11); 2543 bp (OPC-06). A third set of 6 DNA fragments were detected with mycelium treated at 3 hr of UV irradiation as following: 2354, 473 and 383 (bp) with OPB-10; 3815 (bp) with OPB-11; 3443 and 2078 (bp) OPC-06. A fourth set of 10 DNA fragments were detected with mycelium treated at 3½ hr of UV irradiation as following: 2242 and 854 (bp) OPA-10; 429 and 325 (bp) with OPB-10; 4857, 3981, 436, 342 and 92 (bp) OPB-11; 1535 (bp) with OPC-06. A last set of 11 DNA fragments were detected with mycelium treated at 4 hr (lethal exposed period) of UV irradiation as following: 2675, 891, 507 and 135 bp (OPA-10), 936

(bp) with OPB-10; 4997, 3925, 2072, 1494, 336 and 77 bp (OPB-11).

OPZ-18 primer out of the six primers gave not clearly differences of amplified product patterns as shown in Table (6) exposed at 4 hr exposure period of UV irradiation gave six unique fragments with OPZ-18 compared to 8 bands among control and other treatments. Within these 16 fragments were detected at 4 hours (lethal exposed period), there were 10 fragments appeared (2675, 891, 507 and 135bp (OPA-10), 936bp (OPB-10), 4997, 3925, 1494, 336 and 77bp (OPB-11) appeared and 6 others disappeared with OPZ-18; 1860, 1333, 1163, 703, 591 and 335 (bp), so they could be considered as inhibited fragments that causing stop of growth and activity completely. Moreover, one fragment from control seedlings and disappeared with all exposed periods and then was initiated again at 4 hours (lethal exposed period) at 715 (bp) with primer OPB-10.

The main aim of this study was to find molecular markers to discriminate between mycelium of *C. heteronemum* at different exposed periods of UV irradiation. Mulligan (1997) reported that UV light is particularly effective at generating primidine dimmers and the conjugated ring systems of adjacent thymine bases in a polynucleotide chain will absorb UV light and from a cyclobutane ring which links carbons 5 and 6 of each primidine ring to another. He was added that adjacent thymine and cytosine bases can also be photoactive to form a 6-4 between the two bases. Averbeck and Dardalhon (1990) and Kiefer and Feige (1993) showed that the occurrence of DNA double-strand breaks during UV post-treatment incubation for yeast and is likely to occur in mammalian cells. Rajappan *et al.* (1996) showed an attempt was made to induce mutant of *Trichoderma viride* by UV radiation and to assessits effect on the groundnut rot pathogen, *S. rolfsii* {Corticium rolfsii}, *in vitro*. The UV treatment altered the genetic make-up of *T. viride* and increased its bio control abilities. Mulligan (1997) reported that mutations can arise randomly or as a result of specific targeting (site-directed mutagenesis) DNA is a chemical molecule and is subjected to reactions which are both random and non-random with either chemical or radiation.

On the DNA level, RAPD-PCR analysis showed genetic variation between samples irradiated with UV irradiated *C. heteronemum*. Pesti *et al.* (2001) revealed that molecular typing methods were applied to characterized four stable morphological mutants mutant colony of Candida albicans, the intermediate unstable mutant and its four morphologically altered derivatives revealed the same electrophoresis karyotyping, in contrast the random amplification DNA polymorphism patterns of mutant strains differed in all cases from that of the parental strains.

Sarachek and Henderson (1988) reported that evidence is presented that

gene conversion and reciprocal recombination contribute to UV-induced segregations of heterozygous markers from both diploid and hybrid strains of Candida albicans and that hybrids also segregate through induced chromosome loss. Heterozygous diploids strains independently derived from the same wild-type diploids responses, and the organization of a 4 gene linkage group identified in diploids from the segregant products of reciprocal recombination was transmitted intact to all hybrids from fusions between diploid of isogonics.

Generally the RAPD-PCR technique was used to detect DNA damage in the *C. heteronemum* exposed to irradiances of UV. To investigate the potential of this method in ecotoxicological assessments, the qualitative and quantitative modifications in RAPD-PCR profiles were compared with changes in a number of physiological and fitness parameters. RAPD detectable modifications in DNA profiles were observed in all UV exposed individuals compared with controls. Changes in chlorophyll fluorescence (F[v]/F[m] ratio), *in vivo* pigment absorptance, thallus growth and RAPD profiles, examined simultaneously, provided a sensitive measure of UV-induced toxicity.

Using scoring procedure, all DNA fragments were scored to generate genetic dissimilarity coefficients (Nei, 1972). This data matrix was used to generate the genetic distance matrix. The dendrogram was generated from the genetic distance matrix according to the UPGMA clustering method using the NTSYS Program. The results revealed how much mutation happen through exposed the mycelia to the different time so the dendogram shows the popular accessions of each mycelium treated with certain exposure time were divided into two groups (Fig. 4). In other words, the obtained dendogram was in accordance with actual grouping of the effect of different exposure time to the DNA genome. All mycelia of *C. heteronemum* exposed to different exposure time except at 4 hr were grouped in one group and all other mycelia exposed to UV and control on other group. Also, mycelia of control, 2 hr, 3 hr and 3½ hr were grouped in subgroup. Mycelia at ½ hr, 2½ hr, 1 hr and 1½ hr were grouped in other subgroup. The 4 hr separated it could be due to presence of a lot of mutations. Similarity of ½ hr and 1 hr and away from control and other treatment may be because they have small number of mutation which could be responsible for stimulation of enzyme and growth (Table 7).

In conclusion, the application of the RAPD-PCR method in conjunction with other suitable physiological and fitness measurements may prove to be a valuable tool for investigating the specific effects of genotoxic agents upon fungal populations. Ultimately, this methodology may allow the ecotoxicological examination of the link between molecular alterations and measurable adverse effects at higher levels of biological organisation.

SUMMARY

In this study the effect of ultraviolet (UV) radiation on cellulose production of *Cylindrocarpon heteronemum* as well as the fungus itself was evaluated associated with some factors such as temperature degrees, pH degrees and incubation periods. Results showed that exposure periods of ½ and 1 hr of UV radiation stimulated the production of carboxymethyl cellulase (Cx), filter paper cellulase (C1), and β-glucosidase (β-G) enzymes at 20°C, pH 5.0 and 7.0, 9 days post incubation, and then the activity was decreased to reach its lowest with increase temperatures, pH, incubation periods and exposure period of radiation. The genetic variation between wild type and the UV treated-mycelium of *C. heteronemum* was determined by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Through the analysis of genomic DNA of *C. heteronemum*, the wild type was distinguished by presence of 9 unique fragments that disappeared in the DNA extracted from UV-treated mycelia. Some specific fragments were produced only at ½ and 1 hr exposure times which disappeared in the DNA of control. It might responsible for causing induction of growth and enzymatic activity or correlated to them. A number of specific fragments were detected with the lethal period of UV and disappeared with control and others; it could be correlated to the inhibition of growth.

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Table (1): Primers used for RAPD-PCR analysis.

Primer codes	Sequences (5'----------3')	% GC
OPA-10	GTG ATC GCAG	60
OPB-10	GGT CTA CACC	60
OPB-11	GTA GAC CACC	60
OPC-06	GAA CGG ACTC	60
OPZ-04	AGG CTG TGCT	60
OPZ-18	AGG GTC TGTG	60

Table (2): Effect of UV rradiation on cellulase production by *C. heteronemum.*

Ultraviolet exposure period (hour)	Mean Diameter of clear zone (mm)
0	8.0
½	7.6
1	7.0
1½	6.0
2	5.2
2½	4.6
3	4.0
3½	2.8
4	2.0

Table (3): Effect of UV radiation on cellulolytic activities (U/L), on dry weight (g/L) and content (mg/L) of *C. heteronemum* at various temperature degrees.

Temperature degrees (°C)	Parameters	UV irradiation periods (hr)								
		0	½	1	1½	2	2½	3	3½	4
20	DW	2.5	2.1	1.6	1.4	1.3	0.9	0.7	0.7	0.6
	PC	76.9	68.6	56.4	53.8	52.3	53.8	52.3	49.2	43.1
	Cx	145	148	152	141	125	125	120	117	106
	C1	129	131	141	127	109	107	103	95	91
	β-G	159	195	205	185	173	160	149	135	124
	T.A	469	474	498	448	416	392	369	346	319
	S.A	6.0	6.7	8.3	7.9	6.9	6.6	6.4	6.6	8.0
30	DW	1.3	1.3	1.0	1.0	0.8	0.7	0.6	0.5	0.4
	PC	90.7	84.6	87.7	73.9	70.8	69.2	66.1	61.5	53.9
	Cx	125	118	122	106	105	92	90	87	84
	C1	125	128	132	104	108	94	84	82	80
	β-G	142	145	148	113	106	105	95	91	87
	T.A	392	392	409	323	319	291	269	260	251
	S.A	4.3	1.6	4.7	4.4	4.5	4.2	4.1	4.2	4.7
40	DW	1.0	0.9	0.9	0.8	0.7	0.6	0.5	0.4	0.3
	PC	111	92.8	83.1	63.1	53.9	49.2	43.1	38.5	36.5
	Cx	120	120	125	103	100	88	79	74	72
	C1	105	107	111	89	82	78	69	66	59
	β-G	118	127	127	112	105	102	100	96	88
	T.A	353	354	363	314	287	260	228	206	219
	S.A	3.1	3.7	4.4	5.0	5.3	5.3	5.3	5.3	6.0
50	DW	0.8	0.7	0.7	0.6	0.5	0.5	0.4	0.3	0.2
	PC	65.2	60	65.2	54.8	52.2	39.1	36.5	33.9	26.2
	Cx	110	109	112	101	98	86	77	73	70
	C1	57	57	60	45	42	35	35	32	29
	β-G	91	93	95	85	80	74	70	62	54
	T.A	258	259	267	231	220	185	167	151	153
	S.A	3.8	4.1	4.1	4.4	4.2	4.2	4.1	4.1	5.8

DW: Dray weight, PC: Protein content Cx: Carboxymethyl cellulose, C1: Filter paper cellulose, β-G: β-glucosidase (Salicine), T.A: Total activity, S.A: Specific activity.

Table (4): Effect of UV radiation on cellulolytic activities (U/L), on dry weight (g/L) and content (mg/L) of *C. heteronemum* at various pH degrees.

pH Degrees	Parameters	UV irradiation periods (hr)								
		0	½	1	1½	2	2½	3	3½	4
3.0	DW	0.8	0.8	0.7	0.7	0.7	0.6	0.5	0.4	0.3
	PC	57.4	62.6	60	53.8	37.6	40	32.9	25.8	24
	Cx	125	120	129	119	116	111	110	95	93
	C1	94	97	98	90	90	88	80	78	75
	β-G	98	94	90	93	82	80	74	71	69
	T.A	317	311	317	302	288	279	264	244	237
	S.A	5.5	5.0	5.3	5.0	7.7	8.0	8.6	9.5	9.9
5.0	DW	2.5	2.1	1.6	1.4	1.3	0.9	0.7	0.7	0.6
	PC	76.9	68.6	56.4	53.8	52.3	53.8	52.3	49.2	40
	Cx	145	148	152	141	125	128	120	117	106
	C1	129	131	141	127	109	107	103	95	91
	β-G	195	195	205	185	173	160	149	135	124
	T.A	469	473	477	418	362	355	335	323	319
	S.A	6.0	6.7	8.3	7.9	6.9	6.6	6.4	6.6	8.0
7.0	DW	1.1	1.0	1.2	1.0	0.9	0.9	0.8	0.7	0.6
	PC	65.2	70.4	55.4	55.4	52.2	44.7	40	37.5	35.3
	Cx	144	142	155	137	130	121	119	115	105
	C1	118	113	120	110	107	111	102	99	94
	β-G	187	185	157	167	158	117	115	103	96
	T.A	449	440	432	417	395	349	336	317	295
	S.A	6.9	6.1	7.8	6.7	9.6	9.5	9.3	8.5	8.4
9.0	DW	0.9	0.9	1.0	0.9	0.8	0.8	0.6	0.6	0.5
	PC	60	60	64	55.4	40	37.6	35.3	35.3	30.6
	Cx	135	131	138	127	123	113	112	105	102
	C1	111	118	117	109	105	103	100	94	88
	β-G	180	185	185	170	156	141	118	107	103
	T.A	426	434	440	406	384	357	330	306	293
	S.A	7.1	7.2	6.8	6.7	9.6	9.5	9.3	8.7	9.6
11.0	DW	0.8	0.8	0.8	0.7	0.7	0.7	0.5	0.5	0.4
	PC	44.6	54.7	40	40	35.3	30.6	24	24	20
	Cx	128	124	134	121	119	113	105	97	95
	C1	102	102	101	100	100	98	95	88	80
	β-G	119	127	131	116	109	107	103	98	89
	T.A	349	353	366	337	328	318	303	293	264
	S.A	7.8	6.4	6.7	8.4	9.3	10.4	12.6	12.2	13.2

DW: Dray weight, PC: Protein content, Cx: Carboxymethyl cellulose, C1: Filter paper cellulose, β-G: β-glucosidase (Salicine), T.A: Total activity S.A: Specific activity.

Table (5): Effect of UV irradiation on cellulolytic activities (U/L), on dry weight (g/L) and content (mg/L) of *C. heteronemum* at different incubation periods (days).

Temperature degrees (°C)	Parameters	UV irradiation periods (hr)								
		0	½	1	1½	2	2½	3	3½	4
5	DW	2.1	2.1	1.8	1.6	1.5	1.1	0.8	0.7	0.6
	PC	60	52.2	60	54.8	35.3	30.6	30.6	28.3	24
	Cx	123	126	127	113	100	103	95	90	87
	C1	107	107	112	99	93	89	82	78	71
	β-G	121	122	130	115	105	99	92	80	75
	T.A	351	355	369	327	298	291	269	248	233
	S.A	5.9	6.8	6.2	6.0	8.4	9.5	8.8	8.8	9.7
7	DW	2.3	2.2	2.0	1.9	1.7	1.4	0.9	0.8	0.7
	PC	65.2	57.4	60	60	54.8	37.7	32.9	30.6	26.2
	Cx	126	133	135	117	110	107	97	95	88
	C1	115	118	126	105	101	94	89	83	80
	β-G	160	165	170	151	143	136	124	113	102
	T.A	401	416	431	373	354	337	310	291	270
	S.A	6.2	7.2	7.2	6.2	6.4	8.9	9.4	9.5	10.3
9	DW	2.5	2.1	1.6	1.4	1.3	0.9	0.7	0.7	0.6
	PC	76.9	68.6	56.4	53.8	52.3	53.8	52.3	49.2	40
	Cx	145	148	152	141	128	125	120	117	106
	C1	129	131	141	122	115	106	99	94	89
	β-G	195	195	205	185	173	160	149	135	124
	T.A	469	473	498	418	362	355	335	323	319
	S.A	6	6.7	8.3	7.9	6.9	6.6	6.4	6.6	8
12	DW	2.2	2.1	1.9	1.8	1.5	1.1	0.8	0.6	0.5
	PC	75.6	62.6	57.4	52.2	40	35.3	32.9	30.6	26.2
	Cx	146	149	157	140	128	120	114	103	96
	C1	124	129	135	120	113	105	98	91	85
	β-G	150	154	162	145	133	122	111	105	100
	T.A	420	432	454	405	374	347	323	299	281
	S.A	5.6	6.9	7.9	7.8	9.4	9.8	9.8	9.8	10.7
15	DW	1.8	1.8	1.7	1.6	1.4	1.2	1.0	0.8	0.7
	PC	56.9	56.9	53.8	52.2	40	40	35.3	30.6	23.1
	Cx	120	123	130	116	105	100	93	88	84
	C1	105	110	118	102	94	90	86	83	78
	β-G	135	135	142	130	125	119	110	101	96
	T.A	360	368	390	348	324	309	289	272	258
	S.A	8.3	6.5	7.2	8.7	8.3	7.7	8.2	8.9	11.7

DW: Dray weight, PC: Protein content, Cx: Carboxymethyl cellulose, C1: Filter paper cellulose, β-G: β-glucosidase (Salicine), T.A: Total activity S.A: Specific activity.

Table (6): RAPD-PCR analysis of DNA genome of *C. heteronemum* exposed to different periods of UV light using six primers.

DNA fragment (bp)	Control	UV exposure time (hr)							
		½	1	1 ½	2	2 ½	3	3 ½	4
OPA-10									
3714	0	0	0	1	0	0	0	0	0
2743	0	0	1	1	0	0	0	0	0
2675	0	0	0	0	0	0	0	0	1
2608	0	0	0	0	0	1	0	0	0
2242	0	0	0	0	0	0	0	1	0
2186	0	1	0	0	0	1	0	0	0
2131	1	0	1	0	1	0	1	0	1
2026	0	0	0	1	0	0	0	0	0
1742	0	0	0	0	0	0	1	1	0
1193	1	1	1	1	1	1	1	1	1
891	0	0	0	0	0	0	0	0	1
872	0	0	0	0	1	0	0	0	0
854	0	0	0	0	0	0	0	1	0
837	1	1	0	0	1	0	1	0	0
803	0	0	1	1	0	1	0	0	0
507	0	0	0	0	0	0	0	0	1
492	1	1	1	1	1	1	1	1	0
328	0	0	1	1	1	1	0	0	0
141	0	0	0	0	0	1	1	0	0
135	0	0	0	0	0	0	0	0	1
58	0	1	1	0	0	0	0	0	0
UF	0	0	0	2	1	1	0	2	4
OPB-10									
2872	0	0	0	0	0	0	0	1	1
2422	0	0	1	0	0	0	0	0	0
2388	0	0	0	0	1	0	0	0	0
2354	0	0	0	0	0	0	1	0	0
2321	0	1	0	1	0	0	0	0	0
2288	0	0	0	0	0	0	0	1	1
2014	0	1	0	0	0	0	0	0	0
1627	0	0	0	1	0	1	0	0	0
1604	1	0	0	0	0	0	0	0	0
1582	0	0	0	0	0	0	1	1	0
1559	0	1	1	0	0	0	0	0	1
1537	0	0	0	0	1	0	0	0	0
1297	0	0	0	0	0	1	0	0	0
1125	1	1	1	1	1	1	1	1	1
936	0	0	0	0	0	0	0	0	1
860	1	1	1	1	1	1	1	1	0
735	0	0	0	0	0	1	0	0	0
715	1	0	0	0	0	0	0	0	9
705	0	0	1	1	1	0	1	1	0
695	0	1	0	0	0	0	0	0	0
593	1	1	1	1	1	1	1	1	1
480	0	0	0	0	1	0	0	0	0
473	0	0	0	0	0	0	1	0	0
429	0	0	0	0	0	0	0	1	0
415	1	0	0	0	0	0	0	0	0
395	0	0	0	0	1	0	0	0	0
383	0	0	0	0	0	0	1	0	0
325	0	0	0	0	0	0	0	1	0
315	1	0	0	0	0	0	0	0	0
310	0	0	0	0	1	0	0	0	0
UF	3	2	1	0	5	2	3	2	1
OPB-11									
4997	0	0	0	0	0	0	0	0	1
4857	0	0	0	0	0	0	0	1	0
3981	0	0	0	0	0	0	0	1	0
3925	0	0	0	0	0	0	0	0	1
3815	0	0	0	0	0	0	1	0	0
3708	0	0	0	0	0	1	0	0	0
3656	0	0	0	0	1	0	0	0	0
2872	1	1	1	0	1	1	1	1	1
2101	0	0	1	0	1	1	0	0	0
2072	0	0	0	0	0	0	0	0	1
2042	1	0	0	0	0	0	1	1	0
2014	0	1	0	0	0	0	0	0	0
1494	0	0	0	0	0	0	0	0	0
1473	1	0	1	0	0	0	0	0	0
1453	0	1	0	1	1	0	0	0	0
1432	0	0	0	0	0	1	1	0	0
1125	1	1	1	1	1	1	1	1	1
963	0	0	0	1	0	0	0	0	0
812	0	0	0	1	0	0	0	0	0
801	0	0	1	0	0	0	0	0	0

Table (6): Cont'

778	0	0	0	0	1	0	0	0	0
593	1	1	1	1	1	1	1	1	1
436	0	0	0	0	0	0	0	1	0
429	0	0	0	0	1	0	0	0	1
415	1	0	0	0	0	0	0	0	0
408	0	1	0	0	0	1	0	0	0
402	0	0	1	0	0	0	1	0	0
395	0	0	0	1	0	0	0	0	0
342	0	0	0	0	0	0	0	1	0
336	0	0	0	0	0	0	0	0	1
325	0	0	1	0	0	0	0	0	0
315	0	0	0	1	0	0	0	0	0
310	0	0	0	1	0	0	0	0	0
92	0	0	0	0	0	0	0	1	0
77	0	0	0	0	0	0	0	0	1
UF	1	1	2	4	2	4	1	5	6
OPC-06									
3531	0	0	0	0	0	0	0	1	1
3443	0	0	0	0	0	0	1	0	0
2675	1	0	0	1	0	0	0	0	0
2608	0	0	0	0	1	0	0	0	0
2543	0	0	0	0	0	1	0	0	0
2480	0	1	0	0	0	1	1	0	1
2299	0	0	1	0	0	0	0	0	0
2186	1	0	0	0	0	0	0	0	0
2078	0	0	0	0	0	0	1	0	0
2026	0	0	0	0	0	1	0	1	0
1742	1	0	0	0	0	0	0	0	0
1656	0	0	0	1	1	0	0	0	0
1615	0	0	0	0	0	1	0	0	1
1574	0	1	1	0	0	1	0	0	0
1535	0	0	0	0	0	0	0	1	0
1193	1	1	1	1	1	1	1	1	1
970	1	0	0	0	1	0	0	0	1
929	0	1	0	0	0	1	0	1	0
910	0	0	1	1	0	0	1	0	0
771	1	1	1	1	1	1	1	1	1
755	1	0	0	0	1	0	0	0	0
740	0	1	0	0	0	0	0	0	1
725	0	0	1	0	0	0	1	1	0
710	0	0	0	1	0	0	0	0	0
553	1	0	1	0	1	1	1	1	1
492	1	=	1	0	1	1	1	1	1
369	0	1	0	1	0	0	0	0	0
328	1	0	1	0	1	1	1	1	1
252	0	0	0	0	1	0	0	0	0
58	0	1	1	1	0	1	0	0	1
UF	3	1	2	0	1	1	2	1	0
OPZ-04									
3554	0	0	1	0	0	0	0	0	0
3514	0	0	0	0	1	0	0	0	0
3320	0	0	0	0	0	0	1	0	0
3245	1	0	0	0	1	1	0	0	1
2615	1	1	1	1	1	1	1	1	1
2256	1	1	1	1	1	1	1	1	1
2060	1	1	1	1	1	1	1	1	1
1818	1	1	1	1	1	1	1	1	1
1465	0	1	1	1	0	1	1	0	0
1323	1	1	1	1	1	1	1	1	1
1141	0	0	0	1	0	0	0	0	0
943	1	1	1	1	1	1	1	1	1
792	1	1	1	1	1	1	1	1	1
637	1	1	1	1	1	1	1	1	1
542	1	1	1	1	1	1	1	1	1
487	1	1	1	1	1	1	1	1	1
394	1	1	1	1	1	1	1	1	1
252	0	0	0	0	1	0	1	1	0
216	0	0	0	0	1	0	1	1	0
UF	0	0	1	1	1	0	1	0	0
OPZ-18									
1860	1	1	1	1	1	1	1	1	0
1333	1	1	1	1	1	1	1	1	0
1163	1	1	1	1	1	1	1	1	0
859	1	1	1	1	1	1	1	1	0
703	1	1	1	1	1	1	1	1	0
591	1	1	1	1	1	1	1	1	0
516	1	1	1	1	1	1	1	1	1
335	1	1	1	1	1	1	1	1	0
UF	0	0	0	0	0	0	0	0	0

Table (7): Similarities matrix of DNA genome of *C. heteronemum* exposed to different UV incubation periods (UVIP) based on RAPD-PCR analysis using 6 primers.

UVIP (hr)	Control	½ h	1 h	1½ h	2 h	2½ h	3 h	3½ h	4h
Control	1.000								
½	0.645	1.000							
1	0.687	0.694	1.000						
1½	0.598	0.688	0.686	1.000					
2	0.718	0.608	0.667	0.604	1.000				
2½	0.660	0.722	0.724	0.641	0.642	1.000			
3	0.693	0.640	0.717	0.596	0.673	0.673	1.000		
3½	0.653	0.600	0.642	0.558	0.636	0.636	0.722	1.000	
4	0.581	0.522	0.531	0.417	0.529	0.545	0.500	0.520	1.000

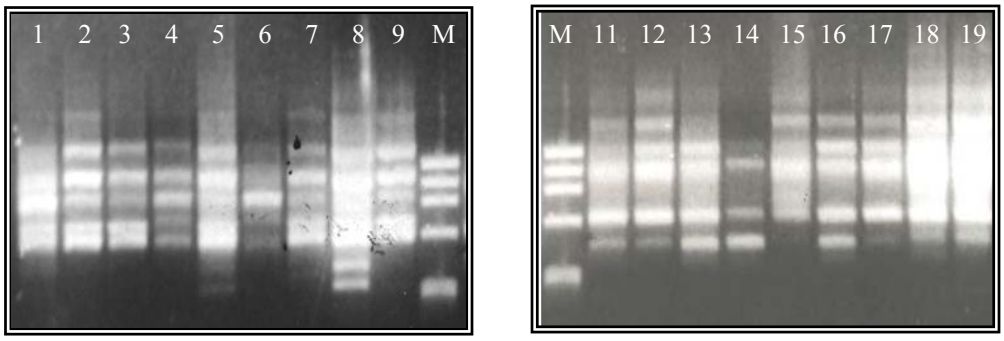

Fig. (1): RAPD-PCR analysis of genomic DNA of *C. heteronemum* exposed to different times of UV. Lane (M) represented the marker used (phi * 174 Hae III), lanes (1) and (11) are represented the control and lanes from (2-9) are represented irradiated *C. heteronemum* at different exposure periods of UV (½, 1, 1½, 2, 2½, 3, 3½ and 4 hrs), respectively, generated by primer OPB-10. Lanes from (12-19) are represented by primer OPB-11.

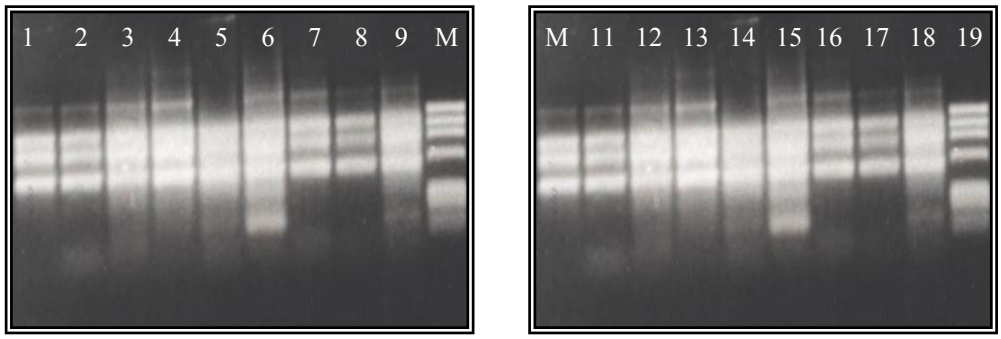

Fig. (2): RAPD-PCR analysis of genomic DNA of *C.heteronemum* exposed to different times of UV. Lane (M) represented the marker used (phi * 174 Hae III), lanes (1) and (11) are represented the control and lanes from (2-9) are represented irradiated *C. heteronemum* at different exposure periods of UV (½, 1, 1½, 2, 2½, 3, 3½ and 4 hrs), respectively, generated by primer OPA-10. Lanes from (12-19) are represented by primer OPC-06.

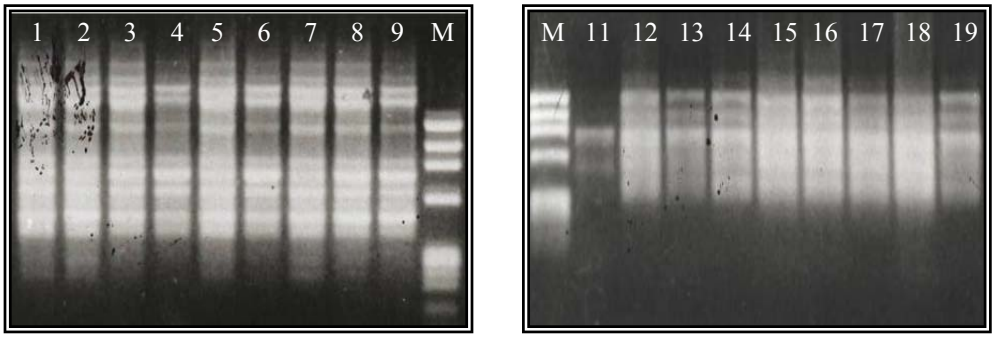

Fig. (3): RAPD-PCR analysis of genomic DNA of *C. heteronemum* exposed to different times of UV. Lane (M) represented the marker used (phi * 174 Hae III), lanes (1) and (11) are represented the control and lanes from (2-9) are represented irradiated *C. heteronemum* at different exposure periods of UV (½, 1, 1½, 2, 2½, 3, 3½ and 4 hrs), respectively, generated by primer OPZ-04. Lanes from (12-19) are represented by primer OPZ-14.

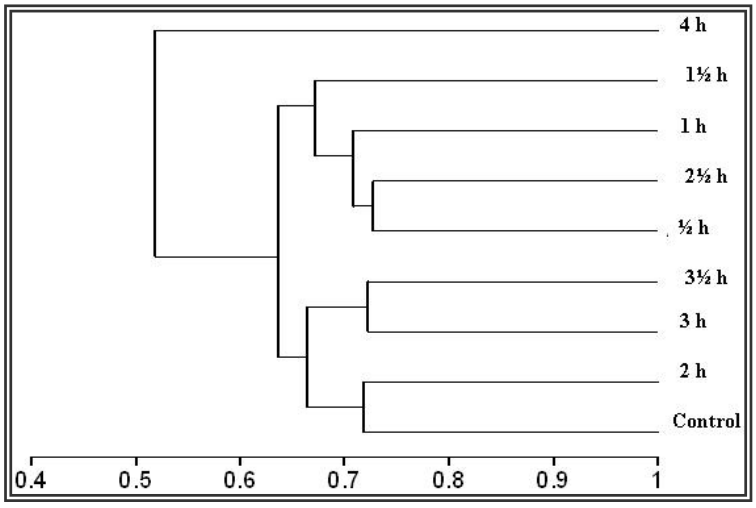

Fig. (4): Phylogenetic tree of DNA genome of *C. heteronemum* exposed to different UV incubation periods based on RAPD-PCR analysis using 6 primers.