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DECOLORIZATION OF SOME TEXTILE DYES BY *PLEUROTUS CITRINOPILEATUS* AND *ASPERGILLUS NIGER* IMMOBILIZED ON WHEAT STRAW

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Abstract: The potential of wheat straw immobilized *Aspergillus niger* and *Pleurotus citrinopileatus* were studied for biodegradation ability of different types of textile dyes. Non exposed and exposed tested immobilized fungi to (1 KGy and 2 KGy) of gamma irradiation were used. The tested textile dyes were Reactive red Bx, Direct Brown BN, Direct yellow 5G, disperse orange 2 RLS, Disperse Violet 3 RS, Sulpher Black Br and Sulpher Brown SG. It was found that immobilized *P. citrinopileatus* exposed to (2 KGy) had more efficiency for decolorization of direct brown and direct yellow dyes. Decolorization percentage of immobilized *P. citrinopileatus* in Direct Brown and Direct yellow reach to 95% and 90% respectively after 8 days at 28°C. Peroxidase activity for (1 KGy) irradiated *P. citrinopileatus* is more than peroxidase activity of unirradiated *P. citrinopileatus*. RAPD.PCR has been performed to know the effect of gamma rays on *P. citrinopileatus*; RAPD profile shows change between unirradiated and irradiated *Pleurotus citrinopileatus*. DNA polymorphism was studied for both irradiated *P. citrinopileatus* exposed to 1KGy and 2 KGy gamma rays.

Key words: Textile dyes, *Pleurotus citrinopileatus, Aspergillus niger*, peroxidase, RAPD-PCR.

INTRODUCTION

Dye-containing effluents represent crucial problem because of their high chemical oxygen demand (COD) and biological oxygen demand (BOD), suspended solids and the content of toxic compounds which causes problems in ecosystem. When dyes are released in the environment, they often exhibit toxic effects on different organisms. They threat ecosystems by reducing sunlight penetration, which reduces photosynthetic activity and dissolved oxygen concentration (Banat *et al.*, 1996). Textile wastewaters are rated as the most pollutant among all industrial sectors. Important pollutants in textile effluents are mainly recalcitrant organics, colors, toxicants and inhibitory compounds, surfactants, chlorinated compounds and salts (Sen and Demirer, 2003). Sudarjanto *et al.*, (2006) stated that some of the dyes and/or products are carcinogenic and mutagenic. Therefore, textile wastewater containing dyes must be treated before discharging into the environment (Kim *et al.*, 2004, Tantak and Chaudhari, 2006). Increase in color fastness, stability and resistance of dyes to degradation have made color removal from textile wastewaters even more difficult (Easton, 1995, Waters 1995 and Robinson *et al.*, 2001). Removal of color from dye-containing wastewaters is a current issue of discussion and regulation in many countries because water is a viable asset that should be protected. Banat *et al.*, (1996) reported that dyed waste-waters are mainly treated by physical and chemical procedures which have many short comings. Degradation of dye by anaerobic bacterial can remove aromatic amines, which are more toxic than the dyes (Hu, 2001, Isik and Sponza, 2003, Nachiyar and Rajakumar, 2004).

Immobilization of ligninolytic fungi on solid supports used for cultivation and enzyme production (Pandey, 2003, Kasinath *et al.*, 2003, Saparrat and Guillén, 2005 and Šnajdr and Baldrian, 2006, 2007), dye decolorization, and pollutant degradation (Yang and Yu, 1996, Shin *et al.*, 2002, Novotný *et al.*, 2004, Šašek *et al.*, 2006 and Svobodová, *et al.*, 2006).

Hatakka (2001) found that white rot fungi (WRF) are the most efficient degraders of lignin in nature. They are able to degrade a broad spectrum of structurally diverse organic pollutants and produce several types of oxidative enzymes, which are useful for remediation of environmental pollutants. *Phanerocheate chrysoporium*, a model organism for lignin and xenobiotics biodegradation studies produces a family of lignin peroxidase (lip) and manganese peroxidase (MnP) isoenzymes (Reddy, 1995). In addition, *P. chrysosporium* is also capable of degrading various azo, heterocyclic and polymeric dyes (Ollikka *et al.*, 1993, Spadaro and Renganathan, 1994). This fungus produces extracellular ligninolytic enzymes such as (Lip) and (MnP) (Hatakka, 2001). The ultimate aim of this study is to identify a fungus able to remove many types of dyes from wastewaters. In order to do that we hypothesized that the exposure to Gamma radiation (source of ionizing radiation) could improve the ability of fungus for decolorization of dye wastewaters. So, in this work dye decolorization, extracellular enzymes abilities of isolate *Aspergillus niger* (Ascomycetes), *Pleurotus citrinopileatus* (Basidiomycetes) exposed and non-exposed to (1 KGy and 2 KGy) of gamma irradiation will studied against different types of textile dyes (one reactive, two direct, two disperse and sulpher) dyes after immobilization two organisms on wheat straw which used as control.

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MATERIALS AND METHODS

Organisms

Pleurotus citrinopileatus MLCC111, was obtained from Central Laboratory for Agricultural Climate, ARC, Cairo, Egypt. It was maintained in Petri – dishes containing PDA (Potato Dextrose Agar) medium with the following compositions by potato extract liquid, 20 g dextrose and 20 g agar.

Aspergillus niger was isolated from the soil and maintained on Potato Dextrose Agar slants at 4°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 according to Moubasher (1993).

Treatments by gamma radiation

Pleurotus citrinopileatus and *A. niger* grown on PDA plates for 6 days were exposed to two doses of gamma radiation in (Atomic Energy Commission, El-Katamya, Cairo, Egypt). The doses were 1 KGy and 2 KGy. The doses rate was 1.0 KGy/12.5 minutes and the length between the gamma radiation source and exposed Petri dishes was constant (10 cm).

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°C for 6 days (Abo-State, *et al.*, 2011).

Biomass immobilization

The Gamma irradiated fungi and their control (non-irradiated) were grown in glass bottles on wheat straw. The moisture content was adjusted to 75%. This substrate was steam sterilized at 121°C for 15 minutes and inoculated with *Pleurotus citrinopileatus* and *A. niger*. Fungi on wheat straw were produced according to the method described by Pant and Adholeya (2006). The cultures were incubated at 28°C until the mycelium covered the wheat straw.

Dyes

Seven dyes represented by four major groups namely reactive, direct, disperse and sulpher. Reactive dyes represented by one sample namely reactive red 6Bx (λmax 295, 545nm), direct dye represented by two samples namely direct brown BN (λ max 290, 240nm) and direct yellow 5G ((λmax 250, 400nm). Disperse dyes represented by disperse orange 2RLS (λ max 288, 444nm) and disperse violet 3RS (λ 291, 499nm). On other hand sulpher dyes represented by sulpher black Br ((λ 220max, 290nm) and sulpher brown SG (λ 250max, 278nm) Absorbance of dyes revealed different λmax after scanning on a spectrophotometer (0-800). All measurements were made on a schimadzo UV visible recording graphicord spectrophotometer. All these dyes were kindly supplied by Cairo dyeing center in industrial zone A2, 10th Ramadan City, Cairo, Egypt.

Reactive dyes represent a major group of dyes employed in textile industry because of their favorable characteristics of bright color and low energy consumption during application (Asku, 2005). Since reactive dyes enter textile effluents after thermal hydrolysis of the reactive groups occurring in dyeing-bath conditions, the dyes were hydrolyzed by a 2-h treatment at 80°C in 0.1 mmol Na_2CO_3 solution, and then neutralized with 1 mmol HCl before use to simulate colored wastewater from a true dyeing bath.

Decolorization of dyes

Decolorization assays were carried out under static conditions with 200 mg/l dye in sterilized tap water. The wheat straw covered with fungal mat was evenly homogenized under sterile conditions and equal amounts (5g of immobilized substrate) were used to inoculate 100 ml of sterilized tap water containing 200 mg/l of different dyes. Control with wheat straw without fungal mat, incubation was carried for 8 days at 28°C (Yang *et al.*, 2003). Dye decolorization was measured spectrophotometrically Dye degradation was reported as decolorization percentage and expressed as follow:

$$\text{Decolorization (\%)} = (A_o - A_t)/A_o \times 100.$$

Where A_o is the absorbance of initial dye solution, which is constant for each dye and equals 0% decolorization, and A_t is the absorbance at cultivation time, t. Decolorization percentage reading refers to the percentage mean of decolorization percentage of three replicas.

Extraction of Extracellular enzymes

Samples of wheat straw covered with fungal mat were extracted in phosphate buffer (pH 6.0) for ligninolytic enzymes. Filtrate of extraction was used for enzyme assay.

Enzyme assay

Lignin Peroxidase activity was determined by monitoring the oxidation of veratryl alcohol to veratraldehyde at 37° C as indicated by an increase in A_{310} (Tien and Kirk 1988). The reaction mixture (2.5 ml) contained 0.5 ml enzyme extract, 0.5 ml of H_2O_2 (2mmol L^{-1}) 0.5 ml veratryl alcohol (10 mmol L^{-1}) and 1.0 ml sodium tartarate buffer pH 3.0 (10 mmol L^{-1}). One unit of enzyme oxidizing 1 µmol of substrate per minute.

Manganese dependent peroxidases (MnP) activity was determined using guaiacol as substrate. The reaction mixture contained 0.5 M Na- tartrate buffer (pH 5.0), 1mM $MnSO_4$, 1mM H_2O_2, 1mM substrate and crude enzymes. The oxidation of substrate at 30°C was followed spectrophotometrically at (A465) (Putter, 1974).

RAPD-PCR analysis

DNA was extracted from fungal samples by Cetyltrimethyl Ammonium Bromide (CTAB) according to Doyle and Doyle (1990). RAPD PCR was performed using 10 random 13 mer primers (table 1) (Metabion, Germany). Polymerase Chain Reaction (PCR) has been performed by mixing 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 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: The nucleotide sequences of the 10 mer primers used for PCR analysis

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 16 (Pipkin, 1984) .

RESULTS AND DISCUSSION

The ability of *Pleurotus citrinopleatus* and *Aspergillus niger* exposed to two doses of gamma irradiation (1 and 2 KGy) and immobilized on wheat straw to decolorize the dyes had been studied. Table 2 shows peaks of wavelengths of different types of dyes before and after decolorization. Peaks of reactive red 6 BX, direct brown BN, direct yellow 5 G, disperse orange 2RLS, disperse violet 3RS, sulpher black Br and sulpher brown SG were at 295, 240, 400, 444, 499, 220 and 250nm were disappeared respectively after decolorization with tested fungi.

Table 2: Wave lengths (λ) of different tested dyes

Dyes	λ max (nm)	
	Bofore decolorization	After decolorization
Reactive red 6 BX	295, 545	545
Direct Brown BN	390, 240	390
Direct Yellow 5G	400, 250	250
Disperse Orange 2 RLS	444, 288	288
Disperse Violet 3 RS	499, 281	281
Sulpher Black Br	220, 290	290
Sulpher Brown SG	278, 250	278

Awan *et al.*, (2011) reported that *Aspergillus niger* is a potent producer of many industrially important enzymes and may genetically 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. The cells of *Aspergillus niger* were subjected to mutagenesis by ultraviolet irradiation, resulting in 45.4% activity of cellulase (Junwei and Shuyun, 1988). Non exposed *A. niger* immobilized in wheat straw decolorized about 47.3% of reactive red 6Bx while *A. niger* exposed to gamma irradiation (1 and 2 KGy) decolorized about 49.5 and 64.9% respectively. On other hand the highest decolorization was reached by exposed *Pleurotus citrinopileatus* at 2 KGy which decolorized 74% of reactive red 6Bx as compared with wheat straw which decolorized about 42.2%. There was a significantly difference between different treatments Fig. 1.

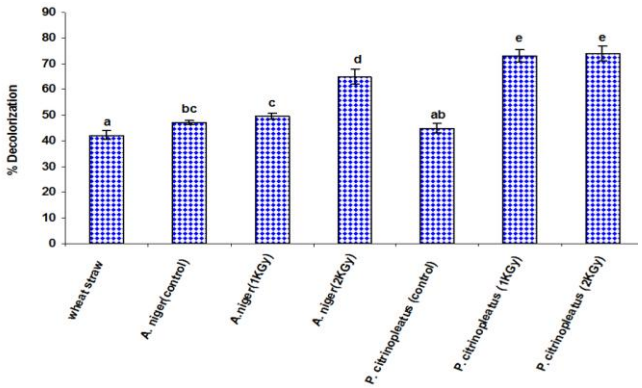

Figure 1: Decolorization percentages of reactive red 6 Bx (0.02% w/v) dye by exposed and non-exposed *Aspergillus niger*, *Pleurotus citrenopilatus* to gamma irradiation immobilized on wheat straw compared with wheat straw as control after 8 days. Each value is the mean ± SD of triplicate decolorization percentage. Letters above the histogram bars represented Analysis of Variance (ANOVA). Bars with different letters are significantly different at p≤ 0.05.

Figure 1 Decolorization percentages of reactive red 6 Bx (0.02% w/v) dye by exposed and non-exposed *Aspergillus niger*, *Pleurotus citrenopilatus* to gamma irradiation immobilized on wheat straw compared with wheat straw as control after 8 days. Each value is the mean ± SD of triplicate decolorization percentage. Letters above the histogram bars represented Analysis of Variance (ANOVA). Bars with different letters are significantly different at p≤ 0.05.

Mahmoud (2008) was used *Aspergillus niger* as a biosorbents for removal of reactive dye (Synazol) from its multi component textile wastewater and found pre-treatment of fungal biomasses with autoclaving increased the removal of dye than pre-treatment with gamma-irradiation. The results obtained revealed that dried autoclaved biomass of *A. niger* exhibited maximum dye removal 88%

A. flavus and *A. niger* degraded (60-65%) of reactive or orange M2R dye at concentration (200mg/l) growing in static Czapek's medium after ten days (Rohilla *et al.*, 2012), *Pleurotus osteratus* and *Trametes pubescens* immobilized on polyurethane foam cubes decolorized (48 and 58%) of reactive R243 dye but increased the efficiency of decolorization of reactive B49 to (97 and 93%) after 6 days 3 sequential cycles at conc. 200ppm (Casieri *et al.*, 2008).

Non exposed *A. niger* immobilized on wheat straw have ability for decolorization of direct brown BN and direct yellow 5 G more than reactive red 6Bx in which it decolorize about (65-71%) of direct brown BN and yellow 5G respectively. Also the decolorization efficiency of non-exposed *Pleurotus citrinopilatus* in these two direct dyes was more than reactive red 6 Bx. The decolorization increased in case of exposed two fungi but was significantly higher than all other treatments in case of *Pleurotus citrinopileatus* exposed to 2 KGy which decolorize (95 and 90%) of direct brown BN and direct yellow 5G Fig. 2.

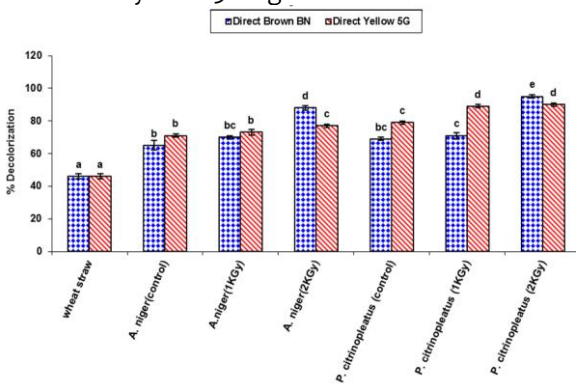

Figure 2: Decolorization percentages of Direct Brown BN and Direct Yellow 5 G dyes (0.02% w/v) by exposed and non-exposed to gamma irradiation (1 and 2KGy) *Aspergillus niger* and *Pleurotus citrinopileatus* immobilized on wheat straw compared with wheat straw as control after 8 days. Each value is the mean ± SD of triplicate decolorization percentage. Letters above the histogram bars represented Analysis of Variance (ANOVA). Bars with different letters are significantly different at p≤ 0.05.

Pleurotus florida EM 1303 immoblized on corncobs powder when applied for decolorization of post anaerobically treated distillery water, 86% decolorization was achieved followed by 50 and 47% reduction in color obtained by *Penicillium pinophilum* TERIDB and *Alternaria gaisen* TERIDB6, respectively (Pant and Adholey, 2007).

In this study (57.3 and 66.3%) decolorization of disperse orange 12 RLS and disperse violet 3RS respectively were achieved after incubation with *Pleurotus citrinopileatus* immobilized on wheat straw to 2 KGy. In our study, it was noticed a low decolorization of disperse dye compared to reactive and direct dyes Fig. 3.

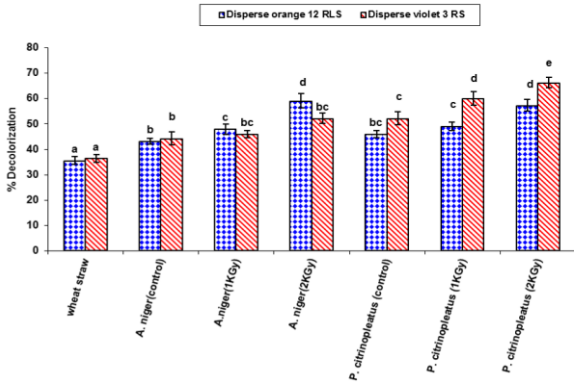

Figure 3: Decolorization percentages of Disperse orange 12 RLS and Disperse violet 3 RS dyes (0.02% w/v) by exposed and non-exposed to gamma irradiation (1 and 2KGy) *Aspergillus niger* and *Pleurotus citrinopileatus* immobilized on wheat straw compared with wheat straw as control after 8 days. Each value is the mean ± SD of triplicate decolorization percentage. Letters above the histogram bars represented Analysis of Variance (ANOVA). Bars with different letters are significantly different at p≤ 0.05.

On the other hand sulpher black Br and sulpher brown decolorization percentages reach to (71.1 and 60.1%) when treated by *Pleurotus citrinopileatus* immobilized on wheat straw and exposed to gamma irradiation at 2 KGy. Also highest decolorization percentages was achieved after exposed to *A. niger* immobilized on wheat straw and exposed to 2 KGy which was recorded 59.6% in case of sulpher black BR Fig. 4.

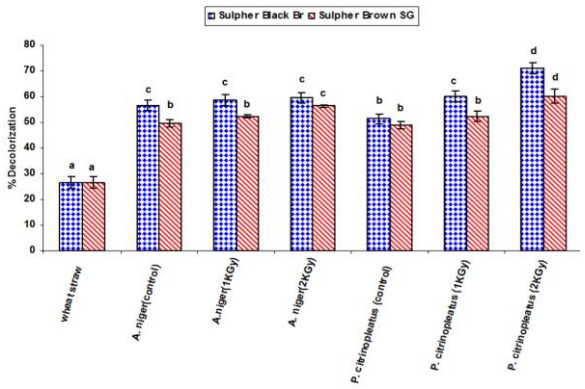

Figure 4: Decolorization percentages of Sulpher Black Br and Sulpher Brown dyes (0.02% w/v) by exposed and non-exposed to gamma irradiation (1 and 2KGy) *Aspergillus niger* and *Pleurotus citrinopileatus* immobilized on wheat straw compared with wheat straw as control after 8 days. Each value is the mean ± SD of triplicate decolorization percentage. Letters above the histogram bars represented Analysis of Variance (ANOVA). Bars with different letters are significantly different at p≤ 0.05.

In this study the exposed tested fungi had more efficiency than non-exposed tested fungi to gamma irradiation. Also immobilization of fungi on wheat straw gave it a high ability for decolorization of the tested dyes. The use of wheat straw had been reported for growth of a number of white rot fungi for bioremediation of polycyclic aromatic hydrocarbon (PAH) contaminated soils (Matsubara *et al.,* 2006). In a study similar to the present one, a novel *Penicillium* isolate was reported by Zheng *et al.,* (1999), which could aerobically decolorize polymeric dye. Halil *et al.*(2012) studied the decolorization of two textile dyestuff (Benazol black ZN and Cibacron black W-NN) by 22 microfungi strains isolated from polluted industrial soil areas and found that Cibacron black W-NN was the best decolorized by *Aspergillus niger* (AN1) (33.0%) at 250 mg l(-1) dye concentration.

Fungal immobilization on inert supports represents several applicative advantages such as treatment of large volumes of waste water and allowing the persistent in competition with faster growing species (Kasinath *et al.,* 2003, Tavcar *et al.,* 2006). Fungal biomass walls are composed of macromolecules which contain carboxyl, amino, sulphates, and hydroxyl groups which act as metal sorption sites (Coulibaly, *et al.,* 2003), and they are able to produce an array of enzymes to enable them to grow under a variety of conditions. The decolorization efficiency of fungi can be due the presence of chitin with hydroxyl and amino groups in their cell wall, which make them an efficient adsorbent of dye effluent (Manikandan *et al.,* 2012). Difference in the capacity of dye decolorization between fungi has been related to inter and intraspecific variations, the molecular complexity of the dye and culture conditions (Ramya *et al.,* 2007).

Activity of lignin and Mn peroxidase enzymes

Pleurotus citrinopileatus and *A. niger* when exposed to two gamma radiation doses (1 and 2KGy) gave different abilities to produce lignin and Mn peroxidase enzymes activity on wheat straw.

Fig. 5 shows the effect of unirradiated and irradiated *Pleurotus citrinopileatus* and *A. niger* on the activities of lignin and Mn peroxidase enzymes on wheat straw. The highest activity of lignin peroxidase was observed in *Pleurotus citrinopileatus* when exposed to 1 KGy dose (0.083 ±0.003 unit/min) and there is significant effect between unirradiated and irradiated *Pleurotus citrinopileatus*. 1 KGy increases the enzyme activity of peroxidase by 2.02 fold relative to control in *Pleurotus citrinopileatus*. On the other hand, there is no significant effect between unirradiated and irradiated *A. niger* on the activity of lignin peroxidase enzyme, this may be indicate to the decolorization efficiency of *A. niger* does not depend on this enzyme; decolorization ability of *A. niger* may be due to absorbance ability of free groups present in *A. niger* cell wall.

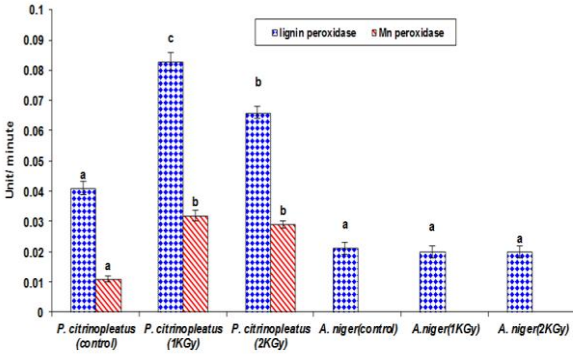

Figure 5: Effect of different doses of gamma radiation on activity of lignin and Mn peroxidases enzyme produced by *P. citrinopleatus and A. niger*. Letters above the histogram bars represented Analysis of Variance (ANOVA). Bars with different letters are significantly different at p≤ 0.05.

In this study, the highest activity of Mn peroxidase (0.032 unit/min) was observed in *Pleurotus citrinopileatus* when exposed to 1 KGy dose and there is significant effect between unirradiated and irradiated *Pleurotus citrinopileatus* where there is not any activity for Mn peroxidase for unirradiated and irradiated *A. niger.* Decolorization of tested dyes by immobilized *P. citrinopileatus* and *A. niger* may be due to utilization of carbon source in dyes molecules. Some reports on the utilization of dyes as a carbon source have been published in the last decade, certain bond in the dye

molecule are cleaved and utilized as carbon source, the chromophore not being affected Knapp (2001). So the increasing Mn peroxidase activity may be the responsible mechanism for dye oxidation and declorization by 1 KGy irradiated *P. citrinopileatus*. In recent years there is a substantial increase in studying the degradative capabilities of fungi for removing the contaminants from wastewaters. Many scientists have highlighted dye decolorization efficiency of various groups of basidiomycetes (Sasek *et al.,* 2006 and Svobodova *et al.,* 2006). It is known that the most of white rot fungi produce at least two of the three highly nonspecific enzymes like LiP, MnP and Lacase which enable the generation of free radicals when conducting a variety of reactions (Pointing 2001). In parallel to our results El-Batal and Abo-State (2006) found that the enhanced productivity in cellulase, Avicelase, xylanase and pectinase by gamma irradiation at dose 1 KGy with increased percent 8%, 20%, 10%, 4%, 31%, 22%, and 34% respectively as compared with unirradiated control. Lee *et al.,* (2000) isolated the enhanced mutants of *Pleurotus citrinopileatus* that enhanced ligninolytic ability induced by using gamma ray irradiated. Abo-State (2011) found that *P. sajor-caju* exposed to 0.75 and 1.0 KGy produced MnP 4.5 fold increase relative to control that of the parent strain. Zhang *et al.,* (1999) observed that MnP played an important role in the decolorization of cotton bleaching effluent by an unidentified white rot fungus, while there was no obvious role for LiP in the decolorization.

Improvement in enzyme activity might be due to photolysis of pyramidines to form dimers, UV irradiation might have caused error at replication and hence, resulted in mutation. There might be an increase in copy number or expression of genes for dye degradation enzymes (Shafique *et al.,* 2009, Gaedner *et al.,* 1991)

DNA analysis of Pleurotus citrinopileatus exposed and non-exposed to gamma irradiation by RAPD- PCR analysis

In a trial to detect whether the effect gamma rays is genetically inherited or not, a RAPD –PCR had been performed. In this regard untreated and treated fungus has PCRed. A non-exposed *Pleurotus citrinopileatus* and *Pleurotus citrinopileatus* exposed to gamma ray (1KGy and 2 KG) were used to study the effect of two doses (1 and 2 KGy) of gamma rays on DNA, using RAPD analysis, it were chosen because of their high dye decolorization efficiency in our study compared to exposed and non-exposed *A. niger*. There 6 primers gave variation out of used 13 RAPD primers (Table 1) leading to a band profiles with a number of amplified DNA bands shown in (Table 3). RAPD profiles for a wild *Pleurotus citrinopileatus* and exposed to the gamma radiation (1KGy and 2KGy) are shown in (Fig. 6 A-F). El-Sherbeny *et al.,* (2005) found that in RAPD profiles for the two fungal yeasts namely *Rhodotorula rubra* and *Hansenula anomala* when exposed to the ionizing radiation had a variation in number of amplified DNA. The number of amplified DNA fragments that are generated by ionizing radiation are also varied depending on type of ionizing radiation used. The method of RAPD has been proved a valuable tool to distinguish different genotypes in valuable tool to distinguish different genotypes in edible mushrooms such as *Ganoderma lucidum* (Hseu *et al.,* 1996), *Lentinula edodes* (Zhang and Molina, 1995) and *Agaricus bisporus* (Khush *et al.,* 1992) and to evaluate genetic similarities. Jones and Kortenkamp (2000) have used RAPD fingerprinting to detect mutation in bacterial and human DNA.

DNA polymorphism may appear as shifts in band migration or missing bands. The polymorphism percentages ranged from zero up to 100 in *Pleurotus citrinopileatus* exposed to1 *KGy* and from 37.5 up to 166.6 in *Pleurotus citrinopileatus* exposed to2 *KGy*. The numbers of amplified DNA fragments that are generated by gamma rays are also varied depending on the doses of gamma radiation (Table 3). El-Sherbeny *et al.,* (2005) found that the polymorphism percentages ranged from 23.1 up to 75.0 in *Rhodotorula rubra* and from 33.3 to 75.0 in *Hansenula anomala* when using 3 M RAD of gamma rays. This means that the means of polymorphism percentages reached 47.9and 51.8 in *R. rubra* and *H. anomala*.

Table 3: DNA polymorphism induced by 1 and 2 KG Gamma rays in *Pleurotus citrinopileatus*.

Primer	*Pleurotus citrinopileatus* (1KGy)		*Pleurotus citrinopileatus* (2KGy)	
	Total bands	% polymorphism	Total bands	% polymorphism
P1	1	10	5	50
P2	3	100	5	166.6
P3	3	37.5	3	37.5
P4	3	50	4	66.6
P5	2	50	5	125
P6	0	0	6	120

Results from RAPD profile which appear in (Table 4) refer to change between control and treatments, while in control showed in total 36 bands results from six primers, number of these bands range from 3 in P2 to 10 bands in P1. The six primers gave specific and stable results, in 1 KGy treatment there are changes in band intensity which was 12 bands compared with control, we can see also polymorphic bands compared with control, in other hand, the results refer to increase in polymorphic bands intensity increase or decrease for bands in 2KGy treatment about both of control and 1 KGy treatment, 2KGy treatment record high increase in band intensity 20 compared with 12in treatment 1 KGy.

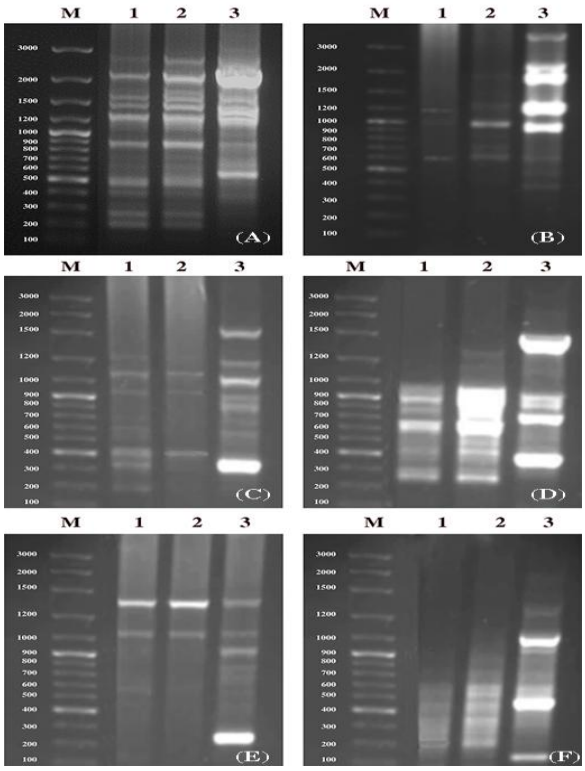

Figure 6A-F: RAPD Profiles of *Pleurotus citrinopileatus* non irradiated and irradiated with (1 – 2) KGy of gamma rays, Lane M, marker. Lane 1, non-exposed to gamma irradiation *Pleurotus citrinopileatus* Lane 2, *Pleurotus citrinopileatus* exposed to gamma irradiation 1KGy, Lane 3, *Pleurotus citrinopileatus* exposed to gamma irradiation 2KGy (A) = P1: primer 1, (B) = P2: primer 2, (C)= P3: primer 3, (D) = P4: primer 4, (E)= P5: primer 5, and (F)= P6: primer 6).

El-Sherbeny *et al.*, (2005) observed that the number of amplified DNA fragments that are generated by ionizing radiation are depending on type of ionizing radiation in which 3 M RAD of gamma radiation induced 81 and 78 amplified framgments in *Rhodotorula rubra* and *Hansenula anomala*. Whereas 3 RAD of fast neutrons resulted in 85 and 84 amplified fragments in the two fungal yeasts. These results revealed that RAPD technique could be easily used to demonstrate DNA polymorphism. Finally, these results indicates that the exposure to Gamma irradiation especially to 1KGy enhanced the decolorization ability of *Pleurotus citrinopileatus and* the effect irradiation genetically as shown in DNA profile.

Table 4: Number of Amplified DNA fragments produced by the effects of gamma ray in *Pleurotus citrinopileatus*.

Primers	No. of bands in control	1KGy				2KGy			
		a	b	c	d	a	b	c	D
P1	10	1	0	2	0	2	3	5	2
P2	3	3	0	1	0	5	0	5	0
P3	8	0	3	0	2	1	2	2	
P4	6	3	0	6	0	2	2	4	0
P5	4	0	2	0	0	5	0	1	0
P6	5	0	0	3	0	4	2	3	1
Total	36	7	5	12	2	19	9	20	3
A+b		12				28			

a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d: decrease in band intensity, a+b : polymorphic band

CONCLUSION

The present study revealed the ability of *Pleurotus citrinopleatus* and *Aspergillus niger* exposed to two doses of gamma irradiation (1 and 2 KGy) and immobilized on wheat straw to decolorize the dyes had been studied. *Pleurotus citrinopleatus* exposed to gamma irradiation (2 KGy) was highly effective in decolourization of reactive red 6 BX, direct brown BN, direct yellow 5 G, disperse orange 2RLS, disperse violet 3RS, sulpher black Br and sulpher brown SG than *Aspergillus niger*.

The highest activity of lignin and Mn peroxidase enzymes was observed in Pleurotus *citrinopileatus* when exposed to 1 KGy dose and there is significant effect between un-irradiated and irradiated *Pleurotus citrinopileatus*. On the other hand, there is no significant effect between unirradiated and irradiated *A. niger* for production of peroxidase enzyme.

Results from RAPD profile show change between un-irradiated and irradiated *Pleurotus citrinopileatus*, the total bands in control was 36 from six primers, number of these bands range from 3 in P2 to 10 bands in P1. We can see also polymorphic bands compared with control; 2KGy treatment record high increase in band intensity 20 compared with 12 in treatment 1 KGy.

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