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EFFECT OF GAMMA IRRADIATION ON CELLULASE ACTIVITY OF *PLEUROTUS PULMONARIUS* DURING THE DIFFERENT GROWTH STAGES ON DIFFERENT PLASTIC POLYETHYLENE WASTES

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ABSTRACT

Pleurotus pulmonarius was excellent growth on determined weights of the wet rice straw, non irradiated plastic, irradiated plastic, mixture of rice straw and non irradiated plastic and mixture of rice straw and irradiated plastic. maximum value of cellulase recorded on rice straw, mixture of rice straw and irradiated plastic, mixture of rice straw and non- irradiated plastic (1.7, 1.5 and 1.3 unit/gm waste), respectively, which recorded after first harvest; but irradiated plastic and non-irradiated plastic giving highest values at promordium stage; (1.14 and 0.79 unit/gm waste).

The spawn of P. pulmonarius was irradiated at the does 0.5, 1 and 2 KGy, and inoculated on each different wastes separately to detect the cellulase activity which was increased in the various wastes by exposing the spawn of P. pulmonarius to gamma irradiation and detectable raises in the cellulase activity was recorded at dose 0.5 KGy, Any further increase in the irradiation dose was accompanied by a decrease in cellulase activity until reach lowest value at 2 KGy.

INTRODUCTION

Accumulation of plastic waste in the environment causes wide scale pollution with long lasting effects making plastic waste management expensive and problematic **(Zafar *et al.* 2013). Yang *et al.* (2007)** reported that plastic material is one of the most serious solid wastes pollution and it's accumulation in the environment is highly resistant to biodegradation and is not able to take part in substance recycle.

Microorganisms utilize polyethylene film as a sole carbon source.These bacteria colonize the polyethylene surfaces forming a biofilm. Cell surface hydrophobicity of these bacteria was found to be an important factor in the formation of biofilm on the polyethylene surface**(Orhan *et al.* 2004)**.Mushrooms are being explored in new ways. That is; they are being used to break down previously harmful materials such as oil and plastic **(Gruber, 2009)**

Exoenzymes from the microorganisms first breakdown the complex polymers giving short chains or monomers that are small enough to permeate through the cell walls to be utilized as carbon and energy sources **(Premraj and Dobley, 2005)**. Enzyme systems in mushroom have the ability to degrade cellulose, hemicelluloses and lignin by the cleaveing of lingo (hemi) cellulosic bonds or through lignolysis **(Neelakantan *et al.*, 1993)**. **Awang *et al.*(1998)** reported that *Pleorotus sajor-caju* and *Coprinus cinereus* can be easily grown on empty fruit bunch and degraded lignocellulosic material by the ability of cellulase enzyme system to break down insoluble cellulose, and hydrolysing salicin such as cellobiose and xylanase for breaking down hemicellulose into simple sugars.

Earlier works have reported that gamma-ray irradiation on various lignocellulosic biomasses such as bagasse **(Kumakura and Kaetsu, 1983)**, rice straw and sawdust **(Bhatt *et al.*, 1992)** were effective to improve enzymatic saccharification rate. All the gamma irradiation were effective in retarding mushroom sensory deterioration **(**Jiang ***et al.* 2010)**.

Benoît et *al.* (1999) reported that ionizing treatments of edible mature mushrooms (*Agaricus bisporus, albidus)* increase significantly the phenylalanine ammonia-lyase, polyphenol oxidase activity and total phenols concentration compared to non irradiated control samples. **Dawoud (2012)** noted that Gradual gamma irradiation (0.5 and 1.0 kGy) on genus *Pleurotus* significantly enhanced high growth, biomass yield,

1,3-β-glucan production and carbon metabolites contents, glucose absorption, 1,3-β-glucan synthase activity and protein content. On the other hand, these low irradiation doses inhibited the accumulation of cellular and extracellular keto acids, free ammonia, extracellular 1,3-β-glucan permeation **Dawoud (2012).**

MATERIALS AND METHODS

Plastic polyethylene wastes

In the present study, plastic polyethylene based wastes stimulates (namely; non irradiated and irradiated plastics) and rice straw were used. In the meantime, equal weights of all waste simulates were mixed together to form the mixture category of the waste simulate. Substrate of rice straw was used to serve as control treatment

Used mushroom

The biological treatment of the stated solid waste simulates were performed using *Pleurotus pulmonarius* (Somy cel 3014, France), This strain was kindly obtained from Agricultural Research Centre, Cairo, Egypt. This strain was obtained as mycelia on 2 % (w/v) malt extract with 1.5 % (w/v) agar media. Plate cultures were prepared for the subsequent work.

Preparation of spawn: -

Wheat spawn was prepared, as described by **Chang(1982)**, as follows: In flask 250 ml capacity, 50 g of wheat grains were added to one g limestone chalk dissolved in 75 ml distilled water. Three equal sets were prepared each was composed of six flasks and autoclaved . Each sets was inoculated with disc (one cm diameter) of *Pleurotus pulmonarius* separately, and incubated at 26 ± 2 ° C for 15 days.

Biodegradation of Plastic polyethylene wastes biologically using *Pleurotus pulmonarius*:

Determined weights of the wet rice straw, non irradiated, irradiated plastics and the mixture of equal ratios of these wastes were mixed with 4% (w/w) of wheat bran and calcium carbonates. Three sets of each waste were then inoculated with the spawn *of P. pulmonarius.* **(Chang 1978).** The spawned waste was then transferred separately to suitable polyethylene clean bags. The inoculated plastic bags of all wastes were incubated at temperature ranged from 20 – 25°C until the mycelia of mushroom have got luxurious growth. Then each bag was opened by tearing to let the fungal growth completed to the fruiting stage.

Gamma irradiation of spawn:

To enhance the capability of the microorganism for the degradation of plastic polyethylene wastes (non irradiated, irradiated) at the end of incubation period, six spawn replicates of *P. pulmonarius* were irradiated at different doses namely (0.5, 1, 2 kilo Gray) in gamma radiation cobalt-60 cell at dose rate 1.56 Gy/min **(El-Sayaad 2008)**. Other set of flasks were not irradiated and used as control. Irradiated and non-irradiated spawn were inoculated separately on the different wastes categories (rice straw, non irradiated, irradiated plastics and their mixture) as indicated previously. Then the determinations of enzymes were done.

Preparation of crude enzymes:-

The plastic bags containing different plastic polyethylene wastes were inoculated with irradiated and non-irradiated *Pleurotus pulmonarus* spawn and were incubated at 20 – 25 ° C. During the mycelia growth, cellulase activity were assayed spectrophotometerically after (3, 6, 9, 12, 15, 18) days of inoculation, at primordia phase, at the beginning of sporophorore formation, and after the first harvest directly.

Tri plicate, each 50 g of the bags containing the mycelia colonized on the wastes (rice straw, non irradiated plastic, irradiated plastic and their mixture) were removed periodically as previously stated. Each sample was then mixed with 50 ml of 0.01 M phosphate buffer (pH 7) and shacked using vortex at 80 rpm for 20 minute, the mixture was squeezed through several layers of cheese cloth and the filtrate was further clarified by centrifugation at 10,000 rpm for 20 min. at - 4°C using Sigma 2k15-USA centrifuge. The resulting clear supernatants were stored at -10°C before being used for all subsequent assays **(Wood and Goodenough, 1977)** then a known weight of ammonium sulphate (5 gm) was added gradually with shaking to measure supernatant until it completely was dissolved to

bring it to 85% saturation then left in refrigerator over night, after that the precipitate was dissolved in 1 ml buffer phosphate (0.01M, pH 7), then it transferred in dialysis bag for one hour in distal water for three time then for half hour in buffer phosphate (0.01M, pH 7) to have a sample of protein for enzymatic analysis.

Enzymes Assay:-

Carboxmethyle cellulase activity (CM cellulase(*:-*

CM cellulase was assayed following **Mandels and Rees, 1964,** where 250 ul of enzyme extract (suitable dilution of enzyme was used) was mixed with 500 ul of 10 mg/ml sodium salt of carboxymethyle cellulose solution in 0.1 M citrate buffer at pH 5. The mixture was incubated at 50°C in water bath with moderate shaking for 30 minutes. Reducing sugars were measured according to **Somogyi (1952)**.The enzyme activity was expressed as ug glucose /g culture.

Somoygi method for determination of the reducing sugar:

Preparation of Somogyi reagent I.

Anhydrous sodium sulphate 288 g was dissolved in one liter of boiled distilled water followed by 24 g Rochelle salt, 48 g sodium carbonate and 32 g sodium bicarbonate. The solution was diluted to 1600 ml with boiled distilled water and stored at 27°C.

Preparation of Somogyi reagent II.

Anhydrous sodium sulphate 27g was dissolved in 300 ml of boiled distilled water followed by 8 g copper sulphate. The solution was diluted to 400 ml with boiled distilled water and stored at 27°C.

Preparation of Nelson reagent.

Ammonium molybdate (100 g) was dissolved in 1.8 liter of distilled water, followed by 84 ml conc. H_2SO_4 and 12 g sodium arsenate dissolved in 100 ml distilled water was added to the mixture. The mixture was stored at 37°C for 24-84 hour in dark glass bottle at room temperature **(El-sayaad 2008)**.

Reducing sugars assay:-

2 ml sample consisting of 4 volume of Somogyi reagent I and one volume of Somogyi II (mixed immediately before use) was pipette into 15 ml tube along with the sample solution containing reducing sugar and completed with distilled water to give a total volume of 4 ml. The solution was boiled in water bath for 15 min, cooled and 2 ml of Nelson reagent. The solution was mixed carefully using vortex mixer. Finally 4 ml of distilled water was added and the solution was mixed by inversion. The absorbance was measured at 540 nm and then translated into glucose equivalent using standard graph obtained by plotting microgram glucose against absorbance. Determination were carried out using photometric colorimeter APEL(AP – 101).

Statistical analysis:

The statistical analysis was carried out using F- test two way followed by Duncan's multiple range test. The level of statistical significance was set at **P≥0.05**.

RESULTS

Pleurotus pulmonarius has excellent growth on determined weights of the wet rice straw, non irradiated plastic, irradiated plastic, mixture of rice straw and non irradiated plastic and mixture of rice straw and irradiated plastic under different gamma irradiation doses in different stages. Table (1) illustrate the CM-cellulase activities (calculated by unit /g dry waste) for non –irradiated and irradiated *P. pulmonarius* cultivated on the different wastes (rice straw, mixture of rice straw and irradiated plastic, mixture of rice straw and non-irradiated plastic, irradiated plastic, non –irradiated plastic). The non-irradiated spawn of *P. pulmonarius* produced the maximum values of cellulase activity after the first harvest directly (1.7, 1.5 and 1.3 unit/gm waste on rice straw, mixture of rice staw and irradiated plastic, mixture of rice straw and non- irradiated plastic but irradiated plastic and non-irradiated plastic giving highest value after promordium stage respectively(1.14, 0.79 unit/gm waste). The fungal cellulase enzyme was detected during cultivation of *P. pulmonarius*, the spawn of which was irradiated at the dose 0.5, 1 and 2 KGy, and inoculated on each different wastes separately.Table (1) illustrates the variation in cellulase activity of *P. pulmonarius* during the

different incubation periods on various wastes simulates inoculated by spawn exposed to different doses of gamma radiation. The cellulase activity was increased in the various wastes by exposing the spawn of *P. pulmonarius* to gamma irradiation. Based on the data stated in Table (1), it could be also stated that the CM-cellulase activity was increased at dose 0.5 KGy than other doses recorded 2.1, 1.8 , 1.6, 0.76 and 0.3 units/ g dry waste respectively for rice straw, , mixture of rice straw and irradiated plastic, mixture of rice straw and non-irradiated plastic, irradiated plastic , non- irradiated plastic. Any further increase in the irradiation dose was accompanied by a decrease in the enzyme contents to reach 1.5, 1.4, 1.16, 0.36 and 0.31 units/g dry waste at irradiation dose of 2 KGy for mixture of rice straw and irradiated plastic, rice straw, mixture of rice straw and non- irradiated plastic, irradiated plastic and non- irradiated plastic respectively. However, it should be mentioned that, the CM-cellulase activities for both irradiated and non-irradiated spawn of *P. pulmonarius* reached to maximum activity after the first harvest phase for all substrate degraded (Table1).

Analysis of variance (F-test) two- way classifications of cellulase activity for doses of gamma irradiation, type of wastes

The statistical analysis using F- test two way in Table (2) illusrated that the variation in cellulase was significant for doses of gamma irradiation and types of wastes. Duncan analysis showed that there was significant value between rice straw, non-irradiated plastic, irradiated plastic, mixture of rice straw and non-irradiated plastic and mixture of rice straw and irradiated plastic,

Table (1): The variation in cellulase activity of *P. pulmonarius* during different incubation periods on various waste simulates after exposing their spawn to different doses of gamma irradiation.

Cellulase activity (U/g)											
waste	Dose(KGy)	Incubation period									
		Mycelial stage						Promordial stage		Fruiting stage	
		3 days	6 days	9 days	12 days	15 days	18 days	Pr1	Pr 2	F1	F2
Rice Straw	Control	0.54	0.77	0.78	0.79	0.91	0.98	1.08	1.3	1.7	1.4
	0.5	0.65	0.79	0.8	0.86	0.91	1.03	1.2	1.27	2.1	1.5
	1	0.57	0.69	0.71	0.87	0.95	0.99	1.01	1.5	1.68	1.49
	2	0.43	0.67	0.69	0.76	0.83	0.91	0.96	1.1	1.4	1.3
Non- irradiated plastic	Control	0.15	0.16	0.27	0.33	0.46	0.53	0.62	0.79	0.65	0.33
	0.5	0.17	0.18	0.26	0.3	0.37	0.49	0.51	0.57	0.3	0.11
	1	0.14	0.19	0.24	0.28	0.35	0.4	0.48	0.51	0.24	0.12
	2	0.16	0.17	0.21	0.25	0.31	0.46	0.47	0.47	0.31	0.17
Irradiated plastic	Control	0.17	0.16	0.19	0.22	0.37	0.4	0.49	1.14	0.94	0.44
	0.5	0.14	0.13	0.26	0.29	0.35	0.44	0.51	1.08	0.76	0.3
	1	0.14	0.17	0.2	0.29	0.36	0.4	0.48	0.96	0.66	0.29
	2	0.13	0.14	0.19	0.21	0.36	0.39	0.48	0.76	0.36	0.21
Rice Straw and Non- irradiated plastic	Control	0.24	0.33	0.38	0.4	0.42	0.44	0.45	0.55	1.3	0.67
	0.5	0.37	0.36	0.41	0.5	0.53	0.59	0.7	1.25	1.6	1.5
	1	0.27	0.3	0.39	0.49	0.51	0.51	0.69	0.95	1.4	1.39
	2	0.26	0.27	0.31	0.44	0.49	0.53	0.56	1	1.16	1.4
Rice Straw and Irradiated plastic	Control	0.26	0.29	0.37	0.45	0.48	0.55	0.81	1.09	1.5	1.06
	0.5	0.29	0.33	0.41	0.52	0.65	0.74	0.86	1.1	1.8	1.5
	1	0.25	0.28	0.39	0.49	0.52	0.63	0.74	0.98	1.7	1.02
	2	0.23	0.29	0.33	0.39	0.45	0.56	0.66	0.73	1.5	0.96

• Cellulase activity expressed by U(mg glucose /g inoculated fresh substrate

•Promordial stage: included Promordium 1,2(Pr1,Pr2) after 20±3days. •After 1st harvest: afer 25±3 days.

Table (2): ANOVA two way and ducan test analysis for the effect of different types of wastes and doses of gamma irradiation on cellulase activity.

F waste		**55.711***			
Duncan waste	Rice straw	Non- irradiated plastic	Irradiated plastic	Rice straw and Non irradiated plastic	Rice straw and Irradiated plastic
	c	**a**	**a**	**b**	**b**
F Dose	**2. 937***				
Duncan Dose	**Control**	**0.5 KGy**	**1 KGy**	**2 KGy**	
	ab	**b**	**ab**	**a**	

*Significant at P≥0.05 , in each column any two means take the same latter have no significant difference between them by Duncan's multiple range test.

But there was no significant between non-irradiated plastic, mixture of rice straw and non-irradiated plastic, also between irradiated plastic, and mixture of straw and irradiated plastic. Duncan showed significant between different doses and non-significant between control and 1KGy.

DISCUSSION

Growing of *P.* pulmonarius on the surface of plastic polyethylene wastes.

It was clear from the present study that *Pleurotus pulmonarius* can grow on the surface of irradiated and non-irradiated polyethylene plastic wastes because it was used as carbon sources which agree with **Orhan,** et al **(2004)** who found that microorganisms utilize polyethylene film as a sole carbon source resulting in partial degradation. Other studies have also shown microbial colonization **(Sudhakar,**et al **2008)**, **(Chiellini,** et al **2007)** biofilm formation for *Rhodococcus rhodochrous*, *Cladosporium cladosporoides* and *Norcardia asteroids* **(Bonhomme,** et al **2003)** and the growth of *Aspergillus flavus*, *Penicillium simplicissium* and *Phanerochaete chrysosporium* **(Ojeda** et al **2009)** in oxo-biodegradable polyethylene.

cellulase activity of *P. pulmonarius* during the mycelial growth and fruiting bodies formation on different wastes (control treatment).

The present part of work was devoted to study the behavior of cellulase secreted by *Pleurotus pulmonarius* during the growth on the stated polyethylene plastic waste simulates, the cellulase enzyme was measured at different time of growth (mycelia, promordium, fruiting) that because some of them increased by growth time and other were decreased according to behavior of enzyme and substrate content. At the same time, the effect of gamma irradiation pretreatment of the mushroom spawn on the released cellulase of *P. pulmonarius* was evaluated.

It should be stated that, cellulase was represented through 18 days from cultivation, during the primordial formation and after the first harvest. This may be explained on the basis that: during mushroom growth more enzymes were consumed for the degradation of cellulose component, while after the first flushing the enzymes may accumulate with less consumption. In the meantime enzyme production was not the same in the different wastes. The same trend was assessed by **Tsang *et al.* (1987) and Jia *et al.*, 1994** who concluded that cellulase production was varied according to type of waste simulates.

It was reported in the present work that, cellulase activity was increased by increasing the incubation period for non-irradiated spawn of *P. pulmonarius* until reached the highest value at fruiting stage recorded (1.7, 1.5 and 1.3 U\g) for rice straw, mixture of rice straw and irradiated plastic, mixture of rice straw and non irradiated plastic respectively, but the highest value of cellulase activity for irradiated plastic and non irradiated plastic recorded at promoradia stages (1.14 and 0.79 U\g, respectively) .

The difference in activity of cellulase for different substrates could be attributed to the presence of cellulosic substrates (straw) which induce the production of ligninolytic enzymes **(Quintero *et al.*, 2006a)**.

The present study reveled also that the highest net value of cellulose after 1st harvest for *P. pulmonarius* grown on rice straw, mixture of rice straw and irradiated plastic and mixture of rice straw and non irradiated plastic may be attributed to cellulosic materials are a plentiful source of nutrients that favor fungal growth and soil colonization. Additionally, cellulosic substrates induce the production of ligninolytic enzymes **(Castillo *et al.*, 2001)**. Additionally plant residues are a plentiful source of nutrients that favor fungal growth and elicit production of adaptative ligninolytic enzymes **(Fujian *et al.*, 2001)**.

Effect of gamma irradiation on cellulase activity of *Pleurotus pulmonarius* during the mycelial growth and fruiting on different cellulosic waste simulates.

In the present investigation, cellulase was followed up during cultivating of *P.pulmonarius* that their spawn were irradiated at the following does; 0.5, 1 and 2 kGy, then inoculated on the various plastic polyethylene waste simulate substrates. **El-sayaad (2008)** observed that irradiation of spawn at dose of 0.75 kGy stimulated the growth rate and activities of extracellular enzymes of *P. pulmonarius*. **Lee *et al.* (2000)** reported that gamma radiation induced the fruiting body formation, growth rate and activities of extracellular enzymes of *P. ostreatus* when mycelia were irradiated at doses of 1–2 kGy. **Benoît, *et al* (2000)** noted that irradiation at doses of 1.5 kGy and 2.5 kGy reduced significantly the rate of respiration of the mushrooms, compared to that of samples irradiated at 0.5 kGy and non-irradiated control samples.

Data of cellulase activity showed very highly significant increasing by exposure spawn of *P.pulmonarius* to gamma irradiation at dose 0.5 KGy giving for each wastes respectively; 2.1, 1.8, 1.6 , 0.76 and 0.3 units/g dry waste for rice straw, mixture of rice straw and irradiated plastic , mixture of rice straw and non-irradiated plastic, irradiated plastic , non- irradiated plastic. Similar results were obtained by **Lee *et al*.(1999)** who stated that, strains of edible mushroom had more highly lignocelluloytic activity after induced by gamma irradiation.

From our study mixture of rice straw and irradiated plastic considered the better waste substrate for *P.pulmonarius* growth than other wastes because it was giving the highest value of enzyme compared with others. This due to rice straw contains highly nutrition value for mushroom and irradiated plastic was break down by irradiation which may be easier for mushroom consumtion.

CONCLUSIONS

From this study *Pleurotus pulmonarius* can grow on plastic polyethylene and use it as nutrient media so that *Pleurotus pulmonarius* can use as environomental biological treatment from plastic wastes pollution. Cellulase enzyme secreted by high level at fruiting stage on rice straw, mixture of rice straw and irradiated plastic, mixture of rice straw and non- irradiated plastic. Gamma irradiation has effective effect on increasing the ability of mushroom (*P. pulmonarius*) for growth, enzymes secreation .

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تأثير أشعة جاما على نشاط إنزيم السيليوليز لفطر عيش الغراب المحارى أثناء مراحل النمو المختلفة على المخلفات البلاستيكية المختلفة

لقد أعطى عيش الغراب المحارى نمو جيد على الأوزان المحسوبة لكل من قش الأرز و البلاستيك الغير مشعع والبلاستيك المشعع والخليط لكل منهما مع قش الأرز تحت جرعات مختلفة من أشعة جاما فى مراحل النمو المختلفة ولقد تم قياس نشاط إنزيم السيليوليز لعيش الغراب المحارى الغير المشعع وا لمشعع النامى على هذه المخلفات ولقد تبين من النتائج أن عيش الغراب المحارى الغير مشعع يعطى أعلى قيمة من نشاط إنزيم السيليوليز بعد الحصاد الأول مباشرة(مرحلة الإثمار) لكل من المخلفات الأتية على التوالى : قش الأرز & خليط من قش الأرز والبلاستيك المشع& خليط من قش الأرز والبلاستيك غير المشع أما البلاستيك المشع & البلاستيك غير المشع يعطيان أعلى قيمة للإانزيم فى مرحلة ما قبل الإثمار. تم تشعيع تقاوى عيش الغراب المحارى بأشعة جاما عند الجرعات الأتية: (٠,٥&١&٢كيلوجراى) ثم حقنها على المخلفات المختلفة لتعين نشاط إنزيم السليوليز ولقد أدى هذا التعرض الى زيادة النشاط الإنزيمى على المخلفات المختلفة خلال مراحل النمو المختلفة و لقد أظهرت الجرعة الأشعاعية ٠,٥ كيلوجراى زيادة فى نشاط إنزيم السيليوليزمقارنة ببقية الجرعات ولذا فقد تبين أن الزيادة فى الجرعة الإشعاعية بعد ٠,٥ كيلوجراى يؤدى الى نقص الأنشطة الإنزيمية ليعطى عند الجرعة ٢ كيلوجراى أقل قيمة.