Arab Journal of Nuclear Sciences and Applications, 45(2) 489-496 (2012)

Gallic acid formation from gallotannins-rich agricultural wastes using *Aspergillus niger* AUMC 4301 or its tannase enzyme

M. Z. El-Fouly[1]*; Z. El-Awamry[2]; Azza A.M. Shahin[1]; Heba A. El-Bialy[1]; E. Naeem[2] and Ghadeer E. El-Saeed[1]

1. National Center for Radiation Research and Technology (NCRRT), Radiation Microbiology Department, Atomic Energy Authority, Egypt.

2. Faculty of Science, Azhar University, Egypt.

ABSTRACT

Gallic acid is used in many fields including dye-making, leather and chemical industries. Seven agricultural wastes were chosen for their high gallotannin content. They were apple bagasse, green tea waste, mango seed kernel, olive mill, palm kernel cake, peat moss and tamarind. Each waste was used as a carbon source instead of tannic acid in the fermentation medium. Some agricultural wastes under investigation were already contain free gallic acid especially mango seed kernel followed by green tea waste, while olive mill, peat moss and tamarind were found to be free from gallic acid. The highest concentration of liberated gallic acid from wastes fermented by *A. niger* AUMC 4301 was occurred at the third day of fermentation. After 72h, a sharp decrease in gallic acid accumulation was noticed. To overcome this sharp decrease, agricultural wastes were treated with extracellular crude *A. niger* tannase directly in stead of tannase producer. The concentration of gallic acid increased gradually and reached its maximum at 18 h incubation in case of apple bagasse, green tea waste and palm kernel cake. On the other hand, gallic acid production was delayed for a lag period (12-18) h depends on the complexity of used agriculture waste.

To increase the tannase productivity by *A. niger* AUMC 4301, the producer fungus was irradiated by different doses of γ rays, D_{10} value was 0.81 kGy. Radiation dose 0.5 kGy shows a positive effect on tannase productivity. An experiment examined the change in amino acid profile between irradiated and unirradiated *A. niger* AUMC 4301 was also conducted.

Key words: Gallic acid formation, Agricultural wastes

INTRODUCTION

Gallic acid (3, 4, 5-trihydroxy benzoic acid) is a phenolic compound. The chemical formula is $C_6H_2(OH)_3COOH$, mainly used in the pharmaceutical industry for the synthesis of antibacterial drugs trimethoprim **(Kar and Banerjee, 2000)**. Gallic acid is also used in food industry as substrate for the chemical synthesis of food preservatives such as pyrogallol and gallates (**Gathon *et al*., 1989)**. It can be produced by hydrolysis of tannic acid with acid or alkali or microbial tannase. Gallic acid seems to have antifungal and antiviral properties. It acts as an antioxidant and helps to protect our cells against oxidative damage. It was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage. It is also used to treat albuminuria and diabetes. Some ointment to treat psoriasis and external haemorrhoids contains gallic acid have been recently described by physicians. Gallic acid finds applications in many fields including dye-making, leather and chemical industries. It can be used in the manufacture of ordinary writing inks and dyes, as photographic developer **(Mukherjee and Banerjee, 2003 a, b)**. Tannase or tannin-acyl-hydrolase (EC 3.1.1.20), is an extracellular and inducible enzyme, which catalyses the hydrolysis of gallotannins releasing glucose and gallic acid **(Barthomeuf *et al*., 1994)**. Microorganisms are the main source for industrial enzymes due to their biochemical diversities, technical and financial advances, Tannase is usually obtained from bacteria, some yeasts and filamentous fungi, mainly from the following species: *Aspergillus*, *Penicillium*, *Fusarium* and *Thichoderma* **(Aguilar *et al*., 2007)**

The exposure of cells to ionizing radiation sets off a chain of reactions giving rise to chemical and then to metabolic or physiological changes. So, irradiation presents an additional stress to the cell, which tends to disturb their organization. Irradiation effects have been shown to be occurring with proteins, enzymes, nucleic acids, lipids and carbohydrate, all of which may have marked effect on the cell. The dose response curve presents the relation between the radiation dose (kGy) and the number of microorganisms surviving. The radiosensitivity of microorganisms is expressed in terms of D_{10} values. The D_{10} value is the doses required to reduce the initial population to 90%. These values are useful in calculating sublethal doses and to know the relative sensitivity of a microorganism to gamma radiation. Ionizing radiation has a mutagenic action on microorganisms. Low-dose irradiation of microorganisms may produce mutations which may conceivably be describe in producing products of ultimate importance such as antibiotics, organic acids, amino acids, vitamins , alcohols and pigments. Previous studies showed the positive effect of gamma rays on the microbial productivity of β-glucosidase **(Macris, 1984)**, β-galactosidase **(El-Fouly *et al.*, 2001)**, phosphatase **(Omar and El-Bialy, 2010)** and volatiles compounds (**El-Fouly *et al.*, 2010)**.

Gallic acid production by *A. niger* tannase using cheap agricultural wastes with high gallotannin content is the main aim of this study. As well as, employing gamma rays to increase the tannase productivity by *A. niger* AUIMC 4301.

MATERIALS AND METHODS

Microorganism

Aspergillus niger AUMC 4301 is locally isolated from soil and identified at Mycological Center (AUMC). Assuit University, Egypt.

Gallic acid production by solid state fermentation

A. niger AUMC 4301 was cultivated in 250 ml Erlenmeyer flask containing 10g of wheat bran as support material for solid state fermentation, 3g of tannic acid as sole carbon source and 10 ml of mineral salt solution as control medium. The composition of the salt solution (g/l) was: NH_4NO_3, 2; KH_2PO_4, 1; $MgSO_4.7H_2O$, 2; $CaCl_2.2H_2O$, 0.002. The pH of the medium was adjusted at 5.7. Seven agricultural wastes named apple bagasse, green tea waste; mango seed kernel, olive mill, palm kernel cake, peat moss and tamarind were collected from local market. The productivity of gallic acid by *A. niger* AUMC 4301 (Producer fungus) was investigated using previous mentioned agricultural wastes as carbon sources instead of tannic acid in the control medium. Gallic acid was assayed during the fermentation process at 0, 3, 5, 7, 10, 12 and 14 days. In individual experiment, gallic acid productivity by crude extracellular tannase (Without producer fungus) was estimated at different time intervals (3, 6, 9, 12, 18, 24 and 42 hours) using previous mentioned agricultural wastes rich in gallotannins as substrate.

Analytical methods

Tannase was extracted from the fermented medium of the cultures cultivated under SSF conditions by adding 80 ml of 0.02 M acetate buffer, pH 5 and shaken for 1 hr at 200 rpm. The buffer containing the enzyme was filtered through cloth filter and whatman No. 1 filter paper. The filtrate was used as the crude extracellular tannase. To avoid the presence of any phenolic compounds that may interfere with the determination of protein by the method of **Lowry *et al.* (1951)**, the extracted crude enzyme was dialyzed against distilled water for 24 hr using dialysis bag (Medicell International Ltd). Tannase activity was determined spectrophotometrically using tannic acid as a substrate according to the method of **Mondal *et al.* (2001)**. One unit of tannase activity is defined as the amount of the enzyme required to hydrolyze 1µmole of tannic acid in 20 min under the standard assay conditions. Specific activity is expressed in units/mg protein. Gallic aid was determined spectrophotometrically depending on the method of **Pinto *et al*. (2006)** using ethanolic rhodanine method. Gallotannin concentration was determined according to the method described by **Bajpai and Patil (1996)**.

Irradiation studies.

To study the effect of increasing doses of gamma radiation on tannase production by *A. niger* AUMC 4301, spore suspension of the fungus under study was exposed to different irradiation doses (0.25, 0.5,

0.75, 1, 1.25 and 1.5 kGy) and inoculated individually to solid state fermentation medium. After 5 days, the crude extracellular tannase was extracted and assayed as previously mentioned before. Cobalt-60 model Indian gamma cell located at the National Center for Radiation Research and Technology, Atomic Energy Authority of Egypt was used as source of γ rays; the dose rate at the time of the experiment was 1.5 kGy/ h. In addition, D_{10} value for *A. niger* AUMC 4301 was investigated using spore suspension irradiated in the range of 1-4 kGy. D_{10} value of *A. niger* AUMC 4301 was calculated from the regression linear equation. Czapek-Dox agar medium was used for the count of fungal spore survivors before and after irradiation. Protein profile of *A. niger* AUMC 4301 before and after irradiated by 0.5 kGy was also studied (Suzanna, 1998). Firstly, 5 ml of performic acid was added to 50 mg of fungal dry mats to avoid methionine and cystine destruction. Then, 5 ml of 6N HCl was added to the oxidized mixture, sealed and put in an oven at 110°C for 24 h before dryness in a rotary evaporator. The dried film of the hydrolyzed sample was re-dissolved in sodium citrate buffer (pH 2.2). Finally, total amino acid composition was estimated using high performance amino acid analyzer. Biochom 20, Pharmacia Biotech located at the NCRRT.

RESULT AND DISCUSSIONS

Production of gallic acid from different agricultural wastes by *A. niger* AUMC 4301

Gallic acid is commonly used in the pharmaceutical industry since it has antioxidant, antifungal and antiviral properties. It is also used to treat albumin urea and diabetes moreover it is used for making dyes and inks. Gallotannin-rich agricultural wastes including apple bagasse, green tea waste, mango seed kernel, olive mill, palm kernel cake, peat moss and tamarind were applied as supports for solid state fermentation of *A. niger* AUMC 4301. Table (1) showed that all the studied agricultural wastes containing gallotannin but with different degrees starting with apple bagasse which had the highest gallotannin concentration followed by green tea waste. Some of these wastes contain free gallic acid especially mango seed kernel followed by green tea waste, while olive mill, peat moss and tamarind were found to be free from gallic acid. The results revealed that *A. niger* AUMC 4301 was able to utilize gallotanin present in all tested wastes as a carbon source and began to liberate gallic acid in the medium after the third day of incubation. The amount of estimated gallic acid for each agricultural waste at different incubation times was hesitated, this could be explained by the fact that produced gallic acid was consumed rapidly as a readily available carbon source by *A. niger* AUMC 4301. This phenomenon was reported elsewhere by many authors. **Mahadevan and Sivaswamy (1985)** explained the decrease in gallic acid by the ability of *A. niger* to degrade gallic acid itself and/or intermediates of its degradation including cis-aconitic, α-ketoglutaric and citric acids. In addition, **Saxena *et al.* (1995)** mentioned that *Aspergillus* and *Penicillium spp.* could utilize catechin, gallotannin and gallic acid as carbon sources. **Nalan and Merih (2009)** recorded that for different species of *Aspergillus* and *Penicillium*, the initial increase in gallic acid production, from gall nuts and sumac leaves, was followed by a clear decrease.

Table (1) Production of gallic acid from different agricultural wastes by *A. niger* AUMC 4301.

		Gallic acid conc. (mg.ml^{-1})						
Wastes / **Days**	**Gallotannin conc.(mg/ml)**	**0**	**3**	**5**	**7**	**10**	**12**	**14**
Apple bagasse	21.0	0.50	1.96	0.40	0.50	0.55	0.56	0.66
Green tea waste	19.3	2.51	3.95	2.40	0.18	0.42	0.86	0.32
Mango seed kernel	11.0	9.60	10.6	3.10	1.21	2.00	0.70	1.62
Olive mill	9.50	0.00	0.18	0.24	0.27	0.35	0.43	0.33
Palm kernel cake	6.53	0.30	0.46	0.18	0.09	0.25	0.20	0.18
Peat moss	4.40	0.00	0.31	0.20	0.00	0.30	0.18	0.10
Tamarind	8.97	0.00	0.45	0.15	0.06	0.09	0.45	0.02

Gallic acid formation of by the action of extracellular tannase of *A. niger* AUMC 4301

The previous experiment cleared that gallic acid production by *A. niger* AUMC 4301 is not ideal for industrial application unless the liberated gallic acid from the agricultural wastes is withdrawn continuously out of the fermentation medium, thus, the following experiment was carried out to overcome this disadvantage by incubating the wastes directly with the crude enzyme produced by *A. niger* AUMC 4301 for different time intervals ranging from 3 to 42 h, thereafter, the amount of gallic acid formed was determined. Gallic acid production didn't start before 12 h in fermented olive mill and tamarind whereas its accumulation didn't observed before 18 h in case of peat moss (Table 2). This variation could be attributed to the complexity of these wastes. It is also evident from the results that the concentration of gallic acid was increased gradually and reached its maximum at 18 h for almost studied agricultural wastes. After that, the level of gallic acid formed was decreased. The decrease in the rate of gallic acid formation may be either due to competitive inhibition of gallic acid or denaturation of the enzyme as a result of prolonged incubation period. Additionally, gallic acid may enter in another pathways catalyzed by other enzymes present in the crude preparation of tannase. Gallic acid (soluble phenolics) may also polymerize to form macromolecule. **Vazquez-Duhalt *et al.* (1994)** stated that the decrease in measurable soluble phenolics could be in response to the polymerization of the released soluble phenolics at the late stage of incubation.

Table (2) Gallic acid formation by the action of extracellular tannase of *A. niger* AUMC 4301

	Gallic acid conc. (mg/g)							
Incubation time (h) / Wastes	**0**	**3**	**6**	**9**	**12**	**18**	**24**	**42**
Apple bagasse	5.00	15.5	19.0	22.0	25.0	43.2	39.5	26.1
Green tea waste	25.1	43.0	49.0	59.0	65.0	76.5	56.5	51.1
Mango seed kernel	96.0	83.0	88.0	91.0	93.3	103.6	96.5	94.5
Olive mill	0.00	0.00	0.00	0.00	20.0	35.7	26.8	21.5
Palm kernel cake	3.00	5.00	6.50	9.00	22.0	39.0	21.9	10.6
Peat moss	0.00	0.00	0.00	0.00	0.00	26.8	8.50	0.00
Tamarind	0.00	0.00	0.00	0.00	3.00	28.1	17.5	10.5

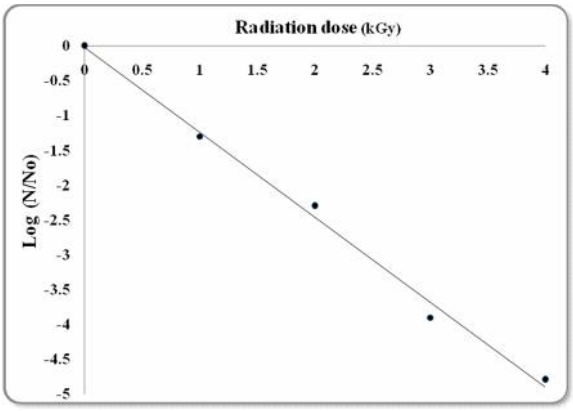
Effect of gamma rays on the productivity of *A. niger* AUMC 4301 tannase

For radiation microbiological view, the over-expression of *A. niger* AUMC 4301 tannase using gamma rays is interesting effort. Gamma rays mean the emission and propagation of energy through space or through a material as a wave motion. Microorganisms differ greatly in their resistance to gamma radiation. Moreover, radiation resistance of the same microorganism differs with species and even with strains. For individual microorganisms, D_{10} values are very important and useful to determine the lethal dose. It is known that any recommended radiation dose which stimulates a function in microorganisms must be less than sublethal dose which may be used to select mutants from the survival organisms after irradiation of microbial strain. Exposure of *A. niger* AUMC 4301 to different gamma radiation doses showed that the count of fungal colonies decreased by increasing radiation doses. The dose response curve was belonged to type A, which indicated simple exponential relationship with a constant slope over the whole dose range (fig 1). The calculated D_{10} value was 0.81 (Table 3) indicating that *A. niger* AUMC 4301 strain is moderately radio resistant. **Sommer (1973)** concluded that the D_{10} values of all fungi were in the range from 0.15 to 1.0 kGy. **Osman (1973)** found that the most radioresistant species of fungi were *A. niger, Cladosporium herbarum, Penicillium notatum* and *Alternaria humucola* as compared with other isolates of fungi. **El-Hadi (1986)** stated that the D_{10} values of *A. flavus, A. niger* and *A. terreus* were 0.438, 0.853 and 0.403 kGy in the same order. **Risk *et al.* (2000)** reported that D_{10} values of *A.niger, A. flavus* and *P. chrysogenum* were o.775. 0.59 and 0.63 kGy, respectively. **Tauxe (2001)** reported that the high energy rays of irradiation directly damage the DNA of living organisms, inducing cross-linkages and other changes that make the organism unable to grow or reproduce. When these rays interact with water molecules in an organism,

they generate transient free radicals that can cause additional indirect damage to DNA. Incomplete inhibition may result from a little injury of cells **(Aubrey, 2002)**, while complete inhibition of fungal growth has been reported that to be due to destruction of DNA structure of the cells by gamma rays, and the cells cannot continue their functions **(Smith and Pillai, 2004).** Since *A. niger* AUMC 4301 is moderately resistant to gamma rays, low dose levels were used to enhance the fungus strain to produced more tannase. Figure (2) showed that the maximum tannase productivity of *A. niger* AUMC 4301 was achieved at 0.5 kGy. Several studies recorded that the stimulative effect of low doses of gamma radiation results in an increase in microbial growth and metabolic activities. In the mean time, high doses of gamma radiation were proved to be inhibitory for both growth and enzymatic activities of microorganisms **(Sadi, 1987)**. **Helal *et al*. (1987)** recorded that maximum metabolic activity of *Trichoderma koningii* and *A. niger* were obtained after exposure to radiation doses of 0.1 and 0.25 kGy, respectively. The study of **Gherbawy (1998)** showed that the lowest dose of gamma irradiation enhanced virulence of *A. niger* by producing more polygalacturonase, cellulase and protease, while the higher doses were inhibitory to the growth of fungi. Also, he showed that production of polygalactronase, pectinmethylgalactironase, cellulase and protease enzymes was enhanced by the low dose of irradiation, but at higher doses, production was significantly reduced. On the other hand, Omar and El-Bialy (2010) demonstrated that irradiation doses equals 1 and 1.5 kGy enhanced phosphatase activety of *Bacillus megaterium* ATCC 19213 and *Bacillus subtilis* ATCC 6633 by nearly three and two folds respectively.

Table (3) Effect of increasing doses of gamma radiation on spore viability of *A. niger* AUMC 4301.

Radiation dose (kGy)	Survivor number	Log (N)	Log (N/N_0)
0.0	1.84×10^6	6.26	0.00
1.0	1.00×10^5	5.00	-1.30
2.0	1.00×10^4	4.00	-2.30
3.0	2.00×10^2	2.30	-3.90
4.0	3.00 x 10	1.48	-4.88
D_{10}	0.81 kGy		



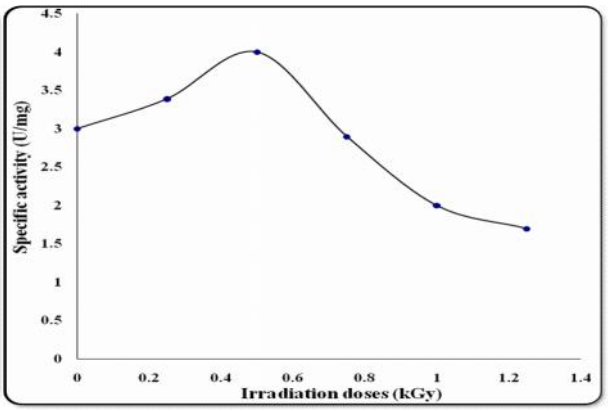


Fig. (1) Dose response curve of *A. niger* AUMC 4301. Fig. (2) Effect of low doses of gamma irradiation the productivity of *A. niger* AUMC 4301 tannase.

Amino acids profile of *A. niger* AUMC 4301 spores before and after irradiation

Gamma radiation is energy. When the cells are exposed to this energy, the organic cell contents usually absorb it causing changes in their chemical bonds, thus leading to changes in the organic molecules either carbohydrates, proteins or lipids. A change in protein molecules is the most important one which affect cell metabolism i.e. the amino acid. Total amino acid profile of *A. niger* AUMC 4301 changed slightly after exposure to 0.5 kGy of gamma radiation (table 4). Thus, it indicates that the fungus showed resistance to gamma radiation due to its contents of cystine, methionine and histidine. Our results agreed with some studies which demonstrated that certain amino acids specially those containing sulphur bond (cysteine, cystine and methionine) and that having double bond (histidine)

gave the organism high resistance to gamma radiation since they work as scavengers to the free radicals occurred due to the effect of ionizing radiation on the water molecules **(Milligan *et al*., 1995** and **Winters *et al*., 1995).** In this respect, **Jacqueline *et al*. (1996)** mentioned that high concentration of sulphur amino acids in bacterial cells enable them to tolerate the effect of high doses of radiation through scavenging the free radical so, protecting DNA against oxidative damage of radiation. **Dahl *et al*. (1988)** demonstrated that histidine and N- alanyl-methyl histidine showed a protective effect through lessing lethality and single strand break in DNA scavenging free radicals making the bacterial strain bear the induction of radiation resistance. Thus the slight changes in the 17 amino acids detected in *A. niger* AUMC 4301 before and after exposure to o.5 kGy of gamma rays indicating that 0.5 kGy change the over-expression of *A. niger* AUMC 4301 tannase positively by altering the amino acids composition slightly.

Table (4) Total amino acid profile of *A. niger* AUMC 4301 before and after irradiation.

	Conc. of amino acid (%)	
Amino acid	**Un irradiated (Control)**	**Irradiated by 0.5 kGy**
Alanine	0.20	0.22
Arginine	0.38	0.40
Aspartic	0.40	0.38
Cystine	0.28	0.26
Glutamic	0.34	0.36
Glycine	0.26	0.24
Histidine	0.40	0.46
Isoleucine	0.50	0.46
Leucine	1.40	1.46
Lysine	0.42	0.44
Methionine	0.04	0.12
Phenylalanine	0.44	0.48
Proline	0.14	0.14
Serine	0.32	0.30
Threonine	0.38	0.36
Tyrosine	0.64	0.66
Valine	0.38	0.38
Total amino acid (%)	**6.92**	**7.12**

REFERENCES

1. **Aubrey, F.M. (2002).** Inactivation by Irradiation, Control of Food borne Microorganisms, MARCEL DEKKER, Inc. p. 535.
2. **Aguilar, C.N.; Rodríguez-Herrera, R.; Gutiérrez-Sánchez, G.; Augur, C.; Favela-Torres, E.; Prado-Barragán, L.A.; Ramírez-Coronel, A. and Contreras-Esquivel, J.C. (2007)**. Microbial tannases: advances and perpectives. Appl. Microbiol. Biotechnol., **76**: 47-59.
3. **Bajpai, B. and Patil, S. (1996).** Tannin acyle hydrolase (EC 3.1. 20) activity of *Aspergillus, Penicillium, Fusarium* and *Trichoderma*. World J. of Microbiol. & Biotechnol. **12**: 217-220.
4. **Barthomeuf, C.; Regerat F. and Pourrat, H. (1994)**. Production, purification and characterization of a tannase from *Aspergillus niger* LCF 8. J. Ferment. Bioeng. **77**: 320-323.
5. **Bhat, T.K.; Singh, B. and Sharma, O.P. (1998).** Microbial degradation of tannins-A current perspective. Biodegradation. **9**: 343-357.
6. **Dahl, T.A.; Midden, W.R. and Hartman, P.E. (1988).** Some prevalent biomolecules as defenses against single oxygen damage. Photochem. Photobiol. **120**: 420-425.
7. **El-Fouly, M.Z.; El-Mongy, T.M.; Abu-Shady, M.R. and El-Shafy, H.M. (2001)**. Effect of environmental factors and gamma irradiation on the production of ß- galactosidase enzyme from *E.coli*. Egypt. J. Biotechnol., **9**: 275-285.
8. **El-Fouly, M. Z.; Shahin, A. A. M. and El-Bialy, H. A. (2010)**. Biological control of sapstain fungi in Egyptian wood stores and infected trees. Ann Microbiol., under press.
9. **El-Hadi, A.F.M. (1986).** Studies on the microbial flora contaminated animal feed and its control by gamma irradiation. M.Sci. Thesis, Faculty of Sci., Zagazig Univ., Egypt.
10. **Gathon A, Gross Z, Rozhanski M**. **(1989)**. Propyl gallate: enzymatic synthesis in a reverse micelle system. Enzyme Microb Technol.,**11**: 604–609.
11. **Gherbawy, A.M.H.Y. (1998)**. Effect of Gamma Irradiation on the Production of Cell Wall Degrading Enzymes by *Aspergillus niger*. Inter. J. of Food Microbiol. **40**:127-131.
12. **Helal, G.A.; El-fouly, M.Z.; El-Zawahry, Y.A. and Shindia, A.A. (1987).** Effect of gamma irradiation on cellulase enzymes production and metabolic activities of *Trichoderma koningii* Oudemuns and *Aspergillus niger* Van Tiegh. Egypt. J. Appl. Sci., **2**: 939-947.
13. **Jacqueline, E.F.; Bran, E.F.; Sarquis, F.; Vincen, T.; Lafleur, M.M. and Retel, J. (1996)**. Effect of sulphydryl compound cysteamine on gamma- radiation induced mutation in double strand M 13. Mut. Res. **364**: 171-182.
14. **Kar, B. and Banerjee, R. (2000)**. Biosynthesis of tannin acyl hydrolase from tannin-rich forest residue under different fermentation conditions. J. Ind. Microbiol. Biotech. **25**: 29-38.
15. **Khanbabaee, K. and Van-Ree, T. (2001).** Tannins: classification and definition. Nat Prod Rep. **18**: 641-9.
16. **Lekha, P.K. and Lonsane, B.K. (1997).** Production and application of tannin acyl hydrolase: state of the art. Adv. Appl. Microbiol. **44**: 215-260.
17. **Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951).** Protein measurement with the Folin-phenol reagent. J. Biol. Chem. **193**: 265-275.
18. **Macris, B.J. (1984)**. Production and characterization of cellulose ß- glucosidase from mutant of *Alternaria alternata*. Appl. Environ. Microbiol., **47**: 560-570
19. **Mahadevan, A.; Sivaswamy, S.N. (1985)**. Tannins and microorganisms. In: Mukerji KG, Pathak NC, Singh VP (eds), Frontiers in applied microbiology. Print House, Lucknow, pp. 327-347.
20. **Milligan, J.R.; Ng, J.Y.; Wu, C.; Agivlera, J.A.; Fahey, R.C. and Ward, J.F. (1995).** DNA repair by thiol in air shows two radicals make a double-strand break. Rad. Res. **143**: 273-280.
21. **Mondal, K.C.; Banerjee, D.; Jana, M. and Pati, B.R. (2001)**. Colorimetric assay for determination of tannin acyl hydrolase (E.C. 3.1.1.20) activity. Anal. Biochem. **295**: 168-171.
22. **Mukherjee, G. and Banerjee, R. (2003a).** Production of Gallic acid. Biotechnological routes (Part 1). Chimica Oggi /Chemistry Today **21**: 59-62.

23. **Mukherjee, G. and Banerjee, R. (2003b).** Production of Gallic acid. Biotechnological routes (Part 2). Chimica Oggi /Chemistry Today **21**: 70-73.
24. **Nalan, Y.S. and Merih, K. (2009).** Isolation of gallic acid-producing microorganisms and their use in the production of gallic acid from gall nuts and sumac. African Journal of Biotechnology. **8**: 1110-1115, 20.
25. **Omar, A. M. and El-Bialy, H. A. (2010).** Agriculture applications for some gamma irradiated bacterial strains. Egyptian journal of radiation science and application, Under press.
26. **Osman, N.E. (1973).** Effect of radiation on soil fungi population. M.Sc. Thesis, Faculty of Sci., Cairo Univ., Egypt.
27. **Pinto, G.A.S.; Couri, S. and Gonçalves, E.B. (2006)**. Replacement of methanol by ethanol on gallic acid determination by rhodanine and its impacts on the tannase assay. Eletronic J. Environ. Agric. Food Chem. **5**: 1560-1568.
28. **Risk, M.I.; El-Fouly, M.Z.; Hassan, A.A. and Abdel Khalek, E.S. (2000).** Effect of radiation on the growth, water activity and nucleic acids of some animal mummies microbial isolates. Az. J. Microbiol. **50**: 283-294.
29. **Sadi, S. (1987).** Irradiation effects on the alcohol fermentation ability of *Saccharomycs cerevisiae*. Majalah-Batkan in (Indonesian), **20**: 40.
30. **Saxena, R.K.; Sharmila, P. and Singh, V.P. (1995)**. Microbial degration of tannins. In: Singh VP (ed.) Biotransformations: Microbial degration of healt-risk compounds. Progress in Ind. Microbiol. Elsevier Science B. V., Amsterdam, **32**: 259-270.
31. **Smith, J.C. and Pillai, S. (2004)**. Irradiation and Food Safety, Food Technol., **58**: 48-54.
32. **Sommer, N.F. (1973)**. The effect of ionizing radiation on fungi. Manual of radiation. Sterilization of medical and biological materials. International Atomic Energy Agency, Vienna.
33. **Suzanna, Nielson, S. (1998)**. Food analysis, Second edition, An Aspen publication, Aspen publishers, Inc., Gaithersburg, Mary Lan P34.
34. **Tauxe, R.V. (2001)**. Food Safety and Irradiation: Protection the Public from Food Borne Infections, Emergent Infection Disease, **7**: 516-521.
35. **Vazquez-Duhalt, R.; Westlake, D.W.S. and Fedorak, P.M. (1994).** Lignin peroxidase oxidation of aromatic compounds in systems containing organic solvent. Appl. Env. Microbiol. **60**: 459- 466.
36. **Winters, R.A.; Zukowshi, J.; Ercal, N.; Mattews, R.H. and Spitz, D.R. (1995)**. Analysis of glutathione and glutathiondi-sulphind, cysteine homocysteine and other biological thiol by HPLC following derivitization by N-(1-pyrenyl) maleimide. Analy. Biochem. **227**: 14-21.