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INFLUENCE OF GAMMA-RADIATION ON THIRD AND FOURTH GENERATION CEPHALOSPORIN ANTIBIOTICS

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ABSTRACT

A total number of 160 pharmaceutical samples of four antibiotic groups were collected, and microbial load was counted by surface plate method in the term of CFU/ml. Total contaminated samples with bacteria and/or fungi were (46) samples with a percentage of (28.7%) where (64) fungal isolates and a single bacterial isolate were detected. Three fungal isolates, which represented the predominant fungal isolates, were selected and identified by 18S rRNA for investigating the effect of gamma radiation as a sterilization method. The dose response curve of the three fungal isolates showed that 6.0 and 7.0 KGy reduced their viable count completely. Three contaminated antibiotics (cefotaxime, cefoperazone and cefepime) were irradiated to determine the sterilization dose. After irradiation, no or slight changes were observed in their odor, clarity, solubility in water, pH and their forms. However, mild changes in their color were detected. The dose 25 KGy was an efficient dose for complete sterilization and could not degrade the chemical structure of the samples as determined by HPLC, which indicated the stability of the drugs after sterilization by gamma radiation. The results of microbiological potency showed that the antibacterial activity generally remained unaffected or slightly decreased for the three antibiotics.

KEYWORDS: Antibiotics, Microbial load, 18S rRNA, Sterilization, Gamma Radiation.

1. INTRODUCTION

Microbial contamination of pharmaceutical preparations is a common problem which has been reported for several medicaments,[1,2] such contaminants include bacteria, fungi, yeasts or molds.[3] Contaminant microorganisms can adapt with least nutritional requirements and are capable of multiplying within both bulk and finished product.[4,5] Some of these contaminants may be pathogenic while others grow as commensals even in the presence of preservatives and spoil products.[6] Presence of microbial contaminants cause physicochemical changes that led to the spoilage of products and proved to be a potential health hazard to the consumer.[7] Antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms.[8] Although antibiotic generally refers to antibacterial, antibiotic compounds are differentiated as antibacterials, antifungals and antivirals to reflect the group of microorganisms they antagonize.[8,9]

Historically the pharmaceutical industry has relied on steam, dry heat, ethylene oxide gas, filtration and chemical processes to reach microbial reduction requirements. Nowadays, several pharmaceuticals, raw materials and finished products are being sterilized successfully with gamma irradiation.[10-14] Gamma radiation is the most important method for sterilization of pharmaceuticals, due to the high ability to penetrate the sterile packaging of pharmaceutical and cosmetic products and the fact that the final heat during the process did not increase their traceability or ability to deliver effective and helpful for the heat-sensitive substances in packaging materials or operations as it has a small temperature rise. Gamma rays are also easier to control, secure, reliable and provide a fast process. The residue used was purified gases and the process does not require post-quarantine measures and the product obtained has no harmful effect on the environment.[15-17] The high effectiveness of drug sterilization by gamma radiation results from the high sensitivity of all microorganisms to these electromagnetic waves.[18,19] It must be said the increase of the dose of radiation in order to get higher sterility assurance level (SAL) ranges may cause change in the chemical structure of sterilized substances.[20] The use of gamma radiation as a sterilization method for medicinal products requires accurate analyses using HPLC to check that the active ingredient has not been modified and no toxic products have been produced.[21-23] Radiation technology is highly recommended for the sterilization of most solid antibiotics.[17]

2. MATERIALS AND METHODS

2.1. Antibiotics collection

A total number of 160 pharmaceutical products (antibiotic vials) were tested. The samples comprised of (64) antibiotics of the third generation cephalosporins, (32) of the fourth generation cephalosporins and (40) of penicillins, which represented Beta-Lactam antibiotics family. Sixteen (16) of aminoglycosides and (8) of macrolides families. Samples were randomly purchased from local pharmacies from different locations in Gharbia, Alexandria and Cairo Governorates, Egypt.

2.2. Microbial load determination

First, two concentrations of each antibiotic were selected, a half gram or one gram of the antibiotic were diluted with 4.5 ml or 9 ml respectively of sterile saline and vortex for 1 min, and then serially diluted with sterile saline as described in the British Pharmacopoeia,[24] thus, the sterile saline was used as a negative control.[25] Then, the viable count was determined using the surface plate method on LB Agar (for bacteria) and Sabouraud's dextrose agar and PDA,[26] (for fungi). According to Abo-State,[27] the aliquot of 100µl for each sample was directly spread on the surface of sterile LB and 100µl for Sabouraud media. In addition, duplicate plates were used for each dilution. The inoculated plates were subsequently incubated at 37°C for 48 hours for bacteria, and at 28 °C for 7 days for fungi. The average of the counts was taken and number of colonies forming unit per ml (CFU/ml) was calculated. Thus, the frequency of contamination by each group (%) = (number of contaminated samples / total number of samples) x100,[28,29]

2.3. Isolation of the microbial contaminants

The separated microbial colonies were isolated from the plates and sub-cultured on LB agar or Sabouraud agar media for purification. Then, the pure separated single colony was finally preserved for identification purpose and further use on agar slants at - 4 °C.[25]

2.4. Determination of the dose response curve

The most three predominant fungal isolates were selected to determine their dose response curves. According to Abo-State (2003),[30] five (250) ml Erlenmeyer flasks containing 100 ml of Sabouraud agar medium were inoculated for each fungal isolate. Then the inoculated flasks were incubated at 28°C for 7 days, and spores were collected by adding 30 ml of sterile saline containing 0.1% (v/v) Tween-80 for each flask. Finally, the spore suspension of each flask was collected in a new sterile flask to form a pool as stock spore suspension. The spore

suspensions of the fungal isolates were distributed in clean sterile screw cap test tubes; thus, each tube contained 5 ml of spore suspension. Then, the spore suspensions of each isolate were exposed to different doses of gamma radiation (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 kGy) from a Cobalt-60 gamma source (Indian chamber at National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority (AEA), Cairo, Egypt). The dose rate was 1.0 KGy/25 min. at the time of experiment; while the control (0 kGy) was left without irradiation. After that, two replicates were used for each dose as well as for the non-irradiated control.[27] The irradiated suspensions and the non-irradiated control were serially diluted and inoculated on the surface of Sabouraud agar plates (surface plate technique). Then, the plates were incubated at 28°C for 7 days before counting. After that, the count (spore/ml) was determined; thus, the log number of survivors was plotted against the absorbed radiation dose in kGy (the dose response curve).[27,30]

2.5. Identification of the selected fungal isolates by 18S rRNA

2.5.1. DNA extraction

DNA was extracted from the samples using the Quick-DNA™, Fungal/Bacterial Microprep Kit (Zymo research catalog number D6007) following manufacturer's instructions.

2.5.2. PCR amplification of fungal 18S rRNA

An aliquot (5µl) of DNA was added to a PCR reagent mix. Following this: ITS regions were amplified in polymerase chain reaction (PCR) using the genomic DNA as template and fungal ITS primers of ITS1 (5-CTTGGTCATTTAGAGGAAGTAA-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3) to amplify an approximately 550bp of small subunit of fungal ribosomal gene. The PCR mixture (50µl) contained 25 µl of Maxima® Hot Start PCR Master Mix (2X) (Thermo K1051), 20µM (1 µl) of each primer, and 18µl of nuclease-free water. The PCR was performed at thermal cycling condition with a hot starting performed at 95ºC for 10 min, followed by 35 cycles of 95ºC for 30 sec., 57ºC for 1 min, and 72ºC for 90 sec., followed by a final extension performed at 72ºC for 10 min.

PCR products were run on a 1% agarose gel electrophoresis, stained with a 10% ethidium bromide solution and then digitally captured using a gel imaging system (Gel Doc, Bio-Rad).The PCR products were purified using GeneJET™ PCR Purification Kit (Thermo K0701), and then were sequenced on GATC Company by use ABI 3730xl DNA sequencer by using forward and reverse primers.

2.5.3. Phylogenetic analysis

Fungal ITS sequences were compared against the GenBank database using the NCBI BLAST program. Sequences were then compared with fungal ITS sequences in the GenBank database using BLASTN. Multiple sequence alignment was done using ClustalX 1.8 software package (http://wwwigbmc.u-strasbg.fr/BioInfo/clustalx) and a phylogenetic tree was constructed by the neighbor-joining method using MEGA (Version 6.1) software. The confidence level of each branch (1,000 repeats) was tested by bootstrap analysis.

2.5.4. Determination of accession numbers

Recovered sequences were deposited in GenBank to access their numbers.

2.6. Sterilization of Antibiotics by Gamma Radiation

The three selected antibiotics (Cefotaxime, Cefoperazone and Cefepime) were exposed to different doses of gamma irradiation (0, 10.0 and 25.0 kGy). After that, the irradiated and non-irradiated samples were tested for organoleptic analysis, chemical and microbiological tests.[31]

2.6.1. Organoleptic Analysis

The irradiated samples were subjected to organoleptic analysis in order to determine the change in its color, form, odor and solubility, compared to non-irradiated sample.[32,33]

2.6.2. Measurements of pH

The measurements of the pH values for the irradiated and non-irradiated active ingredients were performed using the pH meter (Thermo Scientific, Singapore).

2.6.3. Microbiological potency

It was carried out to measure the efficiency of irradiated antibiotics against Gram-positive and Gram-negative bacteria. Antimicrobial activity of samples was determined by disc diffusion method according to the standard method by CLSI, 2012.[34] The clinical pathogenic bacterial isolates were kindly provided by Prof. Abo-State. These isolates were two Gram-positive bacteria: (*Bacillus subtilis* and *Staphylococcus epidermidis)* and six Gram-negative bacteria *(E.coli, Citrobacter* spp.*, Salmonella entero, Salmonella* sp.*, Pseudomonas fluorescens* and *Pseudomonas* sp.*).*

2.6.4. HPLC Analysis

The analytical determination for the irradiated (10 and 25kGy) and non-irradiated antibiotics have been determined by High Performance Liquid Chromatography (HPLC) at the Regional Center for Food & Feed, Agricultural Research Center, Giza, Egypt. The conditions of the experiment were as follows: the system of HPLC (Agilent technologies 1200 series), LC-MS/MS 4000 Q-trap (Applied bio-systems), MDS SCIEX,LC Column (Agilent C18 150x4.6mmx5 um). In addition, the mobile phases were as follows: Mobile Phase A: (Water +0.1% formic acid), and Mobile Phase B: (Methanol +0.1% formic acid) or (Acetonitrile + 0.1% formic acid). Finally, the separation was performed at the ambient temperature at a flow rate of 500µl/min according to the proposed method of Waksmundzka.[35]

3. RESULTS AND DISCUSSION

3.1. Determination of the Microbial Load of the Samples

Microbial load of the samples was counted by surface plate method in the term of CFU/ml. In general, according to the type of isolates, fungi were the most predominant isolates which were recorded in (45) samples of (160) (n= 45/160) and single bacteria (n= 1/160) was recorded in one sample. So total contaminated samples with bacteria and fungi were (46) samples with a total percentage of (28.7%) where (64) isolates were fungi and one isolate was bacteria. The level of fungal contamination in the present study was the most prevalent, where it compromised about 28.1% followed by single bacteria in one sample with a percentage 0.63% as shown in Table (1) and Fig. (1).

Table 1: Presentation of microbial contamination of the collected samples.

Group of antibiotics	Active ingredient	Total no. of samples	No. of samples contaminated with					No. of isolates		
			Fungi	Bacteria	Fungi and Bacteria	Total		Fungi	Bacteria	Total
						No.	%			
Third generation cephalosporins antibiotics	Cefotaxime	16	4	0	0	4	25%	5	0	5
	Cefotaxime	16	3	0	0	3	18.7%	5	0	5
	Cefoperazone	32	12	0	0	12	37.5%	14	0	14
	Total	64	19	0	0	19	29.7%	24	0	24
Fourth generation cephalosporins antibiotics	Cefepime	32	10	0	0	10	31.2 %	15	0	15
	Total	32	10	0	0		31.2%	15	0	15
Penicillin antibiotics	Amoxicillin	24	8	1	0	9	37.5%	11	1	12
	Ampicillin	16	5	0	0	5	31.2%	9	0	9
	Total	40	13	1	0	14	35%	20	1	21
Aminoglycosides	Streptomycin	8	3	0	0	3	37.5%	5	0	5

antibiotics	Amikacin	8	0	0	0	0	0%	0	0	0
	Total	16	3	0	0	3	18.7%	5	0	5
Macrolides antibiotics	Azithromycin	8	0	0	0	0	0%	0	0	0
	Total	8	0	0	0	0	0%	0	0	0
Total		160	45	1	0	46	28.7%	64	1	65

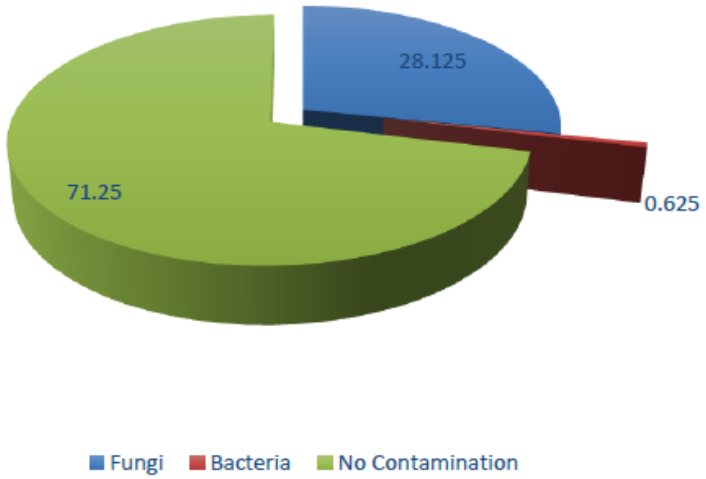


Fig 1: Percentage of total fungal and bacterial contaminated and non-contaminated samples out of the total number of samples.

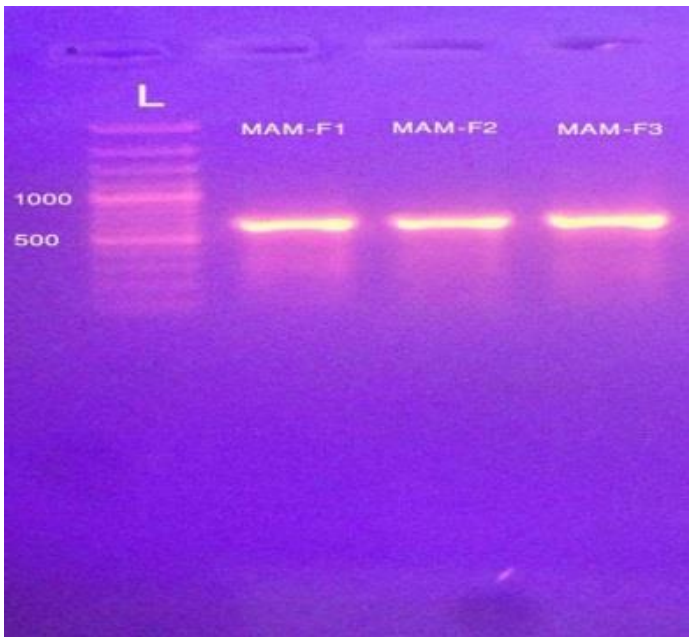


Fig. 2: Agarose gel electrophoresis for PCR products of the amplified ITS fragments of the selected fungal isolates. L, DNA ladder. Base pair (bp) size of two bands are shown on 500 and 1000 bp.

Phylogenetic tree and similarities

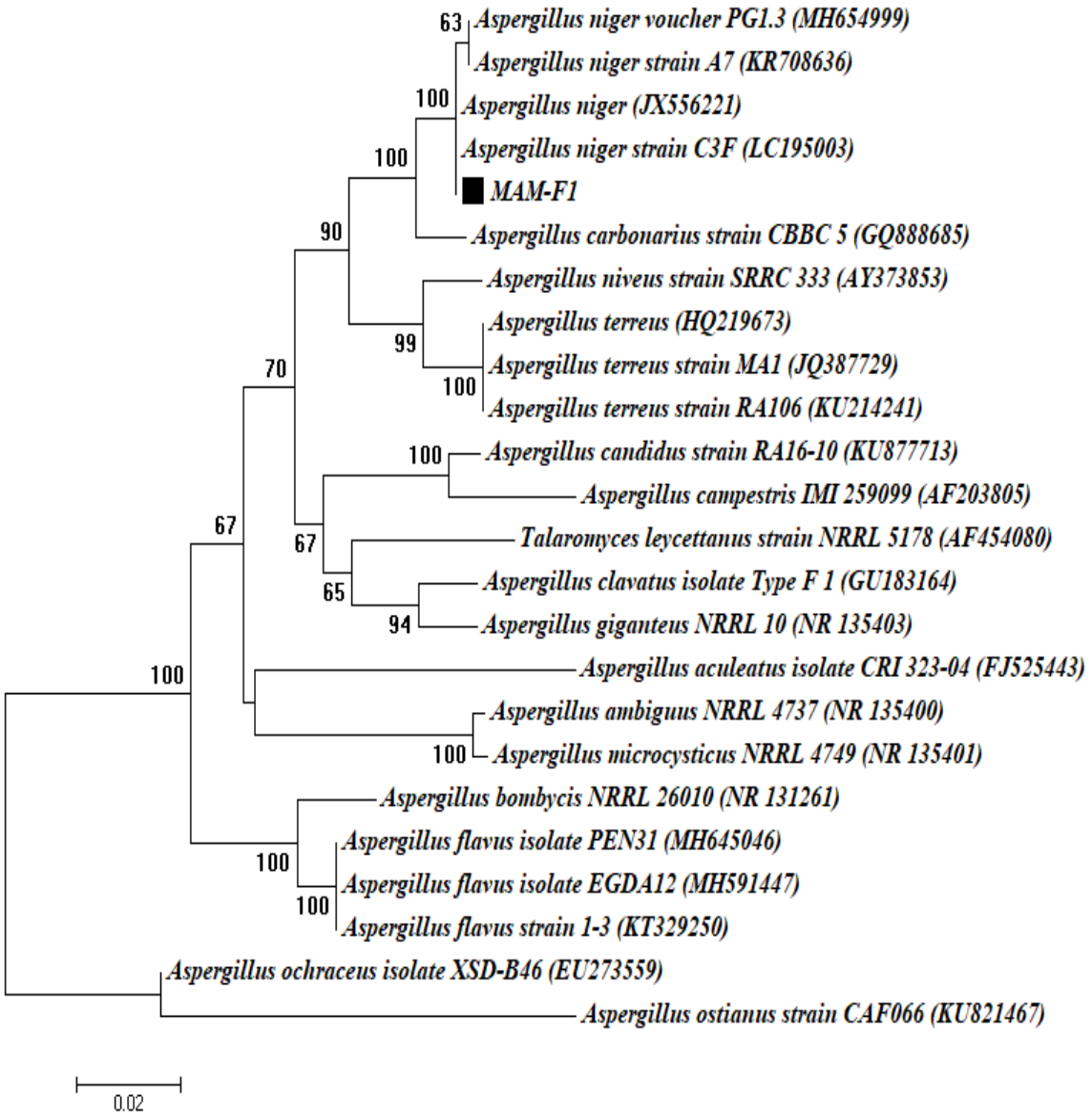


Fig. 3: Phylogenetic tree based on fungal ITS sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura two-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Black squares indicate the stain (MAM-F1) isolated in this study. GenBank accession numbers of reference sequences are indicated.

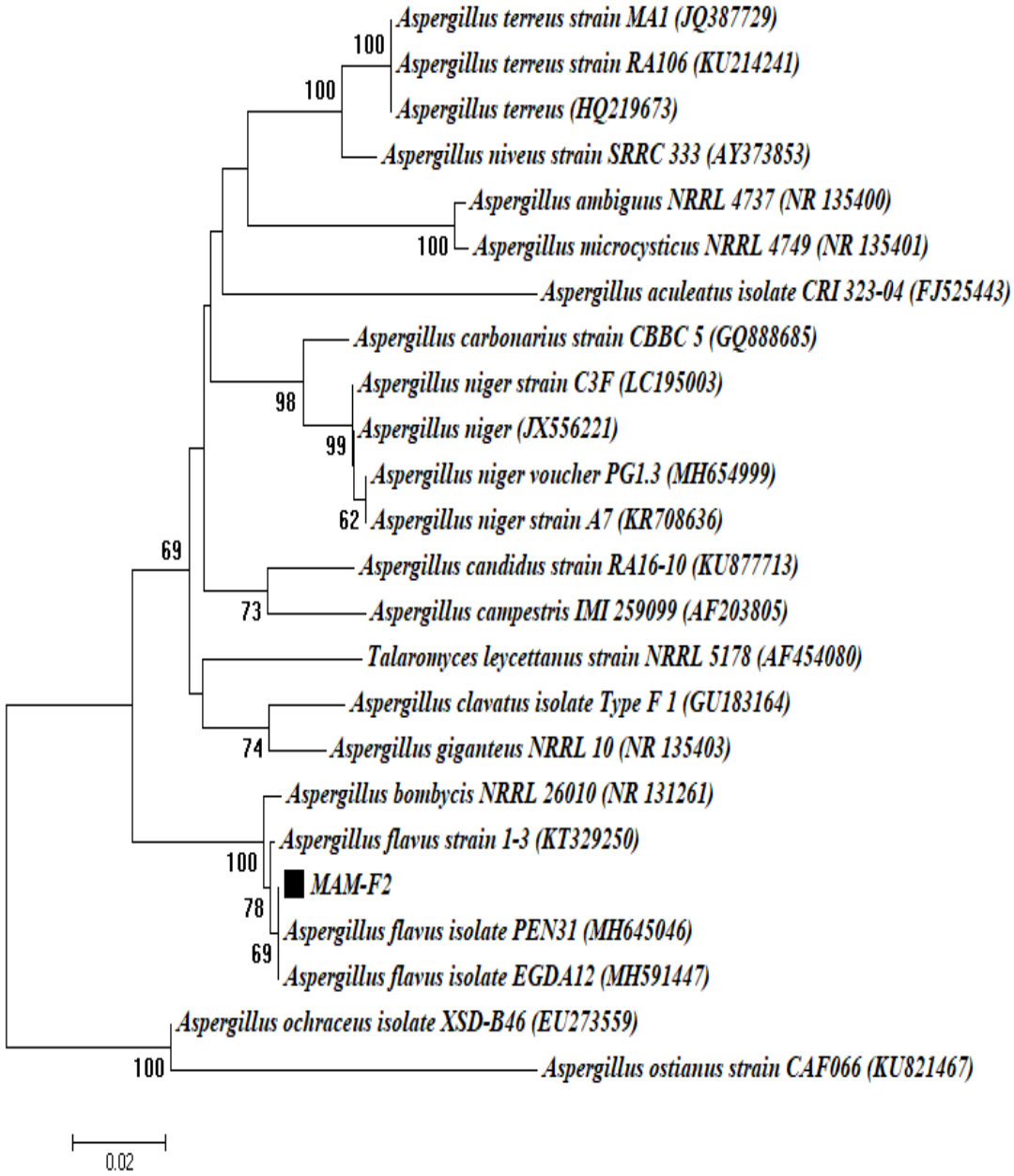


Fig. 4: Phylogenetic tree based on fungal ITS sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura two-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Black squares indicate the stain (MAM-F2) isolated in this study. GenBank accession numbers of reference sequences are indicated.

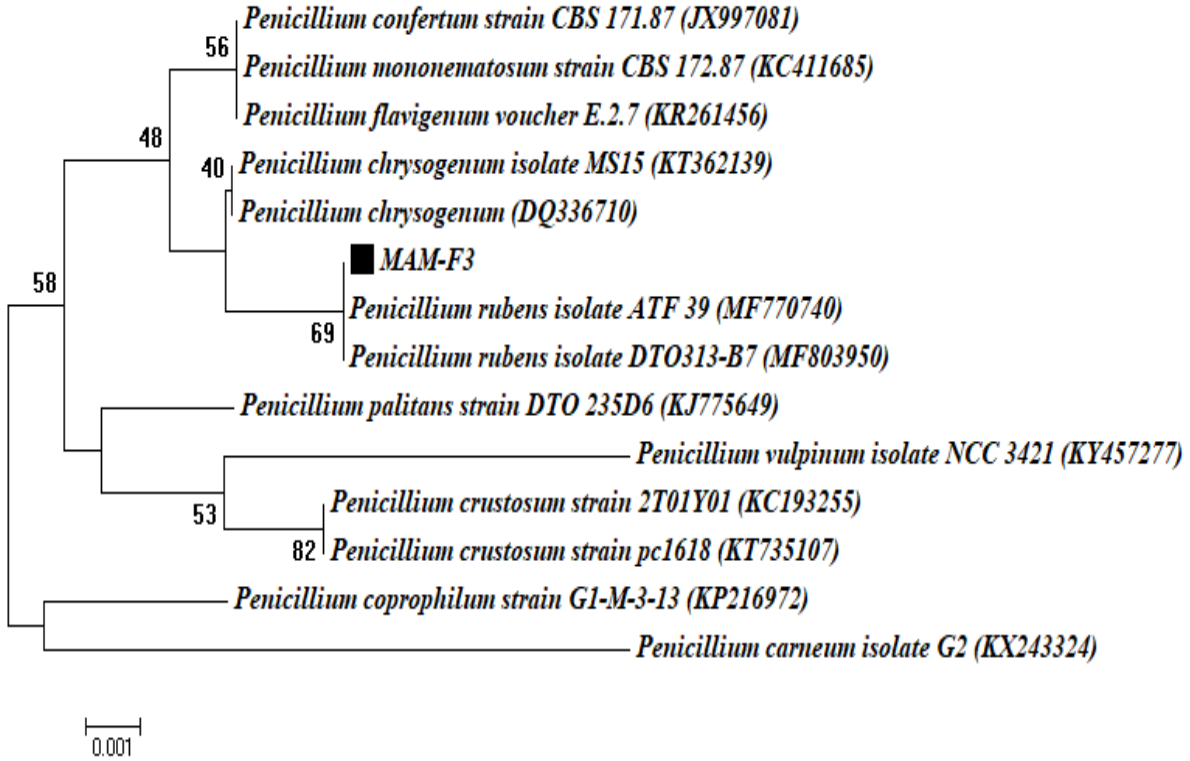
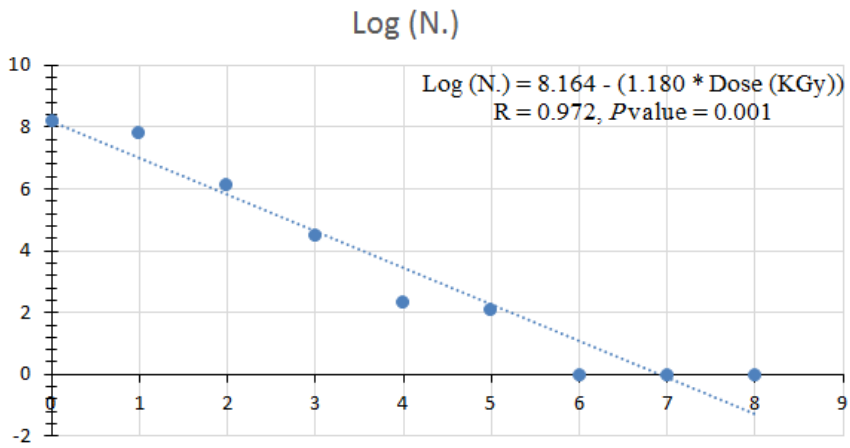


Fig. 5: Phylogenetic tree based on fungal ITS sequences. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura two-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Black squares indicate the stain (MAM-F3) isolated in this study. GenBank accession numbers of reference sequences are indicated.

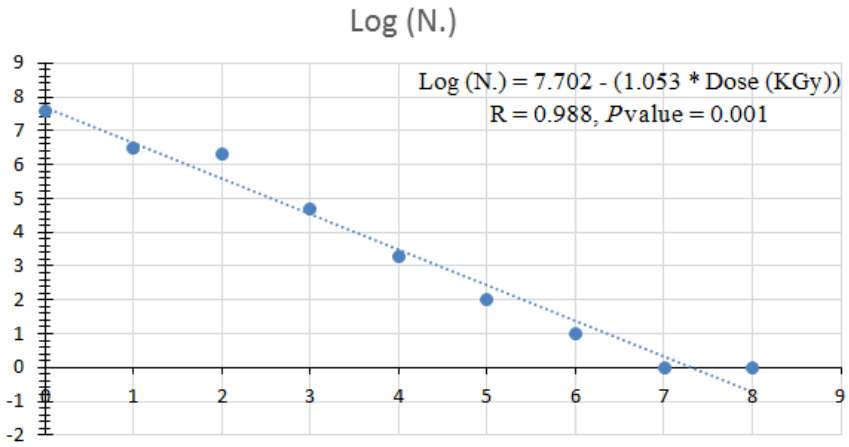
Table 2: Molecular identification based on fungal ITS sequencing data of the isolated strains.

Isolate code	Isolate identification	Closest related sequence (Similarity %)	GenBank accession number
MAM-F1	*Aspergillus niger*	*Aspergillus niger* strain: C3F (100%)	MH828249
MAM-F2	*Aspergillus flavus*	*Aspergillus flavus* isolate NRRL 26010 (100%)	MH828250
MAM-F3	*Penicillium rubens*	*Penicillium rubens* isolate DTO313-B7 (69%)	MH828251



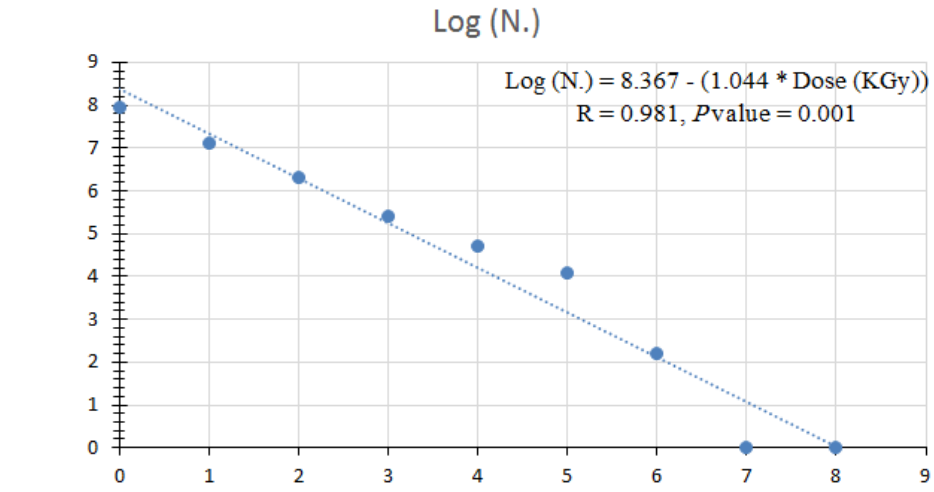
Linear regression between Dose in KGy and Log N of the viable count of the fungal isolate MAM-F1

Figure 6(a).



Linear regression between Dose in KGy and Log N of the viable count of the fungal isolate MAM-F2

Figure 6(b).



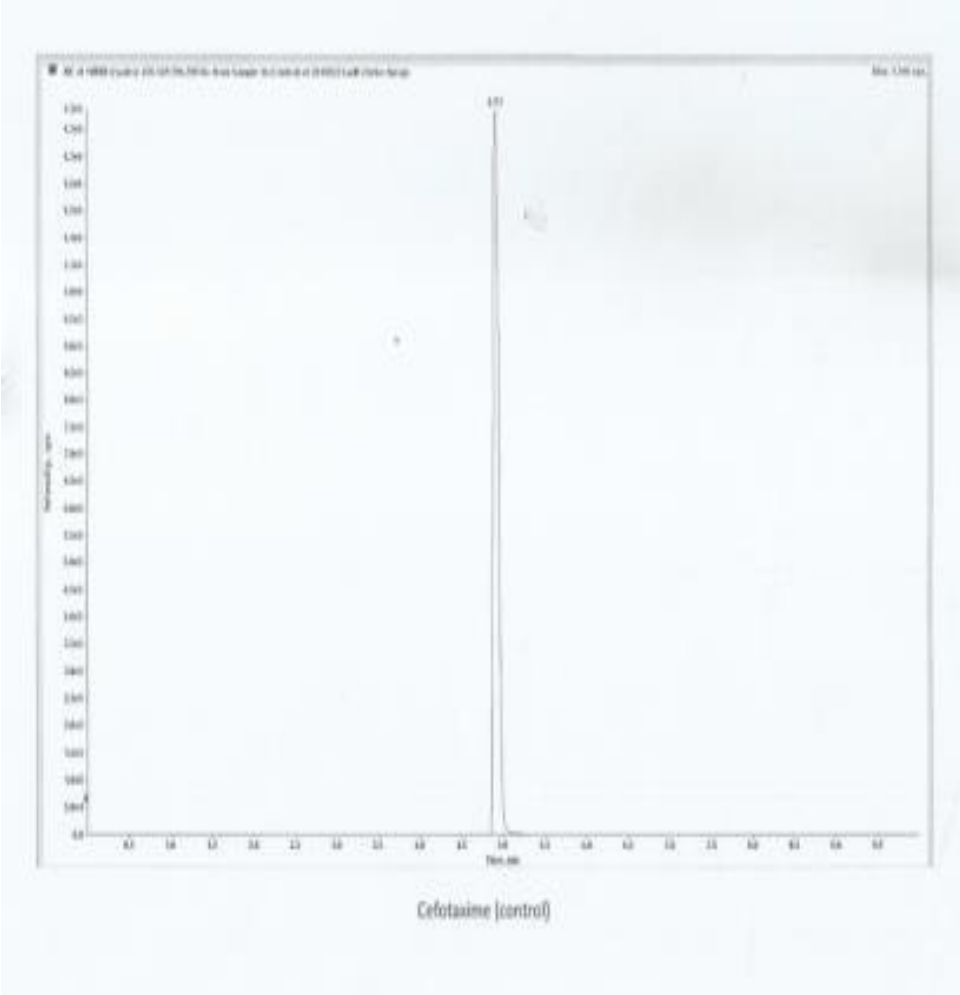
Linear regression between Dose in KGy and Log N of the viable count of the fungal isolate MAM-F3

Figure 6(c)

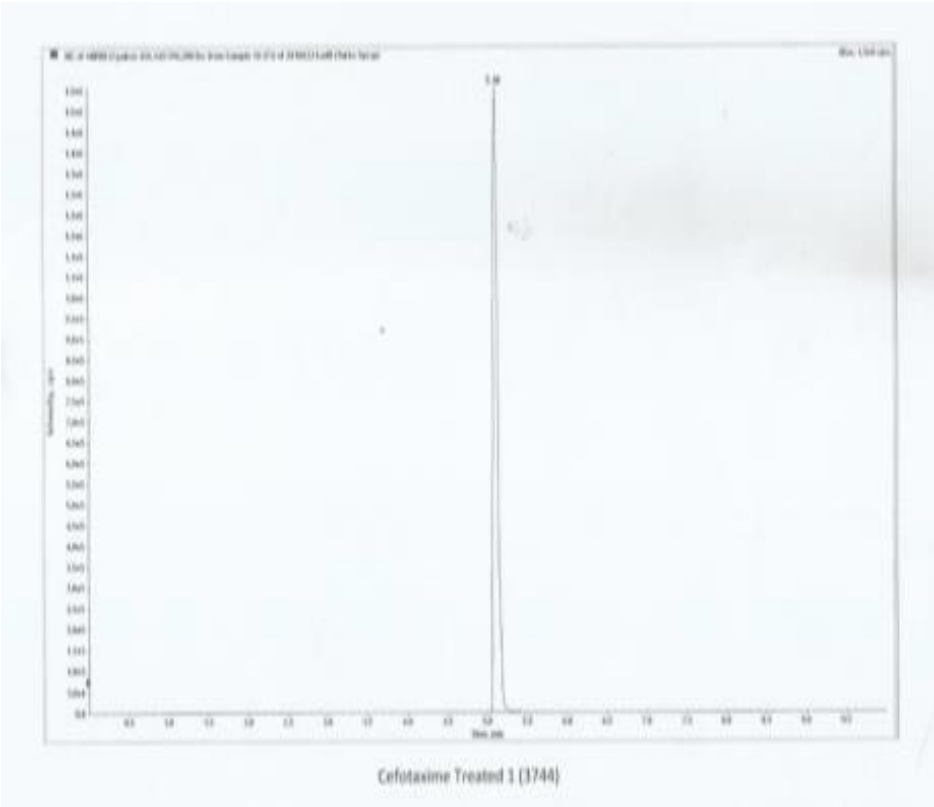
Fig. 6: Dose response curve of a) MAM-F1, b) MAM-F2 and c) MAM-F3.

Table 3: pH change of cefotaxime, cefoperazone and cefepime.

Active ingredient name	pH values		
	Dose (KGy)		
	0	10	20
Cefotaxime	5.35 0.01	5.45 0.006	5.46 0.01
Cefoperazone	4.27 0.006	4.31 0.006	4.33 0.006
Cefepime	4.72 0.01	4.90 0.01	4.92 0.006



Cefotaxime (control)



Cefotaxime Treated 1 (1744)

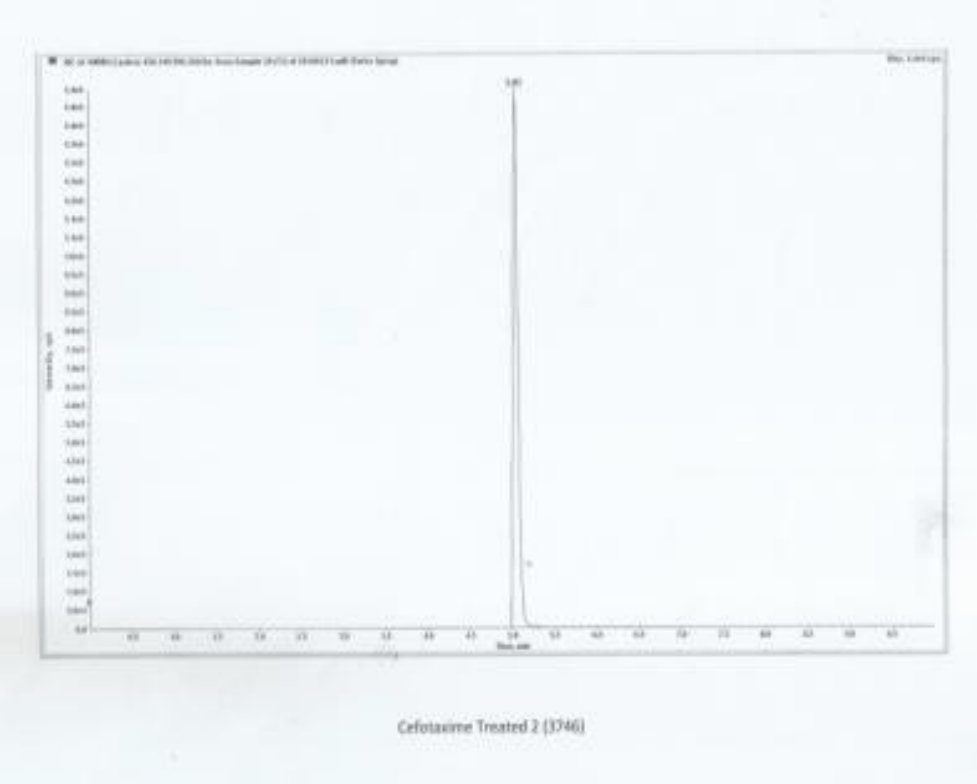
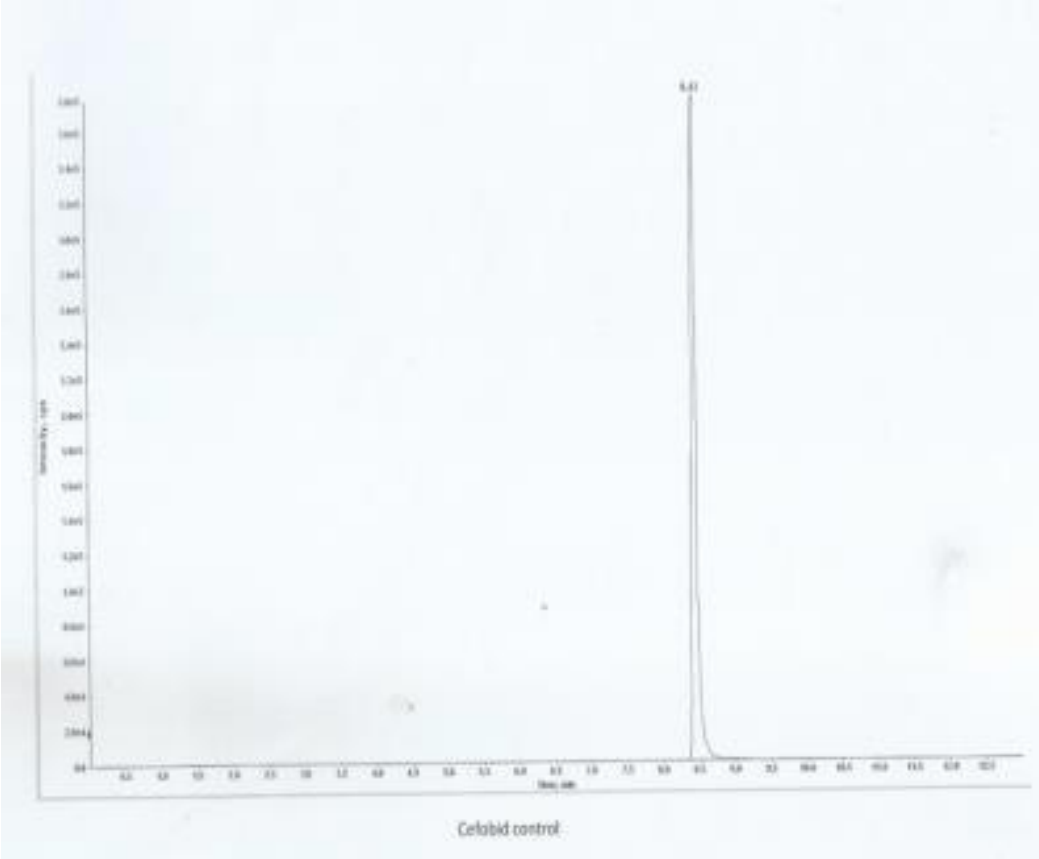


Fig. 7: HPLC chromatogram of Cefotaxime for control and after radiosterilization (10 and 25 KGy).



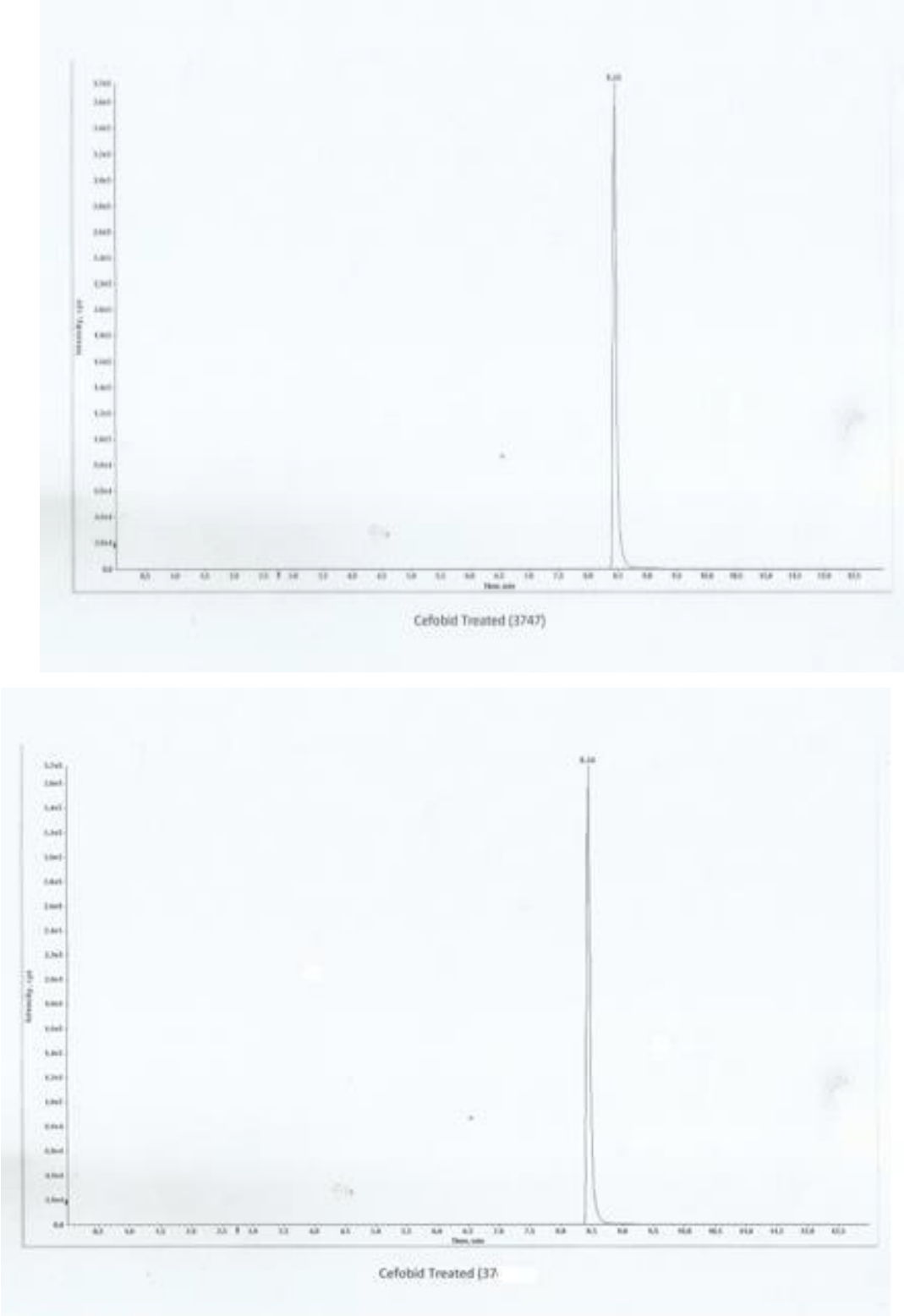
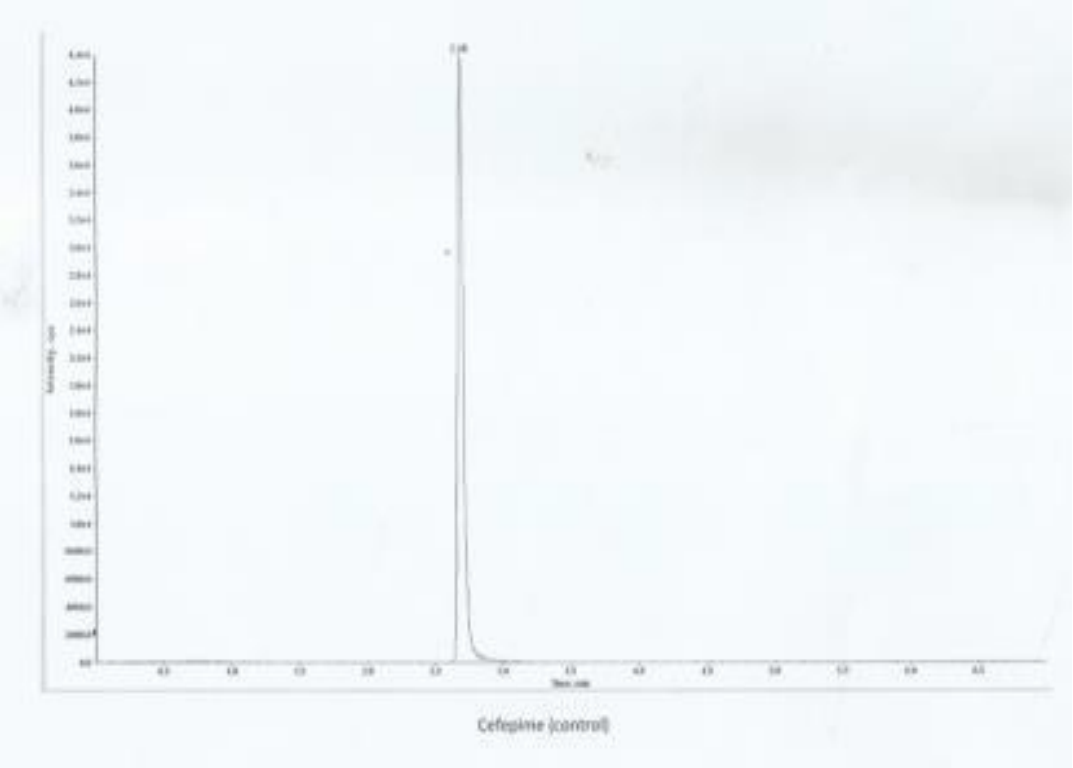
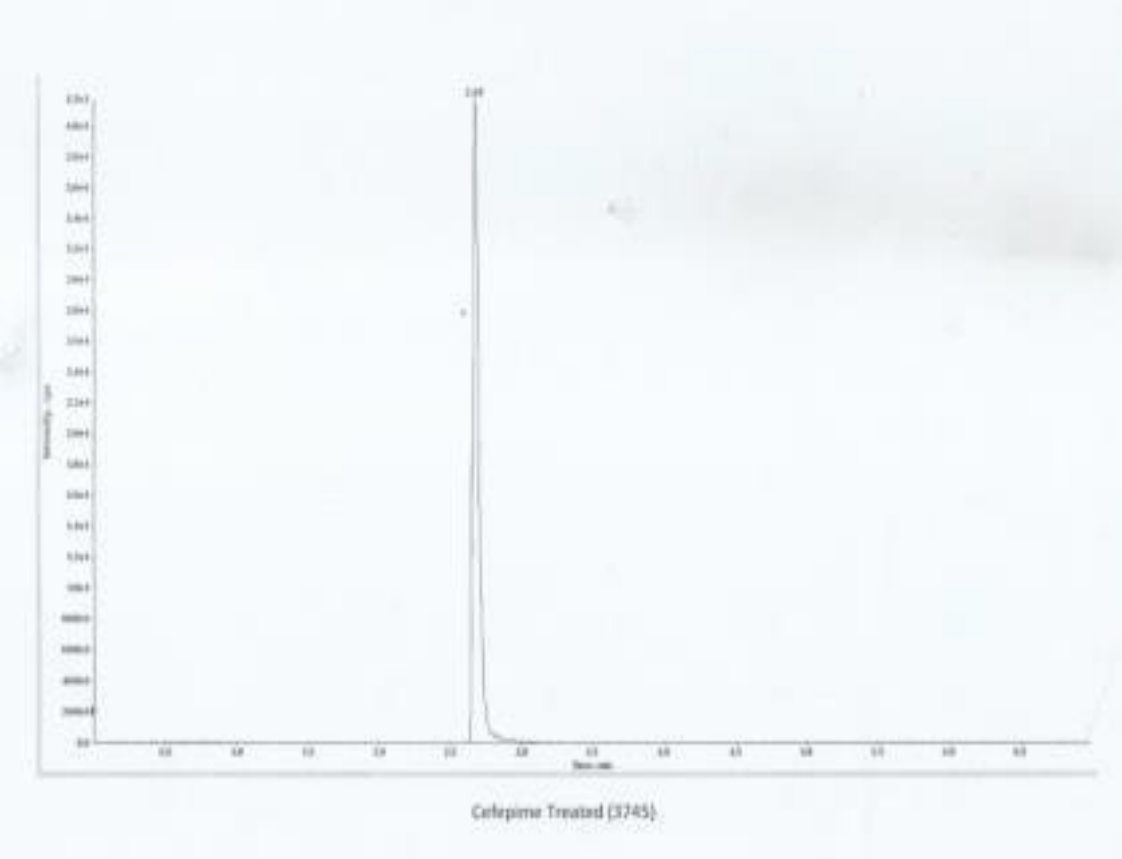


Fig. 8: HPLC chromatogram of Cefoperazone for control and after radiosterilization (10 and 25 KGy).


Cefepime (control)


Cefepime Treated (3745)

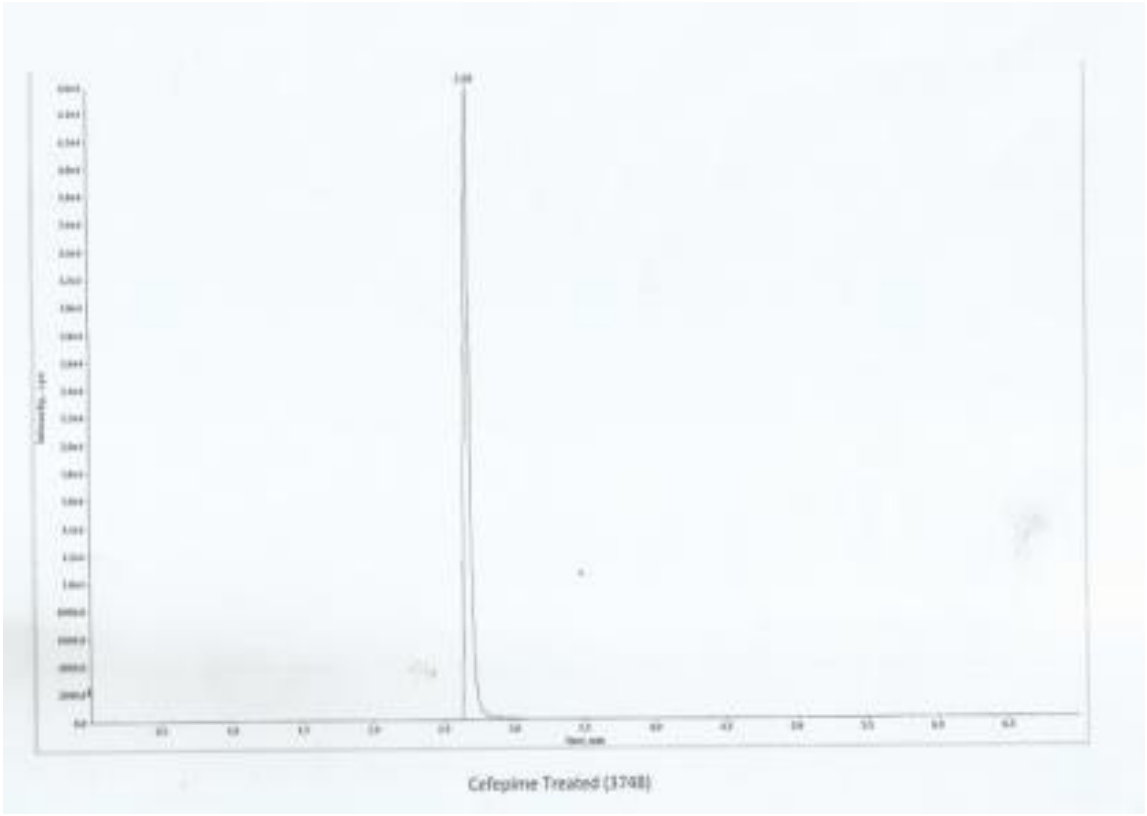


Fig. (9): HPLC chromatogram of Cefepime for control and after radiosterilization (10 and 25 KGy).

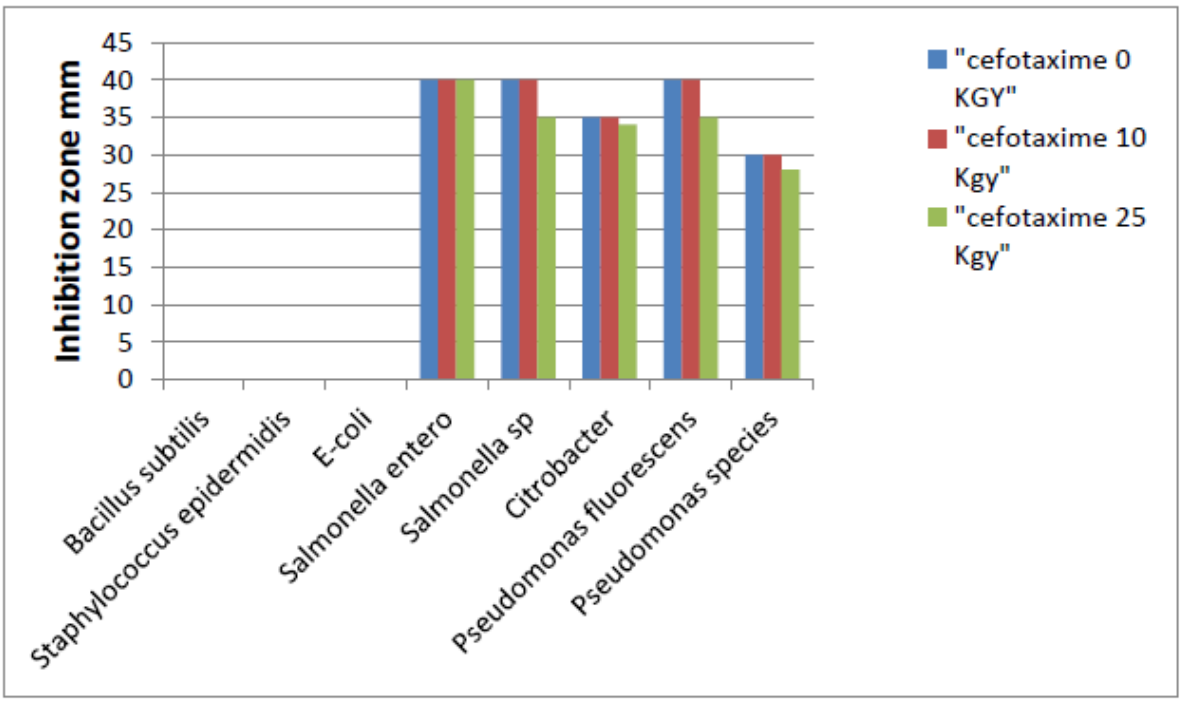


Figure 10(a).

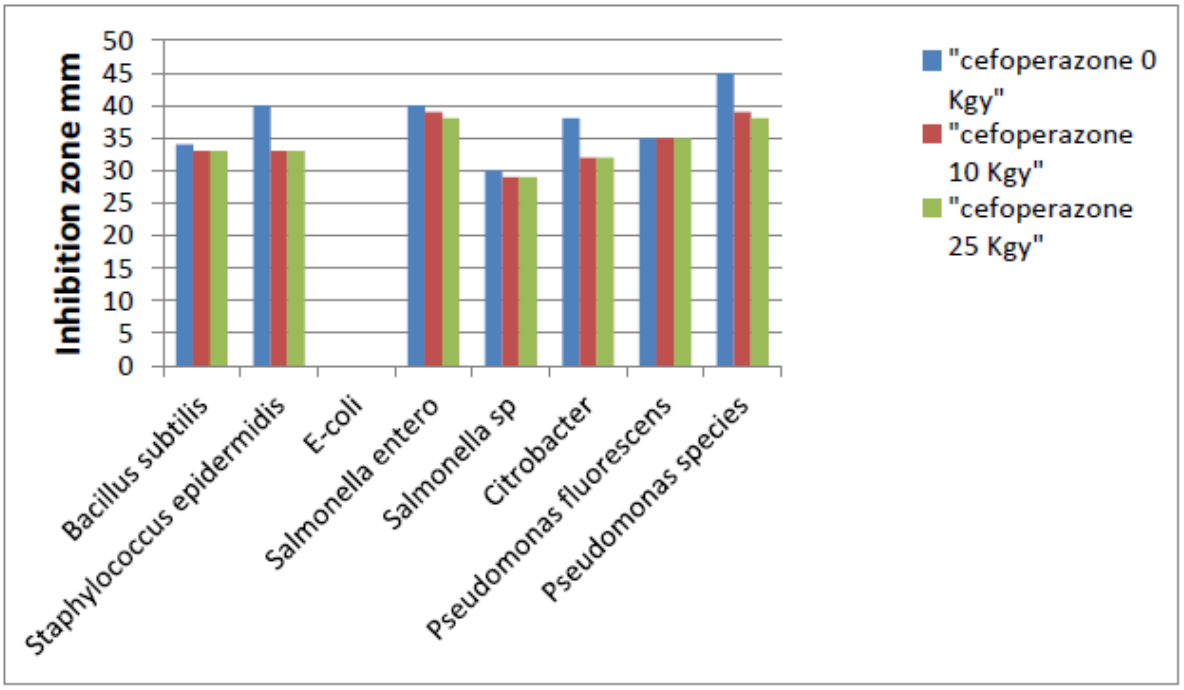


Figure 10(b).

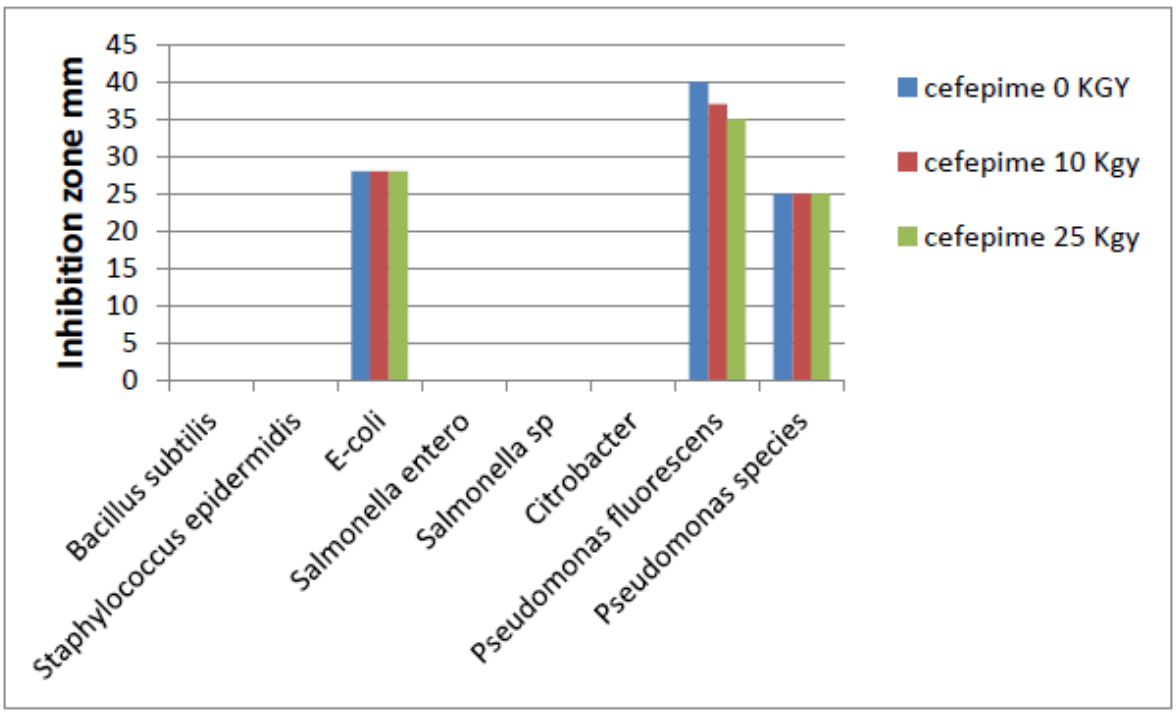


Figure 10(c).

Fig. 10: change in diameter of inhibition zone of a) cefotaxime, b) cefoperazone and c) cefepime at 0, 10 and 25 KGy.

Higher fungal contamination and low bacterial contamination may be related to the nature of the samples, where they are antibiotics having generally antibacterial activity and ability of

fungi to withdraw low humidity. Gad,[28] found that spoilage of the solid raw material itself by mold growth on the surface is due to improper storage with inadequate coverings in a damp environment or under conditions of fluctuating temperature. In the present study mixed growth was observed where a combination of different fungi were isolated from one sample, this is agree with Akerele and Ukoh,[36] who mentioned that spoilage of medicines involved basically, initial or early pioneer invaders of biodegrading microorganisms, whose microbial by products prepare the way for later invaders, by degrading complex nutrients, altering the surrounding pH and making more moisture available, thus creating a more favorable condition for growth of other kinds of microorganisms.

In another study, Mugoyela and Mwambete,[25] found heavy microbial contamination in 50 % of tested pharmaceutical products. *Klebsiella, Bacillus* spp. *and Candida* spp. were the predominant contaminants. Poor handling of the pharmaceutical produts during dispensing or repackaging might have contributed to the observed high rate of microbial contamination. Also**,** Hashem *et al.*,[37] showed that the majority of the initial unirradiated tested compounds (amoxicillin and cefaclor) antibiotics had a slight degree contamination with *Bacillus*, *Micrococcus* genera, and fungi. These results are in accordance with Marciniec *et al.*[38] who found that somepenicillins and their salts, gentamycin and neomycin, had been contaminated to a slight degree by bacteria from genera *Bacillus* and *Micrococcus* and fungi.

Also, Abo-State *et al.*[14] investigated microbial contamination of one hundred samples of cephalosporins and aminoglycosides antibiotics and found that, according to the type of isolates, fungi were the predominant isolates, which contaminated thirty-nine samples of one hundred with a percentage of (39.0%), and bacteria was next to fungi, as they contaminated (6) samples of one hundred with a percentage of (6.0%); in addition, a mixed growth of both bacteria and fungi was observed in one sample only with a percentage of (1.0%) with finally contamination percentage 46.0% of the total samples.

3.2. Identification of fungal isolates

Fig. (2) showed agarose gel electrophoresis for PCR products of the amplified ITS fragments of the selected fungal isolates. Fungal ITS sequences were compared against the GenBank database using the NCBI / BLAST program. Sequences were then compared with fungal ITS sequences in the GenBank database using BLASTN. The confidence level of each branch (1,000 repeats) was tested by bootstrap analysis and phylogenetic tree of MAM- F1, MAM-F2 and MAM-F3 were designed as shown in Fig. (3-5). The result of identification of the

three fungal isolates revealed that isolate MAM-F1 was *Aspergillus niger* with accession No. (MH828249) with 100% similarity with *Aspergillus niger* (JX 556221) and *Aspergillus niger* C3F (LC195003) as in Fig. (3). While MAM-F2 was identified as *Aspergillus flavus* with accession No. (MH828250) with 100% similarity with *Aspergillus flavus* strain 1-3 (KT329250) and *Aspergillus bombycis* NRRL 26010 (NR131261) as in Fig. (4). The result of identification of the MAM-F3 revealed that it was *Penicillium rubens* with accession No. (MH828251) with 69% similarity with *Penicillium rubens* ATF 39(MF770740) and *Penicillium rubens* DT 0313-B7 (MF803950) as in Fig. (5). These results were summarized in Table (2).

3.3. Effect of gamma radiation on the isolated microorganisms

The most prevalent fungal isolates were chosen to investigate the effect of gamma radiation as a sterilizing agent. Lethal dose and sublethal dose were determined then D10 value was calculated as shown in Figures (6 a,b,c). The D10 can be measuredgraphically from the survival curve; the slope of the curve (mostly a straight line) is related to the D10 value. The most sensitive fungal isolate was MAM-F1, while the most resistant fungal isolate was MAM-F2. In the present study, for two isolates, the results revealed that 7.0 kGy reduced the viable count of MAM-F2 by 7.6 log cycles and MAM-F3 by 7.95 log cycles. And 6.0 kGy reduced the viable count of MAM-F1 by 8.2 log cycles. So, this study revealed that a dose of 7.0 kGy could reduce three fungal growths which isolated from the tested drugs completely. The relative sensitivity or resistance of different microorganisms to ionizing radiation is based on their respective D10 value. D10 value is the ionizing radiation dose required to reduce the population by a 10 fold (by one log cycle, 1-log10) or required to kill 90% of total viable number of microorganisms. Lower D10 values indicate greater sensitivity of the organism to ionizing radiation.[39-42] With certain microorganisms, a 'shoulder' may appear in the low dose range before the linear slope starts. This shoulder' may be explained by multiple targets and/or certain repair processes being operative at low doses.[43] D10 value of the MAM-F1, MAM-F2 and MAM-F3 after exposure to gamma radiation was 0.731, 0.921 and 0.880 kGy respectively. Also**,** Abo State *et al.*[14] studied the effect of gamma irradiation on six fungal isolates and the results revealed that 6.0 kGy reduced the viable count of MAM-F97 by 6.27 log cycles and MAM-F109 by 6.30 log cycles. However 4.0 kGy reduced the viable count of MAM-F48 by 3.58 log cycles and MAM-F77 by 2.64 log cycles. For MAM-F15, 5.0 kGy reduced the viable count by 4.48 log cycles and 8.0 kGy prevent the viable count of MAM-F73.

3.4. Sterilization of antibiotics by gamma radiation

3.4.1. Organoleptic properties

After radiation, no changes or slightly changes were observed in their odor, clarity, solubility in water and their forms. All substances were white and/or pale yellow powders before gamma irradiation. However, there were mild changes in their colors. Yellowing was more intense with increasing the dose of irradiation.

3.4.2. Measurement of pH values

Values of pH of active ingredients of antibiotics before and after irradiation showed slight changes as recorded in Table (3). The finding of this study was similar to Özer *et al.*[32] who studied the changing of pH values of raw materials of diclofenacsodium (DFNa) and phospholipids and surfactants after irradiation at four different dose levels 5, 10, 25 and 50 KGy and reported that no significant change was found for all samples after irradiation with different radiation doses.

3.4.3. Evaluation of antibacterial activity

The results of microbiological potency showed slightly decrease in the antibacterial activity for the three active ingredients (cefotaxime, cefoperazone and cefepime) Figure (10 a,b,c). Singh *et al*.[45] demonstrated that irradiation did not produce any change in the antimicrobial activity of both GFX and NFX towards two Gram positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis*; and two Gram negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*. Also, Singh *et al.*[44] indicated that the microbiological potency against *Escherichia coli* and *Staphylococcus aureus* remained unaffected for cefdinir at 25 kGy. The microbiological potency of cefixime against both microorganisms decreased at 25 kGy. Another study by Salem *et al.*[17] showed the effect of ionizing radiation on clarithromycin (CLA) powder at 2, 5 and 25 kGy. The antimicrobial assays revealed that the activity of irradiated clarithromycin at 2 and 5 kGy did not reduced. However, at 25 kGy, the antimicrobial activity of CLA was significantly reduced. In another study, chemical stability of the antibacterial activity of cefozopran hydrochloride irradiated with a dose of 25 kGy was unaltered for Gram-positive bacteria but changed for two Gram-negative strains.[46] Also, the antibacterial activity of cefpirome sulfate for two Gram-positive and three Gram-negative strains was changed at dose 25 KGy.[47]

3.4.4. HPLC Analysis

There were no degradation products detected for the three antibiotics (cefotaxime, cefoperazone and cefepime) at the doses (10 and 25 KGy), which indicated the stability of the drugs for sterilization by gamma radiation as shown in Figures (7, 8 and 9).

Also, Amoxicillin, Amikacin sulphate, Ceftazidime and Cefotaximeime antibiotics were stable and showed no degradation products by HPLC after gamma-irradiation at dose 25 KGy (Abo-State *et al).*[14] Varshney and Dodke,[18] studied two anticancer drugs, cyclophosphamide (CPH) and doxorubicin hydrochloride (DOXO), in powder form. CPH undergoes less than 2% degradation at 30 kGy. DOXO was observed to be quite radiation resistant and did not undergo significant changes in its physicochemical properties and degradation product profile. Salem *et al.*[17] studied the effect of ionizing radiation on clarithromycin (CLA) powder commercially named Zeclar®. The analysis by HPLC confirmed the stability of Zeclar® potency at 2, 5 and 25 kGy and no degradation products were observed. Singh *et al.*[44] determined the chemical potency of two cephalosporin drugs by the HPLC method. The content loss for cefdinir at 25 kGy dose was 4–5%. The content loss for cefixime at 25 kGy dose is 8–9%. Cefdinir has acceptable radiostability, but cefixime is found to be radiosensitive. Although, the lactam ring is susceptible to degradation under thermal, chemical and photochemical stresses, it does not show equivalent sensitivity towards high energy ionizing radiation, as indicated by the nature of radiolytic related impurities and their concentrations. Also, Singh *et al*.[45] studied HPLC content assay and demonstrated that loss of content for the samples irradiated at 25 kGy was less than 1% for norfloxacin (NFX) and 2% for gatifloxacin (GFX). So, the results of both confirmed the radiation resistant nature of GFX and NFX, as the percentage loss of content is not significant at 25 kGy dose, and is within the US pharmacopoeia limit. Another study, Hashem *et al*.[37] applied γ irradiation at 25 kGy on amoxicillin and cefoclar antibiotics and analytical tests results proved that those antibiotics were found to be radioresistant and can be sterilized by gamma-irradiation with a dose of 25kGy, without any detrimental effect on their properties and antibacterial activity. Cefozopran hydrochloride in solid state is not resistant to radiation sterilization and this method cannot be used for sterilization of this compound according to Zalewski *et al.*[46] Results of Zalewski *et al.*[47] concluded that cefpirome in solid state is resistant to ionizing radiation (E-beam) in a standard sterilization 13 dose of 25 kGy.

Although drugs such as antibiotics can attack and destroy bacteria within the human body, they are not self-sterilizing. Pharmaceuticals, and/or their associated adjuvants (materials used to aid the delivery of drugs) can resort bacteria, either from a primary source of origin or introduced during the production process. Their sterilization can present a problem since many such substances react with ethylene oxide to produce toxic chemicals and are unstable to heat. The alternative to manufacture in a sterile environment is expensive. Radiation, therefore, has long offered an imaginative alternative, and it was initially pursued indiscriminately.[48]

CONCLUSIONS

- Fungi were the most predominant isolates contaminated the solid antibiotics tested (28.7 %).
- The dose 6.0 KGy reduced the viable counts of isolates MAM-F1 completely, while 7.0 KGy reduced the viable counts of isolates MAM-F2 and MAM-F3 completely.
- The three fungal isolates were identified by 18S rRNA as *Aspergillus niger* with accession number MH 828249, *Aspergillus flavus* with accession number MH 828250 and *Penicillium rubens* with accession number MH 828251.
- Gamma radiation can be used as a safe technology for sterilization of cefotaximeime, cefoperazone and cefepime antibiotics at the sterilization dose 25 KGy (sterility assurance level).

RECOMMENDATION

The three antibiotics can be sterilized by gamma radiation technology efficiently.

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