Studies on the microbial decontamination of Egyptian bee pollen by γ radiation

Hosny A.S.[a], Sabbah F.M.[b], EL-Bazza Z.E.[c]

[a]Prof. of Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Egypt, [b]Bachelor of Pharmaceutical Science, [c]Prof. of Pharmaceutical Microbiology, Drug Radiation Research Department National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

Correspondence to Bachelor of Pharmaceutical Science, Egypt. Tel: 01005633774; e-mail: fatmasabbah@gmail.com

Received 29 April 2018
Accepted 11 June 2018

Egyptian Pharmaceutical Journal
2018, 17:190–200

Objective
Bee pollen are used as health food ingredients, but may be subjected to microbial contamination; γ radiation technology can offer the process of microbial decontamination as a means of achieving microbiological safety limits.
Materials and methods
Thirty bee pollen samples were collected from Egyptian markets. Detection of contamination and the microbial counts were carried out on nutrient and Sabouraud's agar media for bacteria and fungi, respectively, using the aerobic plate technique. Identification of bacteria was carried out by biochemical methods and analytical profile index. Moreover, identification of fungi was carried out morphologically and microscopically.
Results and conclusion
The order of bacterial contamination was Gram-positive rods>Gram-negative rods>Gram-positive cocci. The order of fungal contamination is *Penicillium* spp.>*Aspergillus flavus*>*Aspergillus niger*>*Aspergillus ochracueus.* Only three strains of *A. flavus* could produce aflatoxin B_1. The microbial counts of bee pollen samples decreased with increasing γ radiation doses. The most radio-resistant bacteria that were isolated at 5.0 kGy were identified as *Bacillus megaterium*, *Bacillus pumilis* and *Bacillus subtilis.* The most radio-resistant fungi were identified as *Penicillium chrysogenum*, *Penicillium expansum* and *Penicillium corylophilum*. Using of γ radiation can decrease the bioburden in bee pollen, and eliminate pathogenic microorganisms including fungi, which can produce carcinogenic aflatoxins.

Keywords:
aflatoxins, bee pollen, γ radiation, micro-organisms

Egypt Pharmaceut J 17:190–200
© 2018 Egyptian Pharmaceutical Journal
1687-4315

Introduction

Bee pollen are produced by flowering plants and collected by bees. Pollen is considered as the primary food source of bees [1]. Bee pollen results from adhesion of flower pollen, nectar and/or honey and the salivary substances of bees [2]. Pollen contains very variable and important components. It contains all of the essential amino acids. It also has vitamins A, D, E, K, C and bioflavonoids as well as B-complex, especially B_5 and niacin [3]. It can be used as an antioxidant, anti-inflammatory, antimicrobial agent, and the great therapeutic action is of clinical value because of its antiprostatic effect [4]. Fresh bee pollen contains about 20–30 g of water per 100 g, which is a very high humidity that helps in the growth of micro-organisms, such as bacteria and yeast [5]. Molds, yeast, aerobic and spore-forming bacteria were found in pollen grains. Moreover, *Aeromonas hydrophilia*, *Salmonella* spp., *Clostridium* spp., *Staphylococcua aureus* and *Streptococcus faecalis*, were detected in pollen samples [6]. Twenty-one fungal species formed 13 genera of microscopic fungi in the pollen samples, while the highest number of mold species was classified in the genera of *Mucor*, *Rhizopus*, *Aspergillus*, *Alternaria* and *Paecilomyces* [7]. Aflatoxin-producing fungi are wide spread worldwide and can produce these toxic compounds either before or after harvest [8]. Aflatoxins are hepatotoxic, teratogenic, mutagenic and carcinogenic. Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxin B_1 is listed as a group I carcinogen by the International agency for research on cancer [9].

γ Radiation is a type of electromagnetic radiation, and it is a cold method for sterilization. The γ radiation process does not create any residuals or impart radioactivity. Complete penetration can be achieved depending on the thickness of the material. It saves energy without the need for chemicals or heat [10]. Irradiation of food using ionizing radiation is used to prevent spoilage and pathogenic microorganisms and

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

also to guarantee the hygienic quality of foodstuffs [11]. Microorganisms resistant are different to ionizing radiation. Bacterial spores are more resistant than yeast, molds and vegetative cells of bacteria [12].

The aim of the present study was to document the microbial contamination of bee pollen, evaluate the effectiveness of γ irradiation on microbial decontamination, identify the most radio-resistant contaminants and suggest the radiation decontamination doses, as well as detection of aflatoxins produced by the contaminated fungi.

Materials and methods

Bee pollen samples

A total of 30 bee pollen samples were purchased from the markets of different localities in Egypt. The samples were packed in sterile closed glass jars.

Micro-organisms

A total of 95 microbial contaminants isolated from bee pollen samples (60 bacterial and 35 fungal isolates) were used in the present study. The contaminants were purified and maintained on nutrient agar for bacteria and on Sabouraud's agar for fungi. All cultures were stored at 4°C and subcultured monthly on the same medium.

Media and ingredients of media

Nutrient broth, Sabrouaud's agar, potato dextrose agar and Czapek's Dox agar were the products of Oxoid (Wade Road Basingstoke, Hampshire, RG24 8PW, United Kingdom). MacConkey agar was the product of LAB. Agar–agar was the product of Adwic (El Nasr Pharmaceutical Chemicals Co, Abu Zaabal, Center Khanka, Qaliubiya, Egypt). Yeast extract was the product of BBL. Peptone was the product of Oxoid.

Chemicals

Methyl alcohol was the product of Piochem (6th of October 6th Zone, Giza, Egypt), dimethyl sulphoxide was the product of Loba Chemie (Jehangir Villa, 107, Wode House Road, Colaba, Mumbai, Maharashtra 400005, India). Other chemicals used in the present study were of reagent grade.

Standard aflatoxins

Standard aflatoxins B_1, B_2, G_1 and G_2 were provided by the applied science division, Milton Roy Company, laboratory group (State College, Pennsylvania, USA).

Chromatographic materials

Silica gel D for thin-layer chromatography was obtained from Riedel-De Haen (AG, Sleez, Hannover, Germany). Silica gel for column chromatography (0.05–0.2 mm) was obtained from ICN Pharmaceuticals (Eschwege, West Germany).

Microbial evaluation of the tested bee pollen samples

Samples were analysed after purchasing. The samples were kept at room temperature and were not incubated, refrigerated, or freezed before analysis. Surfaces of the containers were disinfected with 70% ethanol before opening the containers in a laminar air flow cabinet.

Microbial detection

For detection of contamination of bee pollen samples, aliquots of 1 g of each tested sample were suspended in test tubes containing 9 ml of the sterile diluent (0.1% peptone, 0.1% tween 80, 0.89% NaCl in H_2O), and the test tubes were shaken well on vortex (type paramix II number 65, West Germany). For bacterial detection, 0.1 ml was taken from each test tube and streaked on nutrient agar plates. The plates were incubated at 35 ±2°C for 24 h. For fungal detection, 1 ml was taken from each test tube and mixed with the melted Sabouraud's agar in sterile plates and incubated at 28 ±2°C for up to 5 days. After incubation, the microbial growth was noticed.

Total microbial counts

The contaminated bee pollen samples were exposed to the aerobic plate count technique according to Kacaniova *et al.* [13]. On nutrient agar plates for bacteria and on Sabouraud's agar plates for fungi, the microbial counts were recorded as cfu/g.

Isolation of the microbial contaminants

According to the morphological characters of the microbial isolates, they were separated on nutrient agar for bacteria and on Sabouraud's agar for fungi, purified and then kept on slants of the same medium, at 4°C for further investigations.

γ Irradiation studies

γ Irradiation facility

Cobalt-60 ($^{6^\circ}$Co) 220 Gamma Cell, Canada Co Ltd, located at the National Center for Radiation Research and Technology (Nasr City, Cairo, Egypt), have been utilized as radiation resources. The dose rate was 1.7 kGy/h at the time of experiments.

Determination of the microbial counts in irradiated bee pollen sample

Heavily contaminated bee pollen samples (18) were selected for this study. Aliquots of 1 g of each selected sample of bee pollen in sterile test tubes were exposed to increasing doses of γ radiation from 0.0 to 20.0 kGy, and nonirradiated samples were used as control. After

irradiation, each sample was suspended in 9 ml peptone tween saline. The test tubes were shaken well on the vortex. From each irradiated sample, 0.1 ml was inoculated on nutrient agar in triplicates in a sterile plate for bacteria, and 1 ml was mixed with 20 ml Sabouraud's agar in sterile plates for fungi. Thereafter, the inverted solidified plates were incubated at 35±2°C for 24 h for bacteria and at 28 ±2°C up to 5 days for fungi. After incubation, the bacterial and fungal counts were recorded as cfu/g, and log number of survivors was calculated. Histograms were constructed as log number of bacterial or fungal survivors against the radiation doses [14].

Determination of the microbial sublethal and lethal doses in bee pollen samples

One gram of each pollen sample (30 samples) in sterile test tubes was exposed to γ radiation doses of 0.0–15.0 kGy. After irradiation, each irradiated sample was suspended in 9 ml peptone tween saline. The test tubes were shaken well on the vortex. The microbial growths in each irradiated pollen sample were detected on nutrient agar and Sabouraud's agar for bacteria and fungi, respectively, as mentioned before. The sublethal dose was considered as the highest radiation dose at which the least growth was detected. The lethal dose is the radiation dose at which no growth was detected [14].

Isolation of the most radio-resistant bacterial and fungal micro-organisms

The micro-organisms that could survive the highest radiation doses from the irradiated pollen samples (sublethal doses) were isolated and taken as the most radio-resistant isolates and hence subjected for studying the response to γ radiation.

Identification of the bacterial isolates

Identification of the bacterial isolates involved the following steps:

Examination with naked eye for morphological shape, size and color of colonies. All plates were examined, and morphologically dissimilar colonial types were cultured on MacConkey agar. Microscopical identification using Gram stain of the bacterial isolates was performed before and after exposure to γ radiation.

Identification of the most radio-resistant bacterial isolates

The identification of the most radio-resistant bacteria (MRB) was carried out according to [15] using the biochemical methods and confirmed at Vacsera (Egyptian company for production of vaccines) using Api system (Api CH 50 B) 'analytical profile index' (Biomerieux Inc., BioMérieux Marcy l'etoile, France).

Identification of fungal isolates

The fungal isolates were examined morphologically and microscopically on Czapek's Dox agar and potato dextrose agar according to Moubasher [16] and Nyngesa *et al.* [17].

Identification of the most radio-resistant fungal isolates

The most radio-resistant fungal (MRF) isolates grown on Sabouraud's agar and Czapek's dox agar media were examined morphologically and microscopically according to Moubasher [16].

Study of the response of the radio-resistant bacterial strains to γ radiation

The test was carried out according to El-Bazza *et al.* [18] with some modifications in that the dense suspensions were γ irradiated and used for the study.

Study of the response of the radio-resistant fungal strains to γ radiation

The MRF strains from the irradiated pollen samples were used to study the response to γ radiation according to El-Bazza *et al.* [14] and El-Fouly *et al.* [19].

Construction of dose–response curves of the microbial isolates and calculation of D_{10} values

The survival curves were obtained by plotting the logarithm of the number of microbial survivors versus the radiation doses in kGy. The D_{10} values, which are the measure for the radiation resistance of the microorganisms to γ radiation, were read directly from the curves by finding the γ radiation dose, which reduces the microbial population by one logarithmic cycle.

Detection of aflatoxins

Aflatoxins were detected by using the fungi isolated (35) from bee pollen samples as follows:

Production of aflatoxins by the tested isolated fungal organisms and extraction of aflatoxins (if any) were carried out according to El-Bazza *et al.* [20]. Purification of aflatoxins was carried out using silica gel column chromatography according to A.O.A.C. [21].

Detection of aflatoxins was carried out on thin-layer chromatography. The solvent system used was chloroform–acetone (90 : 10, v/v) according to Younis and Malik [22].

Results

In this study, the contamination of the tested bee pollen samples is summarized in Table 1; 29 (96.7%) samples were found to be contaminated with bacteria, 17 (56.7%) samples were contaminated with fungi and 16 (53.3%) samples were contaminated with bacteria and fungi.

Results reveal that the level of microbial contamination of the examined bee pollen samples differs between the different samples. The level of contamination of examined samples with bacteria ranges between 5.7×10^3 and 3.1×10^7, and a total of 60 bacterial isolates were isolated. The level of contamination of the samples with fungi ranges between 4.3×10^2 and 6.5×10^4, and 35 fungal isolates were isolated.

Table 2 shows the evaluation of the bacteria contaminating the examined bee pollen sample. The order of contamination in bee pollen samples is Gram-positive rods>Gram-negative rods>Gram-positive cocci.

Table 3 illustrates that a total of 35 fungal organisms were isolated on Sabouraud's agar from the studied pollen samples, and the order of contamination was *Penicillium* spp.>*A. flavus*>*Aspergillus niger*>*Aspergillus ochracueus*.

In our study, the detection of aflatoxin production by the fungi isolated from the tested pollen samples was carried out in Sabouraud's broth supplemented with 0.5% yeast extract; the results of the thin-layer chromatographic plates show that only three isolates were aflatoxin B_1 producers. Aflatoxin B_2, G_1 and G_2 were not produced.

Table 1 Survey on the microbial contamination of the tested bee pollen

Number of samples	30
CSB	29
LCB (cfu/g)	5.7×10^3–3.1×10^7 (96.7%)
	$<10^5$ (44.8%)
CSF	17
LCF (cfu/g)	4.3×10^2–6.5×10^4 (56.7%)
	$<1.5\times10^3$ (47.1%)
CSBF (%)	16
	53.3
NCS (%)	ND
	ND

CSB, contaminated samples with bacteria; CSBF, contaminated samples with bacteria and fungi; CSF, contaminated samples with fungi; LCB, level of contamination with bacteria; LCF, level of contamination with fungi; NCS, noncontaminated samples; ND, none detected.

Figures 1–4 illustrate the results of exposure of the selected heavily contaminated bee pollen samples to γ radiation doses that ranged from 0.0 to 15.0 kGy; the results show that the counts of bacteria and fungi in irradiated samples decreased by increasing the γ radiation doses. A dose of 7.0 kGy showed decontamination of the microbial organisms.

The results of exposure of bee pollen samples to γ radiation doses that ranged from 0.0 to 25.0 kGy showed that 37 radio-resistant bacterial contaminants were isolated from 30 irradiated bee pollen samples at sublethal dose levels between 2.0 and 5.0 kGy. The radio-resistant bacteria were identified as Gram positive rods (34), two isolates as Gram negative rods and only one isolate as Gram positive cocci. Percentage of the radio-resistant bacteria are illustrated in Fig. 5. In contrast, 19 radio-resistant fungal contaminants were isolated from 17 irradiated bee pollen samples at the sublethal dose levels of 1.5 and 2.0 kGy. The isolates were identified as *Penicillium* spp.

Table 4 shows that the MRB that survived the highest dose level under treatment (5.0 kGy) were identified, using analytical profile index 50 CH system, as *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus pumilis*. The MRF isolates that survived the highest dose level (2.0 kGy) were *Penicillium chrysogenum*, *Penicillium expansum* and *Penicillium corylophilum* (Table 5).

Table 2 Evaluation of the bacteria contaminating the examined bee pollen samples

Number of CSB	Total number of bacteria	Identification	Number of BI	Percent
29	60	Positive cocci	4	6.7
		Positive rods	45	75
		Negative cocci	ND	ND
		Negative rods	11	18.3

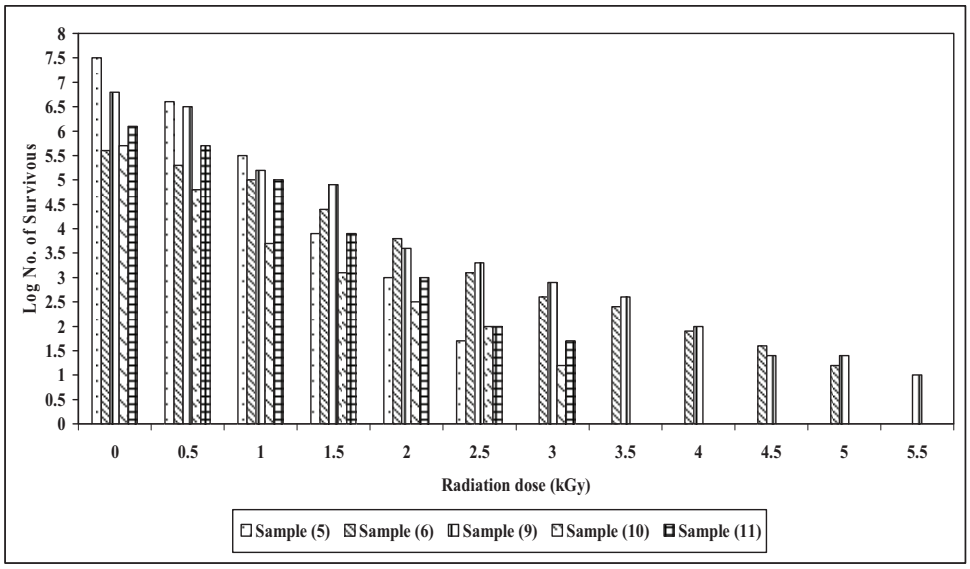
BI, bacterial isolates; CSB, contaminated sample with bacteria; ND, none detected.

Table 3 Evaluation of the fungi contaminating the examined bee pollen samples

Number of CSF	Total number of fungi	Identification	Number of FI	Percent
17	35	*Penicillium* spp.	29	82.9
		Aspergillus flavus	3	8.6
		Aspergillus niger	2	5.7
		Aspergillus ochracueus	1	2.9

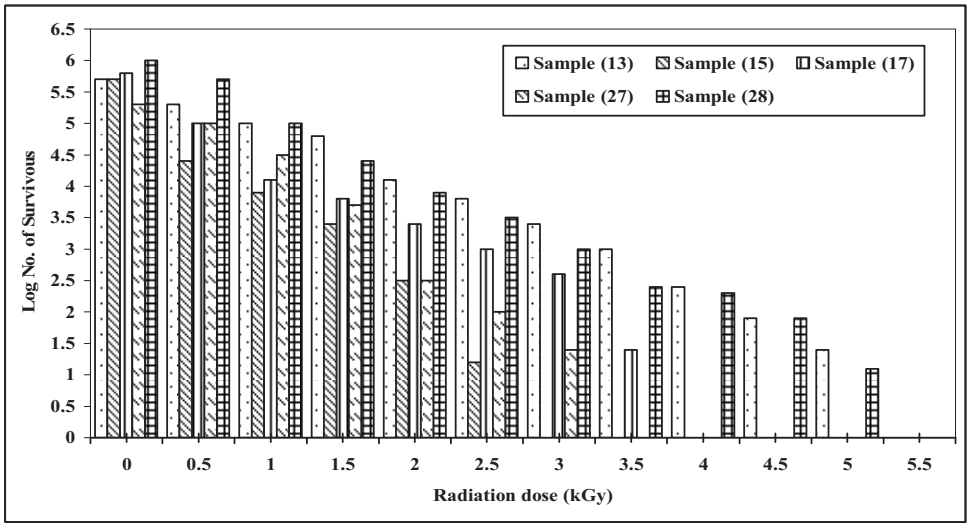
CSF, contaminated samples with fungi; FI, fungal isolates.

Figure 1



Effect of γ radiation on the total bacterial counts (cfu/g) in the heavily contaminated pollen samples (5, 6, 9, 10 and 11).

Figure 2



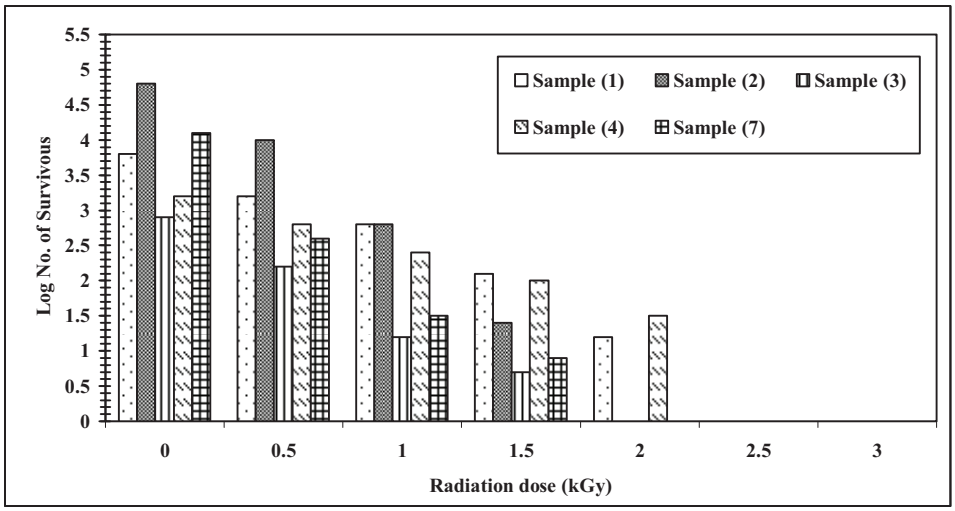
Effect of γ radiation on the total bacterial counts (cfu/g) in the heavily contaminated pollen samples (13, 15, 17, 27 and 28).

In the present study, the MRB and MRF were selected for studying their response towards γ radiation through plotting their dose–response curves and calculation of the D_{10} values from the curves by finding the γ radiation doses that reduce the microbial population by one logarithmic cycle. The dose–response curves of *B. megaterium*, *B. pumilis*, *B. subtilis* and of *Penicillium* spp. were constructed. The curves showed exponential rate of death. The mean of the D_{10} values of *B. megaterium* isolated from samples number 1, 7 and 9 were calculated to be 3.5±0.00, 1.4±0.10 and 3.2±0.15, respectively. While, the mean of the D_{10} values of *B. pumilis* isolated from samples 13 and 19 were 2.4±0.17 and 1.5±0.00, respectively. The mean of the D_{10} values of *B. subtilis* isolated from sample numbers 6, 21, 24 and 28 were calculated to be 2.1±0.12, 1.5±0.00, 2.7 ±0.25 and 2.3±0.15, respectively. Furthermore, the mean of the D_{10} value of *P. expansum* isolated from sample number 4 was calculated to be 0.48±0.03. In contrast, the mean of the D_{10} values of *P. chrysogenum* isolated from sample number 1 were 0.64±0.01 and 0.48±0.05, while the mean of the D_{10} values of *P. corylophilum* isolated from samples number 4 and 21 were found to be 0.54±0.01 and 0.49±0.01, respectively (Figs 6–9).

Discussion

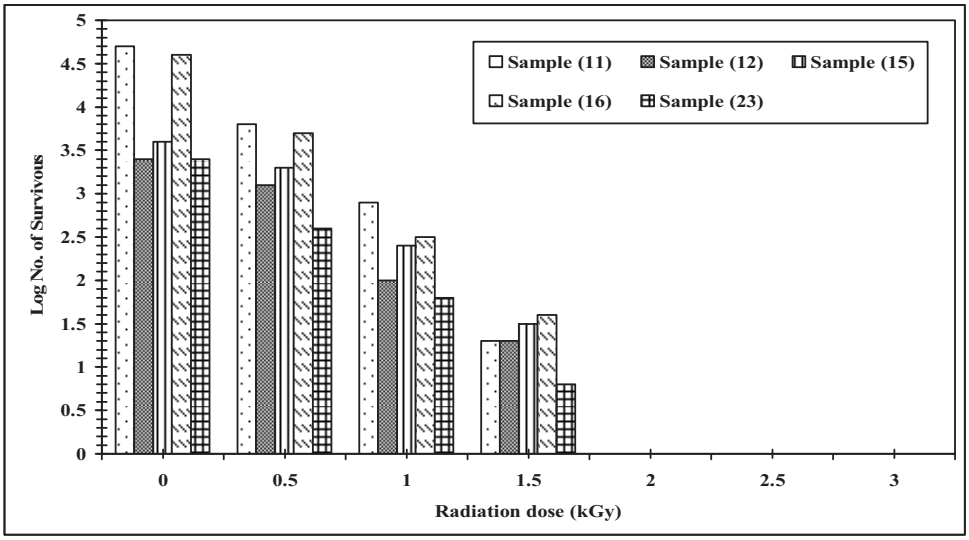
Bee pollen has the image of being natural, healthy and clean. However, the products are produced in an

Figure 3



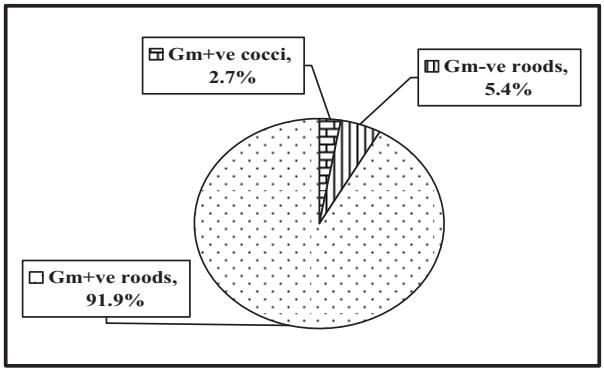
Effect of γ radiation on the total fungal counts (cfu/g) in the heavily contaminated pollen samples (1, 2, 3, 4 and 7).

Figure 4



Effect of γ radiation on the total fungal counts (cfu/g) in the heavily contaminated pollen samples (11, 12, 15, 16 and 23).

Figure 5



Percentage of the different radio-resistant bacteria.

environment, polluted by different sources of contamination. Thus, it is of utmost importance to bee keepers to localize and exclude the different contamination sources [23].

In the present study, the samples were contaminated with bacteria and/or fungi, and detection of the contamination differed between the different bee pollen samples from different localities.

According to Campos *et al.* [24], pollens should have the following microbial aspects: absence of Salmonella/10 g; absence of *Staphylococcus* and *Escherichia coli*/1 g; total aerobic bacterial count could not exceed more than 10^5 cfu/g; total mould yeast count should be less

than 5×10^4 cfu/g; and the maximum count of enterobacteria is 100 cfu/g.

The results obtained by Nogueira *et al.* [2] for the parameters that indicate commercial quality are in agreement with the Argentinian Food Code, which establishes the maximum of 1.5×10^3 cfu/g for moulds and yeasts. In their study, moulds and yeasts were detected in 50% of the samples, while other researchers [25,26] found it in all the samples.

Table 4 Identification of the most radio-resistant bacteria isolated from irradiated bee pollen samples

Sample no.	Sublethal dose	Number of MRBI	Identification
1	5	1	*Bacillus magaterium*
6	5	1	*Bacillus subtilis*
7	5	1	*Bacillus megaterium*
9	5	1	*Bacillus megaterium*
13	5	1	*Bacillus pumilus*
19	5	1	*Bacillus pumilus*
21	5	1	*Bacillus subtilis*
24	5	1	*Bacillus subtilis*
28	5	1	*Bacillus subtilis*
Total			9

MRBI, most radio-resistant bacteria isolates.

Comparing of Campos *et al.* [24] recommendations with our study, 44.8% of the bacteria contaminated bee pollen samples have acceptable criteria ($< 10^5$ cfu/g), while 47.1% have the acceptable criteria for fungi ($< 1.5\times10^3$ cfu/g), when comparing with the Argentinian food code. The highest contamination was seen in bee pollen with the Gram-positive rods and least contamination with the Gram-positive cocci.

Five species belonging to the genus *Bacillus* were isolated from pollen and bee bread. Of the 41 isolates, 33 were *B. subtilis*, which was the only species associated with all pollen and bee bread samples. Possibly because of some role of the organism in the elaboration of bee bread and/or because of the ability of the organism to survive in this particular environment. *B. megaterium*, *B. licheniformis*, *B. pumilus*, and *B. circulaus* were also isolated. Only *B. subtilis* was found in pollen from the flower. As the greatest number of *Bacillus* isolates and species was found in pollen from the trap, the foraging bees may have added these organisms to the pollen, when making a suitable mass to carry back to the hive [27].

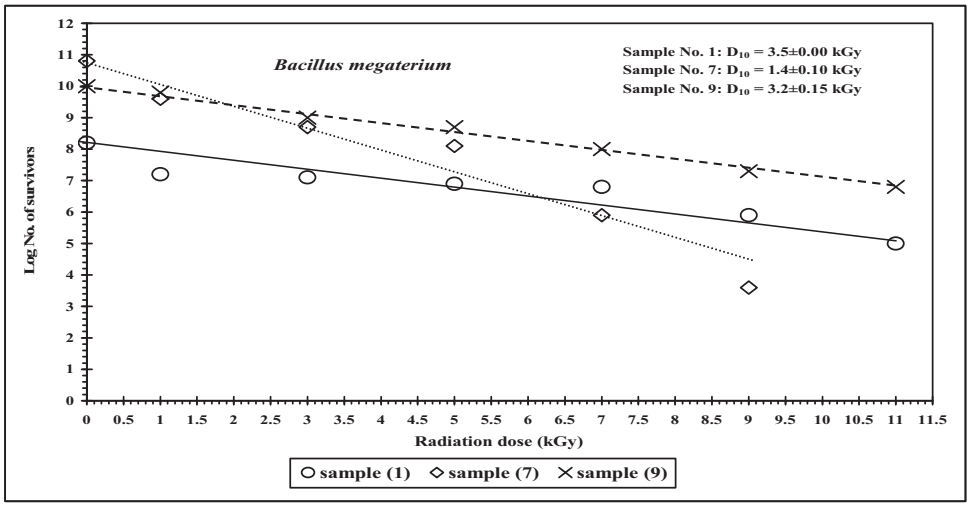
There were 21 species of thirteen genera of microscopic fungi in the pollen samples. Most often the presence of the

Table 5 Identification of the most radio-resistant fungi isolated from irradiated bee pollen samples

Sample no.	Sublethal dose	Number of MRFI	MRFI
1	2	2	*Penicillium chrysogenum* (a and b)
4	2	2	*Penicillium expansum* (c)
			Penicillium corylophilum (d)
21	2	1	*Penicillium corylophilum* (e)
Total	–	5	5

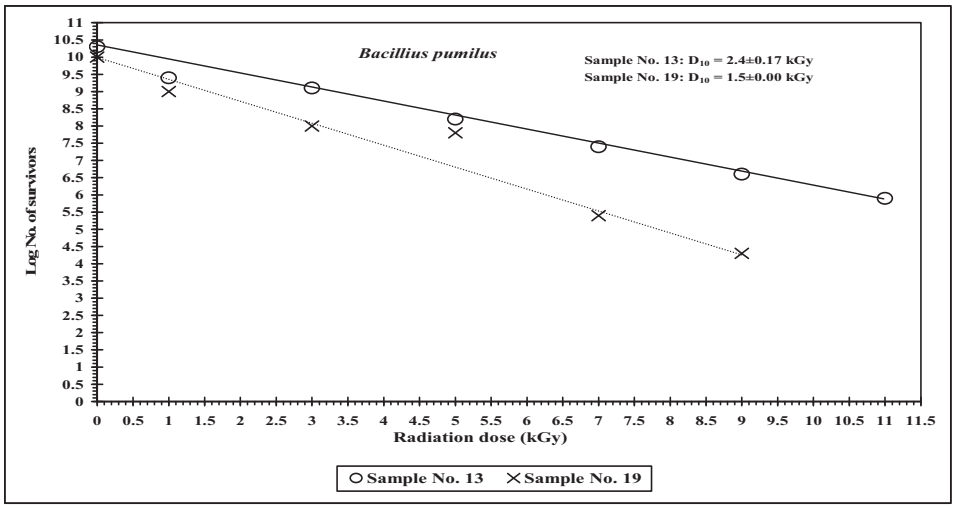
a, b, c, d: most radio-resistant fungal isolates. MRFI: most radio-resistant fungal isolates.

Figure 6



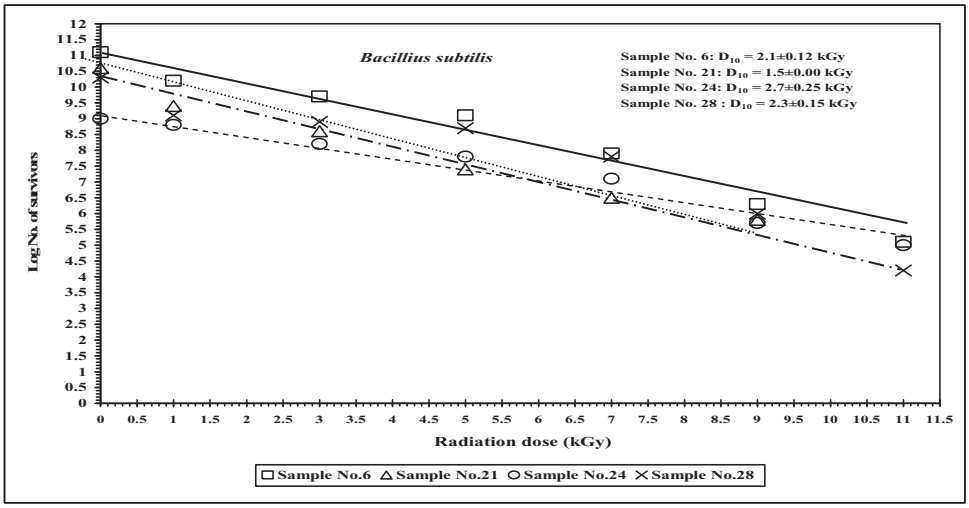
Dose–response study for *Bacillus megaterium* isolated from irradiated pollen samples (no. 1, 7, 9).

Figure 7



Dose–response study for *Bacillus pumilus* isolated from irradiated pollen samples (no. 13, 19).

Figure 8



Dose–response study for *Bacillus subtilis* isolated from irradiated pollen samples (no. 6, 21, 24, 28).

species *Mucor*, *Fusarium* spp., *Rhizopus* (*Rhizopus arrhizus*, *Rhizopus nigricans*) and *Aspergillus* was detected [7].

The aflatoxins are a group of mycotoxins produced by certain *Aspergillus* spp., in particular, *A. parasiticus*, *A. flavus*, *A. nomius*, and *A. pseudotamarii*. Three species of aflatoxin-producing fungi were present, and six pollen samples contained aflatoxins. The microbial and aflatoxin contents of pollen were strongly related to the previous handling and the methods of drying and storage used [28,29].

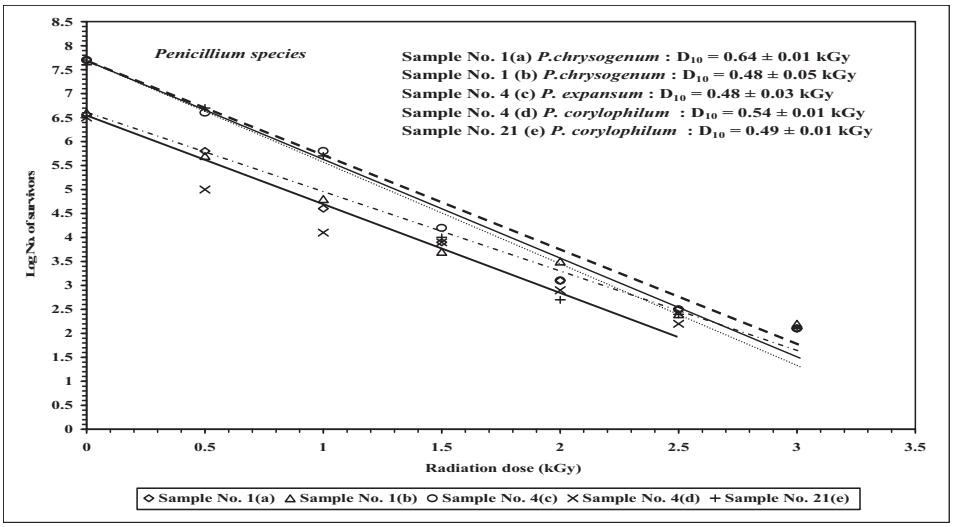
In the present study, aflatoxin B_1 was detected in 8.6% of the tested fungi isolated from the pollen samples, while aflatoxin B_2, G_1 and G_2 were not detected.

In other study, it was reported that 29% of the fungal isolates were *A. flavus* plus *A. parasiticus* produced the aflatoxin B_1 in cultures. Aflatoxin B_2 was detected in only 10% of the cultures. Aflatoxins G_1 and G_2 were not detected in cultures under the assayed conditions [30].

Irradiation of food using ionizing radiation (γ and x rays or electron beam) is used to inactivate both spoilage and pathogenic microorganisms and to guarantee the hygienic quality of several foodstuffs [11].

There was gradual decrease of the microbial counts in the selected heavily contaminated bee pollen samples

Figure 9



Dose–response study for *Penicillium* spp. (a) isolated from irradiated pollen samples (no. 1a, 1b, 4c,4d, 21e).

irradiated at γ radiation doses of up to 7.0 kGy. This maximum dose was enough for decontamination of bacteria and fungi in the bee pollen samples under test.

It was found that ^{60}Co γ radiation had an effective influence on the sterilization of the pine bee pollen and that the intensity of the bacterial contamination decreased with the increase of the radiation doses. The pine pollen without radiation treatment had a high number of bacterial contaminations. The pine pollen radiated with different doses had excellent results, especially with the doses between 6 and 12 kGy [31]. Moreover, γ rays at 7.5 kGy reduced the total microbial loads in bee pollen below 10^2 cfu/g without affecting its physiochemical properties such as amino acid, fatty acid composition also; thiobarbituric acid value, mineral content and pigment were not significantly changed by γ ray [32]. In contrast, exposure of bee pollen to 4 kGy was sufficient for microbial decontamination or reducing the count to less than 10 cfu/g [33].

In 1997, FAO/IAEA/WHO group's study on high-dose irradiation examined and evaluated the results of safety studies carried out on foods irradiated on the dose range 25–60 kGy to achieve the intended technological objective. The conclusion for such foods are both safe to the consumer and nutritionally adequate [34,35].

Irradiation of foods up to an overall average dose of 10 kGy introduced no specific nutritional or microbiological problems [36]. The irradiation dose of 10 kGy is the maximum dose allowed in food according to the Codex standards [37].

Exposure of the contaminated bee pollen samples to γ radiation led to isolation of radio-resistant bacteria that were identified as Gram-positive rods, Gram-negative rods and Gram-positive cocci, at a dose that ranged between 2.0 and 5.0 kGy, while the isolated radio-resistant fungal organisms were identified as *Penicillium* spp. at 1.5–2.0 kGy.

The study of the effect of γ radiation on micro-organisms isolated from contaminated samples showed that the lethal dose level of bacteria and fungi isolates ranged between 2 and 25 kGy [38].

Radiation resistance can be associated with the D_{10} value (the dose of γ radiation required to reduce a microbial population by 90%), and it is the measure for the radiation resistance of the micro-organisms to γ radiation [39,40].

In our study, the mean of the D_{10} values of the MRB organisms, *B. subtilis* (4), *B. megaterium* (3) and *B. pumilus* (2), which were isolated at 5.0 kGy from the irradiated bee pollen samples, were found to be from 1.5 to 2.7 and from 1.4 to 3.5 and from 1.5 to 2.4, respectively. However, for the fungal organisms, the D_{10} values were found to be 0.48 for *P. expansum* (1) and 0.48, 0.64 for *P. chrysogenum* (2), while it was 0.49, 0.54 for *P. corylophilum* (2). All the microbial strains showed an exponential rate of death.

The D_{10} values of *Bacillus cereus* and *A. flavus* were found to be 1.02 and 0.48, respectively [18], while the D_{10} values of four different *Bacillus* spp. ranged from 2.3 to 2.9 kGy [41]. *B. cereus* strains exhibited exponential rate of death, and the D_{10} values were calculated to be 1.9 and 2.2 kGy [42].

Moreover, the D_{10} values of two Gram-positive spore-forming *B. megaterium* were calculated to be 1.7 and 1.8 kGy. Furthermore, D_{10} values of the most radio-resistant strains of *B. sphaericus* (2) were found to be 0.85 and 1.25 kGy, while two strains of *B. pantothenticus* were 0.4 and 0.5 kGy, and six strains of *A. niger* ranged between 0.7 and 1.0 kGy; the D_{10} value for *P. chrysogenum* was 0.95 kGy. The microbial strains exhibited exponential rate of death, while one strain of *B. pantothenticus* and one of *A. niger* exhibited a nonexponential rate of death. In contrast, the D_{10} values of *Bacillus* spp. strains ranged from 0.83 to 0.99 kGy [18,43,44].

Our results and that obtained by other investigators show that the resistance and response of micro-organisms towards radiation differ between the different microorganisms and the different strains of the same micro-organisms. This may be attributed to species and number of micro-organisms, medium suspending the microorganisms, temperature, water activity, oxygen effect and the use of sensitizing compounds during the irradiation process [45,46].

Conclusion

γ Radiation technology can offer the process of microbial decontamination as a means of achieving microbiological safety limits. A dose level of 7.0 kGy was enough to inhibit the growth of micro-organisms on the bee pollen, and eliminate pathogenic micro-organisms including fungi, which may produce carcinogenic aflatoxins.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Broadhurst CL. Bee products: medicine from the hive. Nutr Sci News 1999; 4:366–368.

2 Nogueira C, Iglesias A, Feás X, Estevinho LM. Commercial bee pollen with different geographical origins. A comprehensive approach. Int J Mol Sci 2012; 13:11173–11187.

3 Bruno G. Bee pollen, propolis and royal jelly. 2005; 800:290–4226.

4 Campos RGM, Frigerio C, Lopes J, Bogdanov S. What is the future of Bee-Pollen?. J. of ApiProduct and ApiMedical Sci. 2010; 4:131–144.

5 Moosbeckhofer R. Quality and standards of pollen and beeswax. 1996.

6 Bashandy AS, Taha SMA. Effect of gamma radiation on the microbial contamination and some biochemical properties of pollen grains. Egypt J Microbiol 2004; 8:78–87.

7 Brindza J, Gróf J, Bacigálová K, Ferianc P, Tóth D. Pollen microbial colonization and food safety. Acta Chim Slovaca 2010; 3:95–102.

8 Miller D. Review: fungi and mycotoxins in grain: implications for stored product research. J Stored Prod Res 1994; 30:1–16.

9 IARC, The Evaluation of the Carcinogenic Risk of Chemical to Humans. IARC monograph supplement. Lyon, France: International Agency for Research on Cancer 1982. p. 4.

10 Naki Siviri N, Yekta A, Ozalp M, Atakan N, Polat M. Decontamination of cosmetic products and raw materials by gamma irradiation. J Pharm Sci 2006; 31:198–209.

11 Diehl JF. Safety of irradiated food. New York, NY: Marcel Dekker 1990.

12 Van German SJC, Rombouts FM, van't Riet K, Zwietering MH. A data analysis of the irradiation parameter D_{10}-for bacteria and spores under various conditions. J Food Prot 1999; 62:1024–1032.

13 Kacaniova M, Pavlicova S, Hacsık P, Kociubinski G, Knazovicka V, Sudzina M, *et al.* Microbial communities in bees, pollen and honey from Slovakia. Acta Microbiol Immunol Hung 2009; 56:285–295.

14 El-Bazza EMZ, Farrag AH, El-Mohie EDZ, El-Tablawy SY. Inhibitory effect of gamma radiation and Nigella sativa seeds oil on growth, spore germination and toxin production/Mf fungi. Radiat Phys Chem 2001; 60:181–189.

15 Cowan ST, Steel KI. Manual identification of medical bacteriology. 2nd ed. London, UK: Cambridge University Press; 1974. pp. 67–83.

16 Moubasher AH. Soil fungi in Qatar and other arab countries. The Centre for Scientific and Applied Research, University of Qatar 1993.

17 Nyngesa BW, Okoth S, Ayugi V. Identification Key for *Aspergillus* species isolated from Maize and Soil of Nandi County, Kenya. Advances in Microbiology 2015; 5(04):205–229.

18 El-Bazza ZE, El-Tablawy SY, Mohamed ASE, Nasser HA. Selection of gamma irradiation dose from sterilizing eye make-up preparations. Egypt J Microbiol 2010; 45:131–146.

19 El-Fouly MEZ, Farrag HA, El-Bazza ZE, El- Tablawy SYM. Ultra-structure and metabolic changes of certain pathogenic microorganisms after exposure to gamma rays and Nigella Sativa fixed oil. Azhar J Microbiol 2000; 49:84–99.

20 El-Bazza ZE, Mahmoud MI, Roushdy HM, Farrag HA, El-Tablawy SY. Fungal growth and mycotoxigenic production in certain medicinal herbs subjected to prolonged cold storage and possible control by gamma irradiation. Egypt J Pharm Sci 1996; 37:85–95.

21 A.O.A.C. 'Official methods of analysis of the association of official analytical chemists', chapter 26. William H, editor. 14th ed. Washington, DC: 1985.

22 Younis MHY, Malik MK. TLC and HPLC assays of aflatoxin contamination in Sudanese peanuts and beanut products. Kuwait J Sci Eng 2003; 30:79–94.

23 Bogdanov S. Contaminants of bee products. Apidologie 2005; 37:1–18.

24 Campos MGR, Bogdanov S, AlmeidaMuradian LB, Szczesna T, Mancebo Y, Frigerio C, Ferreira F. Pollen composition and standardization of analytical methods. J Apic Res 2008; 47:156–163.

25 Coronel BB, Grasso SC, Pereira G, Fernández A. Bromatological characterization of Argentine bee pollen. Cienc Docencia Tecnol 2004; 15:141–181.

26 Hervatin HL. Microbiological and physico-chemical evaluation of apiculture pollen in natura and dehydrated under different temperatures. Brazil: Universidade Estadual de Campinas, Campinas; 2009.

27 Gilliam M. Microbiology of pollen and bee bread: the genus *Bacillus*. Apidologie 1979; 10:269–274.

28 Serra Bonvehì J, Lòpez Alegret P. Microbiological studies on honeybee-collected pollen produced in Spain: Total bacteria, coliforms, *Escherichia coli, Staphylococcus, Streptococcus* 'D' of Lancefield, sulfite reducing Clostridia, Bacillus, yeasts, molds, and the detection of aflatoxins by thin layer chromatography (TLC). Ann. Falsif. Expert. Chim 1986; 849:259–266.

29 Bennet JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003; 16:497–516.

30 Gonzalez G, Hinojo MJ, Mateo R, Medina A, Jiménez M. Occurrence of mycotoxin producing fungi in bee pollen. Int J Food Microbiol 2005;105:1–9.

31 Junjie F, Weiqiao S, lianhua Z. Effect of radiation sterilization on pine pollen storage quality. J. Proceedings of the 7th Int. Working Conference on Stored-product Protection 1998; 2:1701–1704.

32 Yook HS, Lim SI, Byun MW. Changes in microbiological and physiochemical properties of bee pollen by application of gamma irradiation and ozone treatment. J Food Prot 1998; 61:217–220.

33 El-Tablawy SY. Influence of gamma irradiation on microbial quality, biological properties and some chemical compositions of propolis and bee pollen in Egypt. Isotope & Rad. Res. 2012; 44:363–377.

34 World Health Organization (WHO). High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Geneva, Switzerland: WHO, Technical Report; 1999.

35 Diehl JF. Food irradiation- past, present and future. Radiat Phys Chem 2002; 63:211–215.

36 WHO: Wholesomeness of Irradiated Food. Report of a Joint FAO/ IAEA /WHO Expert Committee. Geneva, Switzerland: World Health Organization 1981.

37 General Standard for Irradiated Foods Codex Standards. 106-1983, ' 2003. Available at: http://www.codexalimentarius.net/download/standards/16/CXS_106e.pdf. [accessed on 2011 Feb 16].

38 El-Bazza ZE, Toama MA, Taher HA. Study of the microbial contamination of cosmetic creams before and after use. Biohealth Sci Bulletin 2011; 3:37–43.

39 Miller WS, Berube R. Environmental control and bioburden in manufacturing process, in: Sterilization of Medical Products by Ionizing Radiation. (1978) Proceedings of In ternational Conference, Vienna, Austria, 1977 (Gaughran and Goudie, eds.), Multiscience Publication Lts. Montreal, Canada.

40 Russell AD. The destruction of bacterial spores. New York, NY: Academic Press 1982.

41 Bashandy AS, Hassan AA. Induced resistance to hydrogen peroxide, UV and gamma radiation in Bacillus species. Arab J Nucl Sci Appl 2005; 38:261–268.

42 Abostate MAM, Zahran DA, El-Hifnawy HN. Incidence of *Bacillus cereus* in some meat products and the effect of gamma radiation on its toxin(s). Int J Agric Bio 2006; 8:1–4.

43 Atique FB, Ahmed KT, Asaduzzaman SM, Hasan KN. Effect of gamma irradiation on bacterial microflora associated with human amniotic membrane. Biomed Res Int 2013; 2013:586561.

44 El-Bazza ZE, El-Tablawy SY, Hashem AE, Nasser HH. Evaluation of the microbial contamination of some eye-make up products before and after use. Biohealth Sci Bull 2009; 1:68–75.

45 Bridges BA. Effect of chemical modifiers on activation and mutation induction by gamma irradiation in *E.coli*. J Gen Microbial 1963; 405–412.

46 Goldblith SA. Inhibition and destruction of the microbial cell by radiations. In: Hugo WB, editor. InInhibition and Destruction of the Microbial Cell. London and New York: Academic Press 1971. pp. 285–305.

(DUPHAT 2019: www.duphat.ae)