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Application of gamma irradiation in ginseng for both photodegradation of pesticide pentachloronitrobenzene and microbial decontamination

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ABSTRACT

This study investigates the feasibility of using gamma irradiation for photodegradation of a common residual fungicide, pentachloronitrobenzene (PCNB), in ginseng, and for microbial decontamination. American ginseng, *Panax quinquefolius*, was subjected to gamma irradiation. PCNB residues were analyzed by gas chromatography with electron capture detection and mass spectrometry. Eighty percent of PCNB (100 ppm) in a methanol aqueous solution was degraded by 5 kGy irradiation, and the primary degradation product was pentachloroaniline. Furthermore, contaminated PCNB (3.7 ppm) in ginseng were reduced to 0.2 ppm after 20 kGy irradiation. The IC_{50} for treatment of *Sclerotium rolfsii* with 20 kGy irradiated PCNB was about 2.7 times higher than that for treatment with unirradiated PCNB. The survival rate of mouse fibroblast L929 cells treated with 20 kGy irradiated PCNB was about 12.9% higher than that of L929 cells treated with unirradiated PCNB. Additionally, after 20 kGy irradiation, less than 5% reduction of contents of ginsenoside Rb1 and Re were observed, and amounts of ginsenosides Rc, Rd, and Rg1 were not reduced significantly. The minimal gamma dose for microbial decontamination was 10 kGy. Therefore, gamma irradiation can be used for both PCNB photodegradation and microbial decontamination of ginseng without obvious loses of ginsenoside contents.

1. Introduction

Ginseng is the dried root of several distinct species of the genus *Panax* in the family *Araliaceae* [1]. It has been used as an ingredient of traditional Chinese medicine for more than 5000 years in the belief that it is a panacea and can promote longevity. More recently ginseng has gained popularity in the United States and Europe due to its ability to improve energy and vitality [2,3]. However, microorganisms and pesticides pollutions have caused potential hygiene and safety concerns in the use of ginseng. In most ginseng products, ginsenosides are the main bioactive components and are often used as marker components for indicating the type and quality of ginseng products. Until now, more than 30 ginsenosides have been identified, but ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf and Rg1 are the most associated with ginseng's pharmacological activity [4]. It has been proved that the extracts of Asian ginseng improve the immune system by enhancing phagocytosis and the activity of neutral killer cell, increasing interferon production and total lymphocyte count and providing antioxidant compounds [5,6].

Ginseng is susceptible to various fungal and insect diseases, since its ecological cultivation condition is an undergrowth of hardwood mixed deciduous forest. In order to protect ginseng crop, pesticides such as fungicides, insecticides, and herbicides are actively used. Among them, pentachloronitrobenzene (PCNB), also named as quintozene, is a frequently used organochlorine fungicide but also is the frequently found residue in ginseng [7,8]. Due to its cumulative, persistent, hardly degradable characteristics, PCNB can accumulate in the plants and then eventually become hazardous to human health through the incidental intake of contaminated ginsengs [9,10]. Hence, safety issues of ginseng ingestion must always be considered. In the European Union, the legal limit for PCNB is 1 ppm, it also is applied in Taiwan [11].

Several methods have been developed for eliminating pesticide residues from ginseng. A two-phase partition chromatography with the use of soybean oil was developed to eliminate pesticide residues in aqueous extracts of ginseng, but this method only

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could be applied in the aqueous extract of ginseng [12]. Another technique was CO_2 supercritical fluid extraction method to extract organochlorine pesticides from ginseng, but each time only powdered sample can be processed [13]. Therefore, the method is hard to scale up. Radiation-induced degradation for pesticides has been widely applied in water samples for environmental protection, and cobalt-60 radiation has been proved to have a degradative effect on both insecticides and herbicides [14–16]. In addition, PCNB showed a photo-sensitive property, since its half-life value in sunlight irradiation was much shorter than that of non-irradiated control samples [10,17]. Until now, no study has been done for the photolysis of pesticides in ginseng. Therefore, the objective of this study was to investigate the feasibility of gamma irradiation in eliminating PCNB residues from ginseng.

Gamma irradiation has been used for improving food safety for fresh foods and dried raw materials, and is proved as a safe and effective method to eliminate foodborne microorganisms and pests by World Health Organization, U.S. Food and Drug Administration and Institute of Food Technologists [18,19]. Ginseng originally is from natural raw products so it is easily contaminated by microorganisms during harvest, processing, transportation, storage, or post-harvest treatment, resulted in the loss of therapeutic efficiency [20]. In the previous study, Byun's group found that ginsenosides (Rb1, Rb2, Rc, Rd, Re, Rf and Rg1) were very stable to gamma irradiation [21]. Therefore, using gamma irradiation to eliminate microorganisms can be one of optimal decontamination ways to increase shelf life of ginseng without spoiling its ginsenoside contents. Thus, in this study, while gamma irradiation is applied to eliminate pesticides in ginsengs, simultaneously it is an effective process to control microorganisms.

2. Materials and methods

2.1. Materials

Dried *Panax quinquefolius* roots (American ginseng) used in this study were cultured in Wisconsin approximately 4 years old at the time of harvest and were provided by an importer (Juien Guien Trading Co., Ltd.) in Taiwan. Their authenticity was confirmed by Dr. Y.S. Chung in the Institute of Chinese Pharmaceutical Sciences, China Medical University, Taiwan. Acetonitrile, acetone, methanol and dimethylsulfoxide (DMSO) were obtained from Merck (Darmstadt, Germany). Ginsenoside standards (Rb1, Rc and Re) were bought from Sigma–Aldrich Co. (Missouri, USA). Ginsenoside standards (Rd and Rg1) were purchased from Indofine Chemical Co. Inc. (New Jersey, USA). Technical grade pentachloronitrobenzene (PCNB) was purchased from Supelco Inc. (Pennsylvania, USA). Potato dextrose agar (PDA), plate count agar (PCA), plate count broth (PCB) and violet red bile glucose agar (VRBGA) were bought from Difico Laboratories (Michigan, USA).

2.2. Gamma irradiation

PCNB standards were prepared by using 90% methanol/H_2O (90/10, v/v) and were irradiated in a 1.8-mL Pyrex glass vessels at room temperature (25–30 °C). American ginseng contaminated 3.7 ppm of PCNB was ground to a powder and packed in poly vinyl chloride (PVC) bags for gamma irradiation under aerobic condition. Gamma irradiation was performed according to a method published previously [22].

2.3. Analysis of PCNB

PCNB was extracted from contaminated ginsengs by grinding and packing ground material in a disposable column. Subsequently, PCNB residues were eluted with acetone/water (80/20, v/v) and passed through a C_{18} SPE cartridge. The elute was further extracted with 150 mL of acetone/methylene chloride (1/2, v/v) and then 200 mL of acetone/methylene chloride (1/1, v/v). The solvent of the extract was exchanged into acetone and analyzed by a PerkinElmer Auto System XL gas chromatograph (GC) with electron capture detection and a Turbo mass spectrometer. One microliter of extract was injected into a DB-5 MS column (30 m, 0.25 mm I.D., 1.0 μm film thickness) and run temperature conditions were as follows: 150 °C for 3 min, 150–250 °C at 20 °C/min, 250 °C for 20 min, 250–310 °C at 10 °C/min, 310 °C for 20 min. The carrier gas was helium (12 psi) at a flow-rate of 1.5 mL/min. Effluents from the GC column were transferred into a 70-eV electron impact ionization source with the scan speed as 2500 scans/min and ion source temperature as 200 °C. For analyzing PCNB, ions were monitored at m/z of 237 (quantitation ion) and m/z of 230 and 203 (qualifier ions) [23].

2.4. Fungitoxic activity of PCNB to Sclerotium rolfsii

S. rolfsii has been widely used for investigating the activity of pesticides, such as tebuconazole and PCNB [24]. In this study, PCNB was dissolved in methanol and mixed with autoclaved PDA. The final concentration of methanol in plates was 0.05% (v/v) and the PCNB concentrations were 1–8 ppm (μg/mL). Fungitoxic activity of PCNB was based on the inhibition of mycelial development of *S. rolfsii*, and was assessed by measuring the radial growth of mycelial on PDA plates. In petri dishes, 2-mm agar plugs were inoculated on which *S. rolfsii* had been allowed to germinate. Five replicates were used per treatment. Plates were incubated at 26 °C for 72 h, and the diameters of mycelial colonies were measured to calculate the half maximal inhibitory concentration (IC_{50}) value. The IC_{50} value was defined as the PCNB concentration causing a 50% reduction in mycelial growth compared with the control (without adding PCNB) and was obtained from a dose response curve by regressing the percent of mycelial growth against PCNB concentrations [24].

2.5. Cytotoxicity of PCNB

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed with the L929 mouse fibroblast cells (ATCC CCL-1) [25]. PCNB standards were prepared by dissolved in 90% methanol and received 0, 10 or 20 kGy of gamma irradiation, respectively. PCNB solutions were dried at 45 °C and re-dissolved in DMSO. Irradiated and unirradiated PCNB standards were added into fresh culture medium, and cells were cultivated in 24-well culture dishes for 24 h. After replacing fresh culture medium (900 μL/well), 100 μL/well of the PCNB containing media was added. The final concentration of PCNB in media was 0–20 ppm. Each test was processed by repeating 6 times, with samples without PCNB as the control group. After 48 h, cells were subjected to MTT assay. The surviving rate of L929 cells (%) related to the control, was calculated by $[\text{Absorption}_{\text{test}}]/[\text{Absorption}_{\text{control}}] \times 100$.

2.6. Determine the amount of ginsenosides by HPLC

Ginsenosides were extracted from samples by modification of a published method [26]. Five grams of ginseng powders was extracted in 50 mL of 70% methanol by sonication for 1 h at room temperature. After centrifugation at 9500 rpm for 10 min, the supernatants were filtrated by a 0.2-μm nylon filter. Through an optimal dilution, a 20-μL sample was injected into a HPLC (Waters model 2695 Separations Module) with a UV detector (set at 203 nm). An column (Cosmosil $5C_{18}$-MS-II, 5 μm, 4.6 × 250 mm)

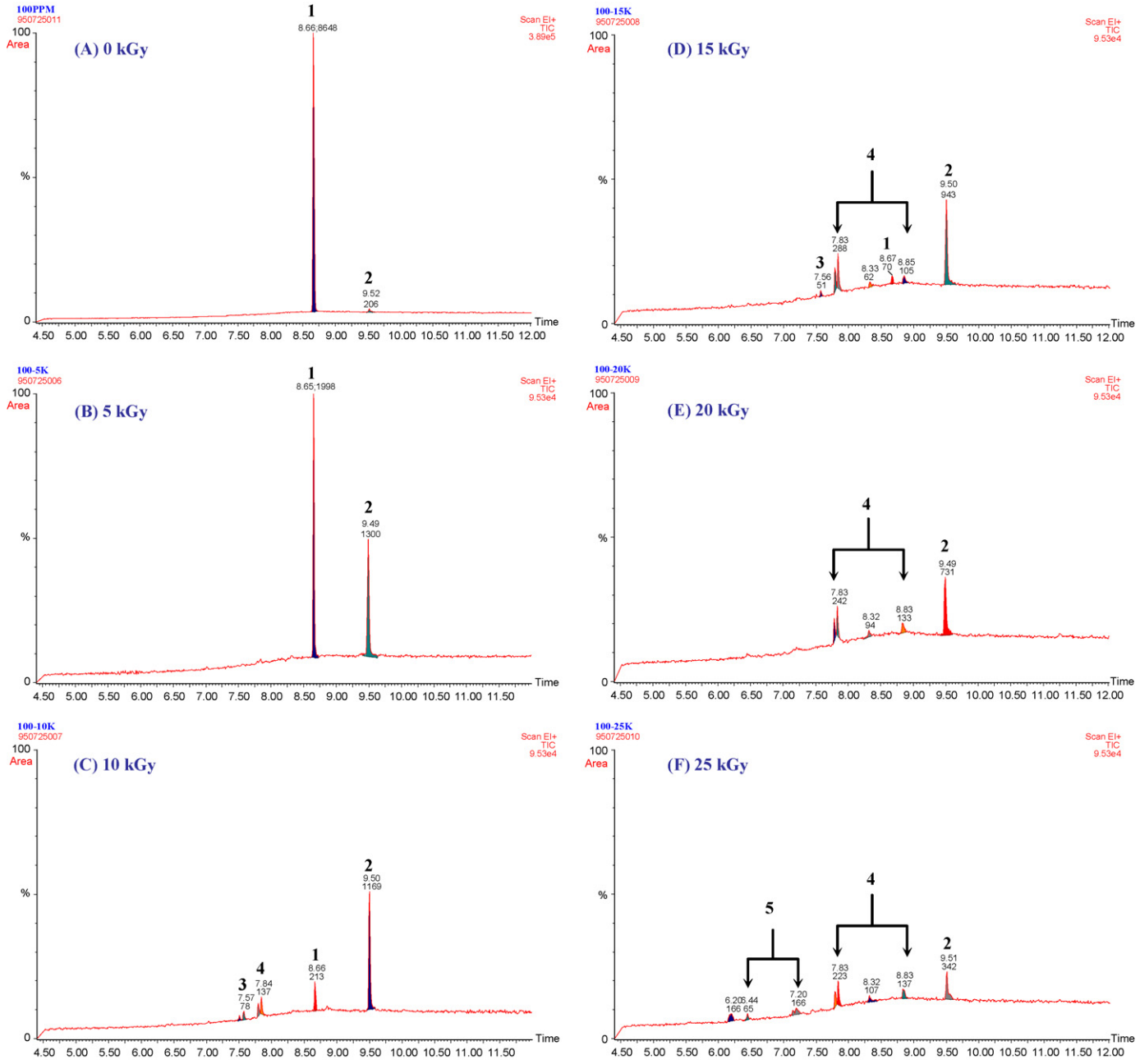

Fig. 1. Gas chromatograms obtained for 100 ppm PCNB with various dosages of gamma irradiation: (A) before irradiation; (B) irradiated with a 5-kGy gamma dose; (C) irradiated with a 10-kGy gamma dose; (D) irradiated with a 15-kGy gamma dose; (E) irradiated with a 20-kGy gamma dose; and (F) irradiated with a 25-kGy gamma dose.

was used with a gradient of two solvents, (A) phosphate buffer (10.3 mM, pH 5.8) and (B) acetonitrile: 0–10 min, 81% A and 19% B; 10–30 min, 81–71% A and 19–29% B; 30–60 min, 71–60% A and 29–40% B, at a flow-rate of 1.0 mL/min at 25 °C. Ginsenoside standards were prepared right before HPLC analysis. Results are reported as milligram of ginsenoside per gram of ginseng on a dry weight basis.

2.7. Enumeration of microbes

Microbial enumeration of was mainly according to the method published previously [22]. Ten samples of American ginseng were purchased and stored under 4 °C before the use. The solid culture media used in this study included PCA, PDA and VRBGA. The spread plate count method was applied to count the microorganisms in ground ginseng samples. The microbial content of the samples was measured immediately and 6 months post-irradiation storage. Counts were recorded in colony forming units per gram (CFU/g).

3. Results

3.1. Observation of radiolytic degradation of PCNB standards by gas chromatography

The feasibility of eliminating PCNB residues by gamma irradiation was investigated by irradiating 100 ppm PCNB in solution with various doses of gamma ray (5–25 kGy) and analyzed by GC–MS. to evaluate PCNB radiolytic degradation efficiency (Fig. 1). In unirradiated one (0 kGy), the main peak showed the retention of 8.7 min was PCNB (peak 1); while a small peak at 9.5 min (peak 2) was pentachloroaniline (PCA) determined through a GC–MS procedure (Fig. 1A). The presence of PCA might be owing to the natural

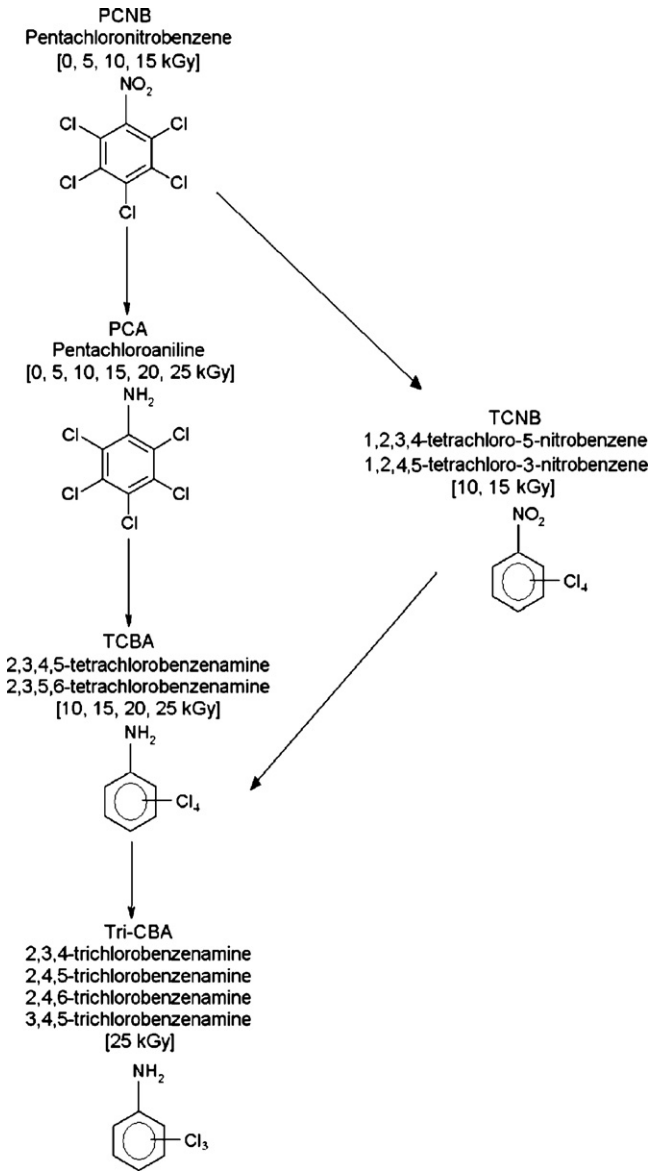

Fig. 2. The radiolytic pathway of PCNB.

degradation of PCNB. Fig. 1B shows the chromatogram of 5 kGy gamma irradiated PCNB sample, the PCNB amount decreased with an increase of PCA concentration, compared with the unirradiated one. At 10 kGy and 15 kGy irradiation (Fig. 1C and D), PCNB (peak 1) is significantly lower than that at 5 kGy, and isomers of tetrachloronitrobenzene (TCNB) and tetrachlorobenzenamine (TCBA) were newly found and labeled as peak 3 (at 7.6 min) and peak 4 (at 7.8 and 8.8 min). At 20 kGy, there is no presence of PCNB (peak 1) and TCNB (peak 3). The main compound of degradation is PCA with small peaks identified as TCBA isomers (peak 4 at 7.8 and 8.8 min) (Fig. 1E). At 25 kGy, a new degraded product was identified at 6.4 and 7.2 min as the isomers of trichlorobenzenamine (Tri-CBA) and labeled as peak 5. Conclusively, the amount of 100 ppm PCNB decreased as irradiation dose increased and was eliminated at 20 kGy. Based on the identified degraded compounds, the pathways of PCNB degradation can be proposed and are shown in Fig. 2. PCNB was firstly degraded to PCA at 5 kGy, subsequently to TCNB and TCBA at 10–20 kGy, and further degraded into Tri-CBA at 25 kGy. About 80% of PCNB was decomposed at 5 kGy, more than 97% of PCNB was decomposed at 10 kGy, and no presence of PCNB was observed at 20 kGy.

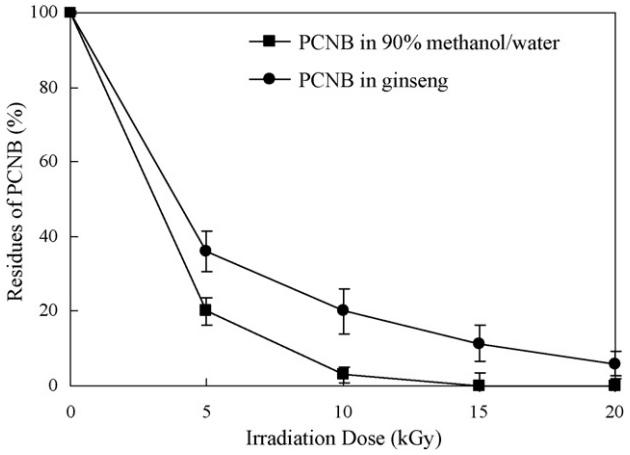

Fig. 3. Radiolytic efficiency of PCNB in ginseng and in the methanol aqueous solution.

American ginseng contaminated with 3.7 ppm PCNB was irradiated with various doses of gamma rays. The samples were analyzed by GC–MS under selected ion monitoring conditions to evaluate the radiolysis of PCNB in ginseng. Results revealed that the radiolytic product of PCNB in 5 kGy gamma ray-irradiated American ginseng was PCA (data not shown). However, the American ginseng extract has a complicated chemical system containing many types of compounds. When 3.7 ppm PCNB-contaminated American ginseng was irradiated with 10 and 20 kGy gamma rays, the signal of the radiolytic product (other than PCA) of PCNB in the extract was not strong enough to be detected. Thus, spiking ginseng powder with a higher concentration of PCNB for irradiation may be effective for studying the radiolytic pathway and identifying the degraded products of PCNB in ginseng.

3.2. Radiolytic efficiency of PCNB in ginseng and in methanol solution

The powder of American ginseng contaminated with 3.7 ppm PCNB was irradiated with 5–20 kGy of gamma doses, with the standard PCNB (3.7 ppm) in 90% methanol aqueous solution as the control. The radiolytic degradation efficiency of PCNB both in ginseng and methanol solution increased as the irradiated dose increased (Fig. 3). At 5 kGy irradiation, PCNB degradation efficiency in methanol solution was found to be 80%, compared to 64% in ginseng. At 15 kGy, more than 99% of PCNB was eliminated in methanol solution, resulted in less than 0.1 ppm PCNB residues. Up to 20 kGy, about 94% of PCNB in ginseng samples was decomposed, resulted in less than 0.25 ppm of PCNB residues; while, PCNB residue in the methanol solution cannot be detected. Hence, this result demonstrated gamma irradiation can be an efficient method to eliminate PCNB from ginseng.

3.3. Effects of gamma irradiation on PCNB fungitoxic activity on the mycelial growth of S. rolfsii

The mycelial growth and colony morphology of *S. rolfsii* after a 72-h growth on PDA were shown in Fig. 4. The mycelia were extensively grown in the PDA plate without the addition of PCNB (Fig. 4A). However, the mycelial growth of *S. rolfsii* was strongly inhibited in 3 ppm of unirradiated PCNB amended PDA plate (Fig. 4B). The mycelial on this PDA plate was condensed and restricted within the central local area of the inoculated plug of agar, and could not spread out to the PCNB contained agar. Among the PDA plates spiked with unirradiated or irradiated PCNB, the mycelial growth was more restricted in the

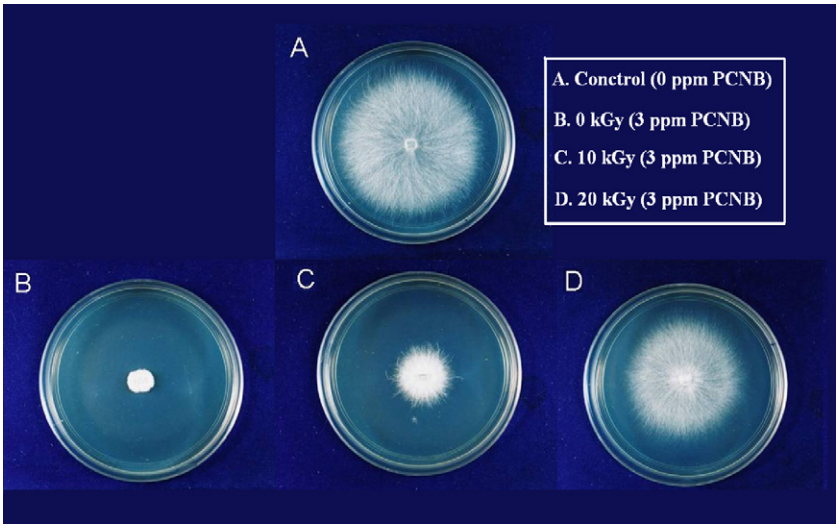

Fig. 4. The mycelial growth and morphology of *S. rolfsii* colonies on PDA plates after 72-h incubation. (A) PDA plate without the addition of 3 ppm PCNB, (B) PDA plate amended with 3 ppm of unirradiated PCNB, (C) PDA plate amended with 3 ppm of 10-kGy gamma irradiated PCNB, and (D) PDA plate amended with 3 ppm of 20-kGy gamma irradiated PCNB.

PDA containing the unirradiated PCNB (Fig. 4B) than that containing irradiated PCNB samples (Fig. 4C and D). The mycelial growth inhibition on 10 kGy irradiated PCNB amended plate was more significant limited than that in 20 kGy irradiated PCNB amended ones. Hence, gamma irradiation could reduce the fungitoxicity of PCNB on *S. rolfsii* and this reduction was dose dependent.

Fig. 5 shows the dose–response curves of *S. rolfsii* grown on the PDA plates with serial dilutions of different irradiated PCNB samples. Mycelial growth was strongly inhibited by the unirradiated PCNB with an IC_{50} value as 1.7 ppm. The irradiated PCNB samples showed less fungitoxicity to *S. rolfsii* than unirradiated PCNB samples, and the level of PCNB fungitoxicity was inversely related to the irradiation does. Compared to the IC_{50} value of the unirradiated PCNB (1.7 ppm), the IC_{50} value of the 10 kGy (3.4 ppm) and 20 kGy (4.6 ppm) irradiated PCNB was 2 and 2.7-fold higher, respectively. In the unirradiated PCNB treated PDA plate, the inhibition of mycelial growth of *S. rolfsii* immediately observed at 1 ppm of PCNB. However, in the 10 and 20 kGy irradiated PCNB treated plates, shoulders were present in the dose–response curves, representing a reduction of PCNB fungitoxicity of these irradiated PCNB. Especially in the 20 kGy irradiated PCNB treated ones, even through PDA plates were treated with 2 ppm irradiated PCNB, no obvious growth inhibition was observed; while, 2 ppm unirradiated PCNB already caused about 70% reduction of mycelial growth. Furthermore, the completely growth inhibition happened at 5.0 ppm for unirradiated PCNB treated curve; while it happened about at 6.0 ppm and 8.0 ppm for 10-kGy and 20-kGy irradiated PCNB samples, respectively. Therefore, gamma irradiation treatment could efficiently reduce the fungitoxicity of PCNB on *S. rolfsii*.

3.4. Effects of gamma irradiation on cytotoxicity of PCNB

PCNB cytotoxicity for L929 cells treated with different concentrations of irradiated or unirradiated PCNB samples was evaluated, with 1% DMSO without PCNB as the control (100% survival fraction), as presented in Fig. 6. All samples affected the metabolic activity in a concentration-dependent manner when they were added in 0–20 ppm of PCNB. However, the cytotoxicity of irradiated PCNB samples to L929 cells was lower than that of unirradiated PCNB. Surviving fraction of L929 cells treated with 10 ppm PCNB

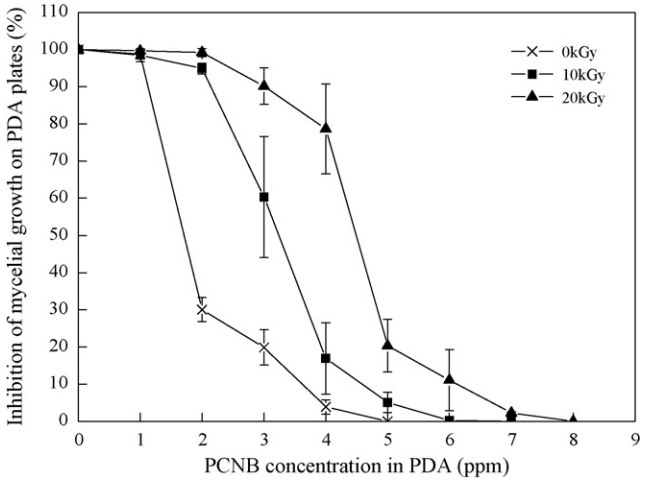

Fig. 5. Comparison of the growth of *S. rolfsii* colonies on (×) PDA plate amended with PCNB, (■) PDA plate amended with PCNB irradiated with 10-kGy gamma radiation, and (▲) PDA plate amended with PCNB irradiated with 20-kGy gamma radiation.

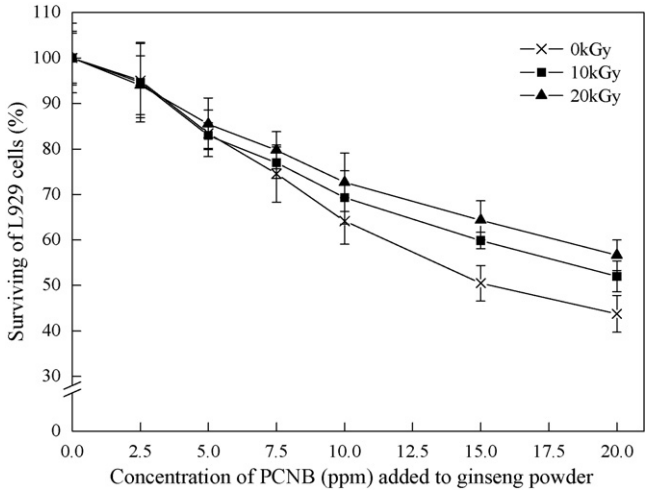

Fig. 6. Cytotoxicity of PCNB before and after 10 and 20 kGy gamma irradiation.

with 20 kGy irradiated, 10 kGy irradiated, and the unirradiated ones were 72.7%, 69.3% and 64.2%, respectively. While the surviving fraction of 20 ppm PCNB with 20 kGy irradiated, 10 kGy irradiated, and unirradiated ones were 56.6%, 52.0% and 43.7%, respectively. Hence, gamma irradiation could reduce PCNB cytotoxicity in L929 cells and the reducing efficiency of PCNB cytotoxicity to L929 cells was increased as the increase of irradiation dose.

3.5. Effects of gamma irradiation on microbiological quality of ginseng

As shown in Table 1, ginseng specimens contained total aerobic bacterial counts of 3.2×10^3 to 7.5×10^4 CFU/g, mold and yeast counts of 1.7×10^2 to 3.8×10^2 CFU/g, and enterobacterial count of 1.3×10^1 to 4.6×10^1 CFU/g. For all of unirradiated samples, total bacterial counts significantly exceeded mold and yeast counts. As the increase of radiation dose, the microbial profiles in ginseng changed significantly. A dose of 4 kGy reduced the total aerobic bacterial counts to 2.1×10^2–4.1×10^2 CFU/g, the mold and yeast counts to 1.1×10^1–3.2×10^1 CFU/g, and the Enterobacterial counts to below the detectable level. Hence, *Enterobacteriaceae* was more sensitive to gamma irradiation and 4 kGy was efficient to eliminate *Enterobacteriaceae* from ginseng. At 6 kGy, the total aerobic bacterial counts were reduced to 1.1×10^1–3.2×10^1 CFU/g, and the mold and yeast counts were reduced to less than 10^1 CFU/g. After 8 kGy of irradiation, 9 of the 10 specimens were decontaminated and the bacterial count in the remained sample was less than 10^1 CFU/g. Finally, 10 kGy of gamma irradiation could effectively reduce bacterial and mold/yeast counts to below the detectable levels. Moreover, unirradiated and irradiated samples were placed at room temperature ($25 \pm 5\,^{\circ}C$) for 6 months and then analyzed their microbial amounts. Counts of molds and yeasts were significantly higher than samples without the 6-month storage. The mold/yeast counts were higher than the total aerobic bacteria counts and the Enterobacterial counts of all ginseng samples after the 6-month storage. No microbial growth was observed in the 10-kGy irradiated samples after the 6-month storage. Thus, 10 kGy of irradiation was sufficient for ginseng decontamination and could maintain sterile condition of ginseng at room temperature for 6 months.

3.6. Effect of gamma irradiation on the contents of major ginsenosides of American ginseng

Quantitative results of five major ginsenosides (Rb1, Rc, Rd, Re and Rg1) were shown in Table 2. Among them, ginsenoside Rb1 was the most abundant, followed by ginsenoside Re, Rd, Rc and Rg1. Ginsenoside Rc, Rd, and Rg1 were quiet stable with gamma irradiation treatment even up to 20 kGy since no significant reduction of their ginsenoside amounts were found between before and after treatment. After 20 kGy irradiation, less than 5% reduction of contents of ginsenoside Rb1 and Re were observed. This small amount of reduction for ginsenosides was acceptable, considering the contributions of gamma irradiation on eliminating PCNB residues and microbial decontamination. Hence, gamma irradiation can be an alternative method to eliminate PCNB residues and microbes from ginseng without the significant loses of ginsenosides.

4. Discussion

In this study, PCNB irradiated by Co-60 gamma ray showed significant reduction in concentration. The main degradation product of PCNB is PCA and such decomposition corresponded to the photodegradation reaction done in referred studies [27]. PCNB has a wider spectrum to against microorganisms and its inhibition to soil microorganisms is at lower concentration than PCA. Thus, the conversion of PCNB into PCA can be considered as a detoxification step [28]. Results showed that at 5 kGy the nitro group of PCNB in solution was firstly reduced to amine group to become PCA, and as the increase of irradiation doses (10–25 kGy) one to two chloride groups were removed from the benzene ring to become isomers of TCNB (1,2,3,4-tetrachloro-5-nitrobenzene and 1,2,4,5-tetrachloro-3-nitrobenzene) at 10–25 kGy, isomers TCBA (2,3,4,5-tetrachlorobenzenamine and 2,3,5,6-tetrachlorobenzenamine) at 10–15 kGy, and isomers of Tri-CBA (2,3,4-trichlorobenzeamine, 2,4,5-trichlorobenzeamine, 2,4,6-trichlorobenzeamine, and 3,4,5-trichlorobenzeamine) at 25 kGy. Moreover, the radiolytic product of PCNB in 5 kGy gamma ray-irradiated American ginseng was also pentachloroaniline (PCA). However, due to that the American ginseng extract has a complicated chemical system containing many types of compounds; the signal of the radiolytic product (other than PCA) of 3.7 ppm PCNB in higher doses of gamma ray-irradiated American ginseng was not strong enough to be detected. Thus, the radiolytic product of PCNB in higher doses of gamma ray-irradiated American ginseng needed further investigation. Moreover, spiking ginseng powder with a higher concentration of PCNB for irradiation may be effective for studying the radiolytic pathway and identifying the degraded products of PCNB in ginseng. In addition, whether an increase in the dose of radiation causes Tri-CBA to be degraded or to be dechlorinated requires further investigation. Although the individual toxicity of each degraded product still needs further investigation, the results of microbial growth inhibition test showed that the total toxicity of residual PCNB and its degraded products after the irradiation was significantly lower than that of untreated PCNB. In addition, PCNB cytotoxicity to the L929 cells decreased after PCNB was irradiated. Hence, the cytotoxicity and fungicide activity of irradiated PCNB were lower than unirradiated PCNB.

The degradation reactions of PCNB by gamma ray might be mainly caused by free radicals that attacked the benzene ring and nitro group of PCNB through the electrophilic addition reaction [29]. The free radical in the degradation process in this study was measured. The radical concentrations that produced the EPR signals detected in non-irradiated, 10 kGy irradiated and 25 kGy irradiated samples were 1.1092×10^{16}, 7.8162×10^{16} and 1.3469×10^{17} spins/g, respectively. For the rise of radicals during irradiation mainly resulted from the reaction between water and radiation, the water content of Chinese herbals also would affect the amount of radicals formed as well as the effectiveness of PCNB decomposition and microbial inactivation. Generally, the water content of dried ginseng roots was about 10–12% (w/w). Thus, in this study, the standards of PCNB solutions were prepared by using 90% methanol/H_2O (v/v) for irradiation. In addition, oxygen molecules may combine with free radicals to form the oxygenized free radicals and extend the lifetime of the free radicals. Thus oxygen molecules inside the packaging might also enhance the efficiency of free radicals on PCNB degradation or microbial inactivation. This is the so-called the effects of oxygen enhancement ratio [30]. Therefore, the water content and oxygen content should be taken into consideration for irradiation. Compared with the extraction methods used for decreasing the pesticides, gamma irradiation is much easier to perform and has no requirement of adding chemicals. For the future work, we will focus on the factors affecting radiolytic efficiency of gamma irradiation on pesticides for ginseng: irradiation dose-rate, packing condition (oxygen or nitrogen concentration in the package), moisture contain of ginseng, and source of radiation.

As seeking the methods of microbial decontamination and PCNB decomposition of Chinese herbals, the major effective ingredients of Chinese herbs should be preserved as much as possible. The results indicated that after 20 kGy of radiation treatment, the

Table 1
Effect of different doses of gamma irradiation on microbial contents in ginseng (CFU/g).

Irradiation dose (kGy)	Total aerobic bacteria		Molds and yeast		Enterobacteriaceae	
	0 month[a]	6 month[a]	0 month[a]	6 month[a]	0 month[a]	6 month[a]
0	3.2×10^3–7.5×10^4	2.2×10^2–3.8×10^2	1.7×10^2–3.8×10^2	8.1×10^4–5.8×10^6	1.3×10^1–4.6×10^1	$<10^1$
2	1.4×10^3–7.4×10^3	1.6×10^2–2.9×10^2	2.1×10^2–2.9×10^2	7.6×10^5–9.7×10^5	$<10^1$	$<10^1$
4	2.1×10^2–4.1×10^2	9.1×10^1–1.4×10^2	1.1×10^1–3.2×10^1	1.8×10^4–4.3×10^4	ND	ND
6	1.1×10^1–3.2×10^1	$<10^1$	$<10^1$	2.7×10^3–7.7×10^3	–	–
8	$<10^1$	ND	ND	ND	–	–
10	ND	–	–	–	–	–

ND indicates no microbe detected on plates; – indicates no determination of microbial growth.
Counts are average of five replication plates, and data are ranges of microbial contents from analyzed of ten samples of American ginseng.
[a] Storage period.

Table 2
Effects of gamma irradiation on major ginsenoside compositions of American ginseng.

Ginsenosides[a] (mg/g)	Irradiation dose (kGy)				
	0	5	10	15	20
Rb1	23.36 ± 0.74ab	23.79 ± 0.28a	23.37 ± 0.09b	22.68 ± 0.36c	22.26 ± 0.21c
Rc	3.28 ± 0.12a	3.33 ± 0.05a	3.30 ± 0.04a	3.27 ± 0.02a	3.21 ± 0.03a
Rd	5.05 ± 0.15a	5.19 ± 0.06a	5.13 ± 0.03a	5.01 ± 0.12a	5.21 ± 0.06a
Re	12.61 ± 0.41ab	12.82 ± 0.12a	12.42 ± 0.05b	12.21 ± 0.27ab	12.03 ± 0.12c
Rg1	1.66 ± 0.14a	1.81 ± 0.03a	1.72 ± 0.03a	1.67 ± 0.09a	1.70 ± 0.03a

[a] Data are means ± standard deviation of three independent determinations, and expressed as mg/g dry basis. Means within each row with the same alphabet are not significantly different $p < 0.05$.

amounts of ginsenoside Rc, Rd and Rg1 did not decrease significantly, and less than 5% reduction for ginsenoside Rb1 and Re were observed that never reported previously. Hence, gamma irradiation is an optimal method to decompose PCNB without obvious reduction of ginsenosides. This preliminary study of using gamma irradiation for photolysis of PCNB not only affords a useful way to study pesticide degradation, but also provides an alternative way for the treatment of other PCNB-contaminated Chinese medicines.

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