

Efficacy assessment of gamma radiation on plant-associated nematodes

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ABSTRACT

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To eliminate unwanted nematodes from import media at the seaport, this study has tested the baseline sensitivity of nematodes to cobalt-60 (⁶⁰Co) gamma irradiation and its effect on killing nematodes in cultivation media. Five plant pathogenic nematodes including *Meloidogyne incognita*, *Radopholus similis*, *Pratylenchus coffeae*, *Bursaphelenchus xylophilus*, and *Aphelenchoides* sp., and one free-living nematode *Rhabditis* sp. were tested. The results revealed that the tolerance of each test nematode to radiation doses varied. However, the application of high dosages (4 to 6 kGy) of ⁶⁰Co could reduce nematode fertility and mortality rates. Of all test nematodes, *Aphelenchoides* sp. was the most susceptible to ⁶⁰Co, followed by *B. xylophilus*. Upon exposure to 2 kGy, *Aphelenchoides* sp. and *B. xylophilus* were eliminated. Both *M. incognita* and *R. similis* were killed by 4 kGy and were moderately tolerant to radiation. *P. coffeae* and *Rhabditis* spp. were highly tolerant to ⁶⁰Co, requiring 6 kGy to kill either species. *Phalaenopsis* orchids, which were grown in cultivation materials after radiation exhibited normal growth and produced flowers like those grown in untreated materials. Our results indicate that irradiation could be a safe and environmentally friendly method to kill nematodes embedded in a cultivation medium at the seaport.

Keywords: gamma irradiation, nematodes, orchids, quarantine, seaport

INTRODUCTION

Orchids belonging to the Orchidaceae family are popular flowering plants worldwide. The plants are commonly cultivated in counties with tropical climates. Taiwan is one of the ideal countries to cultivate orchid plants. Orchid plants grown in Taiwan are exported to more than 36 countries. Various media including sphagnum moss, peat moss, snake wood chips, perlite, vermiculite, foamed stone, coconut shell fiber, and bark are commonly used to cultivate orchid plants. Those plant-based materials are mainly imported in bulk from different countries. One of the biggest problems with the wide-scale use of the import media is the contamination of unwanted nematodes.

Many nematode species are often detected from a wide range of cultivation media imported from other countries. Those nematodes include *Rhabditis* spp., *Aphelenchoides* spp. (leaf nematodes), *Ditylenchus dipsaci* (stem nematode), *Rhadinaphelenchus cocophilus* (red ring nematode), *Meloidogyne* spp. (root-knot nematodes), *Pratylenchus* spp. (lesion nematodes), and *Helicotylenchus* spp. (spiral nematodes). Among them, *Aphelenchoides* spp. are of particular concern to orchid growers. Whether or not the nematodes found in the imported media would infect local crops and lead to yield losses remains largely unknown. Nonetheless, all imported media must not be directly applied in agricultural cultivation and production without any treatments. In addition, according to international trade agreements, a phytosanitary certificate is required when shipping plants or plant products. For these reasons, there is a strong demand for effective sanitation practices to ensure that orchids and cultivation media are free of nematodes and other pests.

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Import media used for orchid cultivation are often sanitized by immersing in bleach, hot water, or ozone for disinfection^(18, 19). However, these sanitation methods can affect the air permeability of the medium, which may lead to a decline in the quality of orchid cultivation. Although fumigation could be effective in inactivating microorganisms present in plant surfaces and cultivation media, the practice could be harmful to operators and environments because of the application of toxic chemicals. Thus, developing a new method to eradicate nematodes from import media is indeed imminent. Radiation has long been considered an environmentally friendly and effective treatment to kill plant pathogenic nematodes^(1, 2, 4). Scientists have tried this kind of research since the 1960s. Myers and colleagues have tried to treat 14 plant pathogenic nematodes and saprophytic nematodes with cobalt-60 (⁶⁰Co) gamma irradiation and found considerable variations in the sensitivity of nematode species to the radiation⁽¹²⁾. Both *Aphelenchoides* spp. and *Pratylenchus vulnus* are highly sensitive to ⁶⁰Co; their reproduction can be completely inhibited after 0.37 kGy treatments. In contrast, of 14 test nematodes, *Paratrichodorus minor* (stubby-root nematode) is highly tolerant to ⁶⁰Co, requiring a dose of 2.98 kGy to kill this nematode^(12, 15). *Ditylenchus dipsaci* can be killed by a dose of 0.48 kGy with gamma rays⁽¹⁴⁾. Experimental tests also have revealed that a dose of 0.02 kGy can completely prevent the egg hatch of *Globodera rostochiensis* (potato cyst nematode)⁽⁶⁾. Irradiation of *Meloidogyne javanica* in Hawaii has revealed that 6.25 kGy is sufficient to prevent nematode eggs from hatching; however, a dose of 7.5 kGy is required to kill second-stage juveniles (J2)⁽³⁾. Those results indicate that irradiation could be a useful method to kill nematodes present in cultivation media imported from other countries.

The objectives of this study were to establish the baseline sensitivity of five plant pathogenic nematodes to radiation, to assess the possibility of using irradiation to eliminate those nematodes in imported cultivation media and to evaluate the practical feasibility of irradiation for commercial use.

MATERIALS AND METHODS

Source and maintenance of nematodes

Nematodes including *Meloidogyne incognita* (root-knot nematode), *Radopholus similis* (Cobb) Thorne (burrowing nematode), *Pratylenchus coffeae* (root-lesion nematode), *Bursaphelenchus xylophilus* (pine wood nematode), and *Aphelenchoides* sp. (leaf and bud nematode) and their origins are listed in Table 1. *M. incognita* was propagated in water convolvulus (also known as water spinach) (*Ipomoea aquatic* Forsk.) Seedlings of water convolvulus were grown in peat moss and perlite mixture (1:1, w/w) for 14 days and inoculated by placing egg masses of *M. incognita* around the roots. The plants were maintained in a greenhouse for 45 days. Newly formed eggs were harvested and incubated in sterile water for 24 h to produce juvenile second stage (J2), which was collected by a sterilized glass straw. Both *R. similis* and *P. coffeae* after being sterilized three times (15 min each time) with a mixture of antibiotics (containing 1000 ppm each of streptomycin, chloromycetin, and penicillin) and rinsed with sterile water were propagated in carrot callus at 25°C for 30 days. *B. xylophilus* and *Aphelenchoides* sp. were propagated using the mycelium of *Ceratomyces* sp. and *Alternaria* sp., respectively. Fungus was cultured on potato dextrose agar (PDA) slants or plates for 7 days, and nematodes were transferred into slants or plates, incubated at 25°C, and re-cultured every 14 days. A free-living nematode was isolated from Sphagnum moss imported from Chile and identified as *Rhabditis* sp. based on its morphological characteristics. *Rhabditis* sp. was maintained on PDA slants at 24°C in the dark and re-cultured every 30 days. All nematodes were identified based on the “Pictorial key to genera of plant parasitic nematodes”⁽¹⁰⁾.

Irradiation of nematodes in tubes

Nematodes (n=500) were placed in 0.5 ml sterile water in a 1.5 ml centrifuge tube. Two different series, each containing three doses of irradiation, were applied to determine the effects on nematode

TABLE 1. The sources of plant-parasitic nematodes used in this study

Common name	Scientific name	Instar	Host	Origin
Southern root-knot nematode	<i>Meloidogyne incognita</i>	Second-stage juvenile	Tomato	Chiayi, Minxiong
Southern root lesion nematode	<i>Pratylenchus coffeae</i>	All instar	Yam	Nantou, Mingjian
Pine wood nematode	<i>Bursaphelenchus xylophilus</i>	All instar	Japanese black pine	Yangmingshan
Leaf and bud nematode	<i>Aphelenchoides</i> spp.	All instar	Import sphagnum	Import
Burrowing nematode	<i>Radopholus similis</i>	All instar	Import sphagnum	Import
Free-living nematode	<i>Rhabditis</i> spp.	All instar	Import sphagnum	Import

mortality. Nematodes were exposed to gamma irradiation (Cobalt-60 source of 4943 curies) by consecutively applying to give doses of 0.5, 1, 2, 4, and 6 kGy. After irradiation, nematodes were incubated for 7 days and examined microscopically to determine their viability and fertility. Nematodes showing motionless after poking with a probe were assumed dead. Nematodes without irradiation were used as no treatment controls. The effect of radiation on nematode fertility was evaluated by transferring 50 nematodes after being radiated to a PDA slant or plate covered with *Alternaria* mycelium. Assays for fertility were conducted three times. After being incubated for two months, a total number of nematodes was determined. Unless otherwise indicated, each treatment contained three replicates, and experiments were repeated two times. Irradiation was conducted at the Institute of Nuclear Engineering and Science, National Tsing Hua University (Hsinchu City, Taiwan). One unit of radiation dose (Gray, Gy) was calculated by the formula: $D = dE/dm$, where D is the Roentgen Absorbed Dose (rad), dE is the radiation energy (joule), and dm is the matter mass (kg). 1 Gy=0.001 kGy.

Irradiation through cultivation medium

In total, eight different cultivation media including Sphagnum moss, peat moss, snake wood (*Piratinera guianensis*) chips, perlite, vermiculite, foamed stone, coconut shell fiber, and bark imported mainly from Chile, Lithuania, Vietnam, and Sri Lanka were found in Taiwan wholesale flower market and tested. Coconut shell fiber and peat moss were sterilized (121°C, 15 psi) by an autoclave for 20 min. Other materials (30 x 30 x 20 cm³) were sterilized by heating in an 85°C water bath for 30 min and dried in an acrylic box for 24 h. *Aphelenchoides* sp. or *Rhabditis* sp. nematodes (n=500) were introduced into the center of a sterilized medium through a syringe, dried, and exposed to gamma-irradiation for varying periods to give 4, 5, and 6 kGy. After irradiation, nematodes were purified

by the Baermann funnel method⁽¹⁷⁾, incubated for 7 days, and examined microscopically to determine their viability. Irradiation was conducted in the China Biotech Corp., Taichung Industry Park (Taichung City, Taiwan). Each treatment contained three replicates and experiments were repeated two times.

Effect of Sphagnum moss after radiation on the growth and flower quality of orchid plants

Sphagnum moss after being radiated (4, 5, and 6 kGy) was soaked in water and used to cultivate *Phalaenopsis* Sogo Yukidian ukid V3 in a 10.5 cm transparent plastic basin (vol. 750 ml). *Phalaenopsis* plants with 6 mature leaves (35-40 cm width) were maintained in an air-conditioned room (20°C) for 30 days to stimulate the formation of the flower spikes and transferred to a greenhouse located at National Chung Hsing University (Taichung, Taiwan). The length of the stalks and the size and shape of the flowers were measured 60 days after transplanting.

Statistical analysis

Each treatment contained three biological replicates and experiments were repeated two times. The variance between treatments was analyzed using Fisher's least significant difference (LSD) test ($p < 0.05$).

RESULTS

Effect of radiation on mortality of plant-associated nematodes in tubes

In-vitro trials revealed that high dosages (6 kGy) of gamma radiation were highly effective in killing nematodes (Table 2). All six test nematodes include *Meloidogyne incognita* (root-knot nematode), *Radopholus similis* (Cobb) Thorne

TABLE 2. Effect of different dosages of radiation exposure on mortality of various plant-parasitic nematodes and free-living nematodes

	M ¹	P	B	A	R	F
Dosage of radiation exposure						
6 kGy	100.00 a ²	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
4 kGy	100.00 a	89.65 b	100.00 a	100.00 a	100.00 a	30.61 b
2 kGy	28.12 b	26.69 c	100.00 a	97.28 a	97.43 a	19.48 cd
1 kGy	8.73 c	26.56 c	90.50 b	100.00 a	28.23 b	8.80 e
0.5 kGy	8.56 c	24.33 c	89.90 c	86.90 b	33.43 b	10.36 ed
Check (No treatment)	6.82 c	17.02 d	11.91 d	24.76 c	35.60 b	25.25 bc
LSD _{0.05}	9.2795	6.32	3.1558	10.228	18.91	10.005

¹ M=Southern root-knot nematode, P=Southern root-lesion nematode, B=Pine wood nematode, A=Leaf and bud nematode, R=Burrowing nematode, F=Free-living nematode.

² Means within a column followed by the same letter are not significantly different at the 5% level by the LSD test.

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(burrowing nematode), *Pratylenchus coffeae* (root-lesion nematode), *Bursaphelenchus xylophilus* (pine wood nematode), *Aphelenchoides* sp. (leaf and bud nematode), and *Rhabditis* sp. (free-living nematode) were completely killed after exposure to 6 kGy gamma radiation in two different experiments. After exposure to 4 kGy, all test nematodes except *P. coffeae* and *Rhabditis* sp. were also completely dead. Both *B. xylophilus* and *Aphelenchoides* sp. showed more than 87% mortality rates after exposure to 0.5 kGy. *Rhabditis* sp., *Pratylenchus coffeae*, and *M. incognita* had less than 30% mortality rates after exposure to 2 kGy and lower gamma radiation.

Effect of radiation of plant-associated nematodes in cultivation media

Aphelenchoides sp. (leaf and bud nematode) and *Rhabditis* sp. (free-living nematode) were commonly isolated from cultivation media imported from foreign countries to Taiwan. The two species were used as targets to determine whether or not gamma radiation could effectively eliminate nematodes from the cultivation medium. No *Aphelenchoides* sp. embedded in peat moss, coconut shell fiber, or Sphagnum moss survived after exposure to 5 kGy or higher gamma radiation (Table 3). Exposure to 4 kGy was also effective in killing *Aphelenchoides* sp. implanted in three test media, resulting in less than 10% survival rates. External exposures to gamma radiation were also very effective in killing *Rhabditis* sp., even though

Rhabditis sp. was relatively less sensitive than *Aphelenchoides* sp. Similar results were observed in both test nematodes embedded in other cultivation media, including snakewood chips, perlite, vermiculite, foamed stone, and bark (data not shown).

Gamma radiation impacts nematode fertility

After radiation, *Aphelenchoides* sp. and *Rhabditis* sp. were incubated on PDA covering with *Alternaria* sp. mycelium for two months. The results revealed that exposures to 5 kGy or higher suppressed nematode reproduction (Table 4). External exposures to 4 kGy and below had little or no effect on nematode reproduction.

Gamma radiation has no impact on the quality of the cultivation medium

Sphagnum moss after being exposed to 4 kGy or higher was used to culture *Phalaenopsis* orchid plants. The length of full spikes, flower width, and total number of flowers per plant were evaluated 60 days after transplanting. The results indicated that orchid plants grown in gamma radiation-treated Sphagnum moss had slightly but not significantly shorter spikes and fewer flowers in comparison with those grown on Sphagnum moss with no radiation treatment (Table 5). All test orchids showed similar flower widths.

TABLE 3. Effect of different dosages of radiation exposure on survival of plant-parasitic nematodes and free-living nematodes in different imported culture media

	Peat moss	Coconut shell fiber	Sphagnum moss
Leaf and Bud nematode			
Treatment			
6 kGy	0 c ¹	0 c	0 c
5 kGy	0 c	0 c	0 c
4 kGy	7.1 b	8.5 b	3.2 b
Check(no treatment)	32.1 a	32.0 a	11.9 a
LSD	3.20	5.37	1.30
Free-living nematode			
Treatment			
6 kGy	0 c	0 c	0 b
5 kGy	6.6 c	0 c	0 b
4 kGy	18.2 b	22.6 b	29.1 a
Check(no treatment)	51.8 a	42.0 a	32.4 a
LSD	6.73	6.05	7.24

¹ Means within a column followed by the same letter are not significantly different at the 5% level by the LSD test.

TABLE 4. Effect of different dosages of radiation exposure on survival of *Aphelenchoides* sp. and *Rhabditis* sp. culturing on fungal mycelium

Test	1	2	3	4	5	6	7	8	9	10
Leaf and Bud nematode										
Treatment										
6 kGy	— ¹	—	—	—	—	—	—	—	—	—
5 kGy	—	—	—	—	—	—	—	—	—	—
4 kGy	+	+	+	+	+	+	+	+	—	+
3 kGy	+	+	+	+	+	+	—	+	+	+
2 kGy	+	+	+	+	+	+	+	+	+	+
1 kGy	+	+	+	+	+	+	+	+	+	+
Check(no treatment)	+	+	+	+	+	+	+	+	+	+
Free-living nematode										
Treatment										
6 kGy	—	—	—	—	—	—	—	—	—	—
5 kGy	—	—	—	—	—	—	—	—	—	—
4 kGy	+	+	—	+	—	+	+	—	—	+
3 kGy	+	+	+	+	+	+	+	+	+	+
2 kGy	+	—	—	—	+	+	+	+	+	+
1 kGy	+	+	+	+	+	+	+	+	+	+
Check(no treatment)	+	+	+	+	+	+	+	+	+	+

¹ “+” means the presence of nematodes. “—” means no nematodes.

TABLE 5. Effect of flowering quality of *Phalaenopsis* Sogo Yukidian ukid V3 on Sphagnum moss treated by different dosages of radiation exposure

	Full Spike length (cm)	Flower width (cm)	Total flower number
Sphagnum moss			
Treatment			
6 kGy	68.9 b	11.3 a	9.6 c
5 kGy	70.8 ab	11.6 a	9.7 bc
4 kGy	70.7 ab	11.5 a	10.3 ab
Check(no treatment)	72.6 a	11.9 a	10.6 a
LSD	2.84	0.84	0.63

¹ Means within a column followed by the same letter are not significantly different at the 5% level by the LSD test.

DISCUSSION

To prevent the invasion of specific diseases, pests, and diseases, quarantine and treatment of plant diseases and insect pests are often required for the import and export of agricultural products from one country to another. Of all the available sanitation practices, phytosanitary irradiation is one of the most effective and feasible methods to kill insects and plant pathogens in the port quarantine^(5, 8, 9, 13). Studies of the effect of radiation on nematodes have revealed that the tolerance of plant nematodes to radiation doses is very different depending on nematode species^(11, 12, 15, 16). For example, 0.3 kGy can completely inhibit the reproduction of the reniform nematode (*Rotylenchulus reniformis*)⁽¹³⁾, and 0.37 kGy can inhibit the reproduction of the foliar nematodes (*Aphelenchoides* spp.) and the root rot nematode (*Pratylenchus vulnus*)^(12, 15). In contrast, 7.5 kGy is required to completely kill the root rot nematode and the root knot nematode (*Meloidogyne javanica*)⁽³⁾. In addition, different radiation treatment methods, sources of radiation, different instars of target nematodes, and the types of cultivation medium could have a profound impact on the effectiveness of radiation on nematode mortality rates. Moreover, the radiation doses required to kill or inhibit the reproduction of plant pathogenic nematodes could also vary considerably among different nematode species⁽⁴⁾.

All of the aforementioned factors should be considered while using radiation as a means to eradicate nematodes from the cultivation media in the port. Thus, experimental trials are required to establish an effective dose of radiation for a range of nematodes. This is particularly urgent for orchid growers in Taiwan as several nematode species have recently been detected from a wide range of cultivation media imported from other countries (unpublished data). Because orchid plants grown in a cultivation medium in Taiwan can

be exported to other countries, using untreated medium poses a great threat to the orchid industry if any nematode species are found in the cultivation medium. Thus, a highly effective treatment is also required for orchid export to ensure that the cultivation medium is nematode-free.

In the current study, we have tested the mortality and reproduction rates of several nematode species to gamma irradiation, also revealing a considerable divergence in the nematode radiation doses. Of six test nematode species, *Aphelenchoides* spp., which could be killed by 1 kGy, is the most susceptible to gamma irradiation. *B. xylophilus* was killed by 2 kGy; both *M. incognita* and *R. similis* were killed by 4 kGy. *P. coffeae* and *Rhabditis* spp. are relatively tolerant to gamma irradiation, requiring 6 kGy to kill either species. Thus, for any given nematode species, the minimum lethal dose (MLD) of radiation could not be predicted until after experimental tests are carried out. The MLD of radiation required to kill nematodes in the water might be very different from that required to kill nematodes in the soil or plant tissues, further complicating the use of radiation to kill nematodes in the cultivation medium. One would expect that the MLD required to kill nematodes in the soil or cultivation medium would be much higher due to the disturbance of the soil environment or plant tissues. As tests with *Rhabditis* sp., we have found that *Rhabditis* sp. present in sphagnum moss or coconut shell fiber could be completely killed by 5 kGy, whereas 6 kGy is required to kill the nematode present in peat moss. Moreover, we have also found that 5 kGy is required to completely kill *Aphelenchoides* sp. present in sphagnum moss, peat moss, or coconut shell fiber. The results support the notion that the type of cultivation medium will impact the MLD of radiation required to kill a nematode.

Overall, irradiation is a safe and environmentally friendly alternative. Our results revealed that the MLDs to kill nematodes embedded in a cultivation medium were around 6 kGy. After radiation, no adverse effects of the cultivation medium on orchid growth and flower quality were observed. Economic assessment may be required to determine whether or not the dose is economically feasible to eradicate nematodes from orchid cultivation media.

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摘要

吳秋燕、許晴情、周鳳英、顏志恒。2024。伽瑪輻射對於進口栽培介質內有害線蟲之滅除效果評估。植物醫學66(1_2): 1-6。

為了滅除進口栽培介質內有害線蟲之存在，準備五種重要植物病原線蟲，包括穿孔線蟲、根瘤線蟲、根腐線蟲、葉芽線蟲、松材線蟲及腐生性線蟲等，測試對於鈷 60 (^{60}Co) 伽馬射線照射的殺死效果。其試驗結果顯示每種測試線蟲對於輻射劑量的耐受性各不相同，而施加高劑量（4至6 kGy）的 ^{60}Co 則可以降低線蟲的繁殖力和提高其死亡率。而在所有測試線蟲中，葉芽線蟲屬於 ^{60}Co 最敏感，其次是松材線蟲，在暴露於 2 kGy 後，葉芽線蟲及松材線蟲就能被消滅。而根瘤線蟲和穿孔線蟲則要在 4 kGy 劑量才能被殺死，因此對輻射具有中等耐受性。另外根腐線蟲和腐生性線蟲則對 ^{60}Co 具有高度耐受性，需要達 6 kGy 才能殺死。而以輻射照射檢疫處理後之水苔作為蝴蝶蘭大苗之栽培介質，蝴蝶蘭表現出正常的生長及抽穗開花，其試驗結果與對照組比較並無顯著差異，因此試驗結果顯示輻照可能是一種安全且環保的殺死栽培介質中線蟲的方法。

關鍵詞：伽瑪輻射、線蟲、蘭花、檢疫、海港