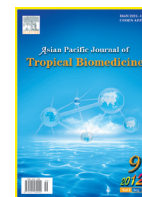




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Effect of germicidal UV–C light(254 nm) on eggs and adult of house dustmites, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Astigmata: Pyroglyphidae)

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ABSTRACT

Objective: To examine the immediate and 24 hours post– irradiation germicidal effects of UV–C lamp on eggs and adults of house dust mites *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) and *Dermatophagoides farinae* (*D. farinae*). **Methods:** This study investigated the immediate and 24 hours post irradiation mortalities of adult mites exposed to UV–C at different exposure times (5 mins, 10 mins, 15 mins, 20 mins, 30 mins and 60 mins) and distances (10 cm, 25 cm, 35 cm, 45 cm and 55 cm). Fresh eggs of the 2 dust mites were also irradiated at 10, 35 and 55 cm for 0.5, 1, 2, 3, and 5 minutes, and observed daily post– irradiation for up to 7 days. **Results:** Highest immediate mortality of 100% occurred with direct irradiation at 10 cm distance from UV–C lamp and for 60 mins, for both species of mites. The post 24 hours mean mortality rates were (58.4±17.4)% for *D. pteronyssinus* and (27.7±9.7)% for *D. farinae* when irradiated for 1 hour at 55 cm distance under UV–C lamp. When mites were irradiated in the presence of culture media, the highest mortality rates were lower compared to the direct irradiation; at 10 cm distance and 60 mins exposure, the mean mortality was (74.0±6.8)% for *D. pteronyssinus* and (70.3±6.7)% for *D. farinae*. Egg hatchability for both species of mites was also notably reduced by greater than 50% following irradiation. **Conclusions:** Ultraviolet C irradiation is lethal to an array of organisms by damaging their nucleic acids (DNA and RNA). This study demonstrates the increasing mite mortalities with increasing exposure times and decreasing distances.

1. Introduction

House dust mites (HDM) are found in most homes. They are microscopic, eight–legged creatures closely associated with us, but they are not parasitic and do not bite. The concern about HDM is that some species produce allergens affecting humans. The HDM allergens cause allergic symptoms such as asthma and atopic dermatitis in atopic humans. A number of the allergen producing HDM belongs to the family Pyroglyphidae. Pyroglyphid mites usually account for >90% of the mite population in houses[1]. *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* are considered among the most important pyroglyphid mites because of their cosmopolitan occurrence and abundance in homes[2].

There are various approaches for the control of house dust mite and their allergen such as by reducing indoor

relative humidity to below 50%, coupled with regular cleaning and use of encasement on mattress and pillows[3]. Several chemicals have been examined in laboratories but their effectiveness in the home is controversial or even if effective, they have not been commercialized for home use because they have potential problems of toxicity to non targets such as humans and pets, produce unpleasant odor, damage household items, and unable to penetrate deeply into carpet and upholstery[4]. Physical strategies like irradiation has become an established technique for controlling aeroallergens because of residue free advantages over chemicals[5]. UV irradiation is widely used as a germicide and as an attractant for insects[6], in embryological physiological studies and for the surface disinfection of insect eggs[7]. Wharton[8] reported that UV irradiation (254 nm) killed nymphs of the American cockroach, *Periplaneta americana*. A number of other investigators also have considered the possibility of using UV rays to control, or at least to suppress development of various aeroallergens and insects[5,9]. Ultraviolet light is known to damage or kill living organisms because it will destroy the DNA by forming covalent

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bonds between certain adjacent bases in the DNA, thereby preventing further replication and growth.

Ultraviolet C (UV-C) is a short wavelength (100–280 nm) radiation and is primarily used for the disinfection of air, surfaces and liquids from microbial contaminants^[10]. To date the UV-C is the wavelength in germicidal applications and is also recommended by the Centre for Disease Control and Prevention. Ultraviolet light air purification has been used for years by the medical field to sanitize rooms and equipment in order to prevent the spread of illness and disease. High intensity UV light modifies proteins as well, so it is possible that UV light might render an allergen non-allergenic. The efficacy of UV-C had been previously demonstrated against some stored product beetle and mite pests^[11–14] with sensitivity varying with species and doses. It is, however, difficult to make direct comparisons between studies as the level of UV dose achieved is not always stated and UV intensities vary with light sources. Long lists of bacteria, viruses and moulds also are often quoted to assert the killing power of UV-C. The implication that goes with those long lists is often made that UV-C will be just as effective on HDM.

The aim of this study is to investigate the mortalities induced by UV-C irradiation on eggs and adults of 2 species of HDM, *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) and *Dermatophagoides farinae* (*D. farinae*).

2. Materials and methods

2.1. Sources of mites

Adult males and females *D. pteronyssinus* and *D. farinae*, and their eggs, were obtained from colonies established since 1960 in the Acarology Unit, Institute for Medical Research (IMR), Malaysia. The colonies are reared in small glass bottles and sterile ground rat chow mixed with fish flake is used as culture medium. All bottles are kept in desiccators at (75±3) % relative humidity (RH) and at an average room temperature of (25±2) °C.

2.2. UV-C radiation source

The radiation source was a 30 watts UV germicidal lamp (G30T8, Sankyo Denki, Japan) measuring 88 cm x 2.5 cm, and emitting radiation at a wavelength of 254 nm. The lamp was fixed to the ceiling of a Laminar Flow cabinet (120 cm x 63 cm x 50 cm) that served as a test chamber; another similar cabinet without the lamp was used for controls. Bioassays were conducted at a room temperature of (25±2) °C.

2.3. Bioassay with adult mites for direct exposure

Sets of 30, 15 – 25 days old adult mites of mixed male and female were placed in Petri dishes of 14 cm diameter and 1.5 cm high. The dishes and mites were next placed inside the UV-C chamber and irradiated for different times (5, 10, 15, 20, 30 and 60 mins) and at different distances (10, 25, 35, 45 and 55 cm) from the UV lamp. Controls were similarly treated in the control chamber. Three replicates were tested and the procedure was repeated 3 times for each irradiation time and distance. The exposed mites were examined immediately

after irradiation under 400x magnifications and the number of dead mites was recorded. Mites that do not move when gently prodded were considered dead. Irradiation mites were maintained at (75 ± 3) % RH and (25 ± 2) °C and mortalities were determined again after 24 hours.

2.4. Bioassay with adult mites in presence of culture medium

Thirty 15 – 25 days old adult mites of mixed male and female were placed in clean glass Petri dishes, 14.0 cm diameter and 1.5 cm high along with 0.25 g of sterile culture medium. The Petri dishes were then placed inside the UV-C chamber and irradiated at different exposure times and distances as above for direct exposure. Controls were similarly prepared but placed in the control chamber. There were 3 replicates for each treatment and the test was repeated 3 times. The number of dead mites after irradiation was examined under 400x magnification and the immediately mortalities were recorded. Irradiated mites were maintained at (75±3)% RH and (25±2) °C and mortalities were determined again after 24 hours.

2.5. Bioassay with eggs

Ten freshly oviposited eggs were collected using fine applicator sticks and placed in glass Petri dishes, 9.0 cm diameter and 1.2 cm high. The Petri dishes with eggs were placed inside the test chamber and irradiated for 0.5, 1, 2, 3 and 5 mins at distances of 10, 35, and 55 cm, from the UV lamp. Control eggs were similarly irradiated in the control chamber. After treatment, eggs were placed individually in clear glass vials measuring 3.5 cm high and 2.0 cm diameter that were secured with snap caps. The eggs were maintained at (75±3)% RH and (25±2) °C; hatchability was monitored daily for a week. All treatments were replicated 3 times, and the experiment repeated once.

2.6. Data and statistical analysis

Mean mortalities were compared and analyzed by independent sample *t*-test and one-way ANOVA at 95% confidence level using SPSS ver 11.0^[15].

3. Results

3.1. Immediate mortalities of *D. pteronyssinus* and *D. farinae* for direct irradiation

Mortality rates for direct irradiation of *D. pteronyssinus* and *D. farinae* at difference exposure times and distances are shown in Table 1. No control mites died. Generally, mortality rates for both species, increased with increasing exposure times and decreasing distances. At 10 cm distance from lamp and 60 minutes exposure, 100% mortality resulted in both species of mites. For similar exposure times at 55 cm distance from lamp, there was significant difference among species ($P<0.05$); the mean mortality rates were (32.5±8.9)% for *D. pteronyssinus* and (11.0 ± 9.8)% for *D. farinae*. Increasing the exposure period at each distance significantly increased

mortalities ($P < 0.01$) of both species. There was significant increase in *D. farinae* mortalities at each exposure period with decreasing distances ($P < 0.01$); similar with *D. pteronyssinus* ($P < 0.03$) except at 15 minutes exposure where the differences were not significant ($P = 0.16$).

3.2. 24 hours post irradiation mortalities of *D. pteronyssinus* and *D. farinae* for direct exposure

D. pteronyssinus and *D. farinae* mortalities at all distances were significantly different between exposure times ($P < 0.05$) (Table 2); 5 minutes exposure caused the lowest mortalities. At each exposure time, there were significant differences in *D. pteronyssinus* mortalities between various distance ($P < 0.01$), however the difference were not significant for 15 minutes exposure times ($P = 0.34$). For *D. farinae* mortalities, there were significant difference for all exposure time ($P < 0.03$). At 60 minutes exposure time and 55 cm distance, the mean mortality rates by species were significantly different from each other ($P = 0.001$).

3.3. Immediate mortalities of *D. pteronyssinus* and *D. farinae* in presence of culture media

Overall the mean mortality rates after exposure to UV-C in the presence of culture media for both mites were lower compared to the direct exposure. It is shown in Table 3 that mortalities increased with decreasing distance from

the UV-C lamp. There was no death in the controls. At the highest exposure time of 60 min and 10 cm distance, there was no significant difference among species ($P = 0.97$); the mean mortality rate were $(74.0 \pm 6.8)\%$ for *D. pteronyssinus* and $(70.3 \pm 6.7)\%$ for *D. farinae*. At the same exposure time but 55 cm distance, the mortalities between *D. pteronyssinus* and *D. farinae* were not significantly different ($P < 0.05$). Increasing the time exposed to UV-C at each distance significantly increased the mortalities of both species of mites ($P < 0.01$). Decreasing distance from the UV lamp at each exposure times significantly increased the mortalities of *D. pteronyssinus* ($P < 0.01$) but for *D. farinae* mortality significantly increased at each exposure ($P < 0.05$) except at 5 minutes ($P = 0.07$).

3.4. 24 hours post irradiation mortalities of *D. pteronyssinus* and *D. farinae* in presence of culture media

It is shown in Table 4 that *D. pteronyssinus* and *D. farinae* mortalities were significantly difference at all exposure times for various distance ($P < 0.01$). At each distance, there was significant differences in *D. pteronyssinus* and *D. farinae* mortalities between various exposure times ($P < 0.05$). However, at the 10 cm distance with the longest exposure time the mean mortality rates by species were not significantly different from each other ($P = 0.40$).

Table 1

Immediate mortalities and 24 hours post mortalities (% mean \pm SD) of *D. pteronyssinus* and *D. farinae* directly irradiated at difference distances and exposure times.

Species	Distance (cm)	Immediate mortalities						24 hours post mortalities					
		5 min	10 min	15 min	20 min	30 min	60 min	5 min	10 min	15 min	20 min	30 min	60 min
DP	55	0.0 \pm 0.0	2.2 \pm 2.8	4.0 \pm 4.0	5.5 \pm 5.5	8.8 \pm 5.5	32.5 \pm 8.9	1.8 \pm 2.3	7.0 \pm 3.8	9.2 \pm 6.8	12.9 \pm 12.2	13.6 \pm 8.5	58.4 \pm 17.4
	45	0.3 \pm 1.1	4.7 \pm 4.4	5.5 \pm 4.0	11.0 \pm 7.0	38.1 \pm 9.7	38.4 \pm 9.8	2.2 \pm 2.8	11.8 \pm 4.7	10.7 \pm 2.7	15.8 \pm 9.8	48.1 \pm 13.5	68.1 \pm 12.3
	35	2.2 \pm 1.6	6.2 \pm 4.2	7.7 \pm 5.9	12.9 \pm 4.2	55.8 \pm 9.6	74.7 \pm 9.7	4.7 \pm 2.3	9.2 \pm 6.8	12.2 \pm 8.9	18.1 \pm 5.0	69.9 \pm 7.9	98.8 \pm 2.3
	25	4.4 \pm 2.8	8.4 \pm 4.1	8.4 \pm 5.2	27.0 \pm 9.6	69.2 \pm 9.5	97.0 \pm 4.2	7.3 \pm 5.2	11.8 \pm 5.2	15.5 \pm 10.4	41.8 \pm 12.4	80.3 \pm 12.4	100.0 \pm 0.0
	10	4.4 \pm 3.3	8.8 \pm 7.6	11.0 \pm 9.7	35.9 \pm 9.3	88.8 \pm 9.6	100 \pm 0.0	8.8 \pm 5.2	15.5 \pm 6.6	16.6 \pm 11.7	54.0 \pm 17.2	96.6 \pm 4.4	100.0 \pm 0.0
DF	55	0.0 \pm 0.0	1.1 \pm 2.3	1.8 \pm 2.3	1.1 \pm 1.6	5.9 \pm 4.6	11.0 \pm 9.8	2.2 \pm 2.3	6.2 \pm 3.5	8.4 \pm 5.0	8.5 \pm 4.4	8.6 \pm 6.4	27.2 \pm 9.7
	45	0.3 \pm 1.1	1.4 \pm 3.3	2.2 \pm 3.3	2.9 \pm 3.5	6.2 \pm 3.5	16.6 \pm 9.9	2.5 \pm 3.2	6.9 \pm 5.1	9.6 \pm 2.6	13.6 \pm 6.9	9.9 \pm 6.6	26.2 \pm 12.3
	35	0.7 \pm 1.4	1.8 \pm 2.3	4.0 \pm 2.2	19.2 \pm 8.2	44.0 \pm 8.6	57.3 \pm 10.5	6.2 \pm 3.1	7.0 \pm 5.6	10.3 \pm 3.8	28.8 \pm 11.0	52.9 \pm 8.4	86.9 \pm 9.3
	25	3.3 \pm 1.6	4.7 \pm 3.3	6.6 \pm 2.3	19.6 \pm 7.8	49.6 \pm 10.9	90.3 \pm 10.1	8.4 \pm 2.9	10.3 \pm 5.3	14.0 \pm 4.9	29.2 \pm 15.0	79.2 \pm 14.1	99.6 \pm 1.1
	10	4.4 \pm 3.3	5.5 \pm 3.3	7.7 \pm 1.7	39.2 \pm 9.8	72.9 \pm 10.9	100.0 \pm 0.0	8.4 \pm 3.7	12.9 \pm 4.8	14.0 \pm 3.6	47.3 \pm 11.4	85.1 \pm 14.7	100.0 \pm 0.0

DP– *D. pteronyssinus*, DF– *D. farinae*.

Table 3

Immediate mortality rates (mean \pm SD) of *D. pteronyssinus* and *D. farinae* in the presence of culture media at different exposure period and distances from UV-C lamp.

Species	Distance (cm)	Exposure time (minutes)					
		5	10	15	20	30	60
<i>D. pteronyssinus</i>	55	0.0 \pm 0.0	0.3 \pm 1.1	1.1 \pm 1.6	1.8 \pm 2.3	2.9 \pm 1.9	12.1 \pm 6.4
	45	0.0 \pm 0.0	0.7 \pm 1.4	1.1 \pm 1.6	5.5 \pm 4.6	5.5 \pm 2.3	26.6 \pm 8.9
	35	1.1 \pm 1.6	2.9 \pm 2.5	4.7 \pm 3.3	10.7 \pm 4.0	22.5 \pm 7.2	37.0 \pm 8.8
	25	2.5 \pm 2.7	3.6 \pm 3.5	7.0 \pm 4.5	17.7 \pm 4.4	40.3 \pm 6.3	69.9 \pm 10.0
	10	2.5 \pm 2.7	4.7 \pm 3.3	7.7 \pm 2.3	31.0 \pm 8.1	64.0 \pm 5.4	74.0 \pm 6.8
<i>D. farinae</i>	55	0.0 \pm 0.0	0.0 \pm 0.0	1.1 \pm 1.6	0.7 \pm 1.4	4.0 \pm 3.2	6.9 \pm 3.8
	45	0.0 \pm 0.0	0.3 \pm 1.1	1.1 \pm 1.6	2.5 \pm 2.2	4.7 \pm 2.9	19.5 \pm 7.1
	35	0.7 \pm 1.4	2.2 \pm 2.8	2.9 \pm 3.0	9.2 \pm 4.6	22.9 \pm 8.2	30.7 \pm 3.2
	25	1.8 \pm 2.9	1.4 \pm 1.7	4.4 \pm 3.7	17.3 \pm 4.3	36.6 \pm 6.2	69.9 \pm 10.0
	10	1.8 \pm 2.3	4.0 \pm 4.0	6.2 \pm 3.5	34.0 \pm 10.8	62.5 \pm 4.9	70.3 \pm 6.7

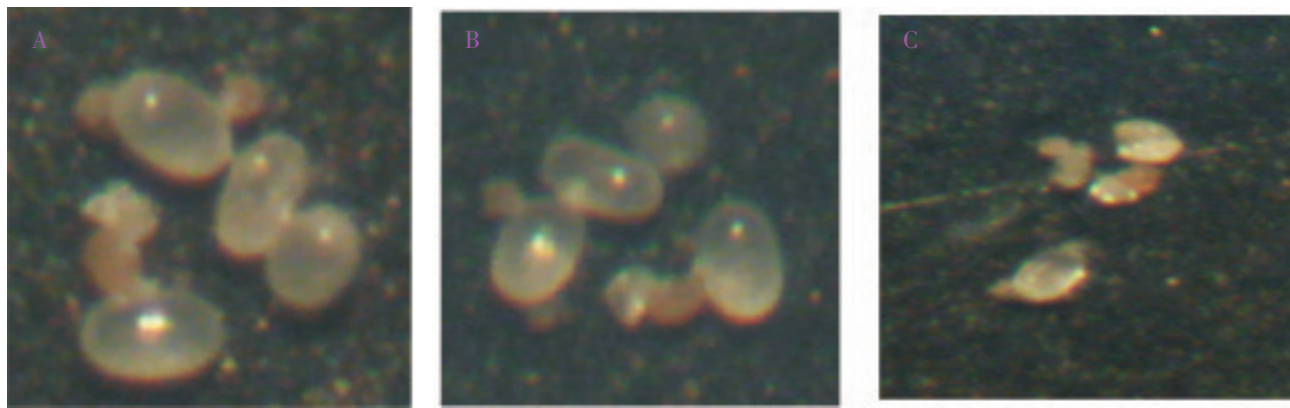


Figure 1. Microscopic examination of UV-C irradiated eggs. A: before irradiation; B: immediately after 5 minutes irradiation; C: on the third day post irradiation.

Table 4
Hatchability (%) of controls and eggs exposed to UV-C for 5 minutes.

Species	Distance (cm)	Eggs	Days post irradiation						
			1	2	3	4	5	6	7
<i>D. pteronyssinus</i>	55	Control	0	0	24	60	76	84	88
		Irradiated	0	0	0	0	0	0	0
	35	Control	0	0	0	30	40	54	82
		Irradiated	0	0	0	0	0	0	0
	10	Control	0	4	14	32	52	68	74
		Irradiated	0	0	0	0	0	0	0
<i>D. farinae</i>	55	Control	0	0	28	56	84	96	96
		Irradiated	0	0	0	0	0	0	0
	35	Control	0	4	4	24	42	52	58
		Irradiated	0	0	0	0	0	0	0
	10	Control	0	10	32	46	52	52	60
		Irradiated	0	0	0	0	0	0	0

3.5. Egg hatchability

No eggs hatched when exposed to UV-C radiation at any of the exposure times and distances; in comparison, >70% of the control eggs hatched. Most (88%) of the treated eggs were dry on day 3 post irradiation. Microscopic examination immediately after irradiation showed no difference with controls and irradiated eggs that fail to hatch; however day 3 post irradiation, treated eggs were wrinkled and dry (Figure 1). This is probably due to leakage of the inner contents when the chorions of treated egg wrinkled. For *D. farinae*, 122 out of 150 (71.3%) control eggs were hatched while 81.3% of *D. pteronyssinus* control eggs were hatched into viable larvae (Table 4).

4. Discussion

The findings demonstrated UV-C radiation affected the egg stages more than the adult mites. It is possible for ultra-violet radiation to stop the development of house dust mites at early stage of its life cycle because none of the treated eggs hatched. This effect probably was due to the thinness of the chorion and the delicateness of the dust mite eggs in general. Needham *et al*[16] came out with similar results and concluded that UV-C has potential to break the life cycle of house dust mites by killing the embryonic stage thus stopping the production of allergens. The nature

of the UV-C effect on early developmental stages of mites is attributed to transmission of UV energy into the tissues. Provided it reaches the cells, UV-C radiation is able to impair cellular functions directly by damaging DNA, or indirectly by inducing increased formation of reactive free radicals leading to oxidative stress[17].

In the present study, the mortality of irradiated mites was directly proportional to the exposure times but indirectly with distances. Similar increase in adult mortality was reported by Faruki *et al*[5] working with UV-irradiated of *A. diaperinus*. This finding agreed also with the results of Faruki *et al*[14] working with the Almond Moth, *Cadra cautella* using UV rays mentioned that larval mortality was positively correlated with radiation exposure periods. Begum *et al*[18] also conducting a similar study with darkling beetle and reported that 100% reduction in the population which was exposed to UV-irradiation at certain times. Germicidal effect of UV-C also would be lethal to mites because of their small size; i.e., their body surface area per weight is large thus accelerate rate of UV absorption and dispersion of damage[19].

Our results also show there was significant difference in immediate and 24 hours post irradiation mortalities between *D. pteronyssinus* and *D. farinae* when directly irradiated at longest distance and times. This would suggest that UV-C treatment may have affected the mites quickly and thus killed them immediately. The recovery of mites from irradiation effect was very slow in most cases, which agrees well with other studies[20]. In case of post 24 hours mortality for the same treatment, the mean mortality rates of *D. pteronyssinus* were significantly higher than *D. farinae*; it could be due to *D. farinae* mites more resistance to UV radiation or *D. pteronyssinus* has been in a poorer condition to begin with.

Although UV-C radiation kill dust mites, its application to control of such house dust mites population in their natural habitat such as mattresses or carpet, is not yet practical because it requires a prolonged exposure and has inherent operational drawbacks which are likely to influence its accuracy[21]. One possible application is combating pest infestations associated with the structure of a building and may serve as a potential new hygiene measure[10,22]. The limited penetration however precludes its use as a treatment on bulk commodities. It may also be able to offer potential as a surface hygiene in empty stores. The lack of irradiation effect on lower mortality when food was present

in this finding demonstrates the limited penetration of UV-C through substrates. Adult mites and eggs concealed in carpeting and dense fabrics may escape exposure to the UV-C light radiation altogether. House dust mites are found most of the time inside mattresses or carpet since the microclimate and dampness are often highly favorable to mites. In order for UV-C treatment to be fully effective, the mites must be directly exposed to the UV-C for the required duration; a shorter distance between the mites and the UV-C source is advantageous. Anything that can shield the mites from exposure, e.g., food particles, dust, debris will affect efficacy. Since mortality rates increase with increasing exposure period, irradiation for more than 1 hour may result in higher mortality rates. When radiation kills or suppress, dust mite colony will be minimal and allergen levels may kept below threshold. However, there is need a balance the effect on the mites with the effect on the store products or house materials since prolonged exposure to UV radiation may reduce the quality of the stored products.

The reduced egg hatching and adult mortality of HDM caused by UV-irradiation is promising from control point of view. It may be concluded that irradiation is a clean method to eliminate mite population and consequently allergen reduction. Practical applications of UV-C within the house environment may, therefore, lie in the treatment of structural and equipment surfaces such as conveyor systems^[10]. However, cleaning and weekly vacuuming of carpets and sofas in homes is an important consideration as the presence of particles may affect UV-C efficacy. Repeated UV-C irradiations are probably required because the shorter wavelength rays of UV-C can penetrate only surface of carpets and mattresses – a single UV-C exposure would not eliminate dust mites and its allergen located in deeper layers of mattresses. The cost and safety implications of UV-C irradiation should also be considered and more comprehensive research is needed.

Conflict of interest statement

We declare that we have no conflict of interest.

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