# Acaricidal Activity of UV-C irradiation on *Tetranychus urticae* and eggs using pulsed krypton fluoride excimer laser

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## Abstract

# Background

While pulsed UV-C light sources such as excimer lasers are used in emerging non-thermal food decontamination method, they also have a high potential for a wide range of other microbial decontamination applications. The acaricidal effect of an experimental UV-C irradiation device was assessed using two-spotted spider mite female adults *Tetranychus urticae* and eggs as a model.

# Methods

The UV-C light source was generated by a pulsed krypton fluoride (KrF) excimer laser operating at 248 nm emission wavelength. Pulse energy and pulse repetition rate were 5mJ, and up to 100Hz rate, respectively. Distance from light source to target was 150 mm with a target surface of 21.6 cm². The exposure time of the mite and fresh eggs varied from 1 to 4 minutes at a power of 300 mW. The post-irradiation acaricidal effects (mites' mortality) were assessed immediately and measured 24 h post-irradiation. Effects on the hatchability of irradiated eggs were observed daily post-irradiation for up to 12 days.

# Results

The mortality of mites observed with an energy amount of 5 and 40 mJ/mm² was 26% and 90%, respectively. The mortality at 80 mJ/mm² reached 99%. The effect of exposure duration on mortality was minimal. The effect of irradiation of eggs hatchability was even more important, with about 100% mortality with as little as 5 mJ/mm² for each exposure time.

# Conclusions

A high mite mortality and lethal egg damage was already observed after less than 1 min exposure to UV-C pulsed irradiation. Pending further developments and feasibility studies, the current results and features of the UV-C generator (energy output modulation and applicability to varying spot sizes) open up a large field of germicidal applications requiring long-range radiation.


## Background

New control and decontamination strategy based on physical technologies is a promising way to increase microbial safety. The aim is to improve the control of foodborne pathogens [1, 2], the inactivation of food and environmental biowarfare agents [3, 4, 5] and even in arthropod pest control [6]. One of the main advantages of non-chemical inactivation procedures is to be eco-friendly microbiocidal methods which do not leave potentially toxic residues in the environment.

Among the physical technologies, non-ionising UV-C irradiation is increasingly used, alone or in combination with other cleaning and disinfection methods, in a wide range of applications including food industry and food safety [7, 8], agriculture [9], water decontamination [10, 11], indoor air and surfaces [12, 13, 14], healthcare environments [15, 16, 17, 18, 19], and personal protective equipment [20], the latter application being particularly assessed in the context of the COVID-19 pandemics and subsequent critical shortage of supply chain [21, 22].

Germicidal ultraviolet irradiation (GUVI) encompasses a continuous range of UV wavelengths between 200 to 280 nm. For this purpose, different types of lamps can be used, as reviewed by Bergman [23] and the IES Photobiology Committee [24]. The most common type of lamp is the low-pressure mercury lamp which emits mainly at 254 nm and has been used to disinfect both the room air and surfaces for decades. Pulsed xenon lamps emit very short pulses (of the order of milliseconds) of broad-spectrum white light including wavelengths in the ultraviolet and near infrared range. Pulsed light (PL) technology involves the application of intense light in the form of short pulses of high intensity on a target of interest with high penetration, emission capacity, and peak power distribution. PL has been shown to effectively inactivate microorganisms such as bacteria, yeasts, molds and viruses [25]. Accordingly, they are used as emerging non-thermal food processing method to decontaminate food products or food contact surfaces and packaging decontamination, but their use is not compatible with human presence. In contrast, excimer lamps are quasimonochromatic sources that emit over a wide range of the UV region depending on the gas used. KrF excimer lamps produce significant emission in the UV-C region in a narrow wavelength band around 222 nm. At this wavelength, effect on human skin is reduced, making possible GUVI application in occupied areas. UV-C light-emitting diodes (LEDs) produce a very narrow wavelength band of UV radiation with peak wavelengths at 265 nm, 273 nm, and 280 nm, among others. Compact size, portability, high efficiency, and long lifetime are balanced by their small surface area, heat dissipation issue, the requirement of a short distance to the target which limit their use in germicidal application.

In the current work, we assess the GUVI of an experimental pulsed UV-C light source based on a pulsed KrF excimer laser which operates at 248 nm emission wavelength with more than 5 mJ of pulse energy and up to 100Hz pulse repetition rate. At the laser output, the average power density of the laser radiation is ~ 8300 mW/cm$^2$. The model used was the two-spotted spider mite *T. urticae*. Indeed, this mite is a worldwide pest of many agricultural crops, including fruit, vegetables, ornamentals, and many other agronomic crops [26]. This model was also previously used to assess the killing efficacy of radiation exposure to UV-C illumination using a broad- spectrum-white LED (420–680 nm) and blue LED (460 nm) [27], or a commercial UV-C lamp with a peak UV output of ~ 254nm [28]. The immediate post-irradiation germicidal effects (mites' mortality and eggs hatchability) were assessed using fixed experimental parameters (distance from light source to target: 150 mm, total target surface: 21.6 cm²; power: 300 mW). The exposure time of the mite and eggs varied from 1 to 4 minutes.

# Methods

# Pulsed UV-C non-ionizing irradiation system

The UV-C radiation-dose system consists of several interconnected modules among which the laser and gas refill module, the beam shaping and beam delivery module, the laser energy meter used to assess the radiation levels at target site (Fig. 1) (Additional file: Figure S1). This system is piloted by an embedded computer software for the control of laser frequency and exposure duration, and automatic calculation of the ultraviolet dose received by the target according to specified beam size and beam power.

The laser source is a KrF excimer laser CEX-100 manufactured by LDI Innovation OÜ, Estonia. The excimer laser operates at 248 nm emission wavelength with more than 5mJ of pulse energy at 500 kW (5 mJ / 10 ns) and 10ns duration with up to 100 Hz pulse repetition rate (Additional file 2: Figure S2). The laser requires a built-in excimer container that is used as the gas refilling source.

For monitoring the refilling procedure, the KrF gas cylinder is equipped with a valve, a pressure gauge, a halogen filter for exhaust gas safety, and a vacuum pump enabling to exhaust the used laser gas from the laser chamber. Continuous operation requires a gas refill every 2 weeks. The refill procedure takes less than 10 minutes, a time during which the laser cannot operate.

The beam delivery and shaping module contains various cut-off apertures (1:1, 1:2, 1:6 and 1:10 of the initial beam power) and a removable diverging lens. The latter enables diverging the laser beam to provide a relatively even laser energy distribution on a larger surface area, in a controlled and measurable manner. Both laser and gas modules, and the laser energy meter are installed on a common mechanical frame.

The energy meter is based on a NIST-calibrated GreenTEG B05 thermopile power sensor, the output of which being amplified and displayed on an LCD screen. The energy meter components are the measurement head placed under the laser beam and the display part. Energy levels delivered to the target are calculated by the software. The latter enables controlling laser frequency and exposure time, and automatically calculating target delivered UV doses with pre-set beam size and beam power.

# Exposure set up

*Tetranychus urticae* were collected from infested plants in citrus orchards in Tunisia and transferred to a climate room (26°C, 50–60% RH, 16:8 (L:D)) in our laboratory at the Biodiversity Research Centre, UCL, Louvain-la-Neuve (Belgium) without any contact with acaricides before the experiments. The strain was reared on bean leaves placed on moistened cotton (*Phaseolus vulgaris)* in Petri dishes [26]. Synchronized individuals of known age are obtained by allowing adult females to lay eggs for a maximum of 24 h on a leaf (Fig. 2). After this time, the females are removed and the eggs continue to develop to the desired stage.

Ten adult females of *T. urticae* adult females were collected from leaves by using camel hair brush with the aid of stereomicroscope and transferred in aluminium chamber placed in a Petri-dish to flatten the leaf surface and prevent the two-spotted spider mites to escape the radiation beam (inner diameter of the

dish, 35 mm; inner dimensions of the rectangular chamber: 12 mm x 18 mm x 2 mm) (Fig. 3) (Additional file 3: Figure S3). Only young adult (24 h old) females were chosen for bioassay (Additional file 4: Figure S4).

# Survival study of mites and eggs after UV-irradiation

The effect of the UV-radiation on mites' mortality was assessed on adult female mites. For each laser exposure condition, the mortality rate was assessed as the ratio of the number of dead mites to the total number (n = 10) of mites used. The study consists in 5 independent experiments using fixed and variable parameters. Fixed parameters were the distance from light source to target (150 mm) resulting in a total target surface of 21.6 cm² and the power (300 mW). The variable parameters consisted in three exposure durations (60, 120 and 240 sec), and multiple laser frequency ranging as follows: from 4 to 60 Hz for 60s, from 2 to 30 Hz for 120s, and from 1 to 15 Hz for 240s. The combination of both variable parameters resulted in six amounts of energy per surface (5, 10, 20, 40, 60, 80 mJ/mm²), for each exposure duration.

Mortality curves were computed using a locally-weighted polynomial regression approach using the loess function of the R.3.6.1 software. The resulting mortalities were reported in three distinct curves showing exposure durations and mortalities per surface energy density on the surface.

The lethal effect of the UV-C irradiation was then assessed on eggs 24 h post-exposition by direct observation of egg hatching, using the same experimental design and up to 12 days post-irradiation. The mortality curves were generated from data obtained from exposed and unexposed eggs, the latter being used as a control. For the control, a surface of the leaf similar in size to was cut and put on the top of the leaf with eggs in order to cover it and protect eggs from the UV-C irradiation.

# Results

Immediate microscopic post-irradiation assessment who already visible effects on mite female adults as many of them stop moving. The effect of the UV-C irradiation on the mortality of mite female adults was quantified after 24 h. *T. urticae* was highly impacted by amount of energy delivered. The mortality observed with an energy amount of 5 and 40 mJ/mm² was 26% and 90%, respectively (Fig. 3). The mortality at 80 mJ/mm² reached 99%. The effect of exposure duration on mortality was minimal as depicted by the superposition of the three corresponding curves. The results confirmed that a reasonably high mortality can already be achieved after less than 1 min of exposure to the UV-C-irradiation produced by the laser beam.

The effect of irradiation on the mortality of eggs was even more important, reaching almost 100% mortality irrespective of the delivered energy (from 5 to 80 mJ/mm²) for each exposure time (Fig. 4). After UV-C irradiation, the eggs conserved their shape during the first few days but turned progressively yellow, dried and collapsed (Additional file 5: Figure S5).

# Discussion

UV germicidal inactivation (UVGI) is a disinfection method which involves killing and inactivation of microorganisms as a direct effect of UV exposure and absorption of high energy photons, especially for short wavelengths UV-C (200 to 280 nm) known as germicidal wavelengths. The rationale for using the 248 nm wavelength in the current study is the strongest light absorbance of nucleic acids around this wavelength [7]. The mechanism of cell alteration following the exposure to high energy photons is manifold. Firstly, UV-C irradiation causes photobiologic effects proportional to prolonged and repeated light exposure, i.e. to the amount of energy delivered to uni- and multicellular organisms [29]. When all photon energy is transferred to absorbing atoms and molecules, the resulting excited state may lead to proteins alterations and enzyme inactivation hampering key biologic systems, e.g. through the split of peptide, alteration of sulfide and disulfide bonds and photochemical oxidations [30, 31]. In the DNA, pyrimidine dimers and cross-linking between cytosine and thymine are considered the most important UV photoproducts formed between two adjacent pyrimidines in the same strand of DNA, resulting in the production of cyclobutane pyrimidine dimers which cause local denaturation of the DNA helix, prevention of DNA repair, inhibition of DNA replication and protein synthesis, RNA damage, loss of essential metabolic activities, and cellular death [7, 8]. Secondly, photons emitted by UV-C may induce oxidative processes through photooxidation reaction, depending on the number of incident photons and absorbed photons by molecules, varying according to their structure [7].

Information on the effects of UV radiation on *T. urticae* are scarce. While biological impact of UV-B (280–315 nm) radiation on spider-mite populations was recently reviewed [32], the use of UV-C was not considered. Nevertheless, this review commented on the sensitivity of spider mites to UV-radiation, underlying that *T. urticae* individuals remain mostly on the lower leaf surfaces as UV-protection mechanism, excepted during diapause when they seemed to be more protected by the synthesis of keto-carotenoid, and notably astaxanthin. Using a 300 W Xenon light source with band-pass filters, Sakai and Osakabe exposed eggs of *T. urticae* with a UV wavelength from 260 to 320 nm during one hour [33] and showed that eggs exposed to 280–300 nm radiation never hatched while hatchability was not affected at any other wavelength. In our case, eggs were particularly susceptible to the treatment as 100 % mortality was observed. In a more recent study, Short and colleagues used UV-C irradiation to control the *T. urticae* on whole plant on strawberries in small phytotronic units [28]. Infested plants that underwent nightly 60-s exposures at low doses (0.237 W/m²) showed a decreased *T. urticae* population by 97% over the entire plant, and by 99% in the middle and upper portions of the canopy compared to untreated plant. No phytotoxicity effect was observed. Moreover UV-C irradiated plant were not covered by the spider mite web whereas 65 % of the control plants were webbed. Albeit obtained using a different technological set up, their results regarding UV-C effects on *T. urticae* corroborate our findings and show clearly the potential of this new technology even if additional studies on the mechanisms of action and the conditions of implementation are still needed [28]. It is also clear that mite mortality and lethal egg damages are proportional the total amount of UV-C energy delivered in one minute. Similar results were obtained on the dust mites *Dermatophagoides pteronyssimus* and *D. farinae* using a 30 watts UV germicidal lamp measuring 88 cm x 2.5 cm, and emitting radiation at a wavelength of 254 nm in the UV-C

range but with longer time of irradiation ranging from 5 to 60 min [34]. The latter study clearly showed an increase in mortality that was proportional to time of exposure and proximity to the radiation source. Lah et al. observed 100 % mortality observed after 60 min at a 12 cm distance while eggs mortality was 100% irrespective of the distance and time of exposure compared to 70 % hatchability in eggs used as a control.

In our study, the ~ 100% mortality of two-spotted spider mites *T. urticae* and eggs after less than 1 min exposure to UV-C pulsed irradiation compares favourably with existing data. The experimental UV-C generator with pulsed KrF excimer laser has key features such as output modulation, applicability to small or bigger spot sizes and long-distance applications whenever needed. These features offer a great potential compared to other UV-C radiation methods [35, 36]. They indeed open up new control possibilities in fields as varied as food infestation by mites or the fight against red mites in poultry (*D. gallinae*). While being a challenge in terms of radiation efficacy and size of the surface to be decontaminated, the capacity to deliver UV-C radiation at long distance is mandatory for this application. Furthermore, choosing a robust excimer laser gives the researchers flexibility to use the laser system in experiments in most industrial settings, without potential issues in terms of vibrations or temperature conditions, though keeping in mind the potential synergistic associations with other decontamination thermal and non-thermal treatments [37], as well as the potential occurrence of UV-C induced resistance among microbial agents.

# Conclusions

The KrF Excimer laser is a robust and relatively cheap high-intensity deep-ultraviolet light source. As shown in this study with *T. urticae*, the mask-adapted beam shaping enables the users to target precisely prokaryotic or eukaryotic cells and expose e.g. biothreat and pest agents to the beam to kill them. The choice of a laser as the source of ultraviolet light enables repeatable beam shaping, comparison of thermally induced versus UV-induced damage, and long-range UV application. However, the use of this system for bio-control applications on distant and large surfaces must still be the subject of further development and research to better understand the mechanisms, and find the best application techniques and optimal set-up. In future perspectives, the focus will also be set on the germicidal effects of this UV-C generator on a range of microbial pathogens using various matrices. There is indeed a need to refine the field of applications and potential limitations, on extending the UV-C exposed area as well as on exploiting the capacity of laser system to irradiate at long distance and to be included in a portable handheld system.

# Abbreviations

KrF: Krypton Fluoride; PL: Pulsed light; UV: Ultraviolet; UV-B: Ultraviolet type B; UV-C: Ultraviolet type C; UVGI: Ultraviolet germicidal irradiation.

# Declarations

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## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable

## Availability of data and materials

The datasets supporting the findings of this article are included within the paper.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

JLG, SB, JA and TH conceived the study. TH provided *Tetranychus urticae* mites and eggs. TH carried out microscopic examination. TH, JA and JLG carried out the experimental work on mites and eggs mortality post-UV-C irradiation. . JA, ON and JLG wrote the first draft of the paper. JA, ON, JLG, SB and TH contributed to the final manuscript which they approve.

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# Figures

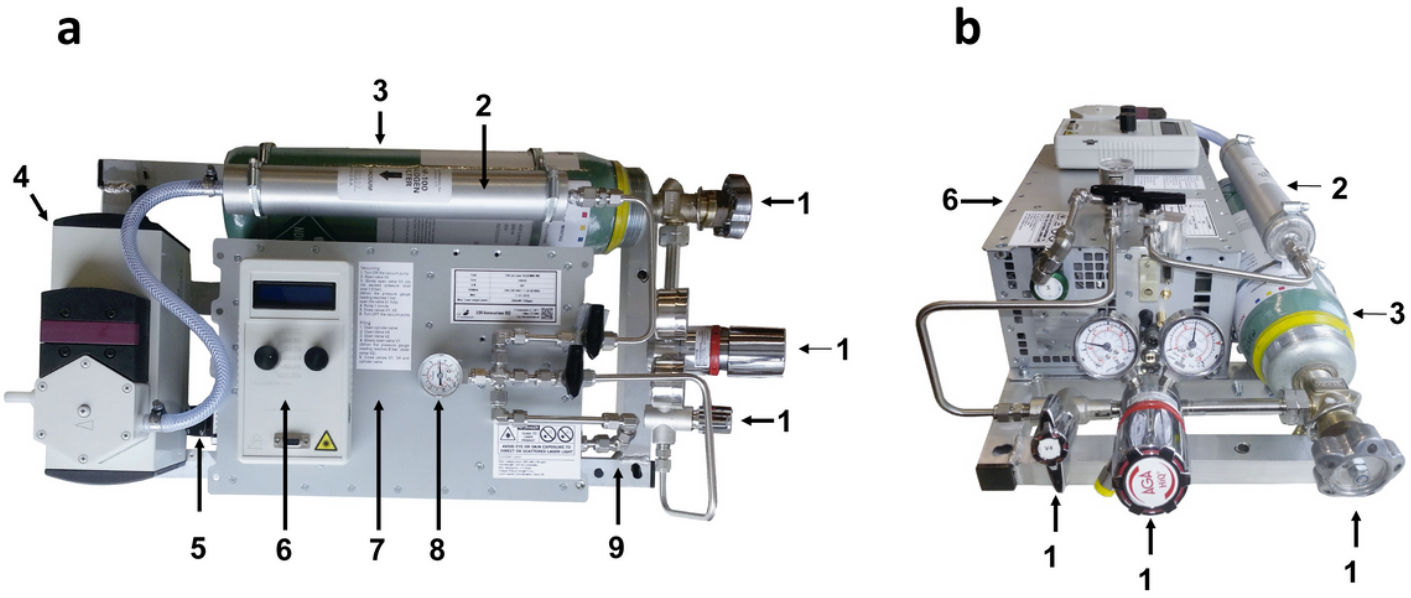
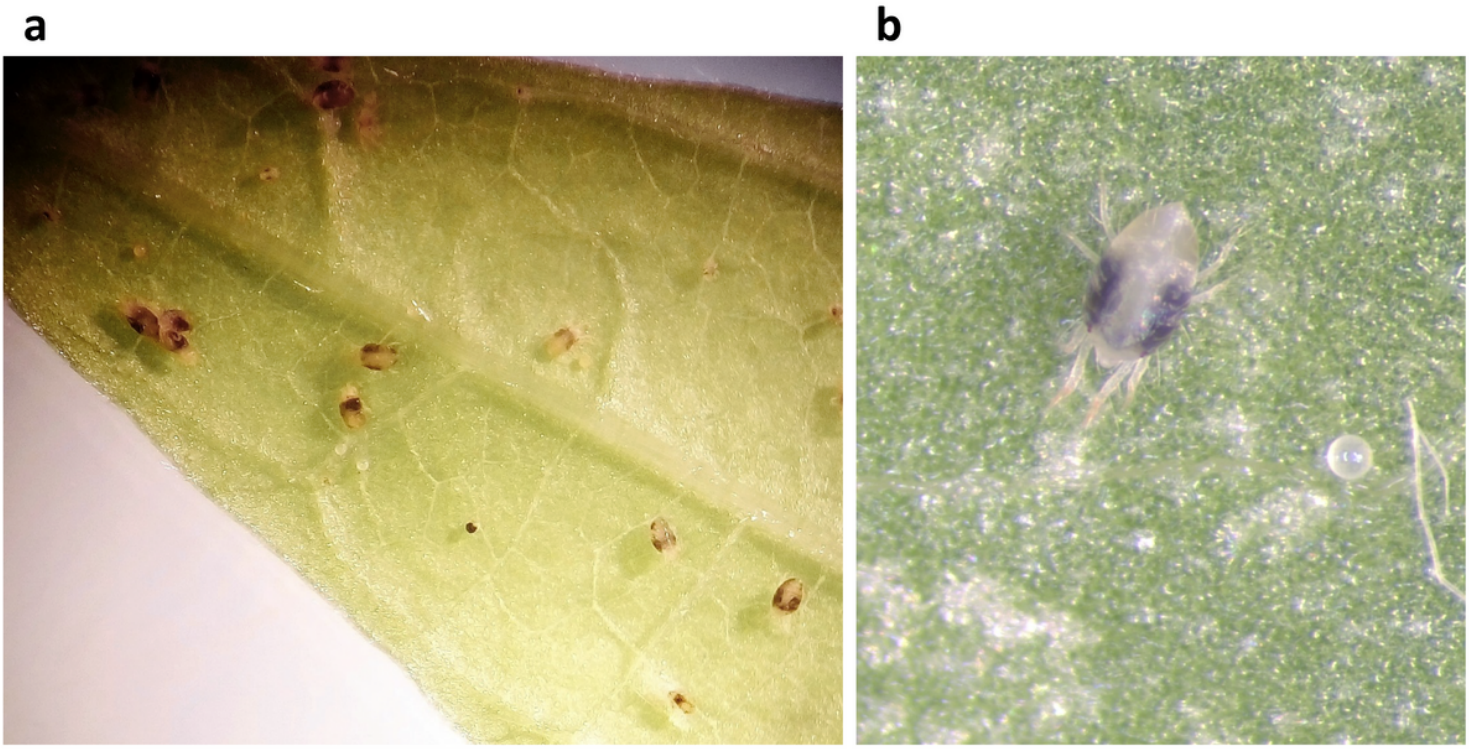



**Figure 1**

UV-C generator components on the top (a) and front views (b). Valves (1), halogen filter (2), krypton fluoride gas refill cylinder (3), vacuum pump (4), optical system (5), energy meter display (6), excimer laser CEX-100 (7), pressure gauge (8), mechanical frame (9)




## Figure 2

Leaf with two-spotted spider mites Tetranychus urticae (a) and egg (b)

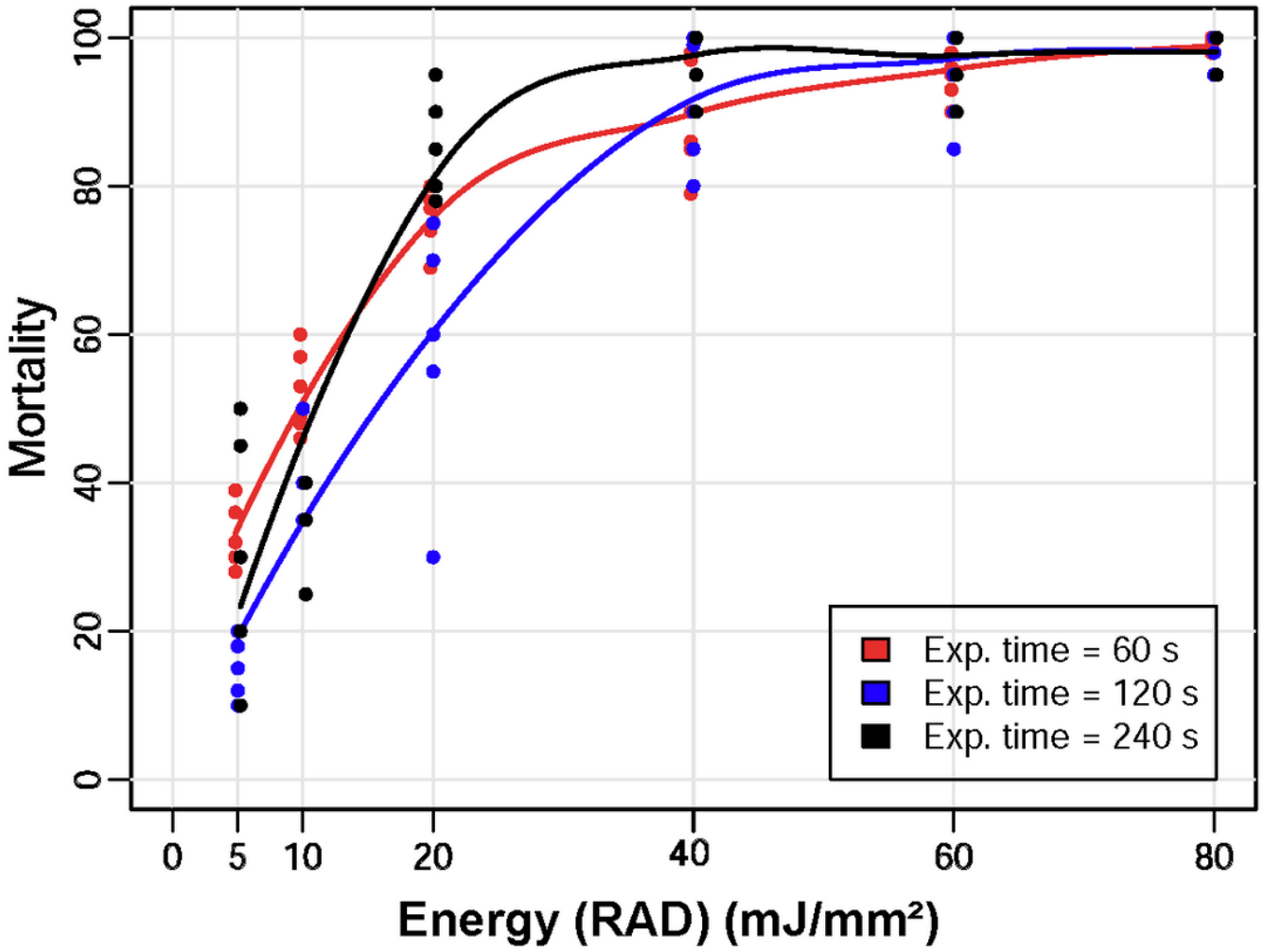



**Figure 3**

Relation between the energy delivered to adult mites and the mortality observed after irradiation at three different exposition times (60, 120, and 240 s) generated by the pulsed krypton fluoride excimer laser

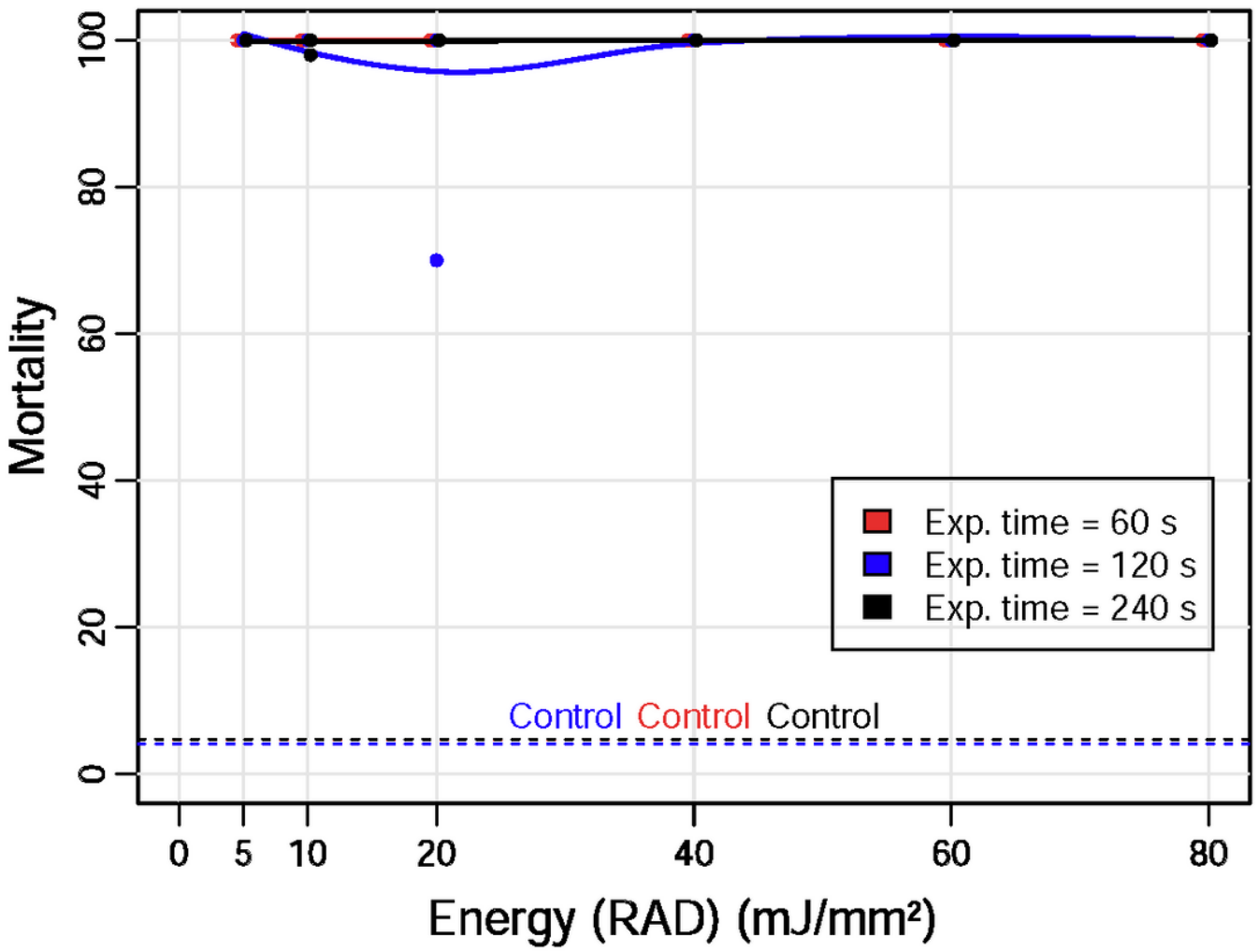



**Figure 4**

Relation between the energy delivered to mite eggs and the mortality associated with three irradiation exposure times (60, 120, and 240 s) generated by the pulsed krypton fluoride excimer gas laser. Dashed line corresponds to the mortality in controls unexposed to UVC-irradiation

## Supplementary Files

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- Additionalfile1FigureS1UVCTridimentionalview.pdf
- Additionalfile2FigureS2UVClaserbeam.mov
- Additionalfile3FigureS3PetriDishChamber.pdf
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