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Effect of ultraviolet - C on the nematode *Caenorhabditis elegans* and plant pathogenic nematode *Meloidogyne incognita*

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Abstract
Caenorhabditis elegans is a model organism to test the various xenobiotics, neurotoxicants, age related studies, magnetic effects and various parameters related to growth, development and life cycle and life span are well studied. In the present study this model organism has been utilized to understand the effect by exposing to Ultraviolet - C (UV – C) on the nematode *Caenorhabditis elegans* and Plant pathogenic nematode *Meloidogyne incognita* with the exposure time of 0,30,45,60 and 120 Seconds with the UV-C device fitted with 8W UV-C tube generating 253.7 nm wavelength and 250 mJ/cm² irradiance energy was utilized. Theire was cent percent survival recorded till 60Seconds of exposure but at 120 second of exposure worm recorded the highest mortality in *C elegans*. Similarly the cent percent mortality in *M. incognita* was recorded on exposure to 180Sec. Hence, the study concludes that the for the plant pathogenic nematode higher time is required for the cent percent mortality. Further this UV-C exposure can be studied for the root infestation during seedling stage or sterilizing soil upper surface can be utilized.

Keywords: C elegans, UV-C, *Meloidogyne incognita*, survival and mortality

Introduction
Caenorhabditis elegans is a model organism that has the potential to bridge the gap between *in vitro* and *in vivo* approaches by virtue of being amenable to high-throughput technologies while providing physiologically relevant data derived from a whole-animal setting. Several features of *C. elegans* make it a powerful tool for the pharmaceutical industry. These include being easy to culture; undergoing rapid reproduction with a short generation time enabling large-scale production of nematodes; small size, which allows assays of more than a hundred animals in a single well of a 96-well plate; transparency, which enables the use of fluorescent markers to study biological processes *in vivo*; and cellular complexity - *C. elegans* is a multicellular organism that has many different organs and tissues (Kaletta and Hengartner, 2006) [12].

Some of the research that has been carried out in the areas of neurotoxicology, genetic toxicology and environmental toxicology, as well as high-throughput experiments with *C. elegans* including genome-wide screening for molecular targets of toxicity and rapid toxicity assessment for new chemicals. An increased role for *C. elegans* in complementing other model systems in toxicological research has been well established (Maxwell *et al.,* 2008) [13].

Root-knot nematode (*Melaidogyn* spp.) is an economically important polyphagous obligate plant parasite, distributed worldwide, and is known to parasitize nearly every species of higher plants causing a damage of 3-24 percent. These are sedentary endoparasites and their parasitism life depends on the success to induce feeding sites in the roots of host plants. *Meloidogyne incognita, M. javanica* and *M. arenaria* are the most common in the tropical regions while *M. hapla, M. fallax* and *M. chitwoodi* are prevalent in temperate and cooler regions. Damage is more pronounced in tropical climates than in temperate because of the favorable conditions for nematode survival and multiplication. The direct and indirect damage caused by various *Melaidogyn* species results in delayed maturity, toppling, reduced yields and quality of crop produce, high costs of production and therefore loss of income (Feyisa, 2021) [14].

Chemical and biological nematicides are commonly used against plant-parasitic nematodes with variable efficacy. Although most chemical nematicides show high nematicidal efficacy under field conditions, their impact on human health and on the environment has resulted in enforced regulation or the total ban of the different products. To control plant-parasitic nematodes, grafting, biofumigation, heat treatment, soil compost and crop rotation with poor hosts, catch crops or antagonistic microorganisms have been intensively studied. However, further management methods, such as plant-based metabolites are being investigated to search for alternative human and environment friendly biopesticides (Eder *et al.,* 2021) [15].

The chemical nematicides such as carbofuran, aldicarb etc. are used to control the plant parasitic nematodes (Varaprasad and Mathur, 1980) [19]. The chemical nematicides may be useful and effective in the control of plant parasitic nematodes, but they do harm and cause environmental pollution. Besides, the chemical nematicides are so expensive that small farmers cannot afford it. The organic amendments based nematicides are used to control such plant parasitic nematodes (Singh and Sitaramaiah, 1970; Akhtar and Abdul Malik, 2000) [17, 16].

Light toxicity, or phototoxicity, is a phenomenon that has been well described for the nonvisible, shorter wavelengths of the light spectrum, especially ultraviolet (UV) and gamma radiation upon organismal physiology. Hence, wavelengths shorter than 200 nm are considered as ionizing radiation because the energy carried by photons of this wavelength is sufficiently powerful to dislodge electrons from their orbital, creating an ionic molecule that is highly reactive. UV light defines wavelengths falling between 200 nm and 400 nm. Following UV photon absorption, DNA bases are excited which can result in pyrimidine dimers causing a DNA damage response and mutagenesis (Filho *et al*., 2018) [1].

Since the cost of available nematicides are prohibitive and many of them being phyto-toxic. These products are costly, having negative impact on environment and polute the water and soil bodies. To minimise the usage of chemicals that pollute the environment in many ways with an eco-friendly approach.Hence, the present study was undertaken to understand the effect of UV-C exposure on the nematodes both non-pathogenic and pathogenic by UV-C handheld device of 253.7 nm wavelength and 250 mJ/cm² irradiance energy, fitted with 8W UV-C tube. As the nematodes are wide spread in nature, survives in various climatic conditions, and causes the damage to the plants around 20 – 60 percent based on the range infestation.

UV-C exposure on the nematodes both non pathogenic and pathogenic by UV-C handheld device of 253.7 nm wavelength and 250 mJ/cm² irradiance energy, fitted with 8W UV-C tube. As the nematodes are wide spread in nature, survives in various climatic conditions, and causes the damage to the plants around 20 – 60 percent based on the range infestation.

Material and Methods

Preparation of bacterial food source

Using *Escherichia coli* strain OP-50 as a food source in the laboratory. *E. coli* OP-50 is an uracil auxotroph whose growth was limited on NGM (nematode growth media) plates. A limited bacterial lawn was desirable because it allows for easier observation and better mating of the worms. A starter culture of *E. coli* OP-50 is obtained from *Caenorhabditis Genetics center, University of Minnesota, USA*. Used the starter culture to isolate single colonies on a streak plate of a rich medium such as Luria Broth agar [10 g Bacto-tryptone, 5 g Bacto-yeast, 5 g NaCl, 15g Agar, H_2O to 1 litre, pH 7.5]. Using a single colony from the streak plate, aseptically inoculated a rich broth, such as Luria Broth. The bottles of media were stored at room temperature for several months. Allowed inoculated cultures to grow overnight at 37 °C. The *E. coli* OP-50 solution was then ready for use in seeding NGM plates. The *E. coli* OP-50 streak plate and liquid culture was stored at 4 °C and used for several months.

Culturing of *C elegans* and preparation of nematode growth media (NGM)

C. elegans were maintained in the laboratory on NGM agar, which was aseptically poured into Petri plates and lawn of *E. coli* OP-50 strain was grown overnight at 37 °C. For solid NGM, mix 3 g of NaCl, 2.5 g of peptone, and 20 g of agar and bring to 1 L with H_2O and autoclaved and cooled for 1 h in a 55 °C water bath. (Add 1 mL of cholesterol (5 mg/mL in ethanol), 1 mL of 1 M $CaCl_2$, 1 mL of 1 M $MgSO_4$, and 25 mL of 1 M (pH 6.0) KPO_4 and mixed after each addition). Worms of L4 stage were allowed to grow on the OP-50 lawn for the days of experiment. The L4 stage worms were obtained by bleaching for the experiments to perform.

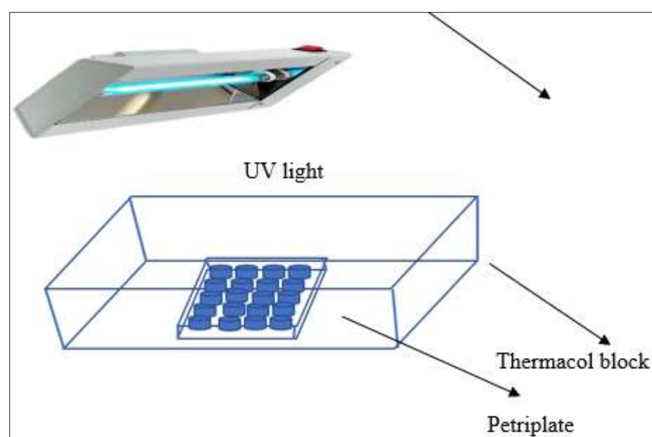
Culturing and maintenance of *Meloidogyne incognita*

A population of *Meloidogyne incognita* was reared on tomato plants in pots and watered regularly. Extraction of nematodes was carried out the soil of infected plants by Cobb's sieve and Baerman funnel method. *M. incognita* infected roots were harvested from the 3–4 months old tomato plants after extraction juveniles were inoculated. To isolate second-stage juveniles (J_2), roots with galls were collected and shaken in 15% bleach for 5 min to release the eggs, and the eggs were isolated by successively passing the liquid through stainless steel sieves with pore sizes of 25, 150 and 850µm. Eggs were collected from inside the 25 µm sieve and hatched in 100 ml of water with 50 µg ml^{-1} of ampicillin shaken at 28°C. After three to four days hatching, J_2 were isolated from the eggs using the 25 µm sieve. These J_2's were used in the study.

UV-C exposure on *C. elegans* and *M. incognita*

The study was performed by UV-C handheld device of 253.7 nm wavelength and 250 mJ/cm² irradiance energy, fitted with 8W UV-C tube obtained from UNICORN DenMartcompany. Approximately 100 ± 10 worms in triplicates were released into 3 cm transparent Petri plates containing NG Media. The plates were exposed to UV-C light for 15, 30, 45, 60, 120 and 180 seconds and non-exposed control. The exposed plates were immediately observed under microscope for its behaviour. The experiments were repeated on three different days. (Fig-1)

Graphical representation of UVC light of 253.7 nm wavelength and 250 mJ/cm² irradiance energy set up



Protocol of the UV - C exposure

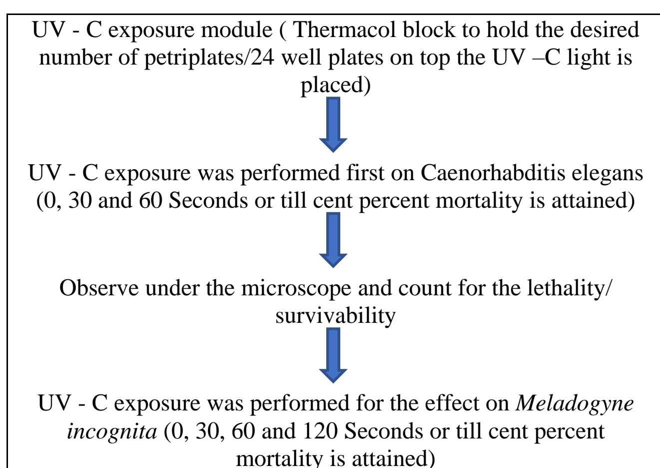


Fig 1: UV - C exposure protocol for the survivability/mortality of nematodes

Live v/s dead counting

Soon after exposing to the UV-C light the worms were counted for live v/s dead worms based on its movement. The worms, which were immobile and straight in posture, were counted as dead and the worms making movement of its body was counted as live.

Results and Discussion

The present investigation was envisaged to study the effect of UV-C exposure on the nematodes both nonpathogenic and pathogenic by UV-C handheld device of 253.7 nm wavelength and 250 mJ/cm² irradiance energy, fitted with 8W UV-C tube for 0. 30. 60, 120 and 180 Seconds of exposure in a thermacol box specifically desighned for the exposure.

The worms tolerated and moving normally in c elegans till the 60 seconds of esposure duration but the 100 percent mortality was recorded under the microscopic observation at 120 seconds (Fig.1). Similar result was found with Meyer *et al.* (2007) [3] Young adults of *C. elegans* (24 hours after L_4 stage N2 wild-type nematodes exposed to 50, 100, 200, or 400 J/m^2 UVC (254 nm) irradiation exhibited a dose dependent increase in lesions, as detected by QPCR. Lesions were induced with a slope of 0.4 to 0.5 lesions/10 kb per 100 J/m2 UVC, with some loss of linearity evident at the higher dose, with some loss of linearity evident at the higher doses.

Similar evidence was founded by Hartman *et al.* (1984) [8]. The effects of UV radiation on the brood sizes of wild-type and "mutant self-progeny broods were examined on *C. elegans*. A comparison of F_{37}, values, where F_{37} was defined as the influence necessary to reduce fertility to 37 percent of unirradiated controls, revealed that rad-I, rad-2, rad-3 and rad-7 were 1.2, 7.1, 8.5 and 0.7 times as sensitive as the wild type, respectively. No shoulders were present in the inactivation curves, this was the only instance for which shoulders were absent in the wild type.

Prasanth *et al.* (2016) [9] reported wild type worms survived up to 15, 13, and 14 days in liquid and 15, 15, and 10 days in solid media whereas *daf-2* mutants of *Caenorhabditis elegans* survived up to 40, 35, and 30 days in liquid and 42, 38, and 33 days in solid media, after 2, 4, and 6 h of UV exposure, respectively. These observations clearly indicate that UV-A significantly reduced the lifespan of the nematode.

Rajasekharan *et al.* (2018) [10] describe an innovative counting strategy that employs light-emitting diode (LED) technology. They found that the nematodes stopped moving under white light (360–760 nm) and when administered with sub-lethal dosage (LC_{50}) of a toxic drug, whereas they responded rapidly to blue (450–490 nm) and ultraviolet (UV) (100–400 nm) LED lights. Furthermore, paralyzed nematodes responded in less than 5 seconds to a LED pulse. The response to the LED stimulus was distinctively noted in *C. elegans* dauers, which squirmed away from illuminated sites within seconds.

Effect of UV-C light on *Meloidogyne incognita*.

There was no mortality up to 120 seconds and 100 percent mortality at 180 seconds was observed under the microscopic observations for the *Meloidogyne incognita* (Fig.2).

Klass *et al.* (1977) [11] reported related studies the percentage survival with varying doses of U.V. for *C. elegans* worms at 4, 18, 42 and 66 hours old was recorded. The younger worms were more sensitive to UV radiation. The life shortening effects of UV light on worms of various ages. Older worms show less of a life-shortening effect than do younger worms. A UV dose of 192,000 ergs/cm 2 reduced the life span of worms 20-day old worms by only 2 percent while the life span of worms 5-day old worms was reduced by 37.7 percent for the same dose. Similar results were obtained with UV doses from 32,000 ergs/cm 2 to 960,000 ergs/cm ^{2}.

Infective L3s of the trichostrongyloid nematodes *Haemonchus contortus, Teladorsagia circumcincta* and *Nematodirusbattus*, suspended in water, were exposed to direct UV irradiation in two experiments. In the first, during six days of constant illumination with UVA lamps at intensities simulating sunlight at ground level, the mortality rate was increased up to 100-fold compared with controls. Significant differences in mortality rates were detected between the three species, with *H. contortus* the least sensitive. In the second experiment, larvae were exposed to natural sunlight during the temperate spring and summer, for 24-h periods on seven separate days representing a range of weather and UV doses. Mortality was again increased by UV exposure in all species, but was less in *H. contortus* than in *T. circumcincta*or *N. battus*. At higher daily UV doses, the mortality rate was on average 2.27 times higher in exposed larvae than in sheltered controls (Dijk *et al.*, 2009) [1].

About 10 percent of the electromagnetic energy emitted by the Sun is between 100 and 400 nm. At the Second International Congress on Light in Copenhagen in 1932, William Coblentz suggested that these wavelengths are subdivided into UV-A, UV-B and UV-C. As recommended by the range of UV-A is from 315 to 400 nm, UV-B is from 280 to 315 nm and UV-C is from 100 to 280 nm. Before reaching the uppermost layer of the Earth's atmosphere, UV-A, UV-B and UV-C constitute 6.8, 2.4 and 0.8% of the solar radiation energy, respectively. UV absorption by the stratospheric ozone reaches its maximum at about 260 nm. Together with Rayleigh scattering, it limits the amount of UV radiation at the Earth's surface, with UV-C becoming completely absorbed. As a consequence, around 5.7 and 0.3 percent of sunlight energy at sea level is in the UV-A and UV-B range, respectively, but their ratio depends on several factors, including latitude, altitude, day of the year, time of day and clouding. UV-B is the part of solar radiation with the shortest wavelengths, i.e., the highest energy that reaches the Earth's surface (Fischer *et al.*, 1995) [2].

The action spectrum of DNA damage from 200 nm, proposing an almost flat shape in the 200–260 nm range, where the curve reaches its maximum and then falls at wavelengths above 260 nm. Due to this curve, the level of DNA damage induced by 260 nm (UV-C) is about 106 times higher compared with irradiation at 360 nm (UV-A). The direct absorption of UV by DNA leads mainly to the formation of pyrimidine dimers between adjacent pyrimidines in a DNA strand (Cockell. 1998) [7].

Quantitative polymerase chain reaction assay to characterize repair of DNA damage induced by ultraviolet type C (UVC) radiation in *C. elegans*, and then tested whether DNA repair rates were affected by age in adults. As a result UVC radiation induced lesions in young adult of *C. elegans*, with a slope of 0.4 to 0.5 lesions per 10 kilobases of DNA per 100 J/m2, in both nuclear and mitochondrial targets. L1 and dauer larvae were more than fivefold more sensitive to lesion formation than were young adults. Nuclear repair kinetics in a well-expressed nuclear gene were biphasic in non-gravid adult nematodes: a faster, first order (half-life about 16 hours) phase lasting approximately 24 hours and resulting in removal of about 60 percent of the photoproducts was followed by a much slower phase (Meyer *et al.*, 2007) [3].

C. elegans lifespan was inversely correlated to the time at which worms were exposed to visible light. While circadian control, lite-1 and tax-2 do not contribute to the lifespan reduction, they demonstrated that the visible light creates photo oxidative stress along with a general unfolded-protein response that decreases the lifespan. Transparent nematodes are sensitive to visible light radiation and highlighted the need to standardize methods for controlling the unrecognized biased effect of light during lifespan studies in laboratory conditions (Filho *et al.*, 2018) [1].

Deactivation of *Caenorhabditis elegans* nematodes in drinking water by using peroxymonosulfate (PMS)/UV-C indicated that 100 percent deactivation efficiency was obtained under optimal conditions and demonstrated that HSO−5HSO5− was activated by UV-C to produce OH and ·SO−4·SO4−, which resulted in oxidative stress and stimulated the occurrence of cell apoptosis, leading to nematode deactivation (Chen *et al.*, 2021) [6].

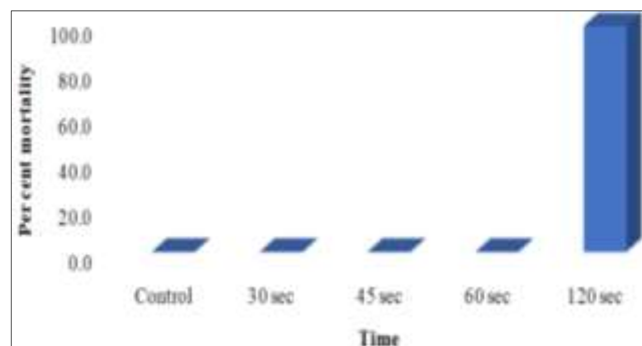


Fig 2: Observations were recorded for exposure of UV-C light on *C. elegans* with different time intervals

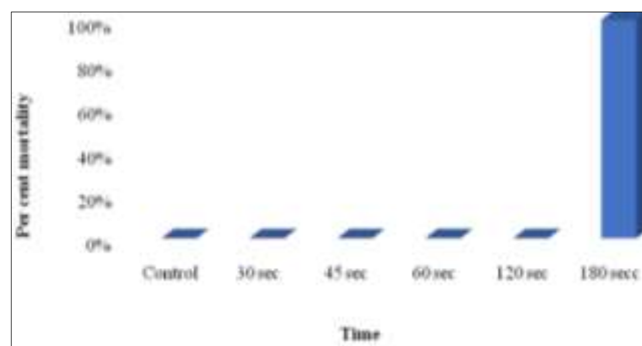


Fig 3: Observations are recorded for exposure of UV-C light on *Meloidogyne incognita* with different time intervals

Conclusion

The effect of UV –C has been understood to lead to mortality in a time response exposure both c elegans and plant pathogenic nematode *Meloidogyne incognita* were achieved cent percent mortality on exposure. Hence, further studies needs to be undertaken to disinfect or sterilize the soils from nematode infestation.

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