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The effects of short UV radiation to the egg hatchability of the soil-dwelling nematode, *Acrobeloides nanus*

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Abstract. Over the years, the risks of ultraviolet radiation (UV) exposures have been growing widely. Here, we tested the egg hatchability of nematodes exposed to different durations of UV (14, 7, 3.5, 1.75 minutes) via a UV germicidal lamp. After logarithmic analysis, the result gave a median effective concentration (EC_{50}) value of 0.7951 minute. This means, at 0.7951 minute of UV exposure, half of the nematode eggs will fail to hatch after 48 hours. However, despite exposing these nematode eggs at different durations to short UV radiation, the yielded EC_{50} value is much smaller compared with the lowest test concentration. Still, statistically, no significant differences were yielded between the 1.75, 3.5, 7 and 14 minute exposures. UV exposure highly affected the hatchability of the nematode's eggs. Although the mechanisms that is responsible for the inhibition of egg hatching following UV exposure is still unknown, it is clear though that only a small dose of radiation can already deny hatching of the eggs.
Key Words: ultaviolet radiation, *Acrobeloides nanus*, egg hatchability, EC_{50}.

Introduction. Pollution has over the years, accumulated and has started to affect the environment on a global basis. Reports from previous studies have already shown the extent of damage that various pollutants have posed to the environment such as increased greenhouse gas emission and stratospheric ozone (O_3) layer depletion. These damages consequently brought about global changes in the climate and increased ultraviolet (UV) light exposures (Krupa & Kickert 1989; Kerr & McElroy 1995; McKenzie et al 2011).

In a more specific level, it has been concluded that the immediate risks of UV light exposure involve mutations among populations of organisms. For instance, it has been reported in other organisms that UV exposure damaged gametes, decreased organism viability and increased population mortality (Nahon et al 2008; Fujiie & Yokoyama 1998; Kiesecker & Blaustein 1995; Gurdon 1960). In humans, UV light exposures are associated to sunburns and onset of skin cancers (Setlow 1974; Pfeifera & Besaratinia 2012). Interestingly, the immediate concern of the risks of UV light exposure has been addressed in the past by banning chlorofluorocarbons (CFC's) which damage the O_3 layer. Despite the banishment of CFC's, O_3 depleting chemicals are still reported to continue to linger in the environment for a few more decades which still places organisms under risk until present (Nahon et al 2008; McKenzie et al 2011).

With this issue at hand, scientists have been widely conducting studies on the various effects of UV radiation on model organisms (Hollaender & Claus 1936; Caldwell et al 2003; Hader & Sinha 2005; Hansson & Hylander 2009). A model organism is a non-human species that is extensively studied to understand particular biological phenomena, with the expectation that discoveries made in the organism model will provide insight into the workings of other organisms (Fox 1986). Model organism reduced the complexity of the task. Over time and with more knowledge to hand, biological research expanded to the study of more complex systems, which required the increasing use of higher organisms, including *Caenorhabditis elegans, Drosophila, Arabidopsis*, zebrafish (*Danio rerio*) and mice (*Mus musculus*) (Hunter et al 2008).

Due to its sustainability and persistence in polluted environments, one of the most promising models for experimental studies is the soil-dwelling nematode, *Acrobeloides nanus*. Like *C. elegans* which is a widely used species as model organism, a single *A. nanus* can lay eggs on its own via asexual reproduction and the rate of reproduction of these species made them ideal for studies to understand biological phenomena. However unlike *C. elegans* (which is hermaphroditic), *A. nanus* actually reproduce by parthenogenesis which means there is no male present and females or the organism themselves does not require a sperm to fertilize their egg (Lahl et al 2006).

Presence of a toxic substance or a change in the magnitude of a critical environmental factor (e.g. temperature) creates conditions close or beyond the range tolerated by a population (the population's niche). Under such conditions, organisms respond with the changes on molecular and physiological level, which might affect their survival, reproduction or other individual life-history characteristics (Doroszuk 2007). Most of the experiments conducted on animal eggs exposed to UV radiation were on marine organisms and amphibians. So far, very little is known with the effects of UV on nematode eggs. Herein, we aim to know the effects of UV at different time exposures on the egg hatchability of the nematode *A. nanus*.

Material and Method. The study was conducted in May 2015 in the Nematology Laboratory of MSU-Iligan Institute of Technology (MSU-IIT). Laboratory cultures of the *A. nanus* (obtained from Ghent University, Belgium and maintained in MSU-Iligan Institute of Technology, Philippines) were used. *A. nanus* is parthenogenic and it typically starts laying eggs around 10 days after hatching. Thirty (30) adult nematodes were chosen based on size morphology. Three adult nematodes were individually placed into a 5-mL Petri plate with 2.5% bacto-agar medium until they started to lay eggs (~4-8 hours). The laid eggs were then prepared for experimentation (Alvarez et al 2006).

Artificial UV radiation via a UV germicidal lamp (built-in with a fume hood with a wavelength of ~254 nm) was induced at different durations (Dahms & Lee 2010). A total of five set-ups were prepared in triplicates. Ten newly laid eggs were placed in a 5-mL Petri dish with 2.5% bacto-agar media for each set-up. Each set-up were exposed to UV-light at four different durations (14, 7, 3.5, 1.75 minutes) with the exception of the control set-up (Fujiie & Yokoyama 1998; Gaugler & Boush 1978) which were not exposed to UV radiation. The hatchability of the eggs were then observed for forty eight (48) hours in each set-up under room temperature, counting hatched eggs as those containing no embryo with lysed shells or by counting the number of emerged larvae per set-up (Bird et al 1993).

The median effective concentration (EC_{50}) or in this case the duration, to UV light exposure in nematode egg hatchability was determined via logarithmic analysis using OriginPro 2015 software.

Results and Discussion. After Regression Analysis (Figure 1), the result of the study yielded an EC_{50} value of 0.7951 minute. This means that the effective duration in which half of the nematode eggs will fail to hatch after 48 hours is 0.7951 minute. Herein, despite exposing these nematode eggs at different durations to short UV radiation, the yielded EC_{50} value is much smaller compared with the lowest test concentration. Numerical differences were observed across all treatments however, it should be noted that upon one-way ANOVA, no significant differences were yielded between the 1.75, 3.5, 7 and 14 minute exposures. This implies that the expected EC_{50} value should fall below the lowest test concentration in this study i.e. the effectiveness of short UV radiation to inhibit egg hatching would be found in lower doses.

The low UV exposure time for the inhibition of nematode egg hatching may be due to the direct exposure of the eggs to unfiltered UV which in a similar study, caused inhibitions of photolyase (an enzyme responsible for DNA repair) activity (Sancar 1994, cited by Dahms & Lee 2010). In the study by Blaustein et al (1994), they reported a decline in egg development among species of anurans (*Rana cascadae* and *Bufo boreas*) which were exposed under unfiltered UV-B light which caused decreased photolyase activity. In the same study, they found out that the eggs of other anuran species (salamanders) which developed strategies to combat UV-B exposure (such as hiding their eggs via submergence in bodies of water) were able to generate higher photolyase activities compared with the two aforementioned species. Furthermore, Bonaventura et al (2005) suggested that sensitivity to UV-B was more common during the early developmental stages than later stages of marine invertebrates and some fishes which could also partly explain the relatively low EC_{50} value of the said stressor towards these nematode eggs.

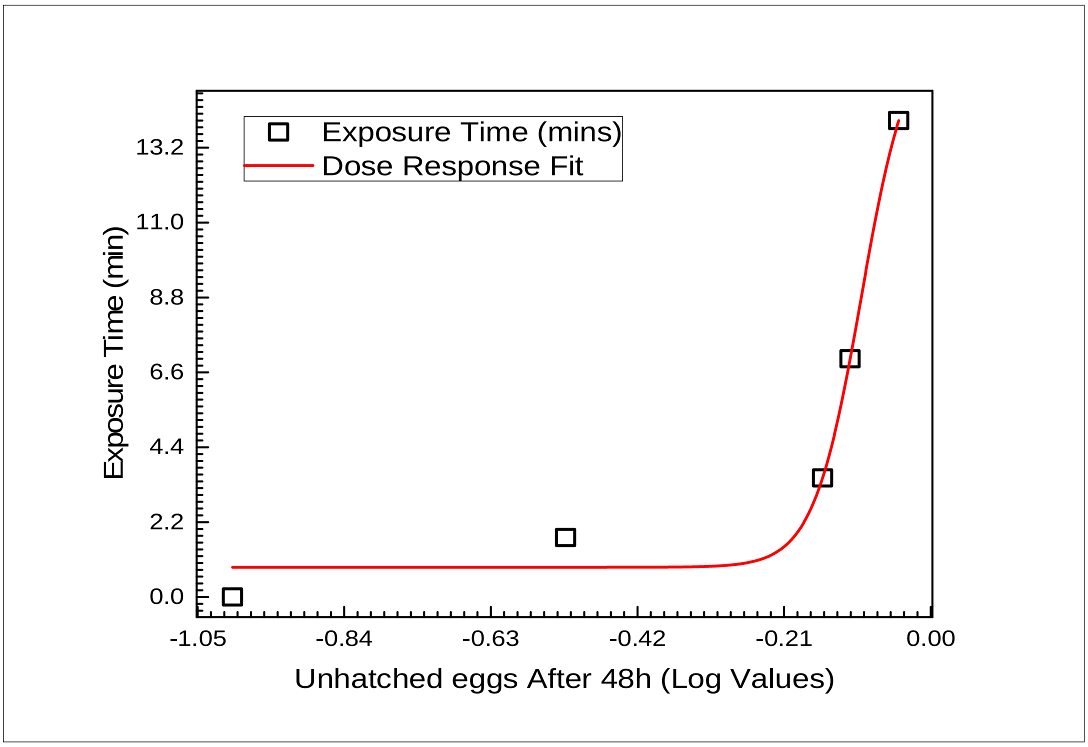

Figure 1. Regression line graph showing the different logarithmic values of the generated data from the hatchability of *A. nanus* eggs 48 hours after exposure to UV-C at different durations.

Some species of parasitic nematode were documented to be sensitive to UV radiation exposure, one of which is *Neoaplectana carpocapsae*, an entomopathogenic nematode (EPN) which after 7 minutes exposure of short UV radiation (254 nm), had its pathogenicity reduced, denying the nematode to cause lethal infection. Complementarily, this nematode's reproduction and development is inhibited after 2.45 and 5 minutes of short-UV radiation exposure respectively (Gaugler & Boush 1978).

Conclusions. The results of this study strongly suggest that *A. nanus* is highly sensitive to UV exposure. To be more specific, UV exposure highly affected the hatchability of the nematode's eggs. Although the mechanisms that is responsible for the inhibition of egg hatching following UV exposure is still unknown, it is clear that only a small dose of radiation can already deny hatching of the eggs. Surprisingly, the minimum dose that is used in this study is far more disruptive than expected and suggests that the dose of UV radiation necessary to deny hatching is lower than the minimum dose used in this study. Observing the result's trend and using logarithmic analysis for approximation, EC_{50} is almost twice smaller than the minimum dose and further experiments should be conducted to confirm the estimate.

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