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Which light colour is better for *Caenorhabditis elegans* research

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ABSTRACT

Caenorhabditis elegans, due to its short life cycle, transparency and known complete genetic map, is a frequently preferred experimental animal in recent years. Standardization of the laboratory condition is very important; these conditions may have unknown or unpredictable effects on the experimental animal we use. Light is a factor that is often overlooked while conditions such as temperature and humidity of the environment are always in the foreground. This study aimed to identify the most suitable light color for maintaining the lifespan of *Caenorhabditis elegans* and to evaluate how different light spectra impact survival. Eggs were collected from adult *Caenorhabditis elegans* for standardization and waited for each group to hatch under their own light color conditions (dark, day light, red, blue, green). After adulthood, they were subjected to life span analysis under their own light colors. The experiments continued using ImageJ program until the last nematode died and the results were reported via statistical methods for survival analyses using an online application. Red and blue light significantly reduced the nematodes' lifespan, whereas nematodes exposed to green light exhibited no mortality during the initial phases. Nematodes were found to be more successful in survival experiments in dark and light environments that were close to their natural habitats; green light showed similar results with daylight and darkness. Survival rates of adult nematodes that completed their development under red and blue light were found to be statistically low when compared to other light colors. The color that really surprised us was the green light; the survival rates of nematodes that completed their development under green light were quite successful in the first two series compared to red and blue colors. Findings highlight the importance of selecting appropriate light conditions to optimize *Caenorhabditis elegans* experiments, suggesting potential for using green light in stress-related studies.

Keywords: *Caenorhabditis elegans*, colour, life span, light

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Introduction

A widely used and a sensitive model organism *Caenorhabditis elegans*' environmental responses remain an area of active research. This rising animal model has been attracting attention in recent years which is one of the easiest to go on with low costs that provides fast results. Its life cycle is so short such that an embryo becomes an adult within approximately five days. Hermaphrodite individuals can produce approximately 300 offspring at one time which makes it extremely asset that one can create experimental groups in a short time under similar conditions (Meneely et al., 2019). However, since it is a transparent worm, it is affected by external factors, especially the light intensity and temperature of the laboratory environment. Even the temperature of the environment affects the determination of gender. These nematodes naturally exhibit primitive behaviours such as avoiding bright environments and staying safe in dark environments (Ward et al., 2008). This behaviour protects them from their predators and is one of the behaviours that do not regress in the evolutionary process.

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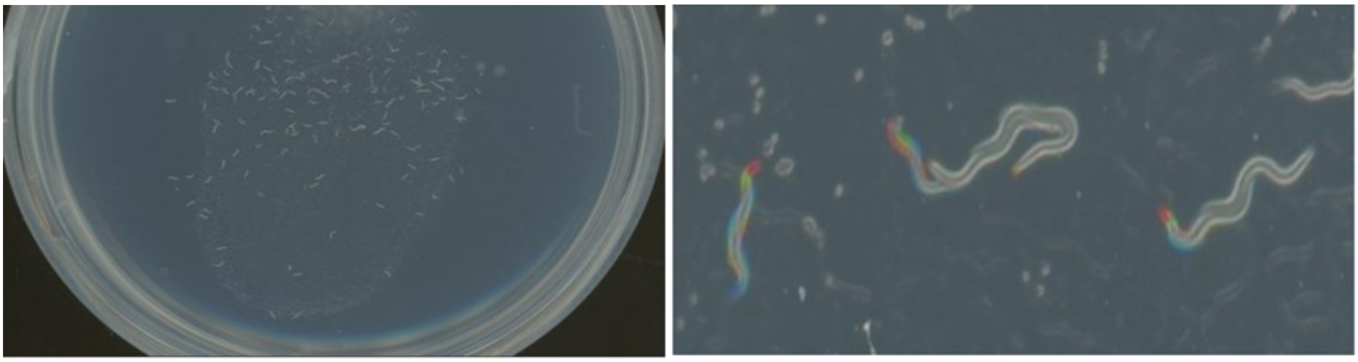

Figure 1. Images out from the scanner. The colors seen at both ends of the nematodes (right image) are due to the movement of them during scanning process

Visible light, also known as the visible spectrum, is the part of the electromagnetic spectrum that corresponds to wavelengths between 380 nm and 780 nm and the difference at the wavelengths creates the colour diversity. Red (700 nm), green (520 nm) and blue (400 nm) light are visible; UV or infrared wavelengths are non-visible for human eye (Abdullah, 2008). Caenorhabditis elegans typically do not have eyes, but they can change the way they approach or behave according to the light; can react to short wavelength lights (Ghosh et al., 2021) and can change their behaviour based on UV lights which is an electromagnetic radiation of wavelengths ranged between 10 and 400 nm (De Magalhaes Filho et al., 2018).

Stress factor is a subject that has been studied in *Caenorhabditis elegans*, and it is known that nematodes that are constantly exposed to bright light have short life spans. According to the literature, as the amount of light per unit area (µW/mm2) increases, their average lifespan decreases significantly (De Magalhaes Filho et al., 2018). Suppose that light is a stress factor for *Caenorhabditis elegans* do different light colours or durations of illumination give the same result? In this present study, we specifically focused on different light colours in the laboratory environment. Accordingly, we examined the survival rates of *Caenorhabditis elegans* in the dark, light and even 12-hour dark and light cycles, as well as the unusual red, blue and green light environments. In this regard, we tested them after they had lived under the mentioned light condition from the very beginning, the egg stage until they have died during the life span assays.

Materials and methods

Group design

Here in this study, we used wild-type N2 strain *Caenorhabditis elegans*. For the synchronization of the study, eggs were collected from individuals in the same stage and groups were designed to have 100 individuals in each. The nematodes were grown in petri dishes under their own light conditions. They were fed up with E. coli OP50. The groups were named as follows and kept under the conditions they were named, provided that they remained the same for the end of the assays. Light intensity was measured and standardized at 40µW/mm2 for groups which were involved in light research. Red, green, blue LED lights (1,325 Lumens, equivalent to 4100 Kelvin) were used, and each light was placed in its own lightproof box from the same distances. Group 1 always remained in dark, Group 2 remained in a 12-hour light/dark cycle, Group 3 always remained in white light (1,325 Lumens), Group 4 always remained in red light, Group 5 always remained in blue light and the last Group 6 always remained in green light lightproof box environment.

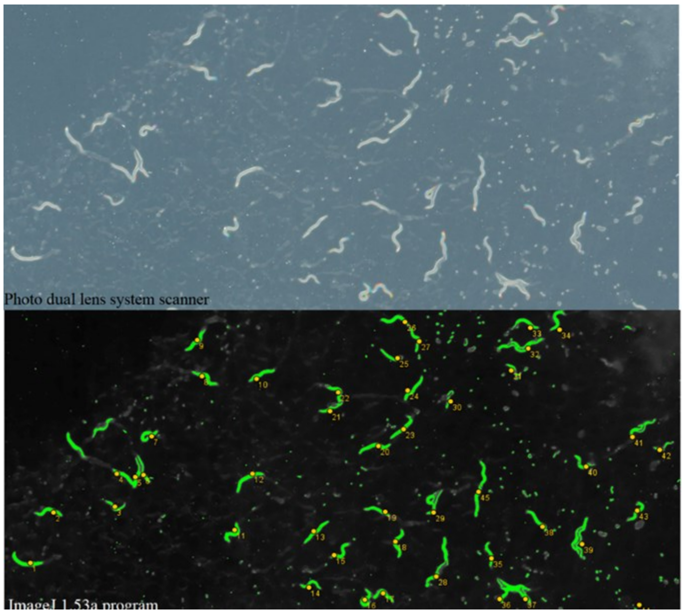

Figure 2. An area bare from scanner is observed as at the top. Same area designed with ImageJ program makes it easier to see and also performs automatic counting.

Life span analysis and scoring

The ambient temperature was 35 °C degrees which helped to speed up the life span analyze process. Photographs were taken with Epson Perfection V800 Photo dual lens system scanner within our laboratory

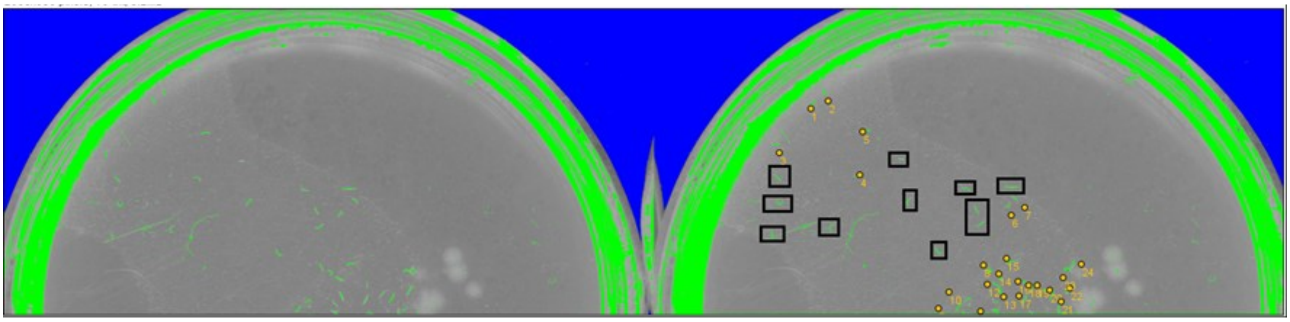

Figure 3. In order to see the nematodes clearly, counts were first made in the upper half of the petri dishes and then in the lower half. Here, the time difference between the petri dish on the left and the petri dish on the right is about 3 minutes, which is the time it takes for the scanner to scan the images. Thus, nematodes that do not move between two images (box) are counted as dead. Nematodes moving between two images are counted as alive (spot)

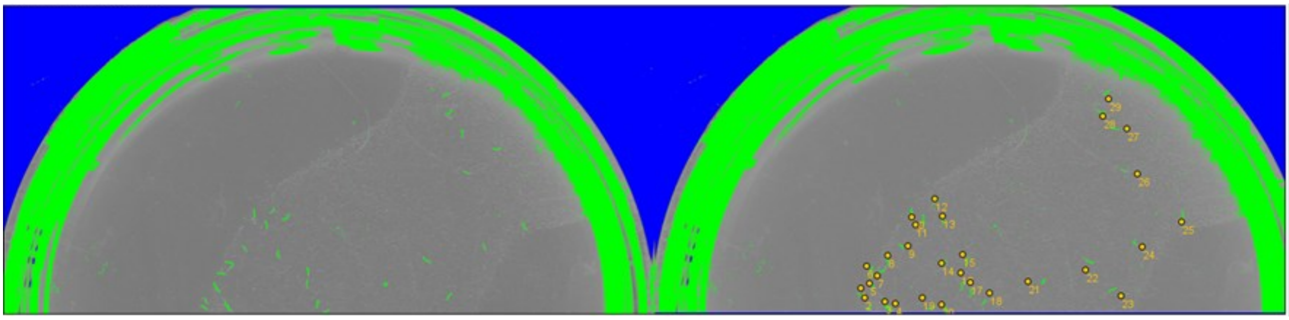

Figure 4. All the nematodes in this petri dish are alive due to displacement

where the nematodes were at their own cabins at every 20-minute interval (Figure 1). In order to distinguish alive and dead worms from each other, two consecutive shots were taken every period. Thus, the live ones change zones between each picture and the dead ones appear in the same place (figure 2). The number of the initial nematodes in the petri dishes were known and we counted the nematodes as dead, missing, and alive using ImageJ 1.53a program. For each paired picture, we entered the following commands as: Image - Type -16 bit to prepare for threshold. Image-Adjust-Threshold and using default approximately 14% below and 15% above so the Caenorhabditis elegans are colored green for us to count and observe easily (Figure 2). Plugin-Analyze-Cell counter notice for numbering the Caenorhabditis elegans.

The nematode considered "dead" was immobile (Figure 3); the zone which the nematode was in between two images were the same. "Missing" was considered as it was buried in the agar, not found in the petri dish, or escaped to the outermost area of the petri dish where there was no food. Motile nematodes that changed zones between two images were considered as "alive" (Figure 4).

For life span analysis and scoring, counting in petri dishes was continued using ImageJ program until all nematodes were dead. Data were plotted as Kaplan-Meier curves and analyzed using an online application for survival analysis of lifespan assays (Yang et al., 2011).

Results

Exposure to red and blue light shortened lifespan

When the effects of different colored lights on lifespans were examined, it was found that there was no significant difference between the dark environment and other light colors, except red ($p > 0.05$). A significant difference was found between the 12-hour light/dark environment and blue light ($p < 0.05$), but there was no significant difference with other light colors ($p > 0.05$). There was a significant difference between the survival rates of nematodes that remained in the white light all the time and those that remained in the red and blue light environments. Although being in the constantly white light environment did not increase the life span as much as in the dark environment. A significant difference was found between the blue light environment and all other lighted and unlighted environments, except red light. When we looked at the results of the blue light environment, there was a significant difference between always white and green light environments ($p < 0.05$); no differences between dark.

We were surprised when we saw the sharp decrease in the survival rates belongs to the red (67.28% mortality) and blue light (57.48% mortality) within the first 40 minutes. The green light results were also interesting; the survival rate was very similar to the result obtained under the 12-hour light/dark environment, which is suitable for a normal habitat of nematodes. The most similar survival percentages/ timeline were between 12-hour light/dark environment, always white light and green light environments (p values for 12-hour light/dark environment and always white light is 0.9179; always white light and green light is 0.8514; 12-hour light/dark environment and green light is 0.7690).

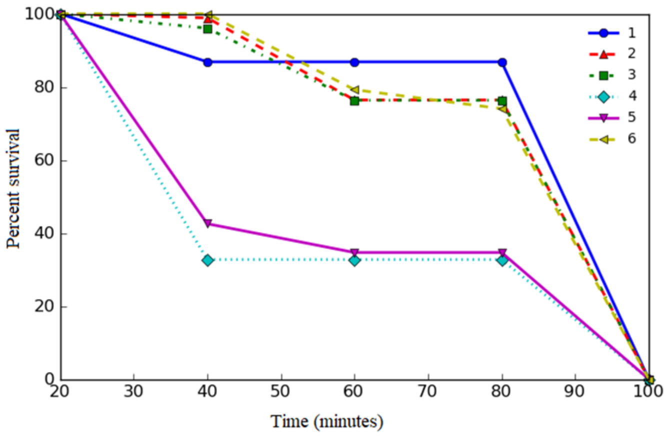

Figure 5. Survival / log cumulative hazard plot graph. 1: dark, 2: 12-hour light/dark, 3: always white light, 4: red light, 5: blue light, 6: green light group

Discussion and Conclusion

Even though nematodes have limited their vision over evolution they can perceive short-wavelength lights, but their perception does not mean they like and support (Ghosh et al., 2021). Studies indicates that Caenorhabditis elegans avoid from UV. Their photoreceptor cells are affected from UV irradiation, and is electro physiologically alerted (Ghosh et al., 2021; Lee and Lee, 2021; Ozawa et al., 2022). During the larval period, a decrease in ATP levels and oxygen consumption was detected while UV radiation exists in the incubation tubes (Leung et al., 2013). Here, we searched the survival rates in different lights from the nematodes' hatching phase to their final destination. Our results were similar to the literature in terms of the short life spans we obtained under red and blue light (De Magalhaes Filho et al., 2018). Apart from that, we noticed that the decrease in the number of individuals of the nematodes exposed to green light occurred much more later in terms of phase than the other light colours; this phenomenon is also valid for continuous light and darkness. Studies on plants claim that green light has positive effects on the growth of some parts of plants (Johkan et al., 2012) pigment is involved at this situation, but we can think analogous mechanism according to Caenorhabditis elegans organization could also have been happen. They felt they were in a less stressful environment; perhaps this result was due to the response of the cells in the epidermal layer to this wavelength of light or the fact that this colour of light was close to natural daylight.

It is still a mystery why nematodes avoid certain colours of light. The fact that they do not have real eyes means that only the light source can stimulate the receptors in the skin. This leads us to prepare a working plan integrated with the skin in future light experiments.

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Conflicts of Interest

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research report.

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