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SHORT COMMUNICATION

Low intensity light effects on survivability, biomass, tissue protein and enzyme activities of the earthworm *Eudrilus eugeniae* (Kinberg)

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Abstract

The biological activities of invertebrates are influenced by environmental factors. Surface feeding soil animals such as epigeic earthworms are likely to be influenced by the type and intensity of light unlike deep dwelling species which live in dark. This study reports the effects of low intensity light exposures on the survivability, biomass, vermicomposting efficiency, tissue protein, lipid peroxidation and activities of three stress enzymes, acetylcholinesterase, lactate dehydrogenase and catalase of the vermicomposting earthworm, *Eudrilus eugeniae.* Consistent exposure of the animal to low intensity white, blue, green and red LED lights for 42 days in semi-decomposed organic substrate indicated decreased protein level, increased lipid peroxidation and enzyme activities in the animal with respect to those kept in dark. The study further indicated that darkness favours survivability, biomass gain and vermicomposting efficiency in this earthworm.

Key Words: earthworm; *Eudrilus eugeniae;* light; biomass; protein; enzymes

Introduction

The lives of many animals including the invertebrates are influenced by light. It has been reported (Nuutinen *et al*., 2014) that the native dew earthworm *Lumbricus terrestris was* most active under ambient light. Earlier studies (Von Frisch and Kupelwieser, 1913) on *Daphnia* had indicated that the animal swims towards red light source and away from a blue light source. The onychophoran *Euperipatoides rowelli* was not attracted by light but instead significantly avoided illumination with wave lengths ranging from UV to green light (Beckmann *et al*., 2015). It has been further reported that sensitivity to blue light is wide spread among invertebrates with monochromatic vision which has been attributed to the maximum distribution of energy of solar radiation at 480 nm (Menzel, 1979; Bowmaker and Hunt, 1999; Wang *et al*., 2003; Kelber and Roth, 2006).The nematode *Caenorhabtidis elegans* when exposed to high energy blue light indicated stress with a spurt in the production of Reactive Oxygen Species (ROS) (Abdel-Rahman *et al*., 2017). Among the invertebrates, behavioural and physiological responses of annelids to light intensity and colours have not been adequately investigated.

Earthworms are extremely sensitive to environmental changes and have been widely used as model bioindicators. They have also been proved useful to evaluate soil contamination due to elevated concentrations of xenobiotics (Samal *et al.,* 2017, Nayak *et al.*, 2018). The effects of environmental factors such as soil moisture, pH, pesticides, animal manure and temperature on the bioactivities of earthworms had been studied earlier by several workers (Grant, 1955; Roots, 1956; Barker, 1959; Lee, 1959; Wheatley and Hardman, 1968; Madge, 1969; Reynolds, 1973; Edwards and Lofty, 1977; Viljoen and Reinecke, 1992). Mishra *et al.* (2018) have reported that *Eudrilus eugeniae,* exposed to variable temperatures for different time durations exhibited significant alteration in tissue protein and activities of some stress enzymes.

Earthworms show unconditioned response to light which is a complex function of a number of variables including light intensity and number of prior presentations. *Eisenia fetida* when exposed to UV radiation shows depressed growth rate with reduction in fecundity, also decreased cocoon fertility by as much as 70% (Hamman *et al.,* 2003). Owa *et al.* (2008) evaluated the suitability of various light colours on an African species of earthworm *Hyperiodrilus africanus*. Results indicated that red

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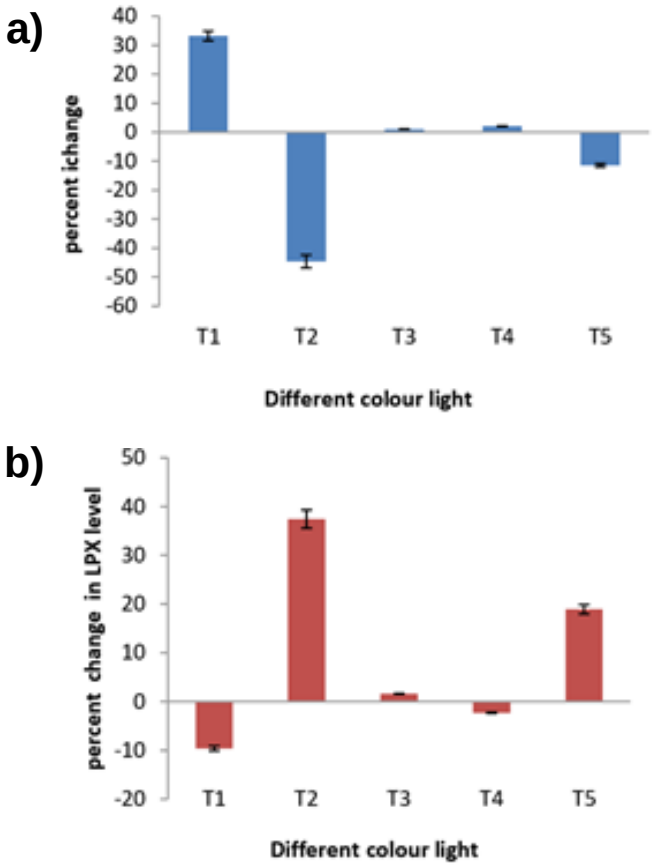

Fig. 1 Percent change in biochemical parameters of *E. eugeniae* with different light exposures. a) Protein content b) Lpx. The bars in graph indicate Mean ± SD values. T1 (No light/dark), T2 (White light), T3 (Blue light), T4 (Green light), T5 (Red light)

light was the most suitable which enhanced the bioactivity of the animal relative to white, blue and green lights. *E. eugeniae,* a detritus feeder and common vermicomposting earthworm is native to Africa but now widely distributed in certain regions of the tropics and subtropics including the agricultural fields in India and Sri Lanka (Sivasankari *et al*., 2013). With the above back drop, this experiment was designed to study the effects of low intensity lights of various colours on the survivability, biomass, tissue protein, lipid peroxidation and activities of the enzymes, acetyl cholinesterase, (AChE), lactate dehydrogenase (LDH), catalase (CAT) of *E. eugeniae*.

Materials and methods

Experimental set up

The earthworm *Eudrilus eugeniae* was procured from the vermiculture unit of the Government Quality Control Laboratotry, Odisha, India and then acclimatized in semi-decomposed cattle dung in a rectangular earthen pot (40 cm × 40 cm) in dark for 7 days. Then earthen pots of size (30 cm × 30 cm) were labelled as T1 (No light/ dark), T2 (White light), T3 (Blue light), T4(Green light), T5 (Red light) respectively as treatment pots. The treatment pots for each light source was taken in triplicate. Each pot was filled with 1 kg of semi decomposed cattle manure. Twenty clitellated pre weighed earthworms of identical size were transferred to each treatment pot. Thermocol sheets (Thick sheets made of pulp) were used to cover the pots. 0.5 watt LED lights of white, blue, green and red colours were fixed at the center of the covered thermo cool sheet on the inner side for uniform illumination of the treatment pots except T1, taken as control with no light source. On the day 1, one earthworm was randomly sampled from each treatment pot for biochemical analysis. This procedure was repeated every seventh day up to 42 days.

Biochemical analysis

For biochemical studies the earthworms were washed properly with distilled water after removal of the gut content and then cut into small pieces. The whole body tissue was homogenized with phosphate buffer (in 1:4 ratio, pH 7.4, 0.05 M) using a tissue homogenizer (REMI) and then centrifuged at 10,000 rpm for 10mins using a table top refrigerated centrifuge (REMI). The supernatant was

collected and stored at -20 °C in a deep freezer (Celfrost) for further use. Lowry's method (Lowry *et al.,* 1951) was followed for estimation of protein at 700 nm by taking bovine serum albumin as standard. The level of lipid peroxidation (LPX) in the tissue sample was measured as malondialdehyde (MDA) at 532 nm in an acidic medium according to the method of Ohkawa *et al.* (1979). LDH was determined in a medium containing phosphate buffer (pH 7.4, 0.05 M), 7.5 mM NADH and 30 mM sodium pyruvate at 340 nm as per Cabaud and Wroblewski (1958). The activity of AChE was determined through Ellman *et al*. (1961) assay by taking 2 mM DTNB and 1 mM Acetylcholine iodide substrate. CAT assay was done as per Cohen *et al.* (1980) at 340 nm using UV-Vis Spectrophotometer (Systronics). Protein was expressed in mg/g tissue, LPX in nmol/mg protein whereas all the enzymes were expressed as U/mg protein. Enzyme kinetics was done for each enzyme by calculating Km value from Line-Weaver Plot. The reciprocal of activity of LDH was plotted against the reciprocal of 5 different concentrations of substrate (NADH) such as 0.04, 0.09, 0.13, 0.19 and 0.25 mM. Kinetics activities were measured by taking reciprocal of 5 different concentrations of substrate (0.02, 0.04, 0.06, 0.08 and 0.1 mM) for AChE and 4 substrate concentrations (0.06, 0.18, 0.22, 0.27 mM) for catalase.

Earthworm biomass, survivability and vermicompost yield

On the day 42, the surviving earthworms from each treatment pot were collected, counted and weighed for recording the final biomass and survivability. Percent survivability and change in biomass were assessed with respect to the number and biomass of the earthworms released at the beginning of the experiment. Percent vermicompost production was calculated with respect to the quantity of organic substrate. Statistical analysis of the data for ANOVA was done with the help of SPSS 6.0 software.

Results and discussion

The amount of tissue protein indicated a non-significant variation between treatments. Fig. 1a presents the percent change of protein over the experimental period of 42 days. Maximum variation was observed in T2 with 44% decrease in protein level followed by T5 with 11%, on day 42. The protein level increased by 33% with respect to T1. However T3 and T4 did not show much variation in protein levels. Highest protein level (268.25 mg/g tissue) was observed in T4 on the 28th day and the minimum (92.49 mg/g tissue) in T5 on the 21st day.

Although variation was observed in the LPX levels of the earthworms between treatments it was not statistically significant.T2 showed a maximum percent increase of 37.42% in the LPX level. A decrease of 9% in LPX level was observed in T1. T5 showed 18% increase in the level of LPX (Fig. 1b). Minimum LPX of 0.0184 n mol/mg protein was recorded in T1 on the 28th day. LPX was found to be maximum (0.263 n mol/mg protein) in T2 on the 28th day of observation.

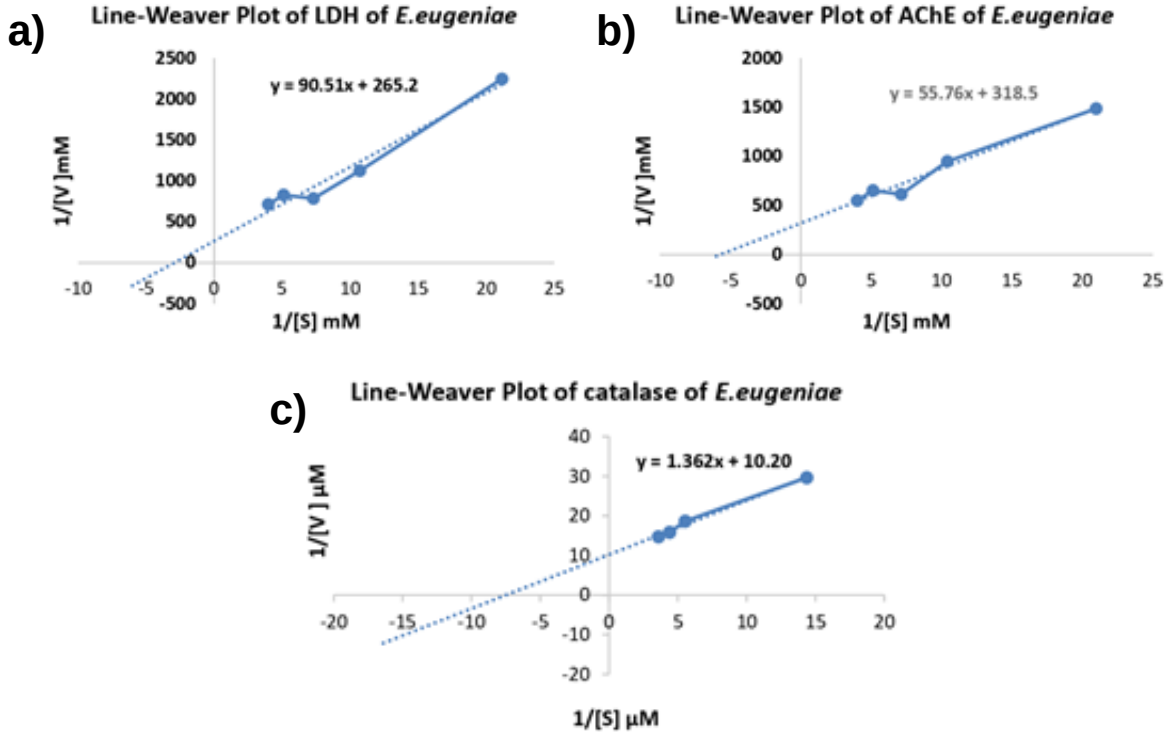

Fig. 2 Line-Weaver Plot indicating Enzyme kinetics of a) LDH activity b) AChE activity and c) catalase activity in *E. eugeniae*

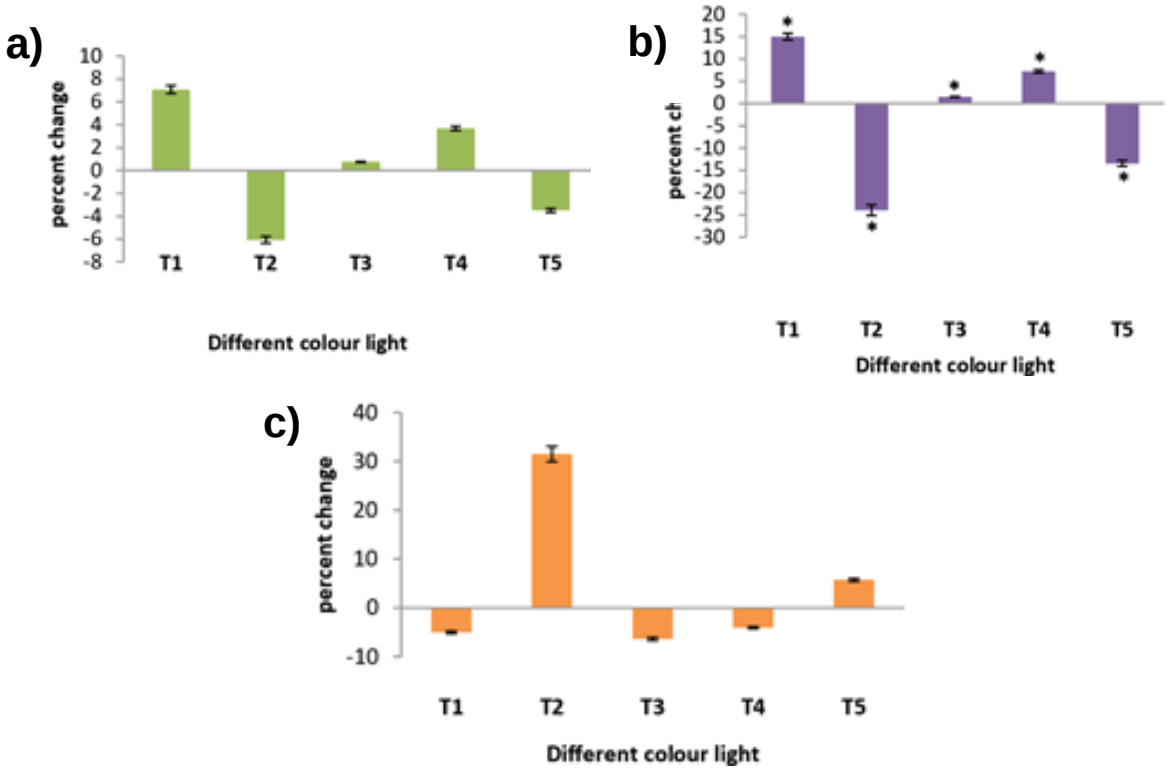

Fig. 3 Percent change in biochemical parameters of *E. eugeniae* with different light exposures. a) LDH b) AChE and c) catalase . The bars in graph indicate Mean ± SD values. The star mark "*" indicates significant variation, $p < 0.05$. T1 (No light/dark), T2 (White light), T3 (Blue light), T4 (Green light), T5 (Red light)

The results of enzyme kinetics for LDH, AChE and catalase were demonstrated in Fig 2. Km value of LDH, AChE and catalase were calculated from the Lineweaver-Burk plot. The values for LDH, AChE and catalase in *E. eugeniae* were found to be 0.34 mM, 0.17mM and 3.92µM respectively.

In T1 the percent change in LDH activity was found to be maximum with 7% increase. However, T2 showed 6% decrease in this enzyme activity (Fig.3a). T3 did not show much variation in the activity over the experimental period. T1 showed the maximum LDH value of 0.247 U/mg protein on the day 1. Substantially lower activity of 0.011 U/mg protein was observed in T2 on the 35th day. Statistical analysis however did not indicate significant variation in LDH activity between treatments.

AChE activity (Fig. 3b) showed a wide variation among different treatments. While 23% and 13% decreased activity were observed in T2 and T5, T1 showed 15% increase in its activity over a period of 42 days. The maximum AChE activity (0.147 U/mg protein) was recorded in T4 on the 28th day and minimum activity (0.007 U/mg protein) in T5 on day 42. Significant variation ($P<0.05$) in the AChE activities was observed in the earthworm between treatments.

The percent change in CAT activity over the period of 42 days is presented in Fig 3c. The percent change in the enzyme activity was the highest in T2 with 31% increase over the experimental period. T5 also indicated an increase of 5% during the same period. T1, T3 and T4 showed a decrease in activity by 5%, 6% and 4% respectively. The minimum CAT activity (0.040 U/mg protein) was observed in T1 on day 1 and the maximum (0.233 U/mg protein) in T2 on the day 28. The variation in CAT activity between treatments however was not statistically significant.

The survivability rate was found to be maximum of 85% in T1 followed by T3 and T4 with 70%and 60% respectively. T2 showed a minimum percent survivability of 20%. Results showed that darkness was the most suitable environment for survival of the earthworm (Fig. 4a). A maximum of 57% increase in biomass was observed in T1 and maximum decrease in T2 over the experimental period. T3 and T4 did not show noticeable variations (Fig. 4b). This indicates that the earthworms were most stressed under white light with increased loss in biomass and underwent the minimal stress at dark with increase in biomass. The maximum earthworm cast was observed in T1 (87.5%) and the minimum (12.5%) in T2. This signifies that earthworms in dark were the most efficient in converting the organic substrate into vermicompost whereas earthworms exposed to white and red lights were the least efficient (Fig. 4c).

Earthworms in general are highly sensitive to environmental changes and indicate wide fluctuations

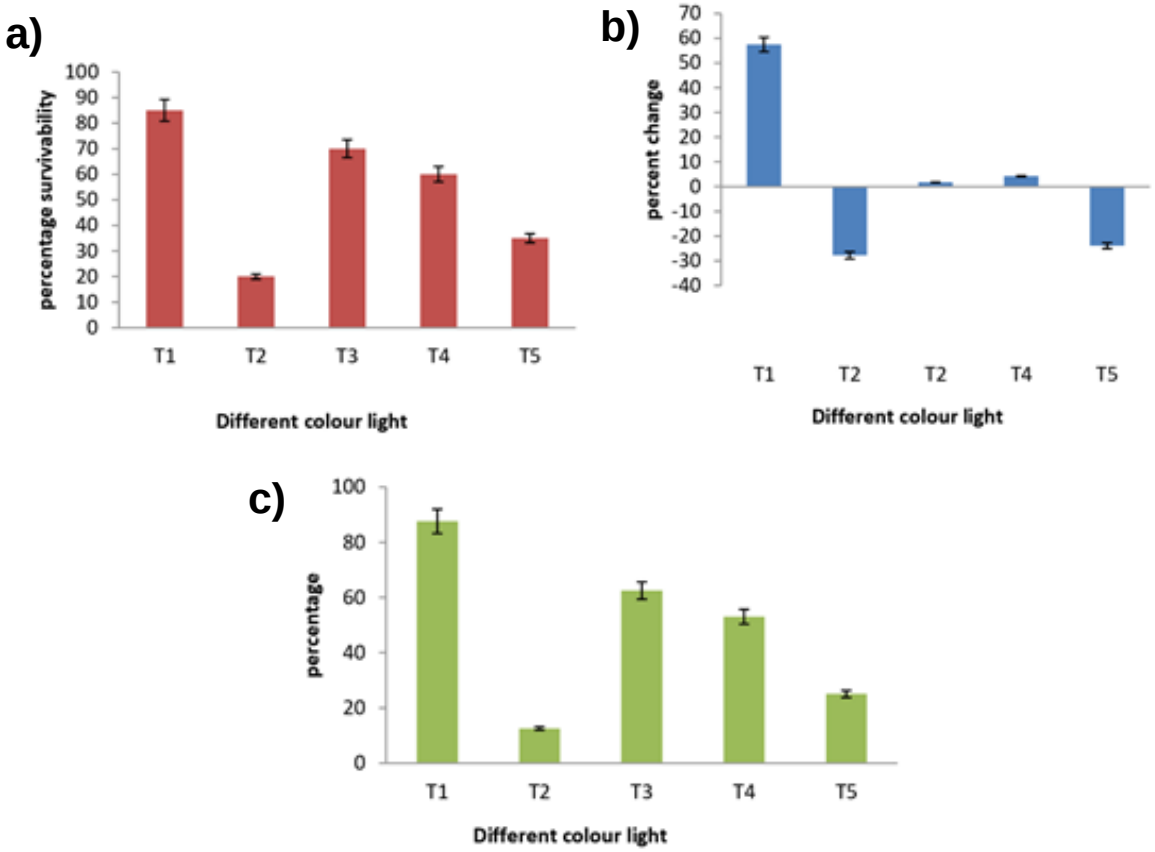

Fig. 4 Percent a) Suvivability, b) biomass, c) vermicompost yield of *E. eugeniae* with different light treatments. The bars in graph indicate Mean ± SD values. T1 (No light/dark), T2 (White light), T3 (Blue light), T4 (Green light), T5 (Red light)

in their number, biomass and metabolism in response to alterations in soil chemical quality. *E. eugeniae*, because of its epigeic nature is likely to be influenced by variations in ecological factors such as light and temperature (Mishra *et al.,* 2018). In the present study, a variation was observed in vital biochemical parameters such as protein, LPX, activities of LDH, AChE and catalase in the earthworm exposed to low intensity LED lights with darkness as control. Owa *et al.* (2008) conducted an experiment to evaluate the suitability of various light colours on an African species of earthworm *Hyperiodrilus africanus* and found the bioactivity and vermicompost production were maximal in earthworms exposed to red light relative to dark, blue, green and white lights. However, our results indicated that, differently from *H. africanus,* the most favoured condition for all the parameters evaluated in *E. eugeniae* is complete darkness.

Saint-Denis *et al.* (1998) have worked on four antioxidant enzymes *i.e*., catalase (CAT), glutathione peroxidase (GPX) , glutathione reductase (GR) and glutathione-S-transferase (GST) in earthworm *E. fetida* exposed to different concentration hydrogen peroxide (H_2O_2). They have reported a significant relation between stress inducing hydrogen peroxide and the enzyme activities. Hamman *et al.* (2003) reported that *E. fetida* when exposed to UV radiation, showed depressed growth rate with reduction in fecundity, also decreased cocoon fertility by as much as 70%. Our results corroborate these earlier findings which support the hypothesis that earthworms in general are photophobic and perform their maximal bioactivity in dark. Light even at low intensity increase the physiological stress on this earthworm resulting in lesser survivability, biomass, vermicomposting efficiency. Light stress could also cause depletion in protein, metabolic enzyme activities with enhanced lipid peroxidation. This finding is supported by the observations of Ravikiran and Aruna (2010) who have reported that *E. eugeniae* shows age-related variations in these parameters with higher LPX in aged earthworms relative to young ones. Jayanti *et al.* (2016) have made a comparative study of various biochemical responses in three earthworm species exposed to pesticide and metal contamination in soil. They have observed that when the earthworms *E. eugeniae*, *Perionyx ceylanensis* and *Perionyx excavates* were exposed to rising concentrations of the pesticide carbaryl and the heavy metal lead for different periods of time, there was an initial increase in protein content in response to low pesticide and

metal concentrations. When the concentrations increased there was subsequent decrease in tissue protein level in all the three earthworms tested. Also the present study indicated a depleted protein level in earthworms exposed to lights relative to dark. Abdel-Rahman *et al.* (2017) conducted an experiment on the nematode *C. elegans* to study the impact of exposure to light emitting diode (LED) domestic lighting and found that high-energy blue light (blue and black emitting lights) induces stress in the animal, affecting egg hatching, development, population, progeny, locomotion and survival. The stress response was also associated with the production of reactive oxygen species (ROS). Our results are in line with these findings. Mishra *et al.* (2018) have reported that *E. eugeniae* exposed to variable temperatures from 4 °C to 40 °C for different time durations exhibit significant alteration in tissue protein and activities of stress enzymes. Light exposure like non optimal temperature, could cause stress on this earthworm thus influencing its survival and bioactivity. It is further established that different light colours could affect the earthworm differentially.

On the whole, our data indicate that *E. eugeniae* is most active under dark condition with maximum tissue protein and minimal stress enzyme activities. Darkness too favours maximal survivability, biomass gain and vermicompost production. White and red lights exert the maximal stress resulting in an increase in LPX level and CAT activity. Blue and green lights are relatively less stressful in comparison to red and white lights. Behavioural and physiological responses of other epigeic species of earthworms to light colors and intensity need further investigation.

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