

# EFFECT OF TEMPERATURE ON REGENERATION OF *Eisenia fetida*

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## ABSTRACT

Earthworms are eucoelomate, vermiform and metamerically segmented animals belonging to Phylum Annelida. They are a powerful model organism for use in studying the mechanism of regeneration. Regeneration is a complex phenomenon to restore lost or damaged parts. Such kind of regeneration is naturally vested in earthworms and regeneration capability varies among different species. In this present study *Eisenia fetida* species was taken. Groups of earthworms were kept in different temperatures and their regeneration capability was examined. The earthworms were exposed to three different temperature ranges: below 24°C, 24°C - 30°C, and above 30°C. The earthworms those were exposed to low (<24°C) and high (>30°C) temperatures showed decreased rate of regeneration. This shows the influence of environmental factors on regeneration rate.

**Keywords:** Earthworms, Eucoelomate, Vermiform, Annelida, Model organism, Regeneration, *Eisenia fetida*, Environmental factors.

## 1. Introduction

Earthworms are hermaphrodite, bilaterally symmetrical, segmented worms bearing setae on all segments except the first two (Edwards *et al.*, 2022). They are scientifically classified under the phylum Annelida belonging to the class Oligochaeta (Yvonne *et al.*, 2016).

Earthworms are considered as ecosystem engineers, providing a variety of imperative ecosystem functions and services such as nutrient cycling, decomposition and climate regulation (Helen R. P. Phillips *et al.*, 2021). Earthworms are essential soil organisms in maintaining soil fertility and thus regarded as biological indicators of soil fertility and commonly known as farmer's friends (Yvonne *et al.*, 2016).

The richness of earthworms in the soil can be affected by several factors including temperature, moisture, soil pH, aeration, carbon dioxide, soil type, organic matter, food supply and the interactions between all these factors (Edwards and Bohlen, 1996). The activity, metabolism, growth, respiration, reproduction and regeneration of earthworms are greatly affected by temperature and they can be killed by temperature outside their survival limits. Therefore an optimum level of temperature is required for various earthworm activities in common species (Edwards and Bohlen, 1996).

Regeneration is a natural process of restoring or replacing lost or damaged parts. Now-a-days scientists are studying regeneration for its great possible potential uses in medicine. Earthworm, *Eisenia fetida* has a good regenerative capacity and has been used in many studies to understand the regenerative process. In earthworms, it is a continuous or sequential process starting with blastemal formation by dedifferentiation and followed by redifferentiation to restore the lost or damaged parts. Regeneration efficiency is dependent on the position of amputation and site specific. Therefore the growth and regenerative potential were compared and correlated. Environmental factors such as temperature, pH and nutrition have a great effect on regeneration capacity (Ray Subarna *et al.*, 2020). In this study we found the optimal temperature range that showed the highest regenerative capacity by exposing earthworms to three different temperature ranges. Earthworms cultured at 25°C regenerated faster as compared to those that were cultured at 20°C and 30°C.

## 2. Methodology

The experiment was conducted to study and examine the effect of temperature on regeneration and regeneration percentage of earthworm, *Eisenia fetida* outside the laboratory conditions. Earthworms were procured from a vermiculture unit. Then 30 healthy earthworms of approximately equal size were acclimatized. Using fine sterilized blade all the 30 earthworms was amputated. The earthworms used were not anaesthetized for amputation. Three groups of 10 earthworms were kept in three different boxes. Those boxes were filled with dark garden soil, and some amount of compost and manure. Three boxes were exposed to three different temperature conditions. The moisture level was maintained throughout by regular sprinkling of sufficient amount of tap water. They were also regularly fed by dry leaves, dried vegetable waste etc.

Experiment was conducted in May and the ambient temperature was above 30 °C. Group A was maintained in ambient temperature. Group B was maintained in a temperature range of 24°C – 30°C in an air conditioned room and group C was kept in a temperature below 24°C by maintaining the AC temperature. The experiment was carried out till 25 days, after that the effect of temperature on regeneration and regeneration percentage were examined.

Regeneration percentage was calculated using the formula given:

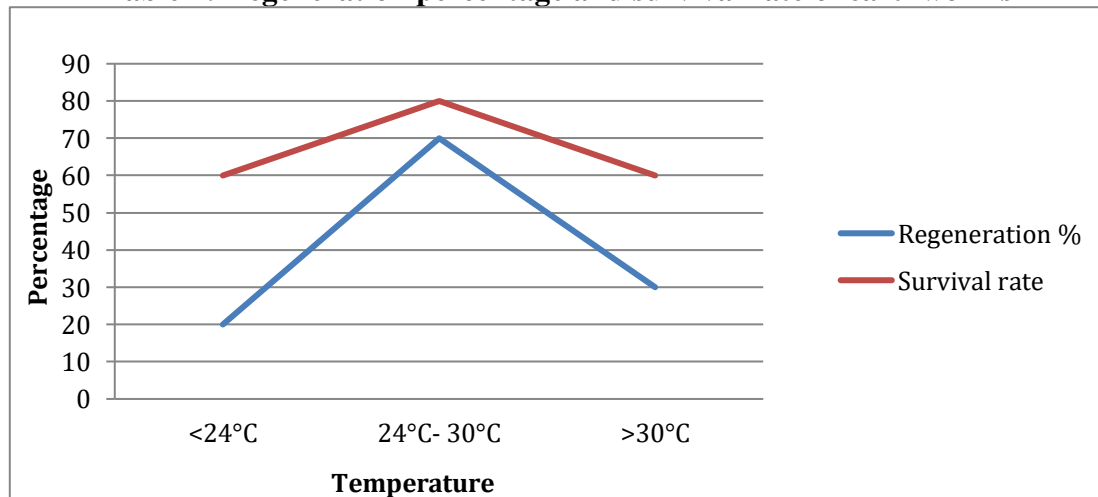
$$\text{Regeneration \%} = (\text{Number of earthworm regenerated} / \text{Total number of earthworms}) * 100$$

## 3. Results and Discussions

In this study, the influence of temperature on *Eisenia fetida* was studied. The groups of earthworm, *Eisenia fetida* were kept in different temperature like <24°C, 24°C – 30°C, >30°C and the regeneration percentage was 20%, 70% and 30% respectively. The earthworms those were exposed to low (<24°C) and high (>30°C) temperature showed decreased rate of regeneration. But group B (24°C – 30°C), regeneration percentage was 70% after 25 days. The earthworms showed more survival and regeneration rate at 24°C – 30°C. The fluctuations in the regeneration capacity of earthworms at different temperatures show that earthworms require an optimal temperature for survival, regeneration and other physiological activities. Hence a temperature range of 24C- 30C is the ideal temperature for the earthworm to activate its regenerative function.

| Group                  | A (<24°C) | B (24°C – 30°C) | C (>30°C) |
|------------------------|-----------|-----------------|-----------|
| No.of worms used       | 10        | 10              | 10        |
| No. of worms survived  | 6         | 8               | 6         |
| No.of worm regenerated | 2         | 7               | 3         |
| Regeneration %         | 20%       | 70%             | 30%       |
| Survival rate          | 60%       | 80%             | 60%       |

**Table 1: Regeneration percentage and survival rate of earthworms**



**Graph 1: To show the effect of temperature on regeneration and survival rate of earthworms**

Regeneration is a natural phenomenon in earthworms. They are considered as regenerative models due to the presence of metameres and segmentation. Different organisms follow different kind of regenerative pathways. Earthworms are particularly well suited to study regenerative capacity in vertebrates because they undergo a regenerative path of dedifferentiation and redifferentiation at the amputation sites (Myohara, M *et al.*, 1999). Certain environmental factors such as temperature have a great effect on regeneration and survival. Temperature is one of the most important factors for most of the biological processes. The overall range of temperature that support life on Earth ranges from -1.8C in polar regions to around 113C in extreme high temperature regions (Stetter, K O., 2006). Most organisms have their specific temperature range which is optimal for their growth; this is because even small changes in the temperature can lead to great changes in metabolism of animals. The regeneration process in *Eisenia fetida* begins with wound healing and blastema formation at the amputaion site which is then followed by head or tail bud formation. After the formation of blastema, segmentation of the regenerative parts occurs and is terminated after a certain number of days. The occurrence of segmentation and termination is also found to be different in different cases and conditions (Jamieson *et al.*, 1981).

Nagavallema *et al.* (2006) reported that earthworms can tolerate temperatures ranging from 0 to 40°C but regeneration efficiency is more at 25 to 30°C. Neurosecretory pathways are also important in initiating the regeneration process in *Eisenia fetida* (Moment, G.B., 1953). Temperature plays an important role in neurosecretory activity of *Eisenia fetida*, which was affected by both cold and warm temperatures. This indirectly affects the regeneration, as the intensity of neurosecretory material is lowered due to temperature variations (Ajit Wakale and Suresh Kulkarni., 2021). The neurosecretory cells in the supraoesophageal ganglia produces clitellum factor which plays a role in sexual reproduction and rates of regeneration (Bilej, M., 1994). Perturbations in the temperature may induce heat shock response pathway and also have an influence in regeneration pathway.

## CONCLUSION

This study confirms that the temperature and moisture strongly influence the survival and regeneration capability of earthworms. Temperature is one of the most important factors for most of the biological processes. Most organisms have their specific temperature range which is optimal for their growth; this is because even small changes in the temperature can lead to great changes in metabolism of animals. The temperature mainly acts on regeneration prompting hormone, neurosecretory cells and neurotransmitter inhibitor. The neurosecretory materials of earthworms and coelomocyte have indirect and direct effect on regeneration. Earthworm shows sluggish movements and body lesions due to alteration in temperature that affects the earthworm. Therefore earthworms require an optimum and favorable conditions for its normal function.

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