



EFFECT OF TEMPERATURE ON CAUDAL REGENERATION OF EARTHWORM, *EISENIA FETIDA*

Ajit Wakale^{1*} and Suresh Kulkarni²

¹Department of Zoology,
Jawahar Arts, Science and Commerce College,
Anadur, Dist. Osmanabad (M.S.), India

²Department of Zoology,
Adarsh Mahavidyalaya Omerga, Dist. Osmanabad (M.S.), India

*Corresponding author: ajit.wakale316@gmail.com

Article Info:
Research Article
Received
15.08.2021
Reviewed
31.08.2021
Accepted
10.09.2021

Abstract: Earthworms are renowned for their ability to regenerate lost parts. The groups of earthworm, *Eisenia fetida* were kept in different temperatures like 20°C, 25°C, 30°C and 35°C and their regenerating efficiency was examined. After 30 days, authors found 44.44%, 66.66%, 66.00% and 50.00% regenerating efficiency respectively with the control group as 70.00%. The earthworms exposed to low (20°C) and high (35°C) temperatures, showed decreased rate of caudal regeneration as compared to other experimental and control groups.

Keywords: Caudal regeneration, Earthworm, *Eisenia fetida*, Environmental factors.

INTRODUCTION

Earthworms are eucoelomate, vermiform, metamerically segmented animal belong to phylum Annelida (Verma and Prakash, 2020). Earthworms are significant biotic resources having antioxidant activity and play a significant role in agro ecosystems (Deswal *et al.*, 2020). A balanced ecosystem is needed for the survival of entire biotic components including humans (Verma, 2017; 2018). The earthworms can live in cold temperature by hibernating or burrowing deeper into soil, often lose weight or enter diapause when soils are too dry (Booth *et al.*, 2000; Holmstrup, 2001).

Earthworms are the friends of farmers as they increase the soil fertility. Vermicompost obtained with the help of them has many benefits to soil, plants and the environment. Increasing global temperatures will change the composition and

functioning of ecosystems (Harte and Shaw, 1995; Melillo *et al.*, 2002; Lambrecht *et al.*, 2007, Prakash and Srivastava, 2019). Earthworms are known to constitute more than 80% of the soil invertebrate biomass in subtropical and tropical, as well as in temperate zones (Kale, 1997; Nainawat and Nagendra, 2001). Earthworms have the ability to improve soil physical structure, contribute to the breakdown of organic matter and release plant nutrients (Edwards and Bohlen, 1996).

The activity, metabolism, growth, respiration, reproduction and regeneration of earthworms are greatly influenced by temperature and they can be killed by temperatures outside their survival limits. The earthworm population in soils is declined due to high surface temperature, absence of ground cover, and dry soils. These are much more limiting to them than low

temperatures and waterlogged soils (Edwards and Bohlen, 1996).

Discovered centuries ago, regeneration is a biological phenomenon that attract even the common people and it is a fascinating field in novel biology that aim to solve the impossible lost structure to regain again. Such type of regenerative ability is naturally vested in earthworm. Fragmentation in Annelida follows several different approaches; one of the well-known ways is spontaneous fragmentation or autotomy that occurs in an ordinary situation for the earthworm in the soil (Tian *et al.*, 2000). In induced fragmentation, when decapitation and electric shock are done, autotomy starts after 24 hours (Tian *et al.*, 2000; Inomata *et al.*, 2000). In autotomy (induced or spontaneous) in the fission zone, the circular body wall muscles contract and as a result the worm divides into two halves (Yoshida-Noro *et al.*, 2000). To avoid autotomy in the samples, the cuts were performed between 20 and 21 segments from the head and 20 segments from the tail. In this fragmentation, the importance of seven segments from the head, which is considered as a head part of the body with the reproductive organs for regeneration, were taken in to consideration, even more the existence of growth zone and pygidium in the posterior region was considered in order to evaluate the external factors in renewing a lost part. A little comprehensive work has been carried out on the regeneration in the earthworm, *Eisenia fetida*. In the present exploration, authors studied the effect of temperature in the process of tolerance and regeneration.

MATERIALS AND METHOD

The experiment was conducted to examine the effect of temperature on regeneration and regenerating efficiency of earthworms, *Eisenia fetida*. Initially experimental group was maintained in beakers and 200 g wet garden soil was filled in the beaker for group (1) 20°C, (2) 25°C (3) 30°C and (4) 35°C temperatures and group (5) was served as control. Acclimatized 10 earthworms looking healthy and having approximately equal size and weight were selected. Usually 10 caudal segments amputated

of each earthworm using fine sterilized scissor under dissecting microscope and inserted in beakers the day of the experiment. For amputation, the earthworms used were not anaesthetized. Same procedure followed as per survivability experiment. All the experimental beakers were kept in B.O.D. incubator at respective temperatures and provide various temperature conditions. The moisture level was maintained throughout the study period by periodic sprinkling of adequate quantity of tap water. The experiment was carried out till 30 days, after that the effect of cold and hot temperature on regeneration and regenerating efficiency, development of new segments and physiology of earthworms, *Eisenia fetida* were examined. The counting of regenerated segments is easy due to their vascularized state, transparency and dimensions. Such counting, however, is possible up to a period of 30 days, after which new segments assume normal dimensions and colour. For calculating the number of segments regenerated, the earthworms were lightly anaesthetized (because of their extreme agility) in 0.5% ethanol (V/V) in tap water and were held on paraffin tray. Dissecting microscope and hand lens were used to confirm body structures that could not easily be seen with naked eye during the identification process.

The percentage of regenerating efficiency was calculated using the formula given:

$$\% \text{ of Regenerating efficiency} = \frac{A}{B} \times 100$$

Where, A= Number of worms regenerating more than 50% caudal segments and B= Number of regenerants.

RESULTS AND DISCUSSION

In the present work effects of temperature on earthworm, *Eisenia fetida* were studied. Differences between control and experimental groups of earthworms were compared and conclusion is drawn. During the experimental period, earthworms showed progressive signs and symptoms as mentioned.

The groups of earthworm, *Eisenia fetida* were kept in different temperatures like 20°C, 25°C, 30°C and 35°C and the result of regenerating efficiency was

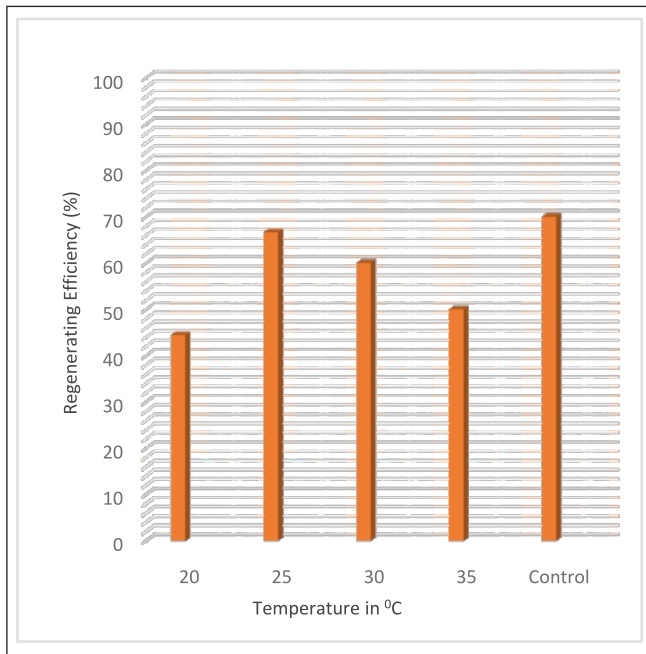
44.44%, 66.66%, 66.00% and 50.00% respectively. Further reduced regeneration rate at 20°C and 35°C indicates that the low and high temperature inhibit temperature tolerance and leads to physiological changes. The control group regenerating efficiency was 70.00% after 30 days (Table 1 and Graph 1). The earthworms showed more survival and regenerating efficiency at 25°C and 30°C temperature. However, the control group showed highest regenerating efficiency. The optimum temperatures were 25°C to 30°C for earthworms, *Eisenia fetida* for their natural habit and habitat. The difference in the regeneration characteristics of earthworms, *Eisenia fetida* at

different temperature confirms that the worms need an optimum temperature to survival, regenerating efficiency and their physiological activities.

The present study therefore finds that the temperature at a range from 25°C to 30°C is an ideal temperature to activate regeneration activity and lead to maximum regenerating efficiency. Authors noticed an influence of temperature on biological characteristics like survival, regenerating efficiency and their physiological activities in earthworm.

Table 1: Effect of temperature on the caudal regeneration in earthworm, *Eisenia fetida*.

	Temperature in °C				
	20°C	25°C	30°C	35°C	Control
Groups	1	2	3	4	5
Number of worms used	10	10	10	10	10
Number of worms survival after 30 days	10	10	10	09	10
Number of worms displaying regeneration	09	09	10	08	10
Number of worms displaying non vascular pygidium	01	01	00	01	00
Number of worms displaying vascular pygidium	00	00	00	00	00
Number of worms regeneration segments.					
1	01	00	00	00	00
2	01	00	01	01	00
3	01	02	02	02	01
4	02	01	01	01	02
5	01	02	01	02	02
6	01	02	01	01	02
7	01	01	02	01	01
8	01	01	02	00	01
9	00	00	00	00	01
10	00	00	00	00	00
Regenerating Efficiency	44.44%	66.66%	60.00%	50.00%	70.00%



Graph 1: To show the effect of temperature on the caudal regeneration of *Eisenia fetida*.

Earthworms have the power to regenerate the lost segments. As most of the important organs are concentrated at the anterior region, regeneration takes place at the posterior end more easily in comparison to anterior end. A worm is cut into two, the anterior half will generate a tail, but the posterior half usually cannot form the heart part. The animal is cut lengthwise; it dies, as regeneration does not take place in this case.

An earthworm is cut by a spade or bitten off by a bird, centipede or mole, it does not necessarily die, but can regenerate, the lost parts of the body in due course of time. The formation of albumen-filled cocoons for fertilization and development of eggs are obviously the adaptation for reproduction under adverse environments. After that, the experiment was conducted to examine the effect on caudal regeneration and the selected range were 20°C to 35°C. The data presented in table 1 showed that the caudal regeneration is affected by lower and higher temperatures *i.e.* 20°C and 35°C. The temperature lower than 20°C and higher than 35°C retarded the caudal regeneration. Jamshidi *et al.* (2014) reported the regeneration in *Aporrectodea caliginosa* is regulated by both internal and external environmental factors. It is significant that earthworms possess the temperature-dependent capacity to regenerate their grossly depleted

coelomocyte community after experimental extrusion, with amoebocyte numbers recovering within a few weeks whilst eleocyte numbers were fully recovered much later, depending on the ambient temperature (Homa, *et al.*, 2008; Klimek, *et al.*, 2012). Nagavallemma *et al.* (2006) reported that earthworms can tolerate temperatures ranging from 0 to 40°C but the regeneration capacity is more at 25 to 30°C. Some workers like Rao and Saroja (1963) reported that the amount of neurosecretory materials was not depleted during low temperature exposure. The temperature appears to play an important role in the neurosecretory activity of *Eisenia fetida*, which was affected by both cold and warm temperatures. This indirectly affects the caudal regeneration, as neurosecretory material intensities lowered due to temperature variations. The present experiment confirms that soil temperature and moisture strongly influence earthworm survival and regeneration activities.

In conclusion, authors noticed that the neurosecretory material of earthworms and the coelomocytes act in concern to regulate fundamentally important functions such as tissue regeneration. The temperature mainly acts on neurosecretory cells, regeneration prompting hormone (RPH) and neurotransmitter inhibitors. Predictable signs and symptoms such as sluggish movement's and bloody lesions are due to the changes of temperature that affects earthworms. Fluctuations in environmental temperature impair the activity of the earthworms concerned. In general, the earthworm, *Eisenia fetida* prefers optimum and favourable conditions for normal functioning.

REFERENCES

1. Booth L. H., Heppelthwaite V. and McGlinchy A. (2000). The effect of environmental parameters on growth, cholinesterase activity and glutathione S-transferase activity in the earthworm (*Aporrectodea caliginosa*). *Biomarkers*. 5 (1): 46-55. [10.1080/135475000230532](https://doi.org/10.1080/135475000230532).
2. Deswal P., Yadav Y., Khushbu and Shukla V. (2020). Evaluation of Antioxidant Activity of *Eisenia fetida*. *International Journal of Biological Innovations*. 2(2): 109-116. <https://doi.org/10.46505/IJBI.2020.2205>.

3. **Edwards C. A. and Bohlen P. J.** (1996). Biology and Ecology of Earthworms. 3rd Edition, Chapman and Hall, London. 426p.
4. **Harte J. and Shaw R.** (1995). Shifting dominance within a montane vegetation community: results of a climate-warming experiment. *Science*. 267(5199):876-880. 10.1126/science.267.5199.876.
5. **Holmstrup M.** (2001). Sensitivity of life history parameters in the earthworm *Aporrectodea caliginosa* to small changes in soil water potential. *Soil Biol. Biochem.* 33(9):1217-1223. 10.1016/s0038-0717(01)00026-8.
6. **Homa J., Bzowska M., Klimek M. and Plytycz B.** (2008). Flow cytometric quantification of proliferating coelomocytes non-invasively retrieved from the earthworm, *Dendrobaena veneta*. *Dev. Comp. Immunol.* 32 (1): 9-14. 10.1016/j.dci.2007.04.007.
7. **Inomata K., Kobari F., Yoshida-Noro C., Myohara M. and Tochinal S.** (2000). Possible neural control of asexually reproductive fragmentation in *Enchytraeus japonensis* (Oligochaeta, Enchytraeidae), *Invert Report. Develop.* 37: 35-42.
8. **Jamshidi P., Pishkahi Z., Karimzadeh L., Masoumehzaman Alizadehnohi M. and Nabiuni M.** (2014). The study of regeneration in posterior part of *Aporrectodea caliginosa*. *Journal of Medical and Bioengineering.* 3(1): 45-49. 10.12720/jomb.3.1.45-49.
9. **Kale R. D.** (1997). Earthworms and soil. *Proc. Nat. Acad. Sci. India.* 67: 13-24.
10. **Klimek M., Kruk J. and Plytycz B.** (2012). Restoration of coelomocytes in the earthworm *Dendrobaena veneta*. *Acta Biol. Cracov. Ser. Zool.* 54: 11-17.
11. **Lambrecht S.C., Loik M.E., Inouye D.W. and Harte J.** (2007). Reproductive and physiological responses to simulated climate warming for four subalpine species. *New Phytol.* 173(1):121-134. 10.1111/j.1469-8137.2006.01892.x.
12. **Melillo J.M., Steudler P.A., Aber J.D., Newkirk K., Lux H., Bowles F.P., Catricala C., Magill A., Ahrens T. and Morrisseau S.** (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*. 298(5601):2173-2176. 10.1126/science.1074153.
13. **Nagavallema K. P., Wani S. P., Stephane L., Padmaja V. V., Vineela C., Babu Rao M. and Sahrawat K. L.** (2006). Vermicomposting: Recycling wastes into valuable organic fertilizer. *SAT eJ* 2(1):1-16. <http://ejournal.icrisat.org/agroecosystem/v2i1/v2i1vermi.pdf>.
14. **Nainawat R. and Nagendra B.** (2001). Density and distribution of earthworms in different localities of Jaipur. *J. Eco-physiology.* 4: 9-13.
15. **Prakash S. and Srivastava S.** (2019). Impact of Climate Change on Biodiversity: An Overview. *International Journal of Biological Innovations.* 1(2): 60-65. <https://doi.org/10.46505/IJBI.2019.1205>.
16. **Rao K. P. and Saroja K.** (1963). Physiology of low temperature acclimation in tropical poikilotherms V. Changes in the activity of neurosecretory cells in earthworm, *Lampito mauritii* and evidence for a humoral agent influencing metabolism. *Proc. Indian Acad. Sci. B.* 58: 14-18.
17. **Tian G., Olimah J. A., Adeoye G. O. and Kang B. T.** (2000). Regeneration of earthworm populations in a degraded soil by natural and planted fallows under humid tropical conditions. *Soil Sci. Soc. Am. J.* 64 (1): 222-228. <https://doi.org/10.2136/sssaj2000.641222x>.
18. **Verma A.K.** (2017). Necessity of Ecological Balance for Widespread Biodiversity. *Indian Journal of Biology.* 4(2):158-160.
19. **Verma A.K.** (2018). Ecological Balance: An Indispensable Need for Human Survival. *Journal of Experimental Zoology, India.* 21 (1): 407-409.
20. **Verma A.K. and Prakash S.** (2020). Status of Animal Phyla in different Kingdom Systems of Biological Classification. *International Journal of Biological Innovations.* 2 (2): 149-154. <https://doi.org/10.46505/IJBI.2020.2211>.
21. **Yoshida-Noro C., Myohara M., Kobari F. and Tochinal S.** (2000). Nervous system dynamics during fragmentation and regeneration in *Enchytraeus japonensis* (Oligochaeta, Annelida). *Dev Genes Evol.* 210 (6): 311-319. 10.1007/s004270050318.