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Effect of moisture on caudal regeneration of earthworms, *Eisenia Fetida*

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Abstract
Earthworms are considered as the friends of farmers. Vermicompost obtained with the help of them has many benefits to soil, plants and in all to the environment. The groups of earthworms, *Eisenia fetida* were exposed to different percentage of moisture contents like 25, 30, 35, 40, 45 and 50 for caudal regeneration and their regenerating efficiency was 57.14%, 64.28%, 66.66%, 68.75%, 62.50% and 53.84% respectively and the control group regenerating efficiency was 70.58% after 30 days. When worms exposed to low (25%) and high (50%) moisture, showed decreased rate of caudal regeneration as compared to others.

Keywords: moisture, caudal regeneration, *Eisenia fetida*

Introduction
Terrestrial earthworms are well adapted to moist conditions. Explanations may be found in optimum ranges or species differences. The earthworms have 75% to 90% water of their body weight (Grant, 1955) [4]. Water constitutes 75-90% of the body weight of earthworms, so prevention of water loss is a major problem of earthworm survival. Nevertheless, they have considerable ability to survive adverse moisture condition, either by moving to more suitable area or by aestivating, if they cannot avoid dry soil. They can survive the loss of a large part of the total water contents of their bodies (Edwards and Lofty, 1977) [3]. *Lumbricus terrestris* can lose 70% and *Allolobophora chlorotica* 75% of their total body water and survive (Roots, 1956) [10].
Earthworms generally require the presence of adequate moisture for growth and survival, and also their activity is strongly determined by moisture content of the soil. This is mainly because they breathe through their epidermis (moist skin) and the blood capillaries on the surface should get sufficient moisture to perform their respiratory activity (Eckert and Randal, 1988) [2]. As a result, most earthworms are more active in moist soils than dry ones, and prevention of desiccation or water loss is the major factor in earthworm survival (Mary, 1982; Kretzschmar and Bruchou, 1991) [8, 5]. However, not all species of earthworms have the same moisture requirements. The present study was carried out and identified that the earthworm, *Eisenia fetida* as a novel model system to study the regeneration and their physiology.

Materials and Method
The experiment was conducted to see the effect of moisture contents on regeneration and their regenerating efficiency of earthworms, *Eisenia fetida*. The moisture-free soil collected from garden and used for experiment. The experimental group were maintained in 250 ml beaker. In each beaker water added in 50, 60, 70, 80, 90, 100 ml and thoroughly mixed with 200 gm soil and the beaker was filled for group (1) 25, (2) 30, (3) 35, (4) 40, (5) 45, (6) 50 percentage of moisture contents and group (7) was served as control. Acclimatized 20 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 beaker, the day of the experiment. For amputation, the earthworms used were not anaesthetized. Record of progress in each beaker was thus maintained. All the experimental beakers were kept in a laboratory and provide natural conditions. The experiment was carried out till 30 days. After 30 days to observed the effect of moisture contents on regeneration and their regenerating efficiency, development of new segments and physiology of earthworms, *Eisenia fetida*. 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 given formula.

$$\% \text{ of Regenerating efficiency} = \frac{\text{Number of worms regenerating more than 50\% caudal segments}}{\text{Number of regenerants}} \times 100$$

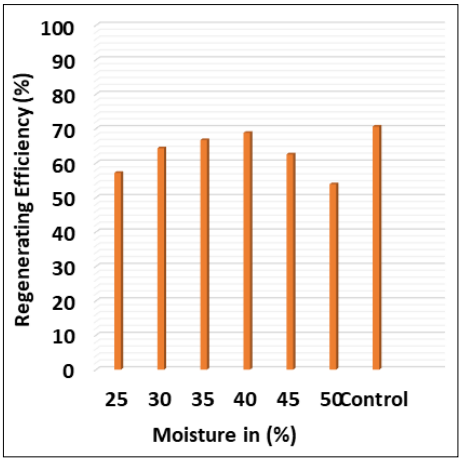
Observations and Results
In the present work on adult earthworm, *Eisenia fetida*, moistures and their effects were studied. If the conditions dry or highly wet they are coming outside of the soil they showed some changes such as coiling, curling and excessive mucous secretion with fast movements. Swelling of the clitellum was also seen after amputated the posterior segments. Extrusion of coelomic fluids resulting in bloody lesions within 48 hours of exposure was observed. Earthworms showed degenerative changes at the posterior

part. Disappearance of metameric segmentations and loss of pigmentations were observed. It was observed that in all the earthworms, the regenerating parts clearly found. The control earthworms showed excellent burrowing movements and exhibited no extra ordinary behavior. This suggests that moisture conditions is associated with behavior and physiology. It showed inhibitory effects on regeneration in earthworms.

The groups of earthworms, *Eisenia fetida* were exposed to different percentage of moisture contents like 25, 30, 35, 40, 45 and 50 for caudal regeneration and their regenerating efficiency was 57.14%, 64.28%, 66.66%, 68.75%, 62.50% and 53.84% respectively and the control group regenerating efficiency was 70.58% after 30 days (Table-1 and Graph-1). Their regenerating efficiency was more at 35-40% percentage of moisture contents in optimum moisture level and the control group compared with experimental groups for regenerating efficiency. The optimum moisture is 35 to 40% for *Eisenia fetida* for their natural habit and habitat.

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

	Moisture %						
	25	30	35	40	45	50	**Control**
Number of worms used	20	20	20	20	20	20	20
Number of worms survival after 30 days	17	17	18	19	19	17	19
Number of worms displaying regeneration	14	14	15	16	16	13	17
Number of worms displaying non vascular pygidium	02	02	02	02	02	02	01
Number of worms displaying vascular pygidium	01	01	01	01	01	02	01
Number of worms regeneration segments. 10 Segments amputated from each worm.							
1	00	00	00	00	00	00	00
2	02	01	01	01	02	02	00
3	02	02	02	02	02	02	02
4	02	02	02	02	02	02	03
5	02	02	03	02	02	02	03
6	02	02	02	02	02	02	03
7	01	02	02	02	02	02	02
8	01	01	01	02	02	01	02
9	01	01	01	02	01	00	01
10	01	01	01	01	01	00	01
Regenerating Efficiency	57.14%	64.28%	66.66%	68.75%	62.50%	53.84%	70.58%



Graph 1: The chart has demonstrated the effect of moisture on earthworm, *Eisenia fetida* caudal regeneration

Discussion

Earthworms can survive the loss of a large part of the total water content of their bodies. *Lumbricus terrestris* can lose 70% and *Allolobophora chlorotica* 75% of their total body water and still survive (Roots, 1956) [10]. Moisture plays an important role in the earthworm, therefore in this investigation the effect of moisture on regeneration were studied and the soil moisture of 35-40% were optimum for good survival and which is good regenerating efficiency in the earthworms, *Eisenia fetida* (Table-1 and Graph-1).

Moisture plays an essential role in the earthworm life not only as an important factor in its embryogenesis and regeneration but also as an inevitable factor for breathing and excreting Sherman (2003) [11]. Therefore, in this investigation the effect of moisture on regeneration of the lost segment were studied and the 35-40% of moisture in the soil were defined as an optimum condition for reproducing the lost segment. On the contrary, the significant reduction has shown in the group of 25% and 50% moisture of the soil which, the rate of regeneration.

Kulkarni (1989) [6] studied the responses of neuroendocrine centers to some environmental factors like photoperiod, temperature, salinity, desiccation, starvation and to some pharmacological drugs. Csoknya *et al.*, (1996) [1] have reported the occurrence of Octopamine in the central nervous system of Oligochaeta. Patil (2002) [9] studied the responses of neuroendocrine centers to some agrochemicals like Ammonium sulphate, Urea, Suffala and Sampurna in the worm *Perionyx excavatus*. Kulkarni (1998) [7] observed and reported that minimum latent period of 24 hours is essential for the activation of brain neurosecretory apparatus, which in turn synthesized and secretes the regeneration prompting hormone (RPH). The endocrine status of brain as the source of RPH was histologically confirmed by the histomorphological changes in the brain neurosecretory cells during caudal regeneration.

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