Impact of Cold Stress and Toxicants on Urea Excretion of Earthworm

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Abstract— Urea excretion in P. posthuma was analyzed with reference to five different media like distilled water, NaCl, Na2CO3, CuSo4, ZnSo4, 1 hour after exposure. Earthworm excreted 76-83 mg/100 ml urea in distilled water at room temperature and 57.14mg/100ml of urea at 5-10˚c in another, 58.33 mg/100 ml urea was excreted in 1% NaCl solution at 5-10˚c and 55.55 mg/100 ml urea in 1% Na2CO3 solution at 5-10˚c, 54.54 mg/100 ml urea in CuSo4 solution which contain 0.001g of CuSo4 in 100 ml distilled water at 5-10˚c, 71.48 mg/100 ml urea was excreted in ZnSo4 at 5-10˚c. At 5-10˚c earthworm live for 1hour in distilled water, 1% NaCl and 1% Na2CO3, but exhibited discomfort after 1 hour the same solutions at normal temperature. In CuSo4 and ZnSo4 they were stressed after 15-16 minutes because of toxicity intolerance, decline in values of urea excreted under different stress situations was noted. Severe stress was caused by ZnSo4 and CuSo4 toxicants. This is probably due to alterations in body metabolism, this may affect growth, water and salt balance and reproduction of this detrivore. So, conservation of worms against toxicants is suggested for maintaining the soil fertile.

Key words: P. Posthuma, Toxicants, Cold Stress, Urea, Conservation

I. Introduction

Earthworms are more than fish bait, they are the main contributors to enriching and improving soil for plants, animals and even humans, they create tunnels in the soil by burrowing, which aerates the soil to allow air, water and nutrients to reach deep within the soil. Earthworms eat the soil and organic matter, after it is digested, the earthworm releases waste as castings, which contain many nutrients that the plant can use. Some people even use earthworm castings as garden fertilizer.The excretory system consists of small, coiled tubes with walls that are glandular and richly supplied with blood vessels, are known as nephridia. They occur in all the segments of the body starting from the third segment downwards, and are named according to their location in the body. Nephridial excreta improve soil fertility.

Integumentary nephridia, are located on the inner side of the body wall in all segments except the first two. Septal nephridia, are attached to both sides of the septa behind the 15th segment. Pharyngeal nephridia, these are located only in the 4th, 5th and 6th segment. (The septal nephridia may be considered as a typical nephridia for detailed description.) Most earthworms don't tolerate temperatures below freezing, nor do they tolerate high temperatures. Prolonged exposure to temperatures above 95°F kills them. They can move down into the soil to escape these adverse temperatures. Optimum temperatures are between 50 and 60°F.

Earthworms are distributed in agricultural fields and at neutral pH, but can tolerate a pH from 5.0 to 8.0. Quantity, quality, and flourish food influence earthworm populations. Earthworms consume organic residue. Residue with a high carbon to nitrogen (C:N) ratio is not very palatable, manure can help make it more palatable. In some cases, residue has to undergo some weathering before earthworms are able to digest it. Top soil dwellers need smaller particles than subsoil dwellers, which can use large leaves but surface dwellers use surface residue in soil.

II. Material and Method

Adult worms ranging in length from 8-10 cm and 14-15 cm and weight from 2-6 gm. were 15taken for the experiments. Worms were collected from Ambazari garden, Nagpur, acclimatized, washed and blotted to remove moisture on the surface of the body. The weight of the worm was taken using electronic balance.

The worms were taken in a group of three for assessing excretory products. Urea is separately estimated for each of the excretory products since earthworms are reported to survive in soil medium and it is easy for the sampling, research and experimental setup. After experiments the earthworms were released in soil.

5 ml of each of the experimental media were taken in 500 ml beaker. The worms were let into each of the media in groups of three and the excretory products were analyzed following the standard procedures. The study was conducted at 5-10˚c temperature. In order to study the changes in the excretory response in the experimental media, sampling was also done during the first hour of observation and at the end till the earthworms died in the toxic material.

To study the response to stress condition, the excretion was monitored at increased temperature (5to10˚c) inside thermacol box and frizzed jelly was used to maintain the lower temperature. Sampling was done at the end of 60 minutes and 18 to 20 minutes of exposure of stress. Sample were analyzed for urea.

III. Preparation of Chemicals

Reagent 1: Zinc sulphate solution: Crystalline zinc sulphate solution 1.56 gm + 16 ml Sulphuric acid 0.25N Reagent 2: 0.75 N Sodium hydroxide: 3 gm in 100 ml distilled water. Reagent 3: p-dimethylaminebenzaldehyde: 2.5gm in 125 ml of 96% alcohol and add 12.5 ml concentrated Hcl. Reagent 4: Urea: 0.15 mg dissolved in 12.5 ml distilled water.

IV. Procedure

24 ml of first reagent was taken in 100 ml bottle to this add 3 ml of mucus secretion. Shake bottle vigorously to obtained complete heamolysis. Add 3 ml of reagent 2 and shake it. Then it is filtered through filter paper (5,8,and 9) to remove protein precipitate. Take 100 ml of filtered solution in 25 ml of measuring flask and add 10 ml of reagent 3 and dilute it with distilled water up to the mark. After 10 minutes mixture is ready for readings.

V. Readings

The reaction were recorded using colorimeter at 420 nm. Take blank prepared in the same way as the reaction with 3 ml distilled water instead of 3 ml of mucous.The urea i.e standard reagent four is treated in the same way as mucous.If p, s and b are the extinctions of the sample, the standard and the blank respectively, the urea concentration was recorded 420 nm.

VI. FORMULA

$$\frac{100(p-b)}{(s-b)}\ \text{mg/100ml}$$

VII. OBSERVATION TABLE

	Distilled Water at Room Temp. (Control)	Distilled Water at 5-10˚c	NaCl in distilled water	Na_2CO_3 in distilled water	$CuSO_4$ in distilled water	$ZnSO_4$ in distilled water
Length	14-15cm	8-10 cm	8-10 cm	8-10 cm	8-10 cm	8-10 cm
Weight	5.91 gm	2.41 gm	2.08 gm	2.14 gm	2.29 gm	2.31 gm
Temperature	29˚c	5-10˚c	5-10˚c	5-10˚c	5-10˚c	5-10˚c
Readings mg/100 ml	76-83	57.14	58.33	55.55	54.54	71.48

Table 1: showing values of urea excreted by P. posthuma under varying experimental conditions

VIII. RESULTS AND DISCUSSION

After 60 minutes of the experiment, the control worms excreted 76-83 mg/100 ml urea in distilled water at the normal room temperature. 57.14 mg/100 ml of urea in distilled water in cold stress i.e 5 to 10˚c temperature. We also studied the impact of cold stress under some conditions that is NaCl, Na2CO3, CuSo4, ZnSo4 in which worms excreted 58.33 mg/100ml urea in 1% NaCl at 5-10˚c, 55.55 mg/ 100 ml urea in 1% Na2CO3 at 5-10˚c , 54.54 mg/100 ml urea in CuSo4 concentration 0.001g in 100 ml distilled water at 5-10˚c, 71.48 mg/100 ml urea in ZnSo4 (see the graph). But worms showed severe discomfort after 18 to 20 minutes in CuSo4 and ZnSo4 and after 1 hour in other experimental conditions.

The present study demonstrates that worms Pheretima posthuma were unable to survive in cold stress but they excreted urea in stressed condition. The given amount of urea excreted by the worms clearly indicate that the worms Pheretima posthuma is primarily ureotelic. The impact of stress situation on excretion in the earthworms has been studied in relation to living conditions and temperature control.

Septal and supraintestinal nephridia are richly supplied with capillaries, nitrogenous wastes are removed from the blood and coelomic fluid.Urea, ammonia and remains of dead cells, the pharyngeal and integumentary nephridia extract only waste matter from the capillaries and water is conserved. Carbon dioxide and nitrogenous wastes is excreted out from the body through moist skin by the process of diffusion. The nitrogenous wastes are excreted out of the body by special excretory organs called nephridia (singular nephridium). The nephridial tubule opens the body surface by a small opening called nephridiopore.

Tillinghast EK, O'Donnell R, Eves D, Calvert E, Taylor J, observed high levels of proteases, amylase, protein and calcium ions in the gut luminal contents of the first two regions, and a significant decline of all four variables in region III. Conversely, ammonia was low in the gut contents of regions I and II but rose sharply in region III, which was also the region to which the tissue enzymes GDH and SDH were localized. The ammonia content of earthworm casts was observed to be much higher than that of the surrounding soil. These data are partial evidence for the proposal that the excretory ammonia produced by feeding earthworms is a product of the luminal epithelium of region III of the gut, and that ammonia and calcium may function as ion-exchangers in the absorptive function of the earthworm gut. Earthworms are primarily ammonotelic and ureotelic under stress situation, urea excretion change under different conditions of the environment like change in temperature, availability of water, food and stress conditions. The nature of excretory products also varies from species to species.

Sodium chloride has a positive and negative effects on human health. “Taste, habit, environment, genes, and behavior probably all influence sodium intake” (Alderman, 2002). “If we do not consume sufficient sodium, our metabolism, driven by specific feedback mechanisms, goes into a sodium-sparing mode so that the circulatory system can maintain osmotic balance and adequate blood pressure” (Satin, 2012). If we consume too much salt, it can raise one’s blood pressure. Worms are important to the environment. They help fertilize the soil, have setae that sense their environment. “The skin of the earthworm secretes mucous, it keeps the skin moist always, which is vital for the survival of earthworm. Earthworms use moist skin for respiration”. Chemistry of earthworm (Eisenia fetida), worm casts and worm body fluids has a high protein content in the range of 54.6 -71% of dry matter.

The growth and weight of this species, Pheretima posthuma and the tissue components (protein, lipid and glucose) vary according to length and weight. Mason et al. (1990) and Pennino et al. (1991) have observed that this variation is proximately associated with specific ecology, food, season, life style, reproductive state etc. Our result show a good seasonal variation in excretion of urea in the worm’s P. posthuma. This is related to protein content was always proportional to the growth of the worm, body weight and reproductive activities of worms.

Impact of triazine herbicide, organophosphate insecticide toxicity to the earthworm, Eisenia fetida was done in 2003 by Lydy MJ, Linck SL. Long-term effects of nitrogenous fertilizers on grassland earthworm in relation to soil acidification, agriculture, ecosystem and Environment had been done by Ma W, Brussaard L, Ridder, JA and Mackay, AD, Kladivko J, (1990), also rate of breakdown of soybean and maize residues in soil. Soil Biology and Biochemistry. Sub-lethal effect on earthworms like growth, reproduction and metabolism is needed. Use of urea at higher dose may cause severe mortality of earthworm. Sub-lethal doses of urea in the agricultural field, must be calculated. Mahajan S, Kanwar SS and Sharma SP.(2007), studied Long-term effect of mineral fertilizers and amendements on microbial dynamics in an alfisol of western Himalayas.Marhan S, Scheu S.(2005).

Studied influence of mineral and organic fertilizers on the growth of the endogeic earthworm Octolasion tyrtanum (Savigny).Kale, R. D., (1985), studied effect of starvation on ammonia and urea excretion in three pecies of tropical Earthworms. Hansen S, Engelstad F. (1999), reported earthworm populations in a cool and wet district as affected by tractor traffic and fertilization.

Panda S, Sahu SK. (1999), reported effects of malathion on the growth, reproduction of Drawida willsi, and found that due to bioaccumulation, worms may disrupt the soil biota and also the soil health. Shabeera Begum, H. (1989), studied the nitrogen excretion in the earthworm, Octochaetona serrata and impact of certain stress factors and found that P. excavatus is primarily ureotelic and also capable of excreting ammonia. In the present study P. posthuma survive for seven hours in 1% solution of NaCl but showed stress to toxicant. This may be due to switching ammonotelism excretion, as reported in P. excavates.

Continues exposure of earthworms to various toxicants like sodium chloride, sodium carbonate, copper sulphate and zinc sulphate in relation to temperature alter physiology. Alteration in reproductive capacity due to disturbed life cycle due to the aqueous toxicants may decrease population of P. posthuma indirectly affecting soil fertility and production of agriculture, gardens, forests, etc. Conservation of earthworms at global level may be achieved by preventing and controlling soil pollution, because pesticides alter acetylcholine esterase activity, thus after burrowing and locomotion which is turn may impair their feeding and reproductive activity.

IX. Conclusion

Earthworms regulate soil fertility and yield of agriculture ecosystem. Humans are continuously adding toxic pollutants like pesticides and metal salts which kill the earthworms. It is high time humans must sincerely abate pollution of environment conserve 70% earthworms. India barry on agricultural country must stringently conserve earthworms by reducing pollution of soil.

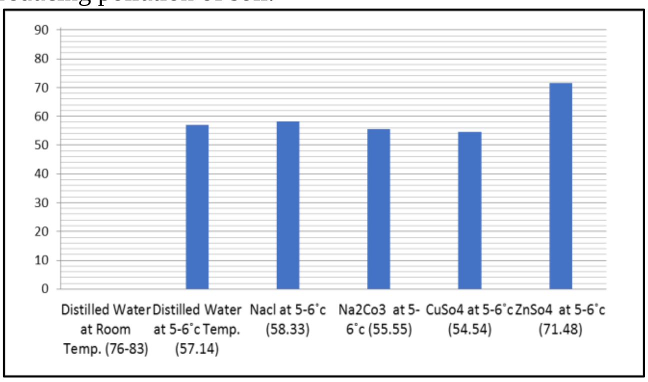

Fig. 1: Series graph: Showing urea excretion in earthworm P.posthuma in cold stressed and toxic solutions.

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