Mortality of earthworm, *Eisenia fetida* to urea fertilizer

Ms. Mamta Passi
Assistant professor, Government post graduate college for women, Department of Zoology, Rohtak (Haryana)-124001, India
Corresponding author* Email: -mamtakhokhar2016@gmail.com

Abstract
Soil is a natural habitat to wide variety of life forms including insects, arthropods and annelids. It is also a vital non-renewable resource for human, too. Chemical fertilizers have been used frequently to feed the mouth of millions and boost productivity. Overdose of chemical fertilizer has posed threat to various soil dwelling microorganisms and earthworm, too. In managed agro-ecosystem, Urea is widely used fertilizer used in crops as nitrogenous supplement. The present study outlines the detrimental effect of nitrogen supplements to earthworm. To determine dose response relationship earthworms *Eisenia fetida* were subjected to varying urea concentrations between 300 and 1500 mg/kg with a standard urea gap of 100 mg/kg. After 14 days, the percentage mortality in each treatment was noted. Ten adult gut cleaned worms were inoculated in artificial soil mixed along with testing chemicals. Experimental set-up was maintained as per the recommendations of OECD guidelines no 207 (1984, ISO, 2020) for testing of chemicals Earthworm. Results of this study indicate 300 mg/kg of urea as NOEC (No observed effect concentration) i.e highest Tested concentration for urea causing no mortality. In this experiment, all adult earthworms perished/were found dead at a urea concentration of 1500 mg/kg. The study therefore, recommends to use fertilizers of organic origin in the agricultural fields and minimizes, if cannot stop the use of chemical fertilizers in agricultural practices.
Keywords: Earthworm, *Eisenia fetida*, Chemical fertilizers, Mortality, Urea.

DOI Number: 10.48047/nq.2019.17.10.2580 **Neuroquantology 2019; 17(10): 32-36**

INTRODUCTION

Earthworms are found in every soil type. Darwin referred them as 'Intestine of earth'. They help to till the soil and create space for plant roots. Through soil aeration, better drainage, and elevated nutrient content, earthworms contribute to improving the quality of soil structure. Earthworms are a vital member of the soil fauna and contribute significantly to improve soil structure by producing biogenic structure (Culy and Berry, 1995). They unintentionally become susceptible to the negative effects of agrochemicals, such as fertilizers, pesticides, herbicides, etc., because they reside close to plants. European Economic Community recommend (EEC, 1985) earthworm's as model experimental animals for toxicity estimation in soil-toxicological investigations.

Chemical fertilizer has become necessary component of agricultural operations. Fertilizer inputs are now crucial for both agricultural productivity and financial gains. Applying fertilizer aids in restoring the nutrients that the previous crop has absorbed. These are either applied directly to soil or as a foliar application. Each artificially synthesized substance especially pesticides or fertilizers have their impact not only on crop quality but also on soil-fauna. Earthworm is a major soil macro fauna therefore affected either directly or indirectly (Reinecke *et al*., 1992).

In literature, there are various instances to study the effect of pesticides on soil, but to feed growing population farm managers are in maximal use of hybrid seeds, herbicides and inorganic fertilizers (Laucka *et al.*, 2020).Various studies have found a positive correlation between use of chemical fertilizers and abundance of earthworm population as far their food supply is concerned (Curry *et al.,* 2008, Estevez *et a*l., 1996). Whalen *et al* (1988) in their study on corn fields demonstrated that Inorganic fertilizers increase the quantity of herbage residue returned to soil, thus increase in food abundance for earthworms increase their population. Contrary to this few studies also emphasized the negative effect of chemical fertilizers on earthworm dynamics (Tinadaon *et al.,* 2011; Bhattachacharya and Sahu, 2014). However, Lofs-holmin (1983) in their study inferred that use of mineral fertilizer on clay soils reports no significant effect on earthworm population. Other studies show that infusion of crop plots with nitrogen and phosphorus based fertilizers causes a significant positive difference in earthworm population dynamics (Iordache and Borza, 2010). Nitrogen, Phosphorous and potassium are the major macronutrients for any plant growth. Urea is widely used artificially synthesized fertilizer used in almost every crop for nitrogen supplement. In view of the above, present study was undertaken with on toxicity induced by urea fertilizers to earthworm *Eisenia fetida*.

Materials and Method

Collection of earthworms

Eisenia fetida were garnered from Ganpati unit, Bahadurgarh (Haryana). It is a private firm engaged in production of vermi fertilizer. Identification of collected earthworms was reconfirmed with an online taxonomic key and digital library (Thakur and Yadav, 2018).

Culture of earthworms

Earthworms were kept in big culture pots for 5-6 months to make them acclimatized in laboratory conditions. Rearing of worms was done in earthen pots. Experimental set-up was maintained as per the recommendations of OECD guidelines no 207 (1984, ISO, 2020) for testing of chemicals “Earthworm, Acute toxicity tests”. To start well mature, healthy (gut cleared) earthworms of approximately the same body weight and length were used in the experimental model. Cow dung (35-40 day old) was used to supplement the nutritive medium (Neuhauser *et al*., 1990). Humidity was maintained by watering on alternate days. Temperature of the medium was maintained at 27 ± 20. Worms were acclimatized in a set up for 24 hours before urea infusion.

Test Chemicals

Urea (N-46%)-White**,** granular Urea was purchased from local market to use as a test chemical. Upon application urea is converted to ammonia; it further reacts with H_2O to form ammonium ions and then into nitrite.

Experimental set-up

This experiment employed an artificial soil testing approach using epigeic worms, E. fetida with range of concentration treatments (300-1500) with urea fertilizer. Earthworms *Eisenia fetida* were subjected to varying urea concentrations between 300 and 1500 mg/kg with a standard urea gap of 100 mg/kg. After 14 days, the percentage mortality in each treatment was noted. To guarantee adequate airflow and moisture levels and also to prevent the earthworms from escaping, a net fabric was placed over each pot. Initially, pots were routinely inspected for abnormal activity. Each treatment set was maintained in triplicate set. Control was also maintained side by side. Mortality was the end point.

Ethical committee approval

The study was an observational, experimental procedure on invertebrate earthworms, *E. fetida* thereby not requiring any clearance from the Ethical committee.

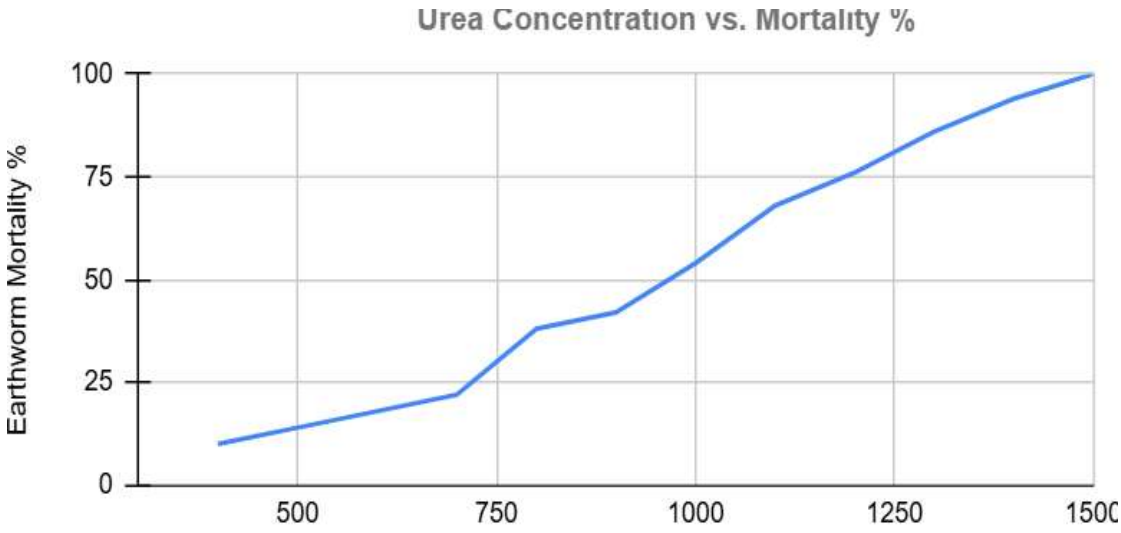

Fig1. Mortality of adult *Eisenia fetida* at different concentrations of urea fertilizer after 14 days.

RESULTS AND DISCUSSION

Results show earthworm mortality rate rose in tandem with the urea concentration in experimental clay pot setup. The higher concentration of urea added to the artificial soil was positively correlated with earthworm mortality. In the present experiment earthworm *Eisenia fetida* shows no mortality at urea concentrations of 300 mg/kg and 100% mortality at urea concentrations of 1500 mg/kg (**Fig1)**. Earthworms exhibit minimal mortality at low urea concentrations, with 1000 mg/kg of urea concentration treatment, the mortality rate increased to ≤50% (**Fig1)**.

The current results are consistent with Xiao *et al*. (2004) who found that urea was advantageous to earthworms, *E. fetida* when given at concentrations less than 500 mg/kg. But in the current setup, urea at concentrations greater than 1000 mg/kg also exhibited significant harmful consequences and the mortality rate increased to ≤50% (**Fig.1)**. In the current experiment, all adult earthworms perished/were found dead at a urea concentration of 1500 mg/kg.

Rai *et al*. (2014) demonastrated that earthworm (*E. fetida*) mortality rate increase when the urea dosage raised from 0.75 g/kg to 1.5 g/kg and 2.25 g/kg and specified on urea dose 3.48gm/kg all worms in culture were dead after 24 hrs (Rashid, 2019). Rai *et al* 2014 also confirms that all adult worms were able to survive with urea conc of 750mg/kg upto day 60.

Rani, 2016 mentioned 79.04 mg/kg of urea as safe conc for earthworm survival .In another experiment all juvenile earthworms die on exposure to 800 mg/kg of urea conc (Rani, 2016).In contrast, mortality rate peaked to 48% at 250 mg/kg of urea (Long *et al.*, 2017). Long *et al.* (2017) indicates that the percentage mortality was at its lowest in treatment with 200 mg/kg concentration day 14 and continued until day 60.

The current experiment shows a positive correlation between earthworm mortality and increased urea concentrations applied to soil. NOEC (No observed effect concentration) i.e highest tested concentration for urea causing no mortality was 300 mg/kg of soil. The dose response relationship is an important

criterion in laboratory conditions on artificial soil helps in figuring out safe application rate of urea fertilizer in an agricultural situation to prevent harm to earthworm populations, which are essential for the fertility and health of the soil. Results of present study differ from earlier studies in the species of earthworm, soil type, fertilizer dose duration, and types of fertilizers employed.

Globally, the synthetic urea amendment is used at higher doses on crops to increase production (Moonilall, 2015). The current study's findings showed that increased urea concentrations had drawbacks to earthworm such as a poor survival rate and higher mortality; earthworms are seriously impacted by the use of synthetic fertilizers in excess.

Conclusion

The finding's of study shows that synthetic fertilizers are hazardous to earthworm survival raises severe concerns about the usage of synthetic fertilizers in agriculture. One of the significant soil fauna, earthworms, has been found to be susceptible to increased dosages of the commonly used nitrogenous fertilizer urea. Results of the present study would be beneficial in directives India's agricultural laws on the usage of urea on farmlands. Additionally, it will inform farmers about the impacts of urea on earthworms, allowing them to modify their operations accordingly. Therefore, enough organic manure should be applied to crops in place of synthetic fertilizers to promote earthworm activity in the soil to establish a healthy agro-ecosystem.

References

Bhattacharya, A., & Sahu, S. K. (2015). Toxic effect of superphosphate on soil ecosystem using earthworm Drawidawillsi as test specimen. *Journal of Biodiversity and Environmental Sciences (JBES).* ISSN: 2220-6663 (Print) ,2222-3045 (Online) 6 (4), 220-226.

Culy, M. D. & Berry, E. C. (1995). Toxicity of soil-applied granular insecticides to earthworm populations in cornfields. *Down to Earth* ,50, 20-25.

Curry, J.P., Doherty, P., Purvis, G. & Schmidt, O. (2008). Relationships between earthworm populations and management intensity in cattle-grazed pastures in Ireland. *Applied soil ecology*, 39(1), 58-64.

Estevez, B., N'Dayegamiye, A. & Coderre, D. (1996). The effect on Earthworms abundance and selected soil properties after 14 years of solid cattle manure and NPKMg fertilizer application .*Canadian journal of soil science,* 76(3), 351-355.

European Economic Community (1985). Directive79/8331, V, Part C: Methods for the Determination of Ecotoxicity – Level 1. DG XI/127-129/82, Rev. 1: Toxicity for Earthworms. Commission of the European Community, Brussels

Hassan, D .(2024). Effect of urea fertilizer on the vitality of the earthworm Octolasion cyanieum**.** ***NTU Journal of Pure Sciences*** 3(2).DOI**:** **10.56286/yhbkj416**

Iordache, M., & Borza, I. (2010). Relation between chemical indices of soil and earthworm abundance under chemical fertilization. *Plant, Soil and Environment*, 56(9), 401-407.

ISO, 2020. Qualit´e du sol - D´etermination des effets toxiques des polluants sur la germination et les premiers stades de croissance des v´eg´etaux sup´erieurs. ISO 18763. Organisation International de normalisation, Gen`eve, Suisse.

Laucka, A., Andriukaitis, D., Valinevicius, A.,Navikas, D., Zilys, M.,Markevicius, V.,Klimenta, D., Sotner, R.&Jerabek, J. (2020). Method for Volume of Irregular Shape Pellets Estimation Using 2D Imaging Measurement. *Appl. Sci,* 10, 2650.

Lofs-Holmin, A. (1983). influence of agricultural practices on earthworms (Lumbricidae), *Acta. Agric. Scand.*, (33), 225 234.

Long, W., Ansari, A. & Seecharran, D. (2017). The effect of urea on epigeic earthworm species *(Eisenia foetida*). *Cell Biology and Development*, *1*(2), 46-50.

Moonilall, N. I. (2015). Impact of Amendments on Soil Properties and Agronomi Productivity in Guyana .Doctoral thesis, The Ohio State University.

Neuhauser, E.F. & Callahan, C.A. (1990). Growth and reproduction of the Earthworm Eiseniafoetida exposed to sublethal Concentration of Organic chemicals. *Soil biology and Biochemistry,* 31,363-366.

Organization for Economic Co-operation and Development (1984).Guideline for testing of chemicals No. 207, Earthworm Acute-toxicity Test (*Eisenia fetida/ andrei*).Organization for Economic Co-operation and Development. Paris, France.

Rai, N., Ashiya, P. & Rathore, D.S. (2014). Comparative study on the effect of chemical fertilizers and organic fertilizers on *Eisenia foetida*. *International J Innov Res SciEngTechnol*, 3 (5), 12991-12998.

Rani, S. (2016). Effect of urea on soil macro fauna, juvenile earthworms. *journal of international academic research for multidisciplinary*. ISSN: 2320-5083, 4(5), 15-19.

Rashid, A. (2019). Comparative study of effect of chemical fertilizers or organic fertilizer on earthworm *Eisenia fetida. Journal of Emerging Technologies and Innovative Research ,* 6(6), www.jetir.org (ISSN-2349-5162) JETIR1908300.

Reinecke, A.J., Viljioen, S.A. & Saayman,R.J. (1992). The suitability of *Eudrilus eugeniae, Perionyx excavatus and Eisenia fetida* (Oligochaete) for vermicomposting in Southern Africa in terms of their temperature requirements. *J. of Soil Biology and Biochemistry*, 24: 1295-1307.

Saxena, P.N., Gupta, S.K. & Murthy, R.C. (2014).Comparative toxicity of carbaryl, carbofuran, cypermethrin and fenvalerate in *Metaphire posthuma* and *Eisenia fetida*-a possible mechanism. Ecotoxicol Environ Saf,100,218–225.

Thakur, S. S. & Yadav,S (2018). Exploration of earthworms of India through Online Digital Library, Earthworms - The Ecological Engineers of Soil, Sajal Ray, IntechOpen, DOI: 10.5772/intechopen.75666. Available from: https:// www.intechopen.com/books/earthworms-the-ecologicalengineers-of-soil/exploration-of-earthworms-of-indiathrough-online-digital-library.

Tindaon, F., Benckiser, G. & Ottow, J.C.G. (2011). Side effects of nitrification inhibitors on non-target microbial processes in soils. *Journal of Tropical Soils,* 16(1), 7-16.

Whalen, J.K., Parmelee, R.W & Edwards C.A. (1998). Population dynamics of earthworm communities in corn agroecosystems receiving organic or inorganic fertilizer amendments. *Biology and Fertility of Soils* ,27(4),400– 407.

Xiao, H., Zhou , QX & Liang, JD. (2004). Single and joint effects of acetechlor and ureaon earthworm *Eisenia foetida* population in Phaiozem. *Environmental Geochemistry and Health*, 26(2), 277- 283.

Zhou, S., Duan, C., Wang, X., Michelle, W.H.G., Yu, Z. & Fu, H. (2008). Assessing cypermethrin-contaminated soil with three different earthworm test methods.J. Environ. Sci, 20(11), 1381-1385.