Biological Forum – An International Journal **14(2): 236-239(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Residual Toxicity of Post Emergence Nonselective Herbicide Glyphosate on Earthworm Survival, its Population Dynamics and Reproduction Rate under *in vitro* condition

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(Received 24 January 2022, Accepted 30 March, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Weeds are the important biotic constraints to agricultural production. It has been estimated that on an average, weeds caused 5% loss in agricultural production in most developed countries, while loss is 10% in developing countries. Therefore, the weed management is the necessary operation to reduce the yield loss. Manual weeding is considered as efficient method, but its use has reduced due to shortage and cost of labor. Mechanical weeding is suitable only for a limited number of crops and sowing methods. Hence the choice of chemical weed management is an intelligent and cost effective one. For increasing labour cost and quick weed control in cropped and non-cropped areas, herbicides use is increasing throughout the globe. In India, currently 51 herbicides are registered for use in various crops. Excess use of herbicide may lead to residue problems in soil, phytotoxicity to crop plants, adverse effects on non-target organisms and ultimately health hazards to humans and animals. The objective of this study was to find out the effect of different concentrations of glyphosate on earthworm population, body weight and juvenile count. Results showed that different concentrations of glyphosate did not significantly affect the earthworm population and earthworm body weight. There is no mortality of earthworms observed during this experiment. The lower concentration at the rate of 10 ppm increases the earthworm population and juvenile count at 60 days after herbicide application due to the hormesis effect.

Keywords: Earthworm, herbicide, nonselective, glyphosate, juvenile and body weight.

INTRODUCTION

Earthworms are an important soil organism that has the capability of maintaining soil fertility and playing a key role in sustainability. It converts the low quality organic matter into a nutrient rich product by developing a mutualistic relationship with soil microflora (Singh *et al*., 2014). It improves soil aeration, infiltration, soil structure, nutrient cycling, water movement and plant growth.The combined activity of earthworms and microbes helps to the retention of nitrogen and gradual release of phosphorus from the soil (Bajsa *et al*., 2003). They are also known as farmer's friend, ploughman of the field, intestines of the earth, ecological engineers and biological indicators. They are hermaphrodite animals and reproduction normally occurs through copulation and cross-fertilization. Cocoons hatch after an incubation period that varies according to the earthworm species and environmental conditions. In *Eisenia fetida* cocoon production started during the 6th week and ceased after the 12th week. The incubation period of *Eisenia fetida*is about 18-26 days and the number of hatchlings from one cocoon is 2-4 (Fetida, 2018).

Herbicides are used to control the weeds in agricultural fields but the heavy use of herbicides without considering the long or short term effects in soil medium degraded the soil. Most of these herbicides may cause harm to nematodes, earthworms and other biological organisms (Kumar and Kumawat 2018). In India about 6704 tons of herbicides were applied to the agricultural field in the year 2010. Of these herbicides, glyphosate is the most commonly used one in tea plantations and non-cropped fields.Over the last decade, about 6.1 billion kilograms of the herbicide glyphosate have been applied worldwide (Benbrook, 2016).

Glyphosate is a broad-spectrum, non-selective and post-emergence herbicide, directly applied to plant foliage.

In plants, glyphosate disrupts the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. The shortage in EPSP production leads to reductions in aromatic amino acids that are vital for protein synthesis and plant growth. It degrades at a relatively rapid rate in most soils, with a half-life estimated between 7-60 days (Giesy *et al*., 2000). Many studies have indicated that the presence of glyphosate in the soil may be toxic to the earthworms, which some studies have also shown glyphosate did not affect the earthworm population in the soil but it gradually reduce the bodyweight of the earthworms (Correia and Moreira 2010). Glyphosate has been considered an environmentally safe herbicide because it is assumed tobe inactivated quickly after spraying due to rapid sorption onto particles in the soil and its fast degradation by microbes (Hagner *et al*., 2015). Hence this trial was conducted to find the effect of glyphosate on earthworm population and reproduction under laboratory conditions.

MATERIALS AND METHODS

The laboratory experiment was conducted from November 2021 to January 2022 at the Department of Agronomy, Tamil Nadu Agricultural University. For this experiment, 15 *Eisenia fetida* earthworms were selected and weights of fifteen worms were measured and introduced into each pot. The known weights of earthworms were placed on each potcontaining 500 grams of soil. Four levels of glyphosate viz., 1000 ppm (T_1), 500 ppm (T_2), 100 ppm (T_3) and 10 ppm (T_4) were prepared from glyphosate 46% SL potassium salt with one absolute control (T_5). Ten ml of glyphosate at different concentrations (1000, 500, 100 and 10 ppm) were added to each pot containing fifteen earthworms. The experiment was conducted in a completely randomized design (CRD) with three replications. Earthworm population and juvenile count were observed up to 60 days at an interval of 15 days.

Observation on earthworm count, earthworm body weight and juvenile count were recorded during this experiment. The experimental data were statistically analysed by adopting Fischer's method of "Analysis of variance" as per Gomez and Gomez (1984). Wherever the treatment differences were found significant, the critical differences were worked out at a 5 percent probability level.

RESULTS AND DISCUSSION

Effect of different concentration of glyphosate on earthworm Eisenia fetidapopulation and earthworm juvenile

Different concentrations of glyphosate did not significantly affect the earthworm population (Table 1) and earthworm juveniles (Table 2). Glyphosate at the rate of 10 ppm has the highest earthworm (21.3) and juvenile population (6.3) at 60 days after herbicide application (DAHA) compared to control (21 and 6, respectively). There is no mortality in the earthworm population during this experiment at the same time the reproduction capacity of the earthworm did not affect by glyphosate. A similar finding was reported by Correia and Moreira, 2010 the different rates of glyphosate (0, 10, 100, 500 and 1000 mg kg^{-1}) did not kill the earthworm *Eisenia fetida*.

Pochron *et al*. (2021) reported that twice the recommended dose of glyphosate (12 ml of Roundup Ready in 4 kg of soil) did not impact on earthworm mortality rate. Glyphosate 100, 10 and 1 ppm did not effect on earthworm *Allolobophora caliginosa* population when herbicide mixed with soil (Martin, 1982). In the treatments with glyphosate (6 litres per ha and 12 litres per ha), the number of cocoons per adult is higher than control but the survival of juveniles and adults and the fertility are lower than in the control treatment (Santadino *et al*., 2014). Morse (1998) points out that hormesis orhormoligosis takes place when small quantities of astressful agent, among them pesticides, can be useful for an organism in suboptimal environments. This occursin numerous invertebrates (Abdullah *et al*., 2006) and *E. fetida*, much like other earthworms, usuallyresponds to different stress factors, increasing their reproductiverate.

Table 1: Effect of different concentration of glyphosate on earthworm population.

Treatments	Initial	15 DAHA	30 DAHA	45 DAHA	60DAHA
T_1- Glyphosate 1000 ppm	15.0	15.3	16.0	17.7	18.7
T_2- Glyphosate 500 ppm	15.0	16.3	17.0	17.3	18.0
T_3- Glyphosate 100 ppm	15.0	16.7	17.0	17.7	18.3
T_4-Glyphosate 10 ppm	15.0	17.3	19.0	19.7	21.3
T_5- Control	15.0	17.3	18.7	19.3	21.0
SEd	**0.00**	**0.94**	**1.23**	**0.87**	**0.89**
CD (*P*=0.05)	**NS**	**NS**	**NS**	**NS**	**1.99**

Table 2: Effect of different concentration of glyphosate on earthworm juveniles.

Treatments	Initial	15 DAHA	30 DAHA	45 DAHA	60DAHA
T_1- Glyphosate 1000 ppm	0	0.3	1.0	2.7	3.7
T_2- Glyphosate 500 ppm	0	1.3	2.0	2.3	3.0
T_3- Glyphosate 100 ppm	0	1.7	2.0	2.7	3.3
T_4-Glyphosate 10 ppm	0	2.3	4.0	4.7	6.3
T_5- Control	0	2.3	3.7	4.3	6.0
SEd	**0.00**	**0.94**	**1.23**	**0.87**	**0.89**
CD (*P*=0.05)	**NS**	**NS**	**NS**	**NS**	**1.99**

Effect of different concentration of glyphosate on earthworm body weight: Earthworm body weight did not significantly affected by different doses of glyphosate (Table 3). Fifteen earthworm's weights were measured before introduction into each pot. Earthworm body weight was reduced gradually up to 30 days after herbicide application including control treatment. This is might be due to earthworms inside the closed potcreatinga stressful environment. After 45 days after the application of herbicide, the weights of the worms were increased because thenumbers of juveniles in each pot were increased.

Roundup Ready-to-Use III® (12 ml glyphosate into 4 kg of soil) contaminated soil did not impact on earthworm *Eisenia fetida* body mass after 7 days of exposure. Both contaminated and uncontaminated soil has a mean body weight of 0.13 g. At 21 days of exposure, the contaminated soil has 0.14 g of mean body weight and uncontaminated soil has 0.12 g of mean body weight (Pochron *et al*., 2021). Pochron *et al*. (2020) reported that soil treated with pure glyphosate lost 14.8- 25.9% of their earthworm *Eisenia fetida* biomass. At the same time soil treated with Roundup Ready-to-Use III® and Roundup Super Concentrate® (26.3 mg of glyphosate per kg soil) did not lose body mass of the earthworms (0.28 and 0.27 g of mean body mass respectively) at 40 days after glyphosate exposure compare to control (0.25 g of mean body mass). The soil treated with glyphosate at different concentration (10, 100, 500 and 1000 ppm) reduce the 50% of the earthworm *Eisenia fetida* body weight. The decrease in mean weight may indicate a chronic effect of this herbicide (Correia and Moreira 2010).

Table 3: Effect of different concentration of glyphosate on earthworm body weight(fifteen earthworms) (g).

Treatments	Initial	15 DAHA	30 DAHA	45 DAHA	60DAHA
T_1- Glyphosate 1000 ppm	3.6	3.4	3.1	3.2	3.4
T_2- Glyphosate 500 ppm	3.7	3.6	3.4	3.4	3.6
T_3- Glyphosate 100 ppm	3.4	3.3	3.1	3.2	3.2
T_4-Glyphosate 10 ppm	3.8	3.6	3.4	3.5	3.5
T_5- Control	3.8	3.6	3.4	3.5	3.5
SEd	**0.32**	**0.35**	**0.34**	**1.13**	**0.32**
CD (*P*=0.05)	**NS**	**NS**	**NS**	**NS**	**NS**

CONCLUSION

Glyphosate herbicide formulations are commercially available in various salt forms, such as isopropylamine, ammonium, potassium, and trimesium salt. It is used to manage annual broadleaf weeds, grasses, and sedges in various field and row crops around the globe. Based on the experiment conducted it may be concluded that the application of glyphosate directly into earthworms did not affect the earthworm population, body weight and reproduction. The body weights of the earthworms are reduced up to 30 days after glyphosate application later the weights of the worms were increased because the numbers of juveniles in each pot were increased.A low concentration of glyphosate at the rate of 10 ppm created the hormesis or hormoligosis effects on earthworm reproduction. Therefore, the number of juvenile count was increased in 10 ppm of glyphosate compared to control.

FUTURE SCOPE

Earthworm anatomy may be studied when glyphosate was added to soil.

Acknowledgement. I gladly acknowledge my sincere and heartful thanks to Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore for providing laboratory facilities to conduct this research work.
Conflict of Interest. None.

REFERENCES

Abdullah, N. M. M., Singh, J., & Sohal, B. S. (2006). Behavioral hormoligosis in oviposition preference of *Bemisia tabaci* on cotton. *Pesticide Biochemistry and Physiology*, *84*(1): 10-16.

Bajsa, O., Nair, J., Mathew, K., & Ho, G. E. (2003). Vermiculture as a tool for domestic waste water management. *Water Sci. Technol., 48*: 125–132.

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, *28*(1): 1-15.

Correia, F. V., & Moreira, J. C. (2010). Effects of glyphosate and 2, 4-D on earthworms (*Eisenia foetida*) in laboratory tests. *Bulletin of environmental contamination and toxicology*, *85*(3): 264-268.

Fetida, E. (2018). Life cycle of vermicomposting earthworms *Eisenia fetida* and Eudrilus Eugeniae under laboratory controlled condition. *Biomedical Journal*, *1*: 4.

Giesy, J. P., Dobson, S., Solomon, K. R. (2000). Ecotoxicological risk assessment for Roundup® herbicide. *Reviews of environmental contamination and toxicology*, 35-120.

Gomez, K. A and Gomez, A. A. (1984). Statistical procedure for agricultural research. Wiley Inter-science Publications. New York. pp. 680.

Hagner, M., Hallman, S., Jauhiainen, L., Kemppainen, R., Rämö, S., Tiilikkala, K., & Setälä, H. (2015). Birch (*Betula* spp.) wood biochar is a potential soil amendment to reduce glyphosate leaching in agricultural soils. *Journal of environmental management*, *164*: 46-52.

Kumar, K., & Kumawat, P. (2018). A review of the effect of herbicides on the earthworms. *International Journal of Zoology Studies*, *3*(2): 120-125.

Martin, N. A. (1982). The effect of herbicides used on asparagus on the growth rate of the earthworm *Allolobophora caliginosa*. In *Proceedings of the New Zealand Weed and Pest Control Conference*, *35*: 328-331).

Morse, J. G. (1998). Agricultural implications of pesticide-induced hormesis of insects and mites. *Human & experimental toxicology*, *17*(5): 266-269.

Pochron, S. T., Mirza, A., Mezic, M., Chung, E., Ezedum, Z., Geraci, G., & Zafar, S. (2021). Earthworms *Eisenia fetida* recover from Roundup exposure. *Applied Soil Ecology*, *158*:103793.

Pochron, S., Simon, L., Mirza, A., Littleton, A., Sahebzada, F., & Yudell, M. (2020). Glyphosate but not Roundup® harms earthworms (*Eisenia fetida*). *Chemosphere*, *241*: 125017.

Santadino, M., Coviella, C., & Momo, F. (2014). Glyphosate sublethal effects on the population dynamics of the earthworm *Eisenia fetida* (Savigny, 1826). *Water, Air, & Soil Pollution*, *225*(12): 1-8.

Singh, J., Kaur, A., & Vig, A. P. (2014). Bioremediation of distillery sludge into soil-enriching material through vermicomposting with the help of *Eisenia fetida*. *Applied biochemistry and biotechnology*, *174*(4): 1403-1419.

How to cite this article: M. Keerthana, C.R. Chinnamuthu, P. Murali Arthanari, N. Sakthivel, V. Rajasree and R. Shanmugasundaram (2022). Residual Toxicity of Post Emergence Nonselective Herbicide Glyphosate on Earthworm Survival, its Population Dynamics and Reproduction Rate under *in vitro* Condition. *Biological Forum – An International Journal*, *14*(2): 236-239.