International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

The Effect of Two Pesticides on the Earthworm *Eisenia Fetida*, (Savigny1826) Under Laboratory Conditions

***Faiza S Albakoush[a],, Hammad M. Ahallil[b], Walid A. Awgie[c], Abdalla I. Mohamed[d] Salmin A. Omar[e]**

[1]Department of Zoology, Faculty of Science, Benghazi University, Alkufra, Libya

[2]Faculty of Agriculture, Benghazi University, Alkufra Camous, Libya

[3]Faculty of Medical Technology, Benghazi, Libya

[4]Department of Zoology, Faculty of Science, Benghazi University, Benghazi, Libya

[5]Department of Zoology, Faculty of Science, Benghazi University, Benghazi, Libya

Abstract: ***Earthworms are the soil dwelling invertebrates, which have great agricultural importance, they influence the soil structure by ingestion, which leads to the breakdown of organic matter and its ejection as a surface or subsurface cast. The 3545DQ effect of the chemicals on earthworms depends on the used substance, but regardless the substance nature, it must be considered in the ecological risk assessment both the direct and the indirect effects of it on soil life. The effect of selected pesticides on the earthworm E. fetida its survival and reproduction are estimated in this research. Some parameters such as changes in the density, mortality, biomass, hampered life activity and the number of laid cocoons and hatching are evaluated. Selected pesticides contained the following active chemical compounds: the type of pesticides used is Glyphosate (Round - up) (herbicide), Chlorpyrifos (Salute) (insecticide). We found that, earthworms responded to soil pesticides in different ways. Round - up caused were greater than that of control mean body weight and Salute similar that of control mean whereas, The Ru seems to have greater impact on the juvenile production whereas, Sal has low effect on the juvenile production. This work will be helpful for future evolution of soil ecological.***

Keywords: *Eisenia fetida*, Herbicide, Insecticide, sublethal dose, Soil

1. Introduction

An important factor in boosting agricultural sustainability is soil biodiversity. According to [1]. Soil organisms are incredibly diverse and enable the best possible use of the resources found in the varied habitats at different resolution levels. Pesticide residue prevalence in environmental matrices is always a problem for the environment, even though organic farming is becoming more and more popular. Synthetic pesticides are still applied in agriculture and for sanitation reasons [2]. By transforming organic waste and biodegradable material into nutrient - rich vermicast, earthworms play a significant role in the growth and upkeep of the soil's nutritional value [3]. [4] refer to them as ecological engineers as well. Earthworms constitute a larger portion (>80%) of the biomass of terrestrial invertebrates and are important for organising and improving soil nutrients. Because they function as useful bioindicators of chemical pollution of the soil in terrestrial ecosystems, earthworms therefore offer an early warning of decline in soil quality [5] [6]. Earthworms became one of the basic ecotoxicological study objects to evaluate. environmental changes [7] focussed particularly on various herbicides which are now inseparable part of agricultural production [8] [9].

Earthworms are important organisms in soil communities and are known for sustaining the life of the soil. They are used as a model organism in environmental risk assessment of chemicals and soil toxicology. Soil provides physical and nutritive support to agriculture system by regulating biogeochemical cycles, nutrient cycle, waste degradation, organic matter degradation etc.

The soil environment contains many organisms from different trophic levels that may be all exposed even if only a few members of the soil food web are exposed. If the contaminated organisms belong to the lower trophic levels, the probability of a widespread contamination and the possibility of bioaccumulation and biomagnification of the contaminants along the food chain are even higher because they are food for several other organisms in the web, acting as a route to contaminants transference [10]. Earthworms are one of these groups of animals that belong to the very complex soil food web. They may be exposed to pesticide residues that are directly applied or reach the soil, either because this is their main source of food or because the contaminants may be absorbed by their body surface [11] [12]. Due to their ecological relevance, earthworms have been used as bioindicators and, as they are also biosensors of sublethal concentrations, they could serve as a warning sign for the early effects of soil contamination [11].

The pesticides used in agriculture land cause morphological, behavioral and physiological changes in reproductive, nervous, respiratory and osmoregulatory organs of many soil organisms and contaminate the soil which exerts a harmful impact on various invertebrates [13] [14] [15]. Depending upon the chemical nature of pesticides and soil properties organs undergo a series of chemical pathways, transport, adsorption and desorption processes [16] [17].

Earthworms are vulnerable to such chemicals and therefore act as model organisms in the evaluation of the impact of the pesticides [18]. These are a vital component of soil biomass and act as an important indicator of total soil metabolism as well as soil pollution and toxicity [19] [20]. In fact, several earthworm species have been used as bio - indicators [21] and hence these are used in the evaluation of chemical environmental [22]

The pesticide effect on earthworms depends on the used chemical substance. Generally, herbicides manifest low toxicity on earthworms, but indirectly can produce the reduction of the populations by decreasing the organic matter input and weed coverage. The fungicides and fumigants are very toxic substances for earthworms [23] [24] [[25]. The methods used for pesticide application could have various effects on the ecological groups of Scientific literature addressing the influence of pesticides on the growth and reproduction of earthworm is reviewed. Earthworms are considered as important bioindicators of chemical toxicity in the soil ecosystem. Studies on this aspect are important because earthworms are the common prey of many terrestrial vertebrate species such as birds and small mammals, and thus they play a key role in the biomagnification process of several soil pollutants. Majority of the studies have used mortality as an endpoint rather than subtler endpoints such as reproductive output. It is now emphasized that, whereas higher concentrations of a pollutant can easily be assessed with the acute (mortality) test, contaminated soils with lower (sublethal) pollutant concentrations require more sensitive test methods such as reproduction test in their risk assessment. [26].

2. Materials and Methods

2.1. The Test Organism

The earthworm *Eisenia fetida* was chosen for this experimental work, because of their important contribution for their role in soil components, both soil structure and functions as well as their use as biological indicators in toxicity.

Eisenia fetida was obtained from a stock culture that maintained in the zoology department lab of the faculty of science for more than five years on an artificial universal pot ground soil (Egmond, spain,) which contains all required nutrients along with enough organic matter for earthworm growth, and PH of 5 - 6.5 and EC of 0.8 - 1.5mm Sh/cm. This soil is the guidelines for testing set by the Organization for Econmic Coorperation and development (OECD, 1984).

2.2. The Pesticides

Two different pesticides were chosen for the experiment including an OP insecticide Salute, the herbicide Round up all are well known for their wide use in Benghazi open agroecosystem against different insect, weeds and plant pathogens, (fungal diseases).

2.2.1. Chlorpyrifos (formulation Salute)

The insecticide salute is an organophosphours consisting of two compounds used at 0.05ml/Kg soil.

1) Chlorpyrifos acting against insects, Nematods and mites.
2) Dimethoate mostly works against insects.

Is a contact and systemic organophosphours insecticide and acaricides effective against a large number of insect pests of different crops.

The compound is a potent cholinesterase inhibitor in both vertebrates and invertebrate even at slightly high dose [27].

2.2.2. Glyphosate (formulation Round up)

Glyphosate (Round - up) herbicide is a non selective and systemic against broad leaf weeds used at 0.5ml/Kg.

A non - residual phosphonic acid herbicide effective againest both annual and perennial weeds in and around several crops. This compound is widely used for several years in most agroecosystems of Benghazi as well as many other regions of Libyan farms.

The compound can irritate eyes and skin if it come in direct contact, otherwise it is safe when used as recommended for each weed [27].

2.3. Worm Rearing

The worms were reared in medium size plastic containers approximately 41cm long, 35cm wide and 20cm deep heaving approximately 15 - 17cm of the sieved artificial soil which moistured up to 65 - 70% water holding capacity, frequent barley flour was added to the soil surface as food supplement.

All rearing containers were held under the laboratory conditions where, both temperature and humidity were monitored but not controlled. However, their range were about 18 - 22± 2C^{o} and 60±5 RH during the experiment.

Containers monitor of the culture indicated that the worms were growing and breeding normally and no mortality was observed.

2.4 The Experiment

The experiment was based on finding the possible interaction of the insecticide Salute, the herbicide Round - up when they come together in the soil habitat of the earthworm.

Consequently – one concentration of each pesticide was chosen on the bases of the used field use with the consideration of the remaining deposit of the pesticide and the limited free space of the treated worms.

Three replicats were set for each pesticide as well as for separately three replicates of same soil quantity at 400ml of H_2o and without pesticide were designed as control (Table1).

Table 1: Pesticides treatments in the earthworms *Eisenia fetida* pesticide ml/liter and 8 worms per replicate.

Treatment	ml/Liter	R1	R2	R3
Salute	0.05	8	8	8
Round up	0.5	8	8	8
Control		8	8	8

The pesticides were original field use Emulsifiable concentration (EC) formulation obtained from a known farmer at Benghazi.

2.5 Data Collection and Statistical Analysis

After the end of the test duration data of both period 4 and 8 weeks were collected sorted and subjected to statistical analysis.

All statistical analysis were done by SPSS software package, where two way analysis of variance for mean separation of the different treatments.

3. Results

3.1 Body weight of treated adult worms

After eight weeks of the worm exposure to the pesticide Table 2 and Fig 1 showed the impact of the insecticide salute and Round up on the worm body weight significant differences ($F=6.19$, $P<0.05$) were reported between the different pesticides treatments as compared to that of control worm body weight. As can be seen in Table (1), this results indicated that Salute**8.7±1.69** and Control**8.5±2.65** respeclively came close whereas, with mean of Round up mean of**9.7±2.22** were greater than that of control mean 8.54±2.6.

Table 2: The mean ± SD in grams of *E. fetida* body weight exposed to Pesticide treated soils after eight weeks of exposure

Weight	Mean ±SD
Control	8.5±2.65
Sal	8.7±1.69
Ru	9.7±2.22

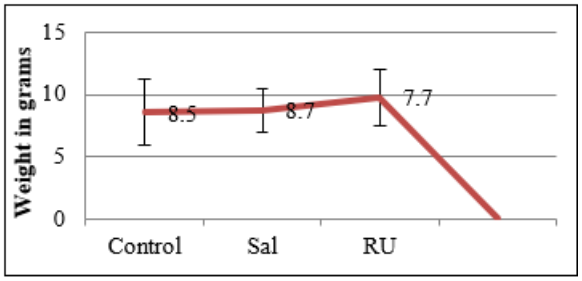

Figure 1: The mean ± SD in grams of *E. fetida* body weight exposed to Pesticide treated soils after eight weeks of exposure.

The Means± SD value of juveniles produced from Sal and Ru treated worms reveled significant difference ($F=29.809$, $P< 0.05$) (Table13 - Fig13) Although Sal is considered insecticides, however, revealed the highest mean number**450±192.5** followed by control with 436. ±54.83, whereas, the least mean value was that of Ru alone with 133.3±89.04

The Ru seems to have greater impact on the juvenile production whereas, Sal has low effect on the juvenile production.

Table 3: The mean ± SD of juveniles number in grams of *E. fetida* exposed to Pesticide treated soils after eight weeks of exposure

Juveniles	Mean ±SD
Control	436±54.83
Sal	450±192.5
Ru	133±89.04

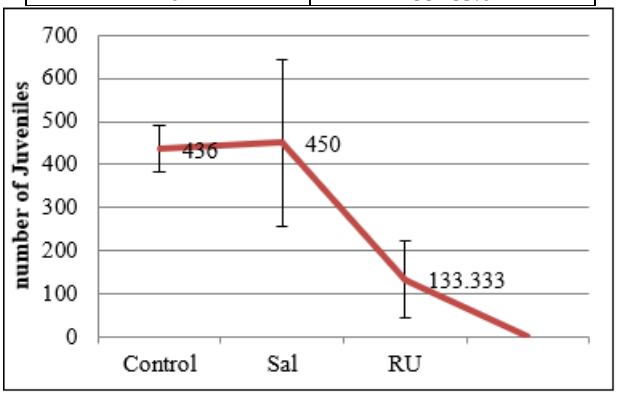

Figure 2: The mean ± SD of juveniles number in grams of *E. fetida* exposed to Pesticide treated soils after eight weeks of exposure.

4. Discussion

The present study focused on the toxicity of two pesticides the insecticide Salute, the herbicide Round up on the growth and reproduction of the earthworm species *Eisenia fetida* under Lab conditions.

Earthworms ingest organic matter of the soil particle and excrete this in the form of casts, which get deposited on the soil surface and this cast acts as a natural fertiliser for the soil and boosts the soil fertility. Loss of fertility is directly proportional to stable aggregate formed by earthworms. Heavy metallic factor present in the soil directly affects the various physiological activities of *E. fetida*.

Application of pesticides in agriculture is a major danger to wildlife and ecosystems, which has led to growing concern in the world due to the indiscriminate use of these chemicals [28] [29]. Furthermore, Pesticides, even when used in small quantities, their multiplicity their toxicity and persistence have an adverse effect on the ecological systems [30].

Negative impact of pesticide on earthworm growth has been reported by various researchers [31] suggested that growth can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms.

Several workers have assessed and found that chlorpyrifos had an adverse effect on growth in earthworm exposed to 5 mg/kg chlorpyrifos after eight weeks whereas, some studies have shown that growth of earthworms appeared to be more severely affected at juvenile stage than at adult stage. [24] [32].

The observation in the present study agrees with the finding of the [33] and with the work of [34] who reported that *E. fetida* exposed to different concentrations in soil tented to lose more weight than those in control.

This result slightly contradicted with the finding of [35] who reported juvenile reduction with Salute and Round up and an increasing number with the fungicide Rubajan.

In the present study, insignificant reduction in *E. fetida* was observed when the Op - salute was used at sublethal dose. However, [12] has reported that a reductions in growth of earthworm were observed in several carbmate and organophosphate insecticides.

Reduction in growth of earthworm by sub - lethal doses of insecticides has also been observed in Eisenia fetida [12] exposed to several carbamate and organophosphate insecticides.

[35] has reported that the insecticide salute, the herbicide Round up and the fungicide Rubigan has proven negative effect on the earthworm *E. fetida* and *A. caliginose* in terms of growth rate and reproduction potential and concluded that these two worms can be endorsed as biomonitrs for chemical pollutants.

The observed reduction in the worm weight can be attributed to the worm avoidance of feeding on the treated diet and this confirm the finding of [36] [37] who added that the earthworm *E. andrei* can be used to measure soil pollutant due to avoidance response.

5. Conclusion

Based on the observations of the present study and previous studies, it can be concluded that the growth and reproductive parameters of earthworms exposed to pesticides seem to be useful bioindicators of soil pollution. The result has also showen that pesticides Salut and Roundup all have negative impact when used as sub lethal concentrations pronounced effect on growth and reproduction. Further study is highly recommended for the field assessment of soil fauna including earthworms which is considered bioindicatores of chemical toxicity and other pollutants Research should be extended to ecologically relevant species of earthworms, and also to other soil fauna to get a comprehensive knowledge on the malfunction in the soil biological processes due to pesticide pollution. Numerous studies indicate negative impact of pesticides on earthworm growth and reproduction. So, there is a need to acquire more knowledge on the chemical nature, mode of action, and means of degradation of pesticides in soil.

References

[1] Ferris, H., & Tuomisto, H. (2015). Unearthing the role of biological diversity in soil health. Soil Biology and Biochemistry, 85, 101 - 109.
[2] Srimurali, S., Govindaraj, S., Kumar, S. K., & Rajendran, R. B. (2015). Distribution of organochlorine pesticides in atmospheric air of Tamilnadu, southern India. International Journal of Environmental Science and Technology, 12 (6), 1957 - 1964.
[3] Jansirani, D., Nivethitha, S., Singh, M. V. P. (2012). Production and utilization of vermicast using organic wastes and its impact on Trigonella foenum and Phaseolus aureus. International Journal of Research in Biological Science, 2, 187–189.
[4] Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69: 373–386
[5] Culy MD, Berry EC. Toxicity of soil - applied granular insecticides to earthworm populations in cornfields, Down to Earth.1995; 50: 20 - 25.
[6] Sorour J, Larink O (2001) Toxic effects of benomyl on the ultrastructure during spermatogenesis of the earthworm Eisenia fetida. Ecotoxicol Environ Saf 50 (3): 180–188.
[7] Cortet J., Gomot - De Vauflery A., Poinsot - Balageuer N., Gomot L., Texier Ch. and Cluzeau D.: Eur. J. Soil Biol.1999, 35 (3), 115–134.
[8] Bauer C., Rombke J. and Edwards C. A.: Soil. Biol. Biochem.1997, 29 (3/4), 705–708
[9] Farenhorst A., Topp E., Bowman B. T. and Tomlin A. D.: Soil Biol. Biochem.2000, 32 (1), 23–33.
[10] Vasseur, P.; Cossu - Leguille, C. Linking molecular interactions to consequent effects of persistent organic pollutants (POPs) upon populations. Chemosphere 2006, 62, 1033 - 1042.
[11] Andréa, M. M. O uso de minhocas como bioindicadores de contaminação de solos. Acta Zoológica Mexicana (nueva serie) 2010, 26, 95 - 107.
[12] Yasmin, S. and D'Souza, D. (2007). Effect of pesticides on the reproductive output of *Eisenia fetida*. *Bulletin of environmental contamination and toxicology*, *79* (5): 529 - 532.
[13] Fingerman M. (1984). Pollution our enemy. Proc. Symp. Physiol. Resp. Anim. Poll. pp.1–6
[14] Mangala P, De Silva CS, Pathiratne A, Van Gestel CAM. (2009). Influence of Temperature and Soil Type on the Toxicity of Three Pesticides to Eisenia Andrei. Chemosphere.76: 1410–1415.
[15] De silva PMCS. (2009). Pesticide effects on earthworms: A tropical perspective, Ph. D. Thesis. Department of Ecological Science, VU University, Amsterdam, The Netherlands. pp.117.
[16] Thapar A, Zalawadia A, Pokharkar OV, Satam SS. (2015). Classification of pesticides and its damaging effects: a review. Biolife 4 (1): 13–24.
[17] Baishya K. (2015). Impact of agrochemicals application on soil quality degradation—a review. ICSTM. pp.778–786.
[18] Edwards C. A. and Bohlen P. J. (1993). The effects of toxic chemicals on earthworms. Rev. Environ. Contam. Toxicol., 125, 23 - 99.
[19] Peakall D. B. (1994). The role of biomarkers in environmental assessment (1) Introduction. Ecotoxicol., 3 (3), 157 - 160.
[20] Brady N. C. and Weil R. R. (2004). Elements of the Nature and Properties of Soil (Second Edition). Pearson Prentice Hall Publ., Upper Saddle River, New Jersy, USA.
[21] Scott - Fordsmand J. J. and Weeks J. M. (2000). Biomarkers in earthworms. Rev. Environ. Contam. Toxicol., 165, 117 - 159.

[22] Bustos - Oberg E. and Goicochea R. I. (2002). Pesticide soil contamination mainly affects earthworm male reproductive parameters. Asian J. Andro., 4, 195 - 199.
[23] BOOTH, L. H., O'HALLORAN, K., 2001 – A comparison of biomarker responses in the earthworm Aporrectodea caliginosa to the organophosphorous insecticides Diazinon and Chlorpyrifos. Environ. Toxicol. Chem., 20, 2494 - 2502;
[24] Booth, L. H., Hodge, S., and O'halloran, K. (2001). Use of biomarkers in earthworms to detect use and abuse of field applications of a model organophosphate pesticide. *Bulletin of environmental contamination and toxicology*, *67* (5): 633 - 640.
[25] ABDUL RIDA, A. M., BOUCHÉ, M. B., 1997 – Earthworm toxicology: from acute to chronic tests. Soil Biology and Biochemistry, 29: 699 - 703;
[26] Shahla, Y., & D'Souza, D. (2010). Effects of pesticides on the growth and reproduction of earthworm: a review. *Applied and Environmental soil science*, *2010*.
[27] Whitehead, R. (1995). *The UK pesticide guide 1995*. The university press, Cambridge, Great Britain.
[28] Rao, J. V. (2006). Biochemical alterations in euryhaline fish, Oreochromis mossambicus exposed to sub - lethal concentrations of an organophosphorus insecticide, monocrotophos. *Chemosphere*, *65* (10): 1814 - 1820.
[29] Kathuria, V. (2007). Informal regulation of pollution in a developing country: evidence from India. *Ecological Economics*, *63* (2 - 3): 403 - 417.
[30] Reddy, N. C., and Rao, J. V. (2008). Biological response of earthworm, *Eisenia foetida* (Savigny) to an organophosphorous pesticide, profenofos. *Ecotoxicology and Environmental Safety*, *71* (2): 574 - 582.
[31] Xiao, N., Jing, B., Ge, F. and Liu, X. (2006). The fate of herbicide acetochlor and its toxicity to *Eisenia fetida* under laboratory conditions. *Chemosphere*, *62* (8): 1366 - 1373.
[32] Zhou, S., Duan, C., Michelle, W. H. G., Yang, F. and Wang, X. (2011). Individual and combined toxic effects of cypermethrin and chlorpyrifos on earthworm. *Journal of Environmental Sciences*, *23* (4): 676 - 680.
[33] Berthelot, Y., Valton, É., Auroy, A., Trottier, B., and Robidoux, P. Y. (2008). Integration of toxicological and chemical tools to assess the bioavailability of metals and energetic compounds in contaminated soils. *Chemosphere*, *74* (1): 166 - 177.
[34] Žaltauskaitė, J. and Sodienė, I. (2010). Effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm *Eisenia fetida*. *Ekologija*, *56* (1 - 2): 10 - 16.
[35] Awgie, W. A and Mohamed, A. I. (2012). Avoidance behavior, Growth and Reproduction of the earthworm *Aporrectodea caliginosa* and *Eisenia fetida* exposed to chemical pesticide under laboratory condition –Msc. Thesis faculty of science, University of Benghazi, Libya.
[36] Garcia, M., Römbke, J., de Brito, M. T. and Scheffczyk, A. (2008). Effects of three pesticides on the avoidance behavior of earthworms in laboratory tests performed under temperate and tropical conditions. *Environmental Pollution*, *153* (2): 450 - 456.
[37] Loureiro, S., Soares, A. M. and Nogueira, A. J. (2005). Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. *Environmental pollution*, *138* (1): 121 - 131.