# Effects of NPK fertilizer on the earthworm, *Aporrectodea caliginosa* (Savigny, 1826): growth inhibition, biochemical composition and biomarker of oxidative stress (GPx).

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***Abstract*—**

**Organisms living in the soil are subject to regular fluctuations of abiotic parameters, and chemical contamination of the environment due to human activities. Thus, they are subject to multiple stressors, which influenced their biochemical profile. To assess the hazards involved in the action of chemicals on soil organisms, several biological methods have been developed on nematodes, earthworms, collembolans and snails. The toxicity endpoints of most of the bioassays are survival, growth and reproduction (Leung et al. 2008; Roh et al. 2010; ISO 10872:2010; ISO 15952:2006). The present study was conducted to study the effect of chemical fertilizer, NPK, at two agricultural doses on juveniles of *Aporrectodea caliginosa*. Growth, biochemical composition (lipid, protein, and carbohydrate content) and a biomarker of oxidative stress, glutathione peroxidase (GPx) were evaluated each week for four weeks. The NPK showed inhibitory effects in the growth of *A. caliginosa*. Moreover, this fertlizer resulted in a significant reduction in the energy reserves (carbohydrate, lipid and protein) after treatment. The enzymatic measurements performed in treated juvenile revealed a stimulation of the detoxification system as evidenced by an increase of GPx activity**.

***Keywords* *Aporrectodea caliginosa*, NPK, Growth, Biochemical composition, GPx**.

## I. INTRODUCTION

In agriculture, chemical fertilizers are administered in order to increase crop yield. They are responsible for massive soil pollution, but are primarily the major cause of air and water pollution and affect not only the target organisms, but also the wild (non-targeted) species such as earthworms that represent one of the most important biological indicators in the terrestrial environment. They are the main drivers of the physical, chemical and biological characteristics of soils. Earthworms constitute more than 80% of the invertebrate biomass in most of the agroecosystems of the world [25] and plays important role in improving the structure and fertility of the soil through their feeding, casting and burrowing activities [9] [16]. Due to their beneficial role in agroecosystem, earthworms are used as indicator species for monitoring the impact of pollutants, changes in soil structure and agricultural practices [12] [25]. Earthworms recognized as 'ecosystem engineers' are naturally in contact with the solid and aqueous phases of the soil, ingest large amounts of soil and are therefore directly exposed to contaminants coming from the intensive use of biocides (herbicides and insecticides) in agriculture, industrial activities and atmospheric deposition. A number of studies were conducted on the acute toxicity of NPK on the eartworms such as *Eisenia foetida* [1] [2] and *Drawida willsi* [4]. The use of biochemical biomarkers to investigate the contaminant toxicity, metabolization, and detoxification in earthworms is nowadays becoming a current practice [10] [22]. The importance of antioxidant enzymes is generally emphasized in the prevention of oxidative stresses by scavenging of reactive oxygen species (ROS) [17]. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (GPx). Superoxide radicals that are generated are converted to $H_2O_2$ by the action of SOD, and the accumulation of $H_2O_2$ is prevented in the cell by CAT and GPx.

Our objective was to study the effects of a fertilizer, the NPK, which is widely used in Tebessa area (North-East Algeria). This compound was applied at the recommended agricultural dose and recommended agricultural dose X2 (mg/kg) for field spreading, on the earthworm, *Aporrectodea caliginosa*, used as a bioindicator species of soil contamination. The effects of NPK were tested on the biochemical composition of the whole body and also the enzymatic biomarkers, by a study of GPx activity. The earthworm was also the subject of a standardized test based on the effects of this fertilizer on the growth.

## II. MATERIALS AND METHODS

### *A. Soil and earthworm*

Population samples of the endogenic juvenile's earthworm's *A. calliginosa* L. were collected by hand from a field in Tébessa (East of Algeria). Animals were acclimated

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for 7 days before use. The earthworm and soil were collected from an upland non-irrigated paddy field which had no record of input of agrochemicals.

*B. Fertilizer*

Mixtures containing all the three principal nutrients (N, P and K) are termed complete fertilizers. NPK fertilizer contains 15% N + 15% $P_2O_5$ + 15%$K_2O$. Two doses of NPK were applied corresponding to the recommended agricultural dose and recommended agricultural dose X2 (mg/kg). These were added to the soil surface and then mixed thoroughly with enough water to ensure a homogeneous mixture. The same procedure with only water was applied to prepare the control set.

*C. Growth inhibition*

The earthworms were washed, dried on filter paper, weighed, and then ten earthworms were placed per plastic container on the soil surface. Test containers were covered with perforated plastic lids. Their weight was monitored weekly. Before weighing, all of the earthworms were sorted, washed with tap water, and blotted with filter paper. Then the earthworms were weighed using an electro-balance and after that returned to the soil. The weights of earthworms in each concentration reported from the various exposure periods were then used to calculate the growth inhibition as follows Shi et al. (2007) [23]: $GIn= (W_0-W_t / W_0) X 100$

Where **GIn** is the growth inhibition for concentration n, **W0** is the weight on day 0 and **Wt** is the weight after t days of exposure.

*D. Biochemical studies*

Protein, carbohydrate and lipid were extracted following the procedure of Shibko et al. [24]. Pooled samples (100-200mg) were extracted in 1ml of TCA (20%). In brief, quantification of proteins was carried following the Coomassie Brilliant Blue G-250 dye-binding method [6] with bovine serum albumin as a standard. The absorbance was measured at 595 nm. Carbohydrates were determined as described by [11] using anthrone as reagent and glucose as standard. Lipids were measured by the vanillin method [14]. Data were expressed in µg per mg of fresh tissue and assays conducted with 3 replicates per treatment.

*E. Determination of Glutathione peroxidase activity*

GPx assay was performed according to [13]. Fragments of the body (100-200 mg) were homogenized in 1 ml of phosphate buffer (pH 7.8). The homogenate was centrifuged (3000 rpm for 10 min), and the supernatant was recovered as an enzyme source. The assay was performed on an aliquot of 200 µl of supernatant added to 400 µl of the GSH solution (0.2 mM, pH 10). Absorbance reading was performed after 5 minutes at 412 nm.

*F. Data Analysis*

The mean and standard deviation were calculated in triplicate from independent experiments. Descriptive statistics were given as box plots to describe the effect of NPK on biochemical responses of earthworms. The data pertaining were also analyzed with Tukey's post hoc analysis and ANOVAs, to test the effect of time exposure, dose, and treatment on biochemical components and antioxydant defense level. ANOVAs were considered statistically significant when p value <0.05.

## III. RESULTS

*A. Effects on growth*

The effect of NPK was assessed at different intervals (1, 2, 3 and 4week). The weight of control and treated individuals are reported in Fig. 1. This fertilizer resulted in a significant reduction in physiological parameter such as the fresh weight of earthworm with a dose-response relationship during the tested period.

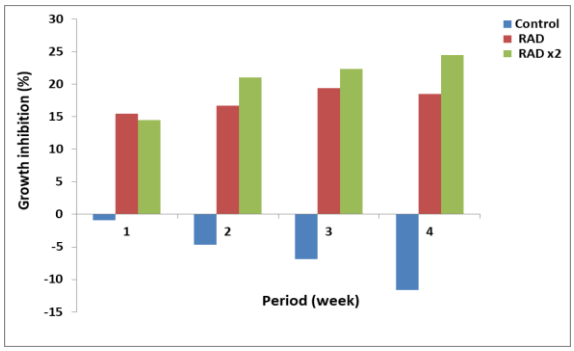


Fig.3. Effects of NPK (RAD and RADx2) on growth inhibition (%) in juvenile of *A. calliginosa*.

*B. Effects on biochemical components*

The levels of carbohydrates, lipids and proteins have been estimated in the body extracts from juvenile stage of earthworm using two doses (RAD and RADx2).The comparison of mean values shows that the lipid content was reduced significantly with the two tested doses at diiferent periods, without dose- response relationship.

Concerning the carbohydrate levels, a significant reduction was observed in first, second, third and fourth week with the two tested doses compared to controls.

Lastly, protein content decreased significantly with the two doses at the third and fourth week. No effect of the product on the protein contents was reported with the two doses applied ($P > 0.05$) at the first and the second week (Table 2).

TABLE I
Effects of NPK (RAD and RADx2) on lipids, carbohydrates and proteins in juvenile of *A. calliginosa* (mean ± SD, n = 3 pools each containing 50-100mg of fresh tissue).

| Periods (Week) | Biochemical components | Control | NPK (RAD) | NPK (RADx2) |
|---|---|---|---|---|
| **1** | Lipids | 4.79±0.28a | 4.01±0.08b | 4.21±0.00b |
| | Carbohydrates | 22.46±1.58a | 13.87±0.02b | 13.59±0.10b |
| | Proteins | 5.61±0.54a | 4.72±0.13a | 5.31±0.43a |
| **2** | Lipids | 5.08±0.20a | 4.18±0.03b | 4.08±0.02b |
| | Carbohydrates | 19.20±0.10a | 13.47±0.11b | 13.11±0.09b |
| | Proteins | 6.97±0.39a | 6.31±0.42a | 5.39±0.83a |
| **3** | Lipids | 6.41±0.21a | 4.04±0.00b | 4.02±0.01b |
| | Carbohydrates | 17.99±0.01a | 12.94±0.08b | 13.01±0.01b |
| | Proteins | 10.96±0.11a | 9.51±0.07b | 10.19±0.61a |
| **4** | Lipids | 6.66±0.02a | 4.12±0.00b | 4.01±0.03b |
| | Carbohydrates | 17.80±0.04a | 12.97±0.03b | 13.16±0.04b |
| | Proteins | 15.96±1.04a | 13.00±0.10b | 13.29±0.49c |

### *C. Effects on GPx activity*

Results of the specific activities of antioxidant defense enzyme (GPx) in *A. calliginosa* are shown in Fig. 1. These results show that values found in antioxidant are very similar in different tested periods. No effect of the product on the GPx activity was reported with the two doses applied ($P > 0.05$).

TABLE II
Effect of NPK (RAD and RADx2) on GPx activity (µM/min/mg of protein) in juvenile of *A. calliginosa* (mean ± SD, n = 3 pools each containing 50-100mg of fresh tissue).

| Periods (Week) | Control | NPK (RAD) | NPK (RADx2) |
|---|---|---|---|
| **1** | 0.571±0.018a | 0.621±0.011a | 0.631±0.009a |
| **2** | 0.590±0.052a | 0.623±0.006a | 0.632±0.078a |
| **3** | 0.593±0.031a | 0.624±0.060a | 0.635±0.007a |
| **4** | 0.596±0.034a | 0.626±0.031a | 0.635±0.027a |

## IV. DISCUSSION

### *A. Effects on growth*

Earthworms provide key soil functions that favour many positive ecosystem services. These services are important for agroecosystem sustainability but can be degraded by intensive cultural practices [21]. [8] did not show any effect of glyphosate on survival and growth of earthworms at recommended field concentrations. Toxicity of glyphosate has been reported but at higher rates of application than here. [26] showed a decrease in growth of earthworms (*E. fetida*) but only for a high concentration in soil (8 mg/kg) of a glyphosate-based formulation (Glycel®). Weight loss has also been reported for organochlorine pesticides intoxication [18] and for the effects of fungicides and herbicides in *Eisenia fetida* and *Lumbricus terrestris* [3] [15]. [20] found endosulfan did significantly reduce the weight of juvenile *Aporrectodea trapezoides* within 5 weeks when applied to soil at normal application rate in both the field and laboratory while fenamiphos did so at normal application rate in the field only.
[27] have reported that the weight of the earthworms was a more sensitive index compared to the mortality in indicating toxic effects of acetochlor and methamidophos. Some studies have shown that growth of earthworms appeared to be more severely affected at juvenile stage than at adult stage [28].

### *B. Effects on biochemical composition*

The increase of total protein in earthworm might indicate physiological adaptability to compensate stress and the development of cellular defenses induced by the pesticide and phosphate fertilizer impact. Furthermore, protein accumulation could be necessary to restore enzymes or lost in tissue necrosis induced by sekator or TSP exposure [20]. Paris-Palacios et al. (2000) reported a significant increase in protein content in zebrafish (Brachy danio rerio) exposed to copper sulfate. Similarly, [20] observed changes in protein content among aquatic worms Tubifex tubifex exposed to copper. Moreover, treated earthworm, *Lumbricus terrestris* with sekator showed a non-significant increase of protein content. In contrast, protein content increased significantly after exposure with TSP and the mixture [19].

### *C. Effects in GPx activity*

The GPx activity increased in response to decreased concentrations. The results observed for GPx activity indicated that increasing the concentration resulted in an increase in enzyme capacity. A similar result was obtained for lymphocytes in a report by Liu (Liu, 2001).

## V. CONCLUSION

Soil organisms are exposed to a wide variety of environmental pollutants and earthworms are one of the most important organisms. They are significantly influenced by environmental stress, and because of their sensitive metabolic and physiological changes, earthworms are useful as test organisms to assess the toxicity. The results obtained showed that the treatment inhibited the increase in weight of *A. calliginosa*, which could be due to the repulsion of the contaminated food. The insecticide also reduced content in proteins, lipids and carbohydrates in the whole body and caused the activation of the system of detoxification, traduced by an increase of the specific activity of GPx. In Perspective, this work can be completed by detection of bioaccumulation of insecticide residue in *A. calliginosa* via HPLC technique.

## ACKNOWLEDGMENT

This study was supported by the National Fund for Scientific Research to Pr. N. Soltani (Laboratory of Applied Animal Biology, Badji Mokhtar University, Annaba) and the Ministry of High Education and Scientific Research of Algeria (CNEPRU Project to Pr. F. Tine-Djebbar and PRFU project to Dr. Tine Samir).

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