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THE LEAVES OF ACER PLATANOIDES: POTENTIAL FOOD FOR EARTHWORM EISENIA FETIDA

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ABSTRACT

This study aimed to establish if the dried shredded leaves of *Acer platanoides* could represent an adequate food for earthworm *Eisenia fetida* (Savigny 1826) (red wiggler worm). The study was carried out in laboratory conditions and its aims were to determine the influence of this food on three biological parameters of earthworm *Eisenia fetida* (survival, weight, and prolificacy), and to reveal the changes appearing into the soil in the presence of this earthworm species, fed or not with shredded leaves of *Acer platanoides*, regarding the concentration of several parameters: the total nitrogen, available potassium, and available phosphorus. The results showed that the leaves of *Acer platanoides* could be an appropriate food for earthworms *Eisenia fetida*. This experiment showed that the leaves of *Acer platanoides* represent an adequate food for the earthworms *Eisenia fetida* because a low mortality was recorded (16,7% in controls, respectively 10% in the boxes with earthworms fed with dried shredded leaves of *Acer platanoides*, at the end of the experiment). The leaves of *Acer platanoides* represent an adequate food for the earthworms *Eisenia fetida* in order to increase the prolificacy of this species reared in microcosm conditions, to increase the pH of the adjacent soil toward alkaline domain, to increase the content in mobile phosphorus of the soil. The earthworms *Eisenia fetida* fed with dried leaves of *Acer platanoides* recorded statistically significant ($p<0.01$, $p<0.05$) positive correlations between the parameters body weight and prolificacy and the content of mobile phosphorous of the soil, and statistically significant ($p<0.01$) negative correlations between the parameters body weight and prolificacy and the content of mobile potassium of the soil. The achieved data have been statistical processed using Spearman and Kendall Correlations, the test One Way ANOVA, and Pearson Correlation ($p<0.01$, $p<0.05$).

Keywords: mortality, prolificacy, *Eisenia fetida*, *Acer platanoides*, soil

INTRODUCTION

The tree foliar preference of earthworms as food has been previously investigated by many researchers in several studies including different tree species and various earthworm species, in order to highlight its direct impact on soil quality and the implication in the biological processes in the soil [1-6; 8; 10]. Thus, Ashwood et al. (2017) demonstrated that earthworms *Aporrectodea longa* and *Allolobophora chlorotica* display preferences for specific types of leaf litter while investigated the effect of a reclaimed soil quality on earthworm growth, survival and feeding preferences [3]. Butt (2011) demonstrated that the food quality/type may be a factor to be considered in construction of production models for earthworm *Lumbricus terrestris* in ecosystems with contrasting food quality [4]. Doube et al. (1997) examined the food-preference behavior of four earthworm species (*Aporrectodea caliginosa, A. longa, Lumbricus rubellus, L. terrestris*) with four types of organic matter (cow dung, sheep dung, decomposed leaf litter, sewage sludge) and have revealed a strong preference for these earthworms for the pure mineral soil over the pure organic matter and a clear preference for mineral-organic mixtures to pure organic sources, the combination leaf litter + soil being the preferred mixture for all four earthworm species tested [5].

The feeding behaviours of earthworms, and as a result their roles in this process, differ between species [6].

Studies regarding the effects of organic matter availability on the growth and hatchling behaviour of *Aporrectodea longa, Lumbricus terrestris* and *L. rubellus* showed for all species changes with time, which may be important in the development of sustainable earthworm populations and hence their role in soil amelioration [6].

The motivation for developing this study was the potential of earthworms *Eisenia fetida* in consuming a wide range of vegetal wastes, and the lack of data about the potential of leaves of *Acer platanoides* in being food for earthworms, generally. In plus, in Romania, especially in the western side (in Banat), the trees of *Acer platanoides* are numerous, and their leaf litter is quantitatively significant as a deciduous species.

MATERIALS AND METHODS

The experiment was conducted as a microcosm experiment and lasted three months. There was chosen the chamber method because this is a simple, rapid and inexpensive test for studying food preferences of earthworms, as selected before by other researchers [5]. The microclimate conditions consisted of 20°C ± 2°C environmental temperature and the natural photoperiod was characteristic to the research interval, respectively November, December, and January of the region (45°45'N21°14'E). The experiment has been carried out in three replicates in control boxes (*Eisenia fetida* not fed with dried shredded leaves of *Acer platanoides*) and in test boxes (*Eisenia fetida* fed with dried shredded leaves of *Acer platanoides*). The experimental soil was a cambic chernozem (FAO System) collected from the first 30 cm of topsoil, cleaned off by vegetal and other rests and without earthworm cocoons and other impurities (Table 1). Each experimental box was filled with soil in amount of 1660 g.

Table 1. Physical, physical-chemical and chemical properties of the experimental soil

Soil layer	Ap	Atp	Am	AB	BC	Cca g_1	Cca g_1	Cca g_2
Depth (cm)	0-20	20-35	35-50	50-65	65-85	85-110	110-130	130-200

pH in water (pH unities)	6,00	6,60	6,70	6,90	7,75	8,10	8,15	8,25
$CaCO_3$%	-	-	-	-	0,60	15,50	17,70	10,80
Total organic carbon (%)	2,97	2,79	2,42	2,23	1,73	0,93	-	-
Total nitrogen (%)	2,58	2,48	2,20	2,11	1,73	0,93	-	-
P_{AL} (ppm)	35,0	7,2	6,7	-	-	-	-	-
K_{AL} (ppm)	322,0	262,3	259,8	-	-	-	-	-
Base saturation (% of total)	87,0	89,0	91,2	94,9	100	100	100	100
Cation exchange capacity (meq · 100 g^{-1} soil)	29,0	30,3	29,4	28,9	-	-	-	-
Total acidity (meq · 100 g^{-1} soil)	3,64	3,32	2,58	1,47	-	-	-	-
Rough sand (2.0-0.2 mm) (%)	0,2	0,6	0,2	0,6	0,6	0,5	0,3	1,1
Fine sand (0.2-0.02mm) (%)	28,2	27,0	29,6	29,6	30,7	32,2	32,5	20,1
Dust (0.02-0.002 mm) (%)	27,5	29,3	24,8	24,7	23,4	26,3	28,1	33,9
Clay (<0.002 mm) (%)	44,1	43,1	45,4	45,0	45,3	41,0	39,0	44,9
Physical clay (<0.01 mm) (%)	55,2	56,4	59,3	55,2	59,4	55,6	55,9	63,9
Density (g · cm^{-3})	2,51	2,51	2,62	2,57	-	-	-	-
Bulk density (g · cm^{-3})	1,44	1,54	1,36	1,52	-	-	-	-
Total porosity (%)	41,04	38,65	48,09	40,86	-	-	-	-
Wilting coefficient (%)	13,35	13,65	13,20	13,20	-	-	-	-
Water field capacity (%)	28,10	28,18	29,06	26,06	-	-	-	-
Useful water capacity (%)	14,75	14,53	15,86	14086	-	-	-	-

In each box there were introduced ten adult earthworms *Eisenia fetida*, with body weight ranging between 5.29 – 5.86 g, without significant differences between weight values. In the test boxes was added nutritive substrate as dried shredded leaves of *Acer platanoides* at surface, in amount of 10 g per box. The moments when the nutritive substrate has been added in the test boxes were: at six days from the moment when earthworms have been introduced in boxes (from the start of the experiment); after 45 days from the moment when earthworms have been introduced in boxes, (at the halfway of the experiment). The moisture content was maintained between 70 and 80% by adding distilled water one time a week.

There were monitored the following parameters: the body weight of earthworms at the start and at the end of the experiment, in control boxes and in test boxes, respectively; the earthworm mortality (as earthworms found alive) at the start and at the end of the experiment, in control boxes and in test boxes; the earthworm prolificacy (hatched larvae) at the end of the experiment, in control boxes and in test boxes; the variation of values for the following parameters of soil: pH, total nitrogen, available potassium, and available phosphorous, in all boxes (control and test);

The number of earthworms and hatchlings were found by hand sorting. The parameters of soil (pH, total nitrogen, available potassium, and available phosphorous) were established using the following methodology: the pH values have been established by potentiometric method in aqueous suspension (pH_{H2O}), ratio soil:solution was 1:2.5; the content in total nitrogen of soil – by the Kjeldahl method; the mobile (plant available) phosphorus and potassium of soil have been determined by spectrophotometry and flame spectrometry methods, respectively, by the Egner-Riehm-Domingo method [7]. The statistical processing was realized using the software SPSS (Statistical Package for the Social Sciences).

RESULTS AND DISCUSSION

Data regarding the mortality, the body weight and the prolificacy of earthworms *Eisenia fetida* within experiment are shown in Table 2.

Table 2. Mortality, body weight, and prolificacy of the species *Eisenia fetida* within experiment

Experimental variant	Mortality		Body weight		Prolificacy
	Earthworm number at the start of experiment	Earthworm number at the end of experiment	Earthworm weight at the start of experiment (g)	Earthworm weight at the end of experiment (g)	Number of hatched larvae after 3 months of experiment
Control	10	8.33 ± 2.08	5.07 ± 0.51	2.09 ± 0.63	6.66 ± 4.04*
Standard Error	0	1.47	0.36	0.44	2.33
Leaves of *Acer platanoides*	10	9	5.55 ± 0.27	2.50 ± 0.39	9.66 ± 3.05*
Standard Error	0	0	0.15	0.22	1.76

*Paired Sample T-test ($p < 0.05$): $t(2) = -5.196$, $p = 0.035$.

There was found a low mortality of earthworms in the experimental boxes: 16.70% in controls at the end of experiment, and 10% in the boxes with earthworms fed with leaves of *Acer platanoides* at the end of experiment.

Generally, the body mass of earthworms fed with organic matter registered increases within experiments, but in this study the body weight of earthworms recorded a decrease. The results achieved by Cesarz et al. 2016 [8] concluded that for the earthworm species *Aporrectodea caliginosa* and *Octolasion tyrtaeum* the mass gain is driven by both soil and litter quality (*Fagus sylvatica*, *Acer platanoides*, *Acer pseudoplatanus*, *Carpinus betulus*, *Tilia spp.*, *and Fraxinus excelsior*) and determined soil microbial biomass C as a potential food source of these species, even this last is a poor predictor of relative earthworm mass gain. Overall, their results indicated that leaf litter quality effects on endogeic earthworm mass gain were more important in low quality soil for both earthworm species [8]. In this study, the mass loss of earthworms is considered to appear because these were moved from a moisturized environment into another environment (the experimental soil) less moisturized.

There was found after 3 months of experiment that the prolificacy of earthworms fed with leaves of *Acer platanoides* considerably increased, with 45,04% as compared to control (paired sample T-test, p = 0.035). Other studies reported the same concerning the earthworms, although in different species. Thus, Ashwood et al. (2017) revealed that earthworms *Aporrectodea longa* and *Allolobophora chlorotica* preferred the higher degraded leaves of *Alnus cordata* over *Acer platanoides*, and that fresh leaves are a suitable food source for earthworms in chamber experiments, recording high survival rates of both A. *longa* and *A. chlorotica* and demonstrated that these earthworm species are suitable candidates for inoculation to reclaimed land [3]. Butt (2011) studied the growth and reproduction of laboratory-reared *Lumbricus terrestris* when fed with birch leaves, and the overall results showed that food quality can have a significant influence on somatic and reproductive production of *L. terrestris* [4; 9].

The analysis of several physical-chemical and chemical properties of the soil is listed in Table 3 and shows a slightly increase of pH and of content in mobile phosphorus of soil containing earthworms fed with leaves of *Acer platanoides* (p<0.05).

Table 3. The physical-chemical properties of the soil of the experimental variants

Experimental variant	Indicator	Values
Soil without *Eisenia fetida*	pH (pH units)	7.89 ± 6.08
	Nt (%)	0.15 ± 2.51[1]
	P ($mg \cdot kg^{-1}$)	18.14 ± 0.13[2]
	K ($mg \cdot kg^{-1}$)	220.66 ± 6.11
Soil with *Eisenia fetida*	pH (pH units)	7.79 ± 6.02[3]
	Nt (%)	0.21 ± 1.52[1,4]
	P ($mg \cdot kg^{-1}$)	36.02 ± 5.07[2]
	K ($mg \cdot kg^{-1}$)	188.33 ± 20.00
Soil with *Eisenia fetida* fed with leaves of *Acer platanoides*	pH (pH units)	8.03 ± 0.04[3]
	Nt (%)	0.19 ± 0.02[4]
	P ($mg \cdot kg^{-1}$)	30.55 ± 0.54
	K ($mg \cdot kg^{-1}$)	223.33 ± 15.69

[1]One-Way ANOVA ($p < 0.05$): $F(1.4) = 12.462$, $p = 0.024$; Paired Sample T-test ($p < 0.05$): $t(2) = -6.000$, $p = 0.027$;

[2]One-Way ANOVA ($p < 0.01$): $F(1.4) = 37.376$, $p = 0.004$; Paired Sample T-test ($p < 0.05$): $t(2) = -6.211$, $p = 0.025$;

[3]One-Way ANOVA ($p < 0.01$): $F(1.4) = 31.905$, $p = 0.005$; Paired Sample T-test ($p < 0.05$): $t(2) = -4.266$, $p = 0.051$;

[4]Paired Sample T-test ($p < 0.05$): $t(2) = 5.000$, $p = 0.038$.

Within the study there were found statistically significant ($p<0.01$, $p<0.05$) positive correlations between the weight of earthworms fed with leaves of *Acer platanoides* and the content in phosphorus of the soil [10], as well as between the weight of unfed earthworms and the pH and the content of total nitrogen of soil. The prolificacy of the fed earthworms was found also to be positive correlated to the mobile phosphorus in the soil, and the prolificacy of the unfed earthworms was found to be positive correlated to the mobile phosphorus in the soil and pH (Table 4).

Statistically significant ($p<0.01$) negative correlations there were established between potassium in the soil and the body weight and prolificacy of earthworms, regardless their feeding way (Table 4).

Table 4. Correlations between body weight and prolificacy of earthworms *Eisenia fetida* fed with leaves of *Acer platanoides* and the concentrations of mobile phosphorous and potassium of the adjacent soil (Spearman and Kendall Correlations: $p < 0.01$, Pearson Correlation: $p < 0.05$).

Correlations between body weight of the earthworms fed with leaves of *Acer platanoides* and the concentration of the mobile phosphorous of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of earthworms fed with leaves of *Acer platanoides* and the concentration of the mobile potassium of soil	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms not fed with leaves of *Acer platanoides* and the concentration of the mobile potassium of soil	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms not fed with leaves of *Acer platanoides* and the concentration of the total nitrogen of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between body weight of the earthworms not fed with leaves of *Acer platanoides* and the pH of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms fed with leaves of *Acer platanoides* and the mobile potassium of soil	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms fed with leaves of *Acer platanoides* and the mobile phosphorous of soil	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000

Correlations between the prolificacy of the earthworms not fed with leaves of *Acer platanoides* and the mobile potassium of soil	Spearman's Correlation Coefficient	–1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms not fed with leaves of *Acer platanoides* and the mobile phosphorous of soil	Pearson's Correlation Coefficient	0.999*
	Sig. (2-tailed)	0.030
	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
Correlations between the prolificacy of the earthworms not fed with leaves of *Acer platanoides* and the pH of soil	Pearson's Correlation Coefficient	0.999*
	Sig. (2-tailed)	0.030
	Kendall's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000
	Spearman's Correlation Coefficient	1.000**
	Sig. (2-tailed)	0.000

**Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

Through this experiment, there was concluded that the leaves of *Acer platanoides* represent an adequate food for the earthworms *Eisenia fetida* because a low mortality was recorded (16,7% in controls, respectively 10% in the boxes with earthworms fed with dried shredded leaves of *Acer platanoides,* at the end of the experiment).

The achievements of this study can be concluded as follows: the leaves of *Acer platanoides* represent an adequate food for the earthworms *Eisenia fetida* if the following objectives need to be reached: the increase of the prolificacy of this species in laboratory conditions (microcosm experiments); to increase the pH of the adjacent soil and to bring it in the alkaline domain, to increase the content in mobile phosphorus of the soil.

The earthworms *Eisenia fetida* fed with dried leaves of *Acer platanoides* recorded statistically significant ($p<0.01$, $p<0.05$) positive correlations between the parameters body weight and prolificacy and the content of mobile phosphorous of the soil, and statistically significant ($p<0.01$) negative correlations between the parameters body weight and prolificacy and the content of mobile potassium of the soil.

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