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EXPERIMENTAL ASPECTS REGARDING THE FEEDING BEHAVIOUR OF THE EARTHWORM SPECIES *Eisenia fetida* – QUICK TEST OF FOOD LOCATION AND SELECTION

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Abstract

This research aimed to study in a laboratory experiment some aspects regarding the feeding behaviour of the earthworm Eisenia fetida: food location and selection, time towards food, time to access food. The experiment has been carried out as a quick test: earthworm released and placed at 15 cm distance away from a food source consisting of two adjacent substrates in order to observe the ability to locate food and to select a certain substrate of the food source: soil and respectively, soil mixed with earthworm food commercially purchased. The results of this experiment showed that earthworms Eisenia fetida possess the ability to locate the organic food source used within the experiment. A proportion of 90% of tested earthworms were able to locate the food source, and 70% of earthworms were able to choose a specific food substrate from two available. A proportion of 30% of earthworms reached the food source in the first 1-2 minutes after exposure, 20% during 2-3 minutes, 30% after 4-5 minutes, and 10% after 6-7 minutes of exposure. The statistical interpretations of the achieved results (Chi-square Goodness-of-fit-test) showed that earthworms Eisenia fetida are able to rapidly identify the organic food sources and chose between food sources, and these are not random behaviours.

Key words*: earthworm, food location, food choice, food access, feeding behaviour.*

INTRODUCTION

The question how earthworms are able to locate and select their food in soil and what implications are these abilities was often asked (Bonkowski & Schaefer, 1997; Amoji et al., 1998; Bonkowski et al., 2000; Zaller & Saxler, 2007; Rief et al., 2012; Euteneuer et al., 2020) and it concerns all three ecological groups of earthworms: epigeic, endogeic, anecic. Due to its particularities of feeding, the epigeic group of earthworms has been questioned frequently: are or are not able these earthworms to locate their food in soil using structural features, like, for example, olfactive structures? Or the feeding behaviour is randomly manifested in soils? To find answers is important because the earthworms dominate the invertebrates in soils (Philips et al., 2021) and their role is extremely important in turning the organic matter back to simple inorganic, plant available, chemical elements, which means implications in soil fertility (Curry & Schmidt, 2007). All the major benefits of earthworms in soil fertility are strongly related to their digestive and locomotion behaviours, and this is the reason why this investigation about food location and selection on *Eisenia fetida* has been carried out. But, there are also other important aspects and implications of earthworms' behaviour of food choice, for example the mechanism of heavy metal resistance which has been demonstrated to be expressed in earthworms through their ability to choose the food (Depta et al., 1999), or the foraging strategies which are based on food choice preferences among others (Zirbes et al., 2011), or ecotoxicological implications both in soil chemistry (Lowe & Butt, 2007; Butt et al., 2020) or in soil biota (Zirbes et al., 2011), and even ecological implications in vegetation distribution (Rajapaksha et al., 2013; Ashwood et al., 2017).

Although the behaviour of earthworms to locate and choose the food and to detect

chemical substances have been demonstrated in various experiments, remain uncertain the structural, physiological and behavioural mechanisms involved in these earthworm actions, and these represent future research directions aiming to detail the feeding ecology of earthworms.

MATERIALS AND METHODS

The experiment has been set up as a laboratory quick test in order to observe if the earthworms *Eisenia fetida* (Savigny, 1826) possess the immediate ability to find the food located at 15 cm distance away. This species has been choose because of its easily purchase and because aspects like food location and choice have been insufficiently studied in this species and generally insufficiently studied in earthworms. The experiment (Figure 1) used ten adult, clitellate earthworms, which were individually tested on a glass plate containing a moisturised (70%) source food made by two components, adjacent, placed together on the glass plate without any space or physical barrier between them: half of the food source was represented by ordinary plant cultivation soil, commercially purchased, and the other half consisted of mixture between this type of soil and specific earthworm food in 3:1 ratio, in order to detect the earthworms ability to find the food measured as time (minutes) spent from the start of the testing until earthworm is reaching the food, and also the earthworms ability to select one of the two adjacent substrates which made up the whole food source.

Figure 1. Aspect during the experimental test

The specific food for earthworms has been commercially purchased and it was based on dried plants of *Aspergillus,* with the following nutritional proportions: 12% proteins, 3% gross fat, 10% fibres, minimum 0,6 - maximum 1,4% calcium, minimum 0,4% phosphorus, minimum 0,1% salt, minimum 0,02 ppm selenium, minimum 25 ppm zinc, minimum 0,9 copper, minimum 9,50 I.U./kg vitamin E, minimum 1,1 I.U./kg vitamin A, minimum 0,11 I.U./kg vitamin D_3. Important to be mentioned is the specific smell of this food as compared with the other half of the food source.

The environmental temperature was 20°C. The moisture of the food substrates was 70%. The level of substrate humidity is an important factor in earthworms' locomotion, which also is strongly related to the nutrition function, both functions being important to be maintained in optimal conditions in our experiment. Based on these arguments, the glass plate was also humidified, to facilitate the earthworm movement according the physiological requirements of the species.

Each earthworm has been individually tested, orientated with the anterior (head) side towards food source. The distance between earthworm and food source was 15 cm. The achieved data has been statistically processed using Chi-square Goodness-of-fit-test, IBM SPSS Statistics 28.0.

The tests have been performed as rapid tests, meaning the release of the earthworm and its positioning on the glass board containing the food source, followed by an waiting time assigned to each worm to locate and reach the food.

RESULTS AND DISCUSSIONS

The main objective of the study was to establish if the earthworms *Eisenia fetida* are able to find a food source located at 15 cm distance away immediately after earthworm release by measuring two parameters: food location expressed as the number of earthworms able to move in the direction of the food source and measured as time (minutes) necessary to reach the food, and respectively the ability of food selection/choice expressed as the number of earthworms choosing one or another of the two available food substrates.

In Table 1 are shown the moving times towards food source of ten earthworms *Eisenia fetida,* individually tested.

A proportion of 90% of tested earthworms was able to identify the food source and 70% of earthworms have chosen the substrate containing special earthworm food. In Table 2 are listed the time measurements of food accession, categorised by time intervals achieved by earthworms starting with the beginning of the test and ending with the reach of the food source.

Regarding the food location ability, there was observed that 30% of earthworms have reached the food source within 1-2 minutes after exposure on the glass board, 20% during 2-3 minutes, 30% during 4-5 minutes, and 10% during 6-7 minutes (Figure 2).

Table 1. The moving times towards food source of earthworms *Eisenia fetida* counted from the test start until the food source is reached by earthworms

Tested earthworms *(Eisenia fetida)*	Moving time (minutes and seconds)	Action type: food source reached/failure		
		Soil with earthworm food	Soil without earthworm food	Failure, movement out of the test board
Eisenia fetida 1	1m15s	x		
Eisenia fetida 2	1m56s	x		
Eisenia fetida 3	2m10s	x		
Eisenia fetida 4	2m0s		x	
Eisenia fetida 5	0			x
Eisenia fetida 6	6m25s		x	
Eisenia fetida 7	2m20s	x		
Eisenia fetida 8	4m18s	x		
Eisenia fetida 9	4m58s	x		
Eisenia fetida 10	4m56s	x		

Table 2. Time intervals achieved by earthworms *Eisenia fetida* in reaching the food source

Tested earthworms *(Eisenia fetida)*	Moving time (minutes andseconds)	Type of the reached food substrate	Time interval
Eisenia fetida 1	1m15s	Soil with earthworm food	1-2 minutes
Eisenia fetida 2	1m56s	Soil with earthworm food	1-2 minutes
Eisenia fetida 3	2m10s	Soil with earthworm food	2-3 minutes
Eisenia fetida 4	2m0s	Soil without earthworm food	1-2 minutes
Eisenia fetida 5	0	Failure, movement out of the test board	0
Eisenia fetida 6	6m25s	Soil without earthworm food	6-7 minutes
Eisenia fetida 7	2m20s	Soil with earthworm food	2-3 minutes
Eisenia fetida 8	4m18s	Soil with earthworm food	4-5 minutes
Eisenia fetida 9	4m58s	Soil with earthworm food	4-5 minutes
Eisenia fetida 10	4m56s	Soil with earthworm food	4-5 minutes

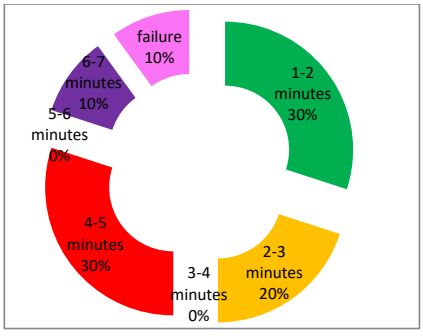

Figure 2. Proportions of earthworms (%) able to locate the food source by time intervals necessary to reach the food source

Regarding the earthworm ability to choose between two types of nutritive substrate (namely ordinary plant cultivation soil and respectively mixture of this type of soil and specific earthworm food in 3:1 ratio), there was found the following results: 20% of earthworms have chose the mixture of soil and special earthworm food in 1-2 minutes after the experiment beginning; 20% of earthworms have chose the mixture of soil and special earthworm food in 2-3 minutes after the experiment beginning; 30% of earthworms have chose the mixture of soil and special earthworm food in 4-5 minutes after the experiment beginning.

The statistical processing of data (Chi-square Goodness-of-fit-test, IBM SPSS Statistics 28.0) showed that earthworms *Eisenia fetida* are able to detect organic food sources, and this is not a random behaviour (Figure 3), the observed results being significantly different from those expected (Chi-square Goodness-of-fit test, $\chi^2(1)=6,40$, $p=0,011$). Also, the Chi-square Goodness-of-fit-test showed that earthworms significantly preferred the food substrate containing special earthworm food versus the substrate of simple soil (Chi-square Goodness-of-fit test, $\chi^2(1)=6,20$, $p=0,045$) (Figure 4).

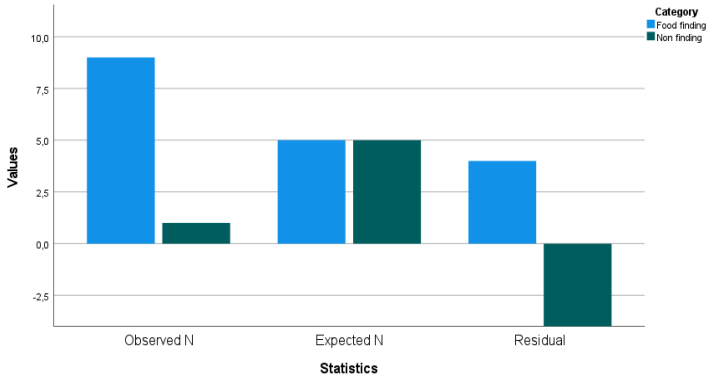

Figure 3. Distribution (earthworm number - N) of the ability of earthworms *Eisenia fetida* to locate the food source: the observed results are significantly higher than those theoretically expected (Chi-square Goodness-of-fit-test: $\chi^2(1)=6,40$, $p=0,011$)

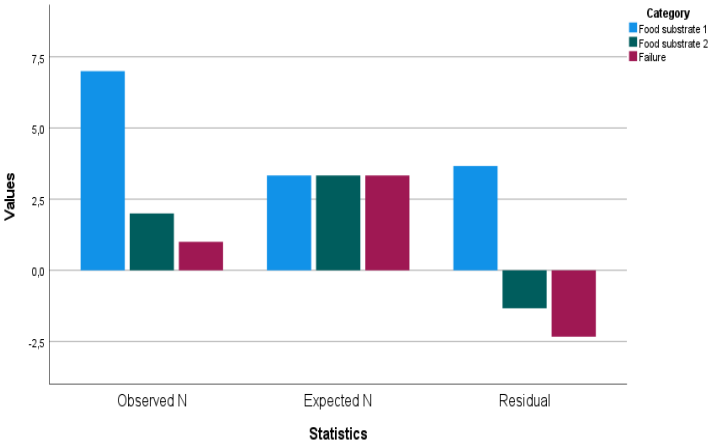

Figure 4. Distribution (earthworm number - N) of the ability of earthworms *Eisenia fetida* to chose a certain food source (Food substrate 1 = food substrate containing special earthworm food; Food substrate 2 = simple soil; Failure = movement out of the test board): the observed results are significantly higher than those theoretically expected (Chi-square Goodness-of-fit-test: $\chi^2(1)=6,20$, $p=0,045$)

The results achieved through this quick test clearly showed that earthworms *Eisenia fetida* are able to rapidly identify the organic food sources tested within this experiment.

However, although this study represents an initiative in trying to demonstrate the ability of earthworms to locate and choose their foods, it is necessary to extend researches with additional factors to be considered, and moreover to explore research directions able to reveal the structural and physiological implications of this behaviour, which currently still remain mostly unknown.

It is cert however, as possible explanation of the results achieved through this study, the presence of specialized sensitive cells – chemoreceptors located on the prostomium (mouth) and in the buccal epithelium of earthworms (Laverack, 1960), which can detect sucrose, glucose, and quinine and other many chemicals (Laverack, 1960; Satchell, 1983). Other studies emphasize that the volatile emissions emanated by fungi and other microorganisms at soil level are the main substances detected by earthworms using chemoreceptors (Zirbes et al., 2011). There are available also other several researches which demonstrate the obviously orientated movement of earthworms towards organic food sources (Satchell, 1967; Doube et al., 1997; Neilson & Boag, 2003; Curry & Schmidt, 2007), and which support the findings achieved through this study.

CONCLUSIONS

The results of the experiment showed that a large proportion (90%) of earthworms *Eisenia fetida* have located the food source and also 70% of the tested earthworms have chosen a specific food substrate within a time interval no longer than 5 minutes.

The statistical interpretations of the achieved results (Chi-square Goodness-of-fit-test) showed that earthworms *Eisenia fetida* are able to rapidly identify the organic food sources and chose between food sources, and these are not random behaviours.

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