Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 9 (20): 3746-3751, 2009
ISSN 1812-5654

Identification of Earthworms species in Sari Township in Northern Iran, 2007-2008

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Abstract: The aim of this study is identification of earthworm species gathered from the geographic regions of Sari Township. Samples gathered from different regions of Sari Township fixed by formaldehyde and alcohol solution in several stages and placed into formalin tubes (5%), in addition to providing sample characteristics tabled by Graff identification key, fixed earthworms are recognized. After recognition, analysis obtained data from geographical information of sampling regions and from original and microscopical observations of morphological characteristics were performed using SPSS with statistical tests (Chi square, ANOVA). From 644 samples gathered, 345 adult samples were recognizable and identified. Five species were recognized, including: *Eisenia fetida* (120 samples), *Dendrobaena byblica* (67 samples), *Allolobophora caliginosa* (58 samples), *Allolobophora kaznakovi* (52 samples) and *Allolobophora jassyensis* (45 samples). Meanwhile, two samples were regenerating and one sample was unknown. The average weight in adult earthworms was 0.54±0.23 (g), while 69.23±16.24 (mm) for their length and the number of segments averaging 100.48±18.96. The greatest weight, length and number of segments belonged to the *Allolobophora caliginous*. This study showed that the dominant species in Sari Township with 34.8% amplitude (120 samples), belonged to *Eisenia fetida*. This species obtained from all regions of Sari Township, included: plains (45%), forests (31%), littorals (18%) and mountains (6%).

Key words: Species identification, earthworm, Sari Township

INTRODUCTION

Nowadays, earthworms are used practically in compost production from organic wastes and garbage (Vermi-compost), feeding of birds and soil reformation (Edwards and Lofty, 1977). According to Gupta (2004), earthworms are used for sewage sludge stabilizing and organic wastes in developed countries such as the USA, Germany and Australia. FAO and related researchers conducted research on the extensive use of these worms in farms of the developing countries such as Philippines, Cuba and India (Hardouin, 1995; Branckaert, 1995). Research was conducted in locating the treatment benefits of these worms because of lumberokinase enzyme and their effects in fibrinolysis and also in the cancer cure (Tang *et al*., 2000; Li *et al*., 2008). These enzymes are used in preparation of drugs (Kim *et al*., 1998). Research was also preformed regarding the preparation of vermicompost and garbage disposal methods by these worms (Tripathi and Bhardwaj, 2004; Maboeta and van Rensburg, 2003; Kaviraj and Satyawati, 2003; Nair *et al*., 2006). Finally, some researches are utilizing the earthworm in aquarium feeding (Kostecka and Paczka, 2006). All of the research proved the crucial importance regarding earthbound animals in different scientific fields.

In Iran, there has been limited research on these animals than in other countries, especially Eisenia fetida, in preparation of vermicompost (Peyvast *et al*., 2007; Azarmi *et al*., 2008; Malek, 2007; Shahmansouri *et al*., 2005; Sludge *et al*., 2008; Parvaresh *et al*., 2004; Alidadi *et al*., 2005).

On basis of obtained informations from creditable texts, Iranian researchers started earthworm identification (Sims and Gerard, 1985; Carter and Gregorich, 2007). The identification of these worms in Iran was researched in 1998, which concluded in eight species being identified from four genera in the Mazandaran Province (Omrani *et al*., 2005). This research wasn't complete because (1), special attention to Eisenia fetida species (2) the extensive size of different townships of sampling site and (3) Non-sampling from different geographic areas. The exact identification of earth-worms species in the center of Mazandaran Province (Sari) has an appropriate

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situation, due to the distance between sea and forest and large plain spaces, which have crucial role in the next step for social, economic and scientific development of the township.

In this research, we want to identify different species of the current earthworms in various geographic regions of Sari township, including the littorals, plains, forests and mountains to utilize them in improvement of earth fertility, transformation of garbage to compost, management and preservation of forest resources, feeding of fish and poultry, fishing industry and even in human feeding and treatment of human diseases, for future researches and use them in the related centers.

MATERIALS AND METHODS

This study was conducted from Oct. 2007 to Jun. 2008. Studied areas were different regions of Sari Township. Sari is center of Mazandaran province in northern Iran and is a coastal province situated by the Caspian Sea. In this research, each of the geographic regions was recognized and then sampling was done. These regions were selected in a way that included all geographic directions. The samples were transferred to the lab after numbering of samples, record of location and time of sampling. We fixed the adult worms after separating the adult worms having the reproductive belts (Clitellum) from the juvenile worms, including graff identification key, characteristic of each were recorded and the fixed worms were identified according to the arranged table (Omrani *et al*., 2005).

According to map and geographic data, Sari Township consist of four regions:, forest, plain, littorals and mountainous and each one divided in to a few district and sectors, according to the size and different geographic directions, with sampling done from three spots of each district or sector.

Two methods were used for sampling and collection of earthworms:

- In the first way, determined spots were dug by spade and trowel and earthworms were separated manually after digging
- In the second that was used for excavator species first the prepared solution of formalin, 55% was poured in the determined location. The earthworms are sensitive to a very small amount of this solution, which stimulates them. Consequently, they receded out of their homes (under ground holes) and moved to the surface of the earth, so we could gather them

Samples were put in a large plastic bag together with earth from the same area, along with some decayed leaves from the surrounding trees were added. For each sample or group of samples, the characteristics of the location, sampling data and details of the observed activity were recorded in a note book.

The bag samples were transferred to special vases for the interim preservation of the worms and then we placed the worms inside a plastic pan to study their morphological characteristics and fixing stages. We fixed them after separation of the adult samples (with reproductive belts) from non- adult ones. We prepared a table in which the approved morphological characteristics were recorded in the next stages for each sample.

For sample fixation, we put sample on a dry tissue after washing with water and then weighted with an exact scale. The following stage, the earthworm is taken slowly from the scale surface with forceps and put inside first Petri-dish (containing formaldehyde solution of 10% and alcohol at 60% with the ratio of 1 to 2). The sample died in this stage and secreted a liquid from itself, these secretions were usually yellow, white or colorless and this was the identification characteristic of the earthworms (Raw, 1959).

The sample is put inside second petr-idish (containing formaldehyde 5%) and then was taken out and laid on a wooden board. To keep the body sample straight, we took one end of its body with forceps and saturated with a swap in third Petri- dish (containing water and formaldehyde, 10% with a ratio of 10 to 1) and rubbed the free end of its body. After the worm was conditioned, its length was measured by a ruler. Finally, the sample was placed inside a tube containing 5% formalin and a number tag was placed.

Then, we surveyed the morphologic characteristics of samples, with Stereo Microscope (Graff, 1953). These characteristics included a kind of prostomium, chaetae condition, along with a number of Clitellum segments. Peripheral glands was conditioned on Clitellum, the first dorsal opening, position of male genital pores, earthworms color (in life situation), secretion color (in dead situation). Other characters were identified such as weight, length and total earthworm segments. Finally, species and genus of each sample were identified with use of recorded characteristics, with data being analyzed with Chi square and ANOVA.

First, frequency tables of adult specimens prepared for different variables with SPSS. Then for data analysis with Chi square tests, relation between different variables and kind of species diagnosed and prepared crosstabs. Finally, ANOVA table prepared for variables (weight, length, number of segments) in each species.

RESULTS

From the 345 samples, (53.6%) were adult worms from 644 gathered samples, related to 108 spots within two seasons, fall (2007) and spring of (2008) (Fig. 1). From the

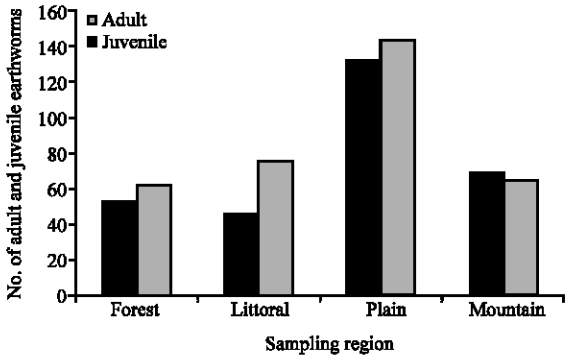

Fig. 1: Number of adult and juvenile earthworms in sampling regions of Sari Township, Iran

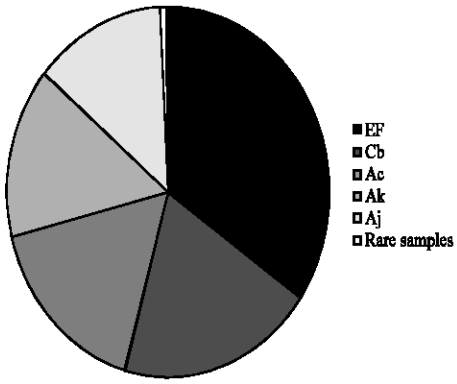

Fig. 2: Frequency percent for identified species of earthworms in Sari township

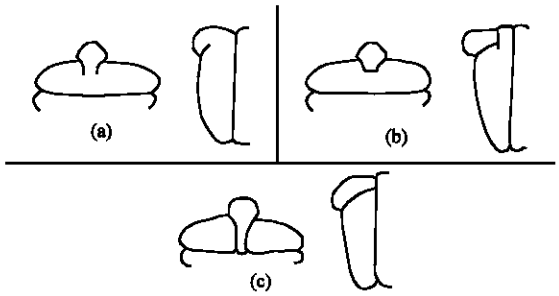

Fig. 3: Types of prostomium among fixed adult samples were, (a, b) Epilobe and (c) Tanylobe

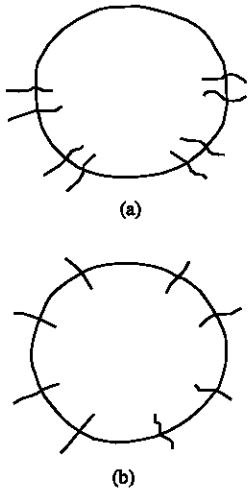

Fig. 4: Kinds of Chaeta among fixed adult samples. (a) Closely paired and (b) separate paired

total gathered adult samples in Sari Township, 143 (41.5%) belonged to the plain region, 75 samples (21.7%) to the littoral region, 62 samples (17.9%) to forest and 65 samples (18.9%) to the mountainous regions.

Five species were identified among fixed adult samples, with all from the Lumbricidae family, which included *Eisenia fetida* (Ef). *Dendrobaena byblica* (Db), *Allolobophora caliginosa* (Ac), *Allolobophora kaznakovi* (Ak) and *Allolobophora jassyensis* (Aj). 120 samples to Ef, 67samples to Db, 58 samples to Ac, 52 samples to Ak, 45 samples to Aj and 3 samples were unknown and regenerate from 345 adult samples (Fig. 2).

There was a meaningful relationship between species and four identification keys including secretion color, life color and type of prostomium and chaeta condition ($p<0.05$).

The secretion color from 55.7% samples was yellow, 28.4% samples were colorless and 15.9% samples were white. Striped yellow with a dark red color belonged to Ef species were allocated the most from 35.1% life color of fixed samples. There were two kinds of prostomium including, Epilobe (64.3%) and Tanylobe (35.1%) among the reported samples. Ef species have Tanylobe and other species have Epilobe (Fig. 3a-c).

Two kinds of Chaeta were observed, including to closely paired (80.6%) and separated (19.4%) that Db species has a separated form and other species have closely paired forms (Fig. 4a, b).

There were a meaningful relation statistically between kinds of species and two other kinds of identification keys; first dorsal opening and male genital pore position. In relation to first dorsal opening, 63.2% of samples include 4/5 condition (in Aj, Ak, Ef species), 16.8% in 8/9 position (in Ac species), 16.2% in 10/11 position (in Db species), 3.2% in position of 11/12 segments (in Db species) and 0.6% were lock of certain condition (in pressed samples). In relation to male genital pore position, 54.5% in 15 segment (in Ef, Db species and one rare case), 19.4% in14-15 condition (in Aj, Ac species), 11.6% in ½14-15 condition (in Ak species), 10.4% in 15-16

Table 1: Statistical specialties of three variables in identified earthworm species: Weight, Length, and Number of segments

Variable-species name	Statistical specialty	No.	Average	SD	Minimum	Maximum
Weight (g)	Ef	120	0.38	0.11	0.1	0.7
	Db	67	0.57	0.16	0.2	0.9
	Ac	58	0.72	0.18	0.5	1.5
	Ak	52	0.71	0.33	0.2	1.9
	Aj	45	0.48	0.14	0.2	0.8
	Rare samples	3	0.23	0.06	0.2	0.3
	Sum	345	0.54	0.23	0.1	1.9
Length (mm)	Ef	120	65/81	15.10	39	107
	Db	67	65/09	13.25	38	94
	Ac	58	80.98	14.96	60	152
	Ak	52	73.60	17.41	37	122
	Aj	45	66.16	14.85	30	102
	Rare samples	3	42.67	6.66	37	50
	Sum	345	69.23	16.24	30	152
Number of segments	Ef	120	83.09	14.77	57	120
	Db	67	102.75	10.87	78	129
	Ac	58	117.09	13.13	97	142
	Ak	52	113.15	12.96	91	149
	Aj	45	108.40	12.21	73	139
	Rare samples	3	79.00	10.54	68	89
	Sum	345	100.48	18.96	57	149

Table 2: Morphological characteristics of identified earthworm species in Sari township

Secretion color	Live color	Male genital pore position	First dorsal opening position	Peripheral glands on Clitellum	Clitellum segments	Chaetae situation	Kind of prostomium	Species name	Row
Yellow	Dark red with yellow stripes	|15|	4/5	27-31½ *28-31 27-30,29-31, 29-32	26-31 *26-32, 26-33,27-33	Closely paired	Tanylobe	Ef	1
Colorless	Pale red*, mat red, bright red	|15|	*10/11,11/12	29,25-29⅛-*25 25,28-29⅛-24⅛ 29-25⅛	24-29,25-29* 24-29½	Separated	Epilobe	Db	2
White*, colorless	Dark brown to grey	*14-15,15-16	8/9	*31-33,30-32,29-31	*27-34,26-34	Closely paired	Epilobe	Ac	3
Yellow*, colorless	Cream to grey	14-15½* 16½-,15	4/5	35⅛-32*31-34 34-,32 35⅛-,31	*27-35,28-35	Closely paired	Epilobe	Ak	4
Yellow*, colorless*	Cream to grey	*14-15,15-16	4/5	35⅛-,*32 35⅝-32, 32-35	*27-35,29-35, 28-35	Closely paired	Epilobe	Aj	5

*Show dominant character

condition (in Aj, Ac species), 3.5% in 15-½16 condition (in Ak species) and 0.6% (in two pressed cases) were lack of certain conditions.

The weight, length and number of segments were important factors for identification of earthworms. There were meaningful relations statistically between each of these variables with species kinds. Average and standard deviations of samples weight were 0.54±0.23 gram, length of them were 69.23±16.24 mm and number of segments was 100.48±18.96. The most average of weight, length and number of segments belonged to Ac species and almost the least average related to these variables belonged to Ef species (Table 1).

Key characteristics of five identified species are also shown in Table 2.

DISCUSSION

The earthworms are useful and suitable animals for humans, also these animals are important factors in land ecosystems (Kurien and Ramasamy, 2006) and however, its taxonomy was not carefully observed in Iran. Previous studies in Iran conducted on a special species or samplings didn't conduct in all regions and niches, but this study was not in this manner. The original research resources on earthworm taxonomy in Iran pointed to identification of 16 species belonging to 6 genera including, Allolobophora (five species), Dandrobaena (six species), Eisenia (two species), Eiseniella, Lumbricus, Octolasium (each of them were one species) (Gupta, 2004; Malek, 2007). Most of these cases were gathered in North, Central and South-Western Iran.

Researcher identified eight species from four genera including, Eisenia, Allolobophora, Dendrobaena and Pheretima in the Mazandaran Province. Common species included, *Allolobophora caliginosa* (23.7%), *Eisenia fetida* (18.1%) and *Dendrobaena byblica* (17.7%), however, this identification was not done with regard to geographical regions.

In this study, almost sampled from all niches and regions of Sari Township and identified all adult specimens except three ones. Therefore gain access the search aims to over 99 %. (Total specimens: 345). In this study, we identified three genera of Eisenia,

Allolobophora and Dendrobaena. On basis of the results, concluded that dominant species in every region including: Plains (*Eisenia fetida*-37.1%), Forests (*Eisenia fetida*-59.7%), Littorals (*Eisenia fetida*-29.3%) and Mountains (*Dendrobaena byblica*-38.5%).

The most frequent percent in fixed species belonged to *Eisenia fetida*, thus, we can conclude the dominant species in Sari. The frequency percent of this species in fixed samples was 34.8%, 45% were in plains, 31% in forests, 18% in littorals and 6% in the mountainous regions. This species is a processing factor in many organic materials and holds a key role in fertility of soil. While there were common kinds of vermin-compost because of the frequency of worms in animal manure. They related to Anesic worms that dig vertical, permanent and depth of holes, along with holes absorbing water and aerate soil. For this reason, plant growth and fertility of soil in forest region is rich. These worms have other names such as, Tiger worm, Garlic worm, Manure worm and Brandling worm (Suthar, 2007, 2008; Lakshmi *et al*., 2005).

The second frequency percent in fixed species belonged to *Dendrobaena byblica* with 19.4%. This worm\was not located in the forest region, but was 40% in plain region, in littoral region with 37% and in mountainous region with 23%. This species is fleshy and used for fishing bait, including/a good vermin-compost for converting kitchen garbage to compost. Meanwhile, the observation in sampling showed birds liked to eat these worms.

The third frequency percent was 16.8% among the fixed species, related to Allolobophora caliginosa, were 43% in forest regions, 21% in littoral, 19% in plain and 17% in the mountainous regions. This species are important in reformation of soil structure, improvement of drainage and holds maximum aerating.

The fourth frequency percent between fixed species belonged to the Allolobophora kaznakovi with 15.1%. This species also were not found in the forest region but existed in plain, especially in citrous gardens (50%), in mountains (29%) and the littoral regions (21%).

Allolobophora jassyensis with 13.1% have the least frequency in identified species. This species weren't in a forest region, 51% of these worms were found in plain regions, 33% in littoral regions and 16% in the mountainous regions.

Two species of Ak and Aj that are found mostly in gardens, fields and farms, have an important role because of their digging depth for aerating and increasing water penetration coefficient. But this characteristic creates problems in accessibility.

Two regenerating samples were not identified in plain region in Sabze-meydan and Aftab Park, because they don't have a head.

One sample found in plain region, (Mashhadikola village) that almost have Eisenia fetida characteristics, but the end of the animal tail was yellow and this characteristic belongs to Dendrodrillus rubidus, but because it was one sample, this species was identified unknown.

CONCLUSIONS

Five species *Eisenia fetida*, *Dendrobaena byblica*, *Allolobophora caliginosa*, *Allolobophora kaznakovi* and *Allolobophora jassyensis* were recognized in the study field. This study showed that the dominant species in Sari Township with 34.8% amplitude belonged to *Eisenia fetida*. This species obtained from all regions of Sari Township, included: plains (45%), forests (31%), littorals (18%) and mountains (6%).

In addition to positive points of this study, we had negative points such as inability for sampling from arduous routes and also inability for identification of three specimens in plain region.

Importance of earthworms is ever-increasing in now days, thus suggest that country scientific centers increase studies about these animals and effort to prepare atlas books of earthworms.

ACKNOWLEDGMENTS

We would like to acknowledge my co-workers in Payame noor university of Tehran, Health faculty of Mazandaran medical sciences university, Agricultural university of Mazandaran (Mr Mardanshah), Mazandaran Environmental research unit (Mr. Kanani). We are respectively grateful to Mr. Movahedi for sampling, Mrs. Eslamifar for laboratory activities and Ms. Shirzad for computer affairs.

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