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Biotechnology of Organic Animal Waste Processing Based on *Eisenia fetida* (Savigny, 1826)

Xafiza Isroilovna Ergasheva[1*], Zafar Fayzullayevich Ismoilov[1], Alimardan Yusupovich Raxmatullayev[2], Yorqin Shokirovich Raxmatullayev[2], Barno Orziyevich Davronov[2], Yulduz Baxtiyor Qizi Raxmatillayeva[2]

[1] Department of Genetics and Biotechnology, Faculty of Biology, Samarkand State University, 140104, Samarkand, Uzbekistan

[2] Department of Zoology, Faculty of Chemistry and Biology, Karshi State University, Kuchabag 17, 180100, Kashkadarya, Uzbekistan

* Corresponding author's e-mail: ergashevahafiza1990@gmail.com

ABSTRACT

Currently, the use of vermicompost in organic soil nutrition is highly efficient. The efficiency of vermicompost production at an industrial level is directly related to the correct implementation of this biotechnological process. In the conducted research, a local earthworm species, *Eisenia fetida* (Savigny, 1826), was used in the preparation of vermicompost from animal organic waste, which accumulates in large quantities in the livestock sector of agriculture. In the conducted experiments, local species of earthworms suitable for vermiculture were selected, and the optimal conditions of the substrate for their survival were studied. As a result of the research, it is possible to increase the efficiency of agricultural production by localizing low-cost biohumus production technology in Uzbekistan based on the use of local earthworms. In the conducted experiments, earthworms started feeding when the moisture level of the substrate was 30%. Their further development processes were manifested differently at different humidity levels. The earthworm hybrid breed had good development indicators at a relatively high humidity of 70–80%. In local species, this indicator was 60–70%. In the experiments, the optimal living conditions of local earthworms were studied and used in biotechnological processes.

Keywords: *Eisenia fetida, Eisenia andrei*, organic waste, vermiculture, vermicompost.

INTRODUCTION

The use of natural organic fertilizers to prevent contamination with pesticides in agrocenoses is a cause of ecological safety (Lishchuk et al., 2023). In nature, earthworms are distributed in waste-contaminated soil and feed on any waste through their intestines (Byambas et al., 2019). Although earthworm species are well studied, there are still unexplored species in tropical regions (Brown et al., 2013). In tropical and subtropical regions, vermicomposting with earthworms is well established (Araneda et al., 2016). In studies, the amount of antioxidants increased in the soil treated with compost mixtures, and the soil structure was improved (Mixtures et al., 2022). Biofertilizers are rich in nitrogen-fixing bacteria, phosphorus-assimilating bacteria, and potassium-dissolving bacteria (Ramy et al., 2022). In the cultivation of agricultural plants, vermicompost is being used very effectively to improve the health of plants and obtain abundant harvests from them. In this case, the harvest gathered from plants is superior in quality and has a long shelf life (Zuo et al., 2018). Restoration of soil structure is urgent. During their life activities, earthworms pass food along with any waste in the substrate through their intestines. In this process, processed waste loses its harmful properties. Granules in the form of caprolites create favorable conditions for the growth of plants and the development of soil microfauna (Hu and Chen, 2014). Biomass of earthworms fed with food waste can be an alternative food source rich in amino acids (Joanna et al., 2022). Earthworms are very sensitive to abiotic and biotic factors of the external environment. Each native species is more adapted to its environment. Earthworms breed intermittently or continuously during their reproductive period. Their cocoon formation takes place at a favorable time of the year, in accordance with temperature, soil moisture, food supply and other environmental factors. Large numbers of cocoons are produced in spring and early summer. The size of the cocoons depends on the size of the earthworms, and their shape varies among species. Under unfavorable conditions, earthworms enter into facultative diapause. In this case, they do not feed, and stop moving. During diapause, it loses weight as a result of using the reserve food in its body for life activities (Clive and Norman, 2022).

MATERIALS AND METHODS

Collection of earthworm species

Local species used in the process of making vermicompost from animal waste were collected from nature, and their classification was studied. An area of 1 m^2 was allocated from the selected place for collecting earthworms. First, a vertical pit was dug to a depth of 50 cm at the selected place. Soil samples were taken from every 10 cm layer along the wall of one side of the pit, and earthworm species were collected there. To determine the species classification of the collected earthworms, several adult individuals were separated and fixed. To fix the earthworms, they were first cleaned of soil particles as well as other impurities and then washed with clean water in a special container. Cleaned earthworms were placed in a Petri dish and killed in a 2% formalin solution. Earthworms become round under the influence of formalin. Therefore, earthworms should be collected individually in a special container and covered with gauze moistened with formalin. Gauze moistened with formalin prevents worms from heating or spoiling in hot weather. After the worms had slightly hardened (2–3 hours), they were sealed and labelled in 0.5 L glass bottles containing 5% formalin for preservation. The collected earthworms were ready for laboratory testing for classification, biomass and other characteristics (Atlavinite, 1990).

Preparation of substrate for vermiculture

Earthworm vermiculture methods are simple and fully automated, using low-tech bioreactors to control the oxygenation of the process, substrate temperature and humidity to create vermicompost. In this case, a substrate with organic residues is prepared at a depth of 15 cm, and a thin layer of new food is spread upwards from time to time. Earthworms always move upwards towards new food. Adding thin layers prevents the substrate from becoming too

Figure 1. The process of studying the classification of local species of earthworms

dense. In winter, the thickness of the layer increases. This provides warmth for earthworms to live under cold conditions. One of the most widely used methods of vermicompost preparation is technology in the form of piles separated by various barriers in open areas. In this case, the thickness of the piles should not exceed 2.4 meters. Different air-permeable building materials can be used to create a pile wall. The length of the piles is not very important. It is not recommended to place piles directly on the ground. In this case, soil particles may be added when receiving recycled waste. A concreted area is convenient for placing the substrate, but measures are taken to prevent water accumulation. Bamboo and straw covers or other air-permeable materials can be used to cover piles. In 1980, the Wedge vermicomposting system was developed by Edwards and his colleagues in Great Britain. This method is based on the successive addition of thin layers to the substrate (Edwards et al., 2010).

Ensuring substrate moisture levels

At a vertical angle of 45° (5–10 cm), the inner surfaces of the fences are filled with organic waste. In such a system, the height of organic waste should be limited to 1.2–1.5 meters. This system needs to be installed on concrete or other hard surfaces. In a layer 15–20 cm deep, the humidity is 80%. Each time new feed is added, metal meshes of a specific size are placed over the old recycled feed. Moisture is provided twice a day with the help of water sprinklers. Earthworms move quickly on old layers that have been completely recycled. All earthworm populations were collected on the new substrate on the surface of the metal mesh. The old layer was dewormed as much as possible, dried to a moisture level of 35–45 and sifted through a special sieve. The application of the layer-by-layer wedge system in the wedge method takes 3–4 months and requires less labor than the open field (Edwards et al., 2010; Tedesco et al., 2019). Layers with different moisture levels were used to ensure substrate moisture. In this case, humidity levels were determined in the form of 70%, 75%, 80%, 85%, and 90%. The process is monitored in the laboratory, and measurements are made every week. Cattle manure for the substrate was obtained from livestock farms, washed and cleaned. Manure cleaned of uric acid was dried in an oven at 60 °C to 0%. A total of 150 g of dry substrate was taken in small sizes, and moisture levels were provided as needed. The species Eisenia fetida (Savigny, 1826) is used for organic waste processing (Gunadi et al., 2003).

Statistical analysis

The statistical significance of data was tested by the analysis of variance of Microsoft Excel 2010 package. Mean comparisons were conducted using the least significant difference (LSD) test (p=0.05).

RESULTS AND DISCUSSION

The process of studying the classification of local species of earthworms. At the beginning of the experiments, local species were collected and those suitable for the biotechnological process were selected. The classification of earthworm species used in the research is as follows:

- Type: *Annelsidis*
- Subgenus: *Clitellata*
- Class: *Oligachaeta*
- Family: *Lumbricidae*
- Family: *Lumbricomorpha*
- Species: *Eisenia fetida* (Savigny, 1826)
- Control: *Eisenia andrei* (Iogonen, 1995)

(California worm) breed

The research was conducted on the basis of the 4-step sequence of vermicompost preparation based on the identified local species (Fig. 2).

The research was conducted in the Vivarium (Biophysiological and Biochemical Research) scientific laboratory of Samarkand State University, Samarkand Region, Republic of Uzbekistan. The object of research was to monitor the response reactions to the humidity of the nutrient environment under laboratory conditions. In this case, the effect of different humidity levels on the biomass and reproduction rate of earthworms was compared to the control *Eisenia anderei*.

Effect of different environmental moisture levels on vermiculture biomass of earthworms

The humidity of the nutrient environment is a factor that directly affects the life activity of earthworms. In the conducted experiments, the *Eisenia andrei* breed was used as a control. In the

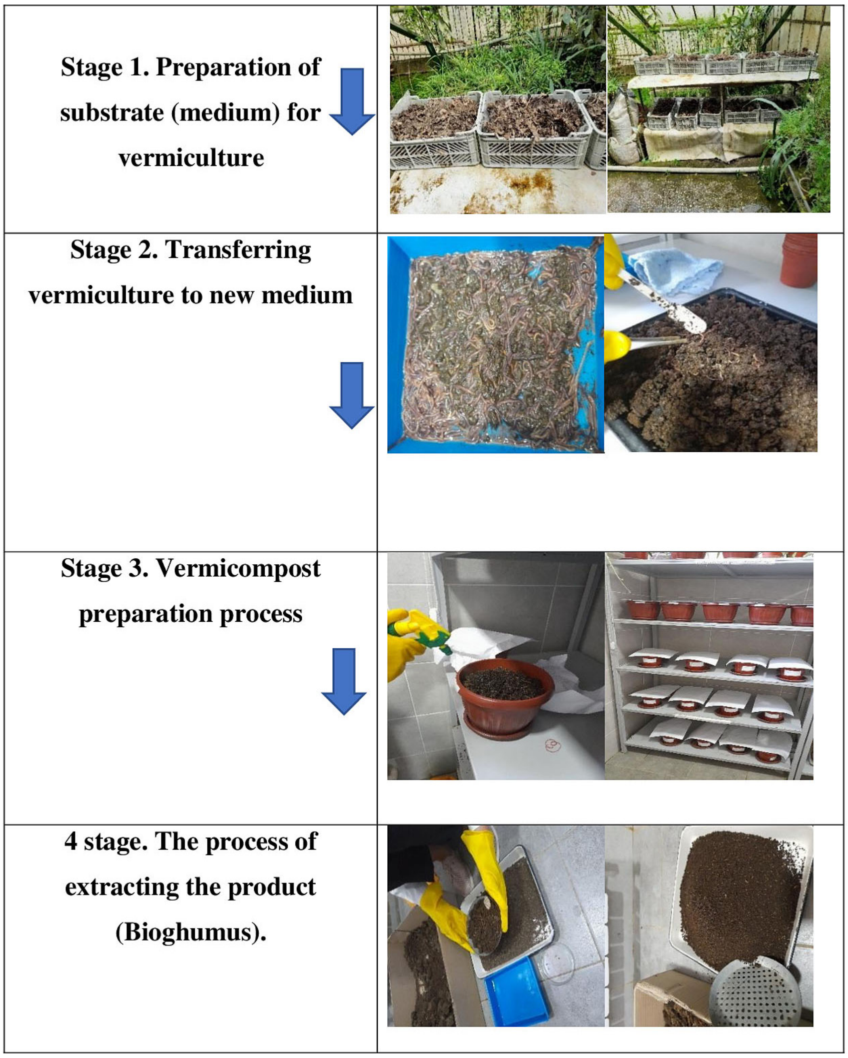

Figure 2. Sequence of the biodegradation process under laboratory conditions

experiments, the optimal level of humidity was determined (Table 1).

Five young individuals weighing 26–28 mg were selected from the F1 generation of the control *Eisenia andrei* and the local species *Eisenia fetida* with the indicators shown in Table 1. They were grown in substrates with the same composition (cattle manure) and different humidity levels of 30%, 40%, 50%, 60%, 70%, 80%, and 90%. In this case, the optimal moisture level for the control *Eisenia andrei* was 80% ($p < 0.001$), and its biomass reached 1280 mg by the 14th week. *Eisenia andrei* biomass increased at relatively low humidity levels of the substrate: 960.4 mg at 70% humidity and 790 mg at 60% humidity. At the 50%, 40%, and 30% moisture levels, the mass did not change ($p < 0.05$). At 30–50% humidity, development was low, and mortality was observed from the 8th week of the experiment. For the native species *Eisenia fetida*, the optimum humidity level was 60–70% ($p < 0.001$), and its biomass reached 1124 mg by week 14 (Fig. 3). At the lowest humidity level of 30% of the experiment, adaptation of the native species was better and continued to survive until the 18th week of the experiment.

The larvae selected for the experiment started laying cocoons from the 10th week under optimal conditions. Larvae emerged from the cocoons after 30–35 days under favorable conditions. Each worm lays 1–5 cocoons depending on the conditions. From each cocoon, depending on the conditions, 5–18 larvae were formed. In experiments, the optimal humidity levels for the growth of worms also determined the optimal conditions for their reproduction. Under relatively dry conditions, worm maturation was delayed, and cocoon fertility was reduced (Table 2). When the growth of native species was observed at different humidity levels, it was noted that native species became

Table 1. Effect of different humidity levels on earthworm biomass (mg)

No	Names	Substrate humidity, %	Biomass, mg													
			Starting	2nd week	4th week	6th week	8th week	10th week	12th week	14th week	16th week	18th week	20th week	22nd week	24th week	The diffe-rence
1	*Eisenia andrei* (hybrid breed)	30	27.3 ±0.2	29.4 ±1.4	38.6 ±4.8	28.4 ±1.8	x	x	x	x	x	x	x	x	x	11.3
		40	27.2 ±0.6	33.6 ±1.6	42.4 ±4.4	54.6 ±4.8	41.8 ±5.3	34.6 ±1.3	x	x	x	x	x	x	x	46.9
		50	27.4 ±0.9	38.4 ±1.8	49.6 ±3.9	62.8 ±4.9	74.3 ±8.4	68.1 ±2.8	52.4 ±8.3	x	x	x	x	x	x	46.9
		60	26.6 ±0.4	44.8 ±1.4	53.8 ±5.3	182.4 ±7.4	372 ±4.1	520 ±5.4	702.4 ±29.3	790 ±61.3	720 ±34.4	683 ±28.4	640 ±26.6	x	x	763.4
		70	27.7 ±1.0	53.2 ±4.63	64. 2±4.8	190 ±5.1	480 ±6.1	630 ±10.1	850 ±28.9	960.4 ±38.1	980.6 ±28.1	890 ±24.3	830 ±22.3	780 ±21.2	760 ±12.13	933.2
		80	27.8 ±0.2	110 ±0.5	310 ±4.64	530 ±4.93	883 ±5.86	1058 ±8.89	1198 ±32.77	1280 ±44.83	1220 ±36.82	1180 ±31.1	1090 ±24.83	1060 ±22.33	1030 ±2011	1152.2
		90	26.3 ±1.3	98.6 ±0.5	294 ±5.6	506 ±5.1	680 ±12.1	890 ±54.2	930 ±62.1	1190 ±52.2	1260 ±41.8	1080 ±38.4	1048 ±33.4	1012 ±29.2	970.4 ±21.2	1163.7
2	*Eisenia fetida* (local type)	30	26.3 ±1.2	32.2 ±2.8	47.4 ±3.2	61.8 ±4.2	76.2 ±4.2	91.8 ±8.9	118.4 ±29.2	130 ±7.4	122 ±8.4	114.6 ±9.4	x	x	x	103.7
		40	27.3 ±0.2	38.4 ±2.4	58.4 ±5.8	62.8 ±4.8	93.6 ±9.3	120 ±13.2	133.6 ±28.1	120.4 ±6.2	113 ±28.4	90.6 ±29.4	74.2 ±30.2	x	x	106.3
		50	27.2 ±0.8	44.8 ±2.8	74.2 ±8.4	108 ±5.1	403 ±28.4	548.4 ±54.2	600.6 ±58.2	584.2 ±44.8	470.4 ±52.4	420.4 ±384	405 ±26.6	390 ±23.6	360 ±25.3	573.4
		60	27.4 ±1.0	53.7 ±3.2	86.2 ±8.4	194 ±3.1	452 ±33.4	680 ±62.4	900 ±64.4	940 ±31.2	890 ±54.4	873.4 ±34.3	863 ±52.3	834.6 ±39.2	820.5 ±38.4	918.6
		70	27.4 ±0.9	66.6 ±2.1	98.6 ±0.6	216 ±1.3	530 ±2.6	888 ±22.9	1092 ±22.9	1124 ±26.2	1080 ±7.1	1010 ±6.6	980 ±5.4	960 ±5.2	952 ±4.9	1096.6
		80	26.8 ±1.1	44.8 ±3.2	68.2 ±9.3	160.4 ±15.1	420 ±54.2	790.2 ±28.4	1010 ±68.4	1024 ±54.2	960.4 ±68.2	930.6 ±33.4	840.4 ±29.8	780.6 ±25.2	753 ±19.2	997.2
		90	26.6 ±0.4	38.4 ±3.8	52.6 ±7.8	140 ±13.2	330 ±29.1	580 ±54.8	723.4 ±52.2	980.2 ±88.2	930.4 ±74.2	870.6 ±62.2	830.4 ±58.4	x	x	953.6

Note: x is the death rate, * – statistically significant at p ≤ 0.0.

more adapted to local conditions. In the control (*Eisenia andrei breed*), viability in substrate moisture was shown in a short range (Fig. 4).

Low humidity levels are disastrous for earthworms. Avoiding dehydration is a key factor in maintaining earthworms (Baker and Whitby, 2003). The life activity of earthworms depends on a sufficient level of soil moisture. Moisture requirements are not the same in all species, and even within a species, moisture requirements may vary from region to region (Edwards and Lofty, 1977). Representatives of the family *Lumbricida* begin developing above 30% humidity (Berry and Jordan, 2001). Earthworm cocoons survived up to 2 months in air-dried soil (Baker and Whitby, 2003). When the water potential is below 12 kPa, the process of cocoon formation slows down. When the potential is below 40 kPa, cocoon formation stops completely (Holmstrup, 2001). Earthworms have the potential to increase nutrient mineralization as well as benefit plant growth by absorbing the physical and chemical properties of soil. However, this effect depends on many factors, including larval density and ecological strategies. In addition to influencing density, soil environmental conditions can limit earthworm activity to certain times during the growing season, reducing their impact on nutrient availability and crop growth (Walsh et al., 2019). Earthworm species density is higher in the areas with higher humidity (Schlaghamerský et al 2019). A drier environment slows its recycling by earthworms (da Silva et al., 2020). Adult earthworm species are more tolerant of low temperatures and humidity than juveniles (Görres et al., 2016). Earthworm growth is affected by changes in soil temperature and moisture; therefore, it can be used as an indicator of earthworm activity under field conditions. There is no standard methodology for measuring earthworm growth, and results obtained with different food sources, soil amounts, and container shapes can be easily compared in the laboratory or used to estimate earthworm growth in the field. In some experiments, the growth rates of endogeic earthworms were determined at different temperatures (5–20

Table 2. The number of cocoons produced by earthworms at different substrate moisture levels and the number of larvae emerging from the cocoons

No.	Names	Substrate moisture, %	Number of cocoons/larvae (piece)									
			10-week	12-week	14-week	16-week	18-week	20-week	22-week	24-week	Total number of cocoons	Total number of worms
1	Eisenia anderii (hebridean breed)	30	0	0	0	0	0	0	0	0	0	0
		40	0	0	0	0	0	0	0	0	0	0
		50	0	2/0	0	0	0	0	0	0	2	0
		60	0	8\15	0	9\23	0	7\22	0	0	24	60
		70	19\302	0	20\221	0	22\268	0	17\149	0	72	940
		80	23/328	0	17/241	0	24/302	0	19/186	0	83	1057
		90	17\292	0	18\193	0	19\258	0	18\116	0	72	859
2	Eisenia fetida (native species)	30	0	0	2\0	0	3/0	0	0	0	5	0
		40	0	4\9	0	0	4\12	0	0	0	8	21
		50	0	6\62	0	8\93	0	6\72	0	0	20	299
		60	0	19\286	0	15\221	0	18\226	0	18\202	70	935
		70	22\302	0	16\216	0	21\292	0	20\274	0	79	984
		80	18\274	0	15\198	0	19\202	0	17\106	0	69	780
		90	0	14\106	0	16\122	0	11\69	0	0	41	297

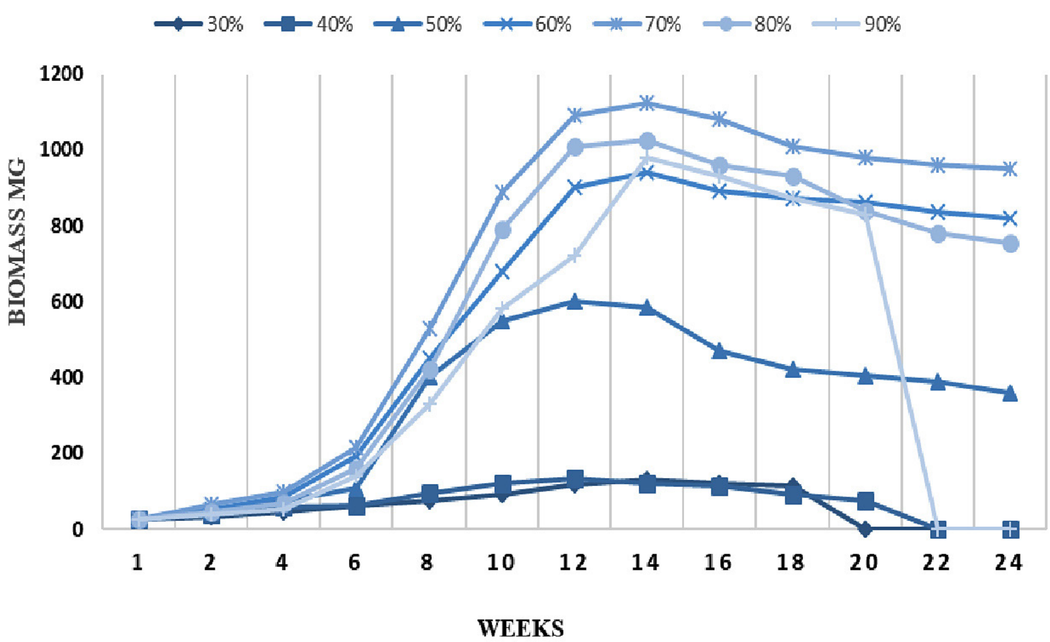

Figure 3. The effect of different humidity (%) levels of the substrate on the biomass of *Eisenia fetida*

°C) and different soil structures and water potentials (from 5 to 54 kPa) in the soil column. The tests were carried out in a sitting structure with dimensions (diameter 6 cm, height 15 cm) and soil of different structures. All containers contained approximately 500 g of moist soil (Eriksen-Hamel and Whalen, 2006). Juvenile earthworms were grown in all possible combinations of soil moisture (10, 15, 20 and 25%, dry weight basis) and temperature (5, 10, 15 and 20 °C). Logistic regression was used to determine the relationship between soil moisture and temperature and the proportion of surviving individuals and time. Stepwise regression analysis was used to identify various possible relationships between temperature, humidity and time on earthworm growth. Soil moisture accounted for 48% of the variation in earthworm survival,

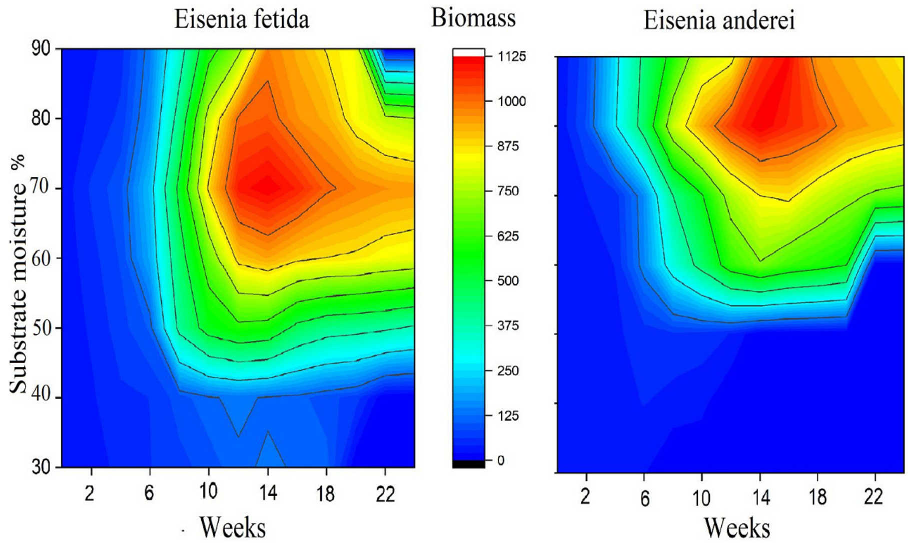

Figure 4. Comparative graph of the biomass changes of the local species *Eisenia fetida* and the hybrid *Eisenia anderei* in substrates at different moisture levels. Note: 0 – no cocoon formation was observed

with the lowest survival associated with 10% soil moisture. The interaction terms time × temperature × moisture and time × temperature accounted for 63% of the variation in earthworm growth. There is an intrinsic relationship between temperature + moisture + time in the processing of waste by earthworms (Wever et al., 2001). The earthworm's skin is its organ of oxygen exchange, allowing earthworms to survive in flooded environments if there is sufficient dissolved oxygen in the water (Kiss et al., 2021; Kiss et al., 2021). It was found that the abundance and biomass of earthworms were strongly related to soil moisture. However, there was little evidence that large-scale variation in soil invertebrate abundance could be explained by spatial variation in climate. Given the importance of earthworms to soil processes, periods of dry weather may reduce their nutrient cycling and other vital capacities, such as food for species. In the future, variables such as rainfall frequency, rather than the commonly used monthly or seasonal averages, may be useful in analyzing the impacts of climate change on soil invertebrates (Martay and Pearce-Higgins, 2018). Valuable biochar has been obtained by adding different types of plastic waste to cattle manure as feed for earthworms (Sanchez-Hernandez et al., 2021). In another study, good results were obtained using fruit and vegetable waste as food for earthworms (Tedesco et al., 2019). Organic waste in the process of making pathogenic microorganism vermicompost is lost during passage through the gut of earthworms (Soobhany et al., 2017). On the basis of the conducted experiments, the authors are planning to study the effect of organic fertilizers on plants in the next stages of research.

CONCLUSIONS

Because local species of earthworms are easily adapted to the climatic conditions of this region, various biotechnological processes can be effectively carried out with their participation. It was observed that earthworms start feeding when the moisture level of the substrate is 30%. Their further development processes differ at various humidity levels. The earthworm hybrid breed had good development indicators at a relatively high humidity of 70–80%. In local species, this figure was 60–70%. Therefore, in the current globalization of the water problem, it is appropriate to use local species in the process of biodegradation.

When biohumus is applied to soil, a large number of earthworm cocoons fall into the soil. Later cocoon development at low moisture levels was better in native species. This determines the viability of earthworms under natural conditions when biohumus is applied to the soil when local species are used.The climate of Uzbekistan is relatively dry, and the amount of precipitation is low compared to that in tropical regions. It is desirable to process tons of collected organic waste on the basis of local species adapted to these conditions.

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