

Identification and Screening of Earthworm Species from Various Temperate Areas of Kashmir Valley for Vermicomposting

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Abstract

Earthworms are very important organisms in different ways, they are both environmentally and economically beneficial and hence their proper identification and screening for vermicomposting is very vital. The present study was carried out during the year 2011-2012 at Organic Farming Research Centre Wadura Campus, SKUAST Kashmir, with an objective to identify the local earthworm species from various areas of Kashmir valley and then screen for their vermicomposting potential. The earthworms were collected from three different locations (Wadura, Shalimar and Gulmarg) of Kashmir Valley using hand sorting method, cultured and then carefully examined. The worms were washed with water and preserved in 10% formalin solution. Based on Taxonomic characteristics, the species *Eisenia Foetida*, *Apporectodea rosea* and *Apporectodea caliginosa* were identified from Wadura Sopore, *Eisenia foetida* and *Apporectodea caliginosa* were identified from Shalimar Srinagar and *Eisenia foetida* and *Apporectodea caliginosa* were identified from Gulmarg. Screening of the identified worms from each location was carried out on the basis of maturation of vermicompost and nutrient contents (N, P, K, Ca, Mg and S) to select the outstanding isolate. Since the isolate *Apporectodea rosea* from Wadura, *Eisenia foetida* from Shalimar and *Apporectodea caliginosa* from gulmarg selected for vermicomposting. *Eisenia foetida* in terms of reduction in the overall maturation period of vermicompost, therefore may be considered as the most outstanding one out all the isolates.

Keywords: Earthworm; Identification; Screening; Vermicomposting

Introduction

Vermicomposting involves use of earthworms as versatile natural bioreactors for effective recycling of non-toxic organic wastes to the soil resulting in soil improvement and sustainable agriculture. Unscientific disposal causes an adverse impact on all components of terrestrial ecosystems dominating the biomass of soil invertebrates in different soils [1]. They have been recognized as the most important soil ecosystem engineers [2]. During the Cleopatra era (69-30 BC), the earthworm was declared as sacred animal in the ancient Egypt. They remarked that earthworms have played more roles throughout the history of the world than any other animal. More than 4,200 species of oligochaetes are known in the world [3]. Of these, 280 are microdrill and remaining 3,920 belong to megadrill (earthworms). In the Indian sub-continent, earthworms also form bulk of the oligochaete fauna. They are represented by 67 genera and 509 species, indicating a high degree of diversity in this region as compared to other areas. The activities of earthworms in soils have been shown to have profound impact on the soil ecosystem functioning as well as on the types and numbers of micro-flora and microfauna [4]. Approximately 4,400 different species of earthworms have been identified worldwide; *Eudrillus eugeniae* and *Eisenia foetida* are being used as composting earthworms in most of the countries. In India, in addition to these, two more species, namely *Perionyx excavans* and *Perionyx sansibaricus* are also used for the purpose. *Eisenia foetida* is perhaps world's most widely used earthworm for vermicomposting [5]. Ref. [6] reported 590 species of earthworms from various parts of India, but at the same time there is paucity of information regarding the distribution and composting potential of the earthworms of Kashmir Valley, India.

Vermicomposting in recent years has gained importance because of its more economic value over traditional methods of composting. Earthworms are conveniently being employed for bioremediation like degrading and decomposing the agricultural and industrial wastes [7] Since Kashmir Valley is a temperate region and experiences harsh winters and cold autumn season. The activity of exotic worms drastically reduces under such climatic conditions. Therefore, the present study was undertaken with an objective to identify and screen local worms that can perform better even under cold climatic conditions.

Materials and Methods

The experiment was conducted in vermibeds of 3 × 10 feet at organic Farming Research Centre, Wadura Campus of Sher-e-Kashmir university of Agricultural Sciences and Technology of Kashmir, India. Earthworms were collected from three different locations are Wadura Sopore, Shalimar Srinagar and Gulmarg Baramulla (Table 1).

Mature earthworms and their cocoons collected from ten sites at each location were pooled together thereby forming a composite sample. The earthworms from each location were identified at Centre of Research for Development, PG Department of Environmental Science, University of Kashmir on the basis of taxonomic characteristics, screening of the identified worms from each location was carried out on the basis of vermicomposting potential to select the most outstanding isolate for vermicomposting. *Apporectodea rosea*, *Eisenia foetida* and *Apporectodea caliginosa* from Wadura, Shalimar and Gulmarg were selected for vermicomposting process.

Preparation of waste samples

The waste samples were first washed in running tap water to remove adhering soil and other foreign material followed by dipping in dilute 0.1N HCl. Washing was repeated with single and double distilled water. After washing, the waste samples were air dried on filter paper and then oven dried at 60 ± 5°C for 24 hours. The dry matter weight

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Location	Altitude (Above Sea level)	Temperature
Wadura Sopore	1589 meters	-05 to +35°C
Gulmarg Baramulla	2650 meters	-20 to +35°C
Shalimar Srinagar	1680 meters	-10 to +35°C

Table 1: Location.

for each waste sample was recorded before crushing the material. The crushed sample materials were passed through 2 mm mesh sieve and stored in air tight polythene bags for chemical analysis.

Pre-decomposition of wastes

All the Organic wastes (kitchen wastes, municipal wastes, crop residues, sheep manure, poultry manure and apple pomace) were shredded and chopped into small pieces and mixed with cow dung (one week old) in its required proportions as per the treatment details. The mixed substrates were kept for three weeks for pre-decomposition under shade and rain proof shed.

An experiment was conducted to screen out the best earthworm isolate for vermicomposting process on the basis of following characteristics as under:

Time of maturity of vermicompost (days): Maturation was done on the basis of physical appearance of the vermicompost.

pH: The pH was determined in 1:2.5 suspension with glass electrode pH meter [8].

Electrical conductivity: After determining pH, vermicompost sample were kept overnight in undisturbed condition and electrical conductivity was measured by electrical conductivity meter [8].

Organic carbon: Organic carbon was determined by rapid titration method described by Ref. [9]. Vermicompost were treated with potassium dichromate, concentrated sulphuric acid and ortho-phosphoric acid before titrating with Ferrous Ammonium Sulphate solution using diphenylamine as an indicator.

Total nitrogen and available nitrogen (N)

Before determination of total nitrogen, vermicompost were digested in digestion mixture of potassium sulphate, ferrous sulphate, copper sulphate and Sellinium powder, with the addition of concentrated sulphuric acid. Then nitrogen determination was carried out by Kjeldhal's method described by Ref. [8]. Known volume of samples were taken in jeltack tube with addition of Boric acid and 40% NaOH and titrated against 50% H₂SO₄. Estimation of available nitrogen was done following the procedure given by Ref. [10]. Potassium permanganate (0.32%) and 2.5% sodium hydroxide was added to samples and dilution was carried out in 4% Boric acid containing mixed indicators for the estimation of available nitrogen.

Total phosphorus and available phosphorus (P)

Known weight of vermicompost was digested in diacid mixture of HNO₃:HClO₄ in the ratio of 10:3 as per procedure described by Ref. [8]. Phosphorus in the extract was estimated by Vanadomolybdate method. Available phosphorus was extracted with extractant (0.5 NaHCO₃) described by Olsen and the intensity of colour developed from ammonium molybdate and stanous chloride was measured by spectrophotometer at 660 nm wavelength as described by Ref. [8].

Total potassium and available potassium (K)

Known weight of vermicompost was digested in diacid mixture of HNO₃:HClO₄ in the ratio of 10:3 as per procedure described by

Benjamin. Potassium was determined by use of Flame Photometer (Digital FPM-125) Available potassium was extracted from samples (organic waste) with the help of suitable extractant (CH₃COONH₄) by shaking, followed by filtration and determination was carried out with the help of Flame Photometer [8].

Total calcium and available calcium (Ca)

Total calcium was determined from diacid digestion extract by use of Atomic Absorption Spectrophotometer (AAS) as described by Ref. [11]. Available calcium was determined by the addition of ammonium acetate solution to the known weight of sample by shaking followed by filtration and determined was carried out with the help of EDTA titration method described by Ref. [8].

Total magnesium and available magnesium (Mg)

Total magnesium was determined from diacid digestion extract by use of AAS (Atomic Absorption spectrophotometer) as described by Ref. [11]. Available magnesium was determined by the addition of carbamate crystals and Eriochrome Black-T indicator to the known volume of extract (sample+ammonium acetate solution) and the determination was carried out with the help of EDTA titration method described by Ref. [8].

Total sulphur and available sulphur (S)

Total sulphur was determined from diacid digestion sample with the addition of buffer solution, gum acacia and barium chloride, and determination was carried out with the help of spectrophotometer at 420 nm wavelength described by Ref. [12]. Available sulphur was determined from organic waste sample by the addition of 0.51% CaCl₂ solution by shaking followed by filtration with the addition of barium chloride and gum acacia and determined with the help of spectrophotometer at 660 nm (Transmittance) described by Ref. [12].

Statistical analysis

The data collected on different observations were analyzed statistically using the standard procedures followed by Ref. [13].

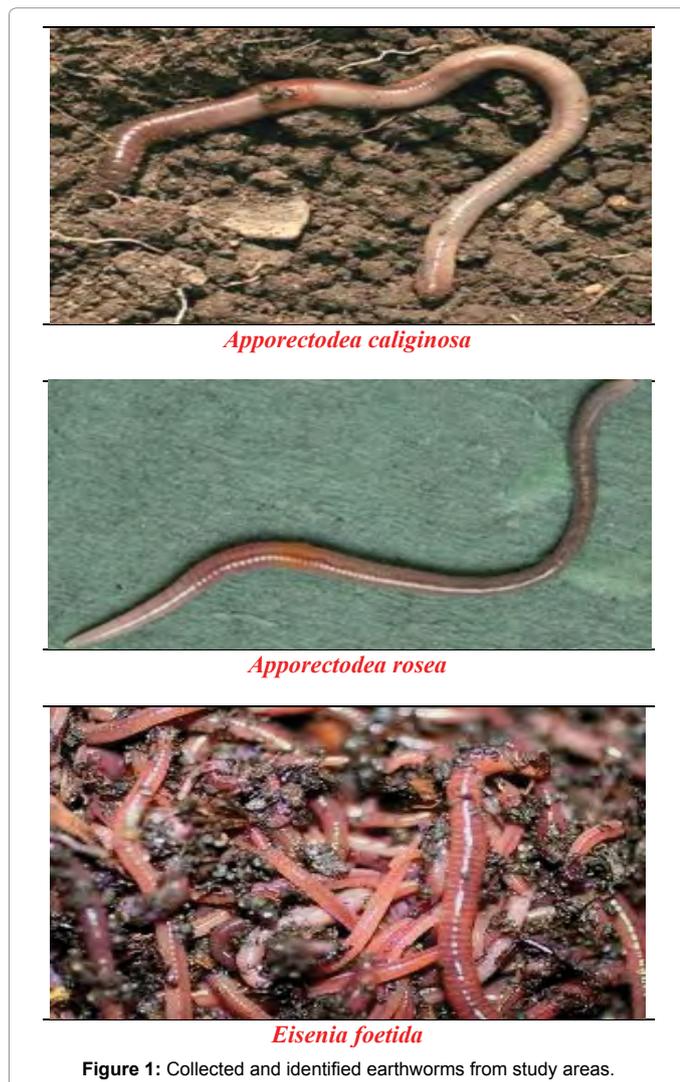
Results and Discussion

Earthworms were collected from three different locations (Wadura, Shalimar and Gulmarg). Mature earthworms and their cocoons collected from various sites at each location were pooled together forming a composite sample. The earthworms from each location were identified on the basis of taxonomic characteristics and mass multiplied in appropriate media. On the basis of body shape, colour, body length and number of segments, *Eisenia foetida*, *Apporectodea rosea* and *Apporectodea caliginosa* were identified from Wadura; *Eisenia foetida* and *Apporectodea caliginosa* were identified from Shalimar and *Eisenia foetida* and *Apporectodea caliginosa* were identified from Gulmarg (Table 2). Similar findings were reported by Ref. [14] who identified the earthworm *Eisenia foetida* from the state of Jammu and Kashmir. *Apporectodea caliginosa*, *Apporectodea rosea* and *Eisenia foetida* (Figure 1).

The results were obtained on the basis of Maturation time, pH, Electrical conductivity, Organic carbon, Total and available Nitrogen (N), Total and available Phosphorus (P), Total and available Potassium (K), Total and available Calcium (Ca), Total and available Magnesium (Mg), Total and available Sulphur (S) are discussed as under.

Time of maturity of vermicompost (days)

The earthworm isolate *Apporectodea rosea* (Wadura) where the mature vermicompost was harvested after 37 days only as compared



to other isolates. *Eisenia foetida* (Shalimar) where the mature vermicompost was harvested only after 35 days. *Apporectodea caliginosa* (Gulmarg) harvested after 40 days. So the earthworm isolate *Eisenia foetida* (Shalimar) showed the best efficiency as compared to all other isolates. It may be due to the preferred temperature showed the potential of earthworms (Table 3).

pH: Use of *Eisenia foetida* (Shalimar) recorded maximum pH (7.45) in vermicompost and was significantly more than other worms. Similarly *Apporectodea caliginosa* and *Apporectodea rosea* from Wadura and Gulmarg recorded also recorded maximum pH of 6.75 and 7.12 as compared to other worm isolates (Table 3). The reason for high pH in vermicompost may be due to release of various bases during the process of composting. Similar findings were also obtained by Ref. [15], who reported that the earthworm species *Perionyx excavatus* performs well in a wide range of substrate pH. Near neutral initial substrate pH was found to be optimal for stabilization of waste with minimal processing time. The substrates having strong acidic initial pH were found to be less suitable for vermicomposting.

Electrical conductivity ($d\text{ Sm}^{-1}$)

Use of *Apporectodea rosea* and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum electrical conductivity of

0.49 $d\text{ Sm}^{-1}$ and 0.48 $d\text{ Sm}^{-1}$. Use of *Eisenia foetida* (Shalimar) recorded maximum electrical conductivity (0.50 $d\text{ Sm}^{-1}$) in vermicompost and was significantly more than other worms (Table 3). The reason for high electrical conductivity in vermicompost may be due to loss of organic matter and release of different mineral salts in available forms (such as phosphate, ammonium and potassium). These results are in conformity with the findings of Ref. [16], who reported that the electrical conductivity (EC) increases during the period of the composting and vermicomposting process.

Organic carbon (%)

Use of *Apporectodea rosea* and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum Organic carbon of 25.98% and 25.75%. Use of *Eisenia foetida* (Shalimar) recorded maximum organic carbon content (26.54%) in vermicompost and was significantly more than other worms (Table 3). Lower organic carbon in vermicompost is possibly due to incorporation into earthworm tissue as well as leaching of nutrients into the bedding material. Thus the organic carbon content exhibited a declining trend with the advancement of vermicomposting process. Similar findings were reported by Ref. [17-20] whose results showed that organic carbon in vermicompost release the nutrients slowly and steadily into system and enables the plant to absorb these nutrients.

Total nitrogen (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum Total nitrogen of 1.48% and 1.20%. *Eisenia foetida* (Shalimar) recorded maximum total nitrogen content (1.51%) in vermicompost and was significantly more than other worms (Table 3), and the most probable reason for the presence of significantly higher quantity of total nitrogen might be due to the nitrogen rich waste, and could be because they contain nitrogen supplements in the form of organic waste (kitchen waste, municipal waste, crop residues, poultry manure, sheep manure) which are rich sources of nitrogen. The enhancement of N in vermicompost was probably due to mineralization of the organic matter and increased rates of conversion of ammonium into nitrate [21]. Similar findings in vermicompost produced by different feed substrates have been recorded earlier by Ref. [18,19,22,23]. Moreover, *Eisenia foetida* (Shalimar), treatment of which produced maximum nitrogen content, might be due to its individual capability and better adaptability to the local temperate conditions.

Available nitrogen (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum available nitrogen of 1.02% and 1.01%. *Eisenia foetida* (Shalimar) recorded maximum available nitrogen content (1.03%) in vermicompost and was significantly more than other worms (Table 3). The most probable reason for the presence of significantly higher quantity of available nitrogen in vermicompost might be due to the sufficient quantities of organic matter worked and decomposed by earthworms as well as by enhanced microbial activity which causes transformation of the soluble nitrogen in the microbial protein thereby preventing nitrogen loss. Moreover, *Eisenia foetida* (Shalimar), treatment of which produced maximum available nitrogen content, might be due to its adaptability to the local temperate conditions.

Total phosphorus (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum Total phosphorus of 1.13% and 1.12%. *Eisenia foetida* (Shalimar) recorded maximum total phosphorus

S No	Site of Isolates/Collection Number	Taxonomic characteristics	Identified
1	Wadura	W ₁ Body shape: Cylindrical Colour: purple Body Length: 35-130 mm No. of Segments: 80-120	<i>Eisenia foetida</i>
		W ₂ Body shape: Cylindrical Colour: Reddish Body Length: 20-110 mm No. of Segments: 100-150	<i>Apporectodea rosea</i>
		W ₃ Body shape: Cylindrical Colour: Brown Body Length: 50-150 mm No. of Segments: 100-180	<i>Apporectodea caliginosa</i>
2	Shalimar	S ₁ Body shape: Cylindrical Colour: Red Body Length: 30-120 mm No. of Segments: 80-110	<i>Eisenia foetida</i>
		S ₂ Body shape: Cylindrical Colour: Brown Body Length: 20-100 mm No. of Segments: 100-145	<i>Apporectodea caliginosa</i>
3	Gulmarg	G ₁ Body shape: Cylindrical Colour: Reddish/Purple Body Length: 35-130 mm No. of Segments: 80-110	<i>Eisenia foetida</i>
		G ₂ Body shape: Cylindrical Colour: Grey Body Length: 50-145 mm No. of Segments: 100-140	<i>Apporectodea caliginosa</i>

Table 2: Collection and identification of earthworms.

Collection Site	Isolates	Maturation of vermicompost (days)	pH	Electric conductivity (dS ⁻¹)	Organic carbon (%)	Total N (%)	Available N (%)	Total P (%)	Available P (%)
Wadura	<i>Eisenia foetida</i>	45	7.10	0.43	22.00	1.32	0.99	1.03	0.044
	<i>Apporectodea rosea</i>	37	7.12	0.49	25.98	1.48	1.02	1.13	0.065
	<i>Apporectodea caliginosa</i>	40	7.00	0.45	23.03	1.20	1.00	1.08	0.035
Shalimar	<i>Eisenia foetida</i>	35	7.45	0.50	26.54	1.51	1.03	1.14	0.067
	<i>Apporectodea caliginosa</i>	40	6.90	0.46	25.00	1.21	1.01	1.12	0.05
Gulmarg	<i>Eisenia foetida</i>	45	6.68	0.41	24.01	0.98	0.98	1.09	0.03
	<i>Apporectodea caliginosa</i>	40	6.75	0.48	25.75	1.20	1.01	1.12	0.064

Table 3: Screening of earthworm isolates for vermicomposting based on chemical characteristics of vermicompost.

content (1.14%) in vermicompost and was significantly more than other worms (Table 3). The most probable reason for the presence of significantly higher quantity of total phosphorus might be due to the combustion and protein contents of earthworm tissues which enhance phosphorus content during decomposition. Similar results were also reported by Ref. [24-26], while studying the effect of different feed substrates on phosphorus during vermicomposting.

Available phosphorus (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum available phosphorus of 0.065%

and 0.064%. *Eisenia foetida* (Shalimar) recorded maximum available phosphorus content (0.067%) in vermicompost and was significantly more than other worms (Table 3). The most probable reason for the presence of significantly higher quantity of available phosphorus could be attributed to organic carbon combustion, presence of mineralogical composition and protein contents of earthworm tissues, and might be due to the additional supplement of phosphorus in the form of organic waste (kitchen waste, municipal waste, crop residues, cow dung, sheep manure, poultry manure). These results are in accordance with those of Ref. [26,27].

Total potassium (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum Total potassium of 4.85% and 4.82%. *Eisenia foetida* (Shalimar) recorded maximum total potassium content (4.87%) in vermicompost and was significantly more than other worms (Table 4). The results were in accordance with Yadav who reported that the potassium content showed significant increase in vermicompost. Results were also supported by Ref. [19,28].

Available potassium (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum available potassium of 1.66% and 1.56%. *Eisenia foetida* (Shalimar) recorded maximum available potassium content (1.77%) in vermicompost and was significantly more than other worms (Table 4). The most probable reason for the presence of significantly higher quantity of available potassium might be due to the additional supplements of potassium through poultry manure, kitchen waste and sheep manure. These results were in conformity with the results of Ref. [18] who reported that there was a transformation of organic waste products into vermicompost by the additional supplements of nutrients. Similar findings have been reported by Ref. [24,28].

Total calcium and available calcium

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum total calcium of 0.62% and 0.65%. *Eisenia foetida* (Shalimar) recorded maximum total calcium content (0.73%) in vermicompost and was significantly more than other worms. However, *Apporectodea rosea* and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum available calcium of 0.0007% and 0.0005%. *Eisenia foetida* (Shalimar) recorded maximum available calcium content (0.0008%) in vermicompost and was significantly more than other worms (Table 4). These results are in conformity with the findings of Ref. [18,29,30].

Total magnesium and available magnesium

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum total magnesium of 0.41% and 0.43%. *Apporectodea rosea* and *Eisenia foetida* (Shalimar) recorded maximum total magnesium content (0.44%) in vermicompost and was significantly more than other worms, The most probable reason for the presence of significantly higher quantity of total magnesium might be due the additional supplement of magnesium through organic waste. However, *Apporectodea rosea* and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum available magnesium of 0.0001%. *Eisenia foetida* (Shalimar) recorded maximum available magnesium content (0.0001%) (Table 4). The presence of significantly higher quantity of available magnesium might be due the calcareous nature of organic residues used as feed to earthworms. Magnesium

has chemical association with calcium hence attributed the similar cause in vermicompost. Similar findings were also reported by Ref. [29] reported that the earthworms worked best with the supplement of nutrients through organic wastes. The results are in conformity with the findings of Ref. [18,30].

Total sulphur and available sulphur (%)

Apporectodea rosea and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum total sulphur of 2.36% and 2.35%. *Eisenia foetida* (Shalimar) recorded maximum total sulphur content (2.38%) in vermicompost and was significantly more than other worms. The most probable reason for the presence of significantly higher quantity of total sulphur might be due to the additional supplements of kitchen waste, sheep manure and poultry manure.

However, *Apporectodea rosea* and *Apporectodea caliginosa* from Wadura and Gulmarg recorded maximum available sulphur of 0.52% and 0.48%. *Eisenia foetida* (Shalimar) recorded maximum available sulphur content (0.53%) in vermicompost and was significantly more than other worms (Table 4). The high range of available sulphur in vermicompost was due to the additional supplements of organic wastes. The results are in accordance with the findings of Ref. [18,29,30]. Moreover, *Eisenia foetida* (Shalimar), treatment of which produced maximum total and available sulphur content, might be due to its individual capability and better adaptability to the local temperate conditions.

Conclusion

On the basis of results obtained during the present investigation following conclusions could be drawn:

- The earthworms collected from three different locations (Wadura, Shalimar and Gulmarg) were identified as given below:-

- Eisenia foetida*, *Apporectodea rosea* and *Apporectodea caliginosa* from Wadura;
- Eisenia foetida* and *Apporectodea caliginosa* from Shalimar;
- Eisenia foetida* and *Apporectodea caliginosa* from Gulmarg.

- The nutrients like N, P, K, Ca, Mg and S resulted the best isolates *Apporectodea rosea*, *Eisenia foetida* and *Apporectodea caliginosa* from Wadura, Shalimar and Gulmarg for vermicomposting process.

- Eisenia foetida* (Shalimar) considered as the most outstanding one out all the isolates for vermicomposting.

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Site of Collection	Isolates	Total K (%)	Available K (%)	Total Ca (%)	Available Ca (%)	Total Mg (%)	Available Mg (%)	Total S (%)	Available S (%)
Wadura	<i>Eisenia foetida</i>	4.76	1.53	0.58	0.0001	0.22	0.0001	2.05	0.43
	<i>Apporectodea rosea</i>	4.85	1.66	0.62	0.0007	0.41	0.0001	2.36	0.52
	<i>Apporectodea caliginosa</i>	4.83	1.54	0.43	0.0007	0.24	0.0001	2.28	0.45
Shalimar	<i>Eisenia foetida</i>	4.87	1.77	0.73	0.0008	0.44	0.0001	2.38	0.53
	<i>Apporectodea caliginosa</i>	4.80	1.65	0.48	0.0003	0.42	0.000	2.32	0.51
Gulmarg	<i>Eisenia foetida</i>	4.79	1.48	0.34	0.0002	0.21	0.000	2.07	0.43
	<i>Apporectodea caliginosa</i>	4.82	1.56	0.65	0.0005	0.43	0.0001	2.35	0.48

Table 4: Screening of earthworm isolates for vermicomposting based on chemical characteristics of vermicompost.

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