Journal of

Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 6 (3): 303-311, 2011
ISSN 1816-496X / DOI: 10.3923/jpt.2011.303.311

Diversity of Bacteria and Fungi in the Gut and Cast of the Tropical Earthworm *Glyphodrillus tuberosus* Isolated from Conventional and Organic Rice Fields

[1]Debasmita Chhotaray, [1]C.S.K. Mishra and [2]P.K. Mohapatra

[1]Department of Zoology and Biotechnology, College of Basic Sciences and Humanities, Orissa University of Agriculture and Technology, Bhubaneswar-751003, India
[2]Department of Botany, Ravenshaw University, Cuttack-753003, India

Corresponding Author: P.K. Mohapatra, Department of Botany, Ravenshaw University, Cuttack-753003, India Tel: +919437356901 Fax: +916742581598

ABSTRACT

A comparative study of the bacterial and fungal diversity in the gut and cast of *Glyphodrillus tuberosus*, isolated from organic and conventional rice fields in Orissa state, India was done in different seasons for a period of two years from 2007 to 2009. Isolation of the strains was done by serial dilution method on incubation of the inoculated plates at 28°C for 72 h for fungi and at 37°C for 24 h for bacteria. Isolated strains were identified as *Bacillus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Bacillus subtilis, Bacillus lentus, Azotobacter* sp., *Micrococcus* sp., *Flavobacterium* sp., *Acinetobacter* sp., *Brevibacterium* sp. and *Thiobacillus* sp., while the molds identified were *Aspergillus niger, Aspergillus flavus, Fusarium* sp., *Penicillium* sp. and *Rhizopus* sp. Microbial load was observed significantly higher ($p<0.01$) in gut sections and cast of the worm than in un-ingested soils. The earthworm was found to constitute a microhabitat enriched in microbes capable of growth and activity. Numbers of bacteria and fungi were observed significantly higher ($p<0.01$) in the gut section, worm cast and undigested soil of organic rice field in comparison to conventional one. Soil pH and percent moisture did not show significant differences between the two management systems. Season wise comparison showed that irrespective of farming systems bacterial and fungal load were maximum in rainy season than in the other and was attributed to high soil moisture content, which is an important limiting factor in tropical soil.

Key words: Bacteria, fungi, *Glyphodrillus tuberosus*, gut content, worm cast

INTRODUCTION

Earthworms are the members of the class Oligochaeta of phylum Annelida. These are one of the major macrofauna of soil and are considered as natural bioreactors since they redesign the physical structure of soil environment by ingesting litter and soil particles by depositing casts on the soil surface (Ansari, 2011; Pederson and Hendrikson, 1993; Verma and Shweta, 2011). Earthworms ingest soil microorganisms along with organic residues from the soil and during passage through the worm's intestinal tract, their population may increase. Therefore earthworm casts have been reported to be much more microbiologically active and richer in microflora than their surrounding undigested soils (Parthasarathi *et al.*, 2007; Scheu, 1987). Gut microbial population plays an important role in earthworm nutrition by helping in the breakdown of organic matter. It also determines the microbial density in the cast and microbial dynamics in soil (Hornor and Mitchell, 1981; Lattaud *et al.*, 1999; Prabha *et al.*, 2007).

Glyphodrillus tuberosus is an epigeic species of earthworm commonly found in agricultural fields of tropical and subtropical regions (Barik and Gulati, 2009). Earlier studies on gut microflora of other species of earthworms have indicated superior bacterial and fungal population in the worm casts in comparison to undigested soil (Idowu *et al.*, 2006). Despite these reports, it has remained unclear whether the differences observed in microbial population were as a result of growth during gut transit or as a result of selective consumption of organic matter richer in microorganisms among other possible factors.

Although studies have found that alternative management practices may improve soil quality compared with conventional management practices (Araujo *et al.*, 2009), few studies have specifically compared the effects of conventional and organic agriculture on residing earthworms. Given the ecological benefits of soil biodiversity, soil organisms are crucial for the sustainability of agroecosystems. Soil chemical and biological properties such as earthworm abundance and their relationship with microorganisms are considered to be major soil quality bio-indicators (Ayeni and Adeleye, 2011; Idowu *et al.*, 2006). However, there is no report comparing the distribution of microorganisms in native earthworms in organic and conventional farming systems in India. Therefore, the present study was undertaken with a view to compare the distribution pattern of microbes in *Glyphodrillus tuberosus* obtained from both the systems. The study also aimed at the observations on the seasonal variation in microbial load, pH and moisture content in various sections of earthworm, casts and surrounding soil.

MATERIALS AND METHODS

Experimental design and sampling procedure: The study was conducted during March 2007 to January 2009 in three seasons i.e. summer (March-May), rainy (July-September) and winter (November-January). Soil, cast and earthworms were collected from rice field under both organic and conventional farming systems located in Khurda district of Orissa state. Sampling was done at an interval of 15 days for each season and by hand sorting the worms. Each time five worms were collected at random from every sampling site and the plot was sampled from 15 such sites thus becoming a collection of 75 worms on the sample day. Fresh casts lying on the soil surface were also collected. All samples in triplicates were collected in sterile polythene bags from five sub plots using sterilized soil diggers. Earthworms from each location were collected along with some surrounding soil. The soil samples were moistened with sterile distilled water in labeled specimen bowls, the earthworms were placed on the soil samples before covering the bowls with wire mesh. The samples were then kept in a shaded, cool place in the laboratory at an average temperature of 27°C for further studies.

Dissection of earthworms: Each specimen to be dissected was washed in sterile distilled water, placed across the second, third and fourth fingers of the left hand (gloved) with the anterior end pointing forward. The fine edge of a flamed pair of scissors was inserted into the ventral surface at the region of the clitella and with the body wall slightly raised up with the scissors; an incision made longitudinally along the earthworm. Sterile dissecting pins were used to hold the earthworm down on a board, stretching out the body wall to expose the internal structures. The gut was then freed from surrounding blood vessels and nephridia with a flamed forcep and separated into three sections: foregut, midgut and hindgut. The gut sections were washed in sterile distilled water to free their contents before being suspended in other bottles containing clean sterile distilled water.

Physicochemical measurements: Replicate soil and cast samples were mixed thoroughly in distilled water to supernatant samples in the ratio 1:5 (w/v) soil or cast: water. The suspensions were used to determine soil pH by a combined glass electrode and a pH meter. Moisture contents (as %) of soil and cast samples were estimated by drying the samples at 105°C for 24 h in a hot air oven (AOAC, 1990).

Microbiological analyses: Three replicates of samples and distilled water in the ratio of 1:5 (w/v) were made for soils, casts and contents of the three gut regions of the earthworm specimens. The preparations were homogenized. Subsamples (1 mL each) were taken from each of the above preparations and homogenized in 9 mL sterile distilled water to make 10 fold serial dilutions of up to 10^4. A 0.1 mL aliquot of the 10^3 and 10^4 dilutions were each inoculated in duplicate by the spread plate method on potato dextrose agar supplemented with 0.01% (w/v) streptomycin sulphate and nutrient agar for isolation and enumeration of fungi and bacteria, respectively. Fungal plates were incubated at 28°C for 72 h while bacterial plates were incubated at 37°C for 24 h. The total numbers of bacteria load per milliliter or gram of sample was estimated by multiplying number of bacteria with dilution factor (ICMSF, 1978) and expressed as total colony forming units per gram (cfu g^{-1}) of soil. A combination of cultural, morphological and biochemical characteristics were used in identifying the bacterial isolates (Sneath *et al*., 1986). Identification of fungi was done on microscopic examination (Barnett and Hunter, 1972; Onions *et al*., 1981).

Statistical analysis: The statistical analysis of all the data has been done using M Stat C Software (Michigan State University, USA). The data were analyzed through ANOVA to determine significance of differences (0.05 and 0.01 levels). Standard statistical procedures were followed for data analysis (Gomez and Gomez, 1984).

RESULTS

Undigested soil had lowest pH and moisture content, followed by cast samples and gut contents with the hind gut having the highest pH and moisture. The highest average pH value (6.5±0.03, organic; 6.4±0.12, conventional) was observed in the hind gut section in rainy season and the lowest (5.4±0.03, organic; 5.4±0.02, conventional) were found in undigested soil in summer (Table 1). The bacterial and fungal counts in undigested soil samples, casts and gut sections of the earthworm specimens collected from all the study sites showed an increased bacterial counts from fore gut section to hindgut sections with the latter recording the highest counts (Fig. 1) (3.6±0.51, organic; 3.31±0.76, conventional), in rainy season and minimum (1.8±0.13, organic; 1.6±0.23, conventional) were observed in summer for undigested soil. Similar results were noticed for fungi, where higher counts were obtained from hind gut section (2.71±0.22, organic; 2.41±0.05, conventional) in rainy than undigested soil (1.5±0.23, organic; 1.3±0.27, conventional) in summer (Fig. 2). Comparison between microbial counts of the worm obtained from conventional and organic rice fields showed that in all seasons, microbial density of the former was remarkably lower than of latter. The density of bacteria of the worm from conventional field in winter and rainy seasons was found to be 91% of that of organic field, whereas the fungal densities were 94, 86 and 88% (of the organic field) in winter, summer and rainy season respectively. Thus the seasonal variation

Table 1: pH and moisture levels in soils, casts and gut sections of earthworm *Glyphidrillus tuberosus* from rice fields

		Undigetsed soil			Fresh cast			Fore gut			Mid gut			Hind gut		
Parameters	Field	W	S	R	W	S	R	W	S	R	W	S	R	W	S	R
pH	O	5.8±0.01	5.4±0.03	5.5±0.11	6.2±0.03	6.1±0.02	6.3±0.05	6.3±0.04	6.1±0.02	6.4±0.01	6.4±0.02	6.3±0.04	6.4±0.05	6.5±0.03	6.1±0.14	6.4±0.01
	C	5.7±0.02	5.5±0.11	5.4±0.02	6.1±0.01	6.1±0.04	6.2±0.11	6.1±0.34	5.9±0.31	6.3±0.24	6.2±0.22	6.2±0.01	6.2±0.11	6.4±0.12	6.1±0.15	6.2±0.06
Moisture (%)	O	21.8±0.24	6.83±0.13	24.1±0.12	21.4±0.24	5.7±0.34	22.3±0.17	35.3±0.12	11.5±0.03	37.5±0.19	37.4±0.01	12.4±0.23	38.2±0.14	38.7±0.01	13.5±0.05	38.8±0.01
	C	21.4±0.02	6.8±0.24	23.1±0.21	21.2±0.12	5.6±0.11	22.2±0.24	34.1±0.24	12.3±0.18	37.8±0.02	36.2±0.03	12.1±0.12	38.5±0.16	38.6±0.25	13.7±0.03	38.7±0.22

O: Organic; C: Conventional; W: Winter; S: Summer; R: Rainy

Table 2:: Biochemical characterization and identification of bacteria isolates of the gut content of *Glyphodrillus tuberosus* of rice fields

Gram's	TSI			Citrate								Indole	Starch	Protein	DNA	
staining	Glu	Lac	Suc	utilization	Urease	Oxidase	Catalase	MR	VP	Motility	Mannitol	formation	hydrolysis	hydrolysis	hydrolysis	Identification
NR	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	*Thiobacillus* sp.
NR	+	-	-	-	-	+	+	+	-	+	-	-	-	-	-	*Brevibacterium* sp.
NR	+	-	-	-	+	+	+	-	+	+	-	-	-	-	-	*Pseudomonas* sp.
PC	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	*Staphylococcus* sp.
PC	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	*Micrococcus* sp.
PR	+	-	-	-	+	+	+	+	-	+	-	-	+	+	-	*Bacillus lentus*
NR	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	*Acinetobacter* sp.
PR	+	+	+	-	+	+	+	-	+	+	+	-	+	+	-	*Bacillus subtilis*
NR	+	+	+	-	+	+	+	-	+	+	-	-	+	-	-	*Flavobacterium* sp.
PR	-	-	-	-	+	+	+	+	-	+	-	-	+	+	-	*Bacillus* sp.

Glu: Glucose; Lac: Lactose; Suc : Sucrose; PR: Positive rod; PC: Positive cocci; NR: Negative rod

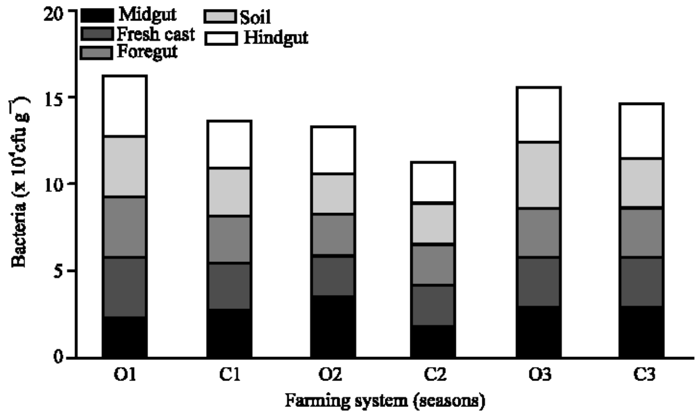

Fig. 1: Bacterial count (x 10^4 cfu g^{-1}) in gut sections, cast and undigested soil in earthworm *Glyphodrillus tuberosus* of Organic (O) and Conventional (C) rice fields, 1: Winter season, 2: Summer season, 3: Rainy season

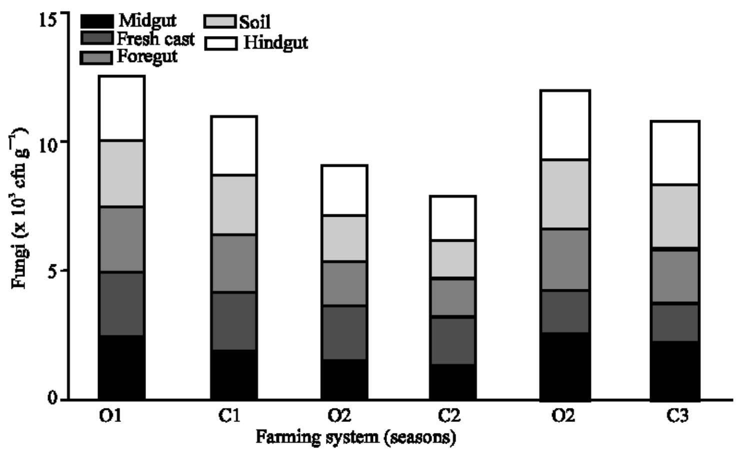

Fig. 2: Fungal count (x 10^3 cfu g^{-1}) in gut sections, cast and undigested soil in earthworm *Glyphodrillus tuberosus* of Organic (O) and Conventional (C) rice fields, 1: Winter season, 2: Summer season, 3: Rainy season

in case of fungi was not as remarkably different as bacteria. The bacterial densities of undigested soil in winter, summer and rainy season were 68, 58, 70% (conventional) and 78, 51, 77% (organic) than that of bacterial densities of hindgut section respectively. Similarly the respective densities of fungi was correspondingly 96, 83, 96 (organic) and 77, 87 and 92% (conventional) in winter, summer and rainy seasons respectively. Thus a greater difference was observed for fungal density than those of bacteria between the two farming systems. Counts were higher in the fresh casts than in un-ingested soil both in organic and conventional farming systems. Density of microflora was found to increase during gut passage of the worms.

Eleven bacteria (Table 2) and four fungi (mold species) (Table 3) were isolated from the samples. From the percentage of occurrence of fungi (Table 3) and bacteria (Table 4) on seasonal basis rainy season showed the maximum value followed by winter and summer. The mold

Table 3: The diversity and density (% of total) of fungi isolated from *Glyphodrillus tuberosus* of rice fields

Seasons (Fields)	Fungus identified with % of occurrence				
	Aspergillus niger	*Aspergillus flavus*	*Fusarium* sp.	*Penicillium* sp.	*Rhizopus* sp.
W (O)	21.0	25.0	22.0	17	10
W (C)	16.0	23.0	14.0	15	8
S (O)	14.0	18.0	15.0	11	9
S (C)	17.3	12.3	11.1	11	7
R (O)	27.0	24.0	18.0	21	11
R (C)	25.1	18.1	13.0	15	10

W: Winter; S: Summer; R: Rainy; O: Organic; C: Conventional

Table 4: Percentage of occurrence of identified bacterial isolates in gut of *Glyphodrillus tuberosus* of fields

Seasons (Fields)	Bacteria isolates									
	Staphylococcus sp.	*Brevibacterium* sp.	*Thiobacillus* sp.	*Pseudomonas* sp.	*Micrococcus* sp.	*Bacillus lentus*	*Acinetobacter* sp.	*Bacillus subtilis*	*Flavobacterium* sp.	*Bacillus* sp.
W (O)	15	17	16	22	13	33	17	27	10	31
W (C)	12	13	14	18	11	27	13	24	10	28
S (O)	11	12	11	10	12	25	11	14	9	21
S (C)	8	10	9	9	8	22	10	15	8	18
R (O)	22	13	18	23	17	34	18	28	11	34
R (C)	21	12	15	21	14	31	16	26	12	32

W: Winter; S: Summer; R: Rainy; O: Organic; C: Conventional

Aspergillus sp., was found to appear in higher density followed by *Fusarium* sp., throughout the year in all the gut sections. Among the bacteria, *Bacillus* sp., occurred in maximum percentage followed by *Pseudomonas* sp.

DISCUSSION

Significant differences ($p<0.01$) were observed in physicochemical and microbial counts among the two (Table 5) farming systems with samples from organic field recording higher values than of conventional fields. An increase in bacterial and fungal count from the foregut to hindgut region in *Libyodrillus violacious*, an epigeic Nigerian earthworm species (Idowu *et al*., 2006) has been reported. Our observation on *G. tuberosus* corroborates the results of these earlier findings that there is an increase in bacterial and fungal count from foregut to hindgut region. In the present study the increase in the fungal count from the foregut to the hindgut indicated that this species of earthworm is not able to digest the fungal spores. Therefore, the present observation contradicts earlier reports that earthworms in general are able to selectively digest fungi. Higher microbial load was related to high moisture content in rainy season in the gut of earthworm. The results of total bacterial loads obtained from soils, earthworm cast and gut content of earthworm indicated that the numbers were higher in casts and gut sections than in the uningested soil samples (Bettiol *et al.*, 2002). Further, the total microbial load was higher in organic field than in conventional fields. An increase of over100% in the total plate counts of actinomycetes and bacteria when samples of the gut were compared with surrounding soil samples was found in similar studies (Daniel and Anderson, 1992; Tangjang *et al.*, 2009). The gut and their contents also had higher moisture and pH levels than of soils.

Table 5: Analysis of variance of parameters in *Glyphodrillus tuberosus* of rice fields

	pH			Moisture			Bacteria			Fungi		
Variables	C.D	F	C.V (%)	C.D	F	C.V (%)	C.D	F	C.V (%)	C.D	F	C.V (%)
Season (S)	0.06	23.23*	2.46	0.06	327817.0*	0.58	0.09	175.84*	7.62	0.04	564.8*	4.64
Parameters (P)	0.09	65.83*		0.09	48301.0*		0.14	51.46*		0.06	54.02*	
S and P	0.17	4.16*		0.16	3993.0*		0.24	0.97ns		0.11	46.48*	
Farming System (OC)	0.05	21.13*		0.05	659.06*		0.07	127.85*		0.03	261.34*	
S and OC	0.09	1.56ns		0.08	1129.2*		0.13	5.37*		0.03	2.18ns	
P and OC	0.14	0.94ns		0.13	1079.2*		0.19	0.83ns		0.09	1.36ns	
S, P and OC	0.24	0.53ns		0.23	967.1*		0.34	0.67ns		0.16	1.59ns	

*: $p<0.01$; ns: Not significant; OC: Organic- conventional; C.D: Critical difference; C.V: Coefficient of variation

Earthworms have been shown to greatly influence the chemical, physical and microbiological properties of the soils they inhabit (Tiwari and Mishra, 1993). The findings of the present study thus confirm the concept that the earthworm gut might be a specialized microhabitat of enhanced microbial activities in soils (Karsten and Drake, 1995). The increase in microbial population in the gut of earthworms might be due to the utilization of additional nutrients available as a result of enzymatic breakdown of ingested materials (Lee, 1985). In the present study, the high moisture contents recorded in the earthworm's gut may also have stimulated microbial activity. The selective feeding of earthworms on the materials with higher microbial counts accounted for the population increase in casts produced (Brown, 1995; Winding *et al*., 1997). This is doubtful in the present study, as the microbial flora of undigested soil samples was less when compared with those obtained for the earthworm gut and casts.

The great similarity observed in the types of microorganisms identified in soil samples and those identified in various gut sections of earthworm implies that *G. tuberosus* ingests with soil and detritus, resident microorganisms which might be of dietary importance to the earthworms without any bias. It has also been reported that earthworms depend on microorganisms such as bacteria, yeast and fungi for nutrients which are extracted from the large quantities of microorganisms ingested with materials as they pass through the worms gut.

It was further stated that earthworms derive their nutrition from organic matter in the form of plant materials, living protozoa, rotifers, nematodes, bacteria, fungi and other microorganisms and decomposing remains of large and small animals most of these extracted from the large quantities of soil that pass through the gut (Edwards and Lofty, 1972). In the present study significantly higher ($p<0.01$) bacterial and fungal populations were observed in undigested soil, gut contents and worm casts isolated from organic rice fields in comparison to conventional fields (Table 5). In previous studies, rice based cropping system showed superior microbial biomass and enzyme activities after organic manuring (Araujo *et al*., 2009). Similarly soil fertility potential has also been found to be restored by organic manuring mainly due to greater biological activities and nutrient mobilization (Chukwuka and Omotayo, 2009). In another study higher bacterial, fungal and actinomycetes count were also found in organic agriculture field soil than in conventional farming system (Bettiol *et al*., 2002). However, suitable application rate of organic matter in relation to crop growth and soil moisture level is essential to minimize anaerobic activity (Affendy *et al*., 2011). In the present study, significantly higher bacterial and fungal load in the gut of *G. tuberosus* isolated from organic rice field irrespective of seasons are likely due to consumption of soil rich in organic matter which accelerated microbial growth.

CONCLUSION

To conclude, the excessive use of chemical fertilizers, characteristics of modern farming, could also have contributed to the low microbial loads observed in the conventional fields. Greater use of synthetic fertilizers in conventional farming systems could adversely affect soil microbial load, which is vital for decomposition of organic matter. Most changes in agricultural technology have ecological effects on soil organisms that can affect higher plants and animals, including man.

The findings of the present study reinforce the general concept that gut and casts of earthworms tend to be much more microbiologically active than the surrounding soil. Enhancing the growth of these soil organisms can serve as a basis for the development of 'living soils' by optimizing the potentials of the beneficial biotic populations identified in this study.

ACKNOWLEDGMENT

The authors thank University Grants Commission, New Delhi, for financial assistance under UGC-SAP (DRS) Programme to carry out this research. The assistance of Mr. J.K. Mohanty, Statistician, OUAT, Bhubaneswar for statistical analysis of the results is also duly acknowledged.

REFERENCES

AOAC, 1990. Official Methods of Analysis. Association of Analytical Chemists, Washington DC.

Affendy, H., M. Aminuddin, M. Azmy, M.A. Amizi, K. Assis and A.T. Tamer, 2011. Effet of organic fertilizer applications to the growth of *Orthosiphon stamineus* Benth. Intercropped with *Hevea brasiliensis* Willd. and *Durio zibethinus* Murr. Int. J. Agric. Res., 6: 180-187.

Ansari, A.A., 2011. Worm powered environmental biotechnology in organic waste management. Int. J. Soil Sci., 6: 25-30.

Araujo, A.S.F., L.F.C. Leite, V.B. Santos and R.F.V. Carneiro, 2009. Soil microbial activity in conventional and organic agricultural systems. Sustainability, 1: 268-276.

Ayeni, L.S. and E.O. Adeleye, 2011. Soil nutrient status and nutrient interactions as influenced by agrowaste and mineral fertilizer in an incubation study in the Southwest Nigeria. Int. J. Soil Sci., 6: 60-68.

Barik, T. and J.M.L. Gulati, 2009. Use of vermicompost in field crops and cropping systems: A review. Agric. Rev., 30: 48-55.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect Fungi. APS, Saint Paul, Minnesota, USA., pp: 218.

Bettiol, W., R. Ghini, J.A.H. Galvao, M.A.V. Ligo and J.L.D.C. Mineiro, 2002. Soil organisms in organic and conventional cropping systems. Sci. Agric., 59: 565-575

Brown, G.G., 1995. How do earthworms affect microfloral and faunal community diversity. Plant Soil, 170: 209-231.

Chukwuka, K.S. and O.E. Omotayo, 2009. Soil fertility restoration potentials of tithonia green manure and water hyacinth compost on a nutrient depleted soil in South Western Nigeria. Res. J. Soil Biol., 1: 20-30.

Daniel, O. and J.M. Anderson, 1992. Microbial biomass and activity in contrasting soil materials after passage through the gut of earthworms *Lumbricus rubellus* hoffmeister. Soil Biol. Biochem., 24: 465-470.

Edwards, C.A. and J.R. Lofty, 1972. Biology of Earthworms. Chapman and Hall Ltd., London.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agriculture Research. John Wiley and Sons, New York, pp: 680.

Hornor, S.G. and M.J. Mitchell, 1981. Effects of earthworm *Eisenia foetida* (Oligocheata) on fluxes of volatile carbon and sulfur compounds from sewage sludge. Soil Biol. Biochem., 13: 367-372.

ICMSF., 1978. International Commission on Microbiological Specification for Foods. Microorganisms in Foods-I. Their Significance and Method of Enumeration. 2nd Edn., University of Toronto Press, Toronto.

Idowu, A.B., M.O. Edema and A.O. Adeyi, 2006. Distribution of bacteria and fungi in the earthworm *Libyodrillus violaceous* (Annelida: Oligochaeta), a native earthworm from Nigeria. Rev. Biol. Trop., 54: 49-58.

Karsten, G.R. and H.L. Drake, 1995. Comparative assessment of the aerobic and anaerobic microflora of earthworm guts and forest soils. Applied Environ. Mirobiol., 61: 1039-1044.

Lattaud, C., P. Mora, M. Garvin, S. Locati and C. Roulad, 1999. Enzymatic digestive capabilities in geophagous earthworm-origin and activities of cellulolytic enzymes. Pedobiologia, 43: 842-850.

Lee, K.E., 1985. Earthworms: Their Ecology and Relationship with Soil and Land Use. Academic Press, Sydney, Australia, pp: 441.

Onions, A.H.S., D. Allsopp and H.O.W. Eggins, 1981. Smiths Introduction to Industrial Mycology. Edward Arnold, London, pp: 398.

Parthasarathi, K., L.S. Ranganathan, V. Anandi and J. Zeyer, 2007. Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. J. Environ. Biol., 28: 87-97.

Pederson, J.C. and N.B. Hendrikson, 1993. Effects of passage through the intestinal tract of detritivore earthworms (*Lumbricus* spp.) on the number of selected gram-negative and total bacteria. Biol. Fertil. Soils, 16: 227-232.

Prabha, M.L., I.A. Jayaraaj, R. Jeyaraaj and S. Rao, 2007. Comparative studies on the digestive enzymes in the gut of earthworms, *Eudrilus eugeniae* and *Eisenia fetida*. Indian J. Biotechnol., 6: 567-569.

Scheu, S., 1987. Microbial activity and nutrient dynamics in earthworm cast (Lumbricidae). Biol. Fertil. Soils, 5: 230-234.

Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt, 1986. Bergeys Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.

Tangjang, S., K. Arunachalam, A. Arunachalam and A.K. Shukla, 2009. Microbial population dynamics of soil under traditional agroforestry systems in Northeast India. Res. J. Soil Biol., 1: 1-7.

Tiwari, S.C. and R.R. Mishra, 1993. Fungal abundance and diversity on earthworm casts and in undigested soil. Biol. Fertil. Soils, 16: 131-134.

Verma, D. and Shweta, 2011. Earthworm resources of western himalayan region, India. Int. J. Soil Sci., 6: 124-133.

Winding, A., R. Ronn and N.B. Hendriksen, 1997. Bacteria and protozoa in soil microhabitat as affected by earthworm. Biol. Fertil. Soils, 24: 133-140.