INNOVARE JOURNAL OF AGRICULTURAL SCIENCE

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 4, Issue 3, 2016

ISSN - 2321-6832

Research Article

INVESTIGATION OF MICROBIAL COUNT IN THE SOIL AND EARTHWORM GUT (*EUDRILUS EUGENIAE*)

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Received: 30 May 2016, Revised and Accepted: 01 June 2016

ABSTRACT

Objective: This study was undertaken to find out the soil microbes are highest in compared with earthworm gut of *Eudrilus eugeniae*.

Methods: Microbes are soaring numbers of present in middle gut, and several nutrients are gift within the soil. Soil includes pH, nitrogen, phosphorus, potassium, zinc, manganese, iron, and copper. Earthworm gut from *E. eugeniae* compared with soil contains the highest bacterium and fungi.

Results: *E. eugeniae* gut and soil present in the bacterial colony (total count - 787.33; percent - 78.7%); fungal colony (total count - 610.66; percent - 61.0%). From the results, it can be terminated the bacterial populations was a lot of within the soil and midgut region of *E. eugeniae*.

Conclusion: The results indicated that the physico-chemical properties present in the soil. Physic-chemical properties used to the soil capacity in this area and growth, potential for earthworms. It is also acted as an organic nutrient for the rapid bacteria and fungi colonization whereby increases the microbial activity.

Keywords: Soil, Earthworm gut, Bacteria, Fungi and *Eudrilus eugeniae.*

INTRODUCTION

Agriculture is the backbone of Tamil Nadu, India, and the agriculture product is not solely used for domestic consumption, however, conjointly it is a mean that currency is obtained for varied functions. Few soil ecology studies are centered on the likelihood of connecting microbes and fauna [1]. Soil fauna plays a prominent role in regulation soil processes, and among these earthworms, it plays an important role in maintaining the soil quality and managing efficient nutrient cycling. Agricultural actions such as cultivation, intercropping, rotation, drainage use of pesticides, and fertilizers have vital implications for the microorganisms, number of fungi, bacteria, actinomycetes, yeast, and protozoa present in the soil [2]. Earthworms are vital drivers of soil biogeochemical processes as they modify soil physico-chemical belongings and microbial groups by feeding, burrowing, and casting activities [3]. In soil, microorganisms are delicate to alters in the encompassing soil [4] and have been shown that the microbial populations changes once fertilization [5]. Fertilizers can directly stimulate the growth of microbial populations as whole by provision nutrients and should have an effect on the composition of individual microbial communities within the soil. The most significance soil factors affecting the distribution of various species of earthworms square measure the C/N quantitative relation, pH, and contents of Al, Ca, Mg, organic matter, soil, and coarse sand. Although molecular methods have been accustomed study soil bacterial communities, very little analysis has been undertaken for soil fungi.

Earthworm's profusion is altered by some of the chemical properties of soil, *viz*., temperature, moisture, regime, pH, soil organic content, and litter inputs. Earthworm's account for the lofty biomass between torrid soil macrofauna. The part of earthworm in the action of decomposition, edifice, and maintenance of soil structure has been well documented for soils temperature region [6]. Decomposition and organic process of perishable organic waste materials are preponderantly meted out by microorganisms within the soil, however, the few recent studies have shown that earthworms too have roles in the organic process [7-9]. The intestine of the worm includes microorganisms and gut enzymes. The microflora in the intestine of nightwalker decomposers these triturated and particulated particles. Recent studies by reference have shown the occurrence of a selection of species of microorganisms within the gut of earthworms within the gut of earthworms. Similar to the occurrence of greater variety of microbes within the gut of earthworms, *Lampito mauritii, Eudrilus eugeniae, and Eisenia fetida* the cast conjointly contains a lot of microorganisms [10]. Interactions between earthworms and microorganisms seem to be advanced. Earthworms are rumored to have an association with such lifestyle soil bacteria and represent the drilosphere [11]. In this method, it is known that microbial biomass and movement are typically increased within the drilosphere, with considerable numbers of microbial colony forming units (CFUs) within the tunnel walls and earthworm casts than in the parent soil [12].

METHODS

Selection of suitable species

Selection of suitable species for vermiculturing was done according to the requirement, for composting, poultry, and animal graze. In general, some species are "vermicultured" that have other possible commercial utilization like *E. eugeniae*.

Collection of samples

Soil and earthworms were collected in the surface of Thiruvaikavur village near at Thanjavur district, Tamil Nadu, in India. They are paddy field soil. The soil type is clay loam soil. In this course, soil particles are grinded to uniform sizes before experimentation process.

The enumeration of the total bacteria in vermicomposts samples was accomplish by following "Serial dilution plate technique Martin [13] using nutrient agar medium for bacterial and potato dextrose agar (PDA) medium for fungi." The population of microbes was examined in various treatments and expressed as CFU/ml.

Sterilization of glass wares

All the glass wares (Petri plate, test tubes, slides conical flask, inoculam needle, measuring jar, and beaker) were kept in cleaning solution for 12 hrs. Then, they were washed in soap water and tap water. Finally, they were cleaned with distilled water and dried. The dried glassware and sterilized at 120°C for 15 minutes in hot air oven. All the chemical media were sterilized using the autoclave.

Serial dilution method

Then, sterilized test tubes taken and the first test tube was taken 10 ml of distilled water, and remaining test tubes were with 9 ml distilled water. 1 g of sample was dissolved in a first test tube with distilled water (10 ml) and its dilution factor 10^{-1}. Then, 1 ml of sample was transferred from first tubes (10^{-1}) and second test tubes and its dilution factor is 10^{-2}, similarly samples were transferred to remaining test tubes, and dilution factor obtained as 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} (Martin, 1950). From these diluted samples, 1 ml of sample was taken and transferred to a separate nutrient agar media (NAM) and PDA plates and incubator and room temperature for 24 hrs for bacterial culture and 72 hrs for fungal culture.

Preparation of nutrient agar

The nutrient agar medium is a liquid medium. It is prepared by mixing the following components.

Peptone	50 g
Beef extract	3.0 g
NaCl	5.0 g
Agar	15.0 g
Distilled water	1000 ml

About 1000 ml of distilled water is measured and poured into a large conical flask. The various ingredients of the medium are weighed accurately and are dissolved in water one after another with content stirring. The content is gradually heated to boiling over a Bunsen burner or a hot plate. The mouth of the conical flask is plugged with absorbent cotton and wrapped with a Kraft paper. The flask with the content is sterilized within an autoclave for 15-20 minutes at a pressure of 15 lbs and at a temperature of 121°C. After sterilization, the medium is cooled and stored at 4°C. The medium is distributed to culture plates allowed to solidify. The medium is now ready for inoculation. The medium is used to bacteria culture.

Preparation of PDA

The PDA medium is a liquid medium. It is prepared by mixing the following components.

Peeled potato	250 g
Glucose	20.0 g
Agar	15.0 g
Distilled water	1000 ml

All the steps are similar to the preparation of nutrient agar. The sterilized PDA liquid medium is directly distributed into culture plates. The medium to fungal culture.

Pour plate technique

About 1 ml of sample was poured into a sterile Petri plate and adds 10-15 ml of NAM and PDA then gently rotated it to spread the sample by hand. The Petri plates were kept in room temperature. After 24-72 hrs, pour bacterial and fungal colonies were obtained and counted.

Enumeration of microbial population of soil (bacteria and fungi)

About 1 g of soil is added into the distilled water taken in one of the test tubes. The content is mixed and allowed to sterile. If necessary, clear suspension is obtained by repeated soil extraction. Using a sterile, 1.0 ml pipette 2 drops of clear soil sample is spread on the nutrient agar and PDA surface of the plate. The sample is spread with a sterilized pour plate method. The culture plate is inoculated at room temperature for 24 hrs at bacteria colony culture and 72 hrs fungal colony culture.

Enumeration of microbial population in earthworm gut (bacteria and fungi)

A healthy worm was selected for enumeration of microbes by pore plate method. The mid gut of earthworm (*E. eugeniae*) content gut ranging from of the three stages of worms (preclitellate, earlyclitellate, and lateclitellate). The earthworm gut is separately using sterile scissors. They are grinded in using pistol and mortar and taken to the sterile test tubes and mixed 2 ml of distilled water. The satisfied is mixed and permitted to achieve. The aforesaid serial dilution method involves in this experiment. Using the sterilized 1.0 ml pipette two drops of clear earthworm gut samples spread on the nutrient agar and dextrose agar surface of culture plates.

The sample is expansion on the sterilized pour plate method

The lids are replaced and after solidification. The agar plates are incubated 30°C for 24-48 hrs in bacterial, and fungal colony forms counter and expressed as CFU × 10^6/g. Data represented in the table.

Statistical analysis

The result obtained in the present investigation was subject to statistical analysis present for mean and standard deviation by following the method for one-way ANOVA in SPSS 16.0 version. The bacteria and fungi count of all treatment shows significant at $p<0.05\%$ level.

RESULTS AND DISCUSSION

Earthworms are known to play important roles in the structure and chemical properties of soil. In this study, the total numbers of bacterial and fungal in the soil and gut content of *E. eugeniae*. In present investigation, it shows on physico-chemical properties of soil (Table 1 and Fig. 1). The soil type was clay loam soil in nature. pH of the sample was taken using an electronic digital pH meter in 1:5 soil-water suspensions. Total nitrogen (N), available phosphorus (P), and exchangeable potassium (K) were determined by Kjeldahl distillation [14], respectively, result from the present experiment demonstrated that soil microbial population. Ramsay and Dunbrack [15] reported increased in soil microbial colony count in response to fertilization. Soil nutrients are induced to microbe's culture.

Tables 2 and 3 show the bacterial and fungal colony counts of samples in soil and gut content of *E. eugeniae*. Many more bacteria and fungi gut content of each species than from the soil (Figs. 2 and 3). Idowu *et al*. [16] reported that the aerobic bacterial counts in midgut of the earthworm; *Libyodrillus violaceous* was higher than that of foregut, whereas the hindgut region recorded maximum. In this present study,

Table 1: Physico-chemical properties of soil

Serial number	Soil characteristics	Percentage
1	Soil pH	7.5
2	Nitrogen	70.0
3	Phosphorus	13.0
4	Potassium	14.0
5	Zinc	0.26 ppm
6	Manganese	6.5 ppm
7	Iron	6.5 ppm
8	Copper	7.8 ppm

Table 2: Bacterial colony count in soil and earthworm gut *E. eugeniae*

Serial number	Sample	Total count	Percentage
1	Soil	787.33±2.51	78.7
2	Earthworm gut	692.00±1.73	69.2

E. eugeniae: *Eudrilus eugeniae*

Table 3: Fungal colony count in soil and earthworm *E. eugeniae*

Serial number	Sample	Total count	Percentage
1	Soil	610.66±2.08	61.0
2	Earthworm	545.33±0.52	54.5

E. eugeniae: *Eudrilus eugeniae*

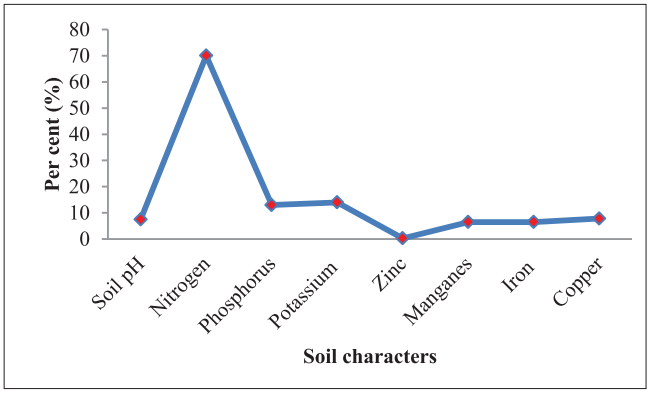

Fig. 1: Bacterial and fungal colony count in soil and earthworm gut of *Eudrilus eugeniae*

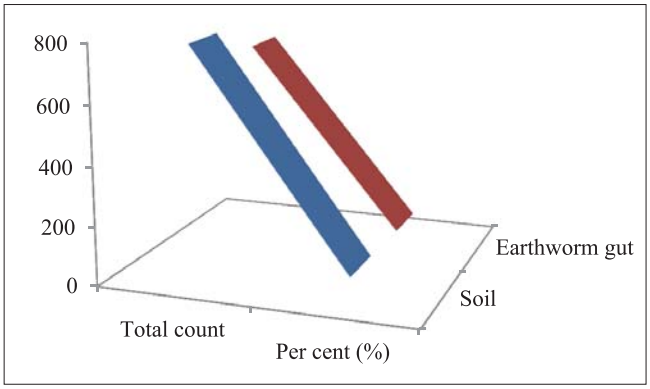

Fig. 2: Bacterial colony count in soil and earthworm gut *Eudrilus eugeniae*

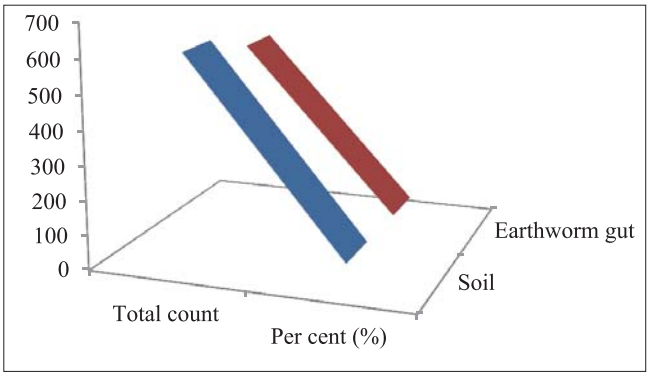

Fig. 3: Fungal colony count in soil and earthworm *Eudrilus eugeniae*

bacterial colony count in soil is 787 × 10^7 cells/ml. Earthworm gut is 690 × 10^7 cells/ml. Bacterial colony is high in soil compared with earthworm gut.

A symbiotic relationship between earthworm and their gut microflora has been proposed by Lavelle *et al.* [17]. The difference between the soil and gut microbial colony counts was highly significant, in spite of the large variation between the samples. Bacteria increased greatly in the gut of *E. eugeniae* as with the other species, but their worm also contained more fungi in soil. The present study has been undertaken to find out the microbial population present in the soil and earthworm gut. In this recorded, very helpful to the future study of soil growth and crop production.

CONCLUSION

In the present investigation, soil and midgut of *E. eugeniae* earthworm contained a lot of numbers bacteria and fungi. The results indicated that the physico-chemical properties present in the soil. Physico-chemical properties used to the soil capacity in this area and growth, potential for earthworms. It is also acted as an organic nutrient for the rapid bacteria and fungi colonization whereby increases the microbial activity.

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