Analysis of Effects of Caffeine on *Eisenia fetida* and its impact on physical and chemical properties of the soil after composting.

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Abstract

Tons of organic waste is produced every day as coffee grounds. The common, concentrated chemical found in these wastes is caffeine which is also a nervous stimulant. Earthworms, especially, Red worms (*Eisenia fetida)*, break down the organic material in wastes through the process of vermicomposting. The effects of caffeine on these Earthworms were studied. Worms were exposed to different concentrations of caffeine and the LC50 was found. It impacted the worm's composting ability. Different chemical and physical tests were carried on the soil to study its quality. There was an increase in the nutrients of the soil like carbonate and nitrate with increasing concentrations. A significant increase in water holding capacity and humus content can also be viewed from the tests. The study indicated that caffeine with in lethal dose has a positive impact on the worm's ability to form vermicompost.

Key Words:- *Eisenia fetida***,** Caffeine, humus, vermicompost.

Introduction

Coffee is one of the most popular drinks in the world. It is prepared and consumed in variety of ways across geographies. It contains Caffeine which has stimulating effects on human beings. It was concluded by F.H.Orozco (1996) that after ingesting coffee pulp by the earthworm there is a decrease in K and increase in P,Ca andMg content of compost is seen.Though Sara Fink et. al.(2012)states that there is no evident effect of coffee on biomass of earthworm still the soil quality needs to be assessed. This study is conducted to understand the effect of Caffeine which mixes with the soil as part of coffee waste on Red Worms and composting.

Eisenia fetida, commonly known as red worms, are surface dwellers which are often found in organic wastes. They are hermaphrodites as they contain both the male and female sex organs on the clitellum. They have a tube-shaped cylindrical body which is divided into segments and respiration is carried out through the skin.

Red worms mainly feed on organic matter such as dried leaves, animal faeces, paper or any other biodegradable material. They pass the food material through their digestive tract and excrete it in the form of castings. The chemicals secreted in their digestive tract help break down the organic matter into simpler forms. Hence, the excreta contains nutrients which are then directly available to the plants. It also improves the physical and chemical properties of the soil such as the texture, moisture content, humus and maintains a pH of around 7. Due to the burrowing habit of *Eisenia fetida*, the soil also becomes more porous. According to J.Haimi and V.Huhta (1986) the waste gets converted into odourless casting of good physical structure.

Materials and Methods

Primarily there are 2 aspects of this study –

- Effects of Caffeine on *Eisenia fetida*
- Subsequent impact physical and chemical properties of the soil after composting

Behaviour of *Eisenia fetida*:

The following behavioural aspects are generally observed on subjecting *Eisenia fetida* to different physical conditions: They are sensitive to UV light/Sunlight and appears on the surface only in the dark to avoid desiccation.

Toxicity test

To study the effect of a chemical substance on an organism, we need to determine how it affects the physiological functions and mortality of the organisms. One such measure of toxicity is the LC50 test. It is the lethal concentration which is needed kill 50% of the population.

To test the mortality of an organism, a population is subjected to different concentrations of the chemical or toxin. The test is only validated if multiple replicates of each concentration and the mortality in the control does not exceed 10%.

Extraction of Caffeine

The coffee powder was dissolved in distilled water to form a saturated solution and boiled for 10 mins. The mixture was then poured in a separating funnel and chloroform was added to it.

The contents were mixed and the separating funnel was left undisturbed for 12 hours. The organic solvent absorbs the caffeine and forms the bottom layer in the funnel. The knob is opened and the organic solvent is collected in a Container.

Three extractions were carried out for each batch of coffee solution. The organic solvent is evaporated and the caffeine in powder form is retrieved. The procedure is repeated multiple times to collect the required amount of caffeine.

Preparation of Artificial Soil

The soil was made by mixing clay and coco peat in the ratio 2:1. Distilled water was added to add moisture to the soil. Cow dung, dried leaves, vegetable peels and shredded papers were added as feed in the soil.

Toxicity Test LC50

LC 50 test was carried out for three concentrations – 0.5g/400 g of soil, 1g/400g of soil, and 3g/400g of soil. For test of a concentration, 5 Containers are filled with 400 g of soil each and 100 earthworms were put in each Container.

A solution of the test material in distilled water is made and poured over four containers while one is kept as a control. Test duration was one week and then the mortality rate was observed. The same procedure was repeated for each concentration.

Assessment of the soil quality

a) Water Holding Capacity

Water-holding capacity refers to a soil medium's ability to hold water. The water-holding capacity of a soil is controlled by its texture, composition, and amount of organic matter content it contains.

A funnel was attached to a vertical stand and a filter paper was put inside. The soil sample was put in the funnel. A Container was placed under the funnel. 50 ml water was poured in the funnel and allowed to be collected in the Container. The amount of water collected in the Container was measured. It was repeated for all the samples.

b) Chemical Properties of Soil

i. Carbonate Test

Carbonate test is performed for detecting the presence of carbonates in the soil. For this purpose 1mg of soil sample was taken in a test tube and 1ml of conc. HCl was added to it. The production of effervescence indicates the presence of carbonate.

ii. Nitrate Test

Nitrate is the primary nutrient form of nitrogen in the soil, so Nitrate test is performed to detect presence of Nitrates. A soil water suspension was made in a test tube. 2 drops of diphenylamine was added into it. Blue colour indicates the presence of nitrates in the soil.

Results and Discussion

a) LC50 Test

LC 50 test was carried out for three concentrations – 0.5g/400 g of soil, 1g/400g of soil, and 3g/400g of soil. 3 different observations were recorded for each of these concentrations as below -

i. First Observation

Results observed are available in **Table 1.**

ii. Second Observation

Results observed are available in **Table 2.**

iii. Third Observation

Results observed are available in **Table 3.**

The results of 3 observations **(Table 1, Table 2 and Table 3)** indicate that mortality rate increases with increase in concentration of caffeine. However it still remains very far from eliminating 50% of the population of earthworms. The result of toxicity test LC50 for 3 samples of above concentration is negative. Matthew M.Bulman (2012)also stated that sublethal effects of caffeine is seen with higher quantities.

b) Chemical Tests:

On a scale of 1-5 (1 being the lowest amount and 5 being the highest amount), the following observations were made for carbonate and nitrate tests:

i. Carbonate Test

As part of Carbonate test 3 observations were carried out with concentrations 0.5g /400g, 1g/400g and 3g/400g. As indicated in the results captured in **Graph 1**, more effervescence was observed in the third concentration (3g/400g of soil) as compared to the previous two while least was observed in the first concentration (0.5g/400g of soil). Lee (1985) showed that wormcasts ingested soil often have much higher content of soil organic carbon and nutrients than the surrounding soil.

ii. Nitrate Test

For Nitrate test, 3 observations were carried out with concentrations 0.5g /400g, 1g/400g and 3g/400g. As shown in **Graph 2** darker blue colour was observed in the third concentration (3g/400g of soil) while it was almost the same in the first (0.5g/400g of soil) and second concentration (1g/400g of soil).

The result of both carbonate and nitrate tests show that the amount of carbonate and nitrate increases progressively with each concentration, and increased amount of caffeine certainly improves concentration of nutrients like Carbonates and Nitrates in the soil. It could not be confirmed that whether increased amount of caffeine affected the soil directly by adding more nutrients or affected the earthworms by increasing their activity. A detailed research with Anatomical study of earthworms could take this study to its logical conclusion.

c) Physical Properties:

i. Water Holding Capacity of Soil

Similar to Chemical Tests 3 observations were carried out with concentrations 0.5g /400g, 1g/400g and 3g/400g to analyze the change in Water Holding Capacity of the soil with increase in concentration of caffeine. The results as indicated in **Graph 3** clearly show that the water capacity also increased with caffeine concentration.

ii. Humus Content

Physical observation of the soil samples with 3 different concentrations of caffeine indicate that the colour of the soil of third concentration (3g/400g of soil) was darker than the second (1g/400g of soil) and first concentration (0.5g/400g of soil) indicating an increase in the presence of organic matter in the soil. An increase in water and nutrient retentention by adding coffee waste is also reported by R.K.Kasongo et.al.(2011).

Conclusion

Hence, it can be concluded that the addition of caffeine to the soil helps in formation of a better quality of vermicompost due to the following reasons:

It resulted in the increase in amounts of nutrients present in soil (as tested for carbonate and nitrate) which could either be due to direct effect on the soil or indirectly by stimulating the earthworms and improving their activity which coincide with the findings of S.Suthar and S.Singh (2007).

It also improved the physical properties of the soil such as the water holding capacity of the soil and increasing the humus or the organic content will enhance the overall growth of plants as reported by Edward and Burrows (1988). These parameters lead to improvement in soil fertility. Overall a positive effect of caffeine (at concentrations below LC50 level) was observed in the present study.

References

1) Orozco, F.H., J. Cegarra, M. Trujillo, and A. Roig, Vermicomposting of coffee pulp using the earthworm Eisenia fetida: Effects on C and N contents and the availability of nutrients, *Biology and Fertility of Soil*, 22(1996) 162.
2) Sarah Fink, Lauryn Hong, Izzy Morrison, Yishi Wang, The Effects of Varying Coffee Ground Amounts on earthworm Eisenia fetida Biomass in Vermicomposting, *Biological Station, University of Michigan*, (2012)
3) J. Haimi, V. Huhta, Capacity of various organic residues to support adequate earthworm biomass for vermicomposting, *Biology and Fertility of Soil*, 2 (1986) 23.
4) Matthew M. Bulman, *The Toxicity of Acetaminophen, Caffeine and Carbendazim in Earthworms (Eisenia fetida)*, Ph. D. Thesis, Eastern Illinois University, Charleston IL, (2012)
5) Lee K E, Earthworms: There ecology and relationships with soils and land use, *Earthworms: There ecology and relationships with soils and land use*, Sydney : Orlando : Academic Press (1985).
6) Kasongo, R.K., Verdoodt A., Kanyankogote P., Baert G, and Ranst E. Van, Coffee waste as an alternative fertilizer with soil improving properties for sandy soils in humid tropical environment, Soil Use and Management, 27(2011) 94.
7) S. Suthar, S. Singh, Vermicomposting of domestic waste by using two epigeic earthworms (Perionyx excavatus and Perionyx sansibaricus), *International Journal of Environmental Science and Technology*, 5 (2008) 99.
8) Edwards CA, Burrows I, The potential of earthworm composts as plant growth media, *In: Edwards C A Neuhauser E (eds)Earthworms in waste and Environmental Management*, SPB Academic Press, The Hague, 22(1988)

Tables

S.no.	Concentration (g/400g of soil)	Earthworms Taken	%Mortality
Control	0	10	0
1	0.5	10	0
2	0.5	10	0
3	0.5	10	0

Table 1 : Results of LC50 Test with concentration 0.5g/400 g of soil

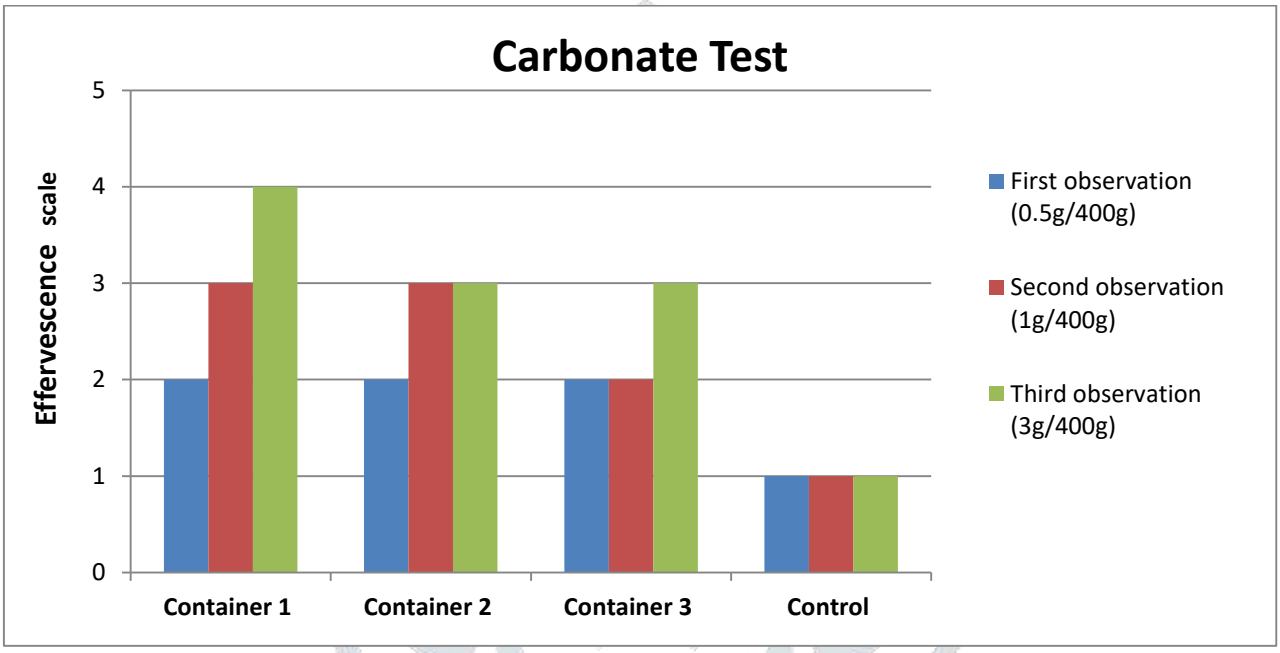
S.no.	Concentration (g/400g of soil)	Earthworms Taken	%Mortality
Control	0	10	0
1	1	10	1
2	1	10	0
3	1	10	1

Table 2 : Results of LC50 Test with concentration 1g/400 g of soil

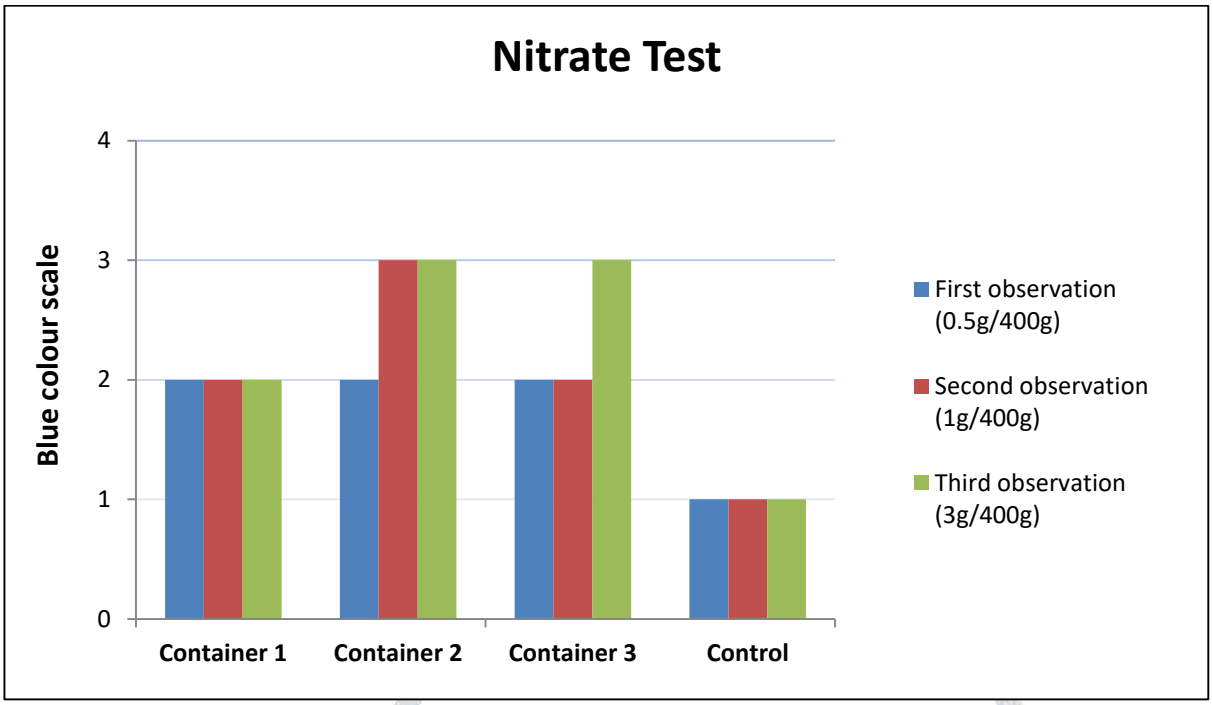
S.no.	Concentration (g/400g of soil)	Earthworms Taken	%Mortality
Control	0	10	0
1	3	10	5
2	3	10	4
3	3	10	6

Table 3 : Results of LC50 Test with concentration 3g/400 g of soil

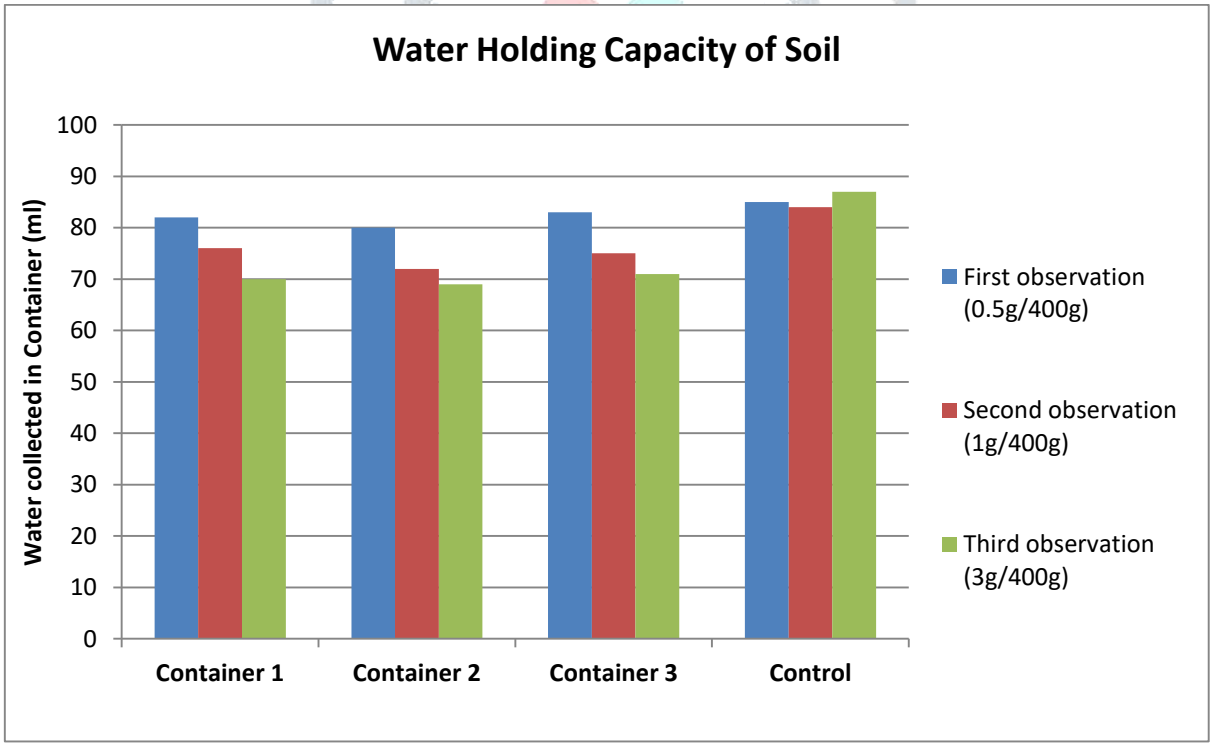
Illustrations



Graph 1 : Observations of Carbonate Test



Graph 2 : Observations of Nitrate Test



Graph 3 : Observations of Water Holding Capacity Test of Soil