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Vermiwash Production From Some Types of Earthworms

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VERMIWASH is a dark colored liquid fertilizer, which is collected after water passes via an earthworm culture column. It is rich in plant growth hormones, micro- and maco-nutrients like N, P, and K. An experiment was conducted using three earthworms' type; *Allopophora spp*, *Eisenia fetida* and *Perionyx excavates* to produce vermiwash. Some chemical and microbial analyzes were performed (pH, EC, macro- and micro elements, toxic metals, total count of aerobic bacterial, fungi, actinomycetes, total coliform, *salmonella* and *shigella*). The results indicated that pH of vermiwash was slightly alkaline and recorded 7.14 followed by 7.35 and 7.46 with the *Allopophora spp*, *Eisenia fetida,* and *Perionyx excavatus*, respectively. The electrical conductivity (EC) recorded insignificant difference between *Perionyx excavatus* and *Eisenia fetida* it was 3.7 and 3.6 dS m^{-1}. Using *Perionyx excavatus* and *Eisenia fetida* provided the highest total N values; 9044 and 9040 mg L^{-1} respectively, while the total P and K were the highest content (2627 and 2607mg L^{-1}, respectively) when *Perionyx excavatus* was used. The concentration of Fe and Cu were the highest when using *Perionyx excavatus* with values of 16.4 and 0.21 mg L^{-1} respectively. The Mn recorded its highest concentration in M, *Allopophora spp*as 0.50 mg L^{-1}. The vermiwash content of Cd, Ni, and Co was not detected, while the low Cr concentrations were recorded with the vermiwash resulting from the three earthworms. As well, the vermiwash obtained from the *Perionyx excavatus* was the highest in the total aerobic bacterial count,while *Allopophora spp* provided the best values for each total numbers of fungi and actinomycetes with an absence of total coliform, *Salmonella* and *Shigella* in all tested samples.

Keywords: Vermiwash; Earthworms; *Allopophora spp*; *Eisenia fetida*; *Perionyx excavatus*

Introduction

Earthworms are groups of soil fauna and derive their nutrients from the decomposing organic matter. Its role in soil formation and fertility is well recognized and documented (Sugapriya and Mahalingam, 2016). Earthworms, the closest companions and benefactors of soil, have long assisted soil in nutrition, respiration, excretion, and a variety of other essential tasks. An earthworm has shown to be the soil's mouth, stomach, and intestine by its distinctive functions of cutting, crushing, cranking, assimilation, and tunneling (Garg et al., 2019) .

Vermiwash, a foliar spray, is a liquid fertilizer assembled following the water passage through a worm activation column. It is a series of earthworms excretory, accompanied by the major soil micronutrients and organic molecules which are valuable for plants (Meghvansi et al., 2012, Aboelsoud and Ahmed, 2020). Vermiwash is abundantin nutrients (such as N, K, Cu, Zn, Ca, Fe, and Mg), amino acids, vitamins, and some

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growth hormones such as auxins and cytokines (Suthar, 2010). In addition to plant nutrients, vermiwash contanis certain organic acids and mucus of both of earthworms and microbes (Shivsubramanian and Ganeshkumar, 2004). Vermiwash acts an important role in growth and development of the plant; contributing to rooting initiation, root growth, promoting crop growth rate, plant development, and improvement in crop production. It also increases the soil organic matter (SOM) and nutrient content which are easily available for the plants, causing higher crop yields. Vermiwash leads to better plant growth and higher yields through slow release of nutrients for uptake by the application of organic inputs such as vermicompost in association with vermiwash (Singh and Sharma, 2002). Furthermore, vermiwash has excellent growth promoting impacts, as well as it serves as a biopesticide (Sundararasu and Jeyasankar, 2014). It appears to have an intrinsic property to act not only as a fertilizer but also as a slight biocide (Pramoth, 1995). Moreover, vermiwash improves the wastewater treatment effieciency, therefore, vermifiltration could be the alternative approach for wastewater treatment due to its low-cost and effectiveness in wastewater treatment, as well due to its ecologically sustainable properties for developing countries (Mungruaiklang and Iwai 2021).

Therefore, the aim of this study is to recycle agricultural waste using vermiwash technology and produce a bio-compound that is efficiently used as fertilizer and stimulant in organic and sustainable agriculture.

Materials and Methods

Organic wastes

The organic wastes that wereused to feed the earthworms were horse manure (HM) and kitchen wastes (KW) , which were collected from the houses and local urban farms from Tanta, El Gharbia Governorate, Egypt. The macro- and micro- elemnets in the organic wastes are presented in Table 1.

Earthworm types

Three types of earthworms, two exotic types; Tiger Worm (*Eisenia Fetida*) and Indian Blue (*Perionyx Excavatus*), and one indigenous type *(Allopophora* spp) are used in this study.

Soil used

The soil used in this study was collected from surface layer 0- 30 cm from agricultural soil in El Gharbia Governorate. The soil was air dried, ground, sieved through a 2 mm sieve, and kept in plastic bags until analyzed. Soil pH was determined in 1:2.5 soil: water (W/V) suspensions (Page, 1982). Electrical conductivity (EC) was measured in 1:5 soil: water extract (Jackson, 1973). Soluble anions and cations were measured in (1:5) soil extract (Richards, 1954). The physiochemical soil properties are presented in Table 2.

Pre-composting of organic wastes

The experiment was conducted to produce vermiwash at the Organic Farm in the Department of Environment and Bio-Agriculture, Faculty of Agriculture in Nasr City, Al-Azhar University – Egypt. Pre-composted was done for organic wastes (HM and KW) to avoid the thermophilic stage of composting that may cause the death of earthworm in vermicomposting systems and for the acceleration of the composting process through augmenting the earthworm population (Frederickson et al., 2007). The organic KW were cut into pieces of 3 - 4 cm length and mixed with HM at 60% and 40% additional rate of HM and KW respectively. This mixture is arranged in piles with sprinkling water, regular mixing, and turning of the substrates for decomposition of organic wastes. The organic materials were used to feed earthworms after pre-composted for 30 days.

Vermiwash preparation

For vermiwash production, a method of Gopal et al., (2010) was adopted with minor modification. A plastic barrel (50L) with a tap fixed above the barrel base was used for vermiwash production. In the barrel, coarse gravel was put up to 15 cm height at the bottom followed

TABLE 1. Macro- and micro elements of the different organic wastes used in this study

Waste types	N (%)	P (%)	K (%)	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})
Horse manure	1.23	0.40	1.39	66	169	15.2	3000
Kitchen wastes	0.41	0.21	0.54	95	145	20.1	5020

TABLE 2. Some physiochemical properties of the investigated soil

Property	Sand ($g\ kg^{-1}$)	Silt ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)	Texture Class	pH	EC ($dS\ m^{-1}$)	Soluble cations (cmolc kg^{-1})				Soluble anions (cmolc kg^{-1})			
							Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	CL^-	SO_4^{-2}	CO_3^-
Value	199	204	597	Clay Loam	7.9	0.64	1.2	0.5	6.2	0.6	1.1	3.2	4.1	0.15

by 5 cm of clean sand and 5 cm of soil-forming the vermiwash filtration and accumulation system compartment. Water was then let to flow through these layers and drain without any blockage from the tap. This permits the filtration layers to settle properly, and then the tap was closed. Above the filtration strata, a layer of 30 cm matured HM was added. Water was addedin enough quantities to save the substrates at moisture content of 40%. Then half kg of each of the three earthworms was added to the barrel to degrade the HM. The unit was remained without further adding of water for 10 days, letting the earthworms to move to the partially decomposed feedstock, and start the active decomposition of the substrates. After 10 days, 0.5 L plastic bottle was hung over the barrel. Cotton wick was inserted in the bottom of the plastic bottle permitting water to trickle and regulated in such a way to refill the bottle once a day (Fig. 1). After one month, the vermiwash had collected at the barrel bottom, drawn from the tap, and kept until analysis. Chemical and microbiological properties were performed on the obtained vermiwash (as in section 2.6). Next collections were done weekly for a month to calculate the overall quantity produced from each of the three systems.

Studied parameters of vermiwash

Chemical and microbiological properties of freshly collected vermiwash was analyzed. .

Chemical parameters of vermiwash

The pH was determinedin vermiwash-water suspension (1:10) using a glass electrode of Orion Expandable ion analyzer EA920. Electrical conductivity (EC) was determined in vermiwash-water suspension (1:10) using EC meter ICM model 71150. Soluble N (i.e. NH4+ and NO3-) were measured in vermiwash according to Page, (1982), while available N was determined according to Cottenie et al., (1982). Available K was determined using flame photometer (model ILAE 201 Fisher Scientific company) according to Cottenie et al., (1982). Available P was analyzed using a spectrophotometer (model 670 SUV/VIS Jen way company) at wavelength 650 (Jackson, 1973). The metals Fe, Zn, Mn, and Cu in addition to toxic HMs (Cd, Cr, Co and Ni) were measured in vermiwash using atomic absorption spectrophotometer (model GBCA vantaE,Victoria, Australia) (Chapman and Pratt, 1961).

Microbiological parameters of vermiwash

The standard dilution technique was used in this porpuse; 10 ml of vermiwash were suspended in 90 ml of sterile water and shaken for 20 min. Ten-fold serial dilutions were performed for counting bacteria, actinomycetes, fungi, total coliform bacteria, *Salmonella* and *Shigella*as follows: Total count of bacteria was determined by plate count on nutrient agar media after two days of incubation at 30 ℃ (Allen, 1950), the total count of fungi was determined by plate count on Potato-dextrose agar media after five days of incubation at 28 ℃ (Allen, 1950), the total count of actinomycetes was meaured by plate count on Jensen media after three days of incubation at 28℃ (Allen, 1950). The total count of the coliform group was determined by plate count on Mac-Conekey'smedia, the plates were incubated at 37℃ for 24 hr for counting total coliform bacteria. Red, pink or nearly colorless with a pink center colonies were measured as coliform group bacteria (Difco,1977). The inoculated plates containing *Salmonella* and *Shigella* (SS) agar media were incubated for 24 hr at 37 ℃. Black centered colonies were counted as *Salmonella*, while colorless colonies without black centered were counted as *Shigella* microorganisms (Difco, 1977).

Statistical analyses

The obtained results were statistically analyzed using one way analysis of variance (ANOVA) for the randomized complete blocks design (RCBD) using SPSS statistics package 20 (IBM, Armonk, NY, USA). Treatments' mean were compared by Duncan's multiple range tests at *P* level =5 %.

Results and Discussion

pH and EC of produced vermiwash

The results in Table 6 reveal that the *Allopophora spp* recorded the lowest pH value

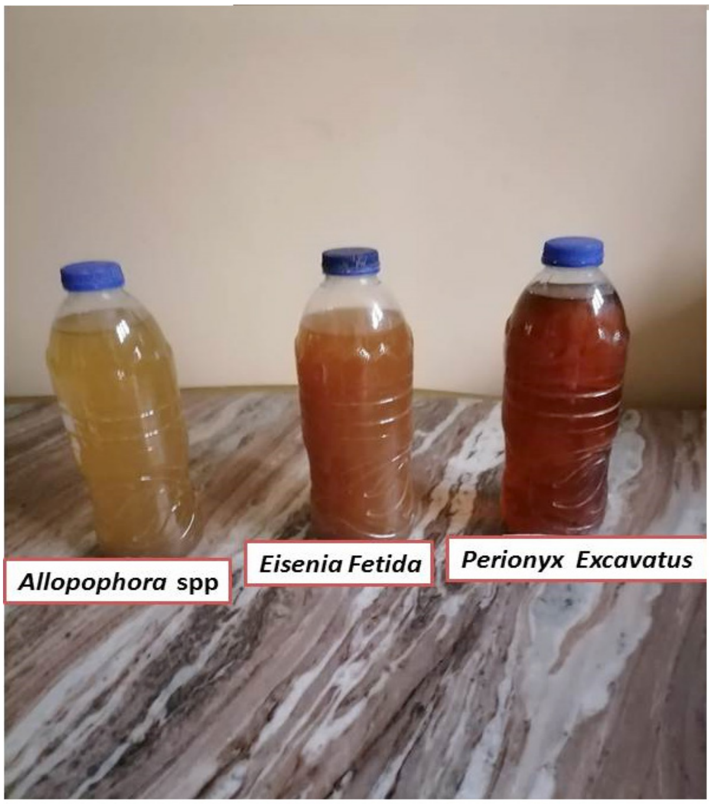

Fig. 1. Different types of vermiwash that produced in this experiment

of 7.14, while the *Eisenia fetida and Perionyx excavatus* recorded 7.35 ad 7.46 , respectively. These findings are in agreement with those obtained by Nayak et al., (2019). With regard to EC, there were no significant differences between *Perionyxexcavatus* & *Eisenia fetida* which recorded 3.7 and 3.6 dSm^{-1}, respectively. Nath and Singh (2016) produced vermiwash from different combination of water hyacinth and gram bran with buffalo dung by *Eisenia fetida*, they found that pH values ranged from 6.71 (which is acidic) to 7.86 (kaline and higher than our pH values in the vermiwash of three worms), while EC values ranged from 1.20 to 1.55 (which is lower than our results). Bidabadi et al. (2017) produced vermiwash by *Eisenia fetida* with cow manur (25 g worms:1 kg manure) and its pH and EC values were 7.56 and 5.42 dS m^{-1} respectively which is higher than our values especially in EC (Table 3). Thus the variation of vermicompost's pH and EC values may be attributed to the variation in waste sources that used with the earthworms to produce the vermiwash.

Macro-nutrients in vermiwash

The results of the three vermiwashes produced were varied either in its color (Fig. 2) or in different caracteristics. Data in Table 4 show that the total N value was the highest in the vermiwash produced by *Perionyx excavatus* and *Eisenia fetida*, which was 9044 and 9040 mg L^{-1} respectively, while in the vermiwash produced by *Allopophora spp* it was (8085 mg L^{-1}). The NH_4-N was the highest in *Allopophora spp* where it was 11.5 mg L^{-1}, while in its corresponding *of Eisenia fetida and Perionyx excavatus* it recorded 9.4 and 9.3 mg L^{-1}, respectively. TheNO^{-3}-N in the vermiwash of *Eisenia fetida* was the highest; 36.6 mg L^{-1}, followed by this of *Perionyx excavatus*; 34.0mg L^{-1} and *Allopophora spp*; 30.3 mg L^{-1}. On the other hand, *Perionyx excavatus* recoded the highest values of total Pand K (8627 and 2607mg L^{-1}, respectively) followed by that of *Eisenia fetida*, which was 6748 and 2459 mg L^{-1} in total P and K respectively, then *Allopophora spp* which was 2223 and 1491 mg L^{-1} total P and K, respectively. These results are in agreement with Buckerfield et al., (1999), who indicated that vermiwash contains several enzymes, plant growth hormones (IAA, cytokinin, GA3), vitamins, macro and micro nutrients.Also, Ruiz-Lau et al. (2020) and Deepthi, et al. (2021) statedthat vermiwash has different contents i.e., N, P, K,

TABLE 3. The pH and EC of vermiwash resulting from the three types of earthworms

Parameter	*Allopophora spp.*	*Eisenia fetida*	*Perionyx excavatus*
pH(1:10)	7.14 ^{c}	7.35 ^{b}	7.46 ^{a}
EC(1:10) dsm^{-1}	2.4 ^{b}	3.6 ^{a}	3.7 ^{a}

Mean with the different letter is significantly different at 5% level of probability

TABLE 4. Comparison of macro-nutrients in the vermiwash of the three investigated types of earthworms (mg L^{-1})

Parameter	*Allopophora spp.*	*Eisenia fetida*	*Perionyx excavatus*
Total N	8085 ^{b}	9040 ^{a}	9044 ^{a}
NH^{+4}	11.5 ^{a}	9.4 ^{b}	9.3 ^{b}
NO^{-}_{3}	30.3 ^{b}	36.6 ^{a}	34.0 ^{ab}
Total P	2223 ^{c}	6748 ^{b}	8627 ^{a}
Total K	1491 ^{c}	2459 ^{b}	2607 ^{a}

Mean with the different letter is significantly different at 5% level of probability

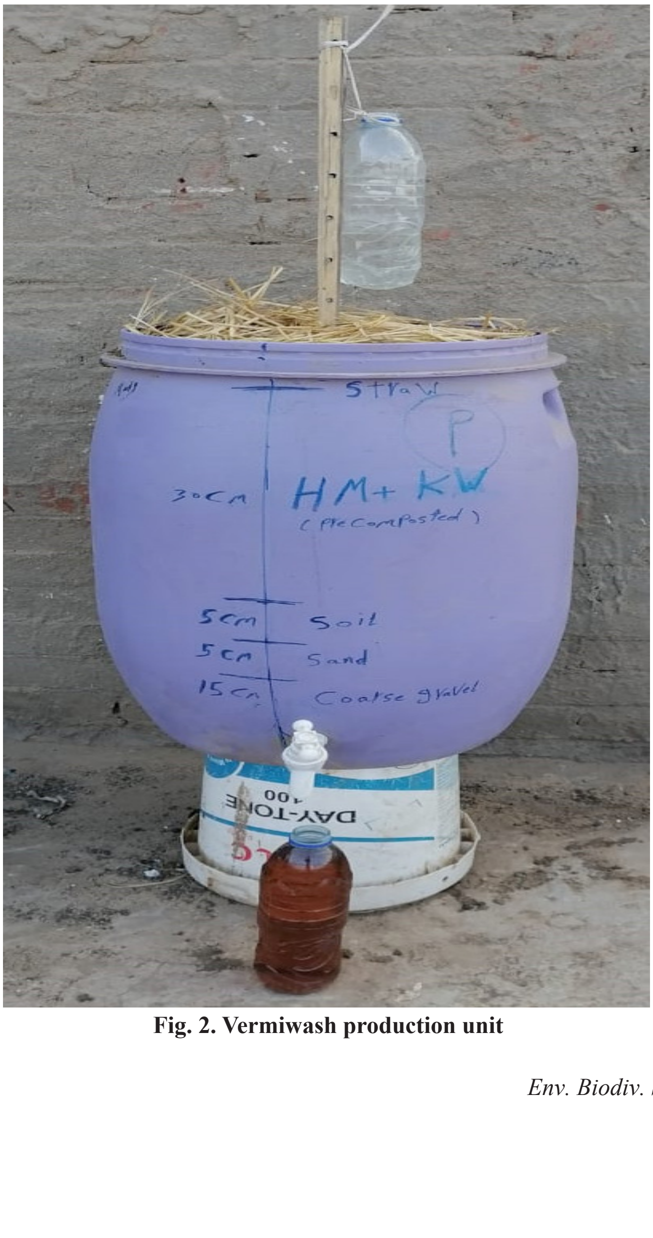

Fig. 2. Vermiwash production unit

Ca, hormones (such as auxin, cytokine) and some other secretions. Makker et al. (2017) and Grag et al (2019) found that vermiwash has total N, P and K of 0.5, 1.2, and 0.8 mg L^{-1}, respectively. Which is lower so much than our results. In addition, Deepthi et al. (2021) confirmed that vermiwash represents an eco-friendly nutrient enricher for the agricultural sustainability. Mishra et al. (2014) mentioned that the vermiwash as a final product of vermicompost reavles significant changes in its physico-chemical properties, especillay when different animal manures are used. The reason of that is increasing the beneficial soil microflora and destroying the soil pathogen and converting organic wasteto valuable products. Through this process, important nutrients (i.e. N, P, K, and Ca) in the feed materials are converted to greatly soluble nutrients by the action of earthworm that is in this time easily utilizable for plants. Furthermore, Garg et al. (2019) stated that earthworms promote the microorganisms growth in their gut which provides ideal conditions there. They also mentioned that fresh vermiwash contains a large quantityof beneficial microorganisms that supportsplant growth. Deepthi, et al. (2021) reported also that vermiwash has many useful microorganisms such as heterotrophic bacteria, fungi, and others. Chatterjee et al. (2020) stated that the microorganisms that found in the vermiwash resupplies the soil with more K ions.

Micro-nutreinets of vermiwash:

The results in Table 5 show that the highest values of Fe and Cu obtained in the vermiwash of *Perionyx excavatus*, which was 16.4 and 0.21 mg L^{-1} respectively, while it was 9.0 and 0.09 mg L^{-1} respectively when using *Eisenia fetida.*. The Fe and Cu values in the vermiwash of, *Allopophora spp* are 3.4 and 0.04 mg L^{-1} respectively. The Mn contentin the vermiwash of *Allopophora spp* was the highest (0.50 mg L^{-1}), while for *Perionyx excavatus* and *Eisenia fetida*, it was 0.29 and 0.17 mg L^{-1}, respectively. These findings are in agreement with those obtained by Buckerfield et al., (1999). Zarei et al. (2018) reported that the vermiwash liquid is rich in Mn, Zn, Fe, Cu and other elements and benifecial constituents for plants. In the same manner, Makker et al. (2017) found that vermiwash has Mn, Cu, and Zn of 0.01, 0.04, and 0.9 mg L^{-1}, respectively. Their results of Mn was lower than ours, while that of Cu was similar ourresults in *Allopophora spp.* On the other hand, their results of Zn was higher than ours.

The content of toxic heavy metals in the vermiwash

According to Table 6 the vermiwash content of heavy metals (Cd, Ni, and Co) was not detected, while Cr was recorded in the vermiwash of the three types of earthworms and the highest concentration resulted from the vermiwash of *Allopophora* spp, which recorded 0.22 mg L^{-1}, and the lowest one was in that of *Perionyx excavatus* as 0.13 mg L^{-1}. The Cr concentration in vermiwash is considered lower than its cocentraion in fertilizers and in agricultural soil (12 and 50:200, respectively) (Kabata-pendias, 2011). Abul-Soud et al. (2009) reported that vermicompost help in reducing the availabilty of heavy metals through the bio substances of earthworm beside the micro-organizms.Therefore, there some of studies that have used vermiwash as Fermi Filtration to treat wastewater (Mungruaiklang and Iwai, 2021).

Microbial content of vermiwash

The total number of aerobicbacteria, fungi, and actinomycetes resulting from the three types of earthworms are shown in Table 7. The vermiwash obtained from the *Perionyx excavatus* has the highest total aerobic bacterial countsand acheviednumbers (289× 10^{8} C.F.U) while, that of *Allopophora spp.* is the highest in total aerobic fungi and actinomycetes count (59 × 10^{5} C.F.U) and (118 × 10^{4} C.F.U), respectively. These findings are conferred with those of Pati et al., (2007). On the contrary, the results indicated the absence of total coliform, *Salmonella* and *Shigella* in all tested vermiwashes (Table 8). The lack of appropriate information on the presence of human pathogens in vermicompost products,

TABLE 5. Comparison of micro nutrients (mg L^{-1}) with vermiwash obtained from the three types of earthworms

Parameter	*Allopophora spp.*	*Eisenia fetida*	*Perionyx excavates*
Fe	3.4 ^{c}	9.0 ^{b}	16.4 ^{a}
Mn	0.50 ^{a}	0.17 ^{c}	0.29 ^{b}
Zn	Nd	Nd	Nd
Cu	0.04 ^{c}	0.09 ^{b}	0.21 ^{a}

Mean with the different letter is significantly different at 5%level of probabilityNd: not detected that made from various organic materials, is a significant roadblock to their acceptance to use as an organic solid waste management alternative. If vermiwash does not have *Salmonella*, human viruses, infective parasitic helminthic eggs, or more than 5 x 10^4 faecal Coliforms/ 100 g of the sample, it is considered hygienic (Ansari et al. 2020).

Feasibility of vermiwash production

Fixed costs for one unit of production of vermiwash

- 50 liter plastic barrel = 50 Egyptian pounds (3 US dollars)
- Sand & gravel = 10 Egyptian pounds (0.6 US dollars)
- Organic waste =10 Egyptian pounds (0.6 US dollars)
- Earthworms = 200 Egyptian pounds (12 US dollars)

Total costs per unit = 270 Egyptian pounds (16.2 US dollars)

Unit productivity

Average each type of earthworm per month

Allopophora spp = 7.8 L / month
Eisenia fetida = 9.32 L / month
Perionyx excavatus= 10 L / month

Annual production per unit

The annual production per unit of the three types of worms ranges from 93.6: 120 liters.

Suggested price per liter of vermiwash

20 Egyptian pounds (1.2 US dollars)

TABLE 6. The content of toxic heavy metals (mg L^{-1}) in the vermiwash resulting from the three types of earthworms

Parameter	*Allopophora spp.*	*Eisenia fetida*	*Perionyx excavatus*
Cd	Nd	Nd	Nd
Ni	Nd	Nd	Nd
Co	Nd	Nd	Nd
Cr	0.22 ^{a}	0.17 ^{b}	0.13 ^{c}

TABLE 7. Total aerobic bacterial, fungal and actinomycetes count of vermiwash resulting from the three types of earthworms

Parameter	*Allopophora spp.*	*Eisenia fetida*	*Perionyx excavatus*
Bacteria (- × 10^{8} C.F.U)	235 ^{c}	264 ^{b}	289 ^{a}
Fungi (- × 10^{5} C.F.U)	59 ^{a}	37 ^{c}	47 ^{b}
Actinomycetes (- × 10^{4} C.F.U)	118 ^{a}	89 ^{b}	90 ^{b}

Mean with the different letter is significantly different at 5% level of probability. C.F.U.: Colony Forming Unit

TABLE 8. Total coliform, Salmonella and shigella count of vermiwash resulting from the three types of worms used

Parameter	*Allopophora spp.*	*Eisenia fetida*	*Perionyx excavatus*
total Coliform (- × 10^{2} C.F.U)	Nd	Nd	Nd
Salmonella (- × 10^{2} C.F.U)	Nd	Nd	Nd
shigella (- × 10^{2} C.F.U)	Nd	Nd	Nd

Mean with the different letter is significantly different at 5% level of probability C.F.U.: Colony Forming Unit; Nd: not detected

Conclusion

Vermiwash is a dark colored liquid fertilizer, which is collected after water passes via a worm culture column. Vermiwash contains excretory products and excess secretions of earthworms plus micronutrients from soil organic molecule, contains high amounts of nitrogen, phosphorous, potash, calcium, magnesium, and zinc, Many beneficial microbes helping plant growth and preventing infections, sugars, phenols, amino acids and hormones promoting plant growth like indole acetic acid, gibberellic acid, and humic acid.

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