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Research Article

Biopesticide and biofertilizer potential of tropical earthworm vermicast tea

Ebenezer O. DADA*, Simeon O. NWANI, Sekinat M. YUSUFF, Yusuf O. BALOGUN

*University of Lagos, Faculty of Science, Department of Cell Biology and Genetics, Environmental Biology Unit, Akoka, Yaba, Lagos, Nigeria; eodada@unilag.edu.ng (*corresponding author); onyebuchinwani11@gmail.com; sekinat15@gmail.com; balogsy01@gmail.com*

Abstract

The adverse effects of chemical pesticides have continued to drive the search for safe, biological alternatives. Studies on biopesticide potential of earthworm casts have remained largely limited to those of temperate earthworms. We evaluated the insect pest repellency and growth-promoting potential of tropical earthworm-derived vermicast tea on the seedlings of *Arachis hypogaea* (groundnut), *Zea mays* (maize) and *Phaseolus vulgaris* (bean). Field-sourced earthworm casts were soaked in water for 48 hours, routinely stirred every 6 hours, and filtered through a fine mesh cloth. The filtrate was the vermicast tea. Seedlings grown in garden soil were sprayed with vermicast tea every four days. The seedlings were monitored for insect pest-induced leaf damage and growth performance for 5 weeks. Vermicast tea exhibited insect pest repellency effect on groundnut and bean seedlings, as evidenced by the significantly lower ($p<0.01$) insect pest attack on the treated seedlings, as against the untreated that recorded high pest infestations. However, leaf damage was relatively low in maize seedlings, and the differences in percentage leaf damage among the treated and untreated were not significant ($p>0.05$). The effect of vermicast tea on the physical growth of seedlings was positive, but marginal. This result calls for increased research on tropical earthworms.

Keywords: chitinases; earthworm casts; proteases; pest repellency; plant growth

Introduction

A pest is an organism whose activities adversely affect a farmer, their crop plants or livestock. Plant pests are typically insects and non-insects, but insect pests are a major group of the most serious pests of agricultural concern (Pureswaran *et al*., 2018). Insect pests of agricultural concern are varied and globally distributed. Depending on the location and type of crops, insect pests may include grasshoppers and locusts, termites, beetles, weevils, aphids, caterpillars, crickets. Some others, which are not insects by taxonomy, are sometimes also categorized together with insect pests; such include snails, slugs, mites (Sorensen *et al*., 2003; Barua *et al*., 2021).

Because of the huge loss pests can cause farmers, pest control remains a major challenge they often have to contend with. Chemical pesticides are the most effective, timely acting, and easy-to-use plant pests control

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options. But in spite of their effectiveness and ease of use, chemical pesticides have been associated with environmental pollution, depletion of nutritional values of soil and crops, human health risks like cancer, nervous and endocrine systems disorder (Aktar *et al*., 2009; WHO, 2018; Dada *et al*., 2018).

With the increasing awareness of the potential adverse effects of chemical pesticides, several safer pest-control alternatives have been suggested, used, or researched; these include cultural, physical (mechanical), biological pest control methods (Penn State Extension, 2016). Cultural pest control is the deliberate alteration of the planting system in order to reduce pest infestation and pest cycles. These include practices like crop rotation, intercropping, companion planting of pest repelling crops. Physical and mechanical pest controls are targeted at directly killing pests or making the farm area unsuitable for them by using traps, barriers, radiation, ultrasonic vibration that mimics the presence of predators. Biological pest control is the use of living organisms or their products to kill, reduce, or repel pests (Datta *et al.*, 2016). This may include introducing, augmenting, or conserving natural enemies of plant pests, or the use of biopesticides. Biopesticides are pesticides derived from naturally occurring materials or living organisms like plants, animals, microorganisms (McGrath and Gardener, 2010; Hossain *et al*., 2017; Shelton, 2020).

It has been discovered that some earthworm products, bye-products, or wastes have the potential to act as biopesticides, capable of suppressing or repelling plant pests (Gudeta *et al*., 2021). Renčo and Kováčik (2015) in their study found that *Eisenia fetida*-derived vermicompost and vermiwash (vermicompost tea) have suppressive effects on the development and survival of two potato-cyst nematodes. Singh and Chauhan (2015) reported that foliar applications of vermiwash obtained from vermicomposting bed of *E. fetida* and buffalo dung significantly reduced okra pod borer in okra plant (*Abelmoschus esculentus*) seedlings. Hussain *et al*. (2018) explored the potential of vermicompost derived exclusively from vermicomposting actions of *E. foetida* (*E. fetida*) on ipomea for use as an organic fertilizer. Soil application of the vermicompost led to improved crop growth and a reduction in the incidence of disease and pest attacks. Arancon *et al*. (2005) in their study found that *E. fetida* vermicompost significantly suppressed the populations of both aphids and mealy bugs on peppers, and mealy bugs on tomatoes. Akinnuoye-Adelabu *et al*. (2019) also reported suppressed Fusarium root rot, caused by *Fusarium graminearum*, in wheat (*Triticum aestivum* L.) watered with *E. fetida* extacts (mucus, vermicompost tea, and vermiwash). The vermicompost also had positive effects on the growth of these plants.

However, studies on biopesticide and pest repellency potential of earthworm casts have remained largely limited to those of temperate species, particularly *E. fetida*, with no study to demonstrate pest repellency capability of tropical earthworm casts. In this study therefore, we evaluated the pest repellency and growth-promoting potential of tropical earthworm-derived vermicast tea on the seedlings of *Arachis hypogaea* (groundnut), *Zea mays* (maize) and *Phaseolus vulgaris* (bean). Vermicast tea is the filtrate of field-sourced earthworm casts soaked in water, and passed through a filtering cloth of fine mesh.

Materials and Methods

Collection of vermicasts and processing into vermicast tea

Field vermicasts (earthworm casts) were collected within the main campus of the University of Lagos, Nigeria. The vermicasts were turret (funnel/finger-like) type, typically produced by *Hyperiodrilus africanus* and *Ephyriodrilus afroccidentsalis* (Aladesida *et al*., 2014). The casts were handpicked into a plastic container, until enough was collected. Vermicasts weighing 5 kg were soaked in 5 litres of distilled water, contained in a plastic bowl. The mixture was thoroughly stirred until the casts were dissolved and homogenized in water. The mixture was allowed to stay for 48 hours, but routinely stirred every 6 hours. The mixture was thereafter, sieved through a fine mesh cloth. The filtrate was retained as the stock verimcast tea, while the cast residue was discarded. Vermicast tea was analyzed for physicochemical properties [pH, Conductivity, Total Dissolved

Solids (TDS), Ca, K, Na, B, Mb, Mg, Fe, Cu, Zn] and enzyme contents (protease, invertase, urease, lipase, amylase, dehydrogenase, chitinase) in the laboratory of the Federal Institute of Industrial Research, Lagos, Nigeria. Physicochemical properties were determined following the methods of the American Public Health Association (APHA, 1998). Invertase, urease, lipase, amylase and chitinase were analysed after Devi *et al*. (2009). Lipase and dehydrogenase analyses were adapted from Ogbolosingha *et al*. (2015).

Experimental set-up

Serial dilutions of vermicast tea (20%, 50%, 100%) were prepared with the appropriate volumes of distilled water, where necessary. The experiment was set up in a vegetated location within the main campus of the University of Lagos, Nigeria. Fifteen (15) pots were filled with 3 kg garden soil, to three-quarter (¾) capacity. The pots were perforated with three holes at the bottom to allow excess water to drain off. Seven seeds each of groundnut, maize, and bean were separately sown and germinated into seedlings, in the garden soil contained in the experimental pots. The seeds were sown with equal spacing and at uniform depth (2 cm). The experimental pots were arranged in a Randomized Complete Block Design (RCBD) of five rows of three columns. The first row consisted of groundnut seedlings treated with urea (+ve control), distilled water (-ve control), 20% vermicast tea, 50% vermicast tea, 100% vermicast tea. The second and third rows consisted of bean and maize seedlings respectively, with the same treatment arrangement as in the first row (Figure 1). The seedlings were sprayed with the respective vermicast tea dilution, urea solution, or distilled water every four days, for a period of five weeks. The seedlings were monitored for growth performance and insect pest-induced leaf damage, throughout the experimental period. The set-up was surrounded, perimeter-wise, with a 2 mm net of height of 75 cm, to screen off rodent pest, reptiles and other big animals. The experiment was replicated in three different vegetated locations.

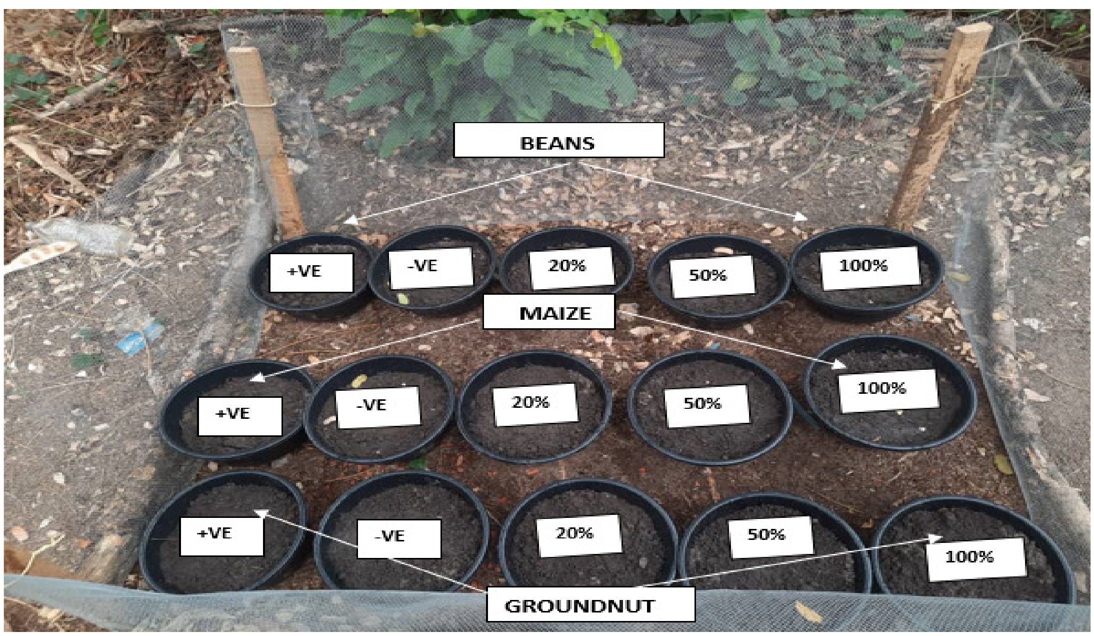

Figure 1. (Experimental layout). +VE = Urea treatment (positive control); -VE = Distilled water treatment (negative control); 20% = 20% Vermicast tea treatment; 50% = 50% Vermicast tea treatment; 100% = 100% Vermicast tea treatment

Determination of pest repellence potential of vermicast tea

The seedlings were observed for insect pest-induced leaf damage every seven days starting from the first week of the experiment to the last day of experiment. To determine insect pest repellence, the number of leaves of the seedlings in each pot was counted and recorded. The number of damaged leaves on each of the three seedlings was also counted and recorded. Percentage leaf damage was calculated as shown in the equation below (Equation 1). The lower the percentage leaf damage, the more the insect pest repellence effect of the treatment (vermicast tea, urea, or distilled water).

$$\% \text{ Damage} = \frac{\text{Number of damaged leaves}}{\text{Number of leaves}} \text{ x } 100\% \quad (1)$$

Measurement of physical and biochemical growth of seedlings

Physical growth was assessed by measuring shoot length and leaf width, using a meter rule, and counting the number of leaves. At the end of the experiment, seedlings were harvested and analyzed for biochemical growth parameters (chlorophyll a, chlorophyll b, total chlorophyll, starch content, total sugar, total protein) in the laboratory of the Federal Institute of Industrial Research, Lagos, using standard procedures.

Determination of chlorophyll a, chlorophyll b, and total chlorophyll

To determine chlorophyll a, chlorophyll b, and total chlorophyll, fresh leaf samples were weighed, put separately in 80% N/V acetone (15 ml for each gram) and homogenized with the Phillip type harmonizer at 1,000 rpm for one minute. Thereafter, 0.5 g of pure sand, plus 1.0 g of anhydrous sulphate was added to the sample. The homogenate was filtered through a two-layered cheesecloth and centrifuged using the Signs centrifuge, at 2,500 rpm for ten minutes. The supernatant was separated, and the absorbance were read at spectrophotometer. Chlorophyll a was read at wavelength 663 nm and 645 nm, while chlorophyll b was read at 645 nm and 663 nm (Dere *et al*., 1998). Chlorophyll a, chlorophyll b, and total chlorophyll were calculated as stated below.

$$\text{Chlorophyll a (Ca)} = 12.7(\text{A663}) - 2.69\ (\text{A645}) \quad (2)$$

$$\text{Chlorophyll b (Cb)} = 22.9\ (\text{A645}) - 4.68\ (\text{A663}) \quad (3)$$

$$\text{Total chlorophyll} = (\text{Ca} + \text{b}) \quad (4)$$

Determination of total sugar

The amount of total sugars (total dissolved sugars) in the seedling leaves was estimated by phenol sulphuric acid reagent method (Meena *et al*., 2014). Fresh seedling leaf sample weighing 500 mg was homogenised with 10.0 ml of 80 percent ethanol. Homogenized sample was centrifuged at 2,000 rpm for 20 minutes. The supernatants were collected into 1.0 ml of alcoholic extract; 1.0 ml of 5% phenol was added and mixed. Then, 5.0 ml of 96% sulphuric acid was added rapidly. The test tube was gently agitated during the addition of sulphuric acid and then allowed to stand in water bath at 26-30 °C for 20 minutes. The optical density of the characteristic yellow orange colour that developed was measured at 490 nm in a spectrophotometer, after setting for 100% transmission against the blank. Standard curve was prepared by using known concentrations of glucose. The quantity of total sugar was expressed as mg/g fresh weight of sample.

Determination of starch content in the seedlings

The starch content in seedling samples was estimated by the method of McCready *et al*. (1950), and as described in Meena *et al*. (2014). The residual mass obtained after the extraction of total sugars, described above, was suspended in 5.0 ml of distilled water. Then, 6.5 ml of 52% perchloric acid was added to the residue and stirred. After stirring, the mixture was centrifuged for 20 minutes at 2,000 rpm. The supernatant was decanted and collected, and the whole procedure was repeated thrice. The supernatant of each step was then poured and the total volume was made up to 100.0 ml with distilled water. The mixture was thereafter filtered through Whatman filter paper (No.42). Thereafter, 1.0 ml aliquot of this filtrate was analysed for starch content following the same procedure as that of total soluble sugars. The amount of starch was calculated in terms of glucose equivalent and factor 0.9 was used to convert the value of glucose to starch. The amount of starch was expressed in terms of mg/g fresh weight of leaf tissue.

Determination of protein content in the seedlings

The protein content in the seedlings was estimated by adapting the Lowry's method as described by Mæhre *et al*. (2018), using UV-Vis spectrophotometric technique. Fresh seedling leaves were cleaned by using tap water, followed by double distilled water, to remove all the dust and dirt. 1 g of finely chopped fresh leaves was crushed to paste in a mortar with a pestle; 20 ml of freshly prepared phosphate buffer saline of pH 7.4 was added, and pasting continued until a clear plant solution was observed. The solution was centrifuged at 10,000 rpm for ten minutes and the final supernatant was collected into a test tube. To quantify the protein content, 4.5 ml of reagent one (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1 ml of 1% sodium potassium tartrate + 1 ml of 0.5% copper sulphate) was added to the sample extracts and incubated for 15 minutes. Thereafter, 0.5 ml of freshly prepared reagent two (1-part Folin-Ciocalteau: 1 part water) was mixed with the sample and left in dark incubation for 30 minutes. Bovine Serum Albumin (BSA) was used as standard reagent for preparing the standard curve, against which the unknown concentration of proteins was estimated. A standard curve was made of bovine serum albumin (BSA; 0, 0.0625, 0.125, 0.25, 0.5 and 1 g/l) and absorbance was read at 650 nm.

Statistical analysis

The data generated from the study were subjected to descriptive analysis using the Analysis of Variance (ANOVA). Mean differences were separated using Duncan Multiple Range Test, at 5% level of significance ($p < 0.05$). All analyses were done using IBM SPSS version 26 (IBM Corporation, New York).

Results

Physicochemical properties and enzyme activities of vermicast tea

The results of the physicochemical and enzyme analysis of the vermicast tea used in this study indicated the presence of potassium, calcium, boron, and zinc among others (Table 1). Of the enzymes in the vermicast tea, proteases recorded the highest concentration of 20.47±6.44 units/ml/min, followed by amylase (17.85±0.35 units/ml/min), invertase (13.43±0.46 units/ml/min), urease (9.69±0.04 C), lipase (2.50±0.04 units/ml/min), dehydrogenase (1.71±0.0313.43±0.46 units/ml/min), and chitinase (1.01±0.02 units/ml/min).

Table 1. Physicochemical properties and enzyme activities of vermicast tea

Physicochemical parameters	Result, as mean ± S.E.
pH value	6.92 ± 0.02
Conductivity (ms/cm)	1566.67 ± 1.53
TDS (mg/l)	1.14 ± 0.02
Calcium (mg/l)	3.55 ± 0.05
Potassium (mg/l)	5.21 ± 0.07
Sodium (mg/l)	0.69 ± 0.03
Boron (mg/l)	0.25 ± 0.02
Molybdenum (mg/l)	0.12 ± 0.01
Magnesium (mg/l)	0.06 ± 0.02
Iron (mg/l)	1.04 ± 0.03
Copper (mg/l)	0.08 ± 0.01
Zinc (mg/l)	1.16 ± 0.02

Enzymes activities (units/ml/min)	
Protease	20.47 ± 6.44
Invertase	13.43 ± 0.46
Urease	9.69 ± 0.04
Lipase	2.50 ± 0.04
Amylase	17.85 ± 0.35
Dehydrogenase	1.71 ± 0.03
Chitinase	1.01 ± 0.02

Insect pest repellency effects of vermicast tea on seedlings

Insect pest repellency effects of vermicast tea was determined by the percentage of leaves damaged by insects. The lower the number of damaged leaves, the more the insect pest repellency effects of vermicast tea. No leaf damage was recorded in the first two weeks of the experiment. Leaf damage appeared from the third week, and increased through the end of the experiment, the fifth week (Table 2). Groundnut and bean seedlings treated with vermicast tea recorded significantly lower leaf damage ($p < 0.01$), relative to the control seedlings treated with urea and distilled water. Percentage leaf damage was lowest in seedlings treated with 100% vermicast tea, followed by seedlings treated with 50% and 20% vermicast tea, respectively. However, leaf damage was generally low in maize seedlings, and the differences in percentage leaf damage among treated and untreated were not statistically significant ($p > 0.05$). Representative photograph of seedlings with damaged leaves is shown in Figure 2.

Table 2. Percentage leaf damage in seedlings treated with vermicast tea, urea and water

Treatment	Percentage (%) leaf damage#		
	Week 3	Week 4	Week 5
Phaseolus vulgaris			
Distilled water	47.88 ± 10.11 a	54.92 ± 7.29 b	58.58 ± 5.33 a
Urea	44.81 ± 11.30 a	44.91 ± 24.29 ab	56.67 ±11.23 a
20% vermicast tea	39.39 ± 7.04 a	44.15 ± 8.26 ab	47.49 ± 6.22 b
50% vermicast tea	24.69 ± 9.18 b	33.03 ± 12.03 a	37.91 ± 9.92 c
100% vermicast tea	20.84 ± 8.84 b	30.11 ± 13.90 a	28.39 ± 8.47 d
F	14.94**	4.29**	20.14**
Zea mays			
Distilled water	2.78 ± 8.33 a	25.55 ± 23.09 a	42.00 ± 17.48 a
Urea	3.70 ± 11.11 a	20.37 ± 25.19 a	43.33 ± 18.05 a
20% vermicast tea	3.70 ± 11.11 a	11.85 ± 19.73 a	32.68 ± 14.14 a
50% vermicast tea	0.00 ± 0.00	8.70 ± 13.48 a	29.62 ± 18.57 a
100% vermicast tea	0.00 ± 0.00	5.56 ± 11.02 a	24.94 ± 14.08 a
F	0.51 ns	1.68 ns	2.07 ns
Arachis hypogaea			
Distilled water	8.11 ± 7.47 a	18.27 ± 9.26 a	29.09 ± 18.99 a
Urea	8.15 ± 5.53 a	15.55 ± 8.42 a	19.06 ± 14.10 ab
20% vermicast tea	4.86 ± 4.02 ab	6.25 ± 5.71 b	14.84 ± 9.16 bc
50% vermicast tea	3.80 ± 4.29 bc	6.46 ± 1.01 b	10.89 ± 4.83 bc
100% vermicast tea	0.93 ± 1.84 c	4.16 ± 3.93 b	5.84 ± 3.79 c
F	3.39**	8.75**	5.41**

#The lower the percentage leaf damage, the higher the insect pest repellency effect of vermicast tea. Values with different letters are significantly different (ANOVA; Duncan multiple range test, **$p < 0.01$, *$p < 0.05$); ns = no significant difference.

Figure 2. Cross section of experimental seedlings zoomed in to show bean seedling leaves damaged by pests

Physical growth in seedlings treated with vermicast tea, water, and urea

Seedlings treated with vermicast tea showed increased performance in all the parameters used to assess growth (Table 3). The numbers of leaves in seedlings treated with vermicast tea were significantly higher ($p < 0.01$) in bean and groundnut seedlings, relative to those treated with urea and water. In bean seedlings, the number of leaves was highest in 50% vermicast tea treatment. In groundnut seedlings, the number of leaves was highest in 50% and 100% vermicast tea treatments. However, the differences in height and leaf length of seedlings with vermicast tea, urea, and water were not statistically significant ($p > 0.05$).

Table 3. Physical growth of seedlings treated with vermicast tea, urea, and water

Treatment	Height (cm)	No of leaves week	Length of leaves (cm)
Phaseolus vulgaris (Bean)			
20% vermicast tea	14.78 ± 4.12 a	18.78 ± 3.53 b	9.33 ± 0.50 bc
50% vermicast tea	15.00 ± 3.87 a	26.11 ± 8.33 c	10.33 ± 2.50 ab
100% vermicast tea	16.89 ± 4.83 a	19.89 ± 2.47 b	10.67 ± 2.18 a
Distilled water	14.44 ± 4.72 a	13.00 ± 3.35 a	9.67 ± 0.50 ab
Urea	14.39 ± 5.36 a	22.22 ± 8.70 bc	9.00 ± 0.87 c
F	0.45 ns	5.97**	1.76 ns
Zea mays (maize)			
20% vermicast tea	14.78 ± 4.12 a	5.22 ± 1.64 a	26.33 ± 5.52 a
50% vermicast tea	15.00 ± 3.87 a	5.78 ± 1.20 a	28.67 ± 5.24 a
100% vermicast tea	18.00 ± 6.87 a	5.56 ± 1.01 a	32.89 ± 6.95 a
Distilled water	14.44 ± 4.72 a	5.11 ± 1.45 a	26.33 ± 622 a
Urea	14.39 ± 5.36 a	5.11 ± 1.36 a	26.22 ± 7.61 a
F	0.8 ns	0.43 ns	1.83 ns
Arachis hypogaea (Groundnut)			
20% vermicast tea	15.67 ± 5.52 a	61.56 ± 9.89 ab	2.22 ± 0.57 a
50% vermicast tea	17.67 ± 6.20 a	74.67 ± 15.49 ab	2.44 ± 0.68 a
100% vermicast tea	18.56 ± 6.37 a	74.67 ± 9.22 b	2.89 ± 0.74 a
Distilled water	15.56 ± 6.21 a	53.56 ± 18.13 a	2.21 ± 0.73 a
Urea	14.22 ± 5.52 a	58.89 ± 15.14 a	2.33 ± 0.83 a
F	0.77 ns	4.21**	1.37 ns

Values with different letters are significantly different ($p < 0.01$)

Biochemical growth in seedlings treated with vermicast tea, urea, and water

The differences among the biochemical growth parameters of seedlings treated with vermicast tea, urea, and water were not significant ($p > 0.05$) except in groundnut seedlings where chlorophyll a was significantly higher in vermicast tea treatment, relative to urea treatment (Table 4). Representative photographs of seedling growth at second and fifth weeks of the experiment are shown in Figures 3 and 4.

Table 4. Biochemical growth of seedlings treated with vermicast tea, urea, and water

Treatment	Biochemical growth parameters (mg/g)					
	Protein	Total starch	Total sugar	Chlorophyll a	Chlorophyll b	Total chlorophyll
Arachis hypogaea (Groundnut)						
20% vermicast tea	26.25 ± 0.99 a	40.09 ± 3.37 a	67.44 ± 5.07 a	30.85 ± 2.99 a	16.81 ± 0.79 ab	47.57 ± 2.50 a
50% vermicast tea	24.42 ± 3.33 a	37.09 ± 7.58 a	56.71 ± 18.34 a	28.36 ± 7.35 a	16.13 ± 0.48 ab	44.49 ± 7.25 a
100% vermicast tea	25.29 ± 3.56 a	37.01 ± 4.82 a	60.68 ± 20.65 a	29.01 ± 8.53 a	16.86 ± 0.62 ab	45.99 ± 8.58 a
Urea	24.74 ± 2.83 a	35.05 ± 8.03 a	56.08 ± 16.00 a	26.01 ± 7.37 a	16.44 ± 1.78 a	42.00 ± 7.46 a
Distilled water	25.91 ± 1.03 a	40.12 ± 2.20 a	64.71 ± 5.11 a	28.47 ± 3.48 a	17.92 ± 2.70 b	50.06 ± 11.55 a
F	0.79 ns	1.04 ns	0.96 ns	0.67 ns	1.76 ns	1.30 ns
Zea mays (Maize)						
20% vermicast tea	22.55 ± 2.00 a	53.03 ± 4.28 a	92.95 ± 5.28 b	31.28 ± 2.99 a	18.15 ± 2.58 a	49.43 ± 2.22 a
50% vermicast tea	22.29 ± 2.50 a	48.77 ± 9.94 a	76.5 ± 23.84 a	29.22 ± 6.07 a	17.99 ± 0.71 a	47.22 ± 6.53 a
100% vermicast tea	22.65 ± 2.63 a	49.49 ± 10.20 a	76.00 ± 21.12 a	29.73 ± 7.38 a	17.76 ± 1.62 a	47.57 ± 6.85 a
Urea	21.43 ± 1.97 a	47.40 ± 8.58 a	77.30 ± 22.66 a	27.06 ± 5.30 a	19.02 ± 2.46 a	46.07 ± 4.28 a
Distilled water	22.26 ± 1.72 a	53.15 ± 4.12 a	95.40 ± 0.79 b	29.29 ± 1.83 a	18.75 ± 2.04 a	48.04 ± 0.37 a
F	0.44 ns	0.97 ns	2.70 ns	0.78 ns	0.63 ns	0.60 ns
Phaseolus vulgaris (Bean)						
20% vermicast tea	26.68 ± 1.16 a	43.51 ± 8.94 a	75.27 ± 4.63 a	31.87 ± 3.97 a	15.16 ± 1.07 a	47.01 ± 3.28 a
50% vermicast tea	25.54 ± 3.31 a	44.11 ± 9.27 a	67.87 ± 19.06 a	29.63 ± 7.98 a	16.09 ± 1.28 a	45.72 ± 7.03 a
100% vermicast tea	26.01 ± 3.68 a	44.25 ± 9.65 a	69.92 ± 19.94 a	30.26 ± 9.03 a	15.55 ± 0.95 a	45.82 ± 8.74 a
Urea	25.01 ± 2.78 a	43.05 ± 8.84 a	65.76 ± 18.86 a	27.56 ± 6.38 a	17.03 ± 2.41 a	44.59 ± 5.77 a
Distilled water	26.69 ± 1.23 a	47.31 ± 4.90 a	77.38 ± 5.71 a	31.74 ± 2.36 a	15.71 ± 2.83 a	47.43 ± 1.67 a
F	0.68 ns	0.35 ns	0.93 ns	0.68 ns	1.30 ns	0.3 ns

Chl = Chlorophyll; ns = not significant. Values with different letters are significantly different ($p < 0.05$)

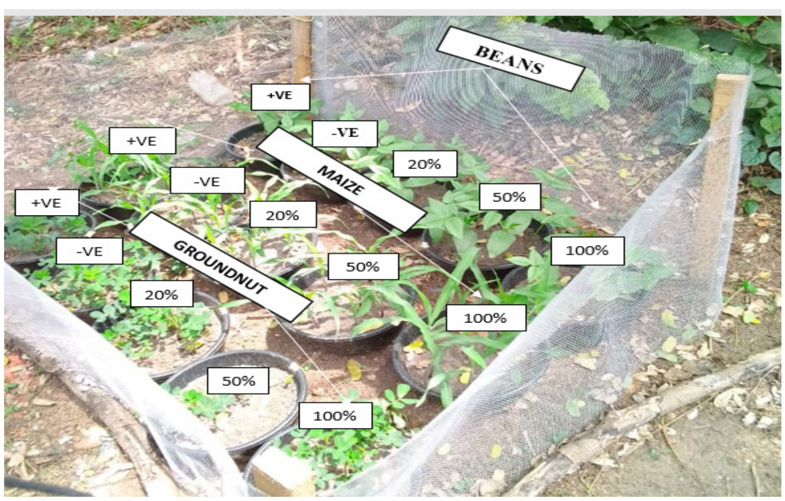

Figure 3. Representative photograph of seedling growth at week two of the experiment
+VE = urea treatment (positive control); -VE = distilled water treatment (negative control); 20% = 20% vermicast tea treatment; 50% = 50% vermicast tea treatment; 100% = 100% vermicast tea treatment.

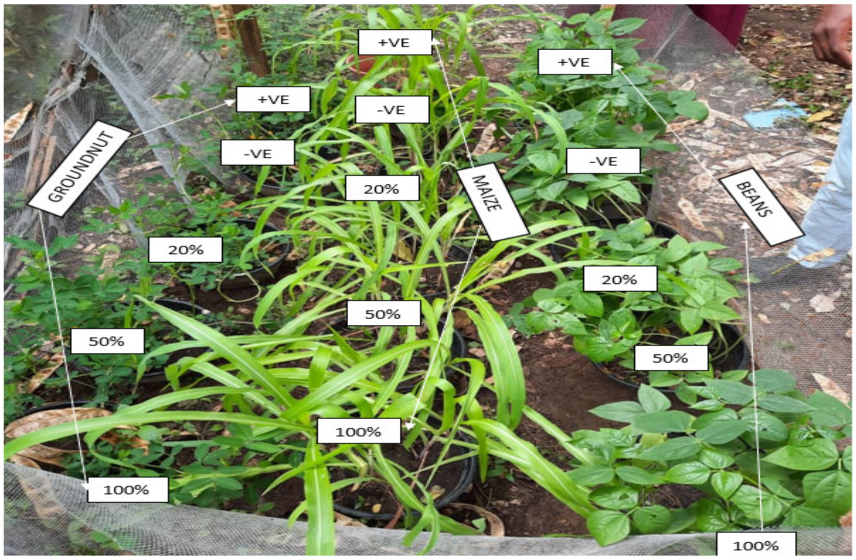

Figure 4. Representative photograph of seedling growth at week five of the experiment
+VE = urea treatment (positive control); -VE = distilled water treatment (negative control); 20% = 20% vermicast tea treatment; 50% = 50% vermicast tea treatment; 100% = 100% vermicast tea treatment.

Discussion

The vermicast tea assessed in this study significantly protected crop seedlings against insect pest attack. The effect of the vermicast tea on the physical growth of seedlings was positive, but marginal. The pest repellency potential displayed by the vermicast tea is in agreement with some other studies which worked with temperate earthworm-derived vermicast tea (Renčo and Kováčik, 2015; Singh and Chauhan, 2015). The dual action of insect pest repellency and growth boosting potential exhibited by the vermicast tea also readily agrees with the findings of Arancon *et al*. (2005) who observed a similar twofold action in vermicompost obtained from *E. fetida*, a temperate earthworm.

The insect pest repellency effect of the vermicast tea apparently stemmed from the enzymes and other compounds contained in it. Among other enzymes contained in the vermicast tea, chitinases and proteases occurred in substantial concentrations. These enzymes have been documented to have the ability to repel and destroy insect pests (Harrison and Bonning, 2010; Kim *et al*., 2016; Yasir *et al*., 2009). Chitinases and proteases are hydrolytic enzymes that catalyse, respectively, the digestion of chitin and protein, which are major components of the exoskeleton of insects (Edwards and Burrows, 1988; Jadhav and Sayyed, 2016).

Chitinases are naturally present in soil and plants, and when plants grow out of the soil, some amount of chitinases are present in their tissues, including the leaves. However, with time and increasing shoot mass, the concentrations of chitinases become too low, and its pest repellence mechanism is overwhelmed (Kumar *et al*., 2018), making the plant prone to insect pest attack. The spraying of vermicast tea, therefore, served as chitinase booster, resulting in the observed resistance of treated seedlings to insect pest attack. The absence of pest attack on the treated and untreated seedlings recorded in the first two weeks of the experiment is an indication that the plant's self-defence mechanism was stronger in the early days of growth.

Proteases are a large group of enzymes primarily associated with digestion, but have also been shown to contribute to plants' resistance and repellence against pests and pathogens (Harrison and Bonning, 2010). The activities of proteases and chitinases in the vermicast tea must have, therefore, complemented each other in providing resistance and repellence against insect pests attack by the test seedlings. Since the actual pest repelling protease(s) were not assayed in this current work, we encourage future studies to identify and isolate the proteases that confer pest resistance on seedlings.

Of the three seedlings used in this study, maize is a monocot while the remaining two are dicots. The observed insignificant leaf damage in maize seedling could be due to the extra insect pest-resistance believed to be associated with monocots, because of the toughness and folding of their leaves (Grubb *et al*., 2008). Other reasons could be that the seedlings activated higher Induced Resistance (IR) to pest attack (Balmer *et al*., 2013), or the maize variety used in the study had been genetically edited to resist insect pest attack.

The findings of this study are potentially beneficial to agriculture, environment, and health. The vermicast type tested in this study has exhibited insect pest repellent effects on crop seedlings, and the casts are known to be produced by two earthworm species, *Hyperiodrilus africanus* and *Ephyriodrilus afroccidentsalis* (Aladesida *et al*., 2014). For practical field application of the vermicast tea, there will be no need to harvest castings from the field as done in this study, as doing so will be environmentally unsustainable and counter-productive to soil nutrient stability. Instead, efforts should be geared towards mass-breeding or culturing these species. Earthworms harvested from the cultured stock can subsequently be engaged in vermicomposting, using appropriate organic food substrate. Vermicompost and vermitea harvested from such composting media can then be applied as biopesticide and biofertilizer in farms, to boost food production.

Conclusions

This study evaluated the insect pest repellency and growth-promoting potential of tropical earthworm-derived vermicast tea on some crop seedlings. The vermicast tea exhibited significant insect pest repellency on the seedlings. The effect of the vermicast tea on the physical growth of seedlings was positive, but marginal. For practical field application, the earthworm species from which the tea was derived should be engaged in vermicomposting, using appropriate organic food substrate. Vermicompost and vermitea harvested from such composting media can then be applied as biopesticide and biofertilizer in farms, to boost food production. Although, tropical earthworms are under-researched, and enjoy less publicity, the findings of this study is an indication that they also have the potential to perform many important roles currently ascribed to some of their temperate counterparts. There is therefore the need to encourage more research on tropical earthworms, in order to identify the species that are potentially useful in agriculture and other applications.

Authors' Contributions

EOD: Conceptualization, experimental design, field experiment, data curation, manuscript writing, review and editing. SON and SMY: Field experiment, data collection. YOB: Field experiment, data curation, manuscript writing. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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