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Earthworm Urine Used as Foliar Spray on *Amaranthus hybridus* (Green amaranth) Boosts Growth and Stimulates Stomata Opening

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ABSTRACT: The advent of spray drones for agrochemical applications calls for continuous exploration of potentially affordable and sustainable liquid biofertilizers. Thus, we evaluated the effects of earthworm urine foliar spray on the growth performance (height, number of leaves, leaf area, stem girth) and stomata opening of *Amaranthus hybridus* (Green amaranth). Earthworm urine was obtained by placing 500 g of *Libyodrilus violaceus* in one litre of earthworm saline, for one hour. *Amaranthus hybridus* seeds were planted into 90 pots, divided into three treatment groups of 30 pots each. Upon germination, the first group was sprayed with earthworm urine, the second and third groups with saline water and ordinary water, respectively. Earthworm urine significantly ($p < 0.05$) increased all the measured growth parameters, and stimulated the opening of stomata, relative to saline water and ordinary water. This result is a strong indication that earthworm urine contains hormones that boost plant growth, and is potentially suitable for use as a liquid biofertilizer, for increased production of *Amaranthus hybridus* and related vegetables.

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Earthworms have long been established to play significant roles in soil nutrient cycling and plant fertilization. They constitute a substantial proportion of soil macrofauna biomass in most soil ecosystems (Bhadauria and Saxena, 2010). Their burrowing activities create air spaces within the soil, thereby improving aeration of soil, microbes, and plants roots. They are important in maintaining soil structure regulation and organic matter dynamics (Lemtiri *et al.*, 2014). Earthworms facilitate the decomposition of organic matter through their physiological activities and the actions of numerous microorganisms that inhabit their gut (Medina-Sauza *et al.*, 2019). Earthworm are regarded as ammonotelic and ureotelic, secreting nitrogenous compounds like urea, uric acid, ammonia and hormones, which aid the rapid growth of plants (Schmidt and Curry, 1999; Hidalgo *et al.*, 2002). Mucus secreted by *Eisenia fetida* has been shown to effectively stimulate maize growth by increasing stem height and diameter (Zhang *et al.*, 2021). A fluid obtained from a tropical wetland earthworm, referred to as vermifluid, has also been

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shown to induce mitotic index in onion roots, even at low concentrations (Dada *et al*., 2021). *Amaranthus hybridus* is a vegetable commonly found in the Tropic. Its leaves and seeds are highly valued as a source of nutrition, rich in carbohydrates, protein, vitamins, minerals and dietary fibre (Nwaogu *et al.*, 2006; Akubugwo *et al.*, 2007). Across Africa, the leaves of the vegetable are included in the preparation of indigenous delicacies, soup and salad, with beneficial effects in reducing cholesterol levels in the blood, due to its constituent therapeutic phytochemicals (Okunlola *et al.*, 2017). The seeds of *Amaranthus hybridus* have been demonstrated as suitable for making a local beverage or drink that can compete favourably with other Nigerian native drinks (Isaac-Bamgboye *et al.*, 2019). The nutritional significance of *Amaranthus hybridus* as a staple leafy vegetable, and its recognised therapeutic properties, make it potentially suitable for commercial production. The adverse effects associated with the conventional chemical fertilizers, and the extra sensitivity of vegetables to environmental contaminants (Vwioko *et al.*, 2018), make the use organic fertilizers or biofertlizers a preferred option for commercial production of *Amaranthus hybridus*. Organic fertilization is widely encouraged due to its eco-suitable nature and ability to recycle organic matter. However, a major drawback is that most organic fertilizers are applied to the soil and are susceptible to erosion and leaching. In addition, short tenure plants like vegetables do not often require long time presence of organic manure, which are often locked in the soil material. Hence, the preference for foliar bioferlilizer over soil fertilization for vegetable crops. This study therefore aimed to evaluate the effects earthworm urine as a foliar spray on the growth performances such as height, number of leaves, leaf area, stem girth and stomata opening of *Amaranthus hybridus* (Green amaranth).

MATERIAL AND METHODS

Earthworm Collection: The earthworms (*Libyodrilus violaceus*) used in this study were collected from the main campus of Olabisi Onabanjo University, Ago-Iwoye, and Oru Town, both in Ogun State, Nigeria. Earthworm collection was by digging and hand sorting. The Earthworms were carefully transported to the laboratory, minimizing their exposure to light, especially sunlight.

Preparation of earthworm urine: Earthworm urine was obtained by briefly soaking the worms in water to wash adhering soil and other particles off their body. Then, five hundred grams (500 g) of live earthworms were carefully weighed and placed in one litre (1L) of 0.6% Sodium Chloride (NaCl) (6 g NaCl dissolved in 1 L water) for 1 hour with slight agitation. The extract was filtered through a Whatsman filter paper into sterilized beakers. The extract was labelled as earthworm urine, and stored under cool conditions (<10^{o}C).

Experimental set-up: Previously harvested stock of dry *Amaranthus hybridus* seeds were obtained from a local farmer. *Amaranthus hybridus* seeds were planted into 90 pots filled with garden soil. The pots were divided in a Randomized Complete Block Design (RCBD), into three treatment groups of 30 pots each. Upon seed germination, the vegetable seedlings in each pot were thinned out, leaving five seedlings per pot. The first treatment group was sprayed with earthworm urine, the second and third treatment groups with saline water and ordinary water, respectively. The soil in each pot was kept moist by wetting with water daily, throughout the experimental period. Each treatment fluid (earthworm urine, saline water, or ordinary water/water) was administered with a handheld spray bottle, targeting the leaves and stems of vegetables directly.

Measurement of growth and stomatal opening: The parameters used to assess vegetable growth were shoot height, numbers of leaves, leaf area, stem girth, and stomata diameter. The parameters were assessed at the end of the experimental period. Shoot height, leaf width, leaf widest width and leaf length were measured using a meter rule. Leaf area was estimated using the formula:

$$A = \frac{3}{4} \times W \times L \qquad 1$$

Where A = leaf area; W= leaf widest width; L = leaf length

To determine the diameter and degree of stomata opening, the leaves of *Amaranthus hybridus* were painted with nail varnish to obtain the print of stomata. The nail varnish was applied to the abaxial surface of the leaves and left to for five minutes, to dry. The nail varnish was carefully peeled off the leave surface. The stomata print was set up on a glass slide and viewed under a microscope that consisted of a graticule eye piece (Medline Scientific™ CETI - MAX BINO). The stomata prints were carefully observed at 400x magnification and stomatal aperture size measured in µm (Wu and Zhao, 2017; Abba, *et al.,* 2018; Millstead *et al.,* 2020).

Statistical analysis: The data generated from the measurement of growth parameters were subjected to descriptive analysis by means of the one way Analysis of Variance (ANOVA). Mean values were compared for significance by Duncan post hoc test. All statistical analyses were done using IBM SPSS (version 25). Graphs were prepared with Microsoft Office Excel software, 2016 edition.

RESULTS AND DISCUSSION

Shoot growth of Amaranthus hybridus sprayed with earthworm urine, saline water, and distilled water: Earthworm urine significantly ($p < 0.05$) improved the shoot growth of *Amaranthus hybridus* relative to other treatment liquid. The mean shoot height of earthworm urine-treated *Amaranthus hybridus* was 31.76 ± 9.53 cm, while the mean shoot heights of saline water and distilled water-treated *Amaranthus hybridus* were 28.83 ± 7.36cm and 26.65 ± 6.38cm, respectively (Fig 1).

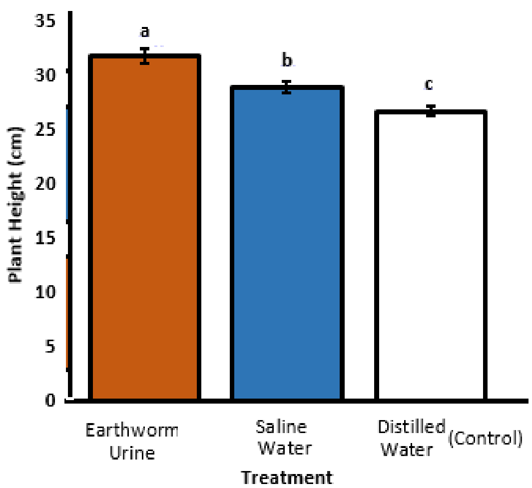

Fig 1: Shoot growth of *Amaranthus hybridus* sprayed with earthworm urine, saline water, and distilled water.

Number of leaves of Amaranthus hybridus sprayed with earthworm urine, saline water, and distilled water: Earthworm urine-treated *Amaranthus hybridus* had a mean number of leaves of 11.74 ± 2.33. This was significantly higher ($p < 0.05$) than the mean leave numbers of 11.15 ± 1.78 and 11.02 ± 2.00 recorded by saline water and distilled water-treated *Amaranthus hybridus*, respectively (Fig 2).

Leaf area of Amaranthus hybridus sprayed with earthworm urine, saline water, and distilled water: Earthworm urine-treated *Amaranthus hybridus* had the widest mean leaf area of 17.12 ± 9.32 cm^2. This was followed by saline water and distilled water-treated *Amaranthus hybridus* with respective leave areas of 15.90 ± 7.28 cm^2 and 14.29 ± 7.77 cm^2 (Fig 3).

Stem girth of Amaranthus hybridus sprayed with earthworm urine, saline water, and distilled water: The stem girth (stem circumference) of earthworm urine-treated *Amaranthus hybridus* was 3.11 ± 0.76 cm. The stem girth of saline water and distilled water-treated *Amaranthus hybridus* were 1.55 ± 0.34 cm and 1.26 ± 0.09 cm, respectively (Figure 4).

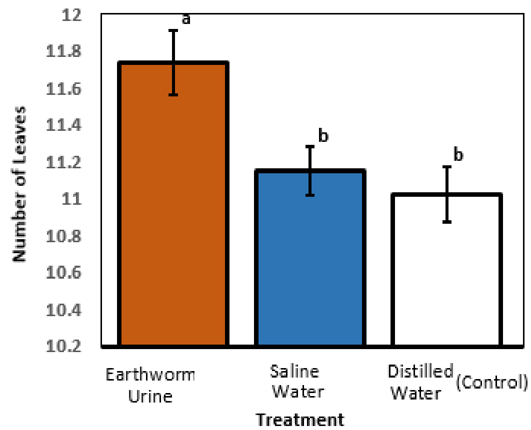

Fig 2: Leaf number of *Amaranthus hybridus* sprayed with earthworm urine, saline water, and distilled water

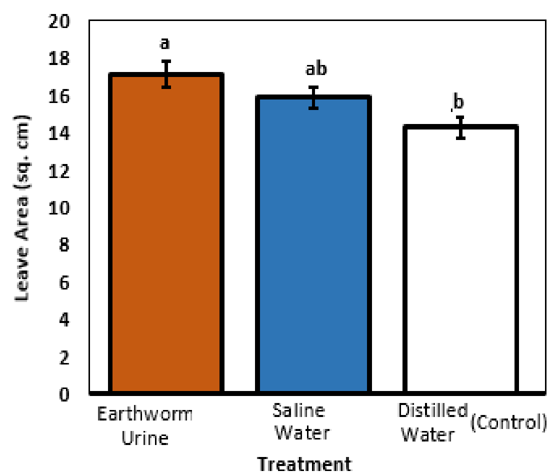

Fig 3: Leaf area of *Amaranthus hybridus* sprayed with earthworm urine, saline water, and distilled water

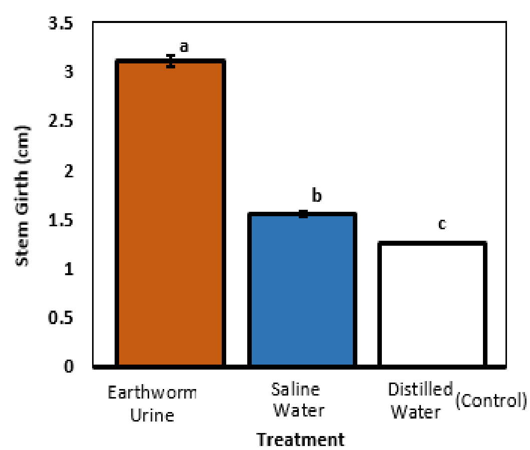

Fig 4: Stem girth of *Amaranthus hybridus* sprayed with earthworm urine, saline water, and distilled water

Leaf stomatal diameter of Amaranthus hybridus sprayed with earthworm urine, saline water, and distilled water: The stomatal diameter (stomatal opening) of *Amaranthus hybridus* treated with earthworm urine, saline water and distilled water were 6.5 ± 1.9 µm, 5.9 ± 1.6 µm and 5.4 ± 1.2 µm, respectively (Fig 5).

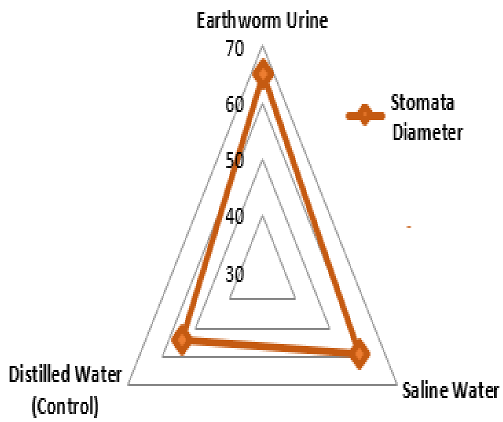

Fig 5: Leaf stomatal diameter (µm) of *Amaranthus hybridus* sprayed with earthworm urine, saline water, and distilled water

The fact that earthworm urine improved all the measured growth parameters, relative to the controls (saline water and distilled water) affirms the efficacy of earthworms and earthworm-derived products to improve plant growth. Earthworm products that have been documented to positively impact plant growth include vermicompost (Dlamini *et al.* 2020), vermicompost leachate (Ngoroyemoto *et al*., 2019), vermicompost tea (Bako *et al.*, 2021). However, in this study, earthworm urine was applied by foliar spray, and not through the soil. Foliar application of biofertilizers has a potential advantage of efficiency and faster action, relative to soil application that is not target-specific and requires longer time to get assimilated by plants. Vegetables, especially leafy, like *Amaranthus hybridus*, are known to have a short life cycle; therefore, shoot application of fertilizers, as done in this study, will affect their growth better and faster than soil-applied fertilizers. Since *Amaranthus hybridus* has a history of high demand (Ogwu, 2020), earthworm urine presents an affordable and efficient biofertilizer tool to increase its production. For commercial application of earthworm urine, we recommend mass breeding of earthworms through laboratory vermiculture. Earthworms can then be harvested from the vermiculture stock, and used to process earthworm urine in large volumes.

The increased number of leaves recorded in earthworm urine-treated *Amaranthus hybridus* is an indication that the earthworm urine contained compounds that promote budding, as observed by Bako *et al*. (2021) in their study. Similarly, the increased stomata diameters recorded in earthworm urine-treated *Amaranthus hybridus* suggests that earthworm urine has the potential to stimulate photosynthetic metabolic activities in the plants, thereby increasing stomatal conductance (Roy and Mathur 2021). Given the positive result of this study, earthworm urine harvested from *Libyodrilus violaceus*, or possibly, other earthworm species, could be developed into a foliage organic biofertilizer, for application on vegetable farms, using appropriate devices like drone spray. Such earthworm-derived organic foliar biofertilizer can be utilized in nursery farming. Earthworm urine provides a cheaper and more accessible form of fertilization in grassroots farming.

Conclusion: In this study, the effect earthworm urine on the growth of *Amaranthus hybridus* was assessed. The result established the capability of earthworm urine to effectively promote vegetable productivity through foliar application. However, there is the need for further studies to determine and characterise the growth-promoting compounds contained in earthworm urine.

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