Journal of Materials and Environmental Science
ISSN : 2028-2508
CODEN : JMESCN

J. Mater. Environ. Sci., 2021, Volume 12, Issue 12, Page 1516-1523

http://www.jmaterenvironsci.com

Vermifluid's Impact on Plant Growth is Evidently Concentration-Dependent

E. O. Dada[1]*, Y. O. Balogun[1], E. O. Oludipe[2], S. O. Owa[2], M. O. Akinola[1]

[1]*Department of Cell Biology and Genetics, Environmental Biology Unit, Faculty of Science, University of Lagos, Akoka, Yaba, Lagos, Nigeria.*

[2]*Department of Food Science and Microbiology, College of Pure and Applied Science, Landmark University, Omu-Aran, Kwara State, Nigeria.*

Received 28 Aug 2021, Revised 12 Dec 2021, Accepted 13 Dec 2021

Keywords
- ✓ *Alma millsoni,*
- ✓ *Chlorophyll,*
- ✓ *Phaseolus vulgaris,*
- ✓ *Phosphatase,*
- ✓ *Zea mays.*

eodada@unilag.edu.ng
Phone: +234-9075414242

Abstract

Vermifluid is an earthworm fluid exudate that positively influences plant growth and mitotic activities. However, more information on the concentrations at which they exert maximum impact is desirable. In a previous work, a tropical earthworm vermifluid was found to impact mitotic activities and root growth of *Allium cepa* better at low concentrations. In this present study, we evaluated the influence of the vermifluid on seed germination and seedling growth of *Zea mays* (maize) and *Phaseolus vulgaris* (bean) using the same dilutions as in the previous work. Seed germination and seedling growth were measured using physical and biochemical parameters. Maize and bean seeds had higher germination in vermifluid, relative to those germinated in water and urea. Maize and bean seedlings sprayed with vermifluid recorded significantly higher ($p<0.01$) leaf and shoot growth, relative to the seedlings sprayed with urea and water. Shoot growth generally increased with rising vermifluid concentrations. Total sugar, starch, protein, chlorophyll a and chlorophyll b were significantly higher ($p<0.05$) in seedlings sprayed with vermifluid. The linear relationship between shoot growth and vermifluid concentrations in this study, as against the inverse association between vermifluid concentrations and onion root growth in the previous work indicates that vermifluids contain plant growth compounds, which can either promote or moderate root or shoot growth, depending on their concentrations. We therefore emphasise the need for farmers to carry out preliminary baseline tests to determine the optimum vermifluid dilutions that will best impact growth, before field application.

1. Introduction

Earthworms are generally essential to sustainable agroecosystems. They are important terrestrial invertebrates, facilitating efficient soil nutrient cycling, drainage, enzyme and microbial activities [1-3]. For a long time, earthworms have been directly utilised in promoting sustainable crop and fish production. However, attention is shifting to the use of earthworm products as biofertilisers and livestock feed supplements. Among earthworm products and derivatives that are used in agriculture are vermicompost and vermifluid. Vermicompost, also referred to as earthworm casts or vermicast, are egested organic matter or organic matter-rich soil that has passed through the alimentary canal of earthworms. They are potentially rich in digestive and plant growth-promoting enzymes and microorganisms, deposited onto them in the course of passage through the alimentary canal of earthworms. Vermicompost is widely used in organic farming in place of chemical fertilizers, with good results [4].

Vermifluid is an earthworm fluid exudate that positively impacts plant growth and mitotic activities [5, 6]. The use of earthworm products or derivatives potentially confers a number of advantages. It reduces the potential threat of ecological imbalance that may arise from direct harvest of wild or field earthworms. In addition, the use of vermifluid or earthworm fluid as biofertilizer has the advantage of flexibility as it can be applied in the nursery and on the farm, in controlled dilutions or concentrations, to improve growth and germination.

Vermifluid contains plant nutrients and growth hormones that impact plant aerial growth in the natural ecosystem. When applied as a foliar spray on the farm, vermifluid increases the rate of photosynthesis in crop plants. Vermifluid is also reported to initiate flowering and long-lasting inflorescence [7, 8]. It can be used as a liquid fertilizer, applied to the rhizosphere to increase the density of microorganisms involved in decomposing soil organic matter [7-9]. Freshly collected vermifluid may be active against plant pathogens, thereby preventing infections [7].

Phaseolus vulgaris (bean or common bean) is a relatively affordable, staple food crop, rich in plant protein, essential vitamins and minerals, soluble fibre, starch and phytochemicals [10]. Of all the edible legumes, *P. vulgaris* has the widest geographical distribution [11, 12]. The common bean is a traditional food for many people in Africa, Asia and Latin America [12, 13]. *Zea mays* (maize or corn) is a globally cultivated annual cereal grain that has become one of Africa's dominant food crops [14]. Maize is used as a staple human food, as feed for livestock, and as a raw material for many industrial products like corn ethanol, corn starch, and corn syrup [15].

Although vermifluids have been found to positively influence plant growth, research emphasis has not been laid on the concentrations at which they exert maximum impact. In an earlier study [6], the effects of serial dilutions of *Alma millsoni* vermifluid on the mitotic index and root growth of *Allium cepa* were assessed. The results showed that vermifluid impacted mitotic activities and root growth better at low dilutions. In this present study, we aimed to evaluate the influence of graduated concentrations of *A. millsoni* vermifluid on seed germination and seedling growth of *Zea mays* (maize) and *Phaseolus vulgaris* (common bean) using serial dilutions as in the previous work.

2. Methodology

2.1 Collection of Earthworms and Preparation of Vermifluid

The adult *Alma millsoni* used for this study were collected at the main campus of the University of Lagos, Nigeria. Earthworms were collected by digging and hand sorting. Care was taken to minimise exposure of the earthworms to light, especially direct sunlight. Vermifluid preparation was as described in [6].

2.2 Seed Germination and Seedling Growth Experiment

Certified seeds of *Z. mays* and *P. vulgaris* were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The seeds were pre-treated with a 0.1% magnesium chloride solution to remove surface contaminations. The germination solutions prepared were vermifluid (10%, 20%, 50%, and 100%), urea (0.05%), and distilled water. The seed germination experiment was performed in sterilized petri plates containing absorbent cotton and blotting papers, wetted with germination solutions (vermifluid, urea, and distilled water). Twenty visibly uniform and healthy seeds were put into the wetted absorbent cottons. The petri plate for each experimental solution was replicated three times. The petri plates containing urea and distilled water served as controls. Seeds were allowed to germinate in a dark room condition, at an ambient room temperature of 29.1 ± 1.2˚C. After 72 hours of dark incubation, the percentage seed germination was calculated for each experimental petri plate:

$$\% \text{ Germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds in plate}} * 100 \quad (1)$$

2.3 Seedling Growth Experiment

Seven germinated seeds were carefully removed from each germination treatment media [vermifluid (10%, 20%, 50%, and 100%); urea (0.05%); distilled water], and transplanted in sieved garden soil contained in plastic containers/pots of 2-Litre capacity. Each seedling growth medium was replicated thrice. Each experimental container was irrigated weekly with distilled water. Germinated seedlings were allowed to grow under illuminated conditions until they reached a length of 7–10 cm. Afterwards, experimental solutions [vermifluid (10%, 20%, 50%, and 100%); urea (0.05%); distilled water] were applied as foliar spray for 15 days, at 3-day intervals, to the seedlings' leaf's surfaces, using a manual jet hand sprayer. After 15 days, three plants were randomly harvested from each experimental container for measurement of physical (shoot length and numbers of leaves) and biochemical (total sugar, starch, protein, chlorophyll a, chlorophyll b) growth parameters. Physical growth parameters were measured by a metre rule. Biochemical growth parameters were measured using standard procedures as described in [16].

2.4 Statistical Analysis of Data

The data generated from the study were subjected to descriptive analysis using the General Linear Model (GLM) of IBM SPSS (version 25). The differences among the means were separated using the Duncan Multiple Range Test (DMRT). Differences between means were considered significant at $p < 0.05$.

3. Results and Discussion

3.1 Germination of Bean and Maize Seeds in Vermifluid, Urea, and Water

Bean and maize seeds in vermifluid generally had higher germination, relative to those germinated in urea and water (Figure 1). Bean seeds in 100% vermifluid recorded the highest significant ($p < 0.05$) germination (75% germination), while maize seeds in 50% vermifluid had the highest significant ($p < 0.05$) germination (65% germination).

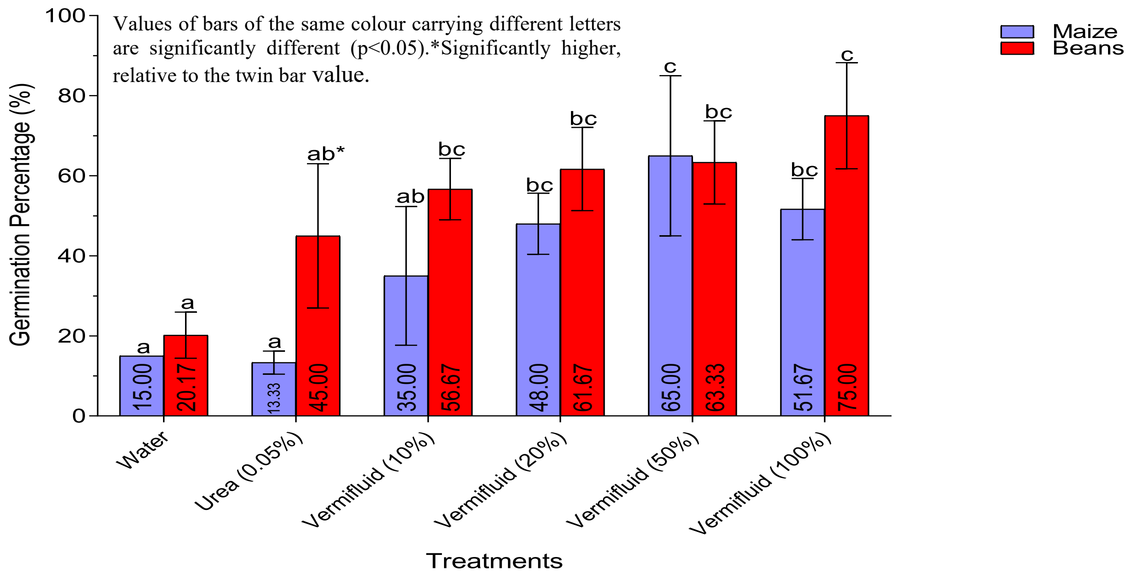

Figure 1. Germination of bean and maize seeds in vermifluid, urea, and water

3.2 Leaf Growth of Bean Seedlings Sprayed with Vermifluid, Urea, and Water

All the bean seedlings sprayed with different treatment fluids had a consistent increase in the number of leaves throughout the 24-day experimental period. However, bean seedlings sprayed with vermifluid showed significantly higher ($p < 0.05$) increases in leaf number, with seedling leaf number increasing with rising vermifluid concentrations. The highest increase in leaf number was recorded in seedlings sprayed with 100% vermifluid, on day-24 (last day) of the experimental period (Figure 2).

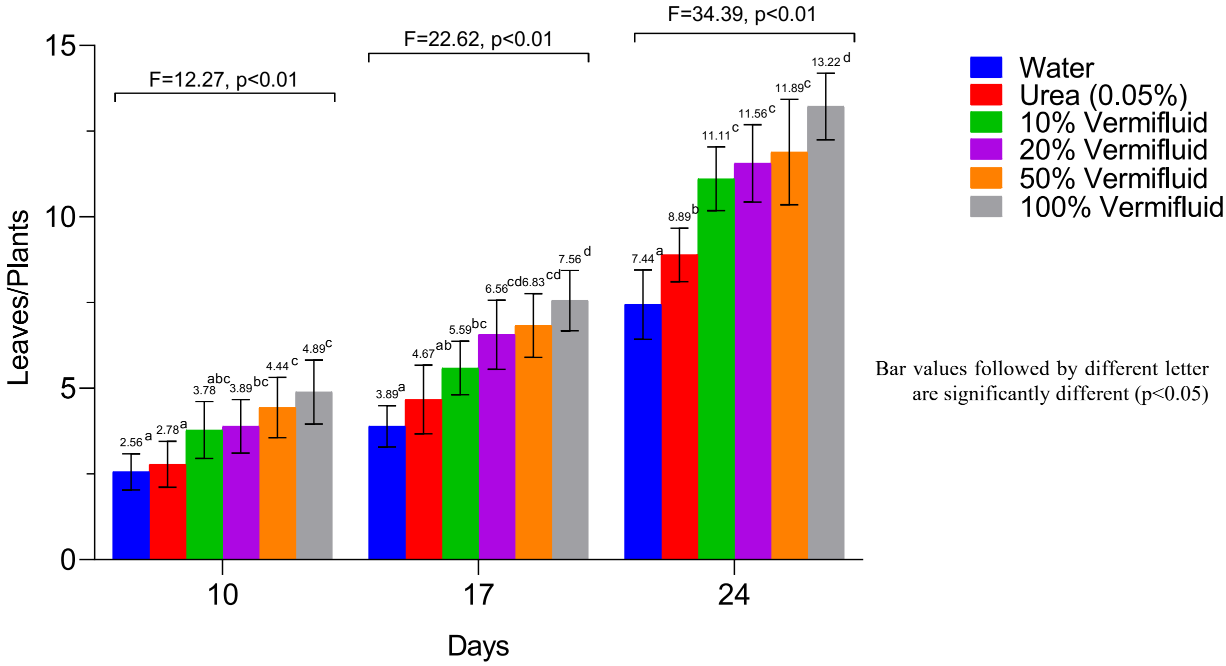

Figure 2. Leaf growth of bean seedlings sprayed with vermifluid, urea, and water

3.3 Leaf Growth of Maize Seedlings Sprayed with Vermifluid, Urea, and Water

Maize seedlings sprayed with vermifluid recorded a significantly higher ($p < 0.05$) increase in leaf number except on day-10 when maize seedlings sprayed with 0.05% urea outgrew those sprayed with vermifluid, in terms of leaf numbers (Figure 3).

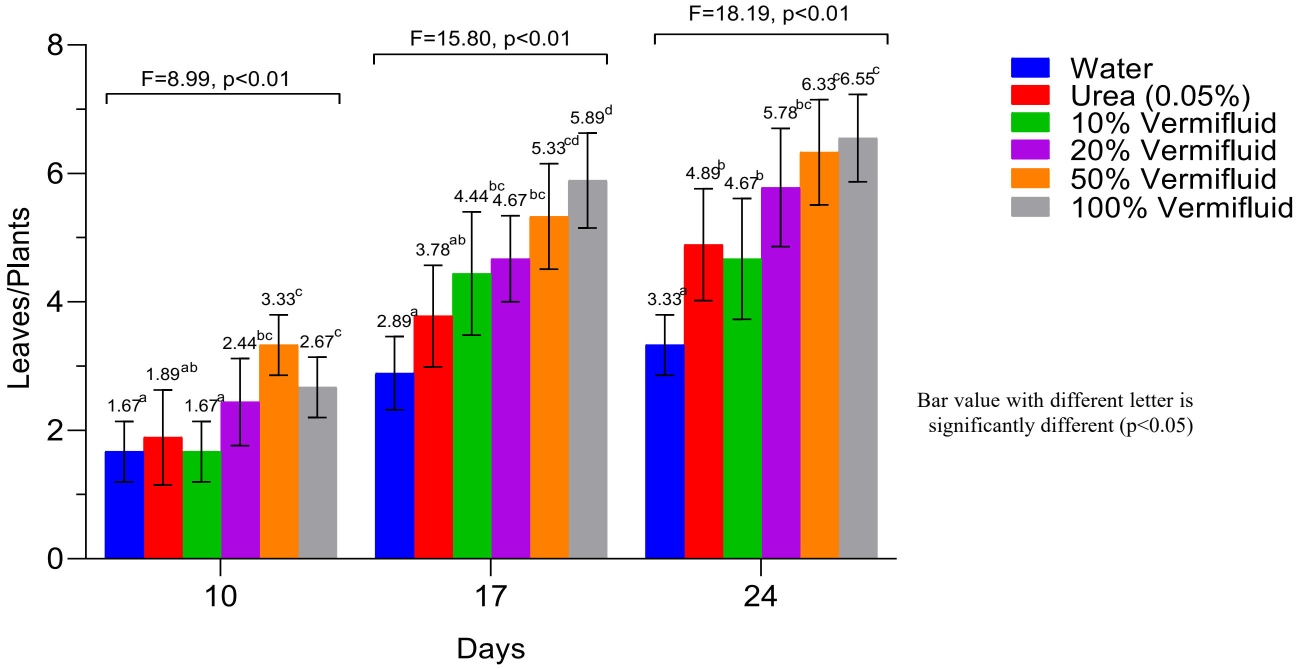

Figure 3. Leaf growth of maize seedlings sprayed with vermifluid, urea, and water

3.4 Shoot Growth in Bean and Maize Seedlings Sprayed with Vermifluid, Urea, and Water at the End of the Experiment

At the end of the experiment, maize and bean seedlings sprayed with vermifluid generally recorded significantly higher ($p < 0.05$) shoot growth, relative to those sprayed with urea and water. Maize and bean seedlings sprayed with 100% vermifluid recorded the highest shoot growth at the end of the experiment. The differences between shoot growth of maize seedlings and bean seedlings were not statistically significant ($p > 0.05$), except for seedlings sprayed with 0.05% urea and 50% vermifluid (Table 1).

Table 1. Shoot growth in bean and maize seedlings sprayed with vermifluid, urea, and water at the end of the experiment

Treatment	Maize (cm)	Beans (cm)	t-test	Sig.
Shoot				
Water	10.24±0.87a	9.09±1.30a	2.21	0.65
Urea (0.05%)	8.99±1.85a	11.89±1.88b	3.30	0.003*
Vermifluid (10%)	13.44±0.64b	12.26±1.89b	1.77	0.63
Vermifluid (20%)	16.17±0.98c	16.34±2.11c	0.22	0.99
Vermifluid (50%)	20.47±1.10d	17.39±2.44c	3.45	0.002*
Vermifluid (100%)	21.32±0.95d	21.19±3.01d	0.12	0.99
F	165.1**	37.30**		

Values followed by different letter are significantly different ($p<0.05$).

3.5 Biochemical Growth Parameters of Maize and Bean Seedlings

At the end of the experiment, bean and maize seedlings treated with vermifluid recorded higher biochemical growth parameters. Bean seedlings treated with 100% vermifluid contained the highest starch (121.39±0.36 mg/g), total sugar (133.51±0.20 mg/g), protein (3.14±0.09 mg/g), chlorophyll a (1.85±0.03 mg/g) and chlorophyll b (0.44±0.02 mg/g). Similarly, maize seedlings treated with 100% vermifluid contained the highest starch (127.98±0.26 mg/g), total sugar (143.53±0.18 mg/g), protein (3.17±0.71 mg/g), chlorophyll a (1.85±0.06 mg/g) and chlorophyll b (0.46±0.01 mg/g). The lowest biochemical parameters were recorded in seedlings treated with water (Table 2).

A number of previous studies have demonstrated the plant growth-promoting potential of vermifluids obtined from other earthworm species [17-20]. Therefore, the present study lends further credence to the plant growth-promoting ability of earthworm-derived fluids. Seeds may be pre-treated with vermifuids to boost germination rates. Vermifluid sprays may be applied to seedling shoots to boost physical and biochemical growth parameters.

However, in a previous study [6], like a number of others [21, 22], vermifluid was found to impact onion root growth better at lower concentrations, as against the linear relationship between vermifluid concentrations and shoot growth in this present study. These divergent effects of vermifluid are an indication that vermifluids contain plant growth compounds, which can either promote or moderate root or shoot growth, depending on their relative concentrations and other prevailing factors. The growth compounds in the vermifluid seem to behave similar to auxins and some mineral nutrients. Auxins are well-known plant growth regulators that may promote or inhibit growth in the shoot or root region, depending on their relative concentrations in that region. Similarly, it is on record that a restriction of mineral nutrients like N, P, S may favour root growth relative to shoot growth, while a limitation of K, Mg, Mn may favour shoot development relative to root growth [23]. Hence, further studies are needed to ascertain the plant growth regulators inherently present in vermifluid.

Table 2. Biochemical growth parameters of maize and bean seedlings

Samples	Biochemical growth parameters (mg/kg)				
	Total sugar	Starch	Protein	Chlorophyll a	Chlorophyll b
Maize seedling					
Water	76.52±0.11a	85.32±0.34a	1.30±0.15a	0.98±0.01a	0.12±0.01a
Urea (0.05%)	98.75±0.23b	108.32±2.23b	2.73±0.36bc	1.20±0.17b	0.28±0.00b
10% Vermifluid	98.90±0.31b	100.8±0.12c	2.20±0.33b	1.27±0.00b	0.28±0.02b
20% Vermifluid	106.80±0.24c	105.31±1.45b	2.40±0.28b	1.30±0.04b	0.34±0.03c
50% Vermifluid	127.41±0.10d	117.42±0.24d	2.52±0.22b	1.72±0.02c	0.43±0.03d
100% Vermifluid	143.53±0.18e	127.98±0.26e	3.17±0.71c	1.85±0.06c	0.46±0.01d
F	38570.00*	3622.00*	16.96*	56.93	112.9*
Bean seedling					
Water	65.13±0.21a	64.01±0.12a	1.72±0.14a	0.96±0.08a	0.10±0.02a
Urea (0.05%)	76.46±0.19b	85.82±1.40b	2.90±0.17d	1.26±0.06b	0.20±0.03b
10% Vermifluid	98.64±0.29c	100.83±0.25c	2.15±0.06b	1.29±0.02b	0.30±0.02c
20% Vermifluid	107.01±0.29d	104.26±0.43d	2.44±0.07c	1.33±0.03b	0.33±0.02c
50% Vermifluid	127.65±0.34e	116.54±0.23e	2.63±0.11c	1.64±0.05c	0.39±0.03d
100% Vermifluid	133.51±0.20f	121.39±0.36f	3.14±0.09e	1.85±0.03d	0.44±0.02e
F	33128.61*	3364.63*	62.79*	126.32*	104.90*

Values followed by different letter are significantly different ($p < 0.05$).

Based on the foregoing, when the procedure and results of the earlier study [6] are juxtaposed with these current ones, it is evident that the effects of vermifluid on plant growth are concentration-dependent. In the former work [6], onion roots were directly immersed in dilutions of vermifluid, which would have impacted high concentrations on the roots; hence, root growth was better in the lower vermifluid concentration. On the other hand, in this current work, vermifluid was applied to the shoot of test plants only in sprays (apparently low concentrations, when compared to direct immersion in vermifluid), and growth was impacted better at higher concentration applications. The seemingly contrasting results of [6] and this present study can therefore be explained in terms of the differences in concentrations at which vermifluid was made available to the test plants. The practical implication of this deduction is that farmers and horticulturists wishing to use vermifluids to improve plant performance and boost crop production should pay attention to optimum concentrations. We therefore emphasise the need for farmers to carry out preliminary baseline tests to determine the optimum vermifluid dilutions that will best impact growth, before field application.

Conclusion

In this study, we evaluated the influence of vermifluid on seed germination and seedling growth of *Z. mays* and *P. vulgaris*. Improved germination, physical and biochemical growth were recorded in seeds and seedlings treated with vermifluid. The linear relationship between shoot growth and vermifluid concentrations in this study, as against the inverse association between vermifluid concentrations and onion root growth in a previous study indicates, among other things, that vermifluid contains plant growth compounds whose effect is concentration-dependent. While this result reinforces the opportunity presented by vermifluids for use as crop growth boosters, we recommend that farmers carry out a preliminary baseline test to determine the optimum dilutions or concentrations that will best impact plant growth, before field application of vermifluid.

Conflict of Interest: The authors declare that there are no conflicts of interest.

References

[1] M. Blouin, M. E. Hodson, E. A. Delgado, G. Baker, L. Brussaard, K. R. Butt, J. Dai, L. Dendooven, G. Peres, J. E. Tondoh, D. Cluzeau and J. J. Brun, "A review of earthworm impact on soil function and ecosystem services," *European Journal of Soil Science*, 64(2) (2013) 161–182. https://doi.org/10.1111/ejss.12025

[2] I. M. Lubbers, K. J.van Groenigen, S. J. Fonte, J. Six, L. Brussaard and J. W. van Groenigen, "Greenhouse-gas emissions from soils increased by earthworms," *Nature Climate Change*, 3(3) (2013) 187–194. https://doi.org/10.1038/nclimate1692

[3] E.O. Dada, M. O. Akinola, S. O. Owa, G. A. Dedeke, A. A. Aladesida, F. O. Owagboriaye and E. O. Oludipe, "Efficacy of vermiremediation to remove contaminants from soil," *Journal of Health and Pollution*, 11(29) (2021) 210302. https://doi.org/10.5696/2156-9614-11.29.210302

[4] D. Maji, P. Misra, S. Singh, and A. Kalra, A., "Humic acid rich vermicompost promotes plant growth by improving microbial community structure of soil as well as root nodulation and mycorrhizal colonization in the roots of Pisum sativum," *Applied Soil Ecology*, 110 (2017) 97–108. https://doi.org/10.1016/j.apsoil.2016.10.008

[5] A. Chattopadhyay, "Effect of vermiwash of Eisenia foetida produced by different methods on seed germination of green mung, Vigna radiate," *International Journal of Recycling of Organic Waste in Agriculture*, 4(4) (2015) 233–237. https://doi.org/10.1007/s40093-015-0103-5

[6] E. O. Dada, T. Abdulganiy, S. O. Owa, Y. O. Balogun, E. O. Oludipe and M.O. Akinola, "Tropical wetland earthworm vermifluid promotes mitotic activities and root growth in Allium cepa at low concentrations," *Chiang Mai University Journal of Natural Sciences*, 20(3) (2021) e2021064. https://doi.org/10.12982/CMUJNS.2021.064

[7] S. K. Das, R. K. Avasthe and R. Gopi, "Vermiwash: use in organic agriculture for improved crop production," *Popular Kheti*, 2(4) (2014) 45-46. https://doi.org/10.1007/s40093-017-0168-4

[8] P. Kaur, M. Bhardwaj and I. Babbar, "Effect of vermicompost and vermifluid on growth of vegetables" *Research Journal of Animal Veterinary and Fishery Science*, 3(4) (2015) 9-12. http://www.isca.in/AVFS/Archive/v3/i4/3.ISCA-RJAVFS-2015-007.pdf

[9] P. Bhardwaj and R. K. Sharma, "Effect of Vermiwash and Vermicompost on the Growth and Productivity of Moong Dal," *Journal of Chemical, Biological and Physical Sciences*, 6(4) (2016) 1381-1388.

[10] H. Høgh-Jensen, F. M. Myaka, D. Kamalongo and A. Ngwira, "The Bean – Naturally Bridging Agriculture and Human Wellbeing" In: Muzzalupo, I (Ed.) *Food Industry*. IntechOpen, chapter 2, pp. 23-38, (2013). DOI: 10.5772/53164. https://www.intechopen.com/books/food-industry/the-bean-naturally-bridging-agriculture-and-human-wellbeing

[11] A. I. Adesoye and O. A. Ojobo, "Genetic diversity assessment of Phaseolus vulgaris L. landraces in Nigeria's mid-altitude agroecological zone," *International Journal of Biodiversity and Conservation*, 4(13) (2012) 453-460. https://doi.org/10.5897/IJBC11.216

[12] C. Nwadike, A. Okere, D. Nwosu, C. Okoye, T. Vange and B. Apuyor, "Proximate and nutrient composition of some common bean (Phaseolus vulgaris L.) and cowpea (Vigna

unguiculata L. Walp.) accessions of Jos- Plateau, Nigeria," *Journal of Agriculture and Ecology*, 15(1) (2018) 1-9. DOI: 10.9734/JAERI/2018/42138

[13] C. R. S. Camara, C. A. Urrea and V. Schlegel, "Pinto beans (Phaseolus vulgaris L.) as a functional food: implications on human health," *Agriculture*, 3(5) (2013). 90-111. https://doi.org/10.3390/agriculture3010090

[14] L. S. O. Liverpool-Tasie, B. T. Omonona, A. Sanou and W. O. Ogunleye, "Is increasing inorganic fertilizer use for maize production in SSA a profitable proposition? Evidence from Nigeria," Food Policy, 67: 41–51 (2017). https://doi.org/10.1016/j.foodpol.2016.09.011

[15] Y. A. Abayomi, A. Arijenja-George and I. A., Kolawole, "Comparative leaf growth and grain yield response of hybrid and open pollinated maize genotype to nitrogen fertilizer application," *Agrosearch*, 8(2006) 13-25. DOI: 10.4314/agrosh.v8i1.39435

[16] A. A. Abdel Latef and M. M. Sallam, "Changes in Growth and Some Biochemical Parameters of Maize Plants Irrigated with Sewage Water," *Austin Journal of Plant Biology*, 1(1) (2015) 1004. https://austinpublishinggroup.com/plant-biology/fulltext/ajpb-v1-id1004.php

[17] S. Suthar, R. Choyal, R. Singh and R. Sudesh, "Stimulatory effect of earthworm body fluid (vermiwash) on seed germination and seedling growth of two legumes," *Journal of Phytological Research*, 18(2) (2005) 219-222. https://pdfs.semanticscholar.org/a38a/9d86611e5373834c370a560a2f01fccbb952.pdf

[18] S. Suthar, "Evidence of plant hormone-like substances in vermiwash: An ecologically safe option of synthetic chemicals for sustainable farming," *Ecological Engineering*, 36 (2010) 1089 1092. https://doi.org/10.1016/j.ecoleng.2010.04.027

[19] M. Fathima and M. Sekar, "Studies on growth promoting effects of vermiwash on the germination of vegetable crops," *International Journal of Current Microbiology and Appllied Sciences*, 3(6) (2014) 564-570.

[20] C. Makkar, J. Singh and C. Parkash, "Vermicompost and vermiwash as supplement to improve seedling, plant growth and yield in Linum usitassimum L. for organic agriculture," *International Journal of Recycling of Organic Waste in Agriculture*, 6(3) (2017) 203–218.

[21] P. Pandurangan, U. Prasad, S. Sunkar, N. M. Saikrishna, A. Atul and A., Kumar, "Formulation of vermiwash and humic Acid and its application on Allium cepa," *Biosciences Biotechnology Research Asia*, 13(1) (2016) 523-529. http://dx.doi.org/10.13005/bbra/2064

[22] S. Senthilmurugan, G. Sattanathan, P. P. K. Vijayan and V. Tamizhazhagan, "Evaluation of different concentration of vermiwash on seed germination and biochemical response in Abelmoschus esculentus (L.)," *International Journal of Biology Research*, 3(1) (2018) 228-231. file:///C:/Users/user/Downloads/3-1-56-714.pdf

[23] T. Ericsson, "Growth and shoot: root ratio of seedlings in relation to nutrient availability," *Plant and Soil*, 168 (1995) 205–214. https://doi.org/10.1007/BF00029330

(2021) ; http://www.jmaterenvironsci.com