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Short Communication: The effects of vermicompost and vermiwash from the medicinal plants, neem (*Azadirachta indica*) and lime (*Citrus aurantifolia*), on the growth parameters of lettuce in a hydroponic system

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Abstract. *Jaikishun S, Hoosein A, Ansari AA. 2018. The effects of vermicompost and vermiwash from the medicinal plants, neem (*Azadirachta indica*) and lime (*Citrus aurantifolia*), on the growth parameters of lettuce in a hydroponic system. Nusantara Bioscience 10: 91-95.* The farming community is confronting critical issues on the usage of chemicals and rapidly decreasing arable lands from salinity, flooding and drought. This study was designed to compare the effectiveness of vermiwash produced from medicinal plants, as a potential substitute for chemicals, and standard hydroponic solution in supporting the growth of lettuce in a hydroponic system, a soilless medium. Vermicompost and vermiwash were produced from *Azadirachta indica*, and *Citrus aurantifolia* using *Eisenia fetida* and physiochemical analyses done indicated the presence of adequate amount of nutrients for plant growth. Therefore, using vermiwash and vermicompost as a standard hydroponic solution is an efficient and effective method of growing more food for the world's growing population in soilless medium, thereby reducing the dependency on land. Plants grown in the vermiwash and vermicompost in the hydroponic system had higher productivity than the one produced by commercial hydroponic solution.

Keywords: plant nutrients, hydroponic, vermiwash, physicochemical properties, vermiculture

INTRODUCTION

The agriculture sector will have a great challenge to meet the demand of over 9 billion people by 2050. In addition, the farming community is currently confronting critical issues on the application of chemicals and rapidly decreasing arable lands due to salinization, inundation, and desertification (Bennet and Khush 2003; Flowers and Colmer 2008; FAO 2011). Hence, we must take the necessary strides to overcome these problems by finding alternative means of food production to satisfy the increasing demand. Hydroponics can play an important role in finding solutions to this increasing demand. It is a system that allows plants to grow in soilless medium, and it was developed years ago from laboratory experiments carried out by scientists who were devoted to identifying which substances plants were composed of and how plants grew and developed (Mathieu 1998; Murali 2011). Vermicomposting is the term given to the process of conversion of biodegradable matter by earthworms into vermicast (Aira et al. 2002). Vermicomposting refers to the production of plant nutrient-rich excreta of worms (Zambare et al. 2008). Vermiculture is a culture containing soil bacteria mixed with an effective strain of earthworms (NIIR Board 2008). Leachate derived from vermicomposting units can be used as a liquid fertilizer as it contains large amounts of plant nutrients. Apart from its large nutrient content, vermiwash contributes to plant development due to its humic acid composition (Tallini et al. 1991; Makkar et al. 2017). Vermiwash was found to contain enzyme cocktail of proteases, amylases, urease, and phosphatase. Microbiological study of vermiwash has revealed that it contains nitrogen-fixing bacteria such as *Azotobacter* sp., *Agrobacterium* sp. and *Rhizobium* sp. and also some phosphate solubilizing bacteria (Zambare et al. 2008).

Nath and Singh in 2009 evaluated the impact of vermiwash on the growth, flowering, and productivity of okra (*Abelmoschus esculentus*), lobia (*Vigna unguiculata*) and radish (*Raphanus sativus*). Vermiwash was extracted from units containing different animal wastes, kitchen, and agro wastes. It was observed that treatment of vermiwash showed a significant increase in growth and productivity and decrease in flowering period. Phukan and Savapondit (2011) used vermiwash to determine its effectiveness as organic nutrient amendment for foliar spray in tea cultivation. Vermiwash from different organic waste with cow dung as feedstock available in and around tea garden was developed. Microbiological study of vermiwash revealed that it had nitrogen-fixing bacteria like *Azotobacter* sp., *Azospirillum* and phosphate solubilizing bacteria. Foliar application of vermiwash showed remarkable improvement in the growth, nutrient contents of shoots and yield of young and mature tea compared to the untreated plants.

Earthworms (*Eisenia fetida*) are terrestrial invertebrates originated about 600 million years ago, during the Precambrian era (Piearce et al. 1990). Earthworm has the ability to consume all types of organic materials including vegetable, industrial and other organic wastes (Zambare et al. 2008). It is quite possible to make quick changeover for sustainable agriculture by harnessing brand new vermicompost technology to the soil (Zambare et al. 2008). Earthworms have important influence on soil structure, forming aggregates and improving the physical conditions for plant growth and nutrient uptake (Ansari and Sukhraj 2010). During vermicomposting, earthworms eat, grind, and digest organic wastes with the help of aerobic and some anaerobic microflora, converting them into a much finer, humified, and microbial active material. The generated product is stable and homogeneous; having desirable aesthetics such as reduced levels of contaminants, and this converted product can be used as a fertilizer or as a source of nitrogen for microbial populations which can be beneficial to plant growth (Ravindran et al. 2008).

Neem (*Azadirachta indica,* Meliaceae) is recognized as a highly potent bio-pesticide with no known non-specific toxicity effect on mammals. Moreover, all components are known to have effective internal chemical defense mechanisms that prevent pest infestation. Harnessing these chemicals can contribute to the development of potent natural pesticides to prevent pest infestation of other plants (Chaudhary 2017). Sufficient evidence has also been provided in support of neem plants being effective in treatment of cancer, microbial infection, having inflammatory properties and antifertility agent (Kaushic 2004; Paul et al. 2011; Raut et al. 2014; Kumar et al. 2015).

MATERIALS AND METHODS

Experimental design

The field work was conducted both at the University of Guyana and in Lamaha Gardens. The vermiwash was produced in the Research Room at the University, and the hydroponically grown lettuce was grown in Lamaha Gardens. The microbial analysis was done at the University of Guyana laboratory whereas other chemical analyses were done at the Guyana Sugar Corporation Central Laboratory, Georgetown, Guyana.

Units were set up and after stabilization samples were collected from the three different unit combinations and applied to the crop for a period of four weeks. The physicochemical properties analyzed were pH, organic carbon (OC), nitrogen (N), available phosphate, potassium (K), exchangeable calcium (Ca), exchangeable magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn).

Unit preparation

A tap was affixed on the lower side of each container in such a way for the wash to be easily drained. Each unit was placed on a stand in such a way to facilitate collection of vermiwash, and 18 cm of broken pebbles were placed at the bottom of each container followed by 18 cm of coarse sand. Water was allowed to flow through these layers enabling the settling of the basic filter unit. A 22 cm layer of loamy soil was placed on top of the filter bed, and 25 earthworms were introduced into the soil of each of the containers. Substrates used in the units were 200g of cattle dung + 150g Neem leaves, 200g cattle dung + 150g Lime leaves and 200g cattle dung + 75 g Neem leaves + 75g lime leaves**.** These units were irrigated and drained every day and were also monitored, and specified quantity of organic materials was added as required.

Preparing the hydroponics unit

White sand and paddy husks were mixed together in the ratio of 2:1/2. The mixture of husks and sand was placed in plastic bags with holes and arranged in rows that are parallel to each other at a distance of 8 inches. At two weeks lettuce seedlings were transplanted in each bag and irrigated daily with 100 mL of 5 different treatments: water {control} (A), standard hydroponics nutrients mixed with water (B), lime based vermiwash (T1), neem based vermiwash (T2), lime plus neem based vermiwash (T3), respectively. The seedlings receiving different washes were administered to their respective vermicompost on their substrates together with spraying of neem weekly.

After six weeks, the morphological measurements were done for the number of leaves, height of plants and status of leaves during growth, and after harvest, the height of plants, number of leaves, shoot biomass (fresh and dry), root biomass (fresh and dry), leaf surface area and number of branches of roots were recorded.

Chlorophyll analysis

About 10g of leaf sample was weighed and thoroughly ground in a mortar. After which, 10 mL of solvent (70% ethanol) was added and ground to fine paste in a dark place to avoid chlorophyll disintegration. Another 10 mL of solvent (70% ethanol) was added and thoroughly mixed. It was then filtered, and filtrate centrifuged. Absorbance was measured using a UV-VIS spectrophotometer at 10nm intervals from an initial setting of 480 nm wavelength to 700 nm wavelength. Absorbance was obtained at 536nm, 647nm, 655nm, 664 nm and 666nm for calculations.

RESULTS AND DISCUSSION

Plant growth parameters

The plants for all of the designed treatments had significant percentage increases in their height as time progressed. T3 had the highest percentage increase in height (329.1%), followed by T2 (313.52%), B (275.16%), A (223.24%) and T1 (33.05%). T3 with the combination of neem and lime resulted in higher growth (20.94±0.75cm) than the commercial hydroponic treatment (16.55±1.81cm). Treatment 1 (17.82±1.39 cm) and Treatment 2 (20.1±1.46 cm) had also exceeded the commercial hydroponic treatment (Figure 1). Two ANOVA test indicated a significant impact of different treatments on number of leaves, with Fcal of 3.42×10^{-5} for the duration of the experiment. Earthworms' excreta provide plant growth hormones which promote growth and work as organic pesticides. Vermicompost served as an impetus for growth and development of lettuce when used in combination with vermiwash as they both make available more nutrients for the plant (Ansari 2008; Tharmaraj et al. 2011).

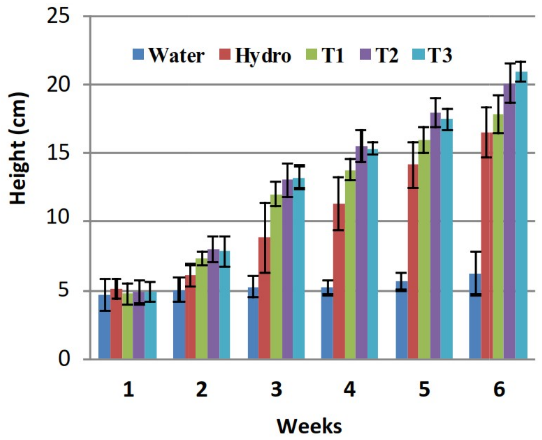

Figure 1. Plant height for each week

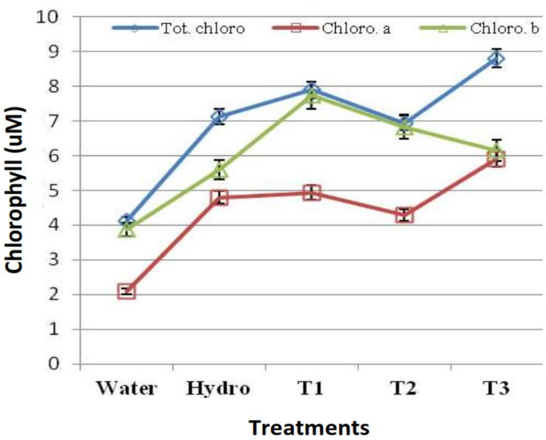

Figure 2. Chlorophyll content

Chlorophyll analysis

For total chlorophyll content, T3 (8.8±1.23) had the highest, followed by T1 (7.98±2.30), hydroponic solution (7.12±1.9), T2 (6.94±1.0) and water (4.12±1.74). Iron (Fe) is one of the main nutrients needed for the biosynthesis of chlorophyll. This indicated that the three different vermiwash treatments {T1 4.92±2.1mg/kg, T2 1.49±0.29mg/kg and T3 2.50±1.25mg/kg) [Table 2] had adequate amount of iron content to foster chlorophyll synthesis. Shlrene et al. (2012) revealed that 10% vermiwash has potential to be used in hydroponic culture and also exhibits superiority in growth as well as an increase in chlorophyll.

Harvest day data

The table below gives the results collected on harvest day on all the different parameters per plant under each of the designed treatments for a period of six weeks.

The shoot with the highest height was found in T3 (20.98±0.75cm), and the shoot with the lowest height was in water (6.24±1.55cm). All the treatments that contained vermiwash indicated better growth than control and standard hydroponic solution. Lime+neem combination of vermiwash and vermicompost had the best results for the parameter of height of shoot. Treatment B (7.67±1.63) had the most number of shoots while A (4.50±0.55) had the least number of shoots followed by T2 (6.12±2.48), T1 (6.00±1.09)and T3 (5.67±0.82), respectively. Branches of root were highest in T2 (41.67±14.72) with A (23.20±1.60) having the least number of roots followed by treatments T3 (37.67±5.16), T1 (35.00±13.78). T3 (23.75±0.51) had the highest length of root, while A (10.35±1.83) had the lowest one, followed byT2, T1 and B.Treatment 2 had the highest total fresh biomass. This was followed by T3, T1, B (commercial hydroponics) and lastly A (Table 1). This shows that the plants with the most weight came from the treatments of vermiwash and vermicompost. While with the leaf surface area, it can be seen that T2 had the largest leaf surface area followed by T3, T1, B (commercial hydroponics) and C (control). Plants treated with vermiwash/vermicompost treatments had better growth for all the parameters, even better than the standard hydroponics. The T2 (neem) treatment was the best because of the six growth parameters mentioned above; T2 lead in four parameters and was followed by T3 (neem+lime) which lead to the other two parameters. ANOVA analyses indicated a significant impact of different treatments on all the growth parameters on harvest day, with Fcal of 0.0025. Atiyeh et al. (2001) suggested that the greatest response from the plants could be observed only when the vermicompost was used at 10-40 % of the volume of plant growth medium. Hence, the 10% vermiwash used for each treatment was effective. Vermiwash exhibited growth-promoting effects on the morphological characters such as plant height, length, and diameter of the internode, number of leaves, leaf surface area, root length, wet and dry weight of the shoot and root (Kaur et al.2015).

Table 1. Data collected at harvest (mean±SD)

Treatment	Height of shoot (cm)	No. of shoots	No. of roots	Length of root (cm)	Total fresh biomass (g)	Leaf surface area (cm^2)
A (water)	6.24±1.55	4.50±0.55	23.20±1.60	10.35±1.83	1.37±0.41	5.56±1.57
B (hydro)	16.55±1.81	7.67±1.63	28.33±3.73	20.35±2.12	5.22±1.29	22.00±3.93
T1 (lime)	17.82±1.40	6.00±1.09	35.00±13.78	21.34±1.08	6.55±2.06	24.67±11.87
T2 (Neem)	20.10±1.46	6.12±2.48	41.67±14.72	23.15±1.27	9.74±3.38	41.84±5.22
T3 (comb)	20.98±0.75	5.67±0.82	37.67±5.16	23.75±0.51	8.65±3.46	41.28±11.15

Physicochemical analysis

All the treatments containing vermicompost had close to neutral pH, suitable for most plants. In most of these treatments, the pH values fluctuated around a neutral pH (7) for vermiwash solutions (Ansari 2015). Organic carbon in T2 and T3 was more than double that of T1. There was a difference of approximately 2000 mg/kg between T3 and T1, and an approximate difference of 1000 mg/kg between T1 and T2 for N. T2 had the most K, followed by T3 and then T1. T1 had the most Ca, followed by T3 and T2. There was not much difference for Mg, Zn, Fe, and Cu among the three treatments. Notably, the three treatments of vermicompost had N, P, K, Ca and Mg in significant quantities (Table 2). Adhikary (2012) said that vermin casts contain available nitrogen 5 times, available potassium 7 times and calcium 1.5 times as high as that found in 15 cm of top soil and remains stable with a nutrient self-life of 6 times as high as that of other types of potting mixes. These casts are rich in both micronutrient and macronutrients needed for healthy and highly productive plants (Kaur et al. 2015). Hence, vermicompost is enriched with more nutrient than soil and can be an efficient substitute when used in hydroponics.

Analysis of the nutrient composition of lettuce leaves showed that treatment B had the most N and least K and Na. T2 had the most P, whereas T3 had the most K and Na. T1 had the least Na and P (Figure 3). These quantities are similar to the results of analyses done by Marchi and colleagues in (2015). This means that the nutrients are all standard; therefore, growing it hydroponically using vermiwash and vermicompost did not alter its nutrient content. Fresh and dried biomasses for plants treated with vermiwash were higher than those with the standard hydroponic solution and water (Figure 4). Vermiwash and vermicompost contain all the nutrient requirements for plant growth and development and productivity (Kaur et al. 2015).

In conclusion, vermiwash produced from the different medicinal plants, namely lime, neem and their combination was very effective for supporting the growth of hydroponically grown lettuce (*L. sativa*). These nutrient solutions produced from medicinal plants gave better results than the commercial hydroponics solution and water, an indication that vermicompost and vermiwash can be implemented as an alternative method of plant growth. Plants can be grown hydroponically without soil to add nutrients and by just using the vermiwash and vermicompost in a medium made of paddy husks and white sand, thereby reducing the need for much space in growing crops.

Table 2. Nutrient analysis of the different types of vermicompost

Parameter (mg/kg) except for pH	**Treatment** 1 (Lime)	2 (Neem)	3 (Lime +Neem)
pH	7.54±1.26	5.84±0.25	6.82±1.12
Organic carbon	12.6±2.23	29.2±3.36	29.5±5.16
Nitrogen	13625±100.23	14725±456.55	11175±687.31
Phosphorus	123±12.12	183±21.85	99.8±18.79
Potassium	28450±901.23	50700±1405.23	35850±1320.25
Calcium	940±12.12	478±14.89	503±56.10
Magnesium	239±21.55	210±13.87	156±25.81
Zinc	38.1±5.69	31.0±12.12	41.4±10.19
Iron	4.92±2.16	1.49±0.28	2.50±1.25
Copper	0.65±0.02	0.15±0.01	0.28±0.00

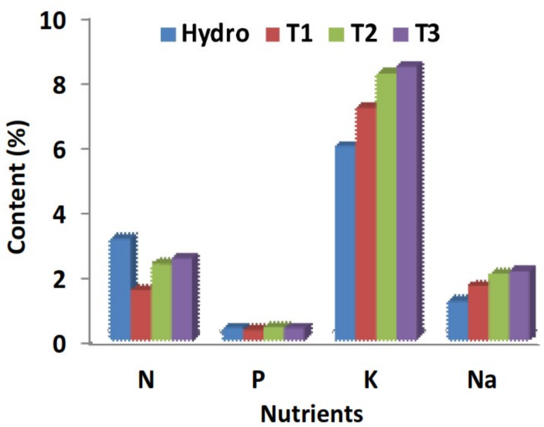

Figure 3. Nutrient content of leaf tissue

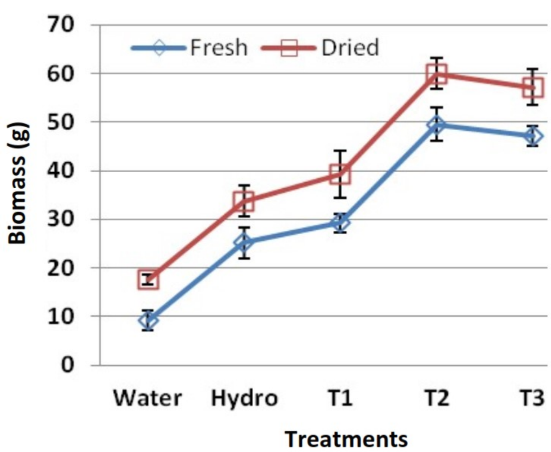

Figure 4. Total biomasses: fresh and dried

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