

## GROWTH IMPACT, PHOTOSYNTHETIC PIGMENTS AND HEAVY METALS CONTENT OF *COLEUS AROMATICUS*: A VERMIPONIC APPROACH

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**Abstract:** This study was undertaken to evaluate the effects of vermiwash and vermicomposting leachate that used pre-composted cow dung, as a substrate on plant growth through soil-less culture (vermiponics). Characterizations of vermiwash and vermicomposting leachate were carried out. Both liquids were diluted before using in hydroponic culture of *Coleus aromaticus*. The pH of these liquids is  $7.23 \pm 0.15$  and  $7.40 \pm 0.20$  for vermiwash (VW) and vermicomposting leachate (VL) respectively. Both earthworm derived liquids show a high concentration of plant nutrients. Nutrients such as potassium are high in both liquids but highest in vermiwash ( $215.33 \pm 5.37 \text{ mg L}^{-1}$ ). *Coleus aromaticus* that was treated with vermiwash showed significantly high value in total chlorophyll content ( $0.374 \pm 0.03 \text{ mg g}^{-1}$  fresh wt.) followed by vermicomposting leachate treatment ( $0.240 \pm 0.02 \text{ mg g}^{-1}$  fresh wt.). Shoot length and root length of both treatments are significantly higher if compared with control samples. Heavy metals content of Cr, Cu, Mn, Ni, Pb and Zn were analyzed in roots and leaves of all treatments. This study confirmed that both liquids showed the potential to be used as nutrient solution in vermiponics.

**KEYWORDS:** Vermiwash, vermicomposting leachate, hydroponic culture, vermiponics, *Coleus aromaticus*.

### Introduction

Diverse research works have been carried out on vermicomposting. Earthworms possess the ability to condition the substrate to encourage microbial activity and decomposition. Substrates get broken down and surface area gets increased, this therefore hastens the process of waste processing (Domínguez *et al.*, 1997). It thereby enhances the decomposition process by increasing the rate of soil organic matter decomposition (Brown *et al.*, 2000). The research focus of vermicomposting mainly lies in its ability to transform various types of waste into value-added products such as vermicompost. Limited studies have been carried out on investigating the potential of the liquid “by-product” that is produced along with this process. Vermiwash (VW) and vermicomposting leachate (VL) are reported to contain valuable plant nutrients in high concentration (Ismail, 2005; Garcia-Gomez *et al.*, 2008). Vermicomposting leachate is often known as “worm-tea”. The leachate

produced is released during the decomposition of organic material by microorganisms and as a result of constant application of water in order to maintain the moisture level of the substrate. Chemical composition of substrate that is used, plays a vital role in chemical composition of leachate produced (Tejada *et al.*, 2008).

Several studies have been carried out on vermicomposting leachate as foliar fertilizer as well as liquid fertilizer (Garcia-Gomez 2008, Gutierrez-Miceli *et al.*, 2008; Singh *et al.*, 2010; León-Anzueto *et al.*, 2011). The vermiwash unit designed by Ismail (2005) is based on the idea of collecting beneficial plant nutrients and microorganisms by percolating water through the drilosphere. Drilosphere as defined by Brown *et al.* (2000), consists of components including earthworms casts, surface that earthworms are in contact with soil, surface of the casts and the ground below, middens and burrows. Nitrogen (N), Phosphorus (P) and potassium (K) are the major components of plant nutrients. They are

known as macronutrients as the amount needed for plant growth are high. In drilosphere, the microenvironment that is “designed” and “conditioned” by earthworm activities provides a favorable site for microorganism activities. Mineralization rate increases, organic matter degradation occurs and therefore enhances the releasing of more plant-available N and P (Edwards and Bohlen, 1996; Barois *et al.*, 1999). Nitrogen is also excreted into drilosphere through earthworm urine and mucus. Water is produced when microorganisms break down the organic matter. When this liquid leaches out, it carries together nutrients that present in the vermicompost. Hence liquids derived from worm-processed substrate are believed to have collected all these plant available nutrients.

One of the uses of *Coleus aromaticus* that is cited regularly is its medicinal properties. The synonyms that are closely used for *Coleus aromaticus* include *Coleus amboinicus*, *Plectranthus amboinicus* and *Plectranthus aromaticus*. The species is widely distributed around the world, including North, East, Central Africa, Asia, South America, Caribbean and Pacific (Lukhoba *et al.*, 2006). This species is mostly being used to treat digestive and skin problems and infections (Morton, 1992; Ruiz *et al.*, 1996). Propagation of *Coleus aromaticus* is via stem cutting, making it a suitable choice to be used in soil-less culture study. Studies have been conducted on utilizing vermicomposting which derived liquids as fertilizers. However, there is little or no literature available showing comparison of vermiwash and vermicomposting leachate on growth impact as well as its potential as nutrient solution. This study was undertaken to study on the effect of vermiwash (VW) and vermicomposting leachate (VL) as nutrient solution towards growth impact, photosynthetic pigments and heavy metals content of *Coleus aromaticus*.

## Methodology

Vermiwash was prepared as recommended by Ismail (2005). A 50L plastic drum was used with

draining tap installed. Earthworms *Eudrilus eugeniae* were used. Vermiwash collection was started on the 16<sup>th</sup> day of setting up the unit as proposed by Ismail (2005). For vermicomposting leachate preparation, a vermicomposting unit was set up in a rectangular plastic container (410mm x 520mm x 160mm). The container was tilted and a leachate collection tap was installed close to the bottom of the container. A layer of gravels was placed at the bottom to prevent water saturation. Partially composted cow dung was used as feed to earthworms *Eudrilus eugeniae* in both units. Water was applied constantly for maintaining the moisture level at 65-70% (Tejada *et al.*, 2008). Liquids collected were stored in separate containers and characterization was carried out. The pH was measured with pH meter (HACH Sension 3) standardized with pH 4.01, 7.01 and 10.01 reference buffers. Electrical conductivity was measured by using conductivity meter (HACH Sension 5). Carbon and nitrogen content were determined using CHNS/O analyzer 2400 Series II, Perkin Elmer. For heavy metals analysis, tri-acid (HNO<sub>3</sub>: H<sub>2</sub>SO<sub>4</sub>: HClO<sub>4</sub> 5:1:1) digestion method was used as suggested by Allen *et al.*, (1986). The filtrates were then used for analyzing the sample with Atomic Absorption Spectrophotometer (Shimadzu AA-7000). Plantlets of *Coleus aromaticus* with an average height of 1-3cm were used in the experiment with 10% diluted vermiwash, and 10% diluted vermicomposting leachate (v/v) as experiments with distilled water as control in hydroponic culture. Photosynthetic pigments concentrations were measured in a spectrophotometer and were calculated from the standard formulae (Duxbury and Yentsch, 1956; Machlachlan and Zalik, 1963).

Statistical analysis of vermiwash (VW) and vermicomposting leachate (VL) was carried out by independent-samples t-test. One-way ANOVA and Duncan's Multiple Range Tests were used to evaluate significant differences between means at  $\geq 95\%$  level of confidence for photosynthetic pigments and morphological characteristics using SPSS 17 software.

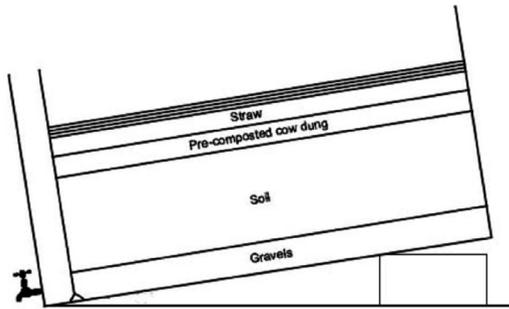


Figure 1: Illustration of Vermicomposting Leachate Reactor.

## Results and Discussion

An independent-sample t-test was conducted to compare the physico-chemical properties of vermiwash (VW) and vermicomposting leachate (VL). The physico-chemical properties of vermiwash and vermicomposting leachate are summarized in Table 1.

There is no significant difference between pH and the concentration of manganese (Mn) of the VW and VL. VL exhibits higher electrical conductivity than VW. For nitrogen content, VW shows statistically higher of content ( $0.03 \pm 0.01$ ) than VL ( $0.01 \pm 0.01$ ). The carbon content is significantly higher in VW ( $2.857 \pm 0.19$ ) than in VL ( $0.668 \pm 0.11$ ). Total phosphorus in VW ( $4.36 \pm 0.00$ ) is significantly higher than in VL ( $3.53 \pm 0.00$ ). For potassium, the concentration presence in VW ( $215.33 \pm 5.37$ ) is significantly higher than in VL ( $90.20 \pm 7.01$ ). There is a significant difference in the iron content of VW ( $8.910 \pm 0.01$ ) and VL ( $7.456 \pm 0.03$ ). Magnesium content in VW ( $0.030 \pm 0.00$ ) is significantly higher than in VL ( $0.028 \pm 0.00$ ). For zinc, VL ( $0.248 \pm 0.01$ ) shows significant difference compared to VW ( $0.114 \pm 0.01$ ). The concentration of copper in VL ( $0.030 \pm 0.00$ ) is significantly higher than in VW ( $0.344 \pm 0.01$ ). The physio-chemical properties of vermiwash listed in the table are in line with the work carried out by Ismail (2005). Plant nutrients are classified into macronutrients and micronutrients based on the amount needed in plants. C, H, O, N, P, S, K, S, K, Ca, Mg, Na and Si are defined as macronutrients. Examples of micronutrients are Fe, Mn, Cu, Mo, Bo and Ni.

Table 1: Characterization of Vermiwash (VW) and Vermicomposting Leachate (VL). The Values are Given as Mean  $\pm$  1SD.

	VW	VL
pH	$7.23 \pm 0.15$	$7.40 \pm 0.20$
Electrical conductivity ( $\text{ds m}^{-1}$ )	$1.482 \pm 0.07^*$	$1.659 \pm 0.02^*$
Nitrogen (%)	$0.03 \pm 0.01^*$	$0.01 \pm 0.01^*$
Carbon (%)	$2.857 \pm 0.19^*$	$0.668 \pm 0.11^*$
Total phosphorus ( $\text{mg L}^{-1}$ )	$4.36 \pm 0.00^*$	$3.53 \pm 0.00^*$
Potassium ( $\text{mg L}^{-1}$ )	$215.33 \pm 5.37^*$	$90.20 \pm 7.01^*$
Iron ( $\text{mg L}^{-1}$ )	$8.910 \pm 0.01^*$	$7.456 \pm 0.03^*$
Magnesium ( $\text{mg L}^{-1}$ )	$0.030 \pm 0.00^*$	$0.028 \pm 0.00^*$
Zinc ( $\text{mg L}^{-1}$ )	$0.114 \pm 0.01^*$	$0.248 \pm 0.01^*$
Copper ( $\text{mg L}^{-1}$ )	$0.025 \pm 0.00^*$	$0.030 \pm 0.00^*$
Manganese ( $\text{mg L}^{-1}$ )	$0.344 \pm 0.01$	$0.280 \pm 0.01$

VW= vermiwash, VL= vermicomposting leachate. Significant differences between vermiwash and vermicomposting leachate according to t-test: \*P < 0.05.

Though the classification is somehow arbitrary, the differences between the concentration of macro and micro nutrients are less well defined (Mengel and Kirkby, 2001). Therefore, the presence of macro and micronutrients in vermiwash and vermicomposting leachate show the potential of being used as a fertilizer as well as nutrient solution for plant.

Table 2 shows that there is a significant difference in the total chlorophyll content of *Coleus aromaticus* between control ( $0.160 \pm 0.02$ ), vermiwash ( $0.374 \pm 0.03$ ) and vermicomposting leachate ( $0.240 \pm 0.02$ ) treatment. *Coleus aromaticus* in vermiwash shows significantly higher total chl content. There is no significant difference in carotenoids content of control ( $0.080 \pm 0.01$ ) and VL ( $0.100 \pm 0.01$ ). However, VW ( $0.110 \pm 0.02$ ) shows significantly higher carotenoids content compared to control and VL. For

total chlorophyll: Car, there is no significant difference comparing control ( $2.04 \pm 0.14$ ) and vermicomposting leachate ( $2.40 \pm 0.31$ ) treatment. Vermiwash shows significant higher value ( $3.51 \pm 0.8$ ) in total chl: Car. Photosynthetic pigments act as indicators of plant health. One of the main target sites of heavy metal toxicity is photosynthetic pigments. Chloroplast may be damaged through multiple mechanisms including protein denaturation and oxidative damage (Hall, 2002). Heavy metal such as zinc may cause losing of chlorophyll in higher levels (Woolhouse 1978). The results obtained indicated that trace elements content present in diluted vermiwash and vermicomposting leachate did not pose a threat to the plant health as total chlorophyll content of both treatments are significantly higher compared to control.

For morphological characteristics of *Coleus aromaticus*, initial shoot and root length were measured. The results are presented in Table 3.

There is a significant difference in shoot length between control and treatments. Shoot length of VW ( $7.10 \pm 1.3$ ) and VL ( $5.97 \pm 1.5$ ) are significantly higher than in control ( $2.77 \pm 0.7$ ). Shoot growth (%) of *Coleus aromaticus* is significantly higher in VW ( $208 \pm 36$ ) and VL ( $158 \pm 40$ ) compared to control ( $28.5 \pm 12$ ). However, there is no significant difference in shoot growth between VW and VL treatments. Both VW ( $15.7 \pm 1.3$ ) and VL ( $13.2 \pm 4.0$ ) shows significant difference in root length compared to control ( $6.67 \pm 1.4$ ). The presence of valuable plant nutrients in VW and VL (Table 1) may be the reason of growth promoting in both treatments compared to control which is distilled water.

Alloway (1995) has reported the concentration of metals that are present in plants in normal range. For chromium, the normal range is  $0.03\text{-}14 \text{ mg kg}^{-1}$ , for copper  $5\text{-}20 \text{ mg kg}^{-1}$ , for manganese  $20\text{-}1000 \text{ mg kg}^{-1}$ , for nickel  $0.02\text{-}5 \text{ mg kg}^{-1}$ , for lead  $0.2\text{-}20 \text{ mg kg}^{-1}$  and for zinc  $1\text{-}400 \text{ mg kg}^{-1}$ . Chromium content in roots and leaves that grew in VW and VL are within the normal range as reported by Alloway (1995).

There is no significant difference between chromium content of control samples and the samples with treatment for root and leaf. This may due to the presence of Cr in the plant itself. It has been reported that chromium is involved in mammalian glucose metabolism and appears to be necessary to man and animal. Nevertheless, there is no evidence showing its significance in plant metabolism according to Huffman and Alloway (1973). For copper, the concentrations are very low in all portions of both treatments. There are no significant differences between control and treatments for root and leaf. Copper is taken up by plants in minute quantity as in most plant species (Mengel and Kirkby 2001).

Manganese content in roots and leaves of VW treatment, as well as root and leaf portion of VL treatments are lower than normal range as reported by Alloway (1995). There is no significant difference between Mn concentration in leaf of control ( $0.12 \pm 0.01$ ), VW ( $0.11 \pm 0.01$ ) and VL ( $0.11 \pm 0.01$ ) treatments. This may due to only minute traces of Mn is reported present in VW and VL as shown in Table 1. There is no significant difference between the Ni content in root for control, VW and VL. For Ni in leaf, there is no significant difference in all treatments. Nickel concentrations in root portion of VW and VL treatments are both lower than normal range, whereas the leaves portion of both treatments are within range. Crooke and Inkson (1955) reported that high Ni concentration that leads to toxicity of the plant will reduce the nutrients uptake of plants. Root portion is the one damaged by it and hence affect the nutrient uptake (Knight and Crooke 1956).

There are no significant differences in concentrations of Pb in roots for control and treatments. However, lead concentration in leaf portion of VW treatments ( $0.16 \pm 0.03$ ) is significantly higher than the concentration of Pb in leaf portion of control ( $0.02 \pm 0.03$ ) and VL ( $0.02 \pm 0.00$ ) treatment. Lead toxicity is one of the major human health concerns and it is one of the major pollutants to human. Root retardation in spruce seedlings was reported in low concentration of lead (Gobold et al. 1988).

Table 2: Total Chlorophyll Content (chlorophyll a + chlorophyll b), Carotenoids Content and Total Chlorophyll: Carotenoids. The Values are Given as Mean  $\pm$  SD; N=3.

Sources	Total chl (mg g <sup>-1</sup> fresh wt.)	Carotenoids (mg g <sup>-1</sup> fresh wt.)	Total chl: Car
Control	0.160 $\pm$ 0.02 <sup>a</sup>	0.080 $\pm$ 0.01 <sup>a</sup>	2.04 $\pm$ 0.14 <sup>a</sup>
VW	0.374 $\pm$ 0.03 <sup>c</sup>	0.110 $\pm$ 0.02 <sup>b</sup>	3.51 $\pm$ 0.8 <sup>b</sup>
VL	0.240 $\pm$ 0.02 <sup>b</sup>	0.100 $\pm$ 0.01 <sup>a</sup>	2.40 $\pm$ 0.31 <sup>a</sup>

VW= vermiwash, VL= vermicomposting leachate.

Different letters in each group show significant difference at  $p < 0.05$ .

Table 3: Morphological Characteristics of *Coleus aromaticus*.

Sources	Initial Shoot Length (cm)	Shoot Length (cm)	Shoot Growth (%)	Root Length (cm)
Control	2.13 $\pm$ 0.3 <sup>a</sup>	2.77 $\pm$ 0.7 <sup>a</sup>	28.5 $\pm$ 12 <sup>a</sup>	6.67 $\pm$ 1.4 <sup>a</sup>
VW	2.30 $\pm$ 0.3 <sup>a</sup>	7.10 $\pm$ 1.3 <sup>b</sup>	208 $\pm$ 36 <sup>b</sup>	15.7 $\pm$ 1.3 <sup>b</sup>
VL	2.30 $\pm$ 0.4 <sup>a</sup>	5.97 $\pm$ 1.5 <sup>b</sup>	158 $\pm$ 40 <sup>b</sup>	13.2 $\pm$ 4.0 <sup>b</sup>

VW= vermiwash, VL= vermicomposting leachate.

Different letters in each group show significant difference at  $p < 0.05$ .

Table 4: Concentration of Heavy Metals in Root and Leaf of *Coleus aromaticus* in Control, VW and VL Treatment.

(mg kg <sup>-1</sup> )	Control		VW		VL	
	Root	Leaf	Root	Leaf	Root	Leaf
Cr (chromium)	0.03 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.04 $\pm$ 0.02 <sup>a</sup>	0.04 $\pm$ 0.02 <sup>a</sup>
Cu (copper)	0.11 $\pm$ 0.12 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.06 $\pm$ 0.01 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>	0.05 $\pm$ 0.02 <sup>a</sup>	0.05 $\pm$ 0.00 <sup>a</sup>
Mn (manganese)	0.12 $\pm$ 0.01 <sup>b</sup>	0.13 $\pm$ 0.01 <sup>b</sup>	0.13 $\pm$ 0.03 <sup>b</sup>	0.11 $\pm$ 0.01 <sup>ab</sup>	0.09 $\pm$ 0.01 <sup>a</sup>	0.11 $\pm$ 0.01 <sup>ab</sup>
Ni (nickel)	0.01 $\pm$ 0.01 <sup>a</sup>	0.04 $\pm$ 0.00 <sup>b</sup>	0.01 $\pm$ 0.00 <sup>a</sup>	0.04 $\pm$ 0.00 <sup>b</sup>	0.01 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>b</sup>
Pb (lead)	0.08 $\pm$ 0.00 <sup>b</sup>	0.02 $\pm$ 0.03 <sup>a</sup>	0.08 $\pm$ 0.05 <sup>b</sup>	0.16 $\pm$ 0.03 <sup>c</sup>	0.00 $\pm$ 0.01 <sup>b</sup>	0.02 $\pm$ 0.00 <sup>a</sup>
Zn (zinc)	2.12 $\pm$ 0.04 <sup>b</sup>	2.08 $\pm$ 0.02 <sup>ab</sup>	2.08 $\pm$ 0.02 <sup>ab</sup>	2.12 $\pm$ 0.01 <sup>b</sup>	2.03 $\pm$ 0.06 <sup>a</sup>	2.04 $\pm$ 0.02 <sup>a</sup>

VW= vermiwash, VL= vermicomposting leachate.

Different letters in each group show significant difference at  $p < 0.05$ .

However, the findings (Table 3) show that root growth of *Coleus aromaticus* in VW and VL is significantly higher than in control. There is no significant difference in zinc concentration between leaf of control, leaves of VW and leaf of VL treatments. Zinc concentration in leaf of VW (2.12  $\pm$  0.01) is significantly higher than the concentration in leaf of VL (2.04  $\pm$  0.02). However, the concentration of Zn in leaf treated

with VW has no significant difference compared to control (2.08  $\pm$  0.02). Although zinc is known as one of the heavy metals, it is also needed in minute amount for plant growth. Application of Zn is reported to improve root growth up to the application of 120mg kg<sup>-1</sup> (Frageria, 2009). This may explain on the significantly higher root growth for *Coleus aromaticus* treated with VW and VL compared with control (Table 3) as zinc

is present in VW and VL. High priority is given to Zn deficiency among other micronutrients deficiency Graham (2008).

### Conclusion

Our result concludes that vermiwash and vermicomposting leachate contain valuable nutrients that are beneficial to plants. Despite the low concentration in N and C, vermiwash and vermicomposting leachate showed their potential as nutrients solution as they have significantly higher shoot growth, shoot and root length compared with control samples. The results from vermiwash analyses showed superiority in nutrient content of K, N, C, Fe, P and Mg, as well as its positive impact towards photosynthetic pigments content.

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