Biochemical Characterization of Vermiwash and its Effect on Growth of *Capsicum frutescens*

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ABSTRACT

Vermiwash is a rich source of vitamins, hormones, enzymes, macro- and micronutrients when applied to plants help in efficient growth. The present investigation was focused on the assessment of the potency of selected earthworm species *Eudrilus eugeniae* with respect to their biodegradation of jackfruit waste (*Artocarpus heterophyllus*) into a liquid biofertilizer vermiwash. The present study was undertaken to evaluate the physicochemical characteristics, micronutrients and macronutrients, proteins and carbohydrates present in vermiwash before and after inoculation of earthworm species and also a comparative study was done on the effect of vermiwash on growth parameters such as root and shoot length, number of leaf count in *Capsicum frutescens* after day 30 of planting. The results obtained revealed that treatment with vermiwash showed a decrease in total organic carbon and C/N ratio than control. The total macronutrients (N, P, K and C) and micronutrients (Fe, Cu, Mg and Zn) showed elevated levels in vermiwash treated when compared with control. Treatment with vermiwash in *C. frutescens* showed increased root and shoot length as well as number of leaves than the vermiwash untreated plant. The present study suggests that the quality liquid manure vermiwash obtained from degradation of jackfruit waste by *E. eugeniae* is an effective biofertilizer which would facilitate the increased uptake of the nutrients by the plants resulting in higher growth and yield and also improves soil health.

**Keywords:** Vermiwash, *Eudrilus eugeniae*, *Artocarpus heterophyllus*, *Capsicum frutescens*

1. INTRODUCTION

India produces about 3,000 million metric tons of organic wastes annually which are disposed off by ocean dumping, incineration and land application. Wastes from domestic, agriculture, urban and industrial sources are the main cause of organic soil pollution [1]. The process of vermiculture farming involves utilization of earthworms (natural versatile bioreactors) to cleaning up the environment with cost effective waste management technology for sustainable agriculture (also known as worm farming) [2]. Vermicompost, a by-product of earthworm mediated organic waste re-cycling, which is rich in nutrients and possess growth promoting substances (bio-fertilizers) [3].

Vermiculture is a mixed culture which contains soil bacteria and effective earthworms. The ability of earthworms to recycle organic wastes into organic manure by biological degradation process is widely recognized [4]. Earthworm has efficiency to consume all types of organic rich waste material including wastes from vegetables as well as from industrial origin [5]. Selection of earthworm species is very important factor because only few species are able to survive and adjust to a particular type of environment. The exotic species of
earthworm namely *Eudrilus eugeniae* (Family: Eudrilidae) is commonly used for vermicomposting. The tropical earthworm commonly called African night crawler is large in size, grows rapidly, breeds fast and is capable of decomposing large quantities of organic materials into usable vermicompost [6]. Earthworms contribute to decomposition of organic matter and nitrogen mineralization by influencing soil moisture and aeration through soil structure by fragmenting and redistribution of plant materials and provide nutrient rich excreta [7].

Vermicomposts are produced from organic wastes through interactions between earthworms and microorganisms, and can be utilized as plant growth media or soil amendments [8]. The vermicomposting technology can be utilized for generating a bio-liquid termed as vermic wash or vermiwash [9]. Vermiwash is a liquid leachate collected by allowing excess water to saturate the actively vermicomposting substrate in which the water washes the substrate in such a way that the vermicast excreted by the earthworms feeding on the substrate as well as the earthworm's body surface. It is well known, vermiwash is a rich source of vitamins, hormones, enzymes, macronutrients and micronutrients which when applied to plants help in efficient growth [10]. Application of vermiwash showed high growth rate due to increased uptake of macronutrients and micronutrients present in it. This leads to increased root length, shoot length and number of leaves in the vermiwash applied plants. This bio-liquid is rich in nutrients and plant growth hormones.

*Capsicum frutescens* (family: Solanaceae), also known as red pepper, bird pepper or chili pepper. The short-lived evergreen shrub which is normally 1 to 1.5 m in height and 1 to 3 cm in basal stem diameter. Red pepper is used as a condiment as well as principal ingredient in sauces. The pharmacological aspects of red pepper is used to relieve muscle pain, to treat cough, asthma, and sore throat and also to treat stomach ache [11]. *Artocarpus heterophyllus* (Jack fruit) belongs to family Moraceae which is native to parts of Southeast Asia. The succulent, aromatic and flavorful fruit is eaten fresh or myriad ways. The application of vermiwash may increase the yield and biological productivity of *C. frutescens*. With this hypothesis, the present study was carried out to evaluate the composition of vermiwash by considering physiochemical, biochemical parameters for sustainable development of plant growth promoting factor and the potential earthworm *E. eugeniae* used to convert *A. heterophyllus* into high quality organic manure which holds promise to play a significant role both in cleaning the environment and building up soil fertility for sustainable agriculture as well as ecofriendly soil conditioner.

### 2. MATERIALS AND METHODS

#### 2.1. Collection of wastes

The fruit waste (*A. heterophyllus*) were collected from local market shops from Thiruvalla, Kerala, India. The collected wastes were allowed to partial decomposition for 20 days. Then the wastes were mixed with cow dung in 3:1 ratio.

#### 2.2. Collection of earth worm and extraction of vermiwash

The earthworm *E. eugeniae* were collected from CARD, Thelilyoor, Kerala and cultured in laboratory conditions for proper growth and survival. Vermiwash is extracted through vermiwash collecting device by using the collected wastes.

#### 2.3. Assay of physicochemical parameters in vermiwash

The various physicochemical parameters such as pH [12], Electrical conductivity [12], Organic carbon [13] and C/N ratio have been determined by using standard methodologies.

#### 2.4. Analysis of macronutrients and micronutrients present in vermiwash

Vermiwash have higher content of macro and micro nutrients like nitrogen, phosphorus, potassium, calcium, sodium, magnesium and micronutrients namely iron, copper, zinc and manganese respectively [14]. The estimation of total Nitrogen was carried out by Pellett and Young method [15]. The total phosphorous, pottasium and calcium were estimated using methodology described by Jackson [16]. The estimation of micronutrients such as iron, manganese, zinc and copper was carried out by using Lindsey and Norwell method [16].

#### 2.5. Total carbohydrate and protein content in vermiwash

The total carbohydrate in the vermiwash as well as control was estimated by method described by Hedge and Hofreiter [17]. The protein content in vermiwash and control was estimated by Lowry et al., [18].

#### 2.6. Study on the effect of vermiwash on growth of *C. frutescens*

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**Effect of vermiwash on growth of *Capsicum frutescens***
The stem cuttings of C. frutescens were grown in two different pots named as (T1 and T2). T1 served as control (stem cuttings without vermiwash). T2 served as stem cuttings treated with 1:5 dilution of vermiwash. The following parameters were observed on day 30 after planting. Root and shoot length of stem cuttings and also leaf counts were measured. After treatment, stem cuttings were carefully removed from the soil without any damage and washed in running water to remove the adhering soil particles. Then, the length of the root, shoot and leaf counts were measured.

3. RESULTS AND DISCUSSION

3.1. Physicochemical parameters in vermiwash

Table 1. Physicochemical parameters in vermiwash.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Vermiwash</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.6</td>
<td>6.9</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>0.36</td>
<td>0.39</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>41.6</td>
<td>18.51</td>
</tr>
</tbody>
</table>

Electrical conductivity (Micromhos) Organic Carbon and C/N Ratio in (%). All experiments were conducted in triplicate.

Analysis of various physicochemical parameters was performed. The results were depicted in Table 1. The level of pH was increased in vermiwash treated when compared to control. Variation of control in percentage is 23.21%. Increase of pH in vermiwash might be due to participation of microbes in the degradation of organic wastes representing aerobic metabolism. The present results were concurrent with the studies of Gajalakshmi and Abassi reported [19], which showed that earthworms are sensitive to changes in pH and prefer conditions of neutral reaction. The level of electrical conductivity was found to be more in vermiwash when compared to control. Variation of control in percentage is 8.3%. The increase of electrical conductivity in the vermiwash in relative to that of control might be due to the loss of weight of organic matter and release of different mineral salts in available forms (such as phosphate, ammonium and potassium). Our results are in agreement with studies reported by Bai and Vijayalakshmi [20] that the electrical conductivity was increased in vermiwash after the inoculation of earthworm E. eugeniae.

The decrease in carbon content in vermiwash may due to earthworms modify the substrate condition which consequently promotes the carbon losses from the substrate through microbial respiration in form of CO₂ and even though mineralization of organic matter. It is evident from the results that the level of C/N ratio was found to be significantly decreased in vermiwash when compared to control. Variation of control in percentage was found to be 28.24%. Decrease of C/N ratio in the vermiwash relative to that of control may due to the combustion of carbon by the earthworms during respiration. Loh et al [21] showed that C/N ratio of cattle and goat manures were lower after vermicomposting that might be probably due to the organic carbon loss as CO₂.

3.2. Macronutrients and micronutrients present in vermiwash

The macro and micro nutrients present in the vermiwash were analyzed. The results were shown in Figure 1 and 2. In the present study the nitrogen content in vermiwash were found to be increased and the variation of control in percentage was found to be 68.75. Increase in nitrogen content in the vermiwash may due to the fact that earthworms enhanced the nitrogen cycle which attributed to the increased levels of nitrogen in vermiwash. Tripathi and Bharadwaj [22] reported that the losses of organic carbon might be responsible for nitrogen addition in the form of mucus, nitrogenous excretory substances, growth stimulatory hormones and enzymes from the gut of earthworms. The level of phosphorous present in vermiwash were higher when compared to control. The variation in control was found to be 26.02%. Studies by Lee [23] suggested that the enhanced phosphorous level in vermiwash may due to mineralization of phosphorous during vermicomposting. The release of phosphorous in the available form is performed partly by earthworm gut phosphatases and further release of phosphorous might be attributed to the phosphorous-solubilizing microorganisms present in vermiwash.

The total potassium present in vermiwash were found to be higher (40.19%) than the control. The increase in potassium content in vermiwash which might be due to changes in the distribution of potassium between non exchangeable and exchangeable forms. Our results are in accordance with studies reported by Suthar [24] suggests that earthworm processed waste material contains high concentration of exchangeable potassium due to enhanced microbial activity during the vermicomposting process, which consequently enhanced the rate of mineralization. The effect of worm action on vermiwash was found to be more when compared to control. The variation of control in percentage was found to be 31.07%. Earthworms possess calciferous glands that are involved in the production of calcium carbonate that might favour the calcium availability in the vermiwash. Garg et
al [25] reported that the increased level of calcium may due to the gut process associated with calcium metabolism which is primarily responsible for enhanced calcium content in worm cast.

Figure 1. Macronutrients Present in Vermiwash

All experiments were conducted in triplicate.

It is evident from the results that the iron content in vermiwash was increased when compared to control. The presence of enzymes and co-factors in the earthworm gut increased the iron content in the vermiwash. Our results are supported by Mall et al [14] who reported that vermiwash contains micronutrients like iron. Higher a content of copper was seen in vermiwash when compared to control. Increase of copper content in vermiwash might be due to the increased content of several Cu containing oxidizing enzymes. Our results are in accordance with Lee [23] who reported that the copper was found to be increased in worm casts. The manganese content in vermiwash was found to be elevated in relative to that of control. Increase of manganese content in vermiwash might be due to the fact that earthworms enhanced the mineralization of this element due to microbial and enzymatic activity of earthworm intestine. Our results are supported by Vasanthi and Kumaraswamy [26] who stated that micronutrient like manganese are rich in vermiwash. The results revealed that the Zn content in vermiwash is higher when compared to control. The findings of the present study were in accordance with the study of Graff [27] reported that zinc content was increased in vermiwash.

3.3. Total carbohydrate and protein content in Vermiwash

The carbohydrate level was found to be lower in the vermiwash while protein content was higher in vermiwash when compared to control. The reason for increase in protein content in vermiwash might be due to the presence of proteolytic enzymes that are secreted by the gut of earthworms.

Our results are in accordance with Zambare et al [5] who reported that the presence of protein and carbohydrate in vermiwash. In contrast to protein content the carbohydrate content was found to be lower in vermiwash when compared to control. The reason for decrease of carbohydrate content in vermiwash is due to decline in substrate content.

Figure 2. Micronutrients present in vermiwash

All experiments were conducted in triplicate.

Figure 3. Protein and carbohydrate content in vermiwash

*Protein (mg/ml); Carbohydrate (mg/ml). All experiments were conducted in triplicate.

3.4. Study on the effect of vermiwash on growth of C. frutescens

Based on the data obtained shown in Figure 4, 5 and 6, reveals the pronounced effects could be noticed in root length, shoot length and number of leaf count when the cuttings were planted with vermiwash. The selected plant namely C. frutescens showed the maximum root length (9 cm), shoot length (45 cm) and leaf count (22) in vermiwash treatment on day 30 after planting when compared to control. Vermiwash contains macro and micro plant nutrients in an available form that plants can easily assimilate for their growth and development.

Effect of vermiwash on growth of Capsicum frutescens
Effect of vermiwash on growth of Capsicum frutescens

**Figure 4.** Root and shoot length of *C. frutescens* treated with vermiwash

![Graph showing root and shoot length comparison between Control and Vermiwash](image)

All experiments were conducted in triplicate.

**Figure 5.** Leaf count of *C. frutescens* treated with vermiwash

![Graph showing leaf count comparison between Control and Vermiwash](image)

All experiments were conducted in triplicate.

This quality manure also contains some of the secretions of worms and its associated microbes, which acts as growth promoters along with other nutrients. Because of all these vital substances, vermiwash has multifarious effects that influence the growth of *Capsicum frutescens*. The results of the present study are in bar with Nath and Singh [28] who reported that the impact of vermiwash on the growth, flowering and productivity of Okra. Gopal *et al* [29] stated that application of vermiwash paddy showed the maximum growth and yield.

**4. CONCLUSION**

Our present study suggests that, vermiwash revealed potential application in sustainable development in agriculture biotechnology with respect to its origin, cost effectiveness, availability, reproducibility, reliability as well as biopesticide and ecofriendly soil conditioner.

**Conflict of Interest**

The authors declare that they have no conflicts of interest.

**Acknowledgement**

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**References**

Effect of vermiwash on growth of Capsicum frutescens