Research Journal of Forestry

ISSN 1819-3439

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Forestry

ISSN 1819-3439
DOI: 10.3923/rjf.2016.15.22

Research Article
Foliar Spray with Coffee Husk Vermiwash Enhances Seedling Growth of *Moringa stenopetala* (Baker F.) and *Jatropha curcas* (L.)

[1]Solomon Girmay, [2]Kaba Urgessa and [3]Gezahegn Berecha

[1]Department of Natural Resource Management, Jimma University, Ethiopia
[2]Federal Ministry of Education, Addis Ababa, Ethiopia
[3]Department of Horticulture and Plant Sciences, Jimma University, Ethiopia

Abstract

Background: Vermiwash, a brownish to pale yellow liquid extract collected during vermicomposting of organic wastes is often used as biofertilizer for the production of many crops globally. However, the effect of coffee husk vermiwash on seedling growth of economically important Agroforestry trees and shrubs in Ethiopia is not yet evaluated. **Materials and Methods:** The present study was conducted to evaluate the effects of coffee husk vermiwash foliar spray on seedling growth of *Moringa stenopetala* (Bak.) and *Jatropha curcas* (L.) under lath house condition in South Western Ethiopia. For this, the responses of the aforementioned tree species to different rates (0, 10, 20, 30 and 40% (v/v)) of coffee husk vermiwash was tested in 2×5 factorial arrangement in Randomized Complete Block Design (RCBD) with three replications. Seedling growth attributes were recorded as response variables and data were subjected to analysis of variance (ANOVA) procedure of SAS v.9.2 computer soft ware. **Results:** The results of the study revealed significant positive effect of foliar spray of coffee husk vermiwash on growth and seedling quality attributes. Higher rate of vermiwash foliar spray generally resulted in maximum leaf chlorophyll b, plant height, leaf number, collar diameter, shoot fresh and dry weight. **Conclusion:** Application of coffee husk vermiwash foliar spray at 30 and 40% (v/v) would be an interesting option for the production of quality seedlings. Generally, owing to its low labor demand and simple technology input, coffee husk vermiwash is an opportunity in coffee growing areas of Ethiopia for better seedling growth and performance in nursery and back yard garden. The study also confirmed coffee husk vermiwash as effective biofertilizer that can be promoted for wider use in Ethiopia and beyond for quality seedling production.

Key words: Coffee byproducts, vermiwash, biofertilizer, vermiculture, *Moringa stenopetala*, *Jatropha curcas*

Received: May 03, 2016 Accepted: May 24, 2016 Published: June 15, 2016

Citation: Solomon Girmay, Kaba Urgessa and Gezahegn Berecha, 2016. Foliar spray with coffee husk vermiwash enhances seedling growth of *Moringa stenopetala* (Baker F.) and *Jatropha curcas* (L.). Res. J. For., 10: 15-22.

Corresponding Author: Gezahegn Berecha, Department of Horticulture and Plant Sciences, Jimma University, Ethiopia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Agriculture is the dominant industry in many developing countries including Ethiopia and byproducts from this industry are known cause of serious environmental pollution[1]. Attempts made thus far to convert these wastes to various usable products in different parts of the globe didn't result in much success. These among others are associated to high costs of collection, transportation and handling of these byproducts. As a result, land disposal and direct combustion remain the cheapest way to get rid of agricultural residual wastes in most countries of the world[2].

Coffee cultivation is a deep rooted culture among many societies of Ethiopia. Both solid and liquid byproducts from coffee industry are continuously generated year after year and commonly disposed on surrounding land and aquatic ecosystem causing environmental pollution, fresh water eutrophication and increased spread of water-borne diseases, since they constitute a source of severe contaminants in Ethiopia[3]. Coffee husk, the major focus of the present study, particularly in the coffee growing regions of South Western Ethiopia, is the hazardous pollutant responsible for the contamination and degradation of the ecosystem[4]. The rational utilization of this byproduct has some ecological and economic implications that may become evident as its use increases[5]. Some of the main alternative uses of coffee byproducts include animal feed[6], production of alcohol and biogas[7], soil mulch[8], charcoal and heat energy[9,10], mushroom production[5], compost[3], vermicompost[11] and toxic chromium adsorption[12]. Generally, the application of environmentally sound coffee husk disposal methods requires an understanding of the range of waste utilization, treatment and recycling options.

Mulching and composting coffee husk are the common conventional agronomic utilization methods among few coffee farmers in Ethiopia. However, direct and inappropriately-timed application of coffee husk mulching to agricultural fields often causes serious environmental problems, including the release of excessive amounts of tannins and phenols in soils, which could inhibit plant root growth[3]. Moreover, odor emission and the presence of pathogens and its plant growth impairment are some considerable limitations of mulch, which make it less suitable for land application. On the other hand, coffee husk compost maturity may take more than 6 months time for use[13], which can be considered as a limitation to exploit its nutritive potential as biofertilizer for soil conditioning and plant growth.

Coffee husk can be converted to useful products through vermicomposting[11] which transforms wastes in to a safer and more stabilized product that can be used as a source of nutrients and soil conditioner in agricultural applications while eliminating environmental pollutants[14,15]. Vermicompost as a product of vermicomposting contains a considerable amount of some essential plant micronutrients responsible for better plant growth and productivity and can considerably contribute to nutrient recovery[6,16] as it has much finer structure, outstanding chemical and biological properties with 'Plant growth regulators' and nutrients.

Vermiwash is a brownish/pale yellow colored liquid extract collected during vermicomposting of organic wastes[17]. It contains excretory products and mucus secretion of earthworms rich in amino acids, vitamins, nutrients like nitrogen, potassium, magnesium, zinc, calcium, iron and copper and some growth hormones like auxins and cytokinins. It also contains plenty of nitrogen-fixing and phosphate solubilising bacteria (nitrosomonas, nitrobacter and actinomycetes). Vermiwash, therefore has great 'Growth promoting' as well as 'Pest killing' properties[18]. Many studies have shown that vermiwash application improves plant growth, yield and nutritional quality[7,19]. Application of vermiwash in the form of foliar spray is more advantageous from economic and environmental point of view due to the absence of nutrient leaching which is often the case when performing soil amendments.

Multipurpose agroforestry trees and biofuel crops can address the issue of food insecurity, high energy costs associated with grain food deficit and import based fossil fuel energy security in Ethiopia. *Moringa stenopetala* (Bak.) and *Jatropha curcas* (L.) are among the most important trees/shrubs to be considered vital in response to food insecurity problems in Ethiopia. Because, *M. stenopetala* is one of a unique strategic multipurpose indigenous agro forestry tree in drought prone areas of Ethiopia with an extremely valuable source of nutrition for people of all ages[20]. It is also a contingency crop in frequently drought prone areas which plays a vital role for household food security, as source of income, medicine, fodder, fuel and shade tree all year round[21]. Similarly, *J. curcas* is one of the perennial shrubs producing seeds with high oil content for biofuel. It is a fast growing, drought tolerant source of biofuel plant without compromising food security[13]. Successful establishment of these important crops in the field depends on quality seedlings production in the nursery. Seedlings growth and development in the nursery depends on the extent of managements, environmental factors (temperature, rainfall and humidity etc.) prevailing in the area and the variety/species under consideration. Although, growers use different sources of nutrition to enhance seedling growth and development, the use of vermiwash as foliar biofertilizer for seedling production of *Moringa* and *Jatropha* has not been well studied in Ethiopia. Therefore, the present study aimed at evaluating vermiwash foliar spray effect on seedling growth of *Moringa stenopetala* (Bak.) and *Jatropha curcas* (L.) under lath house condition in South Western Ethiopia.

MATERIALS AND METHODS

Description of the study area: The experiment was conducted at Jimma University College of Agriculture and Veterinary Medicine, Ethiopia under lath house condition. The area is geographically located at an elevation of 1710 m a.s.l. and at latitude of 7°42' 9''N and 36°47' 6'' E longitude. The average total annual rainfall of 1500 mm with mean maximum and minimum temperatures of 26.8 and 13.6°C, respectively characterize the study area[22].

Experimental materials: Seeds of *Moringa stenopetala* were collected from Ethiopian Institute of Agricultural Research (EIAR), Forestry Research Center (FRC), Addis Ababa, Ethiopia. While *J. curcas* seeds were collected from Alamata district office of Agriculture, Tigray, Ethiopia. Coffee husk was collected from coffee processing plants around Jimma, Ethiopia. Earth worms used for composting were collected from Jimma University soil laboratory which initially were introduced from Canada.

Vermiwash preparation: A handful of *Eisenia fetida* earth worms which fed on coffee husk were immersed in 100 mL warm distilled water (temperature = 40°C) and kept for 30 min on a 10 cm diameter petri plate holding in a slanting position and keeping earthworms pointing downwards as described by Suthar[8] and Rameshguru *et al.*[23]. The vermiwash was collected from the body cavity of earthworms at the lower side of the petri plate without causing any harm to them. Secreted enzyme containing extract was centrifuged to remove the insoluble materials at 3000 rpm for 10 min. Then the filtrate was made cell free using 0.2 µ membrane filtration.

Treatments and experimental design: The experiment consisted of two factors namely, tree species (*M. stenopetala* and *J. curcas*) and vermiwash rate with five levels (0, 10, 20, 30 and 40% (v/v)). Distilled water was used for dilution and as control treatment. The basic soil used for growing the seedlings of both species was top soil. Therefore, the treatments were arranged in 2×5 factorial in Randomized Complete Block Design (RCBD) with three replications. Fifteen pots per treatment per replication were maintained. All management practices, such as weeding and watering were done as per the general recommendations for tree nursery.

Seed sowing: Pure, defect less and large size seeds were used for the study after removing light weighted seeds using water floating method. Seeds of *M. stenopetala* were decocted for easy germination and to avoid decaying. Prior to sowing qualified seeds were soaked for about 6 h to speed up germination. Two seeds from each species were sown independently per pot at about 2 cm depth under lath house condition. The pots were mulched and watered immediately after sowing. Pots were watered twice a day till the final emergence and once per day then after.

Data collection and analysis: The following seedling growth parameters were collected 60 days after seed emergence: Leaf chlorophyll a and b (mg g^{-1}), plant height (cm), collar diameter (mm), number of leaves, root length (cm), shoot fresh dry weight (g), root fresh and dry weight (g). Leaf chlorophyll a and b were estimated following the procedure described by Baskaran *et al.*[24]. Five hundred milligram of Fresh Leaf (FW) material was taken and ground with the help of pestle and mortar with 10 mL of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was filtered and utilized for chlorophyll estimation. Absorbance was read at 645 and 663 nm in the UV-spectrophotometer (UV-4000, Germany).

$$\text{Chlorophyll a (mg g}^{-1}\text{ FW)} = (0.0127)\times(\text{OD } 663) - (0.00269)\times(\text{OD } 645)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ FW)} = (0.0229)\times(\text{OD } 645) - (0.00468)\times(\text{OD } 663)$$

The number of leaves per plant was counted above the cotyledon scar (for *M. stenopetala*) while in the case of *J. curcas* leaves were counted excluding the cotyledon leaves. Plant height was measured from shoot tip of the plant to the root collar while, stem diameter was measured immediately 1 cm above the root collar with vernier calipers perpendicularly. Root length was measured from the tip of the root collar to the tip of the longest root. Consequently, seedlings were clipped off and separated in to root and shoot parts. For analysis, average values of 10 sample plants per treatment of plant height, stem diameter, number of leaves, root length, shoot and root fresh weight were assessed. Whereas, shoot and root dry weight were recorded after oven drying at 60°C for 48 h and weighed using sensitive digital weighing machine.

The data were checked for all the assumptions of analysis of variance (ANOVA) and the data fulfilled ANOVA model assumption. Then all data of seedling growth parameters were subjected to ANOVA procedure of SAS version 9.2. Least Significant Difference (LSD) test at 5% level of significance was employed for means separation of significant response variables.

RESULTS AND DISCUSSION

Leaf chlorophyll content: The effect of vermiwash on leaf chlorophyll a and b content of *Moringa stenopetala* and *J. curcas* was tested and presented in Fig. 1 and 2. Leaf chlorophyll a and b content was not affected by the interaction effect of the two tree species and vermiwash rate. However, leaf chlorophyll a and b content of *M. stenopetala* was significantly higher than *J. curcas* ($p<0.0001$) owing to the inherent genetic botanical differences (Fig. 1). Seedling chlorophyll a content was not significantly affected by different rates of coffee husk vermiwash tested.

Nevertheless, chlorophyll b content was significantly ($p<0.0001$) affected by increased rate of coffee husk vermiwash (Fig. 2). The observed increase in chlorophyll b content could be attributed to plant growth hormones present in the vermiwash that enhanced the leaf growth and help capture solar energy while consequently increasing the photosynthetic efficiency of the seedlings. For instance, researchers elsewhere[25] have observed in their findings that foliar spray of 100, 150 and 200 ppm vermiwash consistently increased leaf chlorophyll and nitrogen contents in soybean. The positive effect of vermi extract on plant growth is largely associated with its N (NO_3^-) content and the presence of gibberellins and increased nutrient uptake by plants[26].

Seedling height: Seedling height was not affected by the interaction effect of tree species and vermiwash rate. However, seedling height was significantly influenced by the main effect of tree species ($p = 0.004$) and vermiwash rate ($p = 0.0056$) (Table 1). *Moringa stenopetala* seedling was taller than that of *J. curcas* (Table 1). The observed difference in seedling height could be explained by the difference in the response of the two species to different growth promoting substances which is the function of their genetic differences. It has been reported by similar study that vermiwash application enhances nutrient uptake and growth promoting substances[27,28].

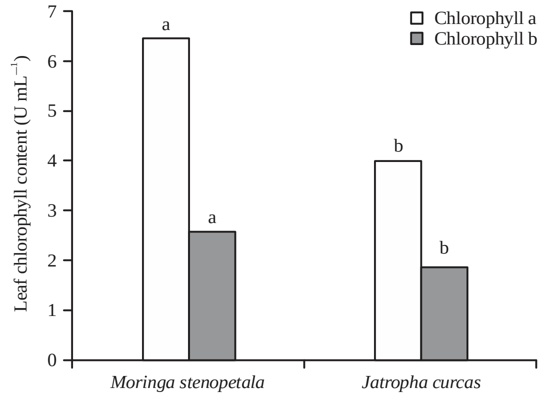

Fig. 1: Effect of coffee husk vermiwash foliar spray on chlorophyll a and b content of *Moringa stenopetala* and *Jatropha curcas*. Bars with the same color but capped with different letter are significantly different at $p<0.05$

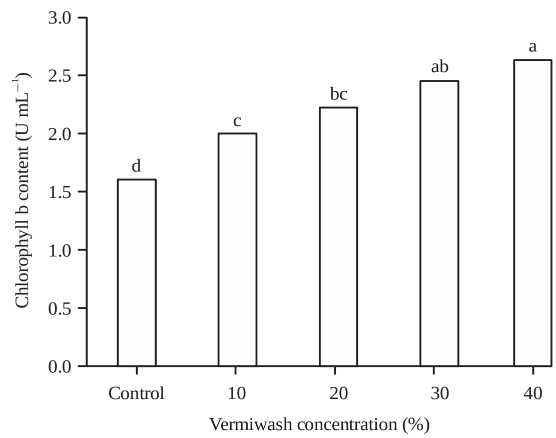

Fig. 2: Effect of different rates of coffee husk vermiwash foliar spray on chlorophyll b content. Bars capped with the same letter are not significantly different at $p<0.05$

Seedling height was increased with increased rate of vermiwash. Application of 10, 20, 30 and 40% (v/v) of coffee husk vermiwash improved plant height by about 4.3, 4.8, 19.7 and 32.2% over the control. These differences could be attributed to increased level of nutrients translocated in

Table 1: Effect of coffee husk vermiwash foliar spray on seedling growth attributes of *Moringa stenopetala* and *Jatropha curcas* under lath house condition

Tree species	PL (cm)	LN (n)	SG (mm)	SFW (g)	SDW (g)	RFW (g)	RDW (g)
Moringa stenopetala	21.88[a]	8.16[a]	6.67[a]	6.17[b]	1.09[b]	8.53[a]	0.88[a]
Jatropha curcas	18.75[b]	3.71[b]	5.34[b]	11.26[a]	1.83[a]	1.53[b]	0.23[b]
CV (%)	12.82	8.87	2.97	16.60	16.54	5.10	8.86
LSD (5%)	2.00	0.40	0.14	1.11	0.19	0.20	0.04

Values are aggregate of the different vermiwash concentrations tested, means within a column represented by same letter are not significantly different at p<0.05, PL: Plant height, LN: Leaf number, SG: Stem girth, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight and RDW: Root dry weight

Table 2: Seedling growth attributes of *Moringa stenopetala* and *Jatropha curcas* as influenced by coffee husk vermiwash rate

Rate (%) (v/v)	PL (cm)	LN (n)	SG (mm)	SDW (g)	RFW (g)	RDW (g)
Control (0)	18.11[c]	5.19[c]	5.58[d]	1.21[c]	4.57[d]	0.48[c]
10	18.89[bc]	5.57[bc]	5.83[c]	1.34[c]	4.88[cd]	0.52[bc]
20	18.97[bc]	5.98[ab]	6.05[b]	1.40[bc]	5.02[bc]	0.57[ab]
30	21.68[ab]	6.47[a]	6.23[ab]	1.65[ab]	5.27[ab]	0.59[a]
40	23.94[a]	6.48[a]	6.35[a]	1.72[a]	5.42[a]	0.61[a]
CV (%)	12.82	8.87	2.97	16.54	5.10	8.86
LSD (5%)	3.16	0.64	0.22	0.29	0.31	0.06

Means followed by the same letter(s) are not significantly different at p<0.05, PL: Plant height, LN: Leaf number, SG: Stem girth, SDW: Shoot dry weight, RFW: Root fresh weight and RDW: Root dry weight

to plant tissues owing to increased rate of vermiwash application. Similar results have been reported by Ansari and Sukhraj[27] indicating that vermiwash application resulted in maximum plant height of okra (*Abelmoschus esculentus*) as compared to the control. The increase in seedling height could be associated with the fact that vermiwash contains a considerable level of plant growth promoters such as auxins and gibberellins like substances[27], high level of macro and micro nutrients[28], which positively influence nutrient uptake by roots and plant physiological function as a whole by improving soil structure and fertility.

Leaf number: Leaf number per plant was not significantly affected by the interaction effect of tree species and coffee husk vermiwash rate tested. There was a significant difference (p<0.0001) between the two tree species tested in terms of leaf number per plant (Table 1). On average more leaf number per seedling was observed for *M. stenopetala* (8.2) than did for *J. curcas* (3.7). This might be attributed to the genetic difference in leaf formation and/or ability to uptake the nutrients provided via vermiwash application.

Moreover, application of different rate of coffee husk vermiwash significantly affected (p = 0.001) leaf number of *M. stenopetala* and *J. curcas* seedlings (Table 2). Coffee husk vermiwash at 10, 20, 30 and 40% increased leaf number by 7.3, 15.2, 24.7 and 24.9%, respectively, over the control. This result is in agreement with the study of Suthar[8], who reported maximum number of leaves per *Cyamopsis tertagonoloba* seedling due to application of 100% vermiwash (72.5%) compared to distilled water (control). Ansari and Sukhraj[27] also reported that application of vermiwash to okra (*Abelmoschus esculentus*) increased the number of leaves per plant than the control. This could be attributed to the presence and impact of auxins like earthworm derived substances and macro and micro nutrients which improve the physical conditions of the medium for plant growth and nutrient uptake[28] and consequently, improved level of chlorophyll pigment and leaf development[6].

Seedling collar diameter: Seedling collar diameter didn't show significant variation due to the interaction effect of species and coffee husk vermiwash rate. There was a significant variation between *M. stenopetala* and *J. curcas* in terms of seedling collar diameter (Table 1). Compared to *J. curcas* and *M. stenopetala* seedling had thicker stems (6.67 mm). The difference in collar diameter could be linked with and influenced by the fact that *M. stenopetala* has thick swollen tap root system, a strategy to store and transport more water from its root tissue to the upper vegetative organ in order to have healthy plant growth in the future than does *J. curcas*.

Table 2 shows that, seedling collar diameter was significantly (p<0.0001) influenced by coffee husk vermiwash rate. Stem girth improved by 4.5, 8.4, 11.7 and 13.8% with increased vermiwash rate from 10, 20, 30 and 40% (v/v). The positive response to elevated vermiwash rate could be attributed to stimulatory effect of growth promoting substances and microorganisms within the coffee husk vermiwash. Earlier study by Suthar[6] reported optimum plant growth (root length, plant height, shoot/root ratio and leaves per plant) achieved due to application of 100% vermiwash compared to 50% vermiwash. According to

Pant *et al*.[29] and Peterson and Graves[30], vermi extracts serve both asasupplemental source of plant nutrients and an enhancer of soil biological properties which consequently give better plant biomass development.

Shoot fresh and dry weight: The interaction effect of species and coffee husk vermiwash rate was not significant for shoot fresh and dry weight of seedlings. Similarly, coffee husk vermiwash rate didn't significantly contribute to shoot fresh weight. However, the main effect of tree species ($p<0.0001$) significantly affected seedling shoot fresh and dry weight (Table 1). Larger shoots fresh and dry weight was observed for *J. curcas* due to its larger leaf area than *M. stenopetala*.

The coffee husk vermiwash at different rate, affected seedling shoot dry weight significantly ($p = 0.0082$) (Table 2). The increased shoot dry weight response with increased vermiwash rate might be due to the incorporation of high amount of micro and macro nutrients in to the plant tissues[28]. Dhiraj and Kumar[31] have also explained that foliar nutrients are readily mobilized and easily utilized directly in to plant leaves which consequently increase nutrient efficiency and uptake, increase cellular activities at all levels while, increasing the rate of photosynthesis.

Root length: Root length was significantly affected by the interaction between tree species and vermiwash rate as well as the main effects of species and coffee husk vermiwash rate ($p<0.0001$) (Table 3). The maximum seedling root length was recorded for *M. stenopetala* at highest rate of vermiwash application (30 and 40%), compared to the other treatment combinations (Table 3). In contrast, the shortest root length was obtained due to the control (without vermiwash treatment) in *J. curcas*. Significant difference in root length of both species could be explained by the contribution of vermiwash as a beneficial seedling growth enhancer. *Cyamopsis tetragonoloba* treated by 100% vermiwash showed about 47.4% more root length than urea 5% solution in the study by Suthar[6]. According to Hatti *et al*.[28] the vermiwash contains high level of macro and micronutrients and growth promoting hormone like substances. This could be the case for more root elongation with increasing rate of vermiwash in the present study.

Root fresh and dry weight: Both root fresh and dry weight were not significantly affected by the interaction effect of tree species and vermiwash rate. The main effects of species ($p<0.0001$) and vermiwash rate ($p = 0.0014$), however, significantly affected seedling root fresh and dry weight

Table 3: Root length of *Moringa stenopetala* and *Jatropha curcas* seedling as influenced by the interaction effect of species and coffee husk vermiwash rate

Species	Vermiwash rate (%) (v/v)	Root length (cm)
Moringa stenopetala	0	8.16^{d}
Moringa stenopetala	10	10.21^{c}
Moringa stenopetala	20	11.39^{b}
Moringa stenopetala	30	12.90^{a}
Moringa stenopetala	40	13.66^{a}
Jatropha curcas	0	4.48^{g}
Jatropha curcas	10	5.70^{f}
Jatropha curcas	20	6.58^{e}
Jatropha curcas	30	8.43^{d}
Jatropha curcas	40	9.53^{c}
CV (%)		1.26
LSD (5%)		0.81

Means followed by same letter(s) are not significantly different at $p<0.05$

(Table 1 and 2). Hence, *M. stenopetala* seedling root fresh and dry weight was higher than that of *J. curcas* (Table 1). This might be linked with its thick stem diameter and swollen root system. Moreover, root fresh and dry weight improved along with increased rate of coffee husk vermiwash (Table 2). As a result, maximum root fresh and dry weight was observed due to application of higher rate of vermiwash compared to the other treatments. The observed root fresh and dry weight improvement following application of different rates of vermiwash might be attributed to the enrichment of growth promoting substances such as cytokinins, auxins, amino acids, vitamins and enzymes[8] and high level of macro and micro nutrients[28] which in turn contributed for more water absorption. Moreover, study by Suthar[8] indicated that *Cyamopsis tetragonoloba* seedlings treated with 100% vermiwash foliar spray had the maximum level of total protein, total soluble sugars and starch in their tissues, compared to the control. Therefore, the presence of higher root biomass might be an indication of good establishment in the field.

CONCLUSION

The findings of the present study revealed the existence of significant influence of coffee husk vermiwash on seedlings growth. Different rates of coffee husk vermiwash impacted seedlings growth and development of *M. stenopetala* and *J. curcas* differently. Higher rate of vermiwash foliar spray generally resulted in maximum leaf chlorophyll b, plant height, leaf number, collar diameter and shoot fresh and dry weight. Accordingly, application of coffee husk vermiwash foliar spray at 30 and 40% (v/v) was found to be beneficial among the tested rates. Therefore, coffee husk vermiwash which demands low labor and simple technology input can be an opportunity in coffee growing areas of Ethiopia for better seedling growth and performance in nursery and/or back yard garden. Besides its use as a source of biofertilizer, vermicomposting of coffee husk reduces environmental pollution associated with coffee processing byproducts.

SIGNIFICANCE STATEMENTS

- Both solid and liquid byproducts from coffee industry are commonly disposed on surrounding land and aquatic ecosystem causing environmental pollution, freshwater eutrophication and increased spread of water-borne diseases in Ethiopia
- Coffee husk vermiwash collected during vermicomposting of coffee processing byproducts was found to be a potential alternative biofertilizer for organic plant/crop production
- Foliar spray of coffee husk vermiwash had significant effect on seed germination and seedling growth of commercially important agroforestry trees tested
- The impact of coffee husk vermiwash on seedling growth differed depending on application rate and the effects were higher at higher rate of application
- The possibility of managing byproducts from coffee industry by utilization is demonstrated
- Recycling/management of coffee industry byproducts minimize environmental pollution and public health concern thereby ensure ecosystem health

ACKNOWLEDGMENT

Funding for this study was provided by Jimma University and authors are very grateful to the University.

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