RESEARCH ARTICLE



Growth evaluation of several types of energy crops from

tropical shrubs species [version 1; peer review: 2 approved]

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Abstract

Background: Few species of tropical shrubs potentially produce biomass to replace fossil fuels for heat production and electricity. The aims of this study were to determine the growth and nutrient status of leaves of several types of energy crops from tropical shrub species with NPK fertilizer application.

Methods: Randomized block design was used with ten replications of four levels of fertilizer treatment: T0 = 40 g, T1 = 80 g, T2 = 120 g and T4 = 160 g per plant.

Results: The results indicated that fertilization increased plant growth and the quantity of nutrients in leaves. The plants accumulated a lot of potassium, followed by nitrogen and phosphorus. The species of tropical shrubs with the best growth were *Vernonia amygdalina*, *Calliandra calothyrsus and Gliricidia sepium*, which are all potentially cultivated as sustainable energy crops.

Conclusions: Serious attention must be paid to the availability of soil nutrients in order to sustain the cultivation of these plants.

Keywords

Pioneer species, Tropical shrubs, Energy crops



This article is included in the Energy gateway.

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Introduction

The Indonesian government has issued a national energy policy to encourage the development of alternative energy. This policy targets the replacement of diesel and gasoline with biodiesel and bio-ethanol by 5% in Indonesia by 2025. The Government commissioned the Ministry of Forestry to play an active role in the development of biomass production. The use of raw material biomass to replace fossil fuels for heat and electricity production is prioritized, including permits for the utilization of plantations in the form of unproductive areas and permits for natural forest utilization^{1,2}.

Different types of forest plants and lignocellulose weeds have the potential to be developed as biomass feedstock for electricity production. Woody shrub species, such as *Vernonia amygdalina* Delile, *Piper aduncum* L., *Gliricidia sepium* (Jacq.) Kunth ex Walp., *Calliandra calothyrsus* Meissner., *Bridelia tomentosa* Blume, *Vitex pinnata* L., *Vernonia arborea* Buch.-Ham. and *Bauhinia purpurea* var. corneri de Wit., are suitable for use as sustainable raw materials for electrical energy³. This type of bush plant is a pioneer species that can easily be grown in secondary forests and open lands that have formerly been cultivated and logged. Energy crops are defined as cultivated plants that are developed and grown specifically for fuel and are rapidly growing, resistant to pests and droughts and can quickly be harvested, so they could have competitive prices if used as fuel⁴.

Wood is a renewable resource as it is sustainable and its supply will always be available. Meanwhile, cellulotic bio-fuel is produced from non-food (feed) stocks that play a critical role in reducing dependence on oil imports⁵. Woody biomass is produced, among others, by establishing plantations of energy crops. Coppice is a tree process regenerated with new shoots from the stumps that have been harvested and the Short Rotation Coppice (SRC) from hardwoods is more promising in generating biomass for bio-energy because it consists of fast-growing tree species and high-yield planted varieties (5000-15000 stems per ha), harvested in a two to six year rotation cycle.

This study focused on the growth and nutrient status of leaves of several types of energy crops from tropical shrub species with NPK fertilizer application, as a first step in the preparation of ready-to-plant if they are cultivated in the future as raw materials for biomass for renewable electricity.

Methods

Site and time

This study was conducted from January to September 2018 in a secondary forest located at Suka Damai Village, Muara Badak Sub-district, Kutai Kartanegara District, East Kalimantan province, Indonesia (00°17' to 18°2" S latitude and 117°14' to 14°39.5" E longitude). The wet season varies from 9 to 12 months and the dry season varies from 0 to 3 months. The average monthly temperature is 27.5°C and average air humidity is $82\%^6$.

Plant materials

Seedling plants (Vernonia amygdalina, Piper aduncum, Gliricidia sepium, Symplocos fasciculata, Vitex pinnata, Bauhinia

purpurea, Melastoma malabathricum, and *Calliandra caloth-yrsus)* were taken from the Mulawarman University Botanical Garden. Seedlings are included in polyethylene bags and maintained for 3 months. Those with uniform height are selected before being planted in the research plot.

Experimental design

Complete randomized block design (CRBD) was used as the experimental design. There were 8 plant species and their growth responses were evaluated based on 5 treatments using NPK fertilizer (commercial by YARA International ASA, Oslo, Norway; 16% N, 16% P₂O₅, 16% K₂O₅, 1.5% MgO, and 5% CaO). Five groups in five plots (each plant species): T0: with no fertilizer as control group (0 g/plant); T1: supplemented with NPK 40 g/plant; T2: supplemented with NPK 80 g/plant; T3: supplemented with NPK 120 g/plant; T4: supplemented with NPK 160 g/plant. The fertilizer treatments were conducted 2 weeks after sowing. There were 10 sample plants (as replication) for each treatment, a total of 50 plants for each species (divided into 5 experimental plots). Therefore, in total, there were 40 experimental plots, each plant was separated 1 m in length within each plot while it was separated 3 m in length between each plot (Figure 1).

Measured parameters

All data were recorded after 5 months of planting. Stem height was measured by retractable tape measure (Shiro, Japan), basal stem diameter was measured by Vernier digital caliper (Mitutoyo, Japan), and the number of leaves and branches of each plant were counted.

All 10 replications of each treatment were pooled into one composited plant sample. Plant materials were analyzed at Forest Soil Science Laboratory, Faculty of Forestry, Mulawarman University to determine the total nitrogen, phosphorus, potassium, calcium and magnesium concentration. Total nitrogen was estimated using the Kjeldahl method⁷. Briefly, leaves were extracted using the wet destruction method using concentrated H_2SO_4 (MERCK, Germany). The extract was distilled and added to 20 ml of 0.05 N NaOH (MERCK, Germany).

To measure the elements of P, K, Ca and Mg, the plant materials were extracted using high pressure digestion method at a temperature of 180° C for 10 hours with HNO₃ 65% (MERCK Milipore, Germany) as a reductant. The calorimetric technique used nitric acid-molybdate-vanadate (MERCK, Germany) as a coloring agent and it was determined by spectrophotometer (GENESYSTM20 Visible Spectrophotometer, Thermo ScientificTM, Thermo Electron North America LLC, 14-385-445) at a wavelength 470 nm. Meanwhile potassium, calcium and magnesium concentration were measured by Atomic Absorption Spectrophotometer (Trace 1800, Aurora Biomed, Canada) at wavelengths of 766.5, 489.5 and 245.2 nm, respectively⁵.

Data analysis

The plant growth data were expressed as mean ± standard error. The data were subjected to ANOVA, followed by Duncan's Multiple Range Test (DMRT) to evaluated significant differences among the groups of treatment. All analysis was done using SPSS 22 (SPSS Inc. USA) and all significant tests

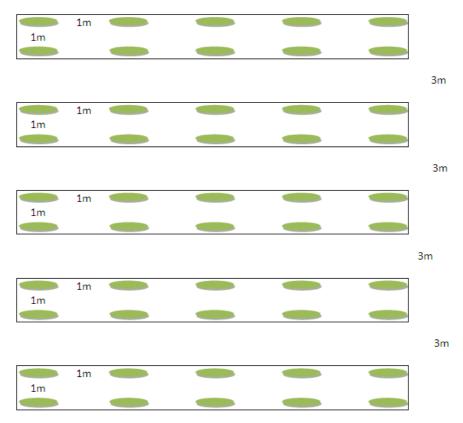


Figure 1. Seedling planting diagrams for each treatment.

were set at $p \le 0.05$. The data of leaves' nutrient concentration were analyzed descriptively.

Results

The first part was the result of planting several types of tropical shrubs, including height growth, stem diameter, number of branches and leaves, while second part included the results of the analysis of nutrient content that was accumulated on the leaves of several types of tropical shrubs, namely nitrogen, phosphorus, potassium, calcium and magnesium.

Based on Table 1, the best stem height was measured from 67.00 cm to 320.50 cm in V. pinnata and V.amygdalina, respectively, where both results were observed with T4 treatment. The best stem diameter ranged from 0.68 cm to 1.60 cm in B. purpurea and P. aduncum, respectively. The B. purpurea result was observed in T4 treatment, whereas the P. aduncum result was in T1 treatment. The highest leaf number ranged from 34.10 in B. purpurea to 271.50 in V.amygdalina. The first result from B. purpurea was observed in T1 treatment while the second from V. amygdalina was in T4 treatment. The highest branch number varied from 2.90 in B. purpurea to 12.00 in V. amygdalina. Similar to the leaves, B. purpurea result was observed in T1 treatment while V. amygdalina was different, because it was obtained from only T2 treatment. Other results on several plant species also show highest numbers however they are not significant within each species.

The results show that fertilization treatment affects the growth of superior energy tropical shrub plants. In plants *V. amygdalina, P. aduncum, V. pinnata, C. callothyrsus, S. fasciculata* and *G. sepium*, the best growth was found in T4 treatment, whereas in *M. malabathricum* and *B. purpurea*, the best growth was obtained by T1 treatment. This indicates that the response of plants varies according to the fertilization treatment. Stem height and stem diameter of the various plants after 5 months of planting are presented in Figure 2.

According to Figure 2, the highest stem height was observed in *V. amygdalina* while the lowest was in *V. pinnata, S. fasciculata* and *B. purpurea*. Moreover, the large stem diameter was measured in *P. aduncum*, while the lowest was in *V. pinnata* and *B. purpurea*.

The accumulation of nutrients in the leaves of the tropical shrub plants varied widely. The highest nitrogenous nutrients accumulated in the leaves of *M. malabatricum, C. calothyrsus* and *V. pinnata,* while the highest phosphorus and potassium nutrients accumulated in the leaves of *V. amygdalina, P. aduncum* and *G. sepium.* Calcium nutrients accumulated the most in the leaves of *P. aduncum, G. sepium* and *S. fasciculata.* On the other hand, the highest accumulation of magnesium nutrients occurred in the leaves of *B. purpurea, V. amiqdalina* and *G. sepium* (Figure 3). Full data of the effect of fertilizer applications on tropical shrubs growth (height, diameter, and

Table 1. Fertilization effect on the average number of plant height, stem diameter, and leaf and branch number. T0 = 0 g (control), T1 = 40 g, T2 = 80 g, T3 = 120 g and T4 = 160 g of NPK. Number followed by the same letter in the same column show no significant difference in the DMRT test (p=0.05) after analysis by ANOVA.

Plant species	Fertilizer treatment	Stem height	Stem diameter	Leaf number	Branch number
Vernonia amygdalina	TO	$197.90^{a} \pm 49.99$	$1.3020^{a} \pm 0.66$	$103.30^{a} \pm 38.16$	$6.50^{a} \pm 1.43$
	T1	302.10 ^{bc} ± 63.39	$1.3640^{a} \pm 0.56$	185.70 ^b ± 55.00	10.80 ^b ± 2.53
	T2	283.60 ^{bc} ± 59.95	$1.2710^{a} \pm 0.38$	230.10 ^{bc} ± 63.71	12.00 ^b ± 4.03
	T3	260.20 ^b ± 55.61	$1.0600^{a} \pm 0.26$	194.90 ^b ± 51.68	10.90 ^b ± 4.23
	T4	320.50° ± 51.15	$1.3480^{a} \pm 0.36$	271.50° ± 53.09	$10.80^{b} \pm 4.76$
Piper aduncum	TO	$61.60^{a} \pm 17.12$	$0.97^{a} \pm 0.41$	$44.10^{a} \pm 17.67$	$6.80^{a} \pm 2.62$
	T1	98.20 ^{bc} ± 31.52	1.60 ^b ± 0.66	89.80 ^b ± 70.00	11.00 ^b ± 4.08
	T2	91.80 ^{bc} ± 17.73	$1.24^{ab} \pm 0.40$	$59.30^{ab} \pm 47.45$	$6.70^{a} \pm 2.98$
	T3	$80.60^{ab} \pm 17.46$	$0.93^{a} \pm 0.22$	$33.90^{a} \pm 13.08$	$5.00^{a} \pm 2.31$
	T4	107.40° ± 36.69	$1.33^{ab} \pm 0.48$	$47.50^{a} \pm 30.10$	$5.30^{a} \pm 2.45$
Vitex pinnata	TO	$35.90^{a} \pm 14.40$	$0.44^{a} \pm 0.13$	$20.30^{a} \pm 9.55$	$1.40^{a} \pm 1.35$
	T1	$54.20^{ab} \pm 12.95$	$0.58^{a} \pm 0.15$	$19.00^{a} \pm 7.99$	$1.70^{a} \pm 0.68$
	T2	$55.50^{ab} \pm 16.47$	$0.53^{a} \pm 0.11$	$17.90^{a} \pm 6.57$	$1.30^{a} \pm 0.48$
	T3	$54.20^{ab} \pm 25.07$	$0.56^{a} \pm 0.16$	$15.00^{a} \pm 4.81$	$1.40^{a} \pm 0.70$
	T4	67.00 ^b ± 46.01	$0.56^{a} \pm 0.21$	$16.50^{a} \pm 7.74$	$1.20^{a} \pm 0.79$
Calliandra calothyrsus	TO	$96.60^{a} \pm 60.24$	$0.69^{a} \pm 0.37$	$13.70^{a} \pm 7.85$	$2.00^{a} \pm 1.41$
	T1	$142.80^{ab} \pm 70.28$	$0.96^{ab} \pm 0.37$	$23.50^{ab} \pm 13.97$	$1.50^{a} \pm 0.97$
	T2	$102.50^{a} \pm 46.97$	$0.71^{a} \pm 0.25$	$20.70^{ab} \pm 12.69$	$1.10^{a} \pm 0.57$
	Т3	163.70 ^b ± 53.25	$1.10^{b} \pm 0.40$	$30.50^{ab} \pm 26.86$	$1.90^{a} \pm 1.66$
	T4	192.10 ^b ± 63.24	$1.00^{ab} \pm 0.34$	38.40 ^b ± 24.56	$1.60^{a} \pm 0.84$
Melastoma malabathricum	TO	$75.70^{a} \pm 12.54$	$0.73^{a} \pm 0.19$	$76.00^{a} \pm 36.62$	$7.30^{ab} \pm 4.24$
	T1	127.10 ^b ± 57.89	$0.97^{b} \pm 0.27$	129.60 ^b ± 78.96	$8.80^{\circ} \pm 3.85$
	T2	$99.30^{ab} \pm 20.04$	$0.85^{ab} \pm 0.24$	$95.00^{ab} \pm 27.38$	$6.10^{ab} \pm 2.69$
	Т3	$89.40^{a} \pm 32.85$	$0.68^{a} \pm 0.28$	$67.30^{a} \pm 42.36$	$4.60^{a} \pm 1.51$
	Τ4	$83.60^{a} \pm 26.65$	$0.71^{a} \pm 0.23$	$66.20^{a} \pm 40.86$	$5.40^{a} \pm 4.17$
Symplocos fasciculata	TO	$39.30^{a} \pm 10.82$	$1.06^{a} \pm 0.29$	$29.20^{a} \pm 14.22$	$3.40^{a} \pm 1.35$
	T1	$51.60^{b} \pm 17.53$	$1.02^{a} \pm 0.38$	$39.50^{a} \pm 10.82$	$3.40^{a} \pm 1.08$
	T2	65.40° ± 13.18	$1.21^{a} \pm 0.30$	$58.50^{\circ} \pm 19.90$	$5.00^{a} \pm 2.21$
	Т3	67.60° ± 14.74	$1.19^{a} \pm 0.34$	$45.90^{ab} \pm 22.16$	$3.30^{a} \pm 2.21$
	Τ4	$77.20^{\circ} \pm 7.41$	$1.05^{a} \pm 0.24$	60.50 ^b ± 25.91	$3.50^{a} \pm 1.58$
Bauhinia purpurea	TO	$44.40^{a} \pm 16.47$	$0.45^{a} \pm 0.13$	$15.90^{a} \pm 10.81$	$2.10^{ab} \pm 1.10$
	T1	$79.50^{\circ} \pm 40.11$	$0.63^{\text{ab}} \pm 0.30$	34.10 ^b ± 23.28	$2.90^{b} \pm 2.69$
	T2	$66.60^{ab} \pm 34.31$	$0.63^{ab} \pm 0.17$	$23.50^{ab} \pm 8.67$	$1.30^{a} \pm 0.82$
	Т3	$46.10^{a} \pm 15.47$	$0.49^{ab} \pm 0.19$	$22.50^{ab} \pm 8.00$	$1.50^{ab} \pm 0.85$
	Τ4	$69.50^{ab} \pm 30.58$	$0.68^{b} \pm 0.27$	$20.10^{a} \pm 13.92$	1.10 ^a ± 1.20
Gliricidia sepium	ТО	$80.00^{a} \pm 11.92$	$1.05^{a} \pm 0.39$	$16.75^{a} \pm 5.56$	$3.50^{a} \pm 1.92$
	T1	162.00 ^b ± 44.53	$1.37^{a} \pm 0.54$	$50.67^{b} \pm 28.57$	$4.67^{a} \pm 1.53$
	T2	179.25 ^b ± 50.61	$1.67^{a} \pm 0.46$	62.00 ^b ± 21.91	$4.75^{a} \pm 2.22$
	Т3	170.33 ^b ± 52.65	$1.65^{a} \pm 0.31$	$47.00^{\circ} \pm 19.47$	$4.67^{a} \pm 0.58$
	T4	192.50 ^b ± 46.88	$1.64^{a} \pm 0.34$	59.25 ^b ± 5.85	$4.75^{a} \pm 1.26$

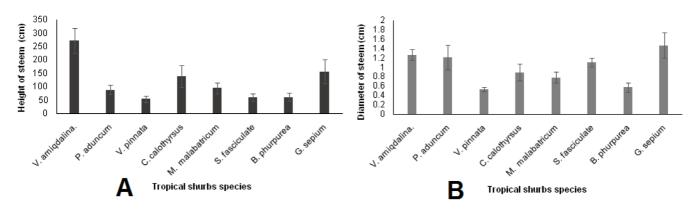
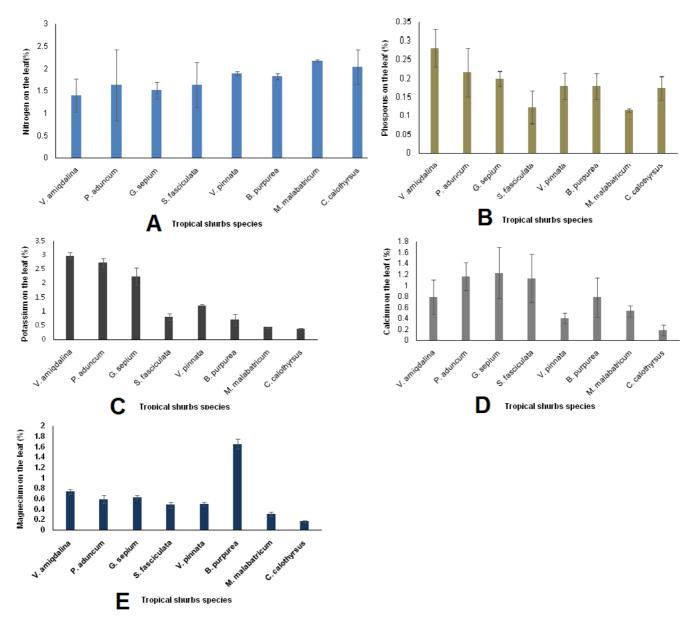
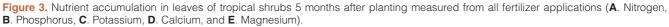


Figure 2. (A) Height and (B) diameter comparison of tropical shrub stems 5 months after planting measured from all fertilizer applications.





leaf and branch number) and leaves' nutrient concentration (N, P, K, Ca and Mg) are available⁸.

Discussion

In this study, the growth and accumulation of nutrients in the leaves of tropical shrub plants varied greatly. The tropical shrubs that grew the best were V. amygdalina, G. sepium and C. calothyrsus, which accumulated mostly phosphorus and potassium nutrients in the leaves, while nitrogen, calcium and magnesium were least accumulated. These plants responded to NPK fertilizer up to 160 g per plant, resulting in best growth and high production of biomass, showing their suitable as raw material for biomass to create electricity. A previous study has shown that V. amygdalina provides 2.25 MWh, G. sepium 2.08 MWh and C. calothyrsus 2.01 MWh per ton of dry biomass³. As reported by Susanto and Amirta, fast-growing pioneer species such as M. gigantea absorbs the most potassium reaching 35% in every ton of plant biomass^{6,9}. For V. amygdalina, growth parameters are positively correlated to rainfall, relative humidity and cloud cover¹⁰. On the other hand, G. sepium can be harvested at a residual height of 70 cm, with better agronomic characteristics and chemical composition occurring in the fall¹¹. C. calothyrsus also has good growth in planting plots in previous research¹², and it was previously reported that mycorrhizae such as Glomus sp. and Acaulospora sp. have significant influence on its height. In Colombia, planted fallows using C. calothyrsus have an additional benefit of producing large quantities of wood for household use¹³. Based on the growth and nutrient analysis in the present study, we believe that these tree plants species can be developed widely to support a sustainable supply of biomass feedstock for the green electricity program in Indonesia.

The growth of five plants species, namely M. malabatricum, P. aduncum, V. pinnata, B. purpurea, S. fasciculata was lower (less than half) than the three plants species discussed earlier (Table 1). In the present study, several plant species that grew slower actually accumulated more nutrients of nitrogen and phosphorus in the leaves, such as S. fasciculata, P. aduncum, V. pinnata, B. purpurea and M. malabatricum, P. aduncum and S. fasciculata also accumulated a large amount of potassium and calcium nutrients in their leaves. Moreover B. purpurea accumulated magnesium mostly in its leaves (Figure 3). For M. malabathricum, the availability of phosphorus and aluminum in the rhizosphere increases its growth, it can also adapt to low soil pH¹⁴ and absorb heavy metals in contaminated soils¹⁵. On the other hand, the mean foliar aluminum concentration in wild plants of M. malabathricum had positive correlation with foliar calcium, total nitrogen, calcium and magnesium concentrations within this species¹⁶ while Symplocos sp. mean foliar aluminum concentrations were detected at 4107 $(\pm 1474 \text{ mg kg}^{-1})$ and 4290 $(\pm 4025 \text{ mg kg}^{-1})$ for seedlings and saplings, respectively¹⁷. P. aduncum can be propagated with seeds and shoot cuttings¹⁸ and can accumulate large amounts of potassium, as previously reported¹⁹; at 23 months, it had accumulated 222 kg N, 50 kg P, 686 kg K, 255 kg Ca, 75 kg Mg, and 24 kg S ha⁻¹. More than half of the P, K, Ca and Mg nutrients were found in the stem (wood). Its leaf litter is significant and

becomes an easily decomposable source of potassium, but *G. sepium's* leaf litter contains much nitrogen¹⁸ while *B. purpurea*, which is a light-demanding tree, only grew 25% in a shady house with full sunlight²⁰. Therefore, each tropical shrubs species in this study varies in accumulating plant nutrients N, P, K, Ca and Mg in their leaves.

Based on this study, it is necessary to pay attention to the fast developing plant type, especially for tropical shrubs that have the potential as raw materials for biomass energy. Many beneficial nutrients also accumulated mostly in the leaves. This accumulation reflects the nutrient requirements of those plants, which will be cultivated as energy raw materials; therefore at the initial stage, the demand for plant fertilizer can be predicted. The carrying capacity of soil nutrients requires serious attention for the sustainment of plant cultivation. These wood shrubs species (*V. amygdalina, G. sepium* and *C. calothyrsus*) were also able to re-grow naturally by generation more than single shoots on their coppice trees. The scheme of Short Rotation Coppices (SRC) was an effort to achieve forest energy plantation using fast growing trees and wood shrubs species for aiming for a sustainable cycle³.

Conclusion

Of the eight types of tropical shrubs in this study, three species, namely *V. amygdalina, G. sepium* and *C. calothyrsus,* had the best growth and could potentially be developed as Energy Crops. The most accumulated nutrients in the leaves of these three species of plants are phosphorus and potassium.

Data availability

Open Science Framework: Growth Evaluation of Several Types of Energy Crops from Tropical Shrubs Species, https://doi.org/10.17605/OSF.IO/3G8FH⁸.

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

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Nagib Ullah Khan 匝

Department of Plant Breeding and Genetics, Khyber Pakhtunkhwa Agriculture University, Peshawar, Pakistan

I have gone through the article entitled "Growth evaluation of several types of energy crops from tropical shrubs species" and found it fit for indexing after minor revision. It is a good study, paper is well written, however, there are quite a few grammatical mistakes. It would be better if the language and grammar were checked throughout the text.

Paper can be Approved after the incorporation of all comments given below:

- 1. The title is good and interesting for readers.
- 2. Rewrite the appropriate abstract, lots of similar work is done on this study. What is new in your paper?
- 3. Overall, the introduction is written poorly. It seems to review the literature. The author failed to justify the need for his research by giving logical arguments. He has cited old references with well-established facts.
- 4. Needs to add some more recent references. I suggested some references below:

Fitmawati, Desti, Juliantari E, Novela D, Kapli H (2022)¹. Abduova AA, Kupriyanov OA, Yessengeldi A, Kupriyanov AN, Sataev MI, Moshkalov BM (2022)². Krishna T.P.A., S. Antony Ceasar, T. Maharajan, M. Ramakrishnan, V. Duraipandiyan, N.A. Al-Dhabi and S. Ignacimuthu. 2017. Haq, I.U., M. Riaz, A. Nawaz, A.U. Rehman, H. Mukhtar and Q.U.A. Syed. 2020³. Asad, W., T. Kiran, F. Saleem, S. Siddiqui and S.A. Rasool. 2021⁴. Nan, L.L. and Q.E. Guo. 2021⁵. Bauyrzhan, T., K. Natalya, I. Zarina, K. Andrey, K. Meruert, A. Karime and B. Aliya. 2022⁶.

5. The author analyzed the nutrient concentration only in the leaves. Did not give about stem

and roots? It is recommended to write a few lines. Also write about the biomass of each plant part.

6. The entire discussion section is lacking an in-depth look at what the driving factors for the results were. Most of this section appears to be a literature review of what others have done and does not draw strong arguments to the data presented. This is in direct contradiction with several statements. This section needs a complete rewrite.

References

1. Fitmawati, Desti, Juliantari E, Novela D, et al.: Molecular phylogeny Of Nibung (Oncosperma tigillarium [Jack] Ridl.) inferred from trnL-F intergenic spacer sequences. *SABRAO Journal of Breeding and Genetics*. 2022; **54** (1): 175-183 Publisher Full Text

2. Abduova A, Kupriyanov O, Yessengeldi A, Kupriyanov A, et al.: Coenoflora of Spiraeanthus Schrenkianus (Fisch. And C.A. Mey.) Maxim.*SABRAO Journal of Breeding and Genetics*. 2022; **54** (1): 184-192 Publisher Full Text

3. Haq I, Riaz M, Nawaz A, Rehman A, et al.: Enzymatic hydrolysis of Saccharum officinarum Lignocellulosic biomass by genetically modified hyperthermophilic cellulases. *Pakistan Journal of Botany*. 2020; **52** (1). Publisher Full Text

4. Asad W, Kiran T, Saleem F, Siddiqui S, et al.: Co-optimization of B. licheniformis 208 biomass and alpha amylase synthesis using response surface methodology. *Pakistan Journal of Botany*. 2021; **53** (6). Publisher Full Text

5. Nan L, Guo Q: Distribution of soil seed reserve and its association with aboveground vegetation in salinized soils of arid regions in northwest China. *Pakistan Journal of Botany*. 2021; **53** (2). Publisher Full Text

6. Bauyrzhan T, Natalya K, Zarina I, Andrey K, et al.: Phytochemical study of Crambe tatarica Sebeok, a promising source of medicinal plant extracts. *Pakistan Journal of Botany*. 2022; **54** (2). Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature? $\ensuremath{\mathsf{Yes}}$

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others? $\ensuremath{\mathsf{Yes}}$

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility? Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Crop Breeding and Research...

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 31 October 2019

https://doi.org/10.5256/f1000research.19754.r46151

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Y. Nornasuha

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- The abstract needs to have a clear result of the metal elements instead of using 'a lot'.
- Introduction: I would suggest the authors add some description or mechanism of plants producing energy or electricity. This is optional.
- The method is fine.
- Results: the author analyses the nutrient concentration in the leaves. How about the stem and roots? Is there any specific reason to not include the nutrient concentrations in the stem and roots?
- How about the biomass of each plant part?
- The conclusion is fine.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others? Partly

F1000 Research

If applicable, is the statistical analysis and its interpretation appropriate? Yes

Are all the source data underlying the results available to ensure full reproducibility? Partly

Are the conclusions drawn adequately supported by the results? Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Ecology, Botany, Biodiversity and Plant Eco-physiology

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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