Solanum scabrum response to application of distilled Cassava with Soluble as Biofertilizer

***Ola E.T.[1], Olaifa G. O.[2], Oyedele O.J.[1], Ibrahim. T.H[1]. Taiwo, F.F.[3], Adeyemo. B. J.[1] and Ewuola. T. N.[1]**

[1]Bioresources Development Centre, Ogbomoso, Oyo State, Nigeria; [2] Aquatic Bioresources Development Centre, Ode-Ajagba, Ondo State, Nigeria
[2] Aquatic Bioresources Development Centre, Ilesha, Osun State, Nigeria

Corresponding Author: Gabriel4olaifa@gmail.com

Abstract
Solanum scabrum is a vegetable widely grown in Nigeria. Its production is affected by the source and quality of nitrogen fertilizer. Plant height and yield vary with the different fertilizers and rates of application. This study aimed at investigating the response of *Solanum scabrum* to the application of Distilled Cassava with Soluble as a biofertilizer. The experiment was laid out in a Complete Randomize Design (CRD) with four treatments: T1: without fertilizer, T2: 160g NPK 15:15:15, T3: whole distilled cassava with soluble (WDCS) 5 kg and T4: Liquid distilled cassava with soluble (LDCS) 10 litres. The experimental site was sandy loam. 20 g of *Solanum scabrum* seeds were planted into the beds of 3m x 3m each using the drilling method. The plant height and yield of T2 were significantly ($P<0.05$) higher than T1, T3 and T4 due to the relative abundance supply of nitrogen from NPK 15:15:15. Plants treated with T3 and T4 produced better results compared to T1 in terms of plant height and yield. Nitrogen application and source were shown to significantly ($P\leq0.05$) affect plant height and yield. Application of WDCS (T3) and LDCS (T4) also produced better results in terms of plant height and yield compared to inorganic fertilizer (T2) and hence can serve as alternative inorganic fertilizer to small scale famers with inadequate capital who may not be able to buy inorganic fertilizers in large quantities as required during production period.

Keywords: *Solanum scabrum*, whole distilled cassava with soluble (WDCS), Liquid distilled cassava with soluble (LDCS), yield

Introduction

The contribution of vegetables universally to the human diet cannot be over-emphasized (Daunay and Hazra, 2012) Vegetable production contributes not in a small amount to the food security of many families of the world (Asongwe *et al*., 2014). Vegetable farming started in some countries of the world about ten thousand years ago, with families producing vegetables for consumption and sale (Thompson, 2007). *Solanum scabrum* otherwise known as African nightshade, is an annual or perennial crop intensively cultivated in many countries of the world including Nigeria (Fontem and Schippers, 2004; Nfor *et al*., 2014). In Nigeria particularly among the Yorubas, *Solanum scabrum* is called Efo Ogunmo. The vegetable plays an important role in the nutritional diet of Nigerians. It can be consumed in different forms either as a principal meal or with other foods. Today, *S. scabrum* serves as an alternative to fish and meat diets which attract very high prices. It is a source of protein, vitamins and iron for many poor families. (Abukutsa *et al*., 2010). *Solanum scabrum* has also been reported to possess medicinal properties and health benefits and this has been documented to cure stomach aches, cancer, diabetes and cardiovascular diseases (Strandhagen, 2000). Presently, the increase in human population and urbanization has increased people's demand for agricultural products with land becoming a constraint. The traditional system of cultivation, such as bush fallowing and shifting cultivation in Nigeria has given way to a continuous system of cropping resulting in the gradual reduction of soil fertility and crop yield (Alimi *et al*., 2006). Fertilizer application from various sources has been reported as one of the ways to solve the problem of a decrease in soil fertility and low productivity in agriculture (Smaling *et al*., 2006). The demand for *S. scabrum* had been reported increasing progressively and its supply has not been able to satisfy demand. To this end, various reasons such as inherently poor and or degraded soils have been attributed to the low supply of *S. scabrum* in many of our local markets in Nigeria (Kaho *et al*., 2001). Producing mature vegetables in commercial quantities in Nigeria required fertilizer application in sufficient quantities.

Thus, the need for the application of fertilizer to enhance soil fertility and at the same time improve land productivity is an important issue among farmers. The Food and Agricultural Organization (FAO 2000) defined fertilizer as any natural or manufactured mineral, which contains at least 5 % of one or more of the three primary nutrients that is Nitrogen, Phosphorus and Potassium (NPK). Fertilizers can be categorized as organic or inorganic. Organic fertilizers are made from natural materials and contain carbon while inorganic are man-made and consist of mined minerals or synthetic nutrients but do not contain carbon. The use of organic fertiliser has been resulting in a doubling of yields and thus contributing to an increase in the food security of a region. Distilled cassava with soluble (DCS) is a residual product from the distillation column of ethanol processes when cassava is used as a feedstock for producing ethanol. (Anil *et al*., 2018). It consists of water, organic matter, organic acids and mineral salts (Moran-Salazar *et al*., 2016). More importantly, DCS is classified as non-hazardous bioresources with greater economic value as a result of the presence of residual sugars, yeasts, and mineral elements including nitrogen, phosphorus, potassium, calcium, magnesium etc. (EPA, 2016; Anil Reis and Hu, 2017; Anil *et al*., 2018).

Presently, the high cost of chemical fertilizers and associated environmental hazards have been great incentives to explore the application of vinasse as organic liquid fertilizer in conservation agriculture (Renato *et al*., 2013; Ratna *et al*., 2017). Studies have been carried out to investigate the use of sugarcane-based vinasse as fertilizers and irrigation (Carpanez *et al*., 2022). The use of inorganic or organic fertilizers is on the increase as a solution to a decrease in soil fertility and crop yield. It had been reported that the use of synthetic fertilizer in agricultural production increased tremendously after the Green Revolution as a panacea to decrease in soil fertility (Goutam *et al*., 2011). The excessive use of chemical fertilizers causes serious health implications and unforeseen environmental Impacts. Also, the misuse of inorganic fertilisers to achieve better yields

without considering the side effects to consumers has caused people to shift demand to more organic vegetables with little or no side effects after consumption and hence better living. There is a dearth of information on distilled cassava with soluble in the literature. This study therefore attempts to assess the response of *Solanum scabrum* to the application of distilled cassava with soluble as Bio fertilizer.

Materials and Methods

The research was carried out at the Bioresource Development Centre, Ogbomoso, Oyo State Nigeria. Ogbomoso is on latitude 8" 10N and longitude 4"10E at 420 meters above sea level and has a bimodal rainfall pattern (1,150mm – 1,250mm per year) with the peak in July and September and a short rainfall break in August. The dry season lasts from November to March. It has a minimum temperature of 280C and a maximum temperature of about 740C all year round except in January when the dry winds blow from the North.

Experimental design

The plot of land 12 m x 30 m used for this study was cleared and raked manually using a hoe and cutlass. The field was then divided into three rows of 8 beds per row (each bed of 3 m x 3 m with 0.5 m spacing in between the plots) and allotted to four treatments in a complete randomized design: T1: without fertilizer, T2: 160kg NPK 15:15:15, T3: WDCS 5 Kg and T4: LDCS 10 litres. NPK fertilizer (15:15:15) used for this study was sourced in one of the farmer's centre in Ogbomoso. Whole distilled cassava with soluble (WDCS) for the study was collected from the Ethanol Plant located at the Bioresources Development Centre Ogbomoso, Oyo State. The WDCS was centrifuged into four (10 litres) buckets out of which the required quantity of Liquid distilled cassava with soluble (LDCS) and WDCS for the experiment were obtained. Vegetable beds 18 cm high were made with hoe. The experimental site was sandy loam. *Solanum scabrum* seeds (20 g) were planted into the beds using the drilling method. NPK fertilizer was applied using the broadcast method while the LDCS was also spread on the vegetable beds using a watering can.

Hand weeding was carried out every week with no herbicide application. Data were collected on plant height using measuring tape every week for 16 weeks while the total yield of *Solanum scabrum* was weighed at the termination of the experiment using a 20kg Camry Kitchen Scale.Test option of SAS (2008) was used to detect significant differences among means.

Statistical Analysis

Data were presented as the mean ± standard error (SEM). Data obtained were analyzed with the General Linear Model of SAS (2008) and the Duncan New Multiple Range The experiment was rain-fed and terminated at the end of sixteen weeks.

Results and Discussion

Table 1 shows the weekly response of *Solanum scabrum* to different fertilizer applications on plant height (cm). There was a consistent increase in the plant heights (cm) of *Solanum scabrum* for all treatments from week 1 to 16 even after the first harvest at week 9 was carried out. Plants treated with T2 had the highest plant height throughout the period of the study. This was followed by T1 and T3. The lowest plant height was recorded for plots treated with T4. At week 9, the plot treated with T2 had a plant height of 26.51cm, this was followed by a plant treated with T1 (22.05cm) and T3 with a plant height of 21.28cm. The lowest plant height 18.36cm was recorded for plots treated with T4. A similar trend was observed in all the treatments from weeks 10 to 15. The result of this study shows that the application of NPK fertilizer improved the vegetative growth of the plant because the fertilizer resulted in an increase in the plant height of the *Solanum scabrum* plant when compared with other treatments. These results corroborate the findings of Overcash *et al*. (2005). They reported that plant growth characteristics such as plant height, number of leaves and leaf area strongly respond to nitrogen applications. It stimulates vegetative growth resulting in large stems and leaves due to its effect on cell division and elongation.

Table 1: Weekly response of *Solanum scabrum* plant height (cm) to fertilizers application.

	Treatment			
Week	T1	T2	T3	T4
Week 1	0.86	0.89	0.78	0.89
Week 2	1.34	1.45	1.27	1.23
Week 3	3.15	4.34	2.83	2.04
Week 4	6.8	10.04	6.47	5.26
Week 5	11.45	21.47	10.48	8.47
Week 6	15.78	27.18	15.59	12.62
Week 7	16.71	34.85	16.12	13.82
Week 8	17.07	20.51	16.29	12.11
Week 9	22.05	26.51	21.28	18.36
Week 10	15.63	20.61	13.54	11.09
Week 11	20.43	29.7	17.4	14.84
Week 12	25.43	41.63	23.35	21.89
Week 13	33.18	52.63	29.7	29.33
Week 14	36.6	59.34	39.45	35.77
Week 15	36.65	54.25	34.93	34.48
Week 16	40.16	61.43	43.25	45.78

T1: Without fertilizer; T2: NPK 15:15:15; T3: Whole distilled cassava with soluble (WDCS) 5 kg; T4: Liquid distilled cassava with soluble (LDCS) 10 litres.

This work agreed with that of Olabiyi and Ojetayo (2010) who reported that the application of fertilizer gave vigorous development in plant vegetative parts due to the release of a considerable amount of nutrients for plant use which is considered to be essential for the formation of plant essential molecules.

In our study, African nightshade plants subjected to organic fertilizers showed signs of nitrogen deficiency during early growth stages due to the slow release nature of the fertilizer. For all the growth indices measured, there appeared to be a dose-dependent inhibitory effect of DCS application on *Solanum scabrum* growth even though most heavy metals (Cu, Zn, Fe, and Cr) in the DCS were below the threshold limits set by the Food and Agriculture Organization (FAO, 1985). The 5 kg / bed of WDCS application had very little positive impact while the 10 L/bed dose actually had negative effect on the plant growth when compared with the control. The dose-dependent inhibition could be attributable to vinasse (cassava) acidity at pH 4.2-4.8 and the fact that excess vinasse usage could cause leaching of nutrients and cationic imbalance due to its high water content (approx. 93%). This observation was corroborated by Maradiaga-Rodriguez *et al*. (2018). Compared to inorganic fertilizers, organic fertilizers are not readily available for plant uptake because mineralization has to take place for nutrients to be available. Inorganic fertilizers are already in a states that make it easy for plants to take up the nutrients.

scabrum treated with the different fertilizers for week 3 to week 16.increase in plant heights (T2) in this study is attributed to NPK fertilizer ability to release nutrient elements quickly for plant uptake. This result also support the findings of Olatunji and Ayuba (2013) who reported that application of NPK fertilizer results in higher plant height and better yield. The result (T3 and T4) obtained in this study also corroborates Maradiaga-Rodriguez *et al*., 2018 who reported that the presence of high organic matter content and carbon to nitrogen ratio in the soil can cause mineral especially nitrogen immobilization and lowers their bioavailability (Parnaudeau *et al*., 2008).

Table 3 shows the ANOVA on the effect of different fertilizers application on the mean plant height (cm), total yield/bed (kg) and total seed harvested (kg) of *Solanum scabrum*. There was significant difference ($p<0.05$) in mean plant height (cm), total yield/bed (kg) and total seed harvested of *Solanum scabrum* treated with different fertilizers. The mean plant height (29.18cm), total yield/bed (5.62 kg) and total seed harvested (6.25 kg) of *Solanum scabrum* treated with NPK 15:15:15 (T2) were significantly ($p<0.05$) higher than plot treated with T1, T3 and T4. The best values indices were associated with the NPK fertilizer application. This could be attributable to the quantity of each of N, P, K, and their respective bioavailability.

Table 2: ANOVA result of the weekly response of *Solanum scabrum* plant heights (cm) to different fertilizers application.

Week	Treatment				
	T1	T2	T3	T4	Probability
Week 1	0.86±0.07^{a}	0.89±0.07^{a}	0.78±0.05^{a}	0.89±0.08^{a}	0.61
Week 2	1.34±0.10^{a}	1.45±0.11^{a}	1.27±0.09^{a}	1.23±0.07^{a}	0.38
Week 3	3.15±0.27^{b}	4.34±0.35^{a}	2.83±0.23^{b}	2.04±0.18^{b}	<.0001
Week 4	6.80±0.58^{b}	10.04±0.89^{b}	6.47±0.58^{b}	5.26±0.44^{b}	<.0001
Week 5	11.45±0.82^{b}	21.47±1.09^{a}	10.48±0.96^{bc}	8.47±0.76^{c}	<.0001
Week 6	15.78±1.16^{b}	27.18±1.33^{a}	15.59±1.50^{b}	12.62±1.48^{b}	<.0001
Week 7	16.71±1.58^{b}	34.85±2.82^{a}	16.12±2.03^{b}	13.82±1.65^{b}	<.0001
Week 8	17.07±1.06^{b}	20.51±1.24^{a}	16.29±1.61^{b}	12.11±0.72^{c}	<.0001
Week 9	22.05±1.01^{b}	26.51±1.47^{a}	21.28±1.79^{b}	18.36±1.29^{b}	0.001
Week 10	15.63±0.58^{b}	20.61±1.14^{a}	13.54±1.14^{bc}	11.09±0.95^{c}	<.0001
Week 11	20.43±0.97^{b}	29.70±1.39^{a}	17.40±1.42^{bc}	14.84±1.176^{c}	<.0001
Week 12	25.43±1.13^{b}	41.63±1.88^{a}	23.35±1.96^{b}	21.89±1.59^{b}	<.0001
Week 13	33.18±2.03^{b}	52.63±1.83^{a}	29.70±2.10^{b}	29.33±1.75^{b}	<.0001
Week 14	36.60±2.04^{b}	59.34±1.75^{a}	39.45±3.14^{b}	35.77±2.13^{b}	<.0001
Week 15	36.65±2.32^{b}	54.25±2.81a	34.93±3.36^{b}	34.48±2.10^{b}	<.0001
Week 16	40.16±2.13^{b}	61.43±2.31^{a}	43.25±2.98^{b}	45.78±2.62^{b}	<.0001

$^{a, b, c}$: Means within each row with different superscript are significantly different ($p<0.05$). T1: Without fertilizer; T2: NPK 15:15:15; T3: Whole distilled cassava with soluble (WDCS) 5 kg; T4: Liquid distilled cassava with soluble (LDCS) 10 litres

Table 2 shows the Analysis of Variance (ANOVA) on the weekly response of different fertilizers application on plant height (cm) of *Solanum scabrum*. There was no significant difference ($p>0.05$) in plant height (cm) of *Solanum scabrum* treated with the different fertilizers at week 1 and week 2. Furthermore, there was significant difference ($p < 0.05$) in the plant height (cm) of *Solanum*

The application of 5kg/bed of WDCS application had very little positive impact although, 10 L/bed of LDCS dose actually had negative effect on the plant growth relative to the control.The dose-dependent inhibition could be attributed to DCS acidity at pH 4.2-4.8 and the excess vinasse usage causing leaching of nutrients and cationic imbalance due to its high water content. (Parnaudeau *et al*., 2008).

Table 3: ANOVA of the effect of different fertilizers application on the mean plant height, total yield/bed and total seed harvested of Solanum scabrum

Parameter	Treatment				Probability
	T1	T2	T3	T4	
Mean plant height (cm)	18.96±0.65^{b}	29.18±0.98^{a}	18.30±0.74^{b}	16.77±0.69^{b}	<0.0001
Total yield/bed (Kg)	1.14±0.62^{b}	5.62±1.64^{a}	1.35±0.57^{b}	0.39±0.16^{b}	0.003
Total seed harvested	2.12±0.20^{b}	6.25±0.79^{a}	2.57±0.83^{b}	1.62±0.41^{b}	0.0001

$^{a, b, c}$: Means within each row with different superscript are significantly different ($p< 0.05$). T1: Without fertilizer; T2: NPK 15:15:15; T3: Whole distilled cassava with soluble (WDCS) 5 kg; T4: Liquid distilled cassava with soluble (LDCS) 10 L

Conclusion

There is an increase in the demand for vegetables and for the vegetable farmers to meet the consumers demand in the face of gradual decreasing in soil fertility, there is need for incorporation of NPK fertilizer along with other organic fertilizers. *Solanum scabrum* performed better with the application of organic fertilizer. The results obtained in this study also revealed that the application of WDCS and LDCS produced good results in terms of plant height and yield compared to inorganic fertilizer and thus can serve as good alternative to small scale famers with inadequate capital who may not be able to purchase inorganic fertilizers in large quantities require during production period.

Recommendation

The application of fertilizers whether inorganic or organic at different rates increases plant height and yield of vegetables by increasing the nitrogen content available for the plant utilization. However, the application of WDCS and LDCS might cause inhibitory effect on plants height and yield at higher dose application. Application of NPK (15:15:15) to *Solanum scabrum* resulted in higher plant heights and total yield/bed (kg). Further research could also be carried out to investigate the effect of combining protein-rich manure (poultry dropping, cow dung, etc.) with DCS as a way of reducing the latter's high carbon to nitrogen ratio.

Declaration of interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgement

We acknowledge Prof. Abdullahi Mustapha, DG/CEO of the National Biotechnology Development Agency (NABDA) Abuja.

References

1. Abukutsa, M.O.O., Kavagi. P., Amoke. P and Habwe. F.O. (2010). Iron and protein content of priority. African Indigenous Vegetables in the Lake Victoria Basin. Journal of Agricultural Science and Technology. 2(1): 67-69.
2. Adesina.S,K.and Gbile.Z.O. (1984). Steroidal Constituents of *Solanum scabrum* subsp. nigericum. Fitoterapia. 55(6): 382-383.
3. Alimi, T., Ajewole, O. C. and Olubode-Awosola, O.O and Idowu. E.O. (2006). Economic rationale of commercial organic fertilizer technology ib vegetable production in Osun State of Nigeria. Journal of Applied Horticulture 8(2): 159-164.
4. Anil, K. M., Amith, A., Kiran, K. M. and Rajeev, K. S. (2018). Lignocellulosic Biorefinery Wastes, or Resources? In Bhaskar, T., Pandey, A., S., V. M., Lee, D.-J. andKhanal, S. K. (Eds.), Waste Biorefinery (pp. 267-297): Elsevier B. V.
5. Asongwe. G., Aaron. S., Bernard. P. and Yerima. B (2014). Vegetable production and the livelihood of farmers in Bamenda municipality, Cameroon.
6. Carpanez, T. G., Moreira, V. R., Assis, I. R. and Amaral, M. C. S. (2022). Sugarcane vinasse as organo-mineral fertilizers feedstock: Opportunities and environmental risks. Science of the Total Environment, 154998.
7. Daunay. M.C. and Hazra. P. (2012). Eggplant. In Peter KV, Hazra P (eds). Handbook on Vegetables, Studium Press, Houston, TX, pp.257-332.
8. EPA. (2016). Resource Conservation and Recovery Act (RCRA) Regulations. Retrieved from http://www.epa.gov/rcra/resource-conservation-and-recovery-act-rcraregulations#nonhaz. on 10/08/2022
9. Food and Agriculture Organization of United Nations (2000): Forestry and Food Security Contribution to Household food Security: The Role of Forest-based income.
10. FAO. (1985). Water Quality for Agriculture. Rome, Italy: Food and Agriculture Organization.
11. Fontem. D. A. Schippers. RR (2004). *Solanum scabrum* Mill. In: Grubben GJH Denton OA (eds). Plant Resources of Tropical Africa 2. Vegetable PROTA. Foundation Wageningen/CTAWageningen/Backhuys Publishers, Leiden, Netherlands, pp493-498.
12. Goutam. B. Chanda. G.K and Chakraborty. S.K. (2011). The effects of Vermicompost and other fertilizers on cultivation of huckleberry plants. Journal of Horticulture and Forestry. 3(2): pp42=45.
13. Khao. F.,Yemefack. M., Feujio-Teguefouet. P. etTchntchoang. J.C. (2011). Effet combine des feuill des thithonia diversifolia et des engrais inorganiques sur les rendements du mais et les proprieties d un sol ferralitique au Centre Cameroun TROPICULTURA. 2011. 29, 1, 39-45.
14. Luz Selene Buller, Romero, C. W. d. S., Lamparelli, R. A. C., Ferreira, S. F., Bortoleto, A. P., Mussatto, S. I. and Forster-Carneiro, T. (2021). A spatially explicit assessment of sugarcane vinasse as a sustainable by-product. Science of the Total Environment, 765, 142717.
15. Maradiaga-Rodriguez, W. D., Pêgo-Evangelista, A. W., Júnior, J. A. and Costa, R. B.-d. (2018). Effects of vinasse and lithothanmium application on the initial growth of sugar cane (*Saccharum sp*. cv. RB 86-7515) irrigated and not irrigated. Acta Agronomica, 67, 252-257.
16. Moran-Salazar, R. G., Sanchez-Lizarraga, A. L., Rodriguez-Campos, J., Davila-Vazquez, G., Marino-Marmolejo, E. N., Dendooven, L. and Contreras-Ramos, S. M. (2016). Utilization of vinasses as soil amendment: consequences and perspectives. Springerplus, 5, 1-11.
17. Nfor, T., Engwali, F. and Ajong D. (2014). Economic analysis of fungicides and fertiliser applications on huckleberry (*Solanum scabrum* Mill). Fresh shoot yield. Journal of Tropical Agriculture 49 (1-2): pp 58-63 on Cam Food Crop Production, Processing and Marketing.
18. Olaniyi. J.O.and Ojetayo, A.E. (2010). The Effect of organomineral and inorganic fertilisers on the growth, yield
19. Olatunji. O., and Ayuba. S.A. (2011). Effect of combine application of poultry manure and NPK 20-10-10 fertiliser on soil chemical properties and yield of maize *Zea may* L.). Proceedings of the 35th Annual Conference of the Soil Science Society of Nigeria. 7th 11th March, 2011, Minna, Nigeria.
20. Parnaudeau, V., Condom, N., Oliver, R., Cazevieille, P. and Recous, S. (2008). Vinasse organic matter quality and mineralization potential, as influenced by raw material, fermentation and concentration processes. Bioresource Technology, 99, 1553-1562.
21. Ratna, D. K., Oktafiani, D. H. and Prima, A. H. (2017). Effects of solid vinasse-based organic

mineral fertilizer on some growth indices of tomato plant. Jurnal Bahan Alam Terbarukan, 6, 190-197.
22. Anil, K. M., Amith, A., Kiran, K. M. and Rajeev, K. S. (2018). Lignocellulosic Biorefinery Wastes, or Resources? In Bhaskar, T., Pandey, A., S., V. M., Lee, D.-J. and Khanal, S. K. (Eds.), Waste Biorefinery (pp. 267-297): Elsevier B. V. Washington: American Public Health Association.
23. Carpanez, T. G., Moreira, V. R., Assis, I. R. and Amaral, M. C. S. (2022). Sugarcane vinasse as organo-mineral fertilizers feedstock: Opportunities and environmental risks. Science of the Total Environment, 154998.
24. Cortes-Rodríguez, E. F., Fukushima, N. A., Palacios-Bereche, R., Ensinas, A. V. and Nebra, S. A. (2018). Vinasse concentration and juice evaporation system integrated to the conventional ethanol production process from sugarcane – Heat integration and impacts in cogeneration system. Renewable Energy, 115, 474-488.
25. EPA. (2016). Resource Conservation and Recovery Act (RCRA) Regulations. Retrieved from http://www.epa.gov/rcra/resource-conservation-and-recovery-act-rcraregulations#nonhaz. on 10/08/2022
26. FAO. (1985). Water Quality for Agriculture. Rome, Italy: Food and Agriculture Organization.
27. Fuess, L. T. and Garcia, M. L. (2017). Anaerobic biodigestion for enhanced bioenergy generation in ethanol biorefineries: Understanding the potentials of vinasse as a biofuel. In Dalena, F., Basile, A. andRossi, C. (Eds.), Bioenergy Systems for the Future (pp. 149-183): Woodhead Publishing.
28. Krajang, M., Malairuang, K., Sukna, J., Rattanapradit, K. and Chamsart, S. (2021). Single-step ethanol production from raw cassava starch using a combination of raw starch hydrolysis and fermentation, scale-up from 5-L laboratory and 200-L pilot plant to 3000-L industrial fermenters. Biotechnology Biofuels, 14, 1-15.
29. Luz Selene Buller, Romero, C. W. d. S., Lamparelli, R. A. C., Ferreira, S. F., Bortoleto, A. P., Mussatto, S. I. and Forster-Carneiro, T. (2021). A spatially explicit assessment of sugarcane vinasse as a sustainable by-product. Science of the Total Environment, 765, 142717.
30. Maradiaga-Rodriguez, W. D., Pêgo-Evangelista, A. W., Júnior, J. A. and Costa, R. B.-d. (2018). Effects of vinasse and lithothanmium application on the initial growth of sugar cane (*Saccharum sp*. cv. RB 86-7515) irrigated and not irrigated. Acta Agronomica, 67, 252-257.
31. Moran-Salazar, R. G., Sanchez-Lizarraga, A. L., Rodriguez-Campos, J., Davila-Vazquez, G., Marino-Marmolejo, E. N., Dendooven, L. and Contreras-Ramos, S. M. (2016). Utilization of vinasses as soil amendment: consequences and perspectives. Springerplus, 5, 1-11.
32. Parnaudeau, V., Condom, N., Oliver, R., Cazevieille, P. and Recous, S. (2008). Vinasse organic matter quality and mineralization potential, as influenced by raw material, fermentation and concentration processes. Bioresource Technology, 99, 1553-1562.
33. Ratna, D. K., Oktafiani, D. H. and Prima, A. H. (2017). Effects of solid vinasse-based organic mineral fertilizer on some growth indices of tomato plant. Jurnal Bahan Alam Terbarukan, 6, 190-197.
34. Reis, C. E. R. and Hu, B. (2017). Vinasse from Sugarcane Ethanol Production: Better Treatment or Better Utilization? Frontiers in Energy Research, 5, 1-7.
35. Renato, d.-M. P., Gustavo, C. and Cid Naud, i. S. C. (2013). Filter Cake and Vinasse as Fertilizers Contributing to Conservation Agriculture. Applied and Environmental Soil Science, 1-7.
36. SAS (2008). SAS User's Guide Statistics, SAS Inc. Cary., North Carolina, 2008 edition.
37. Smaling, E.M.A. (2006). Fertilizer use and the Environment in Africa: Friends of Foes? Bacground paper: African Fertilizer Summit: Nourish the soil, Feed the Content 9-13 June 2006, Abuja, Nigeria, pp. 25.
38. Strandhagen, E., (2006).High fruit intake may reduce mortality among middle aged and elderly men. European Journal of Clinical Nutritio