MPRI-JERT | INTERNATIONAL JOURNAL OF ENGINEERING & RESEARCH TECHNOLOGY
VOL. 10 NO.5 DEC-2019 ISSN: 1074-4741

INVESTIGATING THE PYSIOCHEMICAL AND MICROBIOLOGICAL PROPERTIES OF JATROPHA SEED CAKE WASTE MATERIAL AS A POTENTIAL FOR GREEN ECONOMY SOIL AMENDMENT

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Abstract

The demand for farmers to provide food for increasing populations has made the use of inorganic fertilizer more competitive as it has been utilized frequently to boost farm produce. However its continuous usage is detrimental to natural healthy state of soil. Based on this, alternative environmentally friendly fertilizer which incorporates Jatropha cake with rich vital soil nutrients as carrier material has been identified as a better farm tool. In the current study, investigations were made on the physiochemical and biochemical analysis of Jatropha seed cake waste as source of soil nutrient. Sample of the seed cake was collected from National Research Institute for Chemical Technology (NARICT), Zaria, after biodiesel transesterification process. The cake was air dried for six weeks to stabilize and was ground using a pestle and mortar and then taken to the Soil and Water Laboratory of Institute For Agricultural Research (IAR), Ahmadu Bello University, Zaria for analysis. 2 g of cake sample was digested and Nitrogen(N), Phosphorous (P) and Potassium (K) as well as Carbon (C) contents were analysed in the digestate using N Kjeldahl digestation and distillation technique, absorbance measurement of P using calorimeter, K concentration in the digestate using flame photometer and carbon content using the Walkley and Black procedure. Total metal contents (Copper, Zinc and Iron) were also

KEYWORDS:
Jatropha seed cake, waste, digestate, analysis, tolerable limit

determined by di-acid digestion (nitric: perchloric acid) of seed cake followed by AAS analysis of the digestate. The pH and EC were determined in the sample by inserting digital pH mater and EC meter in the cake paste until the reading in the meters had stabilized. Microbial analysis was carried out using Invic test while microbial count was done with a colony counter. The NPK as well as the C concentrations were 3.33%, 0.46%, 1.2% and 30.52% respectively. These values were within the specified ranges recommended by the Food and Agriculture Organization of the United Nations for organic soil nutrients. The Cu, Zn and Fe concentrations were 0.05%, 0.09% and 0.02% respectively. These values with the exception of Fe concentration were within tolerable limits of trace metals concentrations recommended. The pH value of 4.9 and EC value of 976dsm^{-1} are not within specified ranges recommended. Biochemical analysis and colony count revealed the presence of Bacillius spp, Proteus and Aspergillius niger with colony count 5.2× 10^{7} CFU and 6.0× 10^{5} CFU respectively. The study suggests that the waste may not be safe for direct land application. It is therefore recommended that the waste should be subjected to other forms of treatment to eliminate pathogenic organisms before it is used as a soil nutrient.

Introduction

The application of fertilizers of different levels of Nitrogen (N), Phosphorous (N) and Potassium (K) is a common practice in improving soil fertility, which also improves crop production. Organic substrates such as green manure and animal manure are considered good potential sources of macronutrients and micronutrients apart from chemical fertilizers. The nitrogen, phosphorous and potassium as well as organic contents of Jatropha seed cake are relatively high and has made it a good potential source of soil nutrients (Maylen, 2015). According to Srinophakun *et al.*, (2012) the Jatropha seed cake which is the waste by -product of the biodiesel transesterification process can be used as a rich organic fertilizer. Davappa *et al;* 2010 reported that even though the Jatropha cointains a toxic chemical (pharbol esters), no residue of this toxin is found in soil used with Jatropha based fertilizers.

In the production of biodiesel from Jatropha, about 3 kg of waste seed cake is produced (Srinophakun *et al.*, 2012). The study of Kumar and Schuma revealed that Jatropha seed cake contains 3.2-4.55 N, 1.4-2% P and 1.2- 1.7%K (Kumar and Schuma, 2008). These values exceed the values found in chicken manure or cow manure (Maylen 2015).

The study assess the potential of Jatropha seed cake as a green economy soil nutrient by investigating its physiochemical and microbiological properties.
The study of the value of the parameters in organic soil amendment is fundamental for providing a database for adequate use for crops and pollution control. Fertilizer analysis is important to ensure guaranteed or declared analysis (specifications) It is also necessary that there are no health or environmental hazards in substrates used as soil amendment.

Materials and methods

Collection, Preparation and Analysis of Substrates

The Jatropha seed cake used for this study was collected from the National Research Institute for Chemical Technology (NARICT), Zaria after biodiesel transesterification process. The cake was air dried for six weeks to stabilize and was ground using a pestle and mortar and then taken to the Soil and Water Laboratory of Institute For Agricultural Research (IAR), Ahmadu Bello University, Zaria for analysis as follows : The pH and electrical conductivity (EC) were measured in a solution of dry substrate and water using digital pH and Ec meter. Values were taken after the readings on the meters had stabilized. The organic carbon was determined by the Walkley and Black procedure by wet oxidation using chromic acid digestion (Nelson and Sommers 1996). To determine total phosphorous (P) and potassium (K) the waste material was first digested with a mixture of nitric acid and perchloric acid (9:4). Phosphorus in the digestate was estimated throu by absorbance measurement at 420 nm Whereas, potassium was measured in the digestate using flame photometer (Singh et al., 2007). Total nitrogen (N) was determined using micro-Kjeldahl digestion and distillation techniques (Manab *et al.,* 2011) . Total metal content (Iron: Fe, Zinc: Zn, Cu: cupper and manganese (Mn) was also determined by di-acid digestion (nitric: perchloric acid) of compost followed by AAS analysis of the digestate (Gupta, 2004). C/N ratio was calculated as the quotient of total carbon over total nitrogen in substrate. The results of the analysis are presented in Table 1.

Table 1: Physiochemical analysis

PARAMETERS	RESULT
pH	4.9
EC	976dsm^{-1}
Nitrogen (N)	3.33%

Phosphorous (P)	0.46%
Potassium (K)	1.20%
Carbon (C)	30.52%
Organic Matter (OM)	52.62%
Cupper (Cu)	0.05%
Zinc (Zn)	0.09%
Iron (Fe)	0.02%
Manganese (Mn)	0.01%
C/N	9.2

Biochemical analysis

Total microbial count.

The total microbial counts was carried out on the sample according to the method of (Ghaley and Alhattab, 2013).The total count plate was employed to estimate the numbers of bacteria and fungi in the sample based on the assumption that each viable cell will develop into a colony under specific condition of incubation. The Jatropha seed cake sample was collected in a sterile leather bag. Each sample was diluted to insure that one of the final plates would have 30-300colonies; as the number of colonies within this range would give the most accurate approximation of microbial population. The initial dilution (1:10) was prepared by placing 1 g into a 10 ML dilution blank (physiological saline water). The bottle was shaken vigorously to obtain a uniform distribution of organisms. Further dilutions ($1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, $1:10^8$) were made by pipeting measured aliquots into additional dilution blanks. Sterile petri dishes were first labelled (specimen and dilution). Then, each bottle was thoroughly shaken and 0.1 ML of appropriate dilution was pipetted into a petri dish. Aprroximately 15 ML of cooled melted medium (Manab, 2011) were poured into each petri dish. Immediately after that, the plate was gently spread to distribute the innoculum throughout the medium using a sterile bent glass rod. The plates were allowed to solidify and were incubated in the inverted position in an incubator at 37°C for 24hrs. The plate that contained a number of colonies in the range of 30-300 was selected. An accurate count of these colonies was made by placing the plate on the platform of a colony counter. The instrument facililated the counting process since colonies were illuminated and seen against background. The number of colonies counted on a plate multiplied by the dilution of specimen which the plate represents was equl to the cell count per millimetre of the specimen. For fungal count, plates were

incubated at 27°C for 4 days and fungal growth was counted while for fungal identification, plates were incubated for 7 days and viewed in a microscope. Table 2a shows the results of the analysis for the microorganism counts

Table 2a: Microorganisms Counts in Substrate

Organisms	No. of colonies	Dilution factor	Inoclum (ml)	CFU/ml
Bacteria	52	10^4	0.1	5.2×10^7
Fungi (*A. Niger)*	6	10^4	0.1	6×10^5

Invic test for differentiating Bacteria

Indol Test : One tube of peptone broth was inoculated with the test organism at 37^{OC} for 24hrs . After the incubation period some drops of 40% KOH and few drops drops of Kovces reagent was added to the broth culture and the solution shaken gently and allowed to stand for about 20minutes. A red colour at the reagent layer indicated indole positive while no colour change indicated indole negative

Methyl Red Test

One tube of MRVP medium was inoculated with the test organism at 37^{OC} for 24hrs. After the incubation period some drops of methyl red reagent was added to the broth culture and the solution shaken gently and allowed to stand for about 20minutes. A red ring layer at the reagent indicated methyl re positive while no colour change negative

Vogeous proscauer

One tube of MRVP medium was inoculated with the test organism at 37^{OC} for 24hrs . After the incubation period some drops of napthol reagent was added to the broth culture and the solution shaken gently and allowed to stand for about 20minutes. A red ring layer at the reagent indicated positive while no colour change negative

Citerate Test

One tube of semmon citrate agar was inoculated with the test organism at 37^{OC} for 24hrs. After the incubation period some drops of methyl red reagent was added to the broth culture and the solution shaken gently and allowed to stand

for about 20minutes. A deep blue ring layer at the reagent indicated methyl re positive while no colour change negative
Table 2b shows the results of the biochemical analysis for differentiating bacteria and the fungal identification

Table 2b: Invic test for differentiating Bacteria

Samples	Indole test	Methyl red	Vogeus Proscoeur	Citrate	Genus
JSC_1	Positive	Negative	Negative	Positive	*Proteus*
JSC_2	Positive	Positive	Negative	Positive	*Bascillius spp*

Results and Discussion

Physiochemical Analysis

Nitrogen, phosphorous and potassium are the nutrients which are utilised in the greatest quantities by plants. Fertilizers also contain micronutrients which include iron (Fe), copper (Cu), zinc (Zn), Boron (Bo), Molybdenum (Mo), Manganese (Mn), and Chlorine (CI). These are required in small amounts by the plants but are equally important for plant development as the primary or major nutrients (Kiddar, 1997). Knowledge of the nutrient concentrations of fertilizer is important because the nutrient concentrations can vary widely and also because it allows facility operators to determine an appropriate end use for it (Zethner et al.,2000)
Concentrations of heavy and trace metals metal in an organic soil amendment can constitutes a very important problem from an agricultural and environmental point of view. Hence, it should be mandatory to check the concentration and of metals in an organic manure (Manab,2011)
Table 2 present the results of the analysis made on the Jatropha seed cake waste. The concentrations of Nitrogen (N), Phosphorous (P) and Potassium (K) obtained were 3.33%, 0.40% and 1.20% respectively. These values, with exception of Phosphorous (P) were within the specified range for Jatropha seed cake reported in literature (Maylen, 2015). The low value obtained for phosphorous in this study may be due to difference in species of Jatropha plant. However, these values were within the specifications/standards for organic fertilizer recommended by the FAO, (1994) which are (1-4%), (1.5-5%) and (1.1-5%) for NPK respectively. Concentrations of available Cu, Zn, Fe, and Mn the substrate were 0.05%, 0.09%, 0.02% and 0.01% respectively. These values were within tolerable limits of trace and

heavy metals in organic fertilizer defined by FOA, 1994. Regarding pH and EC, values were higher than tolerable limits (pH: 4.9 , EC : of 976dsm^{-1}) . The result of the organic matter content (OM: 52.62) reveal that the substrate is rich in organic matter. This value exceeds the minimum requirement of at least 20% defined for organic matter content in organic fertilizers by (FAO, 1948). The low C/N ratio (C/N: 9.2) revealed by the result means there is sufficient nitrogen in the substrate for optimal growth of the microbial population. The findings of this study is in contrast with a similar study by Jacub et al., (2016). The difference in the values obtained may be due to difference in the species of Jatropha plant used for studies.

Biochemical Analysis

The presence of pathogens in organic soil amendments can be a serious health and environmental hazard. Hence the specification / standards for organic fertilizer recommended by (FAO, 1994) should be free of pathogenic organisms. Table 2a and 2b presents the results of the microbial counts and biochemical analysis. The results revealed the presence of *Proteus, Bascillius Spp and Aspergillius niger* with counts 5.2× 10^7 CFU/ML and 6×10^5 CFU/ML respectively. The presence of microorganisms in the substrate may be due to contamination as a result of exposure during air drying.

4.0 Conclusion and Recommendation

The physiochemical and biochemical and analysis of Jatropha seed cake waste as a green economy soil amendment was investigated. Upon investigations, it was observed that the the concentrations of Nitrogen (N), Phosphorous (P) and Potassium (K) obtained were 3.33, 0.40 and 1.20 respectively. These values, with exception of Phosphorous (P) are within the specified range for Jatropha seed cake reported in literature. The Cu, Zn and Fe concentrations were 0.05%, 0.09% and 0.02% respectively. These values with the exception of Fe concentration were within tolerable limits of trace metals concentrations recommended. The pH value of 4.9 and EC value of 976dsm^{-1} are not within specified ranges recommended. Biochemical analysis and colony count revealed the presence of *Bacillius spp*, *Proteus* and *Aspergillius niger* with colony count 5.2× 10^7 CFU and 6.0× 10^5 CFU respectively. The study suggest that the waste may not be safe for direct land application. It is therefore recommended that the waste should be subjected to other forms of treatment to eliminate pathogenic organisms before it is used as a soil nutrient.

REFERENCE

Devappa, R. K., Makkar, H. P.S., and Becker K. (2010). Biodegradation of Jatrophacurcasphorbol esters in soil. *Journal of the Science of Food and agriculture*, 90: 2090-2097.

FAO (Food and Agriculture Organization of the United Nations), 1994. Defination and classification commodities, 4 Pulses and derived products.(http:www.fao.org/es/faodef/fdefo4e.htm) Accessed 29 Febuary 2016.

Gupta, P.K., 2004. Soil, Plant, Water and Fertilizer Analysis. Agrobios, India.

Jacub Elbl, Petr Slama, Magdalena D. Vaverkova, Lukas Plosek, Dana Adamcova, Petr Skappa, Jingrich Kynicky, Zdenek Havlick, Helena Ddvorakora, Martin Brtnicky and Elisha Kabourkora (2016). Jatropha sees cake and organic waste compost: the potential for improvement of soil fertility. Ecological Chemical Engineering 23(1)

Kidder, G. "Plant nutrients and Fertilizers for the non-farmer", Institute of food and Agricultural Sciences-University of Florida, pp. 1-6, 1997.

Kumar A. & Sharma S. (2008). An evaluation of multi-purpose oil seed cake crops for industrial uses. (Jatrophacurcas L.). A review: Industrial crops and products. 28, pp 1-10.

Manab Das , H.S. Uppal, Reena Singh, Shanuja Beri, K.S. Mohan, Vikas C. Gupta, Alok Adholeya (2011). Co-composting of physic nut (Jatropha curcas) deoiled cake with rice straw and different animal dung. Bioresource Technology 102 (2011) 6541–6546

Nelson D. W, Sommers L. E.(1996) "Total carbon, organic carbon and organic matter". In: Sparks DL, editor. Methods of soil analysis. Part 3. 2nd ed. SSSA Book Series No. 5. Madison (WI): ASA and SSSA 1996. p. 961–1010.

Singh, D., Chhonkar, P.K., Dwivedi, B.S., (2007). Manual on Soil, Plant and Water Analysis. Westville Publishing House, New Delhi, India.

Srinophakun, P., Titapiwatanakan, B., Sooksathan, I., Punsuvan, V. (2012). Prospect of De-oiled Jatrophacurcas seedcake as fertilizer for Vegetable Crops-A Case study. *Journal of Agricultural Science*. 4(3) pp. 211-219.

Waste Collection – Summary. Available at: <http://www.ubavie.gv.at/ publikationen/Mono/M133s.HTM>.

Zethner, G.B., Götz, F., Amlinger (2000). Quality of Austrian Compost from Derived