# PKFokam Journal of Applied Science & Technology





PKFokam Journal of Applied Science & Technology ; Inaugural Issue; June 2019. ISSN: 2707 - 2843 (Print) 2707 -2851 (Online) Published by the PKFokam University Press. www.pkfokam-cap.org ; journal@pkfokam-cap.org ; PKFokam Institute of Excellence ; PO.Box 11646 Yaoundé-Cameroon Dear reader,

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### Les nouvelles guerres industrielles

# Hakim Ben Hammouda.

Le débat sur les politiques industrielles a connu ces derniers mois des développements majeurs au niveau international tant dans le débat économique que dans les politiques publiques. Le débat économique a connu une rupture majeure dans les positions défendues par les économistes depuis le début des années 1980 et le consensus qui avait prévalu faisant des politiques industrielles les « excommuniés » d'une discipline en quête obsessionnelle de scientificité et de reconnaissance. Ces politiques qui étaient au centre des efforts de reconstruction d'après-guerre et postcoloniale dans le monde depuis la fin de la seconde guerre mondiale a été battu en brèche par la contre-révolution néolibérale du début des années 1980 qui a emporté avec elle toute forme d'interventionnisme étatique en faisant l'héritage désuet d'un monde post-moderne en pleine décrépitude. Désormais, c'est au marché et à la libre concurrence de prendre en charge la répartition des ressources rares, l'Etat devrait se limiter à son rôle de régulateur de l'activité économique.

La contre-révolution néo-libérale s'est également attaquée aux politiques commerciales protectionnistes qui se sont développées dans le sillage des politiques industrielles. Ces politiques seraient, selon les nouveaux prophètes de l'économie, à l'origine du développement de comportement rentiers et d'une grande inefficacité dans la distribution des ressources. Cette contreoffensive ouvrira la porte à la concurrence à l'échelle globale et à la globalisation « heureuse » qui fera du monde un grand marché au profit des grandes multinationales.

Or, ce consensus dans le petit monde des économistes sera rapidement rompu avec les crises à répétition et le recul de la confiance dans la capacité du marché à réguler sans heurts l'ordre marchand. Les débats économiques se sont alors tournés vers les questions de la concurrence et de l'information imparfaite qui ont ouvert la porte à un retour de l'Etat afin de corriger les imperfections du marché.

Parallèlement à cette rupture du consensus chez les économistes, ce débat a été marqué également par une seconde rupture dans les politiques publiques avec la décision de grandes puissances économiques de définir et de s'engager de manière déterminée dans le domaine industriel afin de défendre ces intérêts dans une globalisation 4.0 qui a ouvert la porte à une concurrence acharnée sur les marchés mondiaux. Ainsi, le gouvernement allemand a annoncé le 5 février 2019 le lancement d'une nouvelle politique appelée « Une stratégie nationale industrielle nationale 2030 ». Cette annonce constitue une rupture majeure dans la mesure où dans la tradition allemande l'Etat a toujours gardé une certaine neutralité vis-à-vis de l'économie et particulièrement dans son attitude par rapport aux grands groupes industriels.

Or, cette stratégie a montré ses limites aux yeux des responsables allemands et les entreprises allemandes n'ont pas cessé de perdre pied sur les marchés internationaux face à la concurrence en particulier des entreprises chinoises. L'acquisition en décembre 2016 de la totalité du capital de l'entreprise Kuka, spécialisée dans les bras articulés intelligents et considérée comme l'un des champions de l'industrie 4.0, par le groupe chinois Midea a eu l'effet d'un choc auprès des responsables allemands comme dans l'opinion publique et a préparé les esprits pour un changement majeur dans ce domaine avec la définition d'une nouvelle politique industrielle ayant pour objectif la défense des entreprises allemande face à la concurrence.

La Chine a fait le même choix quelques années plutôt en mettant en place une stratégie industrielle ambitieuse afin d'assurer la nouvelle hégémonie chinoise sur l'industrie mondiale. Cette stratégie a été appelé MIC25 ou « Made in China 2025 » et les travaux préparatoires ont commencé en 2013 avant qu'elle ne soit adopté en 2015 avec pour objectif un retour aux vieilles stratégies d'importsubstitution faisant du remplacement des importations par la production nationale le cœur de l'industrie chinoise 4.0. Cette stratégie s'est fixée des objectifs quantitatifs précis dans les différents secteurs que les entreprises industrielles se doivent d'atteindre. Par ailleurs, le gouvernement chinois a mis des moyens financiers sans précédent estimés à 2000 milliards d'euros pour parvenir à ses objectifs.

Ainsi, les gouvernements chinois et allemand, comme d'autres gouvernements dans le monde, ont rompu la neutralité et la stratégie du « benign neglect » pour s'engager dans la définition de stratégies ambitieuses et agressives afin de reconstruire leur compétitivité au niveau mondial. En dépit de la diversité de ces stratégies, il est possible de distinguer trois grandes caractéristiques communes. La première concerne l'accent mis sur les nouveaux secteurs industriels ou ce que nous appelons « l'industrie 4.0 » pour appuyer les capacités des pays dans ces domaines et les renforcer et maîtriser les transformations technologiques profondes que connaissent les différents secteurs. La seconde caractéristique concerne l'intervention de l'Etat dans ces nouvelles stratégies et le rôle de plus en plus actif qu'il prend dans différents domaines notamment le financement, l'appui à la recherche et le soutien aux exportations. La troisième caractéristique de ces nouvelles politiques industrielles concerne l'appui aux entreprises nationales particulièrement vis-àvis de la concurrence internationale et la mise en place de stratégies de constitution et de soutien aux « champions nationaux ».

Nous traversons aujourd'hui d'importantes mutations et transformations économiques qui ont amené les grands pays à sortir des politiques néo-libérales qui ont marqué les politiques publiques depuis le début des années 1980, et à mettre en place, dans le domaine industriel, des stratégies actives visant à soutenir leur compétitivité dans les nouveaux secteurs industriels. Ces changements doivent nous emmener à réfléchir nos choix de politique économique et sortir de la vision étroite qui prévaut sous nos cieux et qui limite leur rôle dans la gestion des grands équilibres macroéconomiques et donner aux politiques structurelles, particulièrement dans le domaine industriel, toute leur place afin de sortir de la marginalisation, des crises et de cet enfermement sans fin dans la « trappe des pays intermédiaires ».

Les nouvelles guerres industrielles: by Hakim Ben Hammouda PKFokam Journal of Applied Science and Technology, inaugural Edition, June 2019 ISSN: 2707 - 2843 (Print) 2707 - 2851 (Online)

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# Effect of Single Superphosphate and Arbuscular Mycorrhizal Fungi on Growth and Bambara groundnut (Vigna subterranea (L.) Verdc.) Yield

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# Part 1- Scientific Research

### Abstract

The aim of this study was to reduce food insecurity by improving the yield of Bambara groundnut with phosphorus fertilizer. The experiment was carried out in the field at the University of Yaounde I. The experimental design was a split plot with three factors; landraces (V1 and V2), single superphosphate doses (0, 50, 100, 150 and 200 kg  $P_2O_5$ .ha<sup>-1</sup>) and AMF inoculum (M0: -AMF and M1: +AMF).  $P_2O_5$  doses and AMF (composites *Gigaspora margarita, Acaulospora tuberculata* and *Glomus intraradices*) significantly boosted growth (number of branches, shoot height) and yield of Bambara groundnut. The dose 150 kg  $P_2O_5$ .ha<sup>-1</sup> significantly increased the yield by 100% compared to the control (0 kg  $P_2O_5$ .ha<sup>-1</sup>+M0) in V1. AMFs significantly increased the yield by 87.5% compared to the control in V2.  $P_2O_5$  and AMF independently improved the grain yield. However, low doses of  $P_2O_5$  (100 kg.ha<sup>-1</sup>) may be associated with AMF to maximize grain yield of Bambara groundnut.

Key-words: Arbuscular mycorrhizal fungi, Bambara bean, fertilizers, phosphate starvation, phosphorus.

# Introduction

Bambara groundnut (Vigna subterranea (L.) Verdc.) is a neglected legume native to northern Cameroon and northeastern Nigeria (Temegne et al., 2018a). It is used for food and feed because its seeds contain on average 63% of carbohydrates, 19% of proteins and 6.5% of fats (Bamishaiye et al., 2011). They are rich in calcium, potassium, iron (De Kock, 2013), essential amino acids (methionine, leucine, isoleucine, lysine, phenylalanine, threonine, valine, tryptophan) (De Kock, 2013; Yao et al., 2015) and vitamins (E: 3,  $18 \pm 0.15$  mg / 100 g, C:  $1.17 \pm 0.20$  mg / 100 g and A:  $26.05 \pm 0.14$  mg / 100 g). It is used in the traditional pharmacopoeia to treat diarrhea, anemia, abscesses, internal injuries, ulcers, infected wounds, epilepsy, cataracts, menorrhagia during pregnancy, nausea in pregnant women, kwashiorkor and venereal diseases; and prevents heart disease, eye disease and colon cancer (Brink et al., 2006; Jideani & Diedericks, 2014). Bambara groundnut also contains kaempferol, an antioxidant polyphenol, which reduces the risk of many chronic diseases such as cancer (Jideani & Diedericks, 2014; Yao et al., 2015). Bambara groundnut has the ability to tolerate poor soils, drought and salt stress

(Taffouo et al., 2010; Temegne, 2011; Jideani & Diederiks, 2014; Tsoata et al., 2015, 2016, 2017a, b).

Numerous studies have been carried out on optimizing the productivity of Bambara groundnut in Cameroon through biological and chemical fertilization (Ngakou et al., 2012; Temegne et al., 2015, 2017b, 2018b; Temegne, 2018). However, to the best of our knowledge, no work has yet been done on the combined effect of biological and chemical fertilization on the yield of Bambara groundnut. Soils in Cameroon, like most tropical soils, are poor in nutrients (Syers et al., 2008; Mbogne et al., 2015). Arbuscular mycorrhizal fungi (AMF) improve the water and mineral nutrition of plants (Onguene et al., 2001; Nwaga et al., 2010; Temegne et al., 2017a, 2018b). But the extreme poverty of some soils requires additional input (organic or chemical fertilizer) in addition to mycorrhizal fertilizer. Research has shown that high doses of chemical fertilizers inhibit mycorrhization (Bhadalung et al., 2005; Mbogne et al., 2015; Temegne et al., 2017a, b, 2018b). Since chemical fertilizers are the most accessible, it is therefore necessary to determine the minimum dose of chemical fertilizer that does not affect the effectiveness of mycorrhization.

N and P are major nutrients essential for plant growth (Morel et al., 2006). Bambara groundnut has the capacity to fix atmospheric N due to the rhizobial symbiosis (Ngakou et al., 2012; Musa et al., 2016). P is a limiting factor for crop yield on more than 30% of the world's arable land (Vance et al., 2003) and remains a limiting mineral for Bambara groundnut growth. The objective of this work was to evaluate the combined effect of chemical and biological fertilization on the growth and yield of Bambara groundnut.

#### Material and methods

Study Site- The trial was conducted at the Experimental Farm of the Faculty of Science, University of Yaounde I. The site is located in the Centre Region and is characterized by rainfall of 1,617 to 1,800 mm.year<sup>-1</sup>. The average air temperature varies from 23 to 24 °C. It belongs to the humid forest agro-ecological zone with bimodal rainfall pattern, characterized by acidic ferralitic soils. It is governed by a Guinean equatorial climate with four seasons: a long rainy season from September to November, a long dry season from December to February, a short rainy season from March to June and a short dry season from July to August. The soil of the site is sandy clay (Table 1). The low C/N ratio reflects a rapid decomposition of organic matter. It leads to a dysfunction of the clay-humic complex.

Effect of Single Superphosphate and Arbuscular Mycorrhizal Fungi on Growth and Bambara groundnut (Vigna subterranea (L.) Verdc.) Yield, by Temegne et al; 4-13 PKFokam Journal of Applied Science and Technology, inaugural Edition, June 2019 ISSN: 2707 - 2843 (Print) 2707 -2851 (Online) Plant material-The plant material used consisted of two landraces of Bambara groundnut (V1: white seeds, V2: red seeds) bought from the local market.

of same time as the mycorrhizal inoculant (5 g.hole<sup>-1</sup>), i.e. 49 holes for e each unit. One plant per hole was maintained at emergence for a total of 2,940 plants. Plot maintenance was done by hand weeding and hoeing around the plants.

Experimental design- The experimental design of the study was a split plot with three factors; doses of phosphate fertilizer (0, 50, 100, 150 and 200 kg  $P_2O_5.ha^{-1}$ ), landraces (V1 and V2) and mycorrhizal treatment (M-: control, M: mycorrhizae). After clearing and plowing, a plot of 27 m x 18 m was divided into three parallel blocks of 27 m x 6 m. The spacing between the blocks was one meter and 0.5 m between the experimental units. Using a double decameter, a string and stakes, 20 experimental units of 2 m x 2 m were formed per block (60 units in total). Bambara groundnut seeds were sown (two seed.hole<sup>-1</sup>), at the

Ten plants per experimental unit were used for the evaluation of the number of branches and measuring the height of the plants. These data were taken weekly, from the second week after sowing until the tenth week. At harvest, yield parameters were determined and seed P content measured.

### **Data Analysis**

The collected data were subjected to an analysis of variance (ANOVA) using the IBM SPSS software (Statistical Package for the Social

Table 1. Physico-chemical analysis of the soil of the study sites. pH: potential of hydronium ion, OC%: total organic carbon, N%: total nitrogen, C/N: ratio between proportion of total organic carbon and proportion in total nitrogen, P: phosphorus, Ca: calcium, Mg: magnesium, K: potassium, Na: sodium

Sand	Clay	Silt	OC	Ν	C/N	pН	Ca	K	CEC	Mg	Na	P Bray
%							cmol.kg	y <sup>-1</sup>				µg.g <sup>-1</sup>
43.68	43.4	12.92	2.1	0.167	12.6	6.21	6.84	0.23	6.53	0.62	0.04	2.37

Sciences) version 20. The Student-Newman-Keuls test at the 5% threshold allowed to rank the averages.

#### Results

Mycorrhization significantly (p <0.001) favored Bambara groundnut growth in the field. Overall, at high doses, phosphate fertilization reduced the beneficial effect of AMF (Arbuscular mycorrhizal fungi) on the number of branches (Table 2) and the height (Table 3) of Bambara groundnut. Thus, the highest number of branches at V1 (77  $\pm$  12) was observed at mycorrhizal treatment + 0 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup>, eight (8) weeks after sowing (WAS) (Table 2). Two (2) to 6 WAS, the number of branches was significantly (p <0.001) higher at V1, but 8 WAS, it was more important (p <0.001) at V2 (Table 2).

Bambara groundnut (Table 4). Thus, in V1 landrace, the dose 150 kg  $P_2O_5$ .ha<sup>-1</sup> increased the yield by 100% compared to the

control (0 kg  $P_2O_5$ .ha<sup>-1</sup> + M- (without AMF)). In V2, the AMFs

increased the yield by 87.5% compared to the control (0 kg  $P_2O_5$ .ha<sup>-1</sup> + M- (without AMF)).

Overall, high levels of P<sub>2</sub>O<sub>5</sub> decreased the effectiveness of mycorrhization on some yield parameters (Table 4). Indeed, the highest 100-grains weight was observed at mycorrhizal treatment + 0 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> (V1: 111.6 ± 1.7 g). It decreased significantly (p <0.001) at mycorrhizal treatment + 150 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> (V1: 89.6 ± 2.5 g). The highest pod weight (V2: 24.8 ± 5.1 g) was obtained at mycorrhizal treatment + 100 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup>. It decreased significantly (p <0.001) at mycorrhizal treatment + 200 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> (V2: 12.9 ± 2.1 g).

Overall, mycorrhization increased the percentage of double pods (Table 5). The number of double, triple and quadruple pods were

Eight (8) WAS, the highest height ( $35.8 \pm 2.1$  cm) from V2 was obtained at mycorrhizal treatment + 50 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> (Table 3). This height decreased significantly with mycorrhizal treatment + 200 kg P<sub>2</sub>O<sub>5</sub>.ha<sup>-1</sup> ( $34.6 \pm 2.4$  cm). Plant height of V2 was significantly (p <0.001) greater than that of V1 throughout the experiment (Table 3).

Landraces,  $P_2O_5$  doses, AMF and their interactions significantly affected (p <0.05) the yield components of Bambara groundnut (Table 4). Except for the diameter and P content of seeds, mycorrhization significantly (p <0.001) increased the yield components (100-grain weight, number of pods plant<sup>-1</sup>, weight of pods plant<sup>-1</sup>, weight of seeds plant<sup>-1</sup>) of

greater in the V2 landrace compared to V1 where the triple and quadruple pods were non-existent.

# Discussion

The biological fertilizer (AMF) tested does not significantly affect the diameter of Bambara groundnut seeds. Indeed,

plowing and/or weeding would have caused a disruption of the hyphae networks which allow the improvement of water and mineral supply, thus reducing the effectiveness of mycorrhization. This result is similar to those of Bourou et al. (2011) who found that *G. mosseae* had no effect on height and neck diameter of tamarind trees. But these results are contrary to those obtained by the same authors with the strain *G. aggregatum* for the height of the tamarind tree.

AMFs significantly improve the growth, yield and P content of Bambara groundnut seeds. Similar results were obtained by Megueni et al. (2011) on cowpea in the field and Temegne et al. (2017a, 2018a) on Bambara groundnut in pot experiments. In addition, Ngakou et al.

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Effect of Single Superphosphate and Arbuscular Mycorrhizal Fungi on Growth and Bambara groundnut (Vigna subterranea (L.) Verdc.) Yield, by Temegne et al; 4-13 PKFokam Journal of Applied Science and Technology, inaugural Edition, June 2019 ISSN: 2707 - 2843 (Print) 2707 -2851 (Online) (2012) working on the effect of inoculation of Rhizobium and mycorrhizae on Bambara groundnut found that they improved grain yield compared to control. The grain yield obtained in our study varied from 0.6-1.9 t.ha<sup>-1</sup>. This yield is higher than that obtained by Ngakou et al. (2012) who recorded a yield of 0.5-0.9 t.ha<sup>-1</sup>. This result

highlights the importance of using native AMF strains for sustainable Bambara groundnut production (Temegne et al., 2017b). The increase in P contents of cowpea leaves (*Vigna unguiculata* (L) Walp) inoculated with *G. clarum* was observed by Megueni et al. (2011). This result could be

Table 2. Effect of mycorrhization and P2O5 doses on the number of				
Bambara groundnut branches. M-: without AMF, M+: with AMF. For				
each column, the averages followed by the same letter are not				
significantly different at the 5% threshold.				

	Doses P <sub>2</sub> O <sub>5</sub>					
Landraces	(kg.ha <sup>-1</sup> )	AMF	2	4	6	8
	0	M-	4.6±0.6abc	12.3±3.6cde	33.3±7.4bcdef	47.6±8.5a
		M+	4.8±0.4cd	14.0±1.6ef	41.4±6.4gh	77.0±11.1ghij
	50	M-	4.4±0.5abc	12.6±1.2de	30.4±4.5abcd	49.0±6.5a
		M+	4.7±0.5bcd	16.6±2.9h	42.1±7.7gh	64.2±12.9cdef
V1	100	M-	4.6±0.6abc	13.8±1.9ef	35.2±9.5def	58.5±15.8abcd
		M+	5.1±0.6d	14.5±1.9fg	32.4±6.1bcde	58.3±8.3abcd
	150	M-	4.8±0.6bcd	16.1±2.9gh	46.2±10h	76.0±17.9fghij
		M+	5.1±0.6d	14.9±1.4fgh	37.6±4.5efg	65.0±8cdefg
	200	M-	4.6±0.5abc	15.1±2.4fgh	39.3±6.3fg	61.5±11.4bcde
		M+	5.1±0.3d	18.5±2.6i	46.6±11h	75.0±21.0fghij
	0	M-	4.3±0.5abc	8.8±1.9a	24.8±6a	52.2±10.5ab
		M+	4.6±0.5abc	11.3±2.6bcd	27.4±3.2ab	69.4±6.3defghi
	50	M-	4.3±0.4ab	9.70±1.8ab	25.4±5.8a	55.3±11.4abc
		M+	4.5±0.5abc	10.9±1.3bcd	28.3±3.8abc	70.9±7.3efghi
V2	100	M-	4.3±0.4ab	10.4±2 abc	29.2±3.9abcd	65.1±13.6cdefg
		M+	4.6±0.5abc	11.7±1.4bcd	34.1±3cdef	77.4±9.7hij
	150	M-	4.2±0.4a	11.2±1.5bcd	28.7±6.5abc	68.8±15.2defgh
		M+	4.4±0.5abc	11.3±2.2bcd	27.6±4.2ab	64.8±6.5cdefg
	200	M-	4.4±0.5abc	12.7±2.6 de	34.3±6.3cdef	80.9±17.5ij
		M+	4.5±0.5abc	12.3±1.7cde	35.1±5.7def	84.3±14.2j

explained by the fact that mycorrhizae secrete enzymes that hydrolyze mineral substances which are indirectly accessible to the roots. The improvement of water and mineral nutrition as well as the better development of plants amended with the biological fertilizers are due to the development by these fungi of a network of hyphae. These hyphae take water and nutrients to allocate to plants. The work of Tsane et al. (2005) on the growth of banana vitroplants showed that *Glomus* sp improved the neck circumference, the height, the leaf area, the number of leaves emitted, as well as the plant dry matter as compared to the control. The single superphosphate applied significantly improved the growth and the yield of Bambara groundnut. Similar results have been observed by Nweke & Emeh (2013) on *V. subterranea*, by Nkaa et al. (2014) on *V. unguiculata*, and by Temegne (2014) on *Brachypodium distachyon*. P is needed in large amount, and it is involved in the key functions of several plants, including energy transfer, photosynthesis, sugar and starch transformation, nutrient movement in the plant, and transfer of genetic traits (Jemo et al., 2010). This result indicates that phosphate amendment stimulates the vegetative growth of Bambara groundnut. It also emphasizes that soil nutrient depletion slows down and/or reduces plant growth since nutrients are not readily available for plant uptake in control plots (not fertilized with  $P_2O_5$ ). This unavailability of

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nutrients therefore explains the reduction in plant height, leaves and branches development compared to plants fertilized with P. The significant increase observed in these parameters as a function of the increasing doses of P2O5 applied could also be explained by the nitrogen and phosphorus interaction in the soil rhizosphere (Benedyka et al., 1992; Kamchoum et al., 2018). Indeed, P availability increases nitrogen absorption by the plant. In addition, Shaheen et al. (2007) and Nuemsi et al. (2018) have shown that nitrogen is important for the improvement of plant growth because it provides the basic constituent of proteins and nucleic acids. Similarly, Silva et al. (2012) reported that inorganic fertilizer rapidly provides important elements at the early stage of plant growth and development stage. In all legume such as Bambara groundnut, the release of nitrogen from symbiotic nodules and dead legume roots increases the availability of nitrogen in the soil. So, the amount of nitrogen in the soil should be increased in the presence of legumes. The increase in Bambara groundnut growth under phosphate fertilization could also be explained by the fact that nutrient release rates are much higher in phosphate fertilizer. This release would therefore promote better growth of plants fertilized with P2O5 compared to control plants.

High levels of  $P_2O_5$  decrease the effectiveness of mycorrhization. This result is similar to those of Oliveira et al. (2015), who showed that mycorrhization increases plant development at low P supply. The work of Ingleby et al.

(2007) and Temegne et al. (2018b) have shown that large intakes of soluble phosphates decrease the rate of mycorrhizal infection and may result in the elimination of the positive effects of this association on plant yield. However, different degrees of sensitivity can exist between species. This fact could justify the absence of inhibition of mycorrhization by phosphate fertilization on certain parameters. In addition, simple superphosphate is known as a fertilizer that mineralizes slowly.

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### **Authorship Contributions**

Author TNC designed the study, developed the research protocol, performed the data collection and the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Author GWF contributed to the field experiment. Author NFD contributed to the data collection in the field. Authors WGA and NNG corrected the draft and proofread the final manuscript. Author APA translated the draft of the manuscript into English. Authors TVD and YE supervised the work

Doses of P <sub>2</sub> O <sub>5</sub>		f	Time after sowing (Week)					
Landraces	(kg.ha <sup>-1</sup> )	AMF	2	4	6	8		
	0	М-	11.8±2.8 abcde	19.4±2.6 a	22.1±2.8 a	24.0±3.2 a		
		M+	11.9±1.8 abcdef	20.0±1.6 a	23.8±2.2 a	25.7±2.7 abc		
	50	M-	10.4±2.0 a	20.9±2.4 ab	22.8±1.9 a	25.1±3.0 ab		
		M+	10.9±0.8 ab	20.2±1.1 a	24.2±1.0 a	26.6±1.5 abc		
V1	100	M-	11.1±2.1 abc	20.7±2.5 ab	23.2±2.3 a	24.7±2.3 ab		
		M+	12.7±2.3 bcdef	20.8±1.1 ab	22.3±1.9 a	26.1±2.6 abc		
	150	M-	11.5±2.1 abcd	20.1±2.0 a	23.8±2.2 a	25.8±3.3 abc		
		M+	13.5±2.9 ef	22.7±1.1 bc	24.4±1.4 a	28.2±1.4 c		
	200	M-	11.1±1.7 abc	21.1±1.8 ab	22.3±1.6 a	25.6±3.4 abc		
		M+	13.4±2.5 def	21.7±1.7abc	24.4±2.7 a	27.0±3.6 bc		
	0	M-	11.9±1.8 abcdef	22.9±3.7 bc	29.4±1.9 bc	32.2±3.1 de		
		M+	12.9±1.4 cdef	26.5±1.9 de	31.1±2.9 cde	34.1±3.0 ef		
	50	M-	12.1±1.3 abcdef	23.4±4.0 c	28.8±2.0 b	31.2±2.1 d		
		M+	13.4±0.9 def	26.6±1.8 de	30.8±2.6 cd	35.8±2.1 f		
V2	100	M-	12.6±1.9 bcdef	25.5±2.8 d	31.2±3.1 cde	31.5±2.3 d		
		M+	13.3±1.0 def	27.2±3.9 de	33.2±2.6 f	35.7±4.9 f		
	150	M-	12.3±1.5 bcdef	26.3±2.6 de	29.6±2.7 bc	32.8±3.6 de		
		M+	13.9±1.3 f	28.2±2.3 e	32.4±1.9 de	36.1±2.3 f		
	200	M-	13.3±1.9 def	25.8±1.6 d	31.9±3.1 de	32.9±2.9 de		
		M+	13.8±2.1 f	27.3±1.2 de	32.7±2.4 de	34.6±2.4 ef		

Table 3. Effect of mycorrhization and  $P_2O_5$  doses on the height of Bambara groundnut. M-: without AMF, M+: with AMF. For each column, the averages followed by the same letter are not significantly different at the 5% threshold.

Landrace	$P_2O_5$	AMF	100-grain	Seed diamete	rN° of pod	sPod weigh	tSeed weigh	tGrain yiel	dSeed P content
S	Doses (kg.ha <sup>1</sup> )	-	weight (g)	(mm)	plant	plant <sup>-1</sup> (g)	plant <sup>-1</sup> (g)	(t.ha <sup>-1</sup> )	(%)
	0	M-	83.2±1.6c	11.9±0.7ab	10.8±2.0a	9.0±2.7ab	6.4±2.0ab	0.8±0.2ab	0.28±0.1d
	0	M+	111.6±1.7h	12.4±1.4abcd	15.2±5.5ab	14.3±2.4bcde	10.2±1.6bcdef	1.2±0.2bcdef	0.21±0.1ab
	~0	M-	84.2±2.0c	12.2±0.6abc	12.5±4.2ab	10.1±3.8abc	7.2±2.7abc	0.9±0.3abc	0.21±0.1ab
	50	M+	100.4±2.9f	12.4±0.8abcd	17.0±1.5abc	15.4±0.9bcdef	10.7±0.6bcdef	1.3±0.1bcdef	0.22±0.1ab
* 7 4	100	M-	74.2±1.8a	11.1±0.3a	10.5±3.7a	6.9±2.4a	4.5±1.5a	0.6±0.2a	0.22±0.1ab
VI	100	M+	98.8±1.9f	12.5±1.1abcde	13.6±3.8ab	12.0±2.9abcd	8.6±2.3abcde	1.1±0.3abcde	0.21±0.1ab
		M-	92.6±2.6e	12.3±1.2abc	18.7±7.5abc	18.7±7.2def	13.2±5.2fg	1.6±0.6fg	0.23±0.1b
150 200	150	M+	89.6±2.5de	12.1±0.7abc	20.4±3.9bc	16.7±2.2cdef	11.6±1.4	1.4±0.2cdef	0.20±0.1a
	• • • •	M-	108.8±1.4g	12.9±0.9bcdef	14.6±5.9ab	16.5±8.2cdef	11.5±6.0cdef	1.4±0.7cdef	0.21±0.1ab
	200	M+	97.6±2.6f	12.8±0.8bcde	27.0±9.0d	22.1±3.8fg	15.7±2.7g	1.9±0.3g	0.23±0.1b
	0	M-	77.4±4.7b	13.4±1.5bcdef	10.9±2.2a	10.8±2.7abc	6.4±2.0ab	0.8±0.2ab	0.32±0.1ef
	0	M+	83.6±4.3c	12.5±0.9abcde	18.6±2.8abc	20.2±4.0efg	12.1±2.3def	1.5±0.3def	0.32±0.1ef
	-	M-	90.8±2.4de	14.0±1.4ef	16.7±5.2abc	14.3±5.3bcde	8.5±2.3	1.0±0.3abcde	0.29±0.1d
	50	M+	88.4±2.6d	13.0±0.8bcdef	14.2±6.2ab	17.8±5.4def	10.9±3.4cdef	1.3±0.4cdef	0.26±0.1c
		M-	84.0±2.1c	13.2±1.0bcdef	15.4±7.2ab	18.5±8.2def	11.1±5.1cdef	1.4±0.6cdef	0.30±0.1de
<b>V</b> 2	100	M+	91.0±3.5de	13.6±0.8cdef	23.0±6.6cd	24.8±5.1g	12.5±2.6efg	1.5±0.3efg	0.32±0.1ef
		M-	92.6±4.0e	14.3±1.6f	18.0±9.4abc	16.7±8.2cdef	9.2±4.6bcdef	1.1±0.6bcdef	0.32±0.0ef
	150	M+	91.2±3.4de	13.6±0.8cdef	14.0±4.0ab	18.4±1.8def	11±1.2cdef	1.3±0.1cdef	0.34±0.1fg
		M-	76.6±2.2ab	13.9±1.1def	11.1±4.5a	12.4±5.5abcd	7.6±3.6abcd	0.9±0.4abcd	0.39±0.1h
20	200	M+	79.0±4.6b	12.6±1.4bcde	12.4±3.6ab	12.9±2.1abcd	7.9±1.2abcde	1.0±0.1abcde	0.35±0.1g

Table 4. Effect of mycorhization, P2O5 and landrace on Bambara groundnut yield. M-: without AMF, M+: with AMF (5 g.hole-1). For each column, the averages followed by the same letter are not significantly different at the 5% threshold.

#### Conclusion

Mycorrhization significantly improves growth (plant height, number of branches) and the yield of Bambara groundnut. The effectiveness of these fungal species in increasing plant yield and nutrient uptake varies with single superphosphate doses. High phosphate levels (200 kg.ha<sup>-1</sup>) reduce the effectiveness

of mycorrhization. We plan to test the effect of various combinations of local AMF strains found in the Bambara groundnut rhizosphere on yield and build a production unit for this biological fertilizer.

# > The problem solved

This study shows that it is possible to reduce the amount of chemical fertilizer used in agriculture by combination with organic fertilizers. We showed that mycorrhization significantly increases the yield of Bambara groundnut. In landrace V2 for example, mycorrhizae increased the yield by 87.5% compared to the control. A maximum dose of 100 kg P2O5.ha-1 combined with mycorrhizae helped to improve Bambara groundnut yield without inhibiting the effectiveness of mycorrhization.

# ➤ Full description of the methodology used

At first, a field trial was set up. The soil was removed from the Bambara groundnut rhizosphere. This soil was sandwiched between two layers of sterile sand in 500 g bags perforated in the lower third section. Two sorghum seeds were sown in bags and placed in bins to retain moisture. The plants grew under these conditions in a greenhouse until maturity. At this stage, the plants were transferred to the shelter and stressed until the shoot and the substrate were dry. The substrate was poured on dry paper and stirred to homogenize the contents. For each bag, a 100 g sample of substrate taken from each trapping bag was used to extract the spores by wet sieving through a series of sieves ranging from 400 to 38 µm (Gerdemann & Nicholson, 1963). The spore suspension contained in the sieves was centrifuged on a 50% sucrose gradient (Mbogne et al., 2015). After rinsing, it was poured onto a gridded surface filter paper to facilitate counting of the spores. The latter were counted using a binocular loupe according to their size, their colors, their shapes and the presence or absence of a hypha suspensor. Mycorrhizal spores were mounted between slides and lamellae in PVLG (Polyvinyllactoglycerol) with Melzer's reagent (Josserant, 1983) and identified on the basis of morphological descriptions (sizes, colors, shapes, ornamentations, wall characteristics, hypha suspensor) published by INVAM (International Culture Collection of Arbuscular Mycorrhizal Fungi). The retained strains were multiplied by trapping to produce an inoculum needed for the field trial. The average number of spores is expressed per 100 g of dry substrate. A good inoculum must contain at least 30 spores per gram of substrate. The field trial was performed as described above.

# > Description of the technology and know-how required for the successful transfer

For an effective transfer of technology, a production unit will be established in which the inoculum will be produced. Depending on the locality, different types of inoculum may be produced if it is realized that the dominant species are different after spore extraction and identification of native strains. Then, we will organize seminars with the producers not only to make them know the bio fertilizer, but especially to train them on its use. Subsequently, several production units or a distribution network will be set up to ensure the supply of producers in the main Bambara groundnut production areas.

Description of all steps required to achieve marketability- analysis of the target population;- study of competitors;- product analysis (strengths and weaknesses);- study of opportunities by referring to a similar product (other fertilizers);- define the price of the product (mycorrhizae);- to advertise.

# Part 2- know-how transfer

Table 5. Effect of mycorrhization on the distribution of Bambara groundnut pods. M-: without AMF, M+: with AMF (5 g.hole<sup>-1</sup>).

Landraces	P2O5 (kg.ha <sup>-1</sup> )	AMF	% solitary pods	% Double pods	% Triple and quadruple pods
		М-	91	9	0
	0	M+	84	16	0
		М-	97	3	0
	50	M+	90	10	0
		M-	96	4	0
	100	M+	74	26	0
		M-	81	19	0
	150	M+	95	5	0
		М-	85	15	0
V1	200	M+	81	19	0
		М-	59	35	6
	0	M+	38	43	19
		М-	53	45	2
	50	M+	35	60	5
		М-	47	46	7
	100	M+	67	31	2
		М-	61	34	5
	150	M+	29	67	4
		М-	37	46	17
V2	200	M+	38	43	19

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### A decentratlized strong authentication access system based on rfid, face recognition and raspberry pi

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**ABSTRACT**: Access to most secure areas is controlled with use of mechanical lock and key. The objective of this paper is to design a secure door access system using face recognition, raspberry pi and RFID, with capability of data collection and treatment. In this paper, we present three sub-systems that is a system to read, write and protect data on RFID card using raspberry pi and RC522, (The face recognition module has been successfully tested on students attendance) then a decentralized secure access system using the above components with the single addition of a stepper motor which functions as an actuator, finally a web platform to remotely access user data log. Radiofrequency identification (RFID) is a technology that uses radio waves to transfer data from an

# INTRODUCTION

In most African countries and in Cameroon in particular, access to buildings is done through the use of a lock and a key. This system over the years has proven to be ineffective due to a good number of issues it fails to address. For example, entry and exit of building is a process which produces data, which when analyzed latter can help in investigations and provide quick statistics to study and predict human behavior in big businesses so as to increase productivity and equally offer the optimum level of protection.

Again, the keys are easily losable and considering the fact that most keys are out of metals, it is expensive to replicate hence it is not cost effective. It is against this backdrop that we design a decentralized access system based on RFID and raspberry pi to address these issues. Radio frequency identification (RFID) is a matured technology that incorporates the use of electromagnetic or electrostatic coupling in the radio frequency portion of the electromagnetic spectrum to uniquely identify an object, animal, or person.

They are grouped under the automatic identification (Auto-ID) technologies (1). Because RFID offers a high degree of accuracy, we prefer to use it over the other Auto-ID technologies.

Some of these auto id technologies include fingerprint, DNA analyzer, palm, iris and others. The overall block diagram is shown in Figure 1.

### METHODOLOGY

The development of this system was done in three phases, namely programming of tags or cards, development and implementation of a door access system and system management software. electronic tag, called RFID tag or label, attached to an object, through a reader for the purpose of identifying and tracking the object. In our case, it is used to identify a person. When the holder of the tag places the card near the reader, the information is read and cross-referenced in the database for authenticity. If the information is validated, then the door opens and the data timestamped and saved in a local and online server, else the door remains locked. Using RFID tag is more accurate than other biometric means of its high authentication because of its accuracy of detection.

**Keywords:** RFID, Raspberry pi, RC522, stepper motor, biometric data.

# Programming of RFID cards/tags

The goal of this phase is to be able to write user personal information to the tag, again to read and protect it. First, the apparatus to read/write is setup consisting of raspberry pi and RC522 module, and the program to manage the read/write process installed. When the tag come within the reading range of the reader, all information in the tag is read and modified to user personal information. The read range *r*can be calculated using the Friis free-space formula

$$r = rac{\lambda}{4\pi} \sqrt{rac{EIRPGr au}{Pchip}}^{(2)}$$



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# Figure 2: Flow diagram of Programming of RFID cards/tags

Where  $\lambda$  is the wavelength and EIRP is the equivalent isotropically radiated power, usually determined by local country regulations example in Europe EIRP = 3.3W AND 4W in USA. P<sub>chip</sub> is minimum threshold power necessary to activate the RFID chip, G<sub>r</sub> is the gain of the receiving tag antenna, and  $\tau$  is the power transition coefficient between the chip and the antenna.The flow diagram below shows the flow of activities during this phase

#### Development and implementation of a door access system

This phase consists of assembling all the required components (raspberry pi, ULN2003APG, stepper motor, and RC522) with jumper wires, and executing a program to make all these components function to achieve access security. The program is written in python and is configured to run automatically during start-up of the raspberry pi.



Figure 3: Development and implementation of a door access system

The flow diagram below illustrates the sequence of activities in this phase. When the tag comes within reading range of the reader, the information is read and cross referenced in the database. If user is authorized, the door opens else the door remains closed and the system administrator notified. In either of the above cases, the user data is stored in a local and an online server.

#### System management software

In this phase, we develop a web platform using php, html, ajax, JavaScript, jQuery and others, thereby granting the system administrator remote access to the data collected in real time. The platform shall be used to perform queries, and the result of the queries used for investigation.

#### HARDWARE DESIGN

The hardware configuration of this system is mainly composed of a raspberry pi, RC522, UNL2003APG, stepper motor.

### Raspberry pi

Raspberry pi is a credit- card sized computer. It functions almost as a computer (3). It also contains components like SD Card Slot is used to install OS/booting/long term storage, HDMI OUT (High Definition Multimedia Interface) is used with HDTVs and monitors with HDMI input, GPIO allows us to control and interact with real world, Micro USB Power Port, RCA Video Out, Audio Out, Ethernet Port and others.



Figure 4:Raspberry pi Model B

Other boards like Arduino could also be used. But because of the high computational need of our system be prefer raspberry pi. The table below gives a brief comparison.

Variable/ Characteristics	Raspberry pi	Arduino
Internet Connectivity	Very Easy	Difficult
Processing Capability	Run multiple programs simultaneously	Run a single program repeatedly
Battery Support	Difficult	Easy
Complexity Support	Support more complexity	Support less complexity
Cost	Expensive	Less Expensive
Processor Family	ARM	AVR
Software Orientation	Highly oriented	Lowly oriented
Hardware Orientation	Lowly oriented	Highly oriented

Table 1: Comparison between raspberry pi and Arduino

#### RFID tag/card

Radio Frequency Identification (RFID) has a long history and is part of the technological revolution both current and past. RFID enables quick payment of tolls and quick identification of items. In addition, RFID provides benefits, such as tracking assets, monitoring conditions for safety, and helping to prevent counterfeiting. RFID plays an integral part in the technological revolution along with the Internet and mobile devices, which are connecting the world together (4).They are quickly overtaking and replacing the traditional bar-code system because of the numerous advantages they present.



Figure 5: Different shapes and sizes of RFID tags

There are two types of tags namely active tags and passive tags. They operate differently but carry the same information known as EPC-Electronic Product Code. This is a product numbering system that uses an additional set of numbers compared to barcodes and as-signs each item manufactured with a unique product identification number. The EPC system is linked to an online database that increases the opportunity for information sharing (5). Table 2 shows some major comparisons between the two.

Variable/ Characteristics	Passive tag	Active tag
Power	No power needed	Powered by an internal battery with finite lifespan
Range of detection	Short	Long
Interference	Sensitive	Less sensitive
Transmission rates	Low data transmission	High data transmission
Card readability	Can read few at once	Can read many at once
Precision	Reader needs to be aimed at the tag	Tags can be read without precise aiming

Table 2: Differences between Passive and Active tags(5).

#### RC522

A component that can read and write RFID tags with a frequency of 13.56 MHz (6).



### Figure 6:RC522 reader/writer

# ULN2003APG and Stepper Motor

Stepper motors are commonly used in precision positioning control applications. Five characteristics of the stepper motor have been considered while choosing stepper motor for the solar tracker prototype. Stepper motor is brushless, load independent, has open loop positioning capability, good holding torque and excellent response characteristics. A typical controller for a hybrid stepper motor includes:

(i)Logic Sequence Generator: -Generates programmed logic sequence required for operation of stepper motor.

(ii) Power Drivers: -These are power switching circuits which ensure a fast rise of current through the phase windings which are to be turned on at a particular step in the logic sequence. ULN2003 stepper motor driver has been used in the prototype.

(iii) Current limiting circuits: -These are meant to ensure a rapid decay of current in phase winding that is turned off at a particular step in the logic sequence(7).



# Figure 7: ULN2003APG and Stepper Motor

# IMPLEMENTATION

Two main connections are done in this system. One between the raspberry pi and RC522 which permit the reading and writing of data, and the other between the raspberry pi and the ULN2003APG, which in turn is connected to the stepper motor. First the figure below shows the structure and design of the entire system, taking into consideration the online server.

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Figure 8: Structure and Design of the system

The following tables show the interconnection between the GPIO pins and the colored female jumper wire used.

RFID	RASPBERR Y PI	GPIO PHYSICAL PINS	WIRE COLOR
SDA/SS	CEO	24	Green
SCK	SCLK	23	Blue
MOSI	SPI MOSI	19	Purple
MISO	SPI MISO	21	Grey
IRQ	N/C	N/C	N/A
GND	GND	6	Black
RST	GPIO 25	22	Brown
3.3V	3.3V	1	Red

 
 Table 3: Interconnection schematic between RC522 and Raspberry pi GPIO pins

ULN2003APG	GPIO PHYSICAL PINS	WIRE COLOR
IN1	29	Red
IN2	31	Green
IN3	33	Blue
IN4	35	Orange

 Table 4: Interconnection schematic between GPIO Physical pins and wire color

After connecting all the components together, a system like the one below is obtained.



Figure 9: Interconnection of components

# RESULTS AND DISCUSSION

The overall decentralized access security system is programmed with python and its web interface with php, html, JavaScript, ajax and jQuery. We developed an RFID system capable of reading and writing data to tags. After successfully writing user data to a tag, the data blocks are blocked then encrypted for safety purposes. Next, we successfully implemented the locking mechanism based on RFID and raspberry pi. We carried out a series of test on the system so as to observe the performance as shown on table below.

Observa tion	RFID unit	Door Open	Door Close	Data Capture
01	Success	Success	Success	Success
02	Failure	Failure	Failure	Failure
03	Success	Fail	Fail	Success

Table 5: Performance Test

From the above test, we observe that when the tag is registered in the database, the door opens else it remains locked. The second case suggests that the tag is non-functional and undetectable by the reader.

# CONCLUSION

In this paper, a decentralized system is presented that controls access with use of RFID tag and raspberry pi and a stepper motor as an actuator. This system can be further upgraded by using the timestamped log data to control other events such as services in a hotel. Again, it can be easily modified to take attendance of employees or of students in academic environments. The Problem solved should be clearly defined

Access to specific areas should be protected and authorization should be given only to those who are supposed to be there at a specific moment of time. The lock and a key system over the years has proven to be ineffective due to a good number of issues it fails to address. The strong authentication proposed is to use biometric data and also a specific given information that can be overridden at anytime. Fully description of the methodology used

The methodology is to use the Python Open CV library and many other libraries for face recognition based on the list of faces that were pre recorded in a MySQL database. In order to avoid the well-known limitations on such a technique, we add a what you are a what you have leaving a side the what you know that can be added to the project if a customer requires.

Description of the technology and know-how required for the successful transfer

The written scripts are in Python and many other languages specifically for the RFID tag that is issued only to those that should have. A camera that is not always an IP camera is suitable and a firewire or USB interface is acceptable by the Raspberry Pi 3 nanocomputer. The RFID card writer/reader and a card itself is for each user. The server that can use the LAN or WLAN permits to connect to the server where an interface is built an allows to visualize all kind of statistics.

Description of all steps required to achieve marketability

The first step is to identify those who need to protect an asset for access authorization. They can be Bank system, Government documents protection at court, police, military zone, etc.

After we have identified the needs of the customer,

A comparison with the current system can be done and the subject under test by us can be proven to be ineffective. If such a situation occurs, we propose our system that comprises the following.

Description	Price
Server	1500000
Wireless router	100000
RFID Reader	100000
RFID Cards	100000
Camera	50000
Necessary cables	4000000
Workmanship	2000000
Script Licences	2000000
Total Taxes Free	9 850 000

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# Isolation of a pentacyclic triterpenoid from Sceletium tortuosum

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# Abstract

Traditionally, extracts from Sceletium tortuosum have been used, both as a medicine as well as for social and spiritual purposes. The genus is distributed in the southwestern parts of South Africa. Methanolic extracts of S. tortuosum were prepared and fractionated using column chromatography. Indole alkaloids have been reported from S. tortuosum with mesembrine as the most abundant alkaloid and mesembrenone, 4'-odemethylmesembrenol and tortuosamine also present. A triterpenoid, novel to this genus was isolated and reported for the first time. NMR and HPLC-MS/MS analyses were used to confirm the structure of the compound.

Key words: Sceletium tortuosum, alkaloids, triterpenoid, obtusalin.



**Figure 1:** Characteristic skeletonised appearance of old leaves (Gericke and Viljoen, 2008).

The general application of Sceletium (Aizoaceae, subfamily Mesembryanthemoideae) had been revised by various authors since the genus was established in 1925 by N.E. Brown (Gericke and Viljoen, 2008). This group of plants is characterised by the skeletonised leaf venation pattern visible in dried leaves. In 1986, Bittrich argued for a broader application of Phyllobolus which included Sceletium as one of five subgenera (Gericke and Viljoen, 2008). Since Gerbaulet (1996) was unable to find a

# Introduction

The genus Sceletium is classified under the family Mesembryanthemaceae (Aizoaceae) and belongs to the sub-family Mesembryanthemoideae (Patnala and Kanfer, 2009; Smith et al., 1996). The genus name is derived from the word 'sceletus' which means 'skeleton', referring to the prominent vein-like lines, which are easily visible in the old, dry and withered leaves (Figure 1) (Gericke and Viljoen, 2008). The word Mesembryanthemum originates from the name 'midday flower', which refers to the opening of the flowers around noon (as shown in Figure 2). Plants that belong to the Mesembryanthemaceae family are known by most South Africans as vygies (Chesselet et al., 2002)



# Figure 2: Represents that decumbent habit and succulent leaves of *Sceletium* spp. (Gericke and Viljoen, 2008).

synapomorphy (a unique derived character) for Bittrich's broad concept of Phyllobolus, she reinstated Sceletium as a genus (Gericke and Viljoen, 2008). Species of this genus are distinguished on the basis of vegetative, flower, fruit and seed characteristics. Some species are reduced to synonymy including S. joubertii L. Bol., and S. namaquense L. Bol. now considered part of S. tortuosum. Sceletium exhibits a climbing or decumbent habit and has characteristic succulent leaves with "bladder cells" or idioblasts.

The genus Sceletium is distributed in the south-western parts of South Africa and has an affinity for arid environments. It is amongst taxa that have been extensively researched in the past few decades. Traditionally, plants of this genus have been used to relieve thirst and hunger, to combat fatigue, as medicines and for social and spiritual purposes by San huntergatherers and Khoi pastoralists (Gericke and Viljoen, 2008). South Africa can benefit from the scientific evaluation of this indigenous plant and its knowledge base. However; the chemistry and pharmacology of many medicinal plants, such as Sceletium tortuosum, have not

2. Material and Methods

# 2.1 Plant collection

Nineteen kg of Sceletium whole plant material was harvested on 11 – 12 June 2009 under sunny and dry conditions at Kamieskroon in the Northern Cape of South Africa. The plant material was supplied to the Council for Scientific and Industrial Research (CSIR) for this study by Enterprise Creation for Development (ECD). Plant material was collected by an independent contractor who practiced standard operating procedures during harvesting/handling, storage and transportation. This study required harvesting of whole plant material by the supplier. The plant material was positively identified and confirmed to be Sceletium tortuosum N.E. Br. by the South African National Biodiversity Institute (SANBI), batch number: 10025 (Genspec No. ECDMP-100 22).

# 2.2 Plant preparation and extraction

About 6.3 kg of wet plant material was placed in a 60 oC oven to dry. The dry plant material was not ground to a fine powder, but was broken and crushed into smaller pieces. From that, 3.15 kg was transferred to a 1000 ml glass beaker. Two large magnetic stirrers and sufficient methanol (MeOH) solvent, enough to cover the plant material was added. The beaker was covered tightly with a piece of aluminium foil before being placed on a stirring heating plate and heated at 40 oC for 2 days (48 hrs). A mercury thermometer was used to monitor the temperature throughout the extraction process. After extraction, the contents were mixed thoroughly and then filtered through filter paper into a 1000 ml conical flask connected to a vacuum pump. The extract was filtered twice to remove any debris remaining in the suspension. The MeOH was evaporated at 45 oC using a GeneVac® Personal

yet been thoroughly investigated (Harvey, 2000). It is believed that the phytochemical exploration of the genus Sceletium commenced in 1898 when Meiring isolated a crude alkaloid mixture from S. tortuosum. This was followed by the work of Zwicky in 1914 that isolated alkaloids including mesembrine several and mesembrenine (Gericke and Viljoen, 2008). The number of Sceletium species within the Aizoaceae family that have been examined for the presence of alkaloids has been restricted by their geographical inaccessibility. Within the Sceletium genus, a number of alkaloids are produced which mainly belong to the crinane class of compounds (Jeffs et al., 1982), but no compound belonging to the triterpene class has been reported to have been isolated from Sceletium.

evaporator. The crude extract was then stored in a dark, cold room at 4 oC.

# 2.3 Compound isolation and purification

Obtusalin was isolated from the methanol extract of S. tortuosum using Preparative Layer Chromatography (PLC) with a solvent system containing hexane, ethyl acetate and triethylamine [Hex: EtOAc: Et3N (6:4:1)] as eluant. The plate was run twice to improve separation between compounds. A very thick band was observed under UV light and used for further purified using PLC with Hex: EtAc: Et3N (6:4:1) as solvent system. Ultimately, 2 mg of obtusalin was isolated. NMR (600 MHz, Varian,) was used to confirm the structure, using 1H, 13C, COSY, DEPT, HSQCAD and HMBCAD protocols to generate chemical shift data for subsequent comparison with that found in the literature.

#### 3 Results and discussion

The methanol crude extract of S. tortuosum was fractionated and several compounds were isolated, purified and identified. These compounds were confirmed to be alkaloids that have commonly been reported in the extraction of S. tortuosum; namely, mesembrine,  $\Delta 4$ mesembrenone,  $\Delta 7$  mesembrenone (mesembranol) and epimesmbrine. An unexpected finding which later became apparent, was that these compounds degrade over a period of time, verified by the degradation of mesembrine. Consequently; the compounds had to be re-isolated, with more attention being paid to chemical instability problems. A separate High Performance Liquid Chromatography (HPLC) study was undertaken to evaluate the stability and determine if there were gradual changes occurring within the extracts while in storage. Mesembrine and  $\Delta 7$ mesembrenone were re-isolated, with some measure of stability, but the other two compounds had degraded to such an extent within the extract that they could not be reisolated. In addition to these compounds, pinitol and sucrose were also isolated from the plant (Setshedi, 2014).

A pentacyclic triterpenoid confirmed as obtusalin (Figure 3) was isolated for the first time from the methanol extract of S. tortuosum. Triterpenoids are generally very stable and, unlike the alkaloids of this plant material, do not decompose, but were found in low concentrations. Triterpenoids are commonly found in most plants and are produced by arrangements of squalen epoxide in a chairchair-chair-boat arrangement subsequently followed by

Compound 1

condensation (Patočka, 2003). These compounds are isopentenoids composed of thirty carbon atoms and may possess acyclic, mono, di-, tri-, tetra- or pentacyclic carbon skeletons. Pentacyclic triterpenoids are dominant constituents of this class and have been widely investigated (Mahato and Kundu, 1994). Obtusalin is a pentacyclic triterpene belonging to the lupane class of compounds (Zheng et al., 2010; Siddiqui et al., 1989) that was first isolated by Siddiqui and colleagues in 1989 from the leaves of Plumeria obtusa. Obtusalin forms part of is the few naturally occurring pentacyclic triterpenoids possessing a C-27-hydroxyl group in conjunction with a double bond at C-12 in the lupine chain of triterpenoids (Begum et al., 1994 and Siddiqui et al., 1989). Pentacyclic triterpenes are reported to possess a wide spectrum of biological activities, where some may be used as medicines (Patočka, 2003).



Figure 3: Structure of Compound 1

# Table 1: <sup>1</sup>H NMR spectral data of compound 1 (CDCl<sub>3</sub>) in comparison to that found in literature (CDCl<sub>3</sub>) (Siddiqui et al., 1989)

-		
Proton Number	$\delta { m H}$ ( J in Hz)(isolated)	$\delta^{13}{ m H}(J { m in Hz})$ (Literature)
3α	3.16 (br $d$ , $J = 12.69$ )	3.21 ( <i>dd</i> , <i>J</i> = 10.8; 4.9)
5α	0.68 (br $t$ , $J = 12.1$ )	0.72 ( <i>dd</i> , <i>J</i> = 11.6; 1.5)
9	1.54 (m)	1.54 ( <i>dd</i> , <i>J</i> = 10.0, 3.4)
11 <b>a</b>	1.85 (br <i>dd</i> , <i>J</i> = 8.8, 6.4)	1.84 ( <i>ddd</i> , <i>J</i> = 13.2, 3.6, 3.4)
11 <b>β</b>	1.63 (m)	1.61 ( <i>ddd</i> , <i>J</i> = 13.2, 10.0, 3.6)
12	5.07 (brs)	5.13 ( <i>t</i> , 3.6)
23	0.94 (s)	1.01 (s)

24	0.93 (s)	0.98 (s)
25	0.72 (s)	0.78 (s)
26	0.88 (s)	0.94 (s)
27a	3.46 (brd, <i>J</i> = 11.2)	3.52 ( <i>d</i> , <i>J</i> = 10.9)
27b	3.14 ( <i>brd</i> , 11.2)	3.18 ( <i>d</i> ,10.9)
28	1.03 (s)	1.10 (s)
29/30	0.87 ( <i>brd</i> , <i>J</i> = 5.2)	0.93 (d, J = 5.8)
30/29	$0.75 \ (brd, J = 7.0)$	0.80 (d, J = 5.9)

 Table 2: <sup>13</sup>C
 NMR spectral data of compound 1 (CDCl<sub>3</sub>) in comparison to that found in literature (CDCl<sub>3</sub>) (Siddiqui *et al.*, 1989 and Begum *et al.*, 1994)

Compound 1

Carbon Number	$\delta^{13}$ C (Isolated)	$\delta^{13}$ C (Literature)
1	38.8	38.8
2	27.2	27.3
3	79.0	79.1
4	38.0	38.0
5	55.1	55.2
6	18.3	18.4
7	32.8	32.9
8	40.0	40.1
9	47.6	47.7
10	36.9	36.9
11	23.4	234
12	125.0	125.1
13	138.7	138.8
14	42.0	42.1
15	23.4	23.4
16	26.0	26.0
17	38.8	38.8
18	54.0	54.0
19	39.4	39.5

20	39.3	39.4
21	30.6	30.7
22	35.2	35.2
23	28.1	28.2
24	16.8	16.8
25	15.6	15.6
26	15.7	15.7
27	69.9	69.9
28	23.3	23.3
29	21.3	21.3
30	17.3	17.3



Figure 4.: HRTOFMS (ESI+) chromatogram of compound 1

The HRTOFMS (ESI+) spectrum (Figure 4) of compound 1 showed a pseudo-molecular ion signal [M]+ at m/z 441.3689 which corresponds to the molecular formula C30H50O2. There are significant fragments as indicated in Figure 5. This data strongly indicates that the compound is of a lup-12-ene type. 1H NMR, 13C NMR and MS spectral data of compound 1 are in agreement with those reported in literature for obtusalin (Siddiqui et al., 1989

and Begum et al., 1994). One olefinic proton was observed in the proton NMR spectrum (Table 1) and resonated at  $\delta H$ 5.07, while two secondary methyl groups resonated at  $\delta H$ 0.87 (J= 5.2 Hz) and  $\delta H$  0.75 (J= 7.0 Hz), five three-proton singlets indicating tertiary methyls that resonated at  $\delta$  0.94, 0.93, 0.72, 0.88 and 1.03. The 13C NMR spectrum (Table 2) showed the presence of 30 carbon signals: olefinic carbons at  $\delta C13$  125.0 and 138.7; oxygenated carbons at  $\delta$  79.0 and 69.9 and seven methyls at  $\delta$ C13 15.6, 15.7, 16.8, 17.3, 21.3, 23.3 and 28.1. Accordingly, the structure of compound 1 was assigned as obstusalin.

#### 4 Conclusion

Sceletium species have been shown to contain indole alkaloids with mesembrine reported as the most abundant alkaloid in S. tortuosum. A pentacyclic triterpenoid, novel to this species as well as the genus was isolated and reported for the first time. NMR and HPLC-MS/MS analyses were used to confirm the structure of the compound. Pentacyclic triterpenes are reported to possess a wide spectrum of biological activities, with some used as medicines.

5 Acknowledgments

We thank the Department of Science and Technology for their financial support.



Figure 5: HRTPFMS (ESI<sup>+</sup>) fragmentation of compound 1. Showing peak fragmentation correlating to those significant fragments which strongly suggests that the compound is of a lup-12-ene type.

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# Factors affecting the global diffusion of an African animal genetic resource: the case study of the *Cameroon Blackbelly* sheep

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#### Abstract

Sheep farming is a vital component in the food, economic and socio-cultural security of many countries around the world. Sheep are present in most countries of the world, proving their adaptation capacities to different climatic conditions and agro-ecological zones in different countries. Because of these capabilities, they have been widely disseminated in order to enhance their genetic potential. Several elements have contributed to the diversification and dissemination of this biodiversity, including the human slave trade waves. During these movements, Cameroon ovine biodiversity has been exported and distributed throughout the world, particularly the Blackbelly sheep breed. Within the framework of the World Action Plan focusing on the characterization of native biodiversity, it is important to trace the dispersal routes and models of this particular breed across the world in order to demonstrate the impact of slave trade in the dissemination of animal material, but also to collect information on the ways of adaptation and exploitation of the Blackbelly sheep around the world. This could enable the conservation and better improvement of this genetic resource in Cameroon for the benefit of population. From publications collected on the matter around the world, it appears that slave trade and the triangular trades were the main factors of global diffusion of the Cameroon Blackbelly sheep. The Blackbelly has been spread in two continents: America and Europe, but its main settling area was the Caribbean. The Barbaric Island was the main point of adaptation of this breed hence the name Barbados Blackbelly from which several countries have refueled. The Blackbelly sheep has been adopted as an official breed after improvement in 4 countries: Barbaric Island (Barbados Blackbelly), the United States (American Blackbelly), Germany (Kamerun Schafe), and in Martinique (Martinik Sheep). All these varieties share some specific features that have remained very close to that of the sheep of Cameroon notably: the brown coat color with a blackbelly, resistance to diseases (trypanotolerant), hardy, and prolific. Worldwide, the Blackbelly sheep is exploited for meat, leather, prestige, ecotourism and milk in some extent. The current state of information on Blackbelly in Cameroon is limited to descriptive study and some phenotypic assessment. In-depth studies on its mode of exploitation and its molecular genetics will certainly allow better understanding of this breed to plan for methods of conservation and sustainable improvement.

Key words: Blackbelly sheep, colonization, international trade, biodiversity, Cameroon

#### Introduction

Sheep or Ovis aries Linnaeus 1758 is one of the oldest domestic animals. It is widespread throughout the world, with high numbers, demonstrating the ability to adapt to different climates and universal interest (Meyer et al., 2003). The sheep is one of the first species domesticated by humans after the dog and goat, around 9000 - 8500 BC. The domestication of sheep took place in the region of the "fertile crescent" (Turkey, Syria, Iraq, present day Iran) (Meyer et al., 2003). Sheep from Africa and Europe are distributed from this center (Lauvergne, 1979). The sheep appeared for the first time in Europe in 6300 BC to Argissa-Magula. It arrived in Western Europe a few centuries later, by land and by sea. A second wave of sheep arrived in Western Europe during the second and the third century. These animals were larger and had wool fleece. The sheep was already present in North Africa, entering through Egypt around 5000 BC. In Africa, there are trypano-tolerant sheep breeds, indicating the ancient presence of this species locally (Mason, 1951; Meyer et al., 2003). These are smaller sheep adapted to the ecological conditions of West Africa, hence the name "West African dwarf sheep". With the movements of humans and animals, several animal species have moved from one area to another. These movements have been at the origin of several sheep genetic breeds in the world.

This paper finds traces of several very old breeds in areas that are not their zone of origin, for the particular case of Blackbelly (from Cameroon) their traces can be found in America, the Caribbean and even in Europe. Genetic characterization has for some years been the method of describing and classifying livestock breeds using measures of genetic distances between populations (Cavalli-Sforza and Edwards, 1967; Nei, 1972; Nei et al., 1983). Indeed, for sheep, Lauvergne (1979) explains that visible markers could be used to measure quite simply the degree of archaism of a sheep population: composition, coloration and molting of the fleece, type of horn, conformation of the head (chamfer, ears), tail shape. This facilitates the description of sheep genetic diversity in the world. Nijman et al., (2003) supports that mtDNA can also provide a quick way to detect hybridization between farm species and wild sub-species. Of recent, microsatellites became prime markers for the study of diversity (Ruane 1999, Sunnucks 2001) because of their co-dominant nature, ease of amplification and hypervariability while SNPs are becoming more popularized. The blood groups and biochemical polymorphisms of domestic animals have been studied since the early 1960s and have since been used to compare races and study the relationships between them (Grosclaude et al., 1990).

It happens that 5 main breeds were described in Cameroon and are the subject of a summary knowledge of their characteristics: Doutressoule (1947) described the Djallonke, Bardoux (1986) described the Kirdi breed, Dumas (1977) worked on the Peuhl and Uda breeds, Manjeli (1998) described the Blackbelly breed.

Origin and description of the Cameroon Blackbelly sheep

The characterization of animal species consists in the first phase in a classification and description of animal units called races (Gizaw, 2008). The genetics of African pastoralism is controversial and often very underdeveloped (Hanotte et al., 2002). Djallonké sheep include all the most common trypano-tolerant sheep populations in tsetse-infested areas of West and Central Africa; south of the 14th<sup>o</sup> parallel (FAO, 1992). These sheep would come from Fouta Diallon in Guinea (hence the origin of the name Djallonke) and would have for ancestor, the Egyptian sheep Ovis longipes (Devendra and McLeroy, 1982). The most commonly encountered coat patterns are black, black-legged and more rarely white, red, red-legged (Figure 1). In certain regions (particularly in the East of Cameroon), there are animals with a particular tawny coat with belly and legs colored black, which gives them the name of Blackbelly in English (Vallerand and Branckaert, 1975). The Blackbelly sheep is a breed with a fine (smooth) tail, the head is small with a straight profile. The ears are small and slightly drooping (Manjeli et al., 1991). It can reach 55 to 65 cm at the withers. This sub-race is genetically stable and the animals have a more advantageous external appearance. This impression is confirmed by their average weight, which in adult females of this sub-breed exceeds 28 kg (Vallerand and Branckaert, 1975). In Cameroon the Blackbelly sheep is used only for the production of meat, the male can reach 25 to 35kg. Nevertheless, the production of Cameroon's Blackbelly sheep milk is appreciable, ranging from 2.95 to 3.52 liters per week (Manjeli et al., 1991).



Figure 1: Cameroon Blackbelly young ram

Spreading factors and exploitation of Blackbelly sheep

The Blackbelly sheep by its qualities of adaptative production traits has been widely distributed in the world for a hair sheep. This has undoubtedly been favored by several factors such as triangular trade and the slave trade. This demonstrates that the method and chronology of dispersion of African animal breeds also have a non-nuclear cause (Hanotte et al., 2002). These two movements of the western colonial era have had a significant impact on the distribution of animal material, and more specifically on dwarf sheep in West Africa (Naves et al., 2011). One of the determining factors for the spread of Blackbelly sheep in the Caribbean and some Latin American countries were triangular trade with West African ports, simultaneously with human trafficking

(Naves Met al., 2011). It resulted in recurrent introductions of domestic animals from West Africa, between the sixteenth century and the beginning of the nineteenth century (Maillard and Maillard, 1998). For instance, the analysis of mitochondrial DNA of some bovine genotypes in the Caribbean and America has demonstrated their African origin. In Asian chickens, nine different clades of mtDNA have been identified (Liu et al., 2006) that suggest multiple origins in South and Southeast Asia. These results indicate that the knowledge about farm animals' domestication and genetic diversity is still largely incomplete. Therefore, it is established that there is a strong genetic component of African origin in the Caribbean countries' sheep breeds (Blackbelly or Pelibuey), in the Creole goats of the Antilles (Pépin, 1994), and in the Creole cattle of Guadalupe (Miretti et al., 2004; Naves and 2011). Exchanges have also taken place between the Caribbean islands and the American continent, in the North as well as South (Maillard and Maillard 1998; Lucero et al., 2010).

# Expansion to the Caribbean and America (South and North)

The Blackbelly sheep has had a large dispersal in the Caribbean and mainly in the Barbarian Island (Shelton et al., 1990). During the period of the slave trade, the triangular trade allowed the transit of goods between America, Europe and Africa. These movements across the Atlantic Ocean included several Caribbean countries (Cuba, Dominica, Jamaica, Trinidad and Tobago, Martinique, Barbados etc.). The ships from Africa transported slaves but also animals from Africa. This movement allowed the transport of African sheep breeds to the Caribbean. Historic research demonstrated that the *Furry sheep* in the Caribbean is of African origins, namely from Nigeria, Angola and Cameroon during the period of slave trade (Mason, 1980).

# The Barbados

Barbados could be considered as the main area of installation and adaptation of Blackbelly sheep from Africa (later mentioned as *Cameroonian sheep*). According to the BBSAI (2011), the Blackbelly arrived in

the Barbados Island during the period of colonization of the island by the Europeans. Combs (1979) has established that during the first quarter of the century of colonization, a batch of Blackbelly sheep was set up on the island following British colonization in 1627. This sheep adapted to this climate during the exchanges of the slave trade has medium and horizontal ears. It is called Barbados blackbelly with reference to Barbados which is at the origin of its expansion (Shelton et al., 1990). The pattern of Blackbelly color, which seems to be simply inherited, is found in the current populations of Djallonke type sheep in West Africa. This observation, and several other features that the breed shares with the West African sheep, including the shape and size of the ear, of course, the hair (Figure 2), indicates a West African origin for the Barbados Blackbelly sheep breed. However, the latter has two distinctive features, namely: a larger average size of all "West African" populations, and higher prolificacy (Fitzhugh and Bradford, 1980). It is a larger sheep than Cameroon sheep and is generally used for meat production. Ewes raised from 1972 to 1977 at three locations in Barbados produced 1079 litters of milk, with 2194 lambs, an average of 2.03 lambs per litter, and at an average lambing interval of 8.48 months. Rastogi et al (1980) observed 26.8% single birth, 47.3% twins, 22.1% triplets, 3.4% quadruplets and 0.4% quintuplets.



Figure 2: Barbados Blackbelly ram

#### Cuba

According to Mason (1980), the Cuban sheep apparently also came from Africa. It is estimated that about 75% of sheep in Cuba are hair breeds originally called *Pelobuey Carnero* (that is, beef-haired sheep) that has been shortened to *Pelibuey*. These sheep are also called *Criollo*, but they differ from the real *Criollo* of the mainland of Latin America, which are wool sheep of the Churro breed of Spain (Shelton et al., 1990). *Pelibuey* sheep have a variety of colors, including brown (or red), cream whitish and white, black, and more. The first three colors are the most common and are colors that would be inherited from their African origins. *Pelibuey* sheep on state farms are either red or white. Red animals, which are believed to be physically stronger, are preferred. In addition, the red color is dominant in the *Pelibuey*, but not compared to the white types in the European breeds.

#### **Dominican Republic**

There are haired sheep in the Dominican Republic. According to Mason (1980), there are about 52,000 sheep in the Dominican Republic and 90% of them are haired sheep. There is a report of some Cuban *Pelibuey* sheep from the Dominican Republic (Mason, 1980).

# Mexico

There are also traces of Blackbelly in Mexico. In fact, there is a sheep breed in Mexico called *Tabasco* or *Pelibuey* of Mexico whose origins are connected to the Blackbelly sheep of Africa. Figueiredo et al., (1990) in the analysis of sheep origin assume that the Pelibuey of Mexico was introduced from Cuba around the 1930s. In addition to the absence of horns in both sexes, it was found that coat colors patterns are close to those in Blackbelly. Zarazua and Padilha (1983), have shown that this breed is easily adapted to wet climate, with longer rainy seasons, where heat and humidity are high, similar to the humid tropics' conditions. This breed is raised mainly for meat; mature animals are 64-66cm for the height at withers, with live body weight ranging from 35 kg(female) et near 50kg (male).

# **Trinidad Tobago**

Sheep production has been practiced in Tobago for more than 100 years. There were no native sheep in Tobago and the current sheep are descendants of the imported breeds. As early as 1909, a ram and two purebred West African ewes were imported from Barbados. It is unclear as to when Barbados first imported Blackbelly to Tobago occurred. In 1953, the breeding policy of the Tobago Government Farm was directed towards the gradual establishment of a flock of Blackbelly sheep from Barbados and in 1958 three sheep breeds were maintained there, namely: Blackhead Persian, West African Dwarf and Barbados Blackbelly (Fitzhugh and Bradford 1980). Later on, other imports of Barbados Blackbelly took place from the West Indies (in 1977) and Barbados (1977-78). The majority of sheep in Tobago are raised by small farmers in groups of one to five as a ready source of money or meat for a special occasion. In Trinidad Tobago farms, the Blackbelly sheep can weigh 21 to 45kg at maturity.

# Colombia

Colombia has also inherited animal genetic material during the slave trade (Shelton et al., 1990). African sheep in Colombia are similar to Pelibuey from Mexico. The yellow or Sudan type varies from yellow to reddish-brown in color (Otero and Cruz, 1980). African sheep are also known as Pelona or Camura and have existed in Colombia for over 300 years. Some exceptional characteristics of these genotypes include adaptation to warm climates, hardiness and quality of meat. It is generally recognized that these sheep were brought from West Africa on slave ships (Shelton et al., 1990). In Colombia, sheep are generally kept in small herds of less than 50 animals grazing the less productive areas of the farms. They receive special care and derive much of their food from browsing and agro-industries by-products. Their gestation period is 151.7 days for single lambing, with lambing intervals of 213.6 days (Pastrana et al., 1983). Animals

weigh 2.5 kg at birth, 15-18 kg at weaning (4 months), 35-40 kg for one year and 49 and 45kg for male and female fattened animals (12 months old). An adult male under intensive conditions can weigh up to 80kg (Otero and Cruz, 1980). Other studies show that adult females and males weighed 36.7 and 48.7 kg, with 78.0 and 84.0cm for chest girth, and height at the withers 62.9 and 68.7cm respectively (Pastrana et al., 1983). The average age at first lambing is 15-19 months. The fertility rate reaches 98%, with a prolificacy rate of 1.34.

#### United States

The United States have for several years given particular interest in Blackbelly sheep. Genetic analysis of modern American breeds has demonstrated a variety of origins: Europe (Campbell and Lasley, 1985; Davis et al., 1988), Africa and India (Speller et al, 2013). The Barbados Blackbelly come from crosses made from sheep with hair from East Africa. Originally from Barbados, they have extended to the entire West America. According to Combs (1979) 4 ewes and a Barbados Blackbelly ram were imported to the US in 1904 by the USDA. It is from these that the American Barbados Blackbelly or American Blackbelly sheep breed was developed (CODD, 2017). It is assumed that two types of fine (smooth) tail sheep came to America. One, without mane, probably from savannah areas in the Congo region, including northern Angola and Cameroon. These are believed to be the ancestors of the Morada Nova of north-eastern Brazil and other hairless sheep (Mason, 1980; Bradford and Fitzhugh, 1980). The animals that gave origin to these types of sheep were probably selected from flocks with a high frequency of brown color and its combinations. The American Blackbelly sheep type (American variant) looks more like a Cameroonian type of great size. This breed with the Painted Desert, the Texas Dall, the Desert Sand and the Black Hawaiian, is part of the "Corsicans sheep". All these breeds have the particularity of having been obtained in the last century in the United States, by crossing animals from the Barbados with Mouflons (Corsica), but also Rambouillet Merinos and Dorsets (CODD, 2017). The coat is exclusively tawny, from light to reddish brown (Figure 3). This breed is considered mostly for meat. The weight of American Blackbelly is significantly higher than that of the Cameroon type; records in mature individuals show 50 to 60kg for the ewe and up to 90-100kg ram. The prolificacy of the American Blackbelly is close to 200%, doubles or triplets are common, quadruplets are not rare and occasionally quintuplets.



Figure 3: American Blackbelly ram

# The Martinique

Sheep were introduced to the West Indies from colonization and more likely from the shores of the Gulf of Guinea in Africa. They are hairy sheep, closely related to the Djallonke sheep or West African types. The Martinik sheep is selected from Barbados Blackbelly. In 1993, the Martinik sheep benefited from the establishment of a breeding program based on seven private farms grouped together in a Selection Body (Union for the Selection of Martinik Sheep) approved by the Ministry of Agriculture. Carried out in collaboration between the various partners of the genetic sector in France, it was then possible to supply the breeders with sheep of well-known breeds, and evaluated on their results obtained in control of performances (Vertueux et al., 2006). Of recent

creation, the *Martinik sheep* breed includes animals of various coats, but with very similar abilities. The adult sheep weighs on average from 35 to 45 kg and the rams from 50 to 70 kg, according to the conditions of breeding. The height at the withers for ewes is around 55cm. The small ears are borne horizontally and the tail is of medium length (above the hock); fertility is 85% and productivity 1.9 (USOM, 2008). Well-fed adult *Martinik sheep* are generally strong enough not to require anthelmintic treatment, with the exception of a few ewes around lambing, with litters of two or more lambs. Recent comparisons with woolen breeds have also demonstrated higher genetic resistance to parasites in Martinik lambs, making them an interesting breed for valorizing tropical pastures (INRA, 2014).



Figure 4: Martinik ram - (USOM, 2008)

#### **Expansion** to Europe

Europe was the decision point of the spread of Blackbelly sheep to the Caribbean and America through the triangular trade of the colonial era.

#### Germany

Originally, from West Africa, the path that led this wild sheep breed to Europe was unusual. In Germany around the 1900s, Zoological gardens were created. For this reason, ferocious beasts have been shipped from African colonial countries amongst which were lions and leopards. As provisions for wild animals, a breed of African sheep was brought. Some sheep survived these trips and started being reared. Germany empire was established in then called *Kamerun* from where the colonial administration extracted numerous living resources to Europe where traces are still visible. The cradle of Cameroon Blackbelly sheep is East Forest (Moloundou) where a small German colony was established in late 1800s. Sheep in parks and zoos have gained great popularity and today they are mainly found in amateur breeders, because the Cameroon

sheep are convenient and robust landscapers. Called "*Kamerun schafe*" (Figure 5), Cameroonian sheep experienced a very good adaptation in Germany (Ladybug-Farm, 2010). Most black sheep breeders keep their sheep as meat suppliers. The objective is the proper formation of valuable pieces of meat on the back and leg.

Animals slaughtered between 5 and 8 months for about 10-16 kg of meat. The meat has a taste and appearance similar to that of game meat such as the usual lamb meat. The exquisite taste is not lost even in the slaughter of old animals.



Figure 5: Kamerun schafe ram

#### **Other European countries**

The Cameroonian sheep has also been located in several other European countries, particularly in France, England and the Netherlands. Although the mechanisms of diffusion remain very difficult to trace, the authors agree that it was favored by the trade routes favored during the period of colonization. From Combs (1983) findings, Blackbelly sheep was introduced in England and Netherlands during the first quarter of 19<sup>th</sup> century. There have been some early breed associations and relatively interesting literature on this breed in Central and even eastern Europe which could have contributed to the popularization of this sheep genotype to Europe and other directions as pet or zoo animal (Mathieu, 1997).

# Migration waves and spatial distribution Blackbelly sheep

Multiple movements of Blackbelly sheep occurred in several centuries, first northern to Europe and western as shown by the figure 6.



Figure 6: Map of Blackbelly sheep dispersal in the world

#### Primary migration

The very first documented migrations of the Blackbelly from Central Africa (its cradle) to other horizons which dated back from the 15<sup>th</sup> century. This migration was then factored by two main phenomena: the slave trade and the colonization. If the mechanisms are not clearly established on the primary migration of Blackbelly, it is recognized by several authors (Fitzhugh and Bradford, 1980; Mason, 1980; Shelton et al., 1990) that, these two phenomena had a great impact on the spread of this genetic material. The main countries with this primary migration waves were Barbados, Cuba, Brazil, Colombia and Trinidad and Tobago.

# Secondary migration

After the transfer of this genetic resource from Africa to the Caribbean countries, other countries interested in the qualities of production, adaptation but especially disease resistance imported the Blackbelly sheep mainly from the Barbarian islands. These were mainly the United States of America and Martinique, and surprisingly from these sites to Europe (Great Britain and the Netherlands). In these new destinations, new varieties of Blackbelly sheep were developed with emphasis either on crossing or later on selection. The main mechanisms were importation and colonization (Rastogi et al., 1980, Naves et al., 2011).

#### Tertiary migration

The migration of Blackbelly sheep is continuous, within the various the host countries for several purposes, mainly adaptation. In the most recent migration traceable activities, France and Martinique were involved; it resulted in the development of the *Martinik breed* (Mathieu, 1997). Transboundary migration is also very common in Central Africa, though less documented.

# Conclusion

The *Blackbelly sheep*, originated in tropical humid Africa, probably Central Africa and has become very popular throughout years. The breed popularity is based on its ability to adapt and its high prolificacy. Blackbelly sheep has been widely distributed throughout the world through the slave trade and triangular trade. There are various varieties, but all indicating the tendency for meat. In order to enhance its genetic potential, several countries have developed more productive genetic types through selection and double-crossing. The original traits remained the same

in many of these breeds thus favoring the identification of their origin as *Cameroon Blackbelly sheep*. These movements of the colonial era will have had a major impact in the dissemination of animal material between Europe, Africa and America. Deeper molecular

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investigations are to be done to attest the genetic distances and news traits, while Cameroon government should invest more for the protection, ownership and valorization of this important biodiversity resource.

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#### The 3D printing of moulds used in injection moulding processing

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#### Abstract

The possibility of using additive manufacturing methods such as 3D printing for the fabrication of moulds used in injection moulding processing has been discussed in this report. Two case studies depicting the benefits and drawbacks of such a fabrication approach for moulds are presented. The role of materials in creating engineering advantage for the performance of 3D printed moulds is also presented. The case studies showed that there is significant economic value in using 3D printing to fabricate moulds for small series runs on injection moulding machines rather than using moulds fabricated from milling methods.

# Keywords

3D printing, additive manufacturing, injection moulding, carbon fibre, PEEK

# Introduction

One of the most high production volume manufacturing methods in industry is injection moulding. This manufacturing method makes it possible for producers to implement a viable economy of scale in their business strategy because the unit cost of products/articles/parts can be reduced to values which make profits attractive. Most consumer goods are manufactured on the basis of this principle. However a key challenge faced in the field of injection moulding is the rather high cost of tooling; mainly moulds and mould-inserts. Design and fabrication of these kinds of tools are expensive because the designs are often complex and need to be fabricated by machining from relatively expensive metallic blocks using expertise that can be costly to maintain.

With the advent of new manufacturing methods such as 3D printing or additive manufacturing (AM) [1] there is now a chance to explore solutions to industry-based challenges which involve design and manufacturing constraints. A key reason for this is the wide latitude of fabrication ease created by 3D printing technologies even for complex part designs like mould-inserts. Also, by using 3D printing technologies, production cost and time can be markedly lowered compared to conventional manufacturing methods [2-4]. The 3D printing workflow is illustrated in Figure 2.

How 3D printing works

Creating a 3D model. Anything you want to fabricate using 3D printing, must first be modelled in 3D using computer aided design (CAD) tools or generated using 3D scanners to capture the shape of the object to the right measurements. Size and shape of the model will correspond to size and shape of the part which is to be 3D printed.

Digitally Slicing the model. This is done with a software that splits the model to very thin slices. Every slice can be thought of as a (2D) piece of paper. The Slicer draws a path that the tool which lays down the material will follow. It does the same for every layer, until it reaches the top of the objects. It also tells the tool (print head) how fast to go, how much to accelerate. This "printing plan" is called Gcode.

Printing. The G-code is loaded to the printer then the print job starts. Material is deposited on a free surface or printbed in a layer by layer fashion until a full 3D object is built.

While the technical advantages of 3D printing makes it an attractive instrument for tool development in injection moulding, at the core of its efficacy is materials as well as the role of the inherent properties of materials. Hence due to cost considerations, polymers have in recent times been exploited as choice material for the 3D printing of mould inserts. The material extrusion (fused filament fabrication (FFF)) 3D printing technology is the most widely adopted AM method for processing of thermoplastic polymers. Such polymers can be applied in various engineering scenarios; in pure form or filled with different materials so as to create properties previously not found in the pure polymer. One of the key challenges in mould material design is the conduction of heat from the liquid material; which is filling the mould insert, to regions distant from the filling cavity. The reason for this being that if the heat in the melt mass remains latent in the cavity then timely solidification process and form-shape formation by the melt material are negatively affected. Ideally the thermal mass of the melt needs to quickly reduce once the mould cavity is filled and this process is aided by the use of mould insert material that exhibit appreciable thermal conductivity.

Most engineering polymers have thermal conductivity in the range 0.03 to 7.0 Wm<sup>-1</sup>K<sup>-1</sup>[3]. This value is markedly insufficient to quickly transfer heat away from the melt. Even in the absence of cooling-media-assisted processing, the use of mould insert materials which ensure adequate thermal conductivity remains a preferred engineering solution. Therefore mould inserts made from polymeric materials filled with highly thermally conducting materials (such as graphene <1500 to 2500 Wm<sup>-1</sup>K<sup>-1</sup>>, carbon nano tubes <2000 to 6000 Wm<sup>-1</sup>K<sup>-1</sup>>, graphite <100 to 600 Wm<sup>-1</sup> K<sup>-1</sup>> or aluminium <205 Wm<sup>-1</sup>K<sup>-1</sup>>) can provide a viable solution.

# Methods

Two types of materials namely polyetheretherketone (PEEK) and carbon fibre reinforced PEEK (CFR PEEK) were used for the production of the moulds. Designs of the moulds were co-developed with the end-users for manufacture using AM methods. Filament forms of the used materials were separately loaded onto a FFF 3D Printer (P200 machine - Figure 2) manufactured by Apium Additive Technologies GmbH - Germany then used for the fabrication of the moulds. Apium Additive Technologies GmbH have especially developed their 3D printers for high temperature polymeric materials like PEEK and CFR- PEEK. Both materials exhibit relatively high mechanical strength, structural stability up to 250°C. Their thermal conductivities are sufficient for use as mould material in applications requiring the use of low melting temperature materials such as wax, polypropylene, polyethylene and other commodity polymers. The CFR PEEK material is certainly a more technically attractive material to use in a mould system due to its higher thermal conductive property compared to pure PEEK. The heat map indicated below (Figure 3) was obtained from an experiment in which samples of both materials were exposed to a heat source then monitored using a thermal camera.

The 3D printed moulds were then sent to end-users for testing and feedback.

#### End-user-1 Feedback

The mould insert shown in Figure 4 were fabricated from pure PEEK using 3D printing technologies. This mould is a real part deployable for the manufacture of functional parts and it is capable of withstanding up to 700 bars operating loads prior to failure.

The injection moulded material (polystyrene) was processed at a melting temperatures of 210 °C. One of the key challenges associated with this 3D printing mould solution is the ejection time of the moulded part from the mould. Typically a metallic mould insert allows for about 15 sec from mould filling to ejection while this 3D printed PEEK mould insert took about 90 sec to eject the parts (component of a beauty eye brush) shown in Figure 5. Although this time delay is acceptable for this part, it though raises an opportunity to improve on the in-process-time performance of the 3D printed mould insert.

#### Key process data

Mould insert material: Pure PEEK (Heat dissipation designed)

Injection temperature: 210°C

Pressure: 600 bars

Cycling time: 3 – 4 min

 $\rightarrow$  Clamping force on the moulds: 20 tons

Material: Polystyrene

# End-user-2 Feedback

Apium P Series 3D Printing technology was used for the fabrication of moulds made out of pure PEEK (Figure 6a) and out of 30% by weight carbon fibre reinforced PEEK (Figure 6b) for the manufacture of technical bellows. The entire production cost of this fabrication was compared with that of an actual existing process cost based on milling and other post treatment procedures for the country Bulgaria (client location) and Central Europe. The findings are illustrated on Table 1 and Table 2.

This 3D printed mould trial demonstrates that 3D Printing can be used to solve real manufacturing based challenges in the field of injection moulding. By using 3D printing technology to fabricate the moulds used for manufacturing the technical bellows, it was possible to reduce the total production cost of the moulds by about 86% and saving fabrication time by up to 66%. This possibility opens an economic relief and huge savings for small to medium scale enterprises using injection moulding in their manufacturing business.

# Conclusion

The conclusions derived from this study are as follows:

It is evident that moulds and mould inserts used in injection moulding processing can now be fabricated using 3D printing technologies

There is need to develop new materials for 3D printing which can deliver on the optimal operational thermal requirements of mould

3D printed moulds allow for small series production of plastic parts

It cost less money and time to fabricate moulds using 3D printing methods than for conventional milling methods

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# Tables

Author contribution

data from clients and Apium staff.

Brando Okolo entirely authored this report with additional

Conventional mould making milling + post-treatment	Costs Bulgaria	Costs Central Europe (estimated)	Time Required
Prepare inserts	90 €	200 €	1 Day
Electrode milling	330 €	660 €	1 Day
Contour milling	1,100€	1,600€	2 Days
Create CAM-software	120 €	480 €	1 Day
Eroding deep ribs	120 €	280 €	1 Day
Total costs	1,760 €	3,220 €	_
Total time required	-	-	6 Days

# Table 1. List of cost for production of a mould for a technical bellow using milling procedure

# Table 2. List of cost for production of a mould for a technical bellow using Apium P series 3D printer.

Production with Apium P Series 3D Printer	Costs Bulgaria	Costs Central Europe (estimated)	Time Required
Preparing 3D model	120€	250 €	0,5 Days
Printing (material, personnel and depreciation costs)	77 €	77 €	1 Day
Post-treatment	40 €	120€	0,3 Days
Total costs	237 €	447 €	-
Total time required	-	-	2 Days

#### Figure 1. Apium P220 3D printer for industrial application



Figure 2. Schematic workflow of 3D printing process [5]



Figure 3. Heat map of pure PEEK and CF-PEEK indicating faster thermal conduction in CF-PEEK.



Figure 4. (a) 3D printed PEEK mould insert with moulded parts still in the mould.

Figure 5. Moulded parts of a beauty eye-brush handle manufactured from a 3D printed PEEK mould.



Figure 6. 3D printed moulds for manufacture of technical bellows (a) carbon fibre reinforced PEEK moulds (b) Pure PEEK moulds with moulded parts in position. (Images courtesy LIM Technics)







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# Preserving Cameroon's Cultures and Traditions (CAMCT) Using App and Cloud Computing Rochelle Pacio, Nelly-Anne Shurri Ndikum, Russelle Steranova Kenge Department of Computing and Software Engineering PKFokam Institute of Excellence; Yaounde, Cameroon rochellepacio@pkfinstitute.com, anneshurri@gmail.com, kengnesteranova@gmail.com

# ABSTRACT

In today's world, people are addicted to their mobile devices due to its interactive features. Multimedia plays an important role on why people keep using their mobile technologies to gather information through the combination of text with video, animation, audio, graphic and virtual reality. This study aimed to develop an app and use cloud computing for storage. This app will greatly help in disseminating consistent information from one generation to another. Also, it will boost cultural tourism and innovate museums as tourists and locals will enjoy the use of this app while knowing everything about Cameroon. The methodology used for this study was Rapid Application Development (RAD) designed to provide quick software methodology that involves iterative development and quick construction of prototypes.

# **KEYWORDS**

Cultures and Traditions, Multimedia Elements, Apps, Cloud Computing

# 1 BACKGROUND

Cameroon is one of the countries with a very rich and diverse cultural heritage. Locals are very adaptable in technological trends as well. Blending cultures and traditions will make people of Cameroon known to their competitiveness and advancement in using technologies.

Culture is defined as "the characteristics of knowledge of a particular group of people, encompassing language, religion, cuisine, social habits, music and art [1]." Cameroonians have different cultures inherited from their ancestors. They a 1. categorized as expressions of culture which include peopl2. languages, music, cuisine, customs, religions, arts and literature among others.

Mobile technology changes people's lives. They are dependent because of its portability, convenience, and ease of use. Since young people today are adept to technology than learning their culture in a traditional way like making observations and reading books, this study was conceptualized to digitized access to cultures of Cameroon people, to be more accessible and shareable information through the use of an App.

The idea of using cloud computing is another good idea to adapt with the innovative technologies. Cloud storage is an online space where we can store data. Some of its features include (a) all data is stored outside of the network, (b) it can be accessed over the Internet or through built-in synchronization tools, (c) data can be accessed from any device and location by authorized users, and (d) it is based on virtualization technology [2]. Because of the aforementioned features, sharing data with others is easier than ever, making it perfect for globalized world.

To have an indebt understanding of the content of Cameroonian Cultures and Traditions (CAMCT) App, gathered expression of cultures were transformed using multimedia. To access users must download CAMCT application to their mobile devices.

# 1.1 Related Studies

The Cultural Me is an app that helps people to learn about the arts and culture as they would learn new language [3]. The researchers' problem statement is *"Curious people need a way to learn about their interest areas in the arts and culture because they want to increase their knowledge"* is at par to the objective of this study.

CultureGuru is another mobile app with aims to serve as platform which reminds users of traditional Chinese festivals, by digitizing all necessary cultural information. The main reason for this app is to bring cultural awareness to the users [4].

Moreover, Rajasthan app features a glimpse of forts and palaces, temples among others. It has a collection and more 80 plus pictures, music and videos across 7 cities in Rajasthan [5].

The above mentioned studies were very helpful in conceptualizing and developing the CAMCT app. Cameroonians will have accurate and consistent information dissemination. Youths and future generations will not lose their values, cultures, and history.

# 1.2 Objectives

The study has an objective, to develop a CAMCT App that uses cloud computing to preserve the cultures and traditions of Cameroon. Specifically, it sought to achieve the following:

To determine the different cultures and traditions in Cameroon,

To determine the level of likeness of the features of multimedia elements by the end-users and

To determine the perceived level of benefits of the end user using the CAMCT App.

# 1.3 Significance of the Study

The success of CAMCT App set the pace for African countries to follow as it will serve as a model until we can build a new App with the vision that is to preserve African cultures and traditions. CAMCT will be of great help to disseminate consistent information from one generation to the other. Also it boosts cultural tourism and innovate museums. Tourists and locals will enjoy the use of the app while knowing everything about Cameroon cultures and traditions.

# 2 METHODOLOGY

Rapid Application Development (RAD) is an agile project management strategy popular in software development. The key benefit of a RAD approach is its fast project turn around. This rapid pace is made possible by RAD's focus on minimizing the planning

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stage and maximizing prototype development. This results in greater efficiency, faster development, and effective communication [6].

There are four main phases of RAD:



Figure 1: Rapid Application Development Methodology

Phase 1: Requirement Planning: Where planning scoping meeting take place. During this stage, developers, clients and team members communicate to determine the goals and expectations of the project, as well as the current issues that would need to be addressed during the building phase.

Phase 2: User Design: Once the project is scoped out, it is time to jump right into development, building out the user design through various prototype iterations. During this phase, clients work hand in hand with developers to ensure their needs are being met at every step in the design process. This is where the user can test each prototype of the product, at each stage, to ensure it meets their expectations.

Phase 3: Rapid Construction: takes the prototypes and beta systems from the design phase and converts them into the working model. The software development team works together during this stage to make sure everything is working smoothly and that the end result satisfies the client's expectations and objectives. This third phase is important because the client still gets to give input throughout the process. They can suggest alterations, changes, or even new ideas that can solve problems as they arise.

Phase 4: Cutover: This is the implementation phase where the finished product goes to launch. It includes data conversion, testing, and changeover to the new system, as well as user training.

# **3 RESULTS**

#### 3.1 Cultures and Traditions in Cameroon

The CAMCT app can be acquired using QR code or by downloading the said application, the information is stored in the cloud storage. In the CAMCT App, cultures and traditions are categorized according to: People, Languages, Music, Cuisine, Customs, Religions, Art and Literatures, Beliefs, others.

# **3.2** Level of Likeness on the Features of Multimedia Elements by the end-users

Table 1 shows the level of likeness of the features of multimedia elements by the end-users, the overall weighted mean is "Very Much Liked". This implies that the different features of multimedia were effective and interactive.

# Table 1: Level of Likeness of the Features ofMultimedia Elements

Multimedia Elements	Mean	Description
Movie Clips	4.48	Very Much Liked
Audio/Music	4.54	Very Much Liked
Animation	4.35	Very Much Liked
Graphics/Images	4.48	Very Much Liked
Text	4.24	Very Much Liked
Overall Weighted Mean	4.42	Very Much Liked

# **3.3** Perceived Level of Benefits of the end users using the CAMCT App

Table 2 presents the level of benefits of the end-users was individually "Very Much Beneficial" for overall weighted mean of 4.40

#### Table 2: Level of Likeness on the Features of Multimedia Elements

Perceived Benefits	Mean	Description
Portability	4.41	Very Much Benefitted
Accessibility	4.39	Very Much Benefitted
Learnability/Awareness	4.46	Very Much Benefitted
Interactivity	4.35	Very Much Benefitted
Shareability	4.47	Very Much Benefitted
Overall Weighted Mean	4.40	Very Much Benefitted

# 4 CONCLUSIONS & RECOMMENDATIONS 4.1 Conclusions

Based on the results of the study, the following conclusions were drawn:

1) The cultures and traditions were identified and categorized according to: People, Languages, Music, Cuisine, Customs, Religions, Art and Literatures, and Beliefs.

2) The level of likeness of the features of multimedia elements by the end-users was "Very Much Liked".

3) The perceived level of benefits in using CAMCT app was "Very Much Benefitted"

# 4.2 Recommendations

Based on the results and conclusions of the study, the following are recommended:

- 1) The implementation and enhancement of the CAMCT app.
- 2) Acquire Intellectual Property Rights (IPR).
- 3) The use of more 4D multimedia.

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# Secondary metabolites from Desmodium ramosissimum G. Don (Fabaceae) and their radical scavenging properties

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#### Abstract

This work was designed to carry out the phytochemical study and the radical scavenging screening of the MeOH extract from the whole plant of Desmodium ramosissimum used in traditional medicine for the treatment of malaria, fever, diarrhea, lung disorders and venereal diseases. The crude extract was subjected to silica gel open column chromatography (CC) follow by repeated silica gel CC purification and / or Sephadex LH-20 to afford nine compounds, D-pinitol (1), mixture of  $\beta$ -sitosterol (2) and stigmasterol (3),  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside (4), glyceryl-1-tetracosanoate (5), lutein (6), kaempferol (7), catechin (8) and vitexin (9). The structures of these compounds were identified after analysis of their NMR and MS data and comparison with the literature. The crude extract, fractions III, IV and V displayed constituents possessing radical scavenging activities using the 2,2diphenyl-1-picrylhydrazyl (DPPH) bioautography while vitexin (9), and catechin (8) showed activity among the isolated compounds.

Keywords: *Desmodium ramosissimum*; constituents; Radical scavenging, Vitexin; Kaempferol

# Introduction

The human organism permanently produces reactive oxygen species (ROS) that cause several cellular disorders because of their high reactivity with proteins, DNA and membrane fatty acids resulting to different inflammatory and cardiovascular diseases, rheumatoid arthritis, neurodegenerative diseases and the aging process. The physiological production of ROS is regulated by enzymes and low molecular weight compounds with antioxidant properties (Meyer et al., 1998; Hollman and Katan 1999; Moure et al., 2001; Hunt et al., 2001).

*Desmodium ramosissimum* (also named as *D. mauritiamun* or *Hedysarum fruticulosum*) a member of Fabaceae family is an herbal or shrub of 10-150 cm in height, mostly found in tropical Africa (Lebrun and Fotius, 1967). It is used in African ethnomedicine for the treatment of several diseases such as malaria (Benin), headache, diarrhea, dysentery, fever, pulmonary disorders, cough, venereal diseases and jaundice (Nigeria) (Yetein et al., 2013, Alli et al., 2011). Plants species and constituents of the genus *Desmodium* has been reported to possess several pharmacological properties such as antibacterial, antidiabetic, anti-inflammatory, cytotoxic,

antinephrolithic, nootropic, and lipid peroxidation activities (Ma et al., 2011; Tsafack et al., 2018). Previous phytochemical investigations carried out on the genus Desmodium revealed the presence of flavonoids, phenols, anthraquinones, tannins, and triterpene saponins (Alli et al., 2011; Lugudu and Owk, 2016; Thankachan et al., 2017; Tsafack et al., 2017). To the best of our knowledge, no phytochemical study has so far been achieved from this plant species. In the course of our continuing search for bioactive secondary metabolites from medicinal plant species of the family Fabaceae (Awouafack et al., 2015, 2016, 2018; Nguekeu et al., 2017), we investigated the MeOH extract of the whole plant of D. ramosissimum to isolate nine compounds namely D-pinitol (1), mixture of  $\beta$ -sitosterol (2) and stigmasterol (3),  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside (4), glyceryl-1-tetracosanoate (5), lutein (6), kaempferol (7), catechin (8) and vitexin (9). This is the first report on the isolation of secondary metabolites from this plant species.

### 2. Methods

#### 2.1. General experimental procedures

IR spectra were recorded on a SHIMADZU FT-IR 8400S spectrometer. MS data were measured on SHIMADZU LCMS-IT-TOF spectrometer (Japan). NMR spectra were recorded on 500 MHz (<sup>1</sup>H) and 125 MHz (<sup>13</sup>C) on JEOL 500 Spectrometer with TMS as internal reference. Column chromatography was performed on silica gel MERCK 60 F<sub>254</sub> [(0.2–0.5 mm)] 70–230 and 230–400 mesh (Germany) and Sephadex LH-20. Pre-coated silica gel 60 F<sub>254</sub> thin layer chromatography (TLC) plates (Germany) were used for monitoring fractions and spots were detected with UV lights (254 and 366 nm) and further sprayed with 50% H<sub>2</sub>SO<sub>4</sub> or vanillin-H<sub>2</sub>SO<sub>4</sub> reagent followed by heating at 150°C. Solvents used for chromatography were distilled prior to use.

# 2.2. Plant Materials

The whole plant of *D. ramosissimum* were collected in Dschang, West Region of Cameroon, on October 2017 and were identified by Mr. Victor Nana, a botanist at the Cameroon National Herbarium in Yaoundé where our sample was compared to the available specimen having a voucher number 40528/HNC.

2.3. Extraction and isolation

The dried and powdered plant of D. ramosissimum (3.42 kg) was extracted by maceration for three days in methanol (13 L x 3 times) to give the crude extract (287 g) after filtration and removal of the solvent using rotary evaporator. Parts of the extract (150 g and 136 g) were separately subjected to similar column chromatographies (CC) over silica gel (SiO<sub>2</sub>) using solvent systems made of n-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 65 fractions of 600 mL each that were pooled into six main fractions (Fr.I-VI) after TLC monitoring. The combined fraction Fr.V51-53 [26 g, EtOAc/MeOH (8:2)] formed a white powder in methanol to afford 1 (300 mg) after filtration. A part of Fraction II [6 g, n-Hex/EtOAc (4.5:5.5)] was subjected to SiO<sub>2</sub> CC using n-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 35 sub-fractions of 200 mL each. Combined subfractions Fr.II<sub>14-15</sub> formed a white powder in *n*-hexane to give a mixture of 2 and 3 (90 mg) after filtration while combined sub-fractions Fr.II<sub>32-35</sub> was purified by further SiO<sub>2</sub> CC using isocratic solvent system made of n-Hex/AcOEt (8.5:1.5) to afford 7 (0.7 mg). The two major compounds [5 (28 mg) and 6 (17 mg)] of the combined sub-fractions Fr.II<sub>23-31</sub> were separated and purified by successive SiO2 CC using isocratic solvent system [n-Hex/AcOEt (8.5:1.5)] follow by Sephadex LH-20 with MeOH as mobile phase. A part of fraction III [6.5 g, n-Hex/EtOAc (4:6)] was subjected to SiO<sub>2</sub> CC using n-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 162 sub-fractions of 75 mL each. The combined subfractions Fr.III91-127 [2 g, EtOAc/MeOH (8.5:1.5)] formed a white powder in EtOAc to afford 4 (60 mg) after filtration whereas the combined sub-fractions Fr.III<sub>77-90</sub> [0.8.g, EtOAc/MeOH (9.5:0.5)] was subjected to SiO<sub>2</sub> CC eluting with an isocratic solvent system [n-Hex/AcOEt (7.5:2.5)] to give 60 fractions of 50 mL each. The combined sub-fractions 30-39 from the CC of sub-fractions Fr.III77-90 was purified by Sephadex LH-20 using methanol to afford 8 (19 mg). A part of fraction IV [59 g, EtOAc/MeOH (8.5:1.5)] was subjected to SiO<sub>2</sub> CC using n-Hex/EtOAc, and EtOAc/MeOH, in gradient polarities to afford 238 fractions of 75 mL each. The combined sub-fractions Fr.IV172-185 formed a yellow powder in a mixture of acetone/methanol (9.5:0.5) to afford 9 (38.5 mg) after filtration.

2.4. *D-Pinitol (1)*: white powder in MeOH; IR (KBr) cm<sup>-1</sup>: O-H (3502 - 3207) and C-O (1280 – 1080); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\rm H}$  3.63 (br.s, H-1) 3.50 (m, H-2), 3.00 (t, *J* = 9.0 Hz, H-3), 3.34 (m, H-4), 3.44 (m, H-5), 3.59 (br.s, H-6) 3.39 (s, 3-OCH<sub>3</sub>), 4.61 (br.s, 1-HO), 4.45 (d, *J* = 6.0 Hz, 2-OH), 4.50 (d, *J* = 3.5 Hz, 4-OH), 4.32 (d, *J* = 5.0 Hz, 5-OH), 4.70 (br.s, 6-OH) and <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\rm C}$ 83.8 (C-3), 72.6 (C-4), 72.4 (C-1), 72.0 (C-5), 71.9 (C-6), 70.1 (C-2), 59.7 (3-OCH<sub>3</sub>); HR-ESIMS (+): *m*/*z* 217.0631 ([M+Na]<sup>+</sup>), (calcd for C<sub>7</sub>H<sub>14</sub>O<sub>6</sub>Na 217.0688).

2.5.β-Sitosterol (2) and stigmasterol (3): white powder in *n*-Hex; IR (KBr) cm<sup>-1</sup> O-H (3473 - 3325), C-H (2974 - 2835) and C-O (1058).

2.6.  $\beta$ -Sitosterol-3-O- $\beta$ -D-Glucopyranoside (4): white powder in EtOAc; IR (KBr) cm<sup>-1</sup>: O-H (3400), C-H (3000-2800) and C-O (1107).

2.7.*Glyceryl-1-tetracosanoate* (5): white powder in *n*-Hex/EtOAc (8.5:1.5); IR (KBr) cm<sup>-1</sup> O-H (3253), C-H (2956-2848), C=O (1731) and C-O (1180); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\rm H}$  4.65 (dd, J = 5.0; 11.5 Hz, H-1), 4.58 (dd, J = 6.5; 11.5 Hz, H-1), 4.36 (m, H-2), 4.06 (t, J = 5.0 Hz, H-3), 2.29 (t, J = 7.5 Hz, H-2'), 1.58 (m, H-3'), 1.23-1.17 (br.s, H-4' – H-22'), 1.16 (m, H-23), 0.79 (t, J = 6.5 Hz, H-24') and <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta_{\rm C}$  173.8 (C-1'), 70.8 (C-2), 66.6 (C-1), 64.2 (C-3), 34.2 (C-2'), 25.1 (C-3'), 29.1-31.9 (C-4' - C-22'), 22.8 (C-23'), 14.1 (C-24'); HR-ESIMS (+): m/z 465.3846 ([M+Na]<sup>+</sup>) (calcd for C<sub>27</sub>H<sub>54</sub>O4Na 465.3846).

2.8. Lutein (6): orange powder in n-Hex/EtOAc (8.5:1.5); IR (KBr) cm<sup>-1</sup> O-H (3440-3469), C-H (2918), C=C (1666) and C-O (1365); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): 1.74 (m, H-2), 1.46 (m, H-2), 4.16 (ddd, J = 4.5; 9.5; 11.5 Hz, H-3), 2.34 (m, H-4), 6.09 (d, *J* =13.2 Hz, H-7), 6.11 (d, *J* =13.2 Hz, H-8), 6.24 (m, H-10), 6.56-6.65 (m, H-11), 6.35 (m, H-12), 6.24 (m, H-14), 6.56-6.65 (m, H-15), 1.05 (s, H-16), 1.05 (s, H-17), 1.72 (s, H-18), 1.89 (s, H-19), 1.95 (br.s, H-20), 1.34 (m, H-2'), 1.81(m, H-2'), 4.24 (br.s, H-3'), 5.52 (s, H-4'), 2. 40 (br.s, H-6'), 5.41 (dd, J=10.0; 16.0 Hz, H-7'), 6.13 (m, H-8'), 6.13 (m, H-10'), 6.56-6.65 (m, H-11'), 6.34 (m, H-12'), 6. 24 (m, H-14'), 6.56-6.65 (m, H-15'), 0.98 (s, H-16'), 0.83 (s, H-17'), 1.60 (s, H-18'), 1.95 (br.s, H-19'), 1.95 (br.s, H-20') and <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ<sub>C</sub> 37.3 (C-1), 48.5 (C-2), 65.1(C-3), 42.6 (C-4), 126.2 (C-5), 137.8 (C-6), 125.6 (C-7), 138.6 (C-8), 135.8 (C-9), 131.4 (C-10), 124.9 (C-11), 137.6 (C-12), 136.5 (C-13), 132.6 (C-14), 130.3 (C-15), 28.8 (C-16), 30.3 (C-17), 21.7 (C-18), 12.9 (C-19), 12.8 (C-20), 34.1 (C-1'), 44.7 (C-2'), 65.9 (C-3'), 124.7 (C-4'), 138.0 (C-5'), 55.0 (C-6'), 128.9 (C-7'), 137.9 (C-8'), 135.1 (C-9'), 130.9 (C-10'), 124.9 (C-11'), 137.6 (C-12'), 136.5 (C-13'), 132.6 (C-14'), 130.3 (C-15'), 24.3 (C-16'), 29.7 (C-17'), 22.9 (C-18'), 13.2 (C-19'), 12.8 (C-20').

2.9. *Kaempferol* (7): yellow powder in EtOAc; IR (KBr) cm<sup>-1</sup> O-H (3213), C=O (1664) and aromatic ring (1571); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta_{\rm H}$  12.14 (1H, s, 5-OH), 6.24 (1H, d, J = 1.5 Hz, H-6), 6.52 (1H, d, J = 1.5 Hz, H-8), 8.12 (2H, d, J = 8.5 Hz, H-2'/H-6'), 6.99 (2H, d, J = 8.5 Hz, H-3'/H-5'); HR-ESIMS (+): m/z 287.0540 ([M+H]<sup>+</sup>) (calcd for C<sub>15</sub>H<sub>11</sub>O<sub>6</sub> 287.0556).

2.10. *Catechin* (8): white powder in methanol; IR (KBr) cm<sup>-1</sup> O-H (3602), aromatic ring (1625), C-O (1147-1081) and C-H (3049); <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\rm H}$  4.43 (d, J = 7,5 Hz, H-2), 3.77 (m, H-3), 2.60 (dd, J = 5.0; 16.0 Hz, H-4), 2.30 (dd, J = 7.5; 16.0 Hz, H-4), 5.84 (d, J = 2.0 Hz, H-6), 5.64 (d, J = 2.0 Hz, H-8), 6.64 (d, J = 1.5 Hz, H-2'), 6.68 (d, J = 8.0 Hz, H-3'/ H-5'), 6.55 (dd, J = 8.0; 1.5 Hz, H-6'), 4.84 (d, J=5.0 Hz, 3-OH), 9.16 (s, 5-OH), 8.92 (s, 7-OH), 8.84 (s, 3'-OH), 8.79 (s, 4'-OH). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\rm C}$ 81.3 (C-2), 66.8 (C-3), 156.6 (C-5), 95.6 (C-6), 156.9 (C-7), 94.4 (C-8), 155.8 (C-9), 99.6 (C-10), 131.1 (C-1'), 114.9 (C-2'), 145.4 (C-3'/C-4'), 115.6 (C-5'), 118.9 (C-6'); *m*/z 289.0722 ([M-H]<sup>-</sup>) (calcd for C<sub>15</sub>H<sub>13</sub>O<sub>6</sub> 289.0712).

2.11.Vitexin (10): yellow powder in acetone/methanol (95:5); IR (KBr) cm<sup>-1</sup> O-H (3238-3390), C=O (1708) et aromatic ring (1662); <sup>1</sup>H NMR (500 MHz, DMSO- $d_6$ ):  $\delta_{\rm H}$  13.13 (s, 5-OH), 6.75 (s, H-3), 6. 24 (s, H-6), 7.99 (d, J = 8.5 Hz, H-2'/ H-6'), 6.87 (d, J = 8.5 Hz, H-3'/ H-5'), 4.66 (d, J = 10.0 Hz, H-1''), 3.80 (t, J = 9.0 Hz, H-2''), 3.22 (m, H-3''), 3.73 (m, H-4''), 3.13 (m, H-5''), 3.75 (dd, J = 7.0; 11.0 Hz, H-6''), 3.51 (d, J=12.0 Hz, H-6''), and <sup>13</sup>C NMR (125 MHz, DMSO- $d_6$ ):  $\delta_C$ 164.5 (C-2), 103.0 (C-3), 182.6 (C-4), 160.9 (C-5), 98.6 (C-6), 163.1 (C-7), 105.1 (C-8), 156.5 (C-9), 104.6 (C-10), 122.1 (C-1'), 129.5 (C-2'/C-6'), 116.4 (C-3'/C-5'), 161.1 (C-4'), 73.9 (C-1''), 71.3 (C-2''), 79.0 (C-3''), 70.8 (C-4''), 82.1 (C-5''), 61.5 (C-6''); HR-ESIMS (+): m/z 433.1223 ([M+H]<sup>+</sup>), HR-ESIMS (-): m/z 431.1009 ([M-H]<sup>-</sup>), 863.2097 ([2M-H]<sup>-</sup>).

# 2.12. Radical scavenging screening

The radical scavenging screening was evaluated using the precolated TLC silica gel 60 F254 (Merck, Germany), with mobile phases made of n-hexane, EtOAc and MeOH, while a methanolic solution of (0.2%) DPPH (Wako, Japan) was used as spraying reagent to reveal the activity. Briefly, 10 µL of each sample dissolved in MeOH at concentrations of 10 mg/mL (crude extract and fractions), or 1mg/mL [pure compounds and the standard reference, catechin (wako, Japan)] were spotted on TLC plates and eluted with three mobile phases [n-Hex/EtOAc (7.5/1.5), EtOAc/MeOH (9.8:0.2), and EtOAc/MeOH (9.2:0.8)]. Thereafter, the plates were dried and sprayed with the DPPH solution and the appearance of yellow spots as well as the intensity of the color materialized the presence, the number of constituents (for extract and fractions) and the importance of the radical scavenging activity of the sample (Takao et al., 1994).

#### 3. Results and Discussion

The MeOH extract of *D. ramosissimum* was subjected to column chromatography techniques to afford nine compounds that were identified as D-pinitol (1) (Raya-Gonzalez et al., 2008), mixture of  $\beta$ -sitosterol (2) and stigmasterol (3),  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside (4), glyceryl-1-tetracosanoate (5) (Sultana et al., 1999), lutein (6) (Kengne et al., 2016), kaempferol (7) (Xiao et al., 2006), catechin (8) (Almahy and Alhassan, 2011), and vitexin (9) (Cuong et al., 2015) (Figure 1). Their structures were identified by comparison of their spectroscopic data mainly NMR and MS to those reported in the literature.

The radical scavenging activity of the crude extract (CE), some of main fractions ( $F_{II}$ - $F_V$ ), and some isolated compounds (2, 5, 6, 8, 9) was determined using the free radical DPPH. The crude extract, fractions  $F_{III}$ - $F_V$  displayed spots indicating the presence of constituents with good radical scavenging activity while fraction  $F_{II}$  was weakly active (Figure 2). Among the isolated compounds, vitexin (9), and catechin (8) had the highest radical scavenging activity and could be appreciated by the intensity of their spots on TLC after spraying with DPPH (Figure 2).

The potential of compounds 8 and 9 for scavenging free radicals is owed to their flavonoid nature. Several mechanisms have been reported on such class of compounds to elucidate how their radical scavenging activity occurred. The highest activity of catechin (8) and vitexin (9) could be justified by the *o*-disubstitution hydroxyl groups in their B-ring (Amić et al., 2003; Hassan et al., 2014; Grzesik et al., 2018).

Flavonoids have been reported as one of the most important class of secondary metabolites in the genus *Desmodium*, thus our results on the isolation and identification of flavonoids such as kaempferol (7), catechin (8) and vitexin (9) in *D. ramosissimum* have chemotaxonomic significance at the genus level.

# 4. Conclusion

The phytochemical study of the MeOH extract of *D. ramosissimum* was carried out to identify nine secondary metabolites including D-pinitol (1), mixture of  $\beta$ -sitosterol (2) and stigmasterol (3),  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucopyranoside (4), glyceryl-1-tetracosanoate (5), lutein (6), kaempferol (7), catechin (8) and vitexin (9). The good activity of catechin (8) and vitexin (9) could justified the used of this plant species as good antioxidant agent in the treatment of some diseases and further study as cytotoxicity should be conducted in view to associate such plant in food supplement development.

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#### Author contributions

R.A.D.D., and D.H.S.F. carried out the experimental part with the help of Y.M.M.N., while P.T., H.M., and M.D.A. supervised the work. M.D.A. also carried out the NMR and MS analyses and edited the final version of the manuscript.

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Glu = D-glucose Figure 1: Chemical structures of compounds (1-9) isolated from D. ramasissimum



X : negative control, +: positive control, CE: crude extract,

Figure 2: Radical scavenging activity of the crude extract, main fractions and some compounds isolated from D. ramasissimum

#### Urbanization, CO2 Emissions, and Science-Technology-Society in Cameroon

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#### Abstract

Important advances have been made in theoretical and econometric research in the relationship between CO2 and urbanisation. However, scanty attention has been focussed on the role and importance of science and technology as one of the major factors reducing CO2 emissions. For example, to our knowledge, Cameroon has never been chosen as a case study. Therefore, the statistical and econometric analyses undertaken in this paper aims at filling this gap. We begin our analysis by employing statistical tools and econometric tests such as unit root tests, Cointegration tests, Error Correction tests and the Granger short and long run causality tests. Our results related to the causal relationship between CO2, urbanization and science and technology indicate that while there is a bidirectional causality between the latter variables in the long run, there is no causality between the studied variables in the short run.

Keywords: Urbanization, CO2 emissions, science and technology and ecological modernisation

# I. INTRODUCTION

CO2 emissions are currently the greatest puzzling problems of the world. It is not surprising that factors affecting CO2 emissions are one of the most investigated ones in economic literature. The level of CO2 emissions, in Africa, from urbanization is significantly increasing due to the fact that uurbanization is the growth of urban area in a specific country followed with the population growth living in those areas. Urban populations interrelate with the environment through their exhaustion of food, energy, water, and land causing a fast polluted urban environment which will then generate many problems such as excessive air pollution, high demand of housing, poor water quality, climate change, loud noise, waste management and poor health. In response to unregulated urbanization, environmental degradation has been occurring at a rapid rate. For example, the human Development Indices (2018) reported that high human development countries are the biggest contributors to environmental degradation with 10.7 tons average carbon dioxide emissions per capita. Small countries, on the other hand, with 0.3 tons of carbon dioxide emissions per capita have been found to pollute less. According to the World Bank (2019), the population of Cameroon grew at the rate of 3% from 2005 to 2015

while during the same period carbon dioxide emissions per capita has increased at the rate of 4%. Science and technology, on the other hand, appear to exert an inverse effect on CO2 emissions. In the 1970s, contributions on the relationship between CO2 emissions and science and technology were fragmented. The emergence of ecological modernisation in the 1990s provided a common platform to major researchers such as Huber (2004) and Sonnenfeld (2002). Furthermore, utilising the contribution of Beck (1986, 1992) on science and risk, and that of Schot (1992); Geels (2005); Shove (2003) on science and technology studies, ecological modernization theorists nowadays have made significant contributions to the role and importance of science and technology on environmental policy. Mol et al. (2009), on the other hand, also significantly contributed to articles related to the changing role of technology in ecological modernization studies.

Overall, the previous theoretical and empirical studies as will be depicted in the next section, analyzing the relation between CO2 emissions, urbanization and science and technology have concentrated more on the relationship between CO2 emissions and urbanization and less on the relationship between CO2 emission and science and technology. Currently, none of the scholars analyzing the latter relationship focusses on Cameroon and very few focus on Africa. This calls for developing CO2 models for the case of Cameroon that will contribute to environmental policy aiming at reducing CO2 emissions. this paper contributes to the existing knowledge and suggests empirically tested model on the relationship between CO2, urbanization and science and technology. Based on the case of Cameroon with data covering the 2005-2015 period, we endeavour to employ econometric tests such as unit root tests, the augmented Dickey Fuller, Johensen Cointegration tests, Error Correction tests the modified Wald (MWald) test of Toda and Yamamoto (1995) for Granger causality to examine the causal relationship between the above variables of interest.

The present paper is structured as follows: Section 2 outlines methods, results and authorship contributions. Section 3 undertakes major discussions and concludes. Section 4 list relevant tables and Section 5 focusses on selected references.

# II. METHODS, RESULTS AND AUTHORSHIP CONTRIBUTIONS

# 2.1. Literature Review

Three major theories underline the impact of urbanization on the environment. Namely, the Urban Environmental Transition theory, the Ecological Modernisation Theory and Impact City Theory.

The theory of urban environmental transition developed by McGranahan et al (1996) suggests an association between urban environmental burdens and industrial pollution. That is the increase in urbanisation leads to the demand of high energy intensive products which will then contribute to the increase in CO2 emissions. In the present context, we could say that industrial pollution may be reduced via environmental regulations and science and technological innovations. The latter relationship shall be analysed in the present article.

According to Newman and Kenworthy (1989)], the compact city theory supports the increase of urbanization leading to economies of scale related to public infrastructure such as public buses, hospitals, water and electricity supplies. They went on to say that the utilisation of public rather than private services will reduce environmental degradation. Moreover, Ma (2015) explored the energy-saving effect due to increase in urbanization causing the reduction in energy consumption since more public transport, for example, will be used in bigger cities. There will therefore be an increasing utilisation of public services. Although the present theory

economies of scale and U urbanization.

 $\rightarrow$  denotes uni-directional and  $\square$  bi-directional causality.

Overall, the econometric evidence on the causal relationship between Urbanization and CO2 Emissions is very mixed. No tests with regard to the relationship between Science and Technology and CO2 Emissions was carried in Africa. Although sample periods and specification differ somewhat, it is difficult not to conclude that the wide variety of results reflects problems with the data. For example, in developing countries, the low quality of data and lack of sufficiently long series pose degree of freedom problems. It seems, therefore, highly probable that these econometric models were misspecified and thus the estimates presented were unreliable for policy analysis. It may also be argued that specific variables such as Science and Technology are not considered in almost all studies summarised in Table 1. In the next subsection we show that Science and Technology contribute significantly to the reduction of CO2 in Cameroon. The next subsection attempts, therefore, to reduce the above shortcomings by specifying a CO2 model

has been tested in various countries as indicated in our empirical literature survey, such test, to our knowledge has not been carried out in Cameroon. This article endeavours to elucidate this lacuna by examining the causal relationship between urbanization and CO2.

The Ecological Modernisation Theory was developed by Joseph Huber (1982) and Martin Janicke (1985). They argued that ecological disaster should be avoided as the result of the transformation of modern society institution. According to Mol and Spaargaren (1993) the Ecological Modernisation Theory central tenet is the inverse relationship between CO2 and science and technology. Mol and Spaargaren (2013) went on to argue that technologies are motivating drives for environmental fluctuations. The works of ecological modernization theorists such as Beck (1986, 1992), Giddens (1990, 1991), Latour (1993), Urry (2000), Schot (1992), Geels (2005) and Shove (2003) have constituted the basis of the examination of the role and importance of science and technology on the environment. As mentioned earlier, the test of the above theory has never been undertaken in Cameroon. The present article therefore breaks new ground by examining the causal relationship between science and technology and CO2 in Cameroon.

Table 1 indicates that the causal relationship between Urbanization, CO2 Emissions, and Science-Technology-Society has been tested in various countries. However, only two out of ten tests were carried out in Africa on the relationship between CO2 emissions and Urbanization. While no test has been carried out in relation to CO2 and Science and Technology. The present paper therefore aims at feeling the latter gap.

that considers Science and Technology as one of the major factors in Cameroon to reduce CO2 emissions.

2.2. Statistical and Econometric Analyses

Regardless the fact that, important advances have been made in theoretical and econometric research in the relationship between CO2 and urbanisation as indicated in the previous subsections, scanty attention has been focussed on the role and importance of science and technology as one of the major factors reducing CO2 emissions. For example, to our knowledge, Cameroon has never been chosen as a case study. Consequently, the CO2 model for the case of Cameroon may be better specified if its reflects Cameroon specific factors as emphasised in the Ecological Modernisation Theory which argues that increase in science and technology reduce CO2 emissions. In these circumstances, CO2 models that do not include the science and technology effect will fail to capture its fluctuations and could be considered as a specification shortcoming for the case of Cameroon. The following econometric analysis aims at filling this gap by investigating the role of science and technology and

urbanization through estimation of our proposed CO2 model. We begin our analysis by employing statistical tools such as descriptive statistics, interval estimate and hypothesis testing to have preliminary insight into our data. We then proceed with econometric tests such as unit root tests, the augmented Dickey Fuller, Johensen Cointegration tests, Error Correction Model and the modified Wald (MWald) test of Toda and Yamamoto (1995) for Granger causality.

It should be noted that in developing countries, the low quality of data and lack of sufficiently long series pose serious problems. Thus, our results must be interpreted with care. Nevertheless, this potential source of misspecification error also applies to empirical studies in all developing countries. Our data are mainly taken from the World Bank, World Development Indicators (2019). Our analyses for the case of Cameroon cover the period 2005-2015.

#### 2.2.1. Statistical Analysis

The main goal of the present analysis is to provide summaries related to the sample and the measures of location and variability in order to investigate existing instability in our variables. On the basis of the point estimator of the population mean calculated, we use hypothesis testing to determine whether Cameroon could be considered as a big or a small pollutant. We proceed with Interval Estimate to confirm the latter hypothesis results at the 95% confidence that the interval contains the population mean

Table 1: Summaries of Econometric Tests of Urbanization, CO2 Emissions, and Science-Technology-Society

Techr	iology-Socie	ty			
No	Authors	Time Period	Study Area	Variables	Long Run Causality
1	Afawbo and Ntouko (2016)	1960- 2014	142 countries Low- income, Lower-middle- income, Upper-middle- income, High income non-OECD, High- income OECD	CO2 emissions, urbanization, industrialization	No in low and lower income countries Yes in the remaining countries of studies
2	Ameer and Munir (2016)	1980- 2014	Pakistan, India, Bangladesh, Indonesia, Thailand, Malaysia, Philippines, Sri Lanka, Iran, Singapore, and Hong Kong	GDP, openness, urbanization, technology	$E \leftrightarrow C$ $U \leftrightarrow C$ $U \leftrightarrow E$ $T \leftrightarrow C$
3	Çetin and Ecevit (2015)	1985- 2010	Panel data of nineteen Sub Saharan African countries	CO2 emissions, urbanization, Energy consumption	$E \leftrightarrow C$ $U \leftrightarrow C$ $U \leftrightarrow E$
4	Kasman and Duman (2015)	1992- 2010	new EU members	CO2 emissions, urbanization GDP per capita, Energy consumption, , openness	$E \leftrightarrow C$ $U \rightarrow C$ $U \rightarrow E$
5	Begum et al (2015)	1970- 2009	Malaysia	CO2 emissions, Energy consumption, GDP per capita, population	No
6	Shahbaz et al. (2014)	1975- 2011	United Arab	CO2 emissions, Emirates, urbanization, GDP per capita, Energy consumption, exports	E⇔C U⇔C
7	Wang et al (2014)	1995- 2011	30 Chinese provinces	CO2 emissions, Energy consumption, urbanization	$E \leftrightarrow C$ $U \leftrightarrow C$ $U \leftrightarrow E$
8	Hossain (2012)	1960- 2009	Japan	CO2 emissions, urbanization, Energy consumption,	$E \leftrightarrow C$ $U \rightarrow C$

Measures of Location and Variability

	CO2=	Urban	Tech	Cons
Mean	0.292	10,361,445	17.64	7.93
Variance	300.33	1,610,483,294,653	7.37	0.69
standard deviation	18.18	1,330,988.96	2.85	0.87
Coefficient of Variation	0.75	0.13	0.16	0.11

Table 2: Coefficient of Variation Determination

Source: World Development Indicators (WDI)

Where CO2 stands for carbon dioxide emissions per capita, Urban for Urban Population, Tech for Access to Clean Fuels and Technologies for Cooking (% of population) and Cons Construction Industry (% manufacturing value added)

Table 2 indicates that CO2 emissions fluctuate more rapidly than those of the remaining variables. The latter results reflect the existing instability in environmental variables.

#### Hypothesis Testing

In the introductory subsection of the paper, we stated that the Human Development Indices (2018) pointed out that that high human development countries are the biggest contributors to environmental degradation with 10.7 tons average carbon dioxide emissions per capita. Small countries on the other hand with 0.3 tons carbon dioxide emissions per capita have been found to pollute less. We use CO2 emission per capita for the case of Cameroon in order to test the latter claims. 0.292 tons is the estimated average CO2 in Cameroon for the period of 2005 to 2015.

 $H_{o: \mu} \le 0.3$  $H_{a: \mu} > 0.3$ 

Rejection Rule assuming a .05 level of significance,

Reject  $H_0$  if t < -2.262 or if t > 2.262

Df = n-1 = 10 - 1 = 9

$$t = \frac{\bar{x} - \mu}{s / \sqrt{n}}$$

$$t = \frac{.29 - .3}{.04 / \sqrt{10}} = -0.79$$

Since -0.79 > -2.262, we do not reject H<sub>o</sub>.

Conclusion: We are 95% confident that the sample mean CO2 in Cameroon, is 0.3. Therefore, Cameroon, with 0.292 tons carbon dioxide emissions per capita can be considered as a country polluting less. The low pollution level in Cameroon may be due to its environmental policies implemented in sectors such as electricity generation where more focus is placed on renewable energy such as hydroelectricity, solar and gas to power plants.

Interval Estimation

The sample mean of CO2 above for the case of Cameroon has been estimated at 0.292 tons, the present subsection will develop a 95% confidence interval estimate for the population mean of small pollutant.

# $\bar{x} \mp t\alpha/2 \frac{\sigma}{\sqrt{n}}$ 0.292 \ \ 2.262 \ $\frac{0.04}{\sqrt{10}}$ 0.26 tons to 0.32 tons

Conclusion: The present results confirms the hypothesis results since we are 95% confident that the interval contains the population mean of 0.3.

We now turn to the econometric analyses of the above variables in order to explore their causal relationship.

#### 2.2.2. Econometric Analysis

The present Econometrics analysis is an attempt to reduce the shortcomings due to no CO2 models that test the causal

 $CO2_{i} = \alpha_{0} + \alpha_{1}Urban_{i} + \alpha_{2}Tech_{i} + \alpha_{3}Cons_{i} + \varepsilon_{i}$ (1)

Where CO2 is Greenhouse Gas Emissions, Urban is Urban Population, Tech is Access to Clean Fuels and Technologies for Cooking and Cons Industry construction.

Multiple Regression Analysis

The present multiple regression analysis will enable us to assess the significance of the relationship between our variables of interest. The outcome of this analysis will

$$CO2 = -480.89 + 00017 \text{Urban} - 80.03 \text{Tech} + 14.99 \text{Cons}$$

$$(-3.91) \quad (2.90) \quad (-2.85) \quad (3.47)$$

$$R^{2} = 0.82 \qquad \qquad DW = 1.33$$

The results of the proposed CO2 equation presented in equation (2) show that the estimated coefficient on Tech is negative and statistically significant at the 5 per cent level. The performance of this appears to accord with the Ecological Modernisation Theory. The estimated coefficients on Urban and Cons are significant at the 5 and 1 per cent levels respectively and display positive signs as anticipated.

From the Durbin–Watson tables, we find that for 11 observations and one explanatory variable, dL = 0.595 and  $\Delta Yt = \beta Yt-1 + ut$ 

relationship between CO2 emissions and urbanisation and science and technology in Cameroon. We aim at investigating the relationship and the direction of short and long run causalities between the latter variables using the unit root tests, the augmented Dickey Fuller, Johensen Cointegration tests, Granger causality test within Error Correction Model and the Toda and Yamamoto (1995) Granger causality. In line with the theoretical thoughts outlined in our literature review, our CO2 model is specified as follows:

permit us to identify the main factors that affect CO2 in Cameroon by capturing the strength that they exert on CO2 emissions. Our results will be used to forecast the significance of the changes. Equation (2) presents the results of estimation by OLS of CO2 emissions which test the validity of the Ecological Modernisation Theory in Cameroon.

dU = 1.928 at the 5 percent level. Since the calculated d of 1.559 lies between dL and dU, we are in the zone of indecision and cannot therefore conclude about the serial correlation of our error terms in our time series.

(2)

The Unit Root Test

A test of stationarity that has become extensively widespread over the past several years is the unit root test. We estimate the following equation:

Where  $\beta = (\rho - 1)$  and,  $\Delta$  is the first-difference operator. The Ho hypothesis that  $\beta = 0$  is tested. If  $\beta = 0$ , then  $\rho = 1$ , that is we conclude that there is unit root, indicating that the time series employed is non-stationary. Ho:  $\beta = 0$  (unit root) Ha:  $\beta > 0$  (no unit root)

Should the ut is found to be correlated, we shall then employ the augmented Dickey–Fuller (ADF) test using the following regression:

$$\Delta y_t = \gamma + \beta t + \alpha y_{t-1} + \sum_{j=1}^{K} \theta_j \Delta y_{t-j} + u_t$$

Table 3 : Unit Root Tests

	ADF		
Variable	Test Statistic	5% Critical Value	
CO2	-7.163	-3.000	
ΔΔUrban	-2.39	-1.950	
Tech	-3.227	-3.000	
Cons	-22.559	-3.000	

Table 3 presents the unit root tests for the relevant series. The null hypothesis is accepted if the t\*0.05 < critical ADF  $\tau$  value. It can be seen that all t\*0.05 < critical ADF  $\tau$  value = -3.000 for all variables. The null hypothesis is rejected for all variables. We conclude that while CO2, Tech and Cons are I(0), Urban is I(2).

**Cointegration Tests** 

Though some quantitative methods for testing cointegration have been recommended in the literature, we contemplate here the ADF unit root test on the residuals estimated from the cointegrating regression. It should be noted that the ADF test in the existing situation is known as Engle–Granger (EG) and augmented Engle–Granger (AEG) tests. The cointegration test has the following hypothesis.

Ho:  $\beta = (cointegrated)$ 

Ha:  $\beta \neq 0$  (not cointegrated)

The null hypothesis is not rejected if the critical value (t\*0.05) is less than the test statistic of the error term. To implement the test, we first regressed CO2 emission on Urban, Tech and Cons. We obtained the following regression:

Table 4: Cointegrating Regression Estimates

Variable	Coefficient	Test statistic
Dependent (CO2)		
CO2	-480.892*	-3.91
Urban	0.0001736**	2.91
Tech	-80.031**	-2.85
Cons	14.988*	3.57
R <sup>2</sup>	0.82	

Notes: \* and \*\* denote respectively statistical significance at the 1 per cent and 5 per cent level.

Since, CO2, Urban and Tech are individually stationary, there is therefore no prospect that this regression is spurious. Furthermore, when we undertook a unit root

test on the residuals obtained from the cointegrating regression, we obtained the following results:

Table 5: Unit Root Tests on the Residuals

	ADF		
Variable	Test Statistic	5% Critical Value	
Residuals	-3.64	-3.000	

Table 5 presents the unit root tests on the residuals. It can be seen that the augmented Engle–Granger test statistic < t\*0.05 = -3.000. We reject the null hypothesis of unit root since the calculated  $\tau$  (= t) value is lower than -3.000, we conclude that the residuals from the regression of, CO2, Urban Cons and Tech are stationary. We, therefore, conclude that there is a unique cointegrating vector in all CO2 combinations. That is there is a long term, or equilibrium, association between CO2, Urban Cons and Tech.

Error Correction Mechanism (ECM)

Table 6 : ECM Regression Estimates

The error-correction term estimates the deviation of the series from the long run equilibrium relation. It was mostly employed by Sargan and, Engle and Granger who made it widespread. Relating to the Granger Representation Theorem, the association between two variables A and B can be expressed as ECM when they are cointegrated. Statistically, if the error term is negative and significant, we will infer that the changes in A (dependent variable), adjust to changes in B (independent variable) in the short run. Returning to the case presented in this paper, we have the following results:

Table 0. Dewi Regression Estimates					
Variable	Coefficient	Test statistic			
Dependent (D.CO2)					
Constant	49.938	0.73			
D.Urban	0.0001264	1.25			
D.Tech	-114.3558	-1.94			
D.Cons	21.874**	2.92			
Ut-1	-0.8545*	-1.96			
<b>R</b> <sup>2</sup>	0.80				

Notes: \* and \*\* denote respectively statistical significance at the 10 per cent and 5 per cent level. D stands for First Differences of our dependent and independent variables.

Table 6 presents the ECM Regression Estimates where the error term is negative and significant at the 5% level. We conclude that the changes in CO2 emissions, with a negative coefficient of -0.9, do adjust to changes in Urban, Tech and Cons at a speed of 80% in the short term.

The Granger test undertaken in this paper will provide answers to the question of the direction of causality between CO2, Urban, Tech and Cons. For example, the direction of causality inference between W and Z will be made by estimating the following pair of regressions:

The Granger Causality Test

$$= \sum_{i=1}^{n} \propto_{i} Z_{t-i} + \sum_{j=1}^{n} \beta_{j} W_{t-j+u_{1t}}$$
$$Z_{t} = \sum_{i=1}^{n} \gamma_{i} W_{t-i} + \sum_{j=1}^{n} \beta_{j} Z_{t-j+u_{1t}}$$

Direction of causality	F	Df	Prob
$CO2 \rightarrow Urban$	6.15	4	0.0603
CO2→ Tech	0.23	4	0.8044
CO2→Cons	0.11	4	0.9020
Urban $\rightarrow$ CO2	2.93	4	0.1649
Urban $\rightarrow$ Tech	1.07	4	0.4252
Urban $\rightarrow$ Cons	4.35	4	0.0992
Tech $\rightarrow$ CO2	0.45	4	0.6647
Tech $\rightarrow$ Urban	0.50	4	0.6390
Tech $\rightarrow$ Cons	1.27	4	0.3750
$Cons \rightarrow CO2$	0.28	4	0.7667
$Cons \rightarrow Urban$	3.40	4	0.1373
$Cons \rightarrow Tech$	0.08	4	0.9258

Table 7: Short Run Granger Causality Test

Table 8: Long Run Granger Causality Test

Tuble 6. Long Run Grunger Causanty Test						
Direction of causality	Chi-square	df	Prob			
$CO2 \rightarrow Urban$	25.01	4	0.0000**			
CO2→Tech	12.82	4	0.0003**			
Urban $\rightarrow$ CO2	19.24	4	0.0000**			
Urban $\rightarrow$ Tech	13.59	4	0.0002**			
Tech $\rightarrow$ CO2	6.39	4	0.0115*			
Tech $\rightarrow$ Urban	7.90	4	0.0050*			

Notes: \* and \*\* denote respectively statistical significance at the 5 per cent and 1 per cent level.

Tables 7 and 8 report the results about the causal relationship between CO2, urban, Tech and Cons. The above tables suggest that while there is a bidirectional causality between the latter variables (except industry construction) in the long run, there is no causality between the studied variables in the short run. All variables in Table 8 Granger-cause each other in the long run.

#### III. DISCUSSION AND CONCLUSION

# 3.1. Discussion

The bidirectional causality between Tech and CO2 indicates that due to the inverse and significant relationship between the latter variables shown in equation (2), an increase in Tech will reduce CO2 significantly. This may be due to environmental friendly considerations of new technologies in Cameroon. For example, in Cameroon, in terms of electricity generation, more focus is on renewable energy such as solar, hydroelectricity and gas to power plants rather than Diezel plants. The double causality in Table 8 shows that where CO2 is increasing, Science and Technology access is declining due to the neglect of the latter. For example, in Chad where 90% of electricity is generated via diesel power plants, Science-Technology-Society access via renewable energy is merely inexistent. Thus, policies that would help to reduce emissions are undoubtedly required major Science and Technology contribution.

The bidirectional causality between Tech and Urban, together with results in equation (2) indicate that increase in science and technology will create more wealth which will in turn increases urban population. On the other hand, in the long term, more urban population will stimulate science and technology to generate productivity.

The bidirectional causality between Urban population and CO2 Emissions indicates that urban population are in

# IV. TABLES

Table 1: Summaries of Econometric Tests of Urbanisation, CO2 Emissions, and Science-Technology-Society

Table 2: Coefficient of Variation Determination

Table 3 : Unit Root Tests

Table 4: Cointegrating Regression Estimates (2005-2015)

Table 5: Unit Root Tests on the Residuals

Table 6 : ECM Regression Estimates

Table 7: Short Run Granger Causality Test

Table 8: Long Run Granger Causality Test

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# 3.2. Conclusion

This paper has demonstrated that environmental policies implemented in Cameroon to reduce CO2 may not be reliable due to limited or no publication on the causal relationship between CO2 and science and technology supported in the ecological modernisation theory. Our statistical analysis has not rejected the null hypothesis of Cameroon being considered as a small pollutant. Moreover, the unit root tests have indicated that CO2 emissions, Urban population and science and technology are I(0). While, the cointegration tests have found short and long run equilibrium within the latter variables. Furthermore, the Granger and Toda and Yamamoto (1995) causality tests have suggested that while there is a bidirectional causality between the latter variables in the long run, there is no causality between the studied variables in the short run. Our results are amenable to the recommendation that policy makers in Cameroon should design policies controlling urbanization due to it positive and significant effect of CO2 emission. On the other hand, policies encouraging science and technology should be encouraged due to the inverse and significant relation between CO2 emission and science and technology.

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