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Efficacy of *Eucalyptus camaldulensis* leaf extracts against the pea beetle *Callosobruchus maculatus* and their impact on biochemical and microbiological properties of the treated bambara groundnut grains

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Abstract
The present study was aimed to evaluate the efficacy of *Eucalyptus camaldulensis* leaf hexane, acetone and methanol extracts against *Callosobruchus maculatus* and their impact on biochemical, sensorial and microbiological properties of the treated *Vigna subterranea* grains in the laboratory. Each plant extract was applied at doses of 5, 10, 15 and 20g/kg on the bambara groundnut grains and pea beetles were released in each concentration test and *C. maculatus* was monitored 1, 3 and 6 days post-exposure. Effects of the plant products on progeny production *F1* and damages caused by *C. maculatus* after three months post-storage were evaluated. Biochemical, sensorial and microbiological characteristics of the preserved seeds were also assessed. Plant extracts tested caused a significant mortality and reduced progeny production *F1* of *C. maculatus*. Hexane extract (5g/kg) was most effective causing 78.75% mortality of insect, completely inhibited progeny production *F1* and protected seeds for 3 months from insect attack. Kodek bambara groundnut variety was rich in proteins (21.53%), carbohydrates (61.24%) and minerals (3.54%). Antinutrients in treated grains increased but remained below the threshold values. The preservation of bambara groundnut grains with the plant extracts improved the organoleptic properties but affected the taste, tender and the crisp of the derived products. The plant extracts also protected *V. subterranea* flour against the micro-organisms.

Keywords: *Vigna subterranea*, extract, toxicity, nutritional quality, microbiological, sensory characteristics

1. Introduction
The bambara groundnut [*Vigna subterranea* (L.)Verdcourt 1981], the third most important leguminous plant in terms of production and consumption after groundnut and cowpea [1]. Cultivated for its seeds richness in proteins, carbohydrates, lipid, vitamins and minerals [2, 3], this plant is also highly rich in calorie (387 kcal/100 g). That leguminous plant is also rich in lysine and methionine [4, 5] and is considered nowadays as the most recommended food in the denutrition improvement in the chronic malnutrition zones of Cameroun [6]. In the case of significant annual production, bambara groundnut would have a significant consideration in the strategies of food safety promotion in sub-Saharan Africa [7] where more than 239 million persons are suffering from a chronic malnutrition [8]. One of the principal reasons explaining scarcity of this food product for consumption is especially the importance post-harvest losses observed during storage by the devastators [9, 10]. Among these devastating pests, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is known as one of the most detrimental leguminous plant seeds in storage and can cause weight loss ranging from 56 to 69%, an equivalent of 45.6-66.3% protein loss in one year [11, 12].The importance of the damage caused by this devastator in storage remains high and constrains the farmers to resort to several protection measures. Among these measurements, the use of synthetic insecticides constitutes presently, the most used technic to control these ravagers during the post-harvest period [13, 14]. This method although effective releases many disadvantages which limit its employment and this includes the harmful effects on the environment and health of the consumers. Besides, the insecticides are relatively high price, scarce and sometimes with

Doubtful quality on the local markets local [15, 16]. Research of the new molecules, take into account not only the effectiveness, but others criteria becomes currently, a major concern of successful and effective protection of stored food products [17, 18]. It is for these many reasons that the farmers look for others grain protection measures by using substances from plants [19]. The recent works completed on insecticidal evaluation of the plant secondary metabolites extracted using organic solvents have proven their insecticidal effect against *C. maculatus* [20, 21]. However in the Cameroon flora, other plants which their secondary metabolites can be also effective against *C. maculatus* are found including *Eucalyptus camaldulensis*. The leaves of this plant are used to treat diarrhea, dysentery, haemorrhage, digestive troubles and rum [22, 23] and its essential oil against bacteria, parasites and fungi [23]. Different solvent extracts of this plant could have an insecticidal potential against *C. maculatus*. In addition, the preservation of seeds the extracted plant metabolites could modify nutritional and organoleptic quality derived products on the one hand and to improve the microbiological properties of the derived flours in the other hand. In fact, Rose of Lima *et al*. [24] and Mahama *et al*. [20] reported that the conservation of cowpea with plant essential oils belonging to the family of Myrtacea and bambara groundnut with the extracts of *Cassia mimosoides* improved the organoleptic properties with an incidence on the taste and the flavour of the derived products. It would be thus significant to evaluate the anti-insect activity of *E. camaldulensis* extracts against *C. maculatus* and their impacts on the quality nutritional and organoleptic and microbiological of the derived products. The objective of this present work aims to evaluate the efficacy of *E. camaldulensis* leaf hexane, acetone and methanol extracts against *C. maculatus* and their impact on biochemical, sensorial and microbiological properties of the treated *V. subterranea* grains

2. Material and Methods

2.1 Plant material

2.1.1 Harvesting and processing

The green leaves of *Eucalyptus camaldulensis* were collected in September 2014 at Dang, Adamaoua region, Ngaoundéré, Cameroon (1334 longitude, latitude of 728 North and one of average altitude of 1100 m). The plant leaves collected were dried at the shade for three weeks, then crushed in a porcelain mortar and passed through 1mm mesh size sieve. The powder obtained was preserved in the glass bottles then kept in a refrigerator at 4°C until the period of extraction.

2.1.2 Plant Extraction process

The plant extraction process was the cold maceration method performed of Perry *et al*. [25]. Indeed, 1k g of the plant leaf powder was macerated in 3 L of hexane for 3 days in the glass jar (5 L) and the maceration was turned twice a day. Then the maceration was filtrated through Whatman No.1 filter paper to obtain hexane filtrate and residue. Then, the residue was dried and then macerated in 3L of acetone and processed as described previously to obtain acetone filtrate and residue. At last, the dry residue was macerated in the 3 L of methanol solvent as described previously to obtain methanol filtrate and residue. Each filtrate was concentrated separately using rotary evaporator (BÜCHI R-124) to obtain hexane, acetone and methanol extracts. The dry plant extracts were stored at -4°C until use for phytochemical screenings and bioassays. The extraction yield was of each extract was determined using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the extract obtained (g)}}{\text{Weight of the plant powder used (g)}} \times 100$$

2.1.3 Phytochemical screening tests of the plant extracts

The hexane, acetone and methanol extracts of *E. camaldulensis* were subjected to the phytochemical screening test to detect the presence of alkaloids according to Bidie *et al*. [26] method. The presence of total polyphenol compounds was determined following N'Guessan *et al*. [27] method. Debray *et al*. [28] method was used to determine the presence of Flavonoids and Dohou *et al*. [29] method to detect the presence of saponins. The presence of steroids, tannins, coumarins and triterpenoids was assessed according to the method advocated by Fankam *et al*. [30].

2.2 The strain of *Callosobruchus maculatus*

The strain of *C. maculatus* used comes from the infested of *Vigna subterranea* seeds bought from the local market of Ngaoundéré. The adults of *C. maculatus* obtained from the infested grains were reintroduced in 900 mL glass bottles containing 500 g of a sterilized bambara groundnut seeds and kept in the laboratory conditions (25.13±3.03°C, 66.46±10.12 HR). Seven days post-infestation, the insects were discarded from the grains and the infested seeds were incubated until the adults' emergence. For bioassays, 24 hours old adult pea beetles were used for experiments.

2.3 Bioassays

2.3.1 Effect of plant products on *C. maculatus* adults

The method described by Nukenine *et al*. [17] was followed to evaluate the toxic effect of *E. camaldulensis* leaf extracts on *C. maculatus* adults. Each extract was dissolved in the extraction solvent used and different concentrations of 0.25; 0.5; 0.75 and 1g/mL of hexane, acetone and methanol were prepared. Each plant extract doses were mixed uniformly and separately with 50 g of *V. subterranea* grain in the 900 mL glass bottle. The negative control was made by the mixture of 50 g of grains with 1 mL of each solvent used separately constituted the negative control. Test bottles were left in open air for 2 hours to evaporate the solvents from the grains and 20 pea mixed both males and females pea beetles (reared in the same bambara groundnut bought from Ngaoundéré marked), were transferred into each bottle test preparation. Each dose was repeated four times and *C. maculatus* adult mortality was recorded after 1, 3 and 6 days post-treatment.

2.3.2 Effect of plant extracts in the progeny F1 production

Six days post- treatment, the dead and alive pea beetles were discarded from the monitoring bottles and the treated bambara groundnut grain bottles were kept for observation every week until emergence of adults. Every day until 5 last days without emergence of the pea beetles, the number of insects (pea beetle) emerged was recorded.

2.3.3 Effect of *Eucalyptus camaldulensis* extracts on grain damaged by *C. maculatus* during storage

Four stock solutions of 0.25; 0.5; 0.75 and 1 g/mL Concentrations were prepared for each plant extract and 1 mL of each concentration was mixed uniformly with 50 g of pea groundnut in the 900 mL glass bottles and each dose was repeated four times. The negative control consisted of only solvents used were mixed with bambara groundnut. Then, 20 pea beetles adults both sex male and female, 24 h old were added in each test bottle and sealed with muslin cloth. After 3 months post-treatment, the number of *C. maculatus* adults'

dead and alive as well as the number of damaged and undamaged Bambara groundnut grains was recorded. Total damaged and undamaged bambara groundnut grains were also weighted. The formula of Adam and Schulten [31] below was used to calculate weight losses.

$$\text{Weight losses (\%)} = \frac{[(Wu \times Nd) - (Wd - Nu)]}{Wu(Nd + Nu)} \times 100$$

Wu= weight of undamaged grains; Wd= weight of damaged grains; Nd= number of damaged grains, Nu= number of undamaged grains.

2.3.4 Evaluation of nutritional value and organoleptic characteristic of damaged and undamaged grains post-storage

The physicochemical characteristics and nutritional values evaluated before and after storage included: the moisture and dry matter [32], ash content [33], proteins [34], lipids [35] and carbohydrates [36]. Total phenolic content in the extracts and treated seeds was determined according to López-Mejía *et al*. [37] method. Makkar *et al*. [38] method was used to determine total tannin content of extracts and grains.

2.3.5 Sensorial Analysis of Pea Groundnut

As Kodek bambara groundnut variety was the most appreciated and consumed in the various zones of studies, that variety was used in this present study for the sensory analysis. The undamaged grains treated with the lowest effective concentration at the end of three months of storages were considered and divided in two samples. Considering the culinary practices of the population for bambara groundnut processing for consumption, one sample was cooked and the other was roasted according to traditional protocol of preparation obtained from the consumers. The purpose of this sensorial analysis study was to analyze and to interpret the organoleptic characteristics of the processed products as perceived by the sense organs [39] such as the color, odor, texture and the taste of the two processed bambara groundnut. The two form of the processed product were submitted to the hedonic test carried out at the sensory analysis laboratory according to the principle of a classification test. That test consists for one characteristic, to arrange by increasing intensity, the samples presented simultaneously to 20 adult tasters of sex male and female and coming from different zones as well as their familiarity for consuming these products. A numerical output notations ranging from 1 to 7 in which 1 corresponds to "extremely bad" and 7 "extremely good" was analyzed using test Friedman test.

2.3.6 Microbiological analysis

Flours preparation samples, manipulations and materials used were performed according to the norms established by NF ISO 7218. Therefore, total flora, total coliforms, number of yeasts and moulds were determined following AFNOR method.

2.4 Statistical Analyses

Abbott's formula [40] was used to correct mortality percentage when mortality in the control is comprised between 3% and 10%. Data recorded from adult mortality, progeny *F1* production, damages and weight losses percentages were transformed to arcsin√(x/100) and submitted to ANOVA analysis using statistical analysis software SAS followed by mean separation using Tukey test (P<0,05). Probit analysis was also conducted to determine lethal concentrations that cause 50% (LC_{50}) and 95% mortality (LC_{95}) of *C. maculatus* adults. For sensorial analysis, data were analyzed using Friedman test. Fisher coefficient was calculated using Chi-square test (χ^2). Different samples comparison by calculating Fc coefficient and compared with "S" value in the Chi-square table at α=5% and degrees of freedom k-1 [41].

3. Results

3.1 Extraction yield

The extraction yield obtained from cold maceration of 1 kg plant powder in 3 L of hexane, acetone and methanol solvents is presented in Table 1. The extraction yield varied from one solvent to another. Methanol extract presented a high extraction yield of 15.9% compared to acetone extract with 9.4 and 7.1% extraction yield respectively for acetone and hexane extracts.

Table 1: Extraction yield of *Eucalyptus camaldulensis* extracts

Plant extracts	Weight of extract obtained (g)	Extraction yield (%)
Hexane	71	7.1
Acétone	94	9.4
Méthanol	159	15.9

3.2 Phytochemical screening

The phytochemical components revealed in hexane, acetone and methanol extracts of *E. camaldulensis* are presented in Table 2. The plant extracts screened possessed a wide variety of phytochemical components distributed in different solvent extraction used according to their polarity. In the hexane extract of the plant, triterpenoids and steroids were abundantly present. Acetone extract contained flavonoids, coumarins, saponins and abundantly phenolic compounds, tanins and alkaloids. The phytochemical composition of methanol extract of the plant included saponins, alkaloids, tanins and abundantly flavonoids and total phenolic groups.

Table 2: Phytochemical composition of hexane, acetone and methanol extracts of *Eucalyptus camaldulensis*

Phytochemical components	Hexane	Acétone	Méthanol
Total phenolic compound	-	++	++
Flavonoids	-	+	++
Tanins	-	++	+
Coumarins	-	+	-
Alkaloids	-	++	+
Triterpenoids	++	-	+
Steroids	++	-	-
Saponins	-	+	+

+ = present; ++ = abundantly present and - =absent,

3.3 Adult Mortality

Table 3 presents the mortality of *C. maculatus* adults exposed to the hexane, acetone and methanol extracts of *E. camaldulensis*. All extracts of *E. camaldulensis* exhibited a significant adult mortality of *C. maculatus*, compared to the negative control. The mortality of the *C. maculatus* adults depended on the different plant extracts used, the concentrations applied (F= 3.00-289.00) and the exposure period (F= 4.17-748.93 and P < 0.0001). Generally, hexane extract was the most toxic on pea beetle adults compared to acetone and methanol extracts. However, 1 d post treatment with the lowest dose of 5 g/kg, high percentage mortality of 78.75 and 45.00% was recorded with hexane and acetone extracts, respectively. Tested at the highest dose of 20 g/kg, only 75.04% mortality of pea beetle was noticed with acetone

while 100% mortality of *C. maculatus* adults was recorded with hexane and methanol after 3 days post-treatment at the dose applied. Applied at 5g/kg, hexane and methanol extracts of the plant exhibited 100% mortality of *C. maculatus* adults after 6 days post-treatment.

Table 3: Corrected mortality percentage and LD_{50} (g/kg) of *Callosobruchus maculatus* exposed to *Eucalyptus camaldulensis* extracts on *Vigna subterranea*.

Extracts	Conc (g/kg)	Days after infestation			
		1 day	3 days	6 days	F
Hexane	0	0.00±0.00d	0.00±0.00b	0.00±0.00b	
	5	78.75±1.25cB	100.00±0.00aA	100.00±0.00aA	289.00***
	10	86.25±1.25bB	100.00±0.00aA	100.00±0.00aA	121.00***
	15	93.75±2.39aB	100.00±0.00aA	100.00±0.00aA	6.82*
	20	97.50±1.44aA	100.00±0.00aA	100.00±0.00aA	3.00*
	F(4,10)	748.93***	4.17***	4.17***	
	DL_{50} (FL)	0.09 (0.02-0.15)	-	-	
Acetone	0	0.00±0.00c	0.00±0.00e	0.00±0.00	
	5	0.00±0.00cC	28.16±2.30dB	48.54±0.00bA	76.22***
	10	16.25±2.39bC	39.80±2.72cB	54.34±0.00bA	50.70***
	15	26.25±1.25aC	48.75±1.65bB	69.15±0.00aA	126.60***
	20	31.25±1.25aC	58.95±2.13aB	75.04±2.60aA	114.43***
	F (4,10)	118.85***	128.76***	109.26***	
	DL_{50} (FL)	1.35 -	0.73 (0.5-1.0)	0.30 (0.1-0.4)	
Methanol	0	0.00±0.00e	0.00±0.00c	0.00±0.00b	
	5	45.00±2.84dA	65.53±5.29bA	69.28±2.31aA	0.90ns
	10	67.50±1.44cC	76.91±1.52bB	100.00±0.00aA	190.72***
	15	81.25±3.15bB	91.12±4.28aAB	100.00±0.00aA	9.34**
	20	92.50±1.44aB	98.75±1.25aA	100.00±0.00aA	13.29**
	F(4,10)	366.94***	153.73***	17.59***	
	DL_{50}(FL)	0.29 (0.2-0.3)	0.18 -	0.13	

^{ns}p>0,05; * p<0,05; ** p<0,001; *** p<0,0001. FL: Fiducial Limit. For the same product, mean ± standard error in the row followed by the same capital letter and in the column by the same small letter did not differ significantly according to Tukey test (p=0.05). LC50: Lethal concentration that kill 50% of pea beetle. 50. Each datum represents the mean of 3 replicate values.

3.4 Effect of *Eucalyptus camaldulensis* leaf extracts on the progeny *F1* Production

The inhibition percentage of the progeny *F1* production of *C. maculatus* on the bambara groundnut grains tested with different plant leaf extracts is presented in table 4. In results, all plant extracts tested significantly inhibited significantly the progeny F1 production of *C. maculatus* compared to the negative control and this reduction increased with the increasing doses. At all doses tested, hexane extract completely inhibited Progeny *F1*production of pea beetle. Tested at 15 g/kg, complete inhibition of progeny was also observed with methanol extract while acetone extract inhibited 68.27% of progeny production of pea beetles at the same dose.

Table 4: Effect *Eucalyptus camaldulensis* leaf extracts on the reduction of progeny *F1* production

Conc (g/kg)	Hexane extract		Acetone extract		Methanol extract	
	Progeny *F1*	%Inhibition	Progeny *F1*	%Inhibition	Progeny *F1*	%Inhibition
0	99.00±5.40a	0.00±0.00b	99.00±5.40a	0.00±0.00a	99.00±5.40a	0.00±0.00a
5	0.00±0.00b	100.00±0.00a	51.00±1.47b	48.04±3.11b	12.25±0.85b	87.56±0.93a
10	0.00±0.00b	100.00±0.00a	39.25±3.90bc	59.84±4.64bc	6.50±1.32bc	93.36±1.52b
15	0.00±0.00b	100.00±0.00a	30.75±3.50c	68.27±4.99c	0.00±0.00c	100.00±0.00c
20	0.00±0.00b	100.00±0.00a	11.50±2.53d	88.54±2.38d	0.00±0.00c	100.00±0.00d
F	385.20***	902.20***	82.47***	84.47***	285.20***	2902.20***

*** p<0, 0001. FL: Fiducial limit. For the same product, mean ± standard error in the column followed by the same letter and did not differ significantly according to Tukey test (p=0.05). Each datum represents the mean of 3 replicate values.

3.5 Effect of the plant leaf extracts on the reduction of grain damages and grain losses

Table 5 presents the efficacy of hexane, acetone and methanol extract of *E. camaldulensis* on the reduction of grain damages and grain losses caused by C. maculatus. Generally, the different plant extracts reduced significantly the rate of grain damaged and grain weight losses compared to the negative control. Bambara groundnut grains treated with hexane extract was completely protected without damage of grains with doses applied. Tested at 15 g/kg, methanol extract completely protected bambara groundnut grains from *C. maculatus* damages. The efficacy of acetone extract was low compared to the other plant extracts and applied at the highest dose of 20 g/kg, acetone extract exhibited a maximum percentage protection of 83%.

Table 5: Effect of *Eucalyptus camaldulensis* extracts on grain damages and losses reduction caused by *Callosobruchus maculatus*

Conc (g/kg)	% Undamaged grains	% Damaged grains	% losses
	Hexane extract		
0	0.00±0.00b	100.00±0.00a	59.10±1.30a
5	100.00±0.00a	0.00±0.00b	0.00±0.00b
10	100.00±0.00a	0.00±0.00b	0.00±0.00b
15	100.00±0.00a	0.00±0.00b	0.00±0.00b
20	100.00±0.00a	0.00±0.00b	0.00±0.00b
F	∞***	∞***	2062.32***
	Acetone extract		
0	0.00±0.00a	100.00±0.00a	59.46±1.41a
5	0.00±0.00a	100.00±0.00a	57.07±1.39a
10	0.00±0.00a	100.00±0.00a	52.08±0.84a
15	0.00±0.00a	100.00±0.00a	36.64±4.52b
20	3.66±1.50a	93.29±3.41a	22.49±3.18c
F	1.00ns	1.00ns	34.86***
	Methanol extract		
0	0.00±0.00b	100.00±0.00a	59.46±1.41a
5	0.00±0.00b	100.00±0.00a	57.07±1.39a
10	35.39±11.85ab	64.61±2.42ab	21.38±10.61b
15	75.00±5.00a	25.00±0.00a	0.00±0.00b b
20	100.00±0.00a	0.00±0.00b	0.00±0.00b
F	5.72**	5.72**	11.53***

^{ns}p> 0,05; *** p<0,0001. For the same product, mean ± standard error in the column followed by the same letter did not differ significantly according to Tukey test (P=0.05). Each datum represents the mean of 3 replicates values. – F values were not determined because of 100% or no protection at all doses tested.

3.6 Biochemical Characteristics of the treated *Vigna subterranea*

Table 6 presents the biochemical composition of *V. subterranea* variety evaluated showing a significant (P<0.05) difference all biochemical parameters assessed on the treated and untreated bambara groundnut. However, the ash content, proteins, sugars total and lipids varied from 3.54 (BB) to 3.84 (BB Hexane), from 20.17 (BB Methanol) to 22.13 (BB Acetone), from 7.11 (BB Acetone) to 8.68 (BB Hexane) and from 61.24 (BB control) to 62.36 (BB Methanol), respectively. Then, values showed that the treatment of bambara groundnut grains with the hexane, acetone and methanol extracts of *E. camaldulensis* did not deteriorate the biochemical composition of *V. subterranea* grains. Moreover, the increasing in the quantity of the antinutritional factors in the treated grains of bambara groundnut was recorded. Total phenolic compounds and tannins quantity varied from 565.02 mg/100MS to 778.56 mg/100g and from 42.03 mg/100MS to 44.60 mg/100MS, respectively.

3.7 Organoleptic properties of the treated bambara groundnut

The sensorial assessment of the bambara groundnut in cooked and roasted forms previously treated with *E. camaldulensis* revealed changes on the sensory characteristics of the derived products (Figures. 1 and 2). The significant difference (F>9.49 at 5%) between the treated forms and the control product was confirmed in the Friedman test (Table 7). Indeed, the plant extracts increased bitterness, the odor and the color of the two forms of preparation and also decreased the salinity and the crispness of the roasted form and the tenderness for the cooked form of the bambara groundnut grains compared to the control. Similarity regarding the sweet-salty taste of the roasted was also noticed in the control and the treated grain samples. However, the control sample product was more acceptable by the taste volunteers than the treated products.

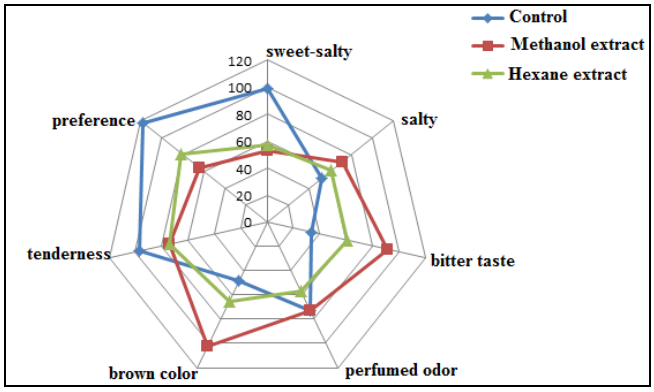

Fig 1: Sensorial profile of the cooked form bambara groundnut treated with *Eucalyptus camaldulensis* extracts

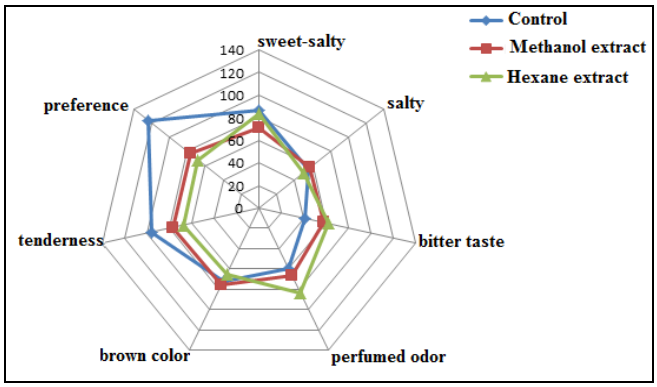

Fig 2: Sensorial profile of the roasted form bambara groundnut treated with *Eucalyptus camaldulensis* extracts

3.8 Antimicrobial quality of the flours

The plant extracts significantly reduced the number of

microorganisms in the treated flours of bambara groundnut compared to the normal number set by Codex Alimentarius (1994) (Table 6). The absence of yeasts and moulds were also registered in the treated flours but coliform are present in the flour treated with acetone extract.

Table 6: Antimicrobial quality of *Vigna subteranea* flour 3 months post-storage

Microorganismes	Norms	control	BB Hexane	BBAcetone	BBMethanol
Total flora	$<10^5$	$5,3*10^3$	$3,5*10^3$	$4.5*10^3$	$4.21*10^3$
Total coliforms	<100	Absent	Absent	Absent	Abs
Yeas and moulds	10^3	Absent	Absent	Absent	Abs
Conclusions		Satisfactory	Satisfactory	Satisfactory	Satisfactory

4. Discussion

All the plant extracts tested (hexane, acetone and methanol of *E. camaldulensis* leaf extracts) against *C. maculatus* have shown their significant insecticidal efficacy on that major bambara groundnut pest.

Compared to the non-polar solvent hexane extract in this present work, a large number and abundant phytochemicals in the acetone and methanol leaf extracts of *E. camaldulensis* was recorded. The variation in quality and quantity of these phytocomponents in different extracts could be attributed to the fact that plants synthesize secondary metabolites during their growth which are implied in the many physiological processes like the rhizome genesis, the cell multiplication, the fruit maturation, the seed germination and the defense against the external aggressions [42,43]. The absence of some compounds in hexane extract could be explained by the fact that hexane is a non-polar solvent incapable to extract some polar compounds. The richness of acetone and methanol extracts in triterpenes and steroids could be explained by the structure of some polar molecules able to bind to the non-polar molecules as triterpenes.

For adult mortality of *C. maculatus*, all plant extracts tested induced a significant toxic effect on the adults of the pea beetle. That insecticidal activity was different from one plant extract to other and also varied with concentration applied and with the exposure period. Similar result was reported by Daniel *et al*. [21], in which a significant adult mortality of *C. maculatus* was recorded with the increasing dose and exposure period of hexane extract of *Gnidia kaussiana*. A strong mortality of *Tribolium confusum*, *Sitophilus zeamais*, *Prostephanus truncatus*, *Rhyzoperta dominica* and *Callosobruchus maculatus* exposed to the terpenic compounds of essential oils was also reported [44, 45, 46]. The efficacy of these plant extracts could be attributed to the presence of the phytochemical secondary metabolites in these different extracts. Indeed, the plant secondary metabolites are responsible for the diverse activities including their insecticidal properties [47]. However, the solvent polarity used and the solubility determine the nature of the compounds present in each plant extract [48]. The higher mortality noted with hexane extract registered a high adult mortality of pea beetles compared to methanol and acetone extracts and could be attributed to the chemical composition and the sensitivity level of the pea beetles towards these various extracts [49]. The phytochemical compounds in these plant extracts might acted like antinutrients hindering assimilation of the nutriments [50], in the insect nervous system by disorganizing ion exchanges sodium and potassium causing insect death [51, 52].

The hexane, acetone and methanol extracts of *E. camaldulensis* reduced significantly the progeny *F1* production depending on increasing concentrations. The grains treated with the plant extracts reduced significant the progeny *F1* production of *Sitophilus oryzae* [53]. A significant reduction of the offspring of Caryedon serratus on *Vigna subterranea* treated with *Cassia occidentalis* and *Calotropis procera* powders was also reported [54]. Similarly, a significant reduction of the progeny *F1* production of *C. maculatus* on seeds treated with *Ocimum canum* and *Gnidia kaussiana* extracts was reported by Daniel *et al*. [21]. The progeny *F1* reduction could be explained by the presence of the antinutritional substances including tannins, phytates, phenolic compounds found in the plant extracts able to supply a complex to nutriments available to the immature stages of *C. maculatus* and also terpenes act directly on the adults in contact by their toxic effect.

All plant extracts reduced significantly grains damaged and grain weight losses caused by *C. maculatus*. A significant reduction of damaged grains and weight losses of maize treated was recorded with Neem Azal, *Azadirachta indica* and *Plectranthus glandulosus* powders against *Sitophilus zeamais* [21].The efficacy of these plant extracts of the damages reduction could be attributed to the presence of the secondary metabolites responsible for the insecticides activity.

Treatment of bambara groundnuts with the extracts of *E. camaldulensis* did not deteriorate the biochemical composition of seeds. No significant variation on the ash, proteins, sugars total and lipids contents was noticed. The ash content obtained in this present investigation is comparable to 3.25% obtained by Mazahid *et al*. [55] with bambara groundnut grains. A range from 3.57 g/100 g and 4.85 g/100 g of dry material of grains was also reported by Amartiefio *et al*. [56]. In contrary, Koffi *et al*. [57] obtained the low values ranging from 2.55 to 2.98 % compared to those obtained in this present work. This difference in the ash content could be attributed to the composition and texture of the soil which could have an effect on mineral absorption of the plants. The carbohydrates and lipid contents obtained in this present study are in agreement with those reported Boateng *et al*. [58]. Similarly, protein content ranged from 17.5 to 21.2 grams of proteins for 100 grams of dry material obtained by Amartiefio *et al*. [56] is also comparable to those obtained in this present study. This slight variation in grain content might be due to the genotypes and the environmental conditions where these seeds were cultivated [59, 60].

In this present study, antinutritional factors level increased in the treated bambara groundnut but are low compared to the critical values of 2700 mg/100g for phenolic compounds and 2000 mg/kg for tannins [61] and are not able to deteriorate the nutriment biodisponibility. However, grain treatments such as soaking or cooking could reduce the antinutritional factor levels [62].

Processed Bambara groundnut treated with plant extracts modified the sensorial characteristics of the grains. Aromatic compounds might be the cause of the increase in the taste, bitterness and low preference of the fried and cooked Bambara groundnut grains previously treated with plant extracts and which could aromatize the derived products compared to the control sample product.

Moreover, tannins and saponins are bitter and might also increase the bitterness of the treated processed grains compared to the untreated bambara groundnut. Similar result was reported by Rose de Lina *et al*. [24] and Mahama *et al*. [20] in which the use of essential oils significantly change the odor and the taste of the stored cowpea and treated Bambara groundnut treated, respectively.

5. Conclusion

From the results of this present investigation, the hexane, acetone and methanol leaf extracts of *E. camaldulensis* exhibited their potential insecticide effect on *C. maculatus* adults. The plant extracts tested, especially hexane extract significantly inhibited the Progeny F1 production of pea beetles and reduced damages and weight losses caused by *C. maculatus*. The plant extracts assessed possess a large range of phytochemical compounds which are responsible of insecticidal, antimicrobial and antioxidant activities. These extracts did not modify the nutritional quality of the derived products but rather improved the sensorial characteristics particularly, the flavour of the treated bambara groundnut grains. Thus, these extracts could be used as an alternative to preserve bambara groundnut from pea beetles attacks and might even replace synthetic insecticides used in the insect pest management program.

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