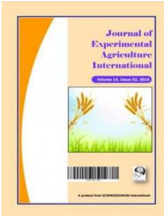

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Physico-chemical Properties and Resistance of Ten Bambara Groundnut (*Vigna subterranea*) Varieties to Attack by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae) in the Sudano-sahelian and Sudano-guinean Zones of Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DK and ENN designed the study and performed the statistical analysis. Author DK wrote the protocol and wrote the first draft of the manuscript. Authors CS and ENN managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This research aimed at selecting indigenous Bambara groundnut varieties with high nutritional value and inherent resistance to insect attack for cultivation in sudano-sahelian (SS) and sudano-guinean (SG) zones of Cameroon.
Study Design: The susceptibility of varieties to insect attack was assessed by adopting the standard evaluation of Dobie index and the experiment was arranged in a completely randomized design (CRD) with five replications.

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Place and Duration of Study: Department of Biological Sciences (laboratory of Applied Zoology), UIT (laboratory of food chemical engineering) at Ngaoundere (SG zone). The work was also carried-out at Maroua (SS zone), starting from Jun to October 2013 for susceptibility assessment and from march 2014 to April 2015 for physico-chemical characterization of varieties.

Methodology: Ten Bambara groundnut genotypes were infested with bruchids for six days. Comparative data on the mean percentage adult emergence, mean developmental period and susceptibility index (SI) were then collected for analysis. Seeds were also analyzed for physical and chemical properties to study the physico-chemical basis of resistance to bruchid attack and the nutritional value.

Results: Varieties were significantly different regarding their physical properties (F = 13.32 – 92.89; P < 0.001), chemical composition (F = 6.57 – 2936.00; P < 0.001 – 0.01) and susceptibility index (F = 107.02 – 152.59; P < 0.001). Overall, SS zone was most suitable for insect development. Biophysical characteristics were not found important to characterize the susceptibility of varieties to insect attack. Carbohydrates were negatively correlated (r = -0.53) with Bambara groundnut SI under SS conditions, while total polyphenolic compounds were positively correlated (r = 0.52) under SG conditions. Galaji, Black eye and Guerade guerlal consistently demonstrated high tolerance to infestation by *C. maculatus* and therefore, may be recommended for relatively longer storage.

Conclusion: The physical parameters of the grains did not linearly relate with varietal resistance to *C. maculatus*. However, it may be inversely or directly correlated with chemical characteristics, depending on the agro-ecological zone. Further analyses of grain biochemical components that may relate to varietal resistance to *C. maculatus* are required.

Keywords: Callosobruchus maculates; bambara groundnut varieties; physico-chemical properties; resistance; soudano-guinean zone; soudano-sahelian zone.

1. INTRODUCTION

Bambara groundnut (*Vigna subterranea* L. Verdcourt) is an indigenous African crop and reported to have originated from north-eastern Nigeria and Northern Cameroon where the wild forms are still found [1]. In the West Africa, Nigeria is the centre of production while Cameroon, Central African Republic and Chad are the largest producers in Central Africa [2]. Far-north is the centre of Bambara groundnut production in Cameroon and account for over 63% of National production, and thus supplies other regions [3]. In Africa, the crop is the third most commonly eaten legume after groundnut and cowpea [4]. This legume makes a balanced food, as it contains sufficient quantities of carbohydrates (49%-63.5%), protein (15%-25%), fat (4.5%-7.4%), fiber (5.2%-6.4%), ash (3.2%-4.4%) and 2% mineral with relatively high proportions of lysine and methionine compared to whole fresh cow milk 88% moisture, 48% carbohydrate, 32% proteins, 3.4% fat, 0.7% ash, and 0.01% cholesterol [5].

To respond efficiently throughout the year to population food request in the arid zones of sub-Sahara Africa food product must be stored, since its production is seasonal. Unfortunately, stored Bambara groundnut is very susceptible to insect attack, with bruchids (Coleoptera: Bruchidae) being the key pests [6,7]. *C. maculatus* was reported as a major pest of Bambara groundnut in Far North region of Cameroon [8]. This bruchid causes substantial quantitative and qualitative loses by perforation, thus taint the taste of the seeds and reducing the degree of usefulness, decreasing value and viability [9,10]. In SG zone of Cameroon, infestation can cause up to 100% grain damage and up to 21% loss in grain weight within four months storage [11]. The control of this pest is then crucial to the increased and sustainable production of Bambara groundnut in tropical Africa.

Since the 1950s, synthetic insecticides are widely available and have been used extensively to fight against stored product insect pests [12]. Nevertheless, their indiscriminate use has resulted in the development of resistance by pest resurgence and outbreak of new pests, toxicity to non-target organisms and hazardous effects on the environment endangering the sustainability of ecosystems [13,14].

In order to reduce both over-dependence on chemicals for control, and seed loss due to bruchid attacks, the search for host plant resistance in Bambara groundnut seeds may be the option of choice. In fact, a plant with resistance offers an attractive alternative pest management approach because it provides an efficient, economical and environmentally responsible approach for effectively managing pests. Hence, it is pertinent that a study of bruchid responses to locally available improved varieties and landraces of different pulse species be conducted periodically in different ecologies. Studies have been reported on the susceptibility of Bambara groundnut [8,9,15] varieties to *C. maculatus*. However, the resistance status of several varieties of these pulse species in Cameroon, is either unknown within an agro-ecological zone and / or across agro-ecological zones in the country. In fact, a resistant variety to insect attack in one locality may be susceptible in another due to the interaction between the genotype and the environment as well as the reproductive performance of the pest in a given environment [16-18]. The physical and chemical basis of resistance of available Bambara groundnut varieties in Cameroon are also not yet elucidated.

Study was then undertaken to evaluate the susceptibility of ten varieties of Bambara groundnut to infestation by *C. maculatus* with the aim to selecting those with high nutritional value and inherent resistance for cultivation or inclusion in breeding programs in SS and SG zones of Cameroon.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Bambara Groundnut Varieties

Bambara groundnut varieties (Fig. 1) used in these experiments consisted of 10 local widely cultivated lines. Seeds were collected from farmers in Far North Region of Cameroon and were identified and named at Department of Annual Crops, IRAD (Institute of Agricultural Research for Development) station of Maroua, Far North Region of Cameroon on the basis of their locality of production, local name, and testa or eyed colour. According to these parameters the adapted names are given in Table 1 and Fig. 1.

The seeds were cleaned by removing manually all foreign matter such as dust, dirt and chaff as well as immature and damaged seeds and then disinfested by keeping them in a freezer at -4°C for 21 days prior to the bio-assays, in order to eliminate the potential forms of any living organism. The seeds were then kept under laboratories conditions (Ngaoundere: 22.73 ± 1.28°C and 82.75 ± 2.61% r.h., Maroua: 27.17 ± 2.54°C and 78.66 ± 6.40% r.h.) for at least 2 weeks before use for experiments. The moisture content of the seeds was preliminary determined by drying at 105°C for 24 h in oven [19].

2.2 Insect Rearing

The research work was conducted at Ngaoundere (SG zone) and Maroua (SS zone) under laboratory conditions. The population of *C. maculatus* used in these studies originated from Mokolo, Far north, Cameroon, in December 2012. At each site of study 250 g of cowpea (Vya Moutourwa) and then 30 *C. maculatus* adults were introduced in glass jars (900 ml) for the insect rearing. The adults were removed after one-week ovipositional period by sieving the

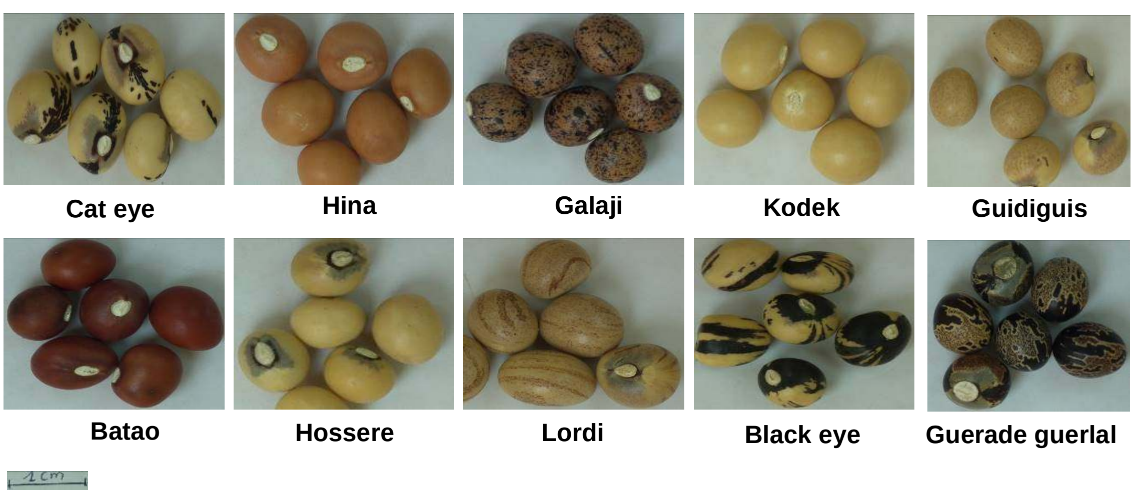

Fig. 1. Bambara groundnut varieties collected from farmers in Far North Region of Cameroon

grains through a 3-mm mesh sieve to separate the grains from the cowpea weevils, after which the grains were returned into the jars. The glass jars containing the infested cowpea (eggs and larvae) were kept to establish the insect colony used for experiment. Seeds were renewed after four generations of *C. maculatus* and infested with adults from different jars to avoid cost of inbreeding [20] and polymorphism induction in culture [21], since only the flightless forms were used in our study.

2.3 Assessment of the Degree of Resistance to the Pest

Experiments were conducted in ambient laboratories conditions at Maroua (SS zone) and Ngaoundere (SG zone). The averages temperature were 28.22 ± 2.10℃ and 23.00 ± 1.37℃ with respective ranges of 22.00 – 34.00℃ and 20.00 – 27.00℃, respectively in Maroua and Ngaoundere. In the same order the relative humidity (r.h.) averages were 77.62 ± 8.72% and 81 ± 2.31% with respective ranges of 41.00 – 94.5% and 70.00–88.00%.

Fifty grams seeds of each variety were individually placed in small glass jar (500 ml) before ten pairs (♂/♀) of newly emerged (not more than 24 h old) adults of *C. maculatus* were introduced. The jars were then covered with gauze cloth and perforated metal lids to allow the weevils to breathe and avoid any external contamination. Six days after infestation, the adults were removed, while observations for emergence started 3 weeks after infestation. The grains were observed daily for adult emergence till the end. The experiment was arranged in a completely randomized design (CRD) with five replications. The SI is given by the formula [22]:

$$\mathrm{SI} = \frac{LogF1}{MDP}$$

where F1 was the total number of emerging adults and MDP was the median developmental period (in day), which was calculated as the time from mid-oviposition period to 50% emergence of F1 generation.

The Dobie SI was used as the criterion to separate varieties into different resistance groups [23] following the scales as follows: scale index of ≤ 4 was classified as resistant; scale index of 4.1 − 6.0 as moderately resistant; scale index of 6.1 − 8.0 as moderately susceptible; scale index of 8.1 − 10 as susceptible; and scale index of > 10 was classified as highly susceptible.

2.4 Evaluation of Grain Physico-chemical Characteristics

Kernel weight (g per 100 kernels) was measured by selecting randomly 100 seeds and weighing them in an electronic balance (Kern&Sohn GmbH,D-72336 Balingen, Germany) to an accuracy of 0.001 g [24]. The length, width and thickness of each variety were determined using a vernier caliper with 0.05 mm accuracy. Fifty grains were randomly selected from each sample of about 2500 seeds. The three principal dimensions of the selected grains were measured and their averages taken. The average diameter (Dg) [25], surface area (S) [26], the sphericity (Sp) [27] and grain volume [28] were determined. The curvature, smoothness, and color of the seeds were determined on a visual basis.

Proximate analyses were performed on two resistant, two moderately susceptible and two susceptible varieties under SG condition. The moisture and crush ash contents of the seeds were determined according to AFNOR methods [19,29]. Carbohydrates and soluble sugars [30], total proteins [31,32], lipids content [33], crude fiber content [34], total polyphenolics and saponins content were quantified [35]. Phytates, tannins, flavonoids, alkaloids, steroids and triterpenoids were screened by using qualitative [36].

2.5 Data Analysis

All data collected were subjected to analysis of variance (ANOVA) procedures [37]. In order to ensure assumptions of ANOVA before analysis, data on adult emergence, mean developmental period and index of susceptibility were tested for normality and heterogeneity of variance. The number of F_1 progenies was log transformed. Then, the transformed and non-transformed data were analyzed using one-way ANOVA. Tukey standardized "Honestly Significant Difference" (HSD) tests were used to differentiate statistically different means at 5% level of significance.

3. RESULTS

3.1 Degree of Resistance to the Pest

Bambara groundnut varieties showed significant difference (P < 0.001) in the number of weevil infestation in each agro-ecological zone (Table 1). The number of adults which emerged range from 2 to 83 in SS zone ($F_{9,40}$ = 201.71) and from 0 to 41 in SG zone ($F_{9,40}$ = 97.14). In the two agro-ecological zones Kodek was the highest infested followed by Hossere (47 emerged adults) in SS zone and Batao (28 emerged adults) in SG zone. Galaji and Black eye were the least infested varieties in SS zone whereas no adult emergence was recorded on Guerade guerlal in SG zone. The ability of *C. maculatus* to produce its progeny on Kodek, Batao, Hossere and Guerade guerlal was strongly influenced by environment (t = 6.06 – 12.20; P < 0.001), while the same statistically performance was recorded on Black eye, Cat eye, Lordi and Guidiguis (t = 0 – 1.60; P = 0.1532 – 1). Kodek, Hossere, Guerade guerlal and Hina varieties recorded respectively higher number of adult emergence in SS zone than in SG zone, while Batao and Galaji varieties recorded respectively the higher number in SG. But overall, means were positively and significantly correlated within environment (r = 0.71, P < 0.05).

Table 1. Mean adult emergence of *Callosobruchus maculatus* infested Bambara groundnut

Variety	Mean number of emerged adult (± S.E.)		*t*
	Sites		
	Maroua	Ngaoundere	
Batao	9 ± 0^{cd}	28 ± 2^{b}	8.32^{***}
Black eye	3 ± 0^{d}	3 ± 0^{de}	00.00^{ns}
Cat eye	7 ± 1^{cd}	7 ± 1^{cd}	0.60^{ns}
Galaji	2 ± 1^{d}	6 ± 1^{de}	5.08^{**}
Guidiguis	15 ± 1^{c}	13 ± 1^{c}	1.60^{ns}
Hina	12 ± 1^{c}	8 ± 1^{cd}	2.51^{*}
Hossere	47 ± 5^{b}	8 ± 1^{cd}	8.25^{***}
Kodek	83 ± 2^{a}	41 ± 3^{a}	12.20^{***}
Lordi	8 ± 1^{cd}	9 ± 1^{cd}	0.75^{ns}
GG	8 ± 1^{cd}	0 ± 0^{e}	6.08^{***}
Mean	19 ± 4	12 ± 2	1.85^{ns}
F value	201.71^{***}	97.14^{***}	-
CV (%)	20.94	23.23	-

*^{ns}: P > 0.05 ; * : P < 0.05 ; ** : P < 0.01 ; *** : P < 0.001*

Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey test

Gg = Guerade guerlal

Analysis of the results (Table 2) showed significant difference among Bambara groundnut varieties with respect to mean developmental period ($F_{9,40}$ = 21.88 – 966.15; P < 0.001). The highest developmental period was recorded on Lordi (33.80 days) and on Black eye (52.60 days) respectively under SS and SG zones, while the lowest was observed on Cat eye (28.40 days) and Guidiguis (45.60 days) respectively in the same order. *C. maculatus* developmental period was strongly influence by environment for each Bambara groundnut variety. Data analysis using t-test applied to compare means with respect to developmental period among the sites of the study varied from 16.74 to 81.00 at 5% level of probability. The increase of developmental period of weevils from one zone to another was not proportional and insignificantly correlated (r = 0.55; P = 0.0977). For example, Black eye and Galaji recorded respectively 31.6 and 33.40 days in SS zone, while 52.60 and 47.00 days were recorded in SG zone.

The SI of the 10 Bambara groundnut varieties ranged from 0.84 for Galaji to 14.73 for Kodek in SS and from 0.00 for Guerade guerlal to 8.00 for Kodek in SG and there were significant difference among them ($F_{9,40}$ = 107.02 and 152.59, respectively at Maroua and Ngaoundere; P < 0.001) (Table 3). Overall, SIs were higher in SS zone than in SG zone (t = 5.09; P < 0.001), but it almost varied randomly from one site to another and thus correlation between means among the sites (r = 0.51) was not significant (P = 0.1304). For example SI (12.03) for Hossere was higher than SI (8.64) for Guidiguis in SS zone, whereas SI (5.53) for the latter was higher than that for the former (4.16) in SG zone; SI for Kodek was the highest in both two agro-ecological zones, while SI for Batao in SS (6.57) and SG (6.77) was not significantly different (t = 0.83; P = 0.4282). Only two varieties (Galaji and Black eye) were regarded as resistant to insect attack in SS zone, whereas, in addition to these two resistant varieties Guerade guerlal was regarded as the most resistant in SG zone. While 50% of the varieties (Lordi, Batao, Guerade guerlal, Cat eye and Hina) tested were regarded as moderately susceptible in SS zone, 50% including Cat eye, Hossere, Hina, Lordi and Guidiguis were regarded as moderately resistant in SG zone. Hossere and Kodek were regarded as highly susceptible in SS, whereas Batao and Kodek were regarded respectively as moderately resistant and moderately susceptible to cowpea weevil attack in SG zone.

3.2 Physical Properties of Bambara Groundnut Varieties Harvested from Different Regions of Northern Cameroon

For all parameters studied there were statistical significant differences between varieties (F =

9.05 – 4758.05; $P < 0.001$). The average values of geometric diameters, sphericities, volumes and geometric surface area were established (Table 4) and varied from 9.14 ± 0.10 to 13.30 ± 0.10 mm, 0.87 ± 0.00 to 0.93 ± 0.00, 406.83 ± 12.83 to 1245.43 ± 27.90 mm^3 and 242.59 ± 4.83 to 502.51 mm^2, respectively. The hundred mass varied from 45.94 ± 0.22 to 151.66 ± 0.84 g. Lordi had so far the higher value for each parameter except sphericity (the least spherical variety) than other varieties. The least heavy, shorter diameter, small volume and surface area varieties were Hina followed by Hossere and Galaji. Seed testa and eye colours were also other variable traits in Bambara groundnut harvested in northern Cameroon (Table 5).

Table 2. Mean developmental period of *Callosobruchus maculatus* infested Bambara groundnut

Variety	Mean developmental period (day ± S.E.)		*t*
	Sites		
	Maroua	Ngaoundere	
Batao	33.00 ± 0.00^{abc}	48.80 ± 0.20^{bc}	79.00***
Black eye	31.60 ± 0.40^{bcd}	52.60 ± 1.03^{a}	19.01***
Cat eye	28.40 ± 0.40^{e}	47.00 ± 0.00^{cde}	46.50***
Galaji	33.40 ± 0.51^{ab}	47.00 ± 0.63^{cde}	16.74***
Guidiguis	31.20 ± 0.20^{cd}	45.60 ± 0.40^{e}	32.20***
Hina	31.80 ± 0.37^{bcd}	50.00 ± 0.63^{b}	24.77***
Hossere	31.80 ± 0.37^{bcd}	48.00 ± 0.45^{bcd}	27.78***
Kodek	30.00 ± 0.00^{de}	46.20 ± 0.20^{de}	81.00***
Lordi	33.80 ± 0.66^{a}	49.60 ± 0.40^{b}	20.40***
GG	28.60 ± 0.51^{e}	£	-
Mean	31.36 ± 0.28	48.31 ± 1.72	5.74***
F value	21.88***	966.15***	-
CV (%)	2.85	2.55	-

*^{ns}: P > 0.05 ; * : P < 0.05 ; ** : P < 0.01 ; *** : P < 0.001*

Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey test

Gg = Guerade guerlal; £ = no developmental period was recorded due to no adult emergence

Table 3. Index of susceptibility of *Callosobruchus maculatus* infested Bambara groundnut varieties

Variety	Index of susceptibility (± S.E.)		*t*
	Sites		
	Maroua	Ngaoundere	
Batao	6.57± 0.18^{dMS}	6.77 ± 0.17^{bMS}	0.83^{ns}
Black eye	3.84 ± 0.23^{eRR}	2.32 ± 0.16^{eRR}	5.43***
Cat eye	6.99 ± 0.31^{cdMS}	4.03 ± 0.23^{dRR}	7.67***
Galaji	0.84 ± 0.84^{fRR}	3.63 ± 0.19^{dRR}	3.23*
Guidiguis	8.64 ± 0.26^{cSS}	5.53 ± 0.12^{cMR}	10.82***
Hina	7.80 ± 0.25^{cdMS}	4.21 ± 0.19^{dMR}	11.42***
Hossere	12.03 ± 0.22^{bVS}	4.16 ± 0.28^{dMR}	21.89***
Kodek	14.73 ± 0.09^{aVS}	8.00 ± 0.09^{aMS}	51.37***
Lordi	6.11 ± 0.27^{dMS}	4.36 ± 0.21^{dMR}	5.06***
GG	6.88 ± 0.50^{cdMS}	0.00 ± 0.00^{fRR}	13.78***
Mean	7.44 ± 0.54	4.30 ± 0.31	5.09***
F value	107.02***	152.59***	-
CV (%)	11.26	9.34	-

*^{ns}: P > 0.05 ; * : P < 0.05 ; ** : P < 0.01 ; *** : P < 0.001*

Means in a column followed by the same lower case letter (s) do not differ significantly at the 5% level by Tukey test

Gg = Guerade guerlal; RR= resistant, MR = moderately resistant; MS = moderately susceptible; SS = susceptible; VS = very susceptible

Table 4. Physical properties (mean ± SE) of ten Bambara groundnut varieties tested for their reaction to *Callosobruchus maculatus* attack

SSt	Variety	Parameters				
		100 GM (g)	GD (mm)	D.Sph.	Vol. (mm3)	GS (mm2)
RR	GG	88.23±0.22^{b}	11.18±0.14^{b}	0.90±0.01^{bc}	748.60±26.29^{b}	363.51±8.80^{b}
	Black eye	58.10±0.08^{fg}	9.81±0.14^{cd}	0.91±0.00^{ab}	511.82±23.20^{cd}	284.12±8.79^{cd}
	Galaji	48.76±0.08^{h}	9.81±0.09^{cd}	0.90±0.01^{bc}	501.58±14.23^{cd}	279.66±5.44^{cd}
	Cat eye	63.88±0.33^{e}	9.86±0.11^{cd}	0.90±0.01^{bc}	512.58±17.84^{cd}	282.98±6.70^{cd}
MS	Hossere	52.92±0.49^{f}	9.39±0.11^{de}	0.91±0.01^{ab}	442.72±15.35^{de}	259.14±6.00^{de}
	Lordi	151.66±0.84^{a}	13.30±0.10^{a}	0.87±0.00^{d}	1245.43±27.90^{a}	502.51±7.77^{a}
	Hina	45.94±0.22^{i}	9.14±0.10^{e}	0.90±0.01^{bc}	406.83±12.83^{e}	242.59±4.83^{e}
	Guidiguis	82.11±1.04^{c}	11.15±0.11^{b}	0.91±0.01^{ab}	735.27±21.71^{b}	363.82±6.84^{b}
SS	Batao	56.35±0.37^{g}	10.09±0.12^{c}	0.88±0.01^{cd}	548.36±18.90^{c}	293.12±6.58^{c}
VS	Kodek	69.61±0.49^{d}	11.51±0.09^{b}	0.93±0.00^{a}	805.58±18.68^{b}	393.02±5.60^{b}
	F	3634.03***	128.31***	9.05***	153.70***	134.02***

****: P < 0.001*

Means within the column followed by the same small letter ('s) did not differ significantly at 5% level by Tukey test.

SSt: susceptibility status, GM: grain mass, GD: geometric diameter, D.Sph.: degree of sphericity, Vol.: volume, GA: geometric surface, RR: resistant, MS: moderately susceptible, SS: susceptible, VS: very susceptible, GG: Guerade guerlal

Table 5. Origin, locality of production and seed colour of ten Bambara groundnut varieties

Variety	Origin	Locality of production	Seed colour	Eyed colour
Kodek	Kossehone market, Mayo Tsanaga, Far North Region	Northern Cameroon	Cream	White
Guidiguis	Guidiguis	Far North and North Regions	Cream with beige red spots	White surrounded by a beige red band
Hossere	Kossehone market	Far North and North Regions	Cream	White surrounded by a grey white patch
Hina	Hina market, Mayo Tsanaga, Far North Region	Far North and North Region	Salmon orange	White
Batao	Rhumsiki, Mayo Tsanaga, Far North	Far North and North Region	Mahogany brown	White
Lordi	Maroua market	Far North Region	Cream with numerous agate grey spots and longitudinal orange brown stripes	White surrounded by an agate grey band
Galaji	Mokolo market, Mayo Tsanaga, Far North Region	Far North and North Regions	Pale brown with irregular black red spots	White
Guerade guerlal	Guili	Bourha and Mogode Sub-Divisions	Agate grey with numerous black red spots and two large ones on both extremities	White surrounded by an agate grey band
Cat eye	Ngong (North Region)	Far North and North Regions	Cream with black grey spots on both extremities	White surrounded by a black grey and an agate grey bands
Black eye	Ngong (North Region)	North Region	Cream with longitudinal black grey stripes	White surrounded by black grey

3.3 Biochemical Profiles of Varieties

Table 6 showed the chemical proximate composition of Bamabara groundnut seeds. The statistical analyses showed significant differences (P < 0.001 – 0.01) for all biochemical parameters studied. Hossere was the least moistened and other varieties had the same statistical means. Galaji, Lordi and Kodek respectively the resistant, susceptible and very susceptible varieties were recorded to have lower ash content, and Black eye the most resistant variety had the higher value. The quantity of total carbohydrates was high in general and varied from 59.32 to 72.17%, respectively for Hossere and Lordi. Hossere, Kodek and Black eye were the same on their total carbohydrates basis; Lordi and Galaji constituted another group. The quantity of soluble sugar was very low except in Hossere and Black eye varieties. Lordi recorded the lower crude fiber content and other varieties had the statistical same value for this parameter. Protein content varied slightly among varieties (17.32 ± 0.03% − 18.00 ± 0.01%). Varieties were different with respect to their fat content ($F_{6,14}$ = 7370.00, P < 0.001) and values were relatively high for all varieties (6.31−7.59%) except for Hossere (2.39%).

3.4 Relationship between Physico-chemical Characteristics of Varieties and their Resistance to Insect Attack

Grain physical characteristics were not correlated with SI in both two agro-ecological zones (Table 7). Total carbohydrates and soluble sugars were negatively correlated with varietal resistance under SS conditions and total polyphenolic compounds positively correlated under SG conditions (Table 8).

Table 6. Chemical profiles (mean ± SE) of seven Bambara groundnut varieties tested for their reaction to *Callosobruchus maculatus* attack

Comp.	Varieties			
	Resistant		Moderately susceptible	
	Galaji	Black eye	Lordi	Cat eye
MC^1	10.84 ± 0.24^A	10.01 ± 0.10^{AB}	10.81 ± 0.07^A	10.53 ± 0.34A^B
As^1	3.08 ± 0.16^B	4.34 ± 0.67^A	3.10 ± 0.22^B	3.75 ± 0.03^{AB}
TS^1	69,41 ± 1,42^{AB}	60,71 ± 1,65^C	72,17 ± 0,83^A	66,97 ± 1,60^B
SS^1	1.99 ± 0.07^D	10.93 ± 0.23^A	0.56 ± 0.07^G	0.99 ± 0.10^F
CF^1	6.59 ± 0.28^A	6.63 ± 0.27^A	4.42 ± 0.28^C	6.74 ± 0.18^A
Fat^1	7.35 ± 0.08^B	7.20 ± 0.04^B	6.49 ± 0.03^C	7.59 ± 0.03^A
Pro^1	17.42 ± 0.04^C	17.32 ± 0.03^C	18.00 ± 0.01^A	17.38 ± 0.01^C
Sap^2	36.16 ± 5.46A^B	21.90 ± 2.20^C	24.06 ± 5.57^{AB}	36.45 ± 5.50^A
TPP^2	17.12 ± 0.55^C	11.08 ± 0.21^E	9.97 ± 0.74^E	15.43 ± 0.21^D

Table 6 continue

Comp.	Varieties			
	Susceptible	Very susceptible		F-value
	Guidiguis	Hossere	Kodek	
MC^1	10.59 ± 0.22^{AB}	9.05 ± 0.29^C	10.57 ± 0.09^{AB}	25.542***
As^1	3.43 ± 0.23^B	3.91 ± 0.27^{AB}	3.47 ± 0.15^B	6.568**
TS^1	55,66 ± 0,83^D	59,32 ± 1,66^{CD}	60,39 ± 2,18^C	47.236***
SS^1	5.37 ± 0.13^C	7.73 ± 0.13^B	1.51 ± 0.69^E	2936.00***
CF^1	5.19 ± 0.34^{BC}	5.97 ± 0.37^{AB}	6.00 ± 0.27^A	24.75***
Fat^1	6.31 ± 0.01^C	2.39 ± 0.05^E	6.95 ± 0.06^B	7370.00***
Pro^1	17.94 ± 0.05^A	17.60 ± 0.05^B	17.55 ± 0.03^B	182.284***
Sap^2	38.38 ± 2.77^A	36.87 ± 4.37^A	33.36 ± 7.89^{AB}	5.05**
TPP^2	23.18 ± 0.73^A	0.97 ± 0.21^F	21.30 ± 0.74^B	575.12***

**: P < 0.01; ***: P < 0.001

Means in a line followed by the same letter(s) do not differ significantly at the 5% level by Tukey test
Comp: component, MC: moisture content, As: ash, TS: total sugar, SS: soluble sugar, CF: crud fiber, Pro: protein, Sap: saponins, TPP: total polyphenolic compounds, DM: dried matter, 1: in g/100 g DM, 2: in mg/1 g DM

Table 7. Correlation between physical properties of Bambara groundnut and susceptibility index (SI) of *Callosobruchus maculatus*

SI/	Physical properties				
Loc	Grain mass	Geometric diameter	Sphericity	Volume	Surface area
SS	0.037^{ns}	0.227^{ns}	−	0.140^{ns}	0.186^{ns}
SG	-0.076^{ns}	0.150^{ns}	−	0.110^{ns}	0.144^{ns}

^{ns}; not significative; Loc, locality; SS, Sudano-Sahelian zone; SG, Sudano-Guinean zone; −, the value of correlation was not determined due to constant value of sphericity

Table 8. Correlation between biochemical constituents of Bambara groundnut and susceptibility index (SI) of *Callosobruchus maculatus*

SI/	Biochemical constituents				
Loc	MC	Ash	TC	SS	Prot
SS	-0.36^{ns}	0.11^{ns}	-0.53**	-0.47*	0.24^{ns}
SG	0.23^{ns}	-0.33^{ns}	-0.29^{ns}	-0.05^{ns}	0.34^{ns}

Table 8 continue

SI/	Biochemical constituents			
Loc	Fat	CF	Sap	TPP
SS	-0.47^{ns}	-0.21^{ns}	0.25^{ns}	-0.01^{ns}
SG	0.144^{ns}	-0.29^{ns}	0.31^{ns}	0.52*

*^{ns}: not significative; *: $P < 0.05$; **: $P < 0.01$*

Loc, locality; SS, Sudano-Sahelian zone; SG, Sudano-Guinean zone; MC, moisture content; TC, total carbohydrates; SS, soluble sugar; Prot, proteins; CF, crud fibers; Sap, saponins; TPP, total polyphenol

4. DISCUSSION

Significant differences among the ten genotypes of Bambara groundnut for seed physical properties indicated the diversity of varieties in pulse species. Variability for pulses physical properties was also reported [26,38−41]. The physical properties of grains are necessary for the design of equipment to handle, transport, processing and store the crop [42] and farmers select for a wide range of characteristics (e.g. size, color, skin texture, shape, vigor, taste). Seed index (weight of 100 seeds) directly influences the productivity and determines grain quality for commercialization and also the seed vigor [38,43]. Lordi, Guerade guerlal and Guidiguis varieties may be most valuable because of their high seed index and seed seize. In addition to their physical properties, chemical profiles of products are very important characteristics to be considered in the evaluation of food quality. The content of carbohydrates, proteins, lipid, crude fibers and ash for all the tested varieties, except the lipid content for Hossere were in the range reported [5]. Accordingly, these varieties may be used by rural farmers as supplements to cereals which are very poor in proteins and lipid. In fact, cereals are the basic diets of rural farmers in northern Cameroon as they are not able to offer themselves food not locally produced, and available legumes consumption with higher nutritional value may be necessary for their balance food.

In addition to their importance in assessment of food quality, physical and chemical properties of grains play an important role in the susceptibility to pest insect attack during storage. Thus, the suitability of legume species for infestation by its insect pest varies among the varieties [44−46] and variables such as adult emergence, developmental period and susceptibility index are the most reliable indicators for resistance of host plant to damage by its pest during storage [47,48].

The least infested Bambara groundnut varieties (i.e.: Galaji, Guerade guerlal and Black eye) termed as resistant to weevil attack may have inherent factors that result in less emerged *C. maculatus* adults than other (the very susceptible one) such as Kodek which lack these factors. The difference in number of adults that emerged were probably related to the number of eggs aborted and the number of larvae that survive to adulthood as a result of varieties variability. It has been suggested that variety that express resistance have physical or biochemical attributes that modify behavioral responses (xenobiosis) or that adversely affect development or survival of the pest insect species through metabolic aberrations (antibiosis) [44]; but in this study physical characteristic such as volume, surface area, sphericity, smoothness or curvature and color which varied among varieties did not influence the susceptibility of Bambara groundnut grains to *C. maculatus* either in SG or SS zones, in contrast to that reported for cowpea grains [48]. However, these physical characteristics are known to affect the number of eggs deposited by *Callosobruchus* spp [44,49] which may be correlated with the rate of grain infestation. The number of eggs deposited was not recorded in our study. In fact, the number of eggs laid by an insect is less important than the proportion of adults that emerged from these eggs in its influence on the rate of multiplication [50]. Hence, this factor of physical resistance may have little interest. However, the thickness and hardness of the seed coat may be partially responsible for differences among varieties with respect to the number of cowpea weevil that emerged. The seed coat, a mechanical barrier may make it difficult for eggs to adhere to the seed or prevent the larva's penetration into the seed when they are hatched [51]. The probably thick seed coat of Batao was very hard in the dry weather conditions of SS zone and this may more prevented larva's penetration into the seed than in SG zone. Thus, beyond some level of seed coat thickness and hardness it may be not possible for larva to bore into the seed cotyledon. It is also reasonable to speculate that the difference in number of *C. maculatus* observed among these varieties are perhaps due mainly to difference in their genetic or biochemical characteristics. Total carbohydrates content may interact with environmental conditions and impart resistance to *C. maculatus* in the SS zone while total polyphenolic compounds may be suitable for insect development in SG zone, in contrast to some findings reported in the literature [52−55]. Indeed, the non-digested oligosaccharides such as raffinose, stachyose and verbascose were reported to have negative effect on herbivores [56] and their quantity may be higher in resistant varieties. Correlation between the moisture content, ash content, proteins, fat and crude fibers and the infestation rate was not significant as reported by Akintola and Oyegoke [50]. In contrast positive correlation between the primary metabolites and the susceptibility of cowpea seeds to *C. maculatus* attack was reported [57]. In fact, evaluating only one or two plant seed components cannot provide an effective way of checking the degree of resistance towards storage pests [58]. Tannic acid the principal anti-nutritional fraction of total polyphenols [56] was not detected in our investigation, except in Galaji variety. The resistance of that variety either in SS or in SG agro-ecological zones may be partially attributed to this anti-nutritional compound and probably to other secondary metabolites such as steroids and triterpenoids which were abundant. There have been suggested that the presence of anti-metabolic compounds in seeds have been associated with resistance to insect attack [57,59]. Further investigations on insecticidal trypsin and α-amylase inhibitors are required to elucidate well more factors responsible for resistance of screened Bambara groundnut varieties in this study.

Under the same experimental conditions the difference in the average duration of the development time of *C. maculatus* observed may be linked to the toxic factors that may display less or more anti-mitotic activity by inhibiting the growth of cells as previously reported [60]. Anti-metabolic compounds may act in two different ways: some may display anti-mitotic activity and hence delay the time of insect pest development without killing them, while other may kill the larvae of their pest without having anti-mitotic activity. Thus, Hossere which recorded higher number of emerged adults and mean developmental period than Guerade guerlal could have anti-mitotic toxicants in its composition while the last one could have non anti-mitotic with larvicidal properties. However, other stages of infestation like eggs, larvae and nymphs were not observed in this study. Both reduction in the number of progeny production and the increase of elapse time from egg to adult emergence might increase the tolerance of a variety to the pest. The interactions between genotype and environment may result in specific or same variety responses to bruchids attack. The longer elapse time of insect development under SG zone than under SS may be explained by both differences in temperature and relative humidity between the two zones. Previous studies [17,18] indicate that temperature and relative humidity are the most important variables influencing generation times (egg to adult) when beetles are raised on preferred host beans and temperature of 35℃ is optimum for egg-laying and progeny development in *C. maculatus*. Thus, within a limited range, increasing temperature will decrease the generation time.

The susceptibility of legume seed to insect pest attack is associated to successfulness of insect growth and to elapse time from egg to adult (generation time). Variety that allows higher percentage adult emergence and short developmental period may suffer from heavy damage; this one that allows less percentage adult emergence and short generation time may suffer from moderate damage.

5. CONCLUSION

From the results of the present finding only two (Galaji and Black eye) and three (Guerade guerlal, Galaji and Black eye) varieties were resistant to bruchids attack respectively in SS zone and SG zone, of the ten varieties tested. Seed legumes suffer from heavy damage by cowpea weevil attack during storage in SS than in SG zone of Cameroon (Northern Cameroon). Nevertheless, Galaji and Black eye may be recommended for relatively longer storage to achieve the goal of long term and sustainable pest management strategies in Northern Cameroon in general.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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