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Insecticidal efficacy of *Veprisheterophylla* leaf extracts against *Callosobruchus maculatus* F. (Coleoptera:Chrysomelidae), and study of the in-vitro digestibility and microbiological of treated seeds

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ABSTRACT
The present study aims to develop an alternative method using hexane, acetone and methanol extracts of*Veprisheterophylla* leaftoprotect*Vignasubterranea* and *Vignaunguiculata*grainsagainst *Callosobruchus maculatus*. For this, toxicity tests were evaluated after 1, 3 and 6 days of exposure at four concentrations (5, 10, 15 and 20g/kg). The effect of the three extracts on F_1progeny emergence,as well as the percentage of weight losscaused by *C. maculatus*after three months of storage were assessed. After the storage period, the microbiological quality of the treated seeds was determined, and the treated seeds were subjected to the in-vitro digestibility study of proteins and carbohydrates. The results showed that, the three extracts of *V.heterophylla* caused a significant mortality of *C. maculatus*. The hexane extract was more active and causedcomplete mortality of *C. maculatus*at all the concentrations after 3 days of exposure for the two commodities.Whileat 20 g/kg, acetone extract caused a maximum of 69.7 and 92.5% of mortality on *V. subterranea* and *V. unguiculata* respectively, and in the same order methanol extract achieved 75.3 and 89.6% of mortality. A complete reduction of F_1progeny was recorded in each commodity treated with hexane extract at all the concentrations, while significant inhibition was recorded in grains treated with acetone or methanol extract. The three extracts of *V. heterophylla* leaf significantly protected Bambara groundnut and cowpea after three months of storage.Microbiological properties showed that, compared to untreated control flours, these extracts contribute to protect the treated flours against yeasts and molds. The percentage of in-vitro protein digestibility ranged from 73.94 ± 0.57 in the seeds treated with methanol extract at 20 g/kg to 75.54 ± 0.60 in untreated control, and that of carbohydrates ranged from 52.81% in treated grains with methanol at 20 g/kgto 56.70% in the untreated control. Based on the results obtained in the present study, it can be concluded that *Veprisheterophylla*leaf extracts effectively protect Bambara groundnut and cowpea in storage, and also improves microbiological properties and has no impact on the digestibility of nutrients in-vitro.

Keywords:*Veprisheterophylla*, extracts, Bambara groundnut, cowpea, storage, digestibility

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I. INTRODUCTION

The rapid population growth in sub-Saharan Africa is undermining the ability of this part of the continent to ensure a stable supply of food products, leading to an increase in the prevalence of malnutrition[1]. The cultivation of grain legumes, a source of vegetable proteins has been recognized as being one of the best and least expensive of the recommended solutions for the recovery of undernutrition in areas of observed chronic malnutrition[2;3]. This is the case of cowpea seeds [*Vignaunguiculata* (L.) Walp] and Bambara groundnut [*Vignasubterranea* (L.) Verdcourt] which indeed contain 2 to 3 times more proteins than cereals and contain the 24 amino acids essential to the human consumption [2], among others.

Nonetheless, due to the short period of production in the fields, whereas the consumption of these foodstuffs is done throughout the year, storage thus ensures their permanent availability for consumption, future seeds for planting or a capital value for small farmers.More than 75% of the postharvest products intended to storage facilities[4]. Unfortunately, in the septemtrional zones, these storage structures are mostly rudimentary[5]and, expose the stored foodstuffs to the wide range ofinsect pests[6].Among these pests, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae) is known to be one of the most destructive of seed legumes in storage and can able to cause a weight loss ranging between 80 to 90% in one year of storage[7]. The extent of the damage caused by this pest to stored seed legumes remains high and forces farmers to resort to several control methods[8]. The use of synthetic insecticides or fumigants is thereforethe most widely used methods to control these pests during the storage period [9].Although the effectivenessthese synthetic insecticides as protectant, they have many adverse effects which should limit their use. These relate in particular to the eco-toxicological effects on the environment, on consumer health, and the development of strains of insects resistant to these insecticides. The search for new molecules, taking into account criteria other than efficiency only, therefore becomes a major concern of the present time to try to overcome the challenge of protecting crops against pests [10].Several studies have been developedthese last years to isolate and identify secondary metabolites extracted from plants responsible of the anti-insect activity[11]. In Cameroon, recent researches carried out on the insecticidal evaluation of secondary metabolites extracted from plants based on organic solvents has proven their insecticidal effects against *C. maculatus*[12;13].However, there are a wide range plants in the Cameroonian flora whose secondary metabolites may also be effective against *C. maculatus*. This is the case of the leaves from*Veprisheterophylla* which is an importantplant specieand has already demonstrated its efficacyin traditional medicine because its leaves are used to treat kidney disease, malaria, arterial hypertension, conjunctivitis, abscess, rheumatism,antihelminth, antibacterial activity[14].Ngamo*et al.*[15] had classified the essential oil of *V. heterophylla* among the species which have a greatest potential in term of protection of crops. In addition, the protection of seeds with insecticidal products based on metabolites extracted from plants could not only modify the nutritional quality of derived products, but also improve the microbiological properties of derived flours. Collilaw[16] reported that the secondary metabolites of plants such as total phenolic compounds, tannins, alkaloids interact with certain nutrients thus causing their complexation and therefore their non-assimilation by organization.Indeed, according to IFN[17], tannins, which are thermostable phenolic compounds, have the property of combining with proteins to form complexes and therefore indigestible. These phenolic compounds bind to both food proteins and digestive fluids, making them inactive [19]. They result nor only in a sharp drop in digestibility, especially of proteins but also of starch and therefore a drop in the available energy of the food [20]. It would therefore be important in view of the foregoing to evaluate the anti-insect activity of extracts from *V. hetroplylla*against *C. maculatus* and their impacts on the nutritional and microbiological quality of the derived products. That is the reason why, the objective of the present study aimed toevaluate the insecticidal activity of extracts from*Veprisheterophylla* leafagainst *Callosobruchus maculatus* in stored*Vignasubterranea* and *Vignaunguiculata*grains.

II. MATERIALS AND METHODS

Insecticidal material: collection and processing of *Veprisheterophylla*

The young leaves of *V.heterophylla* were collected in May 2020 at Goldolalocality (longitude: 13°48'East, latitude: 10°43'North), Far-North region, Cameroon. This locality was characterized by a Sahelo-Sudanese climate with two seasons: a short rainy season ranging from May to September and a long dry season extending from October to April. Once harvested, the leaves were air-dried in the shade in the ambient laboratory conditions for three weeks, then crushed in a porcelain mortar and sieved using a 0.8 mm mesh size stainless steel sieve. The powder obtained was stored in a glass jar and then kept in a refrigerator at 4°C until needed for extraction with solvents.

Extraction of active compounds and Phytochemical analysis of each extract

The extraction was carried out by the soaking method[21]. For that, 1000 g of powder plant was introduced into a 5 L glass jar containing three liters of hexane. The mixture thus obtained was well stirred and left under ambient laboratory conditions, then stirred again two timeper day (morning and evening) for three consecutive days. After 3 days, the solvent was removed by filtration using Wattman N^{o}1 filter paper. The filtrate obtained was then concentrated using a rotary evaporator (BÜCHI R-124). The same procedure was repeated three times with the same solvent to obtain a maximum of the hexane extract. The pasteleftwas air-dried and the same protocol as for hexane extract was

followed to obtain the acetone and methanol extracts respectively. The three extracts obtained were stored in the refrigerator at 4°C until needed for the experiments.

Phytochemical tests

The main phytochemical groups evaluated were: alkaloids according to the method used byBidie*et al*.[22], total polyphenols according to the method used byN'Guessan*et al*.[23], flavonoids according to the method used by Debray*et al*.[24],saponosides according to the method used byDohou*et al*.[25], steroids, anthocyanins, tannins, coumarins, sterols and terpenes according to the method used by Fankam*et al*.[26].

Commodities

Bambara groundnut and cowpea grains used were purchased from farmers in the local markets of Maroua (Far-North region, Cameroon). The difference between the commodities was based on the color of the seed coat, the shape and weight of the grains, the appearance of the hilum. Broken grains, particles, sand, etc. were removed and then disinfested by keeping the grains in a freezer at -20°C for 20 days [10]. The grains commodities were then kept under room temperature (32.5°C) in the laboratory for acclimation duringtwo weeks before use.

Insect rearing

The strain of *Callosobruchus maculatus* used were obtained from the existing culture maintainedin the Applied Zoology laboratory, Department of biological Sciences, Faculty of Science, The University of Ngaoundere, Cameroon. The infested grains were sieved and the adults of *C. maculatus* obtained were reintroduced into 900 ml glass jars containing 400g of sterilized Bambara groundnut or cowpea grains. These jars were stored under ambient laboratory conditions (t ≈ 25.13 ± 3.03°C; r.h. ≈ 66.46 ± 10.12%). Seven days after infestation, the insects were removed and the infested grains were allowed to incubate until the emergence of adults. After emergence, sieves were carried out and the weevils used for the bioassays were not more than 24 hours old.

Effect of each extracts on the mortality of *Callosobruchus maculatus* on *Vignasubterranea* and *Vignaunguiculata*.

Four concentrations (0.25, 0.5, 0.75 and 1 g corresponding to 5, 10, 15 and 20 g/kg) were prepared by dissolving each extract in its original extraction solvent.The concentration was introduced individually into 250 ml glass jars each containing 50g of *V.subterranea*or*V.unguiculata*. Untreated controls were consisted in 50g of Bambara groundnut or cowpea grains treated only with 1ml of each corresponding pure solvent. The glass jars were then shaken manually so that the insecticidal products adhere well to the grains, and left opened in the room temperature for two hours to allowcomplete evaporation of the solvent. After this time, 20 unsexed bruchids aged not more than 24 hours were introduced into each jar. These jars were then covered with fine cotton cloths to prevent the escaping of the weevils, and closed with perforated metal lids to allow ventilation. Each treatment was repeated four times. The count of live and dead weevils was carried out after 1, 3 and 6 days post infestation.

Effect of each extract on F_1progeny production

After mortality counted at the sixth days post infestation, all the weevils in each glass jar were removed and the treated grains and untreated control were reintroduced in the same glass jars for F_1 progeny emergence. Every week, observations were made until emergence. When the first emergence was observed, the number of weevils that emerged was notedonce a week for four consecutive weeks, and the end of emergence criterion was based on the absence of emergence for at least five consecutive days [27].

Assessment of damage caused by *Callosobruchus maculatus*after three months of storage

Four concentrations of each extract (0.25, 0.5, 0.75 and 1g corresponding to 5, 10, 15 and 20 g/kg) were preparedas for mortality test. 50 g of Bambara groundnut or cowpea grains were treated with each individual concentrationin 250 mlglass jars.Untreated control grains consisted to the each commodity grain treated with 1 mL of each corresponding solvent. Then, each glass jar was shaken manually so that the extract or solvent adhere well to the grains. Four replicates were performed for each treatment.Each glass jar was left opened for air-dried during two hours for solvent evaporation. After this time, the glass jars were infested with 20 unsexed adult insects not more than two days old. The glass jars were then covered with fine cotton cloths to prevent insects from escaping and closed with perforated metal lids for sufficient aeration, and left in the ambient laboratory conditions for three months. After this storage period, the number of dead insects and live insects were counted, undamaged and damagedseeds were counted. The undamaged and damaged seeds was separately weighed and recorded for each treatment. The evaluation of weight loss was carried out according to the method of counting and weighing used by Adams andSchulten[28]:

P% = [(U x Ne) - (Uax Ns)] / U(Ne + Ns) x100

Where U = weight of undamagedseeds; Ua = weight of damaged seeds; Ne = number of damaged seeds, Ns = number of undamaged seeds, Ne = weight of damaged seeds

Evaluation of the anti-nutritional compounds of the treated seeds.

The method of López-Mejía*et al*. [29] was used to measure the total phenolic compounds of the treated grains. The total tannins were determined according to the method used byMakkar*et al*.[30]. The aluminum chloride colorimetric technique was used for the estimation of flavonoids according to the method used by Chang *et al*.[31]. As for the alkaloids, they were quantified by the gravimetric method used by Harbone[32]. The oxalates were determined by titrimetry according to the method described by Day and Underwood[33] as reported by Adekanmi*et al*.[34]. These anti-nutritional factors were only evaluated on the methanol and acetone extracts, because after screening of each extract, only these two extracts were positive for these compounds.

Studies of the in vitro digestibility of treated seeds.

In vitro digestibility of proteins

In vitro protein digestibility was determined by the pH method using four enzymes HSU*et al*., [35]. A solution of 10 ml of sample containing 6.25 mg of protein per mL of distilled water was prepared and the pH was adjusted to 7.9 with 1 N NaOH. The resulting mixture was stirred for 1 h and the pH adjusted to 8 with 0.1 N NaOH. One mL of a solution made up of 3 enzymes (pH = 8) containing 1.6 mg of trypsin (Sigma type IX from pig pancreas), 3.1 mg of chymotrypsin (Sigma type II from beef pancreas) and 1.3 mg of peptidase (Sigma type III from pig intestine) per mL of distilled water was added to the mixture and incubated at 37°C for 10 min. After this, in the obtained mixture, 1 mL of protease, which pH was adjusted to pH = 8, and containing 7.95 mg/ml (Sigma type VI of *Streptomyces griseus*) was introduced. The mixture was then incubated at 55°C for 9 min, and then at 37°C for 1 min the pH was measured. In vitro digestibility was calculated using the regression equation established by HSU*et al*.[35]

Y = 210.46 – 18.1Xwhere: Y = digestibility,X = pH

In vitro digestibility of starch

In vitro digestibility of starch was evaluated in the presence of α-amylase pancreatic amylase at pH = 7 at 37°C during 90 min with stirring according to the method of Mbofung*et al*. [36]. Thus, 1g of flour ofeach sampletreated individually with insecticidal extract was mixed with 35mL of a solution (1.5g/l). After incubating of the preparation at 37°C in a water bath with a stirring system for 2 hours, the enzymatic activity was stopped byintroducing the tubes in boiling water (100°C). The mixture was centrifuged at 4000 rpm for 15 min. The residue was washed with 10 mL of phosphate buffer (pH = 7) and centrifuged again at 4000 rpm for 15 min. The supernatant was collected and the sugars released were assayed by the method of Fischer and Stein[37].

Determination of the microbiological profile of treated commodity grains

Preparation of samples

The preparation of the flour as well as the experiments and the use of the materials were carried out in accordance with standard NF ISO 7218 [38].

For the 3 productions that underwent different storage times, 4 glass jars were taken randomly for each treatment and their contents were mixed in a sterile container around a Bunsen burner under a hood.25 g of flour was suspended in 225g of buffered peptone water (EPT) inside a sterile 250mL Erlenmeyer flask. The stock suspension obtained was vigorously stirred and then left to stand for 30 min to ensure the survival of all the microorganisms. Then, a series of dilutions was carried out as follows: 9 ml of EPT were distributed sterilely in a series of tubes and, 1 mL of the stock suspension is transferred to tube N°1. After homogenizing, 1 mL of the contentfrom tube N°1 using a vortex, was transferred to tube N°2 and so on to a dilution factor of 10^9.

Seeding and incubation

Total flora

The method used for the enumeration of the total mesophilic flora is that described by the standard NF V 08-051[39]. On the bottoms of the sterile petri dishes was placed 1 mL of dilution (10^{-4}, 10^{-5}, 10^{-6}). Quickly, about 15 mL of molten culture medium of Plate Count Agar (PCA) brought to the temperature of 45 to 50°C were poured (deep culture). After thoroughly mixing the inoculum into the medium, the petri dishes were placed on a cold horizontal surface to solidify the medium. Incubation was done with overturnedpetri dishes (upside down), for 72 hours at 37°C.

Total coliforms

The count of total coliforms was carried out according to standard NF-V 08 060[40].The inoculation of 1 mL of the inoculum is done with a 10^{-3} and 10^{-4} dilution. Once the inoculum has been deposited at the bottom of a petri dish, approximately 15ml of the EMB medium is poured.The mixture is then homogenized and allowed to cool. EMB medium is used in a double

layer. Once the second layer has become solid, the petri dishes are turned over. Incubation was performed at 44°C for 24 hours.

Yeasts and molds

The enumeration of yeasts and molds was carried out according to standard NF-V 08-022. The inoculum 0.1mL is inoculated with a 10^{-4} and 10^{-5} dilution. Once the inoculum has been deposited at the bottom of a petri dish, approximately 15 ml of the medium is poured and the mixture is then homogenized before being allowed to cool. The medium used is SABOURAUD with the addition of chloramphenicol, the latter inhibits the growth of bacteria. The petri dishes are inverted and then incubated for 5 days at 30°C.

Statistical analyzes

Data on percent mortality, percent F_1 progeny reduction of *Callosobruchus maculatus*and percentage of seeds weight loss were transformed into arcsin√ (x / 100). Abbott's formula Abbott[41] was used to correct for mortality from control prior to application of ANOVA. These transformed data were subjected to the analysis of variance procedure (ANOVA) using the statistical analysis system [42]. For the means separation of all the parameters evaluated, the Tukey test was used.

III. RESULTS AND DISCUSSION

Phytochemical screening of the three plant extracts

Table 1 below shows the results of the phytochemical screening carried out on the crude extracts with hexane, acetone and methanol solvent of *Veprisheterophylla*. This screening revealed the presence of several compounds among which alkaloids, triterpenes, sterols, flavonoids, phenolic compounds, sterols and coumarins. A remarkable presence of triterpenes, steroids and saponins was observed in the hexane extract compared to the acetone and methanol extracts. However, in the acetone and methanol extracts, a significant presence of total phenolic compounds, flavonoids, tannins, alkaloids and saponins was noticed. Apart from the methanol extract, the other two extracts contain steroids. Likewise, the presence of triterpenes was noted in the three extracts. These results are similar to those obtained by Ntchapda*et al*.[43], who noted in the methanolic extract of *V. heterophylla* the presence of alkaloids, polyphenols and flavonoids,according tothe results of the phytochemical screening carried out, and are contradictory to those obtained by Momeni*et al*.[44], who obtained only the presence of phenolic compounds and tannins in the methanolic extract of *V. heterophylla*.

Table 1: Phytochemical screening of extracts of *Veprisheterophylla*

Compounds	Extracts		
	Hexane	**Acetone**	**Methanol**
Total phenoliccompounds	-	++	++
Flavonoids	-	++	+
Tannins	-	+	+
Coumarins	-	+	-
Alcaloids	-	+	+
Triterpenoids	++	+	+
Steroids	++	+	-
Saponins	++	+	+

+++: more abundant, ++: abundant, +: positive, -: negative

Adult mortality

The results obtained are presented in Table 2 and 3. These tables showed that the three extracts of *Veprisheterophylla* leaf caused significantly mortality of *C. maculatus* adults compared to the untreated control. This mortality varied not only according to the type of extracts, but increased with the increase in the concentration of each extract ($F = 1735.38$; $P < 0.0001$) and with exposure period. ($F = 109.85$; $P<0.0001$) (Table 2). In general, the hexane extract showed a highest mortality of *C. maculatus* compared to the acetone and methanol extracts. However, there was no significant difference between the methanol extract and the acetone extract and the highest mortality rates were recorded on *V.subterranea* than on *V.unguiculata*. At 1 day of exposure and at the lowerconcentration of 5g/kg, the least mortality ratesrecorded were respectively 97.5% (*V.subterranea*) and 80.0% (*Vignaunguiculata*) treated with hexane extract, 1.2% *V. subterranea* and*V.unguiculata*treated with methanol extract and 2.5% (*V.unguiculata*) and

1.0% (*V.subterranea*) treated with acetone extract. At the same concentration and at 6 days of exposure, 39.3% adult mortality rates was recorded in *V.subterranea*and 29.9% in *V. unguiculata*treated with acetone extract,29.0% in *V. subterranea*and 27.6% in*V.unguiculata*treated with methanol extract.From the concentration of 10 g/kg and 3 days of exposure, complete mortality rates were recorded in the Bambara groundnut and cowpea grains treated with hexane extract. The maximum mortality ratewere 69.7% (*V. subterranea*) and 92.5% (*V.unguiculata*) at the highestconcentration of 20 g/kg and at 6 days of exposure when the commodities were treated with acetone extract,whilewith methanol extract, 75.3% (*V.subterranea*) and 89.6% (*V.unguiculata*) mortality were recorded at the same concentration and exposure period (Table 3). Lethal concentration 50 (LC_{50}) values vary according to the extract, and decrease with increasing of exposure period (Table 3). The mortality recorded for the different extracts would be due to the presence in these extracts of secondary metabolites. The nature of the compounds present in each extract do not depends only on the solubility of these molecules present in the plant powder, but also on the level of polarity of the solvent used[45].According to Agnès*et al*.[11], the secondary metabolites of plants are involved in various processes, including insecticidal properties. The higher mortality of *C. maculatus*observed with the hexane extract than methanol or acetone extracts could be explained by the fact that the hexane extract contains more triterpenes than the other two extracts. Indeed, the alkaloids, saponins, steroids, phenolic compounds and tannins present in acetone and methanol extracts act much more like antinutrients thus preventing the assimilation of nutrients[46], while the terpene compounds contained in the hexane extract act on the nervous system by disrupting the exchange of sodium and potassium ions, thus leading to the direct death of the insect by contact[47; 48]. This difference in mortality could also be explained by the feeding behavior of *C. maculatus*. In fact, adults of *C. maculatus* do not feed and are therefore not directly influenced by anti-nutritional factors but rather by substances which directly induce their toxicity by contact. The increase mortality with the increasing concentration and the exposure period could be explained by the increase in the quantity of active ingredients responsible of the insecticidal activity. Our results are similar to those obtained by Agnès*et al*.[11], Daniel *et al*.[49] who obtained a high mortality of *C. maculatus* with increasing concentration and period of exposure. Likewise the work carried out by Mahama*et al*.[12]on *C. maculatus*and Fotso *et al*.[50]on *Sitophilus zeamais*revealed a high mortality recorded in the cowpea and maize grains when treated with plant extracts. Adeniyi*et al*.[51] on the contrary, obtained a high mortality of *Acanthoscelidesobtectus*withethanolic extracts. These results could be explained by the feeding behavior of the pest which directly consumes the food.

Table 2: Analysis of variance of the main factors and their interactions for the mortality of *Callosobruchus maculatus* on *Vignaunguiculata* and *Vignasubterranea*

Source	**Df**	**F Values**	**P**
Commodity	1	23.66.50	< 0.0001
Product	9	23889.2	< 0.0001
Day after infestation (DAI)	2	23399.5	< 0.0001
Concentration	5	61235.4	< 0.0001
Product x DAI	18	620.42	< 0.0001
Product x Concentration	36	1735.38	< 0.0001
DAI x Concentration	8	1444.15	< 0.0001
Product x DAI x Concentration	72	109.85	< 0.0001
Variety x product x DAI x Concentration	2266	7.95	< 0.0001

Table 3: Mortality and LC_{50} of *Callosobruchus maculatus* due to each *Veprisheterophylla*leaf extracts on *Vignasubterranea* and *Vignaunguiculata*

Contents g/kg	***V. heterophylla* hexane extract / *V. subterranea***				***V. heterophylla* hexane extract / *V. unguiculata***			
	1 jour	3jours	6 jours	*F*	1 jour	3jours	6 jours	F values
0	0.00 ± 0.00^{bA}	0.0 ± 0.00^{bA}	0.0 ± 0.00^{bA}	-	0.0 ± 0.00^{c}	0.00 ± 0.00^{b}	0.0 ± 0.00^{b}	
5	97.50 ± 1.44^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	3.00^{ns}	80.0 ± 2.04^{bB}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	96.0***
10	100.00 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	-	93.8 ± 3.75^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	2.8*
15	100.00 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	-	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	-
20	100.00 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	-	100.0 ± 0.00^{A}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	-
F values	4743.00***	∞***	∞***		73.1***	421.2***	283.4***	
DL_{50}	0.14(0.08-0.18)				0.19	-	-	
	V. heterophylla* acétone extract / *V. subterranea				***V. heterophylla* acetone extract / *V. unguiculata***			
0	0.0 ± 0.00^{d}	0.0 ± 0.00^{e}	0.0 ± 0.00^{e}	126.2***	0.00 ± 0.00^{cA}	0.00 ± 0.00^{eA}	0.0 ± 0.00^{eA}	-
5	1.2 ± 1.25^{dB}	32.9 ± 0.82^{dA}	39.3 ± 2.76^{dA}	85.5***	1.2 ± 1.25^{cC}	12.8 ± 2.42^{dB}	29.5 ± 0.71^{dA}	76.4***
10	10.0 ± 2.04^{cB}	49.9 ± 2.41^{cA}	50.0 ± 1.20^{cA}	111.1***	11.2 ± 2.39^{bC}	25.5 ± 3.93^{cB}	52.8 ± 3.24^{cA}	42.1***
15	18.8 ± 1.25^{bB}	56.6 ± 0.76^{bA}	$60.6\pm1.88b^{A}$	280.7***	18.8 ± 1.25^{abC}	40.9 ± 3.36^{bB}	76.1 ± 5.90^{bA}	52.7***
20	27.5 ± 1.44^{aC}	64.5 ± 0.84^{aB}	69.7 ± 0.53^{aA}	515.5***	26.2 ± 3.15^{aC}	58.9 ± 2.56^{aB}	92.5 ± 2.93^{aA}	131.6***
F values	73.1***	421.2***	283.4***		33.8***	68.5***	124.5***	
DL_{50}	1.74 (1.25-3.51)	0.89 (0.77-1.07)	0.50 (0.44-0.56)		1.67(1.2-2.8)	0.53(0.4-0.6)	0.43(0.31-0.55)	
	V. heterophylla* methanol extract / *V. subterranea				***V. heterophylla* methanol extract / *V. unguiculata***			
0	0.0 ± 0.00^{cA}	0.0 ± 0.00^{cA}	0.00 ± 0.00^{dA}	-	0.0 ± 0.00^{c}	0.0 ± 0.00^{d}	0.0 ± 0.00^{e}	-
5	1.0 ± 0.00^{cB}	3.8 ± 1.25^{cB}	29.00 ± 2.44^{cA}	99.65***	2.5 ± 1.44^{cB}	12.8 ± 1.29^{cdB}	27.6 ± 5.49^{dA}	-
10	2.5 ± 1.44^{cB}	15.3 ± 1.82^{bB}	46.08 ± 1.25^{bcA}	14.97**	13.8 ± 1.25^{bB}	23.0 ± 3.01^{bcB}	45.4 ± 3.63^{cA}	33.4***
15	20.0 ± 2.04^{bB}	22.9 ± 2.08^{bB}	57.76 ± 0.80^{abA}	24.17***	23.8 ± 1.25^{aB}	31.9 ± 3.39^{abB}	70.5 ± 2.73^{bA}	91.1***
20	28.8 ± 1.25^{aB}	38.3 ± 3.91^{aB}	75.33 ± 1.61^{aA}	93.40***	28.8 ± 1.25^{aC}	42.1 ± 5.11^{aB}	89.6 ± 1.62^{aA}	101.3***
F values					33.8***	68.5***	124.5***	
DL_{50}	1.67(1.2-2.9)	1.43(1.0-2.7)	0.45(0.03-0.8)		1.28 (1.1-1.7)	1.39 (1.1-2.0)	0.52(0.44-0.60)	

Means in the same column followed by the same lower case letter do not differ significantly at P 0.05 (Tukey'sHDS test and t-test of Student). Each datum represents the mean of three replicates of 20 insects for each dosage. ns$P$> 0.05; * P < 0.05; ** P < 0.01; *** P< 0.001.

Effect of each extract on the reduction of F_1progeny emergence

All *Veprisheterophylla*leaf extracts compared to the untreated control significantly inhibited the production of *C. maculatus*F_1progeny emergence, which increased with concentration. This percentage of reduction was highest in Bambara groundnutandcowpea treated with hexane extract, which complete inhibited the *C. maculatus* F_1 progeny emergenceat all the concentration (Table 4). Complete percentage of reduction is only achieved from the concentration of 15 g/kg in the two commodities grains treated with the methanol extract and 20 g/kg in the grains treated with acetone extract.This reduction could be due not only to the anti-nutritional substances such as tannins, phytates, phenolic compounds contained in the extracts, which complexed the availability of nutrients necessary for the immature stages of *C. maculatus*, but also to the presence of substances such as terpenes which act directly on adults causing their death by contact toxicity. *V. subterranea* induced more mortality of the immature stages of *C. maculatus* compared to *V. unguiculata*treated with each plant extract used in this findings. According to Badii*et al*. [52] and, Delobel and Tran[53], these results could be explained by the negative effects that the plant has on the development and reproduction of the insect it hosts (antibiosis), or by the rejection by the insect of the species plant as a food source or egg-laying site, even in the absence of choice by the insect (antixenosis or non-preference). Others authors, on the other hand, believe that the notion of resistance of a variety is more genetic in nature. As a result, they suggest the notion of vertical resistance or genetic resistance due to a gene, and

the notion of horizontal resistance or resistance due to several genes (polygenic resistance) [54]. Similar results were obtained by Fotso *et al.*[50] who reported positive effects of *Hemizygia welwitschii* leaf extracts on *S. zeamais* F_1 progeny inhibition which were greatly inhibitedand Daniel *et al.*[49] who reported that seeds treated with extracts of *Ocimumcanum* and *Gnidiakaussiana* significantly reduced the F_1 progeny emergence of *C. maculatus*. Similar results were also obtained by Mahama*et al.*[12], Thiaw*et al.*[55] who obtained a reduction in the F_1 progeny emergence of *C. maculatus* and *Caryedon serratus* on *V.subterranea* treated with *Cassia mimosoides* extract and powders, and *C.occidentalis* and *Calotropisprocera* respectively. However the findings of Agnès*et al.*[11] showed that acetone extract of *Callistemon rigidus* was not effective in controlling the offspring of *A.obtectus*. This difference could be explained by the composition and the content of the active secondary metabolites contained in the extract.

Table 4: Effect of the three*veprisheterophylla* leaf extracts on the F_1progeny emergence

	***Vepris*Hexaneextract**		***Vepris*Acétoneextract**		***Vepris*Méthanolextract**	
			Vignasubterranea			
Concentrations (g/kg)	**Mean N° of F_1 adult**	**%Inhibition**	**Mean N° of F_1 adult**	**%Inhibition**	**Mean N° of F_1 adult**	**%Inhibition**
0	104.75±4.03^{a}	0.00±0.00^{b}	104.75±4.03^{a}	0.00±0.00^{a}	104.75±4.03^{a}	0.00±0.00^{a}
5	0.00±0.00^{b}	100.00±0.00^{a}	56.75±2.98^{b}	45.92±0.85^{a}	16.50±1.44^{b}	85.78±0.70^{a}
10	0.00±0.00^{b}	100.00±0.00^{a}	12.50±1.50^{c}	88.10±1.22^{b}	6.00±0.41^{b}	94.64±0.83^{b}
15	0.00±0.00^{b}	100.00±0.00^{a}	3.00±0.30^{cd}	97.27±2.73^{c}	0.00±0.00^{b}	100.00±0.00^{c}
20	0.00±0.00^{b}	100.00±0.00^{a}	0.00±0.00^{d}	100.00±0.00^{d}	0.00±0.00^{b}	100.00±0.00^{d}
F values	**447.51*****	**481.39*****	**278.10*****	**956.42*****	**54.94*****	**7868.43*****
			Vignaunguiculata			
0	43.75±1.11^{a}	0.00±0.00^{b}	43.75±1.11^{a}	0.00±0.00^{a}	43.75±1.11^{a}	0.00±0.00^{a}
5	0.00±0.00^{b}	100.00±0.00^{a}	16.50±1.04^{b}	62.36±1.67^{a}	8.00±0.71^{b}	81.74±1.44^{a}
10	0.00±0.00^{b}	100.00±0.00^{a}	7.00±0.41^{c}	83.94±1.20^{b}	0.00±0.00^{c}	100.00±0.00^{a}
15	0.00±0.00^{b}	100.00±0.00^{a}	0.00±0.00^{d}	100.00±0.00^{c}	0.00±0.00^{c}	100.00±0.00^{b}
20	0.00±0.00^{b}	100.00±0.00^{a}	0.00±0.00^{d}	100.00±0.00^{d}	0.00±0.00^{c}	100.00±0.00^{c}
F values	**158.32*****	**2282.88*****	**670.99*****	**2048.61*****	**1042.73*****	**4549.42*****

Means within the column followed by the same small letter do not differ significantly for the same extract at the 5% level according to Tukey's test. *** $P< 0.001$

Effectiveness of the three extracts on reducing damage caused by *Callosobruchus maculatus*

Compared to the untreated control, the three extracts significantly reduced the percentage of weight loss due by *C. maculatus* on each treated commodity grain (Table 5). The hexane extract was more effective and completely reduce weight loss of seeds at all the concentrations. This complete reduction is only achieved from the concentration of 15 g/kg when the commodities were treated with acetone and methanol extracts. At 5 g/kg the percentage of weight losses was lower on *V.unguiculata* than on *V.subterranea* when treated with acetone or methanol extracts (Table 5). In general, the reduction of damaged seeds and percentage of weight losses could be explained by the presence of the secondary metabolites responsible ofthe insecticidal activity. Factors responsible for the differences observed between the two commodities would be also due to the hardness of the seeds, to say that when the hardness of the seed was higher, the greater average development time and the duration of the cycle delayed[53]. This seed hardness would be attributed to the moisture content of the seeds. Indeed, the more a variety is rich in water, the less will be its hardness. Thus, the high moisture content of *V. unguiculata* compared to *V. subterranea* could explain this difference in resistance between *V. subterranea* and *V. unguiculata*. Similar results were obtained by Tofel*et al.*[9] who obtained a significant reduction in the weight losses of corn seeds treated with the powders of Neem(*Azadirachtaindica*) and *Plectranthusglandulosus* against *S. zeamais*.

Table 5: Effectiveness of the three extracts in reducing the damage caused by *Callosobruchus maculatus*

Conc (g/kg)	Percentage of weightloss					
	Vignasubterranea			*Vignaunguiculata*		
	Hexextract	**Ace extract**	**Met extract**	**Hexextract**	**Ace extract**	**Met extract**
0	59.77±1.30[a]	58.26±1.56[a]	59.26±1.56[a]	36.44±1.22[a]	18.32±4.90[a]	24.98±6.18[a]
5	0.00±0.00[b]	5.16±0.70[b]	4.10±1.14[b]	0.00±0.00[b]	2.79±0.54[b]	2.57±0.48[b]
10	0.00±0.00[b]	2.97±1.26[bc]	0.01±0.01[c]	0.00±0.00[b]	1.24±0.11[b]	1.08±0.15[b]
15	0.00±0.00[b]	0.00±0.00[c]	0.01±0.01[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
20	0.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]
F values	**2102.65*** **	**709.29*** **	**916.66*** **	**888.42*** **	**12.61*** **	**15.20*** **

Means within the column followed by the same small letter do not differ significantly for the same extract at the 5% level according to Tukey's test. *** $P< 0.001$. Hex: hexane, Ace: acetone, Met: methanol, Conc: concentrations

Influence of the extract concentration on the content of anti-nutritional compounds in the treated seeds

Table 6 shows the results of the quantitative analyse carried out on grains treated with methanol or acetone of *V. heterophylla* leaf extracts. It appears from this table that, compared to the untreated control, the treatment of the grains with each extract considerably increases the phytochemical characteristics of the different samples. It was also noted that, acetone extract increased the content of anti-nutritional compounds more than methanol extract. This difference would be due to the difference in solubility of these compounds in each corresponding extraction solvents. Thus, in *V. subterranea* the values of total phenolic compounds passed from 94.00 mg/100MS (untreated control) to 270.04 mg/100MS in the samples treated with 20 g/kg, the flavonoids from 7.93 mg/100MS (untreated control) to 26.34 mg/100MS, tannins from 10.54 mg/100MS to 28.63 mg/100MS, alkaloids from 1.25 mg/100MS to 4.3 mg/100MS and total oxalates from 1.7 to 6.6 mg/100MS. The same tendencies were observed in *V.unguiculata*, but the values of these antinutrients are slightly lower than that obtained in *V.subterranea*. The increase in the antinutrient quantities is thought to be due to the increase in the concentration of the two *V. heterophylla* extracts used in this experiment which are the precursor extracts of these antinutrients. These results are close to those reported by Diallo *et al.*[56] who obtained the total polyphenol contents of 141.43 mg gallic acid equivalent/100 g of dry matter in the seeds of *V. unguiculata*. In the present study, the values of the flavonoid content are higher than those obtained in the funding of Diallo *et al.*[56], who obtained a value of 9.27 mg gallic acid equivalent/100 g of dry matter on the seeds of untreated *V. subterranea*. This increase would be due to the fact that the seeds used are not the same variety.

Table 6: Antinutrient content of seeds treated with extracts of *Veprisheterophylla*

commodities	Parameters (mg EAG/100MS)				
Vignasubterranea	CPT	Flavonoids	Tannins	Alcaloids	Oxalates totaux
Untreated control	94.00±24.7d	4.93±0.00d	10.54±0.39c	1.25±0.07c	1.7±0.14c
20 g/kg	270.04±0.39a	26.34±0.18a	28.63±0.4a	4.3±0.14a	6.6±0.14a
15 g/kg	260.05±2.74b	24.56±0.18ab	26.59±3.14ab	3.6±0.28b	5.4±0.28ab
10 g/kg	123.11±0.78c	11.14±0.37b	13.26±3.11bc	1.5±0.14bc	1.9±0.14c
5 g/kg	111.47±0.00cd	7.80±0.04bc	12.58±29.02c	1.3±0.14c	1.7±0.14c
F values	210.24***	121.04***	10.24**	12.24***	10.04**
Vignaunguiculata					
Untreated control	91.43±0.78d	6.73±1.46cd	11.81±1.57c	1.5±0.14c	1.8±0.28d
20 g/kg	288.19±0.78a	23.35±0.29a	28.08±1.96a	6.2±0.28a	8.4±0.28a
15 g/kg	264.82±1.18ab	17.62±0.09b	26.22±5.10ab	5.5±0.14ab	4.7±0.14b
10 g/kg	112.58±3.92c	7.88±0.11c	12.31±1.96b	2.1±0.14b	2.2±0.28c
5 g/kg	100.38±7.84cd	7.55±0.11cd	12.20±9.02b	1.9±0.14bc	2.01±0.28c
F values	194.45***	120.24***	13.24***	14.45***	8.26**

Means within the column followed by the same letter do not differ significantly for the same extract at the 5% level according to Tukey's test. *** $P< 0.001$

In vitro digestibility of proteins and carbohydrates from treated samples.

Table 7 presents the results of the in vitro digestibility of the proteins of treated (with insecticidal plant extracts) and untreated treated samples. It appears from this table that, the percentage of digestibility varies from 73.94 ± 0.57% (M 0.0625) to 75.54 ± 0.60% (B). This value is higher for the untreated sample than the treated one. This reduction observed in the treatment could be due to the presence of bioactive molecules such as tannins and total phenolic compounds which complexed these nutrients. Based on the analysis of variances between the treated samples, we found that their digestibilities are not significantly different, which allows us to say that the treatment does not significantly impact the digestibility of the proteins of our different samples.

The digestibility of carbohydrates ranged from 52.81% (MV 0.0625) to 56.70% (B). It was higher for the untreated samples than the treatedones. This reduction brought about the treatment could also be explained by the presence of molecules such as total phenolic compounds, tannins, alkaloids, flavonoids among others, which would complex these nutrients. Among the treated samples we found that their digestibility is not significantly different.This allows us to say that the treatment does not greatly impact the digestibility of carbohydrates in various samples

Table 7: the in vitro digestibility of proteins and carbohydrates commoditiesgrains treated with *Veprisheterophylla* leaf extracts

Concentrations (g/kg)	***Vignasubterranea***		***Vignaunguiculata***	
	Proteins	**Glucids**	**Proteins**	**Glucids**
Untreated control	75.54±0.60^{ab}	56.70±0.42	73.94±0.57^{a}	54.90±0.42
20	74.01±1.26^{a}	55.20±0.85	73.96±0.21^{a}	54.31±0.42
15	74.44±0.42^{a}	54.01±0.00	73.99±0.24^{a}	53.41±0.00
10	74.40±0.40^{a}	53.71±0.42	74.12±0.75^{b}	53.11±0.42
5	73.99±1.37^{b}	52.81±0.00	87.56±0.25^{c}	53.01±0.75
Caséine	73.94±0.57^{a}		73.94±0.57^{a}	

Means within the column followed by the same letter do not differ significantly for the same extract at the 5% level according to Tukey's test

Microbial load of treated and stored flours with different extracts of *V. heterophylla*

The results of microbiological analyzes of flours of *Vignasubterranea* and *Vignaunguiculata* are presented in the table.The data were compared to the standard for cooking flour (Codex Alimentarius, 1994).The total flora ranged from (1.5–8.5) *10^3 and the high values were observed with untreated or control flours, followed by flours treated with ethanolic, methanolic extracts and finally with hexane.Likewise, regardless of the solvent used, extracts of *V. subterranea* have higher microbial loads than those of *V. unguiculata*.There is also an absence of coliforms and fungal flora.In general, these results reveal that the extracts of *V. heterophylla* contributed to the microbiological stability of the treated flours of *V. subterranea* and *V. unguiculata* after 3 months of storage Similar result was reported by Mahamaet al[57].

in which the use of extract of *Eucalyptus camaldulensis* of the stored Bambara groundnut treated.

Table8: Microbial load of treated and stored flours with different extracts of *V. heterophylla*

Flour	**Solvent**	**Total count**	**Total coliforms**	**Fungi**
V. subterranea	**Control**	7.5 ± 0.3*10^3	Absent	Absent
	Hexane	1.5 ± 0.2*10^3	Absent	Absent
	Acetone	5.5 ± 0.8*10^3	Absent	Absent
	Methanol	3.0 ± 0.4*10^3	Absent	Absent
V. unguiculata	**Control**	8.5±1.1*10^3	Absent	Absent
	Hexane	1.5±0.3 *10^3	Absent	Absent
	Acetone	2.5±0.5*10^3	Absent	Absent

	Methanol	$2.5\pm0.3*10^3$	Absent	Absent
Norms	/	$<10^5$	$<10^2$	10^3

IV. CONCLUSION

*Veprisheterophylla*leaf extracts have strong insecticidal properties to protectBambara groundnut and cowpea against the infestation of *Callosobruchus maculatus*in storage, as they contain a wide range of compounds which are responsible of insecticidal and microbiological activities. These extracts also significantly increased the antinutrient content of the treated grains. However, this increase did not significantly reduce the in vitro protein and carbohydrate digestibility of treated grains compared to untreated grains. They could therefore constitute an effective alternative method in the protection of Bambara groundnut and cowpeas, replacing chemical synthetic insecticides, which have consequences for the health of the consumer.

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