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Kongne Marie Louise
Department of Biological Sciences, University of Maroua, PO Box 814, Maroua, Cameroon

Kouninki Habiba
Department of earth and life Sciences, Higher Teacher's Training College, University of Maroua, PO Box 46, Maroua, Cameroon

Fameni Tope Sidonie
Department of Biological Sciences, University of Maroua, PO Box 814, Maroua, Cameroon

Fernand Nestor Tchuenguem Fohouo
Department of Biological Sciences, University of Ngaoundéré, PO Box 454, Ngaoundéré, Cameroon

Correspondence
Kongne Marie Louise
Department of Biological Sciences, University of Maroua, PO Box 814, Maroua, Cameroon

Management of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) using methanol extracts of *Carica papaya*, *Carissa edulis*, *Securidaca longepedonculata* and *Vinca rosea* and impact of insect pollinators on cowpea types in the Far-North region of Cameroon

Kongne Marie Louise, Kouninki Habiba, Fameni Tope Sidonie and Fernand Nestor Tchuenguem Fohouo

Abstract
In Maroua, Cameroon, from April to August 2016, the methanol extracts of plants were applied at 0, 100 and 200mL per 50g of cowpea seeds obtained from free pollination (FP), from protected flower from any visit of pollinator (PP) and from a selected resistant variety (B125). The higher rate of mortality was obtained with *V. rosea* (93.33±5.77) and the lower one was recorded with *C. papaya* (73.33±5.77) for all the type of cowpea seeds used at 200 mg/mL. The mean larvae were 0±0.0 on FP and B125 with *S. longepedunculata* and *V. rosea* respectively. After three months of storage, the higher emergence of cowpea weevil gave a mean value of adult weevil emergence was (5.64±1.19)FP; (9.15±2.97)PP; (7.24±4.28)B125. The weight loss showed 80.0±2% of PP type, 72.66±1.15% of FP type and 69.33±3.05% within the controls. The FP (88.89±19.25%) cowpea seeds preserved germination power more than those of PP and B125 types.

Keywords: *Callosobruchus maculatus*, stored cowpea, methanolic extracts insecticidal plant, pollination

Introduction
In Cameroon, 15.4% of the population, about 3 millions of person is still on view at starvation and undernourishment and 33% of children suffer from chronic malnutrition [1, 2]. Efforts are being made by the government in order to increase agricultural production, but self-sufficiency is not achieved and episodes of famine still affect certain regions, including sub-Saharan of Africa [1-3]. Among these efforts, investigations carried out for more than 26 years on the relationship between some plants and their floral insects in certain regions of Cameroon among which, the Adamaoua [4, 5], the center [6, 7], the Far-North [8, 9] and the west region [10]. Despite important works done on the interactions between several crops grown in Cameroon and their pollinators which are still unknown however, these insects have benefit role on economic in enabling a better yield and, may participate in the good quality of the fruits, vegetables or seeds (taste, richness of nutrients, size, appearance and germinative capacity) [11, 12]. Likewise, post-harvest losses from insect pests are also a constraint faced by farmers [13, 14]. Among the cultivated plants which can play a role in food security by covering the protein requirements of populations as recommended by the World Health Organization (WHO) [15], cowpea [*Vigna unguiculata* (L.) Walp.] is one of the world's leading protein crops. In Cameroon, cowpea is more and more cultivated, appreciated because all its parts are used from young leaves to the tops, passing by its green pods and its dry seeds. Despite these positions, some constraints remain: the main ones are the relation floral insect, pod yield and seeds are unquestionably the problem of managing pests in storage. The majority of Cameroonian farmers do not know the impact of insect pollinators during plant flowering [16, 11, 17]. Insect pests may cause damage estimated between 25 and 40% of stocks after one month of storage, then 80 to 100% between 5 to 6 months of storage if no measures of control are undertaken [18, 19]. *Callosobruchus maculatus* (Fabricius, 1775) is the major pest of cowpea, which adults appears in crops at the end of the rainy season, reproduces themselves from the period of

fruiting and continues in granary. *C. maculatus* is characterized by its larval stages which develop within the seeds and consume the reserves contained in the cotyledons. To fight against these pests, several methods of control have been developed. Among these methods, we have the preventive methods that include cropping techniques, the picking of crops, the improvement of infrastructures and the hygiene of storage [20]. Physical methods [21]; biological methods using living organisms (virus, fungus, nematode, bacteria and protozoa) and natural enemies (parasitoids and predators) [22, 23], the method of varietal resistance based on physical resistance, chemical and genetic characteristics of the grain [24, 25], the chemical by the use of synthetic chemical insecticides [26, 27, 14] and the use of transgenic or mutant insects which must remain under the control of the public sector [2]. Despite these used methods against insect pests, many problems remain as the persistence of post-harvest losses [18, 19, 28-30], the development of multiple resistances of major pests face to insecticides which are widely used [31, 2]. There is a need to find other fighting methods to support existing ones. It is true that the use of insecticide plants in tropical areas is one of the rational techniques to fight against the multiple infestations in field and in stock [32-37]. Plants products are used against insect pests for their toxic, repellent, growth inhibiting and disruptive effects, which may act by contact or fumigation [38]. One of the possible techniques to reduce the damage due to insect pests will combine the action of insects' pollinators on the resistance of grains of *V. unguiculata* and insecticidal plants to control *C. maculatus* during storage.

The aim of this work was to evaluate the combination action of insects' pollinators and, the insecticidal effects of methanolic extracts of *Carica papaya* Linnaeus 1753, *Carissa edulis* Vahl. 1790, *Vinca rosea* or *Catharanthus roseus* (Linnaeus) G. Don, 1837 and the *Securidaca longepedunculata* Fres. 1819 used by farmers as natural pesticide to manage *C. maculatus* on the mortality, number of eggs laid, number of larvae, emergence, weight loss and the germinative power of grains of *V. unguiculata*.

Materials and Methods

Rearing of *Callosobruchus maculatus*

The experiment was carried out from August to November 2014 and from July to October 2015 in the locality of Dougouf- Maroua (longitude: 14°16' E; altitude: 412 m, and latitude: 10°33'N) for obtaining cowpea type and, storage was done from April to August 2016 at Maroua, in the Far-North region of Cameroon. The strain of adult of *C. maculatus* was harvested by sieving cowpea seeds from the cowpea section of the IRAD of Maroua (Cameroon). Mass breeding was carried out regularly in small 5-liter buckets to the perforated lid of tiny holes. In each of these buckets 2 kg of cowpea seeds and adults of *C. maculatus* were introduced and allowed for oviposition. This rearing took place under laboratory conditions at a mean temperature of 25.61 ± 2.08°C and mean relative humidity of 19.05 ± 2.37%. The cultures were maintained by continually replacing the devoured and infested seeds with fresh and uninfested ones. During the process of replacement, copulating pairs of *C. maculatus* adults were introduced into the containers.

Grain disinfestation

Three types of cowpea seeds of *V. unguiculata* were collected:

- Cowpea seed obtained from flowers left in free pollination open to all visits of pollinating insects (FP);
- Cowpea seeds obtained from flowers protected from any visit of floral insect or protect pollination (PP);
- Cowpea seeds type B125 cultivated with fertilizer, insecticides, herbicides and opening the flowers to any field visits of insects.

The FP and PP were grown without herbicides, insecticides and fertilizers.

These types of cowpea seed were dried in the sun for a week for disinfestation and sieved in order to remove the debris, broken and attacked grains in the field. Each type of cowpea was placed in a freezer at 4 °C for 48 hours in order to eliminate any trace of the different stages of development which may remain inside the grain.

Collection and methanol extraction of the plant samples

The fresh bark of roots and leaves of plant (Table 1) were collected from natural population in the far North region of Cameroon. These parts used were harvested and dried in the shade under normal room temperature and crushed it in a mortar to obtain the various powders. The total extracts were obtained by double extraction in methanol at 95°. Thus, the powders of specific mass of each plant species were introduced into a 5 liter containing 2 liters of methanol at 95 °. The mixture was subjected to mechanical (cold) stirring, then macerated for 48 h, then filtered with a whatman No. 2 paper. The resulting solution was passed through the Heidolph rotary evaporator in a water bath at a temperature of 60 °C, in order to remove the methanol used for extraction. Each of the extracts obtained was weighed and placed in a 1000 ml glass jar previously sterilized and labeled, then stored in a refrigerator at a temperature of 4°C before use [39].

The extraction efficiency of each fraction R was evaluated [39]. as follows: $R = E / MS \times 100$;

Where: E = amount of extract; MS = amount of dry matter in the product.

Table1: The origin, parts of plants used and vernacular names

N°	Plants	Origin	Parts used	Vernacular names
1	*Carica papaya* Linnaeus 1753	Makabaye /Maroua	leaves	Toukoudje (in Foufouldé)
2	*Carissa edulis* Vahl. 1790	Douvangar /Meri	leaves	Madako'ndrom (in Mafa) Tchaboule bali (in Foufouldé)
3	*Securidaca longepedonculata*Fres. 1819	Zouelva/Mayo-Sava	Root bark	Alale or Alali (in Peul)
4	*Catharanthus roseus* (Linnaeus) G. Don, 1837or*Vinca rosea*	Doukoula/Diamaré	leaves	Pervenche de Madagascar (in french)

Experimentation

Preparation of treatment for the evaluation of the insecticidal effect of methanolic extracts towards *Callosobruchus maculatus*

For each test, 100 and 200 mg of extract of each plant were dissolved in 1 ml of methanol at 95°, in order to obtain methanol solutions at different concentrations. The methanolic solution obtained from each type of plant was mixed in a glass jar of 500mL containing 50 g of cowpea seed. The whole were suitably mixed. After being left in the open air for about 15 minutes in order to allow the total evaporation of the methanol, 5 pairs of cowpea weevils of 48 hours old were introduced in each jar and covered with a gauze cloth. The gauze cloth is held in place by numbs in order to disallow the entry or exit any insect and then were placed in the laboratory [40].

The control consists on 50 g of cowpea seed mixed with 1 ml of methanol only.

Three replicates were made for each treatment made.

The efficacy of plant extract against cowpea weevils was evaluated considering parental mortality, number of grain with trace of eggs, number of larvae, adult emergence, seed weight loss and germination from treated and untreated seeds in term of parameters given below:

- **Effect of the different methanol plant extract on the mortality of *Callosobruchus maculatus***

Data on the parental mortality was recorded three days after infestation. The dead weevils were counted, recorded and removed from the jar.

The percentage of mortality is defined as follows [41]:

% of mortality = (Number of dead weevils/Total number of weevils)*100

The corrected mortality percentage was calculated using the [42] formula:

MC = Mo-Mt / 100-Mt

With: MC =% corrected mortality; Mo = natural mortality observed in control jars; Mt = mortality recorded in the batches treated with local insecticidal plants.

- **Number of eggs laid**

Six days after infestation, sample 50 cowpea seeds randomly taken in each jar were carefully examined and seeds with trace of eggs or without eggs were separated. After separation the average numbers of seeds bearing eggs deposited were recorded.

The percentage of seed bearing trace of eggs is defined as follows [43]:

% of seed bearing trace of oviposition = (Number of seeds bearing eggs/Total number of seed)*100

- **Number of larvae**

Twelve days after infestation, 50 cowpea seed randomly were taken from each jar and the cotyledons were gently separated by a cuter and the number of larvae were counted and recorded. The percentage of number of larvae is defined as follows [44]:

% of seed with larvae = (Number of seeds with larvae/Total number of seed)*100

- **Number of F_1, F_2 and F_3 adults after one month, two and three months of storage**

After one, two and three month of infestation, the number of adults from F_1, F_2 and F_3 in each jar were counted and recorded.

The percentage of reduction in emergence is defined as follows [45]:

% of reduction in emergence = (Number of newly emerged adult in untreated jar - number of newly emerged adults in treated jar / Number of newly emerged adult in untreated jar)*100

- **Weight loss**

Three months after storage, the contents of each jar were weighed using an OHAUS scale that was sensitive to the nearest thousandth. Weight loss of cowpea seeds caused by the feeding of *C. maculatus* was determined. The percentage of weight loss is evaluated as follows [45]:

% of weight loss = (Initial weight-weight at the end of the experiment/ initial weight)*100

- **Germination tests**

Three months after storage, twenty-four grains of cowpea were collected randomly and sown in an experimental field in each pole; two grains of cowpea were introduced at the rate of twelve pockets for two lines. One week later, the number of germinated and emerged seeds from the soil was counted per pile, cowpea type, treatment type and recorded. The percentage of germination is evaluated as follows [46]:

% of seed viability = (Number of seeds that germinated/Total number of seed sown)*100

Statistical analysis

The one way ANOVA were performed with Statgraphic 5.0. Data obtained on parental mortality, number of grain with trace of eggs, number of larvae, adult emergence, seed weight loss and germination were analysed by ANOVA. The LSD test was used to rank obtained means.

Results

Yield of extraction

The methanol extraction yields obtained from cold maceration from different mass of powder of plants are presented in Table 2.

Table 2: Extraction yield of the four local plants extraction

Plants	Initial mass / g (MS)	Final mass / g (Residue)	Extracted mass / g	Extraction yield %
Carica papaya	343.30	340.10	3.20	0.93
Carissa edulis	467.11	460.09	7.02	1.5
Securidaca longepedonculata	308.9	306.3	2.60	0.84
Catharanthus roseus or *Vinca rosea*	505.71	503.81	1.90	0.38

Toxicity of plant methanol extracts on adult of *Callosobruchus maculatus*

The results of the mortality of *C. maculatus* adults exposed to the different methanol extract at different concentrations of the four plants used are presented in the Table 3. The adult's mortality rate of *C. maculatus* increased with the plant extracts, the concentrations applied and the type of cowpea seed used. *S. longepedonculata* and *V. rosea* are more insecticides than *C. edulis* and *C. papaya* respectively. For example, for the FP cowpea seeds, The adult mortality rate of *C. maculatus* increased from 40±10 to 73.33±5.77% for *C. papaya* extracts, from 46.66±5.77 to 8.00±10.0% for *C. edulis* extract, from 53.33±5.77 to 90.0±0.0% in *S. longepedonculata* and 56.67±5.77 to 93.33±5.77% with *V. rosea* for the doses of 100mg/mL and 200mg/mL respectively. There was a significant difference at the level of 5% for adult mortality of *C. maculatus* as a function of the different extracts used. All the treatments made with methanol extract of plant were significantly better than control. No mortality was observed for the three types of cowpea seed used.

Table 3: Effect of the plant methanol extracts on the mortality of *Callosobruchus maculatus* adult

Plant extract	Concentration (mg/mL)	Mortality (%)		
		Tpe of *Vigna unguiculata*		
		FP	PP	B125
Carica papaya	0	0±0^{a}	0±0^{a}	0±0^{a}
	100	40±10^{b}	43.33±5.77^{bc}	43.33±11.54^{bc}
	200	73.33±5.77^{b}	76.66±11.54^{bc}	76.67±11.54^{bc}
Carissa edulis	0	0±0^{a}	0±0^{a}	0±0^{a}
	100	46.66±5.77^{bcd}	50.00±10^{bcd}	46.67±15.27^{bcd}
	200	80.0±10b^{cd}	80.0±10^{bcd}	76.67±11.54^{bc}
Securidaca longepedonculata	0	0±0^{a}	0±0^{a}	0±0^{a}
	100	53.33±5.77^{bcd}	53.33±5.77^{bcd}	56.67±5.77^{cd}
	200	90±0^{cd}	86.67±5.77^{bcd}	90±10^{cd}
Vinca rosea	0	0±0^{a}	0±0^{a}	0±0^{a}
	100	56.67±5.77^{cd}	60±10^{d}	60±10^{d}
	200	93.33±5.77^{d}	93.33±5.77^{d}	90±10^{dcd}

In the same line, value followed by the same letter are not significantly different (p>0.05)

Effect of methanol extracts plant on the oviposition of *Callosobruchus maculatus*

Table 4 compares the effect of the four methanol extract plants on the eggs laid by *C. maculatus* on the different type of cowpea seeds. *V. rosea* extract reduces the number of eggs laid more than all the others taken at the doses of 100 mg/mL and 200 mg/mL: the number of eggs laid varies according to the plant extract and the quantity used. For example for FP Type, 10±1 and 3.3±0.58% were recorded at 100and 200mg/mL respectively for the *C. papaya* extract plant. Depending on the type of cowpea in control, this result reveals that, the female of *C. maculatus* lays more eggs on the FP-type grains (32±2.65) than on the PP-type grains (25.67±1.53) and finally on the B125 type grains (15.63±2.89). All the methanol extract of plant used showed significantly less number of eggs laid compared to the control. There is a significant difference at the 5% level for adult oviposition of *C. maculatus* depending on the different extracts and different types of cowpea.

Table 4: Effect of the methanolic plants extracts on the oviposition of *Callosobruchus maculatus*

Plant extract	Concentration (mg/mL)	% of number of grain containing trace of eggs		
		Type of *Vigna unguiculata*		
		FP	PP	B125
Carica papaya	0	32.00±2.65^{h}	25.66±1.53^{g}	15.67±2.89^{f}
	100	10±1 ^{d}	5.67±1.15^{e}	2±1f (4.33±0.58de)
	200	3.33±0.58^{b}	2.33±0.58^{b}	2.0±0.58$^{ab(}$ 2 ± 1cd)
Carissa edulis	0	32.33±2.58^{e}	25.33±1.53i	15.67±2.89^{h}
	100	5±0^{cd}	4.33±0.58^{cd}	4±1 ^{cd}
	200	2.33±0.58^{b}	1.66±0.58^{ab}	2±0^{ab}
Securidaca longepedonculata	0	32.33±2.58^{l}	25.33±1.53i	15.67±2.89^{h}
	100	3.67±0.58^{abc}	3.33±0.58^{abc}	1.66±0.58^{ab}
	200	2.±0ab	1.33±0.58^{ab}	0±0^{a}
Vinca rosea	0	32.33±2.58^{l}	25.33±1.53^{i}	15.67±2.89^{h}
	100	3±1 ^{abc}	3±1^{abc}	1.33±0.58^{a}
	200	2.0±1^{ab}	1.33±0.58^{ab}	0±0^{a}

In the same line, value followed by the same letter are not significantly different (p>0.05)

Effect of methanol extracts plant on the number of larvae of *Callosobruchus maculatus*

Table 5 reveals that the extract of *S. longepedonculata* is more larvicidal than that of *V. rosea*, *C. edulis* and *C. papaya* respectively. This larvicidal effect increases with the increasing of the quantity of plant extract used. For example: Depending on the type of cowpea for the different controls, these results show that the PP cowpea grains (14.67±1.53) and FP cowpea seeds (14.33±1.53) contain more larvae than the B125 cowpea (12.33±1.53). There was a significant difference at the 5% level for the number of *C. maculatus* larvae depending on the different extracts and the different types of cowpea.

Table 5: Effect of the plants methanol extract on the number of *Callosobruchus maculatus* larvae

Plant extract	Concentration (mg/mL)	% of grain containing larvae		
		Type of *Vigna unguiculata*		
		FP	PP	B125
Carica papaya	0	14.33±1.53^{f}	14.67±1.53^{f}	12,33±1,15^{e}
	100	3.66±1.54^{cd}	5±1^{d}	2,67±0,58^{bc}
	200	2±0^{cd}	2.67±1.53^{d}	1,33±0,58^{bc}
Carissa edulis	0	14.33±1.53^{k}	14.67±1.53^{k}	12,33±1,15^{i}
	100	2±1^{ab}	3±1^{bc}	2±0^{ab}
	200	1.66±0.5^{bcd}	1.33±0.58^{bc}	1±0^{bcd}
Securidaca longepedonculata	0	14.33±1.53^{k}	14.67±1.53^{k}	12,33±1,15^{i}
	100	1±1^{a}	2±1^{ab}	1±0^{a}
	200	0±0^{a}	0.67±0.58^{ab}	0.66±0.58^{ab}
Vinca rosea	0	14.33±1.53^{k}	14.67±1.53^{k}	12,33±1,15^{i}
	100	2±1^{ab}	3±1^{bc}	0.66±0.58^{a}
	200	0.67±0.58^{ab}	1±0^{abc}	0±0.0^{a}

In the same line, value followed by the same letter are not significantly different (p>0.05)

Effect of the plants methanol extracts on the emergence of *Callosobruchus maculatus* after after one, two and three months of storage

The insecticidal effect of the methanol extract of the four plants used was evaluated through the reduction of the emergence of *C. maculatus* which emerge in the different treatments during three months (Table 6). The inhibition rate of the emergence increase with the generation and decrease with the augmentation of the quantity of the methanol extract of plantused. Respectively at the dose of 100 and 200 mg/mL, the methanol extract of *S. longepedonculata* was more effective than that of *V. rosea* which in turn was more effective than that of *C. edulis* which itself is more effective than that of *C. papaya*. Also, the dose of extract increased with its effectiveness against *C. maculatus*. The FP cowpea seeds had a higher level of inhibiting rate which varied from 86.34±2.7 to 94.10±2.4 with the methanol extract at 100mg/mL of *C. papaya* and *S. longepedunculata* respectively. The same observation was also made with the other cowpea seeds used (PP and B125) Depending on the insecticidal effect of the plants and the type of cowpea, there was a significant difference at the 5% level.

Table 6: Effect of the plants methanol extracts on the emergence of the adult *Callosobruchus maculatus* after one, two and three months of storage

Plant extract	Generation	Inhibition of the emergence (%)					
		Type of *Vigna unguiculata*					
		FP		PP		B125	
		Concentration mg/mL		Concentration mg/mL		Concentration mg/mL	
		100	200	100	200	100	200
Carica papaya	F1	76.19±4.12^{bcd}	11.90±4.12^{c}	74.56±0.75^{ef}	16.92±7.56^{e}	82.32±0.87^{b}	0±0^{a}
	F2	79.90±3.14^{e}	8.32±0.79^{e}	83.77±4.27^{c}	8.70±1.05^{c}	87.47±1.89^{b}	5.74±2.81^{bc}
	F3	86.34±2.76^{d}	5.64±1.19^{d}	82.94±6.75^{bc}	9.15±2.97^{d}	89.39±1.42^{b}	5.76±3.73^{bcd}
Carissa edulis	F1	85.71±7.14^{cde}	11.90±4.12^{c}	81.73±3.05^{fg}	8.92±2.33^{c}	85.35±4.87 ^{b}	6.06±5.25^{b}
	F2	89.28±7.37^{f}	6.39±2.34^{de}	86.55±4.90^{cd}	6.18±0.62^{cd}	87.48±1.89^{b}	4.78±1.60^{b}
	F3	91.78±4.41e	4.63±2.1^{cd}	87.38±4.03^{bc}	6.61±1.35^{bc}	91.30±1.45^{b}	4.32±1.37^{abc}
Securidaca longepedonculata	F1	90.48±4.12^{e}	0.0±0.0^{a}	88.66±6.84^{g}	3.42±2.96^{ab}	91.16±0.43^{bc}	0.0±0^{a}
	F2	94.10±1.56^{f}	0.0±0.0^{a}	92.43±3.65^{d}	0.0±0.0^{a}	93.27±1.60^{b}	0.0±0^{a}
	F3	94.33±2.4^{e}	2.03±1.54^{abc}	91.69±5.33^{c}	0.0±0.0^{a}	93.27±4.07^{bc}	1.44±0.03^{ab}
Vinca rosea	F1	88.09±4.12^{de}	0.0±0.0^{a}	85.57±1.71^{g}	3.42±2.96^{ab}	94.19±5.04^{bcd}	0.0±0.0^{a}
	F2	89.14±7.44^{f}	1.98±0.88^{ab}	87.78±4.35^{cd}	0.0±0.0^{a}	90.42±1.53^{b}	3.85±1.62^{ab}
	F3	91.72±5.31^{e}	2.57±1.2^{abc}	86.53±5.62^{bc}	6.74±.5.04^{cd}	92.32±2.86^{bc}	7.24±4.28^{cd}

In the same column, value for the same generation followed by the same letter are not significantly different (p>0.05)

Damage assessment of cowpea seeds types treated with the methanol plant extracts after three months of storage

Respectively at the dose of 100 and 200 mg/mL, the effect of methanol extract of the four plants used against *C. maculatus* on the conservation of grain mass in storage varies according to the three types of cowpea (Table 7). The increase in the quantity of the extract increases with its insecticidal effect against *C. maculatus* and consequently increases the conservation of the mass or decreases the mass loss of the grains being stored. For the FP cowpea seeds, with *V. rosea* 47.33±5.03 and 39.33±4.16% were obtained by using 100 and 200 mg/mL of plant extract respectively used. The effects on the three types of cowpea seeds are also noted. Depending on the type of cowpea and the controls, these results revealed that there was more loss of mass of cowpea grains of PP type (80.0±2.0%) than the others cowpea grains of type FP (72.66±1.15%) and type B125 (69.33±3.05%) respectively. There was a significant difference at the level of 5% towards the insecticidal effect of the plants against *C. maculatus* and the loss of mass of the grains of the different types of cowpea during storage.

Table 7: Effect of the methanol plants extracts on the weight loss of the types of *Vigna unguiculata* after three months of storage

Plant extract	Weight loss after 3 months of storage (%)								
	Type of *Vigna unguiculata*								
	FP			PP			B125		
	Concentration mg/mL			Concentration mg/mL			Concentration mg/mL		
	0	100	200	0	100	200	0	100	200
Carica papaya	72.66±1.15gh	39.33±1.15bc	19.33±1.15 bc	80.0±2.0h	68.66±8.08bc	30.0±3.46d	69.33±3.05g	41.33±8.32bcd	12.66±3.05a
Carissa edulis	72.66±1.15 gh	30.0±5.29a	15.33±3.05ab	80.0±2.0h	44.66±5.03cde	22.66±3.05c	69.33±3.05g	52.66±4.16fg	39.33±1.15e
Securidaca longepedonculata	72.66±1.15 gh	37.33±3.06abc	14.0±3.46ab	80.0±2.0h	40.0±3.46bcd	31.33±6.11d	69.33±3.05g	56.66±4.16g	32.66±7.02d
Vinca rosea	72.66±1.15 gh	47.33±5.03def	39.33±4.16e	80.0±2.0h	52.0±3.46efg	30.66±3.06d	69.33±3.05g	36.66±4.16ab	31.33±5.03 d

In the same column, value followed by the same letter are not significantly different (p>0.05)

Impact of methanol plants extracts on germination

When the different types of cowpea seed were in contact with the different methanol extract of the four plants used, the germination rate varied according to the applied concentration and the type of cowpea seed used. The extracts of *V. rosea* and *C. edulis* were more effective in preserving the germinative power of FP grains than those of extracts of *S. longepedonculata* and *C. papaya*. For the PP type, extracts of *V. rosea* and *S. longepedonculata* were more effective in preserving the germinative power of PP-type grains than extracts of *C. papaya* and *C. edulis* where no germination is noted (table 8). For the B125 grains, extracts of *S. longepedonculata*, and *C. papaya* were found to be more effective in conserving the germinative power than those of *C. edulis* and *S. longepedonculata.* The increase in the quantity of the extract increased with its insecticidal effect against *C. maculatus* and consequently retains the germinative power of the grains being stored. The germinative power was almost 0% PP cowpea seeds for 100 and 200 mg/mL of all the plants extract used. The germination rate was better with the FP cowpea seeds where germination rate rise until 88.89±19.25% *V. rosea* and *C. edulis* at 200mg/mL respectively. Depending on the insecticidal effect of plants against *C. maculatus* and the conservation of the germinative power of the grains of the different types of cowpea during storage, there was a significant difference at the 5% level.

Table 8: Impact on the germination of the different types of *Vigna unguiculata* after three months storage methanol plants extracts

	Germination after 3 months of storage (%)								
	Type of *Vigna unguiculata*								
	FP			PP			B125		
	Concentration mg/mL			Concentration mg/mL			Concentration mg/mL		
Plants extract	0	100	200	0	100	200	0	100	200
Carica papaya	0.0±0.0a	33.33±0.0bc	55.56±19.25b	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	33.33±0.0bc	66.67±0.0bc
Carissa edulis	0.0±0.0a	77.78±19.25d	88.89±19.25c	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	55.56±19.25b
Securidaca longepedonculata	0.0±0.0a	44.44±19.25c	77.78±19.25bc	0.0±0.0a	0.0±0.0a	22.22±19.25a	0.0±0.0a	33.33±0.0bc	66.67±0.0bc
Vinca rosea	0.0±0.0a	77.78±19.25d	88.89±19.25c	0.0±0.0a	0.0±0.0a	22.22±19.25a	0.0±0.0a	22.22±19.25b	55.56±19.25b

In the same column, value followed by the same letter are not significantly different (p>0.05)

Discussion

Many studies show that the effectiveness of any plant with an insecticidal effect in the protection of food stuffs in the form of grain against insect pests depends on its rate of toxicity on the target insect, its effects on its development, reproduction and behavior [46, 40, 14]. Several studies have also been carried out on the impact of floral insects on the yield of plants in the field. It emerged from these multiple studies that pollinators not only increased yield but also improved the quality, taste, appearance of the tegument, shape, nutritional value and the germination of the harvested product [11, 15, 12, 47]. The objective of this work was to determine whether entomophilous pollination confered resistance of the grains against the attack of *C. maculatus*, to evaluate the action of methanolic extracts of (*Carica papaya*, *Carissa edulis*, *Securidaca longepedonculata* and *Vinca rosea*) on adult mortality, oviposition, larval numbers, adult emergence of *C. maculatus* during storage of the cowpea grain; to determine the conservation of the mass losses and the germinative power of cowpea grain during three months of storage.

At the dose of 200 mg/mL, the mortality rates of *C. maculatus* obtained after 72 hours infestation increase with the quantity of extract used, vary according to the different extracts and do not vary according to the types of cowpea in the control. All of these extracts would contain secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins which are responsible for their insecticidal, repellent, anti-baiting effect, larvicidal, ovicidal, toxic and dissuasive on the Coleoptera in stock and therefore on the biological activity of *C. maculatus*. They also inhibit food intake, destroy insect cell membranes, causing the death of *C. maculatus* [48, 49, 14]. These results corroborate those obtained by [50] after treatment of cowpea with the methanolic extracts of the roots of *S. Longepedonculata* which would contain the methyl salicylate acid which is a volatile compound at 90% which would be a real natural pesticide against *C. maculatus* [51]. The toxic effects of the extracts may depend on the level of insect sensitivity and the insect groups used for the test [52]. The highest mortalities observed due to extracts of *V. rosea* and *S. longepedonculata* (92.85%) each one, could be explained by the abundant presence of alkaloids (more than 120) and saponins with insecticidal effects [50, 48]. In view of the mortalities in the control, the type of cowpea has no positive or negative influence on adult mortality of *C. maculatus*; they depend solely on the bio-efficacy of the insecticidal plants used.

The mean number of eggs laid on cowpea grains of type FP, PP and B125 after treatment with plant extracts at the 200mg/mL depend with the type of cowpea grain. All the extracts used reduced significantly the laying of egg by the female of *C. maculatus* compared to the control. The lowest numbers of egg laid are obtained with the extracts of *S.*

longepedonculata and de *V. rosea*. Indeed, these plants contain high proportions of phenols, flavonoids, tannins and saponins which insecticidal effects are mentioned by [50, 53] in relation to *C. edulis* and *C. papaya*. Tannins, for example, possess insecticidal, ovicidal and larvicidal properties which inhibit the laying, growth, development and fertility of several phytophagous insects [54-56]. According to [57], this could be due to the direct toxicity of the active molecules which lead to the early death of *C. maculatus* adults when in contact with extracted coated seeds; limiting their fertility or, induced ovarian changes similar to those caused by chemosterilizers which block egg laying by weevils. These results are in line with those of [58] which revealed that extracts of leaves or grains of *C. papaya* contain inhibitors of α-amylases responsible for the reduction in fertility and the lifespan of *C. maculatus*. In the same view [50, 59], showed that the methanolic extract of methyl salicylate contained in the roots of *S. Longepedonculata* reduces the oviposition by females of *C. maculatus*. The number of eggs laid by *C. maculatus* varies depending on the type of cowpea. FP cowpea seed has more number of eggs than that of Type PP and type B125 [60]. have shown a positive correlation between the infestations of *C. maculatus* with the levels of protein, fat, and fiber contained in legumes on the other hand, this correlation is negative with grains that are poor in carbohydrates. On the other hand, the female of *C. maculatus* prefers lay eggs on smooth and of small sizes of grains of *V. unguiculata* than on grains with a tegument that is rough and flattened by what it must hold the seed before depositing its eggs [61, 62]. The difference in laying rates on cowpea seeds used FP, PP and B125 could therefore be explained by the difference in their nutritional composition and by the textural difference of their tegument inherited from the pollinating action of the floral insects. Also, the smooth seed coat observed in FP and wrinkled grains in PP grains corroborates the results of [23] which demonstrated that *C. maculatus* prefers to spawn on the grains of *V. unguiculata* smooth than those of *P. vulgaris* and *C. arietinum* which are swollen. [61] showed that out of 2 varieties of *Phaseolus vulgaris* and 3 varieties of *V. unguiculata*, it is the white cowpea IT97K499-38 having more energy with a high level of protein, fat, sugar and a smooth, thin tegument are favorable to the egg laying and development of the eggs of *C. maculatus* than the others. [60] showed that flattened grains with a rough surface of *Dolichos biflorus* inhibit the egg production of the *C. maculatus* female. In contrast to the results of [63] and [23], B125 seeds although with a smooth tegument are favorable to the laying of the females of *C. maculatus*, have a lower egg mean than FP and PP cowpea seeds. These results could be explained by the fact that these grains may contain residues of the pesticides used in the field to control pests and belong to an improved variety of cowpea seed that has received resistance genes by the insect pollinators' phenomenon against *C. maculatus*. These results could also be explained by the hardness nature of the B125 cowpea seeds which is improved varieties. This is in line with the work of [64] that showed that the females of *C. maculatus* require less hard surface and substrate for the performance of the spawning activity. Similarly, [65] observed that the fertility of *C. maculatus* female varies according to the varieties, to the types of cowpea and according to the exposure of the flowers to the visit of the pollinating insects or not: Pollination by insects would be responsible for the texture, stiffness of the tegument and the nutritional composition of the cotyledons which may play an attractive or repulsive role towards the *C. maculatus* female during laying eggs.

S. longepedonculata is more larvicidal than that of *V. rosea*, *C. edulis* and finally *C. papaya* respectively. This larvicidal effect increases with the increasement of the quantity used for the experimentation. All the plant extracts used significantly reduce larval development. The coating of the cowpea grain with the extracts plant could act on make it difficult to follow and the penetration of neonatal larvae into the grains. These results are similar to [66] which showed that the powder of *Xylopia aethiopica* does not allow larval development of *C. maculatus*. [67] found that the essential oil of *Cinnamon* at low dose, reduce the egg hatching rate of the *C. maculatus* weevils. Contrary to the results obtained with the laying by *C. maculatus*, there are more larvae in the of PP cowpea seeds than in that of FP cowpea seeds. This could be explained by the fact that the FP cowpea produced by free pollination has a diversified genetic material which may be due to the exchange of genes perpetrated by the pollinating insects between the different cowpea plants. The of PP cowpea seeds resulting from the self-pollination has poorly diversified genetic material with very low protein content relative to its carbohydrate content. This diversity in genetic material would therefore be at the origin of the high protein and low carbohydrate content observed in the FP cowpea seeds [60, 62]. In this context [68] have shown that the high protein content and texture of the seed constitute a repellent barrier to the penetration of larvae into the seed and fatal to eggs. In addition, [23] has demonstrated that the seeds of *P. vulgaris* varieties are the most resistant to the attack of the *C. maculatus* weevil, even if eggs laid is important. Indeed, the larvae of the first stage die before the end of their development, because of the toxic substances contained in the seed coats. This is the case was observed with B125 cowpea seeds which shows a low mean of larvae obtained compared to FP and PP cowpea seeds. These results are similaris with those of [69] which have shown that Cistus oil (*Cistus ladaniferus*), affects 90% of the eggs of *C. maculatus* for 60 µl used after 96 h of exposure by reducing the number of larvae which may develop into the grains. In the other hand, the use of pesticides in the fields could have left toxic residues in grain; conferring a quality of "Improved variety" this type B125 could certainly have a high protein which would be detrimental to larval development. The results obtained on the emergence after treatment of the three types of cowpea by the extracts of plants at a dose of 200 mg/mL showed that the mean numbers of emerging *C. maculatus* individuals obtained on the different cowpea seed used FP, PP and B125 are different according to the plant extract. Extracts of *S. longepedonculata* and *V. rosea* remain the most effective because they are also highly larvicidal. The obtained results could be explained by the presence of biologically active molecules in these two plants. These results corroborate those of [50, 70] and [53] who have shown that these plants contain phenols, flavonoids, tannins and saponins acting effectively on the different stages of insect development. The absence or very small number emerging insects in the jars treated with each of the insecticidal plant extracts could be explained by the absence or low mean of the larvae observed in the jars. This corroborates the results of [23] which showed that the low rate of emerging individuals is related to the number of larvae and their susceptibility to phytoinsecticide. The low emergence could also be explained by the increase in the quantity of the active molecules and the larval competition [44] within the same grain leading to the mortality of *C. maculatus* used.

Depending on the type of cowpea, the number of individuals emerging is higher respectively in the jars containing the PP, FP and B125 cowpea seed. The grains of type FP and B125 are obtained by cross-pollination. It may favor the mixing of hereditary traits and the adaptation of the species [71].These results could be explained also by the fact that the high protein content, the grain size and the texture observed with types FP and B125 cowpea seeds may constitute a natural barrier to the penetration of larvae into the grain, lethal to eggs and reduce the emergence of adults. Also, [23] has also shown that larvae of the first stage of development die before the end of their development, because of the toxic substances contained in the teguments of the grains and reduced the emergence. In the PP cowpea seeds, the genetic material could be not well diversified, with very low protein content relative to its carbohydrate content. This composition favors the larval development from which the emergence of the individuals of the F1 observed. For B125 cowpea seeds, the lowest mean of larvae obtained compared to FP and PP types would be linked to pesticides used in the fields during the culture of *V. unguiculata*. The obtained results would certainly have a high rate protein content which would be harmful to the development of eggs laids by *C. maculatus*.

The impact of phytoinsecticides against *C.maculatus* on the loss of grain mass during storage varies according to the three types of cowpea seeds (FP, PP, and B125), the extract used and the quantity of extract used. The increasement in the quantity of the extract increases with its insecticidal effect against *C. maculatus* and consequently during storage. Depending on the type of cowpea and the control, these results revealed that after three months of storage, there is more loss of mass of PP type FP and the B125 cowpea seeds respectively. These results are closely related to those of the emergence observed in these different grain types respectively. [72] showed that for a long period of storage of cowpea grain with bio-pesticides of vegetable origin, there must be a match between the quantity of grains to be stored and the quantity to be applied on the one hand and on the other, take into account the numbers of pests at the beginning of treatment and the evolution of populations during storage. Similarly, [73] on the basis of their work shown that most the concentration of phytoinsecticides rise, more there are lower the number of emerging individuals and the lower the loss mass grains. These results corroborate those of [74] whom work revealed that the loss of mass during storage is also attributed to the lipid degradation of legume seeds on the one hand and damage caused by insect pests such as cowpea weevil [75].

The impact of phytoinsecticides on *C. maculatus* on the conservation of the germinative capacity of grains during storage varies indifferently depending on the three types of cowpea. Respectively, extracts of *V. rosea* and *C. edulis* proved to be more effective in the conservation of the germinative power of FP cowpea seeds than extracts of *S. longepedonculata* and *C. papaya*. For the PP cowpea seeds, extracts of *V. rosea* and *S. longepedonculata* are more effective in preserving germinative power than extracts of *C. edulis* and *C. papaya* respectively. For grains of B125 cowpea seeds, the extracts of *S. longepedonculata* and *C. papaya* were found to be more effective than those of the extracts of *C. edulis* and *V. rosea*. Depending on the type of cowpea and the control, these results show that the conservation of the germinative power is different according to the type of grain. These results corroborate those of [76] which found the highest germination rate recorded with the 45gr / kg powder of *Tephrosia vogelii*, which is explained by the fact that the emergence of *C. maculatus* is weak, most seeds have their albumen intact, which results in a lifting rate of 92% of the total material in 7 days.

Conclusion
The results obtained from this study confirmed that insect pollinator and methanol extract of insecticidal plant can be used for the management of *C. maculatus* during storage and may contribute to the conservation of the mass and the germinative power of cowpea. *S. longepedonculata, V. rosea* are the most promising plants. The combination of the activity of insect pollinators and the use of local insecticidal plants may contribute to increased agricultural yields, the conservation of grains from the attack of pests, allows the availability of post-harvest foodstuffs.

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