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Bioactivity of methanol extracts combination of *Carica papaya* Linnaeus 1753, *Carissa edulis* Vahl 1790, *Securidaca longepedonculata* Fresen 1819 and *Vinca rosea* Linnaeus 1759, against *Callosobruchus maculatus* Fabricius 1775 (Coleoptera: Bruchidae) in the Far-North region of Cameroon

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ABSTRACT: The methanol extracts combination of *Carica papaya* (Ca), *Carissa edulis* (Cas), *Vinca rosea* (V) (leaves) and *Securidaca longepedunculata* (S) (Root bark) were tested against *Callosobruchus maculatus*. Four combination of three (Ca/Cas /V, Ca/Cas /S, Ca/V/S, Cas/V/S) and one combination of four methanol plant extracts (Ca/Cas/V/S) at the ratio of 1/3 and 1/4 were applied at 0, 100 and 200mg per 50g of cowpea seeds to assess the mortality of adults, oviposition, number of larvae, emergence during three generations (F1 F2, F3), weight loss and germination after three months. The effects varied with plant species, quantity and combination**.** At 200 mg / mL, the higher mortality rate (100%) was due to the combination of Ca/Cas/V/S. The higher percentage of eggs laid were due to Ca/Cas/V and Cas/V/S with 8.33±0.58. No larvae and almost no emergence were observed with all the combination made at 100 and 200mg/mL. The mass loss was higher with the Cas/V/S combination and the germination rate was 100% with the combination Ca/Cas/V/S. In control, no mortality, no germination were observed, the percentage of oviposition and larvae were higher than other treatments with 32.00±2.65%, 14.33±2.31% respectively. The emergence obtained increased with the generation (14, 54 and 61.66 individuals for F1, F2 and F3). The mass losses were higher with 72.66±1.15% losses of cowpea grains during three months of storage in control. These results methanol extracts combination of insecticidal plant can be used for the management of *C. maculatus* during cowpea grains storage.

***Keywords*:** cowpea, *Callosobruchus maculatus*, methanol extracts combination, insecticidal plant.

INTRODUCTION

Cowpea is one of the world's major food legumes (FAO, 2016). In Africa, it is a popular staple for its leaves, green pods and dry seeds, which can be consumed and marketed (Moumouni et al., 2013). Its tops are used for animal nutrition and restoration of soil fertility (Folefack *et* al., 2013). In Cameroon and particularly in the Far North region, cowpea occupies a prominent place in the diet of urban and rural populations (Folefack et al., 2013). Unfortunately, all phases of plant development are attacked by insects (Bhubaneshwari et al., 2014). Among the diversity of insects that have adapted to cowpea stocks, the Bruchidae family ranks first (Bhubaneshwari et al., 2014). *Callosobruchus maculatus*, is the main pest affecting the storage of cowpea seeds in northern Cameroon (Ngamo and Hance, 2007). Infestation begins in the field at the pod level and then continues during storage (Bhubaneshwari et al., 2014). The female lays seventy to one hundred eggs, which she sticks with a sticky substance on the cowpea seeds. The larvae of the bruchids feed and grow inside the seeds and emerge as adults after three to four weeks approximatively. Adults mate and give birth to another generation and the cycle is repeated continuously (Bhubaneshwari et al., 2014). Losses due to *C. maculatus* can reach 100% in tropical Africa six months after infestation; making cowpea unmarketable (Chougourou and Alavo, 2011). During their development, the larvae of bruchids produce the nitrogen in the form of toxic uric acid which is accumulated inside the seeds and reduce their quality. *C. maculatus* therefore constitutes a major obstacle to the consumption and development of cowpea production (Doumma et al., 2011, Moumouni *et al.,* 2013, Bhubaneshwari et al., 2014).
To fight against *C. maculatus* and limit its damages, the peasants use several means of struggle like the physical fight which is based on the use of the plastic bags, the hermetic containers, the technique of triple bagging, the humidity, the temperature and anoxia (Folefack et al., 2013). Synthetic chemical control relies on the use of chemical insecticides, that often cause contamination of the biosphere and the food chain, loss of diversity, eradication of non-target species such as auxiliary fauna and the appearance of resistant insects. In addition, the cost of chemical insecticides makes them inaccessible to the users (Ngamo and Hance, 2007). Due to the considerable negative impact of synthetic physical and chemical control, it is necessary to find low-cost or non-polluting control methods that are less expensive, available and effective against *C. maculatus* (Kayombo et al., 2014). In this context, the valorization of plants with insecticidal effect is gaining more and more importance in research programs all over the world and particularly in Africa. These plants are exploited in many forms, like powders and extracts, to limit post-harvest losses (Kouninki et al., 2015; Kongne et al., 2018). Different plants extract may act synergistically to effectively inhibit pest grow and development compared with a single plant extract (Kouninki et al., 2010; Kouninki et al., 2015; Kouninki et al*.,* 2019).
Studies carried out in several agrosystems in Africa and particularly in Northern Cameroon reveal that most of the plants are known by the local populations who, used plants traditionally for their insecticidal or repellent effects to preserve agricultural products, such as the cowpea seeds (Ngamo et al., 2007; Kouninki et al., 2015; Kongne et al., 2018). Because of their safety and low toxicity to humans, these medicinal plants could be used as an alternative to protect cowpea seeds.
The aims of the present work was to evaluate the bioactivity of methanolic extracts combinations of *Carica papaya*, *Carissa edulis*, *Securidaca longepedonculata* and *Vinca rosea* against *C. maculatus* in the Far-North region of Cameroon through adults' mortality, oviposition, number of larvae, emergence after one, two and three generation, weight loss and germination.
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MATERIALS AND METHODS

Rearing of Callosobruchus maculatus

The strain of *C. maculatus* adult 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 bucket with the perforated lid of tiny holes. In each bucket 2 kg of cowpea seeds and adults of *C. maculatus* were introduced and allowed for oviposition. That rearing took place under ambient 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 continuously by replacing the devoured and infested seeds with fresh and uninfested ones.

Grain desinfection

The *V. unguiculata* seeds obtained from and experimental field were used.
The cowpea seed were dried in the sun for a week, desinfected and sieved in order to remove the broken grains and attacked grains in the field. The obtained cowpea seeds was placed in a freezer at 4 ° C for 48 hours in order to eliminate any trace of the different stages of development which might remain inside the grain.

Collection and methanol extraction of the plant samples

The fresh bark of roots of *Securidaca longepedonculata* and leaves of *Carica papaya*, *Carissa edulis* and *Vinca rosea* were collected from natural population in the far North region of Cameroon. The harvested parts were dried in the shade under normal room temperature and crushed separately in a mortar to obtain the various fine powders. The total extracts were obtained by a double extraction in methanol at 95°. Thus, the powders of specific mass of each plant species were introduced into a 5 liters container and 2 liters of methanol at 95 ° were added. The mixture was subjected to mechanical (cold) stirring, then macerated for 48 h, then filtered using whatman No. 2 paper. The resulting solution had 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 utilization.

Experimentation

Preparation of methanolic solutions combination

For a combination of three plants extracts (Ca/Cas/V, Ca/Cas/S, Ca/ V/S, Cas/V/S), 1/3 (33.33mg) of each methanolic plant extract were added to 1 mL of methanol in order to obtained 100mg / mL of solution and for 200mg/mL of solution, 2/3 (66.66mg) of each methanolic plant extract were added to 1mL of methanol.

For a combination of four plants extracts (Ca/Cas/V/S),1/4 (25mg) of each extract were added to 1 mL of methanol in order to obtain 100mg /mL of solution and for the 200mg /mL solution 1/2 (50mg) of each extract were added to 1 mL of methanol in order to obtain 200mg /mL (Table 1).

Table 1. The different formulations of methanolic extract of plant made.

Quantity	Plants extracts combinations	
	of three plants extracts	of four plants extracts
	Ca/Cas /V, Ca/Cas /S, Ca/V/S, Cas/V/S	Ca/Cas/V/S
100mg/mL	1/3 + 1/3 + 1/3	1/4+ 1 /4 +1/4+ 1/4
200mg/mL	2/3 + 2/3 + 2/3	1/2+ 1/2+ 1/2+ 1/2
0mg/mL	0	0

(Ca: Carica papaya; Cas: Carissa edulis; V: Vinca rosea; S: Securidaca longepedunculata)

Evaluation of the insecticidal effect of methanolic extracts towards Callosobruchus maculatus

For each test, 100 and 200 mg of extract of each combined plant extracts were dissolved in 1 mL of methanol at 95°, in order to obtain methanol solutions at different concentrations. The methanolic solution obtained from each plant was mixed in a glass jar of 500mL with 50 g of cowpea seed and sweetly mixed. The glass jar was open 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 muslim cloth. The muslim cloth were held in place by numbs in order to disallow the entry or exit of any insect and then were placed in the laboratory.

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

Three replicates were made for each treatment.

The efficacy of plant extract combination against *C. maculatus* was evaluated considering parental mortality, number of grain with trace of eggs, number of larvae, adult's emergence, seeds weight loss and germination from treated and untreated seeds in term of parameters given below:

Effect of the different methanol plant extract combination 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 after 72 hours of contact.

The percentage of mortality was defined as follows:

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

Number of eggs laid

Six days after infestation, sample 50 cowpea seeds randomly taken in each jar were carefully examined. Seeds with trace of eggs or without eggs were separated. After separation the average numbers of seeds bearing eggs were recorded.
The percentage of seed bearing trace of eggs was defined as follows:
% 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 were randomly taken from each jar and the cotyledons were gently separated by a cuter and the number of larvae were counted and recorded. The percentage number of larvae is defined as follows:
% 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, two and three months of storage.
After one, two and three months of infestation, the number of adults from F_1, F_2 and F_3 which emerge in each jar were counted and recorded.
The percentage of reduction in emergence were calculated and defined as follows:
% of reduction in emergence = (Number of newly emerged adults in untreated jar - number of newly emerged adults in treated jar / Number of newly emerged adults 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 pulse beetle was determined. The percentage of weight loss was evaluated as follows:
% of weight loss = (Initial weight-weight at the end of the experiment/ initial weight)*100

Germination tests
Three months after storage, twenty-four cowpea seeds were collected randomly and sown in an experimental field in each pole, three grains 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 pole, cowpea type, treatment type and recorded. The percentage of germination was evaluated as follows:
% of seed viability = (Number of seeds that germinated/Total number of seed sown)*100

Statistical analysis
Data were analysed using Statgraphic 5.0. The one way analysis of variance was used to compare treatments. Significant differences between data were determined by the least significant difference (LSD) at the level of 5%.

RESULTS AND DISCUSSION

Results
In all experimentations made, considerable differences were noted with the different combination made.

Effect of combined methanolic extracts on adult mortality oviposition and larvae of Callosobruchus maculatus of contact

In all experimentations made, considerable differences in insect mortality, oviposition and larvae were shown with the different combination made.

The action of the insecticidal effect of combined methanolic extracts on adult mortality oviposition and larvae of *C. maculatus* was presented in the table 2.
The percentage of mortality increased with the quantity of methanolic extract used.
The action of the combined extracts was very effective. At a dose of 200 mg/mL, 86.67%; 89.28% and 82.14% of mortality were observed respectively for Ca/Cas/V, Ca/V/S and Cas/V/S combination. The combination of the four plants extract Ca/Cas/S/V induced a maximum mortality rate (100%). Thus, the combination of the extracts of the plant increased the insecticidal activity of the formulation consequently increased the mortality of *C. maculatus*.

The effect of extracts significantly reduced considerably the oviposition by the female of *C. maculatus*. The percentages of oviposition varied with the combination of methanolic extracts of plants used. The rate of oviposition decreased when the quantity of methanolic extract used increased. The higher rate of oviposition was observed in the control.

The plant extract reduced significantly the number of larvae. The higher number of larvae was found with Cas/V/S combination. The higher number of larvae was observed in the control. As for the oviposition, the number of larvae decreased with the increasing of the quantity of the methanolic extract combination.

There was a significant difference at the 5% level for adults' mortality, oviposition by female and larvae.

Table 2. Effect of combined methanol extracts on adults' mortality, oviposition and larvae of *Callosobruchus maculatus.*

Quantity used	Combination of plant extract	%Mortality after 72 hours	% Oviposition	%Larvae
100mg/mL	Ca/Cas/V	66.33±11.55 b	12±1.7e	1±0b
	Ca/Cas/S	70±10c	7.33±0.5d	4.66±0.58c
	Ca/V/S	80±0d	5.33±0.58c	0±0a
	Cas/V/S	73.33±5.77cd	9±1f	9±1e
	Ca/Cas/S/V	93.33±5.77g	3.33±0.58b	0±0a
200mg/mL	Ca/Cas/V	86.67±5.77e	8.33±0.58e	0.67±0.58ab
	Ca/Cas/S	92.85±5.77d	5±1c	0.67±0.58ab
	Ca/V/S	89.28±10f	3.66±0.58b	0.67±0.58ab
	Cas/V/S	82.14±15.26d	8.33±0.58e	8.33±0.58d
	Ca/Cas/S/V	100±00h	1.33±0.33a	0±0a
0mg/mL	/	0.0±0.0a	32.00±2.65g	14.33±2.31f

In the same column, values followed with the same letter are not significantly different (P>0.05) LSD test

Effect of combined methanol extracts on the emergence of Callosobruchus maculatus F1 after one, two and three months of storage

The impact of the different combinations of the methanol plants extracts used on the emergence of F1, F2 and F3 of *C. maculatus* after one, two, and three months was presented in table 3. Respectively at the dose of 100 and 200 mg/mL, the Ca/Cas/S/V, Ca/V/S and Cas/V/S combination were the most effective with 0% emergence since the quantity of 100mg/mL. Almost no emergence was noted in the jar containing methanolic plant combination extract. In the control jar, the number of individual which emerged increased with the generation. The number of emerging individuals was higher in control jar than those treated. These number increased with generation. There was a significant difference at the level 5% for the number of individuals emerging from *C. maculatus* with the different treatment made.

Table 3.The impact of the different combinations of the methanol plants extracts used on the emergence of F1, F2 and F3 of *Callosobruchus maculatus* after one, two, and three months.

Quantity used	Combination of methanol plant extract	Emergence		
		F1	F2	F3
100mg/mL	Ca/Cas/V	1±0b	1.33±0.57a	1.33±0.57a
	Ca/Cas/S	2.33±0.57b	1.66±1.52a	1.66 ±0.57a
	Ca/V/S	0±0a	1±1a	2±0b
	Cas/V/S	0.66±0.57ab	0±0a	1±0a
	Ca/Cas/S/V	0±0a	0±0a	0±0a
200mg/mL	Ca/Cas/V	0.66±0.57ab	0±0a	0±0a
	Ca/Cas/S	0.66±0.57ab	0±0a	0±0a
	Ca/V/S	0±0a	0±0a	0±0a
	Cas/V/S	0±0a	0±0a	0±0a
	Ca/Cas/S/V	0±0a	0±0a	0±0a
0mg /mL	/	14±0a	54±1b	61.66±0.57c

In the same line, values followed with the same letter are not significantly different (P>0.05) LSD test

Evolution of the loss of mass and germination of Vigna unguiculata after three months of storage with the combined extracts of plants

The impact of the different combination of the methanol plant extract used on the loss of mass and germination of *V. unguiculata* was presented in table 4. Respectively at the dose of 100 and 200 mg / mL, the impact of phytoinsecticides on *C. maculatus* on the conservation of grain mass during storage varied indifferently according to the combinations used. The loss of mass of cowpea seeds decreased with the increasement in the quantity of methanol extract plants. Cas/V/S, Ca/V/S, Ca/Cas/S, Ca/Cas/V and Ca/Cas/S/V extracts were effective in preserving the mass of grains. There was more mass loss of the grains with Ca/V/S, Cas/V/S, Ca/Cas /V and Ca/Cas/S/V. The increase in the quantity of the extract combinations increased with their insecticidal effect against *C. maculatus* and consequently increased the preservation of the mass of the grains being stored. The loss of mass of cowpea seeds was higher in control.

Respectively at the dose of 100 and 200 mg / mL, the Cas/V/S, Ca/V/S, Ca/Cas/V, Ca/Cas/S and Ca/Cas/S/V extract combinations had an effective increase effectiveness in the conservation of the germinative power of grains. In control, the rate of germination was lower than those of treatment with methanol plants extracts. 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 level 5%.

Table 4. Loss of mass and germination of *Vigna unguiculata* rate after three months of storage with the combined extracts.

Quantity used	Combination of methanol plant extract	Mass losses (%)	Germination rate (%)
100mg/mL	Ca/Cas/V	28±10g	44.44±19.24b
	Ca/Cas/S	22.66±1.15d	66.66±33.33c
	Ca/V/S	24±5.29e	44.44±19.24b
	Cas/V/S	46±5.29h	44.44±19.24b
	Ca/Cas/S/V	16±5.29c	100±0f
200mg/mL	Ca/Cas/V	4±3.46b	88.88±19.24e
	Ca/Cas/S	12±5.29b	77.77±38.49d
	Ca/V/S	12±8.72b	77.77±19.24d
	Cas/V/S	26±12.16f	77.77±19.24d
	Ca/Cas/S/V	3.33±2.31a	100±0
0mg /mL		72.66±1.15g	0.0±0.0a

In the same column, values followed with the same letter are not significantly different (P>0.05) LSD test

Discussion

These studies shown that the effectiveness of plant with an insecticidal effect in the protection of foodstuffs in the form of grain against insect pests depend on its rate of toxicity on the target insect, its effects on development, reproduction and behavior (Koubala et al., 2013; Kosma et al., 2014; Kouninki et al., 2015, Kongne et al., 2018) . Several studies have also been carried out on the impact of plants during storage. The objective of this work was to determine the impact of a series of methanol extracts plants combinations of four plants (*Carica papaya*, *Carissa edulis*, *Securidaca longepedonculata* and *Vinca roseae*) on adults 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 after three months of storage.

At the dose of 200 mg/mL, the mortality rates of *C. maculatus* obtained after 72 hours infestation increased with the quantity of extract used, varied according to the different extracts. The extracts could contain secondary metabolites such as alkaloids, terpenoids, flavonoids and saponins which was responsible of their insecticidal effect, on the Coleoptera in stock and therefore on the biological activity of *C. maculatus*. They also inhibited food intake, destroyed insect cell membranes, causing the mortality of *C. maculatus* (Kasturi, 2010; Gitahi et al., 2015; Kouninki et al., 2015). These results corroborate those obtained by Stevenson et al. (2009) after treatment of cowpea with the methanol extracts of the roots of *S. longepedonculata* which would contain the methyl salicylate acid. Methyl salicylate acid could be a real natural pesticide against *C. maculatus* (Ojewolé, 2008).The toxic effects of the extracts may depend on the level of insect sensitivity and the insect groups used for the test (Kouninki et al., 2005).The highest mortalities observed due to extracts combination could be explained by the abundant presence of alkaloids (more than 120) and saponins with insecticidal effects (Stevenson et al., 2009; Kastouri et al., 2010) .

The mean number of eggs laid on cowpea grains after treatment with plant extracts at the 100 and 200mg/mL respectively reduced significantly the laying of egg by the female of *C. maculatus*. The lowest numbers of egg laid were obtained with the combination extracts containing *S. longepedonculata* and *V. rosea*. These plants could contain high proportions of phenols, flavonoids, tannins and saponins which have insecticidal effects as mentioned by Stevenson et al. (2009) and Vadeyar et al. (2010) 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 (Vadeyar et al., 2010). According to Schmidt *et al.* (1991), the ovicidal effect could be due to the direct toxicity of the active molecules which lead to the early death of *C. maculatus* adults. These results are in accordance with those of Farias et al. (2007) 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, Kongne et al. (2018), showed that the methanolic extract of the roots of *S. Longepedonculata* reduced the oviposition by females of *C. maculatus*.

The larvicidal effect increased with the quantity used for the experimentation. All the plant extracts reduced significantly larval development. The coating of the cowpea grain with the extracts plant extract combination could act by making difficult the penetration of neonatal larvae into the grains (Kouninki et al., 2010). The mean numbers of emerging *C. maculatus* obtained were different according to the plant extract combination. Extracts which contained *S. longepedonculata* and *V. rosea* plants remained the most effective because they were also highly larvicidal. The obtained results could be explained by the presence of biologically active molecules in the two plants. These results corroborate those of Stevenson et al. (2009) and Vadeyar et al. (2010) 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 of emerging insects in the jars treated could be explained by the absence or low mean of the larvae observed in the jars. This corroborates the results of Kongne et al. (2018) which showed that the low rate of emerging individuals was related to the number of larvae and their susceptibility to insecticidal plant. The low emergence could also be explained by the increase in the quantity of the active molecules and the larval competition (Kongne et al., 2018) within the same grain leading to the death of *C. maculatus*.

The impact of the four plant extracts combination against *C. maculatus* on the loss of grain mass during storage varied according the extract used and the quantity of extract. The increasement in the quantity of the extract increased with its insecticidal effect against *C. maculatus* during storage. These results corroborate those of Bossuet and Vadez (2013) which work revealed that the loss of mass during storage was also attributed to the lipid degradation of legume seeds and damage caused by insect pests such as cowpea weevil.

The impact of plants extracts combinations on *C. maculatus* on the conservation of the germinative capacity of grains during storage varied. Depending on the different treatments made, these results showed that the conservation of the germinative power was different according to the combination made. These results corroborate those of Kayombo et al. (2015) who found the highest germination rate recorded with the 45gr/kg powder of *Tephrosia vogelii*, which was explained by the fact that the emergence of *C. maculatus* was low. 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 shown that methanolic extracts combination of insecticidal plant could be used for the management of *C. maculatus* during storage and might contribute to the conservation of the mass and the germinative power of cowpea

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