

BIOLOGICAL ACTIVITIES OF *CASSIA MIMOSOIDES*; *EUCALYPTUS CAMALDULENSIS*; *VEPRIS HETEROPHYLLA* PLANT EXTRACT TOWARD OLD LARVAE AND ADULTS OF *TRIBOLIUM CASTANEUM* (COLEOPTERA: TENEBRIONIDAE)

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Abstract: In Cameroon and particularly in the Far North region, post-harvest losses are a major problem for rural populations. To reduce losses due to storage pests, the use of biopesticides, remains the recommended method because it provides effective effect against pests without causing health problems to consumers and degrading the environment. This study aimed to evaluate the toxicity and repellent effect of *Cassia mimosoides*, *Eucalyptus camaldulensis* and *Vepris heterophylla* extracts on adults and oldest larvae of *Tribolium castaneum* (Coleoptera, Tenebrionidae). Contact toxicity and repulsion percentages were recorded at different doses (0.125; 0.25; 0.5 and 0.75 g/ml). At the end of the toxicity test, death rates increase with dose, the duration of exposure and the plant used both in adults than in larvae. A significant difference between treatments applied ($P < 0.05$) were noted. Thus the extracts of *Cassia mimosoides* and *Eucalyptus camaldulensis* give a total mortality ($100 \pm 0\%$) in adult for the concentration 0.75g/ml 72 hours after exposure, $97.5 \pm 5\%$ of mortality were obtained with *Vepris heterophylla* extract. In addition to the concentration of 0.125g/ml after 72 hours of exposure, the adult mortality is below 40 % for the three plants extract used. The mortality for larvae at the same concentration and the same exposure period were respectively $50 \pm 4.08\%$; $43.75 \pm 4.79\%$ and $42.5 \pm 9.57\%$ for the three plants. So the toxicity reaction of these extracts is faster for larvae and adults. The repellent effects of the three plants extracts used towards adults and the oldest larvae of *Tribolium castaneum* have a repulsive class IV order (repulsive) with an average of repulsion between 60 and 80%. These results show that the extracts of the three plants possess repellent compounds. This percentage of repulsion increases with the concentration used. Thus plants extract of the different can be use as a novel safe method to protect the grains from the damages caused by *T. Castaneum*.

Keywords: *Tribolium castaneum*; *Cassia mimosoides*; *Eucalyptus camaldulensis*; *Vepris heterophylla*; toxicity; repulsion; plant extracts.

INTRODUCTION

Malnutrition continues to accelerate in developing countries despite works done in the food sciences (OMS, 2011). The food and nutrition security of the population is a major challenge in sub-Saharan Africa because chronic malnutrition affects almost 40% of children under 5

years old (OMS, 2011). There is a need to increase demand of the population; by increasing agricultural production and reduce losses before and after harvest (Delobel and Tran, 1993). In the Sahel, cereals and leguminosis are the staple food of the population (Gueye *et al.*, 2010), deficits in animal and vegetable particular proteins origin are among the most common nutritional deficiencies in Africa (FAO, 2016). In the Far North region of Cameroon (FAO, 2011; Tamgno and Ngamo, 2014), the harvest products are annually while consumption is done all over the year (Kouninki *et al.*, 2007; Tamgno and Ngamo, 2014). Unfortunately between harvest and consumption period, over 30% of the production is lost (Ngamo and Hance, 2007). To ensure the availability of food throughout the year, storage is an indispensable solution (Ngamo 2000). However, this conservation is compromised by the damage due to insect pests (Alzouma 1990; Helbig, 1995; Kouakap 2002; Nukenine *et al.*, 2002; Ngamo 2004; Ngamo and Hance, 2007; Ngamo *et al.*, 2007). In the wide range of pest insects, the best known is *Tribolium castaneum* Herbst (Nanfack *et al.*, 2015). It represents a very important part of the stored product pests (Kouninki *et al.*, 2007) and can cause significant losses by reducing the quality and quantity of stored products (Scotti, 1978; Gwinner *et al.*, 1989; Koussou and Bosque-Perez, 1992; Kouninki 2001; Kouninki *et al.*, 2007; Nanfack *et al.*, 2015, Mahama *et al.*, 2017). Facing to these pests, control methods commonly used by farmers is the use of synthetic chemical pesticides despite the claims brought against their utilization because they selection of resistant strains (Goudoum *et al.*, 2010), poisoning, environmental pollution and ecological disorder (Regnault-Roger, 2002; Afful *et al.*, 2012;. Khaliq *et al.*, 2014). There is a great need to look for alternatives, cleaner and more innovative methods as the use of plants with insecticidal effects (Obeng-ofori, 2007). The potential use of botanical plant in storage pest management system is to be promote.

The Far North Region of Cameroon is an important farming area which many local resources to be used as botanical pesticides and alternatives to hazardous conventional chemical pesticides used in stored product protection (Kouninki *et al.*, 2010, Tamgno and Ngamo, 2014). The use of local plants seems to be economic and environmentally friendly (Kouninki *et al.*, 2010 ; Tamgno and Ngamo, 2014, Kouninki *et al.*, 2015). However little or no work has been conducted on extracts from insecticidal plants. Keeping in view the importance of plant extracts, the present study was carried out to test the efficacy of commonly used plant leaves extracts of *Cassia mimosodes*, *Eucalyptus camaldulensis* and *Vepris heterophylla* on old larvae and adults of *Tribolium castaneum*.

It will therefore specifically to assess the toxicity and repellency of hexane extracts of *C. mimosoides*; *E. camaldulensis* and *V. heterophylla* against the old larvae and adults of *T. castaneum*.

MATERIALS AND METHODS

Insect rearing

The adult beetles of *T. castaneum* were introduced into uninfested flour of *Sorghum bicolor* and allowed for oviposition after which the adults were sieved out 7 days later. The adults that emerged were transferred into another jar such that the F1 adults (which were used as the culturing stock for the experiments) were at the uniform size and age. The set up was kept under laboratory conditions.

Adult insects of 1 day and 5-6mm length of old larvae of *T. castaneum* were used for the contact toxicity and repellency tests.

Collection of the plant samples and extraction

Collection

V. heterophylla (Rutaceae), *E. camaldulensis* (Myrtaceae), *C. mimosoides* (Fabaceae) leaves were collected from natural populations during December 2015 in the far North region of Cameroon.

Preparation of plant powders

The leaves were removed from their branches and were air-dried in shade under normal room temperature for 14 days to ensure that they were well dried. They were pounded using mortar. The different powder obtained were then sieved with a fine mesh.

The obtention of hexane extract of *Cassia mimosoides*, *Eucalyptus camaldulensis*, and *Vepris heterophylla*

For each powder of *C. mimosoides*, *E. camaldulensis*, and *V. heterophylla*, 1kg have been weighed with an electronic balance marked LT lutron GM-300p 300.000g x 0.01 of capacity 300g/0.01g. 1000g of dried powder was then extracted by maceration in 3000 mL of hexane put in a glass bottle of 5L of capacity. After 3 days, the hexane extract have been decanted and filtrated with a wattman paper N° 1. Solution containing the solvent was evaporated by rotary evaporator under reduced pressure which produced the crude hexane extract. The operation was repeated two times for every plant used.

The obtained hexane extract were then kept in the refrigerator at 4°C until the experimentation.

Contact toxicity of the different hexane extract towards old larvae and adults of *Tribolium castaneum*

Four doses were prepared by diluting each time in 1 ml of hexane in the respective volumes of 0.125; 0.25; 0.5 and 0.75 g / mL of the three different extracts of plants used. Each of the prepared solutions was uniformly spread on a filter paper disk (Whatman No. 1) of 9 cm in diameter raised in a glass Petri dish of 9 cm in diameter. The filter paper disc was left at room temperature for 15 minutes to allow the complete evaporation of the hexane dilution.

20 adults of *T. castaneum* and old larvae of 5-6mm in length collected in the rearing milieu were introduced in Petri dish containing a treated disc; then the plates were immediately closed. Four replications were conducted for each dose.

The number of dead insects were counted after 6, 12, 24, 48 and 72 hours of exposure and mortality rate were calculated.

Repellency test on Petri dishes of different extracts towards older larvae and adults of *Tribolium castaneum*

The repellent effect of the three plants extracts of *C. mimosoides*, *E. camaldulensis* and *V. heterophylla* against *T. castaneum* adults was evaluated using the area preference method. Test areas consisted of Whatman No.1 filter paper cut in half (9 cm) of diameter. 0.125, 0.25, 0.5 and 0.75g of the extract of the three plants were diluted in 1 mL of hexane and was evenly applied on half-filter paper discs using a micropipette. The other half filter paper was treated with 1 ml hexane alone and used as control. The filter papers were air-dried for about 15 min in order to evaporate the solvent completely and full discs were subsequently remade by tied treated halves to untreated halves with clear adhesive tape. Each remade filter paper disc was tightly fixed on the bottom of a 9 cm diameter Petri dish. Twenty unsexed *T. castaneum* adults and old larva of 5-6mm in length were then released at the center of the filter paper disc and the Petri dishes were subsequently covered and kept in laboratory at $27 \pm 2^{\circ}\text{C}$, $75 \pm 5\%$ r.h.

Four repetitions were performed for each treatment. The distribution of insects in the treated and untreated part on the filter paper portion was raised after 1h, 2h, 6h, 12h, 24h, 48h and 72h of exposure.

Statistical analyses.

The mortality data obtained for the contact toxicity were adjusted using Abbott's correction. Four replications were performed for each test. For statistical comparison among several means, all the data on larvae and adult mortality were subjected to a

one-way analysis of variance (ANOVA), means were separated using LSD (Least significant difference $P = 0.05$).

The percentage of repulsion towards old larvae and adults of *T. castaneum* is calculated Percentage repellency (PR) values was calculated as follows:

$$PR = [(Nc - Nt) / Nc] 100\%$$

Nc : number of insects present on the control

Nt : number of insects present on the treated area

The mean percentage repellency value was assigned to repellency classes from 0 to V: using the formula of McDonald *et al.*, (1970).

class 0 (PR < 0.1%), class I (PR = 0.1 to 20%), class II (PR = 20.1 to 40%), class III (40.1 to 60%), class IV (60.1 to 80%), class V (80.1 to 100%).

RESULTS

Evaluation of the toxic effect by contact of different plants extract towards olde larva and adults of *Tribolium castaneum*

The results of the toxic effect of different extracts of the three plants towards old larvae and adults of *T. castaneum* are presented in Tables 1 and 2 to differents concentrations used (0.125g / mL; 0.25 g / mL; 0.5 g / mL and 0.75g / mL), and exposure times, there is a statistically significant difference ($P < 0.05$) between the various treatments.

Evaluation of the toxic effect of the different extract plants by contact towards old larva of *Tribolium castaneum*

The results of the toxic effect of different extracts of three plants extract against old larvae of larvae are presented in the table1. For the applied concentrations (0,125g / mL; 0.25 g / mL; 0.5 g / mL and 0.75g / mL), and exposure times, there is a statistically significant difference ($P < 0.05$) between the different treatments.

For *C. mimosoides* extract on old larvae of *Tribolium castaneum* as the results obtained showed that the mortality rate increases with the duration of exposure and concentration of the extract. Indeed, concentration of 0.125 g / mL lead to $50 \pm 4.08\%$ of mortality after 72 hours of exposure. This mortality was $53.75 \pm 8.54\%$ at the concentration of 0.25 g / mL after 24 hours of exposure and reaches $70 \pm 7.07\%$ after 72 hours of exposure to the same concentration. After 48 h exposure, this mortality reached $86.25 \pm 4.79\%$ to $92.5 \pm 5\%$ for concentrations of 0.5 g / mL and 0.75 g / mL. At 0.5g / mL of concentration after 72 hours of duration, the mortality rate is $91.25 \pm 4.79\%$. At the same period for the concentration of

0.75g / mL, the mortality rate is $98.75 \pm 2.5\%$. No effect on old larvae of *T. castaneum* was observed throughout the 72 hours of duration.

For *E. camaldulensis* plant extract on old larvae of *T. castaneum*, 72h after exposure, this mortality reached $43.75 \pm 4.79\%$; $61.25 \pm 7.5\%$; $87.5 \pm 8.66\%$ and $100 \pm 0.0\%$ respectively for the concentration of 0,125g / mL; 0.25g / mL; 0.5g/mL and 0.75g/mL.

The control treatment has no effect on old larvae of *T. castaneum* used throughout the 72 hours of duration.

For *V. heterophylla* plant extract on old larvae of *T. castaneum*, variation of the toxic effect indicates that at the concentration of 0.125g / mL, mortality rate were was $3.75 \pm 2.5\%$; $10 \pm 4.8\%$; $25 \pm 4.16\%$; $32.5 \pm 8.53\%$ and $42.5 \pm 9.57\%$ after 6; 12; 24; 48 and 72 hours of duration respectively. These mortalities were $17.5 \pm 0.45\%$; $26.25 \pm 11.09\%$; $37.5 \pm 6.49\%$; $50 \pm 8.16\%$ and $63.75 \pm 8.54\%$ during the same period of exposure for 0.25 g / ml of concentration. At the 0.5 g / mL concentration it reached $97.5 \pm 2.88\%$ after 72h of duration. At the 0.75 g / mL concentration, this mortality is significantly greater and reached the maximum at 72 h of exposure with $100 \pm 0.0\%$ of mortality.

Table 1 : Contact toxicity (%) of hexane extract of *Cassia mimosoides*, *Eucalyptus camaldulensis* and *Vepris heterophylla* against old larvae of *Tribolium castaneum*

Plant	Dose g /mL	Duration of exposure (hour)				
		6	12	24	48	72
<i>Cassia mimosoides</i>	0.00	0.0±0.0a	0.0±0.00a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	0.125	12.5±2.89c	20±4.8c	33.75±6.29cd	40±4.08c	50±4.08d
	0.25	22.5±2.89f	40±4.08f	53.75±8.54f	61.25±7.5f	70±7.07f
	0.5	51.25±4.78eij	67.5±2.89i	77.5±5hi	86.25±4.79gh	91.25±4.79gh
	0.75	61.24±6.29h	76.25±4.79h	85±7.07jk	92.5±5hi	98.75±2.5ij
<i>Eucalyptus camaldulensis</i>	0.00	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0a
	0.125	0.0±0.0a	10±4.8b	28.71±11.09bc	33.75±6.29b	43.75±4.79c
	0.25	17.5±6.25de	22.5±5d	43.75±2.5e	51.25±6.29e	61.25±7.5e
	0.5	30±14.71f	46.25±4.79h	63.75±4.79g	68.75±4.79f	87.5±8.66g
	0.75	42.5±5g	60±4.08g	76.25±6.29h	82.5±11.90g	100.0±0.0k
<i>Vepris heterophylla</i>	0.00	0.0±0.0a	0.0±00a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	0.125	3.75±2.5b	10±4.08b	25±4.16b	32.5±8.53b	42.5±9.57b
	0.25	17.5±6.45de	26.25±11.09e	37.5±6.49de	50±8.16d	63.75±8.54ef
	0.5	48.75±4.7hi	60±7.07g	83.75±4.79ijk	88.75±4.79ghi	97.5±2.88hi

	0.75	57.5±6.45jk	71.25±4.79j	88.75±2.5k	95±4.08i	100±0.0k
		Fcal=62.54 p<0.05 Ndl=59	Fcal=126.49 p<0.05 Ndl=59	Fcal=115.43 p<0.05 Ndl=59	Fcal=131.89 p<0.05 Ndl=59	Fcal=183.71 p<0.05 Ndl=59

In a same column ; data followed by the same letter are not significantly different at 5% LSD test.

Evaluation of toxic effect of the different extract plants by contact towards *Tribolium castaneum* adults

The toxic effect of the differnt extract plant used against adult of *T. castaneum* is presented in Table 2. The mortality caused by the toxic effect of the extract of the plant increases with the concentration and the period of exposure.

Thus for *C. mimosoides*, the mortality is $33.75 \pm 4.78\%$ after 72 hours of exposure for the concentration of 0,125g / mL. This mortality goes up to $62.5 \pm 6.46\%$ in the same period of exposure for the concentration of 0.25 g / mL. For the concentration of 0.5 g / ml, the mortality was $98.75 \pm 2.5\%$ after 72 hours of exposure. $100.0 \pm 0.0\%$ of mortality were noted for 0.75 g / mL concentration in the same period of exposure. No mortality was observed along the 3day exhibition for the control.

For the extract of *E. camaldulensis*, the variation of the toxic effect indicates that for the concentration of 0.125g / mL, no mortalities were noted afer 6 hours of exposition. After 48 hours of exposure $33.75 \pm 6.29\%$ of mortality were noted. The mortality rate increase to $38.75 \pm 6.29\%$ after 72 hours of exposure. At the concentration of 0.25 g/mL, mortality reached $65.00 \pm 4.08\%$ after 72 hours of exposure. $100 \pm 0\%$ of mortality is obtained with 0.5 g / mL and 0.75 g / mL of concentrations 72 hours after exposure. In the control no mortality along the 3-day exhibition were noted on adults of *T. castaneum*.

For the extract of *V. hetrophylla* against adult *T. castaneum* at 0,125g / mL of concentration, no mortality were noted until 12 hours of exposure); this mortality is $10.00 \pm 4.08 \%$ after 24 hours of exposure and $27.5 \pm 5.5\%$ after 72 hours of exposure. At 0.25g / mL of concentration, the mortality is $48.75 \pm 7.5\%$ after 72 hours of exposure. For 0.5g / mL of concentration, the mortality of adult reached $88.75 \pm 8.53\%$ after 72 hours of exposure. At the 0.75g / mL of concentration, mortality is $60.00 \pm 7.07\%$ after 12 hours of exposure ; this mortality was $82.5 \pm 2.59\%$ after 24 hours of exposure; reached $91.25 \pm 5\%$ after 48 hours of exposure. $97.5 \pm 5.0\%$ of mortality were obtained with 0.75g / mL of concentration after 72

hours of exposure. The control treatment remains without toxic effect on adult *T. castaneum* after 72 hours of exposure.

Table 2: Contact toxicity (%) of hexane extract of *Cassia mimosoides*, *Eucalyptus camaldulensis* and *Vepris heterophylla* against adults *Tribolium castaneum*

Plant	Dose g/mL	Duration of exposure (hour)				
		6	12	24	48	72
<i>Cassia mimosoides</i>	0.00	0.0±0.0a	0.0±0.00a	0.0±0.0a	0.0±0.0a	0.0±0.0b
	0.125	12.5±2.89c	16.25±2.5d	18.75±4.79c	28.75±4.79c	33.75±4.78de
	0.25	18.75±2.5d	28.75±4.79e	47.5±5.0g	52.5±2.89i	62.5±6.46g
	0.5	62.5±2.89f	76.25±2.5gh	88.75±4.79k	96.25±4.79m	98.75±2.5j
	0.75	76.25±4.79g	90.00±4.08i	96.25±4.79l	98.75±2.5n	100.0±0.0k
<i>Eucalyptus camaldulensis</i>	0.00	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	0.125	0.0±0.0a	6.25±2.5b	26.25±2.5d	33.75±6.29d	38.75±6.29e
	0.25	16.25±2.5cd	23.75±4.79e	42.5±2.89f	48.75±4.79f	65.00±4.08g
	0.5	43.75±4.79de	75.00±4.08g	80.00±4.08i	95.00±4.08lm	100.0±0.0k
	0.75	50.00±2.67e	81.25±4.79hi	87.5±2.89j	98.75±2.5n	100.0±0.0k
<i>Vepris heterophylla</i>	0.00	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
	0.125	0.0±0.0a	0.0±0.0a	10.00±4.08b	13.75±8.53b	27.5±5.5c
	0.25	10.00±0.00bc	11.25±2.5c	37.5±2.89e	43.75±4.79e	48.75±7.5f
	0.5	26.25±4.78d	43.75±4.79f	72.5±8.66h	76.25±11.08j	88.75±8.53h
	0.75	35.00±4.08cd	60.00±7.07fg	82.5±2.59ij	91.25±5k	97.5±5.0ij
		Fcal=44.83 p<0.05 Ndl=59	Fcal=298.94 p<0.05 Ndl=59	Fcal=294.46 P<0.05 Ndl=59	Fcal=289.94 p<0.05 Ndl=59	Fcal=152.37 p<0.05 Ndl=59

In a same column ; data followed by the same letter are not significantly different at 5% LSD test.

Evaluation of the repellent effect of extracts from various plants towards old larvae and adults of *Tribolium castaneum*

The percentage of repulsion of different plant extracts towards old larvae and adults of *Tribolium castaneum* varies with the concentration of the plant extract and the exposure time.

Evaluation of the repellent effect of the extracts of the various plants towards old larvae of *Tribolium castaneum*

The repellent effects of different extracts plants used towards old larvae of *T. castaneum* larva are summarized in Table 3.

The repellent effects *C. mimosoides* extract induced two classes repulsion. Moderately repulsive class is induced by 0.125g / mL and 0.25g / mL concentrations with 35.07 and 42.50% of repulsion respectively. 0.5g / mL and 0.75 g / mL of concentrations induced 83.92 and 92.14% repulsion respectively trasucing a very repulsive class.

With *E. camaldulensis* extract, 0.125g / mL ; 0.25 g / mL; 0.5 g / mL and 0.75 g / mL of concentrations; caused respectively 30.35; 52.50; 78.57 and 89.28% of repulsion. Increasing concentrations at different for treatments lead to repulsive classes that differ depending on the dose used (Table 3).

For *V. heterophylla* extract, repulsive effects increase with the concentration. The concentration of 0.125g / mL ; 0.25 g / mL; 0.5 g / mL and 0.75 g / mL; caused respectively 20.35 % (weak repulsive class) ; 51.78% (moderately repusive class) ; 81.78% and 87.71% (Very repulsive class) of repulsion.

Table 3 : Repellent effects of different plant extracts from against old larvae of *Tribolium castaneum* noted after 72 h of exposure.

Plant extract	Concentration (g/ml)	Mean of repulsion (%)	Repulsive class
<i>Cassia mimosoides</i>	0.125	35.07	II
	0.25	42.50	III
	0.5	83.92	V
	0.75	92.14	V
Mean of repulsive class	/	63.41	IV
<i>Eucalyptus. camaldulensis</i>	0.125	30.35	II
	0.25	52.50	III
	0.5	78.57	IV
	0.75	89.28	V
Mean of repulsive class	/	62.67	IV
<i>Vepris heterophylla</i>	0.125	20.35	II
	0.25	51.78	III
	0.5	81.78	V
	0.75	87.71	V
Mean of repulsive class	/	60.41	IV

Evaluation of the repellent effect of the extracts of the various plants towards adults of *Tribolium castaneum*

Repellency percentages of different concentrations of extracts from various plants are summarized in Table 4. It appears that different concentrations of the extract of *C. mimosoides* (0.125g / mL; 0.25 g / mL; 0.5 g / mL and 0.75g / mL) caused 50.12; 59.42; 79.90; and 90.74% of repulsion respectively. So until the concentration of 0.5 g / mL, the repellent effect of all the plant extract used has a mildly repulsive class. This class becomes repulsive when concentration goes to 0.5 g / mL and very repulsive when the maximum dose is 0.75 g / mL.

With *E. camaldulensis* extract plant, the repellent effect at the concentration of 0.25 g / mL has a moderate repulsive class with 53.98% of repulsion. The concentrations of 0.25g / ml and 0.5g / mL cause 63.57 and 79.24% of repulsion respectively. The effect of these two concentrations induces a class called repulsive. When the concentration used is 0.75g / mL we have 88% of repulsion which traduce very repulsive class.

For extracted *V. heterophylla* at 0,125g / mL concentration used, a weak repulsive class is noted with 38.57% of repulsion. This class becomes mildly repulsive with 56.42% of repulsion when the concentration goes to 0.25 g / mL. At 0.5 g / mL of concentration, the percentage of repellency is 80% and for the maximum concentration (0.75g / mL), the percentage of repulsion reached 86.25%, inducing a very repulsive class.

Table 4 : Repellent effects of different plant extracts from against *Tribolium castaneum* adults noted after 72 h of exposure.

Plant extract	Concentration (g/ml)	Mean of repulsion (%)	Repulsive class
<i>Cassia mimosoides</i>	0.125	50.12	III
	0.25	59.42	III
	0.5	79.90	IV
	0.75	90.74	V
Mean of repulsive class	/	70.05	IV
<i>Eucalyptus camaldulensis</i>	0.125	53.98	III
	0.25	63.57	IV
	0.5	79.24	IV
	0.75	88	V
Mean of repulsive class	/	71.20	IV
<i>Vepris heterophylla</i>	0.125	38.57	II
	0.25	56.42	III
	0.5	80	IV
	0.75	86.25	V
Mean of repulsive class	/	65.31	IV

DISCUSSION

This study shows that extracts of leaves of three plants tested induce mortality of *T.*

castaneum, significantly higher than that observed in the controls. These mortalities significantly are depending on the dose and duration of exposure for both adults and old larva.

Extracts of *C. mimosoides* exhibit $12.5 \pm 2.89\%$ of mortality after 6 hours of exposure to 0,125g / mL (minimum dose) and $76.25 \pm 4.79 \%$ to 0.75 g / mL (maximum dose) in adults. These values will increase to 33.75% respectively ± 4.78 and $0.00 \pm 100\%$ after 72 hours. Furthermore larval mortality reached $98.68 \pm 2.63\%$ after 72h of exposure with 0.75 g / mL in adults. For larva the difference is significant between doses, and between the exposure time ($P < 0.05$). These results are similar with the work of Kumar *et al.* (2014) which showed that the extracts of *C. mimosoides* can act as a repellent or as growth regulator and may affect moulting and growth of the larva of arthropods. The observed toxicity may be due to the physicochemical properties of the extract plant used. The leaves of *C. mimosoides* contain alkaloids which are secondary metabolites (Bruneton, 2009) which are metabolically active and may play an important role in the physiology of plants or organisms.

E. camaldulensis extracts showed a significant difference in terms of dose and duration of exposure ($P < 0.05$) both in adults and larva of *T. castaneum*. The results are consistent with the work of other researchers who have demonstrated the insecticidal activity of *E. camaldulensis*. We can list in this case the work of Tapondjou *et al.* (2002) on the genus of *Eucalyptus*, they tested the essential oil of *Eucalyptus smithii* towards six pests of stored products (*Callosobruchus maculatus*, *Callosobruchus chinensis*, *Acanthoscelides obtectus*, *Sitophilus granarius* and *Sitophilus zeamais*), a concentration of 0.4% of concentration caused the death of more than 60% of weevils after two days of exposure.

The observed toxicity will be mainly due to its chemical constituents (tannins, monoterpenes, pinene, cineole 1,8 ...). Monoterpenes have larvicides effect towards these pests (Ngassoum *et al.*, 2007). Kumar (2012) noted in his work that the α - pinene had a toxic effect towards the larvae and adults of *T. castaneum* and *S. oryzae*.

Our studies has also demonstrated the insecticidal and larvicidal extracts of *V. heterophylla* towards adults and old larva of *T. castaneum*. Phytochemical studies have revealed the presence of the compounds wich have repellents effects in both larvae on adults. We can cite for example the alkaloids (Koné *et al.*, 2012.), and oxygenated sesquiterpenes which are also the major compounds of this past (almost 42%) (Ngassoum *et al.*, 2007; Ngamo *et al.*, 2007). Indeed *V. heterophylla* leaves have long been used by farmers to protect their stored product stock (Toumou et al, 2012; Hamawa et al, 2012; Hamawa and Mapongmetsem, 2014;

Nanfack *et al.*, 2015). The results are similar to those of Iliassa and Ngamo (2015) that showed the effectiveness of *V. heterophylla* towards *S. oryzae*.

There is great variation in species susceptibility to the same extract (Delobel, 1994) or even for the same compound (Regnault-Roger *et al.*, 2008). The same molecule of allelochemical does not necessarily have the same activity at different stages of the life cycle of an insect. Our work show that the three extracts used have not the same effect towards *T. castaneum* efficiency. The effectiveness of our extract is variable from one plant to another and from one stage of development to another. For example for adults, after 6 hours of exposure and at 0.75 g / ml dose to a 76.25 ± 4.79 % mortality is noted in *C. mimosoides*; 50.00 ± 2.67 % with *E. camaldulensis* and $35.00\% \pm 4.08$ with *V. heterophylla*. After 72 hours of exposure and at the same dose (0.75 g / ml) this mortality is $100\% \pm 0.00$, respectively; $100\% \pm 0.00$; $97.5 \pm 5.00\%$ for the three plants against old larva. The effectiveness of these hexane extract plants on *T. castaneum* follows the following chronology: *V. heterophylla* ; *E. camaldulensis* and *C. mimosoides*. Indeed the work of Iliassa and Ngamo (2015) on *V. heterophylla* justified that this inferiority is due to the fact that it is mainly composed of oxygenated sesquiterpenes which are less effective than oxygenated monoterpenes. It should also be noted that the response of the three plants is faster toward the larva of *T. castaneum* than adults. This observation was demonstrated by the work of Kouninki *et al.* (2007); Bounechada and Arab (2011) and Chaubey (2012).

In addition, various extracts of our plants are repellent toward both larvae and adults. The three plants extract used showed different repulsion percentages from each other based on the doses used.

Repellency percentages of different doses of leaves extracts of *C. mimosoides*, *E. camaldulensis*, *V. heterophylla* show that after 72 hours of exposure, the different doses of extracts of *C. mimosoides* (0.125; 0.25; 0.5; 0.75 mg / mL) resulted in average 50,12; 59.42; 79,90 and 90,74% of repulsion respectively toward adults of *T. castaneum* and 35,07; 42.50; 83.92; and 92.14% of repulsion respectively towards old larvae of *T. castaneum*. This clearly shows that the percentage of repulsion increases with the dose. From these results, we can note that the extracts of *C. mimosoides* have repellent activity (repellent) against adult and larvae of *T. castaneum* which belong according ranking of McDonald *et al.* (1970) to the repulsive class IV with a mean repellency rate of 70.05% for adults and 63.41% for the larvae. The effect are similar those observed in the work of Kumar *et al.* (2014) who have shown that *C. mimosoides* may act as a phagodissuasif towards insects pests.

Furthermore it is also clear that after 72 hours of exposure the percentage of repulsion obtained with *E. camaldulensis* according to different doses (0.125, 0.25, 0.5, 0.75 mg / mL) are respectively 53.98; 63.57; 79.24; and 88% with an average of 71.24% for adults and 30.35; 52.50; 78.57 and 89.28% with an average of 62.67% for the larvae. These results show that *E. camaldulensis* has a repulsive effect towards adults and larvae of *T. castaneum*. These results are similar to those of Taponjrou *et al.* (2002) on Eucalyptus. Similarly, the repulsive nature of this extract against adults and larvae of *T. castaneum*, could be explained by its high content of eucalyptol (1,8-cineol). Indeed, in a study of the biological activity of 1,8-cineole against weevils food stored, Obeng-Ofori *et al.* (1997) demonstrated the repellent effect of this constituent in respect of *Sitophilus granarius* and *S. zeamais*.

However *V. heterophylla* shows a relatively lower percentage of repulsion to *C. mimosoides* and *E. camaldulensis* with an average of 65.31% for adults and 60.41% in larvae. Despite this, it appears as ranked by McDonald *et al.* (1970) that *V. heterophylla* has a repellency class IV as well as the other two plants. This repellency is the result of its phytochemical properties made essentially with oxygenated sesquiterpenes (Ngassoum *et al.*, 2007). These results are in perfect agreement with the work those of Ngamo *et al.* (2007a) ; Toumou *et al.* (2012) and Hamawa *et al.* (2012) who showed repellent activity of *V. heterophylla*.

However, it would be difficult to think that the insecticidal activity of these extracts would be limited only to some of their major constituents; it could also be due to some minor constituents or to a synergistic effect of several components.

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