



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2020; 8(1): 1148-1154

© 2020 JEZS

Received: 04-11-2019

Accepted: 06-12-2019

**Mohamadou Sali Baïngang**

Department of Biological  
Sciences, Faculty of Sciences,  
University of Ngaoundéré,  
Ngaoundere, Cameroon

**Kouninki Habiba**

Department of Life and Earth  
Sciences Biology, Higher  
Teacher's Training College,  
University of Maroua, Cameroon

**Nukenine Nchiwan Elias**

Department of Biological  
Sciences, Faculty of Sciences,  
University of Ngaoundéré,  
Ngaoundere, Cameroon

**Corresponding Author:****Mohamadou Sali Baïngang**

Department of Biological  
Sciences, Faculty of Sciences,  
University of Ngaoundéré,  
Ngaoundere, Cameroon

## Contact toxicity and repellent effect of three solvent extracts of *Lippia rugosa* A. Chev. leaves on the adults of *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae)

**Mohamadou Sali Baïngang, Kouninki Habiba, and Nukenine Nchiwan Elias**

### Abstract

The use of chemical insecticides in the fight against pests is a real threat to the environment and human health. Thus, insecticidal plants are therefore a serious alternative to cure this issue. In fact, the extracts (hexane, acetone, methanol) of *Lippia rugosa* were tested on the adults of *Tribolium castaneum* to evaluate their insecticidal potential. Toxicity and repellence test were made. These extracts induce mortality of adults of *Tribolium castaneum* varied significantly with doses and duration ( $P < 0.001$ ). After 72h of exposure only the hexane extract caused 100% mortality at the maximum dose. The mortality recorded by the acetone and methanol extract are respectively  $87.50 \pm 3.22\%$  and  $96.25 \pm 2.39\%$  at the highest dose. The repellence test revealed that all the extracts are repellent toward *Tribolium castaneum* with the repellency index of  $50.62 \pm 6.43\%$  for acetone;  $77.55 \pm 4.26\%$  for methanol and  $90 \pm 4.92\%$  for hexane. The  $LD_{50}$  values at 72 hours were 1.63 g (acetone); 1.04 g (methanol) and 1.12 g (hexane). In general, the hexane extract of *Lippia rugosa* was more effective for both toxicity and repellency against the adult of *Tribolium castaneum*.

**Keywords:** *Tribolium castaneum*, extract, *Lippia rugosa*, repellence, mortalities

### Introduction

Food insecurity is a fundamental problem in Africa. This problem is increasingly aggravating for people in the Northern Cameroon where cereals and legumes are the staple food for humans, livestock and poultry<sup>[1]</sup> and the main source of economic input. In this region, the farmers face many difficulties namely: drought, recurrent water deficits, diseases, the progressive depletion of the soil, the lack of supervision of the farmers, the absence of inputs, government disengagement and disruption of some agricultural value chains, seed quality and performance, and irregular rainfall<sup>[1, 2]</sup>, which implies relatively low yields. However, crops are harvested once a year in this agro-ecological zone while consumption is spread all over the year<sup>[3, 4]</sup>. To ensure the availability of food throughout the year, storage remains a key issue.

However, this storage is compromised by insect pests, which are *Callosobruchus maculatus*, *Sytophilus zeamays*, *Sytophilus oryzae*, *Rhizophthera dominica*, *Prostephanus truncatus*, *Caryedon serratus*, *Tribolium castaneum*<sup>[5, 6]</sup>. These insects lead to considerable losses on food during storage. Moreover, IITA,<sup>[7]</sup> estimated these losses at more than 30% per year in Africa during the year 2012. In the very wide range of insect pests, we have the beetles of the family Tenebrionidae, the most vulnerable and insecticide resistant is *Tribolium castaneum*. It can cause significant losses by reducing the quality and quantity of products stored as broken rice and flours<sup>[8, 9]</sup>. In order to reduce losses due to these pests, the control methods commonly used by farmers are essentially focused on the use of physical agents such as fire, sunstroke, anoxia, temperature, humidity, and especially the use of synthetic chemical pesticides. Despite their effectiveness, they lead to the appearance of resistant strains<sup>[10]</sup>, intoxication of mammals, environmental pollution and ecological disorder<sup>[11, 12, 13]</sup>. This is why the search for alternative, environmentally friendly and more innovative solutions such as the use of insecticidal plants is to be encouraged and popularized.

An ethnobotanical survey conducted in the northern regions of Cameroon shown that the peasant populations use a variety of plants daily in the conservation of food. Among these plants of Verbenaceae family, the *L. rugosa* species is widespread in these areas and is

commonly used by peasant populations for its pharmacological properties [14]. Many researches have proved the insecticidal activity of the essential oils of *L. rugosa* towards *T. castaneum*. However, to date, there were no scientific data on the efficacy of non-volatile substances derived from extracts of this plant against *T. castaneum*. In addition, extracts of plants based on organic solvents are more complete than essential oils because they have in addition of volatile compounds also nonvolatile substances. It is in this mind that we have set the main objective in order to test the effectiveness of the hexane, methanol and acetone extracts of *L. rugosa* towards adults of *T. castaneum*. Specifically, to evaluate contact toxicity and the repellent effect of three solvent extracts (acetone, methanol and hexane) of *L. rugosa* leaves towards *T. castaneum* adults.

## Materials and Methods

### Insects rearing

Mass rearing of *T. castaneum* was done in glass jars containing 5 kg of red millet flour infested by *T. castaneum* adults. These jars are then covered with a lid which has been previously perforated to allow ventilation of the enclosure. The set was stored under ambient laboratory conditions until the start of bioassays. In the bioassays, *T. castaneum* adults were obtained by sieving red millet flour using a 2 mm mesh sieve. Their capture was carried out using an entomological forceps.

### Obtention of plants extracts

The green leaves of *Lippia rugosa* used were collected in July 2018 in the locality of Touboro, Mayo-Rey Division, North Region of Cameroon (latitude 07°79.52"N and longitude 15°35.55"E, altitude 3080 meter above sea level. The North region is in the Soudano - Sahelian agro-ecological zone of Cameroon. It was characterized by two seasons: wet (June to September) and dry (October to May). Annual rainfall is low with an average of 70 rainy days per year. The maximum temperature is 45° and the minimum is 18° whereas the R.H. stands at 67% [15]

After harvest in the morning, the leaves of *L. rugosa*, were dried in the shade (in order to avoid the destruction of the active molecules) for three days and afterwards they were exposed to the sun for a few hours before being pounded in a wooden mortar. The obtained powder was collected by sieving the ground material using a fine-mesh screen. Then 1 kg of the powder was weighed using a measuring instrument (sensitive electronic balance brand LT lutron GM-300p 300,000g x 0.01 capacity 300g / 0.01g (Germany)). It was subsequently introduced into a glass bottle with a capacity of 5 liters; where 3 liters of each extract (acetone, methanol, hexane) were added according to the methodology used by Kouninki *et al.* [16].

The mixture was shaken daily, morning and evening with a magnetic stirrer and this for 72 hours under ambient laboratory conditions. Afterwards, the macerate was decanted and then filtered using filter paper Whatman No.1. The clear solution obtained was then introduced into tared jars for evaporation. The operation was repeated twice for each extract. The extracts obtained were then concentrated to dryness under reduced pressure using a rotary evaporator. The calibrated flasks each containing an extract were then weighed to determine the extraction yield. The concentrated extract thus obtained was stored in the refrigerator at a temperature of 4 °C. Before the beginning of the experiments,

he extraction yield was determined with respect to the amount of plant material used according to the formula:

$$R\% = (Me / Ms) \times 100$$

Where Me is the mass of the extract obtained and Ms represents the dry weight mass of dry matter used.

## Bioassays

### Toxicity test

Different masses of 0.5g; 1g; 2g and 3g of the extracts (acetone, methanol, hexane) were weighed using an electronic balance brand LT lutron GM-300p 300,000g x 0.01 capacity 300 g / 0.01 g (Germany) and diluted each time in 1mL of the corresponding solvent. Each of the doses thus prepared was spread uniformly on a 9cm diameter (Whatman N°1) filter paper disc and then placed in a 9cm diameter glass petri dish. The assembly was exposed at room temperature for at least 15 minutes to allow complete evaporation of the dilution solvent. Four repetitions were performed for each of the extracts mentioned above. Controls received only either methanol or only acetone or hexane.

Twenty adult of *T. castaneum* adults not older than two days taken from their breeding medium were introduced into each petri dish containing treated filter paper. The petri dishes were closed immediately with the lid and subsequently sealed with adhesive tape. The dead insects were noted after 24; 48 and 72 hours. Were considered dead all insect which after a dry blow on the back with an entomological forceps does not react.

Mortalities in treated boxes (Mo) were expressed according to Abbott's formula, [17] for corrected mortality (Mc), taking into account the natural mortality observed in the control boxes (Mt) according to the following formula:

$$Mc = \frac{Mo - Mt}{100 - Mt} \times 100$$

### Repellency test on petri dishes

The methodology used is that based on the surface preferably described by McDonald *et al.* [18]. The filter papers 9 cm in diameter were cut into two equals parts. Four doses of extract were weighed (0.5g, 1g, 2g, 3g) and then diluted in 0.5 mL of respective solvent. Then the prepared solution was spread evenly on one half of the disc. While the other half received only 0.5mL of the dilution solvent.

After fifteen minutes, the time required for the complete evaporation of the solvent, the two halves of the disks were put back together and sealed with an adhesive tape. After assembly, the reconstituted filter paper was introduced into the bottom of the petri dishes. Subsequently, 20 adults aged of two days old of *T. castaneum* were placed in the center of the filter paper and covered immediately.

Four repetitions were performed for each treatment. The distribution of insects on the extracted and untreated portions were recorded after 24 hours according to the method used by Nukenine *et al.* [19]. The repulsion percentage (PR) of each extract was calculated according to the formula of McDonald *et al.* [18].

No = number of insects on the untreated portion;

Nc = number of insects on the treated part;

N = total number of insects introduced into the medium.

$$PR = \left( \frac{N_o - N_c}{N} \right) \times 100$$

The repulsion index has been classified according to the method of McDonald *et al.* [18]

Where class 0 (PR<0.1%)

Class I (PR=0.1-20%)

Class II (PR=20.1-40%)

Class III (PR=40.1-60%)

Class IV (PR=60.1-80%)

Class V (PR=80.1-100%)

### Data analysis

The mortality percent were subjected to the Variance Analysis Procedure (ANOVA) using the Statistical Analysis System (SAS Institute 2003). For the comparison of mortality

averages and repulsion percentages, the Tukey (HSD) test at a 5% probability was used. The Probit analysis [21] was conducted to determine lethal doses causing 50% (LD<sub>50</sub>) and 95% (LD<sub>95</sub>) mortality of *T. castaneum* after 24, 48 and 72 hours of treatment with SPSS software, [20] version 16.0. Abbott's [17] formula was used to correct control mortality prior to the application of ANOVA and Probit analysis. The repulsion index was calculated and classified according to the method of McDonald *et al.* [18].

### Results

#### Plant extraction yield

The extraction yields of powders from leaves of *L. rugosa* after 72 hours of maceration are shown in Table 1. It can be seen from this table that the acetone solvent has a higher extraction yield than the others. Hexane has the lowest yield.

**Table 1:** yields extraction of *Lippia rugosa*

Solvent name	Yield of extract (%)
Acetone	8.76% ± 0.2
Methanol	6.24% ± 0.1
Hexane	5.77% ± 0.4

### Mortality

The table 2 shows that acetone extracts of *L. rugosa* had a toxic activity against *T. castaneum* adults. These mortalities varied significantly according to the doses and according to the periods of exposure. There was 11.25 ± 1.25% mortality at the lowest dose (0.5g) after 72 hours of exposure. The highest concentration (3g) induced after 24 hours exposure 36.25 ± 4.26% mortality reach 87.50 ± 3.22% mortality after 72 hours of exposure. There was a highly significant difference between dose-induced mortalities and periods of exposure with respectively F = 23.18 - 339.93 and F = 11.25 - 81.86 for P <0.0001

Compared with the control, the methanol extract of *L. rugosa* induced significant mortality of *T. castaneum* adults. These mortalities increased with dose (F = 37.55 - 201.91, P <0.0001) and duration of exposure (F = 101.125- 289.70, P

<0.0001). At the lowest dose, 16.25 ± 1.25% mortality was recorded after 24 hours of exposure. This mortality was 35.00 ± 2.04% at the same dose after 72 hours of exposure.

The hexane extract also showed insecticidal activity against *T. castaneum* adults after 72 hours of exposure, in contrast to the control where no mortality was recorded. These mortalities varied significantly according to doses and periods of exposure. We then recorded after 24 hours of exposure with the lowest dose 15.00 ± 2.04% of mortality was recorded. This mortality reached 22.50 ± 1.44% at the same dose after 72 hours of exposure; mortality reached 57.50 ± 3.22% at a dose of 2g after three days of exposure. At a dose of 3g, mortality reached 100.00 ± 0.00% after 03 days of exposure.

The mortalities varied significantly according to the doses (F = 82.83 - 381.16, P <0.0001) and exposure periods (F = 32.11 - 231.91, P <0.0001).

**Table 2:** cumulated corrected mortalities (Mean±SE) after 24, 48 and 72 hours of exposure towards *Tribolium castaneum* adults

Product	Doses (g)	% of mortality (Mean ± SE)			
		Time of exposure			
		24h	48h	72h	F
Acetone	0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	
	0.5	3.75 ± 1.25aAB	8.75 ± 2.39aBC	11.25 ± 1.25bC	11.25***
	1	10.00 ± 2.04aAB	13.75 ± 2.39aB	16.25 ± 1.25bB	9.44***
	2	28.75 ± 5.54aBC	40.00 ± 6.45aCD	53.75 ± 2.39bD	18.67***
	3	36.25 ± 4.26aB	85.75 ± 5.15bC	87.50 ± 3.22cD	81.86***
	F	23.18***	37.28***	339.93***	
Methanol	0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	
	0.5	16.25 ± 1.25aB	3.75 ± 2.39aC	35.00 ± 2.04aD	101.25***
	1	23.75 ± 3.14aB	30.00 ± 2.04aBC	37.50 ± 3.22aC	55.71***
	2	36.25 ± 2.39bB	40.00 ± 2.04bB	57.50 ± 3.22bC	121.07***
	3	35.00 ± 3.53bB	81.25 ± 3.14cC	96.25 ± 2.39cD	289.70***
	F	37.55***	184.72***	201.91***	
Hexane	0	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	
	0,5	15.00 ± 2.04aB	17.50 ± 2.50aBC	22.50 ± 1.44aC	32.11***
	1	23.75 ± 3.14aB	36.25 ± 2.39bC	42.50 ± 2.50bC	69.43***
	2	35.00 ± 2.04bB	45.00 ± 2.04bB	57.50 ± 3.22cC	104.07***
	3	60.00 ± 3.53cB	87.50 ± 3.22cC	100.00 ± 0.00dD	231.91***
	F	82.83***	205.35***	381.16***	

In the same column, values followed with the same letter are not significantly different Tukey test.\* p<0,05; \*\* p<0,001; \*\*\* p<0,0001

**LC<sub>50</sub> and LC<sub>95</sub> values for different extracts**

The different extracts used were toxic towards *T. castaneum* adults. These toxicities increases with the doses applied. Lethal doses 50 (LD<sub>50</sub>) and lethal doses 95 (LD<sub>95</sub>) decreased with increasing periods of exposure (Table 3).

The LD<sub>50</sub> of the acetone extract rose from 4.33g at 24 h to 1.63g after 72 hours of exposure. For the DL<sub>95</sub>, was 3.14g at 24 h and 5.58g at 72 h. For the methanol and hexane extracts, the LD<sub>50</sub> values are respectively 7.01 g and 2.63g after 24 h and for 0.65 g and 28.94 g after 72 h. for DL<sub>95</sub>, these values shown the same trends for the other two extracts used.

The slopes vary little according to the periods of exposure. It was  $3.58 \pm 0.38$ ;  $4.22 \pm 0.44$ ;  $2.88 \pm 0.30$  after respectively 24 h, 48 h, 72 h for the acetone extract. For the methanol extract, for the same periods the following gradients were recorded:  $3.11 \pm 0.33$ ;  $4.48 \pm 0.47$ ;  $4.12 \pm 0.43$ . They present the same

trends for the hexane extract. However, the acetone extract seems to act faster towards *T. castaneum* adults because it is on this extract that the greatest slope is recorded at 6h of exposure ( $2.33 \pm 0.24$ ), followed by the hexane extract ( $1.83 \pm 0.19$ ) and then the methanol extract ( $0.95 \pm 0.90$ ).

The effectiveness of the products varied from one extract to another. The coefficient of determination (R<sup>2</sup>) was 0.78 and 0.96 after respectively 24 hours and 72 hours for the acetone extract. It was 0.61 to 24h and 0.90 at 72 h for the methanol extract. For the hexane extract, this value is 0.65 and 0.94 after respectively 24hours and 72 hours of exposure. All values of the coefficients of determination were greater than 0.6.

There was no significant difference for all 2 values regardless of exposure periods.

**Table 3:** LC<sub>50</sub> and LC<sub>95</sub> values for different extracts of *Lippia rugosa* against *Tribolium castaneum* adults

Product	Exposure time (h)	Slope $\pm$ ES	R <sup>2</sup>	LC <sub>50</sub> (g)	LC <sub>95</sub> (g)	$\chi^2$
Acetone	24	$3.58 \pm 0.38$	0.78	4.33	3.40	41.48 ns
	48	$4.22 \pm 0.44$	0.86	2.55	14.47	62.84 ns
	72	$2.88 \pm 0.30$	0.96	1.63	5.58	81.63 ns
Methanol	24	$3.11 \pm 0.33$	0.61	7.01	0.65	21.55 ns
	48	$4.48 \pm 0.47$	0.86	1.65	14.11	102.29 ns
	72	$4.12 \pm 0.43$	0.90	1.04	6.74	147.80 ns
Hexane	24	$2.95 \pm 0.31$	0.89	2.63	28.94	37.18 ns
	48	$4.31 \pm 0.45$	0.90	1.47	7.99	98.72 ns
	72	$3.55 \pm 0.37$	0.94	1.12	4.66	141.02 ns

ns: not significant

**Repellent effect**

The repellent effects of the various extracts of *L. rugosa* towards *T. castaneum* adults are shown in Table 4. The repulsion indices varied according to the doses applied and depending on the solvent used. At the observation, the classification of the repulsive potentialities could be distributed as follows: Hexane >> Methanol >> Acetone.

Indeed, the hexane extract showed a very repulsive activity for all doses, at the smallest dose (0.5g), a repulsion index of  $80.00 \pm 8.16\%$  is recorded. That value reached  $100.00 \pm 0.00\%$  at the maximum dose. All these indices corresponded to the repulsive class V.

For the methanol extract, the repulsion index varied according to the doses. At the smallest dose, the repulsion index was  $52.50 \pm 9.57\%$  corresponding to the repulsive class III. But at the dose (2g), the repulsion index reached  $82.50 \pm 1.25\%$  corresponding to the repulsive class V; the repulsion index Class V did not change before maximum dose.

For extract with acetone, the repulsion index obtained also changed depending the dose used. That index was  $12.50 \pm 1.25\%$ ;  $30.00 \pm 8.16\%$ ;  $70.00 \pm 8.16\%$ ;  $90.00 \pm 8.16\%$  corresponding respectively to the doses 0.5, 1, 2 and 3g applied and corresponded to class I; IV and V. The acetone extract had the lowest repulsion index with an average of  $50.62 \pm 6.43\%$  which corresponded to class III.

**Table 4:** mean of repulsion index of the different extracts

Product	Quantity (g)	Index of repulsion (%)	Repulsive class	Degree of repulsivity
Acetone	0.5	$12.50 \pm 1.25$	I	Très faiblement répulsive
	1	$30.00 \pm 8.16$	II	Faiblement répulsive
	2	$70.00 \pm 8.16$	IV	Répulsive
	3	$90.00 \pm 8.16$	V	Très répulsive
Mean		$50.62 \pm 6.43$	III	Modérément répulsive
Methanol	0.5	$52.50 \pm 9.57$	III	Modérément répulsive
	1	$77.50 \pm 1.25$	IV	Répulsive
	2	$82.50 \pm 1.25$	V	Très répulsive
	3	$97.50 \pm 5.00$	V	Très répulsive
Mean		$77.55 \pm 4.26$	IV	Répulsive
Hexane	0.5	$80.00 \pm 8.16$	V	Très répulsive
	1	$85.00 \pm 5.77$	V	Très répulsive
	2	$95.00 \pm 5.77$	V	Très répulsive
	3	$100.00 \pm 0.00$	V	Très répulsive
Mean		$90.00 \pm 4.92$	V	Très répulsive



## Discussion

The tests performed in this study were mainly experimental. This consist to evaluate the toxic contact and repellent activities of *L. rugosa* extracts towards *T. castaneum* adults. Plants are known to possess a wide range of aromatic or saturated organic compounds. They are often extracted using solvents such as methanol, acetone, chlorophorm and hexane [22, 23]. The extraction yields of *L. rugosa* leaves allowed to note that, yield varied from one solvent to another. For this purpose, the extraction yield of acetone ( $8.76 \pm 0.2\%$ ) was higher than that of methanol and hexane which extraction yields are respectively  $6.24 \pm 0.1\%$  and  $5.77 \pm 0.4\%$ . Several factors could explain this variability, in term of yields of extraction, especially genetic, pedological, climatic and physiological factors [24].

In fact, the different extracts (acetone, methanol and hexane) of *L. rugosa* leaves tested, proved to be effective against *T. castaneum*. These mortalities could be induced by the secondary metabolites contained in leaves of *L. rugosa*. In fact, the secondary metabolites are widely known and are very important for the life of each plants species. They are involved in the protection of plants against solar radiation and phytophagous insects [25]. Many works shown that the hexane extract of *L. rugosa* leaves is mainly composed of terpenoid and triterpenoid [14], while the methanol extract was composed mainly of phenols, flavonoids, tannins, polyphenols, terpenoids and triterpenoids [26, 14]. In addition, the phytochemical screening of the methanolic extracts of some species of the *Lippia* genus has shown the presence of tannins, sterols and triterpenes, alkaloids and flavonoids in the leaves of the plant [26]. All these compounds have proven their toxicity towards insect pests in previous work. That was the case of Kosini *et al.* [27] who tested the toxic and repulsive activity of leaf extracts of *Ocimum canum* on *Callosobruchus maculatus*. That hypothesis corroborates with previous works that have shown that polyphenols such as alkaloids, terpenoids, were the main families of recognized compounds being enzymatic AChE inhibitors [28]. That toxicity was therefore due to the progressive inhibition of AChE in the nervous tissues leading to the accumulation of ACh thus causing neurotoxicity. These observations allow us to say that the mortality induced by the different solvent could be due to their chemical components.

The acetone extracts of *L. rugosa* also induced a higher mortality of adult *T. castaneum* after 72 hours of exposure. These mortalities would be caused by its major compounds namely 3-methoxy-, 3,7-dimethoxy-and 3,7,4-trimethoxyquercetine which had a proven toxic activity [29].

In addition, hexane extracts were found to be more toxic to *T. castaneum* adults than the other two extracts. They induced total adult mortality of *T. castaneum* only after three days of maximum exposure. No information in the literature allows us to justify this high toxicity. Nevertheless, the hexane extracts of *L. rugosa* possessed triterpenoid, terpenoid [14] and phenolic compounds [26] and those phenolic compounds cause disruption of motor function of insects [30]. These compounds could be responsible for those higher mortalities. Furthermore, the hexane extract had a dense structure. This density could help immobilize insects that can no longer move and cause their death because they could no longer feed. However, we reserve the right to state that mortalities were only attributable to the density factor of the extracts or its phenolic compounds.

It was important to note from the above that the three extracts

induce mortality of *T. castaneum* adults after 72 h of exposure. These mortalities varied significantly according to the dose factor and the exposure time factor. With regard to the dose factor, Mortalities increased according to the doses used. At the dose of 0.5 g of the hexane extract,  $22.50 \pm 1.44\%$  mortality was recorded after 72 hours of exposure and then  $42.50 \pm 2.50\%$  (1g) to finally reach  $100 \pm 0.00\%$  mortality at 3 g of extract used. Mortalities recorded with the other extracts also shown the same trends. This gradual increase in mortalities would be due to the increasement of the active ingredient. As for the exposure time factor, the percentages of mortalities had the same trends as in the case of the dose factor. That was explained by the prolonged exposure time of insects to the active compound because the increasement of the exposure time caused the increase of mortality in insects [31]. In the same line, when considering the correlation coefficients  $R^2$ , it was clear that the values obtained in the context of our manipulation are all greater than or equal to 0.6. These values are in agreement with Faraway's postulate [32] which reported that in the biological sciences when the  $R^2$  coefficients of determination are greater than or equal to 0.6; the favorable results found are attributable to the products used. That allowed us to affirm that the mortalities obtained are induced by extracts of *L. rugosa*. Regarding the Chi-square ( $\chi^2$ ) values obtained after 24, 48 and 72 hours were all insignificant for all the extracts tested, which means that the model obtained was close to the theoretical model [21].

With regard to the repellence test, the results obtained indicated that the three extracts were repulsive towards adult *T. castaneum* with repulsion percentages varying from one extract to another and depending on the doses used. The hexane extract exhibited strong repulsiveness at all concentrations with a class V repulsion index according to McDonald's classification. While the extract with acetone and methanol had a relatively low repulsion index but reach the repulsive class V at the maximum dose (3g). These results demonstrate that extracts of *L. rugosa* possess substances with repellent potential against *T. castaneum* adults. In fact, the insect pests of the stocks through to their olfactory sensory receptors were very sensitive to the spectra and to the intensity of the smells released by the repulsive compounds contained in the extracts of the plants. Several authors have proved the deterrent effect of *L. rugosa*. In order to test the toxic and repulsive activity of *Lippia adoensis* essential oils on the main pests of maize *Prostephanus truncatus* and *Sitophilus zeamais*. Nukenine *et al.* [19] proved the repellent effect of the genus *Lippia*. In the same line, Yaouba *et al.* [33] proved the repulsiveness of *L. rugosa* essential oils on fungi of the genus *Penicillium*, *Aspergillus* and *Fusarium*.

## Conclusion

The objective of this study was to evaluate the toxic and repulsive activity of *L. rugosa* acetone, methanol and hexane extracts against *T. castaneum* adults. At the end of our work, it appears that the three extracts of *L. rugosa* shown a toxic and repulsive activity towards *T. castaneum* adults. These toxicities varied according to doses and periods of exposure. The hexane extract showed higher toxic and repulsive activity than the others with 100% mortality after 72 hours at the maximum dose (3g) and a V-repulsion index at any dose according to the classification from McDonald's. However, it should be noted that no information to our knowledge in the literature has confirmed or refuted our results.

## Acknowledgments

The authors sincerely thank Pr. Maponmetsem M. L. for the identification of the plant and Drs. YOUNOUSSA L. and OUMAROU M. for their assistance in extracting the plant.

## References

1. Tamgno BR, Ngamo TSL. Utilisation des produits dérivés du Neem *Azadirachta indica* A. Juss comme alternatifs aux insecticides synthétiques pour la protection des semences de maïs et de sorgho dans la Vallée du Logone. Sciences Technologies et développement. 2014; 15:1-8.
2. FAO. L'état de l'insécurité alimentaire dans le monde: Comment la volatilité des cours internationaux porte-t-elle atteinte à l'économie et à la sécurité alimentaire des pays. Rome, Italy, 2011, 62.
3. Kouninki H. Etude des potentialités d'utilisation d'huiles essentielles pour le contrôle de deux insectes ravageurs des grains *Callosobruchus maculatus* (coleoptera: Bruchidae) et *Sitophilus zeamais* (Coleoptera: curculionidae) au nord Cameroun. Thèse de doctorat, Faculté des sciences, centre de recherche sur la biodiversité, université catholique de Louvain (Belgique), 2007, 319.
4. Ngamo LST, Hance TH. Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical. Tropicultura. 2007; 25(4):215-220.
5. Guèye MT, Seck D, Wathelet JP, Lognay G. Lutte contre les ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale: synthèse bibliographique. Biotechnol. Agron. Soc. Environ. 2010; 15(1):183-194.
6. Nukenine EN, Monglo B, Awason, Ngamo LST, Tchuenguem FFN, Ngassoum, MB. Farmer's perception on some aspects of maize production and infestation levels of stored maize by *Sitophilus zeamais* in the Ngaoundéré region of Cameroon. Cam. J BioI. Biochem Sci. 2002; 12(1):18-30.
7. IITA. Annual report, 2012, 68.
8. Kouninki H, Ngamo LST, Hance T, Ngassoum MB. Potential use of essential oils from local Cameroonian plants for the control of red flour weevil *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). AJFAND 7, 2007, 1-15
9. Nanfack FM, Dongmo YZ, Fogang MAR. Les insectes impliqués dans les pertes post-récoltes des céréales au Cameroun: méthodes actuelles de lutte et perspectives offertes par la transgénèse. Int. J Biol. Chem. Sci. 2015; 9(3):1630-1643.
10. Benhalima H, Chaudhry Q, Mills KA, Price NR. Phosphine resistance in stored-product insect collected from various grain storage facilities in Morocco. J Stored prod. Res. 2004; 40:241-249.
11. Regnault-Roger CJ. De nouveaux phyto-insecticides pour le troisième millénaire In: Philogène B.J.R., Regnault-Roger C. & Vincent C., coord Biopesticides d'origine végétale. Paris, Lavoisier-Editions Tec & Doc, 2002, 19-39.
12. Afful E, Owusu EO, Obeng-Ofori D. Bioactivity of *Securidaca longepedunculata* Fres. Against *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae) and *Sitophilus zeamais* Motsch (Coleoptera: Curculionidea). International Journal of agriculture science research. 2012; 1(3):046-054.
13. Khaliq A, Nawaz A, Ahmad HM, Sagheer M. Assessment of insecticidal potential of medicinal plant extracts for control of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Basic research journal of agriculture science and review. 2014; 3(11):100-104.
14. Momeni J, Tsopmejo JP, Nkouam FT, Ngassoum MB. Antioxidant Activity of the Natural Flavonoid 7-Hydroxy-5, 6, 4'-trimethoxyflavone isolated from the Leaves of *Lippia rugosa* A. Chev. Scientific Research Publishing. 2016; 8:70-78.
15. MNEPAT. Rapport régional de progrès des objectifs du millénaire pour le développement, région du Nord. Yaoundé, Cameroun, 2010, 2-3.
16. Kouninki H, Mfouapon A, Mohamadou Sali B. Biological activities Of *Cassia mimosoides*; *Eucalyptus camaldulensis*; *Vepris heterophylla* plant extract toward old larvae and adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Int. J of Sci., Env. and Tech. 2017; 5(6):3196-3213.
17. Abbot W. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
18. McDonald LL, Guy RH, Speirs RD. Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. Marketing Res. Rep. n° 882. Washington: Agric. Res. Service, US. Dept of Agric, 1970, 183.
19. Nukenine EN, Adler C, Reichmuth C. Toxicity and repellency of essential oils of *Lippia adoensis* from two agro ecological zones in Cameroon to *Prostephanus truncatus* and two strains of *Sitophilus zeamais*. Proceeding of the meeting of IOBC. WPRS study group "integrated protection of stored products"; 20-23 August 2007 poznan, Poland. IOBC bulletin. 2009; 40:221-230.
20. SPSS. SigmaPlot version 6.0. for windows. SPSS Science Technical Support, California, USA, 2000.
21. Finney DJ. Probit analysis. Cambridge University Press, London, United Kingdom, 1971.
22. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants ? Journal of ethnopharmacology. 1998; 60(1):1-8.
23. Krishnananda P Ingle, Amit G Deshmukh, Dipika A Padole, Mahendra S Dudhare, Mangesh P Moharil, Khelurkar VC. Screening of insecticidal activity of *Jatropha curcas* (L.) against diamond moth and *Helicoverpa armigera*. Journal of Entomology and Zoology Studies. 2017; 5(1):44-50.
24. Fatiha D. Composition chimique et activité insecticide de 03 extraits végétaux à l'égard de *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Thèse magister en sciences agronomiques, Ecole nationale supérieure agronomique EL-HARRACH, Département de Zoologie agricole, 2013, 92.
25. Mindiéidiba JB. Etude phytochimique et activités biologiques des tiges feuillées de *Lantana camara* L. et de *Lippia chevalieri* Moldenke: deux verbenaceae du Burkina Faso. Mémoire thèse de doctorat, Université de Ouagadougou, Ecoles doctorales sciences et technologie, Laboratoire de biochimie et de chimie appliquée, 2012, 199.
26. Mevy JP, Bessiere JM, Dherbomez M, Millogo J, Viano J. Chemical composition and some biological activities of the volatile oils of a chemotype of *Lippia chevalieri*

- Moldenke. Food Chemistry. 2007; 101:682-685.
27. Kossini D, Nukenine EC, Tofel KH. Efficacy of Cameroonian *Ocimum canum* Sims (Lamiaceae) leaf extract fractions against *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae), infesting Bambara groundnut. Journal of entomology and Zoology Studies. 2015; 3(5):487-494.
  28. Lee JH, Lee KT, Yang JH, Baek NI, Kim DK. Acetylcholinesterase inhibitors from twigs of *Vaccinium old hamii* Miquel. Archives of Pharmacal Research. 2004; 27:53-56.
  29. Ghisalberti EL. *Lantana camara* L. (Verbenaceae). Fitoterapia. 2000; 71:467-486.
  30. Fatiha D. Composition chimique et activité insecticide de 03 extraits végétaux à l'égard de *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Thèse magister en sciences agronomiques, Ecole nationale supérieure agronomique EL-HARRACH, Département de Zoologie agricole, 2013, 92.
  31. Nukenine EN, Adler C, Reichmuth C. Efficacy evaluation of plant powders from Cameroon as post-harvest grain protectants against the infestation of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Plant Disease and Protection. 2007; 114(1):30-36.
  32. Faraway JJ. Practical Regression and Anova using R, 2002, 212.
  33. Yaouba A, Tatsadjieu NL, Jazet DPM, Etoa FX, Mbofung CM. Antifungal properties of essential oils and some constituents to reduce foodborne pathogen. Journal of Yeast and Fungal Research. 2010; 1(1):001-008.