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RESEARCH ARTICLE

CHEMICAL ANALYSIS AND EVALUATION OF THE LARVICIDAL ACTIVITY OF ESSENTIAL OILS AND CRUDE EXTRACTS OF LEUCAS MARTINISENCIS L. AND CROTON ZAMBESICUS Muell. ON ANOPHELES GAMBIAE Giles

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Abstract

The aim of the present study was to evaluate the larvicidal activities of essential oils and crude organic (petroleum ether, chloroform, ethyl acetate and methanol) and aqueous extracts of *Leucas martinisencis* L. (whole plant*)* and *Croton zambesicus* Muell. (fruits*)*, two aromatic plants of Niger on *Anopheles gambiae Giles*. GC and GC/MS analysis showed that *β*–caryophylene (43,86 %) and *α*–humulene (19,93 %) were the main constituents of the sesquiterpenic essential oil of *L. martinisencis* while two sesquiterpenic compounds *β* –elemol (13,23 %) and *β* –eudesmol (10,50 %) were found as the main constituents of the essential oil of *C. zambesicus* which was a monoterpenic one. Phytochemical analysis of the crude organic and aqueous extracts revealed the presence of terpenes and sterols, tannins and polyphenols, saponosides and alkaloids in the two plants and flavonoids in *C. zambesicus*. The larvicidal tests showed that the essential oil of *L. martinisencis* was more active than that of *C. zambesicus* with DL50 values of respectively 0,050 g/L and 0,083 g/L, but the two essential oils were less active than deltamethrine, the reference larvicide. Of the crude organic and aqueous extracts, only the ethereal extracts of the two plants and the chloroformic extract of *L. martinisencis* showed larvicidal activities after 48 hours of exposure. These results support the use of the two aromatic plants as larvicide against *Anopheles* larvae.

Introduction:-

Females of breeding mosquitoes need blood for egg development and some species have a strong preference for human blood. Among the mosquito species known to transmit diseases to humans, there are members of three well

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now genera: *Culex*, *Aedes* and *Anopheles*. Species of the genus *Culex* transmit parasitic diseases such as filariasis; species of the genus *Aedes* are responsible for yellow fever while species of the genus *Anopheles* transmit malaria, which is one of the most deadly diseases in the world (Ginet and Roux, 1989).

According to the World Health Organization (WHO, 2015), 214 million cases of malaria have been reported, with 438,000 deaths. Children under 5 years of age and pregnant women are the most affected by this disease (WHO, 2005). According to the Programme national de lute contre le paludisme (PNLP, 2015) of Niger, more than 3 817 634 malaria cases were recorded in 2015, with 2 222 deaths. In addition to the existing therapeutic molecules to control parasite, vector control is a very important means of preventing disease transmission. In mosquito's control campaigns, insecticides used belong to synthetic organophosphates, pyrethroids or carbamates (Callec *et al*., 1985). These formulations, although very effective on mosquitoes, have several disadvantages. Indeed, in addition to their high cost, they can cause various environmental problems. Pollution for example by the significant accumulation of their active ingredients in treated aquatic and terrestrial ecosystems (Barbouche *et al.*, 2001). In addition, the active substances in the products used have a broad spectrum of action and do not spare non-target organisms. To all these disadvantages, there is also a major problem in the development of resistance to chemical insecticides in treated mosquitoes (Georghiou *et al.*, 1975; Sinegre *et al.*, 1977). To ensure a better intervention, while preserving environment as much as possible, new preventive methods and new products are constantly being sought. Natural substances that have a broad spectrum of pharmacological action, such as bactericides, fungicides, acaricides, etc., can also be used as alternative insecticides. In Niger, studies on the insecticidal activity of plant extracts against mosquito larvae are very limited. Thus, in constant research of new biopesticides, we screened essentials oils, organic and aqueous extracts of *L. martinicensis* (Lamiaceae) and *C. zambesicus* (Euphorbiaceae) for their larvicidal activities on *An. Gambiae.* Our study also focuses on the chemical composition of the essential oils, and phytochemicals of organic and aqueous extracts of these two aromatics plants.

Material and Methods:-

Plant Material:

Leucas martinicensis L. (Root, leave, stems and inflorescences) was harvested in August 2013 at Garbal, a surrounding village of Niamey. This plant was harvested in a millet field, free of any chemical pesticides treatment. Freshly collected sample was coarsely cut and then dried in shade. Dry fruits of *Croton zambesicus* Muell were purchased in July 2013 at the market of Katako (Niamey, Niger). They have been kept away from dust and moisture. Plants samples were identified in Laboratoire Garba Mounkaila, Département de Biologie, Université Abdou Moumouni de Niamey. Where a voucher specimen was deposit.

Larvae of *Anopheles gambiae Giles*

Second and third instars larvae of *Anopheles gambiae* were collected at Saguiya a district of Niamey with a plastic strainer. Collected larvae were reintroduced into a plastic bucket containing shelter's water, then transported to Centre de recherche medical et sanitaire (CERMES) for identification and to laboratoire de biologie de l'Université Abdou Moumouni for the larvicidal test. Larvae were rinsed abundantly with well's water. They were raised for 24 hours (h) in a plastic bucket by feeding them with cookies (a carbohydrate-rich food) before being used for bioassays.

Extraction Methods:

Extraction of essential oils:

Essential oils of *L. martinicencis* (whole plant) and *C. zambesicus* (fruits) were extracted by hydrodistillation with a Clevenger type apparatus. 100g (*L. martinicensis*) or 300g (*C. zambesicus)* of dry plant material in 0,7L of water were distilled for 2h and 3h respectively. At the end of the distillation, the essential oils were separated from water by decantation and water of distillation was extracted with petroleum ether (10 mL x 3) in a separating funnel. The organic phase (containing the essential oil), less dense than the aqueous phase was collected in a previously tarred bottle. The petroleum ether is allowed to evaporate at room temperature. The mass of the essential oil was determined and aluminum foil is used to cover the bottle, which was conserved at 4°C.

Preparation of organic and aqueous extracts:

Organic and aqueous crude extracts were obtained by sequential extraction using solvents in increasing order of polarity. In this order, we used petroleum ether, chloroform, ethyl acetate, methanol and distilled water. Powder of *L. martinicensis* (50 g) or of *C. zambesicus* (100 g) were placed in a 500 mL Erlenmeyer. The sample was left to macerate, under stirring, for 24 hours, with respectively petroleum ether (300 mL x3), chloroform (300 mL x3),

ethyl acetate (300 mL x3), methanol (300 mL x3), and distilled water (400 mL x3). After filtration on cotton, the ethereal, chloroformic, ethyl acetate and, methanolic crude extracts were dry concentrated using a rotary evaporator and recovered in previously tared bottles. Water was, evaporated using a sand bath from the aqueous crude extract.

Methods of Analysis:

Analysis of Essential Oils:

The essential oils of *L. martinicensis* and *C. zambesicus* were analyzed by gas chromatography and gas chromatography coupled with mass spectrometry (GC/MS).

Gas chromatography was performed on a Varian CP-3380 chromatograph equipped with a flame ionization detector and a capillary column (length 30 m, internal diameter 0.25 mm) with a stationary non-polar methylsilicone phase (DB-1, film thickness 0.25 µ). Nitrogen was used as a carrier gas with a flow rate of 0.8 $ml.min^{-1}$. The injector temperature is 220 °C; the detector is set at 250 °C. Temperature was programmed from 50 °C to 200 °C with a temperature gradient of 5 °C.min-1. The retention indices of the different components have been calculated in relation to the retention times of a series of n-alkanes and their relative percentages calculated by electronic integration, assuming that their response factors are all equal to 1.

The gas chromatography-mass spectrometry coupling was performed on Hewlett Packard HP 5970 A equipment, equipped with an apolar capillary column (30 m x 0.25 mm) of fused silica HP-1 (film thickness 0.25 µ) and a quadrupole detector (ionization energy 70 eV). The temperature of the injector was 220 °C and that of the interface area 210 °C. Injection in split mode (1/100) of 1 µl of a 10% essential oil solution in dichloromethane. Temperature is programmed from 70 °C to 200 °C with a gradient of 10 $°C.min^{-1}$. The carrier gas is helium with a flow rate of 0.6 $ml.min^{-1}$.

Identification of components:

The identification of the constituents of essential oils was based on their retention indices and mass spectra compared with data from the literature (Joulain et al., 1998; Adams, 2001) and the laboratory library.

Phytochemical screening:

Phytochemical analysis of organic and aqueous crude extracts of *L. martinicensis* and *C. zambesicus* were carried out using the standard characterization methods described by Sofowora (1980) and Harborne (1998). These detection tests focused on the following families of phytochemicals: terpenes and sterols, saponosides, flavonoids, tannins and phenolic compounds and alkaloids.

Larvicidal activities:

Larvicidal activities of essential oils, organic and aqueous crude extracts of the two plants and that of deltametrin were carried out using a WHO (1985) protocol. Preliminary bioassays allowed the selection of a set of concentrations. Solutions of the two essential oils from each sample and deltamethrin were prepared in 96° ethanol, those of organic and aqueous extracts were prepared in distilled water and then 3 drops of Tween 80 were added to the solutions of the ethereal, chloroformic and ethyl acetate extracts of the two plants to solubilize them in water. Final experimental concentrations were obtained by dilution in water of the initial solution. The different ranges of concentrations used are, (0.015; 0.030; 0.045; 0.060; 0.075; 0.075; 0.090; 0.105; 0.120 and 0.135 g/L) for essential oils, (0.120; 0.240; 0.360; 0.480; 0.600; 0.720; 0.840 and 0.960 g/L) for organic and aqueous extracts and (0.0003; 0.0006; 0.0012; 0.0015; 0.0030; 0.0060; 0.0090 and 0.0120 g/L) for deltamethrin. Twenty (20) larvae of *Anopheles gambiae* were introduced into each petri dish containing the test solution and incubated for 48 hours at room temperature. The control consists only of water. The dead larvae were counted after 48 hours of exposure. Each concentration was repeated three times.

Mortality of larvae:

The mortality rate of larvae was calculated by Abbott's method (1925).

$$\% m = \frac{NLM - NLMT}{NTL - NLMT} X100$$

% m = percentage of mortality

NLM = number of dead larvae in the test petri dish

NLMT = number of dead larvae in the control

NTL = total number of larvae

Lethal dose 50:

Lethal dose 50 (LD50) is the dose that causes 50% mortality of larvae of *Anopheles*. It was determined using the formula of Dragstedt and Lang (1957).

$$DL_{50}=\frac{50(X2-X1)+X2Y2-Y1X1}{Y2-Y1}$$

X2: higher concentration framing the LD50
X1: lower concentration framing the LD50
Y1: percentage of mortality of larvae corresponding to X1
Y2: percentage of mortality of larvae corresponding to X2

Results and Discussion:-

Yield of essential oils:

Hydrodistillation of the whole plant of *L. martinicensis* produces pale yellow oil with a yield of 0.04%. The essential oil obtained by hydrodistillation of the fruits of *C. zambesicus* yields of 0.03% of dark yellow oil. These results show that *L. martinicensis* and the fruits of *C. zambesicus* have approximately the same essential oil contents. El-kamali et al (2011), reported for the hydrodislation of fruits of *C. zambesicus* from Sudan a yield of 1.3% essential oil.

Yields of organic and aqueous crude extracts:

Examination of the results (Table 1) showed that the yields of organic and aqueous extracts obtained by maceration varied from 3.21% to 11.51% for *L. martinicensis* and from 3.4% to 18.35% for fruit of *C. zambesicus*. These results showed that for this plant, the highest yields are obtained with water (11.51%), chloroform (8.46%) and methanol (8.40%), and the low yields are obtained with petroleum ether (7.56%) and ethyl acetate (3.21%) for *L. martinicensis*, while the highest yields are obtained with methanol (18.35%), water (13.14%) and chloroform (8.7%) and the lowest with ethyl acetate (4.03%) and petroleum ether (3.4%) for *C. zambesicus*. The fact that extraction yields are higher with polar solvents (methanol and water) implies that the main constituents of *L. martinicensis* and *C. zambesicus* were polar compounds. Muhammed et al (2012) have reported 4.08% and 3.51% yield, respectively for chloroformic and methanolic extracts from leaf powder of the *L. martinicensis* from Nigeria. This difference in yield compared to the results of this study could be explained by the difference in the organs used, and the difference in the chemical composition of the plants due to the nature of the soil

Tableau 1:- Yields of organic and aqueous extracts of *L. martinicensis*L. and fruit let of *C. zambesicus* Muell.

Plant species	Part used	Extracts	Color	Returns in %.
L. martinicensis L.	Whole plant	Ethereal	Dark green	7,56
		Chloroformic	Dark green	8,46
		Ethyl acetate	Green green	3,21
		Methanolic	Brown hair	8,40
		Watery	Black	11,51
C. zambesicus Muell.	Fruits	Ethereal	Yellow	3,40
		Chloroformic	Yellow	8,70
		Ethyl acetate	Yellow	4,03
		Methanolic	Red	18,35
		Watery	Black	13,14

Chemical composition of the essential oils of *Leucas martinicensis* L. and *Croton zambesicus* Muell:

Names of the volatile components and yields percentage of the essential oils of *L. martinicensis* and *C. zambesicus* are given respectively in Tables 2 and 3, components being listed according to their order of elution on apolar column.

In the essential oil of *L. martinicensis*, sixty-six (66) compounds have been detected and identified, these compounds are mainly sesquiterpenic hydrocarbon (84,39%), the most prominent being : β-caryophyllene (43.86%); α-humuene (19.93%); germacrene D (7.09%); caryophyllene oxide (2.4%) and β-eudesmol (1.27%). In the essential oil sample *of C. zambesicus*, thirty-two (32) components were detected and identified. This oil was composed mainly of monotrepenes (62.14%), but the main constituents were two sesquiterpenic hydrocarbon: β-Elemol (13.23%) and β-Eudesmol (10.52%). Sesquiterpenic hydrocarbon represented only (33.52%) of this oil. These results are different from those reported in Benin (Block *et al.;* 2004). According to its authors, the essential oil of

the leaves of *C. zambesicus* contains as main compounds caryophyllene oxide (19.5%); β-caryophyllene (10.8%) and α-copaene (6.3%). In addition, the chemical composition of the essential oils of the fruits of *C. zambesicus* is also reported in Sudan (EL-Kamali *et al.;* 2012) According to its authors, the essential oil of the fruits of *C. zambesicus* contains mainly m-cymene (21.56%), linalool (7.21%) and p-mentha-1-en-8-ol (5.28%).

Tableau 2:- Composition of the essential oil of *Leucas martinicensis* L.

Compounds	Retention index	Percentage
Cis-hex-3-enol	854	0,25
Trans- hex-2-enol	865	0,05
N-hexanol	869	0,05
B-pinene	934	1,26
Camphene	951	0,04
Benzaldehyde	-	Tr
B –pinene	978	0,09
Oct-1-en-3-ol	981	0,35
Octan-3-one	984	Tr
Myrcene	991	0,19
Para-cymene	1026	0,08
Limonene	1031	0,54
(z)- β -ocimene	1037	0,07
(e)- β -ocimene	1047	0,39
Γ-terpinene	1060	0,16
Cis-linalol oxyde	1072	0,05
Terpinolene	1086	0,03
Linalol	1100	0,70
Nonanal	1105	0,03
Methylbornylether	1113	0,05
4, 8-dimethylnona-1, 3, 7-triene	1115	0,04
Borneol	1167	0,06
Terpinene-4-ol	1183	0,05
A –terpineol	1197	0,28
Thymylmethylether	1230	0,02
Geraniol	1250	0,18
2, 3-dihydro-1h-inden-1-one	1281	0,30
Tridecane	1300	0,07
Isobutyl benzoate	1330	0,03
Δ –elemene	1337	0,15
A –copaene	1379	0,09
B-boubonene	1387	1,01
B-elemene	1392	0,52
A –barbatene	1421	0,41
B-caryophyllene	1426	43,86
B –copaene	1432	0,15
Γ-elemene	1434	0,37
2-methylbuthylbenzoate	1440	0,18
A –humuene	1461	19,93
6-demethoxy ageratochromene	1464	0,34
Isoledene	1475	0,15
Γ –muurolene	1478	0,26
Germacrene d	1485	7,09
Γ –amorphene	1493	0,22
Bicyclogermacrene	1496	0,12
A –muurolene	1499	0,36
(e,e)- α-farnesene	1504	0,44

Γ –cadinene	1512	0,17
7-epi- α -selinene	1516	0,31
(e)- γ -bisabolene	1528	0,18
Elemol	1551	0,52
Caryophylleneoxideisomer	1556	0,26
Germacrene b	1564	0,65
Nerolidol	1573	0,07
A -caryophyllene alcool (appolanol)	1581	0,33
Caryophylleneoxide	1587	2,40
5-epi-7-epi- α -eudesmol	1611	0,35
Humulene-1, 2-epoxyde	1615	0,89
Caryophylla-4(14), 8(15)-dien-5- ?-ol	1642	0,46
B –eudesmol	1660	1,27
A –eudesmol	1663	0,15
Intermedeol	1670	0,23
A-bisabolol	1668	0,50
Phytol	2107	1,47
B-atlantone	2200	0,05
Alane	2501	0,09
Total		100

Tableau 3:- Chemical composition of *Croton zambesicus* Muell essential oils

Compounds	Retention index	Percentage
1-octen-3-ol	4,517	0,8
P-cymene	4,945	3,02
Eucalyptol	5,026	8,36
Neroloxide	5,348	7,12
Linalooloxide	5,476	5,93
B, β-dimethylstyrene	5,503	0,5
Linalool	5,561	2,82
8-hydroxylinalol	5,593	1,80
Pinocarvone	5,920	0,95
L-trans-pinocarveol	5,949	1,46
Camphor	6,002	10,57
3-nopinenone	6,097	1,25
3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methylcyclohexene	6,147	1,78
Borneol	6,181	4,83
Terpinene-4-ol	6,225	2,51
P-cymen-8-ol	6,266	4,25
Myrtenal	6,349	2,74
2-pinen-4-one	6,437	1,37
Cumaldehyde	6,671	0,88
-nonadien-2-one, 8-methyl-5-(1-methylethyl)-,	7,383	0,56
N-decanoicacid	7,450	1,92
B-elemol	8,593	13,23
Caryophylleneoxide	8,823	2,74
Γ –eudesmol	9,170	1,96
Globulol	9,117	1,35
5-hydroxy-3-methyl-1-indanone	9,173	1,55
B-eudesmol	9220	10,52
Cadiene-1-methanol, .alpha.,.alpha.,4,8-tetramethyl	9,276	1,40
Longiverbenone	9,303	0,55
Pentadecanoicacid	10,577	0,22
0(5,7).0(8,10)]dodecane, 3,3,6,6,9,9,12,12-octamethyl	10,709	0,47

1-vinylcycloheptane	11,342	0,60
Total		**100**

Phytochemical analysis of the organic and aqueous extracts

Phytochemical analysis results (table 4) show that *L. martinicensis and C. zambesicus, contains* terpenes and sterols, saponosides, tannins and polyphenols, alkaloids. Flavonoids were detected only in fruits of *Croton zambesicus*.

Tableau 4:- Phytochemical screening of ethereal, chloroformic, ethyl acetate, methanolic and aqueous extracts of *L. martinicensis* and *C. zambesicus*.

Plant species	Part used	Nature of the extract	Family of characterized phytochemical compounds				
			Saponosides	Tannins and polyphenols	Flavonoids	Alkaloids	Terpenes and sterols
L. martinicensis L.	Whole plant	Ethereal	ND	ND	ND	ND	+
		Chloroformic	ND	ND	ND	ND	+
		Ethyl acetate	-	-	-	-	ND
		Methanolic	+	+	-	+	ND
		Watery	-	+	-	+	ND
C. zambesicus Muell.	Fruits	Ethereal	ND	ND	ND	ND	+
		Chloroformic	ND	ND	ND	ND	+
		Ethyl acetate	-	-	-	-	ND
		Methanolic	+	+	+	-	ND
		Watery	+	+	+	+	ND

ND :Not determined

Larvicidal activities of the essential oil and the organic and aqueous extracts

The percentage of mosquitoes larvae dead as a function of the doses of essential oil of *L. martinicensis*, *C. zambesicus* and deltamethrin (reference) in 48 hours of exposure are represented in Table 5, 6 and figure 1. Analysis of these results shows that the essential oils of *L. martinicensis* and *C. zambesicus* have larvicidal activities on second and third instars mosquito's larvae. Indeed, for each dose of essential oil considered, the percentage of larvae dead is significantly different from the mortality rate in the control, according to the statistical analysis at the 5% threshold. The larvicidal activities of the essential oils of *L. martinicensis* and *C. zambesicus* were strong. Indeed, the doses of essential oil that cause 100% larval mortality were 0.120 and 0.135 g/L for *L. martinicensis* and *C. zambesicus* respectively. The lethal doses 50 of the essential oils of *L. martinicensis* and *C. zambesicus* were 0.050 g/L and 0.083 g/L respectively (Figure 2). Examination of the LD50s values shows that the essential oil of *L. martinicensis* is more active on larvae of the genus *Anopheles* than that of *C. zambesicus*. In addition, deltamethrin used as the reference larvicide in this study has an LD50 value of 0.0023 g/L, so it was more active than essential oils of the two plants. The larvicidal activity of essential oils seems to be linked to the presence of the major compounds that are sesquiterpenic compounds: β-caryophyllene, germacrene D, and β-eudesmol for the essential oil of *L. martinicensis* and β-élémol and β-eudesmol for that of *C. zambesicus*. Larvicidal activities of β-caryophyllene, germacrene D, β-eudesmol and β-elemol have been reported by Hye-Mi and Park (2012) and by Chen et al. (2013). However, a synergetic effet due to the presence of minor's monoterpene and sesquiterpene compound may exist.

Results of the larvicidal activities of organic and aqueous extracts of L. *martinicensis* and *C. zambesicus* on the larvae of *Anopheles gambiae* evaluated showed that the ethyl acetate, methanolic on and aqueous extracts of the two plants have do not activity on the larvae up to a dose of 0.960g/L. Beyond this dose, the larvae are no longer visible in the opaque solutions of the petri test boxes. For *L. martinicensis*, ethereal and chloroformic extracts have a larvicidal activity (Figure 1).

Results of the larvicidal activities of active organic extracts were quite low since at high doses (0.960g/L), the ethereal and chloroformic extracts of *L. martinicensis* cause only 52.6 and 26.5% larval mortality respectively. Indeed, larval mortality rates increase in the same direction as the dose of extracts. The lethal dose 50 of the ethereal extract of *L. martinicensis* is 0.750 g/L and that of the chloroformic extract is greater than 0.960g/L.

For *C. zambesicus*, only the ethereal extract was active, the chloroformic extract having no effect even at high doses 0.960g/L. At a dose of 0.960g/L, the larval mortality rate was 42.1% for the ethereal extract. The larvicidal activity of these organic extracts of *L. martinicensis* and *C. zambesicus* seems to be linked to the presence of terpenes and

sterols in these extracts. Indeed, insecticidal activities on mosquitoes of terpenes and sterols of several plants have been reported by Shaalan et al (2006) and Kovendan and Murugan (2011).

Tableau 5:- Larvicidal activities of the essential oils of *Leucas martinicensis* L., *Croton zambesicus* Muell. and deltamethrin (reference insecticide) in 48 hours of exposure.

Doses in g/l	***L. martinicensis***	***C. zambesicus***		**Doses in g/l**	**Deltamethrin**
0	0	0		0	0
0,015	38,9	0		0,0003	0
0,03	38,9	0		0,0006	0
0,045	44,4	11,1		0,0012	10,5
0,06	61,1	16,7		0,0015	36,8
0,075	72,2	27,8		0,003	63,2
0,09	77,8	66,7		0,006	84,2
0,105	94,4	83,3		0,009	100
0,12	100	94,4		0,012	100
0,135	100	100			

Tableau 6:- Larvicidal activities of organic extracts of *Leucas martinicensis* L. and *Croton zambesicus* Muell. after 48 hours of exposure.

Doses in g/l	**EE**		**EC**		**EAE, EM et EA.**	
	L. Martinicensis	***C. zambesicus***	***L. martinicensis***	***C. zambesicus***	***L. martinicensis***	***C. zambesicus***
0	0	0	0	0	0	0
0,12	5,3	0	0	0	0	0
0,24	10,5	0	5,3	0	0	0
0,36	10,5	0	5,3	0	0	0
0,48	15,8	5,3	10,5	0	0	0
0,6	26,3	15,8	10,5	0	0	0
0,72	26,3	21,1	10,5	0	0	0
0,84	47,4	31,6	15,8	0	0	0
0,96	52,6	42,1	26,5	0	0	0

EE: ether extract, EC: chloroformic extract, EAE: ethylacetate extract, EM: methanolic extract, EA: aqueous extract.

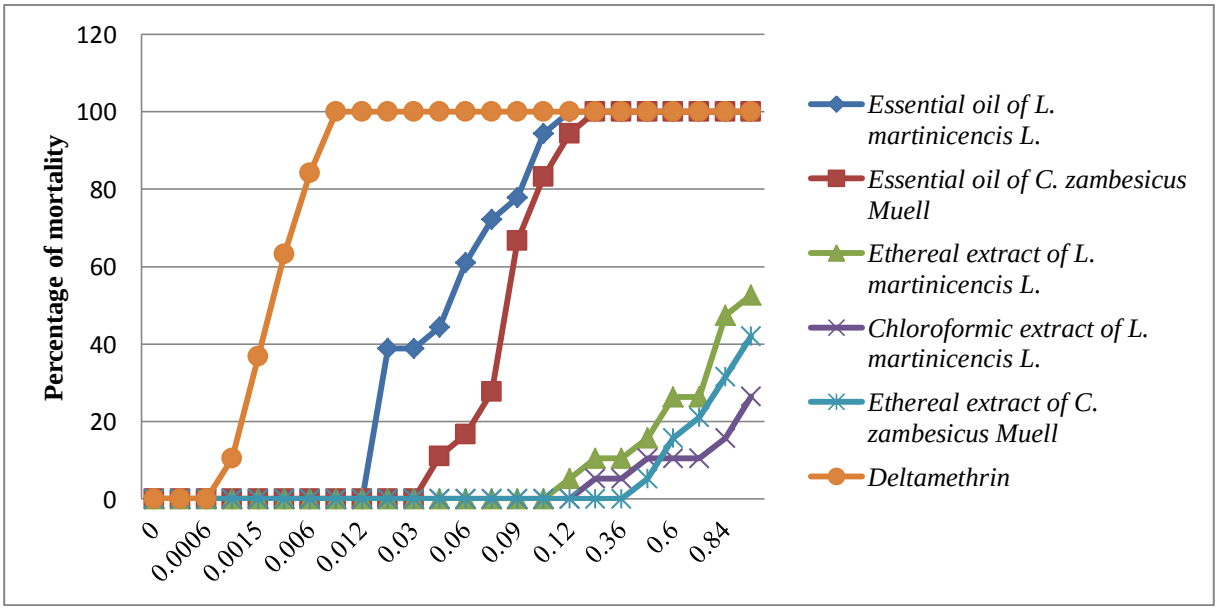

Figure 1:-Larvicidal activity of essential oils and extracts of *Leucas martinicensis* L. and *Croton zambesicus* Muell. and deltamethrin, after 48 hours of exposure.

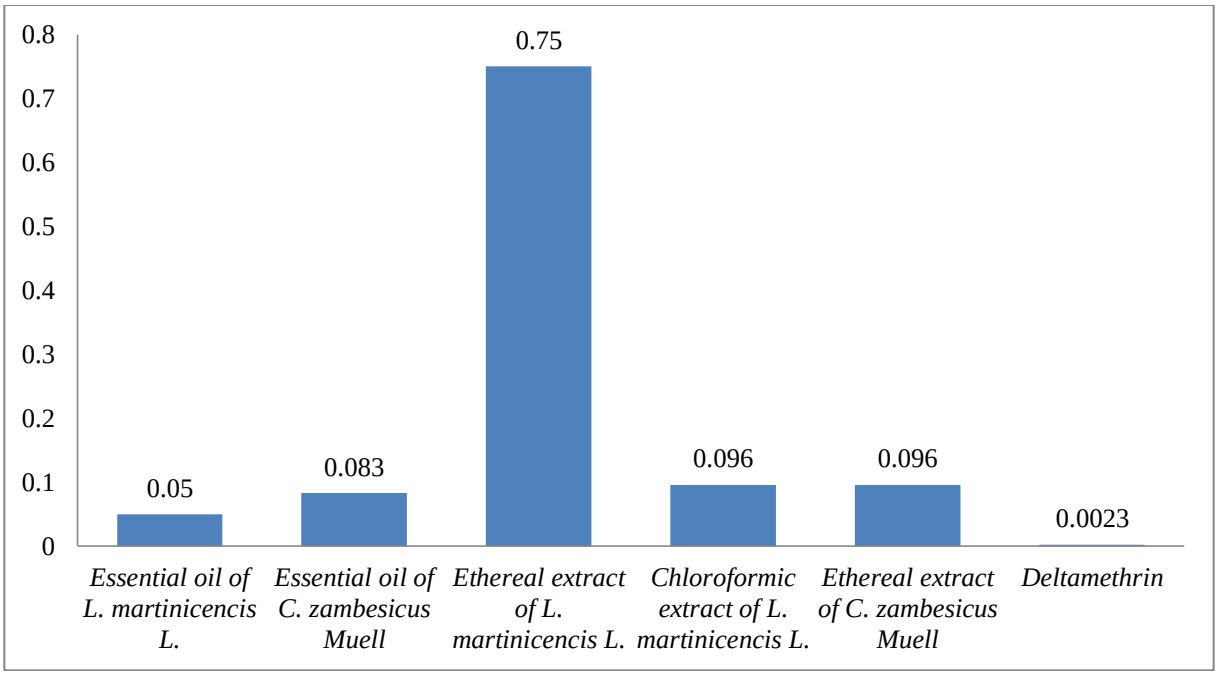

Figure 2:- LD50s in g/L of essential oils, extracts and deltamethrin.

Conclusion:-

Many cases of mosquito's resistance were observed when synthetic insecticides were used for the preventive control of malaria. In this context, the use of natural molecules with biodegradable larvicidal or repellent properties and therefore more respectful of the environment and the ecosystem, is proving to be an alternative approach to the use of synthetic larvicides. In this study, the larvicidal activities of the essential oils and crude extracts of *L. martinicensis* and *C. zambesicus* were evaluated on larvae of *Anopheles gambiae*. The study showed that essential oils have significant activities against larvae of *Anopheles gambiae.* The essential oil of *L. martinicensis* was the most effective, with an LD50 of 0.050g/L. Organic extracts are less active than essential oils. Essential oils and crude extracts are less active than deltamethrin (reference), they could be of great interest in the field of vector control, since as natural substances they will be biodegradable and less polluting than chemical insecticides. These results open up interesting prospects for their use in the production of bio larvicides and insecticides. We plan to continue this study in order to clarify the nature of the compound(s) responsible for this activity by a fractionation conducted in parallel with the biological tests.

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