Phytochemical study and larvicidal activity evaluationof essential oils from *Cymbopogoncitratus* (D.C.) Stapf. and*Cymbopogongiganteus*Chiov. on*Anopheles gambiae*s.l.

Abstract

This study evaluates the larvicidal activity of essential oils extracted from the leaves of *Cymbopogoncitratus* DC.Stapf. and the stems with leaves and inflorescences of *Cymbopogongiganteus*Chiov. against the larvae of *Anopheles gambiae*s.l., the main vector of malaria. The essential oils were obtained by hydrodistillation using a Clevenger-type apparatus and then analysed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). The larvicidal tests were carried out according to the WHO protocol (1985). The highest extraction yields were recorded with *C. citratus* (1.91%), followed by *C. giganteus* (0.46%). GC and GC-MS analysis revealed that the essential oil of *C. citratus* consists mainly of α-citral (44.8%) and β-citral (32.7%). The essential oil of *C. giganteus* was not chemically characterised in this study. Larvicidal bioassays showed significant activity of both essential oils on *An. gambiae* larvae. The essential oil of *C. giganteus* was found to be the most active (LD_{50} = 70.13 ppm), followed by that of *C. citratus* (LD_{50} = 114.36 ppm). Although these essential oils are less active than deltamethrin (LD_{50} = 2.3 ppm), used as a reference product, they appear to be alternatives for the development of more sustainable insecticides. These results also provide scientific justification for the traditional use of aromatic plants in Niger as mosquito repellents.

Keywords:*Cymbopogoncitratus*, *Cymbopogongiganteus*, essential oils, *Anopheles gambiae*, larvicidal activity, malaria, Niger

Introduction:-

Mosquitoes are responsible for transmitting numerous vector-borne diseases, with a considerable impact on public health (Seye et al., 2006; Youssef et al., 2011; Pascal et al., 2011). According to the World Health Organisation (WHO), more than 246 million cases of malaria were reported in 2023, resulting in approximately 569,000 deaths, 95% of which occurred in the WHO African Region, followed by South-East Asia and the Eastern Mediterranean Region with approximately 2% of cases each (WHO, 2024).

Mosquitoes of the genus Anopheles are the main vectors of malaria, a parasitic disease that remains a major public health problem worldwide (Greenwood, 2008). In Niger, a sharp increase in the proliferation of *Anopheles gambiae*(the vector species) has been reported in recent decades (Julvez et al., 1992; Ahadji-Dabla et al., 2014).

The fight against these vectors relies mainly on the use of chemical insecticides, which have proven to be effective. However, their widespread use has several drawbacks, including harmful effects on aquatic fauna and the environment, as well as the emergence of resistance in mosquitoes (Aouinty et al., 2006; Cui et al., 2007; Daaboub et al., 2008). This resistance can be behavioural, physiological or linked to mutations in the proteins targeted by the insecticides (Hamon, 1963; Oppenoorth, 1985; Fournier and Mutero, 1994).

In this context, the search for alternative, safe and environmentally sustainable solutions has become a priority. Natural products, particularly essential oils, are now attracting growing interest due to their biodegradability and popularity (El OualiLalami et al., 2013).

In Niger, despite the availability of a wide variety of medicinal and aromatic plants, studies on the insecticidal activity of their extracts against mosquito larvae remain limited. A comparative study of these bio-insecticides with synthetic products would not only promote local resources, but also offer credible alternatives for vector control.

It is in this context that the present study was conducted, with the aim of evaluating the larvicidal activity of the essential oils of *Cymbopogoncitratus* (DC.) Stapf. and*Cymbopogongiganteus*Chiov., two aromatic plants found in Niger's biodiversity, on the larvae of Anopheles gambiaes.l.

Materials and methods:-

Materials

Plant material

The plant material used in this study consisted of *Cymbopogoncitratus* leaves and leafy stems with inflorescences of *Cymbopogongiganteus*, shadedried for two weeks, as shown in Table 1. These two plant species were authenticated at GarbaMounkaila Laboratory, Biology Department of AbdouMoumouni University in Niamey by comparison with reference specimens preserved in the laboratory's herbarium.

Table 1:-Harvestedplants

Plant species	Botanicalfamily	Part used	Place of harvest
Cymbopogoncitratus(DC.) Stapf.	Poaceae	Dry leaves	Harobandaricefield
CymbopogongiganteusChiov.	Poaceae	Stems + leaves + dried inflorescences	Say (Tillabéry)

Animal material

*Anopheles gambiae*s.l. larvae in stages II and III were used in this study. They were collected by sieving using a plastic strainer in the Saguiyaneighbourhood (Commune V, Niamey, Niger). The collected larvae were then transferred to a plastic bucket containing water from their natural habitat and transported to Biology Laboratory at AbdouMoumouni University in Niamey. Before being used for biological testing, they were thoroughly rinsed with well water, then kept for 24 hours in a plastic bucket and fed with carbohydrate-rich biscuits. The specific identification of the larvae was confirmed at the Centre for Medical and Health Research (CERMES, Niamey, Niger) by MrSadouKadri.

Methods

Essential oils Extraction

Essential oils were extracted from the leaves of *Cymbopogoncitratus* and the leafy stems with inflorescences of *Cymbopogongiganteus,*fresh harvested and previously shade dried for two weeks. Extraction was carried out by hydrodistillation using a Clevenger-type apparatus. To do this, 100 g of dry, unpulverised plant material was placed in a 2 L flask containing 700 mL of distilled water, and the contents of the flask were boiled for two hours from the point at which the water began to boil.

After distillation, the essential oil was isolated from the aqueous distillate by liquid-liquid extraction with diethyl ether (3 × 10 mL) using a separating funnel. The organic phases were combined in a pre-weighed bottle, and the ether was removed by evaporation at room temperature. The essential oil obtained was stored in a bottle covered with aluminium foil and kept in the freezer until use.

Cymbopogoncitratus essential oilanalysis

The essential yellowoil previously extracted from *Cymbopogoncitratus* was analysed by gas chromatography coupled with mass spectrometry (GC-MS).

The analysis was carried out at the AM2N laboratory of the Charles Gerhardt Institute in Montpellier (ICGM UMR 5253 of the CNRS). To do this, 1µl of the sample was injected into the gas chromatograph coupled with a mass spectrometer (SHIMADZU, model QP2010SE), equipped with a 20 m long PhenomenexZebron ZB-5ms column, with an internal diameter of 0.18 mm and a stationary phase film thickness of 0.18 µm. The injector is a split/splitless, Fast type. The regulator is set at 970 kPa, and the controller has 150 positions. The mass spectrum (MS) has an ionisation mode of electron impact, a scan speed of 50 scans/s, and an acquisition speed of 10,000 uma/s. The oven temperature is programmed from 50 °C with a 2-minute plateau to 280 °C with a gradient of 22 °C/min and a final plateau of 2 minutes at 280 °C. The carrier gas is helium with a flow rate of 0.7 ml/min.

Essential oil constituentsIdentification

The various constituents of the essential oil were identified using their retention indices and mass spectra. The results obtained were then compared with reference data available in Shimadzu manufacturer's library (NIST 2008).

Larvicidal activity Studyof essential oil

Biological tests were carried out using a WHO protocol (1985). Preliminary biological tests were conducted, resulting in the following concentration ranges being selected: 0; 25; 50; 75, 100, 125, 150, 175 and 200 ppm and 0.3, 0.6, 1.2, 1.5, 3, 6, 9 and 12 ppm respectively for the essential oil sample and the reference product (Deltamethrin).

Well water was used in the various dilutions. Twenty (20) *Anopheles gambiae* larvae were placed in each 100 ml Petri dish containing the test solution and left to incubate for 48 hours at room temperature. The negative control consisted solely of well water. The dead larvae were counted after 48 hours of exposure. Each experiment was repeated three times.

Mortality percentage

The mortality rate observed in *Anopheles gambiae* larvae was corrected using the Abbott method (1925). This method takes into account the natural mortality recorded in the control groups in order to avoid overestimating the effectiveness of the treatments.

$$\% \text{ m} = \frac{\text{NLM} - \text{NLMT}}{\text{NTL} - \text{NLMT}} \times 100$$

% m = mortality percentage
NLM = number of dead larvae in the Petri dishtest
NLMT = number of dead larvae in the control
NTL = total number of larvae

Lethal dose 50

The lethal dose 50 (LD_{50}) corresponds to the amount of essential oil required to cause mortality in 50% of Anopheles larvae. It was calculated using the method described by Dragstedt and Lang (1957).

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

X_2: upper concentration surrounding the LD_{50}
X_1: lower concentration surrounding the LD_{50}
Y_1: mortality percentage corresponding to X_1
Y_2: mortalitypercentage corresponding to X_2

Statistical analysis

The standardised data were subjected to analysis of variance (ANOVA) followed by Tukey's PLSD test at a probability threshold of 5% for statistically significant means separation. These were used to determine whether there was a significant difference between the different doses of the studied extracts and, if so, which dose was the most effective in terms of mortality.

Results:-

Essential oil extraction yield

The essential oil yields obtained from the hydrodistillation of *Cymbopogoncitratus* leaves and leafy stems accompanied by inflorescences of *Cymbopogongiganteus* are presented in Table 2. The extraction rates obtained were 1.91% for *C. citratus* and 0.46% for *C. giganteus*, respectively. Thus, *C. citratus* leaves had the highest extraction yield.

Table 2:- Essential oil yields

Plant species	Parts used	Yield (%)
C. giganteus	Leafy stems with dried inflorescences	0,46
C. citratus	Driedleaves	**1,91**

Chemical composition of Cymbopogoncitratus essential oil

The results of gas chromatography coupled with mass spectrometry (GC-MS) analysis of *Cymbopogoncitratus* essential oil are presented in Table 3. The analysis shows that the essential oil consists mainly of monoterpene compounds (97.51%). A total of fifteen (15) compounds were detected and identified, the main ones being α-citral (44.8%) and β-citral (32.7%).

The essential oil of *Cymbopogongiganteus* was not analysed in this study. However, several previous studies have described its chemical composition in different countries, notably in Burkina Faso (Bassolé et al., 2011), Benin (Alitonou et al., 2012) and Togo (Nyamador et al., 2010; Ketoh et al., 2004). These studies have all shown that the essential oil of *C. giganteus* belongs to the limonene chemotype.

Table 3: Chemical composition of *Cymbopogoncitratus* essential oil

Retention time	Chemical compounds	Percentage content (%)	Chemical structures
	Monoterpènes	**97,51**	
4544	Méthylhepténone	0,72	
4591	*β*-Myrcène	11,96	
5470	(3Z)-3-Undécen-5-yne	0,28	
5523	Linalol	0,82	
5882	3,3,5-Triméthyl-1,4-héxadiène	0,43	
5952	*β*-Citronellal	0,29	
6016	3-Cyclohéxène-1-carboxaldéhyde, 2,4,6-triméthyl-	1,42	
6155	Carane, 4,5-époxy-, *trans*	1,9	
6622	***β*-Citral**	**32,72**	
6682	3,7-Diméthyl-2,6-octadien-1-ol	1,87	
6830	***α*-Citral**	**44,8**	
6942	2-Undecanone	0,42	
7263	2,7-Octanediol, 2,7-diméthyl-	0,31	
7320	Neric acid	0,28	
7476	*acétate* de *géraniol*	1,75	
Total		**99,26**	

Larvicidal activity of *Cymbopogoncitratus* essential oil on *Anopheles gambiae* larvae after 48 hours of exposure.

Table 4 and Figure 1 show the mortalitypercentage of *Anopheles gambiae* larvae as a function of doses of *Cymbopogoncitratus* essential oil, *Cymbopogongiganteus* essential oil and deltamethrin (reference insecticide) after 48 hours of exposure. Analysis of these results highlights a dose-dependent effect: the larvicidal activity of essential oils increases proportionally to concentration.

The minimum concentration causing total mortality (100%) of *A. gambiae* larvae was estimated at 175 ppm for *C. citratus* and 200 ppm for *C. giganteus*, demonstrating significant insecticidal activity. The essential oil of *C. giganteus* proved to be the most active, with an LD_{50} of 70.13 ppm, followed by that of C. citratus (LD_{50} = 114.3 ppm) (Figure 2).

However, these essential oils remain less active than deltamethrin (LD_{50} = 2.3 ppm), used as a reference product. Despite this lower activity compared to a chemical insecticide, essential oils are of major interest for vector control because they are natural, biodegradable substances that are less polluting and more environmentally respectful than synthetic insecticides.

Table 4: Larvicidal activity of *Cymbopogoncitratus* essential oil after 48 hours of exposure

Concentration	*C. citratus*	*C. giganteus*
Control	0	0
25ppm	3 ± 2[b]	42±5[a]
50ppm	7±3[b]	58±5[a]
75ppm	58±4[c]	84±5[ab]
100ppm	72±10[b]	93±3[a]
125ppm	82±2[b]	95±5[a]
150ppm	82±7[bc]	95±0[ab]
175ppm	100±0[a]	96±2[a]
200ppm	100±0[a]	100±0[a]

Averages in the same row followed by identical letters are not statistically different (Tukey's PLSD test p<0.05).

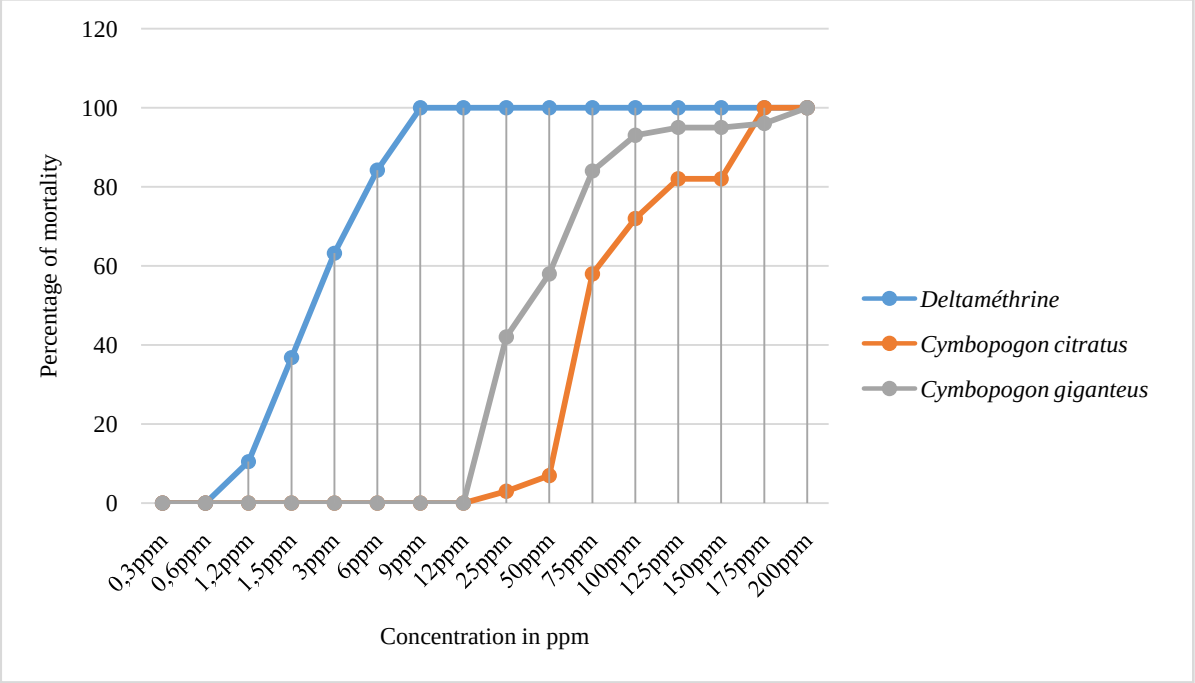

Figure 1: Larvicidal activity of essential oils from *C. citratus*, *C. giganteus* and deltamethrin against *Anopheles gambiae* larvae

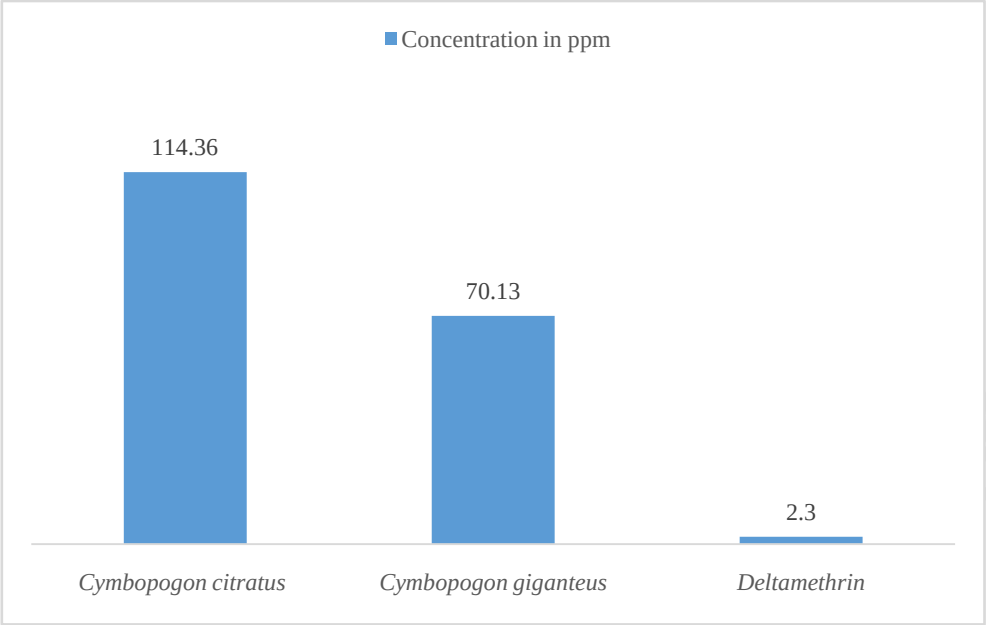

Figure 2:Lethal dose 50 (LD_{50}) of *C. citratus* and *C. giganteus* essential oils and deltamethrin.

Discussion

In the current context of increasing resistance of malaria-carrying mosquitoes to synthetic insecticides and the need to promote environmentally respectfulalternatives, this study was conducted to evaluate the larvicidal efficacy of essential oils from *Cymbopogoncitratus* and *Cymbopogongiganteus*, two aromatic plants widely found in Niger.

Hydrodistillation extraction of dried *C. citratus* leaves using a Clevenger-type apparatus yielded a maximum of 1.91%, highlighting the richness of this species in bioactive compounds. This yield is higher than those reported in other studies: 0.30% in India (Bora et al., 2025), 0.50% in Colombia (Vera et al., 2014) and 0.22% in Rwanda (Kabera et al., 2011). In contrast, stems with inflorescences of *C. giganteus* provided the lowest yield (0.46%), which is comparable to the results observed in Burkina Faso (0.6%, Bassolé et al., 2011). This variability in yields can be attributed to various environmental and biological factors, such as climatic conditions, soil composition, type of secretory organs and the phenological stage of the plants (Mbot et al., 2021).

Gas chromatography coupled with mass spectrometry (GC-MS) analysis of *C. citratus* leaves revealed an essential oil predominantly composed of monoterpenes, with α-citral (44.8%) and β-citral (32.72%) as the main constituents. These results are consistent with those reported in Benin (Kpadonou et al., 2019) and Côte d'Ivoire (Kobenan et al., 2019), where citrals were also among the main components. However, our observations differ from those of Vera et al. (2014) in Colombia, who identified geranial, β-myrcene and neral as the dominant constituents.

These differences can be explained by the variability of soil and climatic conditions, as well as the stage of plant development at harvesttime (Mbot et al., 2021).

The essential oil of *C. giganteus* was not analysed in this study. However, several previous studies have highlighted its high content of limonene, cis-p-mentha-2,8-dien-1-ol and p-mentha-1(7),8-dien-2-ol isomer (14.06%) (Bassolé et al., 2011; Nyamador et al., 2010; Alitonou et al., 2012).

Biological tests carried out on second and third instar larvae of *Anopheles gambiae* revealed significant larvicidal activity of the essential oils, with an LD_{50} of 70.13 ppm for *Cymbopogongiganteus* and 114.3 ppm for *Cymbopogoncitratus*. The essential oil derived from the leafy stems with inflorescences of *C. giganteus* proved to be the most active against the larvae.

This larvicidal activity could be attributed mainly to citrals and limonene, which are known for their insecticidal properties. Indeed, several studies have reported that citrals (α-citral and β-citral) from *C. citratus* are highly toxic to the larvae of *Aedesaegypti* and *Culexquinquefasciatus*, causing dose-dependent mortality (Silva et al., 2014; Regnault-Roger et al., 2012). Limonene, which is mainly present in *C. giganteus*, is effective against various mosquito species, including *Anopheles stephensi*, and acts as both a larvicide and a repellent (Lee et al., 2004; Kostyukovsky et al., 2002).

However, the efficacy of the essential oils studied remains lower than that of deltamethrin, used as a reference product (LD_{50}= 2.3 ppm). Despite this difference, *C. citratus* essential oil is a promising natural alternative in integrated malaria control strategies, offering the advantage of limiting the environmental impacts associated with chemical insecticides.

Conclusion :-

The results of this study indicate that the essential oils of the studiedplants, namely *Cymbopogoncitratus* and *Cymbopogongiganteus* harvested in Niger, exhibit significant larvicidal activity against *Anopheles gambiae*, the main vector of malaria. The best extraction yield was obtained with the leaves of *C. citratus* (1.91%). The main constituents of its essential oil are α-citral and β-citral. The essential oil of *C. giganteus* proved to be the most active, followed by that of *C. citratus*.

Although the larvicidal activity of essential oils remains lower than that of deltamethrin, they are a promising natural alternative in an integrated control strategy, combining biological efficacy and environmental preservation. Furthermore, exploring synergies with other biopesticides appears to be a relevant way to strengthen the fight against malaria in the long term and reduce dependence on synthetic insecticides. In this perspective, the analysis of the essential oil of *C. giganteus* represents an essential step in deepening our understanding of the insecticidal potential of the Cymbopogon species present in Niger.

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