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Effect of Essential Oils of Six Local Plants Used Insecticide on Adults of *Anopheles gambiae*, Giles 1902

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Abstract: The essential oils extracted of six plants have been tested on adults of *Anopheles gambiae* (Diptera: Culicidae) in view to determine their insecticide effect. It is about *Ocimum canum*, *Laggera pterodonta*, *Plectrancthus glandulosus*, *Eucalyptus camaldulensis*, *Hyptis spicigera* and *Pittosporum viridiflorum*. These oils have been diluted in the hexane to different concentrations (5, 10, 15, 20, 30, 40 and 50 mg of active matter by m^2 of screen) in order to expose anopheles in the OMS-cone. The introduction of 20 anopheleses in cones permits to note that alone two plants *Hyptis spicigera* and *Pittosporum viridiflorum* presented a weak insecticide activity. Except *P. viridiflorum*, the five other plants presented of lethals concentrations 50 (LC50) after 24 h of exhibition. Thus, we record for *H. spicigera* and efficient concentration *Laggera pterodonta* to 50 mg m^{-2}. On the other hand *Ocimum canum* is efficient to the concentration 40 mg m^{-2}. Lethal concentrations 50 (LC50) of *O. canum* and *Plectrancthus glandulosus* are, respectively 11.95 and 12.5 mg m^{-2} after 24 h of exhibition. These essential oils also possess the best lethal h 50 (LH50) with lengths of exhibition of 6 h 36 min 16 sec and 8 h 34 min 12 sec, respectively for O. *canum* and of *P. glandulosus*.

Key words: *Anopheles gambiae*, essential oils, LC50, LH50

INTRODUCTION

Bugs, especially mosquitoes are responsible of several parasitic illnesses at the such man the malaria, the yellow fever, the dengue and filariosis 8. The malaria in Africa contributes to a decrease of fruitfulness that affects the raw interior product heavily (Fekam, 2004). In Africa to the South of the Sahara, the malaria represents among the main reasons of morbidity and mortality for the man (Skinner and Johnson, 1980). Of synthesis products and derivative several types have been used without success against these vector agents (Njan Nlôga, 1994). In most cases, these products, not only present limits, but mislead the development of the resistance phenomenon at mosquitoes, giving back so the anti-vectorial chemical struggle little efficient. The program of struggle against mosquitoes only aims to reduce the density of the adult population for one very short time (El Hag *et al*., 1999, 2001). Pyrethrinoïdes have been used extensively to protect populations against stings of mosquitoes because of their repulsive effect and insecticide (Njan Nlôga, 1994). Substances of plant origin in general, the essential oils in individuals constitute this fact, a promising means of struggle against the

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vector bugs of illnesses (Prajapati *et al*., 2005; Strauss *et al*., 1968). Lees essential oils are recognized of a general manner as source natural of struggle against bugs (Gbolade *et al*., 2000; Adebayo *et al*., 1999). Generally, the essential oils are no poisonous for the man and the other mammals (Tripathi *et al*., 2002; Tedonkeng *et al*., 2002). The present research permits to put in evidence the insecticide effects of six oils essential of local plants, on adults of *Anopheles gambiae*.

MATERIALS AND METHODS

Study Area

The study is carried out in Ngaoundere (Cameroon), the headquarters of the Adamaoua province. It is a transition zone between the forested south and the sudan savannas of the north. The region is situated between latitudes 6°20 and 7°40 North and between longitudes 11° and 15° East. Its altitude varies between 900 m and 1500 m. Rainfall stretches over seven months with an average of 1500 mm. Average annual temperature varies between 23 and 25°C (Stranss *et al*., 1968; Tripathi *et al*., 2004).

Materials

The plant material is constituted of six plants *Ocimum canum*, *Laggera pterodonta*, *Plectranclhus glandulosus*, *Eucalyptus camadulensis*, *Hyptis spicigera* and *Pittosporum viridiflorum* on the introverted information basis close to the local populations for their efficiency in the struggle against mosquitoes. Leaves of these plants have been picked and have been dried to the shade during one week. Essential oil extractions have been achieved by the hydrodistillator of Clevenger type. The introverted oils in small bottles, tightly closed to the parafilm, are kept to the refrigerator to a controlled temperature to 4°C until the day of their utilization.

The used bugs are mosquitoes of the species *Anopheles gambiae*. These bugs are raised to the Laboratory of Biology of the University of Ngaoundere from a stock of eggs coming of the OCEAC to Yaounde (Cameroon).

Method

Eggs are soaked in the restrained source water in trays of 40 cm of diameter that are units of mosquito production. Every unit of production receives some pinches of nourishing gunpowder (shrimps+cookies 5 g) (Skinner and Johnson, 1980). This fasting is introduced every morning to 8 h and this counterpart 5 days. The water of trays is renewed every two day. The renewal of the water of source permits to maintain its quality and to avoid its pollution of the fact of the daily introduction of the nourishing gunpowder. Nymphs are withdrawn then of trays and are transferred in the transparent glasses. Glasses also containing the water of source are placed inside of cages made of canvas of volume 8000 cm^3 screen. In every cage, is placed a box of kneaded containing the sugary juice made to basis of sucrose 10%, serving of food for the adult mosquitoes.

Every essential oil is diluted to 7 different concentrations in the Hexane. Concentrations go to 5, 10, 15, 20, 30, 40 and 50 mg m^{-2}. Screens filled of the different oils to the precise concentrations, are fixed then on the basis of the cone-OMS with the help of transparent sticky paper.

In every cone and for all concentrations, 20 anopheleses have been introduced. The effect Knock down has been observed after 3 min of exhibition and the device is maintained until 24 h with observations every 2 h. The number of mosquitoes killed is noted to calculate the death rate. The lethal concentration 50 (CL50) has been gotten after 24 h of treatment. The experience has been repeated for every oil and for every concentration, 5 times.

The regression of the logarithm of the dose as well as the transformation of mortality rates in probit permitted to determine the CL50 (Tedonkeng *et al*., 2002). The second regression is achieved according to hours to get the HL50.

RESULTS

Efficiency of the Essential Oils

Oil essential of *L. pterodonta* possesses a more efficient insecticide effect on adults of *A gambiae*. The maximal mortality of mosquitoes, 100% are observed to the concentration 50 mg m^{-2} after 24 h of exhibition (Table 1). The insecticide effect increases with the concentration of the essential oil used. It comes out again that mortality grows with the concentration. The insecticide activity of this oil begins from the concentration 20 mg m^{-2}. The Table 1 watch that concentrations 5, 10 and 15 mg m^{-2} present a weak activity beyond the 18th h.

Oil essential of *H. spicigera* has a progressive insecticide effect but limited on adults of *A. gambiae*. So, no concentration didn't provoke the death of all mosquitoes. Concentrations 5, 10 and 15 mg m^{-2} have insecticide effect from the 12th h of exhibition (Table 2). After 24 h of exhibition, the concentration 50 mg m^{-2} induced 70% of mortality. The concentration 20 mg m^{-2} has an insecticide efficiency splices the 22nd and the 24th h. The efficient dose was not therefore reached, even to the highest concentration of 50 mg m^{-2} used.

Of the Table 3, it comes out again that the death rate also grows with the concentration and the time of exhibition. With oil essential of *O. canum*, concentrations 40 and 50 mg m^{-2} misled one death

Table 1: Mortality of *Anopheles gambiae* adults according to the length of exhibition to the different concentrations of essential oil of *Laggera pterodonta*

Concentration	Time (h)											
(mg m^{-2})	2	4	6	8	10	12	14	16	18	20	22	24
5	0	0	0	0	1	2	2	2	3	5	17	16
10	0	1	1	1	2	4	4	5	6	11	19	21
15	0	4	4	7	7	7	8	9	15	16	25	28
20	3	6	6	12	22	22	22	24	26	32	37	39
30	7	9	11	20	27	29	29	30	41	45	47	50
40	10	12	22	26	28	34	35	46	55	57	57	68
50	16	19	27	35	35	40	41	57	62	67	83	100

Table 2: Mortality of *Anopheles gambiae* adults according to the length of exhibition to the different concentrations of essential oil of *Hyptis spicigera*

Concentration	Time (h)											
(mg m^{-2})	2	4	6	8	10	12	14	16	18	20	22	24
5	0	0	0	0	0	1	3	5	8	10	13	16
10	0	0	0	0	1	3	4	7	10	12	15	21
15	0	0	0	2	3	5	6	11	13	16	21	26
20	0	1	5	4	5	9	9	9	16	21	26	41
30	2	3	5	6	7	10	13	13	19	25	31	47
40	5	7	10	12	12	13	15	15	27	34	37	58
50	7	10	10	15	15	21	23	23	31	44	53	67

Table 3: Mortality of *Anopheles gambiae* adults according to the length of exhibition to the different concentrations of essential oil of *Ocimum canum*

Concentration	Time (h)											
(mg m^{-2})	2	4	6	8	10	12	14	16	18	20	22	24
5	0	0	0	3	4	6	10	11	13	18	21	17
10	0	0	3	6	8	13	18	19	25	22	28	31
15	2	5	7	13	14	17	20	22	32	35	37	47
20	5	7	10	18	25	28	35	37	38	44	50	72
30	6	11	12	22	35	37	38	42	45	52	61	88
40	15	19	27	32	43	47	54	57	66	72	82	98
50	20	30	33	40	53	66	68	81	84	93	98	100

rate to 100% at the end of 24 h. What translates a more elevated efficiency of this essential oil with regard to *L. pterodonta*. Concentrations 5, 10 and 15 mg m^{-2} of oil essential of *O. canum* could not have provoked a mortality of 50% after 24 h of exhibition. The efficient concentration of *O. canum* on adults of *A. gambiae* is 40 mg m^{-2}.

Of the Table 4, it comes out again that the death rate also grows with the concentration and the time of exhibition. Concentrations 5, 10 and 15 mg m^{-2} of oil essential *P. glandulosus* become efficient from the 18th h. At the 24th h, the concentration 50 mg m^{-2} misled 100% of mortality at adults of *A. gambiae*. What wants to say that the concentration efficient of *P. glandulosus* is 50 mg m^{-2}.

Oil essential of *E. camaldulensis proved out to be efficient as the one of O. canum*. Concentrations 5, 10 and 15 mg m^{-2} begin their insecticide effect from the 12th h (Table 5). One notes that the death rate grows with the concentration. On the other hand no concentration could have provoked one death rate to 100%. To the biggest concentration of 50 mg m^{-2}, 90% of expositions mosquitoes died.

Essential oil of *P. viridiflorum*, possesses a weak toxicity not only, but has an extremely retarded insecticide effect (Table 6). It takes out again the face 6 that, some either concentration understood between 5 and 15 mg m^{-2}, essential oil of *P. viridiflorum* remains inactive on adults of *A. gambiae*. No concentration could not have misled a mortality to 50% after 24 h of exhibition. Only to the biggest concentration of 50 mg m^{-2}, 45% of mortality have been observed after 24 h.

Table 4: Mortality of *Anopheles gambiae* adults according to the length of exhibition to the different doses of essential oil of *Plectrancthus glandulosus*

Concentration	Time (h)											
(mg m^{-2})	2	4	6	8	10	12	14	16	18	20	22	24
5	0	0	0	1	1	1	1	2	2	6	20	31
10	0	2	2	3	4	4	5	5	6	12	24	33
15	1	5	5	8	8	9	9	10	11	17	33	40
20	4	6	7	13	22	23	23	24	27	29	50	50
30	7	10	12	21	28	29	31	33	42	44	70	74
40	12	13	22	27	29	35	38	48	59	64	83	88
50	17	20	28	36	36	42	53	62	71	77	92	100

Table 5: Mortality of *Anopheles gambiae* adults according to the length of exhibition to the different concentrations of essential oil of *Eucalyptus camaldulensis*

Concentration	Time (h)											
(mg m^{-2})	2	4	6	8	10	12	14	16	18	20	22	24
5	0	0	0	1	2	4	5	7	10	12	15	17
10	0	0	1	2	4	5	12	9	16	22	28	32
15	1	2	3	5	11	13	19	19	23	23	32	43
20	3	4	5	9	13	15	20	20	25	30	40	51
30	6	10	11	14	20	22	23	27	37	44	53	64
40	10	12	15	20	22	25	26	31	41	48	55	78
50	13	22	27	30	34	37	40	43	50	53	68	87

Table 6: Mortality of *Anopheles gambiae* adults according to the length of exhibition to the different concentrations of essential oil of the *Pittosporum viridiflorum*

Concentration	Time (h)											
(mg m^{-2})	2	4	6	8	10	12	14	16	18	20	22	24
5	0	0	0	0	0	0	0	0	1	3	4	4
10	0	0	0	0	0	0	0	1	1	2	6	7
15	0	0	0	0	0	0	1	2	5	5	10	12
20	0	0	0	0	2	2	2	5	9	8	16	16
30	1	2	2	2	3	3	6	5	10	9	26	26
40	2	4	4	4	5	5	8	9	13	10	36	36
50	5	7	7	7	8	8	10	10	15	23	43	43

Table 7: Different CL50 of essential oil used on adults of *Anopheles gambiae* after 24 h of exhibition

Plants	Equation of regression	r	dl	CL50 (mg m^{-2})
Ocimum canum	y = 3.8653x +0.8362	0.9151***	6	11.95a
Plectrancthus glandulosus	y = 2.9929x +1.7205	0.8186**	6	12.5a
Laggera pterodonta	y = 3.0691x +1.2189	0.7714*	6	17.06b
Eucalyptus camaldulensis	y = 1.9871x +2.5551	0.9843***	6	16.76b
Hyptis spicigera	y = 1.4586x +2.8378	0.9709***	6	30.36c
Pittosporum viridiflorum	y = 1.6096x +2.0124	0.9864***	6	71.79d

Values on the same column followed by the same letter are not significant different at 5% level; *** Very highly significant (p = 0.001); ** Very significant (p = 0.01); *Significant (p = 0.05)

Table 8: Different HL50 of essential oil used on adults of *Anopheles gambiae* after 24 h of exhibition

Plants	Equation of regression	r	dl	HL50 (h min^{-1} sec^{-1})
Ocimum canum	y=3.03x +2.5143	0.8569***	11	6 h 36 min 36 sec
Plectrancthus glandulosus	y=2.6864x +2.4934	0.7748**	11	8 h 34 min 12 sec
Laggera pterodonta	y=2.4378x +2.6128	0.7256**	11	9 h 31 min 48 sec
Eucalyptus camaldulensis	y=1.5608x +3.1936	0.8677***	11	14 h 22 min 10 sec
Hyptis spicigera	y=1.5404x +2.7128	0.8673***	11	30 h 31 min 48 sec
Pittosporum viridiflorum	y=1.1474x +2.6983	0.7458**	11	101 h 23 min 24 sec

*** Very highly significant (p = 0.001); ** Very significant (p = 0.01)

Assessment of the CL50 (Lethal Concentration 50)

The determination of the essential oil concentrations that provokes 50% of mortality at adults of *A. gambiae* has been done from the right of regression of the essential oil concentrations according to the death rate expressed in values of probit.

Table 7 represent the equation of regression, the coefficient of interrelationship, the degree of liberty and the CL50 gotten for the different essential oils below.

The analysis of the different results watch that, essential oil of oh. *canum*; *P. glandulosuses* were revealed, respectively more efficient with the CL50 of 11.95 and 12.5 mg m^{-2}, follow-up of *E. camaldulensis* and of *L. pterodonta* with the CL50 of 16.76 and 17.06 mg m^{-2}, respectively.

Assessment of the HL50 (Lethal Hour 50)

The determination of hours to leave of which the concentration of induced essential oil 50% of mortality at *A. gambiae* has been done. This parameter has been gotten from the right of the logarithm of hours according to the death rate expressed in value of probit.

It takes out again the Table 2 that *O. canum* possesses a HL50 weak, follow-up of *P. glandulosus* and of *L. pterodonta* (Table 8). One notes that it exists an interrelationship between the efficiency of an essential oil (CL50) and its lethal hour (HL50) at certain plants. For example, essential oil of *O. canum* that is more efficient also has a HL50 weak (6 h 36 min 36 sec). It is some in the same way for oil essential of *P. glandulosus*.

Of general manner, one noted an interrelationship between the efficiency of every essential oil (HL50) and its lethal hour 50 (HL50). The essential oils that presented a weak efficiency also presented a HL50 elevated.

Of all used essential oils, the one of *P. viridiflorum* was revealed least efficient with regard to others, because its CL50 doesn't have can be gotten that to leave 71.79 mg m^{-2} and its HL50 very elevated, is valued to 101 h 23 min 24 sec.

In sum, the poisonous effects of every plant on adults of *A. gambiae* depend on the plant, concentration and the length of exhibition.

DISCUSSION

Essential oil from tested plants possesses some real insecticide properties on adults of *A. gambiae*. Essential oil of *O. canum* revealed the same mortality at lower concentrations. The lethal concentration of 50 (CL50) was obtained at 11.95 mg m^{-2} after 24 h of treatment. An insecticide

activity of essential oil of *O. canum* on adults of *A. stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*, with the more elevated concentrations (75, 82.4 and 115.3 mg of active matter in a volume of 0.50 mL of a volatile solution of acetone) were reported by Rodhain and Perez (1985) and Rao *et al.* (2000). This exhibition dragged in every case, one death rate of 95% after 1 h of exhibition. The difference between these results and Present results, may be attributed to differences in mosquitoes species as well as the selected concentrations used during manipulations.

Results from other authors (Rodhain and Perez, 1985; Chittihunsa and Samngamnim, 1999; Keita *et al.*, 2002) suggested that essential oil of *O. canum* exercises a toxicity and possesses an anorexigen property on other bugs species.

Essential oil of was less efficient than those of *O. canum*, *P. gladulosus and L. pterodonta* due to the fact that 24 h were not enough to provoke 100% mortality for *E. camaldulensis* compared to others. Mark *et al.* (2002) studying the repulsive effect and the remanence of essential oils of 38 plants found that *Eucalyptus* oil was the only product able maintain a long term remanence from more than 6 months.

The ongoing survey on the remanence of these different essential oils of the local plants, will permit the comparison of insecticide effects with regard to the currently used chemicals such as Lambdcyalothrin. This product at a concentration of 15 mg m^{-2} was reported to have a remanence of 7 months closely with a reduction of 69% on adults of *A. moucheti* (Njan Nlôga, 1994). At Kumbantonie in Tanzania, Magesa *et al.* (1991) observed reductions of 70.2% of the aggressive densities, 93% of the daily rate of inoculation for adults populations of *A.. gambiae* and *A. funestus*, for a concentration of 30 mg m^{-2}. Other tests achieved in Tanzania by (Njunwa *et al.*, 1991) indicated that field application of Lambdacyalothrin at 30 mg m^{-2} and 10 mg m^{-2} would provoke a mortality of 100% of adult population of *A. gambiae* for six months. The efficiency of these essential oils can be assigned to the organic solvent used or certain substances present in plants. From other results (Njunwa *et al.*, 1991; Keita *et al.*, 2002; Jirovetz *et al.*, 2002), monoterpeneses hydrocarboneses are very poisonous molecules to mosquitoes. Results by Khan *et al.* (1969); Tripahi *et al.* (2004) have revealed that the presence of thiamine and vitamin B1 in *Eucalyptus* oil may be responsible for the insecticide properties of this plant.

Contrarily to information received from peasants, laboratory tests have revealed that essential oil from *H. spicigera* is less efficient on adults population than others. Nevertheless, the higher concentration used could confirm its efficiency. Works done by Jirovetz *et al.* (2002) and Ngassom *et al.* (2002) on the chemical composition of essential oils of plants sampled in Cameroon showed that *H. spicigera* contains a weak proportion of 13.36% of sesquiterpenes oxygenated and 43.11% of monoterpene hydrocarbon. On the other hand, essential oils of *Annona senegalensis* fairly rich in monoterpene hydrocarbon (32.95%) and in oxygenated sesquiterpenes (31.12%), as well as *Lippia rugosa* with 19.76% of monoterpene hydrocarbon and 71.66% of oxygenated monoterpene were revealed to be more efficient than *H. spicigera*. The very low efficiency of *H. spicigera*, well rich in monoterpene hydrocarbon would be the origin of its weak toxicity on mosquitoes.

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