# EVALUATION OF THE INSECTICIDAL, LARVICIDAL AND OVICIDAL EFFECTS ON *CALLOSOBRUCHUS MACULATUS* OF ESSENTIAL OILS OF *CYMBOPOGON GIGANTEUS* AND OF *XYLOPIA AETHIOPICA*

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**Abstract:** The chemical composition of essential oils extracted from leaves of *Cymbopogon giganteus* (Hochst.) Chiov and fruits of *Xylopia aethiopica* (Dunal) A. Richard has been studied by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The doses of each of these essential oils tested on *Callosobruchus maculatus* at temperatures between 22 °C and 31 °C and a relative humidity of 80% are 0, 10, 20, 30 and 40μL.mL$^{-1}$. The tests of conservation carried out of cowpea seeds (*Vigna unguiculata* (L.) Walp.) of local variety "Chawe" showed that these essential oils have variously exhibited larvicidal and ovicidal effects on larvae and eggs of *C. maculatus* (Fabricius) adult to the highest dose tested (40 μL.mL$^{-1}$).The damage generated by *C. maculatus* and its larvae were evaluated by the percentages of attack (A) and the loss in weight of seeds of cowpea (B).

**Keywords:** *essential oil, Cymbopogon giganteus, Xylopia aethiopica, cowpea, "Chawe" Callosobruchus maculatus, weight loss*

## INTRODUCTION

In the African countries located at the south of the Sahara and in particular Benin, cowpea is one of principal food leguminous plants [1]. With a rate of 25% protein, cowpea appears as a source of protein the least expensive for the poor. In West Africa where more than 70% of the totality of cowpea is produced, it has become gradually an integral part of the farming systems [2].
However, in the farms located in the rural zones of West Africa of which those of Benin, farmers continue to have enormous difficulties for the protection of seeds of cowpea after harvest. In this regard, many efforts to improve yields by the use of pesticides and synthetic chemical insecticides are generally destroyed in post-harvest pests by certain depredators, such as *C. maculatus* which engage their fixed prices since the fields and continue them until in the storage structures. Moreover, the presence in the stored food products of toxic residues and the appearance of stocks of *C. maculatus* resistant to the pesticides and chemical insecticides become increasingly alarming and thus call upon alternative solutions. The bad consequences arising from the use of these insecticides and synthetic pesticides make farmers resort today to phytotherapy by the use of the aromatic plants whose essential oils seem a less expensive solution, without major consequences and impacts on the health of man, on biodiversity and on the environment.
In tropical Africa, and Benin in particular, there are aromatic vegetable species whose populations are useful traditionally for protection of harvests and other medical needs [3]. These vegetable species constitute a potential reserve of identification and natural extraction of biopesticides to the profit of agriculture [4]. Several essential oils extracted from these very varied aromatic plants are, with controlled amount, less toxic to human health, non-persistent in water, soil and nature in general [5]. They constitute by this fact a very significant source of income for the African farmers [6].
This study which lies within the scope of the protection of stocks of cowpea in its two forms (seed and pod) is designed to optimize the use of aromatic plants acclimated in Benin (*C. giganteus* and *X. aethiopica*) by the evaluation of their insecticidal, larvicidal and ovicidal properties of the essential oils on *C. maculatus*.

## MATERIALS AND METHODS

### Plant materials

The leaves of *C. giganteus* were collected in Gbakpodji (commune of Bopa) in September 2008. The fruits of *X. aethiopica* were taken in an agricultural farm located at Banigbe (commune of Ifangni) in December 2007.

Cowpea "Chawe" of an endogenous variety was taken from Dannou Tokpli (commune of Adjohoun). It was kept fresh in two forms: seed pod 20 g of cowpea containing on average 129 grains per sample were placed in each vial.

**Test organisms**

Adults of *C. maculatus* were obtained from a farmer's reserve seeds cowpea in Dannou Tokpli. In the laboratory, they were put in breeding in June 2008 (T = 27.5 ± 0.2 °C; Relative humidity = 80.3 ± 1.6%) for obtaining new generations.

**Phytochemical analysis**

The leaves and fruit were stored in the laboratory at 18 – 20 °C throughout the extraction work. The essential oils were obtained by water distillation of the leaves and fruit (300 g) for 3 hours in a Clevenger type apparatus. They were dried over anhydrous sodium sulphate and analysed by GC-MS. The yield of essential oil from the leaves of *C. giganteus* and the fruit of *X. aethiopica* are relatively important (Table 1).

**GC/MS**

The essential oils were analysed on a Hewlett-Packard gas chromatograph Model 7890, coupled to a Hewlett-Packard MS model 5875, equipped with a DB5 MS column (30 m × 0.25 mm; 0.25 μm), programming from 50 °C (5 min) to 300 °C at 5 °C/min, 5 min hold. Helium as carrier gas (1.0 $mL.min^{-1}$); injection in split mode (1:30); injector and detector temperature, 250 and 280 °C respectively. The MS working in electron impact mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180 °C; mass spectra data were acquired in the scan mode in *m/z* range 33-450.

**GC/FID**

The essential oils were analysed on a Hewlett-Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30 m × 0.25 mm; 0.25 μm), programming from 50 °C (5 min) to 300 °C at 5 °C/min, 5 min hold. Hydrogen as carrier gas (1.0 $mL.min^{-1}$); injection in split mode (1:60); injector and detector temperature, 280 and 300 °C respectively. The essential oil is diluted in hexane: 1/30.

The compounds assayed by GC in the different essential oils were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by GC-MS by comparison of their mass spectra with those of reference substances [7 – 9].

**Insecticidal, larvicidal and ovicidal analysis**

Tests on the various effects (preventive, curative and remanent effects) [10] are conducted in an environment whose temperature varies between 22 °C and 31 °C with a relative humidity located around 80%. Five concentrations (0, 10, 20, 30, 40 $\mu L.mL^{-1}$) obtained by dissolution of essential oil in absolute ethanol were tested.

**Preventive effect**

The vegetable material of variety "Chawe" is treated with each essential oil. After 48 h, the adults of the same age (0 to 48 hours) are taken at random from the breeding ground for sex determination (two females and one male). These insects are selected then

deposited according to the mode of infestation on the vegetable material. On the whole, 5×3×2 = 30 experimental units are installation. The assembly thus carried out is put in observation with intervals of regular times (24 h) for the follow-up of oviposition of the females. The infestation occurs 72 hours at least after the deposit of the insects. During the observation, dead insects are immediately replaced and recorded. At the end of six days, the insects were separated from the grains. The experimental units were subsequently observed for 20 days to follow at regular time interval (24 h) for the rate of emergence of young insects.
The control treatment made up of grain samples untreated by essential oil was infested with *C. maculatus.*
During this period, it followed there rigorous of the various manifestations of the insect to the level of the substrate and the various following parameters were collected:

- the number of insects dead and recorded during six days;
- the number of insects emergent of the various forms of cowpea infested and recorded daily during 20 days;
- the rate of infested grains and the weight loss of cowpea grains.

**Curative effect**
The insects were deposited on the vegetable material. After six (6) days, they were separated and only the vegetable material thus infested was treated with essential oil. The different stages of observation were identical to those of the preventive test.
The methodology is as follows. The insects were deposited on the vegetable material. After six (6) days, they were separated and only cowpea thus infested is treated with essential oil.
The different stages of observation were identical to those of the preventive test. The parameters measured are:

- the number of insects which died and recorded during six days;
- the number of insects emergent of the different forms of cowpea infested and recorded daily for 20 days;
- the number of infested grains and the weight loss of cowpea grains.

**Remanent effect**
Remanence has been evaluated by infesting the samples of cowpea treated with essential oil for 15 days. On the samples beforehand treated, the insects were deposited and put in observation. The parameters measured during this test were:

- the number of insects which died at the end of 16 days;
- the number of emergent insects at the end of 20 days;
- the number of infested grains and the weight loss of cowpea grains.

**Estimated losses after treatment**
To better appreciate the damages of *C. maculatus* on the grains of cowpea, the grains of the substrate were recovered, counted and weighed. The rate of weight loss of dry material of the grains was determined according to MCP (Method of Counting and Weighing). It is the method which presents a more harmonious development of loss of material (subject) by report MPVS (Method of Standard Volumetric Weight) [11 – 12]. Two criteria of appreciation of the damages are commonly used: it is about the

percentage of attack of grains (A%) and percentage weight loss (B%) [13], respectively calculated by the equations:

$$A\% = \frac{N_a}{N_a + N_s} \times 100 \qquad B\% = \frac{P_s N_a - P_a N_s}{N_a + N_s} \times 100$$

*Na* = number of attacked grains;
*Ns* = number of healthy grains;
*Pa* = weight of damaged grains;
*Ps* = weight of healthy grains.

**Statistical analysis**

The results from the observations were treated statistically by analysis of variance method (ANOVA) using S A S software (Statistical Analysis System) Version 9.1 [14]. The raw data underwent the following transformations:

- Arcsin ( $\sqrt{X/n}$ ), X being the number of insects died under the influence of the essential oil and n indicating the total number of insects introduced into each bottle.
- $\sqrt{X+0{,}5}$ (X is the number of young *C. maculatus* having emerged of the substrate).

The masses of grains affected being continuous quantitative data and respecting the conditions of normalization and equal variance were no statistical transformation.
Finally, it was proceeded to a structuralization of the averages by means of the test of Newman and Keuls [14]. The results of statistical tests are considered significantly different, when the probability of the null hypothesis is less than or equal to 5%.

## RESULTS AND DISCUSSIONS

**Yield and chemical composition of essential oil of *C. giganteus* and *X. aethiopica***

The extraction yields compared to the fresh material are respectively 0.15±0.00% for *C. giganteus* and 1.25±0.01% for *X. aethiopica*.
The essential oil of *C. giganteus* contains the following majority compounds (> 5%): *trans*-1- mentha-1(7),8-dien-2-ol (19.9%), *cis*-mentha-1(7),8-dien-2-ol (18.4%), *trans*-para-mentha-2,8-dien-1-ol (17.4%), *cis*-para-mentha-2,8-dien-1-ol (8.9%), limonene (7,8%) and trans-carveol (5.1%). As for that of *X. aethiopica*, the major compounds (>5%) are: 1,8-cineole (15.8%), myrtenal (13.2%), terpinen-4-ol (9.8%) and *cis*-sabinol (6.7%).

**Comparative effects of essential oils of *C. giganteus* and *X. aethiopica* on *C. maculatus* living on cowpea**

The tables below present the results generated by different effects of each essential oil on *C. maculatus* living on a given form of conservation of cowpea.
In these tables, the statistical averages are compared by column. The averages followed by the same letter are significantly not different at the beginning of 5 % (test of Newman and Keuls).
For Tables 1 to 6:

*ns* = not significant difference at the beginning of 5 %
* = significant difference at the beginning of 5 %
** = highly significant difference (1 %)
*** = very highly significant difference (0.1 %)
The Table 1 shows the evolution of the rates of mortality, emergence, the number of grains attacked and loss of weight caused by *C. maculatus* by the presence of the essential oil of *C. giganteus*.

***Table 1.*** *Rate of **C. maculatus** dead, of emergence, number of grains of cowpea affected (A) and the loss of weight (B) caused by the essential oil of **C. giganteus** in curative method*

| Curative method | | | | |
|---|---|---|---|---|
| Doses ($\mu L.mL^{-1}$) | **cowpea grain (without pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.37±0.03(12.96)a | 9.83±0.67(97.00)a | 0.23±0.04(5.43)a | 1.03±0.04(0.70)a |
| 10 | 0.51±0.06(24.07)a | 0.88±0.17(0.33)b | 0.05±0.05(0.77)b | 0.99±0.01(-0.23)a |
| 20 | 0.38±0.09(14.81)a | 0.88±0.17(0.33)b | 0.03±0.03(0.26)b | 1.00±0.00(0.00)a |
| 30 | 0.52±0.09(25.93)a | 0.88±0.17(0.33)b | 0.03±0.03(0.26)b | 1.00±0.00(0.09)a |
| 40 | 0.36±0.06(12.96)a | 0.71±0.00(0.00)b | 0.04±0.04(0.57)b | 1.00±0.00(0.00)a |
| Probability | 0.3421ns | < 0.0001*** | 0.0017** | 0.7061ns |
| CV (%) | 29.21 | 21.67 | 85.43 | 3.15 |
| | **cowpea grain (in pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.44±0.06(18.52)a | 8.93±0.42(79.67)a | 0.22±0.02(4.91)a | 0.99±0.01(-0.23)a |
| 10 | 0.24±0.14(9.26)a | 1.76±0.18(2.67)b | 0.23±0.04(5.43)a | 1.03±0.04(0.70)a |
| 20 | 0.33±0.05(11.11)a | 0.71±0.00(0.00)c | 0.28±0.04(7.75)a | 0.99±0.02(-0.10)a |
| 30 | 0.27±0.03(7.41)a | 0.71±0.00(0.00)c | 0.32±0.03(9.82)a | 1.07±0.04(1.96)a |
| 40 | 0.40±0.09(16.67)a | 1.22±0.00(1.00)bc | 0.27±0.06(8.01)a | 1.00±0.01(0.11)a |
| Probability | 0.4704ns | < 0.0001 *** | 0.4677ns | 0.2362ns |
| CV (%) | 43.72 | 13.41 | 25.54 | 4.58 |

The results of estimating the influence of different concentrations of the essential oil of *C. giganteus* on mortality of *C. maculatus* adult, the emergence of young insects, the rate of grains attacked and the corresponding weight losses are presented in Table 2.

***Table 2.*** *Rate of* ***C. maculatus*** *dead, emergence, number of cowpea grains attacked (A) and weight loss (B) caused by the essential oil of* ***C. giganteus*** *in preventive method*

| **Preventive method** | | | | |
|---|---|---|---|---|
| **Doses ($\mu L.mL^{-1}$)** | **cowpea grain (without pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.36±0.07(12.96)b | 8.49±0.71(72.67)a | 0.43±0.14(19.38)a | 1.01±0.02(0.31)a |
| 10 | 0.59±0.05(31.48)a | 3.52±0.25(12.00)ab | 0.03±0.03(0.26)a | 0.99±0.00(-0.01)a |
| 20 | 0.63±0.04(35.18)a | 3.62±1.75(18.67)ab | 0.18±0.10(5.17)a | 0.99±0.00(-0.01)a |
| 30 | 0.53±0.06(25.93)ab | 4.83±1.67(28.33)ab | 0.26±0.14(9.82)a | 1.01±0.01(0.30)a |
| 40 | 0.65±0.08(37.04)a | 1.83±1.12(5.33)b | 0.09±0.09(2.33)a | 1.00±0.00(0.05)a |
| Probability | 0.0443* | 0.0324* | 0.1606ns | 0.8183ns |
| CV (%) | 19.57 | 48.05 | 95.49 | 1.76 |
| | **cowpea grain (in pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.63±0.02(35.18)bc | 6.05±0.31(36.33)a | 0.26±0.03(6.72)ab | 1.01±0.01(0.31)a |
| 10 | 0.63±0.07(35.18)bc | 1.81±0.32(3.00)b | 0.18±0.03(3.36)b | 1.00±0.00(0.02)a |
| 20 | 0.53±0.04(25.93)c | 1.68±0.09(2.33)b | 0.22±0.04(4.91)ab | 0.99±0.00(-0.00)a |
| 30 | 0.84±0.03(55.55)a | 5.87±0.73(35.00)a | 0.33±0.02(10.34)a | 0.99±0.01(-0.09)a |
| 40 | 0.77±0.02(48.15)ab | 2.08±0.50(4.33)b | 0.03±0.03(0.26)c | 0.99±0.00(-0.07)a |
| Probability | 0.0028** | < 0.0001 *** | 0.0007*** | 0.3416ns |
| CV (%) | 10.59 | 22.11 | 26.87 | 1.05 |

The estimate of the remanent effect of the essential oil of *C. giganteus* on *C.maculatus* has been observed and results are presented in Table 3.

***Table 3.*** *Rate of* ***C. maculatus*** *dead, emergence, number of cowpea grains attacked (A) and weight loss (B) caused by the essential oil of* ***C. giganteus*** *in remanent method*

| **Remanent method** | | | | |
|---|---|---|---|---|
| Doses ($\mu L.mL^{-1}$) | **cowpea grain (without pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.64±0.32(44.44)a | 7.49±0.20(5.67)a | 0.23±0.06(5.68)a | 1.00±0.00(0.06)a |
| 10 | 0.84±0.46(55.55)a | 4.33±0.21(18.33)b | 0.19±0.09(5.43)a | 1.00±0.00(0.07)a |
| 20 | 1.57±0.00(100.00)a | 3.54±0.57(12.67)b | 0.12±0.00(1.55)a | 1.00±0.00(0.03)a |
| 30 | 1.05±0.52(66.67)a | 3.72±0.69(14.33)b | 0.16±0.11(4.65)a | 1.00±0.00(0.08)a |
| 40 | 0.84±0.46(55.55)a | 4.87±0.46(23.67)b | 0.39±0.04(14.47)a | 1.01±0.00(0.29)a |

| | | | | |
|---|---|---|---|---|
| Probability | 0.5508ns | 0.0009*** | 0.1780ns | 0.1136ns |
| CV (%) | 69.84 | 16.95 | 57.73 | 0.49 |
| | **cowpea grain (in pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.84±0.46(55.55)a | 7.71±0.36(59.33)a | 0.28±0.05(8.01)a | 0.99±0.01(-0.07)a |
| 10 | 1.16±0.21(77.77)a | 4.99±0.65(25.33)ab | 0.41±0.05(16.28)a | 1.02±0.02(0.46)a |
| 20 | 0.84±0.11(55.55)a | 3.04±1.2(11.67)b | 0.29±0.04(8.53)a | 1.00±0.00(0.09)a |
| 30 | 1.16±0.21(77.77)a | 2.63±0.78(7.67)b | 0.31±0.08(10.34)a | 0.99±0.01(0.00)a |
| 40 | 1.57±0.00(100.00)a | 3.53±1.42(16.00)b | 0.36±0.07(13.18)a | 1.00±0.01(0.03)a |
| Probability | 0.2810ns | 0.0230* | 0.5560ns | 0.7717ns |
| CV (%) | 38.41 | 38.09 | 31.61 | 2.17 |

The Table 4 summarizes the results of the evaluation of two modes of conservation of cowpea grains in the presence of the essential oil extracted from some fruits of *X. aethiopica* in curative treatment.

***Table 4.*** *Rate of* ***C. maculatus*** *dead, emergence, number of cowpea grains attacked (A) and weight loss (B) caused by the essential oil of* ***X. aethiopica*** *in curative method*

| **Curative method** | | | | |
|---|---|---|---|---|
| **Doses ($\mu L.mL^{-1}$)** | **cowpea grain (without pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.23±0.11(7.4)a | 8.37±0.16(69.67)a | 0.32±0.03(10.33)a | 1.01±0.01(0.27)a |
| 10 | 0.38±0.09(14.81)a | 1.65±0.21(2.33)b | 0.10±0.05(1.55)b | 1.01±0.01(0.17)a |
| 20 | 0.31±0.03(9.26)a | 1.05±0.17(0.67)c | 0.06±0.03(0.52)b | 1.01±0.00(0.18)a |
| 30 | 0.08±0.08(1.85)a | 0.88±0.17(0.33)c | 0.04±0.04(0.52)b | 1.01±0.01(0.35)a |
| 40 | 0.24±0.00(5.55)a | 0.70±0.00(0.00)c | 0.05±0.05(0.77)b | 1.00±0.00(0.00)a |
| Probability | 0.1533ns | < 0.0001 *** | 0.0006*** | 0.8102ns |
| CV (%) | 53.75 | 11.06 | 59.27 | 1.49 |
| | **cowpea grain (in pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.67±0.05(38.88)b | 5.82±0.35(33.67)a | 0.35±0.06(12.14)a | 0.98±0.01(-0.40)a |
| 10 | 0.87±0.05(59.26)ab | 4.82±0.88(24.33)a | 0.35±0.05(12.40)a | 0.98±0.06(-0.32)a |
| 20 | 0.76±0.05(48.15)ab | 1.05±0.17(0.67)b | 0.25±0.04(6.46)a | 0.99±0.02(-0.10)a |
| 30 | 0.78±0.10(50.00)ab | 1.34±0.12(1.33)b | 0.20±0.02(4.13)a | 1.01±0.03(0.27)a |
| 40 | 1.04±0.07(74.07)a | 0.88±0.17(0.33)b | 0.33±0.09(11.37)a | 0.98±0.03(-0.49)a |

| | | | | |
|---|---|---|---|---|
| Probability | 0.03* | < 0.0001 *** | 0.3117ns | 0.9531ns |
| CV (%) | 14.29 | 27.41 | 33.09 | 6.23 |

The results in Table 5 translate the activity of the essential oil of *X. aethiopica* applied in preventive method for conserving cowpea.

***Table 5.*** *Rate of* ***C. maculatus*** *dead, emergence, number of cowpea grains attacked (A) and weight loss (B) caused by the essential oil of* ***X. aethiopica*** *in preventive method*

| **Preventive method** | | | | |
|---|---|---|---|---|
| **Doses ($\mu L.mL^{-1}$)** | **cowpea grain (without pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.36±0.07(12.96)b | 8.94±0.36(79.67)a | 0.31±0.05(9.82)a | 0.99±0.01(-0.02)a |
| 10 | 0.59±0.05(31.48)a | 3.80±0.73(15.00)b | 0.26±0.26(2.51)b | 1.00±0.00(0.00)a |
| 20 | 0.53±0.02(25.93)ab | 2.04±1.08(6.00)bc | 0.11±0.07(2.33)b | 1.00±0.00(0.02)a |
| 30 | 0.47±0.04(20.37)ab | 1.79±0.40(3.00)bc | 0.05±0.05(0.77)b | 1.00±0.00(0.00)a |
| 40 | 0.51±0.04(24.07)ab | 0.99±0.30(0.67)c | 0.22±0.11(7.23)b | 1.00±0.00(0.00)a |
| Probability | 0.0680ns | < 0.0001 *** | 0.001*** | 0.9891ns |
| CV (%) | 17.80 | 31.75 | 82.35 | 0.38 |
| | **cowpea grain (in pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.59±0.05(31.48)a | 7.59±0.05(57.67)a | 0.40±0.03(15.25)a | 0.99±0.01(-0.01)a |
| 10 | 0.69±0.07(40.74)a | 1.77±0.09(2.67)b | 0.18±0.09(4.91)ab | 1.00±0.00(0.06)a |
| 20 | 0.67±0.07(38.88)a | 2.40±0.44(5.67) b | 0.05±0.05(0.77)b | 0.99±0.00(-0.09)a |
| 30 | 0.8±0.04(51.85)a | 1.56±0.18(2.00) b | 0.20±0.04(4.91)ab | 0.99±0.01(-0.13)a |
| 40 | 0.82±0.07(53.70)a | 3.65±1.45(17.00)b | 0.22±0.11(7.23)ab | 1.00±0.01(0.04)a |
| Probability | 0.1152* | < 0.0008 *** | 0.0840ns | 0.8425ns |
| CV (%) | 14.88 | 36.72 | 60.47 | 1.09 |

The statistics reported in Table 6 showed no activity of the essential oil of *X. aethiopica* tested at different doses to control the pest.

***Table 6.*** *Rate of* ***C. maculatus*** *dead, emergence, number of cowpea grains attacked (A) and weight loss (B) caused by the essential oil of* ***X. aethiopica*** *in remanent method*

| **Remanent method** | | | | |
|---|---|---|---|---|
| **Doses ($\mu L.mL^{-1}$)** | **cowpea grain (without pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 0.93±0.32(55.55)a | 6.61±0.31(43.33)a | 0.16±0.02(2.58)a | 0.99±0.01(-0.07)a |

| | | | | |
|---|---|---|---|---|
| 10 | 1.25±0.32(77.77)a | 4.21±0.21(17.33)a | 0.05±0.05(0.77)a | 1.00±0.00(0.04)a |
| 20 | 0.84±0.11(55.55)a | 4.51±0.50(20.33)a | 0.06±0.06(1.03)a | 1.00±0.00(0.03)a |
| 30 | 1.37±0.21(88.88)a | 3.19±1.29(13.00)a | 0.27±0.06(7.75)a | 1.01±0.00(0.20)a |
| 40 | 1.37±0.21(88.88)a | 2.48±1.53(10.33)a | 0.10±0.05(1.55)a | 1.00±0.00(0.00)a |
| Probability | 0.4419ns | 0.0840ns | 0.0616ns | 0.1345ns |
| CV (%) | 36.82 | 38.55 | 68.37 | 0.48 |
| | **cowpea grain (in pod)** | | | |
| | **Died** | **Emerged** | **A** | **B** |
| 0 | 1.16±0.20(77.77)a | 7.09±0.65(50.67)a | 0.38±0.05(14.21)a | 0.99±0.01(-0.24)a |
| 10 | 0.73±0.45(44.44)a | 3.99±0.31(15.67)b | 0.33±0.02(10.85)a | 0.99±0.01(-0.23)a |
| 20 | 1.16±0.20(77.77)a | 3.64±0.67(13.67)b | 0.37±0.05(13.69)a | 1.01±0.01(0.32)a |
| 30 | 0.84±0.45(55.55)a | 3.46±0.66(12.33)b | 0.28±0.07(8.53)a | 0.99±0.00(-0.06)a |
| 40 | 1.36±0.20(88.88)a | 5.11±0.70(26.67)b | 0.28±0.05(8.01)a | 0.99±0.00(-0.10)a |
| Probability | 0.6575ns | 0.0102* | 0.4671ns | 0.2883ns |
| CV (%) | 54.33 | 22.88 | 26.02 | 1.43 |

The results reported in the tables above reflect the nature of the biocidal efficacy of essential oils of *C. giganteus* and *X. aethiopica* on *C. maculatus*. The insecticidal effect of the essential oil of *Cymbopogon giganteus* has not been observed for curative method for the two forms of conservation. This observation results from a lower average of mortality, differently significant ($P < 0.0001$), caused by the essential oil of *C. giganteus* on the adult insect.

In spite of this almost non-existent insecticidal activity of the essential oil of *C. giganteus*, lower values of emergences not significantly different appeared of the two forms of conservation for the concentrations 10, 20, 30 and 40 $\mu L.mL^{-1}$.

The rates of emergence to control are highest. This result would indicate influence of the abundance of the alcohol monoterpenics contained in the essential oil of *C. giganteus* on the appearance of these young *C. maculatus*.

Low emergence induced by the different treatments make it possible to show a curative effect which appeared by the ovicide and larvicide characters of essential oil of *C. giganteus*.

The rates of infestation (A) of grains without pods are weak compared to control marking the activity of essential oil at 10 $\mu L.mL^{-1}$. Otherwise, the cowpea grains in pods were less attacked, this, probably thanks to the defending contribution of the pods.

In the two forms of conservation, the weight loss was not statically significant. The rate obtained for this purpose is practically the same one as that of grains treated with absolute ethanol.

In Table 2, the mortality rates did not vary according to the doses of essential oil of *C. giganteus* (10 to 40 $\mu L.mL^{-1}$) but remained high and different from that calculated for control in mode of conservation of granulates without pods. In mode of conservation of

granulates in pods, the effect of the essential oil of *C. giganteus* on the mortality of the insects became sensitive only starting from the dose 30 µL/mL. The essential oil of *C. giganteus* has showed an insecticidal effect causing average identical statistics for treatments 30 and 40 $\mu L.mL^{-1}$ (P = 0.0028).
In spite of the presence to the essential oil of *C. giganteus* in connection to different doses of two forms of conservation, newly formed larvae were able to penetrate into cowpea grains. This result is translated by statistically identical averages of emergence registered (recorded) for all the concentrations applied in mode of conservation of granulates without pods. The ovicidal (or larvicidal) effect of the essential oil of *C. giganteus* appeared only with the strongest concentration applied (40 $\mu L.mL^{-1}$). On the other hand for cowpea grains in pod, it was observed a fall of the varying rate of emergence of young insects for all the concentrations ranging from 10 to 40 µL/mL except 30 $\mu L.mL^{-1}$ ($P < 0.0001$). Indeed, the average of emergence corresponding to the concentration 30 µL/mL is statistically the same as that of control. This anomaly which does not exclude for showing the ovicide and or larvicide character of the essential oil of *C. giganteus* can be explained by the penetration of the insects deposited during the period of oviposition inside the pods. Of this fact, these insects have deposited eggs which developed into adult insects.
The insecticidal effect of the essential oil of *C. giganteus* was not observed in Table 3 of the remanent method for the two forms of conservation. This observation comes from the weak averages of mortality, statistically identical to that of control, caused by this essential oil at the doses tested (10 to 40 $\mu L.mL^{-1}$) on the adult insect. But this result has not prevented the essential oil of *C. giganteus* to influence by its different concentrations the averages of emergence of young *C. maculatus* causing inhibition of development and the activity of eggs with that of the larvae. Consequently, the essential oil of *C. giganteus* reduced the emergence of young *C. maculatus* contrary to control.
Otherwise, the rate of cowpea attacked (in pod or not) has not changed significantly for all doses of essential oil applied in remanent method. It is the same for the corresponding weight losses (Table 3).
The results recorded in Table 4 concern the effect of essential oil of *X. aethiopica* on the adults (mortality), the evolution of eggs and larvae (emergence) of *C. maculatus*, the rate of cowpea grains attacked and the weight loss produced in curative method.
In the mode of conservation of grains without pods, the average values of adult *C maculatus* dead are not significantly different from control. This result shows that the absence of insecticidal activity observed in the diverse treatments could be reported to the mode of conservation of cowpea which is probably not favorable to the control of the insect by the essential oil of *X. aethiopica*. Nevertheless, the essential oil has subtly, by its effects, modified the frequency of emergences of new pests. These young insects, once outside the pods would be to expose to the fumigant action of the essential oil of *X. aethiopica* probably responsible for reducing the rates of emergence. Consequently, there appeared a difference between the averages of emergence recorded for 0 $\mu L.mL^{-1}$, 10 $\mu L.mL^{-1}$ and values of concentration higher than 10 $\mu L.mL^{-1}$ ($P < 0.0001$).This form of conservation thus facilitated the deposit and the development of eggs, even that of the larvae.
The averages of adults of *C. maculatus* dead in the presence of the volatile extract of *X. aethiopica* therefore did not vary compared to the witness (absolute ethanol) for the

form of conservation grains in pod. The averages induced by the treatments 10, 20 and 30 $\mu L.mL^{-1}$ are not significantly different. But these values are statistically different from those obtained with the concentration 40 $\mu L.mL^{-1}$ and control (0 $\mu L.mL^{-1}$) (P = 0.03). One could conclude that the essential oil of *X. aethiopica* showed an insecticidal activity probably related to the chemical profile of whose abundance in 1,8-cinéole is 15.8%. Moreover, the rates of emergences are weak compared to those recorded with concentrations 0 and 10 $\mu L.mL^{-1}$. These weak rates would explain the appearance of ovicide and/or larvicide effect of the essential oil of *X. aethiopica* which was noticed only with the concentrations $\geq$ 20 $\mu L.mL^{-1}$.
The rates of grains (without pods) attacked decreased starting from dose 10 $\mu L.mL^{-1}$ whereas the corresponding weight losses gave averages practically similar to control. As for grains of cowpea in pod, the rates grains attacked and the corresponding weight losses generated did not bring any information on the effectiveness of the oil essential of *X. aethiopica* against damage caused by *C. maculatus*. The average of mortality recorded at 10 $\mu L.mL^{-1}$ (Table 5) is important (0.59% ±0.05%) inducing a reduction of the rate of emergence of young *C. maculatus* to this concentration on cowpea grains without pod. However, the essential oil of *X aethiopica* expressed an ovicide and or larvicide character by a considerable reduction of the emergence rates produced by the different concentrations contrary to the control. We observed that the rates grains (without pods) treated with essential oil are lower than that of control. This result lets think that the cowpea grains without pods underwent more infestations on behalf of the adult insects and larvae. But the corresponding weight losses did not generate averages different from that of control (0.99% ± 0.01%).
Concerning grains in pods, the averages of dead adults of *C. maculatus* are significantly identical to control. The effect of the essential oil of *X. aethiopica* put in evidence by this method is not effective on the depredate at different concentrations tested. However, low levels of emergence were observed. Consequently, the averages of grains (in pod) attacked and the corresponding weight loss remained statistically identical to the control (pure alcohol). On the level of Table 6, all the averages are significantly identical to that of the control except the emergences noted in the mode of conservation of grains in pod. Indeed, the fall of the emergence rates observed on this level could be linked to fertility reduction of females of *C. maculatus* by the fumigant effect of the essential oil of *X. aethiopica*.
In sum, the essential oils of *C. giganteus* and *X. aethiopica* have produced different effects by acting on *C. maculatus*, their larvae and eggs, especially in curative method. This observation allows us to say that the two essential oils contain active compounds responsible for the activities identified. From the analysis of results, the essential oil of *C. giganteus* produced three effects: ovicidal, larvicidal and insecticidal. These three effects are weakly manifested when the cowpea grains were stored without pod. On the form where the cowpea grains are stored without pods, the essential oil of *C. giganteus* directly wiped developing eggs or larvae in curative method. As for the essential oil of *X. aethiopica*, it was favorable to the control of eggs and larvae of the depredater of curative method.
The effects noticed for essential oils of *C. giganteus* and *X. aethiopica* are in agreement with those obtained during the study of the activity of essential oils of *Melaleuca quinquenervia* (L.) and *Ocimum gratissimum* (L.) in Ivory Coast (Cote d'Ivoire) on *C.*

*maculatus* [15]. Other authors also announced the role of protection played by certain essential oils and extracts of nonaromatic plants on *C. maculatus* [16 – 22].

## CONCLUSION

This work which contributes to the valorization of Benin aromatic plants producers of natural bioactive substances has been devoted to the chemical study of the volatile components extracted from the leaves of *C. giganteus* and the fruits of *X. aethiopica* and evaluation of their biological activity on *C. maculatus*. Different chemical compositions marked by various major compounds were obtained. The two essential oils of *C. giganteus* and *X. aethiopica* are rich in oxygenated monoterpenes, respectively 78% and 76.2% in weight.
During this study, the insecticidal, larvicidal and ovicidal activities of the essential oils of *C. giganteus* and *X. aethiopica* on *C. maculatus*, its larvae and eggs were appreciated in connection with the concentrations applied. The results obtained showed that they lowered by their various effects the level of destruction of cowpea grains by *C. maculatus*. The reduction of the infestation rates of cowpea grain by the depredater became more important probably thanks to the oxygenated monoterpenes identified in abundance (75%) in C. *giganteus* essential oil.
Let us announce that the levels of protection to the concentrations used for two essential oils remain still low.

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