

Original Research Paper

# Ovicide and Larvicide Activities of the Essential Oils Extracted from *Polyalthia longifolia* and *Clausena anisata* on *Sitotroga cerealella*

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**Abstract:** This work has objectives to study the chemical composition of volatile extracts obtained from leaves of *Polyalthia longifolia* and *Clausena anisata* and to evaluate their ovicide and larvicide effects by fumigation against *Sitotroga cerealella*, as alternative to the pesticides of synthesis. The analyses of the extracts by GC-MS showed that the essential oil of *P. longifolia* contains 57 compounds representing 86.1% of oil and 26 compounds corresponding to 97.3% of the essential oil of *C. anisata*. The main compounds identified for the extract of *P. longifolia* are the  $\beta$ -Caryophyllene (24.5%), Allo-Aromadendrene (13.5%), the  $\alpha$ -Zingiberene (9.4%), the  $\alpha$ -Humulene (8.5%) and the  $\alpha$ -Selinene (2.6%). The extract of *C. anisata* mainly composed of methyl chavicol (69.9%) is characterized by the presence of oxygenated monoterpenes (1.4%), hydrogenated monoterpenes (7.8%), hydrogenated sesquiterpenes (16.2%), oxygenated sesquiterpenes (1.7%) and of aromatic compounds (69.9%). The biological test results showed that the treatments by fumigation done with the essential oils reduce meaningfully ( $p < 0.001$ ) the bursting of the ovums on the paddy rice. The essential oil of *C. anisata* has completely inhibited the viability of the larvae and the emergence of adult butterflies from the dose  $0.5 \mu\text{l mL}^{-1}$  contrary to *P. longifolia* that recorded respectively  $10.0 \pm 0.3\%$  and  $50.0 \pm 0.2\%$  at the strong dose  $3 \mu\text{l mL}^{-1}$ . The study has, otherwise, shown that the essential oils delayed the cycle of development "ovums to adult" of *S. cerealella* ( $29.0 \pm 0.4$  to  $35.4 \pm 0.5$  days) in relation to the witness ( $25 \pm 0.1$  days). The essential oil of *C. anisata* proved to be more poisonous for the juvenile stages of *S. cerealella*. These results provide the scientific basis for potential alternatives to the synthetic fumigants in subsistence and commercial agriculture.

**Keywords:** *Polyalthia longifolia*, *Clausena Anisata*, Ovicide, Larvicide, *S. Cerealella*, Fumigation, Essential Oil

## Introduction

Rice has always been a basis common food in many countries in Africa. It is now the food source and its growth is the fastest through the continent (AfricaRice,

2012). Its consumption increases quickly because of changes in the preferences of the consumers and the urbanization. West Africa became an important actor on the world markets of rice, but 40% of her needs in rice are provided by the import. The storage, that is one of

the sure means to answer the needs in seeds and to assure the food security, pose a problem to which an attention all particular must be given, if we want to limit the damages caused by the depredators of the commodities after the harvest (Adjalien *et al.*, 2014). Among the primary devastating insects who attack the cereals in storage, the alucite of the cereals, *Sitotroga cerealella* is considered like a dangerous insect for the stocked grains and difficult to fight. Indeed, the juvenile stages of this species (ovums, larvas, chrysalises) are lodged inside the grain and are transported without possibility of separation of the grains infested from the wholesome grains (Gueye *et al.*, 2011). When the conditions are normal, each female puts down 40 ovums on average, although in favourable conditions, we observed that an individual can lay until 200 ovums (Koleva and Ganeva, 2009). Some important damages can be caused on the stocks of rice as soon as the period of storage lies down from three to four months (Togola *et al.*, 2010). Several methods are used especially those calling on the chemical pesticides of syntheses to limit the losses in the stocks. However, these methods present several limits among which the adaptation of the insects and the resistant stump selection (Benhalima *et al.*, 2004), the food poisonings compromising the human health, the pollution of the environment and the ecological messes (Regnault-Roger, 2002). Considering these ominous effects of the different methods of struggle in particular the one of the use of the chemical substances on the human health, other believable alternatives presenting less negative impacts on the environment and the human health deserve to be explored (Gueye *et al.*, 2011). In this optics, the valorization of the plants with insecticide effect, ovicide or larvicide seems to seduce everywhere in the world. Thus, the exploitation of several organs and parts of the plants at the ends to limit the post-harvest losses constitutes a more built-in alternative (Gueye *et al.*, 2011). The weeping willow, *Polyalthia longifolia* var. is a plant used in the traditional system of medicine for the treatment of the fever, the cutaneous illnesses, the diabetes, hypertension and the helminthiases. The peel is also used like a febrifuge in the district of Balasore of the Orissa (Kirtikar and Basu, 1995). The species *Clausena anisata*, as for it, is a guineo Congolese species that is met in all the intertropicale Africa, in the secondary formations from Guinea to Central African Republic. It is a plant suffrutescens, reaching 2 m of height. The leaves are smelling, alternate. *Clausena anisata* is extensively used like medicinal plant to treat various pains and for its insecticide properties. The pulp of the leaves is used in local applications in the lumbagos (Neuwinger, 2000; Schmelzer, 2001). To this effect, the essential oils of the leaves of *Polyalthia longifolia* and *Clausena anisata* have been studied for their ovicide and larvicide effects on *Sitotroga cerealella*.

In the actual study, the volatile extracts of the leaves of the two species harvested in Benin have been analyzed by gas Chromatography Coupled with Mass Spectrometry (GC/MS), as well as their biological activities that are tested on the ovums and the larvas of the alucite of the cereals.

## Material and Methods

### *Plant Material and Essential Oil Extraction*

The leaves of the two plants *Polyalthia longifolia* (Sonn.) Thwaites and *Clausena anisata* (Willd) J.D. Hook. ex Benth, have been harvested in the south of Benin in 2012 respectively in the localities of Segbohoue and Pahou. They have been identified and have been certified to the national herbarium of the University of Abomey-Calavi and belong in the botanical family of the Annonaceae and Rutaceae. In the laboratory, they have been spread on palliase safe from light at 20°C. The essential oils have been extracted from the leaves (200 to 250 g) by hydro distillation during 3 h thanks to an extractor of Clevenger type. The oil less dense than water has been collected by simple decanting and has been dried on the anhydrous sodium sulphate. The extracted oils have been preserved in 4°C and safe from light in amber small bottles. The efficiencies of oils have been calculated with the help of the following formula:

$$\text{Efficiency}(\%) = \frac{\text{Mass of oil (g)}}{\text{Plant material mass (g)}} \times 100$$

The IR841 variety selected at the International Rice Research Institute (IRRI) and introduced in Benin in the years 70 is well cultivated through the country. It is a variety adapted as well as to pluvial culture of shallow and to irrigated culture and appreciated well by the producers and the consumers for the aroma perfumed of its grain and for its good level of output. It is very sensitive to different stress notably the dryness, the adventitious, the illnesses and the devastators (Agbobli *et al.*, 2004). It is cultivated, in our study, neither with chemical manure nor pesticide of synthesis and is preserved without chemical treatment. For the tests in the laboratory, the rice has been stocked and used like grains of ordinary paddy rice.

### *Insects*

The stumps of *Sitotroga cerealella* used for the mass breeding in this study come from the reserve of the ADRAO (Benin). They have been bred in the laboratory, at the temperature of 29±2°C of relative humidity 70±10% and of natural photoperiod, in globes in glass or in plastics on the paddy rice like substratum. The adult females of a (1) day born from breeding are used for the obtaining of the ovums.

### Analysis of the Essential Oil Compounds

GC/MS: The two 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  $\mu\text{m}$ ), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Helium was used as carrier gas (1.0 mL min<sup>-1</sup>); injection was made in split mode (1:30); injector and detector of temperature were at 250 and 280°C, respectively. The MS working in electron impact mode was at 70 eV; electron multiplier was 2500 eV; ion source temperature was 180°C; mass spectra data were acquired in the scan mode in m/z range 33 to 450.

GC/FID: The two essential oils were analyzed on a Hewlett-Packard gas chromatograph Model 6890, equipped with a DB5 MS column (30 m 0.25 mm; 0.25  $\mu\text{m}$ ), programming from 50°C (5 min) to 300°C at 5°C/min, 5 min hold. Hydrogen was used as carrier gas (1.0 mL min<sup>-1</sup>); injection was in split mode (1:60); injector and detector temperature were 280 and 300°C, respectively. The essential oil was 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 (Rösch *et al.*, 1996; Adams, 1989; Swigar and Silverstein, 1981).

### Test

All tests have been done in the laboratory at a temperature of 29±2°C of natural photoperiod and a relative humidity of 70±10%.

### Obtaining of the Ovums of *Sitotroga Cerealella*

The ovums of *S. cerealella* have been gotten from a device whose main element is formed of two papers in cardboard of black color and of triangular shape "V" Fig. 1 (Ellington, 1930). The black cardboard is chosen to see the ovums that is of whitish or red color according to the age of the ovums. They are maintained at their bases by a staple and to the middle by glue. The set of the two coupled cardboards are arranged vertically in a globe in glass in order to orient the basis of the triangles toward the opening of the globe (face 1). Five (5) males and ten (10) females were set down in every jar. This device allowed the female of *S. cerealella* to introduce its abdomen to put down the ovums there between the cracks of the two coupled triangles. At all for this experience, thirty-five (35) jars of the device of collection of the ovums of *S. cerealella* have been set up for the applied units to infest. The ovums has been set apart (counted progressively) with the help of a fine skewer in order to not to crush them and to be set down on the substratum. Observations have been made every day, while recording the changes (color) at the ovums level until the bursting and the time of incubation.

### Effect of the Treatments by Fumigation on the Evolution of the Ovums

The method of Aiboud (2012) used for this test consisted to study the fumigant effect of the two essential oils on the evolution of the ovums of *S. cerealella*. In globes made in glass of capacity 1 L, a piece of cotton moistened of essential oil at the dose (0, 0.2, 0.5, 1 and 3  $\mu\text{l mL}^{-1}$ ) has been suspended with the help of a thread attached to the internal face of the lid. On 50 g of paddy rice placed on the bottom of the globe, are set down 10 ovums of *S. cerealella* aged from 0 to 24 h with the help of a skewer. Each of the doses is applied in three repetitions and thus the behaviors of the treated ovums have been observed at the end of 72 h. On the whole, 5×3×2 = 30 applied units have been set on. The grains of rices retired from the globes were set down in a box of Kneaded in order to be able to count the comfortably non hatched ovums under a binocular gnarl with an enlargement of ×40 then reintroduce them in their respective globes and count as the larvas emerge from treated grains until the 45 th day after treatment. The following variables have been measured.

The rate of bursting of the ovums at the end of 72 h exhibition for every type and essential oil dose through the formula:

$$\begin{aligned} \text{rate of bursting of the ovums (\%)} \\ = \frac{\text{number of ovums hatched}}{\text{total number of ovums}} \times 100 \end{aligned}$$

The rate of emergence of *Sitotroga cerealella* until the 45th day after treatment:

$$\text{rate of emergence (\%)} = \frac{\text{number of emerged adults}}{\text{total number of ovums}} \times 100$$

The rate of viability of the larvas of *Sitotroga cerealella*:

$$\begin{aligned} \text{rate of viability of larvas (\%)} \\ = \frac{\text{number of emerged adults}}{\text{number of ovums hatched}} \times 100 \end{aligned}$$

The duration of cycle of the insect, from the ovum to the adult, in the conditions of the tests in the laboratory.

### Statistical Analysis

The results gotten from the observations have been treated statistically by the method of variance analysis (ANOVA) using Statistical Analysis System (SAS) software Version 9.1. The data raised during the different tests on the ovums, the larvas and young *Sitotroga cerealella* underwent a logarithmic transformation in order to consolidate the variances and to normalize the population. The masses of the grains attacked being

continuous quantitative data and respecting the conditions of normalization and equality of variance did not undergo any statistical transformation. Finally, it has been proceeded to a structuring of the averages with the help of the test of Newman and Keuls. The statistical test results are considered like meaningfully different, when the probability of the null hypothesis is lower or equal to 5%.

## Results and Discussion

### *Chemical Composition of the used Extracts*

The value of the extraction output of the essential oil from the leaves of *P. longifolia* harvested in Segbohoue is relatively weaker (0.24%) than the one from *C. anisata* (0.54%) harvested in Pahou. In Indonesia the output in essential oil from the leaves of *C. anisata* is of 4.32% (Addae-Mensah *et al.*, 1996). This variability is due to several factors of which the difference of family of the two species and is presumably in relation with abiotic factors as the specific climate in the regions of source of the samples, the geographical factors as the altitude and the nature of soil. *C. anisata* is more aromatic with a more obstinate odor. The results of the chemical analysis by gas chromatography and gas chromatography coupled with the mass Spectrometry (GC/MS) of the essential oil extracted of the leaves *Clausena anisata* harvested in Pahou (Benin) are shown in the Table 1 and 2. From the analysis of these results, it comes out again that this essential oil is characterized by the presence of oxygenated monoterpenes (1.4%), hydrogenated monoterpenes (7.8%), hydrogenated sesquiterpenes (16.2%), oxygenated sesquiterpenes (1.7%) and of oxygenated aromatic compounds (69.9%). The majority compound identified in this essential oil is the methyl chavicol (69.9%). These results are similar to those reported by (Ayedoun *et al.*, 1997; Alitonou, 2006). However, the species met in Cameroon seems completely different since Ngassoum *et al.* (2004) showed that the majority compounds of the essential oil of the leaves of this plant are dominated by acyclic terpenic structures like the (Z)-tagetone (26.8%), the (E)-tagetone (19.2%), the (E)-neralidol (11.5%), accompanied by the D germacrene (9.2%). The analysis of the chemical composition of the essential oil extracted of the leaves of *Polyalthia longifolia* show that it is exclusively rich in-Caryophyllene (24.5%) who constitutes the main majority compound followed by the Allo-Aromadendrene (13.5%), the  $\alpha$ -Zingiberene (9.4%), of the  $\alpha$ -Humulene (8.5%) and of the  $\alpha$ -Selinene (2.6%). In Nigeria, the essential oils of the leaf and the stem peel of *P. longifolia*. (Annonaceae) have been studied. Extracted oil of the leaves is nearly exclusively composed of derivatives sesquiterpenic represented by allo-aromadendrene (19.7%), caryophyllene oxide (14.4%), the  $\beta$ -caryophyllene (13.0%), the  $\beta$ -selinene (7.9%), the  $\alpha$ -humulene (7.0%) and the ar-curcumene (6.8%) (Ogunbinua *et al.*, 2007).

Table 1. Chemical composition of *Polyalthia longifolia* essential oil

N°	Components identified	RI	(%)
2	Tricylene	921	t
3	$\alpha$ -Thujene	924	t
4	$\alpha$ -Pinene	926	1.0
5	Camphene	942	0.4
6	Sabinene	965	1.1
7	$\beta$ -Pinene	970	0.2
8	Myrcene	982	0.3
9	Mesitylene	986	t
10	$\alpha$ -Terpinene	1009	t
11	Para-Cymene	1017	t
12	Limonene	1022	0.2
13	$\beta$ -Phéllandre	1023	t
14	Eucalyptol	1025	t
15	(Z)- $\beta$ -Ocimene	1029	t
16	(E)- $\beta$ -Ocimene	1039	0.7
17	$\gamma$ -Terpinene	1051	t
18	Terpinolene	1078	t
19	2-Nonanone	1083	t
20	Linalol	1092	0.2
21	Nonanal	1096	t
22	Borneol	1166	t
23	Terpinen-4-ol	1174	t
24	Naphthalene	1178	0.2
25	$\alpha$ -Terpineol	1188	t
26	Formate de Bornyle	1222	0.2
27	Acetate de Bornyle	1277	0.2
28	$\alpha$ -Cubebene	1340	t
29	$\alpha$ -Ylangene	1363	0.1
30	$\alpha$ -Copaene	1369	1.1
31	$\beta$ -Elemene	1382	0.1
32	Z-caryophyllene	1398	0.1
33	Z- $\alpha$ -Bergamotene	1405	0.2
34	$\beta$ -Caryophyllene	1415	24.5
35	E- $\alpha$ -Bergamotene	1425	1.2
36	$\alpha$ -Guaïene	1428	0.2
37	Aromadendrene	1433	0.3
38	Z- $\beta$ -Farnesene	1443	0.7
39	$\alpha$ -Humulene	1450	8.5
40	Allo-Aromadendrene	1455	13.5
41	$\gamma$ -Muurolene	1468	1.6
42	ar-Curcumene	1473	2.5
43	E- $\beta$ -Bergamotene	1477	1.5
44	$\beta$ -Selinene	1484	2.6
45	$\alpha$ -Zingiberene	1487	9.4
46	$\alpha$ -Selinene	1490	2.6
47	$\beta$ -Bisabolene	1499	0.4
48	$\gamma$ -Cadinene	1506	0.4
49	$\delta$ -Cadinene	1510	1.1
50	$\beta$ -Sesquiphellandre	1515	0.9
51	Caryophyllenyl alcohol	1571	1.1
52	Caryophyllene oxide	1577	2.1
53	Caryophyllenyl alcohol isomère	1580	0.6
54	Viridiflorol	1589	0.4
55	Epi globulol	1599	1.5
56	$\alpha$ -Cadinol	1649	1.3
57	Neo-Intermedeol	1653	0.9
	Hydrogenated monoterpenes		4.1
	Oxygenated monoterpenes		0.6
	Hydrogenated sesquiterpenes		73.5
	Oxygenated monoterpenes		7.9
	Total (%)		86%

t (traces)  $\leq$  0.1%; RI = Retention Index

Table 2. Chemical composition of essential oil of *Clausena anisata* leaves

N°	Components identified	KI	(%)
1	$\alpha$ -pinene	932	2.2
2	Camphene	946	t
3	$\beta$ -pinene	974	2.1
4	Myrcene	988	0.6
5	p-cymene	1020	t
6	Limonène	1024	0.3
7	(Z)- $\beta$ -ocimene	1032	1.2
8	(E)- $\beta$ -ocimene	1044	1.1
9	-terpinene	1054	0.3
10	M=150	-	0.3
11	Linalool	1095	0.3
12	terpinen-4-ol	1174	1.1
13	methyl chavicol	1195	69.9
14	$\alpha$ -copaene	1374	0.3
15	$\beta$ -elemene	1389	2.6
16	(E)-caryophyllene	1417	3.8
17	$\alpha$ -humulene	1452	2.9
18	germacrene-D	1484	6.0
19	$\beta$ -bisabolene	1505	0.2
20	$\beta$ -cadinene	1521	0.4
21	Spathulenol	1578	0.2
22	Caryophyllene	1582	0.4
23	$\alpha$ -cadinol	1652	0.4
24	(E)-bisabolol-11-ol	1667	0.3
25	germacra-4(15), 5,10(14)-trien-1- $\alpha$ -ol	1685	0.4
26	Not identified		0.3
	Hydrogenated monoterpens		7.8
	Oxygenated monoterpens		1.4
	Hydrogenated sesquiterpens		16.2
	Oxygenated monoterpens		1.7
	Aromatic compounds		69.9
Total			97.3

#### Ovicide and Larvicide Properties of the Extracts

During works in the laboratory, it has been observed that the ovums of *S. cerealella* of one day are of oval shape, streaky, white, turning to the pink or reddish when approaching the hatching, what is in accordance with the observations of (Metcalf and Flint, 1965; Hansen *et al.*, 2004). Other ovums that were not able to hatch themselves remained of black color. These changes of color can explain or not the activity of the essential oils on the development of the ovums on the paddy rice. According to the Fig. 2 the treatments by fumigation done with the essential oils of *P. longifolia* and *C. anisata* reduce meaningfully ( $p < 0.001$ ) the hatching of the ovums on the paddy rice. The average values of the hatching rates varied by meaningful manner and decreased gradually as the doses of the essential oil tested increase global manner. At the dose of  $0.5 \mu\text{L mL}^{-1}$  the essential oil of *C. anisata* inhibited 100% the hatching of the ovums contrary to the oil of *P. longifolia* (93.34%). The essential oil of *Clausena anisata* is more efficient than the oil of *Polyalthia longifolia*. It can be explained by the presence of

oxygenated aromatic compounds (methyl chavicol) responsible of the strong activity of the essential oil of *C. anisata* (Ndomo *et al.*, 2011).

The results of the rate of viability of the larvae and emergence of the adults of the alucite of the cereals as well as the whole cycle time, from the ovum to the adult, according to the doses of the two essential oils has been presented in the Table 3 and 4.

From the Table 3 and 4, one could deduct that the tested essential oils were efficient on the different larvae. It translates the statistically meaningful averages of emergence gotten compared with the witness. Such a reduction of the emergence rates at concentrations  $0.2, 1, 3 \mu\text{L mL}^{-1}$  would result from the demonstration of the ovicide or larvicide effect of the extracts and especially that would have annihilated the development of some ovums or larvae. However the larvicide effects of the essential oil of *P. longifolia* is relatively weaker compared with those of the volatile extract of *Clausena anisata*. The inefficiency of the essential oil of *P. longifolia* at the doses  $0.5$  and  $1 \mu\text{L mL}^{-1}$  had as consequence relatively elevated values of emergence of adults of the alucite. Otherwise, it is necessary to notice also that all ovums hatched could not come to an adult *S. cerealella*. The essential oil of *C. anisata* proved to be poisonous for the larvae because it provoked the meaningful reduction of several larval phases ( $p < 0.0001$ ) even stopped the evolution of the larvae or at different doses. Indeed, the essential oil of the leaves of *C. anisata*, contains some monoterpens hydrocarbonated and oxygenated like the  $\alpha$ -pinene, methyl chavicol of which the ovicide, larvicide and insecticide properties have already been demonstrated against *Tribolium confusum*, *Tribolium castaneum*, *Sitophilus zeamais*, *Prostephanus truncatus*, *Rhyzopertha dominica* and *Callosobruchus maculatus* (Ketoh *et al.*, 2006; Kiendrebeogo *et al.*, 2006; Noudogbessi *et al.*, 2009). The powder of leaves and different excerpts of leaves and roots showed a meaningful anti-palatable activity against the borer *Helicoverpa armigera*. Several recent studies have reported the insecticide, larvicides and ovicides properties of the volatile extracts of plants and in particular the role played by the compounds terpenic during the assessment of these activities (Seri-Kouassi *et al.*, 2004; Kiendrebeogo *et al.*, 2006; Ketoh *et al.*, 2006; Ogunleye and Adefemi, 2007; Noudogbessi *et al.*, 2009). The rates of viability recorded in the Table 4 illustrate that in spite of the presence of the essential oil of *P. longifolia*, some larvae were able to penetrate in the grains of paddy rice. At the weakest concentration of  $0.2 \mu\text{L mL}^{-1}$ , the essential oil of *P. longifolia* inhibited until  $3.33 \pm 0.33\%$  the rate of emergence. This observed effect can be bound to the minority compounds of the volatile extract that would have acted alone or by effect of synergy.



Fig. 1. Device of collection of the ovums of *Sitotroga cerealella*

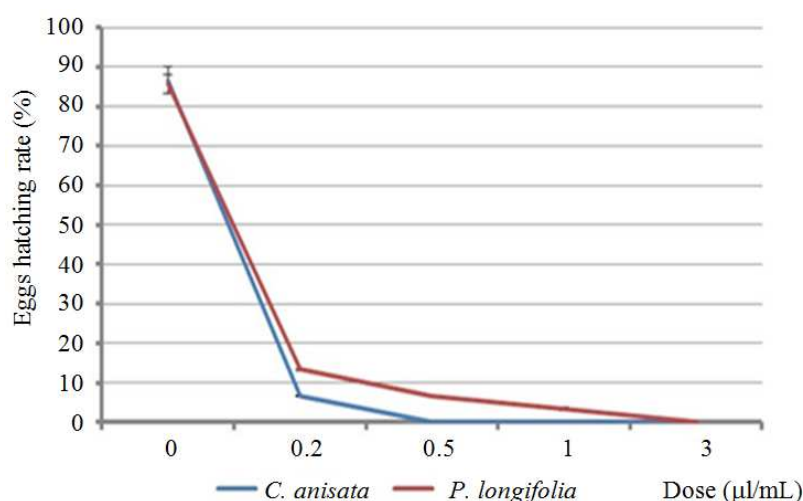


Fig. 2. Evolution of the rates of hatching of the ovums according to the treatments of essential oil

Table 3. Activity of the essential oil of *Clausena anisata* on the viability of the larvas, the emergence of the adults and the length of the development cycle of *Sitotroga cerealella*

Dose (µl/mL)	Date deposit ovums	n	Mean number hatched ovums	Means number emerged adult	Date exit adults	Cycle time average (days)	Rate of emergence (%)	Rate of viability (%)
0	11/03/2014	10	8.6	7.6	05/04/2014	25.0±0.2	76.66±3.33a	88.9±2.5a
0.2	11/03/2014	10	0.66	0.33	15/04/2014	35.4±0.5	3.33±0.5b	50.0±2.1b
0.5	11/03/2014	10	1.33	0	-	-	0b	0b
1	11/03/2014	10	0.33	0	-	-	0b	0b
3	11/03/2014	10	0	0	-	-	0b	0b
Probability						<0.0001***	<0.0001***	
CV (%)							23.79	33.35

Table 4. Activity of the essential oil of *Polyalthia longifolia* on the viability of the larvas, the emergence of the adults and the length of the development cycle of *Sitotroga cerealella*

Dose (µl/mL)	Date deposit ovums	n	Mean number hatched ovums	Means number emerged adult	Date exit adults	Cycle time average (days)	Rate of emergence (%)	Rate of viability (%)
0	15/03/2014	10	9.84	7.66	09/04/2014	25.0±0.1	76.66±3.33	77.77±3.5a
0.2	15/03/2014	10	2.66	1.33	13/04/2014	29.0±0.4	13.33±1.33b	50.0±0.5a
0.5	15/03/2014	10	1.33	1.00	15/04/2014	30.1±0.4	10.0±5.7b	75.18±0.5b
1	15/03/2014	10	1.33	0.66	17/04/2014	33.6±0.4	6.66±0.3b	50.12±0.66b
3	15/03/2014	10	1.00	0.33	18/04/2014	34.3±0.3	3.33±0.3b	33.33±0.5b
Probability							<0.0001***	<0.001***
CV (%)							32.15	38.45

NB: CV: Coefficient of variance; n: Number of initial ovums; 0: Treatment to the ethanol corrected by the control without any treatment; \*\*\* = Very meaningful difference (0.1%). The middle values followed of same letters (a, b) are not meaningfully different at 5% (Newman and Keuls test)

The determination of the average period of the cycle of development of *Sitotroga cerealella*, at an average temperature of  $29 \pm 2^\circ\text{C}$  of relative humidity  $70 \pm 10\%$  and natural photoperiod gave the results indicated in the Table 3. These results show that the essential oil of *C. anisata* provoked on the one hand a delay of 10 days on average at the smallest dose ( $0.2 \mu\text{l mL}^{-1}$ ) in relation to the witness (control); and on the other hand the emergence that is inhibited in a significative manner going up to 100% from the dose  $0.5 \mu\text{l mL}^{-1}$ . The total absence of adult of *Sitotroga cerealella* emerging from the treated applied units ( $0.2$ ;  $0.5$ ;  $1$  and  $3 \mu\text{l mL}^{-1}$ ) didn't permit to calculate the length of the cycle of development of the insect on the 45th day after treatment. The study has, otherwise, shown the average length of the cycle of development "from ovum to adult" of *S. cerealella* is meaningfully longer ( $29.0 \pm 0.4$  days on  $35.4 \pm 0.5$  days) under the action of oils at the doses ( $0.2$ ;  $0.5$ ;  $1$  and  $3 \mu\text{l mL}^{-1}$ ) than the one gotten from the witness ( $25.0$  days). The essential oil of *C. anisata* acted efficiently on the cycle of development of *S. cerealella* at the weakest dose.

## Conclusion

During this study, the ovicide and larvicide activities of the essential oils of *P. longifolia* and *C. anisata* have been appreciated on *Sitotroga cerealella* according to the treatment and the applied dose. The ovicide effect of the two volatile extracts proved to be more interesting. The less effect noticed is observed at the essential oil of *P. longifolia* with a strong rate of emergence and viability in comparison to the essential oil of *Clausena anisata*. The essential oils contributed to delay the evolution of the cycle of development of the alucite of the cereals or even stumped it according to the applied doses. These results provide the scientific basis for the use of the essential oil of the leaves of *C. anisata* as efficient fumigant against the juvenile stages of the devastator and therefore, the potential alternatives to the synthetic smoke in the agriculture of subsistence and the commercial agriculture.

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## Author's Contributions

This work was carried out in collaboration between all authors. Author EA, the PhD student of this work

designed the study and performed biological studies with author PS. Author KD performed the statistical analysis and supervise the work. Authors JN performed the chemical analysis of the oils in the supervision of author CM. authors AE, PS, JN, GA, CM and DS wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors AE, PS, JN, GA, CM and DS managed the analyses of the study and literature searches. DS supervised the work with author KD as Directors of the PhD student. All authors read and approved the final manuscript.

## Ethics

No ethical issues may arise after the publication of this manuscript.

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