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Research Article

Insecticidal activities of enriched sulfur-containing compounds essential oils from *Afrostyrax lepidophyllus* and *Scorodophloeus zenkeri* seeds against two stored grain pests: *Acanthoscelides obtectus* and *Callosobruchus maculatus*

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Abstract

This work consisted of evaluating the insecticidal activities of essential oils of the seeds of two Cameroonian aromatic plants namely *Afrostyrax lepidophyllus* Milbdr. (Huaceae) and *Scorodophloeus zenkeri* Harms. (Fabaceae). These seeds are generally used to flavour the meals in Cameroon and their essential oils are mainly constituted by sulfur-containing compounds (96.1% for *S. zenkeri* and 91% for *A. lepidophyllus*) and with the same major constituent, 2,4,5,7-tetrathiaoctane (52.9% in *A. lepidophyllus* and 51.5% in *S. zenkeri*). Thus, these oils were evaluated for their insecticidal activities against *Acanthoscelides obtectus* Say, (Coleoptera: Chrysomelidae) and *Callosobruchus maculatus Fabricius*, (Coleoptera: Chrysomelidae) main stored grain pests of common bean and cowpea seeds respectively. It has been established that the repellent and toxic effects of the oils vary according to the plant and the insect species. Moreover, production of progeny was completely suppressed on treated common beans and cowpea. Finally, these mixtures of organosulfur compounds from plant origin considerably reduce the weight losses of cowpea and common bean grains, without detrimental effect on their germination. These oils could therefore be used as alternative to some hazardous pesticides currently use for protection of stored grains against insect pests.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L. Walp.) are two legumes cultivated throughout seasons in Cameroon. They play a critical role in the lives of millions of people in Africa and other parts of the developing world, where they are a major source of dietary protein that nutritionally complement staple low-protein cereal and tuber crops and are a valuable and dependable

commodity that produce income for farmers and traders [1-2]. In fact, beans and cowpea are valuable source of the amino acids as lysine and tryptophan (essential amino acids), the minerals iron, copper and zinc, the vitamins (including thiamin, riboflavin, ascorbic acid, niacin, and folic acid), dietary fibre, complex carbohydrates and beneficial phytochemicals, antioxidants, and flavonoids [3-4]. Although low in methionine and cysteine, the dried seeds of these legumes supply those amino acids lacking in diets based on maize, rice, or other cereals [5-6]. In addition, the protein in grain legumes like cowpea has been shown to reduce low-density lipoproteins that are implicated in heart disease [7].

In spite of the importance of these beans on the human health, they are susceptible to many pests and diseases. In combination with sub-optimal growing conditions, common in the low-input scenarios used in developing countries, pests and diseases may act synergistically to cause significant and sometimes total yield losses. In Cameroon and mainly in the western and northern regions which are the main growing areas of common bean and cowpea respectively, crops are regularly attacked in storage by *A. obtectus* for common bean and *C. maculatus* for cowpea.

The organic pesticides registered to deal with are combinations between pirimiphos-methyl, thiamethoxam, permethrin and deltamethrin [8], and the most used combinations are those based on pirimiphos-methyl which contains a sulfur atom. The most sulfur-rich organosulphur pesticides are the dithiocarbamates, some of which are ferbam (**1**), mancozeb (**2**), maneb (**3**), metam-sodium (**4**), metiram (**5**), nabam (**6**), propineb (**7**), zineb (**8**) ziram (**9**) and thiram (**10**). They are generally used as fungicides or additives of pesticides, weed-killers or insecticides. Additional uses are as biocides for industrial or other commercial applications, and in household products. Some are used for vector control in public health [9].

Although there is little work on the insecticidal activities of essential oils rich in sulfur compounds, those carried out on the garlic essential oil have shown strong insecticidal activity against several insect pests [10-11]. In spite of their widespread uses, dithiocarbamates as well as pirimiphos-methyl are not totally healthy for the environment and humans as, most of these compounds rapidly degrade in the presence of oxygen, moisture, etc., to form a number of compounds, some of which are toxicologically important (e.g: ethylenethiourea, propylenethiourea).

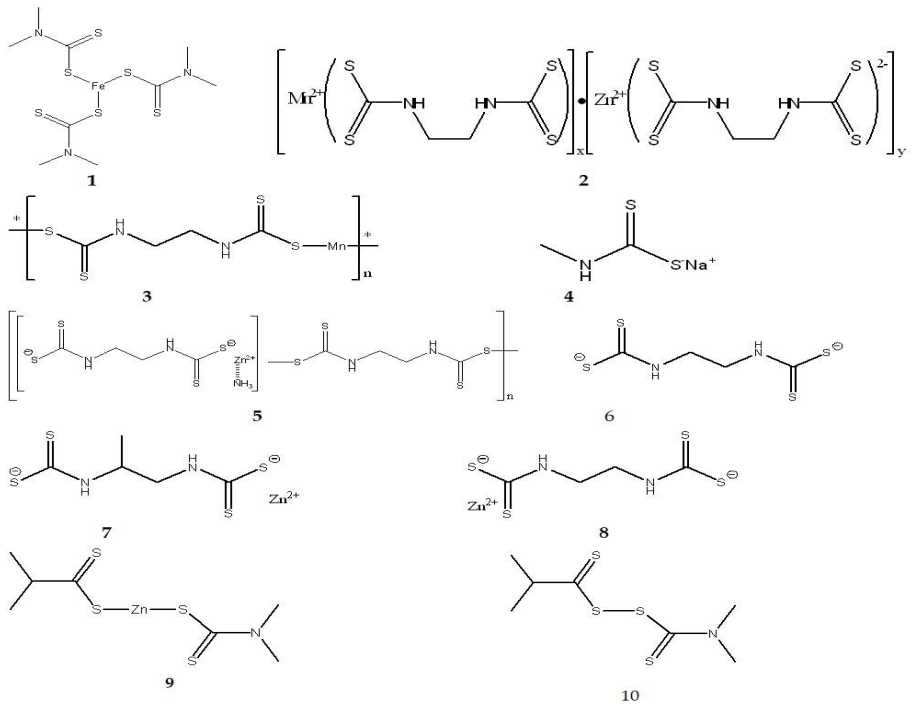

Figure 1 Structures of certain dithiocarbamates showing their sulfur atoms richness.

Moreover, the acute LC_{50} of dialkyldithiocarbamates for fishs is less than 1 mg/L, and that of ethylenebis(dithiocarbamates) (which is another subclass of DTCs) is in the range 1 - 8 mg/L water. In humans, regular contact with dithiocarbamates can cause functional changes in the nervous and hepatobiliary systems, contact dermatitis and sensitization [9]. For example, thiram (100 mg/m^3) has been shown to cause headaches, vertigo, impairment of mental capacity, muscle twitch, and paraesthesia [12]. Moreover DTCs are some of the most frequently detected pesticides and also showed one of the highest frequencies in exceeding maximum residue limits in food commodities [13]. Concerning pirimiphos-methyl particularly, it is very toxic to aquatic life and can cause damage to organs through prolonged or repeated exposure [14]. It is therefore necessary to look for alternative and/or complementary methods and we think that natural products from plant origin and more specifically essential oils can be of good consideration as their efficiency against several insect pests have been documented [15-16].

The main objective of the present research work is the search of essential oils with sulfur-containing compounds as the main constituents and which could be used as alternatives to the synthetic organosulfur pesticides such as DTCs, classified as hightly hazardous pesticides [17]. As such, essential oils extracted from two Cameroonian aromatic plants used as spices namely *A. lepidophyllus* and *S. zenkeri* seeds mainly constituted of sulfur components were evaluated for their insecticidal activities against *A. obtectus and C. maculatus*, respectively pests of common bean and cowpea seeds and the results are herein reported.

2. Materials and methods

2.1 Plant material and extraction of the oils

Oils used for biological assays were obtained from dry seeds of *A. lepidophyllus* and *S. zenkeri.* These spices were bought on the market in Dschang (Menoua division of the west region of Cameroon) and identified at the Cameroon National Herbarium by Mr. Victor Nana (n° 44853/HNC/SRF for *A. lepidophyllus* and n° 3884/HNC/SRF for *S. zenkeri*).

Given that the essential oils from these spices were completely water-soluble, the aromatic water collected was extracted as previously described [18].

2.2 GC-FID and GC-MS analyses

Analyses by GC-FID as by GC-MS were performed with Agilent gas chromatographs equipped with a flame ionization detector (FID) for the former and with a mass selective detector for the latter. The column characteristics, the oven, the injector and the detector temperature programming, as well as the injection, the calculation of the retention index and compounds identification, were carried out as previously described [18].

2.3 Insects rearing.

The objective of this step is to obtain a homogeneous population of insects for the different tests. Adult insects collected from a permanent rearing ongoing at the Research Unit of Environmental and Applied Chemistry of the University of Dschang were reared on local varieties of beans and cowpeas (for *A. obtectus* and *C. maculatus* respectively). 1500g of each bean and adult insects were introduced into a 3 l glass jar. The whole was covered with a piece of porous cloth and kept at 27.1 ± 0.5° C. and 73% ± 3% RH. After 20 days, all the insects were removed from the rearing medium and the bean kept under the same conditions until the emergence of insects used for the bioassays (F1 progeny).

2.4 Clay powder preparation.

The white clay used for powder preparation was chemically characterized by Tonle [19]. It has been sampled at Bambili in the locality of Bamenda (North-west division of Cameroon). In order to obtain a clay with fine particles, it has been sieved with a 0.05 mm-pore-size sieve.

2.5 Bioassay experimental conditions.

Repellency tests were carried out in petri dishes (9 cm diameter) at 24.1 ± 0.6 °C and 76.3% ± 4.1% RH while contact toxicity and fumigation assays were performed in 270 cm^3 glass containers placed in a chamber with a 10 h light, 14 h dark photoperiod at 27.1 ± 0.5°C and 73% ± 3% RH. Two-day-old insects were used for the different tests.

2.6 Repellency test

Since infestation begins before harvest, a repellent oil would not only prevent the insects from attacking the seeds in the field, but also their transport from the fields to the granaries. The ability of sulfur-containing compounds to repel these bruchids was evaluated according to the method described by McDonald et al. [20] and the repellent classes assigned according to the scale of Juliana and Su [21]. Evaluated concentrations were obtained by diluting 2, 4, 8, and 16 µl of oil in 0.5 mL of acetone. After impregnation into a half-disc of 9 cm filter paper, the final concentrations were 0.062, 0.125, 0.251, and 0.503 µl/cm^2.

2.7 Contact toxicity on filter paper.

This test ensures that the toxicity of the oils is linked to the interaction between the constituents of the oil and the normal physiological processes of the insects, and not between the constituents of the oil and those of the seeds. Indeed, oils must protect grains without modifying their quality. Filter paper discs of 9-cm (63.6-cm^2 surface) in petri dishes were impregnated with 4, 8, and 16 µl of oils diluted in 1 mL of acetone and air dried for 10 min to evaporate the solvent. Each dish was inoculated with bruchids (twenty adults of *A. obtectus* or *C. maculatus*) and mortality was noted 1, 2, 3, and 4 days. Each experiment was repeated 4 times.

2.8 Fumigant toxicity.

This test evaluates the insecticidal effect of essential oil vapours. In 270 cm^3 glass containers, twenty adult *A. obtectus* or *C. maculatus* were introduced. Squares of filter paper (4 cm^2) soaked with 0, 4, 8, 16, and 32 µL of oils, corresponding to 0.000, 0.014, 0.029, 0.059, and 0.118 µL/cm^3 respectively, were attached halfway up the container. Each concentration was repeated 4 times and dead beetles were noted after 6, 12, and 24 h.

2.9 Toxicity of oils by contact on seeds.

Tested doses were obtained by diluting 0, 8, 16, 32, and 64 µl in 1 mL of acetone. Each dose was mixed with 50 g of seeds of each legume in 270-cm^3 glass containers and manually mixed before leave for 20 min to evaporate the solvent. Each dose was repeated 4 times, and each jar was inoculated with twenty adult *A. obtectus* or *C. maculatus* before being covered with a porous cloth. Dead beetles were noted daily for 4 days.

2.10 Contact toxicity of aromatized clay powder on grains.

In order to know if the clay can be used to improve persistence of sulfur-containing compounds essential oils and increase their effect, we evaluated the activity of the aromatized clay powder.

Test doses were prepared by mixing 0.05 g of clay powder with 0, 4, 8, 16, 32, or 64 µL of oils and homogenize by stirring manually. Fifty grams of grains were put in 270 cm^3 glass containers and malaxated with the different doses of the aromatized clay powder. Control seeds were treated only with 0.05 g of clay powder. After manual homogenization, final doses were 0.00, 0.08, 0.16, 0.32, 0.64, and 1.28 µL/g (vol/wt), respectively. Twenty *A. obtectus* or *C. maculatus* adults were put into each container, which was then covered with porous cloth. Each dose was repeated 4 times and dead beetles were noted daily for 4 days

2.11 F1 progeny assessment.

The capacity of essential oils and their aromatized clay powder to kill eggs, larvae and adults, and then prevent the emergence of a new generation of *A. obtectus* or *C. maculatus* was evaluated. After the evaluation of mortality in each treatment group, on the 16th day post-infestation, all adults were removed and the experiments were conducted under the same conditions until the emergence of F1 progeny. The recording of F1 progeny was done every day until no more emergence was observed, indicating the end of emergence of this generation. Percentage of reduction (PR) in progeny production was calculated:

$$PR = \{(Cn - Tn)/Cn\} \times 100.$$

Where, Cn is the number of newly emerged insects in the control, and Tn is the number of emerged adults in treated jars.

After the F1 adults in each glass jar were removed, the damaged bean seeds were sieved and weighed to assess weight loss. Percentage weight loss was determined:

[(Initial weight -Final weight)/ Initial weight] × 100.

2.12 Seeds germination test.

Legume seeds are usually treated and stored to be used later either as food or as seedling. In order to ensure that essential oils composed mainly of sulfur compounds protect the seeds against damage caused

by pests while preserving their viability, the germination test of seeds treated and stored for 3 months was carried out as described by Rahman and Talukder [22] and modified by Fogang et al. [23].

2.13 Statistical analysis.

Data obtained from each bioassay were recorded as the mean ± standard deviation (SD) and were submitted to a one-way ANOVA. Differences between means were evaluated with the Tukey test using the SPSS 2000 program [24]. The percent mortality was calculated using the Abbott correction formula for natural mortality in controls [25]. The Bliss [26] method based on the regression of the probits of mortalities [27] was used to determine the 50% lethal dose (LD_{50}) based on the decimal logarithms of the oil doses.

3. Results and discussion

3.1 Composition of the oils

Sixteen (16) and twenty-two (22) compounds, representing 93.9% and 98.2% of the total composition have been identified respectively in the *A. lepidophyllus* and *S. zenkeri* seeds essential oils. Phenylpropanoids, being represented by eugenol (1.2% for *A. lepidophyllus* and 1.6% for *S. zenkeri*) were found in both oils while monoterpene hydrocarbons were present at small amounts and only in *S. zenkeri* oil (Table 1). These seed-oils were characterized in the majority by sulfur-containing compounds (12 representing 91.0% for the former and 16 representing 96.1% for the latter) and possessed the same most abundant compound (2,4,5,7-Tetrathiaoctane; 52.9% for *A. lepidophyllus* and 51.5% for *S. zenkeri*). The number of sulfur atoms in these chemicals varies between 2 for 2,4-Dithiapentane and 6 for 2,4,5,7,8,10-Hexathiaundecane and 5-methyl-2,3,4,6,7,9-hexathiadecane (Table 2).

In this work, the *A. lepidophyllus* fruits oil was pale-yellow and got 16 volatile compounds, unlike the colourless essential oil from the barks with only seven compounds [28]. Though both were characterized mostly by sulfur-containing compounds, they didn't have the same major compound. Other previous work listed on this plant had always been carried out either on the barks or on the extracts and not on the seeds oil [29-31]. Concerning *S. zenkeri* oil, its composition was consistent with that previously reported for the bark oil [32-33].

3.2 Repellency

It appears from table 3 that the two oils have a repellent effect against the adults of these two insects with a more pronounced effect firstly against *C. maculatus* and secondly for the *A. lepidophyllus* essential oil.

Table 1 Chemical composition of the essential oils of *Afrostyrax lepidophyllus* (*A. l.*) and *Scorodophloeus zenkeri* (*S. z.*) seeds

Compound name and class	Content [%]	
	A. l	S. z.
Sulfur-containing compounds		
2,4-Dithiapentane		tr
2,3,4-Trithiapentane		0.6
1,2,4-Trithiolane	0.4	tr
1,3,5-Trithiacyclohexane		tr
2,3,5-Trithiahexane	8.1	24.2
3-Methyl-2,4,5-trithiahexane		0.1
Dimethyltetrasulfide		0.1
Tris(methylthio)methane	0.1	0.5
2,3,4,6-Tetrathiaheptane	1.2	6.2
2,3,5,7-Tetrathiaoctane	1.6	0.3
2,4,5,7-Tetrathiaoctane	52.9	51.5
3,6-Dimethyl-2,4,5,7-tetrathiaoctane	1.6	0.3
2,4,5,6,8-Pentathianonane	2.4	9.0
2,4,5,7,9-Pentathiadecane	11.7	1.0
6-Methyl-2,4,5,7,9-Pentathiadecane	10.8	2.3
2,4,5,7,8,10-Hexathiaundecane	0.2	tr
5-methyl-2,3,4,6,7,9-hexathiadecane	tr	
Monoterpene hydrocarbons		
a-Pinene		0.1
Limonene		0.2
(Z)-b-Ocimene		tr
(E)-b-Ocimene		0.2
Phenylpropanoids		
Eugenol	1.2	1.6
Others		
tetradec-1-ene	0.1	
n –Tetradecane	0.2	
2,4-Bis(1,1-dimethylethyl)phenol	1.4	tr
Total identified [%]	93.9	98.2

This repellency was globally dose-dependent. The average percent repellencies were 68.10% and 46.33% against *A. obtectus* and 91.3% and 82.5% against

Table 2 Structure of sulfur-containing compounds of the essential oils of *Afrostyrax lepidophyllus and Scorodophloeus zenkeri* seeds

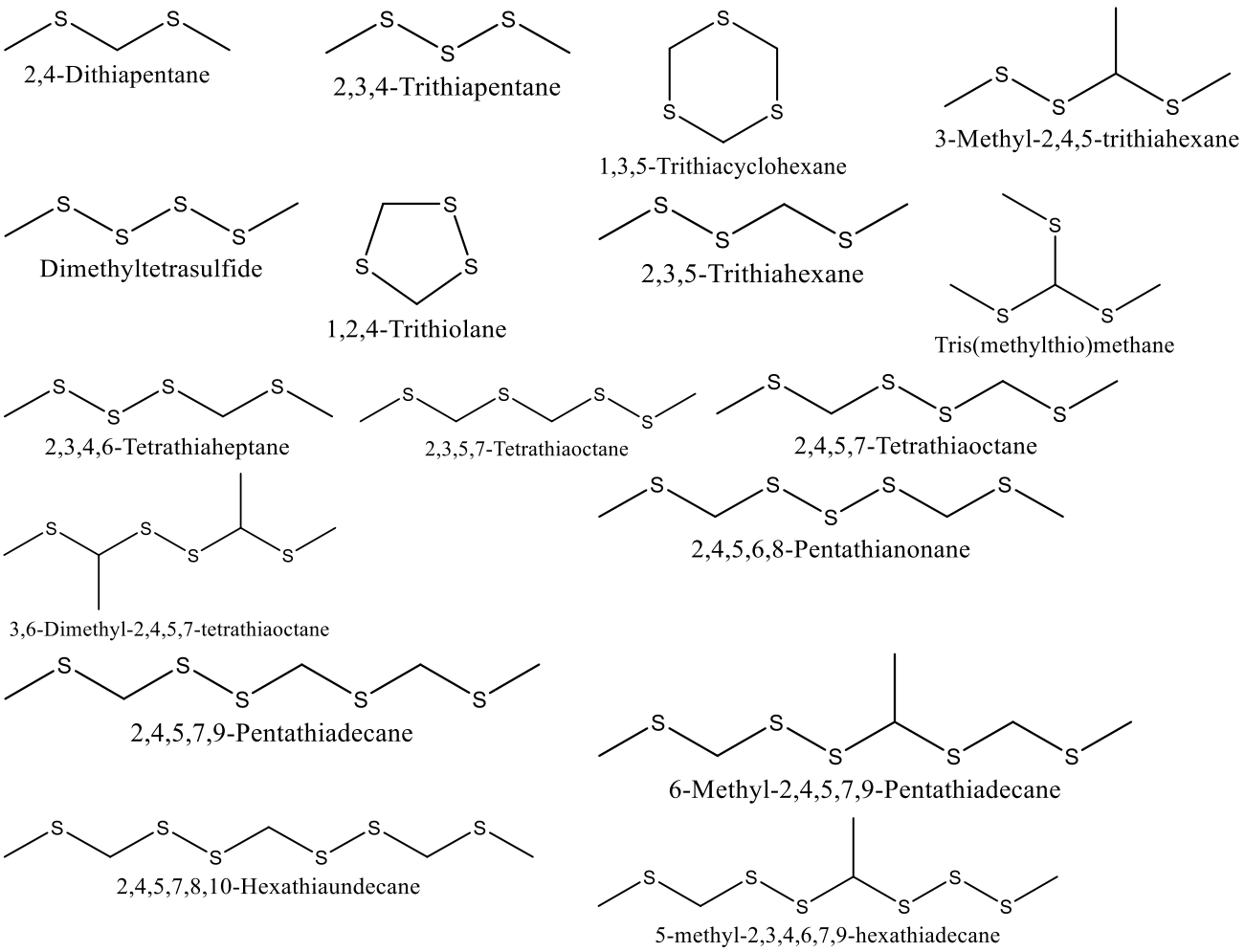

Table 3 Repellency effect of oils from *Afrostyrax lepidophyllus* and *Scorodophloeus zenkeri* seeds against *Acanthoscelides obtectus* and *Callosobruchus maculatus* as a function of concentration.

	Repellency (%)[a]			
	A. obtectus		***C. maculatus***	
Doses (µl/cm²)	***A. lepidophyllus***	***S. zenkeri***	***A. lepidophyllus***	***S. zenkeri***
0.062	47.5±6.3^{b}	27.5±9.2^{a}	65.0±13.2^{b}	55.0±18.4^{a}
0.125	60.0±7.0^{b}	35.0±6.4^{ab}	100.0±0.0^{c}	77.5±4.7^{b}
0.251	82.5±6.3^{c}	47.8±4.7^{b}	100.0±0.0^{c}	97.5±2.5^{bc}
0.503	82.5±2.5^{c}	75.0±6.4^{c}	100.0±0.0^{c}	100±0.0^{c}
Mean	68.1±5.5	46.33±6.6	91.3±3.3	82.5±6.4
Repellency Class	IV	III	V	V

[a] Values are the means ± standard deviation. Within each oil, means with different letters are significantly different (P<0.05) based on the Tukey test.

C. maculatus, which puts the oils in repellency classes IV and III against *A. obtectus* and V against *C. maculatus*, respectively for *A. lepidophyllus and S. zenkeri* oils according to Juliana and Su [21]. Moreover, according to this scale: non-repellent, almost repellent, slightly repellent, moderately repellent, highly repellent and extremely repellent, it could be deducing that both oils were extremely repellent

against *C. maculatus* while the *A. lepidophyllus* oil was highly repellent and that of *S. zenkeri* moderately repellent against *A. obtectus*.

The repellent effect shows that like the monoterpene hydrocarbons [34] and the oxygenated monoterpenes [23], the sulfur-containing compounds also possess repellent properties towards *A. obtectus* and *C. maculatus*.

3.3 Filter paper contact toxicity

Figures 2a, 2b, 2c and 2d illustrate the evolution of the percentages of cumulative and corrected mortalities compared to the control of adults of *C. maculatus* and *A. obtectus* as a function of time and of the dose of essential oils. After 4 days of exposure (except the oil from *S. zenkeri* against *A. obtectus*), the dose 0.251 µl/cm² had caused the death of all the insects. The other doses induced increasing mortalities but which were not total at the end of this exposure time. The LD_{50} obtained were 0.091 and 0.132 µl/cm² for *A. lepidophyllus* oil, and 0.300 and 0.128 µl/cm² for *S. zenkeri* oil against *A. obtectus* and *C. maculatus* respectively

3.4 Fumigation of A. obtectus and C. maculatus with essential oils.

The vapours of these oils have been shown to be toxic against both insects (Fig. 3a, 3b, 3c and 3d). In fact, the

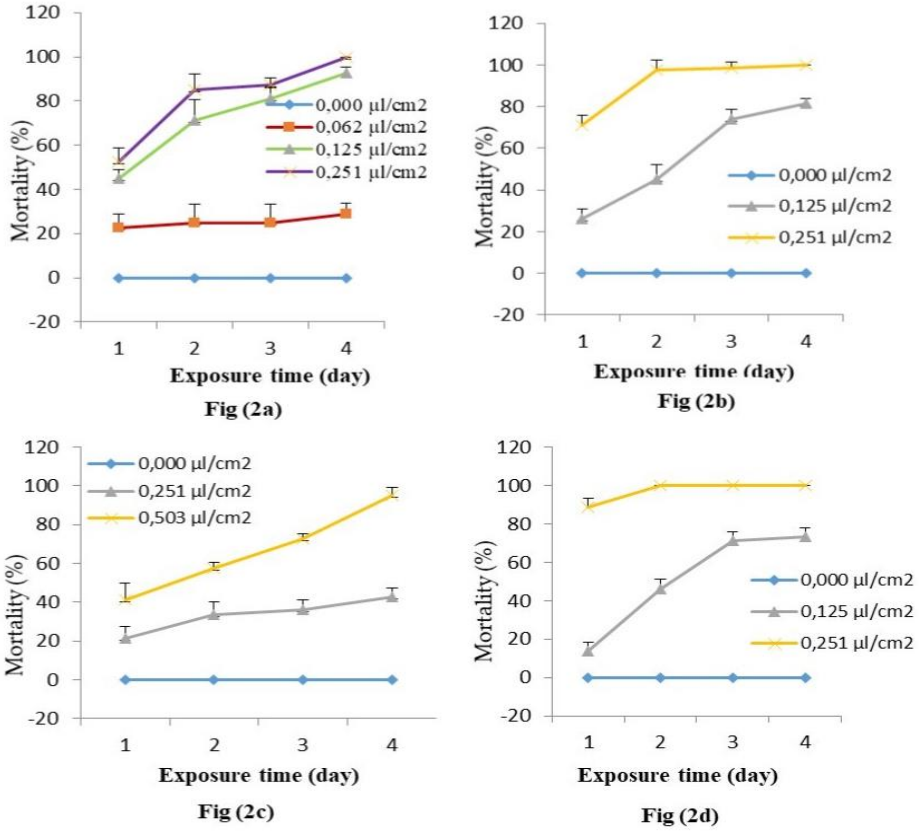

Fig. 2 Percent mortality (CM ± SD) on filter paper discs of:
a: *A. obtectus* as a function of time and dose of essential oil from seeds of *A. lepidophyllus*.
b: *C. maculatus* as a function of time and dose of essential oil from seeds of *A. lepidophyllus*
c: *A. obtectus* as a function of time and dose of essential oil from seeds of *S. zenkeri*
d: *C. maculatus* as a function of time and dose of essential oil from seeds of *S. zenkeri*
CM, corrected mortality; SD, standard deviation.

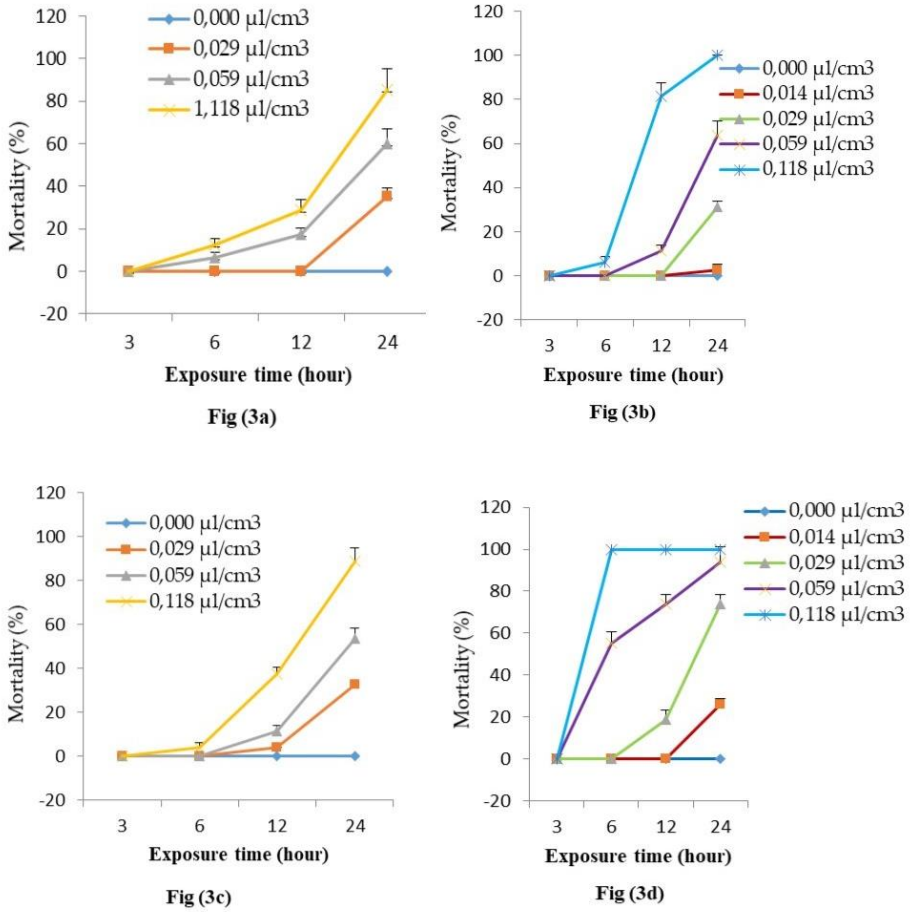

Fig. 3 Percent mortality (CM ± SD) of:
a: *A. obtectus* as a function of time and dose of fumigated essential oil of *A. lepidophyllus*.
b: *C. maculatus* as a function of time and dose of fumigated essential oil of *A. lepidophyllus*.
c: *A. obtectus* as a function of time and dose of fumigated essential oil from seeds of *S. zenkeri*
d: *C. maculatus* as a function of time and dose of fumigated essential oil from seeds of *S. zenkeri*
CM, corrected mortality; SD, standard deviation.

insecticidal activity of these oils was worthless after 3h of exposure and then increased remarkably between 12 and 24 h for all the doses tested. After 6 h of exposure, except in the glass jar treated with *S. zenkeri* oil against *C. maculatus* where mortalities were already total with the highest concentration (0.118 µl/cm^3), mortalities recorded were less than 20% but after 24 h of fumigation, 85.00 and 88.75% of mortality were obtained for the concentration of 0.118 µl/cm^3 against *A. obtectus* for *A. lepidophyllus* and *S. zenkeri* respectively. The mortalities recorded between 12 and 24 h were significantly different ($P<0.05$). The LD_{50} values obtained were 0.316 and 0.159 µl/cm^3 against *A. obtectus*, 0.088 and 0.045 µl/cm^3 against *C. maculatus* respectively for *A. lepidophyllus* and *S. zenkeri*, confirming the high toxicity of these essential oils against adults of *C. maculatus.* The noticed fumigant activity shows that the sulfur-containing compounds essential oils are a source of biologically active vapours that are potentially efficient insecticides.

3.5 Insects mortality in beans and cowpea treated with oils

From figures 4a, 4b, 4c and 4d, it can be seen that all the doses tested caused mortalities which rise with the doses and time toward both insects. The dose 0.64 µl/g of *S. Zenkeri* oil induced total mortality of *C. maculatus* and *A. obtectus* after just 1 and 2 days of exposure respectively (Fig. 3c and 3d) while it's with the dose 1.28 µl/g of *A. lepidophyllus* oil that the total mortality has been observed and after 2 days of exposure (Fig. 3a and 3b). Apart from the dose 0.32 µl/g of *S. zenkeri* oil against *C. maculatus*, others tested doses were certainly effective, but did not induce total mortality after 4 days of exposure (Fig. 4a, 4b, 4c and 4d). The 2-

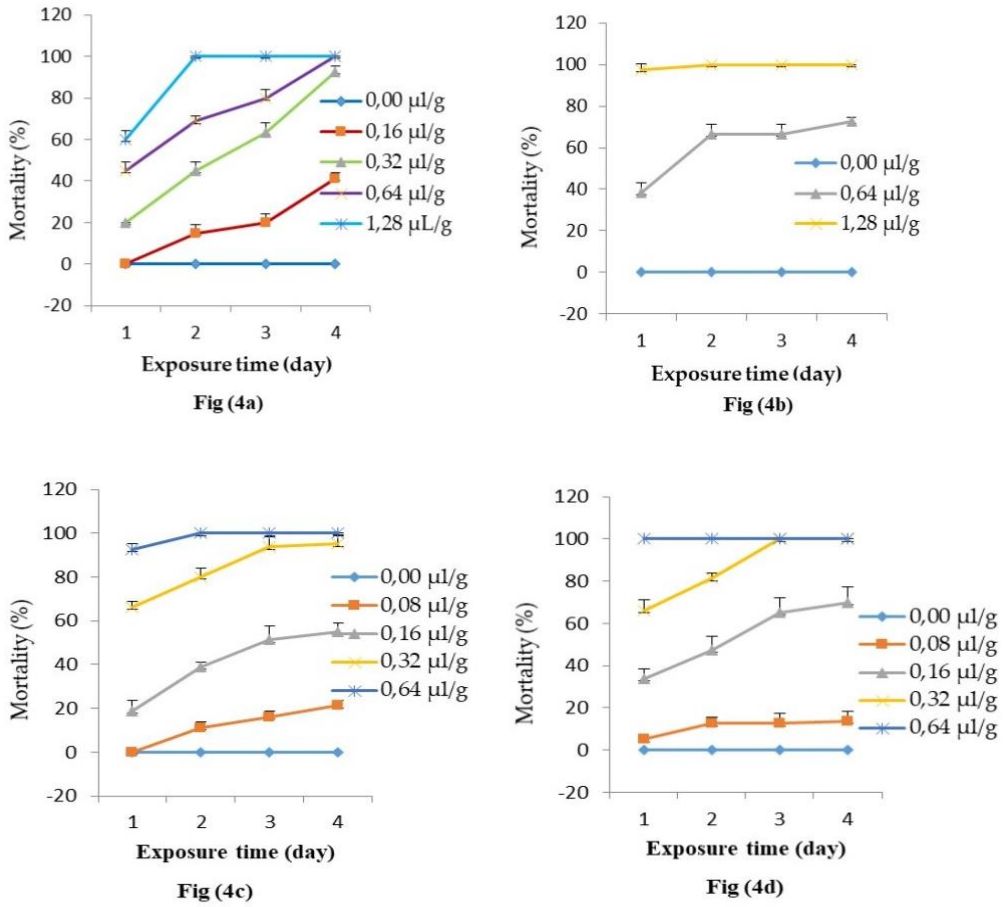

Fig. 4 Percent mortality (CM ± SD) of:
a: *A. obtectus* as a function of time and dose of essential oil of *A. lepidophyllus* on common bean grains.
b: *C. maculatus* as a function of time and dose of essential oil of *A. lepidophyllus* on cowpea grains.
c: *A. obtectus* as a function of time and dose of essential oil of *S. zenkeri* on common bean grains
d: *C. maculatus* as a function of time and dose of essential oil of *S. zenkeri* on cowpea grains.
CM, corrected mortality; SD, standard deviation.

day LD_{50} were 0.27 and 0.19 µl/g against *A. obtectus* and, 0.58 and 0.17 µl/g against *C. maculatus* respectively for *A. lepidophyllus* and *S. zenkeri* oils.

3.6 Insect mortality in beans and cowpea treated with Aromatized Clay Powder (ACP).

It appeared that the mortality increased with time and the complete mortality of *A. obtectus* and *C. maculatus* was obtained with only 0.32 µl/g (except against *C. maculatus* with *A. lepidophyllus* ACP) (Fig. 5a, 5b, 5c and 5d). These formulations have a LD_{50} of 0.33 and 0.12 µl/g against *A. obtectus*, 0.78 and 0.18 µl/g against *C. maculatus* respectively for *A. lepidophyllus* and *S. zenkeri* oils.

The ACP was more active than the essential oil against *A. obtectus*. Indeed, even at the dose 0.08 µl/g, mortality of *A. obtectus* adults was significantly different ($p<0.05$) to the control on the fourth day of exposure with the ACP contrary to the essential oil. Moreover, complete mortality was obtained with the ACP at the dose of 0.32 µl/g whereas it was only 92.5% with the essential oil after 4 days of exposure. From these observations, it can be established that the combination of oil with clay limited its volatility and contributed to the increase in its activity over time.

3.7 F1 progeny production

It was observed that without protection, a farmer could lose up to 9. 20% of the weight of common beans and up to 6.40% of the weight of harvested cowpeas (Table 4). However, protection at doses of 0.64 µl/g for the essential oil, 0.32 and 0.64 µl/g for the ACP (against *A. obtectus* and *C. maculatus* respectively), not only kills adult insects, but also completely suppresses the production of F_1 progeny of these

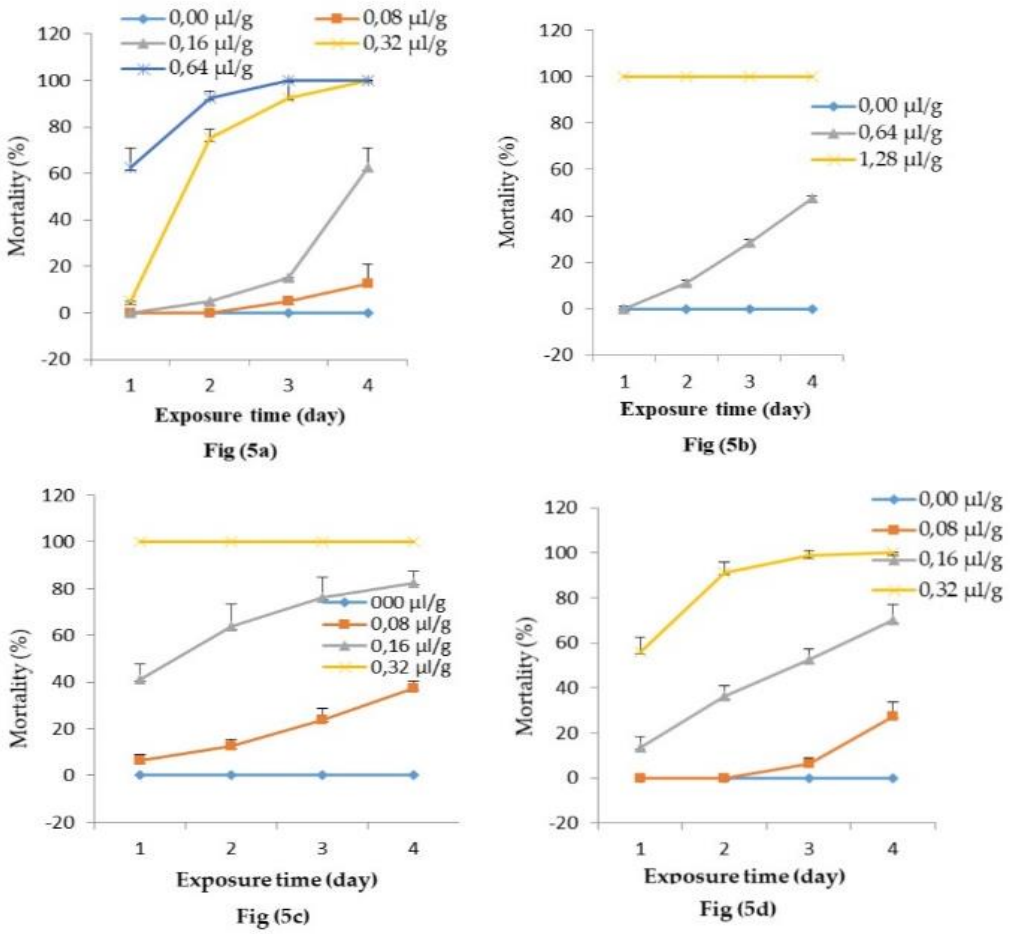

Fig. 5 Percent mortality (CM ± SD of:
a: *A. obtectus* as a function of time and dose of ACP of essential oil of *A. lepidophyllus* on common bean.
b: *C. maculatus* as a function of time and dose of ACP of essential oil of *A. lepidophyllus* on cowpea
c: *A. obtectus* as a function of time and dose of ACP of essential oil of *S. zenkeri* on common bean d: *C. maculatus* as a function of time and dose of ACP of essential oil of *S. zenkeri* on cowpea.
CM, corrected mortality; SD, standard deviation; ACP, aromatized clay powder.

insects. Emergence of adult insects from all control samples indicated that the quantity of clay powder and the volume of acetone used for bioassay was not toxic to the insects.

Concerning *S. zenkeri* essential oil and its ACP, no *C. maculatus* progeny was produced in cowpea treated with the dose 0.16 µl/g. The same effect has been observed in common beans treated with the dose 0.32 µl/g for the EO and 0.16 µl/g for the ACP. The results of F1 progeny inhibition suggested that these two sulfur-containing EOs and their ACP, either suppressed oviposition or killed the larvae hatching from eggs laid on grains, preventing feeding and damage.

The toxicity of the essential oils both by contact on filter paper and by fumigation testifies that it can also be used as a fumigant to protect certain foodstuffs, such as flour, which should not come into direct contact with the pesticide. Moreover, the presence in this oil of eugenol which has antimicrobial [35] and cytotoxic properties [36], would protect the seeds in

Table 4 F1 progeny production by adult *A. obtectus* and *C. maculatus* and percent reproduction inhibition in beans and cowpea treated with essential oils (EO) or aromatized clay powder (ACP) from *A. lepidophyllus* (*A.l.*) and *S. zenkeri* (*S.z.*)

Dosage (µl/g)	*A. obtectus*						*C. maculatus*					
	Nb. of emerged insects		% inhibition of F1 adults		% bean weight loss		Nb. of emerged insects		% inhibition of F1 adults		% cowpea weight loss	
	EO											
	A.l.	*S.z*	*A.l.*	*S.z*	*A.l.*	*S.z*	*A.l.*	*S.z*	*A.l.*	*S.z*	*A.l.*	*S.z*
0.00	172.0± 54.8	221,0± 37,6	0,0	0,0	6.40	8.60	66,0± 41,5	256,0±48,0	0,0	0.0	3.40	6.40
0.08	-	24,0± 3,6	-	89,1	-	3.40	-	11,0± 20,3	-	95,7		0.60
0.16	56.0± 18.9	8,0± 5,3	67,4	96,3	1.60	1.40	-	0,0± 0,0	-	100,0	-	0.00
0.32	16.0± 2.8	0,0± 0,0	90,7	100,0	0.80	0.00	35,0± 32,2	0,0± 0,0	47,0	100,0	1.80	0.00
0.64	0.0±0.0	0,0± 0,0	100,0	100,0	0.00	0.00	0,0± 0,0	0,0± 0,0	100,0	100,0	0.00	0.00
1.28	0.0± 0.0	-	100,0	-	0.00	-	0,0± 0,0	-	100,0	-	0.00	-
	ACP											
0.00	179.0± 2 .1	171,0±19,7	0,0	0,0	4.80	7.54	102,0±52,1	374,0±172,6	0,0	0,0	2.40	11.60
0.08	34.0± 7.4	84,0±17,4	81,0	49,9	1.60	4.60	-	36,0± 43,0	-	90,4	-	4.00
0.16	10.0± 2.2	0,0± 0,0	94,4	100,0	1.20	0.00	-	0,0± 0,0	-	100,0	-	0.00
0.32	0.0± 0.0	0,0± 0,0	100,0	100,0	0.00	0.00	1,0± 0,9	0,0± 0,0	99,0	100,0	0.00	0.00
0.64	0.0± 0.0	-	100,0	-	0.00		0,0± 0,0	-	100,0	-	0.00	-

storage against damage caused by fungi and bacteria whose contamination generally appear after pest infestation.

Previous studies performed with volatile sulfur compounds have shown that thiosulfinates and thiosulfonates have nematicidal effects [37] while disulfides and trisulfides are larvicidal [38]. According to Miller [39], the mechanism of action associated with the pesticidal activity of the dithiocarbamates is the inhibition of metal-dependant and sulfhydryl enzyme systems in fungi, bacteria, plants, and insects, as well as mammals. Moreover, according to many authors, the rapid toxicity of garlic essential oil (which is an enriched sulfur-containing compounds essential oil) and their constituents in insects indicates neurotoxic action as reported to *Delia radicum*, *Musca domestica*, *Cacopsylla* chinensis, and *Diaphorina citri* with hyperactivity, hyperextension of the legs and abdomen and rapid knock-down effect or immobilization [10, 40–41]. Acetylcholinesterase is an enzyme that has been shown to be inhibited by garlic compounds and can act only or in synergism as diallyl disulide, diallyl trisulide, and allicin [42-43]. Halliwell and Gutteridge [44] also demonstrated that the presence of the diallyl sulide in garlic compounds may be responsible for the toxic effect in *T. molitor* and may cause inhibition by cross-linking with essential thiol compounds in enzyme structures, altering the functional shape of the protein and denaturalization. From this mechanism, we can notice that the sulfur atoms play a key role in the organosulfur action and some of our identified molecules contained up to 6 sulfur atoms.

3.8 Effect of oil treatment on the germination of bean and cowpea seeds.

From table 5, it appears that indices increased with time between the second and fifth day, period after which they began to stabilize or change very slowly. After 2 days, the percent germination was already higher than 50% (except with *S. zenkeri* EO at dose 1.28 µl/g, 47.5%) for the seeds treated with the essential oils or their ACP, acetone and clay.

Although not significantly different (except *S. zenkeri* ACP, (P>0.05), the viability indices of the treated seeds, at the end of germination time, were in general higher than those of the controls. The action of the ACP from *A. lepidophyllus* oil on the viability of the cowpea seeds was not carried out because it was not possible to obtain a powdery formulation with 0.1 g of clay and the doses to be tested (doses close to LD_{50}, LD_{100} and $2DL_{100}$). Like synthetic organosulfur pesticides, enriched sulfur-containing compounds essential oils protect bean seeds without affecting their germination.

Table 5 Percent germination of *Phaseolus vulgaris* and *Vigna unguiculata* seeds treated with essential oils or their aromatized clay powders

Germination day	Seeds	Seeds germinated (%) (mean ± SD)[a]											
		Essential oils						Aromatized clay powders					
		Control[b]	0.32µl/g		0.64µl/g		1.28 µl/g		2.56 µl/g	0.10g clay	0.32 µl/g		0.64 µl/g	
			S.z.		A.l.	S.z	A.l.	S.z.	A.l.		A.l.	S.z.	A.l.	S.z.
2	*P.v.*	68.8 ± 8.5^{A}	-		63.8 ± 6.3^{A}	52.5 ± 6.4^{B}	65.0 ± 10.0^{A}	47.5 ± 8.7^{B}	-	72.5 ± 2.9^{A}	75.0 ± 10.8^{A}	-	63.7 ± 8.5^{A}	-
	V. u.	77.5 ± 16.6^{A}	87.5 ± 6.4^{A}		-	87.5 ± 6.4^{A}	73.8 ± 11.1^{A}	-	88.3 ± 6.2^{A}	75,0 ± 4,0^{A}	-	88.8 ± 8.5^{A}	-	87.5 ± 9.5^{A}
3	*P.v.*	77.5 ±11.9^{A}	-		81.3 ± 6.3^{A}	65.0 ± 7.0^{A}	81.3 ± 8.5^{A}	63.8 ± 8.5^{A}	-	76.3 ± 6.3^{A}	78.8 ± 10.3^{A}	-	65.0 ± 7.0^{A}	-
	V. u.	91.3 ± 8.5^{A}	92.5 ± 6.4^{A}		-	96.3 ± 2.5^{A}	91.3 ± 4.8^{A}	-	92.5 ± 6.4^{A}	80,0 ± 4,0^{A}	-	92.5 ± 6.4^{B}	-	96.3 ± 4.8^{B}
4	*P.v.*	82.5 ± 8.7^{A}	-		82.5 ± 6.4^{A}	72.5 ± 9.6^{A}	86.3 ± 4.8^{A}	68.8 ± 10.3^{A}	-	80.0 ± 8.1^{A}	81.3 ± 13.1^{A}	-	80.0 ± 9.1^{A}	-
	V. u.	95.0 ± 4.0^{A}	95.0 ± 4.0^{A}		-	97.5 ± 2.9^{A}	93.8 ± 6.3^{A}	-	93.8 ± 6.3^{A}	83,8 ± 4,8^{A}	-	95.0 ± 4.0^{B}	-	96.3 ± 4.8^{B}
5	*P.v.*	85.0 ± 7.0^{A}	-		86.3 ± 7.5^{A}	77.5 ± 11.9^{A}	90.0 ± 4.0^{A}	80.0 ± 4.0^{A}	-	83.8 ± 9.4^{A}	85.0 ± 9.1^{A}	-	82.5 ± 9.5^{A}	-
	V. u.	92.5 ± 6.4^{A}	95.0 ± 4.0^{A}		-	98.8 ± 2.5^{A}	95.0 ± 7.0^{A}	-	96.3 ± 4.8^{A}	85.7 ± 7.0^{A}	-	97.5 ± 2.9^{B}	-	98.8 ± 2.5^{B}
6	*P.v.*	86.3 ± 6.3^{A}	-		86.3 ± 7.5^{A}	85.0 ± 9.1^{A}	90.0 ± 4.0^{A}	85.0 ± 7.0^{A}	-	86.3 ± 4.8^{A}	86.3 ± 10.3^{A}	-	88.8 ± 6.3^{A}	-
7	*P.v.*	86.3 ± 6.3^{A}	-		86.3 ± 7.5^{A}	88.8 ± 7.5^{A}	91.3 ± 4.8^{A}	81.3 ± 2.5^{A}	-	86.3 ± 4.8^{A}	88.8 ± 7.5^{A}	-	91.3 ± 8.5^{A}	-
8	*P.v.*	86.3 ± 6.3^{A}	-		87.5 ± 6.4^{A}	92.5 ± 6.4^{A}	91.3 ± 4.8^{A}	86.3 ± 4.8^{A}	-	87.5 ± 5.0^{A}	88.8 ± 7.5^{A}	-	91.3 ± 8.5^{A}	-

[a] Within each line, means with the same letter do not differ significantly based on the Tukey test.

[b] Control seeds were treated in acetone at 0.02 mL/g of beans or cowpea. *P.v., phaseolus vulgaris; V.u., Vigna unguiculata; S.z., Scorodophloeus zenkeri; A.l., Afrostyrax lepidophyllus*

4. Conclusions

Essential oils from the seeds of *A. lepidophyllus* and *S. zenkeri* are sources of sulfur-containing compounds and eugenol which possesses antimicrobial activity. These oils have been shown to be extremely repellent to *C. maculatus* and highly repellent to *A. obtectus*. By contact on grains, they not only kill the adults, but also the eggs and larvae of *A. obtectus* and *C. maculatus*, thereby preserving the quality and quantity of common bean and cowpea seeds in storage without affecting their viability. These essential oils being characterized mostly by sulfur-containing compounds, constitute natural organosulfur insecticides and can be used as alternative to synthetic organosulfur pesticides. The use since immemorial time of these two plants as remedy and as spice testifies their harmlessness both for the consumer and for the environment and, make their essential oils natural pesticides that respect the biosphere balance.

Authors' contributions

Methodology, Data acquisition, writing - original draft preparation, H. P. D. FOGANG; Conceptualization, Writing – review, V. WOGUEM; Essential oils analysis and constituents identification, M. FILIPPO; Validation, Writing - review & editing, H. M. WOMENI; Methodology, Validation, Writing - original draft preparation & editing, L. A. TAPONDJOU.

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Conflicts of interest

The authors declare no conflict of interest

References

1. Langyintuo, A. S.; Lowenberg-DeBoer, J.; Faye, M.; Lambert, D.; Ibro, G.; Moussa, B.; Kergna, A.;

Kushwaha, S.; Musa, S.; Ntoukam, G. Cowpea supply and demand in West Africa. Field Crops Res. **2003,** 82(2-3), 215–231.
2. Pathania, A.; Sharma, K. S.; Sharma, N. P. Common Bean. In: Singh, M., et al. (eds.), Broadening the Genetic Base of Grain Legumes. Springer, India, **2014,** 11-50.
3. Murdock, L. L.; Coulibaly, O.; Higgins, T. J. V.; Huesing, J. E.; Ishiyaku, M.; Sithole-Niang, I. *Cowpea*. Compendium of Transgenic Crop Plants: Transgenic Legume Grains and Forages. Edited by Chittaranjan Kole & Timothy C. Hall, **2008.**
4. Dagnew, K. ; Haileselassie, T.; Feyissa, T. Genetic diversity study of common bean (*Phaseolus vulgaris* L.) germoplasm from Ethiopia using inter simple sequence repeat (ISSR) markers. *Afr. J. Biotechnol*. **2014,** 13(36), 3638–3649.
5. Wortmann, C. S. "*Phaseolus vulgaris* L. (common bean): Prota 1: Cereals and pulses/Céréales et légumes secs". **2006**.
6. Hall, A. E.; Cisse, N.; Thiaw, S.; Elawad, H. O. A.; Ehlers, J. D.; Ismail, A.; Fery, R.; Roberts, P.; Kitch, L. W.; Murdock, L. L.; et al. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. Field Crops Res. **2003**, 82(2-3), 103–134.
7. Phillips, R. D.; McWatters, K. H.; Chinannan, M. S.; Hung, Y.; Beuchat, L. R.; Sefa-Dedeh, S.; Saki-Dawson, E.; Ngoddy, P.; Nnanyelugo, D.; Enwere, J.; et al. Utilization of cowpeas for human food. Field Crops Res. **2003**, 82(2-3), 193–213.
8. MINADER. Liste des pesticides homologués au Cameroun au 04 mars **2021**. 2021, 119-123.
9. WHO. Environmental health criteria 78: dithiocarbamate pesticides, ethylenethiourea and propylenethiourea: a general introduction, Geneva, World Health Organization, 1988, 7-12.
10. Prowse, M. G., Galloway, T. S.; Foggo, A. Insecticidal activity of garlic juice in two dipteran pests. Agr. Forest Entomol. **2006**, 8(1), 1–6.
11. Plata-Rueda, A.; Martínez, L. C.; Dos Santos, M. H.; Fernandes, F. L.; Wilcken, C. F.; Soares, M. A.; Serrão, J. E. ; Zanuncio, J. C. Insecticidal activity of garlic essential oil and their constituents against the mealworm beetle, Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae). Sci. Rep. **2017**, 7, 46406.
12. Sprecher, D.; Grigorowa, R. On the maximal concentration of thiuram at the workplace. Z. Gesamte Hyg. **1967,** 13(4), 241-245.
13. Correia, M.; Rodrigues, M.; Paiga, P.; Delerue-Matos, C. Fungicides. Encyclopedia of Food and Health. **2016,** 169-176.
14. National Center for Biotechnology Information. PubChem Compound Summary for CID 34526, Pirimiphos-methyl. Retrieved January 17, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Pirimiphos-methyl.
15. Tripathi, K. A.; Upadhyay, S.; Bhuiyan, M.; Bhattacharya, R. P. A review on prospects of essential oils as biopesticide in insect-pest management. *J. Pharmacognosy Phytother*. **2009,** 1(5), 052-063.
16. Rajendran, S.; Sriranjini, V. Plant products as fumigants for stored- product insect control. J. Stored Prod. Res. **2008,** 44(2), 126–135.
17. Pesticide Action Network Iternatyional (PAN). PAN International List of Highly Hazardous Pesticides. **2019**, 46.
18. Fogang, H. P. D.; Maggi, F.; Tapondjou, L. A.; Womeni, H. M.; Papa, F.; Quassinti, L.; Bramucci, M.; Vitali, L.A.; Petrelli, D.; Lupidi, G.; et al. In vitro biological activities of seed essential oils from the Cameroonian spices *Afrostyrax lepidophyllus* Mildbr. and *Scorodophloeus zenkeri* harms rich in sulfur-containing compounds. Chem. Biodivers. **2014**, 11(1), 161–169.
19. Tonle, K. I. Capteurs électrochimiques à base d'argiles smectiques camerounaises fonctionnalisées par les groupements thiols et amines: élaboration, caractérisation et application au piégeage des métaux lourds à effet polluant. Thèse de Doctorat/Ph.D. Université de Yaoundé I, Yaoundé, Cameroon, **2004**.
20. McDonald, L. L.; Guy, H. R.; Speirs, D. R. Preliminary evaluation of new candidate materials as toxicants, repellents and attractants against stored product insects. *Marketing research report 882*. Agricultural Research Service, U.S. Department of Agriculture, Washington, DC, **1970.**
21. Juliana, G.; Su, F. C. H. Laboratory studies of several plant materials as insect repellents for

protection of several grains. J. Econ. Entomol. **1983**, 76(1), 154–157.
22. Rahman, A.; Talukder A. F. Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*. J. Insect Sci. **2006**, 6, 1–10.
23. Fogang, D. H. P.; Womeni, H. M.; Piombo, G.; Barouh, N.; Tapondjou A. L. Bioefficacy of essential and vegetable oils of *Zanthoxylum xanthoxyloides* seeds against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). J. Food Prot. **2012**, 75(3), 547–555.
24. Steel, R. G.; Torrie, H. J. Principles and procedures of statistics. McGraw-Hill, New York, 1980.
25. Abbott, W. S. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. **1925**, 18(2), 265–267.
26. Bliss, C. The determination of the dosage-mortality curve from small numbers. *Q J Pharm Pharmacol*. 1938, 11(2), 192–216.
27. Finney, D. F. Probit analysis. 3rd ed. Cambridge University Press, Cambridge, New York, **1938**, 333.
28. Yirankinyuki, F. F.; Danbature, L. W.; Silas, V. T. ; Poloma, A. Characterization and Determination of Antifugal Activities of Essential Oil Extracted from the Bark of *Afrostyrax lepidophyllus* "Country Onion or Shirum". *J. Biochem. Mol. Biol*. **2017**, 2(5), 29-33.
29. Ngono, N. A.; Lavault, M.; Séraphin, D.; Landreau, A.; Richomme, P. Three 1-thio-β-D-glucopyranosides from the seeds of *Afrostyrax lepidophyllus* Mildbr. Carbohydr. Res. **2006**, 341(17), 2799-2802.
30. Tamfu, A. N.; Almas, J.; Tagatsing, F. M.; Tariq, A. B.; Farzana, S. Organic and Mineral Composition of Seeds of *Afrostyrax lepidophyllus* Mildbr. and evaluation of ROS inhibition and cytotoxicity of isolated compounds. Chem. Africa. **2019**, 2, 615-624.
31. Moukette, M. B.; Castelão-Baptista, P. J.; Ferreira, L.; Silva, M. A.; Simões, F. R.; Cabral, C.; Pieme, A. C.; Ngogang, Y. J.; Sardão, A. V.; Oliveira, J. P. *Afrostyrax lepidophyllus* Mildbr. and *Monodora myristica* (Gaertn.) Dunal Extracts Decrease Doxorubicin Cytotoxicity on H9c2 Cardiomyoblasts. *Evid.-based Complement. Altern. Med*. **2021**, 8858165.
32. Kouokam, J. C.; Zapp, J.; Becker, H. Isolation of new alkylthiosulfides from the essential oil and extracts from the bark of *Scorodophloeus zenkeri* Harms. Z. *Naturforsch*. **2001**, 56(c), 1003-1007.
33. Amvam-Zollo, P. H.; Dupont Youngo, M. J.; Fekam, B. F.; Menut, C., Lamaty, G.; Bessière, J. M. Etude comparée de la composition chimique des huiles essentielles extraites à partir d'un arbre à ail (*Scorodophloeus zenkeri* Harms, Caesalpiniacee) et de l'ail (*Allium sativum* Linn., Liliacee) du Cameroun. *Actes des 14èmes Journées Internationales Huiles Essentielles*, Digne-les-Bains, **1995**.
34. Tapondjou, A. L.; Adler, C.; Bouda, H.; Fontem, D. A. Bioéfficacité des poudres et des huiles essentielles des feuilles de *Chenopodium ambrosioides* et *Eucalyptus saligna* à l'égard de bruche du niébé, *Callosobruchus maculatus* (F) (Coleoptera : Bruchidae). Cah. Agric. **2003**, 12, 401-407.
35. Marchese, A.; Barbieri, R.; Coppo, E.; Orhan, E. I.; Daglia, M.; Nabavi, F. S.; Izadi, M.; Abdollahi, M.; Nabavi, M. S.; Ajami, M. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. Crit. Rev. Microbiol. **2017**, 43(6), 668-689.
36. Burkey, J. L.; Sauer, J. M.; McQueen, C. A.; Sipes I. G. Cytotoxicity and genotoxicity of methyleugenol and related congeners – a mechanism of activation for methyleugenol. Mutat. Res. - Fundam. Mol. Mech. Mutagen. **2000**, 453(1), 25–33.
37. Tada, M.; Hiroe, Y.; Kihohara, S.; Suzuki, S. Nematicidal and antimicrobial constituents from *Allium grayi* Regel and *Allium fistulosum* L. var. caespitosum. Agric. Biol. Chem. 1988, 52(9), 2383-2385.
38. Amonkar, S. V.; Banerji, A.; Isolation and characterization of larvicidal principle of garlic. Science, 1971, 174, 1343-1344.
39. Miller, D. B. Neurotoxicity of the Pesticidal Carbamates. *Neurobehav Toxicol Teratol*. **1982**, 4(6), 779-787.
40. Mann, R. S.; Rousef, R. L.; Smoot, J. M.; Castle, W. S.; Stelinski, L. L. Sulfur volatiles from *Allium* spp. affect Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), response to citrus volatiles. Bull. Entomol. Res. **2011**, 101(1), 89–97.

41. Zhao, N. N.; Zhang, H.; Zhang, X. C.; Luan, X. B.; Zhou, C.; Liu, Q. Z.; Shi, W. P.; Liu, Z. L. Evaluation of acute toxicity of essential oil of garlic (*Allium sativum*) and its selected major constituent compounds against overwintering *Cacopsylla chinensis* (Hemiptera: Psyllidae). J Econ Entomol. **2013**, 106(3), 1349-1354.
42. Bhatnagar-homas, P. L.; Pal, A. K. Studies on the insecticidal activity of garlic oil II. Mode of action of the oil as a pesticide in *Musca domestica* nebulo Fabr and *Trogoderma granarium* Everts. J. Food Sci. Technol. **1974**, 11, 153–158.
43. Singh, V. K.; Singh, D. K. Enzyme inhibition by allicin, the molluscicidal agent of *Allium sativum* L. (garlic). Phytotherapy Res. **1996**, 10(5), 383–386.
44. Halliwell, B.; Gutteridge, J. M. C. Free Radicals in Biology and Medicine, 3rd edn. Oxford University Press, UK **1999**.