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ORIGINAL ARTICLE

Characterization by GC/MS-FID and GC/MS-HS-SPME and insecticidal activity against *Callosobruchus maculatus* (Fabricius, 1775) of essential oils and powder of *Xylopia aethiopica* (Dunal) A. Rich from Senegal

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Abstract

Today the use of plant extracts, in particular essential oils, is a natural alternative to synthetic insecticides in the fight against crop pests. In this study, the insecticidal activity of essential oils and powder of *Xylopia aethiopica* (Annonaceae) were tested by both fumigation and contact against *Callosobruchus maculatus*. The essential oil of *X. aethiopica,* obtained by steam distillation and the powder, with a particle size of 1 mm, were used for the tests. The analysis of essential oils and powder of *X. aethiopica* by GC-MS/FID and GC/MS-HS-SPME, showed that the main compounds were β-pinene (28.9–19.0%), 1,8-cineole (14.9–7.6%) and α-pinene (9.8–19.4%). Insecticidal activity of essential oils and powder of *X. aethiopica,* respectively, by fumigation (F) and contact (C) against *C. maculatus* showed toxicity LD_{50} = 0.2 ± 0.0 µl · cm^{-3}, LT_{50} = 16.4 ± 1.2 h (F) and LD_{50} = 9.2 ± 0.7 g · kg^{-1}, LT_{50} = 69.6 ± 0.4 h (C). The essential oil and powder of *X. aethiopica* can be considered as bio-insecticides against *C. maculatus* for the protection of cowpeas in Senegal.

Keywords: *Callosobruchus maculatus*, essential oils, GC/MS/HS-SPME, powder, *Xylopia aethiopica*

Introduction

Cowpea is an important staple food in sub-Saharan Africa, particularly in the arid savannas of West Africa. Its seeds represent a valuable source of vegetable protein, vitamins and income for humans, as well as fodder for animals. In Senegal, remarkable efforts have been made to increase national production of cowpea. In 2018, production of cowpea increased by 117,784 tons, an increase of 18% compared to 2016/2017 and an increase of 73% compared to the average of the last 5 years (ANSD 2018). However, despite these efforts, cowpea production faces constraints. Among these, post-harvest losses constitute a major constraint. Stored cowpea is subject to various kinds of deterioration due to many agents including insects which often cause a lot of damage (Guèye *et al.* 2011). The cowpea weevil (*Callosobruchus maculatus* F.) is the major storage insect pest of legumes and peas, especially cowpea (Beck *et al.* 2014). *C. maculatus* is very destructive due to its short life cycle. This insect pest infests cowpea in storage, thereby reducing the quality and quantity of the seeds (Mukendi *et al.* 2016).

Damage caused by *C. maculatus* results from the fact that the females lay their eggs on the surface of the cowpea pods and the neonate larvae penetrate into the healthy seeds. They then continue their development and mature during storage. During their development, the larvae destroy the inner envelope of the seeds where the pupa lodge. In adulthood, insects emerge from the seeds, leaving holes. Thus, the losses of stored foodstuffs can be estimated at 100% in sub-Saharan Africa (Ngamo *et al.* 2007; Chougourou and Alavo 2011; Kayombo *et al.* 2014). For this purpose, synthetic pesticides are often used to fight against these insects (Sahaf *et al.* 2008). However, their progressive use as a fumigant and insecticide generally leads to environmental nuisances and to the development of a certain resistance to parasites, in particular *Tribolium castanum* and *C. maculatus* which may have developed resistance to lindane and phosphine, respectively (Ahmed *et al.* 2002). To deal with this threat to human health, we have resorted to the use of aromatic plants with high or low content of essential oils, in particular *Xylopia aethiopica*. In Senegal, *X. aethiopica* fruit powder is used to flavor certain culinary preparations such as coffee and tea in particular. It is also used to treat the following pathologies: conjunctivitis, bronchitis, dysentery and toothache (Thiam *et al.* 2018). Several studies have been carried out on the chemical composition of essential oils of *X. aethiopica* (Koffi *et al.* 2012; Nguemtchouin 2012). To the best of our knowledge, no study has been carried out at the same time on the chemical characterization of essential oils of *X. aethiopica* by GC/MS-FID and on the powder by Head Space Solid Phase Microextraction Gas Chromatography Mass Spectrometry (GC/MS-HS-SPME). The general objective of this study was to promote plants from the Senegalese flora. In the rest of this work, we will specifically characterize the essential oils of *X. aethiopica* by GC/MS-FID and that of the powder by GC/MS-HS-SPME and then assess the insecticidal activity of essential oils and powder of *X. aethiopica* against *C. maculatus*, the main pest of cowpea, respectively, by contact, fumigation.

Materials and Methods

Materials

Insect rearing

Callosobruchus maculatus adults came from a breeding ground maintained in the Phytosanitary Analysis Laboratory of the Dakar Food Technology Institute at 27 ± 1°C and 70 ± 5% relative humidity (RH) for at least four generations in 1 liter glass jars. Insects used in the tests were young, emerging up to 48 h before the start of the experiment.

Plant material

Cowpea

Seeds of cowpea were purchased from a local market in Dakar (Senegal). To avoid insect infestation, the cowpea seeds were placed in polyethylene bags and stored in a freezer at –4°C for 2 weeks. Finally, they were re-exposed to ambient laboratory conditions before use. Seeds showing any kind of damage were discarded.

Xylopia aethiopica

Fruits of *X. aethiopica* were harvested in the village of Mlomp, a locality located between 12°33'11"N and 16°35'39"W, altitude 30 m, in southern Senegal. A specimen was deposited in the herbarium of "Institut Fondamental d'Afrique Noire" (IFAN) Cheikh Anta DIOP University in Dakar.

Methods

Extraction of essential oils

250 g of dried fruits was submitted to steam distillation for 90 min (with 1.5 l water) using a Clevenger-type apparatus. The essential oil obtained was put in an amber bottle and then stored at 4°C before use.

Xylopia aethiopica powder

Dried fruits of *X. aethiopica* were crushed in a mini electric grinder. The powder obtained was sieved through a 1 mm mesh sieve. It was kept in a glass jar before use at laboratory temperature.

Chromatographic analysis of essential oil by GC/FID

A Trace Ultra GC (Thermo Electron Corporation, Interscience, Milan, Italy) coupled to a flame ionization detector was used. The operation of the oven was the same in GC/FID as in GC/MS. It was first kept isothermally at 40°C for 5 min. Afterwards, the temperature underwent a gradual increase of 8°C · min^{-1} to the limit of 280°C, where it was maintained for 5 min. The injector was operated in splitless mode at 280°C, with a split flow of 30 ml · min^{-1}. The detector temperature was 290°C. Helium was the carrier gas with a constant flow of 1.5 ml · min^{-1}. The capillary column used was of the Optima-5-accent (HP-5MS) 5% phenylmethylsiloxane type (Macherey-Nagel, Germany). It was 30 m long with a diameter of 0.25 mm and a film thickness of 0.25 µm. The volume of the sample injected was 1 µl (10 mg/10 ml n-hexane). The air and hydrogen flows were 350 and 35 ml · min^{-1}, respectively.

Chromatographic analysis of essential oil by GC/SM

The Agilent technologies 5973 Network Mass Selective Detector Quadrupole mass spectrometer was associated with a gas chromatograph, Agilent technologies 6890N (G1530N), USA. The relative abundance of the spectral peaks was between 50 and 550 m/z, for an ionization energy of 70 eV. The identification of the compounds of the essential oils of *X. aethiopica* was carried out by comparing the mass spectra obtained with those of the computerized database (Pal 600K, Wiley 275 L and Nist) and the retention indices with those given in the literature (Joulain and König 1998; Adams 2007). The percentage of each essential oil constituent was calculated by the area normalization method from the GC peak areas calculated as the average value of two injections of each oil. Identification of compounds in the chromatographic profiles was performed by comparing their mass spectra with a library database (Pal 600K, Wiley 275 L and NIST). It was confirmed by comparing retention indices calculated using a series of C7-C30 n-alkanes injected under the same conditions as the essential oils with those of authentic standards (α-pinene, sabinene, β-pinene, myrcene, α-terpinene, p-cymene, 1,8-cineole, γ-terpinene, linalool, trans-pinocarveol and α-terpineol) or with literature values.

Chromatographic analysis of the powder by GC/MS-HS-SPME

The type of SPME fiber used was PDMS/CAR/DVB. Its core was made of fused silica. With a film thickness varying from 50 to 30 µm, the SPME fiber looked like a retractable syringe at its end. It had a 23 gauge needle (OD 0.64 mm) and a length of 10 mm. Two g of substrate (powder) was used for analysis. First, the oven was kept at 50°C for 1 min. Then, the temperature gradually was increased from 4°C · min^{-1} to 200°C for 5 min, then increased from 15°C · min^{-1} to 300°C for 2 min. The sample incubation temperature was 50°C with an incubation time of 5 min followed by shaking at 250 rpm. HS-SPME required operation of the injector in division less mode to allow total desorption of volatile organic compounds in the capillary column type Optima-5-accent, 5%-phenyl-95%--methylsiloxane (Macherey-Nagel, Düren-Germany). It was 30 m long with a diameter of 0.25 mm and a film thickness of 0.25 µm. Desorption lasted 150 s. Helium was the carrier gas with a constant flow rate of 1.5 ml · min^{-1}. The mass spectrometer coupled to the device was of the Agilent technologies 7890A type. It was associated with a gas chromatograph. The relative abundance of the spectral peaks was between 40 and 400 m/z, for ionization energy of 70 eV. The identification of molecules was the same as for GC/MS analysis.

Biological activity

Insecticidal activity of *Xylopia aethiopica* essential oils by fumigation against *Callosobruchus maculatus*

The method used represented a variant to that described by Kouninki *et al.* (2007). Ten adult unsexed insects were used to assess the insecticidal activity of *X. aethiopica* essential oil against *C. maculatus* by fumigation using Petri dishes with a diameter of 9 cm. The upper part of each dish was glued with a cotton ribbon about 3 mm thick, 1 cm wide and 3 cm long. Then, 10 g of healthy cowpea seeds was used as substrate and introduced into each Petri dish. Then, three solutions of essential oils (EO) of *X. aethiopica* were prepared separately in 50 µl of acetone (Ac) EO µl : Ac µl at the following ratios: 12.5 : 50; 25.0 : 50 and 50.0 : 50, and recorded as T2, T3 and T4, respectively. The untreated control and acetone control were recorded as T0 and T1, respectively. Essential oil/acetone mixture was deposited on the cotton attached to the cover of the Petri dishes. After 15 min of evaporation, 10 unsexed adults of *C. maculatus* were added to each Petri dish. The different concentrations of essential oil in addition to the control without treatment and the control treated with acetone were repeated three times. Mortality monitoring was carried out after 24 h for 72 h.

Insecticidal activity of Xylopia aethiopica powder by contact against *Callosobruchus maculatus*

Insecticidal activity by contact was evaluated using the powder of *X. aethiopica* against *C. maculatus*. The tests were carried out in 1 l jars with a moisture content of less than 11%, each containing 100 g of cowpea and *X. aethiopica* powder. To achieve uniform distribution of the powder on the seeds, the jars were agitated manually for 3 min, and then stabilized for 10 min, until all the particles settled. Then, 20 young unsexed adults were added to each jar. Doses of 0.5 : 100, 1.0 : 100 and 2.0 : 100 w/w powder (g) : substrate (g) and a granulometry 1 mm of *X. aethiopica* powder were used during the test.

The treatments included two controls. One was treated with Actellic® and the second was an untreated control. Actellic® insecticide at the recommended dose of 50 g · 100 kg^{-1} was applied at 0.05 g · 100 g^{-1}. 0.5; 1.0 and 2.0 g of powder of *X. aethiopica,* and represented, respectively, T2, T3 and T4 treatments. The untreated control and Actellic® insecticide represented, respectively, T0 and T1. Tests were repeated three times for each treatment. Mortality assessment was made for 7 days after the first day of testing. Dead insects were counted and survivors were returned to their original jars. LD_{50} is defined as the treatment that causes 50%

mortality, and LT_{50} the shortest duration of exposure of insects to different treatments that causes 50% mortality. Abbott's formula (Abbott 1925) was used to correct natural mortality:

$$\%Mcorr = \frac{Mo - Mt}{100 - Mt} \times 100,$$

where: *Mcorr* – corrected mortality (%), *Mo* – mortality in the treated group, *Mt* – mortality in the control group.

Statistical analysis

Statistical analysis was performed with XLSTAT-Pro 6.1.9 software and the treatment data were analyzed and compared by an analysis of variance (ANOVA).

The extraction results of essential oil yield and the insecticidal activity were expressed in mean values ± standard deviation ($n = 3$), and the differences with $p < 0.05$ were considered significant according to the Tukey test.

Results

Essential oil yield

The color of the essential oil was yellow. The extraction of essential oils from dried fruits of *X. aethiopica* obtained by steam distillation gave a yield of 1.19 ± 0.2%.

Table 1. Chemical composition of *Xylopia aethiopica* essential oils obtained by GC/MS-FID analysis

Compounds	RI	*X. aethiopica* EO	Identification	Compounds	RI	*X. aethiopica* EO	Identification
α-Pinene	937	9.8 ± 1.0	MS. RI. Std	α-Copaene	1390	3.1 ± 0.1	MS. RI
Sabinene	976	5.2 ± 1.1	MS. RI. Std	β-Cubebene	1400	0.7 ± 0.1	MS. RI
β-Pinene	982	28.9 ± 2.0	MS. RI. Std	Cyperene	1423	0.8 ± 0.1	MS. RI
Myrcene	989	0.5 ± 0.2	MS. RI. Std	(E)-β-Caryophyllene	1434	2.3 ± 0.8	MS. RI
NI	993	0.3 ± 0.0	–	trans-α-Bergamotene	1440	Tr	MS. RI
NI	997	0.3 ± 0.1	–	γ-Elemene	1445	1.3 ± 0.5	MS. RI
α-Phellandrene	1008	0.5 ± 0.1	MS. RI	Aromadendrene	1460	0.5 ± 0.2	MS. RI
α-Terpinene	1020	0.2 ± 0.1	MS. RI. Std	α-Humulene	1467	0.6 ± 0.2	MS. RI
p-Cymene	1028	2.9 ± 0.2	MS. RI. Std	Germacrene D	1486	1.5 ± 0.5	MS. RI
Limonene	1033	1.2 ± 0.2	MS. RI	α-Amorphene	1496	Tr	MS. RI
1,8-Cineole	1037	14.9 ± 0.2	MS. RI. Std	β-Selinene	1500	Tr	MS. RI
γ-Terpinene	1062	0.3 ± 0.1	MS. RI. Std	α-Muurolene	1508	0.8 ± 0.2	MS. RI
cis-Sabinene hydrate	1075	0.6 ± 0.1	MS. RI	γ-Cadinene	1516	0.5 ± 0.2	MS. RI
Linalool	1100	3.1 ± 0.5	MS. RI. Std	δ-Cadinene	1529	0.7 ± 0.1	MS. RI
n-Nonanal	1105	0.3 ± 0.1	MS. RI	cis-Calamenene	1534	0.3 ± 0.1	MS. RI
α-Campholenal	1133	0.2 ± 0.2	MS. RI	Elemol	1568	0.4 ± 0.1	MS. RI
trans-Pinocarveol	1150	3.9 ± 1.4	MS. RI. Std	Spathulenol	1595	0.7 ± 0.1	MS. RI
5-Undecyne	1165	0.1 ± 0.1	MS. RI	Caryophyllene oxide	1600	Tr	MS. RI
Pinocarvone	1171	1.0 ± 0.3	MS. RI	NI	1603	Tr	–
δ-Terpineol	1176	0.2 ± 0.2	MS. RI	Salvial-4(14)-en-1-one	1613	0.3 ± 0.0	MS. RI
Terpinen-4-ol	1187	1.1 ± 0.1	MS. RI	NI	1631	0.3 ± 0.1	–
Cryptone	1195	0.5 ± 0.2	MS. RI	γ-Eudesmol	1642	2.4 ± 0.4	MS. RI
α-Terpineol	1200	2.0 ± 0.5	MS. RI. Std	β-Eudesmol	1670	Tr	MS. RI
Myrtenol	1203	3.4 ± 1.2	MS. RI	α-Eudesmol	1676	0.3 ± 0.0	MS. RI
α-Phellandrene epoxide	1210	Tr	MS. RI	Monoterpenic hydrocarbons		49.4 ± 4.0	
Verbenone	1216	Tr	MS. RI	Oxygenated monoterpenes		31.4 ± 4.3	
trans-Carveol	1224	Tr	MS. RI	Sesquiterpenic hydrocarbons		14.0 ± 2.1	
Carvone	1251	0.1 ± 0.2	MS. RI	Oxygenated sesquiterpenes		4.1 ± 0.7	
δ-Elemene	1345	0.6 ± 0.3	MS. RI	Not identified		0.9 ± 0.1	
α-Cubebene	1358	0.3 ± 0.0	MS. RI				

RI – retention indices, MS – mass spectrometry, Inj – injection of pure compound, Std – standard, Tr – trace (<0.05%)

Chromatographic analysis of essential oils by GC/MS-FID and powder by GC/MS-HS-SPME

The results showed that the powder had larger amounts of extracted components than essential oils. Qualitative and quantitative differences were noted between the characterization of essential oils (EO) and powder (P) of *X. aethiopica*. 50 and 59 compounds were identified, respectively, in EO and P of *X. aethiopica*, representing 99.3 and 99.8% of total volatile compounds (Tables 1 and 2). Other compounds with percentages greater than 1.0% were identified in the oil: sabinene (5.2%), α-copaene (5.6%), trans-pinocarveol (3.9%), linalool (3.1%), myrtenol (3.4%), α-terpineol (2.0%), γ-eudesmol (2.4%), (E)-β-caryophyllene (2.3%), and p-cymene (2.9%). Nine trace (tr) compounds were also identified and among them we found: verbenone, β-selinene and β-eudesmol which belong, respectively, to oxygenated monoterpenes and hydrocarbon and oxygenated sesquiterpenes. For the powder,

Table 2. Chemical composition of *Xylopia aethiopica* powder obtained by GC/MS-HS-SPME analysis

Compounds	RI	*X. aethiopica* powder	Identification	Compounds	RI	*X. aethiopica* powder	Identification
α-Pinene	934	19.4 ± 0.4	MS. RI. Std	trans-Carveol	1204	Tr	MS. RI
Sabinene	976	8.8 ± 0.1	MS. RI. Std	Carvone	1208	Tr	MS. RI
β-Pinene	983	19.0 ± 0.6	MS. RI. Std	Isopulegol acetate	1217	0.1 ± 0.0	MS. RI
Myrcene	995	0.2 ± 0.0	MS. RI. Std	δ-Elemene	1426	3.3 ± 0.1	MS. RI
α-Phellandrene	1007	0.2 ± 0.0	MS. RI	α-Cubebene	1433	0.6 ± 0.0	MS. RI
δ-3-Carene	1011	2.0 ± 0.1	MS. RI	α-Copaene	1456	4.4 ± 0.0	MS. RI
α-Terpinene	1021	1.5 ± 0.1	MS. RI. Std	β-Cubebene	1464	0.4 ± 0.0	MS. RI
p-Cymene	1025	Tr	MS. RI. Std	β-Elemene	1466	0.5 ± 0.0	MS. RI
Limonene	1032	Tr	MS. RI	Cyperene	1467	Tr	MS. RI
β-Phellandrene	1038	7.6 ± 0.4	MS. RI. Std	α-Gurjunene	1471	2.6 ± 0.1	MS. RI
1,8-Cineole	1041	7.7 ± 0.1	MS. RI. Std	(E)-β-Caryophyllene	1473	Tr	MS. RI
cis-Ocimene	1043	0.2 ± 0.1	MS. RI	trans-α-Bergamotene	1516	Tr	MS. RI
γ-Terpinene	1066	2.0 ± 0.1	MS. RI. Std	γ-Elemene	1523	1.5 ± 0.0	MS. RI
trans-Sabinene hydrate acetate	1074	0.7 ± 0.0	MS. RI	Aromadendrene	1527	Tr	MS. RI
α -Terpinolene	1098	0.9 ± 0.0	MS. RI	α-Humulene	1533	Tr	MS. RI
Linalool	1112	2.1 ± 0.1	MS. RI. Std	γ-Muurolene	1537	2.6 ± 0.1	MS. RI
n-Nonanal	1115	Tr	MS. RI	Bicyclosesquiphellandrene	1556	0.5 ± 0.1	MS. RI
α-Campholenal	1120	0.2 ± 0.0	MS. RI	Germacrene D	1576	6.5 ± 0.1	MS. RI
allo-Ocimene	1124	0.1 ± 0.0	MS. RI	α-Amorphene	1579	Tr	MS. RI
trans-Pinocarveol	1133	0.6 ± 0.0	MS. RI. Std	β-Selinene	1583	Tr	MS. RI
Verbenol	1139	0.1 ± 0.0	MS. RI	α-Muurolene	1590	0.5 ± 0.0	MS. RI
Menthone	1151	0.2 ± 0.0	MS. RI	γ-Cadinene	1607	0.6 ± 0.1	MS. RI
5-Undecyne	1152	Tr	MS. RI	δ-Cadinene	1616	0.6 ± 0.2	MS. RI
Pinocarvone	1156	0.3 ± 0.0	MS. RI	cis-Calamenene 1528	1623	Tr	MS. RI
Cryptone	1160	Tr	MS. RI	Elemol 1547	1640	Tr	MS. RI
α-Terpineol	1171	0.3 ± 0.0	MS. RI. Std	Isoaromadendrene epoxide	1685	0.1 ± 0.0	MS. RI
cis-9-Methyl-2-octalin	1179	0.1 ± 0.0	MS. RI	Spathulenol	1686	0.4 ± 0.1	MS. RI
Linalyl propionate	1186	0.7 ± 0.0	MS. RI	Monoterpenic hydrocarbons		62.0 ± 0.5	
Myrtenal	1190	0.7 ± 0.0	MS. RI	Oxygenated monoterpenes		13.9 ± 0.2	
Myrtenol	1191	Tr	MS. RI	Sesquiterpenic hydrocarbons		24.0 ± 0.3	
α-Phellandrene epoxide	1194	Tr	MS. RI	Oxygenated sesquiterpenes		0.5 ± 0.1	
Verbenone	1203	0.1 ± 0.0	MS. RI				

RI – retention indices, MS – mass spectrometry, Inj – injection of pure compound, Std – standard, Tr – trace (<0.05%)

compounds with relatively low contents included: cis-ocimene (0.2%), verbenol (0.1%), β-cubebene (0.4%) and isoaromadendrene epoxide (0.1%), which, respectively, belong to the hydrocarbon monoterpenes and oxygenated and hydrocarbon and oxygenated sesquiterpenes. Among the 59 compounds identified in the powder, germacrene D (6.5%), α-copaene (4.4%), allo-ocimene (3.1%), α-copaene (2.7%), myrtenol (2.4%), β-selinene (2.2%) and trans-pinocarveol (2.0%) represented the compounds with a percentage greater than 1. Eighteen compounds such as: β-eudesmol, caryophyllene oxide, aromadendrene, 5-undecyne, sabinene were also identified in the powder. The differences between the two techniques were mainly observed in low rates and a few main compounds. Indeed, limonene and p-cymene were detected in trace amounts in *X. aethiopica* powder by the GC/MS-HS-SPME method. On the other hand, analysis of essential oils by GC-MS/FID revealed levels of 2.9 ± 0.2% and 1.2 ± 0.2%, respectively, for p-cymene and limonene. In addition, the majority of compounds were very representative, respectively, in powder and oils for α-pinene (19.4 ± 0.4% – 9.8 ± 1.0%). On the other hand, β-pinene (19.0 ± 0.6% – 28.9 ± 2.0%) and 1,8-cineole (7.7 ± 0.1% – 14.9 ± 0.2%) were identified more in oils than in powder. Thus, qualitatively, analysis by GC/MS-HS-SPME allowed for the detection of an important number of volatile compounds which were relatively similar to those of the essential oil characterized by GC-MS/FID. Four unidentified compounds were also found in the essential oil and none in the powder. The contents of hydrocarbon monoterpenes (49.4% EO and 62.0% P) and hydrocarbon sesquiterpenes (14.0% EO and 24.0% P) were higher in the powder than in the essential oils. δ-Terpineol and terpinen-4-ol were found in oil and but not in the powder. Similarly, β-phellandrene (7.7%) and trans-sabinene hydrate acetate (0.7%) were identified in the powder but were absent in the oil.

Insecticidal activity of *Xylopia aethiopica* essential oils by fumigation against *Callosobruchus maculatus*

Table 3 shows the efficiency of *X. aethiopica* essential oil against *C. maculatus* by fumigation. It reveals that no mortality was observed in the T0 (blank control) and T1 (control with acetone) treatments, indicating that acetone did not have an insecticidal effect against *C. maculatus* for this formulation. T2, T3 and T4 treatments were all effective compared to T0 and T1 treatments where no mortality was recorded (Table 3). T3 treatment, although it had the lowest dose of essential oils, caused a 50% mortality in less than 24 hours with an LD_{50} = 0.2 ± 0.0 µl/cm^3 ($y = -0.0301x^2 + 3.5758x - 2.7273$; $R^2 = 0.9847$) and LT_{50} = 16.4 ± 1.2 h. The T3 treatment (48 h and 72 h, caused 90.0 and 96.7% mortality, respectively) did not present any significant difference compared to T4 (24, 48 and 72 h) where 100% mortality was noted for each duration of treatment.

Table 3. Efficiency of essential oils of *Xylopia aethiopica* by fumigation according to time against *Callosobruchus maculatus*

	Mortality [%]		
Treatments	24 h	48 h	72 h
T0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
T1 (Acetone)	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
T2	30.0 ± 10.0 b	50.0 ± 10.0 bc	80.0 ± 10.0 de
T3	73.3 ± 5.3 d	90.0 ± 10.0 e	96.6 ± 5.8 e
T4	100.0 ± 0.0 e	100.0 ± 0.0 e	100.0 ± 0.0 e

T0 – blank control; T1 – control with acetone; T2 – 12.5 µl; T3 = 25.0 µl; T4 – 50.0 µl

The data are mean ± standard deviation of three replicates. Mortality means in a row followed by a similar superscript are not different ($p < 0.05$). LD50 is defined as the lowest concentration which causes 50.0% mortality and LT50 the shortest exposure time of insects compared to the different concentrations and which causes 50.0% mortality

Insecticidal activity of *Xylopia aethiopica* powder by contact against *Callosobruchus maculatus*

Table 4 shows the efficiency of *X. aethiopica* powder against *C. maculatus* by contact. It reveals that no mortality was recorded in the T0 treatment (blank control), unlike the T1 treatment (Actellic) where 100% mortality was noted (Table 4). It also does not show any significant difference for T2 and T3 treatments during the 1st day compared to T0 treatment with the exception of T4 treatment. On the other hand, from the 2nd day of treatment, a clearly significant difference was noted for T2, T3 and T4 compared to T0. On the 4th day of evaluation, the T2 treatment (0.5 g of powdered *X. aethiopica*), resulted in 50% mortality with an $LD_{50} = 9.2 \pm 0.7$ g · kg^{-1} ($y = 25.538x^2 - 28.300x + 54.467$, $R^2 = 1$) and LT_{50} = 69.6 ± 0.4 h. In addition, treatments T2 (6 and 7 days), T3 (5, 6 and 7 days) and T4 (3, 4, 5, 6 and 7 days) were significantly equal to T1. They resulted in 100% mortality unlike the previous days for the same treatments.

Table 4. Efficiency of *Xylopia aethiopica* powder by contact according to days against *Callosobruchus maculatus*

Treatments	Mortality [%]						
	day 1	day 2	day 3	day 4	dday 5	day 6	day 7
T0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
T1 (Actellic)	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f
T2	3.3 ± 2.9 a	23.3 ± 5.8 b	46.7 ± 7.6 c	58.3 ± 8.7 d	75.0 ± 5.0 e	100.0 ± 0.0 f	100.0 ± 0.0 f
T3	6.7 ± 5.8 a	23.3 ± 5.8 b	51.7 ± 7.6 cd	66.7 ± 5.8 e	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f
T4	30.0 ± 2.9 b	56.7 ± 5.8 cd	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f	100.0 ± 0.0 f

T0 – blank control; T1 – control actellic; T2 – 0.5 g; T3 –1.0 g; T4 – 2.0 g
The data are mean ± standard deviation of three replicates. Mortality means in a row followed by a similar superscript are not different ($p < 0.05$). LD_{50} is defined as the lowest concentration, which causes 50% mortality, and LT_{50} the shortest exposure time of insects compared to the different concentrations and which causes 50% mortality

Discussion

Extraction yield of essential oils

The extraction yield (1.19 ± 0.2%) of essential oils by steam distillation from dried fruits of *X. aethiopica* was less than that from the work of Nguemtchouin (2012) from Cameroon, who obtained 5.2%. However, it was higher than that of Boniface *et al.* (2010) from Benin, who obtained 0.6%. This difference in yield depends on several factors, including harvest period, duration of extraction, organ studied and vegetative stage of species. For example, extraction yields of *Cymbopogon citratus* leaves collected from two different localities in Senegal yielded 0.4 and 0.6% (Diop *et al.* 2017). Furthermore, after 21 days of drying *Eucalyptus alba* leaves, extraction yields were 0.3% after 2 h of extraction and 0.9% after 4 h of extraction (Ndiaye *et al.* 2017). On the other hand, for the same extraction time of *Apium graveolens* essential oils, Thiam *et al.* (2020) obtained yields of 1.1% and 0.4%, respectively, for leaves and stems.

Chemical composition of essential oils and powder

The analysis by GC/MS-FID of the essential oils (EO) of *X. aethiopica* on the one hand and of the powder (P) by GC/MS-HS-SPME on the other hand primarily revealed the following compounds of different proportions: β-pinene (28.9–19.0%), 1.8-cineole (14.9–7.7%) and α-pinene (9.8–19.4%), respectively, for the essential oil and for the powder. These two characterization methods (GC/MS-FID and GC/MS-HS-SPME) made it possible to confirm the chemotype of *X. aethiopica* specie from Senegal. This variation in the chemical composition noted between the essential oil and the powder of *X. aethiopica* can be explained by the use of different analysis methods (GC/MS-FID and GC/MS-HS-SPME) for characterization and distillation (Jirovetz *et al.* 2004). The high rate of hydrocarbon derivatives in the powder is due to the fact that after grinding the dried fruits of *X. aethiopica*, the most volatile molecules (hydrocarbon monoterpenes) are almost completely retained in the vesicles secreting essential oils. These are picked up by the PDMS/CAR/DVB fiber at the time of incubation at 50°C and desorbed entirely in the column, hence the abundance noted in monoterpene hydrocarbon derivatives. In addition, a predominance of oxygenated compounds was noted in essential oils compared to the powder (oxygenated monoterpenes 31.4% EO and 13.9% P, oxygenated sesquiterpenes 4.1% EO and 0.5% P). The high level of oxygenated derivatives can be explained by the fact that during the extraction of essential oils either by hydrodistillation or by entrainment with steam, certain non-oxygenated and unsaturated molecules can hydrate easily, thus increasing the oxygen derivatives (Nguemtchouin 2012).

Insecticidal activity of *Xylopia aethiopica* essential oils by fumigation against *Callosobruchus maculatus*

The use of plant products for the management of food grains against stored product coleopterans (weevils and beetles) infestation is an ongoing practice in many developing countries. Table 3 showed good activity of *X. aethiopica* essential oils against *C. maculatus* for T3 (mean mortality 73.3% for 0.9 g) and T4 (mean mortality 100%) treatments after 24 h. Our results are similar to those of Edwin *et al.* (2018). According to them, *X. aethiopica* gave higher mortality on *C. maculatus* whose mean mortality and LD_{50} was 79.9% and 1.12 g respectively over 96 h exposure. In summary, for each treatment, the mortality was proportional to the dose and the duration of exposure of the insects to essential oils. The mortalities recorded for the various treatments with essential oils were due to the presence

of oxygenated organic compounds (31.4% of volatile compounds), in particular 1,8-cineole (14.9%) which is known for its insecticidal activity (Ilboudo 2009). β-Pinene (28.9%) and α-pinene (8.9%) are known for their insecticidal activities against *C. maculatus* (Edwin and Jacob 2017). They could act in perfect synergy with other terpenes including 1,8-cineole to cause this high mortality. Moreover, although linalool is weakly represented in *X. aethiopica* essential oil from Senegal (3.1%), it can affect the central nervous system of insects by enzymatic inhibition of acetylcholinesterase (Keane and Ryan 1999). Therefore, linalool could be considered as an irreversible inhibitor with neurotoxic effects in the insect by causing paralysis and intoxication in the insect which can lead to its death.

Insecticidal activity of *Xylopia aethiopica* powder by contact against *Callosobruchus maculatus*

In this study, weevil mortality increased with an increase in the concentration of *X. aethiopica* powders. Table 4 shows that *X. aethiopica* powder gave higher kill on *C. maculatus*. The sensitivity of adult *C. maculatus* to various concentrations of powder and extracts of *X. aethiopica* revealed considerable variation in effectiveness. According to the results, the mortalities caused by the T2, T3 and T4 treatments were proportional to the duration of contact and the amount of powder used. Thus, these results corroborate the work of Kayombo *et al.* (2014). The insecticidal activity of *X. aethiopica* powder is due to its high content of α-pinene (19.0%), β-pinene (19.4%) and 1,8-cineole (7.7%). Their insecticidal powers indicate a neurotoxic mode of action against *C. maculatus*. They could also act in perfect synergy to inhibit octopamine which is a neurotransmitter that ensures contact between two different neurons for the transmission of nerve impulses (Enan 2001; Kostyukovsky *et al.* 2002). This would cause paralysis, which could lead to the death of the insects. Moreover, the insecticidal activity of these volatile compounds present in the powder of *X. aethiopica* is similar to that of synthetic insecticides such as organophosphates and carbamates (Isman 2006). Mills *et al.* (2004) reported the possible mode of action of essential oils including 1,8-cineole as a reversible competitive inhibitor of the enzyme acetylcholinesterase. On the other hand, the volatile constituents of the powder are likely to inhibit the ionotropic GABA receptors (Sattelle *et al.* 1988). The mortalities caused can be explained by the fact that volatile substances and powder would exert an abrasive action through their friction with the cuticle of the insect, thus causing dehydration with adsorption of the lipoid film which results in the death of the insect by desiccation (Korunic 1998; Kabir 2013). The insect mortality may equally be due to the blocking of the insects' spiracles by dust particles, causing death by asphyxia (Adedire *et al.* 2011; Fernando and Karunaratne 2012). According to Sarwar *et al.* (2012), the striking effects of plant powders could be attributed to the presence of their toxic components and irritating smell which prevented physical contact of adult weevils with grains and caused suffocation or starvation of the pest. After 7 days of mortality assessment for each treatment, no insect survived. Jars of each treatment were kept in the laboratory under the same conditions as during the tests. Ultimately, the powder of *X. aethiopica* can be considered as a very effective bio-insecticide against *C. maculatus*, the main pest of cowpea in sub-Saharan Africa.

Conclusions

This study allowed us to characterize the powder and EO of *X. aethiopica* by GC-MS/FID and GC/MS-HS-SPME, respectively, and to assess their insecticidal activity against *C. maculatus*, the main pest of cowpea. Thus, analyses by GC/MS-FID (EO) and GC/MS-HS-SPME (powder) mainly revealed the following compounds with different proportions: β-pinene (28.9–19.0%), 1,8-cineole (14.9–7.7%) and α-pinene (9.8–19.4%), respectively, for the essential oil and for the powder. Dried fruits of *X. aethiopica* have an insecticidal activity against *C. maculatus* LD_{50} = 0.2 ± 0.0 µl · cm^{-3} and LT_{50} = 16.4 ± 1.2 h; LD_{50} = 9.2 ± 0.7 g · kg^{-1} and LT_{50} = 69.6 ± 0.4 h, respectively, for EO and powder. Ultimately, the powder of *X. aethiopica* can be considered to be a very effective bio-insecticide against *C. maculatus* and a post-harvest preservative of this legume which constitutes an essential source of our food. It can also replace synthetic insecticides which are reputed to be a potential source of many diseases (cancer and tumors). The results of the present study confirm *X. aethiopica* plant bioactivity against *C. maculatus,* making it effective in protecting cowpea seeds from insect infestation and damage which are important factors for farmers with limited resources and low volume seed storage. Since adult *C. maculatus* do not feed on stored cowpea seeds but only lay their eggs, mixing the seeds with vegetable powders is recommended as an environmentally friendly and non-toxic method of managing *C. maculatus* for short-term storage. Further study is necessary to determine the relative amounts of these materials required for a pest control.

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References

Abbott W.S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265–267.

Adams R. 2007. Identification of Essential Oil Components by Gas Chromatography/Qua-drupole Mass Spectrometry. 4th ed., Allured Publishing Co, Carol Stream IL., USA.

Adedire C.O., Obembe O.M., Akinkurolere R.O., Oduleye S.O. 2011. Response of *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. Journal of Plant Diseases and Protection 118 (2): 75–79. DOI: 10.1007/BF03356385

Ahmed S., Khan M.A., Ahmad N. 2002. Determination of susceptibility level of phosphine in various strains of dhora (*Callosobruchus maculatus* F.). International Journal of Agriculture and Biology 4: 329–331.

ANSD. 2018. Bulletin mensuel des statistiques économiques. Ministère de l'économie, des finances et du plan Sénégal, 140 pp. (in French)

Beck C.W., Bulmer L.S.A. 2014. Handbook on Bean Beetles, *Callosobruchus maculatus*. Texas A&M AgriLife Extension, Cowpea Weevils.

Boniface Y., Jean-Pierre N., Philippe S., Félicien A., Dominique S. 2010. Etude chimique et activités antimicrobiennes d'extraits volatils des feuilles et fruits de *Xylopia aethiopica* (DUNAL) A. Richard contre les pathogènes des denrées alimentaires. Journal de la Société Ouest-Africaine de Chimie 29: 19–27.

Chougourou D.C., Alavo T.B.C. 2011. Systèmes de stockage et méthodes endogènes de lutte contre les insectes ravageurs des légumineuses à grains entreposées au Centre Bénin. in Revue CAMES – Série A 12 (2): 137–141. (in French)

Diop S.M., Gueye M.T., Ndiaye I., Ndiaye E.H.B., Diop M.B., Thiam A., Fauconnier, M.L., Lognay G. 2017. Study of the chemical composition of essential oils and floral waters of *Cymbopogon citratus* (DC.) Stapf (Poaceae) from Senegal. International Journal of Biological and Chemical Sciences 11 (4): 1884–1892. DOI: 10.4314/ijbcs.v11i4.37

Edwin E., Regina A., Ifeoma V. 2018. Insecticidal activity of *Xylopia aethiopica* (Family; Annonaceae) against *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). Journal of Biological Studies 1 (3): 106–115.

Edwin I.E., Jacob I.E. 2017. Bio-insecticidal potency of five plant extracts against Cowpea Weevil, *Callosobruchus maculatus* (F.), on Stored Cowpea, *Vigna unguiculata* (L). Jordan Journal of Biological Sciences 10 (4): 317–322.

Enan E. 2001. Insecticidal activity of essential oils: octopaminergic sites of action. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 130 (3): 325–337. DOI: 10.1016/S1532-0456(01)00255-1

Fernando H.S., Karunaratne M.M. 2012. Ethnobotanicals for storage insect pest management: Effect of powdered leaves of *Olax zeylanica* in suppressing infestations of rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Journal of Tropical Forestry and Environment 2: 20–25.

Guènomy, Society and Environment 15 (1): 183–194. ye M.T., Seck D., Wathelet J.P., Lognay G. 2011. Lutte contre les Ravageurs des stocks de céréales et de légumineuses au Sénégal et en Afrique occidentale: synthèse bibliographique. Biotechnology, Agro

Ilboudo Z. 2009. Activité Biologique de quatre huiles essentielles contre *Callosobruchus maculatus* Fab. (Coleoptera: Bruchidae), insecte ravageur des stocks de niébé au Burkina Faso, Entomologie, Université de Ouagadougou, Burkina Fasso, 150 pp.

Isman M.B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51 (1): 45–66. DOI: 10.1146/annurev.ento.51.110104.151146

Jirovetz L., Wobus A., Buchbauer G., Shafi M.P., Thampi P.T. 2004. Comparative analysis of the essential oil and SPME-headspace aroma compounds of *Cyperus rotundus* L. roots/tubers from South-India using GC, GC-MS and olfactometry. Journal of Essential Oil Bearing Plants 7 (2): 100–106. DOI: 10.1080/0972-060X.2004.10643373

Joulain D., König W. 1998. The Atlas of Sesquiterpene Data Hydrocarbons. E.B.-Verlag, Hamburg, Germany.

Kabir B.G.J. 2013. Laboratory evaluation of efficacy of three Diatomaceous earth formulations against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) in stored wheat. European Scientific Journal 30 (9): 116–124.

Kayombo M.A., Mutombo T.J.M., Somue M.A., Muka M.P., Wembonyama O.M., Tshibangu B.K.E., Kaboko K.J. 2014. Effet de la poudre de Basilic (*Ocimum basilicum*) dans la conservation des graines de Niébé (*Vigna unguiculata* L. Walp.) en stock contre *Callosobruchus maculatus* F. à Mbuji-Mayi (RD. Congo). Congo Sciences 2 (2): 61–66.

Keane S., Ryan M.F. 1999. Purification, characterisation, and inhibition by monoterpenes of acetylcholinesterase from the waxmoth, *Galleria mellonella* (L.). Insect Biochemistry and Molecular Biology 29 (12): 1097–1104. DOI: 10.1016/S0965-1748(99)00088-0

Koffi S.E., Roger H.C.N., Kodjo E., Kokou A.A., Kokouvi D., Honoré K.K. 2012. Chemical composition and insecticidal activity of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) essential oil on *Callosobruchus maculatus*. Journal de la Societé Ouest-africaine de Chimie 34: 71–77.

Korunic Z. 1998. Review Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research 34 (2–3): 87–97. DOI: 10.1016/S0022-474X(97)00039-8

Kostyukovsky K., Rafaeli A., Gileadi C., Demchenko N., Shaaya E. 2002. Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: possible mode of action against insect pests. Pest Management Science 58 (11): 1101–1106. DOI: 10.1002/ps.548

Kouninki H., Hance T.F.A., Noudjou F.A., Lognay G., Malaisse F., Ngassoum M.B., Mapongmetsem P.M., Ngamo T.L.S., Haubruge E. 2007. Toxicity of some terpenoids of essential oils of *Xylopia aethiopica* from Cameroon against *Sitophilus zeamais* Motschulsky. Journal of Applied Entomology 131 (4): 269–274.

Mills C., Cleary B.V., Walsh J.J., Gilmer J.F. 2010. Inhibition of acetylcholinesterase by tea tree oil. Journal of Pharmacy and Pharmacology 56 (3): 375–379. DOI: 10.1211/0022357022773

Mukendi K.R., Ntanga N.R., Kaseba K.S., Tshiamala N., Kamukenji A. and Mpoyi K.G. 2016. Dégâts des bruches sur le pouvoir germinatif des graines de quatre variétés de Niébé infesté pendant 60 jours à Ngandajika. Journal of Applied Biosciences 98: 9323–9329. DOI: http://dx.doi.org/10.4314/jab.v98i1.8

Ndiaye E.H.B., Gueye M.T., Ndiaye I., Diop S.M., Diop M.B., Thiam A., Fauconnier M.L., Lognay G. 2017. Chemical composition of distilled essential oils and hydrosols of four senegalese citrus and enantiomeric characterization of chiral compounds. Journal of Essential Oil Bearing Plants 20 (3): 820–834.

Ngamo L., Hanc T.H. 2007. Diversité des ravageurs des denrées et méthodes alternatives de lutte en milieu tropical. Tropicultura 25 (4): 215–220.

Nguemtchouin M.G.M. 2012. Formulation d'insecticides en poudre par adsorption des huiles essentielles de *Xylopia aethiopica* et de *Ocimum gratissimum* sur des argiles camerounaises modifiées. Thèse doctotat en cotutelle, universités Ngaoundere et Montpellier, 293 pp.

Sahaf B.Z., Moharramipour S., Meshkatalsadat M.H. 2008. Fumigant toxicity of essential oil from *Vitex pseudo-negundo* against *Tribolium vastaneum* (Herbst) and *Sitophilus orzae* (L.). Journal of Asia-Pacific Entomology 11 (4): 175–179.

Sarwar M., Ahmad N., Bux M., Tofique M. 2012. Potential of plant materials for the management of cowpea bruchid *Callosobruchus analis* (Coleoptera: Bruchidae) in gram *Cicer arietinum* during storage. The Nucleus 49 (1): 61–64.

Sattelle D.B., Pinnock R.D., Wafford K.A., David J.A. 1988. GABA receptors on the cell-body membrane of an identified insect motor neuron. Proceedings of the Royal Society B: Biological Sciences 232 (1269): 443–456. DOI: 10.1098/rspb.1988.0006.

Thiam A., Guèye M.T., Ndiaye I., Diop S.M., Ndiaye E.H.B., Fauconnier M.L., Lognay G. 2018. Effect of drying methods on the chemical composition of essential oils of *Xylopia aethiopica* fruits (Dunal) A. Richard (Annonaceae) from southern Senegal. American Journal of Essential Oils and Natural Products 6 (1): 25–30.

Thiam A., Gueye M.T., Sanghare C.H., Ndiaye E.H.B., Diop S.M., Cissokho P.S., Diop M.B., Ndiaye I., Fauconnier M.L. 2020. Chemical composition and anti-inflammatory activity of *Apium graveolens* var. dulce essential oils from Senegal. American Journal of Food Science and Technology 8 (6): 226–232. DOI: 10.12691/ajfst-8-6-1